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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Actinobacteria with heavy metal tolerance from the Sinop coast: A molecular characterization approach

Sinop sahilinden elde edilen ağır metal toleransına sahip aktinobakteriler: Moleküler karakterizasyon yaklaşımı

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Abstract: This study aimed to isolate and molecularly characterize marine actinobacteria tolerant to heavy metals from sediment samples collected from the Meydankapı coast in Sinop, Türkiye. Sediment samples were obtained from three different stations and cultured on five selective media, leading to the isolation of 54 actinobacteria strains. The highest isolation efficiency was achieved on R2A and SM3 media. The tolerance of the isolates to heavy metals, including lead (Pb), copper (Cu), zinc (Zn), mercury (Hg), and cadmium (Cd), was tested. The results revealed that all isolates were sensitive to Hg and Cd but exhibited high tolerance to Pb. Twelve isolates, showing the highest resistance to heavy metals, were selected for genomic DNA isolation. The 16S rRNA gene region was amplified using universal primers and sequenced. Sequence analysis identified the isolates as belonging to the genera *Streptomyces*, *Actinomadura*, *Nocardia* and *Nonomuraea*. The approximately 1500-bp 16S rRNA gene sequences were compared with the EzTaxon database, and the closest species were determined. The obtained sequences were deposited in the GenBank database. The results indicate that marine actinobacteria isolated from polluted sediments of the Black Sea possess significant potential for bioremediation and environmental biotechnology applications due to their resistance mechanisms to heavy metals.

Keywords: Heavy metals, Actinobacteria, Sinop coast, Black Sea, 16S rRNA gene

Öz: Bu çalışmada, Sinop, Meydankapı sahilinden toplanan sediment örneklerinden ağır metal toleransına sahip olan deniz aktinobakterilerinin izolasyonu ve moleküler karakterizasyonu amaçlanmıştır. Sediment örnekleri üç farklı istasyondan alınmış ve beş seçici besiyerinde kültüre alınarak 54 aktinobakteri suşu izole edilmiştir. En yüksek izolasyon verimliliği R2A ve SM3 besiyerlerinde elde edilmiştir. İzolatların kurşun (Pb), bakır (Cu), çinko (Zn), cıva (Hg) ve kadmiyum (Cd) dahil olmak üzere ağır metallere toleransı test edilmiştir. Sonuçlar, tüm izolatların Hg ve Cd'ye duyarlı, ancak Pb'ye yüksek tolerans gösterdiğini ortaya koymuştur. Ağır metallere karşı en yüksek direnci gösteren on iki izolat, genomik DNA izolasyonu için seçilmiştir. 16S rRNA gen bölgesi, evrensel primerler kullanılarak çoğaltılmış ve dizilenmiştir. Dizi analizi, izolatların *Streptomyces*, *Actinomadura*, *Nocardia* ve *Nonomuraea* cinslerine ait olduğunu belirlemiştir. Yaklaşık 1500 baz çifti uzunluğundaki 16S rRNA gen dizileri, EzTaxon veri tabanıyla karşılaştırılarak en yakın türler belirlendi. Elde edilen diziler GenBank veri tabanına kaydedildi. Sonuçlar, Karadeniz'in kirliliği fazla olan sedimentlerinden izole edilen deniz aktinobakterilerinin, ağır metallere karşı direnç mekanizmaları nedeniyle biyoremediasyon ve çevresel biyoteknoloji uygulamaları için önemli bir potansiyele sahip olduğunu göstermektedir.

Anahtar kelimeler: Ağır metaller, Aktinobakteriler, Sinop kıyıları, Karadeniz, 16S rRNA geni

INTRODUCTION

Environmental pollution, primarily driven by industrialization and urbanization, is one of the most pressing global issues of our time. Pollutants are defined as substances that exceed permissible concentrations in the environment and can lead to detrimental effects on ecosystems, human health, and biodiversity (Rahman and Singh, 2019). These pollutants can be categorized into several types, including inorganic, organic, gaseous, metallic, and biological pollutants (Martin and Johnson, 2012). Among these, heavy metals are particularly concerning due to their toxicity, persistence, and ability to bioaccumulate, making them critical pollutants in coastal ecosystems. These metals can enter the marine environment through both natural and anthropogenic activities (Fernandes and Nayak, 2012).

Natural sources of heavy metals include geological weathering, volcanic eruptions, and soil erosion, while human activities such as mining, industrial metal processing, electroplating, pigment production, and petroleum-related operations are the primary contributors to heavy metal contamination (El-Sorogy et al., 2013; Venkatramanan et al., 2014; Masindi and Muedi, 2018).

The accumulation of these metals in coastal waters has severe consequences for marine life, wildlife, and even human populations. These pollutants disrupt the functioning of ecosystems, pose a threat to the food chain, and may lead to various health problems (Pazirandeh et al., 1998; El-Sayed et al., 2024). As heavy metals are highly persistent in the environment, they often create a toxic atmosphere that induces stress on the local microbial communities, which in turn impacts overall ecosystem health.

Over time, microorganisms have developed a variety of mechanisms to adapt to and survive under the stress of heavy metal exposure. These adaptations include biochemical and genetic changes that allow microorganisms to detoxify and tolerate high concentrations of toxic substances. Among these microorganisms, actinomycetes have attracted significant attention for their ability to produce bioactive metabolites that not only enable them to survive in polluted environments but also offer potential for bioremediation (Ashbolt et al., 2013; Mondal and Thomas, 2022). These bioactive metabolites include antimicrobial agents, enzymes, and emulsifiers, which have shown promise in a variety of biotechnological applications.

In recent years, the potential of marine actinomycetes in environmental remediation has been increasingly recognized. These microorganisms, which thrive in the diverse and often extreme conditions of marine environments, possess unique metabolic pathways that allow them to degrade or transform pollutants such as heavy metals, pesticides, plastics, and even radioactive waste (Jagannathan et al., 2021). Their ability to produce diverse and novel metabolites makes them invaluable candidates for addressing a wide range of environmental challenges. Moreover, these metabolites have applications in several industries, including pharmaceuticals, agriculture, and waste management (Bull and Stach, 2007; El-Sayed et al., 2024).

Marine environments, particularly those with high concentrations of pollutants, represent a promising source for discovering novel actinomycete strains with the potential for environmental cleanup. Given the growing interest in marine actinomycetes and their ability to produce valuable bioactive compounds, this study aims to isolate and molecularly characterize actinobacterial strains from the bottom sediments of the Black Sea, specifically from three stations located in Sinop Province. These sites were chosen due to their high levels of heavy metal contamination, making them an ideal environment for exploring the bioremediation capabilities of marine actinomycetes. By studying these strains, the research seeks to uncover new microbial species and metabolites that could be harnessed for environmental remediation and other industrial applications.

