

# Diversity of potential producers of bioactive metabolites having polyketide nature in the Baikal sponge community of *Rezinkovia echinata*

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**ABSTRACT.** In the present study, we analyze the spectra of polyketide synthase (PKS) genes of microorganisms associated with Baikal sponge *Rezinkovia echinata*. PKS are the multifunctional enzymes responsible for the synthesis of biologically active metabolites with low molecular weight. Since the sequences of PKS modules correspond to gene clusters in the genomes of microorganisms, the ability of microbial communities to produce bioactive components can be detected using PCR and subsequent sequencing of these genes. In the microbiome of *R. echinata*, we identified 36 unique sequences of the PKS-gene fragments. A BlastP analysis showed that sequences obtained were by 57.3-99.6% identical to those of various taxonomic groups of bacteria (Cyanobacteria, Verrucomicrobia, Betaproteobacteria, Alphaproteobacteria, Gammaproteobacteria, Chloroflexi, and Acidobacteria) as well as eukaryotic algae (Haptophyta and Ochrophyta). Among the closest homologues, the PKS sequences of cyanobacteria, proteobacteria, and haptophytic algae were widely represented. Some of the discovered PKS genes have been previously identified in the microbiomes of other sponge species (*Lubomirskia baicalensis* and *Swartschewska papyracea*), which indicates the presence of biotechnologically significant microorganisms in the “core” microbiome of the Baikal sponges. The results obtained indicate the presence of a wide variety of microorganisms in the studied community, which are potential producers of bioactive metabolites.

**Keywords:** Lake Baikal, *Rezinkovia echinata*, microbial communities of freshwater sponges, polyketide synthase (PKS) genes, biotechnological potential

## 1. Introduction

Sponges (Porifera) are filter feeders attached to benthic substrates, which inhabit both marine and freshwater ecosystems. Sponges accumulate a wide variety of microorganisms in their bodies, such as heterotrophic bacteria, cyanobacteria, microalgae, archaea, fungi, etc. Symbiotic microorganisms of sponges are involved in the processing of organic material and the accumulation of primary products (photosynthesis) as well as perform protective functions by synthesizing bioactive metabolites, many of which find medical and biotechnological applications (Webster and Taylor, 2012).

Many microorganisms of natural communities are a source of bioactive compounds (polyketides), the biosynthesis of which is carried out by multifunctional enzymes, polyketide synthases (PKSs): a family of multi-domain enzymes or enzyme complexes (Jenke-Kodama and Dittmann, 2009). Some polyketides are used in industry, medicine, and pharmaceuticals. Examples of such substances are the erythromycin

(antibiotic), the avermectin (antiparasitic drug), the lovastatin (cholesterol-lowering agent), the rapamycin immunosuppressant, and the epothilone (anti-cancer agent) (Fischbach and Walsh, 2006). PKS gene clusters encode modular structures of various enzymes that carry out condensation and modification cycles of simple «building blocks», monomers of acetyl-coenzyme A and malonyl-coenzyme A. Each PKS gene cluster produces a specific secondary metabolite. Due to the structural diversity of these clusters, a wide range of low molecular weight substances that are unique in chemical structure and activity are synthesized (Hertweck, 2009; Jenke-Kodama and Dittmann, 2009).

Baikal is the most ancient and deepest lake on the Earth inhabited by 18 species of sponges, 14 of which form the endemic family Lubomirskiidae (Efremova, 2001). Such unique species richness of the spongi fauna is due to the variety of ecological niches and underwater landscapes existing in Lake Baikal as well as the duration of the lake existence (Kozhov, 1962). Despite numerous studies of the biotechnological potential of marine sponge microbiomes (Trindade-

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Silva et al., 2013; Della Sala et al., 2014; Borchert et al., 2016), freshwater sponges remain very little studied at that point (Kaluzhnaya et al., 2012; Kaluzhnaya and Itskovich, 2016). Moreover, bacterial strains obtained from unusual and unexplored communities are often a productive source of new biologically active components (Bode and Müller, 2005).

