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ORIGINAL PAPER

The diversity and antibiotic properties of actinobacteria associated with endemic deepwater amphipods of Lake Baikal

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Abstract The emergence of pathogenic bacteria resistant to antibiotics increases the need for discovery of new effective antimicrobials. Unique habitats such as marine deposits, wetlands and caves or unexplored biological communities are promising sources for the isolation of actinobacteria, which are among the major antibiotic producers. The present study aimed at examining cultivated actinobacteria strains associated with endemic Lake Baikal deepwater amphipods and estimating their antibiotic activity. We isolated 42 actinobacterial strains from crustaceans belonging to *Ommatogammarus albinus* and *Ommatogammarus flavus*. To our knowledge, this is the first report describing the isolation and initial characterization of representatives of *Micromonospora* and *Pseudonocardia* genera from Baikal deepwater invertebrates. Also, as expected, representatives of the genus *Streptomyces* were the dominant group among the isolated species. Some correlations could be observed between the number of actinobacterial isolates, the depth of sampling and the source of the strains. Nevertheless, >70% of isolated strains demonstrated antifungal activity. The dereplication analysis of extract of one of the isolated strains resulted in annotation of several known compounds that can help to explain the observed biological activities. The characteristics of ecological niche and lifestyle of deepwater amphipods suggests that the observed associations between crustaceans and isolated actinobacteria are not random and might represent long-term symbiotic interactions.

Keywords Actinobacteria · Amphipods · Baikal · *Ommatogammarus* spp. · Endemics

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Introduction

Natural products remain the most important source of new pharmaceutical leads, including antibiotics (Doroghazi et al. 2014). The multiple reports on

emergence of pathogenic bacteria resistant to antibiotics increases the need for effective antimicrobials with new modes of action (Haste et al. 2012; Ling et al. 2015). Despite some reduction in interest in the past, actinobacteria still remain one of the most prevalent sources of new drugs. This phenomenon is first of all associated with the diverse and extraordinary creative secondary metabolism of these bacteria. Approximately 8700 of the 16,500 known antibiotics are produced by bacteria from the order *Actinomycetales* (Berdy 2005). The genus *Streptomyces* alone accounts for 80% of the natural products produced by actinobacteria (Manivasagan et al. 2013; Jensen et al. 2005).

Since the 1940s, the main source for the isolation of antibiotics producers has been soil. The rate of discovery of novel biologically active compounds from this source has been declining since then, mostly due to re-discovery of the same compounds. Recently, a transition from the traditional to new sources, such as marine sediments (Fenical and Jensen 2006; Subramani and Aalbersberg 2012), invertebrates (Genilloud et al. 2011), caves (Maciejewska et al. 2016) has been observed. For example, new species of the genus *Micromonospora* were isolated from insects (Fang et al. 2015). Species of the genera *Streptomyces*, *Nocardia*, *Pseudonocardia*, *Amycolatopsis*, and *Micromonospora* with antimicrobial activities were isolated from termite nests (Sujada et al. 2014). The sponge-microbe associations has been a focus of many studies directed on isolation of new compounds (Taylor et al. 2007; Thomas et al. 2010). Many of them have concentrated efforts on marine animals, but several reports on freshwater sponges as a source of new actinobacterial strains and antibiotics are also published (Gernert et al. 2005; Costa et al. 2013). Actinobacteria isolated from aquatic environments are a promising source of novel biologically active compounds, such as antibiotics, enzyme inhibitors, antitumor and antiviral agents (Farnaes et al. 2014; Duncan et al. 2015). One of the unique, ancient and unstudied aquatic ecosystems in terms of actinobacterial diversity is the ecosystem of Lake Baikal and its inhabitants.

Lake Baikal, a UNESCO World Heritage Site, is located in eastern Siberia, Russia. It is one of the most ancient lakes (approximately 25–30 million years old) and the largest (by volume) in the world (Timofeyev 2016). The lake contains approximately 20% of the world's unfrozen surface fresh water. Lake Baikal is the deepest (1642 m) lake in the world, with an average depth of 744.4 m (Kozhov 1963). The lake is inhabited by more than 2500 animal species, 80% of which are endemic (Yoshii et al. 1999; Timoshkin et al. 2001). The lake is well-oxygenated throughout the water column, and is characterized by narrow fluctuations in temperature (3.5–6 °C at depths of 30–100 m). These factors establish specific conditions for ecosystem development. Indeed, a number of endemic groups of macroinvertebrates have evolved during million years in Lake Baikal under these conditions, leading to the formation of a unique ecological system.

Amphipods are the dominant group of macroinvertebrates of Lake Baikal ecosystem (Amphipoda, Crustacea) (Timoshkin et al. 2001). Lake Baikal endemic amphipods form 45.3% of the world's freshwater amphipod fauna. They are represented by 246 species and 78 subspecies with 100% endemicity (Takhteev 2000; Takhteev et al. 2015). They inhabit all types of ecological niches from the littoral to abyssal zones. Amphipods efficiently find and consume dead organisms and detritus. Deepwater amphipods, which are scavengers and necrophages, play a particularly important role in the self-cleaning of the lake. The life style and type of feeding of these organisms suggest constant interactions with microorganisms as competitors and possibly symbionts.

The uniqueness of biotic and abiotic factors makes Lake Baikal and its inhabitants a promising source of new actinobacterial species producing novel biologically active compounds with chemically and pharmaceutically interesting properties. The present study aimed to examine a cultivated actinobacterial population associated with Lake Baikal endemic deepwater amphipods for their antibiotic activity accompanied with the dereplication of secondary metabolites produced by some of them.

Materials and methods

Sampling and location

Two hundred specimens of two endemic deepwater amphipod species of the genus *Ommatogammarus* (*O. albinus* and *O. flavus*) were collected at different depths from the southern part of Lake Baikal near the

Bolshie Koty settlement (51.9053°N, 105.0753°E) in winter 2015. No specific permissions were required for these locations and activities. The studied amphipod species are not endangered or protected species.

Samples were taken at depths of 80, 100 and 200 m. Each sample included 3–5 amphipod specimens. Five samples were collected from each depth. The amphipods were captured in traps baited with sterilized putrescent fish. Immediately after the lifting the traps, the amphipods were rinsed with 70% ethanol following by sterile water (three times) to remove transient bacteria and then homogenized in 20% sterile glycerol at an approximate ratio of 1:10. The obtained samples were stored at −20 °C until plating on the solid nutrient media. Water samples collected from the surface and the suspension of sterile putrescent fish were used as negative controls.

