

Epilithic Biofilms in Lake Baikal: Screening and Diversity of PKS and NRPS Genes in the Genomes of Heterotrophic Bacteria

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Abstract

A collection of heterotrophic bacteria consisting of 167 strains was obtained from microbial communities of biofilms formed on solid substrates in the littoral zone of Lake Baikal. Based on the analysis of 16S rRNA gene fragments, the isolates were classified to four phyla: *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes*. To assess their biotechnological potential, bacteria were screened for the presence of PKS (polyketide synthase) and NRPS (non-ribosomal peptide synthetases) genes. PKS genes were detected in 41 strains (25%) and NRPS genes in 73 (43%) strains by PCR analysis. The occurrence of PKS genes in members of the phylum *Firmicutes* (the genera *Bacillus* and *Paenibacillus*) was 34% and NRPS genes were found in 78%. In *Proteobacteria*, PKS and NRPS genes were found in 20% and 32%, and in 22% and 22% of *Actinobacteria*, respectively. For further analysis of PKS and NRPS genes, six *Bacillus* and *Paenibacillus* strains with antagonistic activity were selected and underwent phylogenetic analysis of 16S rRNA genes. The identification of PKS and NRPS genes in the strains investigated was demonstrated among the homologues the genes involved in the biosynthesis of antibiotics (bacillaene, diffridone, erythromycin, bacitracin, tridecaptin, and fusaricidin), biosurfactants (iturin, bacillomycin, plipastatin, fengycin, and surfactin) and antitumor agents (epothilone, calyculin, and briostatine). *Bacillus* spp. 9A and 2A strains showed the highest diversity of PKS and NRPS genes. *Bacillus* and *Paenibacillus* strains isolated from epilithic biofilms in Lake Baikal are potential producers of antimicrobial compounds and may be of practical interest for biotechnological purposes.

Key words: polyketide synthase genes, non-ribosomal peptide synthetases, epilithic biofilms, Lake Baikal, secondary metabolites

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 repertoire; 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 ‘megasyntases’, including PKS, NRPS and their NRPS/PKS hybrid complexes,

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synthesise 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), acyltransferase (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, acridones, 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 hydrobionts, 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 baicalensis* 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. baicalensis*, PKS and NRPS genes were detected in nine out of 14 cultures of the genera *Bacillus*, *Pseudomonas*, *Variovorax*, *Curtobacterium*, and *Rhodococcus* (Kalyuzhnaya 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 Beryozovy, 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 with 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 agar 15), PCA (HiMedia, India), and TSA (HiMedia, India). The duration of incubation was 5–7 days at 20–22°C. Pure cultures were obtained by depleting inoculations to individual colonies.

The average number of colonies obtained during cultivation on each medium was: R2A – 5.6, NSY – 4.9, PCA – 3.3 and TSA – 1.9 ($\times 10^5$ CFU/cm²). Bacterial colonies with differing morphology were isolated into pure cultures on the nutrient media: R2A – 64 strains, PCA – 48 strains, NSY – 35 strains and TSA – 20 strains. As a result, we obtained a collection of heterotrophic bacteria of 167 strains.

Molecular genetic identification of strains by the 16S rRNA gene fragment. DNA from the day-old bacterial cultures was isolated using the DNA-sorb-B kit according to the manufacturer's protocol (PE CRIE of Rospotrebnadzor, Moscow, Russia). The obtained template was used in the polymerase chain reaction (PCR); target amplicons of the 16S rRNA gene fragment were obtained using the conservative bacterial primers 27L (5'-AGAGTTTGATCATGGCTCAG-3') and 1542R (5'-AAGGAGGTGATCCAGCCS-3') (Brosius et al. 1981). The nucleotide sequences of the 16S rRNA gene fragments 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, LT555292, 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'-GTGCCGGTNC-CRTGNGYYTC-3') and DK-R (5'-GCGATGGAY-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-TAYGTNCC-3') and MT-R (5'-CCNCGDATYTTNACYTG-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-5 α 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 Sintol (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
PCR screening of heterotrophic strains isolated from biofilms of Lake Baikal for PKS and NRPS gene fragments.