MATERIALS AND METHODS

Collection and storage of marine coastal sediment samples

Sediment samples taken from three different stations in the Meydankapı area of the Black Sea coastal bottom sediment of Sinop Province, where heavy metal and other pollutant concentrations are high, were placed in sterile 50 mL falcon tubes and brought to the laboratory, and were cultured using the dilution plate method in five different selective isolation media prepared the day before being stored.

pH measurement of sediment samples

The pH of the sediment samples was measured from the prepared saturation mud using a glass electrode pH meter. The pH values of the sediment samples were determined using a 1:2.5 sediment: water solution (Sağlam, 1978). For this purpose, 10 g of sediment was weighed and transferred into a 50 mL falcon tube, and 25 mL of pure water was added. The falcon tubes were shaken for 1 hour and left to rest for 1 day. The pH values of the samples were measured the next day.

Actinobacteria isolation

Dilution plate technique was used for the isolation of actinomycetes. All media, except marine agar, were prepared with artificial seawater (ASW) (Kester et al., 1967). The media used were M1 (Mincer et al., 2002) Medium (with Cycloheximide (50 µg/mL) and Nalidixic acid (10 µg/mL)), SM3 (Tan et al., 2006) Medium-Gauze's Medium (with Nystatin (50 µg/mL) and Cycloheximide (50 µg/mL)), R2A (Reasoner and Geldreich, 1985) Medium (with Nystatin (50 µg/mL) and Rifamycin (5 µg/mL)), NaST21Cx (Magarvey et al., 2004) Medium (with Nystatin (50 µg/mL) and Cycloheximide (50 µg/mL)), Marine Medium (Difco) (with Nystatin (50 µg/mL) and Cycloheximide (50 µg/mL)). 1 g of sediment samples were weighed and placed in bottles containing 9 mL of Ringer's solution, a sterile isotonic medium containing glass beads. Thus, 10^{-1} dilutions were prepared for each sediment sample. These 10^{-1} dilutions were shaken manually by gently turning them upside down for 45 minutes to separate the spores and mycelia of actinomycetes attached to the sediment colloids from the colloids. Then, these 10^{-1} dilutions were kept in a water bath set at 55° C for 30 minutes in order to reduce contamination that may be caused by vegetative forms, and the 10^{-1} dilutions of each sediment sample were mixed with a vortex mixer to make them homogeneous. 1 mL was taken with an automatic pipette under aseptic conditions and placed in glass tubes containing 9 mL of sterile Ringer's solution, and in this way, 10^{-2} sediment dilutions were obtained. This process was repeated to prepare 10^{-3} sediment dilutions. 0.1 mL of sediment solutions were taken from each of the 10^{-1}, 10^{-2}, and 10^{-3} dilutions with an automatic pipette and transferred to the surfaces of the isolation plates containing antibiotics at different concentrations as mentioned above and inoculated with a sterile loop using the spread plate method. Three plates were prepared for each dilution and incubated at 28°C for 21-30 days.

Selection, purification and storage of isolates

Actinomycete strains were selected from isolation plates incubated for 21 to 30 days in selective isolation media, based on their colony morphology. Selected strains were inoculated onto malt extract-yeast extract (ISP2) medium containing cycloheximide (50 µg/mL) by the streak method. The plates were incubated at 28°C for 21 days. Appropriate isolates were selected and numbered based on their colony morphology. These isolates were obtained as pure cultures. The pure cultures obtained were transferred to autoclaved and screwcapped tubes containing 25% glycerol with the help of a sterile toothpick and stored at -20°C.

In vitro assessment of heavy metal tolerance

Heavy metal resistance of 42 isolates from bottom sediment was evaluated against five elements (Pb, Cu, Zn, Cd, and Hg) at different concentrations (0.05, 0.15, 0.25, and 1 $mg \cdot mL^{-1}$) in triplicate on Duxbury agar. The metals tested were: lead [$PbCl_2$]; copper [$CuCl_2$]; cadmium [$CdCl_2$]; mercury [$HgCl_2$] and zinc [$ZnCl_2$]. Stock solutions of metal salts prepared in distilled water were sterilized by filtration (0.20 µm). The plates were incubated at 28°C for 7 days. The growth of actinobacteria in metal-free culture medium was used as a control. At the end of 7 days, the petri dishes inoculated in Duxbury medium without heavy metal addition were compared and evaluated as a positive control with the other petri dishes (El Baz et al., 2015).

Genomic DNA isolation and amplification of the 16S rRNA gene region

Genomic DNA isolation of 16 isolates with heavy metal resistance was performed using the Invitrogen PureLink Genomic DNA Mini Kit. Universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCCARCC-3') were used for amplification of the 16S rRNA gene region of the isolates (Lane, 1991). The accuracy of the PCR products was confirmed by checking with 1.5% (w/v) agarose gel electrophoresis. TBE was used as buffer.

Sequencing of PCR products of the 16S rRNA gene region, analysis of sequence data and creation of phylogenetic dendrogram

Sequencing of PCR products of the 16S rRNA gene region was performed at Macrogen Inc., Netherlands, using two forward and one reverse primers. Oligonucleotide primers are 518F (Buchholz-Cleven et al., 1997), MG5F (Chun, 1995) and 800R (Chun, 1995). Purification of PCR products was also performed at the same company before sequencing. Approximately 1400 bp of sequence data obtained with three primers of the isolates were aligned and merged using the ChromasPro 1.7.5 program. Then, the 16S rRNA sequence similarity with the most closely related type species was determined using the global alignment algorithms available in EzTaxon Server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). The MEGA X program was used for phylogenetic analyses, and CLUSTAL_W (Kumar et al., 2018) option of the same program was used for alignment. The Neighbor Joining (Saitou and Nei, 1987) method and the Kimura-2 phylogenetic distance matrix (Kimura, 1980) were used to construct phylogenetic dendograms, and bootstrap analyses of phylogenetic trees (Felsenstein, 1985) were performed with 1000 replicates. In addition, Phylogenetic trees of the genus *Streptomyces* were constructed using The Maximum-Likelihood (Felsenstein, 1981) and the Maximum-Parsimony (Kluge and Farris, 1969) algorithms (Supplementary Figure S1 and S2). Phylogenetic trees of the genus *Actinomadura* were constructed using the Maximum-Likelihood (Felsenstein, 1981) and the Maximum-Parsimony (Kluge and Farris, 1969) algorithms (Supplementary Figure S3 and S4).

RESULTS

Sediment samples taken from three different stations in the Meydankapı area of the Black Sea coastal sediment of Sinop Province, where heavy metal and other pollutant concentrations are high, were cultured in five different selective isolation media using the dilution plate method.

