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Microbial Characterization of *Streptomyces* Isolates from Oil Contaminated Soil

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Abstract

In this study, nineteen different *Streptomyces* strains were isolated from oil-contaminated surface soil at Ataş Oil Terminal in Turkey. Biodegradation and Restriction Fragment Length Polymorphism tests (RFLP) were used for selecting isolates. The isolates belonging to the genus *Streptomyces* were confirmed by 16S rDNA analysis. 1592r, 1492r, 800r, 518f, and 27f primers were chosen for the 16S rDNA gene amplifying and sequencing. Then 93-unit characters were used to determine phenotypic, physiological, and biochemical properties. All strains can degrade Tween 80, casein, gelatin, hypoxanthine, RNA, L-Tyrosine, elastin. Nevertheless, EL039, EL045, and EL060 soil isolates have better degradation potentials because of gave positive results to eleven degradation tests. According to the obtained results, EL037, EL039, EL060 could be a member of *S. albogriseolus, S. rochei, S. mutabilis*, respectively, and the phylogenetic tree and characterization tests indicated that especially, EL038, EL045, EL057 could be new members of *Streptomyces* genus. We perceive that the *Streptomyces* isolates would benefit biotechnological studies thanks to the degradation enzyme potentials.

Keywords: *Eco*RI, *Pst*I, *Hind*P1I, Biodegradation.

Petrolle Kirlenmiş Topraktan Elde Edilen *Streptomyces* İzolatlarının Mikrobiyal Karakterizasyonu

Öz

Bu çalışmada, Türkiye'de Ataş Petrol Terminali'nde yağla kirlenmiş yüzey toprağından on dokuz farklı *Streptomyces* suşu izole edilmiştir. İzolatların seçiminde Biyobozunma ve Restriksiyon Parça Uzunluk Polimorfizmi (RFLP) testleri kullanılmıştır. *Streptomyces* cinsine ait izolatlar, 16S rDNA analizi ile doğrulanmıştır. 16S rDNA geni amplifikasyonu ve dizilemesi için 1592r, 1492r, 800r, 518f ve 27f primerleri seçilmiştir. Ardından fenotipik, fizyolojik ve biyokimyasal özellikleri belirlemek için 93 birimlik karakterler kullanılmıştır. Tüm suşlar Tween 80, kazein, jelatin, hipoksantin, RNA, L-tirozin, elastin'i parçalayabilmiştir. Bununla birlikte, EL039, EL045 ve EL060 toprak izolatlarının, on bir bozunma testine de pozitif sonuç verdiğinden daha iyi bozunma potansiyellerine sahip olduğu düşünülmektedir. Elde edilen sonuçlara göre EL037, EL039, EL060 sırasıyla *S. albogriseolus*, *S. rochei*, *S. mutabilis*'in bir üyesi olabilir ve filogenetik ağaç ve karakterizasyon testleri özellikle EL038, EL045, EL057'nin yeni üye olabileceğini göstermiştir. *Streptomyces* cinsine aittir. *Streptomyces* izolatlarının degradasyon enzim potansiyelleri sayesinde biyoteknolojik çalışmalara fayda sağlayacağı kanaatindeyiz.

Anahtar Kelimeler: *Eco*RI, *Pst*I, *Hind*P1I, Biyobozunma.

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1. Introduction

Genus *Streptomyces* Waksman and Henrici 1943 (Approved Lists 1980) is the primary producer for bioactive metabolites and biocontrol agents against a range of pathogens (Arakawa, 2018; Ayed et al., 2018; Benhadj et al., 2020). Green chemistry products are called biopesticides, and agriculturally and medically essential antibiotics are momentous products (Nimaichand et al., 2016; Viana Marques et al., 2018). Also, members of *Streptomyces* are good candidates for bioleaching or bioremediation because of their degradative enzymes (Ferradji et al., 2014; Baoune et al., 2018; Baoune et al., 2019). Therefore, *Streptomyces*, known as the most widespread genus of actinomycetes in the golden age of antibiotics, remains in popularity (Panter et al., 2021).

