Mar. Sci. Tech. Bull. (2024) 13(3): 183-198
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1522501

Marine Science and Technology Bulletin

https://prensip.gen.tr/

RESEARCH ARTICLE

Isolation, molecular characterization and determination of biological activity of alkalitolerant *Streptomyces* members from Van Lake-Çarpanak Island soil

Aysel Veyisoglu[1*] • Demet Tatar[2] • Hunkar Avni Duyar[3] • Ali Tokatli[4,5]

[1] *Sinop University, Vocational School of Health Services, Department of Medical Laboratory Techniques, 57000, Sinop, Türkiye*
[2] *Hitit University, Osmancık Ömer Derindere Vocational School, Department of Medical Services and Techniques, 19500, Çorum, Türkiye*
[3] *Sinop University, Fisheries Faculty, Department of Fishing and Processing Technology, 57000, Sinop, Türkiye*
[4] *Ondokuz Mayis University, Faculty of Science, Department of Biology, 55139, Samsun, Türkiye*
[5] *Ondokuz Mayis University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 55139, Samsun, Türkiye*

ARTICLE INFO

Article History:
Received: 26.07.2024
Received in revised form: 14.08.2024
Accepted: 27.08.2024
Available online: 30.09.2024

Keywords:
Alkalitolerant Streptomyces
Lake Van
Çarpanak Island
16S rRNA gene
Antimicrobial activity
Enzyme production

ABSTRACT

Çarpanak Island is one of the islands in Lake Van, the easternmost and largest lake in Türkiye. In this study, soil samples taken from three different locations of Çarpanak island, Lake Van, were inoculated into 4 different selective isolation media using the dilution plate technique, and 42 actinomycete isolates were stocked. PCR amplification of the 16S rRNA gene of 13 isolates selected according to their morphological differences was performed with 27F and 1525R primers. Phylogenetic trees were constructed with the neighbor-joining algorithm in the MEGA 7.0 software. According to 16S rRNA gene sequence analysis, it was determined that 13 isolates belonged to the genus *Streptomyces*. The antimicrobial activities of 13 isolates against 8 pathogens and the ability of these isolates to produce lipase, amylase, protease and pectinase were determined. Except for CA43 and CA62 isolates, other *Streptomyces* isolates have the ability to produce lipase. Other *Streptomyces* isolates except CA59 and CA94 can synthesize amylase. In addition, 3 of the 13 *Streptomyces* isolates, CA40, CA61 and CA94, do not have the ability to synthesize protease. No isolate is capable of producing pectinase enzyme. As a result of this study, it was observed that most of the *Streptomyces* isolates had higher lipase enzyme production abilities than other enzyme groups.

Please cite this paper as follows:

Veyisoglu, A., Tatar, D., Duyar, H. A., & Tokatli, A. (2024). Isolation, molecular characterization and determination of biological activity of alkalitolerant *Streptomyces* members from Van Lake-Çarpanak Island soil. *Marine Science and Technology Bulletin, 13*(3), 183-198. https://doi.org/10.33714/masteb.1522501

* Corresponding author
E-mail address: aveyisoglu@sinop.edu.tr (A. Veyisoglu)

Introduction

The phylum Actinobacteria is amongst the most prevalent among bacteria, comprising heterogeneous genera of Gram-positive and Gram-variable bacteria, as described by Ventura et al. (2007). The phylum additionally encompasses *Thermoleophilum* sp. (Zarilla & Perry, 1986), *Gardenerella vaginalis* (Gardner & Dukes, 1955), *Saccharomonospora viridis* P101^T (Pati et al., 2009), *Ferrimicrobium acidiphilum* and *Ferrithrix thermotolerans* (Johnson et al., 2009). Actinobacteria are classified according to their oxygen requirements, motility, spore formation, and G+C content (Ensign, 1992). The genome size of Actinobacteria is variable, with values ranging from 0.93 Mb (*Tropheryma whipplei*; Bentley et al., 2003) to 12.7 Mb (*Streptomyces rapamycinicus*; Baranasic et al., 2013). These genomes are typically found in either circular or linear form.

Actinobacteria are found in a variety of ecological niches, including and aquatic (marine and fresh waters) and terrestrial ecosystems, and exhibit a complex life cycle, including the existence of dormant spores or actively growing hyphae (Shivlata & Satyanarayana, 2015).

The genus *Streptomyces* is classified in the family *Streptomycetaceae*, belonging to the suborder *Streptomycineae* (Waksman & Henrici, 1943; Stackebrandt et al., 1997; Kim et al., 2003; Zhi & Stackebrandt, 2009). Currently, the genus contains more than 800 species with validly published names (see https://www.bacterio.net/genus/streptomyces). Members of the genus *Streptomyces* have been identified from different habitats such as soil (Saricaoglu et al., 2014), rhizospheres (Piao et al., 2018; Sujarit et al., 2018), marine sponge (Silva et al., 2016), marine sediment (Veyisoglu & Sahin, 2014), lake sediment (Ray et al., 2016), mangrove sediment (Hu et al., 2012), mangrove soil (Law et al., 2019), desert (Li et al., 2019) and plant tissue (Wang et al., 2018).

It is well established that Actinobacteria are able to thrive in a variety of environments, both normal as well as extreme. These include environments with acidic or alkaline pH, high or low salinity, high radiation, low levels of available moisture or nutrients (Zenova et al., 2011). The diverse physiology and metabolic flexibility of extremophilic and extremotolerant actinobacteria enable them to survive under unfavourable conditions. Alkaliphilic actinobacteria are categorised into three principal groups. The first group is alkaliphilic (optimal growth occurs at pH 10-11), the second group is moderately alkaliphilic (growth occurs at pH 7-10, although poor growth is observed at pH 7.0) and the third group is alkalitolerant actinobacteria (optimal growth occurs at pH 6-11) (Jiang & Xu, 1993). Baldacci (1944) was the first to report alkaliphilicity. Subsequently, Taber (1960) isolated alkaliphilic Actinobacteria from soil. The presence of alkaliphilic and alkalitolerant Actinobacteria has been documented in a range of environments, including deep-sea sediments (Yu et al., 2013), alkaline desert soils (Li et al., 2006), and soda lakes (Groth et al., 1997) (Shivlata & Satyanarayana, 2015).

Alkaliphilic and alkalitolerant actinobacteria have been observed to thrive in high salinity environments, classified as haloalkaliphiles or haloalkalitolerants. Similarly, they are known to inhabit thermobiotic conditions, classified as alkalithermophiles or alkalithermotolerants. Furthermore, halophilic and halotolerant alkalithermophiles and alkalithermotolerant actinobacteria have also been isolated from saline habitats (Zenova et al., 2011; Shivlata & Satyanarayana, 2015). Alkaliphilic *Streptomycetes* are commonly found in soda lakes and alkaline soils (Świecimska et al., 2020). Actinobacteria constitute an important group of microorganisms with an ecological significance. Their involvement extends to a wide range of biological processes, including biogeochemical cycles, bioremediation, biodegradation, and plant growth promotion (Palaniyandi et al., 2013; Cockell et al., 2013; Chen et al., 2015). Moreover, they produce a diverse array of pharmaceutically valuable bioactive compounds (antibiotics, antitumor agents, anti-inflammatory compounds, and enzyme inhibitors) and secrete numerous industrially and clinically significant enzymes.

Following the discovery of streptomycin, the first antituberculosis drug derived from actinobacteria, drug discovery and development programmes have increasingly focused on the identification of antimicrobial agents. A significant number of actinobacterial species have subsequently been investigated for the discovery of clinically valuable compounds. The phylum Actinobacteria includes several genera that include antibiotic-producing species, with the genus *Streptomyces* being of particular importance as an important source of secondary metabolites, especially antibiotics. It is estimated that *Streptomyces* species account for more than 50% of the total known microbial antibiotics, with at least 10,000 examples currently documented (Bull, 2010).

A number of new bioactive compounds with different antimicrobial activities have been identified. These compounds synthesized by various species of the *Streptomyces* genus are albocycline-type macrolides (Gu et al., 2019), benditerpenoic acid (Zhu et al., 2021) and pyrimidomycin (Das et al., 2022). Despite this, bacteria causing nosocomial infections remain a significant public health concern. These bacteria present a

significant public health crisis and health security threat due to the increasing prevalence of antibiotic resistance (Mancuso et al., 2021; Chanama et al., 2023).

Although pharmaceutical companies and research laboratories have access to a wide array of clinical drugs, they remain committed to the search for new therapeutic drugs that can combat microbial pathogens. In order to identify new bioactive compounds with potential pharmaceutical or industrial applications, actinobacteria have been isolated from remote and previously unexplored locations, including deserts (Kurapova et al., 2012), marine environments (Dhakal et al., 2017) and wetlands (Yu et al., 2015). It has been hypothesised that extremophilic actinobacteria may be a source of novel, valuable metabolites containing gene clusters for the synthesis of new biomolecules (Bull, 2010). Consequently, efforts are being made to isolate such organisms from extremely extreme environments.

