NEWLY ISOLATED PLANT-GROWTH-PROMOTING BACTERIA FROM THE RHIZOSPHERE OF *TAMARIX APHYLLA*

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Abstract. *Tamarix aphylla* is an evergreen tree with small leaves that can resist drought and in saline soils. The plant is used in folk medicine in Saudi Arabia and in many Asian, African, and South European countries. Therefore, extensive research has been conducted to investigate its pharmaceutical benefits and antibiotic properties. In addition, *T. aphylla* was also studied for its environmental significance as a soil anti-erosive agent, but little is known about its associated microbiomes. The present study aims to identify and describe the *T. aphylla* microbiome's diversity and to investigate its biological and biochemical properties under abiotic stress. Here, we identified 30 independent bacterial isolates through 16S rRNA gene sequencing analysis. The identified bacteria were from several species and strains of *Bacillus,* which are found in extreme environments and thus could possess biotechnological and environmental potential. Moreover, we analyzed and compared the physicochemical properties of the soil crust and the rhizosphere of *T. aphylla* against a control soil sample to investigate how this plant affects its surrounding environment. Ultimately, this study works towards paving the way for further research on the significance of these plants and their associated microbiomes in Saudi Arabia.
Keywords: *Tamarix aphylla, abiotic stress, microbiome, PGPB, soil chemistry*

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Introduction

Tamaricaceae is a large family comprising various genera, that are primarily small shrubs and trees (DeLoach et al., 2003). They are native throughout Asia, North Africa, and Southeastern Europe (DeLoach et al., 2000). Belonging to the genus Tamarix are more than 54 species, spread broadly in the wild (Pearce and Smith, 2003). Among these species is *Tamarix aphylla* (*Figure 1*), which is an evergreen capable of living in extreme drought, frost, and saline conditions with low transpiration rates (Alshehri et al., 2022; Alhourani et al., 2018; Tambone et al., 2022; Lesica and Miles, 2004). Therefore, it can grow in sandy arid, calcareous, and saline soils. The tree can reach up to 10 to 12m in height and is often multithemed, forming a crown with many stout branches (Alrumman, 2016). Planting of *Tamarix aphylla* (*T. aphylla*) can be employed as an anti-erosion tactic due to the plant's surface cracks and deep root system, which allow it to withstand wind–sand erosion (Marwat and Rehman, 2011). In some countries, *T. aphylla* is planted as a shade tree along roadsides and canals. Its tender branches make it a favored source of fodder among camels and goats, enhancing its role in the ecosystem (ARAZI et al., 2013). Because of its high salt sequestration, *T. aphylla* can be used for the phytoamelioration of salinity-impacted lands (Hussain et al., 2021).

T. aphylla has traditionally been used in folk medicine to cure various ailments, including hepatitis, eczema, tinea capitates, and syphilis (Panhwar and Abro, 2007; Yusufoglu, 2011). Alcoholic extracts of *T. aphylla* have shown antioxidant effects (Shafaghat, 2010). In addition, it has been studied as an antibiotic and a source of other potentially beneficial secondary compounds (Alrumman, 2016). In its phytochemical composition, *T. aphylla* has a high concentration of secondary metabolites, flavonoids, alkaloids, and aromatic chemicals. These substances have a number of phytomedicinal and antibacterial properties, which enable them to be potential medical substitutes (Al-Otibi et al., 2023; Tambone et al., 2022).

Rhizosphere microbiomes of plants usually contain beneficial, neutral, and pathogenic agents. Plants can affect their microbiome diversity by actively selecting specific elements of their bacterial rhizosphere microflora to create a favorable habitat for the plant (Doornbos et al., 2012), known as plant-species-specific rhizosphere communities. Interactions between the different elements of these communities have been widely studied to control harmful agents and enhance plant growth and crop production. Plant-growth-promoting rhizobacteria (PGPR) are beneficial symbiotic bacteria that represent a collection of unrelated microorganisms that are found in the rhizosphere and phyllosphere and within the plant tissue as endophytic bacteria, carrying out several symbiotic interactions with the plant, which include contributing to biomass decomposition, soil fertility, and the cycling of nitrogen, carbon, and other nutrients to improve plant and soil qualities and functioning (Timmusk et al., 2017; del Carmen Orozco-Mosqueda et al., 2018).

