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Halotolerant Plant Probiotic Bacterial Isolates of Mangrove Soils of Chidambaram and Thanjavur, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Halotolerant plant growth-promoting rhizobacteria (HTPGPR) are beneficial microbes that can be exploited to mitigate the negative effects of soil salinity on crops. In the current investigation, eight saline soil samples collected from 2 mangrove ecosystems of Tamil Nadu (Chidambaram and Thanjavur) during December 2022 were used to isolate saline tolerant bacterial cultures at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Using 5 different bacterial growth media, 48 rhizobacterial isolates (ST1 to ST48) were obtained. Salt tolerance ability (0, 5, 10, 15 and 20% NaCl) of these 48 isolates under *in vitro*

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conditions indicated their potential to tolerate up to 10% NaCl (1.71 M). None of the isolates could grow at 20% NaCl (3.42M). Bacterial isolates such as ST11, ST13, ST18, ST20 and ST27 showed minimum growth at 15%NaCl (2.57M). 33 isolates which could grow well at higher salt concentrations were selected. Among 33 isolates, 18 isolates with higher concentration of intracellular sodium content (ST1, ST2, ST3, ST7, ST8, ST9, ST11, ST12, ST13, ST17, ST18, ST20, ST27, ST30, ST32, ST34, ST39, and ST40) were selected and characterized qualitatively for their ability to mineralize phosphate, potassium, and zinc, and to produce HCN. Potential of these18 bacterial isolates to tolerate other abiotic stress factors such as pH and temperature was also studied. Among 18 isolates, the isolates ST7, ST17, and ST30 were found to be multimineral solubilizers. Bacterial isolate ST17 was found to prefer alkaline (pH 9.0) and mesophilic temperature (35ºC) for its growth at both saline (5% NaCl) and non saline condition.

Keywords: Halotolerant; mangrove ecosystem; plant growth promoting bacteria; salinity.

1. INTRODUCTION

Mangroves are distinctive intertidal ecosystems that can be found in tropical and subtropical areas of the world and that are habitats for a wide variety of aquatic and terrestrial species. Mangroves, which are well-known to be extremely productive ecosystems of tremendous ecological significance, encompass roughly 60–70% of the world's tropical and subtropical coastlines. These ecosystems are extremely productive all over the world despite their fragility and limited diversity [1]. Due to the regular tidal flooding, these ecosystems experience extreme conditions such as salinity, poor nutrient availability, etc. According to [2 and 3], significant nutrient changes within mangrove ecosystems are also caused by microbial activity. In tropical mangroves, bacteria and fungi make up 91% of the entire microbial biomass, while algae and protozoa make up just 7% and 2% of the total biomass, respectively [4]. The microbiota of mangrove ecosystem could survive extreme soil conditions like salinity and waterlogging. Plant growth promoting and abiotic stress tolerating microorganisms from the mangrove ecosystem can be exploited for growing crops in saline, sodic, and saline sodic soils [5].

One of the most damaging abiotic stresses for sustainable agricultural operations is salinity. Saline soils are rich in soluble salts such as chlorides, sulfates, and nitrates. These soluble salts negatively impact crop growth, plant physiology, quality, and crop productivity [6]. Long dry spells and high temperatures cause an increase in evapotranspiration, resulting in the formation of saline soils [7]. Low seed germination, low photosynthetic potential, and slow plant growth are the effects of high salinity [8,9], which also lowers soil biodiversity, microbial activity, and the availability of vital nutrients and minerals to plants [10,11].

Many physical and chemical techniques, including scraping, flushing, and leaching [12], have been reported earlier to reduce soil salinity. Leaching soils with good quality water has been advocated for a long time. Gypsum is also used to recover sodic soil. However, it does not boost the rhizosphere microbial population [13], indicating the need for additional microbe-mediated interventions for developing a rhizosphere that is favorable to the successful cultivation of crops [14]. For sustainable production in saline sodic soils, halotolerant plant growth-promoting rhizobacteria (HT-PGPR) have been suggested [15].

HT- PGPR studies have confirmed that they release vast amounts of phytohormones, like gibberellins [16], cytokinins, and auxins [17,18,19], as well as secondary metabolites and osmolytes like exopolysaccharides, trehalose, proline, and glycine betaines [20,21], which activate plants antioxidative enzymes (peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), NR (nitrate reductase), and GR (glutathione reductase))) under salt stress conditions [22,23]. Microbial consortia carry out a number of important functions, including promoting plant growth by acting as an osmoprotectant and antioxidant to lessen salt stress. Salt-tolerant PGPR increased the production of auxin and controlled the uptake of Na^+, K^+, and Ca^{2+} [24]. With this in view, the current research was conducted to develop halotolerant microbial consortia to reduce saline stress, and eighteen different halotolerant plant growth promoting probiotic isolates were obtained.