Lake Van is located in the eastern part of Türkiye's Anatolian peninsula, at approximately 43°E longitude and 38.5°N latitude. The surface area of the lake is 3574 km^2, its volume is 650 km^3, its maximum depth is 450 m and its maximum length is 130 km (Cukur, 2014). In addition to being a closed basin, Lake Van is a salty soda lake and differs from other lakes in the world with its importance. Considering its features and volume, in addition to being the largest lake in Türkiye, it is the largest soda lake and the third largest closed lake in the world (Reimer et al., 2009).

The chemistry of soda lakes is distinctive due to the presence of elevated concentrations of carbonate/bicarbonate in the form of Na_2CO_3 and $NaHCO_3$ (Jones et al., 1998). Soda lakes host a diverse array of microbial communities, and studies on the microbial diversity of these lakes contribute to our understanding of the biology of extreme habitats. At the same time, new microorganisms obtained from these environments and their novel enzymes are of significant interest within the scope of biotechnological applications. Proteases, lipases and cellulases are obtained from alkaliphilic microorganisms in the production of biotechnologically advanced laundry detergents (Horikoshi, 2008). Alkaline protease is also used in the food industry, pharmaceuticals and medical diagnostics (Kanekar et al., 2002). In addition to enzymes, alkaliphilic microorganisms can also be employed for the biodegradation of organic and inorganic pollutants and hydrocarbons (Sorokin et al., 2012; Poyraz & Mutlu, 2017).

The aim of this project is to isolate alkalitolerant *Streptomyces* members obtained from Çarpanak Island in Lake Van, to determine the nucleotide sequences and phylogenetic analysis of the *Streptomyces* isolates obtained by PCR amplification of 16S rRNA gene regions and to determine the antimicrobial activities of the isolates and their capacity to produce enzymes of industrial importance.

Material and Methods

Collection and Storage of Soil Samples

Soil samples taken from three different localities (Table 1) of Çarpanak Island by measuring their coordinates with the eXplorist100 were placed in sterile 50 mL falcon tubes and transferred to the laboratory, dried at room temperature, and then planted in four different alkaline selective isolation media (Table 2) using the dilution plate method. The locality and geographical coordinates of the soil samples are given in Table 1.

Table 1. Locality and geographical coordinates of soil samples

Locality	Geographic Coordinates
From the part of the island close to Lake Van	38°36.366 N 043°05.197 E
From the part of the island far from Lake Van	38°36.531 N 043°05.055 E
One of the regions of the island with dense plant flora	38°36.399 N 043°05.115 E

pH Measurement of Soil Samples

The pH of the soil samples was measured from the saturation mud prepared with the help of a glass electrode pH meter. pH values of soil samples were determined using a 1:2.5 soil:water solution (Sağlam, 1978). For this, 10 g of soil 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 to isolate actinomycetes. 1 g of the soil samples was weighed and placed in bottles containing 9 mL of sterile Ringer's solution containing glass beads. In this way, the 10^{-1} solutions prepared for each soil sample were shaken gently for 45 minutes to separate the spores and mycelia of actinomycetes attached to the soil colloids from the colloids. Then, these 10^{-1} dilutions were kept in a water bath set at 60°C for 45 minutes in order to reduce contamination that could be caused by vegetative forms, and the 10^{-1} dilutions of

each soil sample were mixed with a vortex mixer (Fisons Sientifiv Appartus Ltd., Loughborough, Leicesteshire, England, UK) and made homogeneous. 1 mL soil dilution was taken under aseptic conditions with an automatic pipette (1000: Axygen Inc, 33210 Central Avenue, Union City, CA 94587 USA) and placed in glass tubes containing 9 mL of sterile Ringer's solution, and in this way, 10^{-2} soil dilutions were obtained. This process was repeated and 10^{-3} sediment dilutions were prepared. 0.1 mL of soil solutions were taken from each of the 10^{-2} and 10^{-3} dilutions with an automatic pipette, placed on the surfaces of isolation plates with antibiotics added in different concentrations as mentioned above, and inoculated by the smear plate method with a sterile loop. 3 plates were prepared for each dilution and incubated at 28°C for 25-30 days.

Selection, Purification and Stocking of Isolates

Actinomycete strains were selected from the isolation plates that were incubated in selective isolation media for 21-30 days, taking into account their colony morphologies. The selected strains were inoculated onto the surface of malt extract-yeast extract (ISP2) (Shirling & Gottlieb, 1966) supplemented with cycloheximide (50 µg/ml) by the line sowing method to ensure a single colony, and were incubated at 28°C for 20 days. The isolates selected considering the colony morphology were numbered, pure cultures were made, and transferred to autoclaved screw cap tubes containing 25% glycerol with the help of sterile toothpicks and stored at -20°C.

Determining pH Range

Tolerance ranges were determined at pH 4, 5, 6, 7, 8, 9, 10, 11, and 12 for 20 isolates selected according to color groups. The study employed glucose yeast extract-malt extract agar. The pH values of the agar were adjusted with 0.1 M NaOH and 0.1 M HCl. The inoculation of the isolates into the culture medium was conducted using the point inoculation method with the aid of an automatic pipette. For each isolate, 1.5 mL of Ringer's solution was added to screw-capped bottles and sterilised by autoclaving at 121°C for 15 minutes. The spore and substrate mycelia of the isolates were transferred to small glass vials containing a ringer under aseptic conditions with the help of a sterile loop. This was done to facilitate the point inoculation procedure, which was then carried out using an automatic pipette and a bacterial solution that had been homogenised by mixing with a vortex.

Genomic DNA Isolation

Genomic DNA isolation of 17 out of 20 isolates selected according to color groups was performed with the Invitrogen PureLink Genomic DNA Mini Kit.

Amplification of the 16S rRNA Gene Region

Primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525r (5'-AAGGAGGTGWTCCARCC-3') were used for amplification of the 16S rRNA gene region of the test isolates. PCR products were checked using agarose gel electrophoresis (1%, w/v) (Table 3).

Table 2. List of selective media used

No	Medium	Antibiotics
1	Humic acid vitamin agar (Hayakawa & Nonomuraea, 1987)	Cycloheximide (50 µg/ml), Nalidixic acid (10 µg/ml)
2	Starch-casein agar (Küster & Williams, 1964)	Nystatin (50 µg/ml), Cycloheximide (50 µg/ml)
3	SM3 agar-Gauze's agar (Tan et al., 2006)	Nystatin (50 µg/ml), Rifampicin (5 µg/ml)
4	R2A agar (Reasoner & Geldreich, 1985)	Nystatin (50 µg/ml), Cycloheximide (50 µg/ml)

Table 3. Nucleotide sequences of primers used in 16S rRNA gene region studies

Primer Code	Sequence (5'-3')	Length	Reference
518F	CCAGCAGCCGCGGTAAT	17	Buchholz-Cleven et al. (1997)
800R	TACCAGGGTATCTAATCC	18	Chun (1995)
MG5F	AAACTCAAAGGAATTGACGG	20	Chun (1995)

Sequencing of purified 16S rRNA gene region PCR products was performed by Macrogen Inc., Netherlands, using two forward and one reverse primer.

Analysis of 16S rRNA Sequence Data and Phylogenetic Dendrogram Creation

After the 16S rRNA gene region sequence analysis of the isolates was completed, the sequence data obtained were combined with the ChromasPro 1.7.5 program and using the global alignment algorithms available in Ezbiocloud Server (Chalita et al., 2024; see https://eztaxon-e.ezbiocloud.net/). 16S rRNA nucleotide similarity with the most closely related organisms was determined. MEGA 7 program was used for phylogenetic analysis, and the CLUSTAL_W (Kumar et al., 2016) option of the same program was used for alignment. Neighbor Joining (Saitou & Nei, 1987) method and Kimura-2 (Kimura, 1980) phylogenetic distance matrix were used to draw phylogenetic dendrograms. Bootstrap analyzes of the created phylogenetic trees (Felsenstein, 1985) were performed with 1000 replicates.

Evaluation of Antimicrobial Activities

The isolates were examined for their ability to inhibit the growth of eight pathogenic organisms, including three Gram-positive and three Gram-negative bacteria and two fungi (Williams et al., 1983) (Table 4). The inoculation of the isolates into the culture medium was carried out by the point inoculation method using an automatic pipette. For each isolate, 1.5 mL of Ringer's solution was added to screw-capped bottles and sterilised by autoclaving at 121°C for 15 minutes. The spore and substrate mycelia of the isolates grown on glucose yeast extract-malt extract agar medium at 28°C were transferred to small glass vials containing a Ringer solution under aseptic conditions with the help of a sterile loop.

