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Isolation and Identification of Bacteria from Natural and Planted Mangrove (Avicennia marina) Soils in Bahrain

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***Abstract*— Mangroves are critical coastal ecosystems, particularly in arid regions like Bahrain where Avicennia marina predominates. Studying bacterial diversity in natural and planted mangrove soils is vital because microbes drive nutrient cycling and ecosystem functioning. This study isolated and characterized bacteria from four mangrove sites, examining the influence of soil factors such as pH, salinity, temperature, and conductivity on microbial communities. Soil temperatures ranged from 26.5 to 32.8 ℃, with Ras Tubli showing the highest temperature. Measured salinity and conductivity values were unusually low compared to typical Avicennia marina and Bahrain mangrove conditions, likely due to delays in sample analysis, and therefore are not reliable. Soil pH was consistently alkaline (8.0–8.3), typical of coastal environments.**

Natural mangrove soils appeared to have higher bacterial diversity compared to planted sites, suggesting that ecosystem maturity could influence microbial community composition. Based on biochemical tests including IMViC (for Gram-negative bacteria), isolates were tentatively affiliated with genera such as Staphylococcus among Gram-positive cocci; Bacillus, Lactobacillus, and Clostridium among Gram-positive bacilli; and Citrobacter, Enterobacter, Klebsiella, Acinetobacter, Moraxella, and possibly Neisseria among Gram-negative bacteria. Antibiotic susceptibility testing of coccoid isolates revealed variable sensitivity patterns. However, culture-dependent methods limited the scope of bacterial identification and did not allow for specific species-level identification. These findings contribute to understanding microbial diversity in Bahrain's mangrove soils and highlight the value of molecular approaches such as 16S rRNA sequencing, for further investigation.

Keywords*— *Bahrain; mangrove soil; bacteria; IMViC; isolation; identification; Avicenna marina

I. INTRODUCTION

Microorganisms, or microbes, are the most prevalent and widely dispersed living organisms on Earth and are essential for the survival of all life forms. They can be broadly classified as prokaryotes, including bacteria and archaea, which lack a nucleus, or eukaryotes, such as algae, fungi, and protists, which have a defined nucleus. While only about 1% of bacteria are pathogenic, many others are beneficial or remain functionally unexplored [1]. Microbes can inhabit diverse environments, from extreme habitats such as hot springs, hypersaline waters, polar regions, and deserts to soils, where they play crucial roles in soil formation, fertility, and stability. In soils, microbial communities including bacteria, fungi, algae, and protozoa help bind particles, form aggregates, and mediate nutrient cycling. Their abundance and diversity are influenced by factors such as pH, salinity, temperature, and organic matter, while human, plant, and animal activities continually introduce additional microbial species, further shaping ecosystem productivity [2][3].

Mangroves are coastal ecosystems found in tropical and subtropical regions, recognized for their resilience to harsh saline, waterlogged, and muddy environments [4]. Historical records indicate that mangrove ecosystems were present in regions including Tylos, known today as Bahrain, highlighting their long-standing presence in the region [5]. Globally, mangroves occur in 118 countries and covered approximately 145,000 km² in 2020, with Asia hosting the largest proportion [6][7][8]. In the Persian Gulf, the primary species is Avicennia marina, which is found in Bahrain as well as the UAE, Saudi Arabia, Iran, Oman, Qatar, and Kuwait [9][5].

Mangrove ecosystems play many ecological roles, including supporting marine biodiversity, stabilizing coastlines, filtering sediment runoff, and storing carbon. Their specialized root structures create complex habitats that support fish, crustaceans, birds, and other marine life [10]. However, human activities such as coastal development and pollution continue to threaten mangrove health and biodiversity. In addition, mangroves contribute to natural pollution management, as planted areas can immobilize sediments contaminated with petroleum hydrocarbons, while the rhizosphere hosts diverse hydrocarbon-degrading bacteria, highlighting their potential for bioremediation [11][5]. Mangroves also have cultural, economic and medicinal value; as many mangrove plant parts are used in

