EUROPEAN CULTURE COLLECTIONS' ORGANISATION

THE FEDERAL RESEARCH CENTRE "FUNDAMENTALS OF BIOTECHNOLOGY"

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INSTITUTE OF ECOLOGY AND GENETICS OF MICROORGANISMS RAS

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The success of previous ECCO meetings demonstrates that personal contacts among the active professionals from culture collections are of great importance for exchanging best practices in this field and are not replaceable by the modern technical communication tools.

Activities related to culture collections are multifaceted. Microorganisms are in the focus of attention. Culturing of previously non-culturable microorganisms and gaining insight into microbial associations change our perception of them from a «thing-in-itself» to a «thing-for-us». Cultivable microorganisms and microbiomes should be characterized and preserved with minimal losses in order to provide different customers with standard high-quality biomaterials. Culture collections, having the status of Core Facilities, are expected to follow the commonly accepted and approved practices in biosafety, biosecurity and legitimate transfer of genetic resources.

The ways to achieve the above goals are discussed at the XXXVII ECCO meeting.

Lev Kalakoutskii,
All-Russian Collection of Microorganisms (VKM)

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THE DIVERSITY OF BACTERIA ISOLATED FROM THE EXTREME HABITATS OF INNER ASIA

Elena Abidueva, Elena Lavrentyeva, Erzhena Danilova, Olga Dagurova, Svetlana Zaitseva, Lyudmila Kozyreva, Dulma Tsyrenova, Tuyana Banzaraktsaeva, Aryuna Radnagurueva, Darima Barkhutova

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A unique feature of Inner Asia (the Republic of Buryatia, the Transbaikal Region, the Republic of Tuva (Russia), Mongolia, Inner Mongolia (China) is the wide spread of extreme habitats, which are the places of survival, conservation and active geochemical activity of microbial communities and reservoirs of isolation of extremophilic bacteria with biotechnological potential. Diversity of extremophilic bacteria was studied and collection of microorganisms in Laboratory of microbiology IGEB SB RAS was created.

The collection contains over 150 strains of bacteria, representatives of 7 phyla. The most of them belong to phylum *Firmicutes* (88 strains), family *Bacillaceae* (86 strains). Cyanobacteria is represented by three orders, the most of them belong to *Oscillatoriales.* New types of bacteria: the thermophilic strain "*Anoxybacillus mongoliensis* sp.nov", the alkalitolerant organotrophic strain "*Belliella buryatensis* sp.nov.", the alkaliphilic sulfate reducing strain "*Desulfonatronum zhilinae* sp.nov.", the neutrophilic sulfate reducing strain "*Desulfovibrio alcoholivorans*" were described.

The activity of proteolytic enzymes of alkalothermophilic strains was studied. Their thermal stability (up to 70°C), activity in the alkaline pH range (up to 10.8), wide substrate specificity were detected.

The collection can be used in fundamental microbiology and biotechnology.

The work was supported by IGEB SB RAS Budget Project AAAA-A17–117011810034–9 and grants RFBR No. 18–44–030021 and No. 18–44–030028.

PROKARYOTIC TAXONOMY 3.0

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In the view of this author, modern bacterial taxonomy has witnessed three major shifts from the methodological point of view. In chronological order these are: i) DNA-DNA hybridization (DDH), ii) 16S rRNA sequence analysis (16S) and iii) whole genome sequence analysis (WGS). Stating this does not mean denying the contribution of other developments such as numerical taxonomy, chemotaxonomic methods or multilocus sequence schemes to name a few, but none of those have had such a wide range application to all prokaryotic taxa and none of them has had such a profound impact. It is also important to notice that the contribution if DDH was to provide a gold standard for species delineation whereas 16S provided a backbone for phylogeny and classification. Together they have ruled prokaryotic systematics for about two decades. Currently, WGS can be used on a routine basis for both purposes with greater accuracy. As a matter of fact wet lab DDH is now considered an obsolete approach by many editors and reviewers that will pose objections to accept proposals that rely on this methodology. On the contrary, 16S is still widely in use in prokaryotic

systematics. This is so for pragmatic reasons: its limitations are well-known but they are surpassed by its many advantages and even serves as authenticity control for validation of WGS [1]. The purpose of the talk is to show the impact of these three techniques and introduce the present and future trends in prokaryotic taxonomy.

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ASTROBIOLOGICAL COLLECTION OF BACTERIA FROM EXTREME HABITATS

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Since 2013 the Laboratory of Soil Microbiology of Soil Science Faculty of Lomonosov Moscow State University has been performing creation and replenishment of Astrobiological collection of bacteria isolated from soils, rocks, and sediments of extreme habitats or affected by extreme influence during model astrobiological experiments. The collection is intended to develop astrobiological methods, concretize biomarkers list, and specify astrobiological search criteria in conditions of various planetary models as well as to estimate microorganisms' potential survival rates and effect of mineral matrix on them, to adjust planetary protection protocols, and to reveal technologically perspective strains. The collection's catalogue is available at *depo.msu.ru*.

Today the collection contains more than 1000 strains of aerobic heterotrophic bacteria, belonging to more than 50 genera, isolated from soils, rocks, and sediments of different extreme ecosystems of the Earth: the Mojave Desert (USA), the Namib Desert (Namibia), the Tar Desert (India), the Sahara Desert (Egypt) and the Negev Desert (Israel), Atlas foothills (Morocco), Teide's peak (Spain), Canadian and East Siberian Arctic, Novaya Zemlya archipelago (Russia), Antarctic Dry Valleys, and other ecosystems, as well as bacteria isolated from soils and sediments which were irradiated with different types and doses of ionizing radiation. Based on the nucleotide sequences' similarity to the available data, there are some, which were not previously described, deposited in this collection.

Estimation of metabolic activity of the collection's strains in a wide diapason of factor impacts (temperature, pH, presence of NaCl, KCl, $MgSO_4$, $NaHCO_3$, $MgClO_4$, and presence of antibiotics from different classes of biological action mechanisms) was performed. Resistance of particular strains to ionizing radiation was researched.

High biodiversity, wide diapasons of metabolic activity in different temperatures and pH, moderate tolerance to NaCl and KCl, and high resistance to magnesium sulfate and magnesium perchlorate of bacterial communities of native and irradiated soils samples was shown [1–3]. Different types of stress impacts resistance spectra that were obtained indicate the polyextremotolerance of extreme ecosystems' bacteria.

The collection is open for usage and could be helpful for stress-tolerance physiological-biochemical mechanisms study, specific metabolites producers research, biotechnologically perspective strains search, and astrobiological tests and model experiments performance.

The reported study was funded by RFBR according to the research project No. 18–34–00331 (in part of bacteria metabolic activity characterization) and by RSF according to the research project No. 14–50–00029 (in part of technical support and bacteria deposition into the collection).

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NOVEL CHITINOLYTIC *ACIDOBACTERIA* FROM LICHEN COVERED TUNDRA SOIL: TAXONOMIC CHARACTERIZATION AND GENOME ANALYSIS

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Members of the phylum *Acidobacteria* inhabit a wide variety of terrestrial and aquatic ecosystems [1, 5, 2], and are particularly abundant in diverse soil habitats, where they represent between 5 and 50% of the total bacterial community [3, 4, 6]. Highest relative abundances of *Acidobacteria* are commonly observed in acidic soils and peatlands [6, 4, 7]. In this study, we analyzed acidobacterial diversity in lichen covered acidic (pH 4.1) soils of forested tundra in the Nadym region of northwest Siberia, Yamalo-Nenets AO, Russia. 16S rRNA gene fragments from the *Acidobacteria* comprised 22–24% of total 16S rRNA gene reads retrieved using Illumina pair-end sequencing from tundra soil. Indigenous assemblages of *Acidobacteria* were dominated by uncultivated members of subdivision 1, while members of subdivisions 2, 3 and 6 were also present. An isolate representing one of the abundant groups of sequences (up to 14% of all acidobacterial reads) from as-yet-uncultivated subdivision 1 *Acidobacteria*, strain SBC82^T, was obtained from the soil.

Strain SBC82^T was represented by non-spore-forming, highly polymorphic bacteria that, most commonly, occurred in pairs, in sarcina-like tetrads, and in clusters of 6–8 and more cells. Single cells or short chains of curved cells could also be observed occasionally. The specific feature of cell morphology of these bacteria was the presence of extracellular structures appearing as saccular chambers of a complex structure. Similar structures were earlier described for radiation-resistant

pseudomonads. These chamber envelops could possibly serve as a structural adaptation with protective function for survival in hostile conditions of acidic and cold tundra environments. These bacteria were aerobic, acidophilic and psychrotolerant chemoheterotrophs, which grew at pH values between 4.0 and 7.7 (optimum pH 4.8–7.0) and at 5–36°C (optimum at 20–32°C). Sugars and some polysaccharides, like starch and xylan, were the preferred growth substrates.

The genome of strain SBC82^T is 7.53 Mb in size and encodes a wide repertoire of enzymes involved in degradation of complex polysaccharides, including chitin, cellulose, and xylan. Among those, two secreted chitinases affiliated with the glycoside hydrolase family GH18 were revealed. Given that the ability to degrade chitin has never been experimentally proven for any of the *Acidobacteria*, this particular trait was examined. Strain SBC82^T utilized amorphous chitin as a source of carbon and also a sole source of carbon and nitrogen. Strain SBC82^T was only distantly related (92–96% 16S rRNA gene similarity) to other described members of the family *Acidobacteriaceae* and displayed a number of morphological and phenotypic differences to earlier described members of this family. We, therefore, propose a novel genus and species for these bacteria, *Acidisarcina polymorpha* gen. nov., sp. nov. Members of this genus colonize acidic soils and peatlands and are involved in degrading complex polysaccharides.

This study was supported by the Russian Science Foundation (project No. 16–14–10210).

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STATE COLLECTION OF PATHOGENIC MICROORGANISMS AND CELL CULTURES (SCPM-OBOLENSK) – WORKING IN THE BIG DATA ERA

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SCPM-Obolensk is one of three State collections of pathogenic microorganisms existing in the institutions of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor). The main function of the collection is to collecting, storing and studieng strains of microorganisms of I–IV pathogenicity groups. A significant activity of SCPM-Obolensk is the provision of the test strains. The collection carries out a deposit in three categories – storage, deposition for the national patent procedure and responsible storage.

During the deposition is necessary to verify the claimed and real properties of the strains. Maldi Biotyper (Bruker) is used as an express method for identification of bacterial strains in SCPM-Obolensk. This system allows carrying out the identification of microorganisms in the short time with the minimal cost of experiments.

Modern molecular biological approaches, such as multiple-locus variable number tandem repeat analysis (MLVA), the analysis of single polymorphisms (SNP-typing) using allele-specific PCR allow determining the strain differentiation and identify belonging to clonal groups. It is possible to differentiate closely related strains in many cases. These methods are characterized by low cost and high reproducibility. Another common method of molecular genetics research – multilocus sequencing-typing (MLST) is characterized by a longer experimental procedure and the high cost of the research. For example, the direct cost for carrying out MLST of pathogenic *Escherichia coli* strain including 15 genes (*aspC*, *clpX*, *fadD*, *icdA*, *lysP*, *mdh*, *uidA*, *arcA*, *aroE*, *cyaA*, *dnaG*, *grpE*, *mtlD*, *mutS*, *rpoS*) currently exceeds 10,000 rubles in Russia.

The most informative method for identifying of the pathogenic bacteria is whole genome sequencing (WGS). SCPM-Obolensk is equipped Ion Torrent PGM and MiSeq Illumina. Due to the high productivity and multiplexing capabilities, the cost of full genomic sequencing of a single bacterial strain is currently 30,000 rubles. WGS allows getting the information about all genes of the microorganism – virulence factors, phylogenetic markers, marks of drug resistance. For further bioinformational research can be used the archive of reads in FASTQ format.

At present, we are testing the nanopore sequencing technology on the MinION platform. A specific feature of this technology is the possibility of carrying out the analysis of native DNA. Another feature of using of this equipment is the possibility to obtain extremely long reads – more than 1,000,000 bases. It allows increasing the efficiency of analysis of bacterial genomes. The significant disadvantages of nanopore sequencing are the large numbers of errors and the low efficiency of the analysis of homopolymer sequences. The disadvantages of MinION technology can be compensated of using the additional sequencing in the Illumina platform. The hybrid sequencing approach includes using of both technologies. This approach allows the analysis of the bacterial genome in two stages of the research.

The technology of whole genomic sequencing generates the large amount of the information. Together with traditional catalogs, there is the necessity to use the electronic information systems, in which are combined the data of conducted researches and the history of the passage of the strain.

COMPARATIVE ANALYSIS OF ALPHA-GLUCOSIDASES MAL AND IMA OF SOME ASCOMYCETOUS YEAST GENERA

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The genetics of fermentation of α-glucoside maltose, which is important for the production of bread, kvass, beer, food and technical alcohol, is of great fundamental and applied importance. In eukaryotic microorganisms, an operon-like structure was first observed in the case of maltose polymeric *MAL* loci consisting of a regulatory and two structural genes: maltose permease and maltase (α-glucosidase). Biochemical and genetic analyzes revealed two types of related α-glucosidases in the yeast *Saccharomyces cerevisiae*: maltases and isomaltases. The international project [2] on sequencing and annotation of the genome of the genetic line of *S. cerevisiae* S288C discovered, along with the known *MAL12* and *MAL32* genes, a new family of isomaltase genes *IMA1-IMA5* [1, 3, 4].

Using yeast whole genome databases, we have conducted a comparative analysis of the multiple α-glucosidases MAL and IMA of the genetic line *S. cerevisiae* S288C and other ascomycetous yeasts. Protoploid *Lachancea* and *Kluyveromyces* yeasts share a common ancestor with *S. cerevisiae*, but they diverged before an ancient whole genome duplication from which *S. cerevisiae* arose. While *Debaryomyces hansenii*, *Scheffersemyces stipitis* and *Schizosaccharomyces pombe* are only distantly related to *S. cerevisiae.* Search for homologous α-glucosidases in the yeast genera studied was performed in GenBank using the BLAST software. The diagnostic sites Val216-Gly-Ser and Thr-Ala-Gly allowed us to differentiate the isomaltases and maltases of all yeast studied, except the divergent maltases, which are presented in *Schi. pombe* (NP595063) and *D. hansenii* (DEHA2E00528p). Phylogenetic analysis of amino acid sequences of α-glucosidases demonstrated the existence of several isolated clusters. Only certain MAL and IMA isoforms of *Kluyveromyces* and *Lachancea* yeasts are in close phylogenetic relationship to α-glucosidades MAL12, MAL32 and IMA1–IMA4 of *S. cerevisiae* S288C, while the others are closer to the divergent IMA5 isomaltase of S288C.

Our results suggest that isomaltases and maltases were formed in the common protoploid ancestor of *Saccharomyces*, *Lachancea* and *Kluyveromyces* yeasts, i. e. before their divergence and complete duplication of the *Saccharomyces* genome. Then, in each genus, species and even strain, there was a divergence of its α-glucosidases having both IMA and MAL activity.

The study was supported by the Russian Foundation for Basic Research (project No. 17–04–00309).

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ADVANCES ON THE CONSTRUCTION OF THE EUROPEAN RESEARCH INFRASTRUCTURE MIRRI

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The pan-European Microbial Resource Research Infrastructure (MIRRI) is part of the BioMedical Science Research Infrastructure (BMS RI) ESFRI landscape. By provision of high quality microorganisms, associated data and the broad expertise of its partners, MIRRI aims to support research and development in the field of biotechnology [1].

During its preparation phase (2012–2016) funded by the EU's FP7 program, more than 40 public biorepositories and research institutes from 19 European countries collaborated to prepare the establishment of MIRRI as an European Research Infrastructure Consortium (ERIC) under EU law.

MIRRI is in its construction phase since June 2017. Under the initiative of BELSPO, an interim governance including an Assembly of prospective Members (ApM) and an Interim National Coordinators Forum (INCF) was created. ApM had to engage countries to sign the Memorandum of Understanding (MoU) to commit to the construction of MIRRI. Today, 7 countries have signed the MoU and their representatives will establish the MIRRI statutes: Belgium, France, Greece, Latvia, Poland, Portugal, and Spain. The Netherlands will probably follow soon as prospective Member state, while Romania is planning to participate as an observer. mBRCs from Finland, Italy, Russia and Slovakia are contributing to the development of MIRRI-ERIC while seeking official support from their countries to become a member of MIRRI-ERIC.

In addition, Chile has expressed its will to become a partner of MIRRI-ERIC, showing the important visibility of the RI outside Europe.

Following a tender call, it was accepted that the Central Coordination Unit (CCU) will be hosted by Portugal and Spain in a split configuration with the Statutory Seat located in Portugal (University of Minho, Braga) and the Collaborative Working Environment hub operated from Spain (University of Valencia, Paterna) and supported by LifeWatch-Spain, a closely related e-infrastructure. The MIRRI ICT Task Force, composed of IT specialists from CBS, CECT, CIRM–INRA, USMI and VKM, in charge of the Information System of the RI [2, 3] and has produced a pilot of the future MIRRI database.

MIRRI has elaborated its statutes and is now finalizing the MIRRI-ERIC at scientific and technical levels to accomplish the first submission to the European Commission in September 2018, the formal submission in March 2019 and a planned implementation in December 2019.

During its construction phase, MIRRI is creating synergies with other research infrastructures in H2020 projects, such as CORBEL, EMBRIC, RItrain. In 2018, MIRRI also contributed to the response to three EU cluster project calls involving ESFRI BMS RI, INFRAEOSC-04, INFRASUP-b2 and an EUDON ACTION COST.

Various aspects of MIRRI such as the Information System, communication actions, a legal model on the commercial use of biological material and TransNational Access will be presented and discussed.

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RESOURCE CENTRE "CULTURE COLLECTION OF MICROORGANISMS"

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The Resource Centre "Culture Collection of Microorganisms" of Research Park SPbU was founded in 2011. The main idea was to organize the centre by uniting different SPbU's collections of microorganisms and keeping them at one place and to support them. The centre provides ample opportunities for experimental work with collection's strains and uncultivated natural strains of microorganisms thanks to the presence of modern equipment and highly qualified staff. For example, we conduct various studies using different methods of light microscopy: phase contrast, DIC microscopy, fluorescence microscopy and sample preparation for molecular research. At the moment we have two collections with "free access" in the resource centre.

CALU – Collection of Algae Leningrad University – consists of 446 strains of cyanobacteria, 468 strains of microalgae, 3 strains of endotrophic parasites of algae (Fig.1).

Fig. 1. Cyanobacteria and microalgae from CALU:

Cyanobacteria	Microalgae
Subsection I *Chroococcales* 12 genera, 132 strains Subsection II *Pleurocapsales* 4 genera, 11 strains Subsection III *Oscillatoriales* 13 genera, 175 strains Subsection IV *Nostocales* 7 genera, 119 strains Subsection V *Stigonematales* 2 genera, 9 strains	Phylum *Chlorohyta*: Class *Chlorophyceae* 18 genera, 266 strains Class *Trebouxiophyceae* 15 genera 182 strains Class *Ulvophyceae* 2 genera, 4 strains Phylum *Rhodophyta* Class *Cyanidiophyceae* 1 genus, 2 strains Class *Porphyridiophyceae* 1 genus, 1 strain Phylum *Ochrophyta* Class *Xantophyceae* 4 genera 5 strains Phylum *Charophyta* Class *Conjugatophyceae* 2 genera 2 strains Phylum *Bacillariophyta* Class *Bacillariophyceae* 2 genera 6 strains

RC CCM – Resource Centre "Culture Collection of Microorganisms" – include 520 clones of heterotrophic and 28 clones of autotrophic eukaryotic microorganisms. The major part of this collection are ciliates of the genus *Paramecium* (14 of the currently known species, beside "aurelia" group), including the strains, which contain pro- and eukaryotic symbionts in different cellular compartments. Some other species of free-living ciliates and proteus-like amoebaes are also represented in the collection. Among the autotrophic organisms there are 2 species of *Euglenozoa*, 1 species of *Ochrophyta*, 1 species of *Cryptophyta*, 2 species of *Glaucophyta*, 13 species of *Chlorophyta*, 2 species of *Charophyta* and 6 species of *Dinophyta*.

Apart from that the resource centre supports a number of deposited collections, the largest of which are CCCS (Culture Collection of Ciliates and its Symbionts) and CCHAP (Cultural Collection of the Heterotrophic Amoeboid Protists).

Collection of the resource centre is actively replenished thanks to research work of centre's staff or scientists from other departments of SPbU, mainly departments of microbiology and invertebrate zoology and also by donations from other collections from Russia or foreign countries.

Thus, during the last three years CALU has been replenished on more than 100 strains, RCCCM – on more than 300 clones of microorganisms isolated from different ecotops and geographic areas. Wide spectrum of used culture medium and feed cultures enables us to design new methods of cultivation, such as for difficult to cultivate species of eukaryotic microorganisms. Information base of the centre is constantly updated. Many strains have already been characterized at the molecular-genetic level, including through realization of different research works with the use of collection's materials [6]. Materials from collections are actively used by colleagues from SPbU and other research institutions, including RAS. First of all, it is, of course, research in the field of biodiversity, systematics and phylogeny of various groups of both pro – and eukaryotic microorganisms [2, 8]. Collection of ciliates is extensively used for the studies of different symbiotic associations between ciliates and bacteria [5]. Unique set of Paramecium strains maintained in the RCCCM allows one to address the questions of speciation, molecular phylogenetics, genetics and genomics of this model object of protistological research [3].

Among the actively developing areas of research using the materials of the CALU are: the analysis of pigment composition and the development of new approaches to the identification of cyanobacteria [1, 7], the study of toxigenic strains of cyanobacteria, design of a method of differentiation of strains of cyanobacteria using confocal microspectroscopy [4]. As a result of collaboration with colleagues from Physic and Chemistry faculties of SPbU methods of evaluation of antimicrobial activity of nanomaterials (suspensions and coatings) and also evaluation of total toxicity of samples using different test objects are devised using collection's materials from resource centre. Currently, cyanobacteria and microalgae are again actively used worldwide in the field of biotechnology. We consider this area as one of the most promising for the development of the resource center, given the exceptional variety of collection strains at our disposal.

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AN OUTLOOK INTO THE KEY PRINCIPLES OF A GENOME-BASED TAXONOMY

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Defining evolutionary relationships between microorganisms based on phylogeny is a major goal of microbial systematics and the basis of prokaryotic taxonomy. The continuous growth of genomic data from cultured and uncultured prokaryotes provides a strong foundation to build a comprehensive prokaryotic taxonomy. Using this wealth of data we recently proposed a taxonomic framework where widely used phenotype- and threshold-based classifications are recalibrated with a more natural rank normalization approach using relative evolutionary divergence [4]. Our taxonomy is based on a phylogeny inferred from the concatenation of ubiquitous domain-specific and vertically inherited single-copy proteins. The representative genomes are subject to strict quality filtering, and manual curation of the taxonomy is performed and verified against a range of tree inference algorithms, evolutionary models, marker sets, and genome permutations. During taxonomic curation two major goals are achieved – removal of polyphyletic groups and rank normalization according to evolutionary divergence. This approach resulted in reassessment of both lower and higher taxonomic ranks from Bacteria and Archaea and resulted in modifications to the existing taxonomies for more than half of publicly available genomes. Here we outline the major principles behind this genome-based taxonomy and discuss some of the challenges faced while developing this framework. Nomenclatural adjustments are made based on availability of type material and follow the International Code of Nomenclature of Prokaryotes. However as type material is not yet available for all cultured species and uncultured species are currently lacking any designated type material, we propose to establish types based on genomic sequences [1] following the proposal

of Whitman [4] and Konstantinidis *et al.* [2]. Further challenges associated with type designation and nomenclature issues will be discussed. We believe that the availability of a fully standardized prokaryotic taxonomy will greatly benefit the scientific community and improve communication of scientific results. The taxonomy and all supporting documentation can be found at the Genome Taxonomy Database website (*http://gtdb.ecogenomic.org*).

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PROSPECTION OF ENDOPHYTE MICROORGANISMS FROM *BAUHINIA MONANDRA* LEAVES WITH MAINLY IDENTIFICATION OF *ACTINOBACTERIA*

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There is no investigation regarding the identification of endophytic microorganisms of *Bauhinia monandra*, although some pharmacological studies with this species are reported. The objective of this work was the prospection of microorganisms from *B. monandra* leaves, with the purpose to identify endophytics to obtain strains with biotechnological applications. *B. monandra* leaves, disinfected with hypochlorite solution, were macerated in phosphate buffered saline and seeded in ten selective culture media containing antibacterial or antifungal agents. The use of selective culture media to isolate endophytic microorganisms allowed obtaining non-filamentous and filamentous bacterial strains as well as fungus strains. Relevantly is mentioned the L-arginine agar medium to non-filamentous bacterial colonies; the inorganic salts-starch agar medium to *Actinobacteria* and the potato dextrose agar medium to filamentous fungi. These microorganisms are not epiphytic since sterile controls were performed. The endophytic filamentous fungus strains detected belonged to the genera *Penicillium*, *Curvullaria* and *Aspergillus*. Non-filamentous endophyte bacteria were grouped in the genera *Bacillus*, *Burkholderia* and *Enterobacter*; strains of endophytic *Actinobacteria* were classified as *Streptomyces* and *Nocardiopsis*. There was a predominance of endophytic fungi from

the genus *Penicillium*; also, original data were reported on the identification of non-filamentous endophytic bacteria belonging to the genera *Bacillus*, *Burkholderia* and *Enterobacter*, as well as endophytic *Actinobacteria* from the genera *Streptomyces and Nocardiopsis*; all bacteria are described with biotechnological potential. The isolation of endophytic microorganisms with nine culture media revealed better bacteria development with L-arginine agar; inorganic salt starch agar and potato dextrose agar were superior to *Actinobacteria* and fungus strains, respectively. This work introduces the first data of identification from endophyte *Actinobacteria* in the leaves of *B. monandra.*

Key words: *Bauhinia monandra*, leaves, endophytic microorganisms.

Financial support: CNPq and FACEPE.

ANTI–CANDIDA ACTIVITY OF THE OCOTEA GLOMERATA (NEES) MEZ METANOLIC EXTRACT

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Candidiasis is an infection caused by species of *Candida* that can range from frequent manifestations, such as colonization of the vaginal and oral mucosa, to development in a systemic way via the hematogenous or lymphatic route. Its most frequent etiological agent is *C. albicans*; however, in recent decades, episodes of candidiasis caused by other species of *C.non-albicans* have emerged. The widespread use of antifungal drugs has led to the rapid development of drug resistant strains used in clinical therapy. Thus, natural products have been gaining prominence in recent years for providing a new drug approach for the treatment of pathological processes, among them, infectious. In view of the current scenario, this work had the objective of evaluating the antifungal potential of *Ocotea glomerata* (*Lauraceae* family) extracts and fractions obtained from methanolic extract (ME) against *Candida* spp. resistant. The *O. glomerata* leaves were collected in the municipality of Igarassu (Pernambuco, Brazil). The botanical identification and deposit of species were carried out in the Herbarium of Institute of Agronomic Research of Pernambuco (IPA), Pernambuco, Brazil; IPA voucher 90.944. Leaf flour was mixed with an eluotropic series (hexane, chloroform, ethyl acetate and methanol), and concentrated by evaporation to dryness. Major classes of secondary metabolites (phenols, tannins, flavonoids, alkaloids, saponins, steroids and triterpenoids) was evaluated by phytochemical profile in thin layer chromatography (TLC), exposed to UV light. Methanolic extract (ME) was fractionated on flash chromatography in a C18 column and the compounds of ME and its five fractions (F, FME1 to FME5) were identified by HPLC analysis. The antifungal activity was carried out against *C. albicans* (14053), *C. parapsilosis* (22019), *C. krusei* (6258) obtained from the American Type Culture Collection (ATCC); and *C. albicans* (URM 5901),

C. parapsilosis (URM 4970 and URM 7048) and *C. tropicalis* (URM 6551) were obtained from the Micoteca URM of the Department of Mycology, Federal University of Pernambuco (Pernambuco, Brazil). Minimal inhibitory concentration (MIC) and antifungal sensitivity (MIC50) were determined by microdilution method by twofold serial dilution; afterwards, minimum fungicide concentration (MFC) were determined. Checkboard test for ME was evaluated using as standard antifungal (Ketoconazole, Fluconazole and amphotericin B). Fractional inhibitory concentration (FIC) indexes were calculated as $\Sigma IFC = FIC^A + FIC^B$, where FIC^A and FIC^B represent the minimum concentrations that inhibit fungal growth for samples A and B, respectively: $FIC^A = MIC^A$ in combination/MIC^A isolate and $FIC^B = MIC^B$ in combination/MIC^B isolate. FIC was used to interpret the interactions nature: synergistic (FIC <0.5), additivity (FIC 0.5–1.0), indifferent (FIC > 1) or antagonist (FIC > 4). The possible mechanisms of ME (MIC, 2xMIC and 4xMIC) action were investigated through the tests of ergosterol; sorbitol; cell viability and propidium iodide (PI); intracellularly generated oxygen reactive species (EROS) determined by 2'7'-dichlorodihydrofluorescein diacetate probe (H2DCFDA) and phosphatidylserine externalization (PSE) performed with Annexin V conjugated to FITC (fluorescein isothiocyanate) and PI; the last three methods were analyzed by flow cytometry. Profile phytochemical of extracts and fractions by TLC evidenced the presence of hydrolysable tannins, condensed tannins, flavonoids, triterpenes and steroids, saponins, alkaloids (only in Me and fractions) and sugars. *O. glomerata* extracts showed antifungal activity with MIC ranging from 3.12 to 400 µg/mL or without MIC, but these activities differ according to the microorganism, highlighting the ME; that showed fungal growth inhibition of 6.25 µg/mL against *C. albicans* URM 5901, indicating a promising action of this extract, still, only ME presented fungicidal activity at 400 µg/mL against *C. albicans* ATCC 14053. In relation of *C. Tropicalis* URM 6551, only hexane extract (HE) presented fungicidal activity with MIC of 100 µg/mL; the other extracts presented fungistatic activity, and in relation to *C. krusei* ATCC 6258, chloroform extract (CE) and ethyl acetate extract (EAE) presented fungicidal activity at 200 µg/mL, while ME presented fungicidal activity at 3.12 µg/mL, however, HE presented only fungistatic activity at 200 µg/mL. CE showed fungicidal activity at 100, 200 and 100 µg/mL for *C. parapsilosis* 4970, *C. parapsilosis* ATCC 22019 and *C. parapsilosis* URM7048, respectively, while EAE showed fungicidal activity for *C. parapsilosis* 7048 (100 µg/mL). Once again ME showed fungicidal activity for the three *C. parapsilosis*, *C. parapsilosis* URM 4970 (MIC 12.5 µg/mL), *C. parapsilosis* ATCC 22019 (MIC 6.25 µg/mL) and *C. parapsilosis* URM 7048 (MIC 6.25 µg/mL); HE showed fungistatic activity for *C. parapsilosis* URM 7048. In view of the CIM values demonstrated by ME, an evaluation of the synergistic effect with the known antifungal agents was carried out. According to btained results the addition of ME to the commercial antifungal agents resulted in a reduction of ketoconazole MIC to tested *Candida* spp., *C. albicans* ATCC 14053, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019, with FIC index of 0.51 (additivity), 0.26 (synergistic) and 0.62 (additivity), respectivly. The MIC for amphotericin B decreased to *C. krusei* ATCC 6258 from 0.06 µg/mL to 0.03 µg/mL. In addition, a reduction of fluconazole MIC was observed to *C. albicans* ATCC 14053 and *C. parapsilosis* ATCC 22019, with FIC index of 0.02 (synergistic) and 0.56 (additivity), respectivly. The combinations of ME with amphotericin B showed an indifferent effect against all tested *Candida* spp. Fungicidal and fungistatic activity was showed only to FME1 and FME5, highlighting the activity against *C. krusei* ATCC 6258 with MICs of 200 µg/mL and 25 µg/mL, respectively. These data suggest that when ME was fractionated, probably, there is separation of the compounds that have antifungal activity resulting in an decrease of MIC or antifungal activity absence. In the tests performed with ME and its more active fractions (FME1

and FME5), it was observed that there was no change in MIC values in media with and without addition of ergosterol, indicating that these do not present a mechanism of complexation with ergosterol, whereas, amphotericin B MIC had its value increased in the presence of ergosterol. The same was observed in the tests performed with sorbitol, suggesting that the ME, FME1 and FME5 do not act to inhibit the fungal cell wall synthesis, but probably act on another target. *C. krusei* ATCC 6258 when treated with the ME (MIC, 2xMIC, 4xMIC) showed a significant ($p < 0.05$) decrease in cell viability ($1.35x10^6$ cells ± 0.04, $0.64x10^6$ cells ± 0.04, $0.30x10^6$ cells ± 0.01, respectively) demonstrating a concentration dependent effect. Amphotericin B used as a positive control, after 24 h of exposure, decreased the number of viable cells ($0.28x10^6$ cells ± 0.02). Intracellular production of EROS by *C. krusei* ATCC 6258 treated with ME at different concentrations (MIC, 2xMIC, 4xMIC) showed a significant increase ($p<0.05$) in a concentration dependent manner (26.50 ± 0.59, 44.53 ± 1.05 and 70.13 345 ± 1.39 %, respectively) when compared to the control (0.03 %). After 24 h of exposure ME (MIC, 2xMIC, 4xMIC) was able to induce significant increases ($p<0.05$) in the frequency of externalized PS cells in the isolates of *C. krusei* ATCC 6258 (45.73 ± 2.33, 63.29 ± 1.72, 75.57 ± 1.30 %, respectively) when compared to the control group (1.25 ± 0.03 %), whereas Amphotericin B, positive control, increased 64.02 ± 1.62 %. ME and FME1 and FME5 exhibited a promising anticandida activity, especially against *C. krusei* ATCC 6258 and positively modulating the in vitro action of antifungals, suggesting the future use with adjuvant agent for these drugs. ME was not able to bind ergosterol in the membrane, do not act by inhibiting the synthesis of fungal cell wall, which leads to suppose a different mechanisms of action. ME possibly exerts its antifungal effects through the production of reactive oxygen species, culminating in cell death by apoptosis.

Key words: Candidiasis, fungicidal activity, synergistic effect.

Financial support: CNPq, CAPES and FACEPE.

UNIMORE MICROBIAL CULTURE COLLECTION: A RESERVOIR OF ACETIC ACID BACTERIA FOR FOOD AND NON-FOOD APPLICATIONS

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In the last decades, Acetic Acid Bacteria (AAB) have been extensively investigated not only for conventional food and beverage production but also for others industrial applications that lead to high-value products such as organic acids, ketones, sugar derivates and biopolymers [1, 4]. The industrial exploitation of AAB requires the existence of culture collections, which assure the maintenance of selected microbial cultures for intended biotechnological uses.

The genetic stability of AAB, similar to other organisms, may be affected by casual events as mutations or genomic rearrangements that can cause deficiency in important physiological properties, such as the ability to oxidize ethanol, the resistance to acetic acid or the cellulose production capacity. Therefore a crucial role of AAB microbial collections is to find and validate appropriate methodologies for culture preservation and quality control.

The University of Modena and Reggio Emilia Microbial Culture Collection (UMCC) (*www.umcc.unimore.it*), specialised in the selection of functional microorganisms for both academic and industrial purposes, holds a pool of AAB strains collected from wine vinegars, balsamic vinegars,

cereal vinegars, fermented juices and kombucha tea. Selective strain isolation, molecular typing, polyphasic identification, and technological selection are the core of UMCC activity finalized to build up appropriate AAB starters for different industrial needs.