Subsequently, 7 µL of the bacterial solutions, which were homogenised by mixing with a vortex, were taken with an automatic pipette and inoculated by the point inoculation method in groups of 5 onto the modified Bennett's agar surface (Jones, 1949) without the addition of antibiotics. The inoculated plates were incubated at 28°C for 72 hours. At the end of the incubation period, 3-5 mL of chloroform was poured onto the developing colonies with the help of a sterile syringe. The lid of the petri plates was kept half-open for 45 minutes to allow the chloroform to evaporate. The pathogen test organisms, each of which had been cultivated in 5% nutrient agar for 2 days, were inoculated onto the colonies that had been killed in this way by the spread plate method. The inhibition zones formed around the inoculated plates after 48 hours of incubation at 37°C were measured.

Table 4. Pathogenic organisms selected for antimicrobial activity testing

Number	Pathogenic Organisms
1	*Bacillus subtilis* ATCC 6633
2	*Staphylococcus aureus* ATCC 25923
3	*Enterococcus faecalis* ATCC 29212
4	*Escherichia coli* ATCC 25922
5	*Klebsiella pneumoniae* ATCC 70060
6	*Pseudomonas aeruginosa* ATCC 27853
7	*Aspergillus niger* ATCC 16404
8	*Candida albicans* ATCC 1023

Examination of Lipase Producing Abilities

Lipase activity was determined according to the ability of the isolates to hydrolyze Tween 20. For this purpose, 5 g peptone (Merck, Germany), 2.5 g NaCl (Merck, Germany), 0.05 g $CaCl_2 \times 2H_2O$ (Merck, Germany), 10 g agar and 5 mL Tween 20 (Merck, Germany) in 500 mL distilled water. Then, a single colony was taken from the pure colonies of the isolates in the GYM and they were inoculated in the medium in a spot manner. The media were incubated at 28°C for 5 days. The formation of a zone with a frosted glass appearance around the planting line after incubation was considered positive (Kumar et al., 2012).

Examination of Amylase Producing Abilities

The amylase activity of the isolates was determined by adding 1% lugol to the petri dishes after 7 days of incubation in starch agar medium (1% starch, 0.3% NaCl, 0.1% KH_2PO_4, 2% agar). Lugol dyes the medium in a dark color and creates a transparent zone in the area where amylase production occurs. The presence of activity was determined by measuring the transparent zone formed around the actinobacteria (Fossi et al., 2009).

Examination of Protease Production Abilities

The protease production abilities of the isolates were determined by measuring the transparent zone formed around the actinobacteria after 7 days of incubation in a medium containing 1% casein, 0.5% yeast extract, and 2% agar (Mohamedin, 1999).

Examination of Pectinase Producing Abilities

Pectinase production abilities of the isolates were determined by measuring the transparent zone around the actinobacteria colonies at the end of 7 days of incubation in the medium containing 1% pectin, 0.3% $(NH_4)_2HPO_4$, 0.2% KH_2PO_4, 0.3% K_2HPO_4, 0.01% $MgSO_4$, 2% agar (Raju & Divakar, 2013).

Results and Discussion

Soil samples were taken from three different localities of Çarpanak Island by measuring their coordinates with the eXplorist 100. Dried soil samples were inoculated into four different alkaline selective isolation media using the dilution plate method.

The pH of the soil samples was measured from the saturation mud prepared with the help of a glass electrode pH meter. pH values of soil samples were determined using a 1:2.5 soil:water solution (Sağlam, 1978). For this, 10 grams of soil 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. Three repeated measurements were then made. The average of three measurements is given in Table 5.

Table 5. pH values of soil samples

Locality	Soil pH
From the part of the island close to Lake Van	6.93
From the part of the island far from Lake Van	7.20
One of the regions of the island with dense plant flora	6.88

Soil samples brought to the laboratory were placed in petri dishes and left to dry at room temperature until studied. The dilution plate technique was used to isolate *Streptomyces*.

Actinomycete strains were selected from the isolation plates that were incubated in selective isolation media for 21-30 days, taking into account their colony morphologies. The selected isolates were numbered and pure cultures were made and transferred to autoclaved screw cap tubes containing 25% glycerol with the help of sterile toothpicks and stored at -20°C. 42 isolates were selected and stocked according to colony morphology. Four different media were used as selective isolation media in the study. The highest number of isolates were isolated from R2A medium. 17 isolates were isolated from R2A agar, 14 isolates from starch casein agar, 6 isolates from humic acid-vitamin agar, and 5 isolates from SM3 agar. R2A medium was found to be more efficient than other media we used in isolation from tested soils. Meklat et al. (2020) performed isolation of Alkalitolerant actinobacteria from algerian saharan soils. 29 actinobacterial strains were selectively isolated by the dilution plate method on Complex Medium agar (Chun et al., 2000). Arayes et al. (2022) isolated a haloalkalitolerant *Streptomyces* from Um-Risha Lake in Egypt. Isolation of *Streptomyces* was performed by the spread plate technique using starch casein agar medium (SCA) (Küster & Williams, 1964).

The graph showing the distribution of isolates obtained from the selective isolation media used is given in Figure 1.

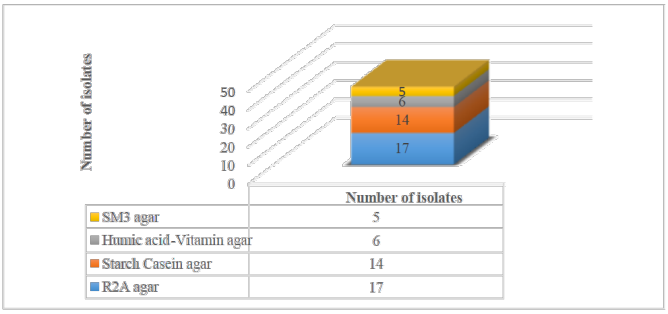

Figure 1. Graph showing the distribution of isolates obtained from the selective isolation media used

Isolation and subculture of some isolates were performed and petri dishes are shown in Figure 2. When the locality distributions of the isolates obtained are examined, it is seen that 18 isolates were obtained from the 2nd locality, 16 isolates from the 3rd locality and 8 isolates from the 1st locality. The distribution of isolates according to localities is given in Figure 3.

Studies continued with 13 isolates selected based on colony morphology differences among the 42 isolates obtained. Glucose yeast extract-malt extract agar was used in the study. The pH values of the agar (4, 5, 6, 7, 8, 9, 10, 11, 12, respectively) were adjusted with 0.1 M NaOH and 0.1 M HCl buffers. The development of 13 spot cultured isolates at each pH value was evaluated. The results are given in Table 6. When the results were evaluated, isolates coded CA43, CA46, CA62, CA68 and CA75 showed growth between pH 4 and 10, while CA59 and CA94 showed growth between pH 4 and 11. The remaining isolates grew between pH 4 and 12. When the pH results were evaluated, it was concluded that the isolates were alkalitolerant, not alkaliphilic.

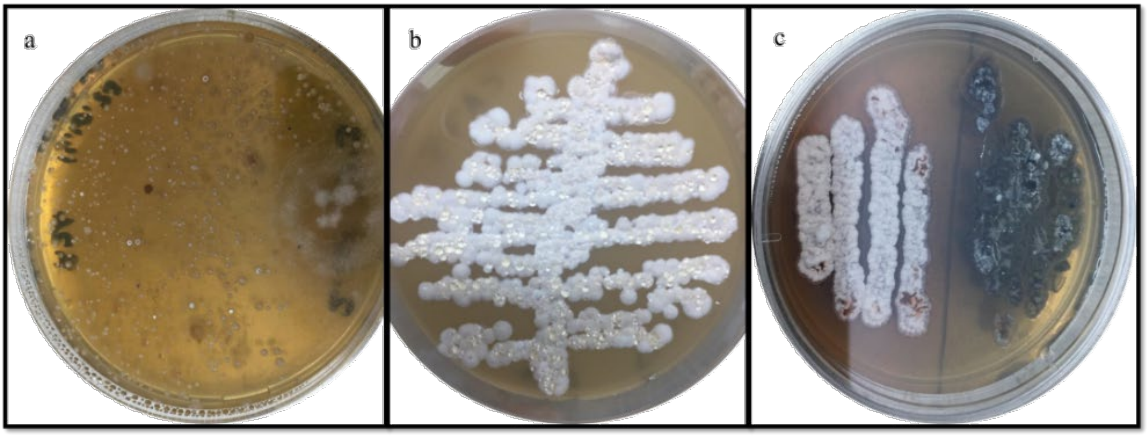

Figure 2. a) Isolation of 1st soil sample obtained from Çarpanak Island (R2A Agar). **b)** Growth of isolate CA46 in ISP2 medium. **c)** Growth of isolates CA94 (left side) and CA40 (right side) in ISP2 medium

Table 6. Development of isolates at different pH values

No	Organism	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11	pH 12
1	CA36	+	+	+	+	+	+	+	+	+
2	CA40	+	+	+	+	+	+	+	+	+
3	CA43	+	+	+	+	+	+	+	-	-
4	CA49	+	+	+	+	+	+	+	-	-
5	CA56	+	+	+	+	+	+	+	+	+
6	CA59	+	+	+	+	+	+	+	+	-
7	CA61	+	+	+	+	+	+	+	+	+
8	CA62	+	+	+	+	+	+	+	-	-
9	CA68	+	+	+	+	+	+	+	-	-
10	CA75	+	+	+	+	+	+	+	-	-
11	CA76	+	+	+	+	+	+	+	+	+
12	CA77	+	+	+	+	+	+	+	+	+
13	CA94	+	+	+	+	+	+	+	+	-

Note: "+" indicate growth; "–" indicate no growth.