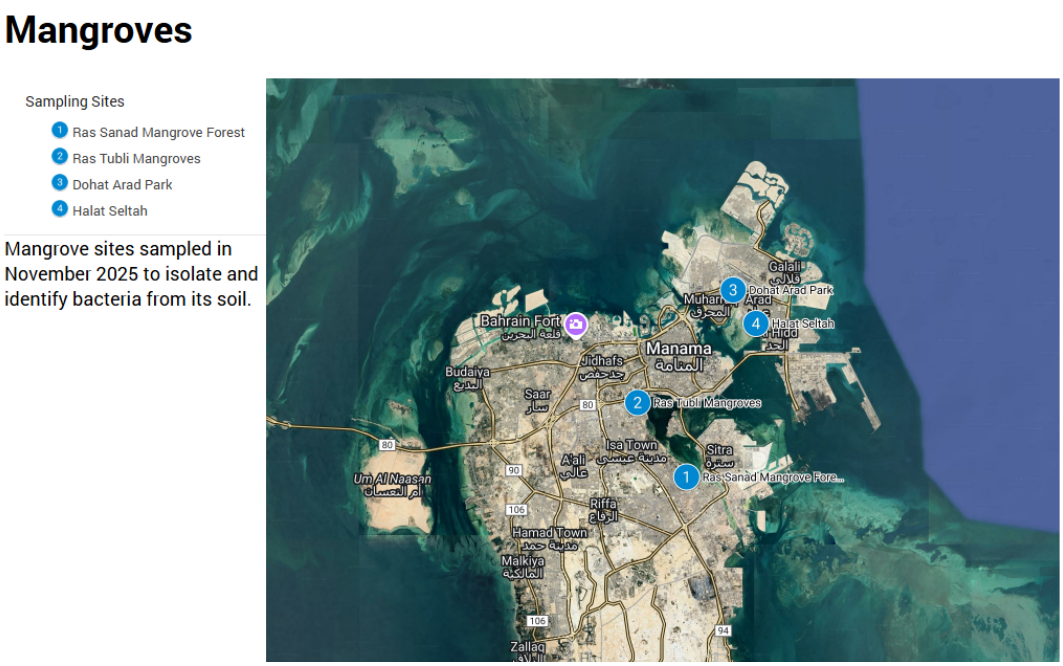

Figure 1. Sampling sites of natural (1&2) and planted (3&4) Avicennia marina mangroves in Bahrain.

traditional medicine, and mangrove-based ecotourism offers opportunities for sustainable income generation. However, such activities must be carefully managed to avoid negative impacts on the ecosystem [12].

Bahrain places a high priority on maintaining and growing mangrove ecosystems. The kingdom contains two natural mangrove sites that are found in Ras Sanad and Ras Tubli. Six planted mangrove areas, including Dohat Arad, Ras Hayan, west of Al Aker, Halat Umm al Bayd, the Mangrove Reserve at GPIC, and Halat Nuaim–Seltah. Additionally, there are mangrove nurseries at Askar and Ras Sanad, both of which have high salinities of about 45 ppt, whereas Tubli is an indoor nursery with a salinity of 0 ppt. The only mangrove species found in Bahrain is Avicennia marina, which is well suited to the salty and variable tidal conditions of the country. Furthermore, the Bahrain Mangroves Initiative reflects the kingdom's commitment to restoring mangrove cover, protecting coastal areas and enhancing marine biodiversity. Under the leadership of His Highness Shaikh Mohammed bin Salman bin Hamad Al Khalifa, the initiative aims to quadruple the number of mangroves in Bahrain by 2035, supporting the country's climate goals. This information was obtained from Appendix 1.

Bacteria are key components of mangrove ecosystems, driving essential processes such as organic matter decomposition and the transformation of ammonia, nitrites, and nitrates. Through these functions, they regulate nutrient availability and help sustain the productivity of the entire ecosystem. Microbial communities in mangrove soils, leaf litter, and surrounding waters are closely interconnected, collectively supporting nutrient cycling and overall ecosystem functioning [2][13][14]. Because soil microorganisms are strongly shaped by the habitats they occupy, understanding them requires considering the broader mangrove environment, which is among the most biologically active coastal systems. Together, this background highlights the ecological importance of mangroves and underscores the relevance of studying their microbial communities.

Despite their ecological importance, bacterial diversity in Bahrain's mangrove sediments remains poorly studied. Thus, this study aims to isolate and identify bacteria from natural and planted Avicennia marina soils in Bahrain, compare their diversity, and examine the influence of environmental factors such as pH, salinity, and temperature. Figure 1 shows the locations of the sampled mangroves for this study.

II. Materials and Methods

A. Sample Collection and Processing

Soil samples were collected from two natural mangrove sites (Ras Sanad and Ras Tubli) and two planted sites (Dohat Arad and Halat Al-Seltah). Sampling was conducted during low tide and the sediments were collected from a depth of approximately 5 cm using sterile, pre-marked spatulas and transferred into sterile glass bottles. The samples were immediately placed in a cooled icebox for transport. All samples were then transferred to the microbiology laboratory and stored at 4 °C until further processing. During sampling, soil temperature of each site was also recorded. [15][16]

B. Physical-Chemical Soil Analysis

Soil pH and electrical conductivity were measured using a 1:5 soil-to-water suspension. Soil was oven-dried at 35 °C for 24 hours and sieved (<2 mm). Then, 20 g of soil was mixed with 80 mL distilled water in a flask, shaken mechanically at 25 °C for 1 hour, and left to settle for 20–30 minutes. Readings were recorded by a calibrated conductivity meter first then pH meter (to avoid KCl leakage from pH reference electrode). Salinity was then calculated from EC readings. [17][18]

C. Bacterial Culturing and Isolation

Prior to sampling day, mannitol salt agar (MSA) was prepared as per manufacturer instructions by dissolving 55.5 g of MSA in 500 mL of distilled water, stirred then sterilized in autoclave at 121 ℃ and 15 lbs pressure for 15 minutes. Then it was poured aseptically into sterile petri dishes after cooling and stored in the fridge. Additionally, 1 L of seawater was collected, filtered using a laboratory vacuum-filtration setup (filter flask, air pump, and membrane filter) and sterilized by autoclaving before use. Part of the seawater was distributed into the serial dilution tubes.