AAB strains from UMCC are investigated for the understanding of the genetic background that is fundamental for assessing the technological stability of industrially useful strains [2, 3]. All the tests performed on strain cultures preserved are recorded in a public database performed by the BioloMICS NET software (*BioAware/ http://umcc.bio-aware.com/*), which acts as a comprehensive platform able to combine phenotypic and molecular traits with industrial strain performance.

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MIRRI–IT JOINT RESEARCH UNIT: THE ITALIAN NETWORK OF MICROBIAL RESOURCES

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The Microbial Resource Research Infrastructure (MIRRI) (*http://www.mirri.org/*) has been launched in 2010 within the Biomedical Research Infrastructures of the European Strategy Forum on Research Infrastructures (ESFRI) initiative.

The current configuration of MIRRI involves several countries (Portugal, Spain, France, Greece, Belgium, Poland and Latvia have already signed a Memorandum of Understanding), which are called to support the implementation of the Central Coordination Unit (CCU), their National Nodes (NN) and the needs of the national communities. While the CCU is clearly defined at the European level by an agreement among interested countries, the NN may follow various forms.

In Italy there are numerous collections of microorganisms disseminated in the country, which can be exploited to boost technological innovations and to face different societal challenges. Indeed, these collections are often not managed according to international standards and their catalogues are not online displayed. Moreover, coordination among these collections is still limited. In this contest, the creation of an effective Italian network of microbial culture collections can be fundamental to implement the recently presented national bioeconomy strategy and to support the needs of the companies acting in different sectors, as well as to foster the signature of the Memorandum of Understanding of MIRRI, which has not yet been done by the relevant National Agency.

For these reasons, a Joint Research Unit for the implementation of the Italian node of MIRRI (MIRRI–IT JRU) (*http://www.mirri-it.it*) has recently been founded with the contribution of the Universities of Turin, Perugia, Modena and Reggio Emilia, the University Hospital San Martino Genoa and the Italian National Research Council. The main goal of the MIRRI–IT JRU is the development of a tight network of Italian collections of microbial resources. The mission is to overcome fragmentation in availability of resources and services, enhancing the quality management system of the collections while focusing on needs and challenges of the stakeholders interested in the biotechnological transfer of these resources.

Therefore, the main activities of the MIRRI–IT JRU are meant to:

a. coordinate and support the operation of the Microbial Culture Collections in Italy according to the established international quality standards;

b. harmonize the procedures of the Collections in order to comply with national and international rules (i. e. Nagoya Protocol, Intellectual Property Rights, privacy, biosafety, etc.);

c. propose actions to relevant State authorities in order to fortify the functioning and sustainability of the Collections;

d. promote interdisciplinary cooperation and represent Italy in relevant national and international Networks and Organizations;

e. provide an unique entry point to quality microbiological services and microbial Biological Resource Centres (mBRC) holdings.

THE PHYLUM *ACIDOBACTERIA*: NEW FINDINGS AND TAXONOMIC REARRANGEMENTS

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The *Acidobacteria* is one of the globally distributed and highly diverse phyla of the domain *Bacteria*. These microorganisms inhabit a wide variety of terrestrial and aquatic habitats, and are particularly abundant in acidic soils, peatlands, and mineral iron-rich environments. The *Acidobacteria* raised considerable scientific interest at the turn of the century, when application of molecular techniques revealed the cosmopolitan distribution and high abundance of these microorganisms in various environments. The corresponding phylum was created in 1997 in order to accommodate a large number of 16S rRNA gene sequences retrieved from a wide variety of environments in cultivation-independent studies [1]. At that time, this phylum encountered only three species and four major subgroups (or "subdivisions", SDs) based on 16S rRNA gene sequences. By 2007, the

number of recognized subdivisions within the *Acidobacteria* increased to 26 [2]; each of them was assumed to be equivalent to a class level.

Due to difficulties of cultivating *Acidobacteria*, the taxonomically described diversity within this phylum remains limited. At present, it includes 28 genera, which represent only 7 out of 26 currently recognized subdivisions (SDs 1, 3, 4, 6, 8, 10 and 23). All characterized representatives are Gram-negative, non-spore-forming bacteria that display a variety of cell morphologies. Most characterized acidobacteria are chemoheterotrophs, although photoheterotrophic members have also been described [3]. Cells of these bacteria contain a number of characteristic lipids, which may be responsible for their environmental adaptations [4]. Genomes of acidobacteria are up to 10 Mbp in size and encode a wide repertoire of carbohydrate-active enzymes involved in breakdown, utilization and biosynthesis of diverse carbohydrates [5]. Recent insights into their functional role in the environment suggest involvement of *Acidobacteria* in decomposition of various biopolymers (cellulose, xylan, chitin, pectin) and participation in the global cycling of carbon, iron and hydrogen [4, 5].

Given the limited characterized diversity within the *Acidobacteria*, the practice of using the category "subdivision" for defining the taxonomic position of a particular representative of this phylum is widely used in the literature. Detailed analysis of the currently explored diversity within the *Acidobacteria* using the candidate taxonomic unit circumscription system (with specific sequence identity thresholds of 78.5 %, 82 %, and 86.5 % for a class, order and family, respectively, [6]) suggests that 26 formerly recognized subdivisions can be assigned to 15 class-level units, five of which contain described members. These include three earlier established classes *Acidobacteriia* [7], *Blastocatellia* [8] and *Holophagae* [9], as well as two as-yet-undescribed classes defined by SDs 6 and 23. A number of orders and families remain to be circumscribed to accommodate the described representatives of SDs 3, 4, 6, 10 and 23, and to fill in the gaps in the taxonomic structure of this phylum.

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NAGOYA PROTOCOL, TIME FOR THE SCIENTIFIC COMMUNITY TO SPEAK OUT

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Culture Collections (CC), professionals underpinning microbial realm exploitation

Comprehensive exploration and structured study of the microbial diversity implies access to huge numbers of specimens. These assets of fundamental scientific importance must be conserved and provided with the highest level of reliability to ensure cumulative research, to build microbiology on solid ground.

Culture Collections have decades of expertise in long term *ex situ* conservation of living microbial material and related data management. These specialised infrastructures provide for facilitated access to technically and legally fit for use microbiological resources and information of consistent quality.

Continuum in Life Sciences R&D – extended role of culture collections

At present day, in microbiology like in other disciplines, the pattern of complementary distinctive basic and applied sciences is replaced by a continuum of upstream and downstream researches producing flows of data and information handled via bioinformatics (Stokes 1997). In Knowledge Based Bio-Economy, the various players in this R&D flow are closely tangled in an interdisciplinary fabric where CCs are providers of raw material as well as infrastructures underpinning scientific activities.

Life sciences practitioners in the arena of political negotiations

It took 16 years from the start of the negotiations initiated at the 4th Conference of the Parties of the Convention on Biological Diversity in 1998 to the entry into force of the Nagoya Protocol on Access and Benefit Sharing (ABS) in October 2014.

The slow progress was caused in part by the context in which the negotiations took place and the lack of effective pre-existing legislation in the field of ABS.

On the one hand some problems have been exaggerated and oversimplified. Although proven, some facts have been so distorted that they betrayed reality. For instance the word "biopiracy" was created to depict the stereotype of the exploitation of poor providers of biological material in southern non-industrial countries by greedy users working for wealthy companies in opulent countries. Such Manichean view was not conducive to smooth and fruitful negotiations anchored in facts.

On the other hand issues of primary importance were neglected in the Nagoya Protocol and must be dealt with afterwards, via applicable implementing regulations at regional and national levels. First, the complexity of the Research & Development processes leading from micro-biodiversity to socio-economic outcomes is underestimated. The oversimplified technical apprehension of the scientific approach produces legal solutions inappropriate to deal with the field complexity.

Then, the cost-benefit and return on investment ratio of an ABS system was overlooked, where the measures may cost more than the benefits it intend to redistribute.

Also, key concepts such as commercialization, placing on the market, R&D, etc. are not defined from the upset, that doesn't help reduce legal uncertainty.

Lack of awareness, *ignorance*, *mistrust*, *disdain*

Notwithstanding extended legal studies and the big media coverage about the Nagoya Protocol, microbiologists do not know the ABS concept by lack of information and awareness.

For life science researchers the debate over ABS seems superfluous because the way they work is based on knowledge sharing and they have always freely shared scientific information with each other. They ignored the ABS concept because they believed the Nagoya Protocol was not of their concern.

On the other hand, the misperception of the situation by the legislators led to inappropriate legal solutions which are considered irrelevant and therefore rejected by the actors in the field. Some case studies are even not representative of the reality in the field. Certain problems are exacerbated while others are neglected.

Ignorance, misperception of the reality and more importantly lack of communication between disciplines tainted the climate of negotiation with mutual mistrust at least, disdain at worst.

Time to stand up and speak

The latest topic that is discussed in the CBD forum is whether the digital sequence information (DSI) should be in the scope of the protocol and how it should be ruled. Putting legal and administrative impediments on one of the fundamentals of open science, open data access, is a mistake that could jeopardize the conservation and sustainable use of the earth biodiversity with no benefit at all.

Excessive legislating will lead to disillusionment and a rejection of the system by its field actors. Furthermore DSI has not yet been defined. Making rules about something you can't describe is just working upside down. Talking about sharing benefit when conservation of biodiversity is not even secured works against the three objectives of the CBD including these of the Nagoya Protocol. Moreover it undermines severely the confidence of the stakeholders in the system. At the end the entire system may collapse because no field actors will support it.

Furthermore, the scientists who attended the first meeting of the Ad Hoc Technical Expert Group (AHTEG) on Digital Sequence Information on Genetic Resources were shocked by the subjective, incorrect and political stances of the non-scientists.

It is time for the scientific community to voice its concerns and to show that many life sciences practices are multilateral non-monetary benefit-sharing actions and that scientists have not waited for someone to tell them how to work and cooperate for the benefit of the scientific community worldwide.

STEROID-TRANSFORMING MICROORGANISMS FROM ALL-RUSSIAN COLLECTION (VKM): DIVERSITY, NOVEL FINDINGS AND BIOTECHNOLOGICAL APPLICATION

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Microbial diversity is an inexhaustible source of bioactive molecules including potent drug candidates and value-added compounds. In spite innovative technologies including high-throughput screening of chemical libraries, *in silico* prognosis and other advanced methods decreased natural product studies, the approach based on analysis of diversity of metabolites by microbial collections is still essential for the discovery of value-added bioactive molecules such as steroids.

Steroid compound superfamily includes sterols (such as cholesterol, sitosterol, ergosterol etc.), bile acids, corticoids, vitamin D and other terpenoid lipids that contain in their structure gonane core of the four fused cycloalkane rings (A-D). These compounds fulfill essential vital functions in all living organisms of the animal and plant kingdom. It is reputed that steroids have originated hundreds millions years ago. They are regarded as chemical fossils and often used as geochemical markers in the sediment maturity estimation in petroleum geochemistry. Throughout evolution microorganisms have been exposed to variety steroid substrates resulting in numerous metabolites and enzymatic activities [1].

Steroid degradation is one of the key processes for biomass decomposition, removal of the pollutants and pathogenesis of *Mycobacterium tuberculosis* and other pathogens. Most steroid transforming bacteria were isolated from soil, but recent metagenomes studies showed global distribution of steroid degraders with prevalence of *Actinobacteria* and *Proteobacteria* also from eukaryote hosts, aquatic environments and other habitats [2]. Spectrum of natural steroid substrates served as carbon source for bacteria includes those of plant origin (e. g. sitosterol, stigmasterol, ergosterol), and vertebrate steroids excreted into the environment such as cholesterol, estrogens, androgens, or bile acids[1].

It is generally accepted that bacteria metabolize steroids as carbon and energy sources via the so-called 9 (10) – secosteroid pathway [3]. Most effective phytosterols degraders have been revealed from mycolic acid rich *Actinobacteria* of *Corynebacterineae* suborder such as representatives of *Mycobacterium*, *Rhodococcus* and *Gordonia*. It was assumed that mycolic acid rich cell wall of these *Actinobacteria* may contribute to the effective transportation of lipophilic substances such as steroids.

Selected strains of *Mycobacterium neoaurum* VKM Ac-1815D, VKM Ac-1816D and *Mycobacterium* sp. VKM Ac-1817D were found to transform natural sterols to androstenedione (AD), androstadienedione (ADD) and 9α-OH-AD, respectively. These androstane steroids are the key intermediates in the synthesis of various steroid drugs. Full genome sequencing and genome-wide transcriptomic profiling of the strains enables identification of the specific genes and gene clusters which are essential for steroid modifications [4]. The data were applied for the generation of engineered mycobacterial strains with improved biocatalytic capabilities for production of the value-added C19 steroids and isoprenoids. Using mycobacterial hosts, the recombinant strains heterologously

expressing eukaryotic steroidogenic systems were created which converted phytosterol to the valuable hormones such as testosterone, 1-dehydrotestosterone and progesterone [5].

Recently, we screened *Actinobacteria* of different taxa from VKM for their transforming activity towards bile acids (lithocholic, – LCA, deoxycholic, – DCA, cholic – CA acids). The strains were found capable of producing clinically important ursodeoxycholic acid and other valuable cholic acids. 7β-Hydroxylating activity towards LCA and DCA was firstly reported for the representatives of *Amycolatopsis*, *Lentzea*, *Pseudonocardia* and *Saccharopolyspora* genera [6, 7], while the capability to 7α-hydroxylation was observed only towards LCA for the selected strains of *Catellatospora*, *Lentzea*, *Nocardia*, *Nocardiopsis*, *Pseudonocardia*, *Saccharopolyspora*, *Saccharothrix* and *Streptomyces* genera. Among wide number of *Actinobacteria* tested, only *Rhodococcus* species were able to transform CA (mainly to 7-keto and 12-keto derivatives) [7]. The results contribute to the knowledge of biocatalytic potential of diverse soil-dwelling actinobacteria towards bile acids, and could be applied at the development of novel bioprocesses for production of the therapeutically important cholanic acids.

Unlike *Actinobacteria*, most fungi are not capable of full degradation of steroid skeleton, but mainly detoxify steroids as fungitoxic molecules. Key feature of filamentous fungi is their capability to steroid oxyfunctionalization such as hydroxylation, or lactonization. Search of microbial strains capable of regio- and stereospecific hydroxylating different type steroids is of great importance since provide generation of the derivatives with high therapeutic potency.

We experimentally estimated over 300 strains of *Ascomycota*, *Zygomycota* and *Mucorales* phyla for their hydroxylase activity towards 3-oxo- and 3a/β-hydroxy-steroids of androstane, pregnane and cholanic acid series. The strains with 11α- and 11β-hydroxylating activity towards pregnane 3-oxosteroids were revealed [8] as well as those providing hydroxylation of DHEA at the allylic 7 (α/β) positions or 7α,15α- dihydroxylation with high efficiency. Bile acids, such as LCA and DCA had not been strongly investigated earlier as substrates for bioconversion by fungi. We revealed the strains with 7α-, 7β- and 15β-hydroxylase activities, and described several new metabolites with potent bioactivity. Effective producers of valuable ursodeoxycholic and ursocholic acids were selected [6]. The results evidence broad capacities of filamentous fungi for hydroxylating different types of steroids and obtaining new steroid metabolites. The findings could be suitable at the production of the valued hydroxysteroids for pharmaceutical industry.

In summary: the results evidence that enzymatic and taxonomic diversity of *Actinobacteria* and filamentous fungi, their biotechnological potential towards different steroid substrates are still underestimated. New whole cell biocatalysts capable of effective performing structural modification of steroids can be applied for new generation industrial bioprocesses.

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NATIONAL BIOBANK OF EMERGING AND EXOTIC ANIMAL PATHOGENS

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Microbiological Biobanking is on the top priority challenge of scientific research. The main tasks of biobanking are deposing, storage and rational use of pathogen biodiversity in human and animal infectious diseases.

For the solution of applied research tasks and in particular in the field of biosecurity of the Russian Federation, according to National Government Regulation on the base of National Research Institute for Veterinary Virology and Microbiology of Russia (VNIIVVIM, currently Federal Research Center for Virology and Microbiology (FRCVM) a biobank of microorganisms causing emerging, transboundary and exotic animal diseases was established. In 2011, according to RF Government Regulation, biobank had been assigned a status of National biobank.

National biobank works on the base of museum complex of FRCVM including strain biobank, laboratory buildings, and animal facilities, equipped with special infrastructure, which are necessary for biological safety when work with BSL3-BSL4 pathogens is carried out.

The main foci of biobank:

– deposing of pathogens of notified, emerging and exotic animal diseases including agents obtained following experimental research and isolated from man-made and natural ecosystems;

– storing in active state the objects of strain biobank of bacterial, mycoplasmal and viral etiology;

– studying the biological features of agent strains of animal infectious diseases including common for human and animal – main identification characteristics, antigenicity, protection potential, immunological propriety of applying vaccine strains and etc.;

– maintenance of strains dedicated for producing biopharmaceuticals and diagnostics kits for animal infectious diseases;

– providing to national diagnostic and pharmaceutical laboratories with testing culture of microorganisms, which are necessary for quality control of culture media, cell culture, validation and verification of diagnostic assays, staff training;

– producing of control samples on the base of biological (pathogenic) specimen of animal origin, infected with animal pathogens of animal infectious diseases for the staff competence verification and quality assessment of diagnostic assays;

– staff training in the field of biobanking activity.

Nowadays strain biobank has more than 2300 strains of notified, emerging and exotic pathogens of animal diseases of 140 nosological entity. The most valuable are *Bacillus anthracis*, Classical Swine Fever Virus, African Swine Fever Virus, Rift Valley fever, Bluetongue, Sheep and Goat Pox, Peste des petits ruminants, rabies, listeriosis, mycoplasmosis of livestock animals. Strain biobank has more than 60 viral and bacterial strains for vaccine production and also candidate strains with a potential for development of diagnostic tools and specific prophylaxis.

Microbiological sampling for the biobank was carried out due to monitoring and diagnostic research from the establishing the Organization and continues at the present time. Over the last ten years within the framework of accomplishment the Government Resolution from September 20th 2016 No 2048-p, National biobank has obtained strains of African Swine Fever, circulating in the territory of the Russian Federation that caused outbreaks of the disease in pig breeding farms of different properties and in wild fauna. In 2016 strains of *Bacillus anthracis* which caused the outbreaks of Anthrax in Yamal-Nenets autonomous district (after 75 years of endemic free period) were deposited the in National biobank.

The stocks of National biobank of microorganisms are regularly used for carrying out fundamental and applied scientific research, also grants of RSF, RFBR and Grants of the President of the Russian Federation, international projects, control of circulation and adaptation of pathogens of transmissible diseases and changing environmental conditions, when developing medical, diagnostic and preventive drugs. Forty-five objects of biobank have formed the basis of patents for developments of studying the pathogenesis of animal infectious diseases, diagnostic tools, and preventive measures.

In 2016–2017 with financial support of FASO of Russia of National biobank of microorganisms, within the framework of additional State project we have generated important results summarized in an article/report entitled “Deposing and storage of microorganisms, studying biological properties of bacterial and viral pathogens”, codes: No. 0615–2016–0001, 0615–2017–0002. Within the framework of this topic we have obtained these main results:

– We determined a genotype and genetic markers of evolutionary variability of ASFV strains, extracted in the territory of the Russian Federation in 2016–2017. The clusters of genetic variant of ASFV localization were defined.

– On the basis of studying molecular-genetic and biological characteristics we carried out an epizootic passportization of *Bacillus anthracis*, caused an outbreak in Volgograd Region and an epizooty among reindeers in Yamal-Nenets district in 2016. We identified genetic clusterization of strains using geographic origin.

– We developed a technological certificate of biobank including a list of SOP, summing up an order of maintaining the biobank in active status and deposition of microorganisms strains.

The basis of the prospects for further development is composed of updating of biobank stocks, implementation of new technologies and methods in biobanking with the use of pathogenic and biological agents, standardization of methods of biological research and procedures of official strain deposit and completing the biobank with the help of high-skilled staff.

BIOLOGICAL RESOURCE CENTER "RUSSIAN NATIONAL COLLECTION OF INDUSTRIAL MICROORGANISMS" (BRC VKPM) OF NRC "KURCHATOV INSTITUTE" IS A KEY LINK OF BIOTECHNOLOGY DEVELOPMENT IN THE RUSSIAN FEDERATION

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Biological Resource Center "Russian National Collection of Industrial Microorganisms' (BRC VKPM) of the National Research Center "Kurchatov Institute" – GosNIIgenetika was founded in 2014 on the base of All-Russian Collection of Industrial Microorganisms (Decree No. 982 of the Ministry of Education and Science of the Russian Federation, 11/08/2014).

The main functions of BRC VKPM are:

- forming a national fund of microbiological genetic resources for biotechnology application (including ones obtained under State Research programs), providing guaranteed storage and regulated availability to a wide range of industrial, research, monitoring, and educational organizations;
- national and international patent deposit services to provide a protection of intellectual property rights;
- standardization of bio-resources (including genetically modified organisms) used in industrial biotechnology in order to provide their bio-safe application;
- providing knowledge-based services to scientific and other companies in the field of microbiological genetic resource application;
- development of methodology for microbiological resource application in industrial biotechnology;
- monitoring and development of normative documentation and standards for microbiological genetic resource (including genetically modified organisms) application;

BRC VKPM is an institution comparable with leading world biological resource centers in terms of collection funds and performed services. Researchers at BRC VKPM perform a large amount of scientific work for developing industrial microbiological producer strains.

The collection fund of BRC VKPM consists of more than 20 000 strains of microorganisms / cell lines. BRC VKPM provide services to more than 1 500 companies annually (approximately 90 % of the main collection strain market in the Russian Federation). BRC VKPM conducts more than 80 % of international patent deposit operations in the Russian Federation.

ISOLATION OF THE LYSOGENIC ACTINOBACTERIUM FROM THE RESERVOIR OF MELTWATER OF ANCIENT ICE WEDGE FROM THE PLEISTOCENE GLACIAL COMPLEX OF MAMONTOVA GORA (YAKUTIA, RUSSIA)

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The most expressed exposures of permafrost ice wedges are in the region of Mamontova Gora on the left bank of the Aldan River (CentralYakutia). Seasonal thawing of relic ice wedge layers is followed by the formation of a reservoir where the meltwater stagnates and then ends up in the Aldan River in the form of water flows. A novel actinobacterium, designated K3–2, was isolated from samples of the reservoir of meltwater. Strain K3–2 formed orange–yellow, circular and smooth colonies that were approximately 1.0–2,0 mm in diameter after 2–3 days cultivation at 28°C on an ISP1or ISP3 agar plate. Cells of the strain were irregular, short rod-shaped (0.3–0.6 × 1.0–3.0 μm), gramstain-positive, non-motile and non-endospore-forming. Cells in older cultures tended to be shorter and rounder. The strain was also catalase- and oxidase-positive. Growth occurred at 20° – 30°C. The pH range for growth was 5.0–7.0. Optimal growth was noted at 28°C and pH 7.0. The strain exhibited good growth with NaCl concentrations of 0–7 % (w/v). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain K3–2 was related to members of the family *Beutenbergiaceae*. The highest 16S rRNA gene sequence similarity value was observed with *Serinibacter salmoneus* Kis4–28^T (97.2 %) and *Serinibacter tropicus* PS-14–7^T (97.2 %). Subsequent analysis of 16S rRNA gene sequences confirmed belonging of the strain K3–2 to the genus *Serinibacter* and detachment from two of its known species *Serinibacter salmoneus* Kis4–28^T and *Serinibacter tropicus* PS-14–7^T. The strain *Serinibacter* sp. K3–2 is deposited in three international collections (VKM Ac-2719, VKPM Ac-2020, DSM 103859).

In the analysis population of the strain *Serinibacter* sp. K3–2 phage plaques were detected in the zone of active growth of some bacterial colonies. Small negative colonies (0.15–0.2 mm) were detected after 3–5 day incubation. The formation of phage plaques was observed visually or under a phase contrast microscope. To elucidate where these negative zones come from, samples taken from the plaques and the filtrate obtained by filtering the culture liquid of the lysogenic isolate *Serinibacter* sp. K3–2. To detect intracellular phage particles u*ltra-thin* sections of *Serinibacter* sp. K3–2 were observed through transmission electron microscope. As a result, phage particles of morphologically spherical type were detected in all variants.

BIODIVERSITY AND ENVIRONMENTAL FUNCTION OF NEW *THERMOPLASMATA*

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Acidophiles are organisms that inhabit natural or man-made (mostly mine impacted) environments that are favourable to their preferences to thrive in the low-pH milieu. Extreme acidophiles have pH optima at 3 or below and are represented by bacteria, archaea and eukaryotes. The most acidophilic organisms known so far belong to the order *Thermoplasmatales (Thermoplasmata*, *Euryarchaeota)* and are represented by moderately thermophilic and mesophilic members. Due to the low numbers of successfully cultured *Thermoplasmata*, their physiological characteristics and roles in the environments have mostly been derived from *in silico* predictions from metagenomic data. *Numerous so-called* "Alphabet plasmas' were predicted to oxidise iron, carbon monoxide and utilise methylated compounds [1]. However, the shortage in experimental verification of such predictions has largely limited our understanding on metabolism, physiology, and environmental roles of these archaeal lineages.

Recently, the rather short list of cultured mesophilic members of the order *Thermoplasmatales* has been extended by new species of the novel family *Cuniculiplasmataceae*, previously identified in metagenomic assemblies to represent the candidate taxon "G-plasma", which appeared ubiquitous in various acidic locations worldwide [1–3]. *Cuniculiplasmataceae* were described as cell-wall-less, acidophilic, organotrophic and facultatively anaerobic organisms, isolated from acidic mine waters formed on copper-ore-containing sulfidic deposits (Spain and North Wales, UK) [2]. The physiological study confirmed the necessity of experimental confirmation of bioinformatic predictions. Metagenomic analysis of available databases from different geographical settings considers that the family *Cuniculiplasmataceae* composed by multiple genera and species.

Isolated and sequenced *Cuniculiplasmataceae* members exhibited largest genomes among *Thermoplasmatales* (1.87–1.94 Mbp), and demonstrated a substantial genome conservation, stable also within their genomic islands, despite their geographically distant emplacements. Being facultatively anaerobic heterotrophs, they secure an ancestral form of A-type terminal oxygen reductase from a recognisably different parental clade [3]. The lack of complete pathways for biosynthesis of a number of amino acids pre-determines the lifestyle of these organisms as scavengers of proteinaceous compounds from surrounding microbial community members. These low-pH ecosystems are generally characterised by poor organic substrate resources and substantial concentrations in inorganic substances, in particular sulfur compounds and iron. Soluble low-molecular weight organic compounds originated from primary producers are considered as credential substrates for *Cuniculiplasmataceae* and other *Thermoplasmatales* in environmental settings. Acidophilic microalgae are probably one of the main carbon-fixing and metabolites'- producing community components in many outward places such as mine drainage waters.

Other globally distributed acidophilic *Thermoplasmata* discovered through metagenomic data analysis, for which it is still not possible to deduce their physiological properties and environmental functions, will also be discussed.

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CULTURE COLLECTION UNIVERSITY OF GOTHENBURG (CCUG): 50 YEARS YOUNG AND GROWING INTO THE 21ST CENTURY, ARCHIVING, TYPING, IDENTIFYING AND RESEARCHING CLINICALLY-RELEVANT BACTERIA

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2018 April, the CCUG passed 50 years in existence and is older than most of the people now working in the Typing Lab and Collection. With the archiving of strain CCUG 1, *Citrobacter freundii*, isolated from a clinical sample by the General Diagnostics Department of the Sahlgrenska University Hospital in Gothenburg, Enevold Falsen "baptized" a new Unit within the hospital Bacteriology Laboratory (BaktLab), dedicated to improving the diagnostics of infectious diseases through systematic analyses of reference strains of pathogenic bacteria and their taxonomic relatives. The foresight of Enevold, as the founder and initial Curator of the CCUG, recognized that establishment of a collection of well-characterized reference strains of clinically-relevant bacteria would provide a valuable resource for understanding bacterial taxonomic relationships and complement strategies for identification and diagnostics of pathogenicity, virulence and antimicrobial resistance.

The CCUG, much as the prokaryotic diversity that Culture Collections try to harness, has undergone also evolution, that is, in workflow pathways for appraising microbial diversity, first, by employing morphological and metabolic phenotypic and chemotypic approaches in analyzing microorganisms, later by adopting genotypic and, more recently, genomic protocols for analytical applications. The CCUG has eagerly accepted the necessity for Collections to expand their roles in the development of improved characterisations of microorganisms and has joined with other microbial Collections, which have participated in more than 60 % of descriptions of all new prokaryotic species [1]. The CCUG has participated in and co-authored more than 300 publications over the past 50 years (an average of a new publication each 2 months), most of which have focused on descriptions of new or reclassifications of bacterial species. However, while the CCUG historically has been working

within a genus- and species-oriented framework that has dominated Collections' traditional focus, the CCUG has begun also to recognize that Collections must accept responsibility for addressing the research and biotechnology communities' imperative that the pragmatic functional "unit" for applications derived from microbial diversity is the "strain". The individual features of microbial strains, that may not be observed as features of a "species", likely will be the future genetic resources of value to researchers [2].

Developments in high-throughput DNA sequencing-based genomics and mass spectrometry-based proteomics analyses have facilitated CCUG efforts forward in developments for addressing clinical concerns in diagnostics, employing cultivation-dependent, as well as cultivation-independent-based analyses of infectious bacteria in clinical samples. The CCUG presents, within more than 70,000 strain entries of bacteria that have passed through the Typing Lab, in conjunction with archiving more than 3,400 Type strains of validly published species, also "special" collections of strains with particularly relevant features of interest to the scientific community, including

Quality Control strains required by reference laboratories, strains of a range of taxa exhibiting antimicrobial resistance, such as all MRSA isolated in western Sweden since 1982, most of which have been PFGE- or *spa*-typed, as well as pneumococcal strains, most of which have been serotyped, by traditional or CCUG-developed "sequetyping" protocols, for applications by developers of vaccines. The future of the CCUG will rely on 3 "legs' of activities: 1) typing and identification for the BaktLab and other microbiology labs, as the priority of the CCUG Lab; 2) archiving, documenting and distributing reference strains, within the regulations of the Nagoya Protocol for Access and Benefit Sharing [3]; and 3) research, with focus on improving identification and diagnostics of infectious microorganisms.

2018 May 24, the CCUG launched (🚀) a new website at *https://www.ccug.se*. We invite colleagues of ECCO to visit the sites and to try out new search tools for accessing the collection of archived strains exhibiting particular features. Please inform us of any encountered problems at *curator@ccug.se*.

2018 September 7, the CCUG consecrated its 50-year anniversary with a celebration at the Institute in Gothenburg. Drinks, food and music were on hand and we invited ECCO colleagues to join the CCUG in marking its march into the next 50 years.

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FROM THE URAL COLLECTION OF ALKANOTROPHIC ACTINOBACTERIA TO INNOVATIVE BIOTECHNOLOGIES

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In recent years, the government agencies of Russia have been actively supporting the collection activities. The current State Coordination Program for the Development of Biotechnology in the Russian Federation until 2020 "BIO 2020" envisages the development of an infrastructure that encompasses national specialized bioresource centers. In order to ensure the stable functioning of existing biological collections and evolving bioresource centers, the Program provides for the long-term financing, tax incentives and improved customs legislation on transfer/exchange of biomaterials. Based on the inventory results of the non-medical microbiological collections in the Russian Federation, there have been selected collections of microorganisms, which have been receiving the special-purpose financial support since 2016, thus giving hope for the successful solution of biotechnologization of the country.

In Russia, there are about a hundred collections of non-pathogenic microorganisms (bacteria, archaea, mycelial fungi and yeasts) housed mainly in research institutes and universities. The largest All-Russian Collection of Microorganisms (VKM), which has a strong scientific reputation at the national and international levels, possesses good research facilities, developed information resources and qualified staff. In 2014, the first National Bioresource Center was established on the basis of the Russian National Collection of Industrial Microorganisms VKPM (WDCM # 588) (BRC VKPM, *http://www.genetika.ru/vkpm*). Smaller specialized collections are distributed unevenly in the European and Asian parts of Russia (80 and 20%, respectively), reflecting the historically formed uneven distribution of the population density, as well as knowledge- and labor-intensive industries, across the country. These collections differ in terms of microorganisms stored with different physiological properties (luminous, hydrocarbon-oxidizing, antibiotic-producers and bioactive compounds, plant symbionts) and ecological confinement (marine, agricultural, extremophilic). These collections are underpinning the formation of appropriate biotechnological clusters of the country, strengthening its food base and biosafety. The bioresource collections successfully functioning in the Urals, Siberia and the Far East are of particular importance for the regional development of the country.

The Urals specialized collection, in which the authors work, may serve an example of how a microbiological collection evolves into a mBRC. The Regional Specialized Collection of Alkanotrophic Microorganisms (IEGM, *http://www.iegmcol.ru*) is focused on the interests of biotechnology and specializes in the maintenance of microorganisms that are capable of natural and anthropogenic hydrocarbon oxidation and thus participate in the biogeochemical processes of the biosphere. The IEGM collection is recognized as a Unique Research Facility; has the status of the Center for Collective Use and is a part of the National Registry of Objects of Research Infrastructure of the Russian Federation (*www.ckp-rf.ru/usu/73559*). The collection is a member of the World Federation for Culture Collections (WFCC, *http://www.wfcc.nig.ac.jp/index.html*), the European Culture Collections' Organization (ECCO, *http://www.eccosite.org*), Microbial Resource Research Infrastructure (MIRRI, *http://www.mirri.org*). It is registered with the World Data Center for Microorganisms (WCDM # 768, *http://wdcm.nig.ac.jp*). Biological information on the maintained cultures from the IEGM collection is presented in the Consolidated Catalog of

Russian Non-medical Collections (*http://www.sevin.ru/collections/*), Pan-European Rhizosphere Resource Network (PERN, *http://www.pern-brio.eu*), and the Global Catalog of Microorganisms (GCM, *http://gcm.wfcc.info*).

The efforts of the research team of the IEGM collection are aimed at achieving the world standards in all areas of collection activities in order to meet the requirements of a national specialized BRC in accordance with the adopted Roadmap. The concept of such BRC profile is justified by the fact that the Perm region is one of the promising oil- and gas-extracting regions in the European part of Russia, which is associated with environmental problems and oil pollution. The main idea of the regional specialized BRC is the creation of the single center for comprehensive studies of a biotechnologically significant group of microorganisms – representatives of hydrocarbon-oxidizing actinobacteria. They occupy the dominant position in biotopes of oil-polluted areas and play the key role in the processes of natural attenuation of oil-polluted ecosystems.

Environmental pollution by anthropogenic hydrocarbons will remain the global environmental problem for a long time. It is due to the unprecedented scale of natural resource exploitation, among which petroleum hydrocarbons are of priority. Today, the oil pollution extent of the natural environment considerably exceeds the existing volumes of remediation efforts. Hence, there is the steadily increasing fundamental interest (especially after the 2010 oil spill in the Gulf of Mexico) in studying the hydrocarbon-oxidizing microorganisms (their genomes, metabolism, adaptive reactions), searching for key biooxidants, novel biodegraders and their biotechnological applications (for pollution diagnostics, oil bioremediation, etc.).

The work program of the IEGM collection includes (i) fundamental studies of biodiversity and functional properties of ecologically significant groups of actinobacteria, adaptation mechanisms for their survival in conditions of long-term chemical contamination of terrestrial and aquatic ecosystems; (ii) the collection-based studies itself, including adequate collection of natural material, classification and taxonomic description of cultures based on the principles of polyphase taxonomy, control of authenticity of objects, assessment of biotechnological usefulness of cultures, optimization of methods for long-term storage of collection cultures and their functional diversity, up-dating the information related to bacterial cultures in accordance with international standards; and (iii) applied research employing the gene pool of alkanotrophs to develop biocatalysts suitable for obtaining products of biosynthesis from hydrocarbon raw material, target intermediates of biologically active compound synthesis, new biologicals and effective bioremediation technologies.