2. MATERIALS AND METHODS

The current research was done in the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

2.1 Collection of Soil Samples

From two different mangrove regions of Tamil Nadu (Chidambaram & Thanjavur), eight saline soil samples were taken. Polythene bags were used to store and transport the collected samples to the laboratory for further studies. The samples were examined for their physico-chemical characteristics and for isolating halotolerant bacterial cultures.

2.2 Isolation of Halotolerant Bacterial Strains

Enrichment of soil samples

Enrichment of salt tolerant microbial population in selected soil samples was achieved by incubation of 10 g soil sample in 15 ml nutrient broth with electrical conductivity of 4.0 and pH of 8.5 for 15 days. This enriched soil sample was used to isolate halotolerant bacteria. Accordingly 1 g soil sample was serially diluted up to 10^{-6} and then cultured on Nutrient agar, Luria Bertani agar, and Tryptic Soy agar, Starch Caesin agar, R2A agar and Soil extract agar media. On sterile Petri plates with a particular medium, 100 microliters of the soil dilution (10^{-3} and 10^{-6}) were applied, spread using a glass spreader, and incubated at 28°C for two days. After the incubation period, colonies with different morphological characters were chosen, purified, and kept as glycerol stocks at -20°C [25].

2.3 Screening Salt Tolerance of Bacterial Isolates

10 ml of nutrient broth was prepared with varying NaCl concentrations (5, 10, 15, and 20%) in test tubes. Then the broth was inoculated with 0.1 ml of cultures obtained from mangrove soil and incubated at 28 °C for 48 hours. During incubation, growth was monitored by measuring optical density at 600 nm using a spectrophotometer (M/s. Shimadzu, Japan) [25].

2.4 Estimation of Intracellular Sodium Ion Concentration

Halotolerant isolates were grown in nutrient broth until they reached 1.0 OD660. Then they were inoculated in nutrient broth of different salt concentrations and incubated for 48 h. Samples were withdrawn after incubation and cells were harvested by centrifugation at 5000 rpm for 10 min. Cell pellets were washed thrice with 0.1mM of isotonic $MgCl_2$ solution. Then the cells were lysed by adding 10% perchloric acid and incubated for 2h. Again centrifuged for 10000 rpm for 10 min. From the supernatant, intracellular ion concentration was measured using flame photometer and the curve was plotted [26].

2.5 Plant Growth Promoting Traits

2.5.1 Phosphate solubilization

The efficiency of selected eighteen bacterial isolates to solubilize insoluble phosphate was assessed using Pikvoskaya's agar medium supplemented with 0.5% tricalcium phosphate and different concentrations of NaCl (0%, 5%, and 10%). Around 10µl of 24 h old culture was spotted in the center of the medium and incubated at 28°C for 48 hours. Clear or halo zone formation surrounding the colony indicates dissolution of precipitated insoluble phosphate. Thus, strains showing a clear zone were scored as positive for phosphate solubilization [27].

2.5.2 Potassium releasing aciivity

The efficiency of selected eighteen bacterial isolates to release potassium was assessed using Alexandrov's agar medium with 0.3% potassium alumino silicate and three different salt (NaCl) concentrations (0%, 5%, and 10%). Around 10 µl of 24 h old culture was spotted on Alexandrov's medium and incubated at 28°C for 48 hours. After incubation, the presence of a clear zone at spot-inoculated sites was considered positive for potassium release [28].

2.5.3 Zinc solubilization

The efficiency of the selected eighteen bacterial isolates in zinc solubilization was assessed using Bunt and Rovira agar with 0.1% zinc oxide and three different NaCl concentrations (0%, 5%, and 10%). Around 10 µl of 24 h old culture was spotted on Bunt and Rovira agar medium. The plates were incubated at 28°C for 48 hours. After incubation, the presence of a clear zone at spot-inoculated sites was considered positive for zinc solubilization [29].

2.5.4 Qualitative assay of HCN production

Using the [30] method, all the isolates were checked for the generation of HCN. Isolates were streaked on Nutrient agar plates enriched with 4.4 g glycine/L. The top of the plate was covered with Whatmann No. 1 filter paper soaked in a solution of 2% sodium carbonate and 0.5% picric acid. Parafilm was used to seal the plates, which were then incubated for 4 days at 28°C. The change in colour of the filter paper from orange to red indicated HCN production.

