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Diversity and Metagenomic Sequence Analysis of Bacterial Strains Isolated from Different Mangrove Environments in Saudi Arabia

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Abstract **Original Research Article**

Mangroves, which dominate tropical intertidal zones and estuaries, are salt-tolerant plants that reproduce through propagules, which are little reproductive units. Because mangroves can grow in seawater, microorganisms capable of interacting with the host and exerting beneficial effects under salt stress should be present. Three mangrove areas in Saudi Arabia yielded sixteen bacterial and actinobacterial species (Thuwal, Jazan, and Farasan islands). The microbial profile of the examined regions was analysed using a 16S rRNA-based metagenomics technique. Based on 16s rRNA and phylogenetic analysis, the selected isolates were identified. The bacterial extracts were tested for antibacterial activity and pharmacological characteristics. The 16S RNA-based metagenomics revealed that eight phyla of bacteria were found in the same region: *Bacteroidetes*, *Firmicutes*, *Chloroflexi*, *Gemmatimonadetes*, *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Planctomycetes*. *Bacteroidetes*, was the most common phyla, followed by *Firmicutes* and *Proteobacteria*. The *Bacillus* genus was found in the majority of samples, followed by *Streptomyces*. The pharmacological characteristics of the bacterial extracts were assessed. Antibacterial testing revealed that 13 of 16 bacterial extracts were having antimicrobial activities against at least one of the microorganisms examined.

Keywords: Mangroves, Metagenomics, Antibacterial, *Streptomyces*.

INTRODUCTION

Mangroves, also known as qurm, are one of the few woody habitats found along the Arabian Gulf's parched coasts (Elmahdy *et al*., 2020). Wetlands found along tropical and subtropical coastlines are known as mangrove habitats. The name mangroves can also refer to a distinct collection of plants and trees that thrive in brackish intertidal areas (Feller *et al*., 2010).

Mangroves have an important role in the environment. Globally, there are approximately 34 main and 20 minor mangrove species belonging to over 20 genera in over 11 families (Tomlinson, 1986). Mangroves are an important aspect of tropical coastal biodiversity, accounting for less than 1% of the world's surface area (Saenger, 2002). They are found mostly between the Tropics of Cancer and Capricorn on all continents, covering an estimated 75% of tropical coastline globally (Sakhia *et al.*, 2016).

Mangrove trees provide habitat for migratory birds and serve as a nursery for major commercial fish species, as well as helping to prevent coastal erosion.The trees help protect nearby marine ecosystems (typically coral reefs) from the effects of terrestrial pollution (Spalding and Parrett, 2019).

The metabolic processes of bacteria from various environments have been investigated all around the world. The mangrove ecosystem is one of the most productive ecosystems due to extremely variable environmental factors such as salinity, temperature, nutrients, and tidal currents. Mangrove trees that are subjected to such erratic environmental fluctuations provide unique biological niches for a variety of endophytic microbial populations (Thatoi *et al*., 2013).

Furthermore, the diverse environmental circumstances found in mangrove habitats may act as good selectors for structurally different secondary metabolites with fascinating pharmacological effects.

Endophytic bacteria have been found to be a rich source of secondary metabolites with interesting therapeutic effects, in addition to their host plants (Ancheeva *et al*., 2018; Cadamuro *et al*., 2021). Many of these compounds have strong biological activity and could be used as lead compounds in future medication development (Dat *et al*., 2021).

The ecosystem of mangroves has been thoroughly investigated because of their adaptive properties. Mangrove molecular features, on the other hand, have received less attention. The higher levels of biodiversity are influenced by genetic diversity. The level of genetic variety among mangrove species has been reliably quantified utilising studies of genetic variability and interspecific connections using molecular markers. Phylogenetic analysis has reached new heights due to recent advances in sequencing technologies. The availability of a variety of phylogenetic methodologies and software packages may appear daunting to an experimental biologist, but phylogenies have pervaded practically every discipline of biology (Sahu and Kathiresan, 2012).

