Int.J.Curr.Microbiol.App.Sci (2022) *11*(04): 215-238

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 11 Number 04 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1104.030

Isolation and Abundance of Different Culturable Microbes from Mangrove Environments in Coastal Areas of Saudi Arabia

Muhammad Sohaib[1*], Fahad N. I. Al-Barakah[1], Hussein M. Migdadi[2] and Mazen Alyousif[1,3]

[1]Department of Soil Science, [2]Department of Plant Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia
[3]King Abdulaziz City for Science and Technology (KACST), Riyadh 11442, Saudi Arabia

**Corresponding author*

Keywords

Coastal areas, Culturable-microbes, Mangrove environments, Saudi Arabia, Arabian gulf, Red sea, Sediment

Article Info

Received:
12 March 2022
Accepted:
05 April 2022
Available Online:
10 April 2022

A B S T R A C T

Mangrove are native to unique stressful habitat and mangrove-associated microbes may be one of the reasons behind the survival of this plant in this habitat. Therefore, it is necessary to explore the microbial diversity from the unstudied mangrove ecosystem because exploration of microbial associates of mangroves may help full to screen such potential specialized microbial strains that are as unique as their habitat. Samples of 8 different locations along the Arabian Gulf and the Red Sea were collected for enumeration and isolation of microbes based on different general and selective agar mediums. The results revealed that the maximum colony counts of total (1.01×10^7 CFU mean /g dry soil), fungi (1.18×10^4 CFU mean /g dry soil), and actinomycetes (5.93×10^4 CFU mean /g dry soil) were observed in L1 (MRS soil), L44 (MRS soil), and L3 (MRS soil) respectively. While minimum colony counts of total (5.32×10^4 CFU mean/g dry soil), fungi (2.59×10^2 CFU mean/g dry soil) and actinomycetes (2.61×10 CFU mean/g dry soil) were observed in L47 (MS soil), L3 (MS soil), and L4 (MS soil) respectively. L3 (MRS soil), L2 (MRS soil and MS soil), L1 (MRS soil), L44 (MRS soil), and L5 (NMRS soil and NMS soil) showed the growth of all studied groups of microbes other than sulfur oxidizers, while water samples, L3 (MSW water), L2 (MSW water), L47 (MSW water), L4 (MSW water), and L1 (MSW water) showed growth of limited groups as compared to soil samples.

Introduction

Mangrove associated microbes colonize the mangrove soil (Al-Amoudi *et al.*, 2016; Ameen *et al.*, 2016), mangrove debris (Hodhod *et al.*, 2012), mangrove rhizosphere (Bibi *et al.*, 2017), roots (Alzubaidy *et al.*, 2016), pneumatophores (Toledo *et al.*, 1995), and leaves (Bhimba *et al.*, 2012). Below a thin layer of oxidized sediment (5-10 mm) at the forest floor surface mangrove soils are often anoxic but rarely sulfidic (Brodersen *et al.*, 2019). Large spatial patchiness in soil characteristics is typical within most mangrove stands. The coefficient of variation for many parameters (i.e., Eh and pH)

varies from 50% to 300%. The ratio of the below-ground to above-ground biomass for mangroves is high compared to other trees. It may be advantageous for arid zone trees to invest a more significant proportion of their biomass to develop an extensive root system to minimize salinity gradients; high soil salinities can pose problems for the water relations of mangroves. Alongi and Dixon (2000) distinguished between live and dead fine roots and found that <10% of fine roots were alive. Fine roots occur in the top meter of soil with equal distribution with soil depth, and dead roots accumulate below the 40 cm depth, which indicates that mangroves continuously produce new fine roots. As in other aquatic soils and sediments, heterotrophic prokaryotes dominate mangrove soils and above-ground roots numerically. Bacterial numbers range from 10^9 to 10^{11} cells per gram of dry sediment weight, while mangrove fungi abundance ranges from 10^3 to 10^7 colonies per gram of dry sediment (Alongi, 2005).

All free-living, rhizospheric, and endophytic mangrove associates play essential roles in host plant survival (Berg *et al.,* 2014; Molina *et al.,* 2012). These microbes feed on the organic nutrients present in the roots exudates and, in return, affect the roots and plant biology in certain ways, i.e., nitrogen fixation, phosphate solubilization, production of siderophores (sonaJanarthine *et al.,* 2011), phytohormones, and antibiotics (Do Carmo *et al.,* 2011; Samuel and Muthukkaruppan, 2011; Vazquez *et al.,* 2000). The relative abundance of the rhizospheric microbial community is highly dependent on the factor of host preference (D'Souza and Rodrigues, 2013). For example, the population of *Azotobacter* is high in the rhizosphere of *Avicennia* than in *Rhizophora*, while the phosphate solubilizing bacteria are more abundant in *Rhizophora* than in *Avicennia* (Selvam and Kathiresan, 2010). The diversity of arbuscular mycorrhizae (AM) fungi in wetland systems is lower than in terrestrial ecosystems (D'Souza and Rodrigues, 2013). Mangrove associated microbes providing the nutrients (i.e. N and P etc) to their hosts by transforming the dead mangroves roots and organic debris (Das *et al.,* 2006; Sahoo and Dhal, 2009). Different groups of bacteria get nutrients from mangrove debris and root exudates (Bano *et al.,* 1997) and, in return, benefit the mangrove ecosystem in so many ways (Holguin *et al.,* 2001). These bacteria take part in; the degradation of organic matter (Behera *et al.,* 2016), degradation of oil pollutants (Ameen *et al.,* 2016), metabolism of aromatic compounds (Alzubaidy *et al.,* 2016), nitrogen fixation (Toledo *et al.,* 1995), phosphate solubilization (Selvam and Kathiresan, 2010), photosynthesis (Vethanayagam, 1991), sulfate reduction (Bharathi *et al.,* 1991), methanogenesis (Mohanraju and Natarajan, 1992; Mohanraju *et al.,* 1997), agarolysis (Shome *et al.,* 2000), production of antibiotics (Bhimba *et al.,* 2012; Jose and Christy, 2013; Sundaram *et al.,* 2010), phytohormones (Ravikumar *et al.,* 2004; Samuel and Muthukkaruppan, 2011) and enzymes, which result in the high productivity (Das *et al.,* 2006; Kathiresan and Bingham, 2001; Tabao and Monsalud, 2010). Mangrove-associated bacteria are capable of many novel halotolerant enzymes, like amylase, cellulase, lipase, and protease (Bibi *et al.,* 2017). Enzyme production and antifungal activity of mangrove-associated bacteria also play an essential role in the defense against different biotic and abiotic stresses (Al-Amoudi *et al.,* 2016; Bibi *et al.,* 2017; Jose and Christy, 2013).

Mangrove forests generate a considerable amount of detritus in leaf litter and woody debris, which constitute an ideal environment for many detritus-dependent fungi (Abdel-Wahab *et al.,* 2016; Abdel-Wahab *et al.,* 2014). Fungal diversity of the mangrove is the best studied for discovering the new species of mangrove`s wood-inhabiting fungi, which constitute almost half of 450 species of known obligate marine fungi (Abdel-Wahab *et al.,* 2016; Purushothaman, 2000). Mangrove associated fungi are reported for biodegradation of diesel fuel hydrocarbons through the activity of Laccase, Catalase, Manganese dependent peroxidase (MnP) and Lignin peroxidase (LiP) (Ameen *et al.,* 2016). These are also reported for Phosphate solubilization, Succinic acid production (Vazquez *et al.,* 2000),

synthesis of nanoparticles (Kathiresan *et al.*, 2009), anticancer and antibacterial activities (Bhimba *et al.*, 2012), Mangrove-inhabiting fungi are categorized as 'manglicolous fungi', as a report of the fossil record from the west coast of India (Kumaran *et al.*, 2004).

Mangrove-associated bacteria and fungi also can tolerate the toxicity of heavy metals like Iron, Zinc, Lead, Magnesium, and Copper (Samuel and Muthukkaruppan, 2011).

There is a requirement to explore the microbial diversity from unstudied mangrove ecosystem because exploration of microbial associates of mangroves may help full of screening such potential specialized microbial strains which are as unique as their habitat (Hodhod *et al.*, 2012; Sahoo and Dhal, 2009), and may help to mitigate the effect of salinity in field crops. Therefore, this part of the study was conducted for enumeration, isolation, and preservation of mangrove-associated microbes for further studies.

Materials and Methods

Evaluation of microbial growth media

To choose the best microbial growth media for further study, a preliminary experiment was conducted to compare different agar media and techniques using mangrove soil, garden soil (as positive control), and sterilized sand (as negative control). Mangrove soil samples (S1 and S2) were obtained from Eastern Region, and garden soil sample (G) was collected from the garden of the Agriculture college.

Two media were evaluated for total counts, i.e., Nutrient Agar (NA) and Casein-peptone Soymeal-peptone Agar (CASO), three media were evaluated for fungi counts, i.e., Rose Bengal Chloramphenicol Agar (RB), Potato Dextrose Agar (PDA), and Czapek-Dox Agar, and three media were evaluated for Actinobacteria counts, i.e., Starch Casein Agar (SCA), Modified Starch Casein Agar (CMA), and Krainsky's Medium were used.

Both pour plate and spread plate techniques were used to evaluate these techniques on the microbial colony counts, total fungi, and actinomycetes under given conditions.

For the pour plate, we inoculated 1 ml of the desired dilution of the sample followed by sterilized media, while for the spread plate, we spread 0.1 ml of the desired dilution on the respective agar media plate (n=3). All the inoculated plates were incubated at 30 °C ± 2, and the number of colonies was recorded daily for up to 7 days. The number of colony-forming units were calculated by following the given equation;

Calculation for CFU were carried out by following;

CFU/g soil = (Number of colonies/volume of sample plated) × Dilution Factor

Sampling

Samples of mangrove rhizosphere soil (MRS), mangrove sediment soil (MS) as control (bulk soil), and mangrove sediment-water (MSW) were collected from different locations along the Arabian Gulf and the Red Sea, namely: Sanabis (L1), ArRabiayah (L2), An Nasim (L3), Saihat (L4), Jeddah (L47), Yanbu (L48), and Al Jamiyin (L5), according to standard methods. Three samples were collected in polythene bags using aseptic tools, then stored in the icebox and transported to the soil microbiology lab, Food and Agriculture College, KSU, for further processing.