This research was fulfilled in the frame of State Task Registration No. 01201353247 and the Program for the Development and Inventory of Bioresource Collections.

YEAST COLLECTION OF THE SOIL BIOLOGY DEPARTMENT IN LOMONOSOV MOSCOW STATE UNIVERSITY (KBP MSU)

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The Yeast collection of the Soil Biology Department in Lomonosov Moscow State University (KBP MSU) was founded by Dr. Inna Pavlovna Bab'eva in 1958. At first the Collection started with strains isolated by I. P. Babjeva, I. S. Reshetova, and co-workers from different soils from the former

USSR. Collection was enriched also with strains from different isolation sources such as phylloplane, tree exudates, fungi, insects and others related with the biogeocenotic approach to the study of yeast ecology [1]. The Collection plays an important role in the students' education process, yeast strains are used in teaching bachelors, masters and PhD-students at the Soil Biology Department.

The Collection holds presently more than 1800 yeast strains from natural environments that make it the third largest yeast collection in Russia, after VKM and VKPM [2]. Today, the KBP MSU contains 355 species of yeast fungi, together with undescribed species, what belonging to 134 genera. The Collection is expanding with scientifically interesting strains in the realization of ecological and biogeographical projects. The geographical diversity of the yeasts maintained in the Collection is reflected by 30 different countries of the strains' origin. Nevertheless, the main part (57%) of the yeast cultures deposited in the KBP MSU now has the Russian origin. The Collection has necessary facilities for sequence-based species identification. The yeasts are stored in glycerol at –80°C.

The KBP MSU is not commercial and shares strains with researches and other collections on the basis of research agreements. The Collection regularly exchanges strains with culture collections, mainly with VKPM, DSMZ, CBS and also with VKM, MUCL, ATCC, NRRL, CCY, IFO, UOFS. The KBP MSU is funded by research grants from the Russian Foundation for Basic Research (RFBR) and the project of Russian Science Foundation (RSF) called "Noah's Ark" for consolidation available university collections into a single depository.

From 2018 the KBP MSU has been listed in the Culture Collections Information Worldwide database (WDCM CCINFO); it has the number 1173. The online version of the KBP MSU catalogue is available at *https://depo.msu.ru*.

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BIODEGRADATIVE POTENTIAL OF MICROORGANISMS OF THE COLLECTION OF THE CENTER "BIOTECHNOLOGY" KUBAN STATE UNIVERSITY

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The working collection of microorganisms of the Department of genetics, microbiology and biotechnology of the Kuban state university was originally created during the researches in field of microbial damage of industrial materials and products under the auspices of the Scientific Council on biological damage of the RAS. Initially, the microorganisms-degraders had been isolated from the drilling mud containing carboxymethyl cellulose and lubricants based on hydrocarbons. Cellulolytic and hydrocarbon-oxidizing bacteria, belonging to different phylogenetic groups were

isolated. Further development of the collection was based on microorganisms isolated from natural and waste waters, soils, grounds, drilling fluids, oil sludge, industrial fluids delivered from the most different regions: Krasnodar, Stavropol, Rostov regions, Adygea, Western Siberia, Sakhalin, Murmansk oblast, Kola Peninsula, etc.

The microorganisms isolated from oil sludge long-term storage (30 to 80 years) constitutes the particular interest. This microorganisms are mainly actinobacteria, capable to degrade heavy fractions of hydrocarbons, including C_{27}-C_{33}, resistant to high concentrations of NaCl and heavy metals, may be cultivated in a wide range of temperature and acidity of environmental conditions.

For optimization and standardization of carrying out bioremediation, hydrocarbon-oxidizing cultures in collection were clustered by their ability to decompose individual hydrocarbons and some commercial oil products. Multi-dimensional scaling was used to group the signs of hydrocarbon degradation depending on the quality characteristics of the spectrum of hydrocarbons consumed. According to the set of features, all isolates were classified by methods of polyphase taxonomy in accordance with modern phylogenetic systematics.

In addition to the hydrocarbon-oxidizing bacteria, in the collection were included strains-satellites, as a rule, presented in natural microbial communities, and bacteria, which may be found in the oil-polluted soil in the bioremediation process. These non-degrading strains are interesting from the point of view of physiology and taxonomy of microorganisms. A wide biological diversity of collection strains of hydrocarbon-oxidizing bacteria, including genera *Arthrobacter*, *Mycrobacterium*, *Nocardioides*, *Micrococcus*, *Agromyces*, *Streptomyces. Pseudomonas*, *Acinetobacter*, *Sphingomonas*, *Dietzia*, *Rhodococcus*, *Kocuria*, *Gordonia*, *Tsukamurella*, *Bacillus*, *Paenibacillus*, *Azotobacter*, *Azomonas*, *Rhizobium*, etc., allowed to use them in the bioremediation of oil-contaminated objects, including detoxification of long-stored oil wastes.

A number of oil-oxidizing biological products, including immobilized products, with the possibility of biological agent changing depending on the object of bioremediation condition, and biological products having phytostimulation properties was created. Scientific approach to the problem, knowledge of physiological and biodegradative properties of collection strains provided a successful solution to the problems of microbiological cleaning of fresh oil-contaminated lands and utilization of oil sludge in the South of Russia. Only in the Krasnodar region was transferred into the category of viable soils over 50 000 tons of long-term stored oil wastes. Territories, contaminated with fresh oil or other petroleum products were returned to agricultural use after microbial remediation. The “marine” microorganisms, isolated from the shelf zone and water estuaries of the Azov Sea significantly enriched the collection. The same criteria for identification and selection as for soil bacteria were applied to them.

The need for recycling food, medical, fat waste and pesticides necessitated and study the biodegradable ability of already existing collections and newly isolated microbes. The majority of collection cultures have proteolytic activity of various degrees. From 124 hydrocarbon-oxidizing cultures, 50 had a pronounced lipolytic activity in relation to hard-to-degrade solid fats, the main pollutants of the environment. Vegetable fats were able to dispose of more than 80 isolates. Thus the lipolytic activity is in no correlated with oil degradation, and was characterized mainly as *Pseudomonas* and *Bacillus*. For a number of strains belonging to the genus *Rhodococcus*, the ability to use as a sole source of carbon and energy of pesticides such as diazinone, metribuzin, imidacloprid, imazamox was found. For imidacloprid, an insecticide of the class of neonicotinoids, the degree of biotransformation was assessed quantitatively.

The collection of cultures of KubSU is constantly updated, maintained on a semi-liquid nutritive agar and in a lyophilized state, periodically monitored for compliance with previously declared physiological and destructive properties. Long-term stored cultures are being audited using MALDI TOF MS and 16s rRNA gene analysis.

SECONDARY METABOLITES OF *PENICILLIUM* STRAINS FROM THE VKM SUBCOLLECTION OF EXTREMOTOLERANT FUNGI

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In the low-temperature ecotopes a group of extreme-tolerant fungi highly adapted to the action of stresses have attracted attention. Fungi of the genus *Penicillium* have a special place among this group. These fungi are often found in the deep Arctic and Antarctic horizons, including the ancient ones dated back to millions of years. They also could be isolated from the surface deposits – both anthropogenically impacted and background sites.

Approximately 900 fungal strains isolated from high latitude areas are maintained in All-Russian Collection of Microorganisms (VKM), 30% of them belong to the genus *Penicillium.*

The qualitative composition of secondary metabolites, mainly mycotoxins, was analyzed in 99 strains of 22 species of this genus. The strains were isolated in low-temperature habitats (26 strains from Antarctica and 73 from the Arctic).

These strains produced 56 secondary metabolites in some chemical subclasses. The most of discovered compounds belong to diketopiperazine alkaloids and clavine alkaloids (21.4% both). Quinoline alkaloids presented 10.7%, benzodiazepine alkaloids, terpenes, amino acid derivatives and quinazoline alkaloids – 7.1% each. Some secondary metabolites are of a group of polycyclic indole alkaloids (3.6%).

The presence of secondary metabolites could be a competitive advantage of the producing organism for survival in extreme habitats. But the production of mycotoxins is not only an aggressiveness tools in competitive relationships. Fungi that produce mycotoxins also demonstrate the ability to disintegrate a wide range of anthropogenically generated hazardous substances. This makes them the excellent candidates for bioremediation. There is an opinion that several regulatory factors of mycotoxin synthesis control also regulate bioremediation abilities of toxigenic strains [3]. It is the fungi of the genus *Penicillium* that are capable to biosorption of heavy metals, biodegradation of halogenated substrates, phenols and other compounds. For example, it has been shown that, from the soils polluted with petroleum, fungi of the genus *Penicillium* are primarily isolated, at the same time, the number of species potentially pathogenic for humans increases [6].

Our studies of Antarctic soils polluted with petroleum products in comparison with original sediments of the same continent showed that oil pollution not only changes the species community structure of the *Penicillium* fungal group presented here, but also affects the amount and composition of the mycotoxins they release. From polluted soils only there were isolated fungi that produce polycyclic indole alkaloids, terpenes and derivatives of amino acids – N-acetyltryptamine, andrastin A and C, fomenon, chaetoglobosin A, communesin B. Many of these substances possess powerful

cytotoxic and biocidal properties. For example, communesin B exhibits cytotoxity against various cell lines, as well as anthelmintic and insecticidal activity [4]. As for the fungi, for them, mycotoxins can be not only assistants in competition, but also participate in the processes of adaptation to different stressors, including stress of parasite in host-organism. It has been shown that chaetoglobosins produced by parasitic fungus *Ijuhya vitellina* may have a helping function in the described parasitism of nematode eggs [2].

The study of secondary metabolites of *Penicillium* strains allows not only to identify new producers of already known substances, but also to discover new low-molecular compounds. Thus, it was shown the strain of *Penicillium citrinum*, isolated from permafrost sediments of the Kolyma lowland, having an age of 1.8–3.0 Ma, formed novel secondary metabolites of the quinoline class, which were named quinocitrinin A and quinocitrinin B [5]. A study of the activity of these compounds on pro- and eukaryotic microorganisms showed that they have broad biocidal activity against gram-positive and gram-negative bacteria, yeasts and fungi [1]. These metabolites were also cytotoxic for tumor cells.

According to the practical needs of biotechnology a special database in Internet had been constructed. The database presents the latest data on VKM fungal strains as well as the secondary metabolites produced. Appropriate links to StrainInfo (*www.straininfo.net*) connect these data to the same strains in other microbial collections. More detailed control of fungal transfer between collections can be inspected with the HISTRI links. Both kind of links – StrainInfo and HISTRI – are presented for all the VKM strains.

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GENETIC RESOURCES OF THE STATE COLLECTION OF PHYTOPATHOGENIC MICROORGANISMS OF THE ALL-RUSSIAN RESEARCH INSTITUTE OF PHYTOPATHOLOGY (ARRIP)

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Successful solution of tasks to ensuring food security requires the creation of cultivars resistant to especially dangerous diseases. For the purpose of prevention of harvest losses for the most dangerous and economically significant pathogenic organisms it is necessary not only to carry out monitoring of virulence gene pool, but also to study the nature of pathogens variability, to define a potential possibility of emergence of the new dangerous genes and races in different populations. For this purpose the centralized collection of pathogens cultures with the stable properties which will provide researches in the spheres of phytopathology, immunology, selection, genetics, toxicology, parasitology and other is necessary. The collection of phytopathogenic microorganisms represents the basic gene pool of races, biotypes, pathotypes of the phytopathogenic fungi, bacteria and viruses which are found in the extensive territory of the Russian Federation. Collections of phytopathogenic organisms are created and successfully function in the majority of the developed countries of the world. In Russia the similar gene pool is created for the first time, until recently in various institutions and laboratories of our institute there were only working collections of different species of phytopathogenic microorganisms.

The collection of phytopathogenic microorganisms in ARRIP was created in 1960 as a result of researches of specific and intraspecific structure of populations of phytopathogens in different zones of the country and was completed mainly by the most virulent strains of causative agents of diseases.

According to the Governmental Regulation No. 725–47c "On the measures for the maintenance and rational use of microorganism collections" (24.06.1996), the collection was named as "The State Collection of Phytopathogenic Microorganisms and Cultivars for Identification (Differentiation) of Pathogenic Strains of Microorganisms (SCPPM ARRIP)" and obtained the status of the State Depository with the function of a depositing of strains of agricultural microorganisms. Under this Regulation Collection is determined as unique in Russia the coordinating center for collecting, deposition and storage of used in agro-industrial complex of the Russian Federation phytopathogenic microorganisms, nonpathogenic for the people and farm animals.

Created in the All-Russian Research Institute of Phytopathology State Collection includes the phytopathogenic organisms causing extensive damage to agriculture and resulting in larger losses of a harvest. This Collection of plant pathogenic microorganisms is the largest in Russia and unique in the sphere of crop production, and it has the status of State collection. Now it contains more than 3500 units of storage of pathogenic for plants strains: fungi (1759 strains), oomycetes (359 strains), bacteria (1319 strains), viruses, phytoplasms (86 strains) and others, affecting the main crops of Russia: wheat, rye, oats, barley, rice, potatoes, vegetables, some commercial crops. Some strains are stored in a collection in stable state more than 50 years. As a part of a collection there are strains of especially dangerous pathogens of plants and also the toxicogenic strains affecting not only plants, but also being dangerous to animals and the humans.

Different microorganism species are stored in low-temperature freezers, in the sterile soil, in nutrient medium, on herbarium samples, on live plants etc. Depending on storage conditions periodically from once a month up to once in 10 years the control of viability and renewing of collection isolates are carried out.

Following operations are conducted regularly in Collection:

– replenishment by stable new species and strains of phytopathogenic microorganisms, their preservation, storage and maintaining in a viable and non-contaminated state;

– perfecting of methods of selection, identification, preservation, storage and genotyping;

– multiplication of biomaterial and providing with it research establishments, selection centers and other consumers

Collection is replenished by new species, strains and isolates of the phytopathogenic microorganisms allocated from the samples of the demaged plants from various regions of the Russian Federation, by strains from working collections of laboratories and groups of institute and also by strains received on exchange from the Russian and foreign collections.

In Collection the modern methods of storage of biomaterial, including a cryopreservation and a lyophilizing are applied; molecular identification of a number of the strains removed from storage is carried out.

Equipment and inventory of the State collection allows to provide reliable storage of phytopathogens strains and conducting of researches at the modern level.

COLLECTION OF THE OPPORTUNISTIC FUNGI ISOLATED FROM THE KOLA PENINSULA SOILS

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The fungi collection of the Laboratory of Microorganisms Ecology of the Institute of North Industrial Ecology Problems – Subdivision of the Federal Research Centre "Kola Science Centre of Russian Academy of Science" (INEP KSC RAS) includes 305 fungi strains. Fungi belong to 2 divisions: *Ascomycota* (90% of collection) и *Zygomycota* (10%) according to *www.speciesfungorum.org*. Division *Ascomycota* is represented by the orders *Eurotiales*, *Hypocreales*, *Erysiphales*, *Pleosporales*, *Microascales*, *Sordariales*, *Dothideales*, *Capnodiales*, *Helotiales*. Division *Zygomycota* is represented by the orders *Mucorales*, *Mortierellales*, *Umbelopsidales*. 40% of the fungi collection is fungi of the genus *Penicillium*, which dominate in the natural environment of the Kola Peninsula.

The collection was established in the 1985thies. Fungi were isolated from native soils and soils polluted by industrial emissions of the copper-nickel and aluminium plants, as well as from oil-contaminated soils of the Kola Peninsula. All strains are preserved on agar slant. Identification of fungi was carried out on the basis of culture and morphological features and molecule-genetic methods in the Laboratory of Ecology of Microorganisms INEP KSC RAS (Apatity) and the Laboratory of Geography and Systematics of Fungi of the V. L. Komarov Botanical Institute of Russian Academy of Science (St. Petersburg).

In total, 62 species of fungi (from 29 genera), which belong to the opportunistic ones, were identified in the polluted soils of the Kola Peninsula. The largest number of the opportunistic fungi species belonged to the following genera*: Penicillium* (11), *Aspergillus* (8), *Mucor* (4), *Phoma* (4), *Lecanicillium* (3) and *Cladosporium* (3). Most fungi belong to the group BSL 1, only 7 fungi species belong to the group BSL2 [1]. We did not isolate the fungi belonging to the BSL3 group. Also 17 species belong to opportunistic fungi according to Satton et al. 2001 and Sanitary… 2008 [3, 2].

We revealed 34 opportunistic fungi species from the polluted soil of the Aluminum Plant emission. In the polluted soils, the share of the opportunistic fungi increased up to 50 % compared to the background soil, where it made 35 % of the total number of the identified species. Among them, there are agents of mycoses and also the fungi causing diseases of respiratory and digestive systems from genera *Acremonium*, *Alternaria*, *Amorphotheca*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Fusarium*, *Mucor*, *Myxotrichum*, *Paecilomyces*, *Penicillium*, *Phoma*, *Rhizopus*, *Sarocladium*, *Scopulariopsis*, *Stachybotris*, *Trichoderma*.

We isolated 30 fungi species belonging to opportunistic ones from the soil, polluted by Copper-Nickel Plant emissions. Their share in the polluted soil is 45 %, and in the background soil is 30 % of the total number identified species. In the soils of both plots, the opportunistic fungi group is represented with the following genera *Acremonium*, *Aspergillus*, *Aureobasidium*, *Collariella*, *Cladosporium*, *Gongronella*, *Lecanicillium*, *Oidiodendron*, *Penicillium*, *Codaphora*, *Phoma*, *Purpureocillium*, *Rhizopus*, *Sarocladium*, *Stachybotris*, *Talaromyces* and *Trichoderma*.

We isolated 24 fungi species from the soil contaminated by oil products. The following genera belonged to the opportunistic fungi group; they were isolated from the soil contaminated with diesel fuel: *Acremonium*, *Alternaria*, *Aspergillus*, *Aureobasidium*, *Fusarium*, *Humicola*, *Lecanicillium*, *Mucor*, *Penicillium*, *Phoma*, *Rhizopus*, *Pseudogymnoascus*, *Rhodotorula*, and *Trichoderma*. The species gg. *Fusarium*, *Mucor*, *Penicillium* and *Trichoderma* occurred in soils both with low and high diesel fuel concentrations; while the species g. *Lecanicillium* was isolated only from the soil with high diesel fuel doses. The genera *Alternaria*, *Aspergillus*, *Aureobasidium*, *Humicola*, *Phoma*, *Pseudogymnoascus* and *Rhizopus* occurred only in the soil with low concentration of diesel fuel. Most species belonged to *Penicillium* (5), *Mucor* (3), *Lecanicillium*, *Phoma* and *Trichoderma* (2), while other genera were presented by one species.

Preservation of the opportunistic fungi collection is necessary for further studies their potential pathogenicity for assessing the risks associated with their share increasing in anthropogenic environments.

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CHANGES IN SOIL BACTERIAL COMMUNITIES DURING CROPLAND -TO- FOREST SECONDARY SUCCESSION

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The land use and management has a significant impact on global biogeochemical cycles of biogenic elements and the Earth's climate.The recovery of ecosystems after their withdrawal from agricultural use is poorly understood. Currently, in Russia, the former arable lands occupy about 20% of the territory and more than half of them are not used in agriculture since the early 90-s.The relationship between plants, soil and microorganisms is the driver of ecosystem functions, and so it can be postulated that any change in plant cover and/or soil properties causes significant shifts in soil bacterial community composition and influence ecological processes. To address this question, this study used high throughput Illumina sequencing of 16S rRNA genes amplified from DNA extracted directly from the soil samples. In examining the dynamics of soil biota, cultivated lands are frequently used as the initial stage of secondary succession when constructing a chronosequence. In order to detect differences in diversity, composition and relative abundance of bacterial taxa from an area which represent different stages of a secondary succession on gray forest soils in Moscow region, Russia, we examined soils from sites covered by 5-, 7-, 10-, 15-, and 25-year-old fallows surrounded by the same forest and wheat field. Several chemical (pH, total C and N, NH_4 -N and NO_3 -N) and physical (moisture content, porosity, water-filled pore space and bulk density) soil properties were evaluated.

The soil properties showed remarkable variability among individual sites. The differences detected in soil pH among the land types the lowest pH in wood site. Significant differences were also observed in soil carbon content, and it was more than three times higher in wood soil as compare with arable soil. Forested site not only showed the highest carbon content, but also the highest $N\text{-}NH_4^+$ and lowest P_2O_5 content as well.

The total of 3,544 OTUs ranging from 1,700 to 2,100 per sample, were obtained. The dominant bacterial phyla (more than 10% in the community) were *Proteobacteria*, *Acidobacteria*, *Verrucomicrobia* and *Bacteroidetes* in all stages, but their abundance ratio varied at different successional stages. It was shown that a total of 60% of the operational taxonomic units (OTUs) were shared between sites and the clustering analysis did not show the occurrence of very distinctive bacterial communities between environments. However, *Proteobacteria* and *Acidobacteria* were overrepresented at late stage of succession. *Alphaproteobacteria* showed the same pattern as the phylum level, but *Gammaproteobacteria* had demonstrated the inverse relation. *Firmicutes* and *Parcubacteria* were found only in early successional stages. The results suggested the prevalence of a resilient core microbial community that did not suffer any changerelated to land use, soil type or edaphic conditions.

Deforestation is known to change the soil from a net sink for CH_4 to a net source as a result of alteration in the activity and composition of the methanotrophic communities. Here, soil CH_4 oxidation rates and associated methanotrophic communities were examined in a chronosequence of *gray forest* soils. CH_4 content was measured by GC and methanotrophic communities were analyzed

by cloning and sequencing of particulate methane monooxygenase key genes (*pmoA*) using the primer pair A189–mb650. Methane oxidation rates were significantly influenced by reforestation and the regenerating soils have the potential to reach those of the native forest. In fallow, scrublands and young forest soil CH_4 oxidation rates were significantly higher as compared with cropland, but not fully stabilized even after 25 years of reforestation. Based on the relative proportion of the *pmoA* clones it was shown the dominance Type II related and uncultured methanotrophs in forest soils. Both Type I and Type II methanotrophs were found in arable and postagrogenic soils, and the relative abundance of Type II methanotrophs increased with the age of regeneration and recovered after 15–25 years to that close to finding in the native forest. In the soils of agrocenosis the share of *Methylocystaceae* representatives, whose is 0.08 %-0.14 % of the community, while in the forest soil their share is significantly reduced and ranges from 0.02 % to 0.07 %. In the soil of forest biocenosis 5 OTE related to *Verrucomicrobia* subphylum 6 were found, to which all currently known methanotrophic verrucomicrobia (*Methylacidiphilum*, *Methyiacidimicrobium*) belong. On the contrary, in soils of agrocenoses and fallows only 2 OTE related to *Verrucomicrobia* sybphylum 6 were found. The proportion of these bacteria varies from 0.06 % to 0.71 % and has a significant positive correlation ($p<0.05$) with the age of the fallow.

The results illustrated that the history of land use might influence present-day community structure. Succession to a forest from former agricultural land is accompanied by changes in plant biodiversity and in the soil community. These changes are the result of a reduction or elimination of management and fertilizer applications. Our findings may be useful in future prediction of changes in methane emissions resulting from reforestation.

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GORDONIBACILLUS KAMCHATKENSIS GEN. NOV., SP. NOV., A NOVEL MEMBER OF *PAENIBACILLACEAE* FROM FROZEN VOLCANIC ASH

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A novel genus and species, *Gordonibacillus kamchatkensis* gen. nov., sp. nov., with the type strain V-9^T (=VKM B-2647^T) is proposed to accommodate endospore-forming, rod-shaped and non-motile bacterium isolated from the frozen volcanic ash sample, Kamchatka Peninsula, Russia.

The cell wall of strain V-9^T contained a lysine-based peptidoglycan as well as mannose, glucose and glycerol. In addition, two teichoic acids were revealed in the cell wall. The polar lipids included phosphatidylglycerol, diphosphatidylglycerol, acylphosphatidylglycerol, and phosphatidylinositol as major components. The fatty acid profile comprised mainly saturated anteiso- and iso-branched acids, with anteiso-C_{15} predominating (about 50 %). The major isoprenologue was MK-7.

The genome size of strain V-9^T was of 6,957,55 bp., with an average G+C content of 55 mol%. The pairwise average nucleotide identity (ANI) [1] between V-9^T and *Paenibacillus* strains with available genomes calculated using IMG/M (*https://img.jgi.doe.gov/cgi-bin/m/main.cgi?section=ANI&page=pairwise*) ranged from 69.2 to 73.7 %, with 69.7 % determined between the target strain and *Paenibacillus polymyxa* ATCC 842^T, the type strain of the type species of the genus. For comparison, the ANI values of 72.85–72.88 % were found between V-9^T and *Thermobacillus composti*, *Fontibacillus phaseoli* and *Cohnella thermotolerans*, where the latter is the type species of *Cohnella.*

The 16S rRNA gene (1537 bp) pairwise similarity calculated using the EzBioCloud server (*http://www.ezbiocloud.net/eztaxon*; [2]) showed that strain V-9^T had the highest similarity to *P. chartarius* [3] (96.5 %) and *P. aestuarii* [4] (94.5 %). The similarity values between V-9^T and type strains of the remaining *Paenibacillus* species, including the type species of the genus, *P. polymyxa* [5], did not exceed 91.5 % that is below 94.5–95 %, a threshold indicative of distinct genera [6, 7].

Most species within the genus *Paenibacillus* also show low pairwise 16S rRNA gene similarity (<92 %), which is evidence of the genus heterogeneity. The species currently included in *Paenibacillus* differ also in chemotaxonomic characters regarded as important markers in the differentiation of genera, including the cell wall diagnostic diamino acids, peptidoglycan composition, and polar lipid patterns [8].

The taxonomic dissection of heterogeneous genera with establishment of better definied novel genera, accompanied by more focused circumscription by genomic and epigenetic characteristics, could improve the taxonomy of this bacterial group (like that done previously in some other heterogeneous genera, including *Bacillus* and *Paenibacillus)*. Kämpfer *et al.* [9] took previously the first step towards improving the taxonomy of the genus *Paenibacillus* when suggested the genus *Cohnella* within the *Paenibacillus* line of descent.

Supporting the above views and based on clear genomic and chemotaxonomic differences of strain V-9^T from the type species of the genus *Paenibacillus, Paenibacillus polymyxa,* and other *Paenibacillus* species that well nested within the genus, we suggest that strain V-9^T should be described as a representative of novel genus within the *Paenibacillus* lineage.

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COLLECTIONS OF MYCOLOGY AND ALGOLOGY DEPARTMENT OF LOMONOSOV MOSCOW STATE UNIVERSITY

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The Department of Mycology and Algology of Moscow University is one of the leading centers in Russia for the study of systematics, ecology, physiology, biochemistry and genetics of fungi, fungi-like organisms and algae, and their use in biotechnology. The Department was organized in the first quarter of the twentieth century and it is the largest institution in the country, preparing highly qualified specialists in the field of Mycology and Algology.

The basis for most scientific research and training are collections and herbariums, which should be represented by organisms of different ecotopes and geographical regions. And therefore, their creation and maintenance is given great attention at the Department. In many ways, this work is based on the study of diversity and ecology of fungi and algae of different systematic and ecological-trophic groups. These research areas have been and remain a priority for the staff of the Department. To study the species composition, the specifics of ecological and geographical distribution of fungi in different natural areas and biogeocenoses, numerous expeditions were carried out in Russia and the republics of the Soviet Union and the countries of the world.

As a result of these research the collections of pure cultures of microscopic fungi, wild, cultivated and edible macromycetes, xylotrophic basidiomycetes, pathogens of solanaceous plants, mycorrhizal agents, endophytes of grasses and algologically pure collection of microalgae were established. In addition to the collections of live cultures, the Department has representative herbariums of lichenized (about 1000 herbarium samples of lichens) and phytopathogenic fungi (2687 herbarium sheets), macromycetes (273 samples), mixomycetes (8794 samples) and algae-macrophytes (807 samples).

The expansion of collections is based on the following criteria – due to strains with unique properties, rare and new species, isolates of widely distributed species that are needed for population and phylogeographic studies, and strains of interest to various biotechnologies.

The collection of soil microscopic fungi (called MSU_FS) is the richest in the number of strains and represented taxa. It includes 1840 cryosaved strains belonging to 527 species and 185

genera. Cultures were isolated from soils and the connected substrates, mainly, from protected territories (natural reserves) of different regions of the European part of Russia, Siberia, Mongolia, Vietnam. For the last 6 years 1614 strain 430 species isolated from soil and litter of tropical forests of Vietnam, among them 6 species were new to science – *Craspedodidymum seifertii* Melnik, A.V. Alexandrova et U. Braun, *Dactylaria mucoglobifera* Melnik, U. Braun et A.V. Alexandrova, *Entoloma flavovelutinum* O.V. Morozova, E.S. Popov, A.V. Alexandrova et Xiao Lan He, *Ityorhoptrum biseptatum* Melnik, A.V. Alexandrova et U. Braun, *Kiliophora novozhilovii* Melnik, U. Braun et A.V. Alexandrova, *Pyricularia contorta* Melnik, U. Braun et A.V. Alexandrova were introduced in the collection.

The large range of fungi that cause various diseases of potatoes and related plants was collected during long-term research by scientists of the Depatrment. The collection of fungi-pathogens of solanaceous plants (MSU_PPO) contains 825 strains of 6 fungal species (*Colletotrichum coccodes* (Wallr.) S. Hughes, *Helminthosporium solani* Durieu et Mont., *Alternaria solani* Sorauer, *Thanatephorus cucumeris* (A.B. Frank) Donk., *Fusarium solani* (Mart.) Sacc.) and one species of oomycetes *Phytophthora infestans* (Mont.) de Bary). They are isolated from the affected by pathogens potato, tomato and wild solanaceous plants collected in Moscow, Kostroma, Leningrad regions, Tatarstan republik, Stavropol and Krasnodar province, and from tubers of seed potatoes imported to Russia from Holland and Germany. The strains are stored on sloped nutrient media in tubes with periodic re-plating. They are used to study the intraspecific diversity, in the development of test systems for rapid diagnosis of pathogens, to assess the resistance to new varieties of potatoes and tomato and the effectiveness of fungicides.

The Department actively studies diversity, phylogeny, mechanisms of adaptation of fungi-extremophiles. Majority of them were isolated from alkaline, saline ecotopes of the European part, Siberia, Mongolia, Kazakhstan, Armenia, Tanzania, and in recent years – from bottom soil, grounds of the northern seas, raised bogs. The collection of extremophilic micromycetes (FEC) includes 150 cryosaved cultures of alkalophilic, alkalotolerant, halotolerant, acidophilic, acidotolerant, psychrotolerant fungi identified to the species on the basis of cultural-morphological and molecular genetic methods. Described New taxa of alcaliphilic and alkalotolerant fungi: 2 genera – *Sodiomyces* A.A. Grum-Grzhim. et al., *Chordomyces* Bilanenko, Georgieva et A.A. Grum-Grzhim; 8 species – *Sodiomyces alkalinus* (Bilanenko et M. Ivanova) A.A. Grum-Grzhim., A.J.M. Debets et Bilanenko, *S. magadii* S.A. Bondarenko, A.A. Grum-Grzhim., A.J.M. Debets et Bilanenko, *S. tronii* S.A. Bondarenko, A.A. Grum-Grzhim., A.J.M. Debets et Bilanenko, *Emericellopsis alkaline* Bilanenko et Georgieva, *Chordomyces antarcticum* Bilanenko, Georgieva et A. Grum-Grzhim, *Alternaria kulundii* Bilanenko, Georgieva et A. Grum-Grzhim, *A. petuchovskii* Bilanenko, Georgieva et A.A. Grum-Grzhim, *A. shukurtuzii* Bilanenko, Georgieva et A.A. Grum-Grzhim; section – *Alternaria* sect. *Soda* Bilanenko, Georgieva et A.A. Grum-Grzhim. The phenomenon of obligate alkalophile was discovered for fungi. The range of proteases secreted by them in alkaline conditions, changes in cytosol and composition of the cytoplasmic membrane were characterized. Phylogenetic analysis showed that alcaliphilic fungi polyphyletic group of ascomycetous affinity.

Collection of wild and cultivated macromycetes, lignicolous basidiomycetes (MSU_FM) and (MGUPI) includes 102 cryosaved strains and about 100 strains maintained on nutrient media strains. These collections preserve 10 species of the genus *Pleurotus*, both fungal cultivated edible strains and natural isolates, strains of *Flammulina velutipes* (Curtis) Singer, *Kuehneromyces mutabilis* (Schaeff.) Singer et A.H. Sm., *Hericium coralloides* (Scop.) Pers. and other cultivated species. Polypore fungi,

which are interesting as producers of enzymes that destroy lignocellulosic complexes of wood and compounds with immunomodulatory activity well represented in the collections.

Collection of mycorrhizal fungi supported in a small number. Endophytes of cereals grasses are represented by several tens of strains of *Claviceps* species.

The algological collection includes 70 algologically pure strains of microscopic algae, mainly are diatoms (phylum of *Ochrophyta*), as well as other taxa – *Chlorophyta*, *Charophyta*, *Haptophyta*, *Ochrophyta*, *Euglenophyta* and cyanobacteria, which are represented by several strains. Cultures of freshwater diatoms are supported on a solid agar medium, and others in the liquid medium at 16°C and mode of illumination day/night.

The Department has a collection of strains of fungi for bio-resistance testing of synthetic polymer plastics, lubricants, leather and other materials according to the many state standards.

Almost all collections are used in the educational process, as well as in the search for producers of promising antibiotics, enzymes and other metabolites, for the development of methods of cryopreservation of cultures. In our collections there are set of strains inhibiting of fungal phytopathogens, effectively oxidizing of oil hydrocarbon, capable to synthesis of antifungals compounds (peptaibols), transformate steroids, produce alkalistable proteases, cyclosporines. Work is underway on the preparation of isolates for the mutually beneficial exchange of strains with a worldwide collection of Russian (VKM, VKPM) and abroad (CBS, VTT).

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THE ROLE OF CULTURE COLLECTIONS AND THE WFCC IN BIOTECHNOLOGICAL ADVANCEMENTS

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The biological resources are vital for the economic and social development of the mankind and their sustainable use and conservation has immense importance. The Convention on Biological Diversity *https://www.cbd.int/* that entered into force on the 29th December 1993 received global level commitment towards sustainable development via ethical use of biological resources. It formed the basis for conservation of biological diversity, use of its components in a sustainable way, and fair and equitable sharing of benefits deriving from the use of natural resources that was further cemented with the implementation of the Nagoya protocol *https://www.cbd.int/abs/about/*. One of the key components of these resources derives from the microbial sources that are preserved in culture collections. In the rapidly changing world, however, the culture collections have now key roles to play and they are now more than repositories. They are *Biological Resource Centres* (BRCs) that are reference centres for sustainable development and biotechnological advancements. In-depth information is now preserved in these collections about each individual microorganism. With the advent of powerful molecular techniques presently, the more the mankind understand about the organization of the genomes of the biological entities the more powerful and equipped it becomes for bio-economy [1].

The World Federation of Culture Collections (WFCC) (*http://www.wfcc.info/)* is a Multidisciplinary Commission of the International Union of Biological Sciences (IUBS) and a Federation within the International Union of Microbiological Societies (IUMS). The WFCC is concerned with the collection, authentication, maintenance and distribution of cultures of microorganisms and cultured cells. Its aim is to promote and support the establishment of culture collections and related services, to provide liaison and set up an information network between the collections and their users, to organise workshops and conferences, publications and newsletters and work to ensure the long-term perpetuation of important collections. WFCC has thus a vital role in connecting the BRCs on a visionary platform leading to future bio-discoveries deriving from the information BRCs hold on biological entities and their taxonomic status [2].