The aim of this study was to investigate the taxonomic composition of microorganisms with the potential ability to produce bioactive metabolites as well as to analyze the diversity of PKS genes in the Baikal sponge community of *Rezinkovia echinata*.

## 2. Material and methods

Sponge specimens of *R. echinata* were collected in the northern basin of Lake Baikal (the Ushkany Islands) in September 2014 from a depth of approximately 25 m during field research using SCUBA diving equipment. Samples were placed in a 70% ethanol solution and stored at 4°C. Total DNA was extracted using a RiboSorb kit (Russia) as recommended by the manufacturer. Fragments of KS domains of PKS genes were amplified using degenerate oligonucleotide primers: DKF (5'-GTGCCGGTNCRCRTGNGYYTC-3') and DKR (5'-GCGATGGAYCCNCARCARYG-3') (Barrios-Llerena et al., 2007). The PCR protocol included activation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 45 s, primer annealing at 62°C for 60 s, and elongation at 72°C for 90 s; and final elongation for 10 min at 72°C. The amplified fragment of the expected size (700 bp) was cloned in a pTZ57R/T vector (Fermentas) and used to transform chemically competent *E. coli* XL1BL cells. Recombinant clones were sequenced on an ABI 3130XL automatic sequencer (Applied Biosystems) (Syntol, Moscow). A comparison of the virtually translated amino acid sequences of the PKS gene fragments with the available data was performed using the BlastP program (Altschul et al., 1990). The obtained sequences of PKS were deposited in the GenBank database with the following accession numbers: MT603533-MT603542; MT863577-MT863602.

## 3. Results and discussion

In the *R. echinata* community, we studied the spectra of polyketide synthase (PKS) genes responsible for the synthesis of biologically active metabolites. A total of 36 unique PKS sequences were identified, showing 54-99% amino acid sequence identity with the PKS of various microorganisms (according to the NCBI genetic database).

BlastP analysis showed that the identified sequences were related to PKS of the following taxonomic groups of bacteria: Cyanobacteria (*Scytonema* sp., *Crocospira watsonii*, *Cyanobium usitatum*, *Anabaena* sp., *Nostoc* sp., *Nostoc punctiforme*, *Trichormus variabilis*, *Synechococcus* sp.), Verrucomicrobia (*Opitutus terrae*, *Pedospira parvula*), Betaproteobacteria (*Piscinibacter aquaticus*), Alphaproteobacteria (*Bradyrhizobium* sp.), Gammaproteobacteria (*Alteromonadaceae*

*bacterium*), Firmicutes (*Paenibacillus polymyxa*), Chloroflexi (*Anaerolineae bacterium*), and Acidobacteria (*Acidobacteria bacterium*); as well as eukaryotic algae Haptophyta (*Emiliania huxleyi*, *Chrysochromulina tobinii*) and Ochrophyta (*Nannochloropsis gaditana*) (Table).

Among the closest homologues of the identified genes, there were the PKS genes of uncultivated microorganisms from various habitats: (Antarctic sediment, Chinese mangrove soil, and the East China Sea) as well as metagenomic communities of sponges (*Lubomirskia baicalensis*, *Swartschewskia papyracea*, and *Theonella swinhoei*) (Table).

It is important to note that some sequences showed a high percentage of identity (89.6-99.6%) with the PKS genes from the microbiomes of the freshwater sponges, *L. baicalensis* and *S. papyracea* (Kaluzhnaya et al., 2012; Kaluzhnaya and Itskovich, 2016). Taking into account that the samples of these sponge species were collected in different years and seasons, it can be assumed that the communities of different species of the Baikal sponges contain microorganisms of the same taxonomic groups with the potential ability to produce bioactive metabolites.