Enumeration (CFU/g and CFU/ml)

In this study, we enumerated the cultivable mangrove-associated microbes. We followed the evaluated media for total, fungi, and actinomycetes for enumeration and isolation. Serial dilutions were prepared by distilled sterilized water by taking 10 g (for soil, i.e., MRS and MS) or 10 ml (for water, i.e., MSW) of each sample (individually) up to 10^6 dilutions. After that, 1 ml of the desired dilution was added to the petri dish, and pour the respective

sterilized cool (45 °C) media into petri dish. The following general-purpose and selective media were used for the enumeration of mangrove associated cultivable microbes: Total Bacterial Counts; by pouring 1/10thstrength of (NA) Nutrient Agar (Saseeswari *et al.,* 2015) in plates containing 10^{-4}, 10^{-5}, and 10^{-6} dilutions, Total Fungi; by pouring (RB) Rose Bengal (Chloramphenicol) Agar (Samuel and Muthukkaruppan, 2011) in plates containing 10^{-2}, 10^{-3}, and 10^{-4} dilutions, Actinobacteria; by pouring (SCA) Starch Casein Agar (Saravanakumar *et al.,* 2016) in plates containing 10^{-2}, 10^{-3}, and 10^{-4} dilutions. For enumeration of actinomycetes in soil, samples were prepared as per the method described by Basavaraj *et al.,* (2010); each sample was dried separately at 45 °C for one hour in hot air oven and then cooled to room temperature. The soil sample (1 g) was added to a conical flask containing 100 ml of sterile water and a few drops of Tween 80. The flasks were shaken for 30 minutes in an orbital shaker incubator at 27 °C, and their contents designated stock cultures, *Azotobacter* spp.; by pouring Winogradsky`s medium (Selvam and Kathiresan, 2010) in plates containing 10^{-3}, 10^{-4}, and 10^{-5} dilutions, *Azospirillum* spp.; by pouring Modified Nfb medium (Bashan *et al.,* 1993; Dobereiner *et al.,* 1976) in plates containing 10^{-3}, 10^{-4}, and 10^{-5} dilutions, Bacillus spp.; by pouring 1/10thstrength of NA in plates containing heat-treating dilutions i.e., 10^{-3}, 10^{-4}, and 10^{-5} at 100°C for 15 min prior to plating as described by Bashan *et al.,* (1993), Cellulose decomposers; by pouring 1/10thstrength of NA supplemented with 1% carboxymethyl cellulose (CMC) in plates containing 10^{-4}, 10^{-5}, and 10^{-6} dilutions. The plates were incubated at 30°C for almost one week. To detect cellulose activity, the plates were flooded with a 0.1% Congo red solution and incubated on an orbital shaker for 15 min, and washed with 1 M NaCl (Ngulube *et al.,* 2018). The positive activity was detected as a halo zone around bacterial colonies on CMC agar, Protease producing; by pouring 1/10thstrength of NA supplemented with 1% skim milk (Bibi *et al.,* 2017) in plates containing 10^{-4}, 10^{-5}, and 10^{-6} dilutions. The plates were incubated at 30°C for almost one week. Bacteria producing proteases, which formed a clear zone on skim milk agar plates, Amylase Producing; by pouring 1/10thstrength of NA supplemented with 1% starch (Bibi *et al.,* 2017), in plates containing 10^{-4}, 10^{-5}, and 10^{-6} dilutions. The plates were incubated at 30°C for almost one week. Amylase-producing bacteria showed starch hydrolysis as a clear zone on starch NA plates, Phosphate Solubilizing Bacteria; by pouring a modified Pikoviskaia Agar (Gupta *et al.,* 1994) in plates containing 10^{-4}, 10^{-5}, and 10^{-6} dilutions. *Pseudomonas* spp.; by pouring. King`s B medium (Samuel and Muthukkaruppan, 2011) in plates containing 10^{-3}, 10^{-4}, and 10^{-5} dilutions, and Sulfur oxidizer; by pouring Thiobacillus medium (Mishra *et al.,* 2012) in plates containing 10^{-3}, 10^{-4}, and 10^{-5} dilutions.

The poured plates were incubated at 30 ± 2 ºC for 24 – 48 hours and a maximum of up to 7 days for Actinobacteria, *Azotobacter*, PSB, Amylase producer, Cellulose decomposers, and Protease producers. Cycloheximide (50 µg/ml) was added to the medium (<55^0 C) before plating to reduce fungal contamination, while Chloramphenicol (0.1g/L) was used as a selective agent to suppress the growth of bacteria in respective media. After incubation, the individual colonies were counted for each plate, and colony-forming units (CFU /g or CFU / ml) were calculated using the following equation (Page, 1982; Somasegaran and Hoben, 1994)

CFU/g or CFU/ml = (Number of colonies/volume of sample plated) × Dilution Factor

For CFU/g dry soil sample:

CFU/g dry soil sample = CFU mean/Dry Weight of Soil

Where:

Dry weight of soil = Wet weight [1-(%H_2O, moist soil/100)]

%H_2O, moist soil = [(wet weight of soil – dry weight of soil)/dry weight of soil] × 100

Isolation

After incubation and counting of colonies, the morphologically different bacterial colonies were picked by sterilized needle from all plates individually and repeatedly cultured by streaking on nutrient agar to get pure isolates.

Storage of isolated culture

Purified bacterial isolates were cultured in Nutrient broth for 24-48 hours until turbidity. The broth culture was stocked in Cryovials (2.0 ml microcentrifuge tubes, Thermo Scientific) after mixing with 50% sterilized glycerol with a ratio of 1:1 and stored for a long time at -80°C to -70°C for further study, while for short period pure cultures were also maintained in Cryovials (2.0 ml microcentrifuge tubes, Thermo Scientific) at -20°C (Alarfaj *et al.*, 2015; Bibi *et al.*, 2017).

Results and Discussion

Evaluation of different media

Results, Table 1 and Figures 1 & 2, show the growth of total colony counts on two different media, nutrient agar (NA) and casein peptone soymeal-peptone agar (CASCO), done with two different techniques pour plate and spread plate. The control sterilized sand (Ctrl) did not show any growth in different media or techniques used. This confirms the success of the sterilization process for the media used and the reliability of the results. In comparison, the garden soil showed more colonies than mangrove sediments (S1 and S2) 80×10^6, 4.96×10^5, and 9.3×10^4, respectively. The pour plate showed uniform colony distribution and was more readable than the spread plate (Figure 1 & 2). NA media gave more diverse colonies based on morphology than the other media. The results also showed no difference in microbial numbers in different media used for counting.

Regarding the fungi total counts on the three different media (RB, PAD, and Czapeke –Dox Agar), in two different techniques, pour plate and spread plate, Table.2, Figures.3, 4 & 5, show that the control sample did not give any growth on any media. The garden soil showed maximum growth in all three media used. The spread plate gives comparatively more counts in all three media used, i.e., 910, 920, and 850 CFU/g soil. There is a significant effect of the technique on colony morphology. Pour plate showed more diverse growth, especially in mangrove samples (Figure 3 & 4).

As for the actinobacteria, Table.3 and Figures.6, 7 & 8 show similar results observed in the total colony counts and the fungi growth. The sterilized control sample showed no growth on any used media (SCA, CMA, Krainsky). However, the garden soil showed maximum growth of 71×10^2 in the SCA media used and the pour plate technique. The technique affects the colony morphology, the pour plate showed diverse and equally distributed colonies, but the spread plate showed uniform and overlapped colonies. The most diverse colony distribution of mangrove sediments was observed in the SCA media, pour plate technique compared to the spread plate (Figures.6, 7 & 8).

Enumeration of culturable microbial load in soil and water of mangrove

Table.4 shows the enumeration of the microbial load in the samples of mangrove plants in the study sites and locations in the Arabian Gulf and the Red Sea.

Total colony counts were developed on the NA media, total fungi developed on the RB media, and actinobacteria developed on the SCA media in CFU mean/g dry soil or CFU mean/ml water. Total counts were observed in all samples, while fungi showed zero growth in all of the samples except L2 (MS soil), L3(MS soil), and L5 (NMRS soil) in the Arabian Gulf and at L47 (MS soil), and L44 (MRS soil) at the Red Sea. Total microbial counts were higher in all tested samples than in actinobacteria and fungi. Maximum colony counts were observed at L1. MRS soil (10.1×10^6 CFU/g dry soil),).

While minimum colony counts (0.21×10^6 CFU/g dry soil) were observed at site L3. Mangrove samples water almost gives the lowest count at all sampling sites and locations. Maximum fungi were also observed at the L44 site (11.80×10^3 CFU/g dry soil). The actinobacteria maximum value was found at site L3 (MRS soil) (59.30×10^3 CFU/g dry soil). In comparison, the actinobacteria minimum value was observed at site L2 (0.005×10^3 CFU/g dry soil). The total colony counts, actinomycetes, and fungi values were always more minor than found in soil. In our study, we found that the order of microbial growth was total colony numbers followed by the actinobacteria and then the fungi. Maybe this is a result of a lack of air from time to time due to water immersion on a plant or the sensitivity of most fungi to salinity compared to some types of bacteria and actinobacteria. By comparing the total numbers between the sites on the east coast of the Kingdom and the west coast, we find that the numbers on the east coast give a higher value than on the west coast. Again the reason for the difference may be related to the effect of water characteristics and quality between the Sea and the Gulf or as a result of the difference in climatic conditions between the two regions.

Table.5 and Table.6 revealed the results regarding enumeration (CFU mean/g dry soil or CFU mean/ml water) of some diazotrophic bacteria (i.e., *Azotobacter*, *Azospirillum*, *Bacillus*, and *Pseudomonas*) and some of the enzymatic producer bacteria (i.e., Cellulose decomposer, Protease producers, Amylase producers, Phosphate solubilizers, and sulfur oxidizers) at the eastern region (Arabian Gulf). *Pseudomonas* was observed in all samples from the soil and water of mangrove plants from the collected samples in the East Region of Saudi Arabia (Arabian Gulf), followed by *Azotobacter*, *Azospirilum*, and *Bacillus*, which was not observed at L2 (MSW water) and L4 (MS soil). Sulfur oxidizers were not observed in any sample under study. L3 (MRS soil), L2 (MRS soil and MS soil), L1 (MRS soil), L44 (MRS soil), and L5 (NMRS soil and NMS soil) showed the growth of all studied groups of microbes other than sulfur oxidizers, while water samples, L3 (MSW water), L2 (MSW water), L47 (MSW water), L4 (MSW water), and L1 (MSW water) showed growth of limited groups as compared to soil samples.