Taxonomy		Number of the strains analyzed	Number of the strains with positive PCR signal	
Phylum	Genus		PKS	NRPS
<i>Firmicutes</i>	<i>Bacillus</i>	42	11	33
	<i>Paenibacillus</i>	4	4	4
	<i>Virgibacillus</i>	1	0	0
	<i>Staphylococcus</i>	2	0	1
<i>Proteobacteria</i>	<i>Pseudomonas</i>	45	9	26
	<i>Aeromonas</i>	29	4	2
	<i>Serratia</i>	3	2	1
	<i>Rhizobium</i>	1	1	0
	<i>Brevundimonas</i>	1	0	0
	<i>Massilia</i>	1	0	0
	<i>Achromobacter</i>	3	0	0
	<i>Stenotrophomonas</i>	3	0	0
	<i>Devosia</i>	1	1	0
	<i>Hydrogenophaga</i>	1	0	0
	<i>Yersinia</i>	1	1	0
	<i>Sphingomonas</i>	1	0	0
	<i>Iodobacter</i>	1	1	0
	<i>Roseomonas</i>	1	0	0
	<i>Actinobacteria</i>	<i>Rhodococcus</i>	2	0
<i>Kocuria</i>		4	2	1
<i>Pseudoclavibacter</i>		3	1	0
<i>Plantibacter</i>		1	0	0
<i>Sanguibacter</i>		1	0	0
<i>Pseudarthrobacter</i>		1	0	0
<i>Microbacterium</i>		4	1	1
<i>Salinibacterium</i>		1	0	0
<i>Streptomyces</i>		1	0	1
<i>Micrococcus</i>		1	0	0
<i>Brachybacterium</i>		1	1	0
<i>Clachiihabitans</i>		1	0	0
<i>Microcella</i>		1	0	0
<i>Bacteroidetes</i>	<i>Flavobacterium</i>	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*, *Devosia*, *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^T (Fig. 2). This strain, isolated from soil, is antagonistic against phytopathogenic bacteria and fungi (Jeong

Table II
Antagonist activity (Zimens et al. 2014), and PKS and NRPS genes in the strains isolated from biofilms of Lake Baikal.

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

* Test cultures used in this work: Bs1 – *Bacillus subtilis* VKPM; Bs2 – *Bacillus subtilis* DSM; Pa – *Pseudomonas aeruginosa* GISK L.A. Tarasevich 190158; Ca – *Candida albicans* ATCC 10231; Sa – *Staphylococcus aureus* (ATCC 25923 and MRSA); Ef – *Enterococcus faecium*; Ec1 – *Escherichia coli* K12 VKPM B-3254; Ec2 – *Escherichia coli* M17-02 VKPM B-8208

