BIOTECHNOLOGY & BIOTECHNOLOGICAL EQUIPMENT
2021, VOL. 35, NO. 1, 1136–1146
https://doi.org/10.1080/13102818.2021.1952898

Diversity and antimicrobial activity of culturable actinobacteria isolated from the sediment of Sarıkum Lake

Aysel Veyisoglu[a] and Demet Tatar[b]

[a]Department of Medical Services and Techniques, Vocational School of Health Services, Sinop University, Sinop, Turkey; [b]Department of Medical Services and Techniques, Osmancık Ömer Derindere Vocational Higher School, Hitit University, Çorum, Turkey

ABSTRACT

In this study, actinobacteria isolated from Sarıkum Lake sediment were characterized (Sinop-Turkey). A total of 48 actinobacteria were isolated using ten selective media with the dilution-plating method. The phylogenetic analysis according to 16S rRNA gene sequencing showed that 40% of the isolated strains belong to the widely distributed genus of *Streptomyces*, 36% belong to genus of *Micromonospora*, 24% of the isolates belong to rare genera such as *Rhodococcus*, *Plantactinospora*, *Nonomuraea*, *Actinomadura* and *Streptosporangium*. Most of the isolated strains belong to the genus *Streptomyces* (40%) and two isolates may be new species. All of the isolates were tested for antimicrobial activity; only 12 isolates exhibited antimicrobial activity. Nevertheless, 11 isolates were active against gram-positive, 5 were potential against gram-negative and no isolates had any effect against pathogenic fungi. All of the 48 isolates were analysed for genes encoding nonribosomal peptide synthetases (NRPS) and polyketide synthases (PKS). In all of the 48 isolates were detected NRPS sequences, PKS-I in 28 isolates and PKS-II in 22 isolates. PKS-I – PKS-II – NRPS genes were identified ın 17 isolates.

ARTICLE HISTORY
Received 12 February 2021
Accepted 5 July 2021

KEYWORDS
Sarıkum; actinobacteria; 16S rRNA; antimicrobial activity; NRPS; PKS-I and PKS-II

Introduction

Actinobacteria are Gram-stain positive, filamentous, unicellular microorganisms and compose one of the greatest phyla within the kingdom Bacteria [1, 2]. These bacteria belong to the order actinomycetales and represent a group of gram-positive bacteria with high GC base pairs in their DNA [3, 4].

The first studies on actinobacteria from freshwater habitats were carried out in 1971. Willoughby [5] isolated *Micromonospora* and *Streptomyces* from the river and stream of Blelham Tarn. Subsequently, six different genera of actinobacteria (*Actinomadura*, *Microbispora, Micromonospora*, *Nocardia*, *Saccharopolyspora*, *Streptomyces*) were obtained from lake water and sediment of Middle Plateau, Yunnan, China [6].

Actinobacteria can be found in both terrestrial and aquatic habitats, and they are one of the most abundant taxa in freshwaters [7]. A number of antimicrobial compounds such as aminoglycosides, anthracyclines, beta-lactams, glycopeptides, macrolides, polyenes, phenazine and tetracyclines have already been isolated and characterized from actinobacteria [8]. However, most of these compounds are either nonribosomal peptides or polyketides which are synthesized by nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) respectively [4, 9].

The 16S rRNA gene is the most widely used marker for molecular identification and phylogenetic analysis of actinobacteria.

Sarıkum Lake is a natural water ecosystem. In 1991, Sarıkum Lake and its surroundings were registered as a Nature Conservation Area covering a 785-hectare complex of a marine, freshwater lake, sand, wetland, peatland, swamp and forest ecosystem. This complex ecosystem has contributed to the biodiversity of Sarikum Lake [10]. Besides, riparian vegetation grows well around the lake. The bottom structure is composed of clay. It is close to the sea and it connects to the sea with a natural water channel. This situation makes the lake an appropriate habitat for birds and other aquatic organisms [11, 12].

To our knowledge, the biodiversity of actinobacteria of Sarıkum Lake (Sinop-Turkey) has not been studed so far. The aim of the present study was to detect the

CONTACT Aysel Veyisoglu aveyisoglu@sinop.edu.tr Department of Medical Services and Techniques, Vocational School of Health Services, Sinop University, Sinop 57000, Turkey

biodiversity of actinobacteria of Sarıkum Lake (Sinop-Turkey) and the presence of the secondary metabolite genes in the isolates. Also, the antimicrobial potential of the isolates was examined against eight different pathogens.

Materials and methods

Collection of lake sediment sample

Sediment samples were collected from the three different stations of the Sarıkum Lake, Sinop, in July 2019 (Table 1). Sediment samples were randomly collected from three different stations of the lake. The labeled samples were placed in sterile tubes (5–10 g), transported to the laboratory and were worked on immediately for the isolation of actinobacteria.

Selective isolation of actinobacterial strains

The collected samples were exposed to a preheated water bath (55 °C for 20 min) to hinder the growth of fastgrowing bacteria and support the growth of actinobacteria [13]. Actinobacteria were isolated using the serial dilution method and the spread plate technique. The stock solution of the sample was prepared with 1 mL of sediment with lake water and 9 mL of Ringer's solution in a test tube, and the solution was mixed for 40 min. The suspension was serially diluted by transferring 1 mL aliquots to a series of test tubes; each containing 9 mL of Ringer's solution to prepare the final volumes of 10^{-1}, 10^{-2} and 10^{-3}, and the diluted suspension was spread over the surface of selective isolation agar. The most common media used for actinomycetes include humic acid-vitamin agar (HV), starch casein agar (SCA), nocardia agar (NA), SM1 agar, SM2 agar, SM3 agar, marine agar, R2A agar, M1 agar and oligotrophic agar. Certain concentrations of antibiotics (see Table 2) were added to selective isolation media to eliminate gram-negative bacteria and fungi. Diluted sediment samples (100 µl) were spread onto the surface of 10 different selective isolation media (Table 2). Isolation plates were incubated at 28 °C for 30 days, and the colonies were observed periodically.

Table 1. Locality and geographic coordinates of sediment samples.

Locality	Geographic coordinates
Mid-lake coastal area	42° 01′ 00″ N 34° 55′ 38″ E
Sea front of the lake	42° 01′ 25″ N 34° 54′ 31″ E
Lake coastline	42° 01′ 34″ N 34° 54′ 34″ E

Maintenance and culture conditions

Bacterial colonies resembling the morphology of actinobacteria colonies were selected and inoculated on prepared yeast extract malt extract medium [14]. The inoculated plates were incubated for 15–21 days at 28 °C. Suspensions of spores and mycelia were maintained on ISP 2 agar slopes at room temperature and as glycerol suspensions (20%, *v/v*) at −80 °C.