Over the last few years, NGS (next-generation sequencing) has proven to be a great method for exploring and answering ecological problems. Several NGS-based studies of mangrove rhizosphere bacterial composition (Alzubaidy *et al.,* 2015), bacterial spatial variation (Jiang *et al*., 2013),

Actinobacteria are the richest suppliers of secondary metabolites and have been considered possible sources of bioactive substances. Actinomycetes having the capacity to create biologically active secondary metabolites can be found in the mangrove ecosystem, which is largely unexplored (Hong *et al*. 2009). Actinomycetes have been found in a variety of mangrove settings, according to several reports from different parts of the world. Several actinomycetes genera, such as *Actinomadura, Microbispora, Actinoplanes, Nonomuraea, Arthrobacter, Micromonospora, Verrucosispora, Isoptericola, Microbacterium, Nocardia*, *Micrococcus*, *Rhodococcus* and *Streptomyces* were reported from mangrove soils and plants in China (Hong *et al*. 2009).

The study of mangrove actinomycetes can aid in the finding of novel isolates, which can lead to the development of valuable antibacterial drugs (Tiwari and Gupta, 2013; Lee *et al*., 2014). Due to its ecological significance and biotechnological benefits, scientists are still exploring the community diversity in the phylum actinomycetes from many countries and habitats (Fu *et al*., 2016). Marine actinomycetes isolated from Red Sea mangrove forests have been shown in recent investigations to be a valuable source of antibacterial, antioxidant, and anticancer compounds (Hamed *et al*., 2021).

The biomass and productivity of mangrove forests are dominated by bacterial flora, which outnumbers trees. Bacterial populations in mangroves are many times bigger than fungal populations (Kathiresan and Qasim 2005).

Nitrogen fixation is the process by which bacteria and cyanobacteria converting gaseous nitrogen (N2) into mixed forms, such as ammonia or organic nitrogen. Diazotrophic microorganisms, both free-living and symbiotic, fix N2 into proteins. Microorganisms that fix nitrogen (diazotrophic) can live in both terrestrial and marine habitats (Thatoi *et al*., 2013). Despite being very productive and carbon-rich, mangrove habitats are considered low-nutrient settings. The nutrients nitrogen and phosphorus have been identified as restricting mangrove growth. Tidal nitrogen export, denitrification, and soil type are all major contributors to nitrogen loss in these ecosystems (Reef *et al*., 2010)

According to studies, microbial N2 fixation could sustain 40–60 percent of the N needs of mangrove ecosystems. Furthermore, research is increasingly focusing on determining how microbial N2 fixation affects mangrove ecosystems. Depending on the geographic location of the mangrove forest, microbial N2 fixation rates can range from 0 to 10.1 mmol N m-2 d-1 (Shiau *et al*., 2021).

Phosphorus (P) is a macronutrient required by plants for the formation of adenosine triphosphate (ATP). Phosphorus levels in the soil are low, ranging from 500 to 10,000 kg per hectare at a depth of 50 cm. The plants were unable to utilise phosphorus adequately due to a lack of availability in the soil, with around 95–99 percent of it present in the form of insoluble phosphates. Because bounded phosphorus is difficult for plants to absorb, it must be converted to a soluble form (Fatimah *et al*., 2021).

Mangrove microbes have previously been shown to be advantageous to agriculture. The beneficial bacteria found in the rhizosphere soil of mangrove plants have a wide range of agricultural applications (Kathiresan and Selvam 2006). These strains can fix nitrogen, solubilize phosphate, generate ammonia, and synthesise the plant growth hormone indole acectic acid, among other things (IAA).

Because of their great production capabilities, low cost, and vulnerability to genetic manipulation, microorganisms are significant in enzymatic manufacturing processes. In numerous disciplines, such as food processing, detergent and textile manufacture, agricultural and pharmaceutical research, medical therapy, and molecular biology, there is a considerable biotechnology interest in microbial enzymes (Sebastianes *et al*., 2017).