2.6 Growth of Isolates at Different pH and Temperature

Bacterial isolates were streaked on nutrient agar plates at different concentrations of NaCl (0%, 5%, and 10% (w/v) and at different pH of 4.0, 7.0, and 9.0 and incubated at room temperature for 24 to 48 hours for identification of the bacterial pH tolerance [31]. At various temperatures, including 20, 30, 35, and 45°C, these plates were incubated, and the growth pattern was monitored.

2.7. Statistical Analysis

Data on soil pH and electrical conductivity were analyzed using Microsoft Excel. Results of intracellular sodium accumulation potential of halotolerant bacterial isolates were analyzed using SPSS software(Version: 16) and the mean values were compared using DMRT(1 and 5%)

3. RESULTS AND DISCUSSION

3.1 Physio-chemical Properties of Soil Samples

Soil samples were taken from eight sampling sites in two different districts of the Tamil Nadu Mangrove ecosystem. Physico-chemical properties like pH, EC, and organic carbon content of the soil samples were analyzed following standard protocols, and the results are given in Table 1. The pH of the samples ranged between 7.52 and 7.89 (slightly alkaline to alkaline). A maximum pH value (7.89) was observed in samples collected from the Chidambaram mangrove forest. Another important soil property is electrical conductivity, which ranges from 2.52 dS/m to 3.90 dS/m. The soluble salt content (EC) of the samples was found to be higher in the soils of Thanjavur's mangrove ecosystem. Organic carbon content was greater in the soils of Chidambaram (0.69%).

3.2 Isolation of Halotolerant Bacterial Isolates

Since the pH and EC of the soil samples were lower than the values of saline soil, pH (8.5) and EC (4 dS/m) were adjusted to enrich saline tolerant microorganisms in the soil samples, and incubated for 15 days at room temperature. After the incubation period, one gram soil sample was serially diluted to 10-6. Dilutents from 10-3,10-4, 10-5 and 10-6 were placed on 5 different media, as mentioned in Section 2.0. There was no growth in10-5 and 10-6 dilutions. Colonies appeared on10^{-3} and 10^{-4} were selected. A total of forty eight bacterial isolates (ST1 to ST48) were chosen from rhizosphere and non rhizosphere soil samples of mangrove ecosystem based on colour, shape and size. Out of 48 isolates, 7 isolates were obtained from Nutrient agar plates at 10^{-4} dilution, 6 isolates were obtained from Luria Bertani agar media at 10^{-4} dilution, 8 isolates from tryptic soy agar media at 10^{-3} and 10^{-4} dilution, 8 isolates from R2A media at 10^{-3} and 10^{-4}dilution; and 19

Table 1. Physico-chemical properties of soil samples used and bacterial isolates obtained

Soil sample	pH	EC (dS/m)	OC (%)	No of isolates
Thanjavur non Rhizosphere -1	7.59 ± 0.03	3.49 ± 0.02	0.32 ± 0.01	4
Thanjavur non Rhizosphhere -2	7.69 ± 0.06	3.90 ± 0.05	0.34 ± 0.02	6
Thanjavur Rhizosphere-1	7.61 ± 0.07	3.19 ± 0.01	0.46 ± 0.01	13
Thanjavur Rhizosphere-2	7.52 ± 0.02	3.51 ± 0.01	0.49 ± 0.02	8
Chidambaram non Rhizosphere -1	7.77 ± 0.02	2.52 ± 0.06	0.52 ± 0.03	4
Chidambaram non Rhizosphere -2	7.74 ± 0.06	2.59 ± 0.02	0.55 ± 0.02	1
Chidambaram Rhizosphere -1	7.89 ± 0.01	2.65 ± 0.01	0.63 ± 0.01	7
Chidambaram Rhizosphere -2	7.81 ± 0.02	2.19 ± 0.03	0.69 ± 0.01	5

Values in each column are the mean of three replications ± SE (standard error)