Isolation of actinobacteria

The actinobacterial strains were isolated by plating obtained homogenate on solid nutrient media. Six media were used: MS (soy flour 20 g/L, D-mannitol 20 g/L, agar 20 g/L, pH 7.2); ISP2 (yeast extract 4 g/L, malt extract 10 g/L, dextrose 4 g/L, agar 20 g/L, pH 7.2); starch-ammonia agar SAA ($(NH_4)_2SO_4$) 2 g/L, K_2HPO_4 1 g/L, $MgSO_4$ 1 g/L, NaCl 1 g/L, $CaCO_3$ 3 g/L, starch 10 g/L, agar 20 g/L); Gauze's synthetic agar (starch 20 g/L, KNO_3 1 g/L, NaCl 0.5 g/L, $MgSO_4 \cdot 7\ H_2O$ 0.5 g/L, K_2HPO_4 0.5 g/L, $FeSO_4 \cdot 7\ H_2O$ 0.01 g/L, agar 15 g/L, pH 7.4); Waksman media (glycerol 3 g/L, K_2HPO_4 1 g/L, $NaNO_3$ 2 g/L, $MgSO_4 \cdot 7\ H_2O$ 0.5 g/L, KCl 0.5 g/L, $FeSO_4 \cdot 7\ H_2O$ 0.01 g/L, pH 7.0); and Czapek media ($NaNO_3$ 2 g/L, starch 30 g/L, $MgSO_4\ 7H_2O$ 0.5 g/L, KCl 0.5 g/L, $FeSO_4 \cdot 7H_2O$ 0.01 g/L, K_2HPO_4 1 g/L, agar 20 g/L, pH 7.2). Media were supplemented with nystatin (50 μg/mL) and phosphomycin (100 μg/mL) (Kieser et al. 2000). Aliquots of collected samples were preheated for 5 min at 50 °C to activate spores germination and inactivate the vegetative cells of other bacteria.

Collected samples were diluted in a 1% sterile saline solution at 1:10, 1:100 and 1:1000 ratios. 150 μL of each dilution was plated on mentioned media in three replicates. The plates were incubated for 30 days at 28 °C and assessed every day for actinobacterial colony appearance. Actinobacteria-like strains were identified based on colony morphology (Kieser et al. 2000). The colonies were transferred from the primary plates to fresh MS plates. Pure cultures were obtained for all colonies identified as actinobacteria on the primary plates. Several isolated strains were deposited in the Russian Collection of Agricultural Microorganisms (RCAM), St. Petersburg, Russia.

16S rRNA gene sequencing and analysis

Strains were cultured in tubes for 3 days in 10 mL of TSB medium at 28 °C at a shaking rate of 180 rpm. Total DNA was isolated by the salting out procedure as described in (Kieser et al. 2000). Amplification of the 16S rRNA gene was carried out with the following primers: 8F (AGAGTTTGATYMTGGCTCAG), 1510R (TACGGYTACCTTGTTACGACTT), ACT235F (CG CGGCCTATCAGCTTGTTG) and ACT878R (CCGT ACTCCCCAGGCGGGG). PCR was performed using a ScreenMix 5X PCR kit (Kat. PK041L, Evrogen, Russia). PCR was performed in a TGradient Thermocycler (Biometra, Germany) in a volume of 25 μL. The parameters of the PCR were as follows: initial denaturation at 95 °C for 5 min, followed by 25 cycles of 95 °C for 40 s, 50–55 °C for 25 s, and 72 °C for 110 s, and final elongation at 72 °C for 5 min.

The amplified PCR products were visualized in 1% agarose gel, purified with Cleanup Standard PCR purification kit (Kat. BC022, Evrogen, Russia) and sequenced with primers used for amplification by SYNTOL (Moscow, Russia). The forward and reverse sequences were assembled with BioEdit software version 7.2.5 (Tom Hall) (Hall 1999).

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The optimal tree with the sum of branch length = 0.47231526 is shown (Fig. 1). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985)—except for values below 50%, which were hidden for readability. The evolutionary distances were computed using the Tamura–Nei method (Tamura and Nei 1993) and are in the units of the number of base substitutions per site. The analysis involved 40 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 423 positions in the final dataset. Evolutionary