The coordinates of three different stations where sediment samples were taken were measured with eXplorist 100, the coordinates and the date of taking the sediment samples are given in Table 1. The pH of the sediment samples was measured with a glass electrode pH meter and the pH values are given in Table 2.

Table 1. Locality and geographical coordinates of marine coastal sediment samples

Locality	Geographic coordinates	Date of collection of sediment samples
Sinop province Meydankapı location 1	42°01'51"N 35°09'06"E	21 October 2023
Sinop province Meydankapı location 2	42°01'49"N 35°09'03"E	21 October 2023
Sinop province Meydankapı location 3	42°01'42"N 35°09'22"E	21 October 2023

Table 2. pH values of sediment samples

Locality	Sediment pH values
Sinop province Meydankapı location 1	7.23
Sinop province Meydankapı location 2	7.62
Sinop province Meydankapı location 3	8.25

In the study, sea bottom sediment was taken from three different localities and cultivation was carried out on five different selective isolation media for the isolation of actinobacteria by the dilution plate method. Thirty-five isolates were obtained from location 1, 5 isolates from location 2 and 14 isolates from location 3. When the selective isolation media used were examined, 19 isolates were isolated from R2A agar, 18 isolates from SM3 agar, 11 isolates from NaST21CX agar, 5 isolates from M1 agar and 1 isolate from Marine agar. R2A and SM3 agar were found to be the most efficient in the isolation of sea bottom sediment. The graph showing the distribution of isolates obtained from the selective isolation media used is given in Figure 1.

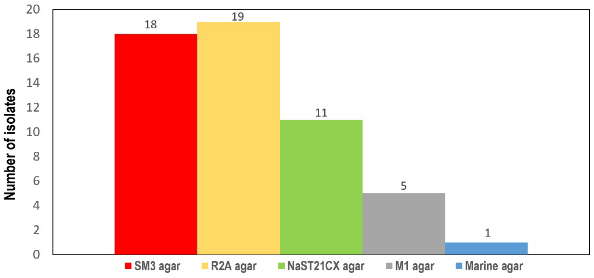

Figure 1. Graph showing the distribution of isolates obtained from the selective isolation media used

Bacterial resistance of 42 strains activated from stocked strains against heavy metals was carried out using modified Duxbury agar. Table 3 shows the differences in heavy metal toxicity levels against the tested strains. While 100 percent of the tested strains grew in the presence of Pb at 0.05 mg·mL^{-1}, 35.71 percent could only grow in the presence of Pb at 1 mg·mL^{-1}. 88% of the tested strains were able to grow in the presence of Pb at 0.15 and 0.25 mg·mL^{-1}. In the presence of 0.05 mg/mL copper, 26% of the isolates showed growth and in the presence of 0.15 mg/mL copper, 11.90% of the isolates showed growth. In the presence of 1 mg/mL copper, only one isolate showed growth. In the presence of 0.05 mg/mL zinc, 59.52% of the isolates grew, 42.85% at 0.15 mg/mL, 26.19% at 0.25 mg/mL, and 19.04% at 1 mg/mL. Mercury and cadmium completely suppressed actinobacterial growth at all concentrations. From these results, we can conclude that the isolates from the region we worked in tolerated lead metal the most, while mercury and cadmium were the most toxic metals. The isolates tolerated zinc metal at a moderate level, while copper metal was tolerated at a lower level.

Table 3. Heavy metal tolerance in actinobacteria isolated from Black Sea coastal bottom sediments

Heavy metal	Number of Actinobacteria isolated	Concentration of heavy metals			
		0.05 mg/mL	0.15 mg/mL	0.25 mg/mL	1 mg/mL
Pb	42	100%	88%	88%	35.72%
Cu	42	26.19%	11.90%	2.38%	2.38%
Zn	42	59.52%	42.85%	26.19%	19.04%
Hg	42	-	-	-	-
Cd	42	-	-	-	-

Genomic DNA isolation of 12 isolates selected by considering the isolates with high heavy metal tolerance out of 42 isolates obtained was performed with Invitrogen PureLink Genomic DNA Mini Kit. The 16S rRNA gene region of the 12 isolates whose genomic DNA was isolated was amplified in Gradient PCR with universal primers 27F and 1525R. 16S rRNA gene region sequence analysis was performed with 3 primers, two of which were universal (518F, 800R and MG5F) for 12 isolates whose 16S rRNA gene region PCR amplifications were performed. After the 16S rRNA gene region sequence analysis of the isolates was completed, the obtained sequence data was combined with the ChromasPro 1.7.5 program and approximately 1500 bp sequence was obtained. It was determined that 7 of them belonged to the genus *Streptomyces*, 3 to the genus *Actinomadura*, 1 to the genus *Nocardia* and 1 to the genus *Nonomuraea.* The obtained 16S rRNA gene nucleotide sequences were deposited in GenBank and the accession numbers are given in Table 4.

The neighbor-joining method (Saitou and Nei, 1987) was used to construct a phylogenetic dendrogram showing the relationships of the 12 isolates to their neighbors. To assess the reliability of the inferred phylogeny, bootstrap analysis was conducted using 1000 replicates, based on a Kimura 2-parameter distance matrix (Felsenstein, 1985) (Figures 2, 3).

The phylogenetic affiliation of seven bacterial isolates was determined by analyzing their 16S rRNA gene sequences. All isolates were identified as members of the genus *Streptomyces*, with sequence similarities to known type strains ranging from 97.22% to 99.79%. Isolate MS23 exhibited the lowest sequence similarity (97.22%) to *Streptomyces althioticus* NRRL B-3981^T, with 40 nucleotide differences over 1440 aligned bases, suggesting it may represent a novel species. In contrast, isolate MS08 showed 99.72% similarity to *Streptomyces coelicoflavus* NBRC 15399^T, while MS31 displayed 99.79% similarity to *Streptomyces malachitospinus* NBRC 101004^T. Isolates MS35 and MS43 were closely related to *Streptomyces aculeolatus* NBRC 14824^T (99.31%) and *Streptomyces althioticus* NRRL B-3981^T (99.79%), respectively. The remaining isolates, MS54 and MS55, showed 99.28% similarity to *Streptomyces nanshensis* SCSIO 01066^T and 99.72% similarity to *Streptomyces phytophilus* PIP175^T, respectively.

