**Introduction**

Microorganisms from various ecological niches are the most important source of antibiotic substances and other bioactive metabolites (Sponga et al. 1999; Lorentz et al. 2006; Wu et al. 2011; Mondol et al. 2013; Palomo et al. 2013). To date, 95–99% of microorganisms in natural biotopes exist in the form of biofilms, since this facilitates access to nutrients, promotes cooperation between microorganisms, and protects cells from negative environmental effects (Costerton et al. 1987). Biofilm is a microbially derived sessile community characterized by the cells that are irreversibly attached to a substratum, interface or to each other, embedded in a matrix of extracellular polymeric substances that they have produced, and it exhibits an altered phenotype with respect to growth rate and gene transcription (Donlan and Costerton 2002). Biofilms are a type of microbial consortia that play an important role in biogeochemical processes in the biosphere.

In the aqueous environment, biofilms exist in several types, depending on the substrate on which they are formed: epilithic (rock surfaces), epipsammic (attached to sediment particles), epixylic (on dead plant material), epiphytic (on living plants), marine or lake snow (on organic and inorganic particles), and biofouling (artificial surfaces) (Romaní et al. 2016). Compared to other biofilms, epilithic biofilms have a more complex heterogeneous structure with a higher algal biomass and a large repertory; they are also more independent of seasonal fluctuations (Romaní and Sabater 2001; Bartrons et al. 2012). Obviously, the search for the biologically active substances (BAS) among the bacteria inhabiting epilithic biofilms is promising.

Multidomain enzymatic ‘megasynthases’, including PKS, NRPS and their NRPS/PKS hybrid complexes,
synthesize a wide range of secondary metabolites of the bacterial origin (Staunton and Wilkinson 2001). A diverse chemical structure and functional activity characterize polyketides, among which there are antibiotics, statins, tumor growth inhibitors, and other pharmaceutically significant compounds. There are three types of PKS (I, II, and III), which differ depending on the structure and mechanism of catalysis. Type I PKS are organized into modules consisting of at least three functional domains: ketosynthase (KS), acyl transferase (AT), and acyl carrier protein (ACP). Each module is responsible for one elongation cycle of the polyketide chain. Type II PKS are a large multienzyme complex of small, discrete enzymes with particular functions. The pivotal component that is responsible for the condensing activity resembles β-ketoacyl synthase II of type II FAS found in bacteria and plants. This class of PKS is responsible for the biosynthesis of bacterial aromatic polyketides, such as oxytetracycline and pradimicin. Type III PKS are self-contained enzymes that form homodimers. Their single active site in each monomer catalyzes the priming, extension, and cyclization reactions iteratively to form polyketide products. Despite their structural simplicity, type III PKS produce a wide array of compounds such as chalcones, pyrones, acidones, phloroglucinols, stilbenes, and resorcinolic lipids (Dayu et al. 2012). NRPS synthesize a few natural compounds with a wide range of biological activity and various medicinal properties. Monomers of amino acids serve as substrates for the synthesis of NRPS peptides. The modules contain an ATP-dependent adenylation domain (A-domain), a peptidyl carrier protein (PCP) domain, and a condensation (C) domain. The assembled molecule is released from the enzyme complex through a thioesterase (TE) domain. The A-domain is the most conservative (Staunton and Wilkinson 2001).

The natural products obtained by these biosynthetic pathways have been widely described for cultured and uncultured strains (Wu et al. 2011; Fickers 2012). Molecular methods have been successfully used to detect and identify target genes in the organism as indicators of the production of novel secondary metabolites (Banskota et al. 2006; Palomo et al. 2013). There have been multiple studies on secondary metabolites synthesized by PKS and NRPS gene clusters in members of the phylum Firmicutes (Lorentz et al. 2006; Wu et al. 2011; Fickers 2012; Mondol et al. 2013; Zhang et al. 2013). Natural strains of the genera Bacillus and Paenibacillus have in their genomes the clusters of genes responsible for the synthesis of several active compounds (antibiotics and biosurfactants), which act synergistically, thus showing high antagonistic activity against various pathogens (Ongena and Jacques 2008; Chen et al. 2009; Kim et al. 2010; Li et al. 2012). Therefore, natural isolates of bacilli represent a rich source of new antimicrobial substances of great importance for biotechnology.

Lake Baikal, one of the largest (area of 31 722 km²) and the deepest (1637 m) freshwater reservoir in the world, has a significant biodiversity and high endemism of microbial communities, unique ecological peculiarities, and rich biotopes. It is a kind of natural laboratory for studying the metabolic potential of microbial communities. Its littoral zone occupies 7% of the total area; the coastline is 2000 km.

Previously, strains of the genera Streptomyces and Micromonospora were isolated from water, sponges, and sediments in Lake Baikal. They showed antagonistic activity against potentially pathogenic microorganisms resistant to a number of antibiotics (Terkina et al. 2006). The authors suggested that Baikal actinomycetes can be used as producers of new BAS. Quite recently, polyketide synthase genes were identified in the metagenome community of the endemic sponges Lubomirskia baikalensis and Swartschewskia papyracea. Among the closest relatives, there were the genes involved in biosynthesis of metabolites, curacin A, stigmatellin, and nostophycin (Kaluzhnaya et al. 2012; Kaluzhnaya and Itskovich 2016). In the genome of the Baikal strain Pseudomonas fluorescens 28Bb-06, PKS genes were identified as 50–66% homologous to the gene clusters involved in biosynthesis of yersiniabactin, rhizoxin, disorazol, and epothilone (Lipko et al. 2012). In strains isolated from the freshwater sponge L. baikalensis, PKS and NRPS genes were detected in nine out of 14 cultures of the genera Bacillus, Pseudomonas, Variorox, Curtobacterium, and Rhodococcus (Kaluzhnaya et al. 2013).

The formation of hydrobiont communities on various geological rocks has been studied in Lake Baikal since 2000 (Timoshkin et al. 2003). These studies showed that the development and activity of organisms depended on the chemical composition of the rocks and their structure. They also showed the high selectivity of these organisms in terms of the occupation of different substrates (Parfenova et al. 2008). For the first time, bacterial communities of water and biofilms formed on a solid substrate in Lake Baikal were studied by pyrosequencing of the 16S rRNA gene fragment. Bacterial communities of biofilms showed high taxonomic diversity, represented by Cyanobacteria, Bacteroidetes, and Proteobacteria; the contribution of other groups did not exceed 1% (Parfenova et al. 2013).

The genomes of bacteria Serratia, Pseudomonas, Rheinheimera, and Flavobacterium isolated from epilithic biofilms in Lake Baikal showed diversity in their PKS genes, which are responsible for the synthesis of antibiotics and cytostatics (Sukhanova et al. 2017). Previously, we determined the antimicrobial activity of Bacillus and Paenibacillus strains isolated from biofilms (Zimens et al. 2014).