Similarly, the diazotrophic bacteria and enzymes producer isolated from the western region locations sites (Red Sea) took the same path, except that the *Azosirillum* spp. were higher than the *Azotobacter* spp. More research is needed to find out the reason for this difference.

Preservation

On the basis of colony morphology, identical colonies were purified by repeated streaking, and these pure isolates were preserved at -80 °C for further studies.

Mangroves grow in unique inter-tidal environments, and mangrove sediments are rich in salinity and other abiotic stress. The high salinity may have restricted the growth of microbial populations compared to other soils. Therefore, choosing the appropriate media for isolation and studying the microbial community for such a habitat requires an appropriate selection from the different media used.

Despite much research in this field, the results are somewhat conflicting and confusing. For example, some use nutrient agar for the growth of total colony counts from mangrove soil (Kumar *et al.,* 2007; Ambeng *et al.,* 2019) while some use Tryptic Soya Agar (TSA) (Pupin and Nahas, 2014).In comparison, others preferred Zobell's marine agar (ZB agar), and synthetic seawater media (Sakhia *et al.,* 2016; Saravanakumar *et al.,* 2016; Schut *et al.,* 1993). Our results suggested that the NA media is suitable for counting and isolating microbes from mangrove soil and sediments. This matches well with what was found by Ambeng *et al.,* (2019), who used nutrient agar media to grow total colony counts from mangroves.

Table.1 Total Counts (CFU/g soil) in mangrove soil vs garden soil

Sample	Pour plate		Spread plate	
	NA	CASO	NA	CASO
	× 10^5	× 10^5	× 10^5	× 10^5
Ctrl	nd	nd	nd	nd
G	800	760	780	770
S1	4.96	5.52	3.50	7.90
S2	0.93	1.20	2.17	1.88

Where; NA= Nutrient Agar, CASO= Casein peptone soymeal-peptone agar, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment and nd= not detected

Table.2 Fungi Counts (CFU/g soil) in mangrove soil vs garden soil

Sample	Pour plate			Spread plate		
	RB	PDA	Czapek-Dox	RB	PDA	Czapek-Dox
Ctrl	nd	nd	nd	nd	nd	nd
G	630	610	900	910	920	850
S1	10	4.00	9.00	1	9	5
S2	1.00	nd	1.00	nd	nd	nd

Where; RB= Rose Bengal Chloramphenicol Agar, PDA= Potato Dextrose Agar, Czapek-Dox = Czapek-Dox Agar, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment and nd= not detected

Table.3 Actinobacteria Counts (CFU/g soil) in mangrove soil vs garden soil

Sample	Pour plate			Spread plate		
	SCA	CMA	Krainsky	SCA	CMA	Krainsky
	× 10^2		× 10^2	× 10^2		× 10^2
Ctrl	nd	nd	nd	nd	nd	nd
G	71.0	60.0	39.5	70.0	25.0	5.25
S1	1.96	2.00	3.63	17.5	70.0	19.0
S2	32.0	nd	0.91	1.10	nd	4.40

Where; SCA= Starch Casein Agar, CMA= Modified Starch Casein Agar, Krainsky = Krainsky's Agar medium, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment and nd= not detected

Table.4 Enumeration of Total, Fungi and Actinobacteria; CFU mean/g dry soil for soil samples and CFU mean/ml water for water sample

Location	Area	Sample Type	CFU mean/g dry soil or ml water		
			Total $\times 10^6$	Fungi $\times 10^3$	Actinobacteria $\times 10^3$
L3	An Nasim, Saihat	MRS Soil	1.76	nd	59.3 0
		MS Soil	5.82	0.26	0.005
		MSW Water	0.21	nd	nd
L2	ArRabiayah, Tarut	MRS Soil	1.42	nd	0.005
		MS Soil	0.66	0.28	0.012
		MSW Water	0.19	nd	nd
L47	As Sahil, Jeddah	MRS Soil	0.49	nd	9.63
		MS Soil	0.053	2.85	nd
		MSW Water	0.22	nd	nd
L4	Khaleej Road, Saihat	MRS Soil	0.37	nd	10.70
		MS Soil	0.29	nd	0.026
		MSW Water	0.40	nd	0.19
L1	Sanabis, Tarut	MRS Soil	10.10	nd	6.94
		MS Soil	0.24	nd	2.27
		MSW Water	0.19	nd	nd
L48	Yanbu, Saudi Arabia	MRS Soil	0.70	nd	12.30
		MS Soil	0.64	nd	0.033
L44	Dahaban, Saudi Arabia	MRS Soil	8.25	11.80	nd
L5	Al Jamiyin, Tarut	NMRS Soil	0.68	6.21	0.033
		NMS Soil	0.59	nd	2.28

Where; MRS: Mangrove Rhizosphere, MS: Mangrove Sediment, MSW: Mangrove Sediment Water, NMRS: Non-Mangrove Rhizosphere NMS: Non-Mangrove Sediment, nd: not detected

Table.5 Enumeration of diazotrphic bacteria and Enzymes producer, CFU mean/g dry soil for soil samples and CFU mean/ml water sample in the Eastern Region, Saudi Arabia.

Location	Area	Sample Type	*Azotobacter*× 10^5	*Azospirillum*× 10^4	*Bacillus*× 10^4	Cellulose decomposer × 10^4	Protease Producers × 10^4	Amylase Producers × 10^4	Phosphate Solubilizes × 10^5	*Pseudomonas* × 10^5	Sulfur Oxidizer
L3	**An Nasim, Saihat**	MRS Soil	3.58	0,240	5.02	1.96	0.873	26.6	0.0437	0.502	nd
		MS Soil	0.236	0.173	0.330	0.472	7.31	9.67	nd	7.26	nd
		MSW	nd	nd	0.167	nd	nd	0.0167	nd	2.03	nd
L2	**ArRabiayah, Tarut**	MRS Soil	0.227	9.07	1.05	18.3	5.27	14.1	3.54	5.47	nd
		MS Soil	0.00161	4.44	0.322	0.921	0.230	0.463	0.253	0.820	nd
		MSW	nd	nd	nd	nd	nd	nd	nd	1.16	nd
L4	**Khaleej Road, Saihat**	MRS Soil	0.00259	0.0259	5.18	nd	nd	0.259	nd	0.308	nd
		MS Soil	0.00522	nd	nd	26.1	nd	nd	nd	0.295	nd
		MSW	nd	nd	0.183	nd	nd	0.00167	nd	3.82	nd
L1	**Sanabis, Tarut**	MRS Soil	5.02	32.4	2.02	20.2	303	36.2	1.44	68.3	nd
		MS Soil	0.00202	0.0809	2.04	2.02	nd	nd	nd	0.285	nd
		MSW	nd	nd	0.0167	1.85	nd	nd	nd	0.647	nd

Where; MRS: Mangrove Rhizosphere, MS: Mangrove Sediment, MSW: Mangrove Sediment Water, NMRS: Non-Mangrove Rhizosphere NMS: Non-Mangrove Sediment, nd: not detected

Table.6 Enumeration of diazotrphic bacteria and Enzymes producer, CFU mean/g dry soil for soil samples and CFU mean/ml water sample in the Western Region, Saudi Arabia.

Location	Area	Sample Type	*Azotobacter* × 10^5	*Azospirillum* × 10^4	*Bacillus* × 10^4	Cellulose decomposer× 10^4	Protease Producers × 10^4	Amylase Producers × 10^4	Phosphate Solubilizes × 10^5	*Pseudomonas* × 10^5	Sulfur Oxidizer
L47	**As Sahil, Jeddah**	MRS	0.0203	0.00452	0.0904	0.678	nd	0.904	nd	0.543	nd
		MS Soil	0.00114	0.00760	0. 07.60	0.190	nd	3.99	0.0190	0.327	nd
		MSW	nd	nd	1.67	nd	nd	nd	1.92	2.05	nd
L48	**Yanbu, Saudi Arabia**	MRS Soil	0.0365	nd	0.153	1.11	0.836	0.0138	nd	5.07	nd
		MS Soil	0.00233	nd	2.35	0.266	0.0115	22.3	0.255	3.60	nd
L44	**Dahaban, Saudi Arabia**	MRS Soil	3.80	61.8	1..3	98.4	115	126	5.48	11.6	nd
L5	**Al Jamiyin, Tarut**	NMRS Soil	0.497	4.35	2.37	6.21	5.77	0.888	2.71	1.70	nd
		NMS	1.23	2.73	4.53	0.601	24.5	2.41	0.220	3.18	nd

Where; MRS: Mangrove Rhizosphere, MS: Mangrove Sediment, MSW: Mangrove Sediment Water, NMRS: Non-Mangrove Rhizosphere NMS: Non-Mangrove Sediment, nd: not detected

Fig.1 Growth of total colony counts of mangrove soil vs garden soil on Nutrient Agar. Where; NA= Nutrient Agar, sediment, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment, P = Pour plate, and S = Spread Plate.