Streptomyces genus is the predominant genus and thrives naturally in the rhizosphere soil. It is also saprophytic and substantial for recycling soil nutrients. It enhances soil fertility in nature with the help of in decomposition of soil organic matter constituting mainly cellulose, chitin, starch, xylene, or even crude oil (Hariprasad 2016; Robl et al., 2019; Chen et al., 2020; Nascimento et al., 2020). Studies on petroleum-degradation potentials of *Streptomyces* species and their metabolites are increasing day by day (Baoune et al., 2018; Baoune et al., 2019; Chen et al., 2020; Elnahas et al., 2021). It contains 691 species taxa with the correct name and validly published now (URL-1). Considering that the discovery of new *Streptomyces* species continues unabated, it is not surprising that scientists focus their studies on this genus. On the other hand, it is a significant challenge to characterize the isolates correctly. Because the genus has a heterotypic structure and 16S rDNA sequencing or physiological characterization alone is insufficient for species identification (Komaki 2021; Komaki & Tamura 2021; Saygın 2021).

We focused our attention on oil-contaminated soils and planned to isolate and characterize *Streptomyces* strains with high degradability from oil-contaminated soil.

2. Materials and Methods

A surface soil sample obtained from Ataş Oil Terminal, Mersin, Turkey, was put in a sterile plastic bag and labeled and stored at 4° C until microorganism isolation. A 20-25 g soil sample was added to a beaker. After enough ddH_2O was added, it was held for 24h; pH was determined with a pH meter three times. We did not use selective isolation procedures in this study. The air-dried soil sample was suspended in ¼ strength Ringer's solution (Oxoid). Then it was heated at 60 °C for 20 min before preparing 10^{-2} and 10^{-3} dilutions in Ringer's solution. We know that when the soil was air-dried, other bacterial numbers decreased, and *Streptomyces* colonies increased (Seong et al., 2001). 100 µL of each soil dilutions were spread over starch-casein agar plates, which were

supplemented with nalidixic acid (10 µg mL^{-1}), nystatin (50 µg mL^{-1}), and rifampicin (5 µg mL^{-1}) (Küster & Williams, 1964). All the agar plates were incubated at 28 °C for 30 days. Single colonies were successively transferred onto glucose yeast extract (GYE) agar and incubated until pure isolates were obtained. Spore mass and mycelium fragments of the pure strains were stored at -20°C as glycerol (20%, v/v) suspension.

2.1. Phenotypic Characteristics of Strains

Production of spore mass and its color, substrate mycelium color, and diffusible pigment production were detected on the 21-day old cultures on oatmeal agar (ISP Medium 3), inorganic salts-starch agar (ISP Medium 4), malt extract agar, and nutrient agar. ISCC-NBS Color Charts was used to record pigment colors (Kelly 1964). Their ability to degradation of hypoxanthine (0.4% w/v), starch (0.1 % w/v), xanthine (0.4% w/v), pectin (0.4% w/v), L-Tyrosine (0.4% w/v), gelatin (0.4% w/v), casein (1% w/v), elastin (0.3 % w/v), guanine (0.5% w/v), RNA (0.3% w/v), tween 80 (1% v/v) as described by Williams et al. (Williams et al, 1983). Their capacity to metabolize Tween 80 degradation test was examined Sierra medium containing tween 80 (Sierra 1957). Hydrolysis of Aesculin (0.1% w/v), allantoin (0.33% w/v), arbutin (0.5% w/v), urea (2% w/v), nitrate reduction (0.1% w/v), lysozyme resistance (0.1% w/v) as described by Gordon et al. (Gordon 1974). Test strains were planted in 22 different sole carbon sources to determine their growth and energy requirements. The basal medium used by Boiron et al. was modified by the addition of NaCl (0.5 g L^{-1}) and $FeSO_4$.$7H_2O$ (0.01 g L^{-1}) and used as the main medium. D(-) Arabinose, L(+) Arabinose, D(+) Xylose, D(+) Galactose, Meso- inositol, Sodium citrate, D(-) Fructose, L(-) Sorbose, α-L (+) Rhamnose, α- D (+) Melibiose, D-Mannitol, L (+) Tartaric Acid, D (+) Sucrose, D (+) Trehalose, D (+) Cellobiose, D(+) Raffinose, D(+) Sorbitol, Arbutin, Dextran, Inulin, Starch, Sodium acetate tests were done (Boiron 1993). We used a 1% w/v ratio in all utilizing carbon sources tests. Only basal medium was used as a negative control and basal media with added glucose (1.0%; w / v) as a positive control. The test strains inoculated by a 12-needle multipoint inoculator were read at 30℃ after 21 days of incubation. Test plates were evaluated by comparison with both positive and negative control plates. The test strains were tested to utilize nine different amino acids as nitrogen sources for growth and energy requirements as described by Williams et al. (Williams et al., 1983). L- Alanine (L-Ala), L-Arginine (L-Arg), L-Hydroxyproline (L-Hyp), L-Histidine (L-His), L-Iso-leucine (L-Ile), L-Methionine (L-Met), L-Proline (L-Pro), L-Serine (L-Ser), L-Valine (L-Val) tests were done. We used a 0.1% w/v ratio in all utilizing nitrogen sources tests. The basal medium was used as a negative control, and the basal medium was added to 1.0% (w/v) L-Asparagine as a positive control. Test inoculation was performed with 12-needle multipoint inoculators, and the plates were read after 21