This work aimed to detect and evaluate of diversity of the PKS and NRPS genes in the genomes of heterotrophic bacteria isolated from epilithic biofilms in Lake Baikal.
Experimental

Materials and Methods

Sampling. Samples of epilithic biofilms were taken from the littoral zone of Lake Baikal near the settlement of Listvyanka (Cape Beryozovoy, 51°50′41.04″, 104°54′05.82″). Biofilms were sampled from plates (rocks and minerals) with a thickness of 0.5–1 cm that had been prepared in advance and were immersed in 2011 by divers at a depth of 7–8 m and exposed under natural conditions of the lake during the year. In May 2012, the plates covered with biofilms were lifted from the bottom of the lake, put in sterile containers with Baikal water and then transported to the laboratory at a temperature of 10°C. Under aseptic conditions, fouling of an area of 2 cm² was scraped, which was used for cultivation in nutrient media.

Isolation of heterotrophic bacteria. The samples of biofilms were suspended in 50 ml of sterile Baikal water and shaken for 30 min on a shaker at 120 rpm. A 1 ml aliquot was added to 100 ml of sterile Baikal water, then 1 ml of the resulting suspension was plated in three replicates using the pour plate method onto solid nutrient media with different contents of organic matter. To isolate pure cultures, the following nutrient media were used: R2A (Fluka analytical, USA), NSY (g/l: nutrient broth 1, soy peptone 1, yeast extract 1 and water, then 1 ml a l. 1981). The nucleotide sequences of the 16S rRNA gene fragment were determined on an ABI PRISM 310A Genetic Analyser automatic sequencer (Perkin Elmer, USA) at the SB RAS Genomics Core Facility (Novosibirsk). Comparative analysis of the sequences obtained with previously published ones was carried out using the FASTA and BLAST software package. Nucleotide sequences of 167 strains were registered in GenBank under the following numbers: HF548373 – HF548383, HF548386 – HF548401, HF678874 – HF678892, HF678894 – HF678990, HF947322 – HF947328, LT55292, and LT601385 – LT601400 (personal results; unpublished data).

The numbers of the strains used in this study were as follows: Paenibacillus sp. 5A (HF678944), Paenibacillus sp. 12A (HF678945), Paenibacillus sp. 7A (HF678946), Bacillus sp. 2B (HF678932), Bacillus sp. 2A (HF678933), and Bacillus sp. 9A (HF678934).

PCR screening of PKS and NRPS genes in the genomes of heterotrophic bacteria. Fragments of the KS-domains of PKS genes were amplified using the degenerate primers DK-F (5′-GTGCCGGTTNC-CRTGNNGYTC-3′) and DK-R (5′-GGATGGGAY-CCNCARCARYG-3′); the fragment length was 700 bp (Ehrenreich et al. 2005). PKS genes were amplified under the following conditions: polymerase activation (5 min at 94°C); 35 cycles, including DNA denaturation (45 s at 94°C), primer annealing (50 s at 60°C) and elongation (60 s at 72°C), as well as final elongation (10 min at 72°C). To screen the A-domain of NRPS genes, we used the primers MT-F (5′-GCNGGYGGYGCN-CRTGNGYTC-3′) and MT-R (5′-CCNCGDATYTTNA-CYTG-3′) (1000 bp); the PCR conditions were the same as those described above (Ehrenreich et al. 2005). PCR products were visualized on 1% agarose gel.

Study of enzymatic activity in members of the genera Bacillus and Paenibacillus. The ability of the strains studied to utilize carbon compounds (Hiss medium) and organic nitrogen-containing substances (amino acids) was assayed. The proteolytic extracellular enzymes were defined on media with casein and gelatine, lipolytic enzymes with tributyrin and lecithin, and amylolytic enzymes with starch (Netrusov 2005). Phosphatase activity was detected using the Alkaline Phosphatase-VITAL kit (Vital Development Corporation, Russia).

Phylogenetic analysis of the 16S rRNA gene sequences from Bacillus and Paenibacillus. For the species identification of Bacillus and Paenibacillus isolates, the sequences were aligned in the Clustal-W program. Phylogenetic analysis of nucleotide sequences of the 16S rRNA gene (length of 1360 bp) was carried out using the Mega 6.06 program, the Maximum Likelihood method, and the Kimura 2-parameter model. Bootstrap support was computed for 1000 replicates.

Identification of PKS and NRPS genes in the genomes of Bacillus and Paenibacillus. Amplicons of the gene fragments were visualized in 1% agarose gel using a transilluminator (VL-6.MC, France). The PCR fragments were cloned in the vector pJET1.2/
blunt (CloneJET PCR Cloning Kit, Fermentas, Lithuania), then amplicons were transformed in the cells of competent *E. coli* DH-5α and XL-1 strains.

Nucleotide sequences were determined on a genetic analyzer (Applied Biosystems, USA) in Irkutsk (Russia) and at the research and production company Sin- tol (Moscow, Russia). To transfer nucleotide sequences of the PKS and NRPS into amino acids, we used the BioEdit 7.2.5. program. A comparative analysis of the sequences obtained was carried out using the BLASTX and BLASTP software package.

Nucleotide sequences were deposited in GenBank under the numbers LT555240-LT555282 for PKS genes (43 pcs.) and LT990671-LT990687 for NRPS genes (17 pcs.).

**Phylogenetic analysis of amino acid sequences of the KS-domain fragments of PKS genes and A-domain of NRPS genes** was carried out using the Mega 6.06 program, the Neighbor-joining method, and the Kimura 2-parameter model. Bootstrap support was computed for 1000 replicates. The sequences were aligned in the Clustal-W program.