Table 2. Growth pattern of 48 isolates at different NaCl concentration

Isolates	0%	5%	10%	15%	20%
ST1	+ + +	+ + +	+	-	-
ST2	+ + +	+ + +	+ + +	-	-
ST3	+ + +	+ + +	+ + +	-	-
ST4	+ + +	+ + +	+	-	-
ST5	+ + +	+	-	-	-
ST6	+ + +	+ + +	+	-	-
ST7	+ + +	+ + +	+	-	-
ST8	+ + +	+ + +	+	-	-
ST9	+ +	+ + +	+	-	-
ST10	+ + +	+ + +	-	-	-
ST11	+ +	+ + +	+	+	-
ST12	+ +	+ + +	+	-	-
ST13	+ + +	+ + + +	+ +	+	-
ST14	+ +	+ + +	-	-	-
ST15	+ +	+ + +	+ +	-	-
ST16	+	+	-	-	-
ST17	+ +	++	+	-	-
ST18	+ +	+ + +	+ +	+	-
ST19	+ +	+	-	-	-
ST20	+ +	+ + +	+	+	-
ST21	+	+ + +	+ +	-	-
ST22	+ +	+ +	+	-	-
ST23	+ + +	+ +	+ +	-	-
ST24	+ + +	+ + + +	+ +	-	-
ST25	+ + + +	+ + +	+	-	-
ST26	+ +	+ + + +	+ + +	-	-
ST27	+ +	+ + +	+	+	-
ST28	+ + + +	+ +	+	-	-
ST29	+ +	+	+	-	-
ST30	+ +	-	-	-	-
ST31	+ +	+ + +	+	-	-
ST32	+ + +	+ +	+	-	-
ST33	+ + +	+	-	-	-
ST34	+ + +	+ +	+	-	-
ST35	+ + + +	+	+	-	-
ST36	+ + +	+ +	+	-	-
ST37	+ +	+	-	-	-
ST38	+ + +	+ +	+	-	-
ST39	+ + + +	+ + + +	+ + +	-	-
ST40	+ +	+ +	+ + +	-	-
ST41	+ + +	+ + + +	+ +	-	-
ST42	+ +	+	-	-	-
ST43	+ +	+ + +	+ + + +	-	-
ST44	+ +	+	+	-	-
ST45	+ +	-	-	-	-
ST46	+ +	-	-	-	-
ST47	+ +	+	-	-	-
ST48	+ + +	+ + +	+	-	-

('++++' confluent growth,'+++' good growth,'++' moderate growth,'+' feable growth,'-' no growth)

Table 3. Colony morphology of 18 halotolerant bacterial isolates

Isolates	Soil	Medium	Morphological characters
ST1	Thanjavur- Non Rhizospere	Nutrient agar	Small size, yellow in colour , less polysaccharide production
ST2	Thanjavur- Rhizospere	Nutrient agar	Small colony size, Light yellow in colour, less polysaccharide production
ST3	Thanjavur- Rhizospere	Nutrient agar	Big colony size, creamish white in colour, high polysaccharide production
ST7	Thanjavur- Rhizospere	Luria Bertani	Smaller in size, dirty white in colour , less polysaccharide production
ST8	Thanjavur- Rhizospere	Luria Bertani	Smaller in size, creamish white in colour, less polysaccharide production
ST9	Thanjavur- Rhizospere	Luria Bertani	Bigger in size, orangish white in colour, spreading type, high polysaccharide production
ST11	Thanjavur- Non Rhizospere	Tryptic soy agar	Bigger in size, yellow pigment producer, smooth colonies, high polysaccharide production
ST12	Thanjavur- Non Rhizospere	Tryptic soy agar	Bigger in size, , Creamish white colonies, high polysaccharide production, spreading type
ST13	Thanjavur- Non Rhizospere	Tryptic soy agar	Bigger in size, white smooth colonies, less polysaccharide production,
ST17	Chidambaram Rhizospere	Tryptic soy agar	Pinkish white smooth solonies, high polysaccharide production
ST18	Chidambaram Rhizospere	Tryptic soy agar	Creamish spreading (tree like) colonies, high polysaccharide production
ST20	Chidambaram Rhizospere	Tryptic soy agar	Reddish orange smooth colonies, high polysaccharide production
ST27	Chidambaram Rhizospere	R2A	White and milky smooth colonies, high polysaccharide production
ST30	Thanjavur Rhizosphere	Starch caesin	Reddish orange pigment producer, high polysaccharide producers
ST32	Thanjavur- Non Rhizospere	Starch caesin	Creamish white spreading colonies, high polysaccharide production
ST34	Thanjavur Rhizosphere	Starch caesin	Brown spreading colonies, less polysaccharide production
ST39	Thanjavur Rhizosphere	Starch caesin	Creamish brown spreading colonies, high polysaccharide production
ST40	Thanjavur Rhizosphere	Starch caesin	Light yellow pigment production, high polysaccharide production

isolates from starch casein agar media at 10^{-3} and 10^{-4} dilution. Similar to the current study, halotolerant plant growth promoting rhizobacteria of saline environments with plant growth promoting effect on wheat under saline conditions were reported [32,33,34].