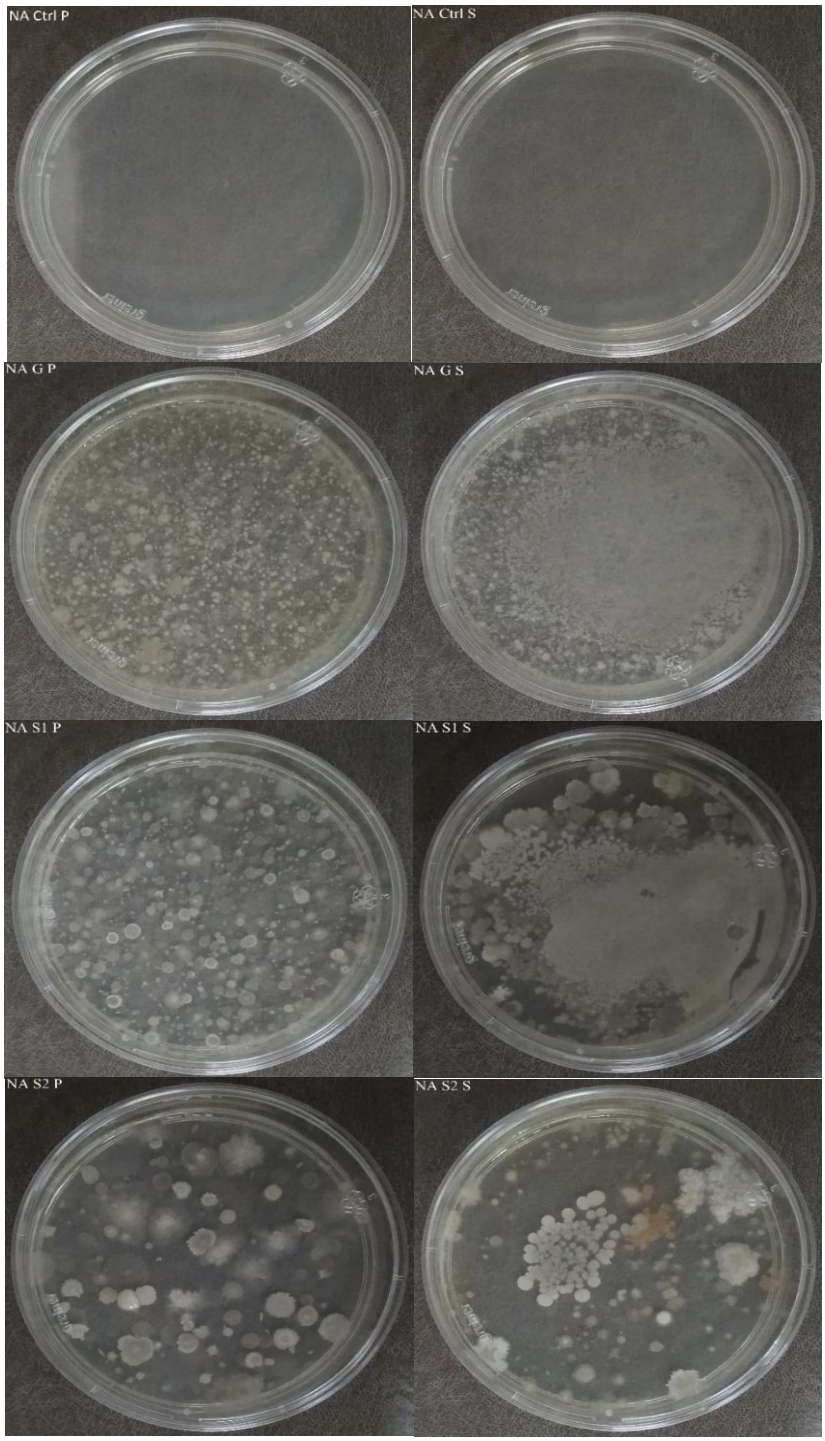


Fig.2 Growth of total colony counts of mangrove soil vs garden soil on CASO. Where; CASO= Casein peptone soymeal-peptone agar, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment, P = Pour plate, and S = Spread Plate.

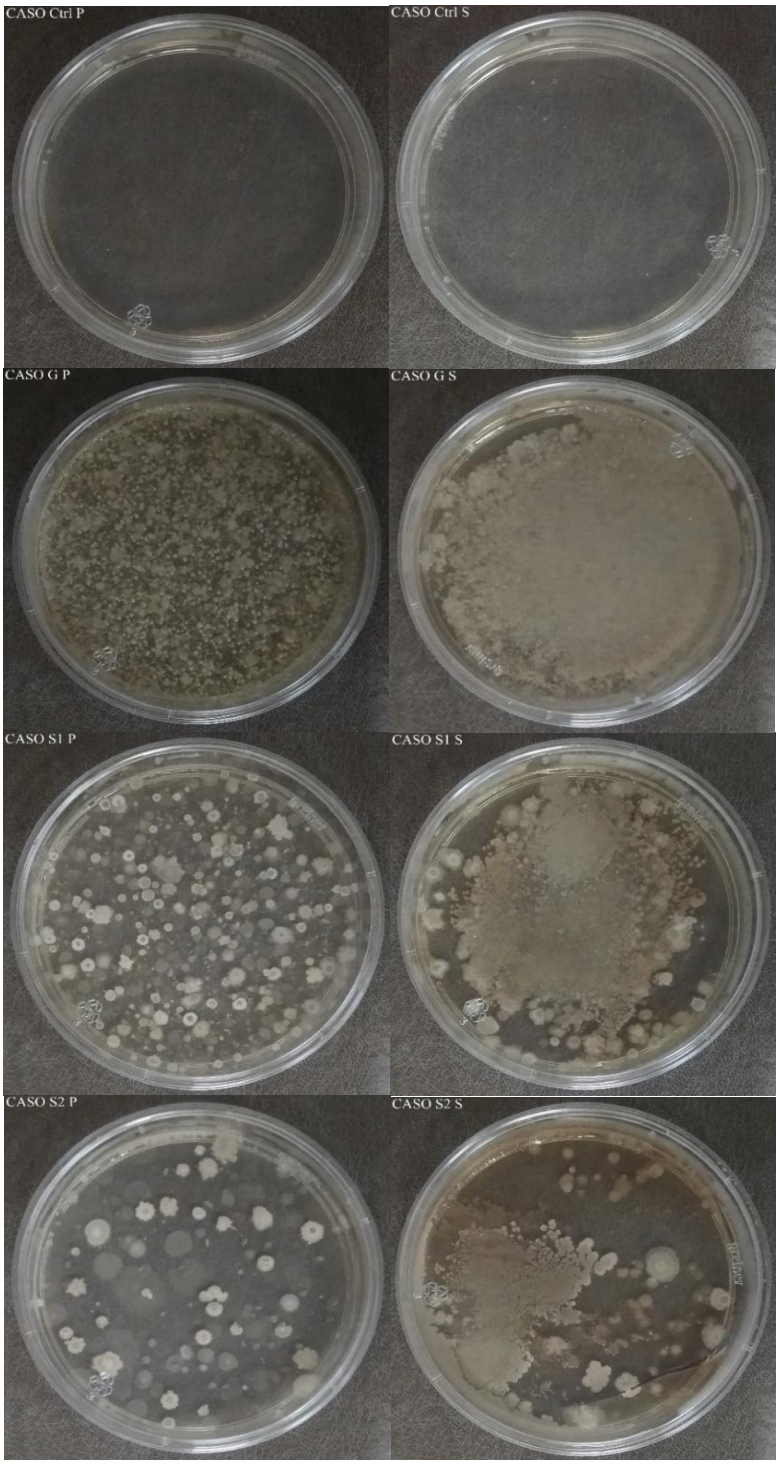


Fig.3 Growth of total fungi of mangrove vs soil garden on RB. Where; RB= Rose Bengal Chloramphenicol Agar, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment, P = Pour plate, and S = Spread Plate.

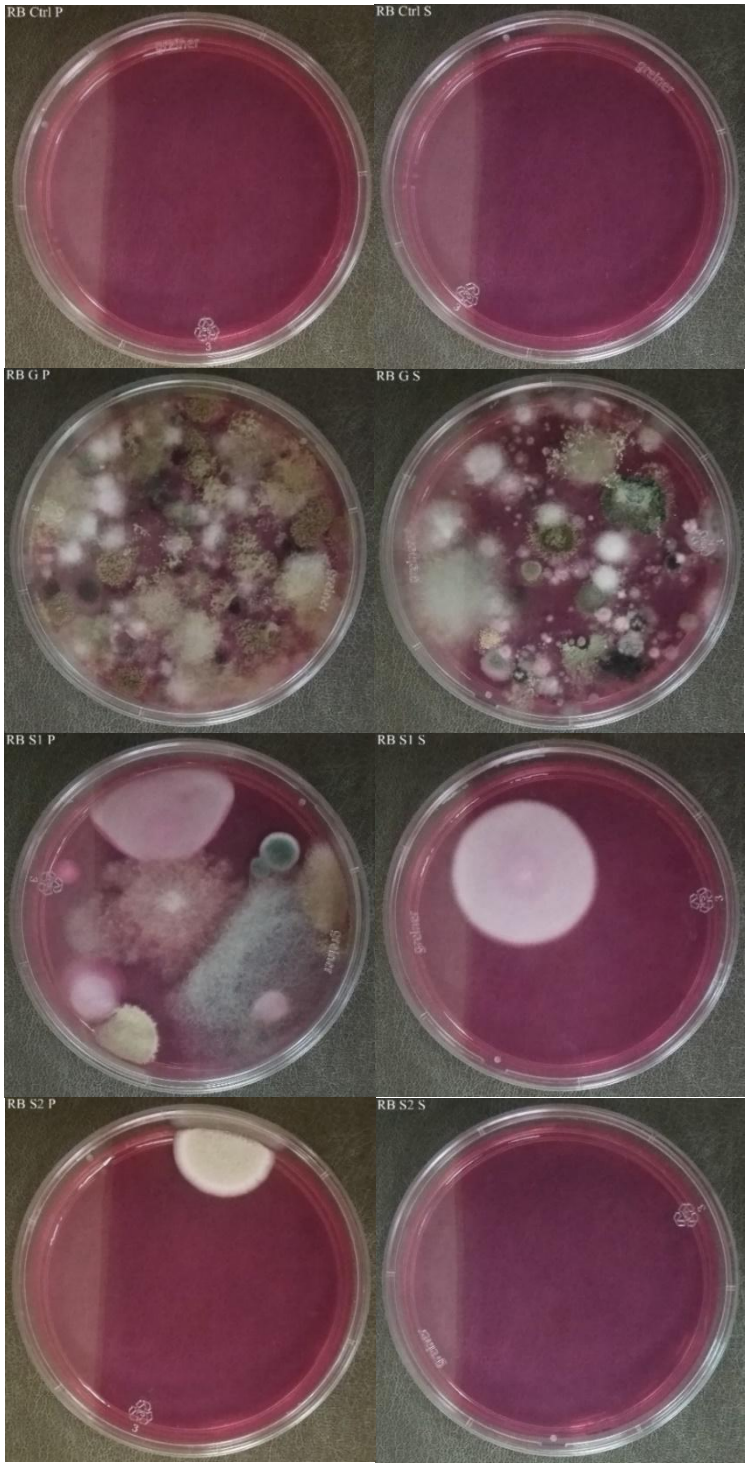


Fig.4 Growth of fungi from mangrove soil vs garden soil in PDA. Where; PDA= Potato Dextrose Agar, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment, P = Pour plate, and S = Spread Plate.

