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Proceeding Paper

Actinomycetes of the Genus *Streptomyces* from the Silty Mud of Tambukan Lake Are Producers of Antibiotic Compounds †

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Abstract: Actinomycetes of the genus *Streptomyces* are members of the phylum Actinomycetota, Gram-positive, filamentous, spore-forming bacteria. Members of the genus *Streptomyces* are known as producers many different bioactive natural products, such as antibiotics, antifungal, antitumor agents. Despite the large number of already known antibiotics, the actinomycetes of the genus *Streptomyces* still occupy an important position due to the rich variety of unique secondary metabolites and are excellent candidates for the search for new antibiotics and antifungal agents for medical purposes. Most species of the genus *Streptomyces* have been isolated from soils. Currently, the attention of researchers is directed to the study of actinomycetes complexes not only in soils, but also in reservoirs, plants, invertebrates. Tambukan is a lake (43°57′37″ N, 43°9′40″ E) with bitter-salt water on Northern Caucasus. The bottom of the lake consists of a thick layer of silty mud containing sulfates and chlorides of sodium and magnesium, with a mineralization of 55–60 g/L. The medicinal water and mud of Tambukan Lake is in pelotherapy offered by health resorts and have been used by famous individuals. Thus, the actinomycetes isolated from the silty mud of Tambukan Lake are of interest not only potential producers of new antibiotics, but also as inhabitants of extreme conditions. The goal of this study was to isolate actinomycetes from the silty mud of Tambukan Lake and to study their antimicrobial and antifungal activity. In the study ten cultures were isolated from the silty mud. The strains were classified as belonging to the *Streptomyces* genus using morphological, physiological, biochemical and molecular 16S rRNA methods. The isolated cultures showed antibiotic activity against the following pathogens: *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* INA 00985, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* INA 00761 (MRSA—Methicillin-Resistant *Staphylococcus aureus*), *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Saccharomyces cerevisiae* ИНА 01042, *Aspergillus niger* ATCC 16404, *Fusarium solani* ВКПМ F-890, *Candida albicans* ATCC 14053, *Fusarium oxysporum* ВКПМ F-148. *Streptomyces fulvissimus* $5T_2$, *Streptomyces globisporus* $20T_2$, *Streptomyces intermedius* $23T_2$ demonstrated strong antimicrobial activities against fungi. These strains can be considered as potential producers of new antifungal agents.

Keywords: *Streptomyces*; antibiotics; antifungal agents; Tambukan Lake

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1. Introduction

Currently, the resistance of pathogenic microorganisms to existing antibiotics is a serious problem in the world. Mortality from some multi-resistant infections is very high. Thus, the search for new antibiotics is an actual problem. Actinomycetes of the genus *Streptomyces* are leaders in the number of produced antibiotics [1,2]. The recent studies have shown that *Streptomyces* species still have high potential as a source of new and interesting natural products including wide diversity chemicals structures such as cyclic and linear peptides, linear polyketides, terpenoids, polyaromatics, macrocycles, and furans. The

unique property of the *Streptomyces* species to synthesize such a variety of compounds is due to the presence of a large number of biosynthetic gene clusters encoding bioactive compounds [2,3].

2. Methods

The samples of silty mud taken from Lake Tambukan were dried in the Petri dishes at room temperature for 2 weeks. The samples were dissolved in sterile water 10^3 times and shaken on a vortex for 5 min. Aliquots of suspension were sown on solid media Gause №2: tripton-3.0 g/L, peptone-5.0 g/L, glucose-10.0 g/L, NaCl-5.0 g/L, agar-20.0 g/L, tap water and incubated at 28 °C for 14 days. Antibiotic activity was investigated using the test-organisms: *S. aureus* INA 00985, *S. aureus* INA 00761 (Methicillin-Resistant *Staphylococcus aureus*), *M. luteus* ATCC 9341, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Sac. cerevisiae* ИНА 01042, *C. albicans* ATCC 14053, *A. niger* ATCC 16404, *F. solani* ВКПМ F-890, *F.oxysporum* ВКПМ F-148. Actinomycetes strains were cultivated on liquid media of the following composition: (1) A4: soy flour-10 g/L, glucose-10.0 g/L, NaCl-5 g/L, $CaCO_3$-2.5 g/L, tap water; (2) 6613: starch-20 g/L, corn extract - 3.0 g/L, KNO_3-4 g/L, NaCl-5 g/L, $CaCO_3$-5 g/L tap water; (3) 330: sucrose-21 g/L, starch-8.5 g/L, pea flour-15 g/L, NaCl-5 g/L, $NaNO_3$-5 g/L, chalk-5 g/L, tap water. The morphology of hyphae and spores was observed by light microscopy OLYMPUS BX-41 (Shibuya-ku Tokyo, Japan). The strains of the actinomycetes were grown on liquid media Gause №2, incubated at 28 °C and 180 rpm for 5–7 days to obtain biomass for the chemotaxonomic and molecular systematic studies. Thin-layer chromatography was used to determine the isomers of diaminopimelic acid and reducing sugars [4,5]. Total genomic DNA was extracted using a Power Soil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). For the amplification of the 16S rRNA gene used the primers 27 F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492 R (5′-TACGGYTACCTTGTTACGACTT-3′) (Syntol, Moscow, Russia) and a set of reagents the «Thermo Fisher Scientific» (Thermo Fisher Scientific Inc. Waltham, MA, USA). PCR (polymerase chain reaction) was performed on a Thermal Cycler 2720 (Applied Biosystems, Beverly, MA, USA). The nucleotide sequences were determinated on an automatic capillary sequencer Genetic Analyzer 3500 (Applied Biosystems, Beverly, MA, USA) using reagents BigDye Terminator v3 Cycle Sequencing Kit (Applied Biosystems, Beverly, MA, USA).