After sampling, 1:10 serial dilution was performed by suspending 1 g of soil sample into 9 mL of sterilized seawater, well mixed and soil particles were allowed to settle down. Serial ten-fold dilutions (10^{-1} – 10^{-6}) of the supernatant using sterilized seawater. 0.1 mL of 10^{-2} - 10^{-6} were spread in MSA plates with a glass spreader. Incubated at 37 ℃ for 18 – 72 h. (plates should be checked because some bacteria grow slowly and the other have overgrowth in less than 24 h). This procedure was performed following general established methods [19], with slight modifications to better simulate the natural saline conditions of the mangrove environment and to select for salt-tolerant bacteria.

After observing grown colonies, different colonies were purified by sub culturing them into MSA media and incubated for ~18 h.

D. Bacterial Identififcation

For the bacterial identification, IMViC biochemical tests were applied to the gram-negative bacteria whereas catalase and lactose fermentation tests were done for the gram-positive ones.

Identification of gram-negative bacteria

Indole production test: 10 mL tubes of tryptone broth were inoculated with bacterial culture, incubated for 24 h at 37 ℃. After incubation, 5 drops of Kovac’ s reagent were added and shaken gently. E. coli was used as positive control and for the negative control, the tube wasn’t inoculated with anything. Positive results show cherry-red ring while negative ones don’t change. [20]

5 mL tubes of Buffered peptone-glucose (MR-VP) broth were prepared and inoculated with bacteria, incubated at 37 ℃ for 24-48 h. then 2.5 mL of each tube was transferred to another tube. One tube was used for MR test and the other for VP test. For the positive controls, E. coli was used for MR test and E. aerogenes was used for VP test.

Methyl red test: 5 drops of 0.05 % methyl red indicator were added to the 2.5 mL inoculated broth. Positive results have shown red color and yellow color was observed for negative ones.

Voges-Proskauer test: 12 drops of 5% α-naphthol (Barritt’s reagent A) and 4 drops of 40% KOH (Barritt’s reagent B) were added to the 2.5 mL inoculated broth. Next, the tubes were gently shacked and allowed to stand for at least 30 min. red color was observed for positive results while negative results did not show any color change. [21]

Citrate utilization test: Simmons citrate agar slants were prepared, and isolated colonies were streaked into the surface of the slant by a needle. Then incubated at 37 ℃ for 24 h. Negative samples remained green whereas positive ones turned blue Positive control was E. aerogenes. [22]

Identification of gram-positive bacteria

For the gram-positive bacteria biochemical tests, bacteria were inoculated from fresh isolates (18-24 h).

Catalase test (slide method): 3% H_2O_2 solution was prepared in the same day of performing test, a drop of the solution was added to the slide and a fresh well isolated colony was added to it by an inoculating loop. O2 bubble formation indicates positive results while nothing changes with negative samples. Staphylococcus aureus was used as a positive control while streptococcus was the negative control [23]

Lactose fermentation test: 10 mL of phenol red lactose broth with Durham tubes were filled in each tube, then bacterial culture was inoculated into them then they were incubated for 24-48 h at 37 ℃. Positive results show a yellow colour and gas production while negative ones remain red without any gas production [24]

All the biochemical tests medias, reagents and procedures were performed according to American society of microbiology protocols.

III. Results

Table 1 shows the physicochemical properties of soil samples from natural (Ras Sanad, Ras Tubli) and planted (Arad, Halat Al-Seltah) mangrove sites. Temperature ranged from 26.5 to 32.8 ℃, with Ras Tubli recording the highest value. Conductivity and salinity varied across sites, while pH remained consistently alkaline (8.05–8.34) in all samples.

Table 1. Soil physicochemical parameters measured at natural and planted mangrove sites in Bahrain.

Soil parameters/ sampling site	Natural		Planted	
	Ras Sanad	Ras Tubli	Arad	Halat Al-Seltah
Temperature (℃)	26.8	32.8	27	26.5
Conductivity (mS)	7.14	3.13	5.57	5.34
Salinity (ppt)	3.92	1.63	3.01	2.87
pH	8.07	8.16	8.05	8.34

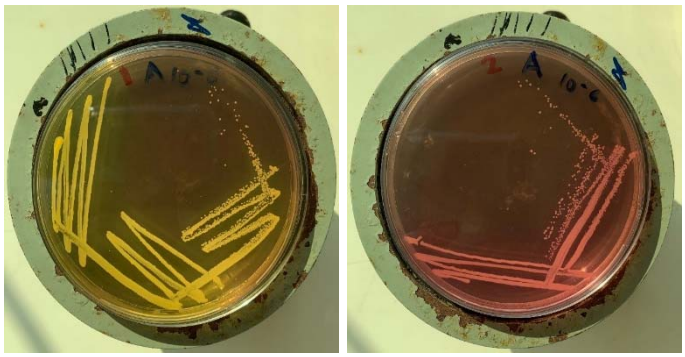

Figure 2. Isolated colonies on mannitol salt agar media (MSA).