Halotolerant bacteria have the ability to thrive in non-saline conditions as well as in salinity levels ranging from 1 to 33% NaCl, while maintaining their PGP function regardless of the high salinity (Khan et al., 2017). PGP properties include phosphorus (P) solubilization and the ability to produce indole acetic acids (IAA). These PGP roles can supply the plant with P in phosphate starvation conditions and with phytohormones such as (IAA) to regulate plant growth and development. IAA is a heterocyclic phytohormone known as plant. The rhizosphere bacteria that produce IAA are associated with enhanced plant growth (Susilowati et al., 2018; Das et al., 2019). IAA stimulates plant growth by breaking the apical dominance that induces the growth of the main stem. Thus, it is

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essential for plant growth and development, including cell expansion and division, flowering, fruit abscission, fruit ripening, root initiation, and vascular tissue differentiation (Nutaratat et al., 2017; Li et al., 2021).

(A)

(B)

Figure 1. *A. Representative photos of the T. aphylla tree collected from an area near an electricity station. B. Sample collection cite map (GPS coordinates; 21.2181343, 39.1749619)*

At a molecular level, microbially mediated abiotic stress tolerance is conferred by PGPRs via the up-regulation or down-regulation of specific genes. For instance, halotolerant PGPRs trigger the up-regulation of stress tolerance genes (Etesami and Beattie, 2017, 2018). *Bacillus* species are a major type of rhizobacteria that can survive in the soil under harsh environmental conditions. A good example is *B. subtilis*, which prevents the absorption of excessive amounts of Na^+ by the plants' roots in highly saline soil by down-regulating the expression of the high-affinity K^+ transporter (HKT1) in the roots (Qin et al., 2016).

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Bacillus subtilis' colonization of roots is advantageous to both the bacteria and the host plant. Bacteria gain a nutrient source, and, in exchange, plants benefit from bacterial compounds and activities that stimulate plant growth and provide protection from biotic and abiotic stress (Allard-Massicotte et al., 2016). These rhizospheric bacteria, with their unique features and relationships with plants, could be exploited to meet the needs of organic and sustainable crop production to combat the increasing abiotic stress resulting from climate change (Hashem et al., 2019).

Levels of plant tolerance to soil salinity vary significantly across different species: glycophytes have a much lower tolerance than halophytes. Although both types of plants are susceptible to salt injury, halophytes can survive at much higher salinity levels due to their ability to implement protective mechanisms (Pan et al., 2020). Halophytes represent less than 1% of the total flora in the world and are mainly spread across arid and semi-arid lands, in addition to high-salinity wetlands (Etesami and Beattie, 2018). Salinity tolerance can be enhanced in both glycophytes and halophytes through the actions of PGPB and arbuscular mycorrhizal fungi (AMF) (Pan et al., 2020).

Because of its chemical and physical characteristics, soil is the main medium for heavy metals in the marine environment. Heavy metal contamination have been connected to the operations of thermoelectric power stations (Stafilov, 2014a).

Recent studies used fingerprinting techniques of group-specific PCR amplified 16S for bacterial rDNA. The employment of these molecular techniques strongly contributed to our understanding of microbial diversity in soil (Doornbos et al., 2012). In this study, we conducted a general survey of microbiome species associated with *T. aphylla* growing in highly saline soil and under other forms of abiotic stress.

Materials and methods

Soil sample collection

Six samples were collected from *Tamarix aphylla* surrounding soil (triplicates from the crust (or surface). To acquire triplicates from the rhizosphere region, the plant was dug up, and samples of the root tissue and dirt (soil) that adhered to the roots were taken and separately placed into sterile tubes to be transported to the laboratory (Kearl et al., 2019). Similarly, triplicates were collected from an area with no plants to serve as the control. Samples were collected from an area near an electricity station (GPS coordinates: 21.2181343, 39.1749619), and the temperature was 38ºC.

The soil surrounding *T. aphylla* was dry and loose at the surface, while it was wet in the rhizosphere area, as the plant possesses mechanisms that hold the water in the root's region (Lee et al., 2016; Pierret et al., 2016).