### Table I

| Taxonomy     | Number of the strains analyzed | Number of the strains with positive PCR signal |
|--------------|--------------------------------|-----------------------------------------------|
| **Phylum**   | PKS   | NRPS   | PKS   | NRPS   |
| **Firmicutes** | Bacillus | 42     | 11    | 33    |
|               | Paenibacillus | 4     | 4     | 4     |
|               | Virgibacillus | 1     | 0     | 0     |
|               | Staphylococcus | 2     | 0     | 1     |
| **Proteobacteria** | Pseudomonas | 45   | 9     | 26    |
|               | Aeromonas | 29     | 4     | 2     |
|               | Serratia | 3      | 2     | 1     |
|               | Rhizobium | 1      | 1     | 0     |
|               | Brevundimonas | 1     | 0     | 0     |
|               | Massilia | 1      | 0     | 0     |
|               | Achromobacter | 3     | 0     | 0     |
|               | Stenotrophomonas | 3    | 0     | 0     |
|               | Devosia | 1      | 1     | 0     |
|               | Hydrogenophaga | 1    | 0     | 0     |
|               | Versinia | 1      | 1     | 0     |
|               | Sphingomonas | 1    | 0     | 0     |
|               | Iodobacter | 1     | 1     | 0     |
|               | Roseomonas | 1     | 0     | 0     |
| **Actinobacteria** | Rhodococcus | 2     | 0     | 2     |
|               | Kocuria | 4      | 2     | 1     |
|               | Pseudoclavibacter | 3    | 1     | 0     |
|               | Plantibacter | 1     | 0     | 0     |
|               | Sanguibacter | 1     | 0     | 0     |
|               | Pseudarthrobacter | 1    | 0     | 0     |
|               | Microbacterium | 4    | 1     | 1     |
|               | Salinibacterium | 1    | 0     | 0     |
|               | Streptomyces | 1     | 0     | 1     |
|               | Micrococcus | 1      | 0     | 0     |
|               | Brachybacterium | 1   | 1     | 0     |
|               | Clachiihabitans | 1    | 0     | 0     |
|               | Microcella | 1      | 0     | 0     |
| **Bacteroidetes** | Flavobacterium | 4    | 2     | 1     |
| **Total** | 167 | 41     | 73    |
Results

Table I shows the results of strain isolation from epilithic biofilms in Lake Baikal. We obtained a collection of heterotrophic bacteria consisting of 167 strains. The isolates classified by a comparative analysis of the 16S rRNA gene fragment belonged to four phyla: Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes and 32 genera of bacteria. The members of the genera Aeromonas, Pseudomonas, and Bacillus were the dominant strains (Table I).

PCR screening of the isolates for the presence of PKS and NRPS genes. Screening of PKS genes in the genomes of heterotrophic bacteria revealed their presence in 41 strains belonging to 14 genera: Bacillus, Paenibacillus, Pseudomonas, Aeromonas, Serratia, Rhizobium, Devesia, Yersinia, Iodobacter, Kocuria, Pseudoclavibacter, Microbacterium, Brachybacterium, and Flavobacterium (Table I). The total percentage of the strains with PKS genes was 25%. The occurrence of PKS genes in members of the phylum Firmicutes (Bacillus, Paenibacillus) was 34%, 20% in Proteobacteria and 22% in Actinobacteria (Table I).

The screening of 167 strains showed a positive PCR signal for the presence of NRPS genes in 73 strains of 11 genera: Bacillus, Paenibacillus, Staphylococcus, Pseudomonas, Aeromonas, Serratia, Rhodococcus, Kocuria, Microbacterium, Streptomyces, and Flavobacterium (Table I). The total percentage of strains containing NRPS genes was 43%. A high percentage of these genes was found in the genus Pseudomonas (57%). At the same time, the occurrence of NRPS genes in members of the phylum Firmicutes (Bacillus and Paenibacillus) reached 78% of the total number of strains from this group. These genes were found in 32% of Proteobacteria and 22% of Actinobacteria (Table I).

The phylogenetic diversity of the genera Bacillus and Paenibacillus isolated from epilithic biofilms in Lake Baikal and the presence of PKS and NRPS genes in their genomes are shown in Fig. 1.

Thus, NRPS genes were more commonly found in Bacillus and Pseudomonas; the members of the phyla Firmicutes (Bacillus, Paenibacillus) also had high percentage of PCR positive strains with both, PKS and NRPS genes.

Physiological and biochemical characteristics of Bacillus and Paenibacillus strains. At the next stage, based on the obtained results of PCR screening, we selected six cultures: Paenibacillus spp. 5A, 12A, and 7A and Bacillus spp. 2A, 2B, and 9A. Previously, these strains showed antagonistic activity (Table II) (Zimens et al. 2014). Among them, there were highly active Paenibacillus spp. 5A and 12A and Bacillus sp. 9A, which simultaneously suppressed the growth of test cultures from different taxonomic groups (Gram-positive and Gram-negative bacteria, as well as fungi). Hence, we can assume that the strains studied can produce several different antimicrobial compounds (Zimens et al. 2014).

The selected isolates were tested for the ability to produce extracellular enzymes (Table III). We found that Paenibacillus spp. strains most actively utilized carbohydrates and polyatomic alcohols, and Bacillus spp. strains used amino acids. All cultures showed the ability to utilize starch and casein (Table III). The data on the physiological and biochemical characteristics of Paenibacillus spp. 5A and 12A and Bacillus sp. 2A and 9A were consistent with the data from the phylogenetic analysis.

Phylogenetic analysis of the nucleotide sequences of the 16S rRNA gene from Bacillus and Paenibacillus strains. Phylogenetic analysis indicated that the nucleotide sequences of the 16S rRNA gene of Paenibacillus spp. 5A and 12A strains formed a separate sister cluster with the type strain Paenibacillus peoriae KCTC 3763* (Fig. 2). This strain, isolated from soil, is antagonistic against phytopathogenic bacteria and fungi (Jeong

| Strain       | Antagonist activity | PCR signal to PKS gene | PCR signal to NRPS gene |
|--------------|---------------------|------------------------|-------------------------|
| Paenibacillus sp. 5A | Ec1*, Ec2, Pa, Bs1, Bs2, Ca, Sa, Ef | +                     | +                      |
| Paenibacillus sp. 12A | Ec1, Ec2, Pa, Bs1, Bs2, Ca, Sa, Ef | +                     | +                      |
| Paenibacillus sp. 7A  | Bs1, Bs2, Ca, Sa, Ef   | +                     | +                      |
| Bacillus sp. 2A      | Ec1, Bs1, Bs2, Ca     | +                     | +                      |
| Bacillus sp. 9A      | Bs1, Bs2, Ca, Sa, Ef   | +                     | +                      |

* Test cultures used in this work: B1 – Bacillus subtilis VKPM; Bs2 – Bacillus subtilis DSM; Pa – Pseudomonas aeruginosa GISK L.A. Tarasevich 190138; Ca – Candida albicans ATCC 10231; Sa – Staphylococcus aureus (ATCC 25923 and MRS); Ef – Enterococcus faecium; Ec1 – Escherichia coli K12 VKPM B-3254; Ec2 – Escherichia coli M17-02 VKPM B-8208
et al. 2012). On the phylogenetic tree, the nucleotide sequence from *Paenibacillus* sp. 7A clusters with the sequence of the nitrogen-fixing bacterium *Paenibacillus graminis* RSA197 isolated from the maize rhizosphere (Berge et al. 2002), which allowed us to preliminarily classify this strain as *P. graminis* 7A.

Nucleotide sequences of the 16S rRNA gene from *Bacillus* spp. 2A and 9A strains formed a joint cluster with the type strain *Bacillus amyloliquefaciens* NBRC 15535T (Fig. 2) isolated from fermented locust bean fruits (Africa) (Meerak et al. 2008). Strains 2A and 9A were preliminarily assigned to the species *B. amyloliquefaciens*.