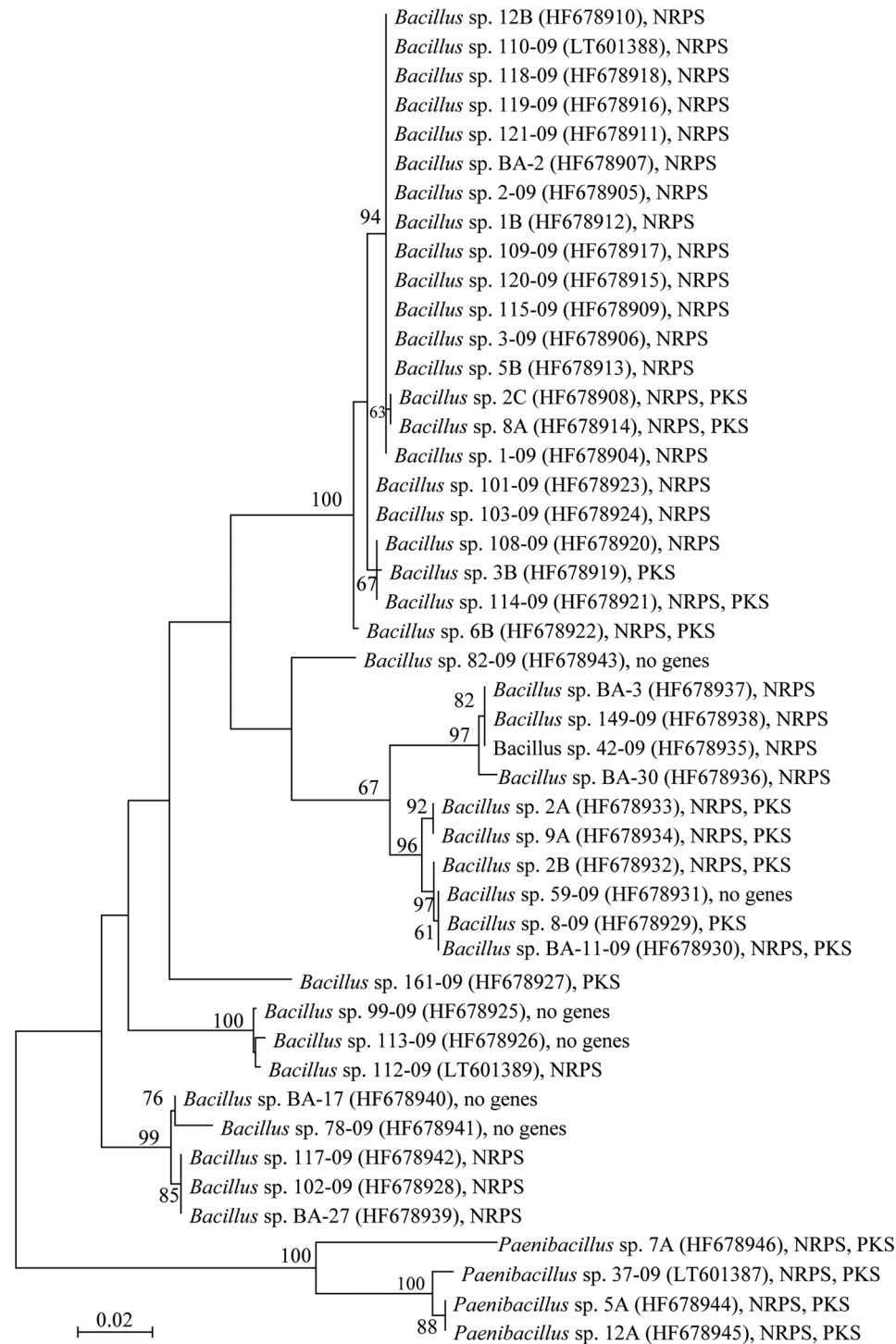


Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences (880 bp), showing the phylogenetic relationship between strains of the genera *Bacillus* and *Paenibacillus* isolated from epilithic biofilms in Lake Baikal. Accession numbers in GenBank are given in parentheses.

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* RSA19^T 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 clus-

ter with the type strain *Bacillus amyloliquefaciens* NBRC 15535^T (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* DSM10^T

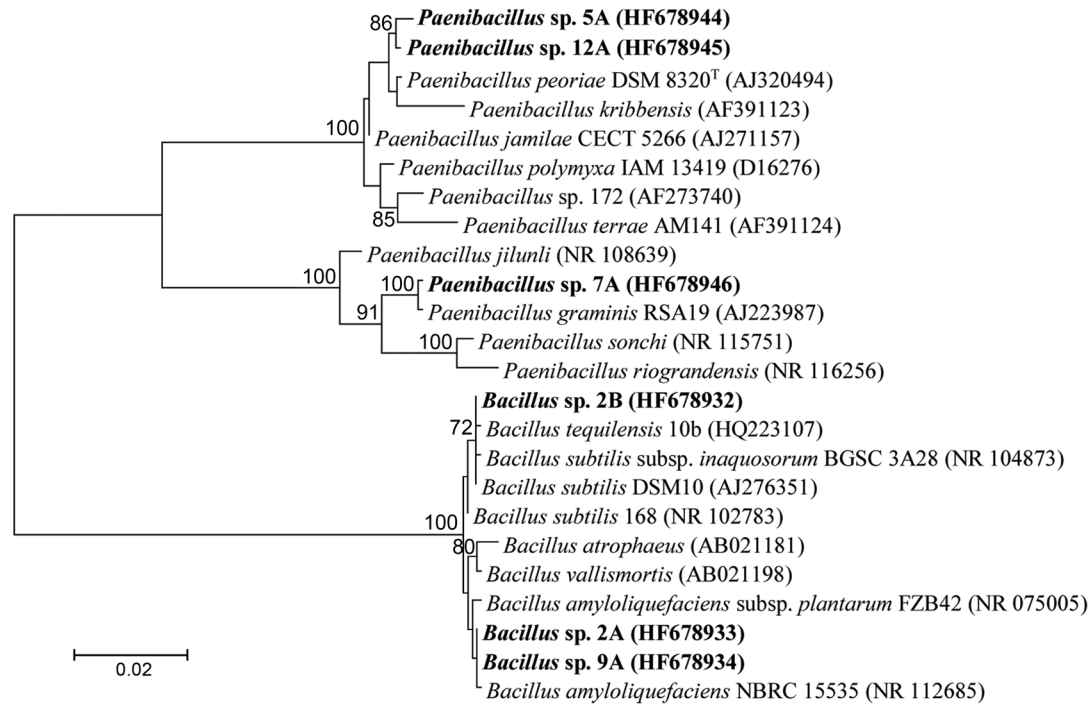


Fig. 2. Phylogenetic tree, based on 16S rRNA gene sequences (1360 bp), showing the phylogenetic relationship between the strains studied and other species of the genera *Bacillus* and *Paenibacillus*. Sequences obtained in this study are in bold, and accession numbers in GenBank are given in parentheses.