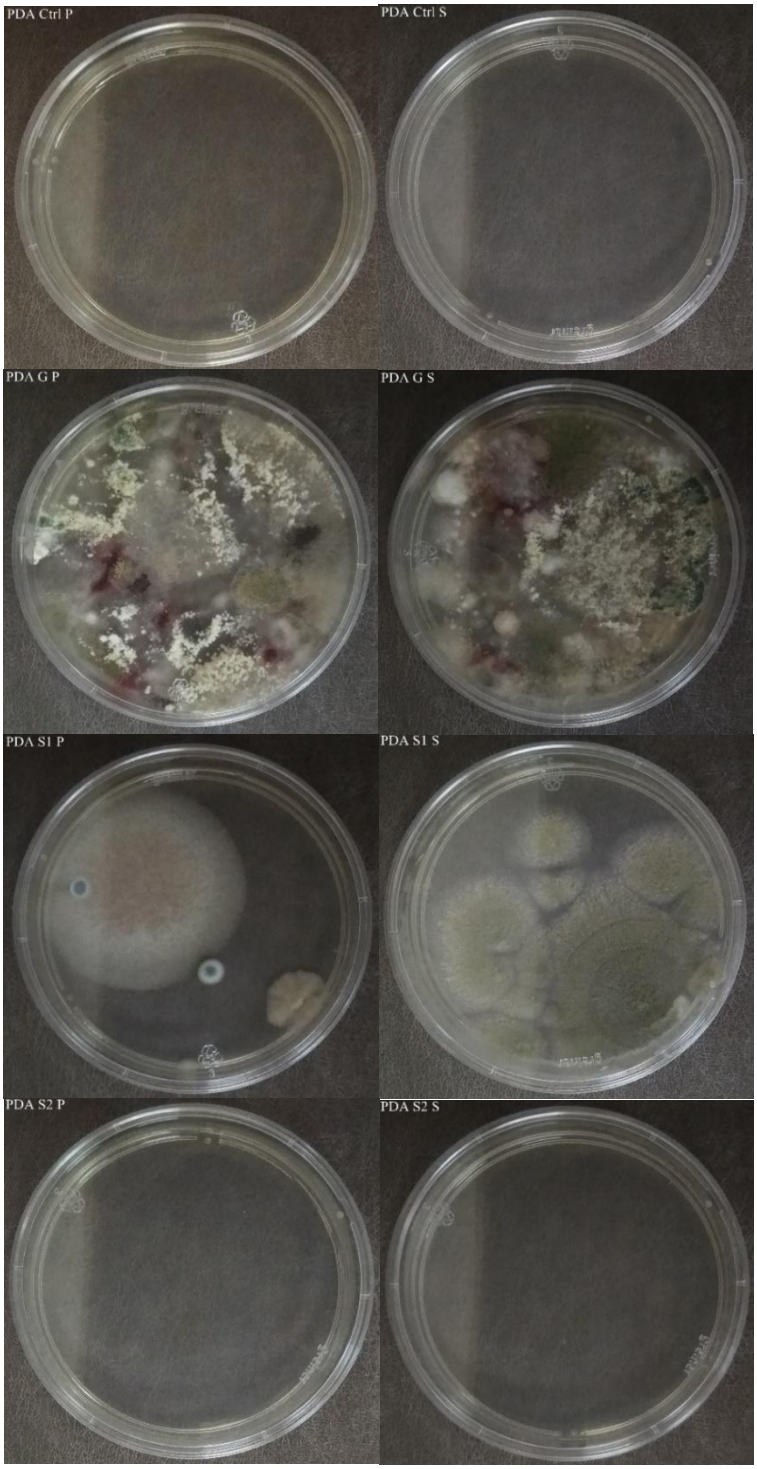


Fig.5 Fungal growth of mangrove soil vs garden soil on Czapek-Dox. Where; Czapek-Dox = Czapek-Dox Agar, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment, P = Pour plate, and S = Spread Plate.

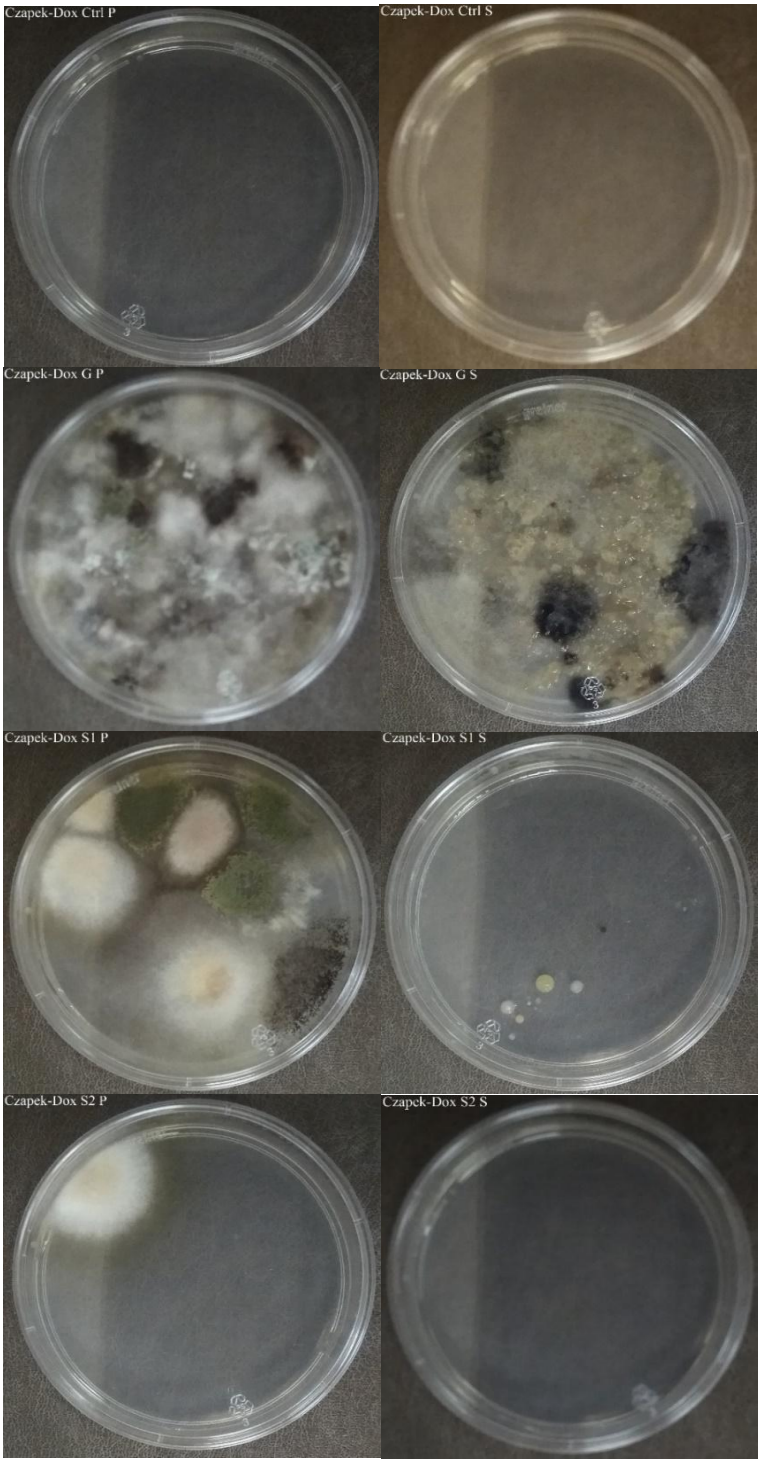


Fig.6 Actinobacteria growth of mangrove soil vs garden soil in SCA. Where; SCA= Starch Casein Agar, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment, P = Pour plate, and S = Spread Plate.

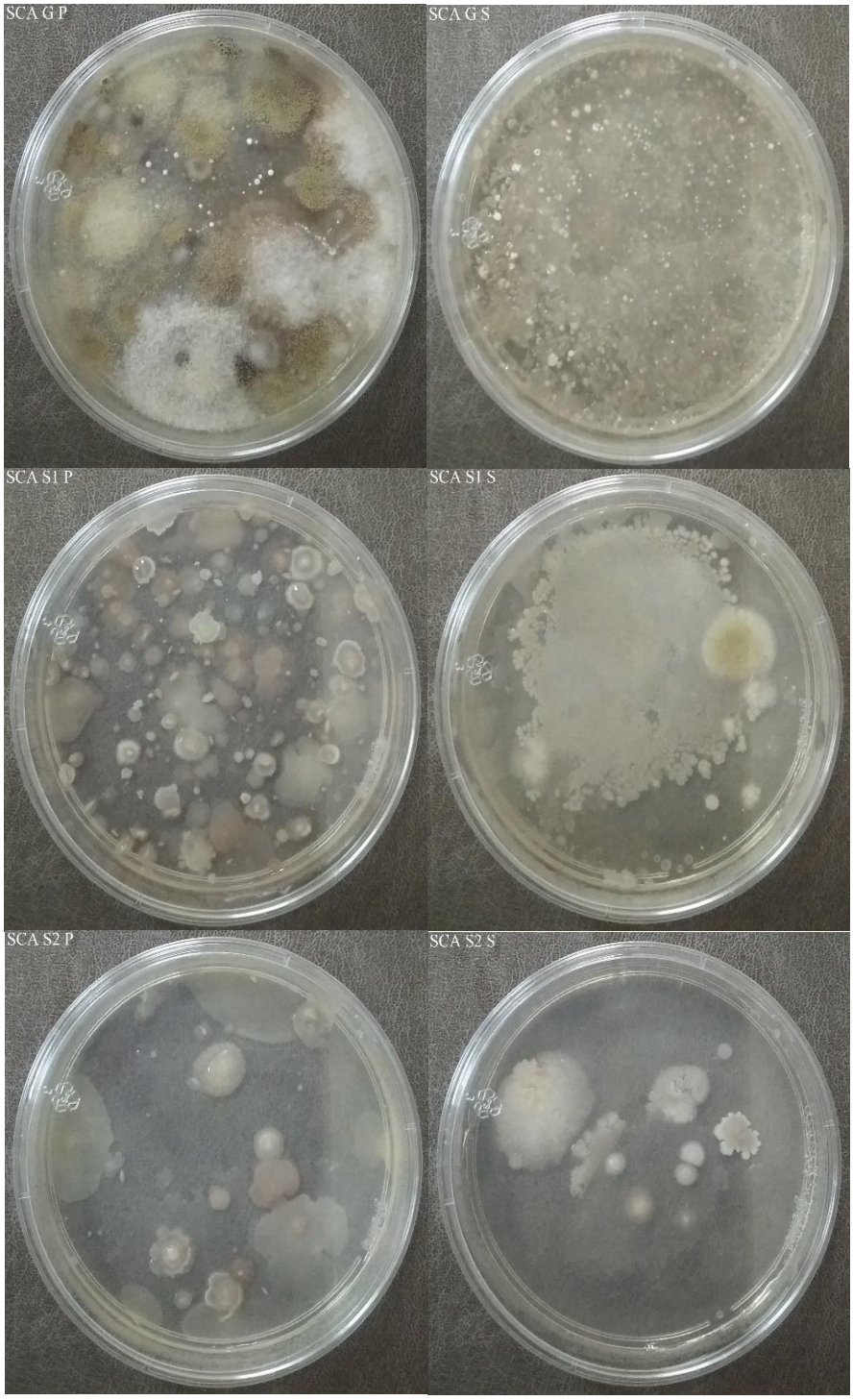


Fig.7 Actinobacteria growth of mangrove soil and garden soil in CMA. Where; CMA= Modified Starch Casein Agar, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment, P = Pour plate, and S = Spread Plate.