Phylogenetic analysis indicated that the nucleotide sequence of the 16S rRNA gene from *Bacillus* sp. 2B clustered with the type strain *Bacillus subtilis* DSM10T.
PKS-NRPS genes in microorganisms

PKS-NRPS genes in microorganisms

(Fig. 2). Additionally, it showed high homology (100%), thus we attributed the Bacillus sp. 2B strain to the species B. subtilis.

Identification of PKS genes in the genomes of Bacillus and Paenibacillus strains. Molecular genetic analysis indicated 43 nucleotide sequences that were 96–100% similar to published sequences. All identified gene fragments were assigned to the modular type I PKS. Table IV shows the closest homologues determined by the BLAST-analysis and Table V presents the polyketide synthase genes identified.

We determined eight nucleotide sequences of PKS genes for both Paenibacillus spp. 5A and 12A (Table IV); the closest homologues were obtained from Paenibacillus polymyxa and P. peoriae. Among the homologous sequences obtained from the 12A and 5A strains, there were genes for the synthesis of antibiotics (difficidin, erythromycin, bacillaene, batumin, and

Table III

| Characteristic | Paenibacillus spp. | Bacillus spp. |
|----------------|-------------------|--------------|
|                | 5A | 12A | 7A | 2A | 9A | 2B |
| Phosphatase    | -  | -   | +  | +  | +  | +  |
| Catalase       | +  | +   | +  | +  | +  | +  |
| Oxidase        | -  | +   | -  | -  | -  | -  |
| Gelatinase     | -  | -   | +  | +  | -  | -  |
| Caseinase      | +  | +   | +  | +  | +  | +  |
| Amylase        | +  | +   | +  | +  | +  | +  |
| Lecithinase    | +  | +   | -  | +  | +  | +  |
| Lipase         | -  | -   | -  | -  | -  | -  |
| Saccharose     | +  | +   | +  | -  | -  | +  |
| Glucose        | +  | +   | -  | -  | +  | +  |
| Maltose        | +  | +   | -  | -  | -  | -  |
| Fructose       | +  | +   | +g | -  | +  | +  |
| Galactose      | +g | +g  | +  | -  | -  | -  |
| Lactose        | +  | +   | +g | -  | -  | -  |

*+g – sugar fermentation together with the formation of acid and gas.*
ogy (97%). In addition, PKS sequences of we detected erythronolide synthase with high homology (bacillaene and difficidine) and antitumor agent (calyculin and bryostatin) (Table V).

Comparative analysis indicated that all the closest homologues were obtained from \textit{Bacillus} \textit{graminis} and \textit{bacillaene} (Table V). Additionally, among the related sequences, there were synthases of the antibiotic responsible for the synthesis of antibiotics (difficidine). Among them, \textit{PKS} genes obtained from \textit{Bacillus} \textit{sp.} 9A strain, we detected four \textit{PKS} genes with high homology (94–98%) that were closest relatives isolated from marine sponges (Zhang et al. 2009). Moreover, we detected genes of enzymes involved in the production of antibiotics (bacillaene and difficidine) and an antitumor agent (calyculin); homology was 70–87% (Table V).

For \textit{Bacillus} sp. 9A strain, we detected four \textit{PKS} sequences (Table IV). They were homologous to the \textit{PKS} genes isolated from \textit{Bacillus} \textit{sp.} 7A strain, we detected four \textit{PKS} genes with high homology (94–98%) that were closest relatives isolated from marine sponges (Zhang et al. 2009). Moreover, we detected genes of enzymes involved in the production of antibiotics (bacillaene and difficidine) and an antitumor agent (calyculin); homology was 70–87% (Table V).

Identification of NRPS genes in the genomes of \textit{Bacillus} and \textit{Paenibacillus} strains. Molecular genetic

### Table IV

| Strain name | Clone number | Results of BLAST analysis |
|-------------|--------------|--------------------------|
| \textit{Paenibacillus} spp. 5A and 12A | 5A-1, 5A-2, 5A-3, 5A-4, 5A-7, 5A-8, 12A-2, 12A-9 | ACN13122 ketosynthase \textit{[Streptomyces sp. G2-4]} |
| | | WP_053325747 polyketide synthase \textit{[P. peoriae]} |
| | | WP_013310977 polyketide synthase \textit{[P. polymyxa]} |
| | 5A-5, 12A-1, 12A-5, 12A-6 | ACX31707 ketosynthase \textit{[Streptomyces sp. 28HAO]} |
| | | WP_013310977 polyketide synthase \textit{[P. polymyxa]} |
| | | WP_053325747 polyketide synthase \textit{[P. peoriae]} |
| | 5A-6 | WP_013310977 polyketide synthase \textit{[P. polymyxa]} |
| | | WP_053325747 polyketide synthase \textit{[P. peoriae]} |
| | 12A-7, 12A-10 | WP_05332574 polyketide synthase \textit{[P. peoriae]} |
| | | WP_023989388 polyketide synthase \textit{[P. polymyxa]} |
| | 12A-8 | WP_05332574 polyketide synthase \textit{[P. peoriae]} |
| | | WP_023989388 polyketide synthase \textit{[P. polymyxa]} |
| \textit{Paenibacillus} sp. 7A | 7A-1, 7A-2, 7A-5, 7A-7 | AIQ67612 erythronolide synthase \textit{[P. graminis]} |
| | 7A-3, 7A-4, 7A-6 | WP_042266339 NRPS/PKS-synthase \textit{[P. graminis]} |
| | 7A-8 | WP_042266418 polyketide synthase \textit{[P. graminis]} |
| \textit{Bacillus} spp. 2A and 2B | 2A-1, 2A-3, 2A-4, 2A-6, 2A-7, 2A-8, 2B-3, 2B-4 | ABR19768 polyketide synthase \textit{[B. subtilis]} |
| | 2A-2 | ABR19764 polyketide synthase \textit{[B. subtilis]} |
| | | ABR19779 polyketide synthase \textit{[Actinomycetales bacterium DA20]} |
| | 2B-1 | WP_032721576 polyketide synthase \textit{[B. subtilis]} |
| | | AGA23985 NRPS/PKS-synthase \textit{[B. subtilis subsp. subtilis BSP1]} |
| | 2B-2 | ABR19775 polyketide synthase \textit{[B. subtilis]} |
| | 2B-5 | WP_043940121 polyketide synthase \textit{[Bacillus sp. YP1]} |
| | | WP_009967299 polyketide synthase \textit{[B. subtilis]} |
| | 2B-6, 2B-8 | ACG70843 polyketide synthase \textit{[Bacillus sp. WPhG3]} |
| | | ABR19767 polyketide synthase \textit{[B. subtilis]} |
| \textit{Bacillus} sp. 9A | 9A-1 | AIO09652 ketosynthase \textit{[Bacillus sp. LX-110]} |
| | 9A-3, 9A-5 | ACG70841 polyketide synthase \textit{[Bacillus sp. WPysW2]} |
| | | AGL92430 polyketide synthase \textit{[B. amyloliquefaciens]} |
| | 9A-10 | WP_016936042 polyketide synthase \textit{[B. siamensis]} |
| | | WP_047474891 polyketide synthase \textit{[B. amyloliquefaciens]} |
PKS-NRPS genes in microorganisms