Table 4. Phylogenetic similarity of test organisms belonging to the Actinobacteria class with the closest type species according to 16S rRNA sequence results

No	Strain code	GenBank no	Closest Type	Similarity %-nucleotide difference
1	**MS08**	PV186786	*Streptomyces coelicoflavus* NBRC 15399^T	99.72% - 4/1448
2	**MS17**	PV187749	*Actinomadura madurae* DSM 43067^T	99.72%- 4/1439
3	**MS21**	PV186843	*Nonomuraea jabiensis* A4036^T	98.82%- 17/1442
4	**MS23**	PV186851	*Streptomyces althioticus* NRRL B-3981^T	97.22%- 40/1440
5	**MS31**	PV187100	*Streptomyces malachitospinus* NBRC 101004^T	99.79%- 3/1448
6	**MS35**	PV187107	*Streptomyces aculeolatus* NBRC 14824^T	99.31%- 10/1440
7	**MS41**	PV187108	*Actinomadura geliboluensis* A8036^T	99.93%- 1/1440
8	**MS43**	PV187302	*Streptomyces althioticus* NRRL B-3981^T	99.79%- 3/1441
9	**MS52**	PV187474	*Nocardia higoensis* NBRC 100133^T	99.17%- 12/1441
10	**MS54**	PV187476	*Streptomyces nanshensis* SCSIO 01066^T	99.28%-10/1390
11	**MS55**	PV187694	*Streptomyces phytophilus* PIP175^T	99.72%- 4/1449
12	**MS56**	PV187695	*Actinomadura hibisca* NBRC 15177^T	98.61%- 20/1435

All isolates demonstrated the ability to tolerate lead at a concentration of 1 mg/mL. Isolate MS23 also tolerated zinc at 0.25 mg/mL. Isolate MS08 was resistant to lead and zinc at 1 mg/mL, and to copper at 0.15 mg/mL. Similarly, MS31 showed high tolerance to all three tested metals (lead, copper, and zinc) at 1 mg/mL concentrations. Isolate MS35 exhibited tolerance to lead (1 mg/mL) and zinc (0.05 mg/mL), while MS43 tolerated lead (1 mg/mL) and zinc (0.15 mg/mL). Both MS54 and MS55 were resistant to lead, copper, and zinc, indicating broad-spectrum metal tolerance.

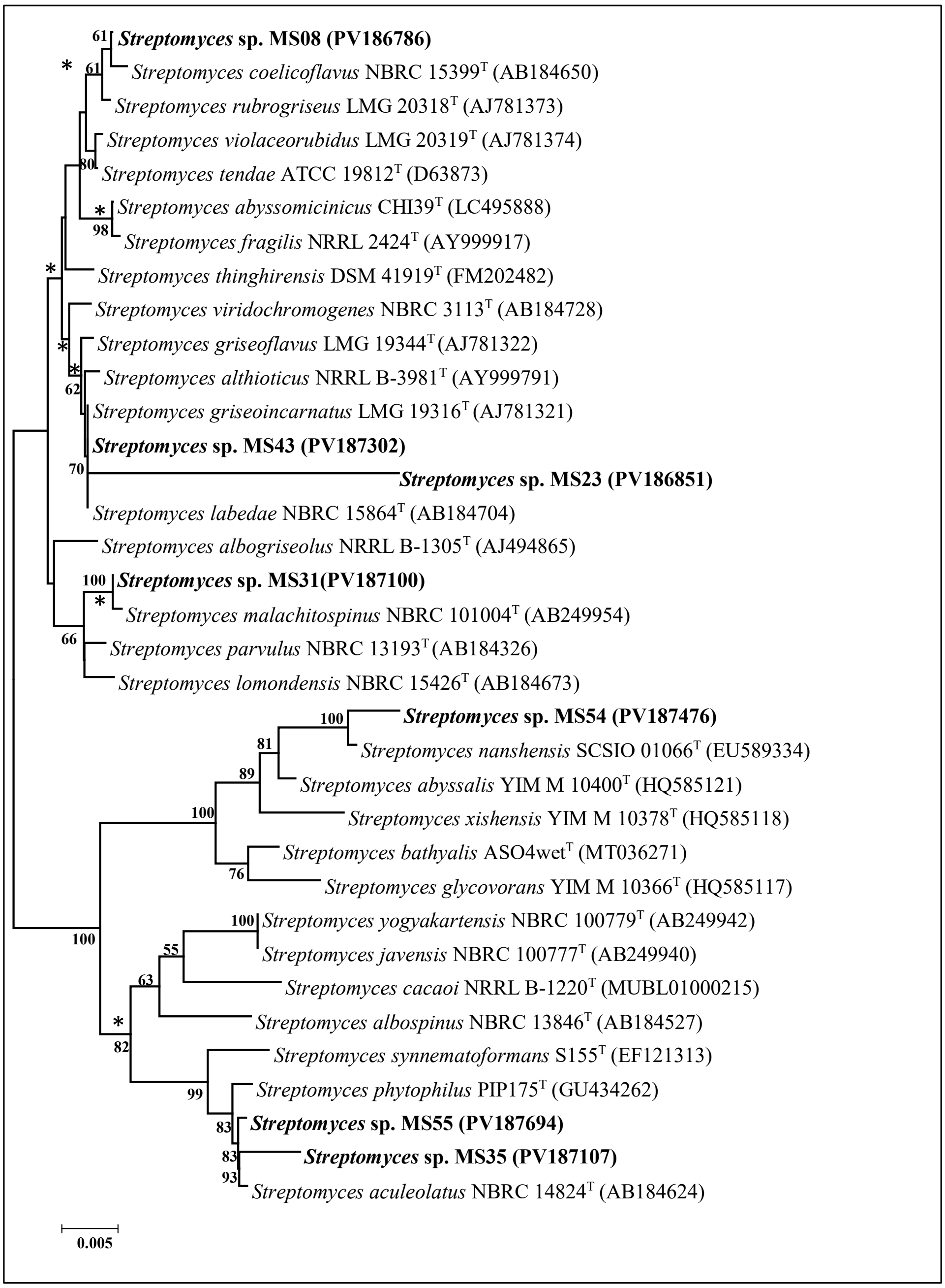

Figure 2. Phylogenetic dendrogram of *Streptomyces* genus isolates based on 16S rRNA gene sequence analysis. The tree was constructed using the neighbor-joining algorithm, with bootstrap values provided for branch points supported by more than 50% of the samples. Asterisks (*) indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge and Farris, 1969) tree making algorithms. The scale bar represents 0.005 genetic distance.

