

The following supplement accompanies the article

# Freshwater *Actinobacteria* from sediments of the deep and ancient Lake Baikal (Russia) and their genetic potential as producers of secondary metabolites

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*Aquatic Microbial Ecology* 84: 1–14 (2020)

Table S1. 16S rRNA gene sequence similarity between freshwater Actinobacteria and the closest type strains.

Strain	GenBank accession number	Closest type strain (GenBank accession #)	Sequence identity	Sampling site	Isolation medium
IB2016P328-1 (F)	MG199054	<i>Streptomyces sampsonii</i> strain ATCC 25495 (NR_025870)	100	Lake Baikal, Russia, 50 m	IPS2
IB2016P328-2 (F)	MG199055	<i>Streptomyces badius</i> strain A14 (MK680817)	100	Lake Baikal, Russia, 50 m	MS
IB2016P328-3 (F)	MG199056	<i>Streptomyces badius</i> strain A14 (MK680817)	100	Lake Baikal, Russia, 50 m	MS
IB2016P328-4 (F)	MG199057	<i>Streptomyces lividans</i> strain T38, (KY411691)	100	Lake Baikal, Russia, 50 m	MS
IB2016P328-5 (F)	MG199058	<i>Streptomyces lividans</i> strain N309 (MK629802)	100	Lake Baikal, Russia, 50 m	MS
IB2016P328-6 (F)	MG199059	<i>Streptomyces parvus</i> strain MC025 (MG016024)	100	Lake Baikal, Russia, 50 m	MS
IB2016P328-7 (F)	MG199060	<i>Streptomyces albogriseolus</i> isolate 1N10 (LT978354)	100	Lake Baikal, Russia, 50 m	IPS2
IB2016P328-8 (F)	MG199061	<i>Micromonospora endolithica</i> strain WA68, (KX354312)	99.83	Lake Baikal, Russia, 50 m	IPS2
IB2016P329-1 (F)	MG199062	<i>Streptomyces albidoflavus</i> strain HQA403, (MH044518)	99.83	Lake Baikal, Russia, 50 m	MS

IB2016P329-2 (F)	MG199063	<i>Streptomyces albidoflavus</i> strain HQA403, (MH044518)	100	Lake Baikal, Russia, 50 m	MS
IB2016P330-1 (F)	MG199064	<i>Streptomyces muensis</i> strain MC-1 (KY753304)	100	Lake Baikal, Russia, 50 m	MS
IB2016P330-2 (F)	MG199065	<i>Streptomyces griseus</i> strain SCSB4 (MG892846)	100	Lake Baikal, Russia, 50 m	MS
IB2016P330-3 (F)	MG199066	<i>Streptomyces sampsonii</i> strain JHC003 (MK564728)	100	Lake Baikal, Russia, 50 m	MS
IB2016P331-1 (F)	MG199067	<i>Streptomyces badius</i> strain A14 (MK680817)	100	Lake Baikal, Russia, 150 m	Czapek
IB2016P332-1 (F)	MG199068	<i>Streptomyces olivaceus</i> strain HQA933 (MH044533)	100	Lake Baikal, Russia, 150 m	IPS2
IB2016P332-2 (F)	MG199069	<i>Pseudonocardia tropica</i> strain SAK-28 (MK526909)	100	Lake Baikal, Russia, 150 m	MS
IB2016P333-1 (F)	MG199070	<i>Pseudonocardia profundimaris</i> strain GY0556 (NR_156947)	99	Lake Baikal, Russia, 150 m	Czapek
IB2016P333-2 (F)	MG199071	<i>Streptomyces lividans</i> strain N309 (MK629802)	100	Lake Baikal, Russia, 150 m	MS
IB2016P333-3 (F)	MG199072	<i>Streptomyces lividans</i> strain N309 (MK629802)	98	Lake Baikal, Russia, 150 m	Czapek
IB2016P334-1 (F)	MG199073	<i>Micromonospora chokoriensis</i> strain B032, (KY858240)	100	Lake Baikal, Russia, 200 m	IPS2
IB2016P334-2 (F)	MG199074	<i>Streptomyces badius</i> strain A14 (MK680817)	100	Lake Baikal, Russia, 200 m	IPS2
IB2016P334-3 (F)	MG199075	<i>Streptomyces caniferus</i> strain YBUF5 (MK212945)	100	Lake Baikal, Russia, 200 m	IPS2
IB2016P335-1 (F)	MG199076	<i>Streptomyces enissocaesilis</i> strain C47 (MK696278)	100	Lake Baikal, Russia, 200 m	IPS2
IB2016P336-2 (F)	MG199077	<i>Streptomyces albogriseolus</i> isolate 1N10 (LT978354)	100	Lake Baikal, Russia, 200 m	ISP2

Table S2. Previously reported PKS-I KS domain sequences most similar to the amino acid sequences of freshwater strain KS domains, based on BLASTP analyses.