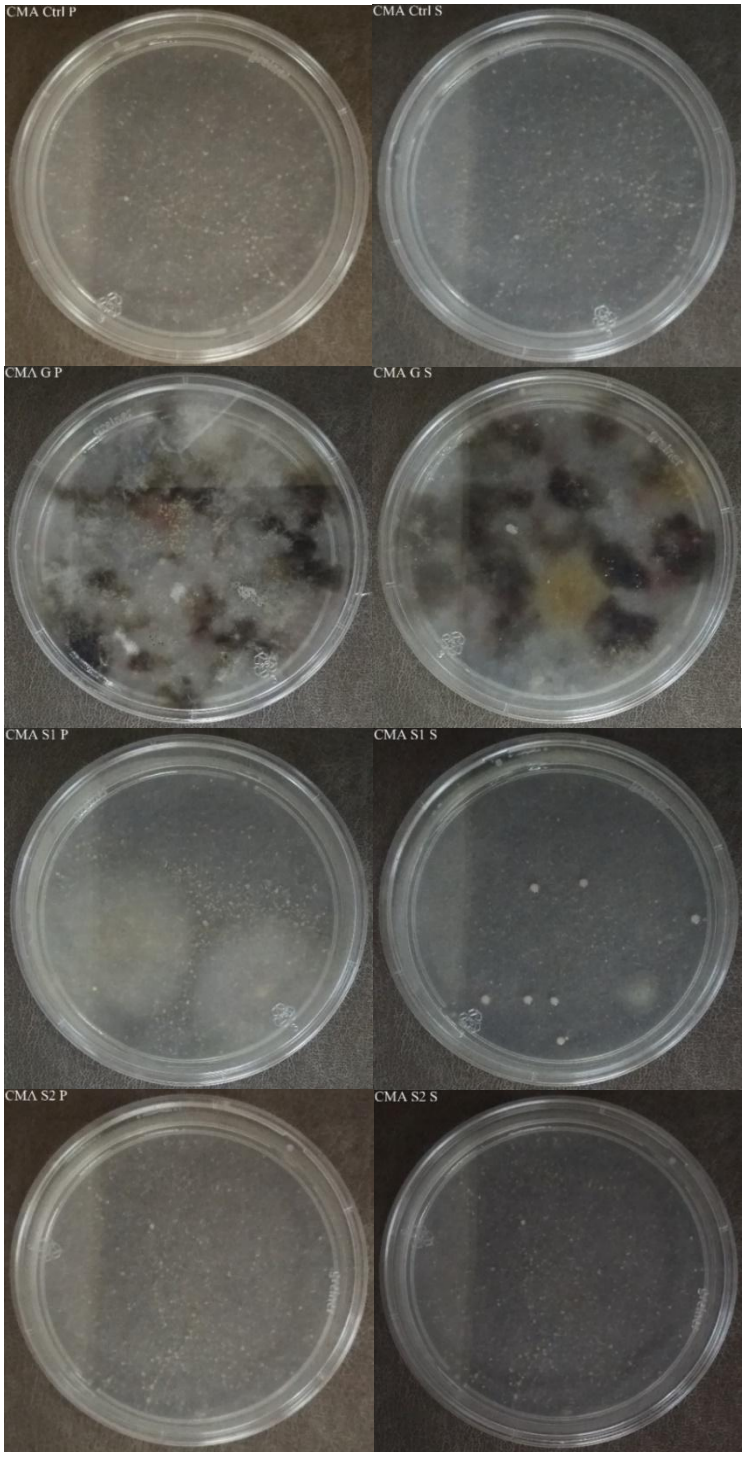
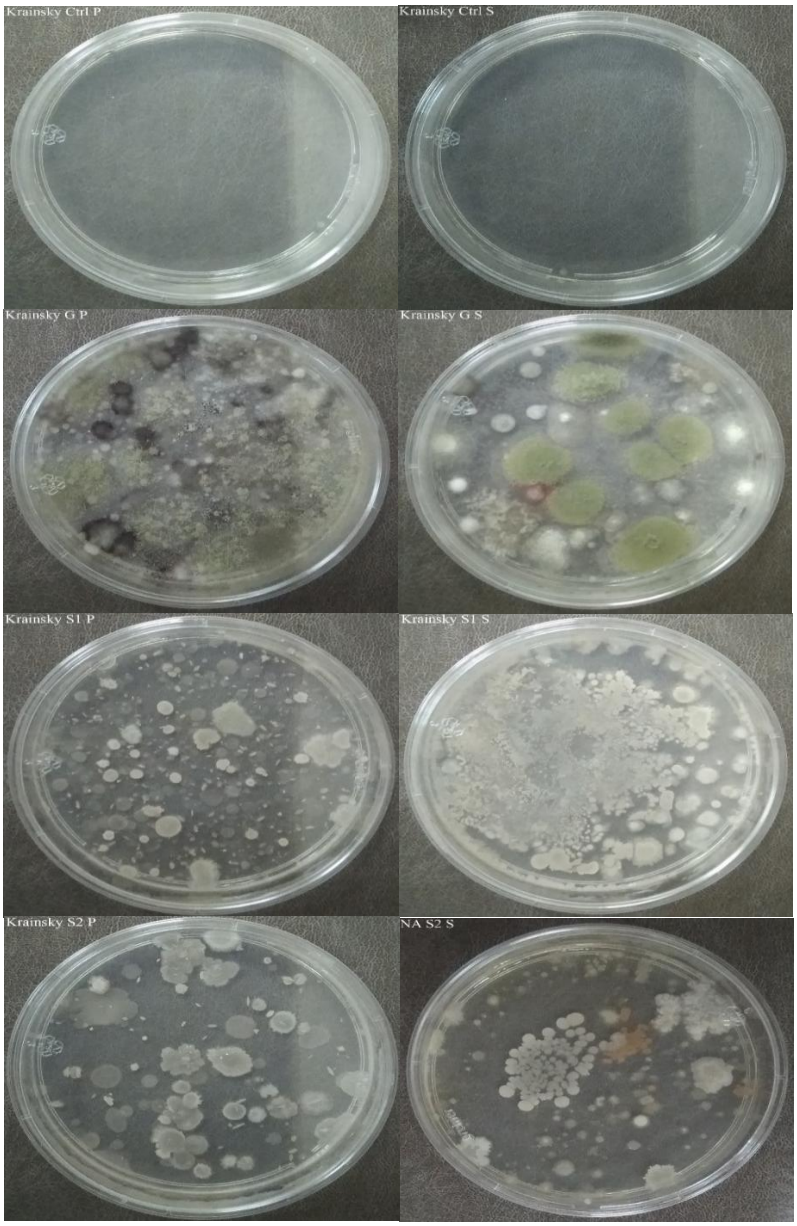


Fig.8 Actinobacteria growth of mangrove soil vs garden soil in Krainsky. Where; Krainsky = Krainsky's Agar medium, Ctrl = Sterilized sand, G = Garden soil, S1= Mangrove sediment, S2=Mangrove sediment, P = Pour plate, and S = Spread Plate.



In order to explore the fungi, the Potato Dextrose Agar medium for fungi was used by Ravikumar *et al.*, (2004), and other researchers used Czapex dox (Thupurani *et al.*, 2016). Pupin and Nahas (2014) also reported regional microbial growth in mangrove sediments compared with Atlantic forests. The abundance of microbial growth was as; bacteria were maximum followed byactinobacteria, and fungi was the least abundant in the studied mangrove habitat.

Hoben *et al.*, (1982) compared the pour plate, spread plate, and drop plate techniques. All techniques showed non-significant results, but the pour plate showed less counts comparatively. In pour plates, agar media cooled to 45°C may result in selective

killing of heat-sensitive strains of bacteria and therefore lower the counts with the pour plates. The effect of quantity and temperature of agar on microbial population is also mentioned by O'Leary (1989). In this study, the variation in microbial diversity and density indicates that the pour plate or spread plate's efficiency also depends upon the type and strain of microbes. Clark (1967) also reported that the *Pseudomonads* did not show any effect of the technique used, whereas some but not all of the *Achromobacter* showed a significant difference between the pour plate and spread plate technique.

The rhizosphere inhabits rich microbial population as compared to bulk soil. The microbial population in the rhizosphere sample can reach 10^{10} to 10^{12} CFU/g of the rhizosphere, while bulk soil often shows $<10^{8}$ CFU/g of bulk soil (Cheng and Gershenson, 2007). Praeg *et al.,* (2019) also reported the variation of prokaryotic population among rhizosphere and bulk soil due to the influence of rhizodeposits and other environmental parameters (i.e., pH and Temperature are the most robust environmental parameters). This study reported the highest number of major groups (total, actinobacteria, and fungi) in rhizospheric soil samples, while the lowest population was observed in mangrove sediments (bulk soil). Water is homogeneous while the soil is a heterogeneous medium; due to more variation, soil carries more diversity of microorganisms, as our results also depict that water samples showed growth of limited groups as compared to soil samples. Jiang *et al.,* (2006) also reported that sediment of the hypersaline lake (32.5% salinity) inhabits more microbial diversity than lake water.