Endophytic microbe compounds having medicinal characteristics have recently sparked a surge of interest in research. Indeed, this group of bacteria has produced a large number of novel compounds having antimicrobial action (Sebastianes *et al*., 2017).

The aim of this study the diversity and characterization of bacterial strains isolated from mangrove environments using the high throughput sequencing in Saudi Arabia.

MATERIALS AND METHODS

1. Study area and Sample collection

Field trips conducted to collect samples from different study places across Saudi Arabia.

2. Evaluation of the bacterial diversity using the high throughput sequencing

DNA was isolated from 19 soil samples directly. Following the manufacturer's instructions, genomic DNA was extracted from roughly 0.5 g of each sample using the FastDNATM spin Kit for dirt. (Sujatha and Swethalatha, 2017). Following the manufacturer's instructions, the DNA purification was measured using a Qubit 3 Fluorometer (Invitrogen). By passing the extracts across a 1% agarose gel, the integrity of the DNA isolated from the samples was validated. (Najar *et al*., 2018).

Identification of strains morphologically was done by Electron microscope using Coverslip culture technique on both ISP2, as well as Hagem media agar (Yaminisudha *et al*., 2015). phylogenetic markers that amplify the V4-V5 hypervariable region, which has been shown to be universal across nearly all bacterial species. The forward primers 515-F and 907-R each had a six-nucleotide barcode that identified the sample. This short targeted gene region can provide enough precision for reliable microbial sequence taxonomy classification. In a 50-l reaction mixture, 25 l of 2X DreamTaqTM Green Master Mix (DNA polymerase, dNTPs, and 4 mM MgCl 2), 22 l nuclease-free water, 1 l sample DNA, and 2 l forward and reverse primers were used to amplify the extracted DNA. The PCR amplification was carried out as follows: initial denaturation at 95 °C for 5 minutes, followed by 30 cycles at 95 °C for 30 minutes, 58 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 10 min. To isolate DNA from other molecules, all PCR results were put into a 1 percent gel electrophoresis. The pure DNA bands were then extracted using the TIANGene universal DNA gel extraction kit, as directed by the manufacturer. On an Illumina MiSeq PE300 platform, the amplicons were purified, quantified, pooled, and sequenced. After obtaining the raw reads, QIIME software was used to assign samples by exactly matching the unique barcode into each sample to eliminate primer mismatches, homopolymers with more than six bases, sequences with ambiguous bases, and barcode errors (Caporaso *et al*., 2010a). Furthermore, low-quality readings with more than 20 low-quality bases should be filtered. Sequences that were noisy or chimeric were filtered out. After that, using Uclust clustering, which clusters sequences with a minimum pair-wise identity of 98 percent, the sequencing was grouped into operational taxonomic units (OTUs). Also removed from the final OUT table were chloroplast, mitochondrial DNA, and singlton OTUs. The most prevalent read was chosen as a sample sequence for each OTU. PyNAST aligner was used to align each of the sequences (Caporaso *et al*., 2010b). For additional investigation, the phylogenetic tree was constructed using the FastTree technique (Price *et al.,* 2010). The unweighted/weighed UniFrac and Bary-Curtis distances between every pair of samples were computed using QIIME to estimate Beta-diversity.