(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 deter-

mined 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 (difficidine, erythromycin, bacillaene, batumin, and

Table III
The strain screening for the presence of enzyme activity.

Characteristic	<i>Paenibacillus</i> spp.			<i>Bacillus</i> 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	-	-	-

Table III
Continued.

Characteristic	<i>Paenibacillus</i> spp.			<i>Bacillus</i> spp.		
	5A	12A	7A	2A	9A	2B
Arabinose	+	+	+	-	-	-
Raffinose	+	+	+	-	-	-
Rhamnose	+g	+g	+	-	-	-
Mannose	+	+	+	-	-	+
Xylose	+	+	+	-	-	-
Sorbitol	+	+	+	-	-	-
Inositol	+	+	+	-	-	+
Dulcete	+g	+g	+	-	-	-
Mannitol	+g	+g	+	-	-	-
Proline	-	-	+	+	+	+
Leucine	-	-	-	+	+	+
Phenylalanine	-	-	-	+	+	+
Alanine	-	-	-	+	+	+

*+g - sugar fermentation together with the formation of acid and gas.

Table IV
Comparative analysis of the sequences of PKS gene fragments from heterotrophic bacteria.

Strain name	Clone number	Results of BLAST analysis	
		Closest homologs	Homology, %
<i>Paenibacillus</i> spp. 5A and 12A	5A-1, 5A-2, 5A-3, 5A-4, 5A-7, 5A-8, 12A-2, 12A-9	ACN13122 ketosynthase [<i>Streptomyces</i> sp. G2-4]	99
		WP_053325747 polyketide synthase [<i>P. peoriae</i>]	
		WP_013310977 polyketide synthase [<i>P. polymyxa</i>]	98–100
	5A-5, 12A-1, 12A-5, 12A-6	ACX31707 ketosynthase [<i>Streptomyces</i> sp. 28HAO]	99
		WP_013310977 polyketide synthase [<i>P. polymyxa</i>]	99
	5A-6	WP_013310977 polyketide synthase [<i>P. polymyxa</i>]	97
		WP_053325747 polyketide synthase [<i>P. peoriae</i>]	97
	12A-7, 12A-10	WP_053325746 polyketide synthase [<i>P. peoriae</i>]	97
		WP_023989388 polyketide synthase [<i>P. polymyxa</i>]	97
	12A-8	WP_053325746 polyketide synthase [<i>P. peoriae</i>]	96
WP_023989388 polyketide synthase [<i>P. polymyxa</i>]		96	
<i>Paenibacillus</i> sp. 7A	7A-1, 7A-2, 7A-5, 7A-7 7A-3, 7A-4, 7A-6	AIQ67612 erythronolid synthase [<i>P. graminis</i>]	96–98
		WP_042266339 NRPS/PKS-synthase [<i>P. graminis</i>]	98–99
	7A-8	WP_042266418 polyketide synthase [<i>P. graminis</i>]	99
<i>Bacillus</i> spp. 2A and 2B	2A-1, 2A-3, 2A-4, 2A-6, 2A-7, 2A-8, 2B-3, 2B-4	ABR19768 polyketide synthase [<i>B. subtilis</i>]	98–99
		ABR19764 polyketide synthase [<i>Bacillus subtilis</i>]	
	2A-2	ABR19779 polyketide synthase [<i>Actinomycetales bacterium</i> DA20]	98
		WP_032721576 polyketide synthase [<i>B. subtilis</i>]	97
	2B-1	AGA23985 NRPS/PKS-synthase [<i>B. subtilis</i> subsp. <i>subtilis</i> BSP1]	97
		ABR19775 polyketide synthase [<i>B. subtilis</i>]	98
	2B-2	WP_043940121 polyketide synthase [<i>Bacillus</i> sp. YP1]	98
		WP_009967299 polyketide synthase [<i>B. subtilis</i>]	98
	2B-6, 2B-8	ACG70843 polyketide synthase [<i>Bacillus</i> sp. WPhG3]	99
		ABR19767 polyketide synthase [<i>B. subtilis</i>]	98
<i>Bacillus</i> sp. 9A	9A-1	AIO09652 ketosynthase [<i>Bacillus</i> sp. LX-110]	99
	9A-3, 9A-5	ACG70841 polyketide synthase [<i>Bacillus</i> sp. WPySW2]	98
		AGL92430 polyketide synthase [<i>B. amyloliquefaciens</i>]	99
	9A-10	WP_016936042 polyketide synthase [<i>B. siamensis</i>]	99
		WP_047474891 polyketide synthase [<i>B. amyloliquefaciens</i>]	99

sorangicin) and antitumor agents (calyculin and bryostatin) (Table V).