Isolation and characterization of bacteria from soil samples

The soil samples were immediately transported to the laboratory for gradient dilution. To isolate soil bacteria, soil samples were vortexed in autoclaved distilled water; for each soil sample, 1 g of sample in 9 ml distilled water was used to produce microbial suspensions (10^{-1} to 10^{-4}). Aliquots were transferred to a suitable growth medium, and the cell concentration was adjusted to $1.3x10^6$ CFU/ml. Next, the samples were propagated for bacterial growth on Luria broth (LB) agar containing an increasing concentration of NaCl (1 M, 2 M, 3 M, 4 M) to determine the maximum salt tolerance of each isolate, and the plates were incubated overnight at 37°C. The next day, random colonies were isolated

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and re-culture/inoculated for further DNA analysis. Meanwhile, gram staining was performed on each isolate, and its microscopic characteristics were vigilantly observed.

Only one colony of each morpho-species was selected for genetic sequencing among the colonies possessing the same growth characteristics and morphology. The growth rate and colony morphologies of the isolated strains, such as size, color, diaphaneity of the colony, and the degree of smoothness, were considered. All isolated strains (*Table 1*) were preserved in a -80°C liquid medium, with glycerol added to obtain a final concentration of 20% (v/v).

DNA extraction, PCR amplification, and 16S dataset processing

Genomic DNA was extracted from each isolated colony and used, with a minor modification to the Azcárate-Peril and Raya (2001) DNA isolation process, to identify the bacteria. First, an appropriate quantity of bacterial pellets from an overnight culture was combined with 200 µl of TES buffer and 20 µl of lysozyme. Each sample received 20 µl of proteinase K, which was added after the mixture had been incubated in a water bath at 37°C for 20 min. Then, 250 µl of 4M sodium acetate was added to the mixture. Chloroform: isoamyl (24:1) was added, then the mixture was stirred gently, then centrifuged at 13000 rpm/2 min. The upper zone was transferred to a new clean Eppendorf tube, and isopropanol was added and then stored at -20 overnight. The next day, the mixture was centrifuged at 13000 rpm/2 min, and DNA was left to be dried at room temperature and then resuspended with 20 µl of distilled water. 5 µl of isolated DNA was loaded in 0.5% agarose gel in 1x of TBE buffer at 100 V for 60 min (Azcárate-Peril and Raya, 2001). For each sample, the 16S ribosomal RNA gene was amplified by PCR using 341F and 534R universal primers for sequence determination; bacterial 16S rRNA V3-V4; Forward (5'-CCTACGGGNGGCWGCAG-3') and 16S rRNA V3-V4 Reverse (5'-GACTACHVGGGTATCTAATCC-3'). The PCR reaction was carried out in 25 µl volumes, including 12.5 µl of master mix solutions 1X (containing *Taq* DNA polymerase, reaction buffer $MgCl_2$, dNTPs), DNA template, and 0.5 µM of each primer was mixed to a final volume of sterile water. The following PCR conditions were used: initial denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation (94°C for 30 s), annealing (60°C for 30 s), and extension (70°C for 1 min) and a final extension step at 70°C for 10 min. A total of 36 PCR products were then resolved by gel electrophoresis on 1% (w/v) agarose gels, stained by ethidium bromide, and visualized using UV light. After confirming the fragment size, the products were sent for DNA sequencing (Klindworth et al., 2013; Kearl et al., 2019).

Sequence analysis and phylogenetic tree construction

The 16S rRNA gene sequences of the isolated strains were compared with sequences in DDBJ/EMBL/GenBank using the basic local alignment search tool (BLAST). The isolates had significant hits to the genus, with the lowest e-value in the results from BLAST. Only the first sequence was selected when more than one sequence had the same e-value. Further, sequences were aligned using the Crustal Omega software (Version 1.2.2. 2016, UCD, Dublin). The phylogenetic tree was inferred using the neighbor-joining method with the Phylogeny.fr software. Bootstrap analysis was based on 1,000 re-samplings.