days at 30℃. All microorganisms were grown over pH (4, 9, and 10) at various temperatures (10, 28, 37, and 45 °C). Antibiotic resistance (Ampicillin 10 µg/ml, Ampicillin 100 µg/ml, Neomycin 10 µg/ml, Neomycin 100 µg/ml, Penicillin 50 µg/ml, Penicillin 100 µg/ml, Polymyxin 50 µg/ml, Streptomycin 10 µg/ml, Streptomycin 100 µg/ml) tests were recorded. Thirteen chemical growth inhibitor tests were performed at 28°C on modified Bennet's Agar medium (Jones, 1949). Copper II sulphate (0.001 w/v), Ferrous sulphate (0.01 w/v), Ferrous sulphate (0.02 w/v), Sodium citrate (0.01 w/v), Sodium citrate (0.02 w/v), Thallium acetate (0.001 w/v), LiCl (0.02 w/v), Potassium tellurite (0.01 w/v), Potassium nitrate (0.005 w/v), Zinc sulphate (0.005 w/v), Zinc chloride (0.005 w/v), NaCl (7 w/v), NaCl (14 w/v).

2.2. DNA Extraction, 16S rDNA PCR Amplification, and Sequence Analysis

Before DNA extraction, biomass was grown in shake flasks containing modified tryptone-yeast glucose extract (TYG) broth; these cultures were incubated for 6 to 8 days at 28°C. Chromosomal DNA was isolated by the "guanidine thiocyanate DNA isolation method" (Pitcher et al., 1989).

The 16S rRNA genes (rDNA) were amplified by using universal primers **27f** and **1525r**. The positions and nucleotide sequence of PCR primers used in the study are given in Table 1.

Table 1. List of PCR primers, positions, and nucleotide sequences used in this study.

PCR primer name	Positions From 5' to 3'	Nucleotide sequence of the PCR primer From 5' to 3'	Reference
27f	8 to 27	AGAGTTTGATCMTGGCTC AG	(Lane, 1991)
1592r	1544 to 1525	AAGGAGGTGWTCCARCC	

PCR mixture contained chromosomal DNA (50-100 ng), 20 pmol forward and reverse primers (Invitrogen, USA), 25 mM dNTPs (Promega), Taq polymerase, and its buffer (Sigma-Aldrich, USA). The DNA thermal cycler (PCR Express, ThermoHybaid, Middlesex, UK) was programmed for 16S rDNA gene sequence amplification as in Table 2.