As observed in figure 2, bacterial colonies were successfully isolated from mangrove soil samples, displaying distinct colony morphologies on agar plates. The 10^{-2} dilution yielded the most suitable colony density, while higher dilutions showed limited growth. Colony appearance varied in size, shape and growth rate, indicating bacterial diversity.

Microscopic examination of bacterial isolates showed variation in Gram reaction and cell shape (Figure 3). Isolate 3(a) was Gram-positive cocci, 3(b) Gram-positive bacilli, 3(c) Gram-negative cocci, and 3(d) Gram-negative bacilli. These results indicate the presence of both Gram-positive and Gram-negative bacteria with diverse morphologies in the mangrove soil samples.

Gram-positive isolates were primarily bacilli and cocci, showing positive catalase and lactose fermentation reactions (Table 2). Gram-positive bacteria were detected at all sites (7T, 10T, A1, and 7H) except Ras Sanad. At Ras Tubli, two bacilli

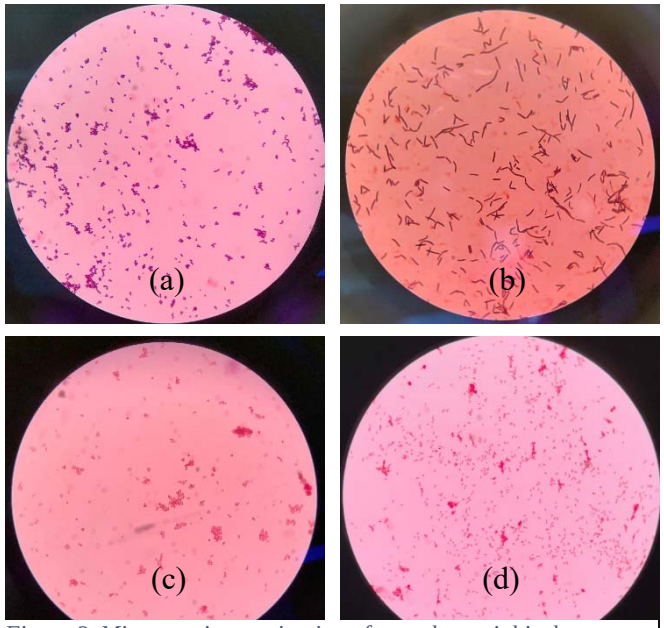

Figure 2. Microscopic examination of some bacterial isolates.

3 [(a).] gram positive, cocci, 3 [(b).] gram positive, bacilli, 3 [(c).] gram negative, cocci, 3 [(d).] gram negative, bacilli.

Table 2. Morphology of gram-positive bacteria and the biochemical tests applied on it (catalase and lactose fermentation).

Sampling site	Isolate / Biochemical Test	Gram staining	Morphology	Catalase	Lactose fermentation
Ras Tubli	**7T**	Gram +	Bacilli	++	+ Orange, no gas
	10T	Gram +	Bacilli	++	+ Orange, gas
Arad	**A1**	Gram +	Cocci	+	+ Orange
Halat Al-Seltah	**7H**	Gram +	Cocci	+	+ Orange

isolates exhibited similar biochemical responses, including catalase positivity and lactose fermentation. Both isolates produced oxygen bubbles in the catalase test, and both fermented lactose as indicated by colour change; however, only isolate 10T produced gas in the Durham tube, while 7T did not.

At planted mangrove sites, Gram-positive cocci were also isolated and showed positive catalase and lactose fermentation tests, but with less vigorous catalase activity compared to bacilli from Ras Tubli. Lactose fermentation produced an orange coloration, intermediate between the positive and negative controls.

Gram-negative isolates from Ras Sanad were mainly bacilli, with some displaying mixed bacilli and cocci morphology. Citrate utilization was negative for all isolates, while indole production was negative in S5, S13, and S14. Methyl Red (MR) and Voges-Proskauer (VP) tests showed no detectable growth, resulting in incomplete IMViC profiles for several isolates. Overall, the biochemical patterns suggest limited differentiation among isolates at this natural mangrove site.

Isolates from Ras Tubli exhibited mixed bacilli and cocci morphologies, with most showing positive citrate utilization (6T, 11T, 12T, 18T, and 19T). Indole production was mostly negative, except for isolate 12T, which showed a slightly positive reaction. MR results were positive only for isolate 6T, while VP results were largely negative or undetermined. These patterns indicate higher metabolic diversity in Ras Tubli compared to Ras Sanad.