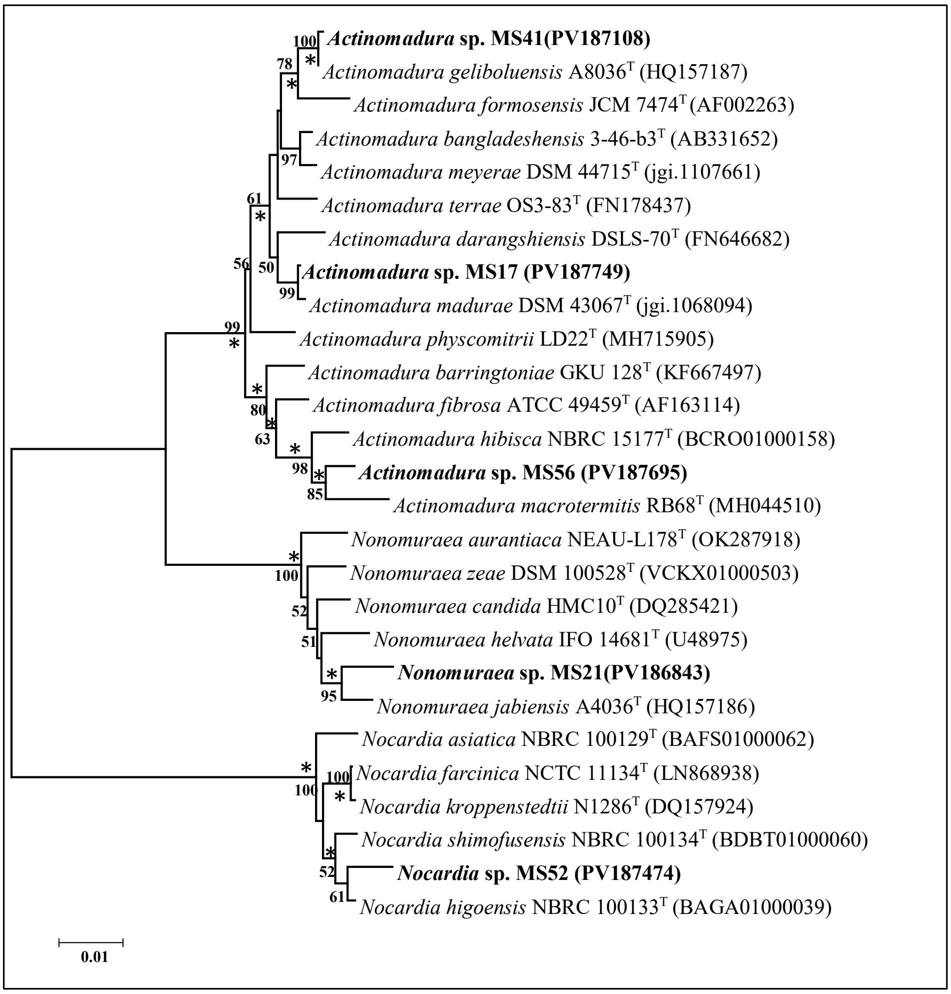

Figure 3. Phylogenetic dendrogram of *Actinomadura, Nonomuraea* and *Nocardia* genus isolates based on 16S rRNA gene sequence analysis. The tree was constructed using the neighbor-joining algorithm, with bootstrap values provided for branch points supported by more than 50% of the samples. Asterisks (*) indicate branches of the tree that were also recovered using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge and Farris, 1969) tree making algorithms. The scale bar represents 0.01 genetic distance

In this study, five bacterial isolates were evaluated in terms of their taxonomic affiliations and tolerance to heavy metals. Isolate MS56 exhibited 98.61% 16S rRNA gene sequence similarity to its closest type strain, *Actinomadura hibisca* NBRC 15177^{T}, with 20 nucleotide differences across 1435 aligned positions. This level of divergence suggests that MS56 may represent a novel species. Additionally, MS56 demonstrated notable tolerance to lead (Pb) and zinc (Zn), indicating potential for environmental bioremediation applications.

Isolates MS17 and MS41 showed high similarity to known species, with MS17 displaying 99.72% similarity to *Actinomadura madurae* DSM 43067^{T} and MS41 showing 99.93% similarity to *Actinomadura geliboluensis* A8036^{T}. Although these levels suggest they are closely related to existing species, both isolates demonstrated considerable tolerance to Pb, copper (Cu), and Zn, reflecting strong adaptive responses to metal stress.

MS21 showed 98.82% similarity to *Nonomuraea jabiensis* A4036^{T}, which is below the generally accepted threshold for species delineation, indicating its potential status as a novel species. This isolate also displayed robust resistance to Pb, Cu, and Zn, highlighting its ecological resilience and potential utility in heavy metal-contaminated environments.

Isolate MS52 showed 99.17% similarity to *Nocardia higoensis* NBRC 100133^{T}.

DISCUSSION

In our study, 42 isolates were isolated from the Meydankapı location of the Black Sea coastal sediments of Sinop province, where heavy metal and other standards are high. 16S rRNA analysis was performed on 12 of 42 isolates. Seven of these were identified as belonging to the *Streptomyces* genus, three to the *Actinomadura* genus, one to the *Nocardia* genus, and one to the *Nonomuraea* genus. The study conducted by Cimermanová et al. (2021) highlights the remarkable microbial potential of technosols-artificial soils formed as a result of anthropogenic activities and characterized by extreme physicochemical conditions. Despite being harsh and often contaminated with toxic substances, these environments were found to harbor diverse populations of *Actinobacteria*, with a dominance of the genus *Streptomyces*. This aligns with previous findings that emphasize the ecological resilience and metabolic versatility of Streptomyces species, particularly their well-known capacity to produce a wide range of bioactive secondary metabolites. Among the nine *Actinobacteria* isolates obtained from various technosol sites in Slovakia, eight were identified as members of the genus *Streptomyces*, with at least one potentially representing a novel species. Another isolate was classified under the genus *Crossiella* and may also constitute a previously undescribed species. These findings suggest that technosols are not only reservoirs of known microbial taxa but also promising sources for the discovery of novel actinomycetes with unique physiological traits. In addition to taxonomic diversity, the isolates displayed considerable resistance to heavy metals such as lead (Pb), zinc (Zn), copper (Cu), and nickel (Ni), with lead being the most tolerated. In a similar manner, our study also revealed that seven out of twelve isolates belonged to the genus *Streptomyces*, exhibiting the highest tolerance to lead among the tested heavy metals.

In line with previous studies, the isolation of numerous heavy metal-resistant *Streptomyces* strains from contaminated environments highlights the adaptive capacity of this genus to harsh ecological conditions (Álvarez et al., 2013). Álvarez et al. demonstrated that heavy metal resistance is phylogenetically widespread among *Streptomyces*, as evidenced by both bibliographic data and 16S rRNA gene sequence availability (Álvarez et al., 2013). This widespread resistance may reflect evolutionary pressure in metal-rich environments, leading to the selection of strains with enhanced survival mechanisms. Furthermore, the presence of diverse *Streptomyces* species in metal-contaminated soils, as supported by several reports (Li et al., 2010; Lin et al., 2012; El Baz et al., 2015; Mo et al., 2017; Li et al., 2019), suggests that such habitats act as reservoirs for resistant and potentially novel strains. Polti et al. (2007) isolated nine chromium-resistant actinobacterial strains from contaminated sites in Tucumán, Argentina, which were previously identified as members of the genus *Streptomyces*. The resistance of actinobacteria to lead (Pb) had already been reported by Sanjenbam et al. (2012).