Nine sequences showed identity with PKS of eukaryotic algae. Of these, eight sequences were related to the PKS genes of the haptophytic algae, *E. huxleyi* and *C. tobinii*, whose genomes were sequenced in 2015. Representatives of the Haptophyta group are phototrophic flagellates widespread in the composition of nanoplankton of marine and freshwater communities (Moon-van der Staay et al., 2000). Previously, the presence of haptophytic algae in the Baikal sponge *Baikalospongia intermedia* was indicated in the study of the spectra of psbA genes in the microbial community (Kaluzhnaya and Itskovich, 2017). *E. huxleyi* is the main fixer of carbonates in oceans, playing an important role in the global biogeochemical equilibrium on the Earth, and not least in the formation of climate (Eikrem et al., 2016). However, this species is not typical of Lake Baikal, which explains the low identity (55.1-67.8%) of the newly obtained sequences with the PKS genes of this species. Nevertheless, the presence of species related to haptophytic algae was shown in the study of high-throughput sequencing of microbial eukaryotes in Lake Baikal (Yi et al., 2017). An example is the *Braarudosphaera* sp. unicellular algae, which, like *E. huxleyi*, belong to the order Coccolithales and provide the formation of the calcium carbonate plates on their surface (Hagino et al., 2013).

A single sequence from the *R. echinata* microbiome shows 67.8% identity with the PKS of another haptophytic alga, *Chrysochromulina tobinii* (Table). Species of the genus *Chrysochromulina* (order Prymnesiales) are phototrophs capable of producing toxic components under certain environmental conditions (Einar et al., 2005). Moreover, stenothermal cryophilic species *Chrysochromulina parva* dominated summer phytoplankton of Lake Baikal in different years (Izmet'eva, 2011).

The PKS gene sequence of eukaryotic algae, *N. gaditana* (phylum Ochrophyta), was found among the closest homologues (88.07% identity) of the sequences

**Table.** Homology between newly identified sequences and known polyketide synthases

Sequence/ Acc. No.	BLAST search result (with EMBL database)		
	Closest homologues (Acc. No.)	Phylum	Per. Ident, %
03_Rez_KS/ MT603533	Uncultured microorganism from East China Sea (ABQ50542) <i>Emiliana huxleyi</i> CCMP1516 (XP_005770941)	Haptophyta	66.67 58.93
05_Rez_KS/ MT863577	Uncultured bacterium from freshwater sponge <i>Lubomirskia baicalensis</i> (AGB58255) <i>Nostoc punctiforme</i> PCC 73102 (AAW55385)	Cyanobacteria	93.39 64.47
06_Rez_KS/ MT863578	<i>Limnoraphis robusta</i> CCAP 1446/4 (AAX44121)	Cyanobacteria	57.27
07_Rez_KS/ MT863579	Uncultured bacterium from freshwater sponge <i>Swartschewskia papyracea</i> (ALP70475) <i>Trichormus variabilis</i> ATCC 29413 (AAX44126)	Cyanobacteria	99.12 60.18
08_Rez_KS/ MT863580	<i>Nostoc punctiforme</i> GU2015 from Lake Baikal (ARE30046)	Cyanobacteria	63.44
09_Rez_KS / MT603534	Uncultured microorganism from East China Sea (ABQ50542) <i>Emiliana huxleyi</i> CCMP1516 (XP_005775470)	Haptophyta	66.67 55.16
10_Rez_KS/ MT603535	Uncultured microorganism from East China Sea (ABQ50542) <i>Emiliana huxleyi</i> CCMP1516 (XP_005764102)	Haptophyta	67.14 56.95
11_Rez_KS/ MT863581	<i>Opitutus terrae</i> (WP_012374783)	Verrucomicrobia	83.63
12_Rez_KS/ MT863582	Uncultured bacterium from freshwater sponge <i>Lubomirskia baicalensis</i> (AGB58263) Uncultured bacterium from freshwater sponge <i>Swartschewskia papyracea</i> (ALP70477) <i>Nostoc</i> sp. LEGE 06150 (AGJ72788)	Cyanobacteria	99.56 98.67 64.16
13_Rez_KS/ MT863583	<i>Opitutus terrae</i> (WP_012374783)	Verrucomicrobia	80.70
14_Rez_KS/ MT863584	Uncultured bacterium from freshwater sponge <i>Lubomirskia baicalensis</i> (AGB58253) <i>Piscinibacter aquaticus</i> (TXC66096)	Betaproteobacteria	97.79 78.32
20_Rez_KS/ MT603536	Uncultured microorganism from East China Sea (ABQ50542) <i>Emiliana huxleyi</i> CCMP1516 (XP_005775470)	Haptophyta	67.14 55.16
21_Rez_KS/ MT603537	<i>Chrysochromulina tobinii</i> (KOO31876)	Haptophyta	94.57
24_Rez_KS MT863585/	Uncultured bacterium from freshwater sponge <i>Swartschewskia papyracea</i> (ALP70471) <i>Synechococcus</i> sp. 8F6 (WP_094559778)	Cyanobacteria	99.13 83.91
25_Rez_KS/ MT863586	<i>Opitutus terrae</i> (WP_012374783)	Verrucomicrobia	85.53
28_Rez_KS/ MT863587	<i>Pedosphaera parvula</i> (WP_007412583)	Verrucomicrobia	75.42
30_Rez_KS/ MT863588	Symbiont bacterium of sponge <i>Theonella swinhoei</i> (AAQ91800) <i>Anabaena</i> sp. WA102 (WP_053539922)	Cyanobacteria	66.20 61.67
31_Rez_KS/ MT603538	Uncultured microorganism from East China Sea (ABQ50542) <i>Emiliana huxleyi</i> CCMP1516 (XP_005775470)	Haptophyta	67.61 57.85
32_Rez_KS/ MT863589	Hydrogenophaga sp. PBC (WP_069902798)	Betaproteobacteria	88.99