At Dohat Arad planted mangrove site, isolate 2A showed negative results for all IMViC tests, whereas isolate 5A was positive for citrate but negative for indole, with MR and VP tests not determined. This limited biochemical variation suggests lower metabolic diversity in planted sites relative to natural mangrove sediments.

Table 3 IMViC (indole, methyl red, Voges-Proskauer and citrate utilization) patterns of gram-negative bacteria found in Natural (Ras Sanad and Ras Tubli) and planted (Dohat Arad and Halat Al-Seltah) planted mangrove reserves.

Sampling Site	Isolate / Biochemical Test	Gram staining	Morphology	Indole production	M.R.	V.P.	Citrate utilization
Ras Sanad (Natural)	S1	Gram -	Bacilli + cocci	ND	ND	ND	-
	S2	Gram -	Bacilli	ND	ND	ND	-
	S5	Gram -	Bacilli + cocci	-	ND	ND	-
	S13	Gram -	Bacilli	-	ND	ND	-
	S14	Gram -	Bacilli	-	ND	ND	-
	S17	Gram -	Bacilli	ND	ND	ND	-
Ras Tubli (Natural)	6T	Gram -	Bacilli + cocci	-	+	-	+
	9T	Gram -	Bacilli + cocci	ND	ND	ND	-
	11T	Gram -	Bacilli + cocci	-	ND	ND	+
	12T	Gram -	Bacilli + cocci	Slightly +	-	-	+
	18T	Gram -	Cocci	-	ND	ND	+
	19T	Gram -	Bacilli	-	ND	ND	+
Dohat Arad (Planted)	2A	Gram -	Cocci	-	-	-	-
	5A	Gram -	Bacilli	-	ND	ND	+
Halat Al-Seltah (Planted)	9H	Gram -	Bacilli	+	ND	ND	-
	10H	Gram -	Bacilli + cocci	-	ND	ND	-
	11H	Gram -	Bacilli + cocci	-	ND	ND	+
	13H	Gram -	Bacilli + cocci	ND	ND	ND	-
	15H	Gram -	Cocci	+	-	-	+
	20H	Gram -	Cocci	+	ND	ND	-
	27H	Gram -	Bacilli	Slightly +	ND	ND	-

Isolates from Halat Al-Seltah exhibited variable IMViC results, with indole-positive isolates including 9H, 15H, and 20H, and citrate-positive isolates including 11H and 15H. MR and VP tests were mostly undetermined, resulting in incomplete profiles. Overall, planted mangrove soils supported bacteria with moderate metabolic diversity. (Table 3)

Antibiotic susceptibility testing was performed on coccoid mangrove soil isolates that grew successfully in broth culture, including isolate S1 (Gram-positive cocci) and isolate S2 (Gram-negative cocci) (Figures 10–11, Table 7). Both isolates showed measurable zones of inhibition against all antibiotics tested. Neomycin and Novobiocin produced the largest mean inhibition zones (21.75 mm and 26 mm, respectively), indicating higher susceptibility, while Penicillin showed the smallest mean inhibition zone (12 mm), suggesting reduced effectiveness. Streptomycin showed intermediate activity (15.25 mm). Low standard deviation values indicate consistent responses between replicates.

Other bacterial morphologies, including Gram-positive and Gram-negative bacilli, did not grow in broth culture, preventing susceptibility testing. Therefore, antibiotic data are limited to coccoid isolates, restricting broader comparisons across bacterial types.

Table 4. Zones of Inhibition (mm) Created by Selected Antibiotics Against Bacteria Isolated from Soil.

Plate	**Neo (mm)**		**St (mm)**		**Nov (mm)**		**P (mm)**	
Replica	Replica (1)	Replica (2)	Replica (1)	Replica (2)	Replica (1)	Replica (2)	Replica (1)	Replica (2)
S 2	23	22	17	16	28	25	13	12
S 1	21	21	15	13	23	28	13	10
Mean	21.75		15.25		26		12	
Median	21.5		15.5		26.5		12.5	
Mode	21		N/A		28		13	
Std Deviation	0.957427108		1.707825128		2.449489743		1.414213562	

**These data of antibiotic susceptibility were provided by Mohammed Faisal (personal communication, 2025.*

IV. Discussion

The physicochemical soil parameters examined in this study are presented in Table 1. The variation in temperature across sites may be attributed to differences in sampling time environmental exposure, explaining the higher value observed at Ras Tubli. Salinity and conductivity varied across sites, with natural mangroves generally showing lower salinity than planted ones. However, the overall salinity values were lower than expected for mangrove ecosystems, likely due to delays between sampling and measurement affecting accuracy. Soil pH was consistently alkaline (8.0–8.3), aligning with typical coastal mangrove conditions [2]. Similarly, [9], along with [25], reported comparable pH (6.1–8.5), electrical conductivity (<36 mS/cm), and temperature (22 – 35 ℃) ranges, but substantially higher salinity values (27 – 38 PSU), where 1 PSU ≈ 1 ppt. This discrepancy highlights potential methodological limitations and emphasizes the importance of immediate sample processing in future studies.