Table 2. 16S rDNA gene sequence amplification condition

	Temperature (°C)	Time (min)	Cycles
Initial denaturation	94	5	1
Denaturation	94	1	35
Annealing	55	**2**	

Elongation	72	**3**	
Final Elongation	72	**8**	1
Storage after reaction	4		

The amplicons were electrophoresed on 1% agarose gel (Serva, Germany) in 1×TBE buffer and were visualized with the Gene Genius Bioimaging system. They were purified (QIAquick purification kits, Qiagen, Hilden, Germany), and 16S rDNA gene sequencing was performed by ABI PRISM BigDye Terminator Cycle Sequencing kits (Macrogen, Netherland). We selected universal oligonucleotide primers listed in Table 3.

Table 3. List of sequence analysis primers, positions, and nucleotide sequences used in this study

Oligonucleotide primer name	Positions from 5' to 3'	Nucleotide sequence from 5' to 3'	Reference
518f	499 to 518	CCAGCAGCCGCGGTAATAC	(Muyzer et al., 1993)
800r	800 to 782	TACCAGGGTATCTAATCC	(Chun 1995)
1492r	1492 to 1474	TACGGYTACCTTGTTACGACT	(Gyobu & Miyadoh 2001)

The resulting 16S rDNA gene sequences (1477-1594 nucleotides) were used to search the GenBank database with the BLAST program and the current version of the EzBioCloud to determine relative phylogenetic positions. MEGA server 6 package provides multiple sequence alignment by CLUSTAL W 1.8 (Thompson et al., 1994; Tamura et al., 2013; Yoon et al., 2017). Phylogenetic analysis was conducted using MEGA 6. An evolutionary distance matrix was generated for the neighbor-joining described by Jukes and Cantor (Saitou & Nei, 1987; Jukes & Cantor, 1969). The resultant tree topology was evaluated by bootstrap analysis (Felsenstein 1985).

2.3. Restriction Fragment Length Polymorphism (RFLP)

16S PCR amplification products of all test organisms were digested using restriction endonuclease enzymes *Eco*RI (Promega), *Pst*I (Promega), *Hind*P1I (New England BioLabs). Purified PCR product (6 µL), ddH_2O (11.5 µL), restriction enzyme buffer (1.25 µL), and restriction enzyme (1.25 µL) were transferred to Eppendorf tubes, and the reaction mixture was carefully pipetted to ensure thorough mixing. The reaction tubes were kept in a water bath set at 37 ℃ for at least 4 hours so that each reaction mixture was 20 µL. At the end of the period, it was kept at 65 ℃ for 20 minutes to inhibit restriction endonuclease enzymes. Products cut with *Eco*RI, *Pst*I restriction endonuclease enzyme were run in 2% MetaPhor agarose gel for 1.5 hours at 100 volts. On the other hand, the products cut with *Hind*P1I restriction endonuclease enzyme were run in 3% MetaPhor agarose gel for 2.5 hours and visualized in the imaging system (GeneGenius, SyneGene; Bio-Imaging Systems, UK).

3. Findings and Discussion

Probably the most popular filamentous Gram-positive bacteria is *Streptomyces*. The popularity of this genus comes from the ability to produce varieties of natural products, including enzymes, antibiotics, etc. (Hashimoto et al., 2020). This study was conducted on Ataş Oil Terminal, Mersin, Turkey (36°81N–92°67E, 34°69N–74°78E). Soil pH was measured as 7.84, and approximately 1.4 x 10^6 CFU was determined for each petri dish. All the strains were aerobic and Gram-stain-positive. At first, we isolated nineteen different *Streptomycetes* colonies. We preferred biodegradation and RFLP tests for preliminary selecting isolates. We select three restriction endonuclease enzymes (*Eco*RI, *Pst*I, and *Hin*dP1I) for digesting the 16S rDNA gene region. The fragment sizes of 16S rDNA gene region amplification products varied between 160 and 960 bp. In the RFLP assay *Eco*RI, *Pst*I enzymes gave the same number and size of restriction fragments, but *Hind*P1I gave better results than EcoRI, PstI. EL060 had three, and the others had four pieces. EL039, EL045, and EL057 had the same number and size of four restriction fragments, and EL037 and EL038 had different RFLP profiles. Gel detectable restriction fragment sizes (in base pairs) of the 16S rDNA gene region were given in Table 4. We selected six isolates that gave at least nine positive results for biodegradation tests and had partially different RFLP profiles.