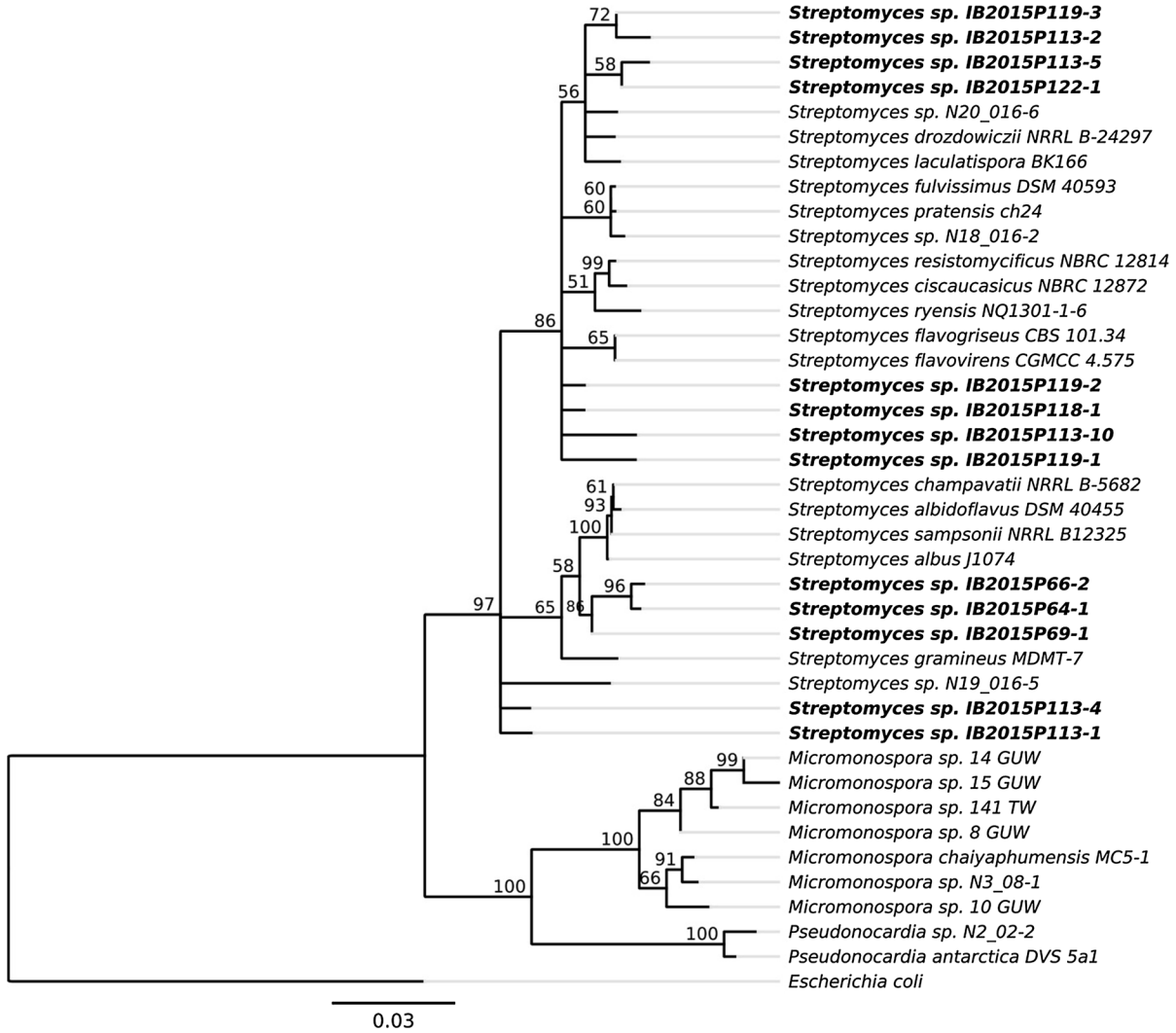

Fig. 1 Evolutionary relationships of actinobacteria isolated from amphipods belonging to the genus *Ommatogammarus* and collected in a gradient of depth 80–200 m

analyses were conducted in MEGA7 (Kumar et al. 2016). The sequences were deposited in the GenBank and their ID presented in Table 1 of the results section.

Cultivation and extraction of secondary metabolites

Strains were cultivated in 10 mL of TSB medium for 2 days at 28 °C at a shaking rate of 180 rpm. 2 mL of each pre-culture was used to inoculate 50 mL of production medium in a 500-mL Erlenmeyer flask with baffles. Three different liquid media were selected for metabolite production: NL19 (soy flour—20 g/L, D-mannitol—20 g/L); SG (dextrose—20 g/L, soy peptone—10 g/L, $CaCO_3$—2 g/L, $CoCl_2$—0.001 g/L); and ISP2 (yeast extract—4 g/L, malt extract—10 g/L, dextrose—4 g/L, pH 7.2) (Kieser et al. 2000) The strains were cultivated at 28 °C for 4 days at a shaking rate of 180 rpm. The cultural liquid and biomass were separated by centrifugation at $3000\times g$ for 5 min. Metabolites from the liquid were extracted with an equal volume of ethyl acetate (Sigma, St. Louis, USA) (Sarker and Nahar 2012). The compounds from the biomass were extracted with 10 mL of acetone:methanol mixture (1:1). The extracts were evaporated on an IKA RV–8 rotovap (IKA, Germany) at 40 °C and dissolved in 500 µL of methanol (Sigma, St. Louis, USA).

Table 1 Actinobacterial strains isolated from deepwater amphipods belonging to *Ommatogammarus* genera

No	Strain	Isolation source	Depth, m	Seguence ID (In GenBank)	Query score, %/ identity, %	Closest homologue strains ID
1	*Streptomyces* sp. IB 2015P60-1	*O. ablinus*	80	KX539267	100/99	CP003990.1
						NR074559.1
						HF935087.1
2	*Streptomyces* sp. IB 2015P60-1HS	*O. ablinus*	80	KX539268	100/99	JF512559.1
						DQ882847.2
						FN687756.1
3	*Streptomyces* sp. IB 2015P60-2	*O. ablinus*	80	KX539269	98/99	NR115669.1
						NR112266.1
						NR042102.1
4	*Streptomyces* sp. IB 2015P60-2HS	*O. ablinus*	80	KX539270	96/99	LC158571.1
						KR817735.1
						LC073310.1
5	*Streptomyces* sp. IB 2015P60-3	*O. ablinus*	80	KX539271	94/86	KP998448.1
						KU500592.1
						KT970739.1
6	*Micromonospora* sp. IB 2015P61-1	*O. ablinus*	80	KX539272	96/90	NR137397.1
						NR108478.1
						NR041265.1
7	*Streptomyces* sp. IB 2015P61-3	*O. ablinus*	80	KX539273	84/87	NR115669.1
						NR114830.1
						NR043816.1
8	*Pseudonocardia* sp. IB 2015P62-1HS	*O. ablinus*	80	KX531081	95/87	KU317912.1
						KP998448.1
						KT970739.1
9	*Streptomyces* sp. IB 2015P63-1HS	*O. ablinus*	80	KX531082	95/87	CP011862.1
						KP338814.1
						KP013381.1
10	*Streptomyces* sp. IB 2015P64-1	*O. ablinus*	80	KX531083	95/99	KF432190.1
						KU317912.1
						KT970739.1
11	*Streptomyces* sp. IB 2015P64-2	*O. ablinus*	80	KX531084	99/99	HQ238398.1
						HQ238340.1
						AB362247.1
12	*Streptomyces* sp. IB 2015P65-1	*O. flavus*	80	KX531085	91/87	GU350493.1
						KU382724.1
						CP014485.1
13	*Streptomyces* sp. IB 2015P66-2	*O. flavus*	80	KX531086	98/99	HQ238398.1
						AB362247.1
						HQ992724.1
14	*Streptomyces* sp. IB 2015P67-2	*O. flavus*	80	KX531087	92/99	KC462527.1
						KU317912.1
						DQ882847.2

Table 1 continued

No	Strain	Isolation source	Depth, m	Seguence ID (In GenBank)	Query score, %/ identity, %	Closest homologue strains ID
15	*Streptomyces* sp. IB 2015P69-1	*O. flavus*	80	KX531088	100/99	EU551698.1 KT844496.1 KU321340.1
16	*Streptomyces* sp. IB 2015P102-1	*O. flavus*	100	KX531089	97/89	HQ238398.1 HQ238340.1 JX047032.1
17	*Streptomyces* sp. IB 2015P104-1	*O. albinus*	100	KX531090	94/87	CP014485.1 KM067288.1 KR817746.1
18	*Streptomyces* sp. IB 2015P113-1	*O. flavus*	200	KX539274	99/99	KU317917.1 KX279642.1 KU324435.1
19	*Streptomyces* sp. IB 2015P113-2	*O. flavus*	200	KX539275	98/99	JN976939.1 FR692070.1 HE617252.1
20	*Streptomyces* sp. IB 2015P113-3	*O. flavus*	200	KX539276	96/99	KP718580.1 KJ781974.1 FJ492842.1
21	*Streptomyces* sp. IB 2015P113-4	*O. flavus*	200	KX539277	98/100	KC978877.1 LC085600.1 KU289083.1
22	*Streptomyces* sp. IB 2015P113-5	*O. flavus*	200	KX539278	98/99	KF803307.1 NR116093.1 NR041424.1
23	*Streptomyces* sp. IB 2015P113-6	*O. flavus*	200	KX539279	95/97	KT717916.1 KM287557.1 KF554192.1
24	*Streptomyces* sp. IB 2015P113-7	*O. flavus*	200	KX539280	97/87	KT958868.1 HM007155.1 KP336669.1
25	*Streptomyces* sp. IB 2015P113-8	*O. flavus*	200	KX539281	98/99	KP718540.1 KF620334.1 JQ924387.1
26	*Streptomyces* sp. IB 2015P113-9	*O. flavus*	200	KX539282	95/99	KF317976.1 KC554304.1 HQ850405.1
27	*Streptomyces* sp. IB 2015P113-10	*O. flavus*	200	KX539283	99/99	KX279563.1 AB907697.1 JQ924389.1
28	*Streptomyces* sp. IB 2015P113-11	*O. flavus*	200	KX539284	94/87	EU906929.1 KF582596.1 AY582733.1