DNA extraction

For molecular identifcation and phylogenetic analysis, genomic DNA was extracted and purifed using a DNA extraction kit (Purelink Invitrogen) as described in past studies [15]. The DNA quantity was checked by the ratio of optical density 260/280 using a spectrophotometer (Thermo scientific Multiskan GO microplate reader).

Amplification and determination of 16S rRNA gene sequence

PCR was carried out on T100 (Bio-RAD) in a 25 µL volume. PCR mixtures (25 µL) included 0.5 µmol/L F (20 µmol/L), 0.5 µmol/L R (20 µM), Taq polymerase buffer (HotStarTaq®) and deoxynucleoside triphosphates mixture (Promega) (12.5 µmol/L). Taq polymerase (2.5 U, HotStarTaq®) and chromosomal DNA (50–300 ng) were added to the solution. The 16S rRNA genes were amplified using the universal primers 27F [5′-AGAGTTTGATC(AC)TGGCTCAG-3′] and 1492R [5′-ACGG(CT)TACCTTGTTACGACTT-3′] [16]. The PCR amplification (MyGenie-96 Gradient Thermal Cycler, Korea) included the following parameters: 95 °C for 5 min (initial denaturation), 35 cycles of 95 °C for 1 min (denaturation), 55 °C for 2 min (annealing), and 72 °C for 3 min (extension) and 72 °C for 10 min (final extension). Then the amplification products were visualized by gel electrophoresis using 4 μL of PCR product in a 1% agarose gel (Merck) and were imaged with the Gene Genius Bioimaging system.

Sequence analysis

The PCR products of the 48 isolates were purified with QIAquick purification kit (Qiagen). PCR-mediated amplification and sequencing of the 16S rRNA gene were performed as described by Chun and Goodfellow [17]

Table 2. List of selective media used and codes of isolates.

Number	Isolate codes	Name of medium	Antibiotic	References
1	SL05, SL19, SL20, SL21, SL51, SL52, SL53, SL78, SL80, SL84, SL92, SL93	Humic Acid-Vitamin Agar	Cycloheximide (50 µg/mL), Nalidixic acid (10 µg/mL)	[35]
2	SL30, SL31, SL32, SL34, SL41, SL42, SL44, SL54, SL62, SL65, SL66, SL86, SL87, SL88, SL97, SL109	Starch-Casein Agar	Cycloheximide (50 µg/mL), Nystatin (50 µg/mL)	[36]
3	SL13, SL27, SL49, SL98, SL100, SL101, SL102, SL103	Nocardia Agar	Cycloheximide (50 µg/mL), Nystatin (50 µg/mL)	[37]
4	–	SM1 Agar	Nystatin (50 µg/mL), Nalidixic acid (10 µg/mL), Neomycin sulfate (10 µg/mL)	[38]
5	–	SM2 Agar	Nystatin (50 µg/mL), Nalidixic acid (10 µg/mL), Neomycin sulfate (10 µg/mL)	[38]
6	–	SM3 Agar	Nystatin (50 µg/mL), Rifampicin (5 µg/mL)	[38]
7	SL35, SL48, SL59, SL77, SL105, SL108	Marine agar	Cycloheximide (50 µg/mL), Nystatin (50 µg/mL)	
8	–	R2A Agar	Cycloheximide (50 µg/mL), Nystatin (50 µg/mL)	[39]
9	SL67, SL68	M1 Agar	Cycloheximide (50 µg/mL), Nystatin (50 µg/mL), Rifampicin (5 µg/mL)	[40]
10	SL37, SL38, SL39, SL64	Oligotrophic Agar	Cycloheximide (50 µg/mL), Nystatin (50 µg/mL), Rifampicin (5 µg/mL)	[41]

using an ABI PRISM 3730 XL automatic sequencer with the previously described oligonucleotide primers (Table 3). Chromatogram files in ABI format were converted to FASTA format using Chromas 1.7.5. An almost complete 16S rRNA gene sequences of the 48 isolates were compared to sequences of type strains in GenBank [18] and EzBioCloud [19] databases. Evolutionary trees were carried out using the neighbour-joining [20] algorithm drawn from MEGA version 7.0 software package [21]. Evolutionary distances were calculated using the Kimura two-parameter [22] and topologies of the resultant trees evaluated by bootstrap analyses [23] based on 1000 resamplings. The 16S rRNA gene sequences obtained in this study were deposited in GenBank (Table 4).

Table 3. Oligonucleotide primers used for 16S rRNA PCR amplification and sequencing.

Primer code	Sequences (5′–3′)	Base length	References
27F	AGAGTTTGATC(AC) TGGCTCAG	21	[16]
518F	CCAGCAGCCGCGGTAAT	17	[42]
800R	TACCAGGGTATCTAATCC	18	[17]
MG5F	AAACTCAAAGGAATTGACGG	20	[17]
MG6F	GACGTCAAGTCATCATGCC	19	[17]
1492R	ACGG(CT) TACCTTGTTACGACTT	21	[16]

Antimicrobial activity

The antimicrobial activities of the 48 isolates to inhibit the growth of eight different pathogenic microorganisms [gram-positive bacteria: *Bacillus subtilis* ATCC 6633^T, *Enterococcus faecalis* ATCC 29212^T and *Staphylococcus aureus* ATCC 25923^T; gram-negative bacteria: *Escherichia coli* ATCC 25922^T, *Klebsiella pneumoniae* ATCC 700603^T and *Pseudomonas aeruginosa* ATCC 27853^T and fungi: *Aspergillus niger* ATCC 16404^T and *Candida albicans* ATCC 10231^T], was observed using an overlay technique described by Williams *et al.* [24]. Spot-inoculated colonies on modified Bennett's agar plates were inverted over 2 mL chloroform for 40 min. Killed colonies were overlaid with 5–7 mL sloppy nutrient broth inoculated with the test organisms. Zones of inhibition were scored as positive results after 24 h at 37 °C.

Amplification of NRPS, PKS I and II gene regions

Non-ribosomal peptide synthetase (NRPS), polyketide synthase I and II gene regions of isolates were investigated with specific primers (Table 4). The amplified PCR products were observed in 1.5% agarose gel *via*

Table 4. Primers used for amplification of NRPS, PKS-I and II gene regions.

Target genes	Primers	Base length	Product length (bp)	References
NRPS	A3F (5′GCSTACSYSATSTACACSTCSGG3′)	23	700–800	[9]
	A7R (5′SASGTCVCCSGTSCGGTAS3′)	19		
PKS-I	K1F (5′TSAAGTCSAACATCGGBCA3′)	19	1200–1400	[9]
	M6R (5′CGCAGGTTSCSGTACCAGTA3′)	20		
PKS-II	KSαF (5′TSGCSTGCTTGGAYGCSATC3′)	20	613	[43]
	KSαR (5′TGGAANCCGCCGAABCCGCT3′)	20		

electrophoresis. Ethidium bromide (EtBr) was added to the gel before electrophoresis to a final concentration of 0.5 µg/mL, followed by separation at 100 V for 1 h.