We identified eight nucleotide sequences of polyketide synthases for *Paenibacillus* sp. 7A strain (Table IV). Comparative analysis indicated that all the closest homologues were obtained from *P. graminis*. Among them, we detected erythronolide synthase with high homology (97%). In addition, PKS sequences of *Paenibacillus* sp. 7A differed in structure from genes isolated from the *Paenibacillus* spp. 5A and 12A strains. Among the similar sequences, there were synthases of the antibiotic plipastatine and the antitumor agent epothilone with 43–53% homology (Table V).

In the genomes of *Bacillus* spp. 2A and 2B strains, we identified seven and eight nucleotide sequences of the PKS gene fragment, respectively (Table IV). Inter-

estingly, PKS sequences obtained from *Bacillus* strains had the 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 *Bacillus* sp. 9A strain, we detected four PKS sequences (Table IV). They were homologous to the PKS genes obtained from strains of the genus *Bacillus* spp., including the species *B. amyloliquefaciens*. Additionally, among the related sequences, there were PKS genes with high homology (94–98%) that were responsible for the synthesis of antibiotics (difficidine and bacillaene) (Table V).

Identification of NRPS genes in the genomes of *Bacillus* and *Paenibacillus* strains. Molecular genetic

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

Strain	Clone number	Results of BLAST analysis		
		Homologs with identified protein	Homology, %	
<i>Paenibacillus</i> 5A and 12A	5A-1, 5A-2, 5A-3, 5A-4, 5A-7, 5A-8, 12A-2, 12A-9	EJD67453 diffidicin synthase, (<i>DfnD</i>) [<i>Bacillus</i> sp. 916]	71	
	5A-5, 12A-1, 12A-5, 12A-6	EIF13796 diffidicin synthase, (<i>DfnG</i>) [<i>Bacillus</i> sp. 5B6]	71	
	5A-6	BAP05593 calyculin synthase, (<i>CalE</i>), uncultured [<i>Entotheonella</i> sp.]	71	
		ADN68476 sorangicin synthase, (<i>SorA</i>) [<i>Sorangium cellulosum</i> So ce12]	71	
	12A-7, 12A-10	WP_004619353 erythronolid synthase, [<i>Clostridium papyrosolvens</i> DSM 2782]	75	
		EIF13279 bacillaene synthase, (<i>BaeL</i>) [<i>Bacillus</i> sp. 5B6]	73	
	12A-8	ADD82940 batumin synthase, (<i>Bat2</i>) [<i>P. fluorescens</i> BCCM_ID9359]	70	
		ABK51300 bryostatin synthase, (<i>BryC</i>) [<i>Endobugula sertula</i>]	69	
	<i>Paenibacillus</i> sp.7A	7A-1, 7A-2, 7A-5, 7A-7	EIF13279 bacillaene synthase, (<i>BaeL</i>) [<i>Bacillus</i> sp. 5B6]	72
		7A-3, 7A-4, 7A-6	ADN68477 sorangicin synthase, (<i>SorB</i>) [<i>Sorangium cellulosum</i> So ce12]	72
AIQ67612 erythronolid synthase, [<i>P. graminis</i> DSM 15220]			97	
7A-8		CUB31962 plipastatin synthase, [<i>B. amyloliquefaciens</i>]	53	
		WP004618786 erythronolid synthase, [<i>Clostridium papyrosolvens</i> DSM 2782]	50	
<i>Bacillus</i> spp. 2A and 2B	2A-1, 2A-3, 2A-4, 2A-6, 2A-7, 2A-8, 2B-3, 2B-4	WP_013663185 erythronolid synthase, [<i>Marinomonas mediterranea</i> MMB-1]	43	
	2A-2	ADB12491 eptothilone synthase, (<i>EpoD</i>) [<i>Sorangium cellulosum</i> KYC3013]	43	
	2B-1	EJD66458 bacillaene synthase, (<i>BaeN</i>) [<i>Bacillus</i> sp. 916]	85	
	2B-2	BAP05593 calyculin synthase, (<i>CalE</i>), [<i>Entotheonella</i> sp.]	71	
	2B-5	EJD67453 diffidicin synthase, (<i>DfnD</i>) [<i>Bacillus</i> sp. 916]	70	
	2B-6, 2B-8	EIF13279 bacillaene synthase, (<i>BaeL</i>) [<i>Bacillus</i> sp. 5B6]	85	
	2B-7	EIF13280 bacillaene synthase, (<i>BaeM</i>) [<i>Bacillus</i> sp. 5B6]	84	
		EIF13279 bacillaene synthase, (<i>BaeL</i>) [<i>Bacillus</i> sp. 5B6]	71	
	2B-7	EJD66458 bacillaene synthase, (<i>BaeN</i>) [<i>Bacillus</i> sp. 916].	87	
		AFZ90784 bacillaene synthase, (<i>BaeL</i>) [<i>B. methylotrophicus</i> AS43.3]	86	
<i>Bacillus</i> sp. 9A	9A-1	WP_049628737 diffidicin synthase, [<i>Bacillus</i> sp. JFL15]	98	
	9A-3, 9A-5	EJD66458 bacillaene synthase, (<i>BaeN</i>) [<i>Bacillus</i> sp. 916]	94	
	9A-10	EIF13279 bacillaene synthase, (<i>BaeL</i>) [<i>Bacillus</i> sp. 5B6]	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 surfactin) (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