Table 1 continued

No	Strain	Isolation source	Depth, m	Seguence ID (In GenBank)	Query score, %/ identity, %	Closest homologue strains ID
29	*Streptomyces* sp. IB 2015P113-12	*O. flavus*	200	KX539285	95/98	KU500358.1 KX389560.1 KU324455.1
30	*Streptomyces* sp. IB 2015P113-13	*O. flavus*	200	KX539286	95/98	KJ781968.1 KC336416.1 JX430822.1
31	*Streptomyces* sp. IB 2015P113-14	*O. flavus*	200	KX539287	97/96	KF620334.1 JQ924387.1 NR112269.1
32	*Streptomyces* sp. IB 2015P113-15	*O. flavus*	200	KX539288	93/88	HQ143637.1 DQ882847.2 KR817746.1
33	*Streptomyces* sp. IB 2015P113-16	*O. flavus*	200	KX539289	100/93	KF803307.1 DQ167410.1 KC856911.1
34	*Streptomyces* sp. IB 2015P114-1	*O. flavus*	200	KX539290	91/87	LC128341.1 KF973309.1 HM235458.1
35	*Streptomyces* sp. IB 2015P114-2	*O. flavus*	200	KX539291	95/99	LC128341.1 EU119191.1 EU722761.1
36	*Streptomyces* sp. IB 2015P117-1	*O. flavus*	200	KX539292	98/99	KP191987.1 KF555351.1 KX056501.1
37	*Streptomyces* sp. IB 2015P118-1	*O. flavus*	200	KX539293	99/99	KX171767.1 KP004442.1 JF728875.1
38	*Streptomyces* sp. IB 2015P119-1	*O. flavus*	200	KX539294	97/99	KX130872.1 KX056510.1 KJ862784.2
39	*Streptomyces* sp. IB 2015P119-2	*O. flavus*	200	KX539295	98/99	KM453011.1 AM889470.1 HE585543.1
40	*Streptomyces* sp. IB 2015P119-3	*O. flavus*	200	KX539296	100/98	KJ957004.1 KR181801.1 KC815756.1
41	*Streptomyces* sp. IB 2015P119-5	*O. flavus*	200	KX539297	95/94	KF803307.1 KJ889187.1 JF806651.1
42	*Streptomyces* sp. IB 2015P122-1	*O. ablinus*	200	KX539298	98/98	JN020523.1 JN020499.1 KT581319.1

Liquid chromatography–mass spectrometry (LC–MS) and dereplication analysis

The strain *Streptomyces* sp. IB 2015P113-12 was cultivated in 1.0 L of NL19 medium. The secondary metabolites from the culture liquid and biomass were extracted using ethyl acetate and the acetone-methanol mixture as described above (Sarker and Nahar 2012). The extract was dissolved in methanol and analyzed on an ultra-high resolution QTOF maXis II mass spectrometry system (Bruker, Billerica, USA) and LTQ XL Orbitrap (Thermo Fisher Scientific, USA). The sample was separated on an Ultimate 3000 HPLC system (Dionex, Sunnyvale, USA) with diode array detector using a C18 column (ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1 mm × 100 mm) and a linear gradient of acetonitrile from 5 to 95% against a 0.1% ammonium formate solution in water at a flow rate 0.6 mL/min for 20 min. Mass detection was performed in positive mode, with the detection range set to 160–2500 Da. Data were collected and analyzed using Bruker Compass Data Analysis software, version 4.1 (Bruker, Billerica, USA) and Thermo Xcalibur, version 3.0.63 (Thermo Fisher Scientific, USA). Dereplication was performed using the Dictionary of Natural Products (DNP) database, version 6.1 (CRC Press, Boca Raton, USA) with the following search parameters: accurate molecular mass, absorption spectra and biological source. Compounds were considered to be similar when the difference in accurate mass was less than 0.005 Da, mass accuracy was less than 5 ppm, MS2 and the UV absorption spectrum were identical (Whittle and Willett 2003).

Antibiotic assay of extracts from isolated strains

The disk-diffusion method was used to determine the antimicrobial activity of obtained extracts. Seven model strains of microorganisms were chosen as the test cultures: *Bacillus subtilis* ATCC 6633, *Pseudomonas putida* KT2440, *Staphylococcus carnosus* ATCC 51365, *Escherichia coli* ATCC25922, *E. coli* K12, *Saccharomyces cerevisiae* BY4742 and *Candida albicans* ATCC 90027. Overnight cultures were used to inoculate fresh plates with LB (for bacteria) or YPD (for fungi) media. The paper disks (5 mm in diameter) were loaded by 25 µL of crude extracts derived either from liquid culture or biomass and dried at room temperature. The dry disks were placed on media with plated test cultures. The extracts were tested against *E. coli* K12 only in the case of activity against *E. coli* ATCC25922. Plates with disks were incubated at 37 °C (*B. subtilis*, *E. coli*, *St. carnosus*, *P. putida*) or 28 °C (*S. cerevisiae*, *C. albicans*) (Grainger et al. 2001). The zones of inhibition were manually measured with ±1 mm accuracy. Disks loaded with methanol were used as negative controls.

Bacterial test cultures were obtained from the Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).

Results

Isolation and phylogenetic characterization of actinobacteria from deepwater endemic amphipods of Lake Baikal

In this study, 42 individual actinobacteria-like strains were isolated (Table 1; Fig. S1). The isolates were selected by their morphological features. In total, 13 cultivated strains of actinobacteria were obtained from *O. albinus*. Eleven of them were obtained from the amphipods samples collected at 80 m depth. One strain was isolated from the amphipods inhabiting the 100 m, and one from 200 m depths. Twenty-nine strains were isolated from *O. flavus* specimens. Twenty-four of them were from the amphipods inhabiting the 200 m depth. From the representatives of *O. flavus* collected at the depths of 80 and 100 m, four and one strains were isolated, respectively.