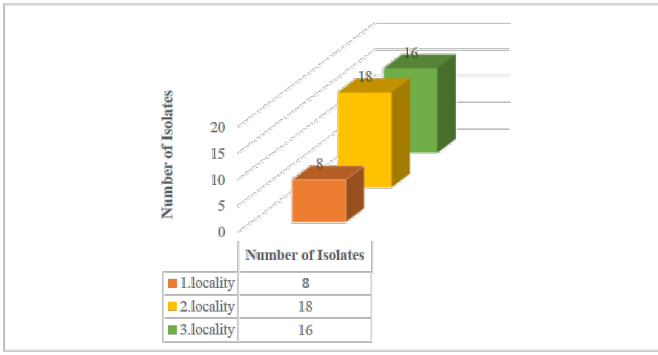

Figure 3. Graph showing the distribution of isolates according to localities

Genomics of 13 isolates, selected according to their colony morphology differences among the 42 isolates obtained, were performed with the Invitrogen PureLink Genomic DNA Mini Kit. The 16S rRNA gene region of 13 isolates, whose genomic DNA was isolated, was amplified in Gradient PCR with 27F and 1525R universal primers. The DNA region, amplified after PCR, with an average size of 1500 base pairs, was visualized on agarose gel electrophoresis on the Mini Lumi imaging device.

16S rRNA gene sequence analysis was performed with 3 primers, 518F, MG5F and 800R, of the 13 isolates for which 16S rRNA gene region PCR amplifications were performed. After the 16S rRNA gene sequence analysis of the isolates was completed, the sequence data obtained were combined with the ChromasPro 1.7.5 program and the sequence data of the most closely related species in international databases were used using Ezbiocloud Server (Chalita et al., 2024; see https://eztaxon-e.ezbiocloud.net/) and their % similarity was determined. According to the sequence analysis results, all 13 isolates were determined to belong to the *Streptomyces* genus (Table 7). The obtained 16S rRNA gene nucleotide sequences have been deposited in GenBank and their accession numbers are given in Table 7.

Table 7. Phylogenetic similarity of test organisms of the *Streptomyces* genus with their closest type species according to 16S rRNA sequence results

No	Strain Code	GenBank No	Closest Type	% Similarity- Nucleotide Difference
1.	**CA36**	PP352073	*Streptomyces exfoliates* NRRL B-2924^T	100% - 0/1447
2.	**CA40**	PP352075	*Streptomyces rectiviolaceus* NRRL B-16374^T	99.31% - 10/1448
3.	**CA43**	PP352171	*Streptomyces sanglieri* NBRC 100784^T	98.96% - 15/1448
4.	**CA49**	PP352271	*Streptomyces himalayensis subsp. Himalayensis* PSKA28^T	95.76% - 60/1414
5.	**CA56**	PP352628	*Streptomyces ambofaciens* ATCC 23877^T	99.45% - 8/1449
6.	**CA59**	PP352629	*Streptomyces seymenliensis* B1041^T	99.10% - 13/1449
7.	**CA61**	PP352630	*Streptomyces aureoverticillatus* NRRL B-3326^T	99.45% - 8/1448
8.	**CA62**	PP352631	*Streptomyces ureilyticus* YC419^T	99.59% - 6/1448
9.	**CA68**	PP352689	*Streptomyces angustmyceticus* NRRL B-2347^T	99.86% - 2/1451
10.	**CA75**	PP352913	*Streptomyces arenae* ISP 5293^T	97.53% -35/1417
11.	**CA76**	PP352914	*Streptomyces ambofaciens* ATCC 23877^T	99.72% - 4/1448
12.	**CA77**	PP353666	*Streptomyces rosealbus* YIM 31634^T	99.78% - 3/1388
13.	**CA94**	PP353683	*Streptomyces violarus* NBRC 13104^T	98.76% - 18/1446

According to 16S rRNA gene region nucleotide sequence analysis, 13 isolates belonging to the *Streptomyces* genus show 95.76% to 100% similarity to their closest type species. CA36 isolate was 100% with its closest type species, *Streptomyces exfoliates* NRRL B-2924^T, CA68 isolate was 99.86% with its closest type species, *Streptomyces angustmyceticus* NRRL B-2347^T, CA76 isolate was 99.72% with its closest type species, *Streptomyces ambofaciens* ATCC 23877^T, CA77 isolate was 99.78% with *Streptomyces rosealbus* YIM 31634^T, the closest type species to the isolate, CA62 isolate was 99.59% to the closest type species, *Streptomyces ureilyticus* YC419^T, CA56 isolate was 99.45% to the closest type species, *Streptomyces ambofaciens* ATCC 23877^T, CA61 isolate was 99.45% with *Streptomyces aureoverticillatus* NRRL B-3326^T has similarity, and CA40 isolate has 99.31% similarity with its closest type, *Streptomyces rectiviolaceus* NRRL B-16374^T. CA59 isolate was 99.10% with its closest type species, *Streptomyces seymenliensis* B1041^T, CA43 isolate was 98.96% with its closest type species, *Streptomyces sanglieri* NBRC 100784^T, CA94 isolate was 98.76% with its closest type species, *Streptomyces violarus* NBRC 13104^T, and CA75 isolate was 97.53% with *Streptomyces arenae* ISP 5293^T, and CA49 isolate was 95.76% with the closest type species, *Streptomyces himalayensis* subsp. *himalayensis* PSKA28^T has similarity.

For the identification of new species, the 16S rRNA gene similarity value has been recommended to be below 97% (Stackebrandt & Goebel, 1994), but recently this value has been increased to the range of 98.7-99% (Stackebrandt & Ebers, 2006).

The neighbor-joining (Saitou & Nei, 1987) method was used to draw the phylogenetic dendrogram containing 13 isolates of the *Streptomyces* genus. Additionally, bootstrap analyses of the phylogenetic tree created using the Kimura-2 phylogenetic distance matrix (Felsenstein, 1985) were performed with 1000 replicates (Figure 4).

A total of 13 isolates were examined for their ability to inhibit the growth of eight pathogenic organisms, including three Gram-positive and three Gram-negative bacteria and two fungi. The *Streptomyces* sp. CA61 and *Streptomyces* sp. CA94 isolates demonstrated the most promising antimicrobial activity against five different pathogens.

The isolate coded CA36 showed antimicrobial activity against four different pathogenic microorganisms. While isolates coded CA56, CA68 and CA76 showed antimicrobial activity against three different pathogenic microorganisms, isolates coded CA62 and CA75 showed antimicrobial activity against two pathogenic microorganisms. While the isolate coded CA40 showed antimicrobial activity against a single pathogenic microorganism, isolates coded CA43, CA49, CA59 and CA77 did not show antimicrobial activity against any of the eight pathogens used in the study. Additionally, it was determined that none of the isolates tested showed activity against the fungi. The petri dish image of the zone diameters of the antimicrobial activity of some isolates against pathogenic microorganisms is given in Figure 5 and Table 8.