In this presentation information will be provided on the vital roles of the BRCs as well as the WFCC for advancement of science and biotechnology and ultimately serving towards the future needs of the mankind.

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CULTURES OF ALL-RUSSIAN COLLECTION OF MICROORGANISMS – BIORECEPTORS OF AMPEROMETRIC BIOSENSORS FOR DETERMINATION OF LOW- MOLECULAR ORGANIC COMPOUNDS

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All-Russian collection of microorganisms is a source (supplier) of aerobic cultures for bioreceptors of amperometric microbial biosensors. These cultures are bacteria, actinobacteria and yeast.

Microorganisms are grown in batch conditions in flasks. The grown biomass was centrifuged, resuspended in buffer. An aliquot of suspension was placed on a carrier (immobilization by physical adsorption), and allowed to dry. The resulting bioreceptor was conjugated with an oxygen electrode of the Clark type. The parameter recorded was the maximum rate of change in the output signal

dI / dt (nA / s). Methylotrophic bacteria *Methylobacterium extorquens* VKM B-2067, *Methylopila musalis* VKM B-2646, *Paracoccus kondratievae* VKM B-2222, *Paracoccus simplex* VKM B-3226 have enzyme systems that oxidize methylamine. Using *Methylopila musalis* strain BKM B-2646 as the basis of the bioreceptor, the methylamine was detected in the range from 4 to 250 μM. The maximum permissible concentration of methylamine in water is 1 mg / l (33 μM) which is within this range. Operational stability was 5 days [1, 2]. Bacterial strain *Chelativorans oligotrophicus* VKM B-2395 is used as a bioreceptor for determining ethylenediaminetetraacetic acid (EDTA) or diethylenetriaminepentaacetate (DTPA). The EDTA determination limit was 0.125 mM, the stability was 4 weeks. The limit of DTPA was 0.50 mM; stability of the bioreceptor was 3 days [3, 4]. Actinobacteria *Rhodococcus wratislaviensis* VKM Ac-2631D were used as a bioreceptor for the sodium salt of 2,2-di- (para-chlorobenzene) acetic acid [5] and disodium orthophthalate salt [6]. The detection limit of 2,2-di- (para-chlorobenzene) acetic acid was 1.0 mM, stability of the bioreceptor was observed for 2 days [5]. Actinobacteria *Rhodococcus wratislaviensis* VKM VKM As-2782 were used as a bioreceptor for disodium orthophthalate salt too [7]. The strain of methylotrophic yeast *Ogatea polymorpha* VKM Y-2559, ealier known as *Pichia angusta* VKM Y-2559, is used for the determination of methanol. The detection limit of methanol was 25 μM. Stability of the bioreceptor was 7 days [8]. Based on immobilized cells of the yeast strain *Arxula. adeninovorans* VKM Y-2676 a laboratory model of a biosensor for determining biological oxygen demand (BOD) was developed [9].

The microorganism-substance pairs were selected, for which the influence of low-molecular organic compounds on the respiratory activity of selected immobilized microorganisms was studied. The study of the interaction between a microorganism and the substance can be useful both for the evaluation of the content of a compound in an aqueous medium and investigation of the properties of a microorganism. Estimation of the content of a compound using immobilized microorganisms is not highly specific, but can be used to solve a number of analytical problems.

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DIVERSITY OF SOIL MICROMYCETES IN THE PERMAFROST PEATLANDS IN THE FOREST TUNDRA AT THE NORTHEAST EUROPEAN

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Assemblages of microscopic fungi were studied in the seasonally thawing and permafrost layers of peat soils at permafrost peatlands. Permafrost peatlands are located at the plain of Bolshezemelskaya tundra (forest tundra) and mountain landscapes of the Subpolar Urals. In order to define the species of microscopic fungi, we used medias enriched by hydrocarbons at different cultivation temperatures (4–5, 25 and 35°C). 76 micromycetes species were revealed from peat permafrost soils. The group of *Zygomycota* contained 17 species genera *Absidia*, *Actinomucor*, *Mortierella*, *Mucor* and *Umbelopsis.* The group of *Ascomycota* contained 59 species from 18 genera. The genus *Penicillium* (30 species) was prevalent. The other positions were occupied by genera *Trichoderma* (8 species), *Mortierella* (7), *Oidiodendron* (5), *Mucor* (4), *Umbelopsis* (3), and *Aspergillus* (3). Peat soils of mountain mires had higher species diversity of micromycetes assemblages (Shannon index 4.16) and alignment (Pielu index 4.07). Higher diversity is caused by the presence of species from genera *Geotrichum*, *Oidiodendron*, *Paecilomyces*, *Actinomucor*, *Gliocladium*, *Monilia* and *Absidia* that were not found in the peat soils at the plain. *Geomyces pannorum* prevailed in the plain and mountain peats. In the permafrost peats at Bolshezemelskaya tundra, *Chrysosporium merdarium*, *Penicillium lanosum*, and *Penicillium simplicissimum* were also abundant. In the peats of mountain at the Subpolar Urals – *Penicillium funiculosum*, *Penicillium spinulosum*, and *Penicillium granulatum.* The report highlights the distribution specificity of soil microscopic fungi to taxa among seasonally-thawing and permafrost peat layers in permafrosr

peatlands of the forest tundra zone. It also deals with distribution of microscopic fungi (namber and species diversity) within seasonally-thawing layers of peat soil in dependence of vegetation.

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PENICILLIA DIVERSITY FROM FOOD IDENTIFIED POLYPHASICALLY, INCLUDING MYCOTOXIN PRODUCTION

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Penicillium is of high importance in food contamination particularly from mycotoxin production. The fungus is diverse and ubiquitous although it can be controlled from "field to fork". Mycotoxins are fungal secondary metabolites that cause disease or death in people or domesticated animals when ingested, inhaled, and/or absorbed. Major mycotoxins associated with penicillia are: ochratoxin A (OTA) (*P. verrucosum* and *P. nordicum*), patulin (*P. expansum*), citrinin (*P. expansum*), cyclopazonic acid (*P. camemberti*), penicillic acid (*P. radicicola*) and secalonic acid D, F (*P. griseofulvum*). Penicillia identification is often subjective based on conventional morphological methods and more objective methods involving a polyphasic approach are recommended, which includes phenotypic, biochemical and genotypic approaches. Identification of *Penicillium* strains isolated from Tunisian apples, Chilean chillis (the traditional *Merkén*), and Italian cheeses, with their capacity to produce patulin or OTA where appropriate is presented herein.

For morphological characterisation, isolates were inoculated in triplicate on different media and temperature conditions: Czapek yeast autolysate (CYA) agar at 17ºC, 25ºC, 30ºC and 37ºC; malt extract agar (MEA), oatmeal agar (OAT), yeast extract sucrose agar (YES), glycerol nitrate agar (G25N) and creatine sucrose agar (CSN) at 25ºC. After seven days, digital images of colonies were obtained and macro- and micro-morphological characters were examined under light optical microscopy and when appropriate scanning electron microscopy. Multilocus sequence analysis was performed through comparison of partial β-tubulin, calmodulin and ITS sequences available in GenBank. Specific primers for genes involved in the mycotoxin pathways were used for PCR amplification. Patulin and OTA were quantified using HPLC-DAD and HPLC-FLD (fluorescence detection) respectively

A novel species was isolated from Tunisian apples with the proposed name, *Penicillium tunisiense* from section *Ramosa*. It is not a patulin producer, since the compound found at the same retention time as patulin has a different UV spectrum and the *idh* gene test was negative, in contrast to the other dominant *P. expansum* isolates that were patulin producers. The new species could not rot apples when tested, implying it may have potential as a biocontrol agent in apple orchards. Ochratoxigenic strains of *P. verrucosum* were rarely isolated from chillis and never from cheese or apples. However, *P. crustosum* was abundantly isolated from chilli and cheese samples in which OTA was detected in the food samples. The isolates were characterised with genes involved in the OTA biosynthesis pathway which demonstrated that key genes were present. The chromatographic analysis indicated that isolates were able to produce OTA. This fungus is not conventionally considered an OTA producer and more work is being undertaken.

Overall, our findings demonstrated that mycotoxigenic *Penicillium* species are important contaminants of food about which more information is required. Special attention needs giving to *Penicillium* species that are not currently considered as important mycotoxin producers, which might produce them in different environmental or food manufacturing process conditions, such as high NaCl concentrations.

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ABUNDANCE AND DIVERSITY OF PROKARIOTIC COMMUNITIES IN SOILS OF OASISES IN EAST ANTARCTICA

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Detailed soil investigations in Antarctica are concentrated mainly on soil genesis, development of relatively precise soil maps and molecular-genetic analysis of microbial components in such soils. Molecular-genetic methods showed the diversity of bacteria in Antarctic soils, but these results don't reveal biological activity, physiological status and stability of microbes in external environment. A combination of culture-depended and independent techniques was used to characterize bacteria community in earlier not investigated soils in East Antarctic Coast. The samples of soils and barren rock with endolithic soil-like bodies were taken from the inter-hill wet valleys area (oasisis) in East Antarctica). The bottoms of inter-hill valleys show maximum biota concentration and highest bio- and soil diversity. Moss, lichen and algae ground covers are formed here, as well as algal-bacterial mats and microorganisms develop various soil profiles in sandy granitoid sediments. The main feature of these soils is that they are formed under the protection of a gravel pavement, or detritus.

Under such "armor" the effect of wind is decreased, the moisture is better preserved, as well as the proportion of direct light with aggressive UV component is reduced. Microbiological studies revealed the following features of examined Antarctic soils:

1. The total number of bacterial cells counted by staining with acridine orange was rather high for Antarctic habitat and varied in the range of more than 10 mln cells/g.

2. Using Live/Dead (L7012) pigment stain it was shown that 60 % of detected total number cells (in some samples about 80 %) have undamaged cell membranes that testify for their viability and stability to extreme environment.

3. The largest number and proportion of viable cells was observed in the fine earth directly under the gravel pavement. Such habitats (sand beddings with moss pads, algae and micromycetes colonies) are the most favourable for the development of bacteria, as sheltered by the gravel pavement from wind corrasion, dehydration and aggressive UV radiation, but at the same time they are close to the surface, well warmed by pavement insolation and are fed by the melting snowfields.

4. Considerable number among viable cells (70–80 %) were nanoforms with cell diameter not higher than 200 nm, that exceed such indexes in temperate soils and represent the specific pool of Antarctic bacteria.

5. The total number of bacteria does not find a strong correlation with a carbon content (correlation coefficient 0.15), while the number of heterotrophic bacteria by the number of CFU, vice versa, show such connection (correlation coefficient 0.78). This indicates that a significant portion of the organic matter is slightly binded to the mineral matrix and primarily contained in labile form, available for heterotrophic bacteria.

6. The concentration of bacterial biomass in studied soils was significantly (ten or more) lower, than that in the soils of temperate zone, and varied depending on the type and extent of vegetation on the surface of the soil and the depth of sampling. The maximum concentration values of microbial biomass were observed in organic horizons (6.4–13.5 mg/g oven-dry soil), the lowest in the mineral horizons (1.1–2.30 mg/g). Stocks of bacterial biomass calculated for the entire soil profile ranged from 0.13 mg/m^2 in endolithic soil to 5.67 mg/m^2 in the soil with abundant growth of hypolithic algae and cyanobacteria.

7. Taxonomic composition at the domain level and phylum determined by molecular biological method revealed representatives of the phylum *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, *Acidobacteria*. Domain *Archaea* was presented in smaller quantities than *Bacteria*, its proportion was less than 20 %, as compared with the domain Bacteria accounted for about 80 % of all cells identified by FISH. Application of FISH method (fluorescence in situ by hybridization) allowed to determine the representatives of different genera – the same among bacteria with common size cells, and nanoforms, that testify for the bacterial transformation to nanoforms under unfavourable conditions.

8. The alternation of 10 to 30 cycles of freeze-thawing of all soil samples resulted in an increase in the number of cultured heterotrophic bacteria (CFU), as compared with the number of CFU, counted immediately after thawing, in the most cases, one order of magnitude higher. Several freeze-thawing cycles can be used for more complete extraction of cultivated forms from Antarctic soils. It is possible, that increase in CFU indexes occurs due to partial destruction of biofilms.

9. Examined soils were characterized by significant discrepancy between indexes of total and viable number of cells, as well as high irregularity in horizons of developing soils. It can be explained both by specification of physical and chemical processes in permanently frozen habitats and by

imperfection of microbial isolation techniques. The basic form of the existence of cryptogamic organisms in investigated soils – are biofilms, which can be detected at the micro and submicro levels and possess an extracellular polymer matrix. It is a natural form of the development of organisms in subaerial conditions on mineral surfaces especially in extreme conditions. Such data indicate the importance of taking into account in Antarctic investigations the microbial biofilms, which may play the leading role both in soil development and modification of the cell metabolism that require special techniques for reactivation of bacteria from such extreme environment.

10. Antibiotic resistance in microbes is widespread and it has been demonstrated earlier that soil bacteria especially in contaminated soils are rich in resistance determinants to both natural and synthetic antibiotics commonly used in clinics (Forsberg et al., 2012). Systematic studies of antibiotic resistance among bacteria isolated from uncontaminated soils have not yet fully been explored. Antarctic soils are considered as the most nonpolluting environment with minimal anthropogenic action. The first experiments were carried out to compare resistance spectrum of bacteria isolated from Antarctic and contaminated soils. It was shown that 70 % of Antarctic bacteria revealed high resistance both to natural and synthetic antibiotics, especially they demonstrated multiple resistance to various combinations of antibiotics. Some of these combinations were unique for particular sites of Antarctic soils. For the first time it was shown that antibiotic multiple resistance does not be attributed only for contaminated soils. Antibiotic resistance in Antarctic soils can be explained by innate resistance formed in extreme Antarctic climate conditions. Collection of isolated Antarctic bacteria can be used to reveal evolution processes in mechanism of antibiotic resistance in nature.

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COMPARATIVE PHYLOGENETICS OF BETA-GALACTOSIDASES LAC OF ASCOMYCETOUS YEASTS

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The enzyme beta-galactosidase is widely distributed in plant and animal tissues, however is extremely rare in yeasts. Due to the presence of the beta-galactosidase enzyme, dairy yeasts *Kluyveromyces lactis* and *K. marxianus* are able to ferment lactose. Polymeric *LAC* loci have a complex structure and consist of two closely linked structural genes *LAC4* (beta-galactosidase) and *LAC12* (lactose permease), and a regulatory sequence [1–3].

We conducted a comparative phylogenetic analysis of amino acid sequences of the beta-galactosidase *LAC4* gene of *Kluyveromyces*, *Debaryomyces*, *Scheffersemyces* and *Sugiyamaella* yeasts. Search for homologous α-glucosidases in the yeast genera studied was performed in GenBank using the BLAST software. The phylogenetic analysis revealed significant differences between LAC4 proteins of *Kluyveromyces* and other three studied genera of *Scheffersomyces*, *Sugiyamaella* and *Debaryomyces*. On the other hand, the LAC4 proteins of *Kluyveromyces* yeasts divided into two distinct subgroups corresponding to ecological origin of strains: dairy products and natural sources.

The group of dairy strains is heterogeneous and includes both *K. lactis* and *K. marxianus* yeasts (98% identity), suggesting the common origin of their *LAC4* genes.

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A GLOBAL CATALOGUE OF MICROBIAL GENOME-TYPE STRAIN SEQUENCING PROJECT OF WDCM

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WFCC–MIRCEN World Data Center for Microorganisms (WDCM, *http://www.wdcm.org*/) has long been committed to facilitating the application of cutting-edge information technology to improve the interoperability of microbial data, promote the access and use of data and information, and coordinate international co-operation between culture collections, scientists and other user communities.

To help plenty of culture collections that cannot make their data available online, WDCM launched the Global Catalogue of Microorganisms (GCM) (*http://gcm.wdcm.org/*) project in 2012. Up to now, GCM (*http://gcm.wdcm.org/*) has become one of the largest data portals for public service microbial collections and several international culture collection networks, providing data retrieval, analysis, and visualization system for microbial resources. Futhermore, GCM gradually developed into a knowledge base linking taxonomy, phenotype, omics data as well as relative scientific papers and patents with its catalogue information, which currently has aggregated 402,778 strains and other holdings (plasmids and antibodys) deposited in 117 collections from 46 countries and regions.

Recently, WDCM announced the launching of Global Microbial Type Strain Genome and Microbiome Sequencing Project in the 7th WDCM Symposium, marking the GCM project has begun to enter a new stage (GCM 2.0). Focused on exploring the genomic information of microorganisms, this project has planned to sequence all uncovered prokaryotic type strains together with select eukaryotic type strains, construct a database for genomics data sharing, and also provide online data mining environment. Working groups responsible for selecting bacteria and fungal strains, drafting SOP, managing intellectual property right and legal issues and construcing database have already embarked on the the pioneer stage of GCM 2.0, scheduled to last until May 2018. The project will establish a cooperation network for type strain sequencing and functional mining, covering more than 30 major culture collections of 20 countries, and complete genome sequencing of over 10000 species of microbial type strains in five years.

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USING OF THE SUCCESSION APPROACH FOR EVALUATION OF DIVERSITY OF BACTERIAL COMMUNITIES IN SOILS OF EASTERN ANTARCTICA (OASIS LARSEMANN HILLS)

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In recent years, intensive studies of the microbial diversity of Antarctic soils have been performed both with using classical approaches for cultivating bacteria on nutrient media and using modern molecular genetic methods [5, 6]. However, the analysis of thawed samples shows a relatively low number and diversity of bacteria on the traditionally used bacteriological nutrient media, with a relatively high number of bacterial cells, determined by luminescent microscopic method [3].

It is known that one of the ways of survival of prokaryotes in inauspicious conditions is the conversion to viable but nonculturable state (VBNC) [1]. Probably, as a result of this, the analysis of newly thawed soil samples reveals a high content of filtering forms of prokaryotes (FFP), which many researchers consider as special resting forms that ensure the viability of bacterial cells in the event of unfavorable environmental conditions [2, 4].

We applied a succession approach for evaluation the taxonomic diversity of cultivated heterotrophic bacteria in two soil samples from oases of East Antarctica, differing in content of organic matter. For this purpose, succession was initiated by moistening and subsequent incubation in two temperatures: + 5° and + 20°C, i. e. in conditions simulating a warm season in Antarctic region. Total number of prokaryotes and FFP as well as the number and taxonomic diversity of the heterotrophic bacterial complex were determined in the course of the model experiment. In all cases, the maximum of the total number of bacteria was fixed on the 14th day, the minimum at the beginning (the 1st day) and at the end (the 160th day) of the experiment. Contrariwise the number of FFP was maximal at the beginning of the experiment and minimal – on the 14th day. The dynamics of the number of bacteria and FFP makes it possible to assume that "revitalization" of Antarctic soils by moistening and incubation at positive temperatures promotes the transition of resting cells forms to an active, viable state. In the course of succession, the number and taxonomic structure of the heterotrophic complex of bacteria cultivated on the glucose-peptone-yeast nutrient medium also changed. The genus *Arthrobacter* dominates at all stages of the model experiment. The diversity of

grown bacteria increased in groups of average abundance and minor components from the 14th day of experiment and further along the succession. During the experiment, Gram-negative bacterial strains were also isolated and identified by 16S rRNA gene sequence analysis. All of them were affiliated to the phylum *Proteobacteria*, genera *Sphingopyxis*, *Bosea*, *Sphingomonas*, *Brevundimonas*, *Ralstonia*, *Delftia*, *Variovorax*, *Stenotrophomonas*, *Pseudomonas*, *Acinetobacter*. A high similarity to a particular species was not found for many studied strains which makes it possible to assume that they belong to new species of bacteria. In previously studied samples of Antarctic soils by fluorescent in situ hybridization (FISH), it was found that more than half of the number of identified bacterial cells within the domain *Bacteria* belonged to the phylum *Proteobacteria* [3], although during the plating of newly thawed samples of Antarctic soils, mainly Gram-positive bacteria grew.

Thus, we suppose that the application of the succession approach allows to more fully characterize the taxonomic diversity of the heterotrophic bacterial complex and to identify a wider range of the genera of Gram-negative bacteria. The use of a succession method can be recommended both for a comprehensive study of bacterial complex of extreme biotopes and for other studies in which it is necessary to obtain the maximum diversity of cultured bacteria from natural samples.

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THE COLLECTION OF BIORESOURCES IBSO IS THE BASE FOR RESEARCHES ON BIOLUMINESCENCE

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The Culture Collection of luminous bacteria in IBP SB RAS (CC IBSO) was laid in the 60 years of the last century after several expeditions to the Pacific Ocean and then to other regions of the World Ocean. The isolated strains of luminous bacteria had become actively used in the study of their metabolism and bioluminescence. The culture collection IBSO includes the unique gene pool. It consists of numerous marine natural glowing bacteria and recombinant E. coli strains carrying the genes of the luminescent system from natural luminous bacteria, mycelial cultures of glowing basidiomycetes isolated in different climatic zones of the globe (Europe, Canada, Vietnam, Malaysia, Siberia, etc.) that are used for a detailed study of the bioluminescence mechanisms.

Using the gene pool of natural luminous bacteria, studies were carried out on the issues of cultivation, the impulse character of bacterial luminescence, the structural organization of luminous bacteria, and the biodiversity of luminous bacteria. For many years, work was successfully carried out to study the mechanisms and regulation of the luminescence of bacteria, the kinetic properties of the luminescent reaction. As a result of these studies, the mechanism of the bioluminescent reaction of bacteria became understandable, the nature and role of the substrates necessary for its course was clarified [1].

In subsequent years, the localization of the luminescence system in the cell was determined [2] and a model of bacterial luciferase was proposed [3]. The role of luminous bacteria in the transformation of natural biopolymers, the features of the formation of aggregated communities in media of different viscosities was clarified. The study of the action of chemical substances of different nature on the metabolism and ultrastructure of luminous bacteria provided an opportunity to develop techniques for using the luminescence of bacteria in applied problems and to offer biotests based on bacteria and the luminescent system isolated from them. The original technologies for obtaining highly purified bacterial luciferases from luminous bacteria were developed, as well as methods and procedures for obtaining reagent kits for bioluminescent analysis (CRABs), which can be used in biochemistry, medicine, in technical microbiology, and in environmental monitoring [4]. Together with the staff of the photobiology laboratory, it was studied the influence of xenobiotics on the bioluminescent system of bacteria, a bioluminescent method for monitoring the toxicity of radioactive isotopes was developed. Among the strains of luminous bacteria promising producers of biologically active substances, in particular, luciferases, restriction ribonucleases, as well as strains with accumulation of a high content of polyhydroxyalkanoates have been identified.

In 2011, new section was created in the Collection IBSO – the Collection of luminous basidiomycetes. The section is represented by cultures that were obtained by the laboratory staff during the collection of fruit bodies of several species of Basidiomycetes growing in Siberia, and also brought from expeditions to the tropical zones of South Asia. Now it includes more than 60 mycelial cultures of glowing fungi of the genera *Armillaria*, *Panellus*, *Neonothopanus*, *Omphalotus*, *Mycena*, unidentified tropical fungi, and about 20 cultures of non-luminous fungi *Pholiota*, *Pleurotus*, etc., which can synthesize the prospective substrate / activator of bioluminescent fungal reaction.

During these years the chemiluminescence of the tissues of the fruit bodies of more than 20 genera of 15 families of higher fungi of Siberia has been studied. Methods to produce biomasses of air and globular mycelium with long and stable luminescence, (they are necessary for studying the characteristics of bioluminescence of fungi) have been developed and optimized. The effect of ionizing radiation on the luminescence of the fungal mycelium *Neonothopanus nambi* has been studied [5]. From the mycelium of the luminous *N. nambi*, the fungal luminescence system was first isolated and investigated, and determined the substrate precursors of luminescent reaction and the enzyme that activates the reaction substrate in the presence of NADPH [6]. The relationship between luminescence and the activity of peroxidase and catalase enzymes in fungi *N. nambi* and *Armillaria borealis* was studied. It is shown that *Armillaria* fruiting bodies don't glow because the components necessary for the bioluminescent reaction are absent in them, or present in very small content. But mycelium has full set of components for bioluminescence [7]. A method has been developed for identifying loci of armillariasis by use glowing sample of wood; the method can be used regardless of the season of the year [8].

The culture collection IBSO is one of the channels for information exchange in the global network. The Web-portal "Bioluminescence and luminous organisms" (*http://bl.ibp.ru*) is developed; the "Catalogue of luminous bacteria cultures" is published [9]. Data on the properties of natural luminescent bacteria from Collection IBSO included in the combined Catalogue of microorganisms of Russian collections (*http://www.vkm.ru*) and are presented on the website of the International Society of Collections of Cultures WFCC–MIRCEN World Data Center for Microorganisms (*http: //www.wdcm.org/*) in the Global Catalogue of Microorganisms (*http://gcm.wfcc.info/*).

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BIODIVERSITY AND BIOACTIVE COMPOUNDS OF MARINE BACTERIA AND FUNGI

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World Ocean is biggest part of biosphere. Marine microorganisms account for a large part of the total biomass of life on Earth. Marine microorganisms are a crucial component of the Earth's life-support system. Therefore, any new information about the marine microorganisms is important and relevant. Marine microorganisms essentially differ from soil bacteria and synthesize various biologically active substances which have not been found among soil microorganisms, despite of more than century history of intensive research. Exploitation of microbial resources of Ocean (and they virtually are inexhaustible) promises wide prospects as technological basis for the future. The biotechnological boom, we can see these days followed by a splash of interest to the problems of biosystematics and ecology. This economical interest undoubtedly will bring a new knowledge about fundamental unit biodiversity-biotechnology. Collections of microbes must play a leading role for these investigations. The Collection of Marine Microorganisms of the G. B. Elyakov Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences was created in 1985. Now the Collection is the member of the *World Federation for Culture Collections* and has official acronym KMM, registration number 644. KMM contains about 4000 strains of marine heterotrophic bacteria (mainly), as well as microscopic fungi (about 1000 strains). The staff of KMM validly described more than 250 new species, about 50 new genera of bacteria, as well as several new species of marine fungi. Several hundred compounds with unusual chemical structure and biological activity has been described (especially with anti-cancer activity) from these microorganisms. These and some other aspects of the study of marine bacteria and fungi are discussed in the presentation.

WIDESPREAD DIVERSITY VS COSMOPOLITISM OF CERTAIN CYANOBACTERIAL STRAINS OF *NOSTOCACEAE* FAMILY

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During several decades a lot of strains of heterocystous nitrogen-fixing cyanobacteria of *Nostocaceae* family (order *Nostocales*) obtained from different public culture collections and different laboratories were collected in the museum of cyanobacterial strains at the Department of Genetics of Moscow State University. This collection includes model strains with sequenced genomes (*Trichormus variabilis* ATCC29413, *Nostoc* sp. PCC7120, *Nostoc punctiforme* PCC 73102) as well

as original *Anabaena/Nostoc* strains from Vietnam, Siberia, Buryatia and several symbiotic isolates from *Azolla* fern with incomplete genetical identification (27 strains).

In 2017 our laboratory in collaboration with the Department of geography and evolution of soils of the Institute of Geography RAS started the new research concerning the isolation of the new cyanobacterial strains from samples of hypolithic horizons within the inter-hill valley in the Larsemann Hills oasis of East Antarctica (69°24'S, 76°14'E, near Russian station "Progress"). These samples of cyanobacterial domination as biofilms were located immediately under the stone pavements from the depth of 1 cm. Similar work on isolation of new free-living and symbiotic/associative cyanobacterial strains was carried out with both aquatic/benthos samples from fresh water reservoirs and the mosses plants in Moscow region. All isolates were cultivated under identical conditions on nitrogen free medium at 22°C and continuous light provided by cool–white fluorescent lamps (50μE/m^{-2} s^{-1}), namely in selective conditions for heterocystous cyanobacteria of order *Nostocales*.

The initial microscopic morphological examination of 40 axenic cultures of newly isolates allowed us to determine the dominant strains as belonging to several main genera of order *Nostocales*: *Nostoc*, *Anabaena*, *Cylindrospermum*, *Tolypothrix*, *Calothrix*, and the obtained collection is a good material for studying of cyanobacterial diversity in different habitats and geographical regions. Since the morphological characteristics do not allow distinguishing closely related strains, PCR-fingerprinting and 16S rRNA sequencing were used for the establishment of taxonomic affiliation of new isolates.

In this work, the subject of special interest were strains of *Trichormus/Nostoc* group due to their ability to intensive nitrogen fixation and their perspectives in genetic engineering and biotechnology. PCR-fingerprinting revealed the minimal genetic polymorphism of independent *Trichormus/Nostoc* isolates from our collection. Whole-genome sequencing allowed us to compare the genomes of five independent strains of *Trichormus variabilis*, isolated in America, Israel and Vietnam from the aquatic environment and from association with different plants (e. g. rice, *Azolla*), and revealed only minor genomic differences between studied strains, such as single insertion of additional copy of IS-element and the loss of the short linear chromosome. These data along with the analysis of such unique elements of *Trichormus* genome as plasmid D and a small linear chromosome in several new isolates from Siberia and Moscow region demonstrates a high degree of genomic stability in the group of *Trichormus* strains and allows us to consider *Trichormus variabilis* as cosmopolitan species. Further genomic studies of new strains of *Trichormus variabilis* will reveal the main adaptation mechanisms and molecular basis of the genetic stability of these cyanobacteria.

The results obtained in this paper indicate that the genome research using modern sequencing methods involving a wide range of strains already available in various collections and newly isolated from different biotopes is a promising approach in studies of the basic mechanisms of the evolutionary reorganization of cyanobacterial genomes.

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PHOTOTROPHIC POTENCIAL OF METHANOTHROPHIC BACTERIA *METHYLOCAPSA PALSARUM* ISOLATED FROM GEOGRAPHICALLY REMOTE HABITATS

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Methane is one of the major greenhouse gases and an alternative fuel. The concentration of methane in the atmosphere tends to increase, thus stimulating the research on microorganisms capable of its biological oxidation, i. e. methanotrophic bacteria. Most known aerobic methanotrophs grow only on methane, methanol, formate and some methylated amines. Our previous study revealed the array of genes providing the ability for anoxygenic photosynthesis by means of photosystem II, typical for purple bacteria, in the genome of an obligate methanotroph from a subarctic Norway wetland, *Methylocapsa palsarum* NE2^T [1]. This array includes the genes encoding the light-harvesting complex *pufABCML*, the *puhA* reaction center, and the genes encoding the synthesis of bacteriochlorophyll a and b and the carotenoids spirilloxanthin and hydroxysferoidene [2]. Most of these photosynthesis-related genes are organized in one large gene cluster. An interesting feature is the presence of *bchE* and *acsF* genes, enabling the synthesis of 13 (1) -hydroxy-Mg-protoporphyrin IX 13-monomethyl ester (precursor of bacteriochlorophyll) both in the presence of oxygen and under anaerobic conditions. Phylogenetic analysis of *pufML* genes showed high similarity to the corresponding sequences in the genomes of many plant-associated *Methylobacterium* species which, similar to *Methylocapsa* species, belong to the order *Rhizobiales*. However, a number of *pufML* sequences from known phototrophic representatives of order *Rhizobiales*, including members of the genera *Rhodopseudomonas*, *Rhodomicrobium*, *Afifella*, and *Rhodoblastus*, form a separate remote cluster, which is consistent with modern ideas of the high influence of lateral transfer on the evolution of these genes [3].

The experimental part of the work was focused on searching for conditions under which *Methylocapsa palsarum* NE2^T is capable of bacteriochlorophyll biosynthesis. Among the factors examined in our study were the presence of various organic (methane, methanol, acetate, succinate, DMSO) and inorganic (sulfur and nitrogen salts) compounds, as well as the effect of oxygen concentration and illumination. Bacteriochlorophyll formation was identified by the monitoring the presence of characteristic peaks in absorption spectra of acetone-methanol extracts of grown cells. As revealed in our study, bacteriochlorophyll could indeed be synthesized by *Methylocapsa palsarum* NE2^T during its growth on a solid medium under day/night light cycle. The biosynthesis of bacteriochlorophyll was not observed during growth in liquid media.

To explore the distribution of genetic determinants of photosynthesis in methanotrophs, a collection of isolates obtained from ecosystems of northern Russia was screened for the simultaneous presence of *pmoA* and *pufML* genes. The genes were identified by PCR using the primer systems 189f / 682r and pufLf / pufM750r for the *pmoA* gene and for the *pufML* operon, respectively. The presence of both genes was revealed in five closely related (99.5–100 % 16S rRNA gene sequence similarity) strains of *Methylocapsa palsarum* isolated from the subarctic *Sphagnum*-dominated wetland in Khanty-Mansi Autonomous Region. Further analyses were performed with a representative strain N8 (99 % similarity of the *pmoA* and *pufML* gene sequences with *M. palsarum* NE2^T). Similar to *M.*

palsarum NE2^T, this strain was also capable of producing bacteriochlorophyll during cultivation on a solid medium under periodic illumination. Our results indicate that bacteriochlorophyll biosynthesis is characteristic for representatives of the species *Methylocapsa palsarum* inhabiting geographically remote habitats.

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THE COLLECTION OF UNIQUE AND EXTREMOPHILIC MICROORGANISMS (UNIQEM COLLECTION)

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Since its foundation, the Collection of Unique and Extremophilic Microorganisms (UNIQEM Collection), having the status Core Facility, has been maintained in Winogradsky Institute of Microbiology, Research Center of Biotechnology. Many microorganisms in the Collection (> 2500 strains), including the representatives of new genera, families and phyla, were isolated from different environments, studied, and described validly by scientists from the Research Center. The UNIQEM Collection also comprises bacteria and archaea which have the uncertain taxonomic position and are difficult for culturing. Some microbial strains are of potential practical importance or have found applications in biohydrometallurgy, enhanced oil recovery, waste processing or as producers of stable enzymes and bioactive substances. Both UNIQEM Collection and laboratories in Research Center of Biotechnology use modern infrastructure and resources to study, maintain, and store microbial strains. More than 200 new strains are annually isolated from different hot, cold, saline, and other environments, characterized and identified with the use of high-throughput molecular diagnostics in the partner Core Facility “Bioengineering”. Besides the major missions, the UNIQEM Collection assists in scientific research or conducts investigations related to physiology and taxonomy of new taxa of prokaryotes; efficient preservation of prokaryotes; long-term survival and dormancy of microorganisms; high-resolution microscopy.

BIOTECHNOLOGICAL POTENTIAL OF THE IBPPM RAS COLLECTION OF RHIZOSPHERE MICROORGANISMS

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Microbial culture collections of microorganisms are the only way to preserve the diversity of natural microbial resources. They are also of great importance for research and development in medical, agricultural, and environmental biotechnology.

The Collection of Rhizosphere Microorganisms of the IBPPM RAS (*www.collection.ibppm.ru*) is a specialized scientific depository focused on selecting and maintaining nonpathogenic bacteria, isolated mainly from the root zone of plants. The collection houses about 500 cultures of bacteria of different taxons, isolated from different ecological niches, but mostly from plant organs and the surrounding zones (roots, seeds, nodules, rhizosphere, rhizoplane, and phyllosphere). Most of the strains are adapted to life in association with plants growing in a variety of environments, including man-made pollution. In this regard, the collection is a promising source of natural microbial resources, which can be used to make effective biological products for agricultural and environmental purposes.

The strains maintained in the collection have a number of properties useful for environmental and agricultural biotechnology and are conditionally assigned to the groups of "biofertilizers' and "bioremediators".