Streptomyces sp. MS23 displayed the lowest similarity (97.22%) to *Streptomyces althioticus* NRRL B-3981^T, differing at 40 nucleotide positions across 1440 aligned bases, indicating its potential novelty. Also, *Actinomadura* sp. MS56 displayed the lowest similarity (98.61%) to *Actinomadura hibisca* NBRC 15177^T, differing at 20 nucleotide positions across 1435 aligned bases, indicating its potential novelty. Another isolate with potential to be a new species is *Nonomuraea* sp. MS21 (98.82% similar to *Nonomuraea jabiensis* A4036^T).

Initially, a 16S rRNA gene sequence similarity of less than 97% was proposed as the threshold for defining a novel bacterial species (Stackebrandt and Goebel, 1994). However, subsequent studies have suggested raising this threshold to a range of 98.7% to 99% to improve taxonomic resolution (Stackebrandt and Ebers, 2006). Notably, Schmidt et al. reported the isolation of highly nickel-resistant *Streptomyces* strains from a former uranium mining site, reinforcing the idea that extreme environments can foster unique microbial communities with biotechnological potential (Schmidt et al., 2007). In our study, all isolates exhibited the ability to withstand lead at a concentration of 1 mg/mL. Isolate MS23 additionally tolerated zinc at 0.25 mg/mL. Isolate MS08 was resistant to both lead and zinc at 1 mg/mL, as well as to copper at 0.15 mg/mL. Likewise, MS31 showed high resistance to lead, copper, and zinc at 1 mg/mL concentrations. Isolate MS35 displayed tolerance to lead (1 mg/mL) and zinc (0.05 mg/mL), while MS43 tolerated lead (1 mg/mL) and zinc (0.15 mg/mL). Both MS54 and MS55 demonstrated resistance to lead, copper, and zinc, indicating broad-spectrum tolerance to these metals. Additionally, MS56 exhibited significant tolerance to lead (Pb) and zinc (Zn), suggesting its potential for environmental bioremediation applications. Isolates MS17 and MS41 showed considerable tolerance to Pb, copper (Cu), and Zn, reflecting their strong adaptive responses to metal stress. The MS21 isolate also demonstrated robust resistance to Pb, Cu, and Zn, underscoring its ecological resilience and potential use in heavy metal-contaminated environments. Similarly, isolate MS52 displayed resistance to Pb, Cu, and Zn, indicating its potential for biotechnological processes related to heavy metal remediation. In their study conducted in 2015, El Baz et al. investigated the resistance and bioaccumulation potential of actinobacteria strains isolated from some abandoned mining areas to heavy metals. They determined the resistance of 59 isolated actinobacteria to five heavy metals. The minimum inhibitory concentration (MIC) recorded was 0.55 for Pb, 0.15 for Cr and 0.10mg·mL^{-1} for both Zn and Cu.

CONCLUSIONS

Based on nucleotide sequence analysis of the 16S rRNA gene region, three isolates exhibiting high tolerance to heavy metals showed sequence similarity levels of 97.22% (*Streptomyces* sp. MS23), 98.61% (*Actinomadura* sp. MS56), and 98.82% (*Nonomuraea* sp. MS21) to their closest type strains. These values fall below the commonly accepted threshold for species identification, suggesting that the isolates may represent novel species. To verify this, whole-genome sequencing, as well as chemotaxonomic and phenotypic characterizations, will be conducted in the near future. Additionally, comprehensive genomic analyses will be performed to identify heavy metal resistance genes and secondary metabolite biosynthetic gene clusters. If such genes are confirmed, the isolates will be considered promising candidates for bioremediation applications. Overall, the findings suggest that some of the isolates studied may represent novel actinomycete species with notable resistance to multiple heavy metals, highlighting their potential in environmental biotechnology, particularly for the remediation of metal-contaminated soils and wastewater.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the concept and design of the study. Sediment samples were collected by Hunkar Avni Duyar and Aysel Veyisoglu, laboratory studies were performed by Aysel Veyisoglu and Ali Tokatlı, analysis of gene sequence data and writing of the article were performed by Aysel Veyisoglu and Demet Tatar. All authors read and approved the article.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS APPROVAL

This article does not contain any studies with human participants and/or animals performed by any of the authors. Formal consent is not required in this study.

DECLARATION OF AI USE

We have not used AI-assisted technologies in creating this article.

DATA AVAILABILITY

All information regarding the current study can be obtained from the corresponding author.