The 16S rRNA gene sequence-based phylogenetic analysis indicated that 40 out of the 42 actinobacteria strains belong to the genus *Streptomyces*. Two other strains were identified as *Micromonospora* and *Pseudonocardia* (Table 1). Both strains were isolated from *O. albinus* sampled at 80 m. The 16S rDNA sequences from isolated bacteria formed a tight clade with 16S rDNA of several representatives of the respective genera (Fig. 1).

Most of the strains including the *Micromonospora* sp. IB 2015P61-1 were selected on MS medium. Only one strain, identified as *Pseudonocardia* sp. IB2015P62-1HS was obtained on starch-ammonia agar (SAA). The frequencies of strain isolation and the

strains distribution in the collected samples of amphipods of the genus *Ommatogammarus* are shown in Table 2. In addition, four of the obtained strains were isolated from *O. albinus* inhabiting the 80 m depth using thermal pretreatment, including the *Pseudonocardia* sp. (marked as "HS").

Analysis of the biological activities of isolated actinobacterial strains

Among 42 isolated strains, extracts of only four (*Streptomyces* sp. IB2015P61-1, *Streptomyces* sp. IB2015P113-15, *Streptomyces* sp. IB2015P114-1, and *Streptomyces* sp. IB2015P122-1) did not demonstrate antimicrobial or antifungal activity against at least one of the test organisms used (Table 3, Tables S1–S6). All other strains were found to produce compounds inhibiting growth of bacteria and/or fungi. The antimicrobial activities of the isolated strains are presented in Table 3 and Tables S1–S6. In total, the growth of Gram-positive bacteria was inhibited by extracts of 22 strains out of 42. The growth of *S. carnosus* and *B. subtilis* was inhibited by extracts obtained from five strains grown in NL19 medium, two strains incubated in SG medium and 17 ISP2 cultures (Table 3, Tables S1–S6).

The growth of Gram-negative bacteria was affected by extracts of 12 strains. *P. putida* and *E. coli* were inhibited by four isolates cultivated in NL19 medium, 1 strain grown in SG medium and eight strains cultivated in ISP2 medium. Extracts of the culture liquid of the strains *Streptomyces* sp. IB2015P113-12 and *Streptomyces* sp. IB 2015P113-2 grown in SG and NL19 media were also able to inhibit the growth *E. coli* ATCC25922 (*tolC* mutant) and *E. coli* K12 (Table 3, Tables S1–S6).

Thirty stains demonstrated antifungal activity. Sixteen of them were able to inhibit growth of both *S. cerevisiae* and *C. albicans*. Twelve strains demonstrated activity against *S. cerevisiae* only. Two strains, *Streptomyces* sp. IB 2015P113-13 and *Streptomyces* sp. IB 2015P61-3, were able to inhibit the growth of *C. albicans* but not *S. cerevisiae*. In total, 18 strains grown in NL19 medium, 25 in ISP2, and 17 in SG were found to produce fungicides.

In summary, the ISP2 medium was found to be the most efficient for production of biologically active compounds by the isolated actinobacteria, followed by NL19 and SG media. Also, it should be mentioned that activity was not always detected for both culture liquid and biomass extracts. In some cases, only one type of extract demonstrated activity against a particular test culture. For example, only culture liquid extracts of the *Streptomyces* sp. IB 2015P113-12 grown on NL19 or SG media were active against *E. coli* strains. On the other hand, the same strain accumulates compounds active against *S. cerevisiae* only in biomass when grown in ISP2 medium.

Table 2 The frequency of strain isolation and strain distribution in collected samples of amphipods belonging to the genus *Ommatogammarus*

Number of isolated strains, per sample	80 m	100 m	200 m
Total	15	2	25
Ommatogammarus albinus	11	1	1
MS medium	7	1	1
ISP2 medium	2	0	0
Gauze's synthetic agar	3	2	0
Waksman's medium	1	1	0
Czapek medium	1	0	1
Starch-ammonium medium	1	0	0
Ommatogammarus flavus	4	1	24
MS medium	3	1	14
ISP2 medium	1	1	11
Gauze's synthetic agar	0	0	1
Waksman's medium	1	0	0
Czapek medium	0	0	0
Starch-ammonium medium	0	0	0

Table 3 Antimicrobial activities of isolated actinobacteria strains grown on different media

Strain	Media	Bacterial test culture					Fungal test culture	
		B. subtilis	*St. carnosus*	*P. putida*	*E. coli*	*E. coli K12*	*S. cerevisae*	*C. albicans*
Streptomyces sp. IB 2015P60-1	NL19							
	ISP2						*BE*	
	SG							
Streptomyces sp. IB 2015P60-1hs	NL19						*BE*	*CLE, BE*
	ISP2		*BE*				*CLE, BE*	
	SG							*BE*
Streptomyces sp. IB 2015P60-2	NL19							*CLE, BE*
	ISP2						*BE*	
	SG						*BE*	*BE*
Streptomyces sp. IB 2015P60-2hs	NL19						*BE*	*CLE, BE*
	ISP2						*CLE*	
	SG							*CLE, BE*
Streptomyces sp. IB 2015P60-3	NL19							*CLE, BE*
	ISP2							
	SG						*BE*	*BE*
Streptomyces sp. IB 2015P61-1	NL19							
	ISP2							
	SG							
Streptomyces sp. IB 2015P61-3	NL19							
	ISP2		*BE, CLE*	*BE*				*BE*
	SG							*BE*
Streptomyces sp. IB 2015P62-1hs	NL19							
	ISP2		*BE*					
	SG							
Streptomyces sp. IB 2015P63-1hs	NL19						*CLE, BE*	*CLE, BE*
	ISP2							
	SG							*CLE, BE*
Streptomyces sp. IB 2015P64-1	NL19						*CLE*	*CLE, BE*
	ISP2	*CLE*	*BE, CLE*	*BE*			*CLE*	
	SG							*BE*
Streptomyces sp. IB 2015P64-2	NL19						*CLE, BE*	*CLE, BE*
	ISP2	*CLE*	*BE, CLE*				*CLE*	
	SG						*BE*	*CLE, BE*
Streptomyces sp. IB 2015P65-1	NL19						*CLE, BE*	*BE*
	ISP2							
	SG							*BE*
Streptomyces sp. IB 2015P66-2	NL19							*CLE, BE*
	ISP2				*BE*		*CLE*	
	SG						*CLE, BE*	*CLE, BE*
Streptomyces sp. IB 2015P67-2	NL19							*CLE, BE*
	ISP2				*BE*		*CLE*	
	SG						*BE*	*CLE, BE*