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Indole acetic acid (IAA) production detection

The ability of the bacterial isolates to produce IAA was tested. Auxin production by the plant-growth-promoting rhizobacteria (PGPR) isolates was tested in the presence and absence of L-tryptophan (L-TRP) and measured colorimetrically. For this purpose, 20 ml of glucose peptone medium (GPM) broth was added to 100 ml Erlenmeyer flasks, autoclaved, and cooled. Then, 5 ml of filter-sterilized (0.2 µm membrane filter, Whitman) L-TRP solution (5%) was added to the liquid medium GPM to reach a final concentration of 1.0 g l^{-1}. The flask content was inoculated by the addition of 1.0 ml of 4-day-old bacterial broth adjusted to an optical density of 0Æ5 (10^7–10^8 CFU ml^{-1}) measured at 550 nm by spectrophotometer (three unrepeated strains were examined). The flasks were incubated at 28 ± 1°C for 48 h under 100 rpm shaking. Two control strains were added to the experiment. The control strains were isolated from the arid areas near the plant in the study region, where there was no plant growth. Following incubation, the mixture was filtered using Whitman filter paper no. 2 before being placed in a new Petri dish with Salkowski's reagent (4.5 gm of $FeCl_3$ per liter in 10.8 M H_2SO_4) and incubated for 30 min. at room temperature and in the dark. According to Sarwar et al. description, the organisms that made IAA gave off a pink to red tint after the incubation time (Sarwar et al., 1992a; Khalid et al., 2004; Khan et al., 2017; Metwali et al., 2020).

Soil physicochemical analysis

Sample preparation and extraction

For soil elemental analysis by inductively coupled plasma–optical emission spectrometry (ICP-OES), 0.2-0.3 g of soil was weighed. Then 3 ml of HNO_3, 2 ml of H_2O_2, 1 ml of deionized water, and 1 ml of hydrochloric acid were added to each sample. As for water samples, 1 ml of HNO_3 was added to 5 ml of water. The samples were left at room temperature overnight. Then, samples were digested using a microwave digestion system (Milestone, Ultra-Wave), which was programmed as follows: t_{max} = 230 C, P_{max} = 1400 (W), t_{time} = 20, and 10 min. Each digested solution was transferred into a 50 ml falcon plastic tube and then the samples were centrifuged at 5000 rpm for 10 min. After this, each sample solution was filtered with a syringe filter and diluted 10-fold prior to the analysis (Roje, 2010). For the measurement of salinity and pH, samples were prepared by adding deionized water to obtain a 1:1 soil–water ratio. Each sample was then stirred for 1 hour and left at room temperature for 2 hours before taking the readings.

Concentrations of heavy metals in soil

All reagents used were of ultrapure grade. Deionized water was obtained from a high-purity Milli-Q-grade system (Elga Veolia, United Kingdom). High-purity nitric acid (65%) (HNO_3) was purchased from Romil Pure Chemistry (Cambridge, United Kingdom), while hydrochloric acid (37%) was purchased from Merck (Darmstadt, Germany). Hydrogen peroxide (30%) was purchased from Scharlau Chemie (Scharlarb s.r.l., Barcelona, Spain). The multi-element standard solution (containing Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, Li, Mg, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, and Zn at 10 – 1000 mg/L), with a mass concentration of 1000 mg/l in 5% nitric acid (HNO3), was obtained from Agilent (Santa Clara, California, United States). Argon (99.999%) was purchased from Gas ACKO (Riyadh, Saudi Arabia) (Gray et al., 2015).

The determination of elements was performed using inductively coupled plasma–optical emission spectrometers (Agilent 710, Santa Clara, California, United States). The

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operating conditions used were those recommended in the manufacturer's manual. Digestion was performed using a (Milestone, Ultra-Wave) microwave, while pH and conductivity were measured with a HANNA Conductivity Meter (Woonsocket, RI, USA). ICP-OES optimization conditions applied to quantify elements referred to the method of Robinson and Calderon (2010a); and Sarwar et al. (1992b). Click or tap here to enter text.

Soil pH and salinity measurement

The soil sample (9 g) was placed in a 50 mL beaker with 9 mL of deionized (DI) water and stirred for 30 minutes. Samples were covered and left to stand for an hour at room temperature before measuring the pH. For salinity measurement, samples were prepared by adding deionized water to obtain a 1:1 soil–water ratio. Each sample was stirred for 1 hour and then left at room temperature for 2 hours before taking the readings (HANNA Conductivity Meter, Woonsocket, RI, USA).