For actinobacterial isolates molecular identification Using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5' GGTTACCTTGTTACGACTT-3'), genomic DNA was isolated as described by Li *et al*. (2007) and utilised as the template to amplify the 16S rRNA gene by PCR. 25µL 2supermix (TransGen, Beijing), 1µ L each of the primers (10 mM, Sangon Biotech, Beijing), 1.5µ L DNA, and 21.5µ L dd H2O made up the reaction mixture (50µ L). The following PCR amplification conditions were used: (i) 95°C for 3 minutes (initial denaturation), (ii) 30 cycles of 94°C for 1 minute (denaturation), 60°C for 1 minute (annealing), and 72°C for 1 minute (extension), and (iii) 72°C for 10 minutes (extension) (final extension). Gel electrophoresis with 5 litres of PCR product in a 1% agarose gel was used to visualise the amplicons. The PCR products were purified and then sequenced on the ABI PRISM^{TM} 3730XL DNA Analyzer (Foster City, CA) (Li *et al*., 2019).

Phylogenetic trees of the aligned sequences were constructed by the neighbor-joining method (Saitou and Nei, 1987) by using software MEGA X. The extracted DNA from each sample was quantified using Nanodrop 8000 spectrophotometer device (Figure 6).

3. Evaluation of the actinobacterial diversity

Unless otherwise specified, all material that was used in this study was sterilized as media, plasticware and glassware etc. by autoclaving for 20 min at 120°C and 1.5 atm (Armada and Simora, 2016).

To eliminate all surface-living organisms, plant portions were surface sterilised. Following this, the plant parts were sequentially immersed in a 5 percent aqueous sodium chloride solution, tissues then were washed with properly sterilized distilled water to wash away the contaminants and surface microflora, followed by treating with 70% ethanol for 5 minutes, then rinsed with sterile water, and plants parts immersed in Sodium hydrogens carbonate (NaHCO3) to inhibit fungal growth. After that, the explants were washed with distilled water (Li *et al*. 2016).

To obtain pure isolates, colonies were picked up and streaked on the same freshly prepared isolation media after incubation at 28°C for 2-4 weeks. The pure cultures were kept on agar slants at 4°C for many weeks and then stored at 80°C in glycerol solutions (20% v/v) (Li *et al.*, 2019).

4. Assay of antibacterial activity

The actinobacterial strains were inoculated in the same media used for isolation for 7 days at 30 °C with 150 rpm shaking, and the cultures were filtered and the cell-free supernatants were used in the antibacterial assay.

The bacterial extracts were examined for antimicrobial activity against *Escherichia coli*, *Pseudomonas aegurinosa*, *Salmonella typhi*, and *Candida cadrola*, which were inoculated in nutrient broth media to create suspension. The suspension of test organisms was seeded on nutrient agar media. The agar well diffusion method was used to perform the antibacterial assay (Isaacson and Kirschbaum, 1986). Each well had 200 µl of fermentation broth, which was added aseptically. The antibacterial activity was measured by measuring the inhibition zone after plates were incubated at 37 °C overnight.

RESULTS

1. Isolation and Identification of bacterial strains

Sixteen bacterial isolates were successfully isolated from marine water and soil samples, collected from the three different isolation sites located in Saudi Arabia. Seven isolates from thuwal, and seven isolates from Jazan region. The bacterial isolates were chosen based on their morphological differences and were purified on the same isolation media used and then stored on slants at 4ºC and in 20% glycerol at -20ºC for further analysis (Figure 1).

The electron microscope image of isolate (SHj88)(Figure 4) show Gram-positive, long, spore-forming rods with a diameter of < 0.9 µm (Figure 3). Colonies are small to medium-sized, semi-transparent, and flat.

Based on a combination of 16s rRNA molecular sequencing and microscopic identification from plant and soil samples; the 16 bacterial isolates were belonging to 12 species in 5 genera. *Bacillus* (50%), *Streptomyces* (21%) and *Larsenimonas* (14.3%) were the most common genera. A total of seven bacterial strains were isolated and identified as belonging to the genera *Bacillus* (5 species), three bacterial strains were identified as members of the genera *Streptomyces* (3 species), *Larsenimonas* (1 species), *Kushneria* (1 species), *Mycolicibacterium* (1 species). (Table 1).

3. Screening the isolated actinobacterial strains for their antimicrobial activities

The antimicrobial assays showed that 13 out of 16 bacterial extracts exhibited antimicrobial activity against at least one of the tested microbes.