Results

Morphological analysis

A total of 48 morphologically distinct actinobacterial isolates were obtained from sediment Sarıkum lake. Ten different selective isolation media were used. Sixteen strains were isolated on starch-casein agar, twelve strains from humic acid-vitamin (HV) agar, eight strains from nocardia agar, six strains from marine agar, four strains from oligotrophic agar, two strains from M1 agar and incubated at 28 °C for about 30 day. No improvement was observed on the other four types of agar (SM1, SM2, SM3 ve R2A agar).

These results clearly showed that starch-casein agar was the most suitable medium for the isolation of actinobacteria from lake sediments and provided 33% of the total isolates followed by humic acid-vitamin (HV) agar (25%) (Table 2). In total, 48 culturable actinobacterial isolates were isolated from the three different stations of the Sarıkum Lake: 20 isolates from the first locality, 21 isolates from the second locality and 7 isolates from the third locality.

16s rRNA gene sequence analysis

The 16S rRNA genes of all 48 isolates were amplified using universal primers (Table 3). Most of the strains belonged to the genus *Streptomyces* (19 isolates) and to the genus *Micromonospora* (17 isolates). Other strains belonged to the genera *Rhodococcus* (5 isolates), *Plantactinospora* (3 isolates), *Nonomuraea* (2 isolates), *Actinomadura* (1 isolates) and *Streptosporangium* (1 isolate) (Table 5).

Phylogenetic analysis

According to 16S rRNA gene sequence analysis, while over 40% of the isolates (19 out of the 48) are members of the genus *Streptomyces*, over 36% of the isolates (17 out of the 48) are members of the genus *Micromonospora*. Members of the genus *Streptomyces* and *Micromonospora* are dominant in sediments lake Sarıkum (Figure 1).

Based on 16S rRNA gene sequence analysis, 19 isolates were identified as *Streptomyces* spp. The phylogenetic tree, according to the neighbor-joining algorithm, indicated that nineteen strains were members of the genus *Streptomyces* (Figure 1; Table 5). According to the 16S rRNA gene sequence analysis, 19 *Streptomyces* isolates showed close 16S rRNA gene sequence similarity with the type strain of *Streptomyces*, which are 100% and 98.41%.

The phylogenetic tree, according to the neighbor-joining algorithm indicated that 17 strains were members of the genus *Micromonospora* (Figure 2). Seventeen *Micromonospora* isolates showed close 16S rRNA gene sequence similarity with the type strain of *Micromonospora,* which are 100% and 99.10% (Table 5).

Five *Rhodococcus* isolates showed close 16S rRNA gene sequence similarity with the type strain of *Rhodococcus* which are 100% and 99.58%, three *Plantactinospora* isolates showed close 16S rRNA gene sequence similarity with the type strain of *Plantactinospora* which are 99.58% and 99.10%, two *Nonomuraea* isolates showed close 16S rRNA gene sequence similarity with the type strain of *Nonomuraea*, which are 100% and 99.65%, an *Actinomadura* isolate showed close 16S rRNA gene sequence similarity with the type strain of *Actinomadura*, which is 99.79%, and a *Streptosporangium* isolate showed close 16S rRNA gene sequence similarity with the type strain of *Streptosporangium*, which is 99.79% (Figure 3; Table 5).

Antimicrobial potential

All 48 isolates were tested for antimicrobial activities against the pathogenic microorganisms *Bacillus subtilis* ATCC 6633^T, *Enterococcus faecalis* ATCC 29212^T, *Staphylococcus aureus* ATCC 25923^T, *Escherichia coli* ATCC 25922^T, *Klebsiella pneumoniae* ATCC 700603^T, *Pseudomonas aeruginosa* ATCC 27853^T, *Aspergillus niger* ATCC 16404^T, *Candida albicans* ATCC 10231^T. Twelve of the forty-six isolates (25%) exhibited activity against at least one of the pathogens tested. Activity against

Table 5. Summary of information on 16S rRNA sequence determination of isolated strains.