Table 3 continued

Strain	Media	Bacterial test culture					Fungal test culture	
		B. subtilis	*St. carnosus*	*P. putida*	*E. coli*	*E. coli K12*	*S. cerevisae*	*C. albicans*
Streptomyces sp. IB 2015P69-1	NL19							*BE*
	ISP2				*BE*			
	SG						*BE*	*BE*
Streptomyces sp. IB 2015P102-1	NL19							*CLE, BE*
	ISP2	*CLE*					*BE*	
	SG						*BE*	*BE*
Streptomyces sp. IB 2015P104-1	NL19		*BE*				*CLE, BE*	*CLE, BE*
	ISP2						*CLE, BE*	
	SG							*CLE, BE*
Streptomyces sp. IB 2015P113-1	NL19		*CLE, BE*		*BE*		*CLE, BE*	*BE*
	ISP2						*BE*	
	SG							*CLE, BE*
Streptomyces sp. IB 2015P113-2	NL19		*CLE, BE*		*CLE*	*CLE*		
	ISP2							
	SG							
Streptomyces sp. IB 2015P113-3	NL19							
	ISP2							
	SG		*CLE, BE*					
Streptomyces sp. IB 2015P113-4	NL19		*BE*				*CLE, BE*	
	ISP2							
	SG							
Streptomyces sp. IB 2015P113-5	NL19							
	ISP2						*BE*	
	SG							
Streptomyces sp. IB 2015P113-6	NL19							
	ISP2						*BE*	
	SG							
Streptomyces sp. IB 2015P113-7	NL19							
	ISP2						*CLE, BE*	
	SG							
Streptomyces sp. IB 2015P113-8	NL19							
	ISP2						*BE*	
	SG							
Streptomyces sp. IB 2015P113-9	NL19							
	ISP2						*CLE, BE*	
	SG							
Streptomyces sp. IB 2015P113-10	NL19							
	ISP2		*CLE, BE*				*CLE*	
	SG							
Streptomyces sp. IB 2015P113-11	NL19						*BE*	
	ISP2		*CLE, BE*				*CLE, BE*	*CLE*
	SG						*BE*	

Table 3 continued

Strain	Media	Bacterial test culture					Fungal test culture	
		B. subtilis	*St. carnosus*	*P. putida*	*E. coli*	*E. coli K12*	*S. cerevisae*	*C. albicans*
Streptomyces sp. IB 2015P113-12	NL19		*CLE*	*CLE*	*CLE*	*CLE*		*CLE*
	ISP2		*CLE, BE*	*BE*			*BE*	*BE*
	SG		*CLE, BE*	*CLE, BE*	*CLE*	*CLE*		
Streptomyces sp. IB 2015P113-13	NL19							
	ISP2		*CLE, BE*	*BE*				*CLE, BE*
	SG							*BE*
Streptomyces sp. IB 2015P113-14	NL19							
	ISP2		*CLE, BE*	*BE*				
	SG							
Streptomyces sp. IB 2015P113-15	NL19							
	ISP2							
	SG							
Streptomyces sp. IB 2015P113-16	NL19							
	ISP2	*BE*						
	SG							
Streptomyces sp. IB 2015P114-1	NL19							
	ISP2							
	SG							
Streptomyces sp. IB 2015P114-2	NL19							
	ISP2	*BE*						
	SG							
Streptomyces sp. IB 2015P117-1	NL19							
	ISP2	*CLE, BE*					*CLE, BE*	
	SG							
Streptomyces sp. IB 2015P118-1	NL19							
	ISP2	*CLE*		*BE*				
	SG							
Streptomyces sp. IB 2015P119-1	NL19							
	ISP2	*CLE, BE*					*CLE, BE*	
	SG							
Streptomyces sp. IB 2015P119-2	NL19							
	ISP2	*CLE, BE*					*CLE*	
	SG							
Streptomyces sp. IB 2015P119-3	NL19							
	ISP2						*CLE*	
	SG							
Streptomyces sp. IB 2015P119-5	NL19				*CLE*			
	ISP2							
	SG							
Streptomyces sp. IB 2015P122-1	NL19							
	ISP2							
	SG							

BE biomass extract, *CLE* cultural liquid extract

Dereplication of the secondary metabolites produced by *Streptomyces* sp. IB 2015P113-12

Strain *Streptomyces* sp. IB 2015P113-12 showed inhibitory activity against both bacterial and fungal test cultures. To gain deeper insights into the nature of compounds possibly contributing to observed phenomenon we conducted a dereplication analysis of the secondary metabolites produced by this strain. High-resolution mass spectrometry methods enable the general analysis of secondary metabolites and facilitate the annotation of individual compounds using information from pre-existing databases. A total of 43 major compounds were detected in the cultural liquid and biomass extracts of the *Streptomyces* sp. IB 2015P113-12 strain cultivated in NL19 medium (Fig. S2). Thirty-seven and twenty-nine compounds (peaks) were found in the biomass and cultural liquid extracts, respectively. Twenty-four of them were present in both extracts.

Twenty-three compounds were preliminarily annotated based on exact mass, source, and absorption spectrum parameters search in the Dictionary of Natural Products (DNP) database (Whittle and Willett 2003). Another 20 did not give positive hits in the DNP and could not be annotated based on the available mass-spec data and might represent new findings. Results of dereplication analysis with a list of the detected adducts and possible database hits are shown in Figs. S2–S5 and Table 4.

The following compounds could be annotated in both culture liquid and biomass extracts of *Streptomyces* sp. IB2015P113-12: N6-Deoxy-nocardimine (terragine E) (*m/z* 585.312 $[M + H]^+$, CRC code—MCG61; 4.6 ppm) and three derivatives of bafilomycin - 2-O-desmethyl-Leucanicidin (*m/z* 769.4738 $[M + H]^+$, CRC code—CJW04; 1.6 ppm), Leucanicidin (*m/z* 783.4859 $[M + H]^+$, CRC code—DON49; 4.5 ppm) and Antibiotic NK 155141 (*m/z* 797.5005 $[M + H]^+$, CRC code—CKN32; 5.8 ppm). Terragine E, that seems to act as a siderophore, originally was obtained by expression of metagenomics DNA in *Streptomyces lividans* (Wang et al. 2000). Finding of terragine E in the producing strain isolated from a deepwater endemic organism confirms the specificity of Lake Baikal ecosystem. Bafilomycins are macrolides, produced by *Streptomyces griseus* and act as inhibitors of membrane ATPases. These compounds have antibacterial, antifungal, antineoplastic and immunosuppressive activities (Bowman et al. 1988). In addition, another polyketide antibiotic BE 67251 (*m/z* 454.2957 $[M + H]^+$, CRC code—KFM90, 0.7 ppm.) was found in the biomass extract of *Streptomyces* sp. IB2015P113-12 (Wu et al. 2009; Conti et al. 2016) (Fig. S2; Table 4).