Table V
Comparative analysis of the sequences of PKS genes from heterotrophic bacteria.

| Strain            | Clone number | Results of BLAST analysis                                                                 |
|-------------------|--------------|------------------------------------------------------------------------------------------|
| *Paenibacillus* 5A and 12A | 5A-1, 5A-2, 5A-3, 5A-4, 5A-7, 5A-8, 12A-2, 12A-9 | EID67453 difficidin synthase, (DfnD) [Bacillus sp. 916] 71% |
|                   | 5A-5, 12A-1, 12A-5, 12A-6 | EIF13796 difficidin synthase, (DfnG) [Bacillus sp. 586] 71% |
|                   |                           | RAP05593 calyculin synthase, (CalE), uncultured [Entotheonella sp.] 71% |
|                   |                           | ADN68476 sorangicin synthase, (SorA) [Sorangium cellulosum So ce12] 71% |
|                   | 5A-6                     | WP_004619353 erythronolid synthase, [Clostridium papyrosolvens DSM 2782] 75% |
|                   |                           | EIF13279 bacillaene synthase, (BaeL) [Bacillus sp. 586] 73% |
|                   | 12A-7, 12A-10            | ADD82940 batumin synthase, (Bat2) [P. fluorescens BCCM_ID9359] 70% |
|                   |                           | ABK51300 bryostatin synthase, (BryC) [Endobugula sertula] 69% |
|                   | 12A-8                    | EIF13279 bacillaene synthase, (BaeL) [Bacillus sp. 586] 72% |
|                   |                           | ADN68477 sorangicin synthase, (SorB) [Sorangium cellulosum So ce12] 72% |
| *Paenibacillus* sp.7A | 7A-1, 7A-2, 7A-5, 7A-7 | AQ67612 erythronolid synthase, [P. graminis DSM 15220] 97% |
|                   | 7A-3, 7A-4, 7A-6         | CUB31962 pilipastatin synthase, [B. amyloliquefaciens] 53% |
|                   |                           | WP004618786 erythronolid synthase, [Clostridium papyrosolvens DSM 2782] 50% |
|                   | 7A-8                     | WP_013663185 erythronolid synthase, [Marinomonas mediterranea MMB-1] 43% |
|                   |                           | AD12491 epothilone synthase, (EpoD) [Sorangium cellulosum KYC3013] 43% |
| *Bacillus* spp. 2A and 2B | 2A-1, 2A-3, 2A-4, 2A-6, 2A-7, 2A-8, 2B-3, 2B-4 | EID66458 bacillaene synthase, (BaeN) [Bacillus sp. 916] 85% |
|                   | 2A-2                     | RAP05593 calyculin synthase, (CalE), [Entotheonella sp.] 71% |
|                   |                           | EID67453 difficidin synthase, (DfnD) [Bacillus sp. 916] 70% |
|                   | 2B-1                     | EIF13279 bacillaene synthase, (BaeL) [Bacillus sp. 586] 85% |
|                   | 2B-2                     | EIF13280 bacillaene synthase, (BaeM) [Bacillus sp. 586] 84% |
|                   | 2B-5                     | EIF13279 bacillaene synthase, (BaeL) [Bacillus sp. 586] 71% |
|                   | 2B-6, 2B-8               | EID66458 bacilaene synthase, (BaeN) [Bacillus sp. 916] 87% |
|                   | 2B-7                     | EIF13279 bacilaene synthase, (BaeL) [Bacillus sp. 586] 86% |
|                   |                           | AFZ90784 bacilaene synthase, (BaeL) [B. methylotrophicus AS43.3] 86% |
| *Bacillus* sp. 9A | 9A-1                     | WP_049628737 difficidin synthase, [Bacillus sp. [FL15]] 98% |
|                   | 9A-3, 9A-5               | EID66458 bacilaene synthase, (BaeN) [Bacillus sp. 916] 94% |
|                   | 9A-10                    | EIF13279 bacilaene synthase, (BaeL) [Bacillus sp. 586] 97% |

Analysis indicated 17 nucleotide sequences of the NRPS gene fragment that were 95–100% similar to published sequences (Table VI).

For the *Paenibacillus* sp. 12A strain, we obtained five nucleotide sequences of the NRPS gene fragment which had homologues isolated from *Paenibacillus polymyxa* (Table VI). The homologous sequences included genes for the synthesis of antibiotics (bacitracin, fusaricidin, tridecaptin, and bacillorin). In *Paenibacillus* sp. 5A, two sequences of the NRPS gene fragment were detected. The homologous sequences included genes for the synthesis of antibiotics (bacitracin and fusaricidin) and low homology to fengycin (Table VI).

We identified two sequences of the NRPS gene fragment in *Bacillus* sp. 2B strain. Among the homologues, there were genes coding for the enzymes responsible for the synthesis of biosurfactants (plipastatin and suraclin) (Table VI).

Three sequences of the NRPS gene fragment were determined *Bacillus* sp. 2A strain. The homologous sequences included genes for the synthesis of an antibiotic (bacillaene) and biosurfactants (plipastatin, fengycin, and surfactin) (Table VI).

We detected four sequences of the NRPS gene fragment in *Bacillus* sp. 9A strain. The homologous sequences included genes responsible for the synthesis...
of antibiotic (bacillaene) and biosurfactants (bacillomycin, surfactin, and iturin) (Table VI).

Phylogenetic analysis of amino acid sequences of the KS-domain fragments of PKS genes (Fig. 3) and A-domain of NRPS genes (Fig. 4) in the bacteria isolated from the epilithic biofilms of Lake Baikal showed that sequences from different strains clustered together. It means that enzyme complexes of such strains as *Bacillus* spp. 2A and 2B, *Paenibacillus* spp. 5A and 12A were similar. On the other hand, different sequences were obtained from one strain. It means that this strain, e.g. *Bacillus* sp. 9A, possessed several enzyme complexes.