Table VI
Comparative analysis of the sequences of NRPS genes from heterotrophic bacteria.

Strain	Clone number	Results of BLAST analysis	
		Closest homologs	Homology, %
<i>Paenibacillus</i> spp. 5A and 12A	5A-4	WP_029514857 non-ribosomal peptide synthetase [<i>P. polymyxa</i>]	95
		ADJ56349 fengycin A [<i>Bacillus subtilis</i>]	56
	5A-5, 12A-1, 12A-2	KOS00083 bacitracin synthetase [<i>P. polymyxa</i>]	97–100
		ABQ96384 fusaricidin synthetase [<i>P. polymyxa</i>]	96–99
	12A-5	AKH45460 tridecaptin, TrbE [<i>P. polymyxa</i>]	96
		KOS03006 fusaricidin synthetase [<i>P. polymyxa</i>]	96
	12A-6	WP_013310169 NRPS/PKS synthase [<i>P. polymyxa</i>]	96
		KOS01021 bacitracin synthetase [<i>P. polymyxa</i>]	94
		AHM66091 bacillorin synthetase b [<i>P. polymyxa</i> SQR-21]	94
		AKH45459 tridecaptin B, TrbD [<i>P. polymyxa</i>]	93
		KTS81295 fusaricidin synthetase [<i>P. jamilae</i>]	92
	12A-10	WP_025721680 non-ribosomal peptide synthetase [<i>P. polymyxa</i>]	99
ABQ96384 fusaricidin synthetase [<i>P. polymyxa</i>]		96	
KOS01669 bacitracin synthetase [<i>P. polymyxa</i>]		94	
<i>Bacillus</i> sp. 2B	2B-1	WP_080466694 plipastatin synthetase [<i>B. subtilis</i>]	100
	2B-3	CAA49816 surfactin synthetase, srfA [<i>B. subtilis</i>]	99
<i>Bacillus</i> sp. 2A	2A-5	WP_019712401 plipastatin synthetase subunit B [<i>B. subtilis</i>]	98
		ACX47457 fengycin synthetase B [<i>B. subtilis</i>].	98
	2A-7	AOA54360 polyketide synthase PksJ [<i>B. subtilis</i>]	98
		WP_009967299 non-ribosomal peptide synthetase [<i>B. subtilis</i>]	99
2A-8	WP_048654751 surfactin synthetase SrfAA [<i>B. subtilis</i>]	97	
<i>Bacillus</i> sp. 9A	9A-1	WP_047474893 non-ribosomal peptide synthetase [<i>B. amyloliquefaciens</i>]	99
		ASB53193 polyketide synthase PksJ [<i>B. velezensis</i>]	94
	9A-4	AHW81970 bacillomycin [<i>B. subtilis</i>]	96
	9A-8	WP_064777945 surfactin non-ribosomal peptide synthetase SrfAA [<i>B. siamensis</i>]	98
	9A-9	WP_045925809 non-ribosomal peptide synthetase [<i>B. siamensis</i>]	98
CCF05308 iturinA synthetase ItuA [<i>B. velezensis</i> CAU B946]		93	