Strain	Freshwater KS accession number	Most similar entry in NCBI database based on BLASTP (Accession #)	Sequence similarity
<i>Streptomyces</i> sp. IB2016P328-2	MG249996	beta-ketoacyl synthase, N-terminal domain protein of <i>Streptomyces ipomoeae</i> 91-03 (EKX63339)	88%
<i>Streptomyces</i> sp. IB2016P328-3	MG249997	polyketide synthase of <i>Streptomyces</i> sp. ID05-A0327 (BAH67922)	91%
<i>Streptomyces</i> sp. IB2016P328-4	MG249998	beta-ketoacyl synthase of <i>Streptomyces</i> sp. FXJ7.329 (ADC68516)	88%
<i>Streptomyces</i> sp. IB2016P329-2	MG250000	polyketide synthase of <i>Streptomyces ascomycinicus</i> (BAH66951)	80%
<i>Streptomyces</i> sp. IB2016P330-1	MG250001	beta-ketoacyl synthase of <i>Streptomyces</i> sp. FXJ6.141 (ADC68463)	98%
<i>Streptomyces</i> sp. IB2016P330-2	MG250002	polyketide synthase of <i>Streptomyces ascomycinicus</i> (BAH66951)	79%
<i>Streptomyces</i> sp. IB2016P330-3 (1)	MG250003	polyketide synthase of <i>Streptomyces blastmyceticus</i> (BAH67200)	81%
<i>Streptomyces</i> sp. IB2016P330-3 (2)	MG250004	beta-ketoacyl synthase of <i>Streptomyces</i> sp. FXJ7.329 (ADC68516)	69%
<i>Streptomyces</i> sp. IB2016P331-1	MG250005	beta-ketoacyl synthase of <i>Streptomyces</i> sp. FXJ6.141 (ADC68463)	98%
<i>Pseudonocardia</i> sp. IB2016P333-1 (2)	MG249995	polyketide synthase of <i>Streptomyces</i> sp. S240 (ABU25213)	99%
<i>Streptomyces</i> sp. IB2016P333-2	MG250006	amino acid adenylation enzyme/thioester reductase family protein of <i>Streptomyces</i> sp. SM8 (EKC91163)	99%
<i>Streptomyces</i> sp. IB2016P333-3	MG250007	polyene macrolide polyketide synthase/candicidin polyketide synthase FscE of <i>Streptomyces</i> sp. IgraMP-1 (SCE26328)	99%
<i>Micromonospora</i> sp. IB2016P334-1	MG249993	polyketide synthase of <i>Micromonospora zamorensis</i> (WP_088951670)	99%

Table S3. Relative abundance of Actinobacteria in sediments collected at three depths (50, 150, 200 m) from Lake Baikal.