Results

Characterization of bacteria from soil samples

In total, ten bacterial isolates were selectively obtained from soil samples collected below the maturing body of a *T. aphyll*a colony in the crust and rhizosphere region. All the isolates grew normally in the tested NaCl concentrations (1 M, 2 M, 3 M, and 4 M). The isolates were selected based on the methods described in the Materials and methods.

Sequencing and phylogenetic analysis of the 16S rRNA gene sequences

The nearly complete 16S rRNA gene sequences of the ten bacterial isolates were amplified using the universal primers for the 16S rRNA and sequenced by comparing the 16S rRNA sequences with those in DDBJ/EMBL/GenBank (*Table 1*). The strains identified in two samples collected from the soil crust surrounding *T. aphylla* (Tc1.2 and Tc2.1) and two samples collected from the rhizosphere of the plant (Tr2.1 and Tr3.1) had significant hits for *Bacillus subtilis* subsp. *Spizizenii*. In addition, samples collected from the rhizosphere (Tr1.1 and Tr2.3) had significant hits for *Bacillus vallismortis,* and those collected from the soil crust (Tc2.3, Tc1.1 and Tc3.1) had significant hits for *Bacillus subtilis* subsp. *Inaquosorum, Bacillus tequilensis,* and *Bacillus licheniformis*, respectively (*Figure 2*).

Indole acetic acid (IAA) production

The presence of IAA in bacterial plates was confirmed after culturing for 120 h, as evidenced by the pink color after adding the Salkowski reagent. Bacterial isolates were screened for plant growth promoting property, production of IAA. Five isolates from ten could produce IAA (*Table 2*). Auxin production by the PGPR isolates was tested in the presence and absence of L-tryptophan (L-TRP) and determined by colorimetry (*Figure 3*). The examination indicated that the ability of the eight isolates to produce IAA varied among different Bacillus species.

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Table 1. *Selected bacterial strains form the crust and rhizosphere region of T. aphylla*

Sample Number	Isolate Code	Bacterial Strain
1	TC1_1	*Bacillus tequilensis* strain 10b 16S ribosomal RNA, partial sequence
2	Tc1_2	*Bacillus subtilis subsp. spizizenii* strain NBRC 101239 16S ribosomal RNA, partial sequence
3	Tc2_1	*Bacillus subtilis subsp. spizizenii* strain, NBRC 101239 16S ribosomal RNA, partial sequence
4	Tc2_2	*Bacillus vallismortis* strain NBRC 101236 16S ribosomal RNA, partial sequence
5	Tc2_3	*Bacillus subtilis* subsp. *inaquosorum* strain BGSC 3A28 16S ribosomal RNA, partial sequence
6	Tr3_1	*Bacillus subtilis* subsp. *spizizenii* strain NBRC 101239 16S ribosomal RNA, partial sequence
7	Tr1_1	*Bacillus vallismortis* strain NBRC 101236 16S ribosomal RNA, partial sequence
8	Tc3_1	*Bacillus licheniformis* strain DSM 13 16S ribosomal RNA, partial sequence
9	Tr2_1	*Bacillus subtilis* subsp. *spizizenii* strain NBRC 101239 16S ribosomal RNA, partial sequence
10	Tr2_3	*Bacillus vallismortis* strain DSM 11031 16S ribosomal RNA, partial sequence