The "biofertilizers" group includes about 200 PGPR (plant-growth-promoting rhizobacteria) strains that stimulate plant growth through improving nutrition (e. g., by nitrogen fixation, solubilization of insoluble phosphates, or production of phytohormones). The main feature of the collection is the largest assortment of bacteria of the *Azospirillum* genus – typical PGPR. Testing of these microorganisms as inoculants for agricultural crops has shown that they increase the productivity of their plant partners and, therefore, can serve as a basis for commercial agricultural bioagents [e.g., "Organit N" (*http://bionovatic.ru*)].

PGPR strains also can be used in phytoremediation, because their activity promotes plant root growth under contaminated conditions. On the one hand, this increases the numbers of useful roots-associated bacteria owing to the appearance of new niches, and on the other hand, this increases the release of plant catabolic enzymes as part of root exudates to degrade persistent organic pollutants (e. g., hydrocarbons or pesticides) or, conversely, improves the extraction of inorganic pollutants (heavy metals and metalloids) from the root zone.

Members of the "bioremediators' group have the characteristic ability to degrade persistent organic pollutants and/or resist to inorganic ones. The global pollution of the environment by petroleum hydrocarbons, pesticides, heavy metals and metalloids, as well as the possibility of improving the ecological situation through such low-cost and eco-friendly technologies as bio- and phytoremediation, based on the use of plant-microbial associations ensure the interest in these microorganisms. The collection's "bioremediators" group includes strains degrading crude oil and petroleum products, polycyclic aromatic hydrocarbons (PAH), and herbicides, as well as strains resistant to arsenic and heavy metals. Our studies on the optimization of phytoremediation led us to develop protocols for the use of several collection strains in this technology [1]. Plant-microbial associations have been developed to restore soils exposed to various technogenic pollutants. The effectiveness of the selected plant-microbial associations in the decontamination of soil from

oil hydrocarbons, glyphosate, heavy metals, and arsenic has been experimentally proved. These developments are protected by Russian patents [2, 3].

A number of strains from the IBPPM Collection of Rhizosphere Microorganisms that can be used as biofertilizers and bioremediators have been included in the PERN (Pan-European Rhizosphere Resource Network, *http://www.PERN-BRIO.eu*) database, organized as a virtual collection containing beneficial microorganisms isolated from the rhizosphere and constituting a virtual, common, and wide-ranging pool of microbial rhizosphere diversity that is exploitable in research and industry [4]. PERN is centrally managed in Brussels by the Belgian Coordinated Collections of Microorganisms (BCCM, *http://bccm.belspo.be/*). The description of each strain in the network is accompanied by data on taxonomy, methods of isolation and identification, storage and cultivation conditions, properties of microorganisms with an emphasis on biotechnological significance, and bibliographic sources. Such integration of the collections allows significant expansion of users access to bioresources of rhizospheric origin and facilitation the search for objects with the necessary characteristics.

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THE ROLE OF CULTURE COLLECTIONS IN FUTURE BIODISCOVERY AND BIOECONOMY

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Despite decades of cultivation and isolation attempts, only about 14,000 species of *Bacteria* and *Archaea*, representing 0.1–0.001 % of the estimated global number of species, have so far been validly described. Even more significantly, laboratory cultivation has returned predominantly isolates from four bacterial phyla, whereas only 10 % of the described species are affiliated to the 29 other bacterial and archaeal phyla that contain cultivated representatives, and no single isolate is available for 85 phyla. Since this pronounced bias of cultivation approaches has profound effects on our current understanding and future applications of bacterial diversity, novel concepts for cultivation are urgently required. The *Acidobacteria* are a representive example for the successful application of novel high-throughput, rationally designed cultivation trials. These permitted in the retrieval of a considerable collection of environmentally important bacterial group which had been grossly underrepresented among cultured representatives. Because of their profound knowledge of bacterial

physiology and cultivation, microbial resource centers are particularly competent to take over such future tasks. Aside of the work with cultured microorganisms, access to extensive taxon-specific (meta) data has become of increasing importance for the utilization of collection holdings. The new bacterial metadatabase Bac*Dive* has been designed to fill part of this gap. It is free, publicly available, offers multiple search functionalities, and is constantly extended by novel features. At present, Bac*Dive* holds >0.5 Mio data points for 64,000 prokaryotic strains. Since October 2014, EU-based collections face the additional challenge of finding suitable solutions for handling cultures under the restrictions of the Nagoya Protocol. In particular, legal interpretations differ significantly between authorities of different countries. This calls for a concerted action of culture collections to ensure interoperability and effective collaboration in the years to come. Finally, culture collections can be foreseen to play a key role in future bioeconomy given the increasing demand for data-rich bioresources.

TERMITICIDAL ACTIVITY OF CULTURE FILTRATES FROM FOUR *STREPTOMYCES* ISOLATES

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Termites are eusocial insects and are divided into morphologically distinct castes. The workers have the function of constructing tunnels and galleries of the nest as well as foraging and feeding the other individuals. The soldiers are responsible for the protection of the nest. Termites act as decomposers of the environment promoting the recycling and mineralization of lignocellulosic resources but some species are considered urban pests. *Nasutitermes corniger* is a species widely distributed in the Americas that damages civil constructions, historical monuments, art collections, documents and ornamental trees. The use of chemical insecticides is still the main strategy used to control pest insects. However, the indiscriminate and continuous use of pesticides has led to environmental contamination and imbalance to the ecosystem, mainly by selecting resistant populations and eliminating natural predators. Insecticide turnover makes more difficult the emergence of resistant populations and, in this sense, studies have been performed aiming to identify new compounds for use in termite control strategies. The Caatinga is an exclusively Brazilian biome (11 % of the national territory) that presents average temperature between 25 and 29ºC and annual precipitation around 300 to 800 mm with long periods of drought. The survival of species in the Caatinga requires adaptive metabolism that includes the biosynthesis of specific secondary metabolites. Therefore, the plants and microorganisms of this biome have been evaluated as sources of antibiotics and insecticides. The genus *Streptomyces* comprises about 600 species and are the most commonly isolated actinobacteria from the soil. Actinobacteria are considered important sources of compounds with antimicrobial, antioxidant and insecticide activities. The objective of this work was to evaluate the termiticidal

activity on *N. corniger* of filtrates obtained from four cultures of *Streptomyces* spp. isolated from the rhizosphere soil of *Caesalpinia pyramidalis*, a Caatinga plant popularly known as *catingueira* (CA). The isolates CA-02, CA-06, CA-07, and CA-17 are deposited in the Collection of Cultures of the Department of Antibiotics from the *Universidade Federal de Pernambuco* – UFPEDA. The isolates were reactivated by culturing them in International Streptomyces Project medium (ISP) 2, 3, 4 and Arginine Yeast Agar (AYA) at 37°C. ISP-2 medium was selected due to best growth and sporulation of the actinobacteria. The cultures were then maintained in test tubes with solid ISP-2 under refrigeration. The strains were inoculated into 250-mL erlenmeyer flasks, each one containing 100 mL of Glucose Yeast Medium (GYM) pH 7.2. After 7 days of incubation at 37°C under rotary shaking at 150 rpm, the cultures were vacuum filtered using Whatman no.4 paper to separate the microbial biomass from the culture filtrate (CF). Artificial diets for the termites were composed by a 20% (w/v) Avicel (microcrystalline cellulose) suspension prepared in CF-CA-02, CF-CA-06, CF-CA-07 or CF-CA-17 at the concentrations of 2, 4, 10, 20, 50 and 100%. The diet (1 mL) was put in a petri dish and incubated at 56°C for 24 h The control diet consisted of 20% Avicel (w/v) in distilled water. Workers (16) and soldiers (4) of *N. corniger* were transferred directly from the nest to each petri dish and the assay was kept in the dark at 28°C. Survival of the termites was evaluated for 8 days. Three independent trials were performed in quintuplicate. The lethal concentrations required to kill 50% of workers or soldiers (LC_{50}) was calculated by probit analysis at a significance level of 95%. CF-CA-02, CF-CA-06 and CF-CA-07 presented high toxicity for workers with LC_{50} of 14%, 4.05% and 6.23%, respectively, while CF-CA-17 did not promote mortality of insects. CF of all strains were toxic to soldiers and the determined LC_{50} values were 38.3%, 64%, 60%, and 2.43% for CF-CA-02, CF-CA-06, CF-CA-07 and CF-CA-17, respectively. The comparison of the termiticidal efficiency of the filtrates reveals that the castes showed different sensitivity to them being the workers more sensitive than the soldiers, except for CF-CA-17. The fact that CF-CA-17 presented the highest termiticide efficiency on soldiers and did not promote worker mortality suggests that it has a profile of metabolites distinct from other strains, being rich in those that act specifically on soldiers. The study proceeds with the determination of the chemical composition of the metabolic liquids of the four strains and investigation of the termiticidal mechanism of action. In conclusion, the results show that isolates of the genus *Streptomyces* isolated from rhizosphere of Caatinga plant are sources of compounds with termiticidal action with distinct toxicity for the castes of workers and soldiers.

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STORAGE OF BACTERIAL CULTURES WITHOUT CRYOPROTECTANTS: FEATURES OF PRESERVATION OF VIABILITY OF POLYSACCHARIDE-PRODUCING BACTERIA

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Storage of bacterial cultures in collections requires stage-by-stage preparation and ends with lyophilization with a protector. This ensures long-term storage of dried cultures at room or small positive temperatures.

From lichens of the north taiga zone, subarctic alpine ecosystems and the forest-steppe zone, bacterial cultures have been isolated that are capable of producing significant amounts of exopolysaccharides of varying degrees of density and viscosity.

The phylogenetic affiliation of the strains was established by molecular genetic methods. About 70 % of the strains belonged to the phylogenetic group *Proteobacteria*. Among the most common genera were *Pseudomonas*, *Paraburkholderia*, *Sphingomonas*, *Collimonas*, *Methylobacterium*, and other taxa, belonging to which is established at the level of new species and genera. Also strains affiliated with *Acidobacteria*, *Actinobacteria* and *Firmicutes* groups were isolated.

When precipitated by centrifugation, they formed a dense or loose gel-like precipitate containing up to 50 % of the exopolysaccharide (dry weight). The biomass was frozen at –20°C. The cultures were then plated at intervals of 1, 3, 6, 8 and 12 weeks. The number of cells in the inoculum aliquot was determined by direct microscopy. Cell survival was assessed by the number of CFUs on agar medium. As a control, cultures that did not form exopolysaccharide were used: "*Lichenibacter ramalinii*" and species of the genus *Methylobacterium*.

The results of the study showed the ability to preserve the viability of most exopolysaccharide-producing bacteria. An important criterion for assessing the degree of viability was the rate of growth of colonies after freezing. Bacteria possessing EPS matrix, formed more colonies and the rate of their growth was higher than in control organisms.

Repeated freezing did not practically change the properties of strains, but somewhat increased the interval between sowing and the appearance of colonies – it increased by 10–20 %. The shelf life of the primary frozen cultures reached 1 year, after which they were able to grow. Control cultures showed less activity and after storage for half a year lost the ability to grow by 70–90 %. Comparison with the storage of the same cultures in glycerol at –20 and –70°C showed that during the first 3 months of storage, the survival situation looks similar. However, with an increase in shelf life, the frozen items with glycerol proved to be much more viable.

Thus, short-term (up to six months) storage of strains producing EPS is possible without the use of cryoprotectants, at a temperature of –20°C. This is especially true for mass screening of cultures, the re-isolation of which is difficult due to a large number of samples.

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METAGENOMIC SEQUENCING OF RAINBOW TROUT GUT MICROBIOTA: SEARCH FOR BACTERIAL TAXA INDICATING FISH INFECTIOUS STATE

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Recently, freshwater fish farming has widely spread in Karelia [1]. Application of industrial methods in aquaculture can lead to complication of ecologic and epizootic situation in exploited water ecosystems. Intensive development of this economic sector requires to focus research on fish diseases since well-timed diagnosis and suitable treatment prevent massive fish mortality on time and decrease financial losses [2].

The aim of the study was to identify the intestinal tract microbiota of rainbow trout under developed bacterial disease and to compare microbiota composition in fish fed by standard diet (control group) and a diet enriched by antioxidant dihydroquercetin (experimental group).

Trout guts were sampled in June, July, and August 2017. The intestinal contents and their mucous tissue have been fixed in aseptic conditions. Total DNA was isolated with "AmpliPrime DNA-sorb-B" reagents. Sequencing of hypervariable regions V3, V4 of gen 16S rRNA on platform Illumina MiSeq was made to analyze microbial community.

On the microbiological and biochemical data such as *Yersinia* sp. and fatty acids of bacterial origin detected in internal organs, the fish were infected at the beginning of the experiment (in June) and obvious symptoms of the disease appeared later (in August). The infection was a disease-provoking factor allowing to confirm efficiency of the dietary supplement on trout survival rate and internal microbiota state.

2374 OTE referred to eubacteria belonging to 15 phyla and 3 phantom groups (OD1, SR1 и TM7) of bacteria have been identified in the intestinal tract of the rainbow trout. There was no difference in microbiota of the control and experimental fish groups, excluding starting points. Microbiota composition was depleted by enrofloxacin antibacterial therapy in both groups but it was readily restored to the control level in the fish fed by dietary supplement. The maintenance of bacteria taxa diversity at the constant level under the effects of exogenous factors, such as temperature, feed content, medicinal drugs, immune status of fish, presumably also depends on the supplement.

Appearance of unique taxa in intestinal microbiota can be associated with any disease, i. e., in our case, the fish infected by yersiniosis also had enterobacteria. The increase in dominant representatives of Mycoplasmataceae has also been revealed. Probably, high occurrence of prokaryotes was caused by their contribution to fight against pathogen organisms, i. e. Lactobacillus found in studied trout can suppress pathogen microbiota.

Development of resistance to the infection and further recovery involve activation of the defense systems in fish. Antioxidant dihydroquercetin added into the feed seems to stimulate both the resistance to the infection and reparative processes in experimental trout. The information on the microbial community of the trout intestinal tract and their cellular defense mechanisms extends our knowledge on fish infectious diseases and allows us to test innovations in feed production such as dietary probiotic complexes. Bacterial species composition in the digestive organs of rainbow trout is tightly related with at type of a disease that allows suggesting more efficient diagnostics of

infectious pathologies. Total microbiota of fish organs and tissues as well as the presence of specific bacterial taxa match the epizootic status of a fish.

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THE SYKOA COLLECTION OF CYANOBACTERIA AND LIVING MICROALGAE STRAINS OF THE NORTHEAST OF THE EUROPEAN PART OF RUSSIA

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The collection of cyanobacteria and microalgae living cultures (*https://ib.komisc.ru/sykoa*) was started in the Institute of Biology (IB) of Komi Scientific Centre in 2010 as a part of Unique Scientific Installation "Scientific Herbarium of the Institute of Biology of Komi Scientific Center of Ural Branch of the Russian Academy of Sciences SYKO". The microalgae collection is registered in GCM (Global Catalogue of Microorganisms) Acronym: SYKOA, WDCM Number: 1125. Today, the collection contains more than 300 algologically pure strains of cyanobacteria and microalgae collected in the European Russian Northeast (Polar and Subpolar Urals, Bolshezemelskaya tundra), isolated from soil and water samples [1, 2]. Also, there are several strains from soils of the Southern Svalbard and other Arctic regions. Green algae and cyanobacteria/ cyanoprokaryota form the main part of the collection. Departments *Ochrophyta* and *Streptophyta* were presented in the collection by 10 species. Collection includes monocultures of dominant species of microalgae from different ecological groups (edaphophilous, cryophilous, nitrophilous, etc.), rare species and taxa with uncertain systematic position. The main aims of the collection are to conserve biodiversity of microalgae from Arctic and northern regions of the European Russia, and to collect new strains for their use in floristic, systematic, evolutionary, molecular, genetic and ecological studies.

Patents has been obtained for a number of strains, including the *Acutodesmus obliquus* (Turpin) Hegoewald et Hanagata SYKOA Ch-055–12 (deposited in IPPAS S-2016) is recommended for wastewater treatment in the communal and pulp-paper industry, and on the *Eustigmatos magnus* (B.-Peters.) Hibberd SYKOA E-001–09 (deposited in VKPM Al-25, in IPPAS H-2027) to obtain eicosapentaenoic acid.

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THE COLLECTION OF BIOTECHNOLOGICAL MICROORGANISMS OF THE ICG SB RAS

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The collection of biotechnological microorganisms of the ICG SB RAS was created in 2016 based on the existing material of the laboratory of molecular biotechnology of ICG SB RAS. It is a unique collection of microorganisms isolated from poorly studied extreme environments.

The increased interest towards extremophiles is caused by the presence of metabolic pathways that may be totally different from those of mesophilic organisms. Therefore, a complex approach using methods of genetics, microbiology, molecular biology, genetic engineering, proteomics, metabolomics, and bioinformatics applied to diverse collections of microorganisms is highly promising.

Our collection currently contains over 2000 pure cultures, over 1500 DNA samples, over 300 metagenome samples, etc. We performed detailed characterization of extreme natural communities, including saline lakes, thermal springs, natural oil sites, acid springs, as well as of axenic cultures isolated from these communities.

We also created an online catalog of our collection: *http://www.bionet.nsc.ru/biocollections/microbelandingpage.html*. We designed a specialized database with a novel data format, and a Web-site. We developed prototypes of the graphic user interface and a program interface for data accession (API) according to the REST technology (*http://cells.biores.cytogen.ru/microbe_api/*). We created an electronic profile of our collection on the portal of Bioresource collections (*http://www.biores.cytogen.ru/*).

For the strains that were included in the electronic catalog we determined growth parameters for various media and substrates, cell morphology, lipid composition of the cell membrane, and physiological characteristics, such as reaction to oxygen, feeding type, the range of temperature, pH, and medium ionic strength, etc. We created mass spectra for 62 strains of our collection and found that the resulting phyloproteomic tree was in accordance with their taxonomy. The obtained characteristic mass spectra were deposited in the database and may be used for identification of microorganisms.

IS THE ISO 9001:2015 A RISK OR AN OPPORTUNITY FOR A MICROBIAL BIOLOGICAL RESOURCE CENTRE? THE EXPERIENCE OF MYCOTHECA UNIVERSITATIS TAURINENSIS – MUT

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The ISO 9001 defines the requirements for a quality management system for an organization. The requirements are generic and can be applicable to any type of organization, including public microbial Biological Resource Centres (mBRCs). Indeed according to ISO provided products and services are not relevant.

The ISO 9001 quality management standards help who would like to subject their production process to quality control in a cyclical manner, starting from the definition of the customer's requirements and ending with monitoring of all phases of the production process.

The quality management system, according to ISO 9001:2015 can support the organization using the process approach, based on the "Plan – Do – Check – Act" (PDCA) and risk-based thinking.

The Mycotheca Universitatis Taurinensis (MUT) is the fungal collection of the Department of Life Sciences and Systems Biology of the University of Turin (Italy). The MUT is one of the most important banks of fungal biodiversity in Italy, and it has a great value from the systematic, ecological and applications point of views. Microorganisms are an untapped source for innovation able to provide solutions to some of today's global challenges and the aims of the MUT are the acquisition, identification, characterization, preservation and distribution of fungi to boost academic research and bioeconomy. The MUT is the first structure, among the Italian collections, which has operated according to the standards of ISO 9001. The decision to undertake the ISO certification already from 2006 has been favoured of the willingness to achieve the standardization of the processes in order become reliable providers of biological resources and services.

Waiting the end of activities of ISO Committee 276 on Biotechnology and the establishment of a new standard on Biobanks, ISO/DIS 20387, in the last year MUT, worked on the transition from ISO 9001:2008 to the new ISO 9001:2015 focusing on the risk analysis of all processes. This process lead to the identification of the strengths and weaknesses of the system. The regular monitoring of internal and external factors are indeed fundamental to improve the quality of services and of the biological material provided to customers.

The experience led to the awareness that MUT, as a mBRC, can consider the ISO 9001:2015 an important opportunity that allow the continuous improvement of the management of the collection to enhance customer satisfaction.

COLLECTION OF ENVIRONMENTAL BACTERIA RESISTANT TO ANTIBIOTICS AND HEAVY METALS OF INSTITUTE OF MOLECULAR GENETICS RAS AND ITS USE FOR RESEARCHES OF HORIZONTAL TRANSFER AND SPREAD OF RESISTANCE GENES

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Creation of the Collection of Environmental Bacteria of Institute of Molecular Genetics (IMG RAS) was began in 1983 due to the initiation by R. B. Khesin of researches of horizontal gene transfer on the model of the mercury resistance operons. This collection includes strains of bacteria isolated from soil, sediments and water samples collected at different regions of the Earth, including the areas of mercury deposits.

Later this collection was supplemented with environmental strains resistant to antibiotics, and resistant bacteria isolated from the permafrost of the Eastern Siberia and Antarctica. Currently, the collection includes about 1500 strains. On the basis of studies of the strains from our collection, it was shown that the main types of mercury resistance operons and transposons appeared and spread in populations of bacteria long before the beginning of anthropogenic pollution. Many of these transposons (Tn*5041*, Tn*5042*, Tn*5044*, Tn*5053*, Tn*5058*, Tn*5070*) were identified and described for the first time. A simple transposon Tn*5060* almost identical in structure to the hypothetical ancestor of modern complex transposons of clinical bacteria was found in the collection of permafrost bacteria.

Indisputable proofs of origin of antibiotic resistance genes of clinical bacteria from environmental strains were received. Plasmids, IS elements and transposons involved in the horizontal transfer of resistance genes in bacterial populations, including the modern clinical bacteria, were identified in ancient strains and studied in detail. For the first time the data were obtained that demonstrated the ancient origin of integrons with genes of antibiotic resistance.

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MICROBIAL COLLECTIONS IN RUSSIA: CURRENT STATE AND OUTLOOKS

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Most Russian microbial collections or banks with different size and diversity of biological resources are now hosted in Institutions of the Federal Agency for Scientific Organizations (Today Ministry of Science and High Education). Twelve major and known collections, including All-Russian Collection of Microorganisms (VKM), treasure the most part of diverse bacteria, archaea, fungi, and algae in Russian Federation, which become important over and over for fundamental science, bioeconomy, and sustainable development of biobased industries. Collections have significantly been supported and fostered due to governmental investments from the Federal Agency for Scientific Organizations. The aim of this communication is not only to review each collection in terms of richness and practical use, as well as the current initiatives to create the Core Microbial Resource Center. Thus, standard operational procedures to be used in different collections and the Core Center were developed and verified experimentally in the results of coordinated recent projects. Special initiatives and activities have a focus on a uniform mechanism of data sharing and collecting. Integration of resources, facilities, and experience of leading microbial collections and host Institutions is now a prerequisite to keep and extend resources of microorganisms and to perform high-level research using classic methods and advanced “omic” technologies.

FUNGI IN THE COLLECTION OF MARINE MICROORGANISMS AT THE G. B. ELYAKOV PACIFIC INSTITUTE OF BIOORGANIC CHEMISTRY (KMM 644)

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Marine fungi involve ecologically defined group of primarily filamentous ascomycetes, basidiomycetes and their anamorphs. The ecological importance of filamentous fungi in marine systems is often underestimated or ignored completely, and yet these organisms represent a diverse range of saprobes, pathogens and symbionts that form an integral part of coastal systems. The interactions between microbial diversity and ecosystem function are not well understood. In particular, it is unclear how population stability and metabolic function are related to diversity. Assessing fungal diversity accurately, encompassing phylogenetic diversity, species richness and evenness, is the first step towards modeling fungal assemblages dynamics in terms of species redundancy, species spatial and temporal distributions, and nutrient cycling. Such models are essential for the efficient management and conservation of marine environments that are economically important. Similar limitations also apply to the identification, isolation and quantification of fungi from marine environments, with the additional complication of distinguishing between transitory and native forms. Fungi isolated from marine environments have been considered traditionally to be either obligate, where growth and reproduction occur exclusively in a marine system, or marine-derived (facultative). The distinction between these states is not always clear. However, as many marine- and maritime-derived fungi can grow in saline conditions. Defining a fungus as an obligate marine species, therefore, has relied upon direct microscopic observation of morphological, particularly reproductive structures, growing on the substrata. Diversity assessments based on the identification of sexual and asexual fruiting bodies alone are likely to be incomplete for several reasons: the inability of some fungi to grow or fruit in culture or on substrata, differential rates of sporulation, the presence of unrecognized multiple life cycle forms, and the limitations of distinguishing between species with similar morphologies. The procedure for distinguishing between transitory and native facultative marine fungi has been developed from the germination of marine isolate in the natural, not sterile sea water and in the fresh water, as well as incubation fungi in the sea. In 1985 the Collection of Marine Microorganisms of the G. B. Elyakov Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences was founded. Now the Collection is the member of the World Federation for Culture Collections and has official acronym KMM, registration number 644, and includes 1000 strain of fungi. At present 32 species of 11 genera ascomycetes, 249 species of 57 genera anamorphic fungi were defined. The main goal these collection is obtaining new producers of substances. More than 300 producers of new antibiotics, anticancer substances, and inhibitors of enzyme were obtained and structures of these substances were elucidated. Taxonomic and chemical studies of marine-derived fungi demand more attention and will be very promising.

ACTIVITIES OF THE COLLECTION OF CELL CULTURES OF VERTEBRATES

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The development of the most important and promising fundamental and applied research in the field of molecular and cell biology, genetics, embryology is inextricably linked with the widespread use of human, animal and plant cell cultures. Cells in the culture are subject to high hereditary variability in long-term cultivation under the influence of changing environmental conditions. But in most cases it is impossible to determine which specific properties have changed. Therefore, when working with cell cultures, measures should be taken to avoid increasing genetic instability. The successful preservation of the original or the modified directional characteristics of the cell lines, as well as obtaining reproducible experimental results is achieved by adhering to strictly adhere to the conditions of cultivation and cryopreservation of cell cultures. Maintaining the original cellular properties and control their condition is carried out by National collections of different countries, the creation of which took place, mainly in the second half of the XX century.

In 1978 in the USSR the All-Union (Russian) collection of cell cultures was created. The initiator and founder of the all-Union, and then the Russian collection of cell cultures (RCCC) is prof. Georgy Petrovich Pinaev (1929–2013), who until the last days of his life was the coordinator of the RCCC activities. The collection of vertebrate cell cultures (CCCV) was approved by the Central Bank of the all-Union (Russian) collection of cell cultures.

Currently in the CCCV contains 145 human cell lines and different species of animals, including immortalized and non-immortalized lines. Also in the funds of the CCCV are 775 author's cell lines and a hybrid deposited in connection with the patenting procedure.

The principles or tasks of the CCCV are as follows.

1. Creation, continuous maintenance and development of collection funds by collecting, removing, certification and storage of human and animal cell lines.

2. Development of common requirements for the quality of collection material: unified passports, methods of analysis, storage and control of cell lines, according to international requirements.

3. Improvement of methods work with cell lines on the basis of long-term research on the effect of cultivation conditions, cryopreservation and contamination on the genetic variability of cell lines; the derivation and characterization of new cell lines and a hybrid.

4. Deposit of author's cell lines and hybridomas in connection with the patenting procedure.

5. Creation of information databases on cell cultures.

6. Providing samples of standard and fully characterized cellular material for fundamental and applied biological, medical, agricultural research.

7. Providing scientific and methodological assistance to the staff of scientific institutions of the country on the methods of cultivation and analysis of cell lines and the publication of methodological guidance.

Each collection cell line has a passport, which presents the main characteristics, according to international requirements for collection lines. The passport is the most important document regulating the methods to be used for the adoption of cell culture in the collections and the assignment of the status of the collection line. A complete passport of the cell line includes the following items:

origin, morphology, mode of cultivation, conditions for cultivation, viability after cryoconservation, sterility, species, karyology, DNA profile (STR), plating efficiency, tumorigenicity, other (additional) properties, applications, collections.

The main research carried out in the CCCV over 40 years of its existence, have 4 main directions: 1. Study of the regularities of karyotypic variability in cell cultures under long-term cultivation under different conditions; 2. Derivation and characterization by a hybridomas widely used for fundamental and applied biomedical research 3. The derivation and characterization of lines of human embryonic stem cells under cultivation in feeder and feeder-free conditions; 4. The derivation and comparative characteristics of human mesenchymal stem cell (MSC) lines isolated from different sources; comparative analysis of the characteristics of different MSC lines under long-term cultivation, including the processes of replicative senescence and karyotypic analysis; study of the differentiation potential of MSC in order to assess the MSC lines of different origin to the predominant differentiation in a certain direction and to study the mechanisms of different differentiations. MSC have unique properties that allow them to be used both to deepen fundamental knowledge about the processes occurring in the cell and to expand applied biomedical research. The importance of the comparative analysis of the characteristics of human MSC, isolated from different sources, follows from the features of the interaction of MSCS with their unique microenvironment (niche), characteristic of a certain tissue, which regulates the main cellular processes by means of intercellular interactions and various bioactive molecules. Thus, the origin or source of the MSCS may determine their functional characteristics.

In addition to scientific research, the CCCV has a wide range of activities as a Center for collective use (CCU). The main services provided by the CCU "Collection of cell cultures of vertebrates" are: 1. Provision of standard and fully characterized cellular material samples for fundamental and applied biological, medical, agricultural and biotechnological studies; 2. Deposit of author's cell lines and hybridomas in connection with the patenting procedure; 3. Providing consulting services on methods of cultivation and analysis of cell lines. As example is the work of the CCU for 2015–2017. For the 1st service 596 samples of cell lines were issued. For the 2nd service it is accepted to Deposit 8 author lines. The 3rd service provided consultations to 11 the staff of different institutions of the Russian Federation.

CCU CCCV has its own page on the website of Institute of Cytology RAS – *www.cytspb.rssi.ru/lab_ckp/ckp_lab_ru.htm* and on the Federal information portal – *http://ckp-rf.ru/ckp/3029/.*

This page contains the following information: list of key methodologies used in the CCCV (information updated periodically); the list of standard works in the CCU; list of scientific equipment of the CCU and the time of its use (information is updated annually); information on the implementation of standard works and the list of user organizations (information is updated annually); the regulation of access to services include: rules of order and issue of collection samples cell lines, Deposit rules copyright cell lines, the rules provide consulting services, application forms; model contracts for the provision of services. Given information on the development of the CCU CCCV and major publications (information updated periodically). On the same page there is Russian version of a catalogue of Collection of cell cultures of vertebrates *http://www.cytspb.rssi.ru/rkkk/katalog1_2017_with_figs_new.pdf.*

English version of a catalogue of Collection of cell cultures of vertebrates – *http://www.cytspb.rssi.ru/rkkk/katalog1_2017_en_new.pdf.*

The information provided in the catalogues is updated periodically.

CELL WALL GLYCOPOLYMERS IN TAXONOMY OF *ACTINOBACTERIA*

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The study of chemical composition of bacterial cell envelope is of scientific and practical importance in many fields such as chemistry, biology, medicine, pharmacology, and environmental science. New data on cell wall glycopolymers in representatives of various bacterial taxa expand our notion on the diversity of natural polymers and biosynthetic potential of microorganisms. The results of comparative study of the cell wall glycopolymers in members of different species and higher taxa are of interest to microbial taxonomy.

Over the years, we have been studying the structural diversity of the cell wall teichoic acids and other glycopolymers in representatives of *Actinobacteria* (orders *Actinomycetales*, *Frankiales*, *Geodermatophilales*, *Kineosporiales*, *Micrococcales*), with focus on seeking for new chemical structures of polymers and exploring the use of such polymers as chemotaxonomic markers for actinobacterial species.

Here we summarize the results of studying teichoic acids from the taxonomic point of view in representatives of some genera, including *Actinomadura*, *Agromyces*, *Brevibacterium*, and *Nocardiopsis.* We also provide recent data on chemical composition and distribution of the phosphate-free glycopolymers in species of the order *Micrococcales* (genera *Clavibacter*, *Curtobacterium*, *Promicromonospora*, etc.) with special attention to various rhamnomannans and teichuronic acids revealed in members of the genus *Rathayibacter* (family *Microbacteriaceae*).

The genus *Rathayibacter* currently comprises eight species with validly published names (*R. rathayi*, *R. agropyri*, *R. caricis*, *R. festucae*, *R. iranicus*, *R. oskolensis*, *R. tritici*, and *R. toxicus*). A few other species within this genus, including *"Rathayibacter tanaceti"* and *"Rathayibacter acroptilonus"*, were revealed but have not been validly described. The species *R. rathayi*, *R. iranicus*, *R. tritici*, and *R. toxicus* are known to be plant pathogens which are transmitted to their host plants by seed gall nematodes of the genus *Anguina*. The *Rathayibacter* species exhibit high 16S rRNA gene sequence similarity (up to 99.7%) and are difficult to distinguish by conventional phenotypic properties. Species of the genus are also characterized by a similar set of cell wall sugars. All species contain mannose, rhamnose, and glucose; some include additionally galactose, xylose or fucose.

We have shown that representatives of different *Rathayibacter* species (*R. rathayi*, *R. festucae*, *R. iranicus*, *R. oskolensis*, *R. tritici*, *R. toxicus*, *"R. tanaceti"*, and *"R. acroptilonus»*) possess the specific composition and chemical structures of the cell wall glycopolymers, and well distinguished from each other by this feature.

All strains used in the work have structurally different rhamnormanns in their cell walls. The rhamnose and mannose residues in these polymers are linked by α-1→2 and / or α-1→3 glycosidic bonds in various combinations; some polymers have lateral branches (residues of mannose or xylose).

Alongside rhamnomannans, most strains contain also teichuronic acids of different structures with monosaccharide constituents characteristic of particular *Rathayibacter* species. The teichuronic

acids of some strains include additionally residues of pyruvic or lactic acid. It is worth noting that all glycopolymers identified in *Rathayibacter* spp. have been revealed in the cell wall of Gram-positives for the first time.

Thus, the cell wall components, both teichoic acids and phosphate-free glycopolymers (as well as their combinations), can serve as chemotaxonomic markers of actinobacterial taxa at species and generic levels.

All the above-stated evidence favors the advisability of further comparative studies of cell wall glycopolymers in actinobacteria and other Gram-positives. Of particular interest to taxonomy are the studies of polymers aiming at phenotypic characterization and differentiation of phylogenetically closely related species.

THE ROSCOFF CULTURE COLLECTION

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The Roscoff Culture Collection (RCC: *www.roscoff-culture-collection*) is hosted at the Roscoff Marine Station (Sorbonne University/CNRS) in France and currently holds over 5000 strains covering a broad range of the biodiversity of marine unicellular photosynthetic plankton (microalgae and cyanobacteria). The RCC also maintains strains of other types of photosynthetic bacteria and microalgal viruses, and has recently started to integrate macroalgal strains. RCC strains originate from worldwide biogeographical locations, including all main ocean basins. Approximately 20% of collection holdings are maintained as cryopreserved stocks. The RCC is an integral part of the EMBRC infrastructure (European Marine Biological Resource Centre: *www.embrc.eu*) and it's French node EMBRC-France (*www.embrc-france.fr*), as well as the infrastructure projects EMBRIC (*www.embric.eu*) and EBB (*www.ebb.eu*). Subsidized access to strains within Europe is provided via the ASSEMBLE-Plus H2020 InfraIA program (2017–2021). The RCC is involved in several applied screening projects, both European (NOMORFILM: *www.nomorfilm.eu*) and national (OCEANOMICS: *www.oceanomics.eu*; POLYSALGUE: *www.polysalgue.fr*). The RCC also collaborates in a R&D project to test and develop methods for the genetic transformation of picoeukaryotes (Moore Foundation, USA). The main strategic focus of the collection remains the isolation, maintenance, characterization and distribution of strains used for fundamental research.