SH88J showed antimicrobial activity against two tested microbes (Figure 5) (Table 2).

Table-1: Sequence similarity of isolated strains based on 16S rRNA sequences compared to the GenBank database.

Sample name	Most related similar strain						Score		Identities	
	Accession	Description	length	start	End	Coverage	Bit	E.Value	Match/Total	PCt (%)
SH 5T	NR_134755.1	*Larsenimonas salina*	1491	16	1468	98	2704	0.0	1470/1473	99
SH 6T	NR_044001.1	*Kushneria avicenniae*	1433	2	1433	99	2623	0.0	1428/1432	99
SH 33T	NR_134755.1	*Larsenimonas salina*	1491	23	1486	98	2687	0.0	1461/1464	99
SH 42T	KX785171.1	*Bacillus licheniformis*	1545	21	1494	95	2660	0.0	1463/1474	99
SH 44T	KX785171.1	*Bacillus licheniformis*	1545	17	1496	95	2671	0.0	1469/1480	99
SH 66T	KY694464.1	*Bacillus velezensis*	1508	17	1506	98	2721	0.0	1485/1490	99
SH 103T	NR_043083.1	*Bacillus Seohaeanensis*	1401	1	1401	100	2579	0.0	1400/1402	99
SH 8J	NR_104873.1	*Bacillus subtilis*	1538	12	1490	96	2724	0.0	1478/1479	99
SH 16J	NR_118438.1	*Bacillus drentensis*	1469	1	1456	99	2684	0.0	1455/1456	99
SH 35J	NR_109303.1	*Streptomyces qinglanensis*	1487	11	1473	98	2697	0.0	1462/1463	99
SH 87J	NR_104873.1	*Bacillus subtilis*	1538	8	1491	96	2734	0.0	1483/1484	99
SH 88J	NR_118467.1	*Streptomyces albus*	1499	5	1475	98	2455	0.0	1434/1482	97
SH 91J	NR_025235.1	*Mycolicibacterium poriferae*	1450	1	1447	99	2595	0.0	1433/1447	99
SH 99J	NR_025722.1	*Streptomyces africanus*	1488	1	1460	98	2636	0.0	1452/1463	99

Fig-1: Geographic map showing location of samples collection Saudi Arabia using Google earth software.

Fig-2: Different isolation sites from thuwal,Farasan and Jazan region

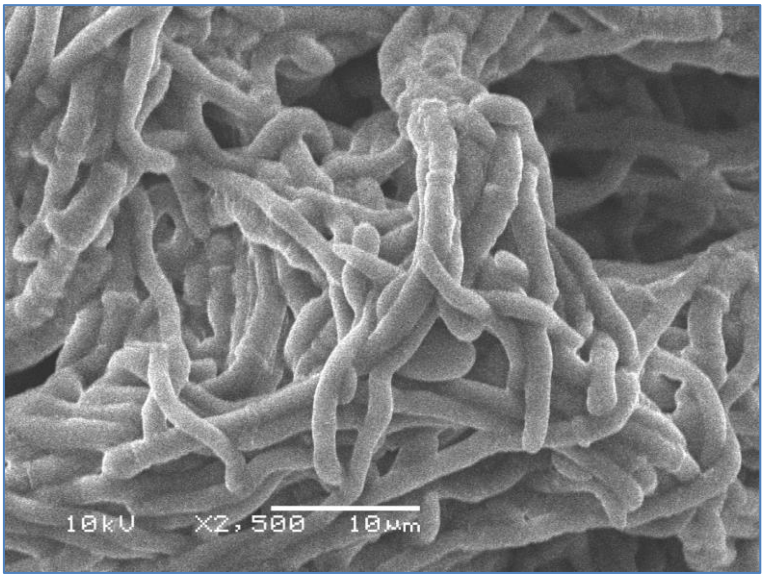

Fig-3: Electron micrograph of isolate (SH 88J)