Sample (depth, m)	MS medium		ISP2 medium		Gauze's medium #1	
	Total cfu/mL	Actinobacterial cfu/mL (relative abundance, %)	Total cfu/mL	Actinobacterial cfu/mL (relative abundance, %)	Total cfu/mL	Actinobacterial cfu/mL (relative abundance, %)
328 (50 m)	12*10 <sup>3</sup>	5*10 <sup>2</sup> (4)	5.1*10 <sup>3</sup>	3*10 <sup>2</sup> (5.9)	25.4*10 <sup>3</sup>	0 (0)
329 (50 m)	16.3*10 <sup>3</sup>	1*10 <sup>2</sup> (0.6)	11.7*10 <sup>3</sup>	1*10 <sup>2</sup> (0.8)	41.8*10 <sup>3</sup>	0 (0)
330 (50 m)	14*10 <sup>3</sup>	3*10 <sup>2</sup> (2.1)	58.2*10 <sup>3</sup>	0 (0)	12*10 <sup>3</sup>	0 (0)
331 (50 m)	9.1*10 <sup>3</sup>	0 (0)	44.2*10 <sup>3</sup>	0 (0)	59*10 <sup>3</sup>	1*10 <sup>2</sup> (0.2)
<b>Σ<sub>50</sub></b>	<b>51.4*10<sup>3</sup></b>	<b>9*10<sup>2</sup> (1.8)</b>	<b>119.2*10<sup>3</sup></b>	<b>4*10<sup>2</sup> (0.3)</b>	<b>138.2*10<sup>3</sup></b>	<b>100 (0.07)</b>
332 (150 m)	17.5*10 <sup>3</sup>	1*10 <sup>2</sup> (0.6)	26.8*10 <sup>3</sup>	1*10 <sup>2</sup> (0.4)	30,2*10 <sup>3</sup>	0 (0)
333 (150 m)	22.3*10 <sup>3</sup>	1*10 <sup>2</sup> (0.5)	37.9*10 <sup>3</sup>	0 (0)	15.4*10 <sup>3</sup>	2*10 <sup>2</sup> (1.3)
<b>Σ<sub>150</sub></b>	<b>39.8*10<sup>3</sup></b>	<b>2*10<sup>2</sup> (0.5)</b>	<b>64.7*10<sup>3</sup></b>	<b>1*10<sup>2</sup> (0.2)</b>	<b>45.6*10<sup>3</sup></b>	<b>2*10<sup>2</sup> (0.4)</b>
334 (200 m)	6.4*10 <sup>3</sup>	0 (0)	30*10 <sup>3</sup>	3*10 <sup>2</sup> (1)	17.8*10 <sup>3</sup>	0 (0)
335 (200 m)	9.7*10 <sup>3</sup>	0 (0)	19.6*10 <sup>3</sup>	1*10 <sup>2</sup> (0.51)	18.4*10 <sup>3</sup>	0 (0)
336 (200 m)	21.3*10 <sup>3</sup>	0 (0)	5.8*10 <sup>3</sup>	1*10 <sup>2</sup> (1.7)	26*10 <sup>3</sup>	0 (0)
<b>Σ<sub>200</sub></b>	<b>37.4*10<sup>3</sup></b>	<b>0 (0)</b>	<b>55.4*10<sup>3</sup></b>	<b>5*10<sup>2</sup> (0.9)</b>	<b>62.2*10<sup>3</sup></b>	<b>0 (0)</b>
<b>Σ<sub>total</sub></b>	<b>128.8*10<sup>3</sup></b>	<b>1.1*10<sup>3</sup> (0.9)</b>	<b>239.3*10<sup>3</sup></b>	<b>1*10<sup>3</sup> (0.4)</b>	<b>246*10<sup>3</sup></b>	<b>3*10<sup>2</sup> (0.1)</b>

Table S4. Evaluation of selected putative type I PKS KS domain sequences from Lake Baikal freshwater strains using NaPDoS. Based on comparison of KS domain sequences between the NaPDoS database and Lake Baikal strains, known metabolites most similar to those genetically encoded by Lake Baikal strains were predicted. KS accession numbers for Lake Baikal strains are provided in Table S3.

Lake Baikal strain from which KS domain was proposed	Pathway product from most closely related KS domain in NaPDoS (producing strain)
<i>Streptomyces</i> sp. IB2016P328-2	Avermectin ( <i>Streptomyces avermitilis</i> )
<i>Streptomyces</i> sp. IB2016P328-3	Candicidin ( <i>Streptomyces</i> sp. FR-008)
<i>Streptomyces</i> sp. IB2016P328-4	Meridamycin ( <i>Streptomyces violaceusnige</i> )
<i>Streptomyces</i> sp. IB2016P329-2	eco-02301 ( <i>Streptomyces aizunensis</i> )
<i>Streptomyces</i> sp. IB2016P330-1	Avermectin ( <i>Streptomyces avermitilis</i> )
<i>Streptomyces</i> sp. IB2016P330-2	Avermectin ( <i>Streptomyces avermitilis</i> )
<i>Streptomyces</i> sp. IB2016P330-3 (1)	Avermectin ( <i>Streptomyces avermitilis</i> )
<i>Streptomyces</i> sp. IB2016P330-3 (2)	eco-02301 ( <i>Streptomyces aizunensis</i> )
<i>Streptomyces</i> sp. IB2016P331-1	Avermectin ( <i>Streptomyces avermitilis</i> )
<i>Pseudonocardia</i> sp. IB2016P333-1 (2)	Candicidin ( <i>Streptomyces</i> sp. FR-008)
<i>Streptomyces</i> sp. IB2016P333-2	-
<i>Streptomyces</i> sp. IB2016P333-3	Candicidin ( <i>Streptomyces</i> sp. FR-008)
<i>Micromonospora</i> sp. IB2016P334-1	Tylosin ( <i>Streptomyces fradiae</i> )

