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FATTY-ACID COMPOSITION OF SEVERAL LAKE BAIKAL STREPTOMYCETES

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The fatty-acid composition of seven strains of aquatic actinobacteria of the genus Streptomyces *was studied. GC and GC–MS showed that the microorganisms produced mixtures of saturated, unsaturated, and branched fatty acids. A producer of dehydroabietic acid was found.*

Keywords: aquatic actinobacteria, *Streptomyces*, fatty acids, dehydroabietic acid.

Actinobacteria of the genus *Streptomyces* are the leading producers of antibiotic, cytotoxic, and other biologically active compounds [1–3]. The aquatic environment has been a rich source of these producers during the course of several decades [4–6]. Until recently, metabolites of streptomycetes isolated from freshwater had been little studied.

Actinomycetes of the genus *Streptomyces* in Lake Baikal are of great interest. Phylogenetic analysis and fluorescent hybridization *in situ* of the microbial community of Lake Baikal showed the presence of bacteria of the phylum Actinobacteria, in particular, the genus *Streptomyces* in the aquatic strata of the lake [7, 8]. Cultivation was used to detect representatives of this genus in sediments and water of Lake Baikal, where they were dominant [9].

We investigated previously EtOAc extracts of several *Streptomyces* strains and showed that streptomycetes of Lake Baikal exhibit rather high antimicrobial activity against pathogenic and facultative pathogenic microorganisms in addition to antitumor and exoglucanase enzyme activites [10]. In continuation of research on biologically active compounds from Lake Baikal streptomycetes, we determined the fatty-acid composition of several of them (Table 1).

According to the results (Table 2), all strains produced saturated, unsaturated, and branched acids whereas strain 7 produced only palmitic acid (16:0). The mass of saturated acids in all obtained fractions was greater than that of unsaturated acids. Palmitic acid made up a large fraction of saturated acids in all strains, where its mass varied from 27.58 to 100%. The exception was only strain 6, where 16:0 acid was missing and the principal component was 15:0 methyl-14 acid (50.3%). A high content of palmitic acid was characteristic for actinobacteria [11–13], in particular, actinobacteria of the genus *Streptomyces* [13]. Actinobacteria are known to produce fatty acids (saturated, unsaturated, and branched) with chain lengths in the range C_{10}-C_{18} [11–14]. Our results agreed with those in the literature.

The strain *Streptomyces* sp. LB T12 synthesized dehydroabietic acid. The mass spectrum of dehydroabietic acid agreed with that of the same compound in the NIST98 database and the literature [15, 16]. Dehydroabietic acid is one of the principal toxic components of extracted wood resins and is an abietane-type diterpenoid. Dehydroabietic acid was obtained earlier from sap of conifers [17]. It was identified for the first time from metabolites of an aquatic actinomycete.

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TABLE 1. Sources for Isolating Actinomycetes

Strain No.	Strain	Source
1	*Streptomyces* sp. LB T178	South Baikal, polygon near Cape Berezovyi, water, 100 m depth
2	*Streptomyces* sp. LB T89	South Baikal, near B. Goloustnoe peninsula, water, surface
3	*Streptomyces* sp. LB T131	South Baikal, polygon near Cape Berezovyi, water, 100 m depth
4	*Streptomyces* sp. LB T12	South Baikal, polygon near Cape Berezovyi, water, 100 m depth
5	*Streptomyces* sp. LB T156	South Baikal, central station of Listvyanka-Tankhoi peninsula, water, surface
6	*Streptomyces* sp. LB T32	South Baikal, polygon near Cape Berezovyi, water, 50 m depth
7	*Streptomyces* sp. LB T125	South Baikal, polygon near Cape Berezovyi, water, 50 m depth

TABLE 2. Fatty-Acid Composition of Fractions from Aquatic Actinomycete Strains, % of Total Fatty Acids

Acid	Actinomycete strain						
	1	2	3	4	5	6	7
				Saturated			
15:0	4.05	–	–	–	–	–	–
16:0	29.70	48.37	34.27	49.33	27.58	–	100
17:0	5.47	–	12.00	–	–	–	–
18:0	12.43	–	11.24	17.49	–	23.38	–
Σsat.	51.65	48.37	57.51	66.82	27.58	23.38	100
				Unsaturated			
16:1	6.64	–	–	–	27.36	–	–
18:1	6.80	15.93	25.73	25.13	20.43	26.30	–
18:2	–	–	–	–	Tr.	Tr.	–
Σunsat.	13.44	15.93	25.73	25.13	47.79	26.30	–
				Branched			
14:0 methyl-9	–	–	–	8.04	–	–	–
14:0 methyl-12	17.44	–	–	–	–	–	–
15:0 methyl-14	11.37	9.90	16.74	–	24.62	50.30	–
16:0 methyl-14	1.35	8.40	–	Tr.	–	Tr.	–
17:0 methyl-16	–	17.38	–	–	–	–	–
Σbranch.	30.16	35.68	16.74	8.04	24.62	50.30	–
Dehydroabietic acid	–	–	–	Tr.	–	–	–
Mass of fatty acids in hexane fraction, %	58.49	53.34	41.96	87.48	83.94	75.84	34.41

EXPERIMENTAL

Cultivation of Microorganisms and Isolation of Fatty-acid Mixtures. Strains were cultivated for 5 d at 22°C in Ehrlenmeyer flasks (300 cm^3) in liquid medium (100 mL) containing glycerin (0.3 g), fish-peptone bullion (0.05), peptone (0.05), NaCl (0.045), $CaCO_3$ (0.035), and H_2O (100 mL) at pH 7.2 on a PSU-20 (Biosan, Latvia) apparatus for mixing biological fluids. Total fractions of fatty acids were obtained from the strains by chromatography of the EtOAc extract over silica gel using hexane:EtOAc (0→10%).

Analysis of Mixtures of Acid Methyl Esters. Methyl esters of fatty acids (MFA) were prepared by methylation with diazomethane of the obtained fractions. MFA were studied by GC and GC–MS. The products were identified by comparing their mass spectra with those of known compounds using the NIST98 database. The methyl esters were analyzed on an HP-5SM GC using an Agilent 6850 column (0.2 mm × 30 m) (Germany). The temperatures were inlet 270°C; 100°C/1 min-10°C/min-270°C/20 min; detector 300°C (He carrier gas). GC–MS of MFA was performed under the same chromatographic conditions on a System 6890 GC chromatograph with an MSD 5973 mass-spectrometric detector (Hewlett Packard, USA) and an HP-5 MS column (30 m × 250 μm × 0.25 μm) with 5% phenylmethylsiloxane and He carrier gas.

Mass spectrum of dehydroabietic acid methyl ester (EI, 70 eV, *m*/*z*): 314.0 (13) $[M]^+$, 299.0 (13) $[M - CH_3]^+$, 239.0 (100) $[M - CH_3 - HCOOCH_3]^+$, 197.0 (10) $[M - CH_3 - HCOOCH_3 - C(CH_3)]^+$.

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