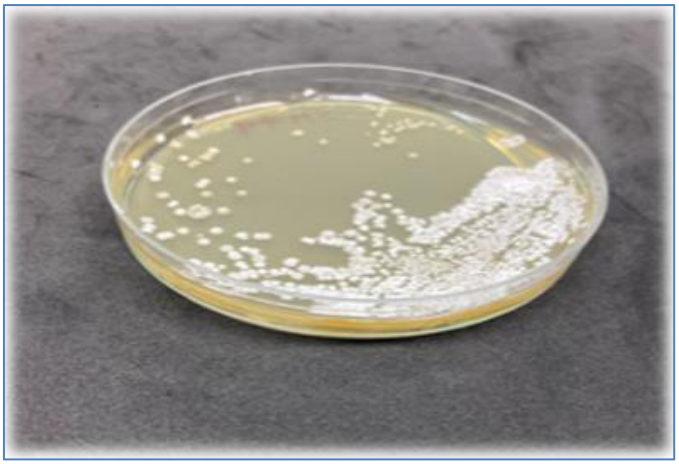

Fig-4: De-replication of isolate SH88J

Table-2: Antimicrobial screening of the bacterial isolates from different mangrove habitat in Saudi Arabia SH 87 J - SH 88 J – SH 91 J – SH 99 J

Bacterial isolate	*E.coli*	*S.typhi*	*C.candida*	*P.aeroginosa*
SH 5 T	+++	-	++	-
SH 6 T	++	-	+	++
SH 15 T	++	-	-	-
SH 33 T	-	-	-	+
SH 40 T	++	-	-	-
SH 42 T	-	-	-	-
SH 44 T	-	-	-	-
SH 66 T	++	-	+	-
SH 103 T	+++	+	+++	++
SH 8 J	-	-	-	+
SH 16 J	+	-	-	-
SH 35 J	-	+++	+++	++
SH 87 J	-	-	-	++++
SH 88 J	-	-	++++	+
SH 91 J	-	-	-	++
SH 99 J	-	-	-	-

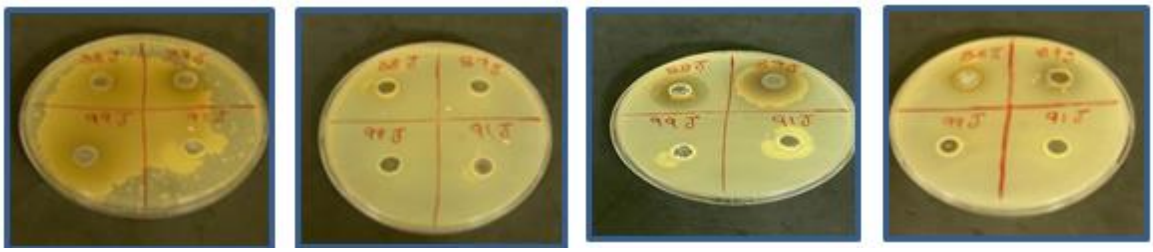

Fig-5: Inhibition zones of bacterial isolates against tested bacterial and fungal pathogens and isolate SH88J within them

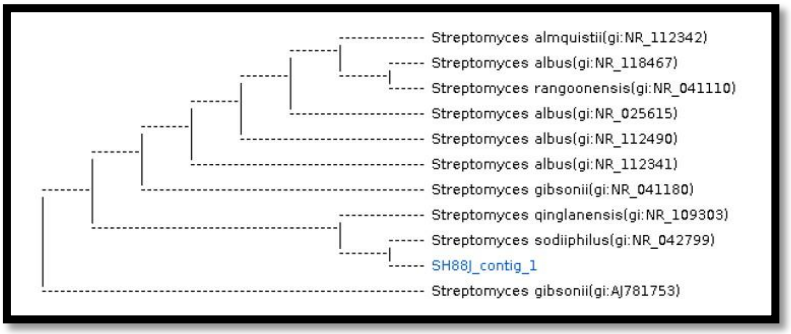

Fig-6: A neighbor joining tree of strain SH88J which show 97% similarity percentage to genus *Streptomyces albus* NR_112341