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,

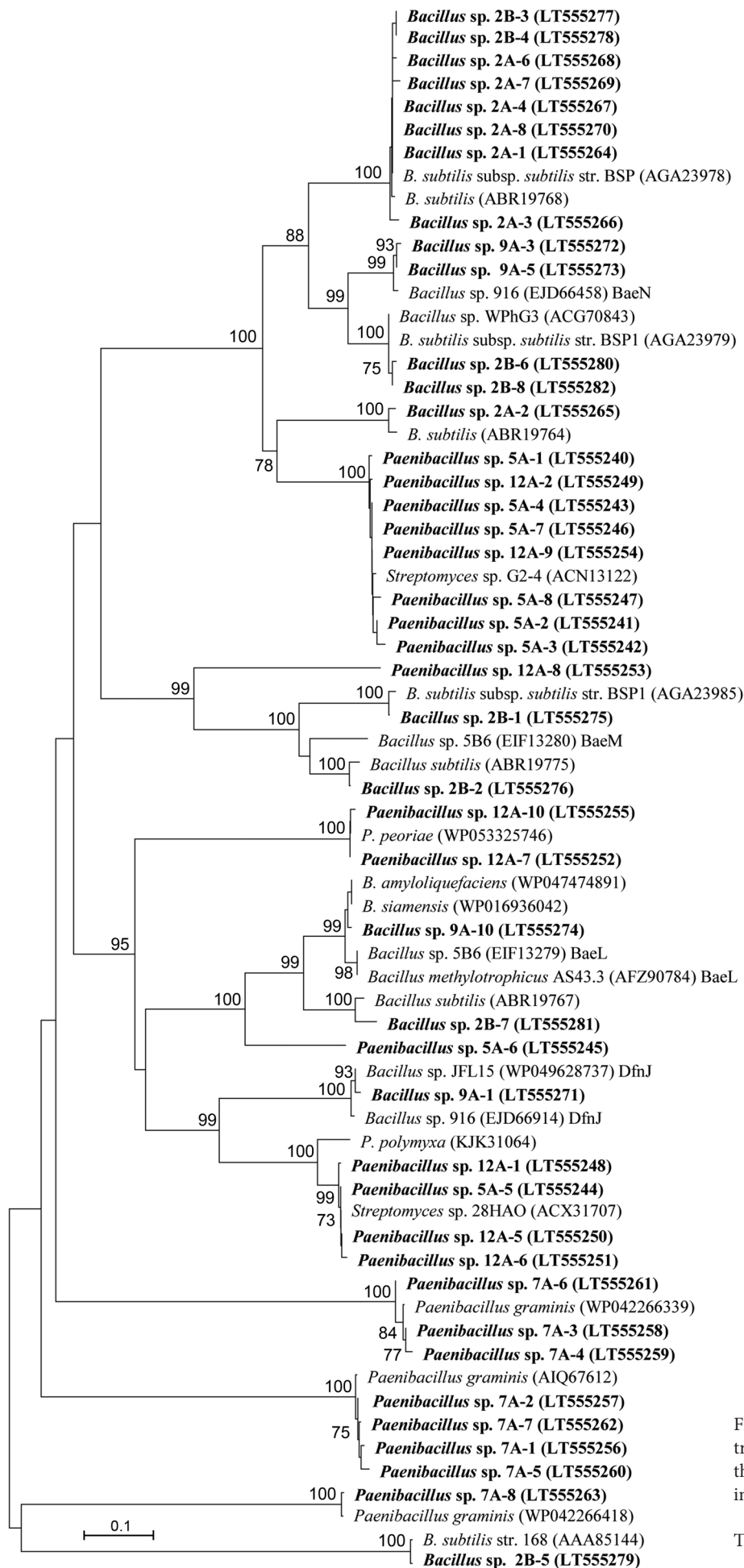


Fig. 3. Neighbour-joining phylogenetic tree based on amino acid sequences of the KS-domain fragments of PKS genes in bacteria isolated from the epilithic biofilms of Lake Baikal (in bold). The scale bar represents 0.1 amino acid substitutions per site.