3. Results

Ten strains from representatives of the genus *Streptomyces* were isolated from the sample of the silty mud of Tambukan Lake in the experiments. All strains of actinomycetes were identified based on the morphological, physiological, biochemical, and molecular features.

We have determined the antibiotic activity of the isolated strains against gram-positive, gram-negative bacteria and fungi. The results of antibiotic activity against gram-positive microorganisms are presented in Table 1. The best antimicrobial activity was detected in the strain *Streptomyces* sp. $5T_2$. None of the strains showed strong antibiotic activity against gram-negative microorganisms: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853.

Only three strains demonstrated strong antimicrobial activities against fungi: *Saccharomyces cerevisiae* ИНА 01042, *Candida albicans* ATCC 14053, *Aspergilus niger* ATCC 16404, *Fusarium solani* ВКПМ F-890, *Fusarium oxysporum* ВКПМ F-148. Zones of antifungal activity were more than 25 mm. These strains were determinated as *Streptomyces fulvissimus* $5T_2$, *Streptomyces globisporus* $20T_2$, *Streptomyces intermedius* $23T_2$. These strains of the genus *Streptomyces* can be considered as potential producers of new antifungal agents.

Table 1. Antibiotic activity of strains of the genus *Streptomyces* against gram-positive microorganisms.

Number	Media	Areas of Suppression of Test-Organisms, mm			
		***M. luteus* ATCC 9341**	***S. aureus* INA 00985**	***S. aureus* INA 00761 (MRSA)**	***B. subtilis* ATCC 6633**
Streptomyces sp. $1T_2$	330	11	11	15	12
Streptomyces sp. $5T_2$	330	15	30	30	15
Streptomyces sp. $7T_2$	330	15	20	20	15
Streptomyces sp. $12T_2$	330	22	20	n/a	22
Streptomyces sp. $13T_2$	330	n/a	11	n/a	13
Streptomyces sp. $14T_2$	330	15	20	20	16
Streptomyces sp. $17T_2$	6613	n/a	13	n/a	13
Streptomyces sp. $18T_2$	6613	20	n/a	n/a	11
Streptomyces sp. $20T_2$	A4	n/a	n/a	n/a	n/a
Streptomyces sp. $23T_2$	A4	14	n/a	n/a	n/a

n/a—antibiotic activity was not detected.

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References

1. Berdy, J. Thoughts and facts about antibiotics: Where we are now and where we are heading. *J. Antibiot.* **2012**, *65*, 385–395. [CrossRef] [PubMed]
2. Antoraz, S.; Santamaría, R.I.; Díaz, M.; Sanz, D.; Rodríguez, H. Toward a new focus in antibiotic and drug discovery from the *Streptomyces* arsenal. *Front. Microbiol.* **2015**, *6*, 46. [CrossRef] [PubMed]
3. Lacey, H.J.; Rutledge, P.J. Recently discovered secondary metabolites from *Streptomyces* species. *Molecules* **2022**, *27*, 887. [CrossRef]
4. Gaillard, B.D. Use of unneutralized hydrolysates in paper chromatography of sugars. *Natures* **1953**, *171*, 1160. [CrossRef]
5. Lechevalier, H.A.; Lechivalier, M.P.; Gerber, N.N. Chemical composition as a criterion in the classification of actinomyces. *Adv. Appl. Microbiol.* **1971**, *14*, 47–72. [PubMed]