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Supplementary Figure S1

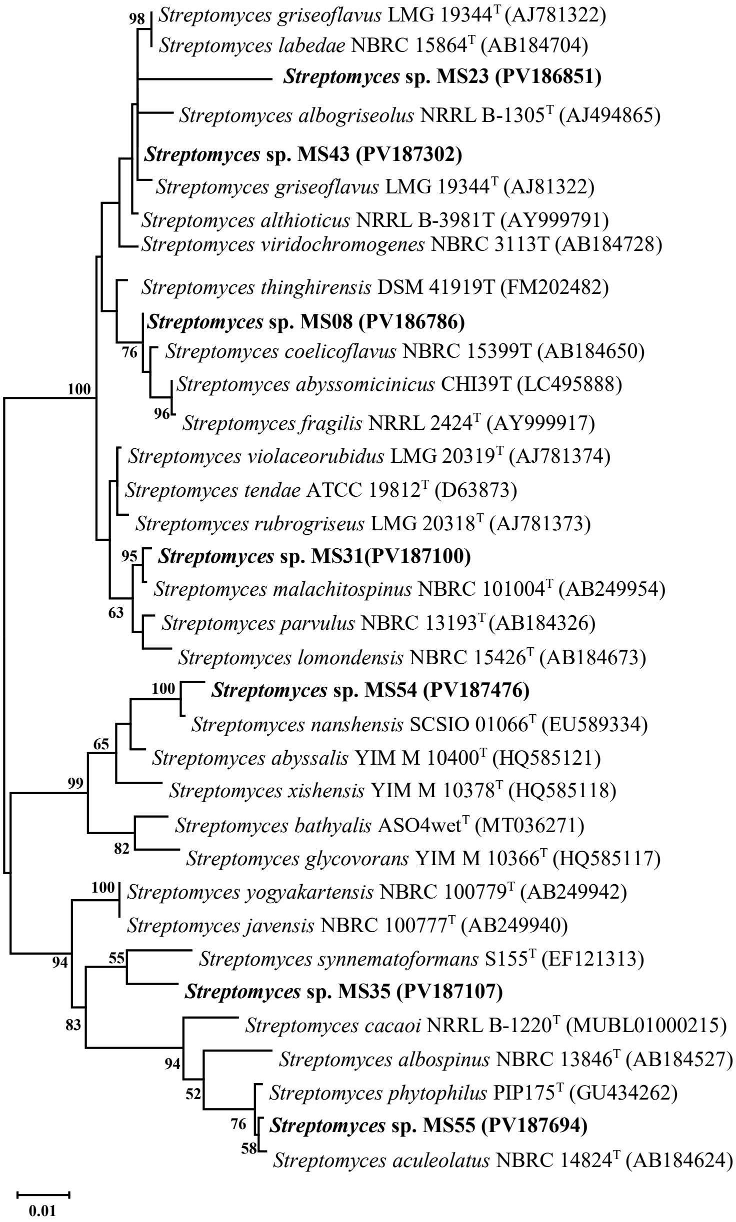

Figure S1. Maximum-Likelihood (Felsenstein, 1981) tree based on 16S rRNA gene sequences showing the position of *Streptomyces* genus isolates among their phylogenetic neighbors

Supplementary Figure S2

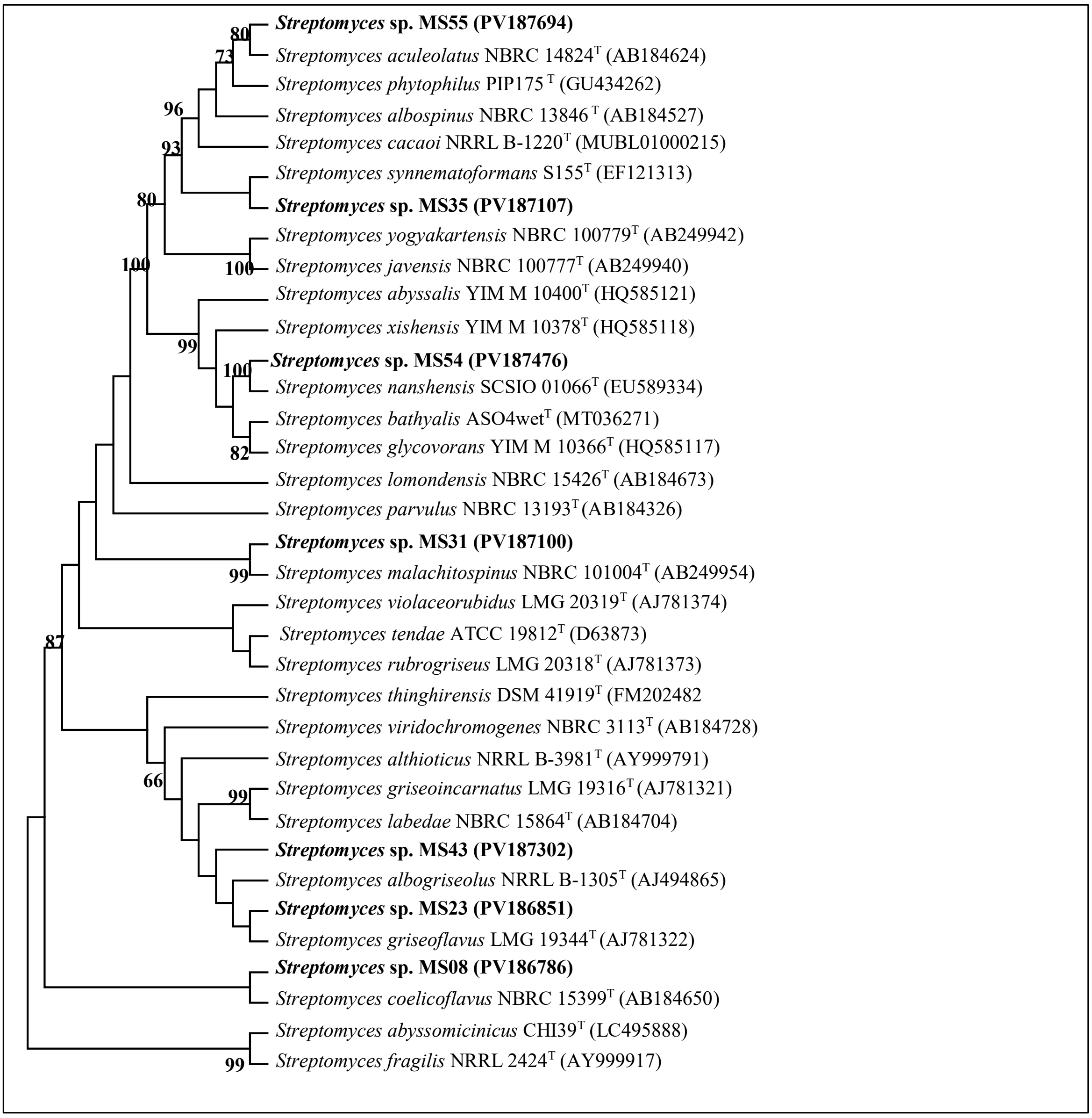

Figure S2. Maximum-Parsimony (Kluge and Farris, 1969) tree based on 16S rRNA gene sequences showing the position of *Streptomyces* genus isolates among their phylogenetic neighbors