Table S5. Bioinformatics evaluation of biosynthetic capabilities for publicly available genome sequenced strains closely related to Lake Baikal strains. Genome sequenced strains were evaluated using antiSMASH v5.0 to predict the number and types of biosynthetic pathways they harbor. Halogenases were predicted from these genome sequences by using SynTax bioinformatics tool to evaluate homology between predicted open reading frames (ORFs) and model FADH<sub>2</sub>-dependent halogenases from *Salinispora* sp. DRTO102 (NCBI Accession #MG249985), *Amycolatopsis mediterranei* DSM5908 (Accession #CAA76550), and *Streptomyces venezuelae* ISP-5230 (Accession # WP\_015032134).

Lake Baikal strain(s)	Closest relative in NCBI database with complete genome sequence (Accession # for genome sequence)	Number of putative biosynthetic gene clusters from sequenced strain	Type 1 PKS	Type 2 PKS	NRPS	Ectoine	FADH <sub>2</sub> -dependent halogenase of <i>Salinispora</i> sp. DRTO102 (percent normalized Blast score)	FADH <sub>2</sub> -dependent halogenase of <i>Amycolatopsis mediterranei</i> DSM5908 (percent normalized BLAST score)	FADH <sub>2</sub> -dependent halogenase of <i>Streptomyces venezuelae</i> ISP-5230 (percent normalized BLAST score)
<i>Streptomyces</i> sp. IB2016P328-1 <i>Streptomyces</i> sp. IB2016P328-5 <i>Streptomyces</i> sp. IB2016P330-3 <i>Streptomyces</i> sp. IB2016P333-2 <i>Streptomyces</i> sp. IB2016P333-3	<i>Streptomyces koyangensis</i> strain VK-A60T (CP031742)	21	4	0	8	1	+ (10.31)	+ (10.4)	-
<i>Streptomyces</i> sp. IB2016P328-2 <i>Streptomyces</i> sp. IB2016P328-3 <i>Streptomyces</i> sp. IB2016P328-6 <i>Streptomyces</i> sp. IB2016P328-7	<i>Streptomyces hundungensis</i> strain BH38 (CP038147)	26	3	2	3	1	+ (20.71)	+ (22.28)	<b>+ (89.59)</b>

<i>Streptomyces</i> sp. IB2016P328-4	<i>Streptomyces</i> sp. SAT1 (CP015849)	27	2	1	6	1	+ (18.66)	<b>+ (55.54)</b>	+ (16.54)
<i>Micromonospora</i> sp. IB2016P328-8, Lake Baikal	<i>Micromonospora tulbaghiae</i> strain CNY-010	18	3	1	3	0	0	0	0
<i>Streptomyces</i> sp. IB2016P329-1 <i>Streptomyces</i> sp. IB2016P330-1	<i>Streptomyces</i> sp. CLI2509 (CP021118)	17	3	0	6	1	0	0	0
<i>Streptomyces</i> sp. IB2016P330-2 <i>Streptomyces</i> sp. IB2016P331-1 <i>Streptomyces</i> sp. IB2016P334-2	<i>Streptomyces griseus</i> subsp. <i>griseus</i> strain ATCC 13273 (CP032543)	26	3	2	5	1	0	0	0
<i>Streptomyces</i> sp. IB2016P334-3	<i>Streptomyces lydicus</i> strain WYEC 108 (CP029042)	34	3	1	5	1	0	0	0
<i>Streptomyces</i> sp. IB2016P335-1 <i>Streptomyces</i> sp. IB2016P336-2	<i>Streptomyces griseoviridis</i> strain F1-27 (CP034687)	31	3	2	9	2	+ (17.1)	+ (21.49)	+ (16.24)

For Lake Baikal strains *Streptomyces* sp. IB2016P329-2, *Pseudonocardia* sp. IB2016P332-2, *Pseudonocardia* sp. IB2016P333-1, and *Micromonospora* sp. IB2016P334-1, no completely genome sequenced strains were found among the 100 most closely related strains in the NCBI database via BLAST.

Fig. S1. Phylogenetic tree of Actinobacteria strains isolated from Lake Baikal sediments in the current study. The Bayesian tree of ~600 bp fragments of 16S rRNA genes supports representatives from three orders within the phylum Actinobacteria. The sequence of *E. coli* K12 was used as the outgroup.