Table 4. Gel detectable restriction approximate fragment sizes (in base pairs) of 16S rDNA gene region digested by *Eco*RI, *Pst*I, and *Hin*dP1I restriction endonuclease enzymes.

Isolate	*Eco*RI	*Pst*I	*Hind*P1I
EL037	900-600	955-450	600-450-340-190
EL038	900-600	955-450	480-460-325-150
EL039	900-600	955-450	480-460-430-160
EL045	900-600	955-450	480-460-430-160
EL057	900-600	955-450	480-460-430-160
EL060	900-600	955-450	500-370-160

The test isolates an extensively branched substrate mycelium, which carries white or grey aerial hyphae and yellow to brown substrate mycelia on oatmeal agar media (Table 5). Sodium citrate, L(-) Sorbose, D-Mannitole, L (+) Tartaric Acid, D (+) Cellobiose, D(+) Sorbitol, Starch and Sodium acetate, L-Met, L-Pro, L-Val test results were negative for all isolates. All isolates in the study grew at pH 9, but none of the isolates grew at pH 4 or 10. All isolates showed antibiotic resistance to Ampicillin 10 µg/ml, Penicillin 50 µg/ml, and Penicillin 100 µg/ml and could tolerate to copper II sulphate (0.001 w/v), ferrous sulphate (0.01 w/v), ferrous sulphate (0.02 w/v), sodium citrate (0.01

w/v), LiCl (0.02 w/v), potassium nitrate (0.005 w/v) but any isolates could not grow at 7 nor 17% NaCl.

The phylogenetic analyses of the 16S rRNA gene sequences of the oil-contaminated soil strains revealed that the isolates were related to *Streptomyces*. The phylogenetic relationship can be inferred by analyzing 16S rRNA gene sequences of the isolates, and it is the closest type strains using the neighbor-joining method. The optimal tree with branch lengths was derived. The percentage of replicate trees was shown next to the branches. (Figure 1).

EL037 (KY860703) had the same 16S rDNA gene sequence similarity (99.65%) and variation ratio with *S. albogriseolus* and *S. viridodiastaticus* type strains. According to partial 16S rRNA gene sequencing, *S. albogriseolus* and *S. viridodiastaticus* type strains were identical. EL037 could not use raffinose, whereas *S. albogriseolus* was good growth, but *S. viridodiastaticus* was very poor. Sucrose utilization test was another identical test. EL037 has not used sucrose for carbon sources like *S. albogriseolus,* but *S. viridodiastaticus* could use it well. Aerial hyphae of EL037 were very compact. The spore color of EL037 was different from highly related type strains. The isolate gave a negative result for xanthine degradation and aesculin hydrolysis tests. EL037 could degrade gelatine as *S. albogriseolus,* but *S. viridodiastaticus* could not. Except for L-Arg and L-ile, amino acid utilization tests were positive. EL037 could tolerate 45 ℃ but *S. albogriseolus* and *S. viridodiastaticus* could until 40℃. According to Komaki (2021), *S. viridodiastaticus* was considered as later heterotypic synonyms of *S. albogriseolus* (Komaki 2021). Our consensus is that EL037 could be a member of this clade.

EL038 (KY860704) had the same 16S rDNA gene sequence similarity (98.13%) and variation ratio with *S. albidoflavus, S. luteus, and S. daghestanicus.* EL038 had light grey aerial mycelium on ISP 3 agar medium as with *S. albidoflavus* and different from other highly related type strains. No sporulation was observed on nutrient agar. EL038 gave two negative results for biodegradation tests (Pectin, starch). EL038 could not use rhamnose as *S. albidoflavus,* but *S. luteus and S. daghestanicus* did it. It also gave a negative result for aesculin hydrolysis tests. Except for L-Ser, the amino acid utilization test was negative. EL038 was mesophilic and proliferated at 28-37 °C. EL038 could not grow with sodium citrate (0.02 w/v), thallium acetate (0.001 w/v) and zinc sulphate (0.005 w/v) and only EL038 could not tolerate Potassium tellurite (0.01 w/v) among the isolates. EL038 could be a new member of this clade.