DISCUSSION

Endophytic bacteria and fungus are found in abundance on mangrove trees. Reviews of microbial secondary metabolites associated with mangrove plants have demonstrated that the secondary metabolites have interesting therapeutic effects (Cadamuro *et al*., 2021; Ancheeva *et al*., 2018; Xu *et al.*, 2014). Because of their bioactive secondary metabolites, mangrove endophytic bacteria have piqued the interest of pharmacological researchers, particularly when their host plants are also employed as native traditional/folk medicines (Bibi *et al*., 2020). Almost 1000 new natural products derived from mangrove associated microbes have been reported in the recent decade, with 850 derived from fungi and 120 derived from bacteria (the majority of which are generated from endophytes) (Ancheeva *et al*., 2018).

At the Arabian Gulf, mangroves can protect shorelines, reduce climate change, and provide natural habitat for wildlife species as well as resources for the local environment (Li *et al*., 2019). Nonetheless, modern activities and natural pressures pose a serious threat to mangrove ecosystems. The mangrove biome is ecologically significant because it contains a range of endophytes that are known to improve edaphic factor and mitigate the negative effects of soil (Ghosh *et al*., 2010).

The structure of the microbial community, including bacterial and archaeal species, is greatly influenced by environmental conditions. Microorganisms influence numerous biogeochemical cycles through their physicochemical and biological processes in the soil environment. A multitude of physicochemical parameters, particularly salt concentration, influence the microbial population in extreme habitats such as hypersaline soils (Canfora *et al.*, 2014). Identification of microorganisms in the soil environment provides a deeper understanding of the relationships between these microbial communities and aids in the decipherment of their members' ecological and/or biological roles.

Traditional methods of studying the microbial community of a saline soil environment, such as isolation, are biassed and do not reflect the true structure of the microbial community. This could be owing to the existence of non-cultivable bacteria, necessitating particular conditions for the cultivation of other microbes. The development of metagenomics techniques made it possible to profile microbial communities and investigate biodiversity in a given environment without relying on culture (Mirete *et al*., 2015). Several research have used this technique to investigate microbial diversity in a variety of soil settings. The field of metagenomics has benefited from the advancement of next-generation sequencing methods (Corneo *et al.*, 2013; Fujita *et al*., 2010; Zarraonaindia *et al*., 2015).

The 16S rRNA gene is best for profiling microbial diversity and establishing phylogenetic links between non-cultivable and undiscovered species. This type of structural metagenomics aids in deciphering and identifying the metabolic pathways and defining the geoecological roles to individual microbes of the entire population. As a result, the 16S rRNA-based metagenomics technique was used in the current work to investigate microbial diversity in three extreme habitats in Saudi Arabia. The research was carried out in Saudi Arabia's Farasan Islands, Thuwal, and Jazan regions.

Eight phyla of bacteria were identified in the same region; *Bacteroidetes*, *Firmicutes*, *Chloroflexi*, *Gemmatimonadetes*, *Proteobacteria*, *Acidobacteria*, *Actinobacteria* and *Planctomycetes*. Of these phyla, members of the phylum *Bacteroidetes* were the most dominant species followed by *Firmicutes* and *Proteobacteria.* The most common bacteria were *Bacteroidetes*, *Firmicutes*, and *Proteobacteria.* Our findings are supported by several metagenomic study studies from various saline soil ecosystems throughout the world. *Bacteroidetes*, *Gemmatimonadetes*, *Nitriliruptoria*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* dominated the microbial makeup of these research (Canfora *et al*., 2014; Ahmed *et al.*, 2018;Vera-Gargallo *et al.*, 2019). It should be emphasised, however, that certain habitats (e.g., severe conditions) may harbour more Eukarya or Archaea cells than Bacteria (Biddle *et al*., 2011).