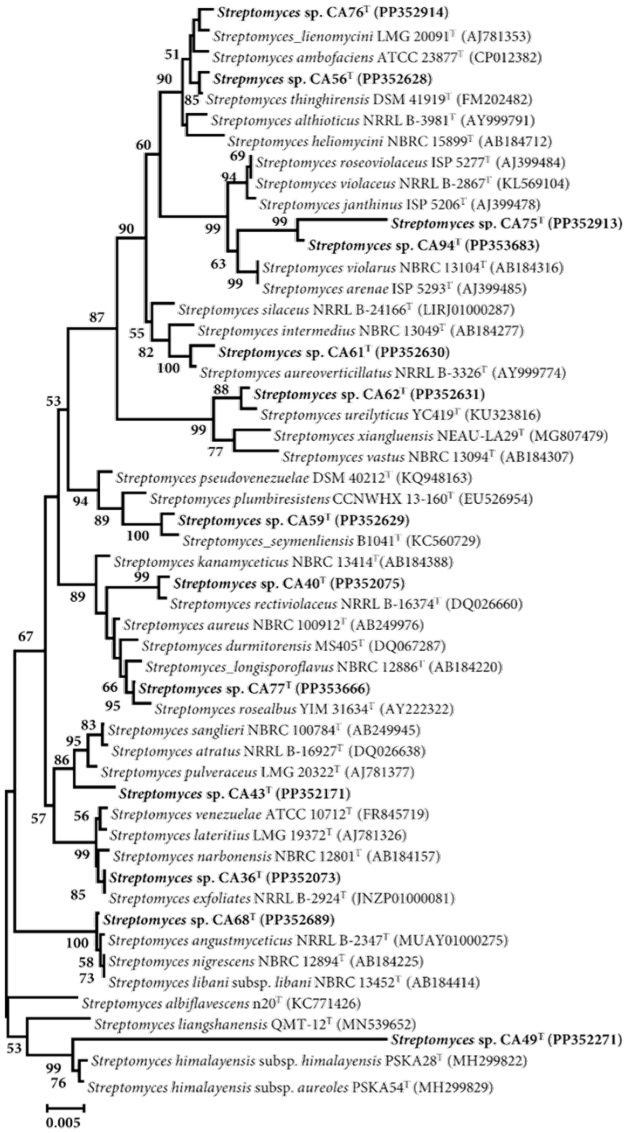

Figure 4 Phylogenetic dendrogram of isolates of the Streptomyces genus based on 16S rRNA gene sequence analysis. In the tree drawn according to the neighbor-joining algorithm, bootstrap values were given for branch points that were supported over 50%. Bar, 0.005

Tween 20 agar was used to test the lipase-producing abilities of the isolates. The zones formed around the isolates, which were sown as dots on petri dishes, as a result of 5-day incubation, were evaluated. When their lipase production abilities were examined, 11 isolates, except CA43 and CA62, had the ability to produce lipase.

The most researched and most efficient sources of lipases are animal, plant and microbial (bacterial, fungal and yeast) sources. However, one bacterial phylum that shows good potential for lipase production is actinobacteria (Mansour et al., 2015; Arumugam et al., 2017; Hamedi et al., 2019; Sharma & Thakur, 2020). Among the lipase-producing actinobacteria, the most studied is the *Streptomyces* genus (Nithya et al., 2018; Panyachanakul et al., 2020).

The amylase activity of the isolates was evaluated on starch agar medium. Spot-seeded isolates were incubated for seven days. After incubation, 1% lugol was added to the petri dishes. The formation of a transparent zone in the area of amylase production was evaluated. While two isolates did not produce amylase, 11 isolates produced amylase.

Studies on amylases are largely of fungal origin, but actinobacteria have also been shown to be good amylase producers. Soil has been an excellent source for obtaining such bacteria. For example, Thampi & Bhai (2017) isolated three species of amylase-producing actinobacteria from rhizosphere soil samples of black pepper plants.

The protease production abilities of the isolates were evaluated by measuring the transparent zones formed around the isolates. While three isolates did not produce protease, ten isolates were determined to produce protease.

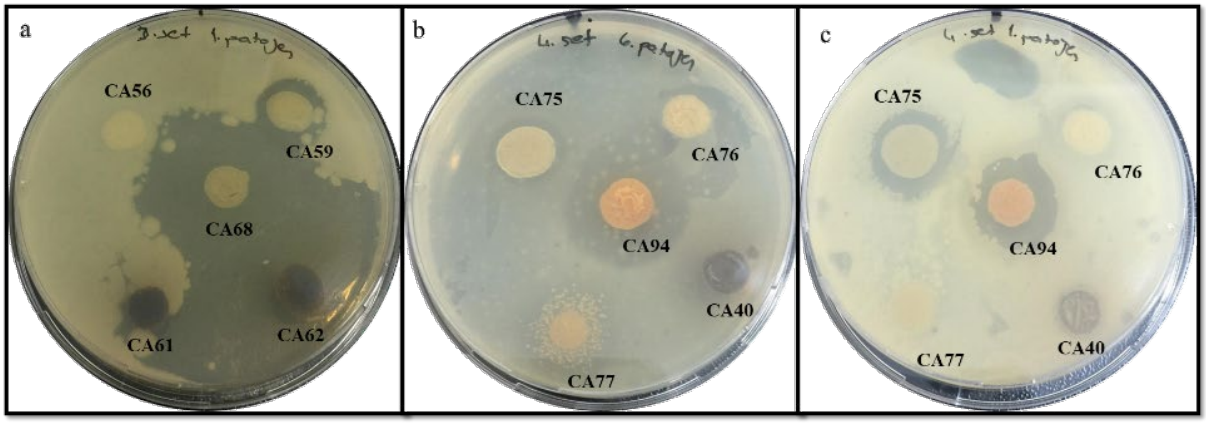

Figure 5. Antimicrobial activity test. **a)** *Bacillus subtilis* ATCC 6633^T-Group 1. **b)** *Pseudomonas aeruginosa* ATCC 27853^T. **c)** *Bacillus subtilis* ATCC 6633^T-Group 2

Table 8. Zone diameters (mm) of the antimicrobial activity of the isolates against pathogenic microorganisms

No	Organism	*B. subtilis*	*S. aureus*	*E. faecalis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*A. niger*	*C. albicans*
1	**CA36**	30	20	-	14	20	-	-	-
2	**CA40**	-	-	-	-	30	-	-	-
3	**CA43**	-	-	-	-	-	-	-	-
4	**CA49**	-	-	-	-	-	-	-	-
5	**CA56**	14	14	-	-	50	-	-	-
6	**CA59**	-	-	-	-	-	-	-	-
7	**CA61**	54	54	40	20	40	-	-	-
8	**CA62**	-	10	-	-	40	-	-	-
9	**CA68**	44	30	-	-	30	-	-	-
10	**CA75**	14	-	-	-	-	20	-	-
11	**CA76**	20	-	16	10	-	14	-	-
12	**CA77**	-	-	-	-	-	-	-	-
13	**CA94**	20	20	16	-	20	26	-	-

Microbial production is an ideal alternative to meet industrial needs. Within the microbiota, the *Bacillus* genus is mainly used for enzyme production. In addition, another new microbial source studied is Actinobacteria members, which are widely used in the production of bioactive compounds such as cellulase and amylase. These are currently under investigation because extremophilic actinobacteria are known to produce thermostable proteases and mesophilic actinobacteria such as *Streptomyces* are known to produce pronase 7 M (*S. griseus*) and fradiase (*S. fradiae*), which are in commercial use (McCarthy et al., 1985; Flores-Gallegos & Nava-Reyna, 2019).

The pectinase production abilities of the isolates were evaluated by measuring the transparent zones formed around the isolates. In the study, it was observed that none of the isolates produced pectinase. Table 9 presents the lipase, amylase, protease and pectinase production abilities of the isolates. The commercial applications of this enzyme include juice purification, vegetable purification, wastewater treatment, degumming, defoaming agent, and fermentation accelerator in the tea and coffee industry. Additionally, it is used as an analytical tool for plant product evaluation (Beulah et al., 2015; Oumer & Abate, 2018).

An alternative and powerful source of pectinase has been identified in actinomycetes, which are known for their production of bioactive compounds and are used for the production of antibiotics (Kumar & Sharma, 2012). In recent studies, it has been reported that *Streptomyces thermocarboxydus* produces thermostable pectinases, which are active at high temperatures and are gaining increasing importance. At elevated temperatures, the process is accelerated, which contributes to economic gain (Priyanka, 2019).

Table 9. Abilities of isolates to produce lipase, amylase, protease and pectinase

No	Organism	Lipase	Amylase	Protease	Pectinase
1	**CA36**	+++	+	+	-
2	**CA40**	+++	+	-	-
3	**CA43**	-	+	+	-
4	**CA49**	+++	+	+	-
5	**CA56**	+++	+	+	-
6	**CA59**	+++	-	+	-
7	**CA61**	+++	+	-	-
8	**CA62**	-	+	+	-
9	**CA68**	+++	+	+	-
10	**CA75**	+++	+	+	-
11	**CA76**	+++	+	+	-
12	**CA77**	+++	+	+	-
13	**CA94**	+++	-	-	-

Note: Symbols: (-) No growth; (+) Little growth; (++) Moderate growth; (+++) Excellent growth.

Conclusion

According to 16S rRNA gene region nucleotide sequence analysis, *Streptomyces* spp. isolates CA49, CA75, CA94 and CA43 show similarities between 95.76% and 98.96% to their closest type species. All genotypic, chemotaxonomic and phenotypic analyzes of these four isolates, which are likely to be new species, will be completed and introduced to the literature in the near future. Members of *Streptomyces* are the most

common microorganisms that have the function of breaking down organic compounds through enzyme activities. Therefore, industrially and agriculturally important enzymes detailed characterization of the industrially important enzymes produced by these isolates to be introduced into the literature is required.