In the crude extract of the *Streptomyces* sp. IB2015P113-12 strain several ionophore antibiotics of the macrotetrolide family could be annotated. We were able to identify nonactin (*m/z* 737.4498 $[M + H]^+$, CRC-code HDN12), monactin (751.4655 *m/z*, CRC code CKH81) and dinactin/isodinactin (*m/z* 765.4819 $[M + H]^+$, CRC code CKH81). These compounds (Figs. S2–S4) are known for their broad spectrum antibacterial activity, as well as for insecticidal, anthelmintic, larvicidal and coccidiostatic activities (Zhan and Zheng 2016). In addition, several less characterized representatives of the macrotetrolide group can be annotated in the extract of the strain, including macrotetrolide G (*m/z* 779.5028 $[M + H]^+$, CRC code CKW16), macrotetrolide B (*m/z* 821.5379 $[M + H]^+$, CRC code CKW13), macrotetrolide C (*m/z* 807.5231 $[M + H]^+$, CRC code CKW14), macrotetrolide D (*m/z* 793.5123 $[M + H]^+$, CRC code CKW15) (Figs. S2, S5). These compounds are derivatives of nactins with partially defined structures and possess antibacterial activity against Gram-positive bacteria (Jizba et al. 1991; Botti et al. 2010). We also managed to identify several precursors of macrotetrolides: homononactic acid, nonactyl nonactoate, homononactyl nonactoate (Table 4; Fig. S2). Based on these findings, we can assume that the activity of *Streptomyces* sp. IB2015P113-12 against Gram-positive bacteria can be explained at least partially by the accumulation of macrotetrolide antibiotics. Also, nonactins could be responsible for the activity against Gram-negative bacteria and fungi (Becerril Espinosa et al. 2012).

Discussion

Previous studies have demonstrated a high abundance of actinobacteria in Lake Baikal water, sediments, and sponge microbial communities (Kaluzhnaya et al. 2012). These observations suggested that the lake endemic animals can be also a source of novel actinobacterial species. Herein, we report isolation and initial characterization of 42 strains of

Table 4 Compounds isolated from extracts of strain *Streptomyces* sp. IB2015P113-12 and annotated with DNP

No	Biomass extract	Cult. extract	RT [min]	CRC-code	Name of compound	AM	Δ (mass), m/z[1]	ppm	Source	Importance	References
1	+	+	12.08	MCG61	Nocardamine, N6-deoxy (terragine E)	584.3534	0.003	4.6	*S. lividans*	Siderophore	Conti et al. (2016)
2	+		12.87	KFM90	Antibiotic BE 67251	453.2879	0.000	0.7	*Streptomyces* sp. *A67251*	Antitumour agent; Active against mycobacteria. Induces morphological changes in insect cells. Antioxidant. Inhibits WNT signalling	Patent US 4252898A
3	+	+	14.36	CJW04	Bafilomycin A1; O21; O21-α-L-rhamnopyranosid	768.4660	0.001	1.6	*S. olivaceus*	Anti-fungal agent; anticancer agent; Protein phosphatase inhibitor	Wu et al. (2009)
4	+	+	14.96	DON49	Bafilomycin A1; O21-(2-*O*-methyl-α-L-rhamnopyranoside) (Leucanicidin)	782.4816	0.004	4.5	*S. halstedii, S. olivaceus*	Strong insecticidal and antifungal props	Wu et al. (2009)
5	+	+	15.52	CKN32	Bafilomycin A1 O19-Me, O21-(2-*O*-methyl-α-L-rhamnopyranoside (Antibiotic NK 155141)	796.4973	0.005	5.8	*Streptomyces* sp. *NK155141*	Antitumour agent, pesticide	Wu et al. (2009)
6	+	+	16.88	HDN12	Nonactin (Werramycin)	736.4398	0.002	3.0	*Streptomyces* sp. *ETH 779*	Active against gram-positive bacteria. Potassium ion membrane transporter	Barazi et al. (2002); Keller-Schierlein et al. (1968); Zhan and Zheng (2016)
7	+	+	17.43	CKH81	Nonactin homologue (R1 = CH2CH3, R2 = R3 = R4 = CH3 (Monactin)	750.4554	0.002	3.0	*Streptomyces* sp. *ETH 779*	Shows antibiotic props. Insecticide, anthelmintic, larvicide, coccidiostat. Smooth muscle relaxant, immunosuppressant. Calcium channel blocker, glycoprotein transportation inhibitor, Antiallergic	Barazi et al. (2002); Keller-Schierlein et al. (1968); Zhan and Zheng (2016)

Table 4 continued

No	Biomass extract	Cult. extract	RT [min]	CRC-code	Name of compound	AM	Δ (mass), m/z[1]	ppm	Source	Importance	References
8	+	+	17.98	CKF55	Nonactin homologue (R1 = R2 = CH2CH3, R3 = R4 = CH3) (dinactin)	764.4711	0.003	4.0	*Streptomyces ETH, S. griseus CB00830*	Ionophore antibiotic. Acaricide	Barazi et al. (2002); Keller-Schierlein et al. (1968); Zhan and Zheng (2016)
				PWD21	Nonactin homologue homologue (R1 = R4 = CH2CH3, R2 = R3 = CH3) (Isodinactin)	764.4711	0.003	4.0	*Streptomyces* sp.	Ionophore antibiotic. Active against gram-positive bacteria. Potassium ion membrane transporter	Barazi et al. (2002); Keller-Schierlein et al. (1968); Zhan and Zheng (2016)
9	+	+	18.48	CKW16	Macrotetrolide. Macrotetrolide G	778.4867	0.002	3.6	*S. flaveolus, S.roseochromogenes*	Active against gram-positive bacteria	Smith (1975)
10	+	+	18.95	CKW15	Macrotetrolide. Macrotetrolide D	792.5024	0.002	2.7	*S. flaveolus, S.roseochromogenes*	Active against gram-positive bacteria	Smith (1975)
11	+	+	19.36	CKW14	Macrotetrolide. Macrotetrolide C	806.5180	0.003	3.3	*S. flaveolus, S.roseochromogenes*	Active against gram-positive bacteria	Smith (1975)
12	+	+	19.76	CKW13	Macrotetrolide. Macrotetrolide B	820.5337	0.004	4.4	*S. flaveolus, S.roseochromogenes*	Active against gram-positive bacteria	Smith (1975)

actinobacteria from Lake Baikal endemic deepwater amphipod species *O. albinus* and *O. flavus*. Among them, 40 were identified as representatives of the genus *Streptomyces* and two belongs to the genera *Micromonospora* and *Pseudonocardia.* This finding correlates with the previous observations of phylogenetic diversity of microbial communities of Lake Baikal water and sediments (Terkina et al. 2002). It is generally accepted that the minor genera of actinobacteria are potentially more interesting in terms of secondary metabolites novelty. Thus, the relatively high frequency of isolation of strains belonging to *Micromonospora* and *Pseudonocardia* (appr. 5% of all isolated specimens) from the sources used is promising and will further stimulate the research in this direction.