EL039 (KY860705) had the same sequence similarity (98.41%) with *S. rochei* strain, *S. enissocaesilis, and S. plicatus* strains. As seen in Figure 1, *S. rochei* strain (AB184237) seems to be the nearest one on the phylogenetic tree. So we only added *S. rochei* test results in Table 5. The isolate EL039 showed cultural and 16S rDNA gene sequence similarity with *S. rochei* but exhibited differences in physiological and biochemical properties. EL039 has dark grey aerial mycelium and

grey substrate mycelium with brown diffusional pigments and well-grown oatmeal agar medium (ISP 3) as *S. rochei.* Sporulation could not form on nutrient agar. First of all, EL039 gave positive results to all degradation tests in this study. The casein test result was different from the nearest neighbor, *S. rochei*. Except for L-His and L-Arg, amino acid utilization tests were negative. EL039 could tolerate pH9 but not pH10. EL039 and *S. rochei* can handle 3–7%(w/v) NaCl but could not be over 7%. For example, the optimum temperature was 28℃ for both highly related type strains, but EL039 could also tolerate 45℃.

Also, EL039 could not grow with sodium citrate (0.02 w/v), thallium acetate (0.001 w/v) and zinc sulphate (0.005 w/v). Only L-Arg and L-His test results were positive for EL039. EL039 could not grow with sodium citrate (0.02 w/v), thallium acetate (0.001 w/v) and zinc sulphate (0.005 w/v) same as EL038. According to Komaki, *S. enissocaesilis and S. plicatus* strains are synonyms of *S. rochei* (Komaki 2021). Our consensus is that EL039 could be a member of this clade.

16S rDNA gene sequence similarity of **EL045** (KY860707) was 98.90% with *S. ambofaciens* (AB184690) and 98.96% with *S. violaceochromogenes* (AB184312). Reverse side pigment on ISP 3 differentiates closely related type strains. *S. ambofaciens'* reverse color on ISP3 agar media was yellow, EL045's yellowish-brown, and *S. violaceochromogenes's* blue tint. Poor sporulation formed on nutrient agar. EL045 gave positive results to all degradation tests in this study. On the other hand, *S. violaceochromogenes* could not degrade starch. EL045 EL045 grew well at 45℃ as *S. ambofaciens*. EL045 could use rhamnose as *S. ambofaciens* but *S. violaceochromogenes.* Aesculin, allantoin, arbutin hydrolysis tests were positive as *S. ambofaciens*. On the other hand, arabinose, fructose, and sucrose test results were negative dislike *S. ambofaciens.* We conclude that EL045 could be a new member of this clade.

Table 5. Phenotypic characteristics to separate the isolates from related type strains and nucleotide similarities (%) and variation ratio based on the 16S rRNA sequences

Characteristic	**Isolates**											
	EL037	EL038	EL039	EL045	EL057	EL060	*S. albogriseolus*	*S. albidoflavus*	*S. rochei*	*S. ambofaciens*	*S. tuirus*	*S. mutabilis*
Spore surface	Warty	Smooth	Smooth	Smooth to warty	Smooth	Smooth	Warty	Smooth	Smooth	Smooth to warty	Smooth	Smooth
Colour of aerial mycelium on ISP 3	Light grey	Light grey	Dark grey	White	White	Light grey	Whitish grey	Light grey	Dark grey	Light grey	white	Light grey
Reverse side pigment on ISP 3	-	Yellow	Brown	Yellowish-brown	Greenish yellow-olive	-	-	-	Brown	Yellow	-	-
Carbon utilization												
L(+) Arabinose	-	-	+	-	-	-	-	+	+	+	+	-
D(+) Xylose	-	-	-	-	+	+	+	+	+	-	+	+
D(-) Fructose	+	+	+	-	-	-	+	+	+	+	+	+
α-L (+) Rhamnose	+	-	-	+	-	+	-	-	-	+	+	+
D (+) Sucrose	-	-	-	-	-	+	+	-	+	+	+	+
D(+) Raffinose	-	-	-	-	+	-	+	-	-	-	+	-
16S rDNA similarity with highly related type strain %	99.65	98.13	98.41	98.90	98.47	98.40						
Variation ratio	5/1448	27/1442	23/1447	16/1448	22/1438	23/1447						