EX SITU CONSERVATION OF MACROMYCETES DIVERSITY IN THE LE-BIN CULTURE COLLECTION

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Fungal conservation is an integral and important part of the biodiversity conservation problem. The best result can be achieved when traditional *in situ* (on site) conservation is complemented with *ex situ* (of site) conservation by maintaining of fungal genetic resources in pure culture. The current number of described fungal species globally is between 80000 and 97000 from which over 20000

species are macrofungi [1, 2]. But the number of species which actually exist is estimated to be much higher. In the beginning of 21st century it was evaluated up to 1.5 million [3], but after the advent of molecular approaches to species delimitation it increased up to 3.8 million [4]. *Ex situ* conservation removes a living sample of the species from its natural ecological context and preserves it out of nature. It may become the only option to preserve the species when the natural habitat is destroyed and the species are endangered. The benefit of *ex situ* conservation is supplemented by possibility of using and multiplying the genetic resources of fungi for fundamental mycological research, biotechnology, medicine and other uses. Fungal Culture Collections play a key role in successful preservation of fungal genetic resources and provide of high quality biological material for research and industry, aspiring to maintain purity, viability and genomic integrity of biological material, avoid selection of variants, and lessen the prospects of strain deterioration. *Ex situ* conservation of macromycetes diversity along with the preservation of producers of biological active substances has become the main goal of the development of the Komarov Botanical Institute Basidiomycetes Culture Collection (LE-BIN) in St Petersburg, Russia. The Collection was established about 60 years ago for study of biologically active compounds and enzymes in basidiomycetes. In the late 1990s the Collection started developing a new project on conservation *ex situ* of macromycetes diversity. The Collection's development plan consisted in conservation ex situ of taxonomical and ecological diversity of macromycetes in Russia with emphases on preserving rare and endangered species, maintaining ectomycorrhizal fungi, and culturing species strains useful for biotechnology and medicine. The objective was to preserve *ex situ* and study in culture as many different macromycetes species as possible, following the 3-step culturing process: collection and identification, isolation and verification, cultivation and investigation. Macromycetes can only be isolated into culture manually from fruitbody tissue, spores or rarely mycelium in or on a substratum. Most of the Collection strains are original isolates which are maintained only in the LE-BIN. They were obtained during field works in various regions of Russia (European areas, the Caucasus, Urals, Siberia, and Far East) mainly in protected zones, i. e. nature reserves and national parks. Also there are strains from territories of the former USSR and other countries both original and exchanged. Species and strain diversity involved in conservation *ex situ* is gradually increasing in the LE–BIN Collection. During field seasons of the last 5 years (2013–2017) over 900 isolates from about 400 macromycete species were cultured. Collecting was carried out in the following regions of Russia: 2013 (Buryatia, Barguzinsky Nature Reserve; Lipetsk Oblast); 2014 (Vietnam, Cat Tien, Bu Gia map and Bidoup Nui Ba National Parks; Vilgograd Region; Republic Adygea, Caucasus Nature Reserve; Orlov Oblast); 2015 (Krasnoyarsk Krai, Sayano-Shushenski Nature Reserve; Bryansk Oblast, Bryansky Les Nature Reserve; Kursk Oblast, Central Black Earth Nature Reserve; Moscow Region); 2016 (St. Petersburg; Belarus, Belovezhskaya Pushcha National Park; Lipetsk Oblast, Galich'ya Gora National Park, Tatarstan Republik, Bolga-Kama Nature Reserve; Republic of Abkhazia); 2017 (Kamchatka Oblast, Vulcanoes of Kamtchatka Nature Park, Bryansk Oblast, Bryansky Les Nature Reserve). Original specimens were identified by taxonomist specialists in various groups of macromycetes. Voucher specimens for maintaining cultures were preserved in the Mycological Herbarium of the Komarov Botanical Institute (LE). Cultures maintained in the Collection are mainly of saprotrophic mushrooms including xylotrophs, litter decomposers, soil saprotrophs, and fungi growing in nature on various other substrata such as buried wood, cones, humus, grass, conifer needles, coal, and mosses. There are also cultures of several ectomycorrhizal fungi (e. g. species of *Amanita*, *Boletinus*, and *Suillus*). In the collection, strains are maintained as sub-cultures in tubes on beer-ale (4°Balling) agar (2%)

slants and in screw-cap vials under distilled water at 4–6°C. Cultures from tropical regions are maintained as sub-culture at 20ºC and in screw-cap vials under distilled water at room temperature. Since 2011 the collection's strains have been also maintaining by cryoconservation method at -80ºC. Now about 1/2 of the LE-BIN fund have already been transferred to cryopreservation. LE–BIN is now the largest living collection of macromycetes in Russia, preserving approximately 10% of the natural diversity of these fungi in Russia – about 3000 strains of over 600 species from about 190 genera, 60 families, 21 orders, and 5 classes from 2 phyla of agaricoid, aphyllophoroid, and gasteroid fungi from *Basidiomycota* and macromyceteds from *Ascomycota*. Names of LE–BIN cultures are regularly updated following modern nomenclature primarily as set out in *Index Fungorum* (*www.indexfungorum.org*). The latest edition of the Collection Catalogue was published in 2007 [5]. A list of the LE-BIN strains, some information about their origination and example of the collection's documentation are available via the Komarov Botanical Institute webpage (*www.binran.ru*). In 2012 the Collection was registered in the WDCM databases as LE-BIN 1015 and in 2015 became a member of the World Federation for Culture Collections (WFCC). In 2016 the Collection joined the WFCC Global Catalogue of Microorganisms (GCM) and transferred there information about 1363 strains (*http://gcm.wfcc.info/*). Verification of the LE–BIN strains is carried out using cultural, biochemical, and molecular methods. Over the past several years, strains of some new interesting and rare species were obtained. *Lignomyces vetlinianus*, *Rogersiomyces malaysianus*, *Sarcosoma globosum* and some other can be mentioned as examples. These species were insufficiently or totally unstudied before for their biological activity, and they might be of interest for practical use in biotechnology and medicine. The progress in *ex situ* conservation of macromycetes diversity will promote not only biodiversity conservation but increase the variety of species and strains available for screening of new biologically active metabolites.

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MICROBIAL VIABILITY: THE UNCERTAINTY THAT NEEDS TO BE MEASURED

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Viability is a key feature of microbial cells as a living matter. It needs to be evaluated quantitatively in many arrears of the basic and applied microbiology including culture collection practice. However, theoretically, the meaning of the microbial viability is uncertain, there is no unified concept on this subject and the debates on it continue.

Nevertheless, it is generally agreed, for practical reasons, to consider microbial viability as an ability of the cells to multiply and to give progeny. However, quantitative assessment of this ability is not strait forward, and there is no any universal method that could be used in all cases. This is due to the two main causes. 1. There is wide genetic, structural, behavioral and metabolic diversity of microorganisms. 2. A microbial cell may be in a range of states termed active, dead, dormant, inhibited, stressed, injured, living, moribund, quiescent, resting, starved, sublethally damaged, viable but not culturable, and vital.

Two groups of so-called direct and indirect methods for the multiplication ability assessment have been developed to date. The direct methods enable assessing it by cultivating microorganisms at appropriate conditions. So, viability is considered as culturability. The main problem here is with the choice of appropriate cultivation conditions. The plate count technique is an example, which is often considered as a "gold standard" in many practical cases. However, an unequivocal interpretation of the results is possible only for restricted number of cultures with well separated single cells. Also, the technique, in its original form, is time- and labor-consuming. Recent improvement of the technique is associated with application of computer based colony counters. For cultures consisting of cell associates/aggregates, only conditional semi-quantitative approaches are available.

The indirect methods are based on the evaluation of some features of the cells which are crucial for life/multiplication. The most basic determinants of viable cells include: an intact and functioning (barrier properties) cytoplasmic membrane; DNA transcription and RNA translation (replication of the DNA); generation of energy to meet requirements for cellular metabolism, synthesis of proteins, nucleic acids, polysaccharides and other cellular components. All indirect methods are predictive, that is they provide data which may reflect multiplication ability of the cells with some probability. For validating an indirect method, in every particular case, correlation between indirect indicator (s) and culturability must be established. In most indirect methods, the viability indicators are assessed with fluorescent probes and labels using fluorimetry, fluorescence microscopy or flow cytometry. Currently, significant progress has been achieved in fluorescence microscopy improvement by coupling it with computer image analysis.

In conclusion it should be stressed that, in spite of the urgent need in many areas of experimental microbiology, the problem of quantitative microbial viability assessment is far from its solution. One potential way to solve it could be via multiparametric characterization of the cells by their viability-related features coupled with validation by direct methods of the multiplication ability assessment.

BIOLOMICS, SCIENTIFIC AND COLLECTION DATA MANAGEMENT AND ANALYSIS SOFTWARE

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BioloMICS is a large and modern software that allows curators, technicians, and sales managers to manage all the complex aspects associated with the collections ranging from data entries, importations, exportations, stock management, label printing, report templates, etc. The beauty of the system is that it is dynamic and flexible. This allows the database to evolve with the needs of the end-users since new tables or fields can be added or removed on the fly, without the intervention of any software developer. The system is secured as the users are belonging to user groups that have the right (or not) to read, write or delete in tables, fields and records. All changes in the database are recorded (user identifier and date and time), can be easily queried, and reverted by the administrators, if needed. The new version of the software has been totally redesigned to make it extremely user-friendly for an everyday usage. The look and feel of the software is identical to Microsoft Excel. Many functionalities are working in the exact same way as Excel (including filtering and highlighting of data, formulas, reporting, graphics, pivot tables, some data analyses, dashboards etc).

The web version of the software allows to display part or the entire database and can create modern websites with searching tools, display of scientific data and collections can sell their strains online. End-users can deposit their data online allowing curators to save a lot of time. Many tools are available with BioloMICS that is certainly the most complete and affordable system for the management, the analysis and the publication of culture collections' data.

BioloMICS has been proposed to be used as the backend system for the future MIRRI–IS system due to its flexibility and large spectrum of tools and features. MIRRI members have to confirm this choice but in the meantime a demo version of the MIRRI–IS central database and website has been created and could be demonstrated during the meeting.

GENOTYPIC DIVERSITY OF *SINORHIZOBIUM MELILOTI* STRAINS FROM ORIGINS OF CULTIVATED PLANTS DETERMINED BY MOLECULAR APPROACHES

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Root nodule bacteria (rhizobia) are forming a highly specific symbiosis with legume plants. Genotypic characteristics of strains-microsymbionts are crucial for the creation of highly productive and stress-resistant symbiotic pairs formed by rhizobium strain with particular variety/species of the host plants. Genetic centers of host plants are the centers of genetic diversity not only of plants,

but also of their microbial symbionts. It is shown that the level of intraspecific polymorphism of genetic markers of core genome and of accessory genome as well is very high precisely in sites of centers of origin. Reliable differences in levels of genetic diversity between sub-populations of rhizobia isolated from nodules of wild plants and from soil samples by the trapping method have been revealed. It is shown that the genomes of natural strains have significant differences in genes involved in the control of metabolism, replication, recombination, and in the formation of protective mechanisms. The natural populations of nodule bacteria represent an unlimited resource of new genes that determine the ability of rhizobia to form a highly effective symbiosis under various agro-ecological conditions. As a result of the analysis of the combination of different alleles of marker genes determining symbiotic effectiveness, competitiveness, and tolerance to stresses, we have selected a set of molecular markers suitable for testing genome characteristics stability and for genotyping of symbiotically active strains. Such marker sets could be recommended for assessing genetic stability of strains of practical significance.

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COMPLEMENTATION OF SYMBIOTIC GENES IN THE TAXONOMICALLY DIFFERENT CO-MICROSYMBIONTS OF A RELIC LEGUME *OXYTROPIS POPOVIANA*

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Symbiotic systems of relic legume plants are the promising models for studying the evolution of plant-microbe interactions, which is still poorly understood. Ten rhizobial strains were isolated from root nodules of the Miocene-Pliocene relict legume *Oxytropis popoviana* Peschkova, originated from the restricted area of the Baikal Lake region. For identification of the isolates obtained the sequencing of 16S rDNA, ITS region and housekeeping genes *recA*, *glnII* and *rpoB* was used. It was shown that nine fast-growing isolates were *Mesorhizobium*-related: eight strains were identified as *M. japonicum* (99.61–100% *rrs* similarity with the type strainMAFF 303099^T) and one isolate

belonged to *M. kowhaii* (100% *rrs* similarity with the type strain ICMP 19512^T). The only slow-growing isolate was identified as *Bradyrhizobium* sp. due to the difficulty of determining at the species level. Two strains Opo-242 and Opo-243 belonged to *M. japonicum* and *Bradyrhizobium sp.*, respectively were isolated from the same nodule and present there in approximately equal proportion. Symbiotic genes of these isolates were searched throughout the whole genome sequences obtained. The common *nodABC* genes necessary for the formation of symbiosis and other *nod*, *noe*, *nif* and *fix* genes required for the plant nodulation and nitrogen fixation were present in the isolate Opo-242. The strain Opo-243 did not contain the principal *nod*, *nif* and *fix* genes, however five genes (*nodPQ, nifL, nolK* and *noeL*) affecting the specificity of the plant-rhizobium interactions and absent in the isolate Opo-242 were detected.

Symbiotic phenotype of the isolates Opo-242 and Opo-243 was studied in sterile plant nodulation assays with *O. popoviana* plants using variants of mono- and co-inoculation. Root nodules were observed in two variants of inoculation: with the isolate Opo-242 and co-inoculation with both strains. It was revealed that the isolate Opo-243 could not independently form a symbiosis, however significantly accelerated the root nodule formation after co-inoculation with the isolate Opo-242. Thus we demonstrated that strains isolated from the archaic symbiotic systems can be co-microsymbionts having complementary sets of symbiotic genes and increasing the efficiency of host plant nodulation. Joint presence of such co-microsymbionts in nodules can promote the recruitment of genes that are important for symbiosis and distributed over different taxa.

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SINORHIZOBIUM MELILOTI NATIVE ISOLATES FROM ORIGINS OF *ALFALFA*: POLYMORPHISM OF GENES RESPONSIBLE FOR VIRULENCE AND SALT TOLERANCE

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Symbiosis between root nodule bacteria (rhizobia) and legume host plants is a result of molecular signaling between macro- and microsymbionts. The process of plant-microbium interaction has been studied in detail under standard conditions, whereas the processes of their interaction can undergo impacts of different stress factors, one of which is salinity. Genomes of native rhizobia strains adapted to various soil-climate conditions are representing a great pool of genetic resources.

The Laboratory of Genetics and Selection of Microorganisms (All-Russian Research Institute for Agricultural Microbiology, ARRIAM) has a unique collection united more than 1000 native isolates of root nodule rhizobia which were recovered from nodules of wild alfalfa, sweet clover and fenugreek plants in two primest gene centers of cultivated plants designated by Vavilov N. I. [1]. They are Caucasian and Uzbekistan (Central Asia) regions. Native isolates were also collected at the Aral Sea region (Kazakhstan) – the modern center of introgressive hybridization of alfalfa, and at European–Siberian gene center (including the southern Baikal region) designated by Ivanov A. I. [2].

The analysis of the structural polymorphism of the two functionally different groups of genes was carried out using 92 native strains of *Sinorhizobium meliloti* from the collection of the Laboratory. The strains were isolated from nodules of wild host plants and from soil samples collected in the two geographically distant locations of cultivated plants centers of origin. One location is at the North of Caucus and another one is next to the Aral Sea area subjected to salinity. It is a modern center of introgressive hybridization of alfalfa.

The structural polymorphism of the *nodABC*, *nodH*, and *betCBA*, *betB2* genes responsible for virulence (synthesis of signal molecules) and salt tolerance (synthesis of osmoprotector – glycine betaine), was evaluated using the PCR-RFLP method. RFLP types (or alleles, according to [3]) of the *nod* and *bet* genes in the reference strain Rm1021 were designated as a "reference" types of alleles. Alleles different in structure from those mentioned above (further "divergent allele") were revealed in genomes of native isolates. Pair combinations of alleles of *nod* and *bet* genes in both populations by the linkage disequilibrium test (LD) were analyzed.

As a result divergent alleles of the *nod* genes were two times more frequent in the population from location at the North Caucasus, while the same type of alleles of the *bet* genes was predominant in the population from Aral Sea region. The disequilibrium was shown between two analyzed groups of genes in genomes of native isolates from the center of the alfalfa diversity located at the North Caucasus. Whereas no disequilibrium was detected for the population of rhizobia native to salinized Aral Sea region [4].

The obtained results clearly show differences in microevolutionary processes occured in geographically different populations of bacteria associated with alfalfa nodules.

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APPLICATIONS OF OXFORD NANOPORE SEQUENCING AT THE CULTURE COLLECTION UNIVERSITY OF GOTHENBURG (CCUG)

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The development of next-generation sequencing (NGS) technologies has triggered a revolution in the field of microbial genomics, enabling the generation of millions of DNA sequence reads in just hours. During the last years, several NGS platforms have been consolidated in the market, all with various advantages and shortcomings. For instance, Illumina and Ion Torrent sequencing platforms provide high quality but short reads (i. e., a few hundred base pairs), thus hampering the determination of complex regions of the genomes. Meanwhile, PacBio sequencers provide long reads (i. e., several kilobases) with a high consensus accuracy, although, the requirements of high quantity and high-quality DNA, make PacBio sequencing tedious and unfeasible for some applications. Furthermore, the Illumina, Ion Torrent and PacBio sequencing technologies have in common large capital costs, complex and time-consuming protocols for library preparations (several hours), as well as relatively long running times.

Recently, Oxford Nanopore Technologies launched the MinION portable sequencer, which has no capital cost, the sequencing library can be prepared in 10 min and the sequencing results are provided in real time. Additionally, the MinION can provide reads of up to 2,000,000 bp. However, initial high error rates have caused doubt and skepticism within the scientific community.

Here, we show the potential of Oxford Nanopore MinION sequencer as a tool to be used for applications by mBRCs and culture collections for rapid determination of complete and error-free bacterial genome sequences, for bacteria typing, as well as a rapid turnover infection diagnostic tool to be used in the clinical laboratories.

Application 1: sequencing complete genome sequences of reference strains. To demonstrate the ability to obtain high-quality genome sequences, *Streptococcus pyogenes* CCUG 4207^T was sequenced, using an Illumina HiSeq platform and an Oxford Nanopore MinION sequencer. A hybrid assembly of sequence reads from both platforms was performed, using SPAdes. The result was a scaffold of 1,914,862 bp. The assembly was mapped with the Illumina reads to search for discrepancies and compared with the complete genome sequence of *S. pyogenes* NCTC 8198^T = CCUG 4207^T, determined with PacBio (1,100-fold coverage), using Nucmer and BLAST. No discrepancies were found when mapping the Illumina reads. Furthermore, analyses showed that the Illumina-MinION hybrid assembly generated the same genome sequence length as the PacBio assembly, thus overcoming concerns of error rate. Furthermore, in a whole-genome sequence determination of an ESBL-*E. coli* strain isolated from a clinical sample, the number and sequences of plasmids were determined (four plasmids ranging from 1.8 to 160 kb), without targeted plasmid preparations.

Application 2: real-time bacterial species identification. Mixtures of genomic DNA from *S. pyogenes* CCUG 4207^T and an *E. coli* clinical isolate were used for model library preparation

and sequencing, using a MinION sequencer. Sequencing reads were processed, using the EPI2ME workflow "What's in my pot?" (Metrichor). The workflow was able to identify both species by assigning 97 % of the 151,769 reads to the correct species.

Application 3: rapid culture-independent detection of infectious bacteria in clinical samples. During the year 2018, we have received funding from Sahlgrenska University Hospital to optimize and evaluate applications of Oxford Nanopore MinION for real-time detection of bacterial pathogens and whole-genome sequence-based analyses of clinical samples.

Conclusions:

- Oxford Nanopore MinION, in combination with Illumina, is able to generate complete and error-free closed genome sequences.
- Oxford Nanopore MinION, by using the EPI2ME workflow "What's in my pot?", is able to accurately identify bacterial species in pure and mixed cultures.
- Oxford Nanopore MinION is a rapid turn-over diagnostic tool for detection and identification of bacterial pathogens directly in clinical samples.

BCCM/ULC: A UNIQUE BIOLOGICAL RESOURCE CENTER OF (SUB) POLAR CYANOBACTERIA

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The BCCM/ULC public collection of cyanobacteria funded by the Belgian Science Policy Office (BELSPO) focusses on the "*ex situ*" conservation of a representative portion of the (sub) polar cyanobacterial diversity with different origins, isolated from terrestrial (e. g. soil crusts, cryoconites, endoliths) and aquatic ecosystems (e. g. limnetic microbial mats, freshwater lakes and marine environments). BCCM/ULC currently holds 175 cyanobacterial strains, including over 100 of polar origin (catalogue: *http://bccm.belspo.be/catalogues/ulc-catalogue-search)*. The strains are available for researchers who study the taxonomy, evolution, biogeography, adaptation to harsh environmental conditions, etc. Morphological and molecular identifications (based on SSU rRNA sequences) indicate that the strains belong to the orders *Synechococcales*, *Oscillatoriales*, *Pleurocapsales*, *Chroococcidiopsidales* and *Nostocales*. This large taxonomic distribution makes the collection interesting for phylogenomic and genomic make-up studies, hence the genome sequencing of several strains is ongoing. Continuous maintenance of living cultures ensures the preservation of strains, whose majority are cryopreserved (as back-up at –70°C) in order to limit the genetic drift.

BCCM/ULC obtained an ISO 9001:2015 certification for public and safe deposits, and distributions of strains, as part of the multi-site certification for the Belgian Coordinated Collections of Microorganisms (BCCM) consortium.

The policies of acquisition and distribution of the collection are translated respectively into contracts called Material Accession Agreements (MAA) and Material Transfer Agreements (MTA). This guarantees safe fit-for-use microbiological material and data compliant with the rules on access

and utilization of the Nagoya Protocol under the Convention on Biological Diversity (12 October 2014).

BCCM/ULC progressively incorporates the most interesting strains from the research collection of the host laboratory into the public collection, whose variety is also enriched by public deposits from other geographical areas (more temperate). The collection is also interested to test new cultivation methods to better reproduce the complex ecological interactions experienced in nature.

In addition, Antarctic cyanobacterial strains are known to produce a range of secondary metabolites with different potential bioactivities [1], as well as the exploration of some unknown gene clusters identified in the first Antarctic cyanobacterial genome ever determined may potentially lead to discover novel peptides which could have biotechnological or biomedical applications [2].

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POLYPHASIC EVALUATION OF LONG-TERM PRESERVATION ON *ASPERGILLUS* (SECTION *NIGRI*) STRAINS

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The implementation of consistent fungal preservation techniques and appropriate quality assurance are key issues for an effective and efficient preservation. The cost and convenience of each method are important aspects to be taken into consideration such as the knowledge of all parameters capable of affecting the procedures [1]. Preservation methods currently used are highly empirical and in many instances, do not provide reliable genetic and phenotypic stability. Freeze-drying is commonly used to preserve fungal strains at room temperature, however, genetic and phenotypic alterations after long term-storage are yet unknown. Therefore, the main goal of the present experimental study is to evaluate the freeze-drying preservation method for the effective long-term preservation of strains belonging to *Aspergillus* section *Nigri*.

Twenty-one strains representative of *Aspergillus* section *Nigri* were selected and preserved by freeze-drying. The strains were subjected to accelerated storage during 4 weeks at 37 ºC. These samples were morphological, physiological and genotypical analysed. In order to detect macro and micro-morphological changes, growth for seven days at 25°C on Potato Dextrose Agar, Malt Extract Agar, Czapek Yeast Extract Agar and Czapek Dox Agar was performed. The physiological changes

were monitored for the detection of ochratoxin A and fumonisin B2 as described elsewhere [2, 3]. In order to identify genotypic changes, DNA fingerprinting techniques using the oligonucleotides M13 and $(GACA)_4$ were performed. All assays were evaluated at 3 points in time: before preservation (I), 2 (II) and 4 (III) weeks after preservation.

For all the methodologies used to evaluate freeze-drying of fungi along time the major results are: 1) no significant changes were observed in the macro and micro-morphological analysis; 2) all strains maintained their mycotoxins production pattern, before and after ageing; 3) after ageing different DNA fingerprinting was observed.

In conclusion, freeze-drying can be considered a technique of excellence to be used on the maintenance of biodiversity within the filamentous fungi, and more accurately for *Aspergillus* section *Nigri*. However, it is recommended to consider possible genetic changes after long shelf-life periods.

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SCIENCE AND PRODUCTION POSSIBILITIES OF STATE COLLECTION OF PHYTOPATHOGENIC MICROORGANISMS FOR DIFFERENT TASKS AND DIRECTIONS OF FOREST PHYTOPATHOLOGY

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The All-Russian Scientific Research Institute of Phytopathology has one of the largest specialized storage of phytopathogenic microorganisms and varieties-identifiers (differentiators) of pathogenic strains of microorganisms in Russia. The collection of pathogenic microorganisms has existed since 1960 and reflects the main areas of research carried out at the institute in plant pathology and plant protection of economically significant crops.

The collection has been defined as “The State Collection of pathogenic micro-organisms and plant varieties identifiers pathogenic strains of microorganisms” at the All-Russian Research Institute of Phytopathology and it defined the status of the State Depository with the function of the deposit of microorganisms for agricultural purposes (Government Decree of the Russian Federation dated June 24 1996 No. 725–47s).

The Center for Collective Use "The State Collection of Plant Pathogenic Microorganisms, Indicator Plants and Differential Cultivars at All-Russian Research Institute of Phytopathology" (SCPPM ARRIP) was created on the basis of a collection for the development of fundamental and applied research in the plant growing, ecology and rational nature management, as well as solving problems in related fields of science (selection, genetics, medicine, biotechnology, etc.) with the involvement of other scientific organizations, representatives of the agro-industrial complex and active business (The order of the Director of All-Russian Research Institute of Phytopathology dated 20 October, 2015 No. 88/1 (*http://ckp-rf.ru/ckp/434813/*).

Years of experience in collecting (monitoring), storing strains and depositing crops have been accumulated now, which became possible due to the use of unique high-tech scientific equipment and biomaterial included in the collection (*http://vniif.ru/vniif/structure/collection/*).

The depositary is replenished with strains of microorganisms and pathogens of the most dangerous diseases of economically important ornamental and medicinal plants since 2015. Sampling, replenishment and guarantee storage of collection strains of microorganisms is carried out when examining urban and garden green plantations and planting materials of domestic and imported origin; inclusion in the catalog of collection samples of phytopathogenic microorganisms with a characteristic of their diagnostic features; improvement of methods for isolating, identifying, multiplying, storing and genotyping phytopathogenic microorganisms; the development of pathogens and the provision of biomaterials of scientific research institutions and research and production centers.

The work on the creation of genetically improved forms of woody plants for a specified purpose, the search for new promising biological means of protecting ornamental, horticultural and medicinal plants from diseases are of great interest in recent years. Therefore, the new sector in the SCPPM ARRIP, on the basis of the Department of Pathology of Ornamental and Garden Crops, conducts research on improving the methods for isolating, identifying, multiplying, storing and genotyping phytopathogenic microorganisms and pathogens of woody and herbaceous ornamental crops.

The demand for such works is confirmed by the high active growth in the production of fruit and ornamental crops from the stage of clonal micropropagation to the nursery, industrial garden and green plantations, as well as the need to develop new ecologized plant protection technologies, including from the standpoint of biodiversity and environmental safety.

Part of the work are performed using equipment of the State Collection of Plant Pathogenic Microorganisms, Indicator Plants and Differential Cultivars at All-Russian Research Institute of Phytopathology (SCPPM ARRP).

COLLECTION MICROORGANISMS OF WINEMAKING "MAGARACH"

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The collection microorganisms of winemaking "Magarach" (CMW "Magarach") functions on the grounds of microbiology department of the Federal State Budget Scientific Institution All-Russian National Research Institute of Viticulture and Winemaking "Magarach" of the Russian Academy of Sciences and is the oldest collection in the post-Soviet space that preserves a unique

gene pool of industrially valuable strains of the wine yeast used in production of all types of wines, including sparkle and sherry wines.

Currently, the collection fund is represented by 895 strains of the yeast microflora (1022 storage units). The collection has 720 yeast strains of the genus *Saccharomyces*, 40 yeast strains of the genus *Schizosaccharomyces* and 135 yeast strains of non-*Saccharomyces* – pests of winemaking. Most strains of the collection fund were isolated from natural sources, such as: wine must at the stage of fermentation, winemaking materials, grape and fruit wines and grapes.

Establishment of the collection began in the late 19th century. It started as the collection of pure cultures at the premises of eno-chemical laboratory at Magarach of the Nikitsky Botanical Garden (Crimea, Yalta), and since 1910 almost all the wines of the experimental wine cellar "Magarach" were made using yeast strains from the collection. Since 1933, the work to maintain and replenish the collection fund has been conducted by the staff of the Microbiology Department of the Research Institute of Viticulture and Winemaking "Magarach". Many cultures are the result of the selection work conducted by famous domestic microbiologists-viniculturalists (Gerasimov M. N., Sayenko N. F., Odintsova E., Kvasnikov E. I., Burian N. I., Tyurina L. V., Kishkovskaya S. A., Skorikova T. K., etc.) and are actually still used in winemaking. The collection is composed of the yeast strains isolated from all the wine-growing regions of the former USSR, as well as of strains obtained from collections abroad.

The activities of the CWM "Magarach" are aimed at gene pool preservation, replenishment and rational use of the biological diversity of microorganisms that are of scientific and practical interest for winemaking. The study of the physiological, biochemical and technological properties of cultures is aimed at selection work, while the use of efficient strains helps develop technologies and new wine brands.

The main objective of the collection is to preserve cultures with characteristic properties in the viable state and provide enterprises of the industry with pure cultures. Another significant focus area of the Collection activity is to organize scientific and practical seminars for microbiologists of the wineries and implement author's supervision over selection cultures being introduced.

Since 2016, the Collection has been receiving support within the framework of the Federal Agency for Scientific Organizations of the Russian Federation interventions to develop bio-resource collections.

THE INEP CULTURE COLLECTION OF ALGAE AND CYANOBACTERIA

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Culture collection of algae and cyanobacteria was established in 2011 at the Institute of North Industrial Ecology Problems (INEP) in Apatity. Our collection is a part of the Herbarium of the Institute. In 2012, it was registered in the international system Index Herbariorum with the acronym INEP *http://sweetgum.nybg.org/science/ih/herbarium_details.php?irn=173572*. In 2017, the Herbarium was registered as a unique scientific installation on the site "Modern Research Infrastructure of the Russian Federation" (registration number 498838) *http://www.ckp-rf.ru/usu/498838/*.

The collection includes strains isolated from various ecosystems of the Arctic region (the Kola Peninsula and Rybachy peninsula). The strains of algae and cyanobacteria isolated in algologically pure cultures from soils samples selected from the Pasvik Nature Reserve and the Rybachy Peninsula (Al-Fe-humus podzols, podburs, dry-peaty, peats low moor, cryogenic, undeveloped soils), as well as from anthropogenic impact zones where the main pollutants are heavy metals (the emissions from "Severonikel" and "Pechenganikel" copper-nickel plants), fluorine compounds (Kandalaksha aluminum plant), oil products (Kaskama mountain) and from anthropogenically altered environments (nepheline sands, quarry waters, etc.).

At present, more than 200 strains of microscopic algae and cyanobacteria are maintained in collection. Eukaryotes are mainly represented by strains of *Chlorophyta* (*Chlorophyceae*, *Trebouxiophyceae*, *Ulvophyceae*), *Streptophyta* and *Ochrophyta* (*Xanthophyceae*, *Eustigmatophyceae*). Cyanobacteria cover about 20% of the total spectrum of strains. Currently, the identification of approximately 15 of strains was verified by means of molecular methods.

The main function of the collection is to collect, maintain and supply cultures of microscopic algae and cyanobacteria important for basic and applied research. Maintained algal isolates are broadly used for scientific and education purposes.

PROKARYOTES FROM ARCTIC PERMAFROST: THE WAY TO PUBLIC COLLECTION

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To date, both cultivation methods and culturally independent methods have shown that permafrost is a habitat for microorganisms of *Bacteria*, *Archaea* and *Eukarya* [9]. Their representatives not only survive in cold but can show significant growth and metabolic activity (respiration and biosynthesis) at temperatures down to –20°C, and even at –39°C [2]. The number of publications describing new taxa isolated from the Polar Regions has been growing steadily and increased approximately eight times between 1995 and 2015. Psychrophilic and psychrotolerant microorganisms and their unique proteins have a host of biotechnological applications [3].These include cold-water detergents, food additives and flavor modifying agents, biosensors, and environmental bioremediation. Cold-active lipases catalyze the hydrolysis of fats and remove fatty acids stains from tissues at low temperatures (for example, [5, 6, 7]. Recent studies have focused on the discovery of new cold-active proteases in psychrophilic microorganisms derived from the Arctic and Antarctic ecosystems [1, 4].

Maintaining strains in a viable state, preserving their valuable properties are important conditions for almost any work with microorganisms – from primary research to using them in the production of various bioactive compounds. This can be done only in the collections of microorganisms responsible for the safety and viability of the strains accepted for storage. A survey of researchers studying the prokaryotic diversity of the Arctic showed that no more than 25% of the initially isolated strains reach storage sites of bioresource centers and collections of microorganisms. These statistics are due to: (1) difficulties in selecting of medium components for the best viability

of psychrophilic and psychroactive prokaryotes; (2) the turnover of scientific personnel in research groups, which leads to the loss of working collections of microorganisms.

The solution to the situation would be the close cooperation of the staff of collections and researchers in the early stages of the investigation, which would combine the knowledge and experience of the two teams to solve different problems. An example of such cooperation is the interaction of the VKM and the laboratory of soil cryology from the Institute of Physicochemical and Biological Problems in Soil Science RAS, which allowed depositing in the VKM found more than 80 bacterial and archaeal strains isolated from permanently frozen grounds. Another way of solution can be the conservation of the permafrost samples from which Arctic prokaryotes were isolated. For now laboratory of soil cryology holding the biggest and unique samples collection which contains more than four thousands of modern frozen soil and ancient permafrost sediments samples from Kolyma lowland (Russian Arctic region) and McKenzie River delta (Canadian Arctic). Each sample in the collection provided with a following information: geographic location of sampling site, the granulometric composition, ice content, redox potential, organic content, and chemical characteristics of water extracts from the samples.

Nature provides a vast source of biocatalysts. However, the probability of finding the right enzymatic activity for a particular application depends on the available technical capabilities to efficiently assess this large biodiversity. This capability is mainly mediated by technologies, such as metagenomic screenings, genome mining, and direct enzymatic exploration [8]. Metagenomic screenings and genome mining require that the search for a novel enzyme is based on genetic sequence homology to already described enzymes. But the discovery of new enzymes in this way does not always give accurate information, especially for less studied organisms like psychrophiles from Arctic permafrost.

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FEDERAL CRYOBANK OF NATURAL SYMBIOTIC MICROBIOCENOSES OF HUMAN, ANIMALS, PLANTS, SOILS AND OPEN RESERVOIRS OF THE RUSSIAN FEDERATION

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In nature, there is neither a single biochemical process, nor a single function, or a behavioral response that would take place without the direct or indirect involvement of microorganisms.

Microorganisms of humans, animals, insects, plants, soil and open reservoirs are the crucial link in the emergence and evolution of the biosphere on our planet. There are many functions of microorganisms. They control the stability and modification of the genome and epigenome of all living organisms and their preservation in specific habitat conditions, health, the risk of infectious and chronic metabolic diseases. Moreover, microorganisms are involved in regulation of soil, hydrochemical and hydrobiological regimes.

Anthropogenic environmental impact, particularly in the last 50–100 years, leads to global contamination of the environment with industrial, domestic waste, drugs, hormones and other xenobiotics, which contributes to the development of profound disturbances in microbiocenoses of animals, plants, soils and reservoirs.