Sequence/ Acc. No.	BLAST search result (with EMBL database)		
	Closest homologues (Acc. No.)	Phylum	Per. Ident, %
34_Rez_KS/ MT863590	Uncultured microorganism from Antarctic sediment (CAM34274) <i>Ideonella azotifigens</i> (WP_141286805)	Betaproteobacteria	84.07 83.86
35_RezKS/ MT603539	Uncultured microorganism from East China Sea (ABQ50542) <i>Emiliana huxleyi</i> CCMP1516 (XP_005775470)	Haptophyta	68.08 58.30
38_Rez_KS/ MT863591	Bradyrhizobium sp. LTSP857 (WP_045001591)	Alphaproteobacteria	93.69
45_Rez_KS/ MT863592	Bradyrhizobium sp. LTSP857 (WP_045001591)	Alphaproteobacteria	95.05
50_Rez_KS/ MT863593	Symbiont bacterium of <i>Theonella swinhoei</i> (AAQ91800) <i>Anabaena</i> sp. WA102 (WP_053539922)	Cyanobacteria	67.13 63.44
54_Rez_KS/ MT863594	<i>Alteromonadaceae</i> bacterium 2753L.S.0a.02 (WP_144694783) <i>Paenibacillus polymyxa</i> (SAJ35020)	Gammaproteobacteria Firmicutes	67.56 54.42
55_Rez_KS/ MT863595	<i>Acidobacteria</i> bacterium (PYS31643) Uncultured microorganism from Chinese mangrove soil (AIE12064)	Acidobacteria	82.96 80.27
61_Rez_KS/ MT603540	Uncultured microorganism from East China Sea (ABQ50542) <i>Emiliana huxleyi</i> CCMP1516 (XP_005775470)	Haptophyta	68.08 58.30
63_Rez_KS/ MT863596	<i>Cyanobium usitatum</i> str. Tous (PSJ07310) Uncultured bacterium from freshwater sponge <i>Swartschewskia papyracea</i> (ALP70470)	Cyanobacteria	90.43 89.61
67_Rez_KS/ MT863597	Uncultured microorganism from Antarctic sediment (CAM34274) <i>Ideonella azotifigens</i> (WP_141286805)	Betaproteobacteria	84.51 84.44
69_Rez_KS/ MT863598	<i>Hydrogenophaga intermedia</i> (WP_009517173)	Betaproteobacteria	86.73
77_Rez_KS/ MT603541	<i>Chrysochromulina tobinii</i> (KOO31876) <i>Emiliana huxleyi</i> CCMP1516 (XP_005775470)	Haptophyta	67.87 63.68
79_Rez_KS/ MT863599	Uncultured bacterium from freshwater sponge <i>Lubomirskia baicalensis</i> (AGB58258) <i>Scytonema</i> sp. LEGE 07189 (AGJ72771)	Cyanobacteria	98.67 69.74
84_Rez_KS/ MT603542	Uncultured bacterium from freshwater sponge <i>Swartschewskia papyracea</i> (ALP70479) <i>Nannochloropsis gaditana</i> (EWM19998)	Ochrophyta	88.07
86_Rez_KS/ MT863600	Uncultured microorganism from Antarctic sediment (CAM34275) Uncultured <i>Paenibacillus</i> sp. (AMY26664)	Firmicutes	80.09 77.78
88_Rez_KS/ MT863601	Bradyrhizobium sp. LTSP857 (WP_045001591)	Alphaproteobacteria	94.59
91_Rez_KS/ MT863602	<i>Anaerolineae</i> bacterium (RIK32670) <i>Crocospaera watsonii</i> WH 0002 (AAW55372)	Chloroflexi Cyanobacteria	63.00 59.39