**Discussion**

The results of PCR screening showed that PKS and NRPS genes in members of *Bacillus*, *Paenibacillus*, and *Pseudomonas* from Lake Baikal were more frequent than in other heterotrophic bacteria isolated from biofilms. The high occurrence of the BAS genes found in Baikal isolates is typical of the members belonging to these genera, since they are well-known producers of various secondary metabolites. For example, many *Bacillus* species produce such antibiotics as bacillaene, difficidine, macrolactin, mycosubtilin, bacillomycin, iturin, bacitracin, and gramicidin C (Fickers 2012). *Paenibacillus* strains isolated from various habitats synthesize antibiotics of a peptide or macrolide nature: polymyxins A-E, paenibacillin, jolipeptin, gavaserin, saltavalin, fusaricidin A-D, gatavalin, paenimacrolidine, paenilamicin, and others (Wu et al. 2011; Aleti et al. 2015). A review by Zhao and Kuipers (2016) represented the analysis of 328 full genomes of 57 species of the family *Bacillaceae*, including 30 species of the genus *Bacillus* and 16 species of *Paenibacillus*. NRPS gene clusters were present in 70% of the tested genomes.
Fig. 3. Neighbour-joining phylogenetic tree based on amino acid sequences of the KS-domain fragments of PKS genes in bacteria isolated from the epilithic biofilms of Lake Baikal (in bold). The scale bar represents 0.1 amino acid substitutions per site.
and only 50% of the analyzed species had genes encoding PKS (Zhao and Kuipers, 2016). In total, 1231 gene clusters for putative non-ribosomal antimicrobials were identified and combined into 23 types of NRPS, five types of PKS, and three types of hybrid synthesized NRPS/PKS compounds distributed across 49 Bacillales species. Previously, other authors also noted the high content of NRPS and PKS genes in bacilli (Aleti et al. 2015). In addition, a high percentage of isolates (85%) containing one or both metabolic clusters were isolated from the rhizosphere (Aleti et al. 2015). The authors noted that this was due to a more detailed study of the rhizosphere as an important subject in agriculture; hence, these genes may be also characteristic of bacilli from other ecological niches. For instance, this study on Bacillus and Paenibacillus strains from freshwater reservoirs has shown that they also contain NRPS and PKS genes.

Metabolites produced by B. amyloliquefaciens and B. subtilis represent a bulk of the studied diversity of polyketides and lipopeptides from the genus Bacillus (Aleti et al. 2015). These two species are used to obtain most of the commercially available substances contributing to the plant growth and biocontrol (against phy-
topathogens) in agriculture. They produce three types of polynye polyketides, including bacillaene, difficidine, and macroactin. At present, two polyketides (paenimacrolidine and paenilaminic) have been described for the genus *Paenibacillus* (Aleti et al. 2015).

In *Bacillus* and *Pseudomonas*, the NRPS genes mainly encode for the synthesis of lipopeptide biosurfactants (LPBS) (Roongsawang et al. 2010). Due to their complex and diverse structures, lipopeptides demonstrate various biological activities, including surface activity, as well as antitubercular and antifungal activity. Lipopeptides are involved in multicellular behaviour, such as swarming motility and biofilm formation. Among the producers, the genera *Bacillus* and *Pseudomonas* are of special interest, since they produce a wide range of effective LPBS, which are potentially useful for agricultural, chemical, food, and pharmaceutical industries (Roongsawang et al. 2010). NRPS clusters of the genus *Bacillus* encode lipopeptide families of surfactin, fengycin, iturin, and kurstatin (Aleti et al. 2015).

The results of this study indicate that heterotrophic bacteria isolated from epilithic biofilms in Lake Baikal are potential producers of secondary metabolites, for which the synthesis involves PKS and NRPS gene clusters.

Identification of PKS genes has shown that *Bacillus* sp. strain 9A contains sequences in the genome that are related to the genes known for the synthesis of antibiotics bacilaene (*baeL*, *baeN*) and difficidine, which can indicate their ability to produce these compounds whereas *Bacillus* sp. 2A and 2B contains only bacilaene (*baeL*, *baeM*, *baeN*). Bacilaene is a polynye antibiotic and it was first found in the culture medium of *B. subtilis* 3610 and 55422 strains (Fickers 2012; Aleti et al. 2015). Its biosynthesis was described in *B. amyloliquefaciens* FZB42 and is encoded by a hybrid cluster of PKS-NRPS genes called bae. This cluster has a similar structure to the pksX cluster of *B. subtilis* 168 strain, which is also likely to encode bacilaene. The bae gene cluster contains five open reading frames, i.e. *baeL*, *baeM*, *baeN*, and *baeR* (Aleti et al. 2015). Difficidine is a macrocyclic polynye synthesized by *B. amyloliquefaciens* ATCC 39320 and ATCC 39374 strains. It is encoded by the dif gene cluster with 14 open reading frames, from *difA* to *difN* and *difY*. Difficidine and bacilaene exhibit antimicrobial activity against a wide range of pathogenic bacteria by inhibiting protein synthesis (Fickers 2012; Aleti et al. 2015).

Another strain, *Paenibacillus* sp. 7A, has genes with high homology to erythronolide synthase responsible for the biosynthesis of macrolide 6-desoxy-erythronolide B, which is the precursor of the well-studied and widely known antibiotic erythromycin. It was first isolated in 1949 from the culture liquid of a *Surchoarayropsora erythraea* strain (Liu et al. 2013). The effect of this antibiotic is due to binding to the 50S ribosome subunit, which disrupts the formation of peptide links between amino acid molecules and blocks peptide synthesis in microorganisms.

Despite the high percentage of similarity (96–100%) with the closest relatives of PKS genes from *Paenibacillus* spp. 5A and 12A strains, the homologues had low similarity with the identified polyketide synthases (69–75%). It is likely that these genes have not been characterized yet, and these strains can produce novel and previously undescribed secondary metabolites.

Identification of NRPS genes showed that the sequences from *Paenibacillus* spp. 5A and 12A had high homology with their closest relatives, among which there were genes encoding for the synthesis of peptide and lipopeptide antibiotics (bacitracin, bacillorin, fusaricidin, and tridecaptin).

Bacitracin is a polypoly peptide antibiotic and a mixture of related cyclic peptides produced by *B. subtilis* strains. Bacitracin is active against Gram-positive bacteria. It was first isolated in 1945. It is usually used for topical treatment of skin, eye or nose diseases, but it can also be used internally in the form of an injection as an intestinal antiseptic. Due to its toxic effect on kidneys, bacitracin is used only when other antibiotics are ineffective. Its action involves breaking the synthesis of the cell wall by inhibiting lipid carriers (Johnson et al. 1945; Karala and Ruddock 2010; Ciesiolka et al. 2014). Moreover, bacitracin degrades nucleic acids, in particular RNA, through a hydrolytic mechanism (Ciesiolka et al. 2014). Bacillorin and bacillomycin L should be considered as synonymous names for a single molecule.