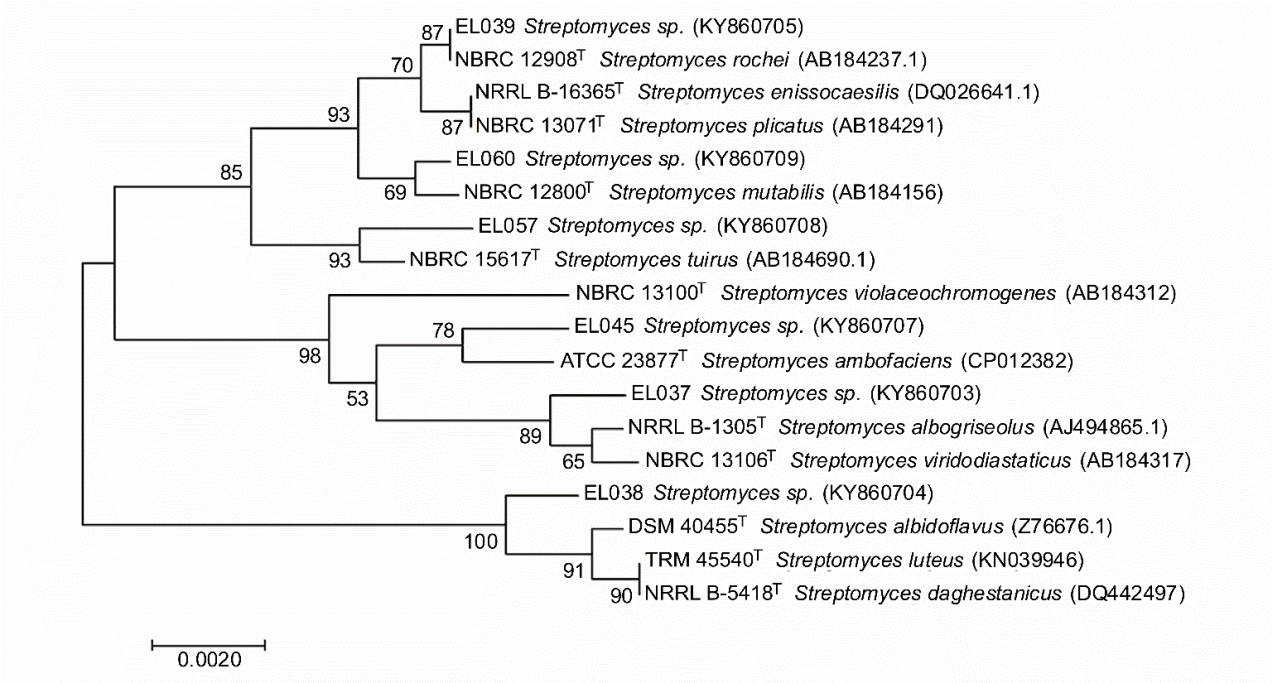

Figure 1. Neighbor-joining tree reconstructed based on 16S rDNA gene sequences showing the phylogenetic relationship between *Streptomyces* isolates and closely related type strains. Bootstrap values (1000 replication) >50% are given at nodes. (Total alignment 1436 bp)

EL057 (KY860708) had 98.47% 16S rDNA gene sequence similarity with *S. tuirus* (AB184690). The phylogenetic tree based on the neighbor-joining algorithm showed that the closest neighbor of EL057 was *S. tuirus* (Figure 1). Aerial mycelium of EL057 was white and had Greenish yellow-olive diffusible pigment on oatmeal agar media. Light grey sporulation was on malt extract agar *S. tuirus* was a mesophilic microorganism. Its optimum growth temperature was 28℃ but, EL057 could also tolerate 45℃. EL057 gave negative results for starch and guanine degradation tests. Aesculin, allantoin, and urea hydrolyze test results were positive, arbutin hydrolyses and lysozyme resistance tests were negative. The nitrogen utilization test and some identical tests like L-Arabinose, fructose, arabinose, and sucrose test results were negative different from the closest type strain *S. tuirus*. We conclude that EL057 could be a new strain of this clade.