Finally, widespread deterioration of microbial ecology, can call into question not only of the human preservation on our planet, but the entire diversity of living organisms. Creation of the Federal "Cryogenic Bank" of natural symbiotic microbiocenoses of the Russian Federation will allow us initially to preserve evolutionally developed microbiocenoses in an unchanged viable form for a long time at low temperatures.

Cryopreservation of microbiocenoses allows to use them not only for a variety of scientific, fundamental and applied research, but also to perform the implementation of high-tech critical methods and techniques in medicine, livestock, crop production and ecology.

Microorganisms and microbiocenoses stored in cryobanks will be applied for the creation of various medicines, biologically active additives, probiotics, functional food products, microbial fertilizers, and other biotechnological products, agricultural, veterinary, medical and other purposes at different scales.

Development of biobanking is a priority direction for Russia. Thus, the funding of the Federal Cryogenic Bank creation is up to 50 million US dollars. The structure of the cryogenic bank and a list of necessary equipment have been established and the commissioning, according to the program, will be carried out approximately in two years from the beginning of financing.

ECOPHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF CYANOBACTERIAL AND MICROALGAL STRAINS FROM THE IPPAS CULTURE COLLECTION

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We have screened 20 strains of microalgae and cyanobacteria from collection IPPAS of Institute of plant physiology RAS and studied their growth characteristics, pigment content, protein, carbohydrate and lipid content in storage conditions and during intensive growth in exponential and stationary phases.

From those screened strains five were chosen as containing high amount of valuable compounds and perspective for biotechnology.

IPPAS B-1200 *Cyanobacterium* sp. Cells of this strain have mostly saturated and monounsaturated fatty acids (FA) with short chains. Among them the total amount of C14 fatty acids reaches 40 %, which is unusually high for cyanobacteria, and it has never been reported before. Such a fatty acid composition, together with a relatively high speed of growth, makes this strain a prospective candidate for biodiesel production. Important advantage of this strain is its ability to grow efficiently in a wide range of temperatures.

We described one more strain, IPPAS B-1201, which is similarly to strain IPPAS B-1200 characterized by high content of short chain fatty acids 14C and a very low unsaturation index of total lipids. Thus, IPPAS B-1201 is also a promising strain for biofuel production. According to morphology and 16S rRNA and ITS region sequences, this strain belongs to the same genus as the previously described IPPAS B-1200 strain, but to a different species, *Cyanobacterium apponinum*. Unlike the IPPAS B-1200 strain, which was isolated from the salt lake Balkhash in October, when the water temperature was 15–17°C, strain IPPAS B-1201 was isolated from the freshwater hot spring Turgen.

IPPAS H-242 *Eustigmatos* sp. This strain is lipid-rich (FA content is up to 16 % of dry weight). Cells of strain IPPAS H-242 had high lipid content in all studied conditions, also they had high carotenoid: chlorophyll ratio. Their lipids contained max 17,5 % of EPA which is valuble longchain ω-3-polyunsaturated fatty acid. Thus strain H-242 is perspective for EPA and carotenoids production and is comparable with most effective producers of EPA. Also the strains had relatively high growth rates (doubling time 20–22 h).

IPPAS C-1210 *Chlorella* sp. We found that strain of green microalga IPPAS C-1210 had store big amount of TAGs in lipid droplets (FA content is up to 15 % of dry weight). This strain was able to grow with high rate having doubling time 8,5–10,5 h.

IPPAS B-1220 *Desertifilum* sp. A new cyanobacterial strain from fresh-water Lake Shar-Nuur (Mongolia) was isolated and characterized by polyphasic approach. According to the 16S ribosomal RNA gene sequence this strain belongs to newly described genus *Desertifilum*. Despite its wide distribution, the genus remains fairly stable genetically as seen from the analysis of 16S rRNA gene and 16S–23S ITS region sequences of strains from distant habitats. The isolated strain is characterized by unusual fatty acid composition (16:1Δ7 and 16:2Δ7,10). The presence of hexadecadienoic FAs have been previously reported in a number of cyanobacterial strains however, this was the first report on the precise identification of the positions of double bonds in this FA. Important advantage of this strain is its ability to grow efficiently in a wide range of temperatures 28–40°C. Analysis of *Desertifilum* sp. IPPAS B-1220 draft genomic sequence reveals the presence of six genes for the acyl-lipid desaturases: two 9-desaturases, *desC1* and *desC2*; two 12-desaturases, *desA1* and *desA2*; one desaturase of unknown specificity, *desX*; and one gene for the bacillary-type desaturase, *desG*, which supposedly encodes an ω9-desaturase. A scheme for a fatty acid desaturation pathway that describes the biosynthesis of 16:1Δ7 and 16:2 Δ 7,10 fatty acids in *Desertifilum* is proposed. The analysis of the draft genomic sequence reveals the presence of, at least, 11 gene clusters that encode non-ribosomal peptide synthases, polyketide synthases, bacteriocins, lantipeptides, etc.

In addition to *Desertifilum* sp. IPPAS B-1220, we described another strain of unicellular cyanobacterium, IPPAS B-1203, which also has a significant amount of 16:2Δ7,10 fatty acid. According to morphological and molecular-genetic characteristics, this strain was identified as *Gloeocapsopsis* sp. In both strains s decrease in ambient temperature caused a decrease in the proportion of saturated FAs in favor of unsaturated FAs. The most noticeable changes were observed in the amounts of 16:0 and 16:2Δ7,10. In contrast to "model strain" *Synechocystis* sp. PCC 6803 which desaturate FAs at positions Δ12 and/or Δ15 while exposed to cold, *Desertifilum* and *Gloeocapsopsis* desaturate FAs at positions Δ7 and Δ10 suggesting that Δ7 and Δ10 desaturase are responsible for adaptation to cold stress in these strains.

We developed methods of intensive growth with high biomass productivity and high viability of cells. For this purpose we optimized growth conditions for each strain (optimal medium and growth temperature were found). We found conditions under which our strains accumulate their valuable compounds most intensively.

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INTERACTION OF FLUORESCENT LABELED BACTERIOPHAGES PHIKZ *PSEUDOMONAS AERUGINOSA* WITH HUMAN ORAL EPITHELIAL CELLS FROM ORAL CAVITY SCRAPING

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Bacteriophages are viruses that can only infect and kill bacteria; they cannot infect eukariotic cells [1]. On the same time they can penetrate cultivated cells of epithelial origin and are transported through epithelial cell layers thus being spread into different organs and tissues [1]. Bacteriophages that penetrated mammary epithelial cells maintain their ability to kill intracellular *Staphylococcus* aureus [2]. Phages transferred into murine phagocytes could significantly reduce the damage caused by cytotoxic effects of intracellular bacteria [3]. In this work we show that fluorescent labeled bacteriophages phiKZ *Pseudomonas* aeruginosa can penetrate human epithelial cells from oral cavity scraping. Possible directions of further investigations are discussed.

P. aeruginosa and bacteriophages phiKZ were deposited in "Scientific-and-production Centre Micro-World" LLC *http://micro-world.ru/* (Russian Federation). Fluorescent labeled bacteriophages phiKZ P. aeruginosa were produced as described [4].

Human epithelial cells were produced by sterile scraping of oral mucous membrane immediately before the experimental procedure and were never stored. About 500 µl of the sample containing living and destroyed epithelial cells, saliva and mucous secretion were suspended in 2 ml of sterile phosphate buffered solution (PBS) and centrifugated by 1000 g during 5 min. The precipitate was resuspended in 2 ml of sterile cell culture medium DMEM containing 10% of fetal bovine serum. 500 µl of the produced cell suspension were introduced into each well of 4-welled chamber slide (Nunc Lab-Tek). 50 µl of PBS were further introduced into each control well, 50 µl of bacteriophage suspension were introduced into each experimental well. Then the chamber slides were incubated by 37ºC, 5% CO_2 during 40 min. Further cell culture medium was discarded from each well, the wells were accurately washed by 500 µl of PBS. After that chamber slides were covered by cover slips, the cells were microscoped in transmitted light and by fluorescence (excitation 350 nm, emission 460 nm), by magnification x400 (AxioImager D1, Zeiss, Germany).

Adhered epithelial cells, conglomerates of destroyed cells, groups of several bacteria were to be seen in each well in transmitted light. No fluorescent objects were observed in control wells. Weekly fluorescent bacteria and adhered epithelial cells with brightly fluorescent nuclei were observed in experimental wells containing bacteriophages. This leads to the conclusion that bacteriophages interact not only with bacterial hosts but also penetrate epithelial cells and are transported into their nuclei. Our data support earlier results published by other authors which give evidences that phages can be transported into eukaryotic cells. These phenomena are to be studied more carefully. The most interesting questions are: 1) can bacteriophages interact with apoptotic and quiescent cells; 2) what are the mechanisms of bacteriophagal transport and in what way do they differ for the cells of different origin; 3) how does the eukaryotic cell react upon the bacteriophage penetration (adhesive properties, expression of physiologically active substances, signaling pathways activation, shift of differentiation, mutagenesis etc.); 4) can bacteriophages exhibit cytotoxic effects.

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DOXORUBICIN UPTAKE BY CULTURED MCF-7 AND NIH3T3 CELLS IS INCREASED IN PRESENCE OF RECOMBINANT HUMAN ALPHA-FETOPROTEIN

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Human mammary adenocarcinoma MCF-7 cell line and murine embryonic fibroblast NIH3T3 cell line are widely used as *in vitro* models to study cancer signaling and biochemical effects of anti-cancer drugs, and to find out possibilities to enhance anti-tumor cytotoxic action [1]. Doxorubicin is an anti-tumor drug which intercalates DNA similar to daunorubicin. It is prescribed to treat leukemia of different origin, sarcomas, mammary cancer, stomach cancer, neuroblastoma etc. [2]. Recombinant human alpha-fetoprotein is a recombinant analogue of embryonic protein which plays an important role in correct fetus phenotype formation and is expressed by various tumors in adults [3]. Cytotoxic effects of intercalating anti-tumor drugs are dependent upon their uptake by tumor cells. Fluorescence properties of doxorubicin being similar to those of rhodamin [4] allow studying doxorubicin uptake by cultured cells *in vitro* using fluorescent microscopy. This work shows that doxorubicin uptake by MCF-7 and NIH3T3 cells is increased in presence of recombinant human alpha-fetoprotein. Possible directions of further investigations and applications of this phenomenon are discussed.

MCF-7 and NIH3T3 cell lines were purchased in Russian Cell Culture Collection *http://www.rccc.cytspb.rssi.ru/ecellbank/index.html* (Russian Federation). Both MCF-7 and NIH3T3 cells were cultured at 37ºC, 5% CO_2 in DMEM cell culture medium supplemented with 10% fetal calf serum (FCS). MCF-7 cells were subcultured once per week by using 0.25% trypsin – 0.53 mM EDTA solution, splitting ratio 1:3. NIH3T3 cells were subcultured every 3–4 days by using 0.25% trypsin– 0.53 mM EDTA solution, splitting ratio 1:6. Growth medium was renewed twice per week, as recommended by the manufacturer. Cells were stored by cryopreservation, $1x10^6$ cells/ml in 1-ml vial, freeze medium containing complete growth medium supplemented with 10% DMSO. Doxorubicin was purchased in Sigma Aldrich (USA). The production of recombinant human alpha-fetoprotein was described previously [5].

Cells were seeded on 4-welled Nunc Lab-Tek chamber slides (Sigma Aldrich, USA) and cultured in DMEM containing 10% FCS. By 40% confluence, doxorubicin (final concentration 5 ng/ml) was introduced into fresh cell culture medium. Recombinant human alpha-fetoprotein (final concentration 100 mkg/ml) was added into experimental wells. Control wells were left without alpha-fetoprotein. After 20 min of incubation (37ºC, 5% CO_2) culture medium was removed, cells were washed with sterile phosphate buffered saline and fixed in 96% ethanol (–20ºC, 10 min). After that chamber slides were covered by cover slips, and the cells were microscoped using fluorescent microscope AxioImager D1 (Zeiss, USA), with excitation 550 nm, emission 605 nm, and magnification x400.

Microphotographs of both cell lines demonstrate that nuclei of cells that were incubated with doxorubicin plus alpha-fetoprotein are stained more brightly than control cells incubated with doxorubicin alone. It leads to the conclusion that doxorubicin is transported more actively into the nuclei if alpha-fetoprotein is present in culture medium of both cell lines. Unstained regions within nuclei may possibly correspond to nucleoli but this assumption needs to be proved by additional experiments. MCF-7 and NIH3T3 cells as well as cells of vast majority of other immortalized cell lines demonstrate properties typical for undifferentiated cells. Thus one can suggest that recombinant alpha-fetoprotein binds *in vitro* to undifferentiated MCF-7 and NIH3T3 cells and transports doxorubicin into their nuclei. Alfa-fetoprotein is shown to interact *in vivo* with low-differentiated tumor tissues and not to affect normally differentiated tissues [6]. Both MCF-7 and NIH3T3 are immortalized cell lines which normally possess characteristics of low-differentiated cells. From this standpoint, it is no wonder that alpha-fetoprotein interacts with them. Thus it is necessary to establish adequate cell models *in vitro* where cells with different status of differentiation are compared in their ability to bind alpha-fetoprotein and to uptake anti-tumor drugs.

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TAXONOMY OF THE GENUS *CLAVIBACTER*

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The genus *Clavibacter* Davis *et al.* 1984 (family *Microbacteriaceae*, class *Actinobacteria*) comprises coryneform bacteria with the B2γ-type cell wall peptidoglycan based on 2,4-diaminobutyric acid and the major respiratory quinone MK-9. As a result of re-classification [5], the genus currently includes six species (*C. michiganensis*, *C. capsici*, *C. insidiosus*, *C. nebraskensis*, *C. sepedonicus* and *C. tessellarius*) and four subspecies (*C. michiganensis* subsp. *michiganensis*, *C. michiganensis* subsp. *californiensis*, *C. michiganensis* subsp. *chilensis*, and *C. michiganensis* subsp. *phaseoli*). The 16S rRNA gene sequence similarities between the type strains of the above taxa are very high, from 99.46 % for the pair *C. nebraskensis – C. sepedonicus* to 100 % for the *C. michiganensis* subspecies *(californiensis*, *chilensis*, and *phaseoli)*.

Except for *C. michiganensis* subsp. *californiensis* and *C. michiganensis* subsp. *chilensis*, the species and subspecies of this genus are known as plant pathogens attacking important agricultural crops. They cause bacterial cancer of tomato and pepper (*C. michiganensis* subsp. *michiganensis* and *C. capsici*), ring rot disease in potato (*C. sepedonicus*), leaf freckles and leaf spots in wheat (*C. tessellarius*), wilt and blight disease in corn (*C. nebraskensis*), wilting and stunting of alfalfa (*C. insidiosus*), and leaf yellowing in bean (*C. michiganensis* subsp. *phaseoli*) [1, 2, 4, 5, 8]. Some species/subspecies are quarantine or regulated plant pathogens in many countries.

Here we report the results of taxonomic study performed on 35 *Clavibacter* strains preserved in the All-Russian Collection of Microorganisms (VKM). Along with representatives of the taxa with validly published names, the working collection contained isolates from wild plants without any symptoms of diseases, including *Ammodendron* sp., *Bromus* sp., *Calligonum* sp., *Ephedra* sp., *Gagea* sp., and *Salsola* sp. collected in the desert Kyzyl-Kum, Uzbekistan, as well as *Festuca* sp., *Carex* sp., and *Galatella punctata* originating from different regions of Russia. The target collection also included strains isolated from the nematode-induced galls on *Agrostis* sp. (Far East, Russia) and *Elymus repens* (Moscow region, Russia). All strains under study showed more than 99 % 16S rRNA gene sequence similarities to the known species of the genus *Clavibacter* and had chemotaxonomic characteristics typical of this genus.

MALDI-TOF mass spectrometry and analysis of DNA gyrase β-subunit gene sequences (*gyr*B) placed the desert isolates and strains from *Festuca* sp., *Carex* sp. and *Galatella punctata* into a few discrete clusters near the species level, while the two strains from nematode galls are more likely to belong to *C. michiganensis* subsp. *michiganensis* and *C. michiganensis* subsp. *californiensis.*

The genomes of *C. michiganensis* subsp. *phaseoli* VKM Ac-2641^T and two isolates from desert plants (VKM Ac-1371 from *Salsola* sp. and VKM Ac-1372 from *Ammodendron* sp.) were sequenced, annotated and compared with representatives of the recognized *Clavibacter* species and subspecies. Strain *C. michiganensis* subsp. *phaseoli* VKM Ac-2641^T shared 90.2–93.2 % average nucleotide identity (ANI) and 42.2–52.8 % digital DDH (dDDH) levels with representatives of the known *Clavibacter* species. The above values were well below the cutoff used to define a bacterial

species [3, 9]. For comparison, the pairwise ANI and dDDH values computed for six recognized *Clavibacter* species with available genomes were within the range of 88.6–94.9 % and 36.4–59.9 %, respectively. The genomic data along with the phenotypic differences reported [2] support the raising of *C. michiganensis* subsp. *phaseoli* to the species rank (*C. phaseoli*).

The ANI (89.6 %) and dDDH (41.9 %) values between VKM Ac-1371 and VKM Ac-1372 and the values obtained for these strains towards the type strains of the recognized *Clavibacter* species (ANI, 89.4–90.4 % and 87.9–88.6 %; dDDH, 41.1–43.5 % and 36–41.9 %, respectively) were also below the accepted species threshold. The strains also differed from the validly described species at the phenotypic level. Unlike the recognized *Clavibacter* species and subspecies, these strains produce a pink-orange pigment and differ markedly by the cell wall glycopolymers, the MALDI-TOF mass-spectra and a number of physiological and biochemical features [6, 7]. All the above-stated evidence indicate that strains VKM Ac-1371 and VKM Ac-1372 represent two novel species.

Thus, the results of our taxonomic study indicate that at least four novel non-pathogenic species should be added to the list of *Clavibacter* species, and the subspecies *C. michiganensis* subsp. *phaseoli* have to be elevated to the species rank (*C. phaseoli*).

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TAXONOMY OF THE GENUS *RATHAYIBACTER*

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Actinobacteria of the genus *Rathayibacter* Zgurskaya *et al*. 1993 (family *Microbacteriaceae*) are characterized by 2,4-diaminobutyric acid (L-isomer) in the cell-wall peptidoglycan and predominant menaquinone MK-10 [3, 9]. Until 2018, the genus included six species with validly published names: *R. rathayi*, *R. iranicus*, *R. tritici*, *R. toxicus*, *R. caricis* and *R. festucae*. More recently, another new species, *R. agropyri*, has been described [5]. The species *R. rathayi*, *R. iranicus*, *R. tritici*, *R. toxicus* and *R. agropyri* are plant pathogens causing gumming disease of wheat and cereal grasses (Poaceae) and are transmitted to their host plants by seed gall nematodes of the genus *Anguina* [3, 4]. *R. toxicus* is also responsible for toxicity of ryegrass (*Lolium rigidum* Gaudin) and some other grasses, which often results in fatal poisoning of grazing animals in Australia and some other countries [4]. The type strain of *R. festucae* was isolated from *Festuca rubra* infected by *Anguina graminis* and the type strain of *R. caricis* was recovered from *Carex* sp. (Cyperaceae) without any symptoms of bacterial diseases or nematode infestation [1].

Here we provide the results of comparative study of *Rathayibacter* strains preserved in the All-Russian Collection of Microorganisms (VKM). The set of 26 strains used in this study contained members of six recognized species (described before 2018) and several novel strains, including those isolated from the nematode galls on *Acroptilon repens* L. (Asteraceae) and from necrotic lesion on *Tanacetum vulgare* L. (*Asteraceae*), as well as from plants without any symptoms of bacterial and nematode infestation, such as *Pedicularis kaufmannii* Pinzger (*Orobanchaceae*) and *Androsace koso-poljanskii* Ovcz. (*Primulaceae*). The generic affiliation of strains was confirmed by the 16S rRNA gene sequence analysis. The strains were preliminary assigned to the recognized and three novel species (*R. oskolensis*, "*R. tanaceti"* and "*R. acroptilonus"*) on the basis of comparative study MALDI-TOF mass spectra and housekeeping genes.

The complete genomes of *R. rathayi* VKM Ac-1601^T (the type strain of the type species of *Rathayibacter*), *R. iranicus* VKM Ac-1602^T and representatives of the recently proposed or revealed new species, *R. oskolensis* VKM Ac-2121^T, "*R. tanaceti"* VKM Ac-2596 and "*R. acroptilonus"* VKM Ac-2630, were sequenced and annotated [6, 8]. The comparative analysis was performed with genomes of 17 *Rathayibacter* strains which included the above and other strains whose genome sequences were available from data bases. The calculated genome relatedness indices, sush as average nucleotide identity (ANI), tetranucleotide signature frequency correlation coefficient (TETRA) and digital DNA-DNA hybridization (dDDH), provided clear evidence in support of separate species status of strains VKM Ac-2121^T, VKM Ac-2596 and VKM Ac-2630.

The revealed physiological and chemotaxonomic characteristics, including cell-wall sugars, cell-wall glycopolymers and polar lipid profiles, allowed clear phenotypic differentiation of strains VKM Ac-2121^{T}, VKM Ac-2596 and VKM Ac-2630 from each other and from the recognized *Rathayibacter* species [2; 7]. Based on the data obtained, a novel species, *R. oskolensis* have been described [2], while strains VKM Ac-2596 from *Tanacetum vulgare* L. and VKM Ac-2630 from *Acroptilon repens* L. are still waiting for their descriptions as two novel species.

Our work also resulted in creating the reference MALDI mass-spectral database for the genus *Rathayibacter*, which allows fast and reliable identification of strains comprising the validly described and putative new species. The genus-specific (3954, 4428 и 6458 m/z) and several species-specific components of mass-spectra were detected which can serve as chemotaxonomic markers of the genus *Rathayibacter* and its species. And finally, the borderline similarity values for housekeeping genes were proposed that allows one to distinguish strains of the recognized and novel species of this genus: gyrB – 94 %, recA – 95 %, rpoB – 97 % and ppk – 95.5 %.

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THE ISOLATION AND DIFFERENTIATION OF PROBIOTIC STRAINS OF LACTIC ACID BACTERIA FROM NATIONAL FERMENTED MILK PRODUCTS

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Lactic acid bacteria (LAB) have been used since time immemorial by people, in particular in some ancient communities, over time, each nation has its own national lactic acid products used not only as food products, but also as therapeutic, preventive and curative agents for the treatment of the cardiovascular system, respiratory organs, gastrointestinal infections, etc. [1, 2]. The LAB isolated from the national lactic acid products draw a special interest among the probiotic correctors of normal intestinal microbiota plays an important role in human ecology. LAB are cultures of strategic importance to the nation's health.

The basic property of LAB, by which they are combined into a separate broad group of microorganisms, is the ability to form lactic acid as the main product of fermentation. Lactic fermentation is carried out by bacterial organisms heterogeneous in morphology, physiological and biochemical properties. Conditions and habitats contribute to the variability of the morphological and cultural properties of these bacteria, which is the cause of greater difficulty in their differentiation and identification. The advantage of the method of natural selection is that nature itself tests the properties of microorganisms. Sometimes even in nature stand out the most adapted form from existing culture conditions. The basis of LAB group are the genus of *Lactococcus* and *Lactobacillus*. The genus *Lactococcus* of serological group was isolated from the genus *Streptococcus*, which includes pathogenic forms, and under a new name *Lactococcus* is classified in the "GRAS" status (absolutely harmless for human health and animals) [3].

The subjects of the study were the microbiota of national home-made fermented products brought from Lebanon (Beirut) – Laban, Leben, but from Buryatia (Ulan-Ude) and Iran (Tegeran) – Kurunga and Doogh (respectively), products of mixed lactic acid and alcohol fermentation.

Isolation of LAB was carried out in stages using elective media. The best environment for the cultivation of LAB is the skim milk. In test tubes, the product under study was introduced for self-fermentation at different temperatures (30°, 37°, 42°C) under steady-state conditions. Then,

the original test tubes were selected, in which a different density of the milk clot formed, which is characteristic of lactococci or lactobacilli. Then, they sowed on the agar medium to obtain unit colonies. The number of acid-forming LAB was recorded by adding of bromocresol magenta indicator to the medium of incubation, forming a clearing zone around the colonies on the agar medium with deep sowing of lactobacilli due to the formation of lactic acid [4].

The main requirements for probiotic cultures are their inhibitory activity against opportunistic and pathogenic bacteria and health benefits [1, 2, 4]. For this, using the replica method, colonies of isolated pure cultures were tested for antimicrobial activity on the main representatives of various taxonomic groups of bacteria that caused infections.

Mesophilic lactococci were grown on agar media for 3 to 4 days, separated from other LAB by colonies on a Petri dish and by microscopy of preparations. To differentiate *L. lactis* subsp. *lactis* from *L. lactis* subsp. *cremoris* and from *L. lactis* subsp. *lactis diacetilactis* took into account the growth pattern on dense media with milk hydrolyzate: *L. lactis* subsp. *cremoris* form dark round colonies on the surface of the medium, and *L. lactis* subsp. *lactis diacetylactis* – deep colonies of irregular shape in the form of pieces of cotton wool. The cultural features were assessed using a list of cultural characteristics for the identification of bacteria [4]. But strains K-205 and IR, isolated from Kurunga and Doogh (respectively) adapted to an alcohol substratum, could ferment sugar alcohols, including mannitol, which is not characteristic for *L. lactis* subsp. *lactis*, but is a differentiating feature for *L. lactis* subsp. *cremoris*. Sixty-eight cultures of LAB were isolated: 18 and 15 acid-forming lactococci clones and mesophilic lactobacilli were isolated from drinks Kurunga and Doogh, of which 3 lactococci strains with high antimicrobial activity up 2900–3100 IU / ml as compared with nisin ("Nisaplin", Aplin and Barrett, LTD). It should be noted that lactococci had a high level of inhibitory activity, effectively inhibited the growth of both Gram-positive and Gram-negative bacteria: *Proteus vulgaris*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* also showed a fungicidal effect. Inhibitory activity against molds and yeasts is a little-known biological property of lactic acid bacteria of the genus *Lactococcus.*

The most active colonies lactobacilli, with a wide spectrum of action, were shown on the agar medium of MRS, which is most often used for the cultivation of lactic acid bacteria. Up to 50 % of colonies of lactic acid bacteria isolated from the microbiota of the Lebanese dairy products Laban and Leben, inhibiting the growth of gram-positive and gram-negative bacteria and up to 30 % possessing fungicidal activity.

The taxonomic position of the isolated cultures by classical microbiological methods for the identification of bacteriocin-forming strains of lactococci was confirmed by a genotypic method based on the analysis of the similarity of the nucleotide sequences of the 16S rRNA gene. The cultures of the genus *Lactobacillus* from Kurunga were identified as *L. paracasei* and *L. rhamnosus* related to the *L. casei* group and as *L. brevis*, *L. buchneri*, *L. diolivorans*, and *L. parabuchneri* (the *L. buchneri* group) using the classical microbiological methods and on the basis of the 16S rRNA sequence analysis. The polymorphism of the nucleotide sequences of the genes *groEL*, *rpoB*, and *rplB* encoding specific proteins was studied for intraspecific differentiation of the lactobacilli. To confirm the belonging of two strains 9 and 2.5 isolated from Laban and Leben to the species *Lactobacillus paracsei* and *Lactobacillus delbrueckii* ssp. *bulgaricus* also used a molecular-biological method of identification. The gene nucleotide sequences of all the genotyped strains of most perspective LAB were deposited in the GenBank database.

The purpose of further research is to study the probiotic properties of these new strains. Compared to other microorganisms used in the manufacture of fermented milk products, lactococci and lactobacilli are much more active in fermenting the main carbohydrate of milk, lactose. Reducing the concentration of milk sugar in the product is combined with the presence of a high number of living microorganisms that contain their own enzymes that digest lactose. This produces a substitution effect in the intestine for people with lactase deficiency. These strains possessed high proteolytic and antioxidant activities, are resistant to the conditions of the gastrointestinal tract.

It should be noted that the new strains of LAB have replenished the collection of cultures and are of scientific and practical interest in terms of expanding biological diversity, their possible use as probiotics, as well as bacterial starter cultures, for the production of lactic acid products.

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VETERINARY FUNGAL STRAINS IN THE SPOTLIGHT AT BCCM/IHEM

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The BCCM/IHEM is a fungi collection focussing on human and animal health. Although the majority of the 15000 strains is related to human mycology, the collection also has about 1000 strains from veterinary cases, isolated in 40 different countries and covering 200 fungal species. The animals involved are farm animals, pets and zoo animals. In 2017 and 2018 we collaborated with Animal Healthcare Vlaanderen (DGZ), the Zoo Antwerp and the veterinary laboratory Zoolyx to validate the Maldi-TOF MS for identification of veterinary strains. We are currently also involved in a project sampling bats for *Pseudogymnoascus destructans* and for other fungal species that might be present.

MICROBIAL METABOLISM OF NATURAL AND SYNTHETIC PHOSPHONATES

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Phosphorus is an essential element for biosphere. Phosphate ester (C-O-P^{5+}) bond forms the backbone of nucleic acids, is a key constituent of ATP and is utilized in cell response mechanisms. However, there is a class of organophosphorus compounds which exact role in biosphere cycle is yet to be established. These are phosphonates (Pn), which contain direct carbon-phosphorus (or C-P^{3+}) bond in their structure. This bond is chemically inert and resistant to hydrolysis by known phosphatases [3, 5].

Pn were of greater abundance on primitive anoxic Earth and constituted the majority of phosphorus pool. With onset of oxygen environment the percentage of Pn diminished. Since the discovery of biogenic Pn in rumen protozoa, such compounds were regarded as minor constituents of membranes with limited functions (McGrath et al., 2013). Recent studies recognized Pn as a significant component of global phosphorus cycle. In some oligotrophic environments reduced phosphorus compounds represent the majority of bioavailable phosphorus [4, 8]. The other major source of Pn is chemical industry. Synthetic Pn are utilized as pesticides, chelating agents, flame retardants, lubricants etc. Synthetic Pn are generally resistant to physical and chemical decomposition [3, 7].

We took special interest in two Pn. The first was methylphosphonic acid (MPA), the simplest and most stable compound of that kind originating both from natural and anthropogenic sources. Recently, biosynthesis of MPA was demonstrated as an abundant process in certain environments [4, 8]. When in soil, MPA may persist for five decades without significant decrease and exert toxic effects on various plant species. The second compound was *N*-phosphonomethylglycine known by its commercial name “glyphosate” (GP). It is an inhibitor of aromatic compounds biosynthesis in plants, bacteria and fungi. GP isopropylamine salt is the most widespread non-selective herbicide formulation [9]. GP has become an essential part of agriculture and forestry; its importance has been amplified by introduction of transgenic GP-resistant crops [2, 9]. GP environmental impact and its toxicity is highly disputed matter. Initially GP was regarded as safe. Earlier works assumed fast GP decomposition into aminomethylphosphonic acid (AMPA). According to later studies, GP may exert toxic and mutagenic effects on animal cells, while its environmental fate is more complex. GP can accumulate in soils and migrate horizontally reaching water bodies resulting in poor crop productivity, emergence of resistant weeds and food contamination. Its primary metabolite AMPA is even more stable and also has phytotoxic properties. GP contamination is a serious environmental risk which still lacks reliable solution [1, 9].

The bulk of natural and synthetic Pn are metabolized by bacteria and fungi, which degradation capabilities depend on presence of specialized enzymes. Some natural phosphonates are degraded by certain hydrolases with unique substrate specificity. C-P bond of MPA, GP and AMPA may be disrupted by multi-protein complex know as “C-P lyase”. In *Escherichia coli* it is composed of 14 proteins with transport, catalytic and auxiliary functions. In other species C-P lyase structure may vary. The mode of action of C-P lyase catalytic core was established only recently [2, 3].

C-P lyase from *E. coli* is unable to break down GP, though. Recently we were able to demonstrate the existence of two types of C-P lyase in soil bacteria *Achromobacter* sp. MPS 12 isolated form

soils polluted with MPA, one of which disrupted the C-P bond of MPA, while the other was induced only in media with GP and converted this compound in sarcosine and inorganic phosphate [7]. The same pathways were later discovered in *Achromobacter* sp. MPK 7 isolated from GP-contaminated soil [1].

The other way of GP decomposition is by action of glyphosate oxidoreductase with formation of AMPA. In some bacteria the AMPA is then disrupted by C-P lyase, but in most cases this compound is excreted (Hove-Jensen et al., 2014). Recently a novel efficient GP degrader *Ochrobactrum anthropi* GPK 3 was isolated. This strain completely utilized GP and AMPA as sources of phosphorus, the putative pathway involving AMPA transamination and hydrolysis of C-P bond by phosphonatase was proposed [7].

There is evidence that in some bacteria GP could be acylated, decarboxylated, phosphorylated etc., though there are little or no data on corresponding enzymes [2]. For example, we have detected a novel pathway of GP detoxification in soil bacteria *Achromobacter* sp. Kg 16, where a portion of GP was transformed into AMPA, while the bulk of the herbicide was acetylated [6]. Acetyltransferase encoded by gene *phnO* is a part of C-P lyase complex in *E. coli* and is essential for C-P bond disruption in 1-aminoalkylphosphonates, but it cannot use GP as a substrate [2].

It is peculiar that *Achromobacter* sp. Kg 16 was unable to utilize its own product of GP transformation, but AcetylGP was readily degraded by other strains, including *O. anthropi* GPK and *Achromobacter* sp. MPS 12 [1]. This is yet another demonstration of the complexity of pathways of microbial metabolism of Pn in the environment. Further studies of these processes and interactions may lead to better understanding of global phosphorus cycle in the biosphere both with respect to natural and synthetic Pn. Such research is of major practical importance as well, being the base for development of efficient techniques of bioremediation of soils and water bodies contaminated by abundant synthetic Pn, such as GP, AMPA, MPA, phosphonomethyliminodiacetic acid.

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DIVERSITY, NOVELTY AND ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC ACTINOBACTERIA FROM MANGROVE PLANTS IN BEILUN ESTUARY NATIONAL NATURE RESERVE OF GUANGXI, CHINA

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Endophytic actinobacteria are one of the important pharmaceutical resources and well known for producing different types of bioactive substances. Nevertheless, detection of the novelty, diversity and bioactivity on endophytic actinobacteria isolated from mangrove plants are scarce. In this study, five different mangrove plants were collected from Beilun Estuary National Nature Reserve in Guangxi Zhuang Autonomous Region, China, including *Avicennia marina*, *Aegiceras corniculatum*, *Kandelia obovota*, *Bruguiera gymnorrhiza* and *Thespesia populnea*. One-hundred and one endophytic actinobacteria strains were recovered by culture-based approaches. They distributed in 7 orders, 15 families and 28 genera including *Streptomyces*, *Curtobacterium*, *Mycobacterium*, *Micrococcus*, *Brevibacterium*, *Kocuria*, *Nocardioides*, *Kineococcus*, *Kytococcus*, *Marmoricola*, *Microbacterium*, *Micromonospora*, *Actinoplanes*, *Agrococcus*, *Amnibacterium*, *Brachybacterium*, *Citricoccus*, *Dermacoccus*, *Glutamicibacter*, *Gordonia*, *Isoptericola*, *Janibacter*, *Leucobacter*, *Nocardia*, *Nocardiopsis*, *Pseudokineococcus*, *Sanguibacter*, *Verrucosispora.* Among them, seven strains were potentially new species of genera *Nocardioides*, *Streptomyces*, *Amnibacterium*, *Marmoricola* and *Mycobacterium*. Above all, strain 8BXZ-J1 has already been characterized as a new species of the genus *Marmoricola*. Sixty three out of 101 strains were chosen to screen antibacterial activities by paper-disc diffusion method and inhibitors of ribosome and DNA biosynthesis by means of a double fluorescent protein reporter. Thirty one strains exhibited positive results in at least one antibacterial assay, notably, the strain 8BXZ-J1 and three potential novel species including strain 7BMP-1,

5BQP-J3 and 1BXZ-J1 showed antibacterial bioactivity. In addition, twenty one strains showed inhibitory activities against at least one "ESKAPE" resistant pathogens. Other important results are *Streptomyces* strains, 2BBP-J2 and 1BBP-1, can produce bioactive compound with inhibitory activity on protein biosynthesis due to translation stalling, meanwhile, *Streptomyces* strain 3BQP-1 can produce bioactive compound to induce SOS-response due to DNA damage. In conclusion, this study proved mangrove plants harbored a high diversity of cultivable endophytic actinobacteria, which can be a promising source for discovery of novel species and bioactive compounds.