Fusaricidins are depsipeptide antibiotics synthesized by the members of the genus *Paenibacillus*. They have a ring structure. These antibiotics have high antifungal activity against plant pathogenic fungi, such as *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus oryzae*, and *Penicillium thomii*. Fusaricidins also have good bactericidal activity against Gram-positive bacteria, such as *Staphylococcus aureus* (Li et al. 2007; Choi et al. 2008).

Tridecaptins are a class of linear cationic lipopeptides exhibiting strong activity against multidrug-resistant Gram-negative bacteria. At the same time, they show low cytotoxicity and hemolytic activity. Tridecaptins are produced by *Paenibacillus polymyxa* strain (Cochrane et al. 2015).

Most NRPS gene sequences from *Bacillus* spp. 9A, 2A and 2B strains were homologous with the sequences responsible for the synthesis of different lipopeptide biosurfactants, such as fengycin, bacillomycin, lipastaatin, surfactin, and iturin. Notably, the closest relatives of the sequences of NRPS gene fragments from *Bacillus* spp. 9A and 2A strains included PKS genes responsible for the synthesis of bacilaene. The identification of PKS genes also indicated the genes responsible for the synthesis of this antibiotic. As mentioned above, a type
I PKS-NRPS hybrid gene cluster is responsible for its synthesis. Therefore, in the strains studied by two different pairs of primers, we detected the genes responsible for the synthesis of bacilamine.

The fengycin family includes fengycin and plipastatin, which are cyclic lipopeptides produced by B. subtilis (Bie et al. 2009). Natural fengycin is a mixture of isoforms, which differ slightly in their physicochemical properties due to variations in the chain length and branching of its hydroxy fatty acid component (Bie et al. 2009). Fengycin specifically inhibits filamentous fungi; its hemolytic activity is 40-fold less than that of surfactin (Bie et al. 2009).

Plipastatin, an antifungal antibiotic, is one of the most important non-ribosomal lipopeptides produced by B. subtilis. Plipastatin is involved in inhibition of phospholipase A2 and biofilm formation (Ratool et al. 2011). It is produced by different strains of Bacillus species and shows moderate surfactant properties. It is an antifungal metabolite and inhibits filamentous fungi, but it has no effect on yeast and bacteria (Romero et al. 2007; Chen et al. 2009).

The iturin family includes compounds of iturin and bacillomycin. Both are cyclic lipopeptides produced by B. subtilis, and they exhibit strong antifungal properties (Peypourx et al. 1981; Zhang et al. 2013). Iturin has low toxicity in mammals and shows strong antibiotic activity, thus making it potentially a useful and effective substance for biological control to reduce the use of chemical pesticides in agriculture (Romero et al. 2007; Ongena and Jacques 2008; Kim et al. 2010; Zhang et al. 2013).

The surfactin family are structurally cyclic peptides with a multiple biological activity produced by some B. subtilis strains (Cosmina et al. 1993; Ongena and Jacques 2008). Surfactin is a strong surface-active compound. It can lyse erythrocytes and protoplasts of bacteria. Additionally, surfactin inhibits the thrombin-fibrinogen interaction, thus slowing the formation of fibrin. This property defines it as a possible component in the development of anticoagulants for the prevention of thromboses and diseases, such as myocardial infarction, pulmonary embolism, etc. Surfactin exhibits anti-cholesterol activity and decreases the level of cholesterol in the plasma and liver. It has antitumor, fungicidal, and antibiotic activity. Many useful physicochemical characteristics of this substance indicate that it can be widely used in the pharmaceutical, technical, and environmental fields.

In this study, we showed the presence of RKS and NRPS genes in the genomes of heterotrophic bacteria isolated from epibiotic biofilms in Lake Bai-kal. The occurrence of these genes in bacteria of the genera Bacillus and Paenibacillus was higher than in other bacterial groups. Comparative analysis of the obtained amino acid sequences showed a wide variety of the genes. These sequences were related to the genes involved in biosynthesis of antibiotics (bacilamine, difficidine, erythromycin, sorangicin, and batumin), biosurfactants (fengycin, bacillomycin, plipastatin, surfactin, and iturin) and antitumor agents (epothilone, calyculin, and briostatin). Bacillus sp. 9A (iturin, bacillomycin, surfactin, bacilamine, and difficidine) and Bacillus sp. 2A (plipastatin, bacilamine, surfactin, fengycin, and difficidine) showed the highest variety of PKS and NRPS genes. Furthermore, the investigated strains exhibited multiple enzymatic and antagonistic activities, indicating that they are potential producers of bioactive metabolites. Therefore, Baikal representatives of the genera Bacillus and Paenibacillus can be of practical interest for biotechnological purposes. To confirm our assumptions, it is necessary to obtain individual compounds and determine their structure, as well as study biological activity.

Acknowledgements

The reported study was supported by the framework of the state task on the topic No. 0345-2016-0003 (AAAA-A16-116122110061-6) ‘Microbial and viral communities in biofilms…’ with partial financial funding from RFBR (research project No. 18-34-00443). The results of PCR screening and the identification of PKS and NRPS genes in the genomes of heterotrophic bacteria were obtained under the RFBR grant.

The authors are thankful to Kirilchik S. for his assistance in sequencing the PKS genes.

Conflict of interest

Author does not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Aleti G, Sessitsch, A, Brader G. 2015. Genome mining: prediction of lipopeptides and polyketides from Bacillus and related Firmicutes. Comput Struct Biotechnol J. 13:192–203.

Banskota AH, Mcalpine JB, Sørensen D, Ibrahim A, Aouidate M, Farnet CM, Zazopoulos E. 2006. Genomic analyses lead to novel secondary metabolites. Part 3. ECO-0501, a novel antibacterial of a new class. J Antibiot. 9:533–542.

Bartrons M, Catalan J, Casamayor EO. 2012. High bacterial diversity in epibiotic biofilms of oligotrophic mountain lakes. Microb Ecol. 65:203–218.

Batool M, Khalid MH, Hassan MN, Hafeez FY. 2011. Homology modeling of an antifungal metabolite plipastatin synthase from the Bacillus subtilis 168. Bioinformation 7(8):384–387.

Berger O, Guinebretière MH, Achouak W, Normand P, Heulin T. 2002. Paenibacillus graminis sp. nov. and Paenibacillus odorifer sp. nov., isolated from plant roots, soil and food. Int J Syst Ecol. 52:607–616.

Bie X, Lu Z, Lu F. 2009. Identification of fengycin homologues from Bacillus subtilis with ESI-MS/CID. J Microbiol Methods. 79:272–278.

Brosius J, Dull TJ, Sleeter DD, Noller HF. 1981. Gene organization
PKS-NRPS genes in microorganisms

and primary structure of a ribosomal RNA operon from *Escherichia coli*. J Mol Biol. 148:107–127.