Bacterial isolation from mangrove soil was performed using serial dilution and plating techniques to determine viable microbial populations. During the initial processing, serial dilutions (10^{-3}, 10^{-4}, and 10^{-5}) were prepared and plated; however, no bacterial growth was observed, likely due to delayed processing. Several alternative methods were tested, including incubated soil broth, double-fold dilutions, and swab sampling in physiological saline. These resulted either in heavy bacterial growth, which complicated isolation, or very low/no growth. When samples were processed on the same day as collection, colonies were successfully obtained. The 10^{-2} dilution produced the most suitable colony density, while 10^{-3} to 10^{-6} showed limited growth. Some bacteria grew rapidly within 18 – 24 hours at 37 ℃, whereas others required up to three days to appear.

Initial cultivation on nutrient agar did not support optimal growth. Improved growth was observed on Mannitol Salt Agar (MSA), likely because its high salt concentration (7.5% NaCl) better mimics the saline conditions of mangrove and seawater environments. Serial dilution was chosen as the primary isolation method because it provides a better representation of soil bacterial diversity compared to swab or broth-based methods, and the use of sterilized seawater helped simulate natural mangrove salinity.

Bacterial isolation and biochemical characterization revealed the presence of both Gram-positive and Gram-negative bacteria across all sampled sites, with Gram-negative bacteria dominating the mangrove soils. Although MSA is primarily selective for Gram-positive bacteria, it does not completely inhibit Gram-negative growth. The higher abundance of Gram-negative bacteria suggests the presence of salt-tolerant and halophilic microorganisms adapted to mangrove conditions [26].

Gram-positive isolates were primarily bacilli and cocci, showing positive catalase and lactose fermentation reactions. As shown in Table 2, Gram-positive bacteria were detected at all sites except Ras Sanad. At Ras Tubli, two bacilli isolates exhibited similar biochemical responses, including catalase positivity and lactose fermentation, with variation in gas production suggesting differences in fermentation pathways or efficiencies between isolates. At planted mangrove sites, Gram-positive cocci were also isolated, displaying catalase positivity and lactose fermentation, although with less vigorous catalase activity compared to bacilli from Ras Tubli. The intermediate colour change observed during lactose fermentation indicates moderate fermentation capacity. These variations likely reflect differences in microbial community

composition influenced by site-specific environmental conditions. The absence of Gram-positive bacteria at Ras Sanad may be attributed to differences in soil chemistry, microbial competition, or sampling limitations.

Comparisons with previous studies show strong agreement with the predominance of catalase-positive Gram-positive bacteria in mangrove environments. Studies [15] and [25] reported multiple Bacillus species, including *Bacillus subtilis, Bacillus licheniformis,* and *Bacillus* coagulans, which are Gram-positive and catalase-positive, consistent with the bacilli isolates observed in this study. Similarly, study [2ambeng] identified Gram-positive cocci such as *Staphylococcus* and *Macrococcus*, which also exhibit catalase activity, aligning with the cocci isolated from planted mangrove sites. Additionally, sediments from Avicennia marina mangroves have yielded Gram-positive genera including *Bacillus, Corynebacterium,* and *Listeria* [14], all catalase-positive, consistent with the biochemical traits observed in the present isolates. In the Ghabban study [27], Gram-positive genera such as *Propionibacterium, Corynebacterium,* and *Staphylococcus* were reported, supporting the presence of catalase-positive Gram-positive bacteria in similar saline environments. Furthermore, Bushra [28] reported *Pseudarthrobacter oxydans,* a Gram-positive, catalase-positive bacterium, which aligns with the traits observed in the present isolates. While these biochemical characteristics provide preliminary insight into isolate identity, they are insufficient for definitive classification. Among all reported genera, *Bacillus, Corynebacterium, Listeria, Propionibacterium, Staphylococcus,* and *Pseudarthrobacter* are most consistent with the observed traits, suggesting the present isolates may belong to similar halotolerant Gram-positive groups. Additional tests, including coagulase assays and molecular approaches such as 16S rRNA sequencing, are required for accurate identification.

Gram-negative bacteria isolated from Bahrain's mangrove soils exhibited site-specific metabolic variability(as shown in Table 3), though the dataset was limited due to incomplete IMViC results. Natural mangrove sites (Ras Sanad and Ras Tubli) showed higher apparent diversity than planted sites (Dohat Arad and Halat Al-Seltah). At Ras Sanad, most isolates were bacilli with negative citrate and mostly negative indole reactions, indicating adaptation to stable, nutrient-rich sediments but limited biochemical differentiation. Ras Tubli isolates displayed slightly higher metabolic diversity, with most isolates citrate-positive (6T, 11T, 12T, 18T, 19T) and one slightly indole-positive (12T), suggesting potential functional versatility. Planted sites exhibited fewer positive reactions and incomplete profiles, reflecting lower metabolic diversity and possibly fewer ecological niches. Overall, these results suggest tentative functional differentiation between natural and planted sites, though small sample size and limited biochemical testing prevent confident genus-level identification.