Supplementary Figure S3

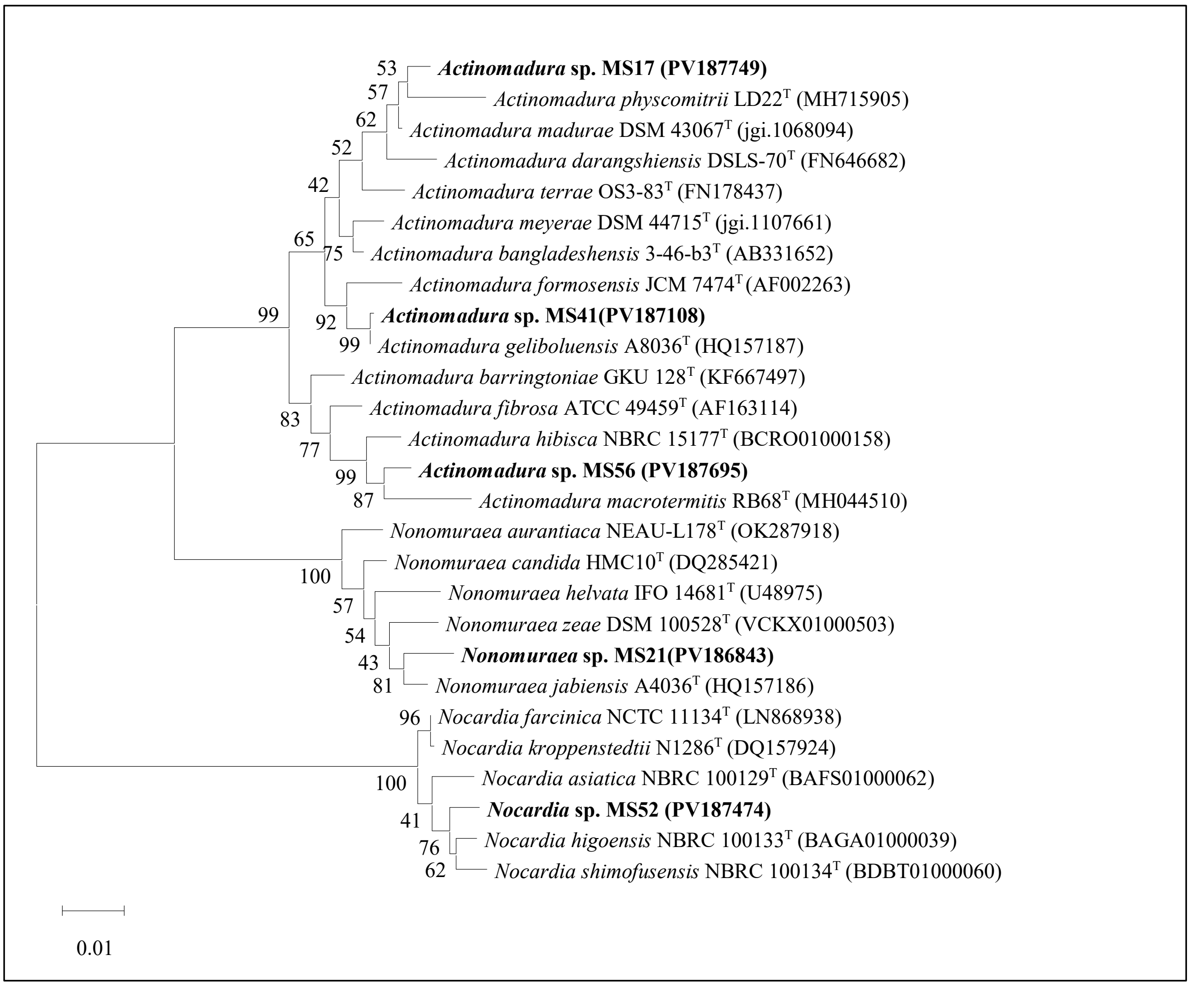

Figure S3. Maximum-Likelihood (Felsenstein, 1981) tree based on 16S rRNA gene sequences showing the position of *Actinomadura, Nonomuraea* and *Nocardia genus* isolates among their phylogenetic neighbors

Supplementary Figure S4

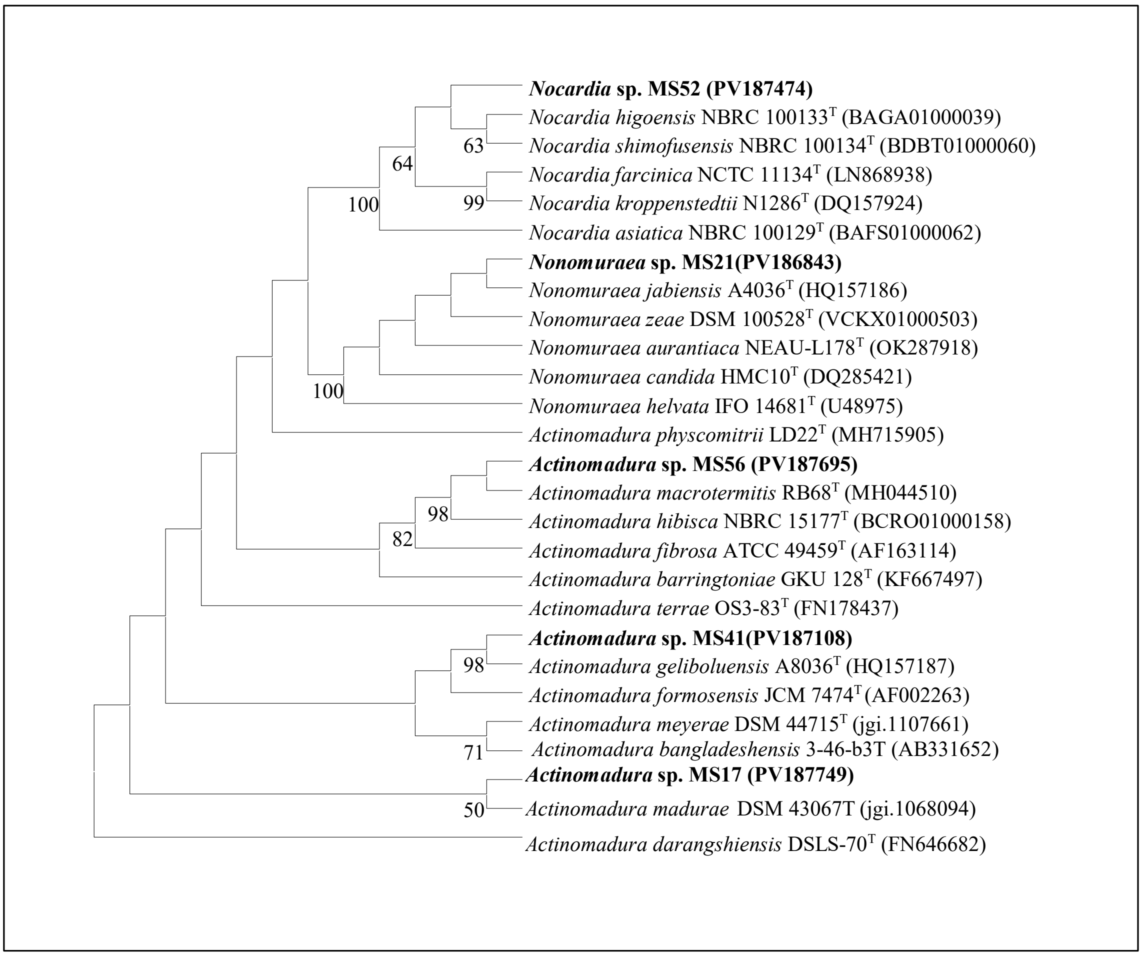

Figure S4. Maximum-Parsimony (Kluge and Farris, 1969) tree based on 16S rRNA gene sequences showing the position of *Actinomadura, Nonomuraea* and *Nocardia genus* isolates among their phylogenetic neighbors