Ciesielska J, Jezowska-Bojczuk M, Wrzesinski J, Stokowa-Soltys K, Nagaj J, Kasperowicz A, Blaszczuk I, Szczepunek W. 2014. Antibiotic bacitracin induces hydrolytic degradation of nucleic acids. Biochim Biophys Acta. 1840(6):1782–1789.

Chen XH, Komoetoussi A, Scholz R, Borris R. 2009. More than anticipated – production of antibiotics and other secondary metabolites by *Bacillus amyloliquefaciens* FZB42. J Mol Microbiol Biotechnol. 16(1–2):14–24.

Choi SK, Park SY, Kim R, Lee CH, Kim JI, Park SH. 2008. Identification and functional biosynthesis of the fusaricidin biosynthetic gene of *P. polymyxa*. E681. Biochem Biophys Res Commun. 365(1):89–95.

Cochrane SA, Lohans CT, van Belkum MJ, Bels MA, Vederas JC. 2015. Studies on tridecapetide B1, a lipopeptide with activity against multidrug resistant Gram-negative bacteria. Org Biomol Chem. 13:6073–6081.

Cosmina P, Rodríguez F, de Ferra F, Grandi G, Peregó M, Venema G, van Sinderen D. 1993. Sequence and analysis of the genetic locus responsible for surfactin synthesis in *B. subtilis*. Mol Microbiol. 8(5):821–831.

Costerton JW, Cheng KJ, Geesey GG, Ladd TJ, Nickel JC, Daga- mpta M, Marrie TJ. 1987. Bacterial biofilms in nature and disease. Annu Rev Microbiol. 41:435–464.

Dai Y, Zhang X, Jia Z, Jin Z. 2012. Type III Polyketide Syntheses in Natural Product Biosynthesis. J UBM Life. 64(4):285–295.

Dolnan RM, Costerton JW. 2002. Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms. Clin Microbiol Rev. 15(2):167–193.

Ehrenreich I, Waterbury J, Webb E. 2005. Distribution and diversity of natural product genes in marine and freshwater cyanobacterial cultures and genomes. Appl Environ Microbiol. 71(11):7401–7413.

Fickers P. 2012. Antibiotic compounds from *Bacillus*: Why are they so amazing? Am J Biochem Biotechnol. 1:40–46.

Gupta M, Marrie TJ. 2007. Use of PCR-targeted Biotechnol. 20(1):138–145.

Hill RT, Vicente F, Reyes F, Genilloud O. 2013. Sponge-derived *Kocuria* and *Micrococcus* spp. as sources of the new thiazolyl peptide antibiotic kocurin. Mar Drugs. 11(8):2846–2872.

Netrusov AI. 2003. Praktikum po mikrobiologii (Practical Course in Microbiology). Moscow (Russia): Akademiya. Ongena M, Jacques P. 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 16(3):115–125.

Palomo S, González I, Cruz M, Martín I, Tormo JR, Anderson M, Hill RT, Vicente F, Reyes F, Genilloud O. 2013. Sponge-derived *Kocuria* and *Micrococcus* spp. as sources of the new thiazolyl peptide antibiotic kocurin. Mar Drugs. 11:8071–1086.

Parfenova VV, Mal’nik VV, Boiko SM, Sheveleva NG, Logacheva NF, Evstigneeva TD, Suturin AM, Timoshkin OA. 2008. Communities of hydrobionts developing at the water-rock interface in Lake Baikal. Russ J Ecol. 39(3):198–204.

Parfenova VV, Gladkikh AS, Belykh OL. 2013. Comparative analysis of biodiversiy in the planktonic and biofilm bacterial communities in Lake Baikal. Microbiology. 82(1):91–101.

Peypoux F, Besson F, Michel G, Delcambe L. 1981. Structure of bacillicin D, a new antibiotic of the iturin group. Eur J Biochem. 118:323–327.

Romani AM, Sabater S. 2001. Structure and activity of rock and sand biofilms in a Mediterranean stream. Ecology. 82:3232–3245.

Romani AM, Guasch H, Dolores Balaguer M. 2016. Aquatic Biofilms. Ecology, water quality and wastewater treatment. Norfolk (UK): Caister Academic Press 230 p.

Romer D, de Vicente A, Rakotoaly RH, Dufour SE, Veening JW, Arrerbole A, Cazorla FM, Kuipers OP, Paquot M, Pérez-Garcia A. 2007. The iturin and fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* toward *Pedobacter fusca*. Mol Plant Microbe Interact. 20(4):430–440.

Roongsawang N, Washio K, Morikawa M. 2010. Diversity of non-ribosomal peptide synthetases involved in the biosynthesis of lipopeptide biosurfactants. Int J Mol Sci. 12(1):141–172.

Spona F, Cavaletti I, Lazzarini A, Borghi A, Ciciliato I, Losi D, Marinelli F. 1999. Biodiversity and potentials of marine-derived microorganisms. J Biotechnol. 71(1):1–12.

Staunton J, Wilkinson B. 2001. Combinatorial biosynthesis of polyketides and non-ribosomal peptides. Curr Opin Chem Biol. 5:159–164.

Sukhanova EV, Zimens EA, Parfenova VV, Belykh OL. 2017. Diversity of polyketide synthase genes in the genomes of heterotrophic microorganisms isolated from epilithic biofilms of Lake Baikal. Mol Plant Microbe Interact. 20(4):430–440.
Gastropoda in the shallow littoral zone of Lake Baikal (East Siberia) as evidenced by underwater macrophotograph analysis. Berliner Palaeobiologische Abhandlungen Berlin. 4:193–200.

Wu XC, Qian CD, Fang HH, Wen YP, Zhou JY, Zhan ZJ, Ding R, Li O, Gao H. 2011. Paenimacroladin, a novel macrolide antibiotic from Paenibacillus sp. F6–B70 active against methicillin-resistant Staphylococcus aureus. Microb Biotechnol. 4:491–502.

Zhang B, Dong C, Shang Q, Cong Y, Kong W, Li P. 2013. Purification and partial characterization of bacillomycin L produced by Bacillus amylobacteriaceus K103 from lemon. Appl Biochem Biotechnol. 171:2262–2272.

Zhang W, Zhang F, Li Z, Miao X, Meng Q, Zhang X. 2009. Investigation of bacteria with polyketide synthase genes and antimicrobial activity isolated from South China Sea sponges. Appl Microbiol. 107:567–575.

Zhao X, Kuipers OP. 2016. Identification and classification of known and putative antimicrobial compounds produced by a wide variety of Bacillales species. BMC Genomics. 17:882.

Zimens EA, Sukhanova EV, Shtykova YR, Parfenova VV, Belkova NL. 2014. Antagonistic activity of heterotrophic microorganisms isolated from biofilms of hard substrates in littoral zone of Lake Baikal. ISU Proceedings Series 'Biology. Ecology'. 7:91–98.