Number	Strain	Accession number	Highest match	Similarity (%)-Nucleotide difference
1.	SL48	MN812720	*Streptomyces qinglanensis* 172205^T	99.93% – 1/1448
2.	SL52	MN818636	*Streptomyces qinglanensis* 172205^T	99.93% – 1/1449
3.	SL59	MN818646	*Streptomyces qinglanensis* 172205^T	100% – 0/1449
4.	SL77	MN822706	*Streptomyces qinglanensis* 172205^T	99.93% – 1/1448
5.	SL92	MN822725	*Streptomyces qinglanensis* 172205^T	99.65% – 5/1448
6.	SL13	MN811627	*Streptomyces wuyuanensis* CGMCC 4.7042^T	100% – 0/1440
7.	SL20	MN812279	*Streptomyces wuyuanensis* CGMCC 4.7042^T	100% – 0/1440
8.	SL31	MN812664	*Streptomyces neopeptinius* KNF 2047^T	99.28% – 10/1398
9.	SL34	MN812677	*Streptomyces neopeptinius* KNF 2047^T	99.28% – 10/1398
10.	SL102	MN823075	*Streptomyces caeruleatus* NRRL B-24802^T	99.52% – 7/1447
11.	SL109	MN932120	*Streptomyces caeruleatus* NRRL B-24802^T	99.52% – 7/1448
12.	SL19	MN811648	*Streptomyces glauciniger* CGMCC 4.1858^T	99.72% – 4/1441
13.	SL30	MN812662	*Streptomyces yangpuensis* fd2-tb^T	100% – 0/1446
14.	SL35	MN812678	*Streptomyces chumphonensis* K1-2^T	99.86% – 2/1453
15.	SL37	MN812679	*Streptomyces scabiei* NRRL B-16523^T	99.79% – 3/1448
16.	SL39	MN812682	*Streptomyces aculeolatus* NBRC 14824^T	99.86% – 2/1440
17.	SL49	MN818640	*Streptomyces xinghaiensis* S187^T	99.93% – 1/1448
18.	SL78	MN809579	*Streptomyces karpasiensis* K413^T	98.62% – 20/1448
19.	SL84	MN809580	*Streptomyces haliclonae* DSM 41968^T	98.41% – 23/1448
20.	SL32	MN812668	*Micromonospora tulbaghiae* DSM 45142^T	100% – 0/1437
21.	SL53	MN818642	*Micromonospora tulbaghiae* DSM 45142^T	100% – 0/1437
22.	SL54	MN818645	*Micromonospora tulbaghiae* DSM 45142^T	99.93% – 1/1437
23.	SL64	MN818651	*Micromonospora tulbaghiae* DSM 45142^T	100% – 0/1437
24.	SL65	MN818654	*Micromonospora tulbaghiae* DSM 45142^T	100% – 0/1437
25.	SL105	MN823085	*Micromonospora tulbaghiae* DSM 45142^T	100% – 0/1437
26.	SL05	MN812168	*Micromonospora taraxaci* DSM 45885^T	99.65% – 5/1437
27.	SL68	MN822705	*Micromonospora taraxaci* DSM 45885^T	99.58% – 6/1436
28.	SL66	MN818670	*Micromonospora chalcea* DSM 43026^T	99.51% – 7/1437
29.	SL101	MN823072	*Micromonospora chalcea* DSM 43026^T	99.58% – 6/1437
30.	SL41	MN812684	*Micromonospora kangleipakensis* MBRL 34^T	99.79% – 3/1413
31.	SL42	MN812695	*Micromonospora halotolerans* CR18^T	99.44% – 8/1429
32.	SL44	MN812709	*Micromonospora chaiyaphumensis* DSM 45246^T	99.10% – 13/1437
33.	SL86	MN932112	*Micromonospora vinacea* GUI63^T	99.72% –4/1418
34.	SL97	MN822727	*Micromonospora schwarzwaldensis* HKI0641^T	99.93% – 1/1437
35.	SL98	MN932114	*Micromonospora gifhornensis* DSM 44337^T	99.65% – 5/1437
36.	SL100	MN823065	*Micromonospora fiedleri* MG-37^T	99.78% – 3/1381
37.	SL21	MN812656	*Rhodococcus coprophilus* NBRC 100603^T	99.72% – 4/1445
38.	SL38	MN812680	*Rhodococcus coprophilus* NBRC 100603^T	100% – 0/1445
39.	SL80	MN822707	*Rhodococcus coprophilus* NBRC 100603^T	100% – 0/1445
40.	SL93	MN822726	*Rhodococcus coprophilus* NBRC 100603^T	100% – 0/1445
41.	SL51	MN932113	*Rhodococcus hoagii* DSM 20295^T	99.58% – 6/1439
42.	SL62	MN818650	*Plantactinospora sonchi* NEAU-QY2^T	99.51% – 7/1438
43.	SL67	MN818671	*Plantactinospora siamensis* CM2-8^T	99.10% – 13/1438
44.	SL103	MN823080	*Plantactinospora endophytica* YIM 68255^T	99.58% – 6/1438
45.	SL27	MN812661	*Nonomuraea coxensis* DSM 45129^T	100% – 0/1425
46.	SL88	MN822710	*Nonomuraea maritima* FXJ7.203^T	99.65% – 5/1441
47.	SL108	MN829436	*Actinomadura sporangiiformans* NEAU-Jh2-5^T	99.79% – 3/1440
48.	SL87	MN822708	*Streptosporangium jiaoheense* NEAU-Jh1-4^T	99.79% – 3/1444

E. faecalis ATCC 29212^T was clearly the most frequent (6 isolates (12.5%)). Activity against *K. pneumoniae* ATCC 700603^T and *B. subtilis* ATCC 6633^T were the least frequent (4.16%), while 10.4% and 6.25% of the isolates were active against *S. aureus* ATCC 25923^T and *P. aeruginosa* ATCC 27853^T, respectively. Two isolates were found to inhibit three pathogens, while two isolates could inhibit two pathogens. None of the isolates could inhibit the growth of *E. coli* ATCC 25922^T, *A. niger* ATCC 16404^T and *C. albicans* ATCC 10231^T (Table 6).

Secondary metabolite genes

The 48 strains were screened for the presence of PKS-I, PKS-II and NRPS sequences by specific with primer sets K1F–M6R, KSαF–KSαR and A3F–A7R, respectively (Table 4). NRPS sequences were detected in 48 isolates (100%), while PKS-I and PKS-II sequences were detected in only 28 and 22 of the 48 strains (58.3% and 45.8%), respectively. Seventeen isolates gave positive amplification products with both the PKS-I, PKS-II and NRPS primers (Figure 4; Table 7).

Discussion

Actinomycetes have been isolated from many different habitats so far. Among these habitats, aquatic environments attract more attention in terms of their new species hosting potential. Zothanpuia *et al.* [25] identified 84 actinobacterial isolates based on 16S rRNA gene sequence analysis in a study from two rivers and