Nowadays, researchers have been working on isolating actinobacteria from the soil, marine, even extreme habitats like the deep sea, desert, cryo environment, or volcanic environment rather than metagenomic approach because of isolating new bioactive compounds (Sivalingam et al., 2019). These consistent efforts may lead to discovering novel natural products obtained from new species of actinobacteria that may reform the field of agriculture and medicine (Hariprasad 2016).

Streptomyces is considered a crucial rhizobacterial genus and play a pivotal role in maintaining rhizosphere ecology and nutrient cycling. Secondary metabolites obtained from *Streptomyces* are used in numerous fields, including medical, biotechnological fields. Therefore, it is probably the essential taxa in secondary metabolite production, vital to the food industry, drug industry, and other industries (Harir et al., 2018). In this study, aerobic, mesophilic *Streptomyces* present in soil were isolated from Mersin Province in Turkey. The study's goal is a phenotypic and phylogenetic analysis of cultivable *Streptomyces* isolated from the ground with a high potential for secondary metabolites.

The 16S rRNA gene is generally used to define bacterial taxonomic positions. It has been reported in previous studies that there may be difficulties in the characterization of isolates of the genus *Streptomyces* due to its heterotypic structure. It was considering many species in the genus, ıt has been recommended using the polyphasic approach for characterization and identification processes by actinomycetologist (Anderson & Wellington; 2021). *Streptomyces* type strains can be assigned to distinct multimembered species groups as exemplified by species classified in *S. albidoflavus* (EL038), *S. ambofaciens* (EL045) clades. 16S rRNA gene-based methods may not be practical to classify the genus by species level.

Baoune et al. reported that endophytic isolate of *Streptomyces* sp. (Bmb4) was highly related to *S. mutabilis* and *S. rochei*, with approximately 98% identity in the same cluster (Baoune et al., 2018). This result is similar to our results. EL039 was closely related to *S. rochei*, and EL060 was highly associated with *S. mutabilis*.

It has been reported in previous studies that *S. ambofaciens*, *S. rochei, S. mutabilis, S. albogriseolus, S. albidoflavus* can be used to obtain many products of biotechnological importance (Hamed et al., 2018; Shao et al., 2019; Zhang et al., 2020; Patel et al., 2020). For example, Wongsariya and Thawai reported that soil isolates closely related to *S. rochei, S. mutabilis*, and *S. tuirus* species were effective against aflatoxin-producing microfungi (Wongsariya & Thawai, 2019). Shao et al. reported that a genome of a polyethylene degradative strain belongs to *S. albogriseolus* (Shao et al., 2019).

The increase in studies on genome mining in recent years shows that this method has become popular among scientists (Aigle et al., 2014; Haas et al., 2017) because of a strain-level strategy for exploring novel natural products from microorganisms provides an alternative way.

4. Conclusions and Recommendations

As a result, in this study, actinomycete isolation from soil samples taken from Ataş Oil Terminal in Mersin province and phylogenetic analyzes were performed. In the study, we saw again; the soil is a good reservoir of *Streptomyces*. According to the phylogenetic analyzes of the isolates whose 16S rRNA sequence analysis has been partially completed, priority is given to the isolates that show the most nucleotide differences with their closest relatives, and it is planned to complete the molecular and chemotaxonomic analyzes and bring them to the literature in future studies. On the other hand, A study on specific biosynthetic gene clusters in *Streptomyces* type strains can be planned.

Although antibiotic production comes to mind first when *Streptomyces* is mentioned, it should not be forgotten that this genus can be used in studies such as bioremediation and bioleaching. The studies for developing effective biodegradation for oils, polycyclic aromatic hydrocarbons, and environmental petroleum degradation with these *Streptomyces* isolates can also be planned. Hopefully, it would be some novel *Streptomyces* strains that are reservoirs of new secondary metabolites.

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Authors' Contributions

All authors contributed equally to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics.

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