Soil and plant samples were collected and put into appropriate isolation media in an attempt to isolate and identify bacteria and actinobacteria. On the basis of 16s rRNA and phylogenetic analysis, 16 isolates were chosen and identified. The majority of identified samples were for the genus *Bacillus* followed by *Streptomyces.* Other identified genera include *Larsenimonas* sp., *Kushneria* sp., and *Mycolicibacterium* sp. Several studies have documented the dominance of these species in hypersaline environments, particularly *Bacillus* spp. (Mukhtar *et al*., 2017). Genome investigations of Bacillus, Streptomyces, and Pseudovibrio have revealed that these genera' genomes contain a wide range of metabolic pathway genes, as well as genes connected to symbiosis and lifestyles, allowing for host switching and adaptation to different environments (Tian *et al*., 2016; Belbahri *et al*., 2017; Hernández-González *et al.*, 2018).

The potential pharmacological effects of endophytic bacterial extracts were investigated in this work. Because their extracts have antibiotic activity, the bioassays suggested that these endophytic bacteria could be viable sources of bioactive secondary metabolites.The antimicrobial assays showed that 13 out of 16 bacterial extracts exhibited antimicrobial activity against at least one of the tested microbes. Several extracts showed antimicrobial activity against multiple tested microbes, for example, *Bacillus Seohaeanensis* exhibited antimicrobial activity against the four tested microbes.

Zhou *et al*. (2019) identified three antimicrobial substances, fusolanone A-B and fusaric acid from the endophytic fungus *Fusarium solani* HDN15-410 from the plant *R. apiculata*. With MICs of 6.25–50 µg/mL, these compounds showed good antibacterial action against a wide range of pathogenic microorganisms, including *P. aeruginosa*, *Monilia albican*, *B. subtilis*, and *Vibrio parahaemolyticus.*

Chaeprasert *et al*. (2010) identified 1921 fungal endophytic isolates from ten mangrove plant species, including *R. apiculata*, *R. mucronata*, *Ceriops decandra*, *Sonneratia alba*, *Lumnitzera littorea*, *Avicennia alba*, *Acanthus ilicifolius*, *Xylocarpus granatum*, *Xylocarpus moluccensis*, and Klaiklay *et al*. (2013) discovered an antibacterial chemical, tremulenolide A, from the plant *R. apiculata* endophytic fungus *Flavodon flavus* PSU-MA201. With MICs of 128 µg/mL, the chemical showed antibacterial efficacy against *S. aureus* and *C. neoformans*.

CONCLUSION

In this study, sixteen bacterial and actinobacterial isolates belonging to five genera *Bacillus*, *Streptomyces*, *Larsenimonas*, *Kushneria*, and *Mycolicibacterium* were isolated from the soil and plant samples of the mangrove sites (Thuwal, Jazan region, and Farasan islands) in Saudi Arabia. In the current study, the 16S rRNA-based metagenomics approach has been applied to analyze the microbial profile of the investigated regions. Eight phyla of bacteria were identified in the same region; *Bacteroidetes, Firmicutes, Chloroflexi, Gemmatimonadetes, Proteobacteria, Acidobacteria, Actinobacteria* and *Planctomycetes*. Of these phyla, members of the phylum *Bacteroidetes* were the most dominant species followed by *Firmicutes* and *Proteobacteria.*

The selected isolates were identified based on 16s rRNA and phylogenetic analysis. The majority of identified samples were for the genus *Bacillus* followed by *Streptomyces.* The bacterial extracts of the were evaluated for their pharmacological properties. The antimicrobial assays showed that 13 out of 16 bacterial extracts exhibited antimicrobial activity against at least one of the tested microbes.

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