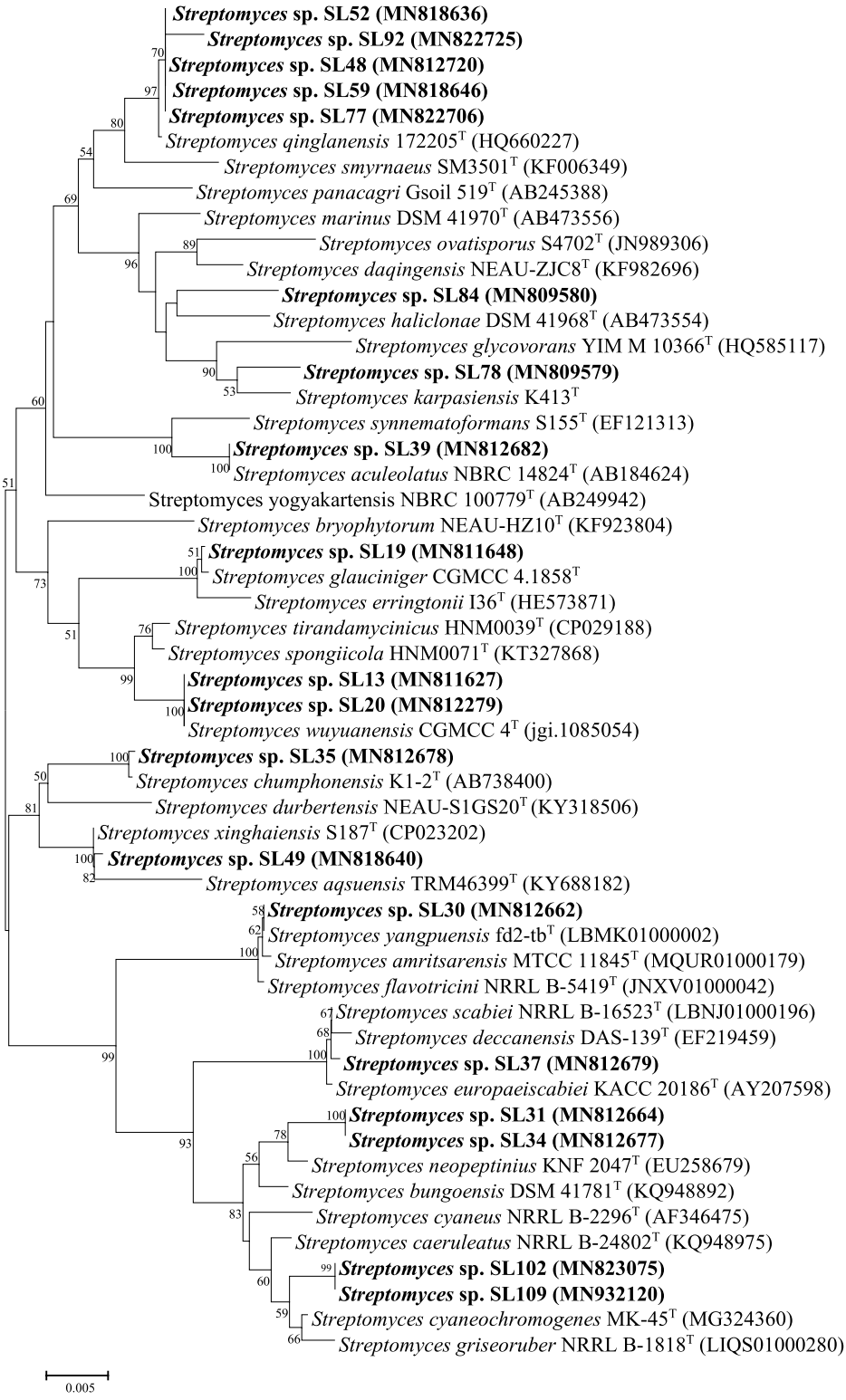

Figure 1. Neighbor-joining tree [20] based on 16S rRNA gene sequences showing the position of isolated *Streptomyces* strains among their phylogenetic neighbors. Numbers at the nodes demonstrate the levels of bootstrap support (%); only values ≥ 50% are shown. GenBank accession numbers are placed in parentheses. Bar, 0.005 substitutions per nucleotide position.

one lake sediment. They concluded that 84 actinobacterial isolates were separated into a common genus (*Streptomyces*) and eight rare genera (*Nocardiopsis*, *Saccharopolyspora*, *Rhodococcus*, *Prauserella*, *Amycolatopsis*, *Promicromonospora*, *Kocuria* and *Micrococcus*). In our study, 48 isolates were obtained

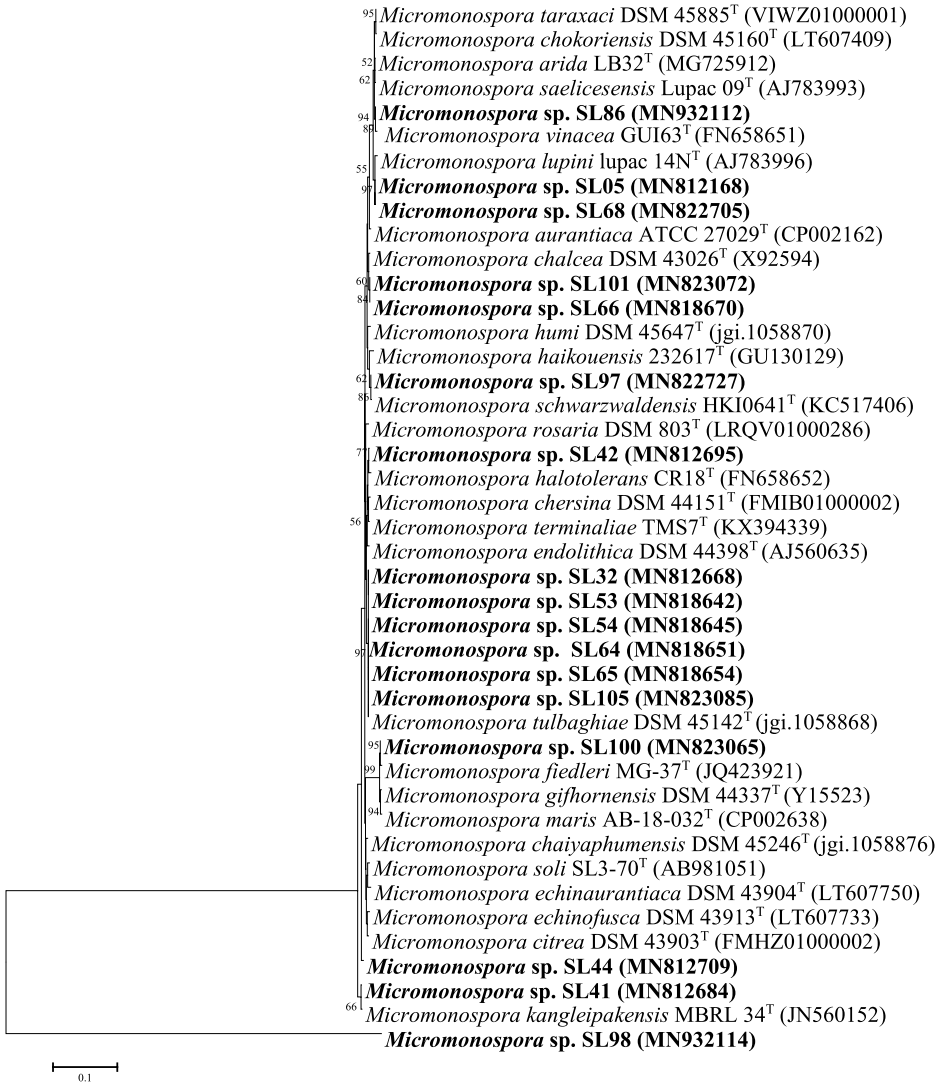

Figure 2. Neighbor-joining tree [20] based on 16S rRNA gene sequences showing the position of isolated *Micromonospora* strains among their phylogenetic neighbors. Numbers at the nodes demonstrate the levels of bootstrap support (%); only values ≥ 50% are shown. GenBank accession numbers are placed in parentheses. Bar, 0.1 substitutions per nucleotide position.

as a result of the isolation we made from Sarıkum Lake. The 48 isolated strains were found to belong to *Streptomyces*, *Micromonospora*, *Rhodococcus*, *Plantactinospora*, *Nonomuraea*, *Actinomadura* and *Streptosporangium*.

According to a study conducted in 2019, 10 mangrove soil samples were taken from Futian and Maoweihai of China, and the culture-dependent method was employed to obtain actinobacteria [26]. A total of 539 cultivable actinobacteria were isolated and distributed in 39 genera affiliated to 18 families of 8 orders by comparison analysis of partial 16S rRNA gene sequences. The dominant genus was *Streptomyces*, followed by *Microbacterium*, *Agromyces* and *Rhodococcus* [26].

In another study carried out in 2020, a total of 32 isolates were isolated from soil samples of different forest locations of Bisle Ghat and Virjapet situated in Western Ghats of Karnataka, India. The isolates were identified as species of *Streptomyces*, *Nocardiopsis* and *Nocardioides* by cultural, morphological, and molecular studies [27].