Compliance With Ethical Standards

Authors' Contributions

AV: Conceptualization, Writing – original draft, Investigation, Formal analysis
DT: Writing – original draft, Investigation, Formal analysis
HAD: Investigation, Formal analysis
AT: Investigation, Formal analysis
All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical 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.

Funding

This research was supported by Sinop University, project no SHMYO-1901-23-001.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Arayes, M. A., Nawar, E. A., Sabry, S. A., & Mabrouk, M. E. (2022). Bioactive compounds from a haloalkalitolerant *Streptomyces* sp. EMSM31 isolated from Um-Risha Lake in Egypt. *Egyptian Journal of Aquatic Biology and Fisheries*, *26*(2), 307-330. https://doi.org/10.21608/ejabf.2022.229723

Arumugam, T., Kumar, P. S., Kameshwar, R., & Prapanchana, K. (2017) Screening of novel actinobacteria and characterization of the potential isolates from mangrove sediment of south coastal India. *Microbial Pathogenesis*, *107*, 225-233. https://doi.org/10.1016/j.micpath.2017.03.035

Baldacci, E. (1944). Contributo alla systematica degli actenomyceti: X-XVI *Actinomyces madurae*; *Proactinomyces ruber*; *Proactinomyces pseudomadurae*; *Proactinomyces polychromogenus*; *Proactinomyces violaceus*; *Actinomyces coeruleus*; cjn un elencj alfabetico delle specie e delle varieta finora studiate. *Atti dell'Istituto Botanico della Università e Laboratorio Crittogamico di Pavia*, *3*, 139-193.

Baranasic, D., Gacesa, R., Starcevic, A., Zucko, J., Blazic, M., Horvat, M., Gjuracic, K., Fujs, S., Hranueli, D., Kosec, G., Cullum, J., & Petkovic, H. (2013). Draft genome sequence of *Streptomyces rapamycinicus* Strain NRRL 5491, the producer of the immunosuppressant rapamycin. *Genome Announc*, *1*, 1-2. https://doi.org/10.1128/genomeA.00581-13

Bentley, S. D., Maiwald, M., Murphy, L. D., Pallen, M. J., Yeats, C. A., Dover, L. G., Norbertczak, H. T., Besra, G. S., Quail, M. A., Harris, D. E., von Herbay, A., Goble, A., Rutter, S., Squares, R., Squares, S., Barrell, B. G., Parkhill, J., & Relman, D. A. (2003). Sequencing and analysis of the genome of the Whipple's disease bacterium *Tropheryma whipplei*. *The Lancet*, *361*(9358), 637-644. https://doi.org/10.1016/S0140-6736(03)12597-4

Beulah, D., Sunitha, E. M., Srilakshmi, T., Degree, P. G., Venkat, R. B., & Reddy, R. (2015). Production, purification and assay of pectinase enzyme from *Aspergillus niger*. *Helix, 2,* 673-677.

Buchholz-Cleven, B. E. E., Rattunde, B., & Straub, K. L. (1997). Screening for genetic diversity of isolates of anaerobic Fe(II)-oxidizing bacteria using DGGE and whole-cell hybridization. *Systematic and Applied Microbiology*, *20,* 301-309. https://doi.org/10.1016/S0723-2020(97)80077-X

Bull, A. T. (2010). Actinobacteria of the extremobiosphere. In Horikoshi, K., Antranikian, G., Bull, A. T., Robb, F., & Stelter, K. (Eds.), *Extremophiles handbook* (pp. 1203-1240). Springer-Verlag GmbH.

Chalita, M., Kim, Y. O., Park, S., Oh, H. S., Cho, J. H., Moon, J., Baek, N., Moon, C., Lee, K., Yang, J., Nam, G. G., Jung, Y., Na, S. I., Bailey, M. J., & Chun, J. (2024). EzBioCloud: A genome-driven database and platform for microbiome identification and discovery. *International Journal of Systematic and Evolutionary Microbiology, 74*(6), 006421. https://doi.org/10.1099/ijsem.0.006421

Chanama, M., Suriyachadkun, C., & Chanama, S. (2023). *Streptomyces antimicrobicus* sp. nov., a novel clay soil-derived actinobacterium producing antimicrobials against drug-resistant bacteria. *PLoS ONE, 18*(5), e0286365. https://doi.org/10.1371/journal.pone.0286365

Chen, M., Xu, P., Zeng, G., Yang, C., Huang, D., & Zhang, J. (2015). Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: Applications, microbes and future research needs. *Biotechnology Advances, 33*(6, Part 1), 745-755. https://doi.org/10.1016/j.biotechadv.2015.05.003

Chun, J. (1995). *Computer assisted classification and identification of actinomycetes.* [Ph.D. Thesis. University of Newcastle].

Chun, J., Bae, K. S, Moon, E. Y., Jung, S. O., Lee, H. K., & Kim, S. J. (2000). *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. *International Journal of Systematic and Evolutionary Microbiology, 50*(5), 1909-1913. https://doi.org/10.1099/00207713-50-5-1909

Cockell, C. S., Kelly, L. C., & Marteinsson, V. (2013). Actinobacteria- An ancient phylum active in volcanic rock weathering. *Geomicrobiology Journal, 30*(8), 706-720. https://doi.org/10.1080/01490451.2012.758196

Cukur, D., Krastel, S., Schmincke, H. U., Sumita, M., Çağatay, M. N., Meydan, A. F., Damcı, E., & Stockhecke, M. (2014). Seismic stratigraphy of Lake Van, eastern Turkey. *Quaternary Science Reviews, 104,* 63-84. https://doi.org/10.1016/j.quascirev.2014.07.016

Das, P., Kundu, S., Maiti, P. K., Mandal, S., Sahoo, P., & Mandal, S. (2022). An antibacterial compound pyrimidomycin produced by *Streptomyces* sp. PSAA01 isolated from soil of Eastern Himalayan foothill. *Scientific Reports, 12,* 10176. https://doi.org/10.1038/s41598-022-14549-4

Dhakal, D., Pokhrel, A. R., Shrestha, B., & Sohng, J. K. (2017). Marine rare actinobacteria: Isolation, characterization, and strategies for harnessing bioactive compounds. *Frontiers in Microbiology, 8,* 1106. https://doi.org/10.3389/fmicb.2017.01106

Ensign, J. C. (1992). Introduction to the Actinomycetes. In A. Balows, H. G. Truper, M. Dworkin, W. Hardeer & K. H. Schleifer (Eds.), *The Prokaryotes 2nd Edition. Vol. II*, (pp. 811-815). Springer-Verlag.

Felsenstein, J. (1985). Confidence limits on phylogeny: An appropriate use of the bootstrap. *Evolution, 39*, 783-791. https://doi.org/10.2307/2408678

Flores-Gallegos, A. C., & Nava-Reyna, E. (2019). Chapter 30 - Plant growth-promoting microbial enzymes. In Kuddus, M. (Ed.), *Enzymes in food biotechnology: Production, applications, and future prospects* (pp. 521-534). Academic Press. https://doi.org/10.1016/B978-0-12-813280-7.00030-X

Fossi, B. T., Tavea, F., Jiwoua, C., & Ndjouenke, R. (2009). Screening and phenotypic characterization of thermostable amylases producing yeasts and bacteria strains from some Cameroonian soils. *African Journal of Microbiology Research, 3*(9), 504-514. https://doi.org/10.5897/AJMR.9000640

Gardner, H. L., & Dukes, C. D. (1955). *Haemophilus vaginalis vaginitis*. A newly defined specific infection previously classified "non-specific" vaginitis. *American Journal of Obstetrics and Gynecology, 69*, 962-976. https://doi.org/10.1016/0002-9378(55)90095-8

Growth, I., Schumann, P., Rainey, F. A., Martin, K., Schuetze, B., & Augsten, K. (1997). *Bogoriella caseilytica* gen. nov., sp. nov., a new alkaliphilic actinomycete from a soda lake in Africa. *International Journal of Systematic Bacteriology, 47*, 788-794. https://doi.org/10.1099/00207713-47-3-788

Gu, C. Z., Yuan, S. H., Lu, J., Qiao, Y. J., Song, Elzaki, M. E. A., Yang, C.-R., Zhang, Y.-Z., & Zeng, R.-S. (2019). Albocyline-type macrolides with antibacterial activities from *Streptomyces* sp. 4205. *Chemistry & Biodiversity, 16*(1), e18000344. https://doi.org/10.1002/cbdv.201800344

Hamedi, J., Kafshnouchi, M., & Ranjbaran, M. (2019). A study on actinobacterial diversity of Hampoeil cave and screening of their biological activities. *Saudi Journal of Biological Sciences, 26*(7), 1587-1595. https://doi.org/10.1016/j.sjbs.2018.10.010

Hayakawa, M., & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *Journal of Fermentation Technology, 65*(5), 501-509. https://doi.org/10.1016/0385-6380(87)90108-7

Horikoshi, K. (2008). Alkaliphiles. In eLS (Ed.). https://doi.org/10.1002/9780470015902.a0000337.pub2

Hu, H., Lin, H. P., Xie, Q., Li, L., Xie, X. Q., & Hong, K. (2012). *Streptomyces qinglanensis* sp. nov., isolated from mangrove sediment. *International Journal of Systematic and Evolutionary Microbiology*, *62*(Pt_3), 596–600. https://doi.org/10.1099/ijs.0.032201-0

Jiang, C., & Xu, L. (1993). Actinomycete diversity in unusual habitats. *Actinomycetes, 4,* 47-57.