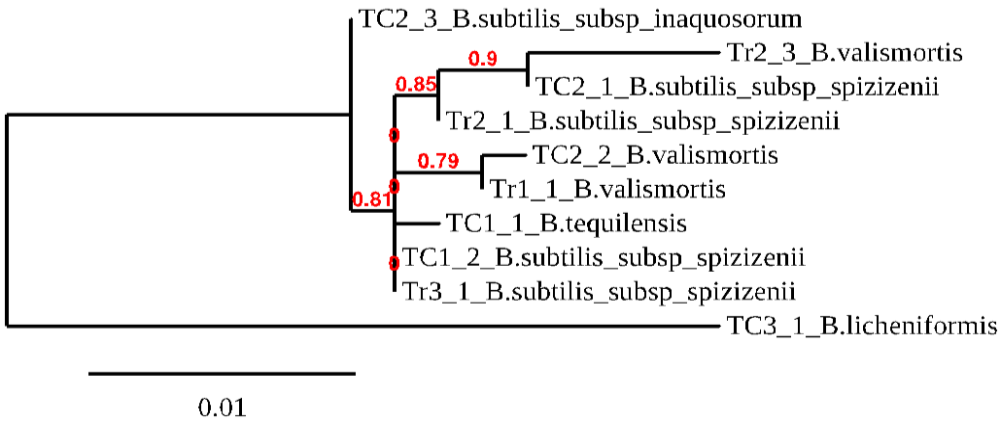

Figure 2. *Phylogenetic tree of the bacterial community surrounding T. aphylla. TC denotes the bacterial strains isolated from the soil crust, while Tr denotes the bacterial strains isolated from the rhizosphere*

Table 2. *Detection of production of IAA and nitrogen fixation by bacterial isolates*

Sample number	Bacteria	IAA production
1	*Bacillus tequilensis* strain	++
2	*Bacillus subtilis subsp.* spizizenii strain	++
3	*Bacillus licheniformis* strain	-+
4	*Bacillus haynesii* strain	++
5	*Bacillus vallismortis* strain	-+

-- = weak producer (growth); -+ = moderate producer (growth), and ++ =good producer) growth)

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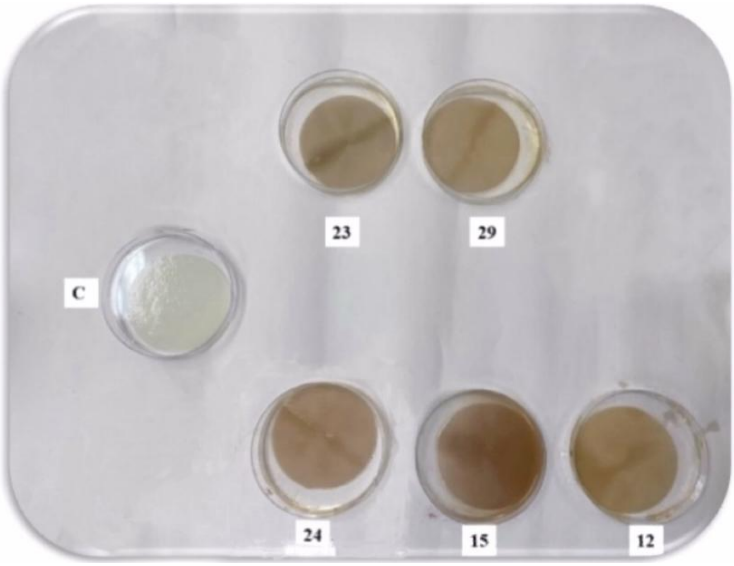

Figure 3. *Determination of auxin production by Salkowiski's reagent method; C = control*

Heavy metal concentration in soil

ICP-OES was used to quantify the heavy metals. *Table 3* shows the concentrations of heavy metals determined in soil samples collected from the crust and below the body of *T. aphylla* compared to a control sample collected from the same region near the Jeddah south thermal power plant.

Table 3. *Heavy metal concentration (mg/kg) in soil samples; the SD of heavy metals results (n = 3)*

Samples	Heavy metals										
	Cu	**Zn**	**Pb**	**Cd**	**Cr**	**Ni**	**Fe**	**Mn**	**V**	**Co**	**As**
T.aphylla crust	6.36 ± 0.42	15.95 ± 1.44	4.27 ± 0.72	0.26 ± 0.03	19.39 ± 0.25	9.09 ± 0.81	11536.45 ± 175.15	173.96 ± 8.32	34.22 ± 0.67	4.15 ± 0.25	4.95 ± 0.78
T.aphylla rhizosphere	8.45 ± 0.37	31.97 ± 0.10	5.63 ± 0.15	0.26 ± 0.03	20.40 ± 1.29	10.56 ± 0.54	12835.67 ± 348.59	206.30 ± 9.09	39.81 ± 1.18	4.60 ± 0.39	5.28 ± 1.90
Control	7.19 ± 0.07	23.08 ± 1.09	7.62 ± 0.38	0.20 ± 0.01	19.40 ± 2.67	9.70 ± 0.79	10396.20 ± 66.47	176.01 ± 13.51	36.77 ± 0.38	3.67 ± 0.12	5.67 ± 1.83

Soil pH and salinity

The measurements of salinity and pH in soil samples collected from the crust and rhizosphere below the body of *T. aphylla* are shown in *Table 4* in comparison to a control soil sample collected from the same region.