The materials obtained in this study are summarized in Table 5. Some correlations could be observed between the number of actinobacterial isolates, the depth of sampling and the species of amphipod used as a source. For example, the number of actinobacteria decreased in the case of *O. albinus* along the depth gradient from 80 (n = 11) to 200 m (n = 1). In the case of *O. flavus* the opposite trend was observed: the number of actinobacteria strains increased with the depth. A deeper understanding of ecology of the studied amphipods can help to explain the observed phenomena. Both species are known for their seasonal migration activities (from depth of 2.5–1313 m in the case of *O. flavus* and from depth of 100–1600 m in the case of *O. albinus*) (Timoshkin et al. 2001). These migrations are caused by factors related to feeding and probably can influence the microbial associations of amphipods depending on the substrates exploited.

We also found that a large proportion (>70%) of strains isolated from amphipods have antifungal activity. This makes us to believe that these associations are not random and could represent symbiotic interactions between bacteria and amphipods. The amphipods consume the organic matter and detritus which are naturally inhabited by fungi as decomposers (Su et al. 2015). Fungi enter the gastrointestinal tract of amphipods together with food and may have undesirable effects on the metabolism and physiology. Actinobacterial produced compounds may therefore be involved in the fungal resistance of amphipods. It is known that fungi are one of the main causes of infections in aquatic crustaceans (Armstrong et al.

Table 5 The strain's activity in actinobacteria isolated from amphipods belonging to the genus *Ommatogammarus* and collected in a gradient of depth 80–200 m

Number of isolated strains, per sample	80 m	100 m	200 m
Number of isolated strains	15	2	25
Number of active strains (N strains/%)	14 str./93%	2str./100%	22 str./88%
Incl. antibacterial activity	8str./53%	2 str./100%	15 str./60%
Incl. Gr^+ bacteria	5str./33%	2 str./100%	15 str./60%
Incl. Gr^- bacteria	5 str./33%	0/0	7 str./28%
Incl. antifungal activity	13str./87%	2 str./100%	15 str./60%
Number of no active strains (strain/%)	1 str./7%	0/0	3str./12%
Ommatogammarus albinus	11	1	1
Number of active strains (N strains/%)	10 str./91%	1str./100%	0/0
Incl. antibacterial activity	5str./45%	1 str./100%	0/0
Incl. Gr^+ bacteria	5str./45%	1 str./100%	0/0
Incl. Gr^- bacteria	2 str./18%	0/0	0/0
Incl. antifungal activity	9str./82%	1 str./100%	0/0
Number of no active strains (strain/%)	1 str./9%	0/0	1 str./100%
Ommatogammarus flavus	4	1	24
Number of active strains (N strains/%)	4 str./100%	1str./100%	21 str./87%
Incl. antibacterial activity	4str./100%	1 str./100%	16 str./67%
Incl. Gr^+ bacteria	0/0	1 str./100%	15 str./62%
Incl. Gr^- bacteria	4str./100%	0/0	7 str./29%
Incl. antifungal activity	4str./100%	1 str./100%	15 str./60%
Number of no active strains (strain/%)	0/0	0/0	3str./12%

1976; Hatai 2012). In some cases, the crustaceans are remarkably resistant to fungal infection. Gil-Turnes et al. described the resistance of embryos of the shrimp *Palaemon macrodactylus* to the action of the parasitic fungus *Lagenidium callinestes* (Gil-Turnes et al. 1989). The cause of the tolerance was the bacteria *Alteromonas* sp. isolated from the surfaces of the embryos. It produces the highly effective antifungal compound 2,3-indolinedione or tribulin. Similar studies led to the discovery of another antifungal metabolite, 4-hydroxyphenethyl alcohol or tyrosol, isolated from the embryos of the American lobster *Homarus americanus* (Gil-Turnes and Fenical 1992). Both compounds have a narrow activity against *L. callinestes*, supporting the idea of the adaptive nature of bacteria-crustaceans' interaction.

Among the animals of Lake Baikal, the microbial communities inhabiting sponges are the most extensively studied. Actinobacteria of the genera *Streptomyces* and *Micromonospora* were found to be permanent components of microbial communities of sponges belonging to the genera *Swartschewskia*, *Baicalospongia*, and *Lubomirskia* (Parfenova et al. 2008; Kaluzhnaya et al. 2011). However, other ecological niches and endemic animals of the lake are less studied. We attempted for the first time to explore the cultivable actinobacterial populations from deepwater amphipods of Lake Baikal. These macroinvertebrates are a key element of the food chains of the Baikal ecosystem. Due to their lifestyle, they are facing a number of challenges, including permanent competition with fungi and bacteria. However, little is known about the protection mechanisms that amphipods are using to fight these competitors and potential pathogens. A significant role in this could be played by the associations with actinobacteria producing biologically active metabolites. Also, this is the first report of isolation of actinobacteria belonging to the genera *Micromonospora* and *Pseudonocardia* from Lake Baikal amphipods. In our previous study, ten strains of the genera *Aeromicrobium*, *Nocardia*, and *Streptomyces* were isolated from the amphipod species *Brandtia* sp. (Axenov-Gribanov et al. 2015). One strain identified as *Nocardia* sp. was obtained from the amphipod *Pallasea cancellus*.

To conclude, in the present study we isolated 42 actinobacteria strains from Lake Baikal endemic deepwater amphipods *O. albinus* and *O. flavus*. The high frequency of isolation of strains producing biologically active and possibly new compounds supports the idea that actinobacteria from unique and extreme ecosystems might be a prominent source of novel metabolites for pharmaceutical and biotechnological needs. This is especially relevant in the light of global problems caused by development and spread of antibiotic resistance among pathogens (Stadler and Dersch 2016).

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Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals *Statement of human rights*: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. *Statement on the welfare of animals*: This article does not contain any studies with human participants or vertebrate animals performed by any of the authors. As stated in the article, Baikalian macroinvertebrate species are not endangered or protected species. No specific permissions were required for sampling of invertebrates species.

Informed consent Informed consent was obtained from all individual participants included in the study.

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