Comparison with literature provides context for observed patterns. A study reported only Gram-positive bacteria, with no Gram-negative isolates detected [15]. Other study [25] documented Gram-negative *Geminicoccus* and *Thermodesulfovibrio* in natural mangrove sediments, which may tentatively correspond to some Ras Sanad isolates. Diverse Gram-negative genera were reported in [14] *(Plesiomonas, Enterobacter, Aeromonas, Actinobacillus, Pseudomonas, Flavobacterium)* in *Avicennia marina* sediments, potentially related to citrate-positive and indole-positive isolates at Ras Tubli. Another study, found *Alteromonas, Vibrio,* and *Escherichia* in coastal mangroves [2], suggesting possible similarity with some planted-site isolates. *Achromobacter xylosoxidans, Citrobacter sp.,* and *Klebsiella quasipneumoniae* were reported in oil-polluted mangroves [5], which may align with some citrate-positive Ras Tubli isolates. Additional Gram-negative genera *(Dehalogenimonas, Dehalococcoides, Anaeromyxobacter, Desulfuromonas, Geobacter, Desulfomonile, Desulfovibrio, Shewanella)* were reported in contaminated sediments [29], highlighting potential functional diversity. Although some findings, such as greater IMViC diversity and clearer metabolic differentiation in natural sites (Ras Tubli), support the hypothesis that natural mangrove soils harbor higher bacterial diversity, the small number of isolates, incomplete profiles, and culture-dependent methods limit definitive conclusions. Overall, the presence of citrate-utilizing and indole-producing Gram-negative bacteria in natural sites emphasizes the ecological importance of undisturbed mangroves in supporting diverse microbial communities.

The observed variation in inhibition zones indicates differences in antibiotic response among the tested isolates, suggesting variability in susceptibility patterns within mangrove-associated bacteria. The larger inhibition zones produced by neomycin and novobiocin suggest higher effectiveness against the tested coccoid isolates, whereas the smaller inhibition zone for penicillin indicates comparatively lower activity. The intermediate response to streptomycin further reflects differential antibiotic activity. Low standard deviation values indicate consistent responses between replicates, supporting reliability.

However, since the isolates were not fully identified, it is not possible to classify them as susceptible, intermediate, or resistant based on established reference standards, which depend on the specific bacterial species. A key limitation is that antibiotic susceptibility testing was restricted to coccoid bacteria due to the inability of bacilli isolates to grow in broth culture, limiting comparisons across morphologies and broader conclusions about resistance patterns. In comparison, [16] reported measurable inhibition zones for multiple antibiotics, including tetracycline, erythromycin, carbenicillin, and aztreonam, demonstrating variability among environmental mangrove isolates. Similarly, [15] found that all tested isolates were sensitive to the antibiotics used, with some showing resistance to Penicillin G. While these studies provide context, we cannot definitively assign susceptibility categories to our isolates, as bacterial identity, prior antibiotic exposure, and culture conditions differ. Overall, the results suggest that mangrove-associated bacteria may display generally high susceptibility, but caution is required due to incomplete species-level identification and limited testing.

V. Conclusion

This study This study demonstrated that mangrove soils in Bahrain harbor both Gram-positive and Gram-negative bacteria, with Gram-negative isolates showing greater apparent metabolic diversity, particularly in natural mangrove sites. Physicochemical parameters were generally consistent across sites, with alkaline pH and comparable temperature and conductivity ranges, although salinity values were lower than expected due to methodological limitations.

Biochemical characterization indicated catalase-positive Gram-positive bacteria and metabolically variable Gram-negative bacteria. Gram-positive isolates may be related to genera such as Bacillus and Staphylococcus, while Gram-negative isolates may correspond to members of Enterobacteriaceae or other environmental groups; however, these identifications remain tentative due to limited data.

Antibiotic susceptibility testing showed variable inhibition patterns, but classification into susceptibility categories was not possible without species-level identification. Overall, the findings suggest functional diversity in mangrove bacterial communities, with natural sites potentially supporting greater variability. However, culture-dependent methods and incomplete data likely underestimated diversity, highlighting the need for molecular approaches such as 16S rRNA sequencing.

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References

[1] A. Gupta, R. Gupta, and R. L. Singh, "Microbes and environment," in Principles and Applications of Environmental Biotechnology, Singapore: Springer, 2016, pp. 43–84.

[2] N. Ambeng, H. Zubair, N. Oka, and A. Tonggiroh, "Isolation and characterization of bacteria from mangrove sediment at coastal area in Pangkep South Sulawesi," Journal of Physics: Conference Series, vol. 1341, no. 2, p. 022016, 2019.