Table 4. *Soil pH and salinity measurement*

Sample	***pH Measurement***	***Salinity (ppm – mg/L)***
T. aphylla crust	7.96	173
T. aphylla rhizosphere	8.8	2279
Control	8.39	381

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Discussion

Arid and semi-arid lands cover a large area of the Earth's surface. The harsh environment of these lands, caused by water scarcity, exposure to high solar radiation levels, and soil salinity, represents a severe challenge for plant growth and survival. Plants growing in such extreme conditions and their associated beneficial microbes have garnered increased attention in recent years (Alsharif et al., 2020). Because of global climate change, there has been increasing interest in studying bacterial diversity in the soil surrounding halophytic plants to search for salt-tolerant PGPB that could be employed to enhance crop yields in saline soils (Egamberdieva et al., 2019). Halophytes, such as *T. aphylla*, are the ideal candidates because they can survive and thrive in saline soil and have a certain tolerance to heavy metals (Li et al., 2022).

This study aimed to identify the soil microbial diversity associated with *T. aphylla*, which grew near an electricity station in Jeddah, Saudi Arabia, to find beneficial strains that promote plant growth and survival in harsh environmental conditions.

The Earth is home to a wide range of microorganisms that researchers are still working to identify and isolate (McHugh et al., 2017; Alsharif et al., 2020). This great diversity has necessitated extensive research to investigate such promising bacteria thoroughly. Saline ecosystems host a specific microbiota, which can be recruited by the halophytes (Behairi et al., 2022). Rhizosphere bacteria promote plant growth directly or indirectly through several mechanisms (Dhungana and Itoh, 2019). Moreover, PGPB play a vital role in farming industries by reducing the use of synthetic fertilizers (Chauhan et al., 2015).

To expand our knowledge of saline ecosystems, the microbiome analysis that we conducted included sampling, processing, sequencing, and bioinformatics analysis to determine the composition of microbiota inhabitants associated with the halophyte *T. aphylla*. For this purpose, soil samples were acquired from *T. aphylla* adjacent soil from the crust or surface and from the rhizosphere.

According to the sequencing results, there were several phyla in the taxonomic distribution of the bacterial populations. The reservoir of bacterial diversity was dominated by the genus *Bacillus*. Salt screening of the isolated strains showed that all species belonging to the genus *Bacillus* were able to grow in all tested salt concentrations (up to 4 M of NaCl). Multiple studies have demonstrated that these bacteria, notably plant-growth-promoting bacteria (PGPB), offer numerous environmental and ecological benefits (Gupta et al., 2015). The Bacillus genus includes strains that are well adapted to hot climates. A significant group of rhizobacteria known as Bacillus species have modest nutritional requirements and are capable of producing spores that can remain in the soil for a prolonged period of time in adverse environmental conditions (Hashem et al., 2019). As a result, they readily colonize oligotrophic environments such as salt marshes, hot springs, and arid soils (Mohammad et al., 2017).

The formation of indole-3-acetic acid (IAA), the solubilization of phosphate, and improved nutrient uptake are only a few of the ways that rhizobacteria that colonize the rhizosphere of plants contribute to plant growth and increase the productivity of agricultural products (Jung et al., 2015). The bacterial isolates were tested for IAA production and we detected eight *Bacillus* species that had the ability to produce IAA, which plays a key role in the development of roots and shoots in plants (Chauhan et al., 2015). Further confirmation and quantification of IAA could be conducted in future work by using techniques such as thin-layer chromatography and gas chromatography–mass spectrometry (Dhungana and Itoh, 2019).