While members of the genus *Micromonospora* were the most frequently isolated actinobacteria from freshwater lakes in past studies, members of the genus *Streptomyces* were more frequently isolated in recent studies [5, 28].

Strains SL78 and SL84 may be new species that belong to the genus *Streptomyces*. Strain SL78 had the closest 16S rRNA gene sequence similarity with *Streptomyces karpasiensis* K413^T (98.62%) [29] and SL84

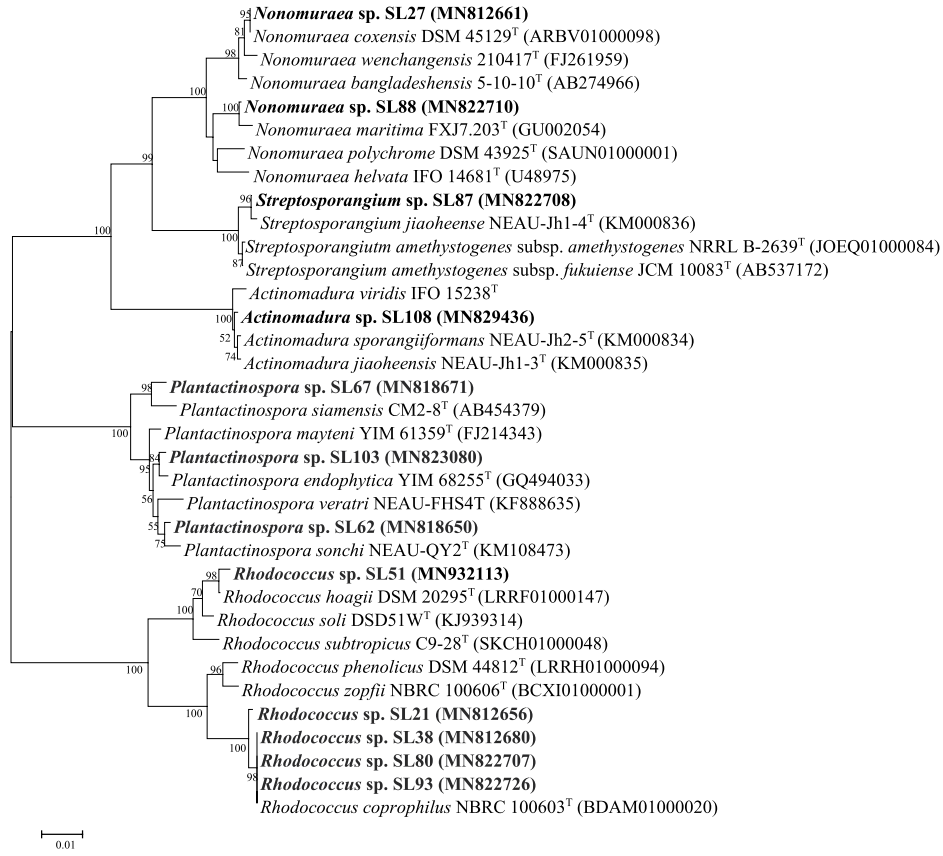

Figure 3. Neighbor-joining tree [20] based on 16S rRNA gene sequences showing relationships between the isolates and recognized species of the genera *Rhodococcus*, *Plantactinospora*, *Nonomuraea*, *Streptosporangium* and *Actinomadura* strains among their phylogenetic neighbors. The numbers at the nodes demonstrate the levels of bootstrap support (%); only values ≥ 50% are given. GenBank accession numbers are placed in parentheses. Bar, 0.01 substitutions per nucleotide position.

indicated the closest 16S rRNA gene sequence similarity with *Streptomyces haliclonae* DSM 41968^T (98.41%) [30]. All values are below the threshold of 98.65% for delineation of a novel species [31–33].

Rare actinobacteria are important sources in the discovery of novel antibiotics [34]. In this study, *Rhodococcus*, *Plantactinospora*, *Nonomuraea*, *Actinomadura* and *Streptosporangium* isolates were obtained as members of the rare actinobacteria.

Aquatic habitats are important environments for the discovery of new and bioactive compounds. A large number of bioactive compounds are producted by type-I polyketide synthases (PKS-I) and nonribosomal peptide synthetases (NRPS) [9]. The elucidation of PKS and NRPS gene regions is important for the discovery of new biologically active secondary metabolites.

Conclusions

Sarıkum Lake is very important in terms of microbial diversity since it is connected to the Black Sea by a natural water channel. Until now, there has been no study on the diversity of actinomycetes in Sarıkum

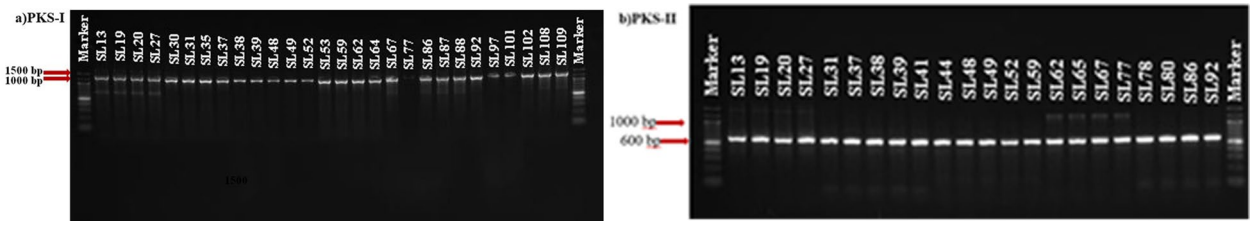

Figure 4. Images of PKS-I and PKS-II gene regions PCR amplification products of positive isolates in 1.5% agarose gel. EtBr was added to the gel before electrophoresis to a final concentration of 0.5 µg/mL, followed by separation at 100 V for 1 h. Marker (Biolabs 1 kb DNA Ladder).

Table 6. Inhibition zone diameters caused by test organisms against different microorganisms (mm).