Johnson, D. B., Bacelar-Nicolau, P., Okibe, N., Thomas, A., & Hallberg, K. B. (2009). *Ferrimicrobium acidiphilum* gen. nov., and *Ferrithrix thermotolerans* gen. nov., sp. nov.: Heterotrophic, iron-oxidizing, extremely acidophilic actinobacteria. *International Journal of Systematic and Evolutionary Microbiology*, *59*(5), 1082-1089. https://doi.org/10.1099/ijs.0.65409-0

Jones, B. E., Grant, W. D., Duckworth, A. W., & Owenson, G. G. (1998). Microbial diversity of soda lakes. *Extremophiles, 2,* 191-200. https://doi.org/10.1007/s007920050060

Jones, K. L. (1949). Fresh Isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *Journal of Bacteriology*, *57,* 141-145. https://doi.org/10.1128/jb.57.2.141-145.1949

Kanekar, P. P., Nilegaonkar, S. S., Sarnaik, S. S., & Kelkar, A. S. (2002). Optimization of protease activity of alkaliphilic bacteria isolated from an alkaline lake in India. *Bioresource Technology, 85*(1), 87-93. https://doi.org/10.1016/s0960-8524(02)00018-4

Kim, S, B., Lonsdale, J., Seong, C. N., & Goodfellow, M. (2003). *Streptoacidiphilus* gen. nov., acidophilic actinomycetes with chemotype I and emendation of the family *Streptomycetaceae* (Waksman and Henrici (1943)^{AL}) emend. Rainey et al. 1997. *Antonie Van Leeuwenhoek, 83,* 107–116. https://doi.org/10.1023/a:1023397724023

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111-120. https://doi.org/10.1007/BF01731581

Kumar, A., & Sharma, R. (2012). Production of alkaline pectinase by bacteria (*Cocci* sps.) isolated from decomposing fruit materials. *Journal of Phytology, 4,* 1-5.

Kumar, R., Sharma, A., Kumar, A., & Singh, D. (2012). Lipase from *Bacillus pumilus* RK31: Production, purification and some properties. *World Applied Sciences Journal, 16*(7), 940-948.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution, 33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Kurapova, I., Zenova, G. M., Sudnitsyn, II., Kizilova, A. K., Manucharova, N. A., Norovsuren, Z. H., & Zvyagintsev, D. G. (2012). Thermotolerant and thermophilic actinomycetes from soils of Mongolia Desert Steppe Zone. *Microbiology, 81,* 98-108. https://doi.org/10.1134/S0026261712010092

Küster, E., & Williams, S. (1964). Selection of media for isolation of *Streptomycetes*. *Nature*, *202*, 928-929. https://doi.org/10.1038/202928a0

Law, J. W. F., Ser, H. L., Ab Mutalib, N, S., Saokaew, S., Duangjai, A., Khan, T. M., Chan, K.-G., Goh, B.-H., & Lee, L.-H. (2019). *Streptomyces monashensis* sp. nov., a novel mangrove soil actinobacterium from East Malaysia with antioxidative potential. *Scientific Reports*, *9,* 3056. https://doi.org/10.1038/s41598-019-39592-6

Li, L. Y., Yang, Z. W., Asem, M. D., Fang, B. Z., Salam, N., Alkhalifah, D. H. M., Hozzein, W. N., Nie, G.-X., & Li, W.-J. (2019). *Streptomyces desertarenae* sp. nov., a novel actinobacterium isolated from a desert sample. *Antonie Van Leeuwenhoek, 112,* 367–374. https://doi.org/10.1007/s10482-018-1163-0

Li, W. J., Zhang, Y. Q., Schumann, P., Chen, H. H., Hozzein, W. N., Tian, X. P., Xu, L. H., & Jiang, C. L. (2006). *Kocuria aegyptia* sp. nov., a novel actinobacteria isolated from a saline, alkaline desert soil in Egypt. *International Journal of Systematic and Evolutionary Microbiology, 56*(4), 733-737. https://doi.org/10.1099/ijs.0.63876-0

Mancuso, G., Midiri, A., Gerace, E., & Biondo, C. (2021). Bacterial antibiotic resistance: The most critical pathogens. *Pathogens, 10,* 1310. https://doi.org/10.3390/pathogens10101310

Mansour, S. R., Abdel-Azeem, A. M., & Abo-Deraz, S. S. S. (2015). A new record of Actinobacteria isolated from soil in Jerusalem and their enzymatic potential. *F1000Research, 4,* 11. https://doi.org/10.12688/f1000research.3257.1

McCarthy, A. J., Peace, E., & Broda, P. (1985). Studies on the extracellular xylanase activity of some thermophilic actinomycetes. *Applied Microbiology and Biotechnology, 21,* 238-244. https://doi.org/10.1007/BF00295129

Meklat, A., Bouras, N., Mokrane, S., Zitouni, A., Djemouai, N., Klenk, H. P., Sabaou, N., & Mathieu, F. (2020). Isolation, classification and antagonistic properties of alkalitolerant actinobacteria from Algerian Saharan soils. *Geomicrobiology Journal, 37*(9), 826-836. https://doi.org/10.1080/01490451.2020.1786865

Mohamedin, A. H. (1999). Isolation, identification and some cultural conditions of a protease-producing thermophilic *Streptomyces* strain grown on chicken feather as a substrate. *International Biodeterioration & Biodegradation, 43*(1-2), 13-21. https://doi.org/10.1016/S0964-8305(98)00061-4

Nithya, K., Muthukumar, C., Biswas, B., Alharbi, N. S., Kadaikunnan, S., Khaled, J. M., & Dhanasearan, D. (2018). Desert actinobacteria as a source of bioactive compounds production with a special emphases on Pyridine-2,5-diacetamide a new pyridine alkaloid produced by *Streptomyces* sp. DA3-7. *Microbiological Research, 207*, 116-133. https://doi.org/10.1016/j.micres.2017.11.012

Oumer, O. J., & Abate, D. (2018). Screening and molecular identification of pectinase producing microbes from coffee pulp. *BioMed Research International. 2018*, 2961767. https://doi.org/10.1155/2018/2961767

Palaniyandi, S. A., Yang, S. H., Zhang, L., & Suh, J. W. (2013). Effects of actinobacteria on plant disease suppression and growth promotion. *Applied Microbiology and Biotechnology, 97,* 9621-9636. https://doi.org/10.1007/s00253-013-5206-1

Panyachanakul, T., Lomthong, T., Lorliam, W., Prajanbarn, J., Tokuyama, S., Kitpreechavanich, V., & Krajangsang, S. (2020). New insight into thermo-solvent tolerant lipase produced by *Streptomyces* sp. A3301 for re-polymerization of poly ($_{DL}$-lactic acid). *Polymer, 204*, 122812. https://doi.org/10.1016/j.polymer.2020.122812

Pati, A., Sikorski, J., Nolan, M., Lapidus, A., Copeland, A., Glavina Del Rio, T., Lucas, S., Chen, F., Tice, H., Pitluck, S., Cheng, J. F., Chertkov, O., Brettin, T., Han, C., Detter, J. C., Kuske, C., Bruce, D., Goodwin, L., Chain, P., D'haeseleer, P., Chen, A., Palaniappan, K., Ivanova, N., Mavromatis, K., Mikhailova, N., Rohde, M., Tindall, B. J., Göker, M., Bristow, J., Eisen, J. A., Markowitz, V., Hugenholtz, P., Kyrpides, N. C., & Klenk, H. P. (2009). Complete genome sequence of *Saccharomonospora viridis* type strain (P101^T). *Standards in Genomic Sciences, 1,* 141-149. https://doi.org/10.4056/sigs.20263

Piao, C., Ling, L., Zhao, J., Jin, L., Jiang, S., Guo, X., Wang, X., & Xiang, W. (2018) *Streptomyces urticae* sp. nov., isolated from rhizosphere soil of *Urtica urens* L. *Antonie Van Leeuwenhoek, 111,* 1835–1843. https://doi.org/10.1007/s10482-018-1072-2

Poyraz, N., & Mutlu, M. (2017). Alkaliphilic bacterial diversity of Lake Van/Turkey. *Biological Diversity and Conservation*, *10*(1), 92-103.