obtained (Table). Species of the genus *Nannochloropsis* are flagellate unicellular microalgae, the primary producer of planktonic microorganisms, can adapt and successfully develop at low temperatures (Konga et al., 2012). *Nannochloropsis* is typical of marine ecosystems; however, it has been rarely reported in freshwater bodies (Fawley and Fawley, 2007). Notably, the species *N. limnetica* was found in Lake Baikal (Fietz et al., 2005), where its presence in phytoplankton samples was observed throughout a year, as well as in the phototrophic community of the Baikal sponge *B. intermedia* (Kaluzhnaya and Itskovich, 2017).

A rather representative group is formed by sequences related to various cyanobacterial PKS (11 sequences). We identified the highest percentage of amino acid identity with the PKS of the picocyanobacteria, *Synechococcus* sp. (83.9%) and *Cyanobium usitatum* (90.4%) (Table) typical of picoplankton communities in Lake Baikal (Belykh et al., 2006) and microbial associations of the Baikal sponge species (Kaluzhnaya et al., 2011; 2012; Kaluzhnaya and Itskovich, 2016; 2017). The biotechnological significance of cyanobacteria in natural communities is associated with their ability to produce a wide range of polyketide metabolites with cytotoxic, antifungal, antiviral, antiprotease, and other activities (Fawley and Fawley, 2007).

Among other related sequences with identity above 80%, we can also note *Opiritus terrae* (Verrucomicrobia), *Bradyrhizobium* sp. (Alphaproteobacteria), *Ideonella azotifigens*, and *Hydrogenophaga intermedia* (Betaproteobacteria) which may indicate the presence of these species in the sponge community as potential producers of bioactive metabolites.

Table shows that 19 of 36 sequences obtained in this work had an identity above 80% with the PKS of known microorganisms. Representatives of these taxonomic groups are most likely present in the studied community as potential producers of natural metabolites. The causes of the low percentage of identity (57.3-75.4%) for the other 16 sequences are as follows: firstly, the database of polyketide synthase gene sequence (unlike, for example, the 16S rRNA database) does not cover all microorganisms, and, usually, the closest homologues are the microorganisms with a known genomic sequence; secondly, we can assume the presence microorganisms producing new biologically active metabolites in the communities of endemic Baikal sponges.

#### 4. Conclusions

The present study revealed a significant diversity of polyketide synthase gene sequences in the freshwater sponge community of *R. echinata* as well as a taxonomic diversity of microorganisms that are potentially capable of producing bioactive metabolites. Among the identified sequences, PKSs of haptophytic algae, cyanobacteria, and proteobacteria were the most numerous. In the microbiome of *R. echinata*, some PKS

sequences belonged to uncultivated microorganisms that were also present in the communities of sponges *L. baicalensis* and *S. papyracea*. This fact may indicate the existence of microorganisms producing bioactive metabolites in the “core” community of the Baikal sponges. This study contributes to understanding the biotechnological potential of the microbial communities of the Baikal sponges and opens up prospects for further search for producers of new bioactive metabolites in freshwater microbiomes.

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