No	Test isolates	1	2	3	4	5	6	7	8
1	SL05	30	–	–	–	–	–	–	–
2	SL13	–	–	–	–	–	–	–	–
3	SL19	–	–	–	–	–	–	–	–
4	SL20	–	–	–	–	–	–	–	–
5	SL21	–	–	–	–	–	–	–	–
6	SL27	–	–	–	–	–	–	–	–
7	SL30	–	–	–	–	30	–	–	–
8	SL31	–	30	–	–		–	–	–
9	SL32	–	30	–	–	20	–	–	–
10	SL34	–	32	–	–	–	–	–	–
11	SL35	–	–	60	–	–	–	–	–
12	SL37	–	–	–	–	–	–	–	–
13	SL38	–	–	–	–	–	–	–	–
14	SL39	–	–	44	–	–	–	–	–
15	SL41	–	–		–	–	–	–	–
16	SL42	–	11	15	–	–	10	–	–
17	SL44	–	–	–	–	–	–	–	–
18	SL48	–	–	–	–	–	–	–	–
19	SL49	–	–	20	–	–	–	–	–
20	SL51	–	–	–	–	–	–	–	–
21	SL52	–	–	–	–	–	–	–	–
22	SL53	–	–	–	–	–	–	–	–
23	SL54	–	–	–	–	–	–	–	–
24	SL59	–	–	–	–	–	–	–	–
25	SL62	–	–	–	–	–	–	–	–
26	SL64	–	–	–	–	–	–	–	–
27	SL65	–	–	–	–	–	–	–	–
28	SL66	–	–	–	–	–	–	–	–
29	SL67	–	–	–	–	–	–	–	–
30	SL68	–	–	–	–	–	–	–	–
31	SL77	–	–	25	–	–	–	–	–
32	SL78	17	30	–	–		36	–	–
33	SL80	–	–	–	–	–	–	–	–
34	SL84	–	42	–	–	–	40	–	–
35	SL86	–	–	–	–	–	–	–	–
36	SL87	–	–	–	–	–	–	–	–
37	SL88	–	–	–	–	–	–	–	–
38	SL92	–	–	–	–	–	–	–	–
39	SL93	–	–	–	–	–	–	–	–
40	SL97	–	–	–	–	–	–	–	–
41	SL98	–	–	–	–	–	–	–	–
42	SL100	–	–	–	–	–	–	–	–
43	SL101	–	–	–	–	–	–	–	–
44	SL102	–	–	–	–	–	–	–	–
45	SL103	–	–	–	–	–	–	–	–
46	SL105	–	–	–	–	–	–	–	–
47	SL108	–	–	–	–	–	–	–	–
48	SL109	–	–	–	–	–	–	–	–

Strains: 1, Bacillus subtilis ATCC 6633^T; 2, Enterococcus faecalis ATCC 29212^T; 3, Staphylococcus aureus ATCC 25923^T; 4, Escherichia coli ATCC 25922^T; 5, Klebsiella pneumoniae ATCC 700603^T; 6, Pseudomonas aeruginosa ATCC 27853^T; 7, Aspergillus niger ATCC 16404^T; 8, Candida albicans ATCC 10231^T.
–, no activity.

Table 7. PCR amplification results of NRPS, PKS-I and PKS-II gene regions of test isolates.

No	Test isolates	NRPS	PKS-I	PKS-II
1	SL05	+	–	–
2	SL13	+	+	+
3	SL19	+	+	+
4	SL20	+	+	+
5	SL21	+	–	–
6	SL27	+	+	+
7	SL30	+	+	–
8	SL31	+	+	+
9	SL32	+	–	–
10	SL34	+	–	–
11	SL35	+	+	–
12	SL37	+	+	+
13	SL38	+	+	+
14	SL39	+	+	+
15	SL41	+	–	+
16	SL42	+	–	–
17	SL44	+	–	+
18	SL48	+	+	+
19	SL49	+	+	+
20	SL51	+	–	–
21	SL52	+	+	+
22	SL53	+	+	–
23	SL54	+	–	–
24	SL59	+	+	+
25	SL62	+	+	+
26	SL64	+	+	–
27	SL65	+	–	+
28	SL66	+	–	–
29	SL67	+	+	+
30	SL68	+	–	–
31	SL77	+	+	+
32	SL78	+	–	+
33	SL80	+	–	+
34	SL84	+	–	–
35	SL86	+	+	+
36	SL87	+	+	–
37	SL88	+	+	–
38	SL92	+	+	+
39	SL93	+	–	–
40	SL97	+	+	–
41	SL98	+	–	–
42	SL100	+	–	–
43	SL101	+	+	–
44	SL102	+	+	–
45	SL103	+	–	–
46	SL105	+	–	–
47	SL108	+	+	–
48	SL109	+	+	–

'+' Represent PCR screening for target genes is positive and '–' is that of negative.

lake. In our study, the diversity and antimicrobial activity of cultivable actinobacteria from the sediment of Sarıkum Lake (Sinop-Tukey) were investigated. Overall, 48 isolated strains were found to belong to *Streptomyces*, *Micromonospora*, *Rhodococcus*, *Plantactinospora*, *Nonomuraea*, *Actinomadura* and *Streptosporangium* by the phylogenetic analysis based on 16S rRNA gene sequencing. *Streptomyces* sp. SL78 and *Streptomyces* sp. SL84 were considered as two different potential new species. Sarıkum Lake was shown to be a valuable source of Actinobacteria strains with a high proportion of putatively new and rare species. A relatively large number of strains showed antimicrobial activities and presence of secondary metabolite genes. Thus, Sarıkum lake has been found to contain many members of actinobacteria with secondary metabolic activity.

Acknowledgements

The authors extend our gratitude to Prof. Dr. Nevzat Sahin (Faculty of Art and Science, Department of Molecular Biology

and Genetics, Ondokuz Mayıs University) for his contributions to the study.

Disclosure of potential conflicts of interest

There is no conflict between the authors.

Funding

This study was supported by Sinop University Scientific Research Projects Unit (MMF-1901-16-16).

Research involving human participants and/or animals

The research does not involve human participants or animals.

Informed consent

I declare that all data in the study is correct. The work presented has not been published elsewhere.

Data availability

All data that support the findings reported in this study are available from the corresponding author upon reasonable request.

References

[1] Ludwig W, Euzèby J, Schumann P, et al. Road map of the phylum actinobacteria. In: Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Suzuki KI, Ludwig W, Whitman WB, editors. Bergey's manual of systematic bacteriology. Vol. 5. New York (NY): Springer; 2012. p. 1–28.

[2] ZothanpuiaPassari AK, Leo VV, Singh BP. Freshwater actinobacteria: potential source for natural product search and discovery. In: Sing BP, Gupta VK, Passari AK, editors. New and future developments in microbial biotechnology and bioengineering. 1st ed. United States: Elsevier; 2018. p. 67–77.

[3] Chavan DV, Mulaje SS, Mohalker RY. A review on actinomycetes and their biotechnological applications. Int J Pharmaceut Sci Res. 2013;4:1730–1742.

[4] Verma E, Chakraborty S, Tiwari B, et al. Antimicrobial compounds from actinobacteria: synthetic pathways and applications. In: Sing BP, Gupta VK, Passari AK, editors. New and future developments in microbial biotechnology and bioengineering. 1st ed. United States: Elsevier; 2018. p. 277–295.

[5] Willoughby LG. Observation on some aquatic actinomycetes of streams and rivers. Freshwater Biol. 1971;1(1):23–27.