The adaptation of bacterial species to the mangrove ecosystem indicates a potential source of biotechnological resources. The mangrove's soil could provide a rich resource for discovering new bacterial and fungal species that produce enzymes and molecules that could be used for human life, agriculture, industry, and bioremediation (Dias *et al.*, 2009; Dourado *et al.*, 2012; Thompson *et al.*, 2013). In the present study, a great diversity of the total microbial composition and the diazotrophic composition is observed in the mangrove rhizosphere. N-fixer and *Pseudomonadaceae* were both ubiquitous and dominant, and this is consistent with what was mentioned by Mishra *et al.,* (2012) that the diazotrophs were dominant community in Bhitarkanika, a tropical mangrove ecosystem in India. Such bacteria play an essential role in fixing N_2 and providing it to plants. In the present study, *Azotobacters* and *Azospirillum* were greater in rhizosphere soil of *Avicennia marina* than other diazotrophs bacteria. A similar trend has been recorded by Selvam and Kathiresan (2010). They reported a high number of *Azotobacters* and *Azospirillum* as some beneficial bacteria from the soil of a tropical mangrove. They have linked this presence to the pattern of the root system. *Avicennia* produces numerous soft aerial roots (pneumatophores) that may flush the soil with oxygen, providing a congenial environment for aerobic *Azotobacters* and *Azospirillum*.

Also, *Pseudomonadaceae* play an important role in K nutrient (Alzubaidy *et al.,* 2016), suggesting that diazotrophs bacteria play an important role in biogeochemical cycles, such as nitrogen, phosphorus, sulfur, and potassium, in the mangrove habitat.

The present study did not show any sulfur oxidizer in the different mangrove locations. These results are the opposite of what Jing *et al.,* (2015) mentioned the detected diazotrophs, composed entirely of sulfate bacteria, were more abundant in the mangrove soil. In the present study, cellulose-degrading bacteria were observed in mangrove soil of the Gulf and the Red Sea. This finding is in line with the reported observation of Behera *et al.,* (2016) for cellulose-degrading bacteria from mangrove soil of Mahanadi river delta, Odisha, India. The phosphate-solubilizing bacteria have already been reported to occur in the present study in the mangrove area. This agrees with what Selvam and Kathiresan (2010) found in phosphate solubilizing bacteria isolated from the rhizosphere of *Rhizophora* and *Avicennia*.

To the best of our knowledge, this is the first study report to screen some of the potential enzymes of cellulose decomposers, protease-producing bacteria, phosphate solubilizing bacteria, and amylase activities from mangroves in both locations the Gulf and the Red Sea. Our results partially agree with what was found by Khiftiyah *et al.,* (2018) and Mamangkey *et al.,* (2021). Despite that, we believe that we need more studies to know more about the enzymatic activity of the mangrove plant in Saudi Arabia to benefit from it in the future.

Nutrient agar, Rose Bengal chloramphenicol agar, and Starch casein agar are the recommended mediums for enumeration and isolating the diverse mangrove-associated isolates. Bacterial counts ranged between 10^4 to 10^6 CFU, while actinomycetes ranged between 10 to 10^4 CFU. The fungi were least abundant (mostly not detectable) in all studied mangrove samples. The highest number of microbes (Bacteria, actinobacteria and fungi) were recorded in rhizospheric soil samples, while the lowest population was observed in sediments (bulk soil). Water samples showed growth of limited groups as compared to soil samples. We obtained 124 different mangrove-associated bacterial isolates based on their morphology and preserved them at -80°C for further studies regarding characterization and evaluation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Credit Roles

Muhammad Sohaib: Conceptualization, Methodology, Roles/Writing - original draft. Fahad N. I. Al-Barakah: Supervision, Conceptualization, Data curation, Writing - review & editing, Resources. Hussein M. Migdadi: Supervision, Data curation, Formal analysis. MazenAlyousif: Writing - review & editing

Acknowledgment

The authors extend their sincere appreciation to the Deanship of Graduate Studies and Deanship of Scientific Research (DSR) at King Saud University for funding and supporting the work through the initiative of DSR Graduate Students Research Support (GSR), College of Food and Agriculture Sciences Research Center.

References

Abdel-Wahab, M. A., A. H. Bahkali, E. G. Jones, A. M. Elgorban, F. A. Abdel-Aziz, M. S. Hodhod, and M. O. Al-Hebshi. 2016. Two new species of *Kallichroma* (Bionectriaceae, Hypocreales) from Saudi Arabian mangroves. *Phytotaxa* 260:66-74.

Abdel-Wahab, M. A., M. S. Hodhod, A. H. Bahkali, and E. G. Jones. 2014. Marine fungi of Saudi Arabia. *Botanica Marina* 57:323-335.

Al-Amoudi, S., M. Essack, M. F. Simões, S. Bougouffa, I. Soloviev, J. A. Archer, F. F. Lafi, and V. B. Bajic. 2016. Bioprospecting Red Sea coastal ecosystems for culturable microorganisms and their antimicrobial potential. *Marine drugs* 14:165.

Alarfaj, A. A., M. Arshad, E. N. Sholkamy, and M. A. Munusamy. 2015. Extraction and characterization of polyhydroxybutyrates (PHB) from *Bacillus thuringiensis* KSADL127 Isolated from mangrove environments of Saudi Arabia. *Brazilian Archives of Biology and Technology* 58:781-788.

Alongi, D. M. 2005. Mangrove-microbe-soil relations. *Interactions Between Macro-and Microorganisms in Marine Sediments, Coastal Estuarine Stud* 60:85-103.

Alongi, D. M., and P. Dixon. 2000. Mangrove primary production and above-and below-ground biomass in Sawi Bay, southern Thailand. *Phuket Marine Biological Center Special Publication* 22:

Alzubaidy, H., M. Essack, T. B. Malas, A. Bokhari, O. Motwalli, F. K. Kamanu, S. A. Jamhor,

N. A. Mokhtar, A. Antunes, and M.F. Simões. 2016. Rhizosphere microbiome metagenomics of gray mangroves (*Avicennia marina*) in the Red Sea. *Gene* 576:626-636.

Ambeng, H. Z., N. Oka, and A. Tonggiroh. 2019. Isolation and characterization of bacteria from mangrove sediment at coastal area in Pangkep South Sulawesi. In Journal of Physics: Conference Series. IOP Publishing, 022016.

Ameen, F., M. Moslem, S. Hadi, and A. E. Al-Sabri. 2016. Biodegradation of diesel fuel hydrocarbons by mangrove fungi from Red Sea Coast of Saudi Arabia. *Saudi Journal of Biological Sciences* 23:211-218.

Bano, N., M.-U. Nisa, N. Khan, M. Saleem, P. J. Harrison, S. I. Ahmed, and F. Azam. 1997. Significance of bacteria in the flux of organic matter in the tidal creeks of the mangrove ecosystem of the Indus River delta, Pakistan. *Marine Ecology Progress Series* 157:1-12.

Basavaraj, K., S. Chandrashekhara, A. M. Shamarez, P.S. Goudanavar, and F. V. Manvi. 2010. Isolation and morphological characterization of antibiotic producing actinomycetes. *Tropical Journal of Pharmaceutical Research* 9:

Bashan, Y., G. Holguin, and R. Lifshitz. 1993. Isolation and characterization of plant growth-promoting rhizobacteria. *Methods in plant molecular biology and biotechnology. CRC Press, Boca Raton* 331-345.

Behera, B.C., R.R. Mishra, S.K. Singh, S.K. Dutta, and H. Thatoi. 2016. Cellulase from *Bacillus licheniformis* and *Brucella* sp. isolated from mangrove soils of Mahanadi river delta, Odisha, India. *Biocatalysis and Biotransformation* 34:44-53.

Berg, G., M. Grube, M. Schloter, and K. Smalla. 2014. Unraveling the plant microbiome: looking back and future perspectives. *Frontiers in Microbiology* 5:1-7.

Bharathi, P., S. Oak, and D. Chandramohan. 1991. Sulfate-reducing bacteria from mangrove swamps II: Their ecology and physiology. *Oceanologica Acta* 14:163-171.

Bhimba, B., D. D. Franco, J. M. Mathew, G. M. Jose, E. L. Joel, and M. Thangaraj. 2012. Anticancer and antimicrobial activity of mangrove derived fungi *Hypocrea lixii* VB 1. *Chinese Journal of Natural Medicines* 10:77-80.

Bibi, F., I. Ullah, S. Alvi, S. Bakhsh, M. Yasir, A. Al-Ghamdi, and E. Azhar. 2017. Isolation, diversity, and biotechnological potential of rhizo-and endophytic bacteria associated with mangrove plants from Saudi Arabia. *Genetics and Molecular Research* 16:1-12.

Brodersen, K. E., S. M. Trevathan-Tackett, D. A. Nielsen, R. M. Connolly, C. E. Lovelock, T. B. Atwood, and P.I. Macreadie. 2019. Oxygen consumption and sulfate reduction in vegetated coastal habitats: effects of physical disturbance. *Frontiers in Marine Science* 6:14.

Cheng, W., and A. Gershenson. 2007. Carbon fluxes in the rhizosphere. In The Rhizosphere. Elsevier, 31-56.

Clark, D. J. C. J. O. M. 1967. Comparison of pour and surface plate methods for determination of bacterial counts. 13:1409-1412.

D'Souza, J., and B. F. Rodrigues. 2013. Biodiversity of Arbuscular Mycorrhizal (AM) fungi in mangroves of Goa in West India. *Journal of Forestry Research* 24:515-523.

Das, S., P. Lyla, and S. A. Khan. 2006. Marine microbial diversity and ecology: importance and future perspectives. *Current Science* 90:1325-1335.

Dias, A. C., F. D. Andreote, F. Dini-Andreote, P. T. Lacava, A. L. Sá, I. S. Melo, J. L. Azevedo, and W. L. Araújo. 2009. Diversity and biotechnological potential of culturable bacteria from Brazilian mangrove sediment. *World Journal of Microbiology Biotechnology Advances* 25:1305-1311.