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BACTERIA OF THE GENUS *AZOSPIRILLUM* – NEW NICHES OF HABITATION AND NEW PROPERTIES

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Azospirillum is one of the best- studied genera of plant growth-promoting rhizobacteria, which are found in various ecological and soil conditions and are closely related to various wild-growing and agricultural plants. Nitrogen fixation and denitrification, as far as the ability to produce phytohormones and other biologically active substances are proposed for explaining the plant growth promotion effects of *Azospirillum* on inoculated plants of barley, wheat, maize, rice, cotton, grasses, etc, mainly under stressing conditions. Attaching to the roots and colonizing plant tissues, azospirilli improve the growth and increase the yield and resistance to pathogens, that attached a constant attention of researchers for several decades.

In natural conditions, *Azospirillum* usually has a phase of active vital activity that coincides with the vegetation of the host plant and a rest phase. Resting cells survive the winter period under the shells of the grain of the host plant, and in the spring they begin to actively multiply, abundantly colonize the root surface, the root intracellular and root hairs of the plant [1]. *Azospirillum* are chemoorganotrophs and use mainly organic acid salts (malate, succinate, lactate and pyruvate) and carbohydrates, which are the main components of root secretions.

Despite numerous environmental studies, such as identifying the preferred habitat, plant-bacterial interactions, fixing nitrogen and producing phytohormones, there is very little information about the existence of *Azospirillum* in the absence of higher plants. Recently the data have appeared

on the isolation of *Azospirillum* from atypical habitats. Thus, *Azospirillum thiophilum* was isolated from a sulfur source (Stavropol Territory, Northern Caucasus, Russia) and found the ability to lithoheterotrophic growth under microaerophilic conditions with the simultaneous use of organic substrates and thiosulphate as an electron donor for energy metabolism [2].

In our study, we hypothesized the use of unusual carbon sources and transition to facultative autotrophy as a mechanism of *Azospirillum* adaptation to environmental conditions in the absence of vascular plants. To test this hypothesis, novel isolates from different water-saturated environments were investigated.

In 2007, *Azospirillum* sp. B2, B21, B22 strains were isolated from the oligotrophic *Sphagnum* peat bog of the Tver region, Russia, from the oligotrophic sphagnum peat bog as a components of the stable methane-oxidizing enrichments [3]. In 2018, a strain Sh 1was isolated directly from a peat sample taken from a dried Shatura bog in the Moscow region. These cultures showed the greatest degree of 16s rRNA similarity with *A. humicireducens* SgZ-5T. Strains AA2 and RA 4–11 were isolated in 2017 from the sediments of Lake Titicaca (Peru) and were closest to *A. brasilense* and *A. formosense*. Based on the genomic analysis, the isolated strains proposed to be the new species in the *Azospirillum* genus. The ability of all these strains to use methanol for growth and fixation of nitrogen has been demonstrated experimentally.

Based on the whole published genome of *A. brasilense* Sp245 primer system the specific detection of the key genes for the oxidation of methanol, a large subunit of the methanol dehydrogenase gene (mxaF) was developed and successfully used for AA2 and PA4–11 strains. No mxaF genes were detected for B2 by PCR analysis, however, whole genome sequencing demonstrated the presence of a homologue xoxF gene [4].

Thus, the distribution of bacteria of the genus *Azospirillum* is much wider than it was known to the present day. The ecological mechanism of survival in water-saturated habitats in the absence of higher vascular plants is realized through the formation of associations with methane-oxidizing bacteria and consists in the transition to the use of methanol. However, this hypothesis requires more detailed study.

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CHANGES IN IJSEM FOR THE DESCRIPTION OF NOVEL TAXA

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Genomics is currently recognized as a reproducible, reliable and highly informative methodology as it provides means to infer phylogenetic relationships among prokaryotes and allows the continuation to natural classification [1]. Wayne and colleagues already noted that the complete DNA sequence of a microorganism would be the reference standard to determine phylogeny and that phylogeny should determine taxonomy [3]. Thus, replacement of DNA-DNA hybridization which indirectly measures the degree of genetic similarity between two genomes in prokaryote taxonomy with pairwise genome-sequence derived similarity has been proposed, and there is sufficient experimental evidence to adopt this proposal. There have been a series of efforts to develop a bioinformatic method to replace DDH for differentiating species. Because the DDH value basically reflects relatedness or similarity between two genomes, these efforts focused on devising values analogous to DDH values. These values, as forms of similarity or distance, were coined as the overall genome related index (OGRI) [2].

Even though there has been a considerable effort in obtaining genome data for type strains, less than 50% of species with validly published names are represented by genome sequences of their type strains. In contrast, an almost complete database of 16S rRNA gene (16S) sequences is available for the type strains of prokaryotic species [4, 5]. Therefore, a combination of 16S similarity and OGRI can be used in a systematic process to identify and recognize a new species [1].

International Journal of Systematic and Evolutionary Microbiology (IJSEM) is the official journal of the International Committee on Systematics of Prokaryotes and the leading forum for publication of new taxa. It is also the official journal of record for bacterial names. As of January 2018, the inclusion of genome sequence data as part of the characterization of new taxa is highly recommended. In this context, the Editorial Board of IJSEM in collaboration with Prof. Chun have recently proposed a set of minimal standards for the quality of genome sequences and how they can be applied for taxonomic purposes. These proposals will be presented and discussed.

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HUMAN ENTEROCOCCI COLLECTION AS SOURCE OF INNOVATIVE MEDICAL AND BIOTECHNOLOGICAL COMPOUNDS

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The *Enterococcus* genus consists of more than 50 species [1] that occur in human gastro-intestinal tracts, as well as in the guts of mammals, birds and insects, dairy products and traditional fermented foods, and in various environments, including fresh and salt water, plants and soil [2]. Enterococci survive or grow over a wide range of temperatures, pH, and they are highly tolerant to osmotic and oxidative stresses, desiccation, antibiotics, disinfectants, and high heavy metal concentrations. Their metabolic versatility drew attention of researchers working in food, pharmaceutical, and biotech industries.

Enterococci, which belong to the group of lactic acid bacteria (LAB), produce antimicrobial compounds that contribute to niche control: bacteriocins, organic acids, activated oxygen metabolites, enzymes, exopolysaccharides, etc. [3]. Our team examined bacteriocin production by enterococci of human intestine microflora and found some new data on biological activity of *Enterococcus faecium* antimicrobial peptides [4–6]. We cultivated *E. faecium* strain on simplified medium to reduce amount of purification steps. This approach allows to purify the novel heavy weight bacteriocin produced by *E. faecium* ICIS 7. The novelty of this bacteriocin, named enterocin-7, was confirmed by N-terminal sequencing and by comparing the structural-functional properties with available data. Purified enterocin-7 is characterized by a sequence of amino acid residues having no homology in UniProt/SwissProt/TrEMBL databases: NH_2 – Asp – Ala – His – Leu – Ser – Glu – Val – Ala – Glu – Arg – Phe – Glu – Asp – Leu – Gly. Isolated thermostable protein has a molecular mass of 65 kDa, which allows it to be classified into class III of bacteriocin classification schemes. Enterocin-7 displayed a broad spectrum of activity against some Gram-positive and Gram-negative microorganisms. Fluorescent microscopy and spectroscopy showed the permeabilizing mechanism of the action of enterocin-7, which is realized within a few minutes [7].

Another novel bacteriocin-like inhibitory substance (BLIS), produced by the *E. faecium* ICIS 8 strain, was purified and characterized using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and N-terminal amino acid sequencing revealed the following partial sequence: NH_2-APKEKCFPKYCV. The proteinaceous nature of purified BLIS was assessed by treatment with proteolytic enzyme. Studies of the action of BLIS using bacteriological and bioluminescence assays revealed a dose-dependent inhibition of *Listeria monocytogenes* 88BK and *Escherichia coli* K12 TG1 *lac:: lux* viability. The interaction of the BLIS with the bacterial surface led to the compensation of a negative charge value, as shown by zeta-potential measurements. Assessments of membrane integrity using fluorescent probes and atomic force microscopy revealed the permeabilization of the cellular barrier structures in both *L. monocytogenes* and *E. coli* [8].

These data strongly support the potential for industrial application of enterococci strains from collection of human body microorganisms. These cultures and/or their metabolites should be seen as components of innovative probiotics and synbiotics.

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MARINE FUNGI WITHIN SPONGES: BIODIVERSITY, CHEMODIVERSITY AND BIOTECHNOLOGICAL POTENTIAL

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With more than 70 % of the planet's surface covered by water, Oceans are at the same time the most common and the less studied environment by microbiologist. Today, more and more attention is paid on microorganisms associated with macroorganisms and in particular with sponges. These animals provided more novel bioactive compounds than any other marine organisms [3]. Nevertheless, different molecules isolated from sponges are structural similar to those produced by the associated

microorganisms, leading to think that microorganisms are involved in the biosynthesis of interesting metabolites [2]. Considering the large gaps in our knowledge on the presence of marine fungi in the oceans, the first goal of this study was to isolate and identify the fungal community associated with four species of sponges *Dysidea fragilis*, *Grantia compressa*, *Pachymatisma johnstonia* and *Sycon ciliatum* isolated in the Atlantic Ocean and still unknown for their associated mycobiota. The second goal of the present research was to study the chemodiversity of the mycobiota associated with *G. compressa*, applying the OSMAC approach (One Strain-Many Compounds, [1]) and isolate promising bioactive molecules.

Sponges revealed an astonishing biodiversity: 97 fungal taxa were isolated and 29% of the identified species were reported for the first time in marine environment, including two new fungal species *Thelebolus balaustiformis* and *Thelebolus spongiae*. The use of different isolation methods, media and growth temperatures allowed increasing the number of cultivable fungi. Sponges demonstrated to host a significantly different fungal community with only three species common to the four studied species.

The fungal community (20 taxa) associated with *G. compressa* was further studied for the production of secondary metabolites, following the OSMAC approach (One Strain-Many Compounds). The results from the chemical fingerprints highlighted the effectiveness of the OSMAC approach on the stimulation of different biosynthetic pathways. *Eurotium chevalieri* (MUT 2316) showed an incredible chemical diversity and was selected as the best candidate for deeper studies. Ten pure molecules were isolated from *E. chevalieri* and showed antiviral activity (against Herpes simplex 1 virus and Influenza virus) and antibacterial activity with emergent pathogens as *S. aureus* methicillin-resistant.

In conclusion, fungi inhabiting sponges represent an incredible source of biodiversity and a promising source of natural products for biotechnological exploitation. On the other hand, due to their nature, they pose new challenges for the long-term preservation, since the traditional techniques (i.e cryopreservation) are not always effective.

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BIOREMEDIATION AND ECOLOGICAL RESTORATION OF CONTAMINATED SOILS BY FUNGAL AND BACTERIAL CONSORTIA

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Soil pollution is a serious problem all around Europe, with 42 potentially contaminated and 5.7 contaminated sites every 10,000 inhabitants. There is growing interest in developing techniques to reduce levels of organopollutants from contaminated soils by means of selected microorganisms. Bioremediation is considered a cost effective solution to this problem even though there are few example of full-scale application. LIFE BIOREST (LIFE15 ENV/IT/000396, *www.lifebiorest.com*) aims to optimize a biomerediation method of a highly contaminated soil, where the transformation made by consortia of fungi and bacteria is finalized by the final step of re-vegetation.

The aim of the first phase of the action is the characterization of the microbial community of the contaminated site. The soil was collected at three different depths from –1 to –3 m. Different methodologies have been set up and carried out simultaneously in order to meet the objectives: i) solid screening for the identification of the fungal and bacterial strains capable of growing in presence of target contaminants as sole carbon source; ii) enrichment in liquid cultures to favor the development of microbial communities adapted and capable of degrading specific target analytes (BTEX, PHAs, alkanes).

About 300 fungal and 140 bacterial strains were isolated from the solid and liquid cultures. Fungi mostly belong to Ascomycetes even though few Basidiomycetes were found (i. e. *Phlebia tremellosa* and *Trametes gibbosa*). The main genera were *Cladosporium*, *Aspergillus*, *Penicillium*, *Fusarium*, *Scedosporium*, *Trichoderma* and *Epicoccum*. The bacterial isolates mostly belonged to the Gram negative genera *Pseudomonas*, *Sohingobacterium*, *Pseudoxanthomonas*, *Rhizobium* and *Acinetobacter*.

Polycyclic Aromatic Hydrocarbons (PHAs) as phenanthrene and pyrene and alkanes as heptadecane and paraffin oil were the best substrates, easily used as sole carbon source, confirming the great adaptation skills of the strains isolated from the contaminated site. All the bacteria and fungi were tested against the pollutants of interest in an innovative miniaturized approach in multiwell plates. Several strains were capable of growing on the pollutants as much as positive controls with glucose, highlighting their capability to exploit complex source of nourishment as far as simple and bioavailable ones. Particular attention was also given to those strains capable of producing oxidative enzymes and biosurfactants.

Biosurfactants production is a great advantage for the microorganisms involved in bioremediation processes due to the enhancement of the pollutants bioavailability. The screening was carried out by four different tests, providing semi-quantitative results for more than 370 strains.

Noteworthy the results of the oil dispersion test, because performed with the crude oil extracted from the Fidenza site: considering the very high concentration of organic pollutants, any positive response could indicate the presence of quite strong biosurfactants. Several fungi (i.e *Purpureocillum lilacinum*, *Fusarium solani*) and bacteria (e. g. *Rhizobiaceae bacterium*, *Pseudomonas stutzeri*) gave clear halo of dispersion comparable to the positive control with Tween 80. Besides the blue agar plates was performed in order to evaluate the production of anionic surfactants. Bacteria response was easy to evaluate whereas fungi showed a plethora of responses that partially interfere with the evaluation of positive results. Among the tested strains, 43 bacteria and 20 fungi were identified as good producers of anionic biosurfactants, as indicated by the formation of a clear dark blue halo around the colony [1].

The most performing fungi and bacteria were used in microcosms (500 mg of soil) and mesocosms (10 kg od soil) trials in order to evaluate their actual capability to transform the pollutants and survive in the extreme conditions of the contaminated soil. Fungi and bacteria were tested singly or as complex consortia to investigate their capability to sinergically work against the pollutants. Since the bioavailability of the pollutants is a serious issue that could limit the effectiveness of a bioremediation process, the addition of biosurfactants was also evaluated.

Fungi and bacteria worked in synergism. Combined (fungi + bacteria) consortia resulted faster and more efficient against the most recalcitrant pollutants. Up to 80 % of pyrene degradation was observed after only 20 days.

In mesocosms trials, the total hydrocarbons content was reduced up to 70 %, but the choice of the microbial consortia came to be a critical issue. Among the six selected consortia, the yields varied from 20 to 70 %. In the optimal condition, the most concentrated pollutants (e. g. phenanthrene, fluoranthene and pyrene) were almost completely transformed. According to germination experiments, three plant species (*Sorghum bicolor*, *Festuca arundinacea* and *Trifolium pretense*) were selected for further treatments of the soil.

Bioaugmentation approach confirmed then to guarantee a high and stable degradation. Ongoing experiments are focused to demonstrate the degradation skills of the selected consortia at biopile level, where the microbial treatment will be followed by the final step of phytoremediation.

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DATA INTEGRATION WITH LIFE SCIENCE DATABASES: GATHERING OF TECHNOLOGY

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Keywords: databases, integration, microorganisms, software, API, screen scraping

This report presents the database integration task, its practical realisation steps, the current state of procedure, position of this data interconnection schema in Strategic Plan of MIRRI-ERIC, in MIRRI–IS proposal, in European biomedical system, in COST EUDOn Action, as well as participating parties.

The technical side presented: the central metabase characteristics, brouther extensions, screen scraping, API, FAIR options, the possible demonstrator with EMBL-EBI in EOSC–Life project.

Abbreviations:

API – application programming interface,

COST is the longest-running European framework supporting trans-national cooperation among researchers, engineers and scholars across Europe,

EMBL-EBI – The European Bioinformatics Institute,

EOSC–Life – European Open Science Cloud,

ERIC – European Research Infrastructure Consortium,

EUDOn – European Digital Objects network,

FAIR – a set of guiding principles to make data Findable, Accessible, Interoperable, and Reusable,

MIRRI–Microbial Resource Research Infrastructure,

MIRRI–IS – MIRRI Information System.

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THE ADVANTAGE OF THE USAGE OF THE LONG "BOOSTED" FUNGAL BARCODE THAT UNCLUDES ITS1/2 REGION AND D1/D3 DOMAIN OF THE 26S LARGE SUBUNIT RIBOSOMAL DNA

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The study of the biodiversity of the kingdom of fungi is a grandiose and most important task for the present and many more generations of scientists, such are the scales of wildlife and its "white spots". Therefore, it is extremely important to increase the effectiveness of methods of study and cognition. The identification of known species and the assessment of the supposedly new and their phylogenetic position is the path that any study of biodiversity takes.

The main objective molecular method for such a study is barcoding. The main barcode of fungi, as it is accepted since 2012 [1], is the area of internal transcribed spacers (ITS), in the middle of which there is a conservative short locus of 5.8S rDNA between the variable ITS1 and ITS2. This bar code works well outside the white spots at the intra-venous level of a relatively well-studied genus, but turns out to be helpless if it leads to a white patch of scale from an unknown genus and up the ladder of the taxonomic hierarchy.

In this case it is recommended to additionally use the first two or three domains (D1, D2 and D3) of the gene for the large ribosomal subunit (LSU or 28S) for the analysis. The moderate variability of these sites allows the construction of phylogenetic trees in a wide taxonomic range and more adequately position candidates for the role of representatives of new taxa.

The idea to use both the ITS area and a part of LSU for barcoding is presentin the air for quite a time. Nature's gift is that these areas are adjacent to each other and can easily and conveniently be amplified with one universal pair of primers, with the typical length of the amplicon 1500 bp.

Modern Sanger sequencing continues to improve and makes it possible to effectively read such amplicons. High-throughput sequencing (HTS) has platforms with very long reads (PacBio and Oxford Nanopore), for which 1500 and much more is not a problem. Thus, methodically, the science is completely ready to unite the "primary" and "secondary" barcodes in a single standard. Using it significantly increases the efficiency of both the molecular identification of new isolates and metabarcoding, which is precisely needed to accelerate progress in studying yet hidden biodiversity.

We have demonstrated the effectiveness of the approach described above by using the example of analysis of high-latitude fungal isolates and close to them collection strains [2]. The analysis of only about ninety samples led to the identification of new species, candidates for them, and even several higher-order taxa.

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LARGE-SCALE GENERATION AND ANALYSIS OF FILAMENTOUS FUNGAL DNA BARCODES BOOSTS COVERAGE FOR KINGDOM FUNGI AND REVEALS THRESHOLDS FOR FUNGAL SPECIES AND HIGHER TAXON DELIMITATION

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Species identification lies at the heart of biodiversity studies that has in recent years favoured DNA-based approaches. Microbial Biological Resource Centres are a rich source for diverse and high-quality reference materials in microbiology, and yet the strains preserved in these biobanks have been exploited only on a limited scale to generate DNA barcodes. As part of a project funded in the Netherlands to barcode specimens of major national biobanks, sequences of two nuclear ribosomal genetic markers, the Internal Transcribed Spaces and 5.8S gene (ITS) and the D1/D2 domain of the 26S Large Subunit (LSU), were generated as DNA barcode data for ca. 100 000 fungal strains originally assigned to ca. 17 000 species in the CBS fungal biobank maintained at the Westerdijk Fungal Biodiversity Institute, Utrecht. In a previous study, the barcoding project resulted in the release of 8 669 barcode sequences of manually validated CBS strains representing 1 351 yeast species [1]. Similarly to the results obtained in Vu et al. [1], in this study, using more than 24 000 DNA barcode sequences of 12 000 ex-type and manually validated filamentous fungal strains of 7 300 accepted species, we showed that ITS and LSU can be used to classify a large portion of all fungi to species level. The optimal identity thresholds to discriminate filamentous fungal species were predicted as 99.6 % for ITS and 99.8 % for LSU. ITS has been shown to outperform LSU in filamentous fungal species discrimination with a probability of correct identification of 82 % vs. 77.6 %, and a clustering quality value of 84 % vs. 77.7 %. At higher taxonomic classifications, LSU has been shown to have a better discriminatory power than ITS. With a clustering quality value of

80%, LSU outperformed ITS in identifying filamentous fungi at the ordinal level. At the generic level, the clustering quality values produced by both genetic markers were low, indicating the necessity for taxonomic revisions at genus level and, likely, for applying more conserved genetic markers or even whole genomes. The taxonomic thresholds predicted for filamentous fungal identification at the genus, family, order and class levels were 94.3%, 88.5%, 81.2% and 80.9% based on ITS barcodes, and 98.2%, 96.2%, 94.7% and 92.7% based on LSU barcodes. The newly generated ITS barcodes were also compared with the "Top 50 Most Wanted Fungi" [2] dataset to reveal the most frequently sampled environmental sequence types that have been difficult to be assigned to meaningful taxonomic levels. The DNA barcodes used in this study have been deposited to GenBank and will also be publicly available at the Westerdijk Institute's website as reference sequences for fungal identification, marking an unprecedented data release event in global fungal barcoding efforts to date.

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GANODERMA LUCIDUM AND *GANODERMA RESINACEUM* STRAINS PRODUCING ANTITUMOR ALKALI-SOLUBLE POLYSACCHARIDE XYLOMANNAN

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Two strains belonging to the *Ganoderma* genus (5–1 and 10) were obtained in the study.

Strain 5–1 is a producer of antitumor alkali-soluble (XM) and water-soluble polysaccharides. (2) It was shown to have a branched molecules containing a backbone of (1→3) linked residues of α-D-mannopyranose, most of which are substituted at position 4 by single β-D-xylopyranose-residues or disaccharide residues β-D-Manp (1→3) β-D-Xylp (1→. (1) XM was obtained from a submerged mycelium. The search of XM has been conducted in other strains of Ganoderma, characterized by high biotechnological indices when using submerged cultivation. Target XM was identified in alkali-soluble extract of mycelium of strain 10 using nuclear magnetic resonance method. Its antitumor effects were demonstrated in vivo by the research group from Laboratory of Pharmacology and Chemotherapy in Gause Institute of New Antibiotics. During the submerged cultivation both strains 5–1 and 10 were growing in the form of pellets. The comparative study of their pellets micromorphology was conducted using scanning electron microscope. The following structures were revealed: clamps, apical and intercalary chlamidospores, mycelial cords. Hypha

connections and anastomose formation between them was observed in the mycelial cord. Molecular phylogenetic analysis of selected strains based on ITS and IGS rDNA sequences revealed that strain 5–1 belongs to the *G. lucidum* clade while strain 10 more likely is within *G. resinaceum* group.

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TREASURE HUNT IN COLLECTIONS AND ENVIRONMENT

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Microbial culture collections compliment collections of museums of natural history and herbaria in the task of preservation of the World's biodiversity. Also, our understanding of evolution, species concepts and development of tools to identify organisms heavily rely on field-collected reference specimens, vouchers and cultures. Preservation of and access to this material are therefore among of most important tasks fulfilled by collections. Across the world, natural-history collections hold thousands of species awaiting identification. Thanks to development of more robust identification techniques and information exchange through databases, collections are becoming increasingly valuable and publications that used material from collections are prevailing over pure field surveys in some fields of study.

Availability of the reference material for future research is crucial for development of stable classification of living organisms. Descriptions provide information about an organism following standards and using techniques of the time of discovery. Because methods and tools for studying species have massively changed over three decades, reexamination of the original material is often needed. Availability of the material is extremely important for rare or threatened species as well as for isolates from undisturbed or disappearing habitats. Safeguarding old collections is an important task for service collections to save cultures from habitats that literally may not exist anymore. It is also important to direct the ongoing exchange of information between collections towards creating lists of the lacking and lost reference material.

Changes in national regulations regarding access to genetic resources are shaking today's research landscape in many countries. As a consequence, a future decline of research activity was repeatedly alarmed by leading scientists. Although this situation already has a negative impact on culture collections, it can also revitalize the interest for studying old material in the future.

NATRONOGRACILIVIRGA SACCHAROLYTICA GEN. NOV. SP. NOV. A NEW ANAEROBIC, ALKALIPHILIC ORGANOTHROPHIC BACTERIUM FROM THE SODA LAKE

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Sample of sediment from one of Tanatar soda lakes (Altai region, Russia) was cultivated on the medium containing sodium formate and ferrihydrite as the electron donor and acceptor, without any additional organic compounds. The enrichment culture was obtained containing three different forms of rods, which were further isolated in pure cultures. Here we present a taxonomic description of one of these isolates designated as strain Z-1702. Cells of new isolate were motile thin flexible rods 0.1–0.2× 1–4 μm with Gram-negative cell wall structure. In stationary phase they formed a sphaerical cyst-like structures at one of the cell endings. Strain Z-1702 is an obligate alkaliphile with a pH range of growth from 8.0 to 10.0 with an optimum at 8.7–9.0, growing in soda brines containing 1.4–4.2 M total Na^+ (optimum at 2.3–2.8 M). It is obligately dependent on sodium carbonate but not sodium chloride ions. Strain Z-1702 ferments sugars and several polysaccharides: glucose, maltose, fructose, mannose, sorbose, galactose, cellobiose and starch. Interestingly, strain Z-1702 is an oligotrophic bacterium and can successfully grow on the medium containing only 50 mg of glucose as a substrate. It does not need yeast extract. It cannot grow on peptides, alcohols or amino acids. Strain Z-1702 does not reduce any tested electron acceptors: nitrate, nitrite, sulfate, sulfite, thiosulfate, elemental sulfur, synthesized ferrihydrite, fumarate or crotonate. It is an obligate anaerobe, and oxygen completely inhibits its growth. 16S rRNA gene sequence analysis showed that strain Z-1702 is a member of a recently proposed phylum *Balneolaeota*. It has <88 % 16S rRNA gene sequence identity with the validly published representatives of this phylum (*Rhodohalobacter* representatives were the closest relatives according to BLAST) and form a family-level lineage on the phylogenetic tree. On the basis of its physiological characteristics and phylogenetic position the novel isolate is considered to be a representative of a novel genus and species in the novel family *Natronogracilivirgulaceae.* The name *Natronogracilivirgula saccharolytica* gen.nov., sp. nov. is proposed.

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A NOVEL BACTERIUM ISOLATED FROM A CHUKOTKA HOT SPRING REPRESENTS DEEP BRANCH OF UNCULTIVATED BACTERIA

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Chukotka Peninsula is an arctic region with permafrost zones and negative annual temperatures. Due to a widespread tectonic disturbances and “young” volcanic activity, Chukotka Peninsula has regions with discharging of thermal springs with water temperature up to 97C [4]. Up to the moment, there was no information about microbial communities in hot springs, located in this region. In July-August 2016, first samples of water, sediments and microbial mats were collected from the hottest

springs at three locations (Mechigmen, Senyavin and Chaplino thermal groups) on the East of the Peninsula. Totally samples from 50 springs were collected for water chemistry analysis, microbial cultivation and NGS.

A pink microbial mat (sample 3753) was obtained from the head of a small hot stream in Chaplino hot springs region. At the sampling site the temperature was 59°C, pH=6,8 and Eh= +120mV, what is characterized as a moderate thermophilic, microaerophilic conditions. The sample used for enrichment cultures preparation on the mineral medium with various polysaccharides as a substrate: xyloglucan, xanthan gum (XG), galactan (Gal) and pectin. Enrichment cultures were incubated at 60°C, pH 7,0 in aerobic conditions for 12 days. No growth was observed in xyloglucan and pectin enrichments while large oval cells were dominating the xanthan gum enrichment and various cell morphotypes, as long thin filaments, ribbon-like ones and small motile rods, inhabit the galactan enrichment.

16S rRNA gene NGS profiles analysis of enrichment cultures with positive growth revealed that in XG enrichment two bacterial genera were dominant: thermophilic planctomycete *Thermogutta* (74% of reads) and *Thermu*s (19%). Accordingly to light microscopy observations galactan enrichment culure has several dominating phylotypes: *Rhodothermus* (42%), *Litorilinea* (24%), *Thermus* (15%), *Caldilinea* (7%) and the rest (11%) represented a novel phylum-level lineage of uncultivated bacteria OPB56 [1, 2], belonged to FCB superphylum [3].

A number of pure cultures were obtained from these enrichment cultures including known species *Thermogutta terrifontis* strain 3753XG, *Litorilinea aerophila* strain 3753C1, *Caldilinea aerophila* strain 3753TK10, all with 99% identity of 16SrRNA genes with the types strains. However, one strain designed 3753F appeared to be the first cultivated representative of the OPB56 candidate division.

Cells of isolate 3753F were motile Gram-negative rods with length 0.4–0.6 μm and width 0.25–0.3 μm. The cells occured singly, in pairs or 5–7 cell chains, were able to form aggregates, motiled by means of one polar flagellum. A special feature of the strain was its ability to produce extracellular matrix and membrane vesicles.

Strain 3753F is a facultative anaerobe, growing optimally at 54°C and pH 7.5. Growth was observed on medium with NaCl concentration 0–0.6% and an optimum was 0.3%. In aerobic conditions strain 3753F grew on yeast extract, maltose, pullulan, starch, guar gum, locust bean gum, xanthan gum and laminarin. Weak growth was observed on D-glucose, galactan and xyloglucan. Yeast extract and xanthan gum were also utilized at microaerophilic (1 and 3% v/v oxygen in the headspace) or anaerobic (without electron acceptor) conditions.

Thus, a representative of a bacterial candidate division OPB56 was enriched and isolated in pure culture. The first cultivated OPB56 strain is being a facultative anaerobe, thermophile, capable of growing on various polysaccharides. The genome of strain 3753F will be sequenced and analyzed in near future. This will allow comparative analysis of phenotypic and genotypic results for better understanding the phylogeny and physiology of this microorganism.

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ISACHENKONIA ALKALIPEPTOLYTICA GEN. NOV., SP. NOV., A NEW ANAEROBIC, ALKILIPHILIC ORGANOTHROPHIC BACTERIUM ISOLATED FROM A SODA LAKE AND ABLE TO REDUCE SYNTHESIZED FERRIHYDRITE

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Anaerobic peptolytic bacterium was isolated from a bottom sediment of Tanatar soda lake (Altai region, Russia) and designated as the strain Z-1701. Cells of isolate Z-1701 were motile short rods 0.3–0.5×0.75–1.5 μm with Gram-positive cell wall. It was an obligate alkaliphile with a pH growth range from 7.5 to 10.2 and the optimum at 9.0–9.3. In natural habitat of strain Z-1701 soda brines contained 1.4–3.3 M total Na^+, so it had its growth optimum at 2.35 M and was obligately dependent on sodium carbonate but not on chloride ions. It was mesophile growing at T range from 15–43°C with an optimum at 35°C. The analysis of substrates supporting the growth of strain Z-1701 revealed that it is capable of fermenting proteinaceous substrates such as yeast and beef extracts, peptone, triptone, soytone and caseine. It could not grow on organic acids, sugars and alcohols or amino acid mixtures. With peptone as an electron donor, it could reduce Fe (III) in the form of synthesized ferrihydrite, and elemental sulfur. Strain Z-1701 could grow in anaerobically prepared medium without the addition of reducing agent. Oxygen was not used as the electron acceptor and inhibited the growth. 16S rRNA gene sequence analysis (based on BLAST) showed that strain Z-1701^T had the representatives of families *Clostridiaceae* and *Peptostreptococcaceae* as its closest relatives, with sequence identity <92 %. 16S rRNA gene phylogenetic analysis placed strain Z-1701 in a separate cluster between *Clostridiaceae* and *Peptostreptococcaceae* indicating it is a representative of a separate family-level branch. On the basis of its physiological characteristics and phylogenetic position the novel isolate is considered to be a representative of a novel genus and species for which we propose a name *Isachenkonia alkalipeptolytica* gen. nov., sp. nov. The name was proposed after Boris L. Isachenko (1871–1948), a Russian microbiologist who in 1927th started the research of microbial communities in Tanatar soda lakes group. *Isachenkonia* is proposed to be a first genus of the novel family *Isachenkoniaceae*, yet this should be first supported by more comprehensive, phylogenomic analysis.

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Extremophilic Microorganisms of Various Physiological Groups (UNIQEM)" (No. 0104–2017–0001), the Basic Research Program of the Presidium of the Russian Academy of Sciences "Evolution of Organic World and Planetary Processes," subprogram 2.

MAPPING AND VALORIZING THE SPANISH MICROBIAL RESOURCES

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The Spanish Type Culture Collection (CECT, Colección Española de Cultivos Tipo, cect.org) leads the Spanish participation in "MIRRI" (Microbial Resource Research Infrastructure), a Research Infrastructure (RI) included in both the European and Spanish RI road maps. The Spanish connection with the MIRRI network requires the construction of a Spanish National Node connecting resources, service providers and experts, to foster R&D&i related to microbiology. In 2015, to start gathering information about the publicly funded research groups working with microbial resources, the Spanish Network of Microorganisms "REDESMI" was established as a CECT initiative funded by the National Institute for Agricultural and Food Research and Technology (INIA, grant number AC2013–00028). The aim of the project is first, to map the Spanish microbial resources isolated and characterized with public funds, which are mostly hidden in research laboratories; second, to give visibility to such highly valuable resources; and third, to preserve selected strains in public collections under high quality standards, ensuring their transfer in the appropriate legal framework (MTA, Nagoya, biosecurity etc.) and facilitating their commercial exploitation.

As a step forward, in 2017, the construction of the MIRRI Spanish National Node was initiated, as a Network of Excellence supported by the Ministry of Economy and Competitiveness, with the National Programme for Fostering Excellence in Scientific and Technical Research. The network is called MicroBioSpain and it is currently composed by the two Spanish Public Microbial Culture Collections, the CECT and the Spanish Bank of Algae (BEA), and six research centers: IPLA (Dairy Research Institute of Asturias), CIAL (Institute of Food Science Research) and IATA (Institute of Agrochemistry and Food Technology) from the Spanish Research Council (CSIC), INIA (National Institute for Agricultural and Food Research and Technology), IRTA (Institute of Food and Agricultural Research and Technology) and CNTA (Research and Technology for the Competitiveness of the Food Industry). They all work with microbial resources covering different aspects such as isolation, characterization, preservation and delivery, and offer other services and access to infrastructures, like pilot plants or specialized equipment for scale up processes, some of them unique in Europe, such as the NOVALINDUS platform or the Dynamic Gastrointestinal Simulator (simgi®) from CIAL. Besides, they keep contact with the industrial sector by means of research contracts and technology or strain transfer for commercial exploitation.

With these actions we aspire to promote communication among the different actors in Spain; i. e. scientists, technologists, bioindustry representatives and OTRIs (Research Results Transfer Offices), to promote information exchange and to add value to the microbial resources, connecting the sector demanding microbiology-related innovation with the providers of isolated and characterized microorganisms.

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