[6] Jiang CL, Xu LH . Diversity of aquatic actinomycetes in lakes of the Middle Plateau, Yunnan, China. Appl Environ Microbiol. 1996;62(1):249–253.

[7] Glockner FO, Zaichikov E, Belkova N, et al. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. Appl Environ Microbiol. 2000;66(11):5053–5065.

[8] Adegboye MF, Babalola OO. 2013. Actinomycetes: a yet inexhaustive source of bioactive secondary metabolites. In: Méndez-Vilas A, editor. Microbial pathogens and strategies for combating them: science, technology and education. Badajoz (Spain): Formatex Research Center.

[9] Ayuso-Sacido A, Genilloud O. New PCR primers for the screening of NRPS and PKS-I systems in actinomycetes: detection and distribution of these biosynthetic gene sequences in major taxonomic groups. Microb Ecol. 2005;49(1):10–24.

[10] Yilmaz C. 2005. Ecosystem of Sarıkum Lake (Sinop)O.M.Ü. Fen Edeb. Fak., Turkey Symposium on Quantum, p. 219–223.

[11] Yardim Ö, Sendogan E, Bat L, et al. Sarıkum Gölü (Sinop) makrobentik Mollusca ve Crustacea faunası (Macrobenthic Mollusca and Crustacea fauna of Lake Sarıkum, Sinop, Turkey). E Ü Su Ürünleri Dergisi (E U J Fisheries Aquatic Sci). 2008;25(4):301–309.

[12] Sivaci ER, Yardim Ö, Gönülol A, et al. Sarıkum (Sinop-Türkiye) lagününün bentik algleri (Benthic algae of Sarıkum (Sinop-Turkey) lagoon). J Fisheries Sci. 2008;2(4):592–600.

[13] Yuan M, Yu Y, Li HR, et al. Phylogenetic diversity and biological activity of actinobacteria isolated from the Chukchi. Self marine sediments in the Arctic Ocean. Mar Drugs. 2014;12(3):1281–1297.

[14] Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol. 1966;16(3):313–340.

[15] Zothanpuia Passari AK, Chandra P, Leo VV, et al. Production of potent antimicrobial compounds from *Streptomyces cyaneofuscatus* associated with fresh water sediment. Front Microbiol. 2017;8:68.

[16] Weisburg WG, Barns SM, Pelletier DA, et al. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol. 1991;173(2):697–703.

[17] Chun J, Goodfellow M. A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. Int J Syst Bacteriol. 1995;45(2):240–245.

[18] Boratyn GM, Camacho C, Cooper PS, et al. BLAST: a more efficient report with usability improvements. Nucleic Acids Res. 2013;41:29–33.

[19] Yoon SH, Ha SM, Kwon S, et al . Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol. 2017;67(5):1613–1617.

[20] Saitou N, Nei M. The neighbor-joining method. A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–425.

[21] Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–1874.

[22] Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16(2):111–120.

[23] Felsenstein J . Confidence limits on PHYLOGENIES: AN APPROACH USING THE BOOTSTRAP. Evolution. 1985;39(4):783–791.

[24] Williams ST, Goodfellow M, Alderson G, et al. Numerical classification of *Streptomyces* and related genera. J Gen Microbiol. 1983;129(6):1743–1813.
[25] ZothanpuiaPassari AK, Leo VV, Kumar B, et al. Bioprospection of actinobacteria derived from freshwater sediments for their potential to produce antimicrobial compounds. BMC Microb Cell Fact. 2018;17:68.
[26] Li F, Liu S, Lu Q, et al. Studies on antibacterial activity and diversity of cultivable actinobacteria isolated from mangrove soil in futian and maoweihai of China. Evid Based Complement Altern.11. 2019;2019:1–11.
[27] Siddharth S, Vittal RR, Wink J, et al. Diversity and bioactive potential of actinobacteria from unexplored regions of Western Ghats, India. Microorganisms. 2020;8(2):225.
[28] Colmer AR, Mccoy E. *Micromonospora* in relation to some Wisconsin lakes and lake populations. Trans Wis Acad Sci Arts Lett. 1943;35:187–220.
[29] Veyisoglu A, Tatar D, Cetin D, et al. *Streptomyces karpasiensis* sp. nov., isolated from soil. Int J Syst Evol Microbiol. 2014;64(Pt 3):827–832.
[30] Khan ST, Tamura T, Takagi M, et al. *Streptomyces tateyamensis* sp. nov., *Streptomyces marinus* sp. nov. and *Streptomyces haliclonae* sp. nov., isolated from the marine sponge Haliclona sp. Int J Syst Evol Microbiol. 2010;60(Pt 12):2775–2779.
[31] Stackebrandt E, Ebers J. Taxonomic parameters revisited: tarnished gold standards. Microbiol Today. 2006;33:152–155.
[32] Kim M, Oh HS, Park SC, et al. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol. 2014;64(Pt 2):346–351.
[33] Chun J, Oren A, Ventosa A, et al. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol. 2018;68(1):461–466.
[34] Tiwari K, Gupta RK. Rare actinomycetes: a potential storehouse for novel antibiotics. Crit Rev Biotechnol. 2012;32(2):108–132.
[35] Hayakawa M, Nonomura H. Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. J Ferment Technol. 1987;65(5):501–509.
[36] Kuester E, Williams ST. Selection of media for isolation of streptomycetes. Nature. 1964;202:928–929.
[37] Sanglier JJ, Whitehead D, Saddler GS, et al. Pyrolysis mass spectrometry as a method for the classification, identification and selection of actinomycetes. Gene. 1992;115(1-2):235–242.
[38] Tan GYA, Ward AC, Goodfellow M. Exploration of *Amycolatopsis* diversity in soil using genus-specific primers and novel selective media. Syst Appl Microbiol. 2006;29(7):557–569.
[39] Reasoner DJ, Geldreich EE. A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol. 1985;49(1):1–7.
[40] Mincer TJ, Jensen PR, Kauffman CA, et al. Widespread and persistent populations of a major new marine actinomycete taxon in ocean sediments. Appl Environ Microbiol. 2002;68(10):5005–5011.
[41] Jiang Y, Li Q, Chen X, et al. Isolation and cultivation methods of actinobactera. In: Dhanasekaran D, editor. Actinobacteria–basics and biotechnological application. London (UK): IntechOpen; 2016. p. 39–57.
[42] Buchholz-Cleven BEE, Rattunde B, Straub KL. Screening for genetic diversity of isolates of anaerobic Fe(II)-oxidizing bacteria using DGGE and whole-cell hybridization. Syst Appl Microbiol. 1997;20(2):301–309.
[43] Metsä-Ketelä M, Salo V, Halo L, et al. An efficient approach for screening minimal PKS genes from *Streptomyces*. FEMS Microbiol Lett. 1999;180(1):1–6.