Priyanka, S. B. (2019). Isolation, purification and characterization of pectinase enzyme from *Streptomyces thermocarboxydus*. *Journal of Biotechnology & Bioresearch, 1*(5), JBB.000523.201. https://doi.org/10.31031/jbb.2019.01.000523

Raju, E. V. N., & Divakar, G. (2013). Screening and isolation of Pectinase producing bacteria from various regions in Bangalore. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, *4*(1), 151-154.

Ray, L., Mishra, S. R., Panda, A. N., Das, S., Rastogi, G., Pattanaik, A. K., Adhya, T. K., Suar, M., & Raina, V. (2016). *Streptomyces chitinivorans* sp. nov., a chitinolytic strain isolated from estuarine lake sediment. *International Journal of Systematic and Evolutionary Microbiology, 66,* 3241–3248. https://doi.org/10.1099/ijsem.0.001176

Reasoner, D. J., & Geldreich, E. E. (1985). A new medium for the enumeration and subculture of bacteria from potable water. *Applied and Environmental Microbiology*, *49*(1), 1-7. https://doi.org/10.1128/aem.49.1.1-7.1985

Reimer, A., Landmann, G., & Kempe, S. (2009). Lake Van, eastern Anatolia, hydrochemistry and history. *Aquatic Geochemistry, 15,* 195-222. https://doi.org/10.1007/s10498-008-9049-9

Sağlam, M. T. (1978). *Toprak kimyası tatbikat notları.* Atatürk Üniversitesi.

Saitou, N., & Nei, M. (1987). The neighbour-joining method: A new method for constructing phylogenetic trees. *Molecular Biology and Evolution, 4*(4), 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Saricaoglu, S., Isik, K., Veyisoglu, A., Saygin, H., Cetin, D., Guven, K., Spröer, C., Klenk, H.-P., & Sahin, N. (2014). *Streptomyces burgazadensis* sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology, 64*(Pt_12), 4043–4048. https://doi.org/10.1099/ijs.0.065870-0

Sharma, P., & Thakur, D. (2020). Antimicrobial biosynthetic potential and diversity of culturable soil actinobacteria from forest ecosystems of Northeast India. *Scientific Reports, 10*, 4104. https://doi.org/10.1038/s41598-020-60968-6

Shirling, E. B., & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology, 16*(3), 313-340. https://doi.org/10.1099/00207713-16-3-313

Shivlata, L., & Satyanarayana, T. (2015). Thermophilic and alkaliphilic Actinobacteria: Biology and potential applications. *Frontiers in Microbiology, 6,* 1014. https://doi.org/10.3389/fmicb.2015.01014

Silva, F. S. P., Souza, D. T., Zucchi, T. D., Pansa, C. C., de Figueiredo Vasconcellos, R. L., Crevelin, E. J., de Moraes, L. A. B., & Melo, I. S. (2016). *Streptomyces atlanticus* sp. nov., a novel actinomycete isolated from marine sponge *Aplysina fulva* (Pallas, 1766). *Antonie Van Leeuwenhoek, 109,* 1467–1474. https://doi.org/10.1007/s10482-016-0748-8

Sorokin, D. Y., Tourova, T. P., Panteleeva, A. N., & Muyzer, G. (2012). *Desulfonatronobacter acidivorans* gen. nov., sp. nov. and *Desulfobulbus alkaliphilus* sp. nov., haloalkaliphilic heterotrophic sulfate-reducing bacteria from soda lakes. *International Journal of Systematic and Evolutionary Microbiology, 62*(Pt_9), 2107-2113. https://doi.org/10.1099/ijs.0.029777-0

Stackebrandt, E., & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today, 33*, 152–155.

Stackebrandt, E., & Goebel, B. (1994). Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic Bacteriology, 44*(4), 846-849. https://doi.org/10.1099/00207713-44-4-846

Stackebrandt, E., Rainey, F. A., & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system. Actinobacteria classis nov. *International Journal of Systematic Bacteriology, 47*(2), 479–491. https://doi.org/10.1099/00207713-47-2-479

Sujarit, K., Kudo, T., Ohkuma, M., Pathom-Aree, W., & Lumyong, S. (2018). *Streptomyces venetus* sp. nov., an actinomycete with a blue aerial mycelium. *International Journal of Systematic and Evolutionary Microbiology, 68*(10), 3333–3339. https://doi.org/10.1099/ijsem.0.002995

Świecimska, M., Golińska, P., Nouioui, I., Wypij, M., Rai, M., Sangal, V., & Goodfellow, M. (2020). *Streptomyces alkaliterrae* sp. nov., isolated from an alkaline soil, and emended descriptions of *Streptomyces alkaliphilus, Streptomyces calidiresistens* and *Streptomyces durbertensis*. *Systematic and Applied Microbiology, 43*(6), 126153. https://doi.org/10.1016/j.syapm.2020.126153

Taber, W. A. (1960). Studies on *Isaria cretacea*: Morphogenesis of the synnema and endogenous nutrition. *Canadian Journal of Microbiology, 6*(1), 53-63. https://doi.org/10.1139/m60-008

Tan, G. Y. A., Ward, A. C., & Goodfellow, M. (2006). Exploration of *Amycolatopsis* diversity in soil using genus-specific primers and novel selective media. *Systematic and Applied Microbiology, 29*(7), 557-569. https://doi.org/10.1016/j.syapm.2006.01.007

Thampi, A., & Bhai, R. S. (2017). Rhizosphere actinobacteria for combating *Phytophthora capsici* and *Sclerotium rolfsii*, the major soil borne pathogens of black pepper (*Piper nigrum* L.). *Biological Control, 109,* 1-13. https://doi.org/10.1016/j.biocontrol.2017.03.006

Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F., & van Sinderen, D. (2007). Genomics of Actinobacteria: Tracing the evolutionary history of an ancient phylum. *Microbiology and Molecular Biology Reviews, 71*(3), 495-548. https://doi.org/10.1128/mmbr.00005-07

Veyisoglu, A., & Sahin, N. (2014). *Streptomyces hoynatensis* sp. nov., isolated from deep marine sediment. *International Journal of Systematic and Evolutionary Microbiology, 64*(Pt_3), 819–826. https://doi.org/10.1099/ijs.0.055640-0

Waksman, S. A., & Henrici, A. T. (1943). The nomenclature and classification of the actinomycetes. *Journal of Bacteriology, 46*(4), 337–341. https://doi.org/10.1128/jb.46.4.337-341.1943

Wang, Z., Tian, J., Li, X., Gan, L., He, L., Chu, Y., Tian, Y. (2018). *Streptomyces dioscori* sp. nov., a novel endophytic actinobacterium isolated from Bulbil of *Dioscorea bulbifera* L. *Current Microbiology, 75,* 1384–1390. https://doi.org/10.1007/s00284-018-1534-9

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A., & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *Journal of General Microbiology, 129*(6), 1743-1813. https://doi.org/10.1099/00221287-129-6-1743

Yu, J., Zhang, L., Liu, Q., Qi, X., Ji, Y., & Kim, B. S. (2015). Isolation and characterization of actinobacteria from Yalujiang coastal wetland, North China. *Asian Pacific Journal of Tropical Biomedicine, 5*(7), 555-560. https://doi.org/10.1016/j.apjtb.2015.04.007

Yu, L., Lai, Q., Yi, Z., Zhang, L., Huang, Y., Gu, L., & Tang, X. (2013). *Microbacterium sediminis* sp. nov., a psychrotolerant, thermotolerant, halotolerant and alkalitolerant actinomycete isolated from deep-sea sediment. *International Journal of Systematic Bacteriology*, *63*(Pt_1), 25-30. https://doi.org/10.1099/ijs.0.029652-0

Zarilla, K. A., & Perry, J. J. (1986). Deoxyribonucleic acid homology and other comparisons among obligately thermophilic hydrocarbonoclastic bacteria, with a proposal for *Thermoleophilum minutum* sp. nov. *International Journal of Systematic Bacteriology, 36*(1), 13-16. https://doi.org/10.1099/00207713-36-1-13

Zenova, G. M., Manucharova, N. A., & Zvyagintsev, D. G. (2011). Extremophilic and extremotolerant actinomycetes in different soil types. *Eurasian Soil Science*, *44*, 417-436. https://doi.org/10.1134/S1064229311040132

Zhi, X. Y., Li, W. J., & Stackebrandt, E. (2009). An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class Actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *International Journal of Systematic and Evolutionary Microbiology*, *59*(3), 589-608. https://doi.org/10.1099/ijs.0.65780-0

Zhu, C., Xu, B., Adpressa, D. A., Rudolf, J. D., & Loesgen, S. (2021). Discovery and biosynthesis of a structurally dynamic antibacterial diterpenoid. *Angewandte Chemie International Edition*, *60*(25), 14163–14170. https://doi.org/10.1002/anie.202102453