Do Carmo, F. L., H. F. Dos Santos, E. F. Martins, J. D. van Elsas, A. S. Rosado, and R. S. Peixoto. 2011. Bacterial structure and characterization of plant growth promoting and oil degrading bacteria from the rhizospheres of mangrove plants. *The*

Journal of Microbiology 49:535-543.

Dobereiner, J., I. Marriel, and M. Nery. 1976. Ecological distribution of Spirillum lipoferum Beijerinck. *Canadian Journal of Microbiology* 22:1464-1473.

Dourado, M. N., A. Ferreira, W. L. Araújo, J. L. Azevedo, and P. T. Lacava. 2012. The diversity of endophytic methylotrophic bacteria in an oil-contaminated and an oil-free mangrove ecosystem and their tolerance to heavy metals. *Biotechnology research international* 2012:

Gupta, R., R. Singal, A. Shankar, R. C. Kuhad, and R. K. Saxena. 1994. A modified plate assay for screening phosphate solubilizing microorganisms. *The Journal of General and Applied Microbiology* 40:255-260.

Hoben, H., P. J. A. Somasegaran, and e. microbiology. 1982. Comparison of the pour, spread, and drop plate methods for enumeration of Rhizobium spp. in inoculants made from presterilized peat. 44:1246-1247.

Hodhod, M. S., M. A. Abdel-Wahab, A. H. Bahkali, and K. D. Hyde. 2012. *Amarenographium solium* sp. nov. from Yanbu mangroves in the Kingdom of Saudi Arabia. *Cryptogamie, Mycologie* 33:285-294.

Holguin, G., P. Vazquez, and Y. Bashan. 2001. The role of sediment microorganisms in the productivity, conservation, and rehabilitation of mangrove ecosystems: an overview. *Biology and Fertility of Soils* 33:265-278.

Jiang, H., H. Dong, G. Zhang, B. Yu, L. R. Chapman, M. W. J. A. Fields, and e. microbiology. 2006. Microbial diversity in water and sediment of Lake Chaka, an athalassohaline lake in northwestern China. 72:3832-3845.

Jing, H., X. Xia, H. Liu, Z. Zhou, C. Wu, and S. Nagarajan. 2015. Anthropogenic impact on diazotrophic diversity in the mangrove rhizosphere revealed by nifH pyrosequencing. *Frontiers in microbiology* 6:1172.

Jose, A. C., and P. H. Christy. 2013. Assessment of antimicrobial potential of endophytic bacteria isolated from *Rhizophora mucronata*. *International Journal of Current Microbiology and Applied Sciences* 2:188-194.

Kathiresan, K., and B.L. Bingham. 2001. Biology of mangroves and mangrove ecosystems. *Advances in Marine Biology* 40:81-251.

Kathiresan, K., S. Manivannan, M. Nabeel, and B. Dhivya. 2009. Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment. *Colloids and Surfaces B: Biointerfaces* 71:133-137.

Khiftiyah, A. M., N. N. Hanifah, M. Bachruddin, M.a. Sholichah, S. Istiqomah, S. R. Dewi, T. Rahayu, I. A. W. Prasetya, L. Marjayandari, and N. 'Aini. 2018. Isolation and screening of potential proteolytic and amylolytic microbes from Wonorejo Mangrove forest soil, Surabaya, Indonesia. In AIP Conference Proceedings. AIP Publishing LLC, 040014.

Kumar, S., A. Hatha, and K. Christi. 2007. Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags. *Revista de biología Tropical*

Kumaran, K., M. Shindikar, and R. B. Limaye. 2004. Fossil record of marine manglicolous fungi from Malvan (Konkan) west coast of India. *Indian Journal of Marine Sciences* 33:257-261.

Mamangkey, J., D. Suryanto, E. Munir, A. Z. Mustopa, M. T. Sibero, L. W. Mendes, A. Hartanto, S. Taniwan, M. J. Ek-Ramos, and A. Harahap. 2021. Isolation and enzyme bioprospection of bacteria associated to Bruguiera cylindrica, a mangrove plant of North Sumatra, Indonesia. *Biotechnology Reports* 30:e00617.

Mishra, R. R., M. R. Swain, T. K. Dangar, and H. Thatoi. 2012. Diversity and seasonal fluctuation of predominant microbial communities in Bhitarkanika, a tropical mangrove ecosystem in India. *Revista de biologia tropical*

Mohanraju, R., and R. Natarajan. 1992. Methanogenic bacteria in mangrove

sediments. In The Ecology of Mangrove and Related Ecosystems. Springer, Dordrecht, 187-193.

Mohanraju, R., B. Rajagopal, L. Daniels, and R. Natarajan. 1997. Isolation and characterization of a methanogenic bacterium from mangrove sediments. *Journal of Marine Biotechnology* 5:147-152.

Molina, G., M. R. Pimentel, T. C. Bertucci, and G. M. Pastore. 2012. Application of fungal endophytes in biotechnological processes. *Chemical Engineering Transactions* 27:289-294.

Ngulube, M., A. M. Mweetwa, E. Phiri, S. Njoroge, H. Chalwe, V. Shitumbanuma, and R.L. Brandenburg. 2018. Effects of biochar and gypsum soil amendments on groundnut (*Arachis hypogaea* L.) dry matter yield and selected soil properties under water stress. *African Journal of Agricultural Research* 13:1080-1090.

O'Leary, W. M. 1989. Practical handbook of microbiology. CRC press,

Page, A. L. 1982. Methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy, Soil Science Society of America,

Praeg, N., H. Pauli, and P. J. F.i.m. Illmer. 2019. Microbial diversity in bulk and rhizosphere soil of Ranunculus glacialis along a high-alpine altitudinal gradient. 10:1429.

Pupin, B., and E. Nahas. 2014. Microbial populations and activities of mangrove, restinga and A tlantic forest soils from C ardoso I sland, B razil. *Journal of applied microbiology* 116:851-864.

Purushothaman, A. 2000. Fungal flora in mangrove ecosystem. *Flora and Fauna in Mangrove Ecosystems: A Manual for Identification* 128-147.

Ravikumar, S., K. Kathiresan, S. T. M. Ignatiammal, M.B. Selvam, and S. Shanthy. 2004. Nitrogen-fixing azotobacters from mangrove habitat and their utility as marine biofertilizers. *Journal of Experimental Marine Biology and Ecology* 312:5-17.

Sahoo, K., and N. Dhal. 2009. Potential microbial diversity in mangrove ecosystems: a review. *Indian Journal of Marine Sciences* 38:249-256.

Sakhia, N., S. Prajapati, V. Shetty, S. Bhatt, and A. Bhadalkar. 2016. Study of bacterial diversity of mangroves rhizosphere. *Open Journal of Marine Science* 6:23.

Samuel, S., and S. Muthukkaruppan. 2011. Characterization of plant growth promoting rhizobacteria and fungi associated with rice, mangrove and effluent contaminated soil. *Current Botany* 2:22-25.

Saravanakumar, K., R. Anburaj, V. Gomathi, and K. Kathiresan. 2016. Ecology of soil microbes in a tropical mangrove forest of south east coast of India. *Biocatalysis and Agricultural Biotechnology* 8:73-85.

Saseeswari, A., G. Kanimozhi, and A. Panneerselvam. 2015. Studies on physicochemical parameter and bacterial populations in sediment soil at Karankadu mangrove forest, Ramanathapuram (Dt), Tamil Nadu. *International Journal of Science, Environment and Technology* 4:886-889.

Schut, F., E. J. de Vries, J. C. Gottschal, B. R. Robertson, W. Harder, R. A. Prins, and D. K. Button. 1993. Isolation of typical marine bacteria by dilution culture: growth, maintenance, and characteristics of isolates under laboratory conditions. *Applied environmental microbiology* 59:2150-2160.

Selvam, M. M., and K. Kathiresan. 2010. Beneficial bacteria from soil of a tropical mangroves. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences* 12:1-2.

Shome, B., S. Rajeswari, S. Ahlawat, and N. Verma. 2000. Agar depolymerizing (agarolytic) bacteria isolated from mangrove soil samples of Andaman. *Current Science* 79:696-697.

Somasegaran, P., and H. J. Hoben. 1994. Handbook for rhizobia: methods in legume-Rhizobium technology. Springer-Verlag New York Inc.,

sona Janarthine, S. R., P. Eganathan, T.

Balasubramanian, and S. Vijayalakshmi. 2011. Endophytic bacteria isolated from the pneumatophores of *Avicennia marina*. *African Journal of Microbiology Research* 5:4455-4466.

Sundaram, R., S. J. Inbaneson, S. Ramasamy, and R. Andy. 2010. Assessment of endophytic bacterial diversity among mangrove plants and their antibacterial activity against bacterial pathogens. *Annals of Biological Research* 1:240-247.

Tabao, N. S. C., and R. G. Monsalud. 2010. Characterization and Identification of High Cellulase-Producing Bacterial Strains from Philippine Mangroves. *Philippine Journal of Systematic Biology* 4:13-20.

Thompson, C. E., W. O. Beys-da-Silva, L. Santi, M. Berger, M. H. Vainstein, and A. T. R. Vasconcelos. 2013. A potential source for cellulolytic enzyme discovery and environmental aspects revealed through metagenomics of Brazilian mangroves. *AMB Express* 3:1-18.

Thupurani, M. K., R. Vadluri, E. M. K. Reddy, and B. Anuradha. 2016. Evaluation of Fungal Flora and Aflotoxins from the Ground Nuts Collected from Various Regions of Warangal District. *Asian Research Journal of Agriculture* 1-11.

Toledo, G., Y. Bashan, and A. Soeldner. 1995. Cyanobacteria and black mangroves in Northwestern Mexico: colonization, and diurnal and seasonal nitrogen fixation on aerial roots. *Canadian Journal of Microbiology* 41:999-1011.

Vazquez, P., G. Holguin, M. Puente, A. Lopez-Cortes, and Y. Bashan. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils* 30:460-468.

Vethanayagam, R. 1991. Purple photosynthetic bacteria from a tropical mangrove environment. *Marine Biology* 110:161-163.

How to cite this article:

Muhammad Sohaib, Fahad N. I. Al-Barakah, Hussein M. Migdadi and Mazen Alyousif. 2022. Isolation and Abundance of Different Culturable Microbes from Mangrove Environments in Coastal Areas of Saudi Arabia. *Int.J.Curr.Microbiol.App.Sci.* 11(04): 215-238. doi: https://doi.org/10.20546/ijcmas.2022.1104.030