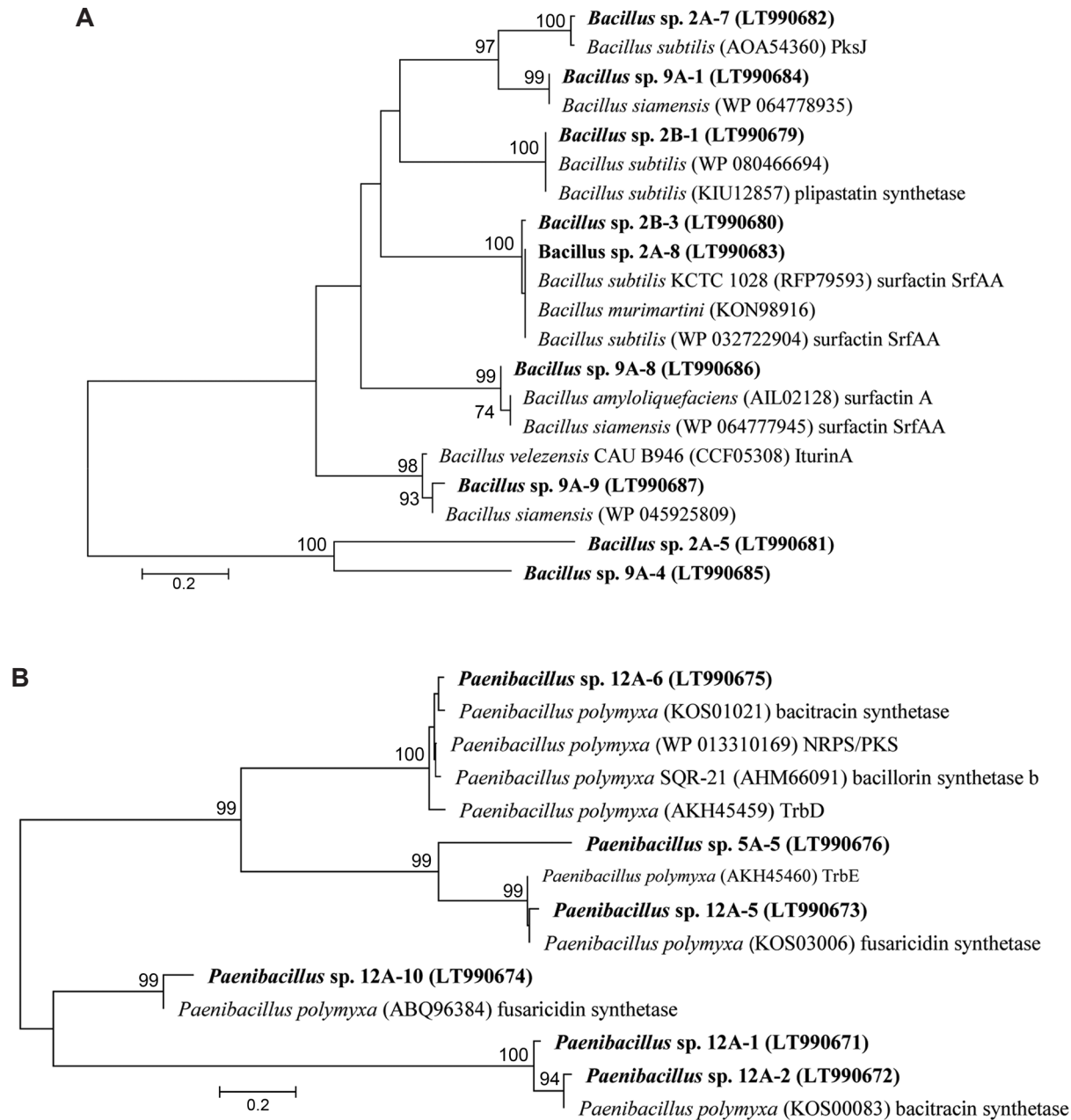


Fig. 4. Neighbour-joining phylogenetic tree based on amino acid sequences of the A-domain fragments of NRPS genes in bacteria isolated from the epilithic biofilms of Lake Baikal (in bold): a) genus *Bacillus* and b) genus *Paenibacillus*.

The scale bar represents 0.2 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 polyene polyketides, including bacillaene, difficidine, and macrolactin. At present, two polyketides (paenimacrolidine and paenilamicin) 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 anticellular and antienzymatic 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 bacillaene (*baeL*, *baeN*) and difficidine, which can indicate their ability to produce these compounds whereas *Bacillus* sp. 2A and 2B contains only bacillaene (*baeL*, *baeM*, *baeN*). Bacillaene is a polyene 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 *pkxX* cluster of *B. subtilis* 168 strain, which is also likely to encode bacillaene. The *bae* gene cluster contains five open reading frames, i.e. *baeJ*, *baeL*, *baeM*, *baeN*, and *baeR* (Aleti et al. 2015). Difficidine is a macrocyclic polyene 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 bacillaene 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 *Saccharopolyspora 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 polypeptide 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; Ciesiołka et al. 2014). Moreover, bacitracin degrades nucleic acids, in particular RNA, through a hydrolytic mechanism (Ciesiołka 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, plipastatin, 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 bacillaene. 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 bacillaene.

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 (Batool 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 (Peypoux 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 epilithic biofilms in Lake Baikal. 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 (bacillaene, diffi- cidine, erythromycin, sorangicin, and batumin), biosurfactants (fengycin, bacillomycin, plipastatin, surfactin, and iturin) and antitumor agents (epothilone, calyculin, and briostatine). *Bacillus* sp. 9A (iturin, bacillomycin, surfactin, bacillaene, and diffi- cidine) and *Bacillus* sp. 2A (plipastatin, bacillaene, surfactin, fengycin, and diffi- cidine) 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.

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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.

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