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Contamination with heavy metals is a significant environmental issue due to their potential remobilization and bioaccumulation in sediments and marine bodies. The sources of metal in sediments are mainly industrious sources, with little contribution from natural sources. Soil is the primary carrier for heavy metals in the marine environment because of its chemical–physical properties, including particle properties, precipitation, and adsorption/desorption processes. Various studies have linked thermoelectric power plants' activities with the rise in heavy metal concentrations in their surrounding soils. It was noted that the sites with the highest concentrations of all determined metals were in the area around the south thermal power plant (Stafilov, 2014b). *Table 3* shows the concentrations of heavy metals determined in soil and samples collected near the Jeddah south thermal power plant. Various studies have related the activities of thermoelectric power stations with increased levels of heavy metals in the soil around the power stations. By studying the soil composition in a region near a thermoelectric power station located in Macedonia, Stafilov was able to find a significant increase in metals in the soil surrounding the power station (Stafilov, 2014b). The maximum and median values of Pb and Zn were 130 and 200 mg/kg, respectively. A study by Mazhaiskii et al. (2000) found average concentrations that were 16 mg/kg for Cu, 50 mg/kg for Zn, 16 mg/kg for Pb, and 0.4 mg/kg for Cd in soils close to a thermoelectric energy station in Ryazan in Russia that uses coal, natural gas, and fuel oil. The soil near the thermal power plant had higher metal levels than in other areas. The concentrations for most metals were similar to those in the control sites (C). The findings for Fe were higher than in another study by Badr et al. (2009), which was conducted in Prince Nife and downtown Jeddah, while the levels of other heavy metals were lower than those of the metals studied by Badr et al. (2009).

The pH measurements (*Table 4*) showed that as the soil sampling descended from the crust to the rhizosphere, the pH rose from 7.96 to 8.8, likely because of salt buildup resulting from the mineralization of the organic matter and the limited leaching brought on by the lack of rainfall. These results are consistent with the finding of a study on *T. aphylla* effect on surrounding soil conducted in Tunisia (Tambone et al., 2022).

Arid regions generally contain higher concentrations of salts as evaporation concentrates salts in the soil by a capillary rise from groundwater, along with insufficient precipitation to remove accumulated salts by leaching. Soil salinity has become one of the most conspicuous environmental issues due to climate change impacts, posing a threat to the environment and the sustainability of food and agriculture (Chaudhry et al., 2013), (Khumairah et al., 2022). Elevated soil and water salinity are generally associated with Tamarix spp. (Glenn and Nagler, 2005). Tamarix can draw salts from underground soils and groundwater, store them in its tissues, excrete them through the foliage, and then deposit them on the soil surface through leaf senescence (Stromberg, 2001). Our results revealed a notable increase in salt concentration in the rhizosphere of *T. aphylla* compared to the control soil sample, which agrees with various previous research. Arazi and co-workers evaluated the effect of *T. aphylla* as a tree windbreaker on the soil salinity of the agricultural lands of Ardakan city, Iran. Their results showed that the rate of soil salts in the region with windbreaks was significantly higher than in lands without windbreaks (ARAZI et al., 2013). Another study was carried out to assess the effects of planting *T. aphylla* trees in a semi-arid dunefield in northern Negev, Israel. This study confirmed that the salt accumulation in *T. aphylla* leaves and litter increases soil salinity under *T. aphylla* shades by 4–5 times, forming "salinity islands" ('Erratum to: Hierarchical Effects of *Tamarix Aphylla* Afforestation in a Sand Dune Environment on Vegetation Structure and Plant Diversity' 2020). This salinity level is well tolerated by all the

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bacterial isolates, as demonstrated by our salt screening results. The charisctarirecs of these strains of Bacillus indicate a great potential for biocontrol and the ability to increase crop productivity under conditions of biotic and abiotic stress.

Conclusions

This study identified the bacterial community in the rhizosphere and soil crust around *T. aphylla*. These microbe communities could have potential halotolerant plant-growth-promoting activities and can be used as effective bioinoculants to improve crop growth under salinity and other abiotic stress conditions, serving the ultimate goal of feeding the exponentially growing population. This study's encouraging findings urge further thorough investigation to confirm these bacterial species plant-growth-promoting properties.

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