[3] F. Buscot and A. Varma, Microorganisms in Soils: Roles in Genesis and Functions. Berlin, Germany: Springer, 2005.

[4] P. Saenger, "Introduction: The mangrove environment," Springer, 2002.

[5] A. Siddique et al., "Diversity of hydrocarbon-degrading bacteria in mangroves rhizosphere," Mar. Pollut. Bull., vol. 205, p. 116620, 2024.

[6] M. Jia et al., "Mapping global distribution of mangrove forests," Sci. Bull., vol. 68, no. 12, pp. 1306–1316, 2023.

[7] H. Thatoi, D. Samantaray, and S. K. Das, "The genus Avicennia: A review," Front. Life Sci., vol. 9, no. 4, pp. 267–291, 2016.

[8] J. Zhen et al., "Mangrove species classification using remote sensing," J. Remote Sens., vol. 4, 2024.

[9] Z. Farshid et al., "Reforestation of grey mangroves (Avicennia marina) along the Persian Gulf," Wetlands Ecol. Manage., vol. 31, no. 1, pp. 115–128, 2022.

[10] B. M. Wolf, "Ecosystem of the mangroves," 2012.

[11] P. Waryszak et al., "Planted mangroves cap petroleum-contaminated sediments," Mar. Pollut. Bull., vol. 171, p. 112746, 2021.

[12] D. A. Friess, "Ecotourism as a tool for mangrove conservation," Sumatra J. Disaster Geogr. Geogr. Educ., vol. 1, no. 1, pp. 24–35, 2017.

[13] M. N. Kannan et al., "Isolation and characterization of bacterial isolates from soil," J. Pharmacogn. Phytochem., pp. 108–110, 2018.

[14] N. Yulma et al., "Identification of bacteria from mangrove forest," IOP Conf. Ser.: Earth Environ. Sci., vol. 564, p. 012020, 2020.

[15] S. Bhat and L. Shewade, "Isolation and characterization of microorganisms from mangrove soil of CBD Belapur creek," Int. J. Environ. Sci., vol. 3, no. 6, pp. 2304–2312, 2013.

[16] J. Singh, S. Yadav, and A. Durve-Gupta, "Isolation and characterization of bacteria from mangrove soil," J. Emerg. Technol. Innov. Res., vol. 6, no. 5, pp. 37–38, 2019.

[17] Land Resources Division, Soil Analysis Methods and Results Interpretation Manual. 2011.

[18] G. Rayment and F. Higginson, Australian Laboratory Handbook of Soil and Water Chemical Methods. 1992.

[19] A. M. Khan, A Book on Experiments of Microbiology. Jaipur, India: Women University Press, Jayoti Vidyapeeth Women's University.

[20] M. P. MacWilliams, "Indole test protocol," American Society for Microbiology, 2009.

[21] S. McDevitt, "Methyl red and Voges–Proskauer test protocols," American Society for Microbiology, 2016.

[22] M. P. MacWilliams, "Citrate test protocol," American Society for Microbiology, 2009.

[23] K. Reiner, "Catalase test protocol," American Society for Microbiology, 2010.

[24] American Society for Microbiology, "Carbohydrate fermentation protocol," [Online]. Available: https://asm.org/ASM/media/Protocol-Images/Carbohydrate-Fermentation-Protocol.pdf

[25] F. N. Hamzah et al., "Characterization of Antimicrobial Properties of Bacillus spp. Isolated from Red Sea Mangrove Sediments in Jeddah, Saudi Arabia," Journal of Pure and Applied Microbiology, vol. 19, no. 2, pp. 1283–1296, May 2025, doi: 10.22207/jpam.19.2.30.

[26] A. Begum, R. Y. Shilpi, and A. Haque, "Isolation and identification of salt tolerant bacteria in Sundarbans mangrove forest," Int. J. Adv. Res. Biol. Sci., vol. 7, no. 5, pp. 40–56, 2020.

[27] H. Ghabban *et al.*, "Investigating the bacterial community of gray mangroves (Avicennia marina) in coastal areas of Tabuk region," *PeerJ*, vol. 12, p. e18282, Oct. 2024, doi: 10.7717/peerj.18282.

[28] R. Bushra, B. Uzair, A. Ali, S. Manzoor, S. Abbas, and I. Ahmed, "Draft genome sequence of a halotolerant plant growth-promoting bacterium Pseudarthrobacter oxydans NCCP-2145 isolated from rhizospheric soil of mangrove plant Avicennia marina," *Electronic Journal of Biotechnology*, vol. 66, pp. 52–59, Sep. 2023, doi: 10.1016/j.ejbt.2023.08.003.

[29] S. M. Alsharif, M. Ismaeil, A. M. Saeed, and W. S. El-Sayed, "Metagenomic 16S rRNA analysis and predictive functional profiling revealed intrinsic organohalides respiration and bioremediation potential in mangrove sediment," *BMC Microbiology*, vol. 24, no. 1, p. 176, May 2024, doi: 10.1186/s12866-024-03291-8.