3.3 Screening Salt Tolerance Potential of Bacterial Isolates

On the basis of tolerance limit, bacterial cultures can be classified as slightly (0.2 to 0.5M) , moderate (0.50 to 2.5 M) and extreme halophiles (2.5 to 5.2M). All the forty eight bacterial isolates were first inoculated in nutrient broth with varying salt concentrations (0%, 5%, 10%, 15%, and 20%) and cultured at 28 °C for 48 hours. Among 48, forty six isolates showed normal growth in 5% (0.86 M) and thirty seven isolates in 10% NaCl (1.71M) and only five isolates in 15% (2.58M) NaCl concentration. This implies that the isolates were moderately halophilic to extremely halophilic in nature [35]. None of the isolates were able to tolerate more than 15% NaCl. PGPR characterization was performed on 18 halotolerant bacterial isolates that could survive in 10% NaCl concentration.

3.4 Intracellular Sodium Ion Accumulating Capacity

According to [36], the most crucial salinity amelioration concept is to lower plant Na^+ absorption and regulating osmotic homeostasis

to reduce the chances of flaccidity and death. The intracellular sodium accumulating capability of 11 of the 33 isolates (ST1, ST32, ST3, ST4, ST5, ST8, ST10, ST11, ST23, ST27, and ST30) increased as the NaCl concenteration increased. Even at low NaCl concentrations, the isolates ST1, ST3, ST5, ST24, ST31, and ST33 were able to accumulate sodium chloride. At a concentration of 5% NaCl, the isolates ST1, ST3, ST13, ST15, ST24, ST25, ST26, ST27, ST28, ST29, ST30, ST31, and ST33 accumulated more sodium chloride than the control. At 5% NaCl concentration the sodium accumulating capacity was high for these isolates (ST13, ST15, ST24, ST25, ST26) than 10%. These isolates able to accumulate more sodium at 5% than 10% NaCl concentration. At 10% the sodium content decreased which may be because the growth was much reduced. Our findings are consistent with a study by [37] which further found that salt stress increased the intracellular Na^+ concentration in *Methylophilus* species and *Methylobacterium* species.

Values in each column are the mean of three replications ± SE (standard error). ND- Not detected. Mean values in each column followed by the same letter(s) are not significantly different at 5% level.(Foot note of Table 4.0).

Table 4. Intracellular sodium ion accumulation capacity of the isolates

Isolate No	0% NaCl	5% NaCl	10% NaCl
1	4.70 ± (0.01)^{ab}	5.30 ±(0.13)^{fg}	4.12 ±(0.10)^{f}
2	3.84 ± (0.10)^{ef}	4.28 ±(0.03)^{k}	5.35 ±(0.05)^{d}
3	4.07± (0.05)^{d}	5.63 ±(0.10)^{ef}	5.19 ±(0.07)^{d}
4	3.71± (0.09)^{fg}	4.59 ±(0.03)^{j}	5.22 ±(0.11)^{d}
5	3.99± (0.11)^{de}	3.85 ±(0.04)^{l}	4.94 ±(0.06)^{e}
6	2.91 ± (0.04)^{jk}	3.04 ±(0.08)^{n}	3.09 ±(0.04)^{i}
7	3.59 ± (0.04)^{g}	4.02 ±(0.05)^{l}	2.73 ±(0.05)^{j}
8	2.91 ± (0.05)^{jk}	2.94 ±(0.03)^{no}	5.22 ±(0.11)^{d}
9	1.74 ± (0.07)^{r}	0.43 ±(0.04)^{t}	0.97 ±(0.04)^{o}
10	2.77 ± (0.02)^{kl}	2.78 ±(0.01)^{op}	6.01 ±(0.07)^{c}
11	1.74 ± (0.11)^{r}	2.14 ±(0.06)^{qr}	7.10 ±(0.26)^{b}
12	1.17 ± (0.02)^{u}	1.14 ±(0.06)^{t}	3.54 ±(0.07)^{h}
13	1.51 ± (0.03)st	4.90 ±(0.17)^{i}	2.48 ±(0.08)$_{n}$
14	2.49± (0.11)^{mn}	2.55 ±(0.07)^{p}	2.33 ±(0.06)^{kl}
15	1.83 ± (0.05)^{qr}	6.68 ±(0.09)^{c}	1.84 ±(0.10)^{n}
16	2.01 ± (0.08)^{pq}	2.18 ±(0.07)^{qr}	1.91 ±(0.09)^{jk}
17	1.43 ± (0.04)^{t}	1.93 ±(0.06)^{rs}	2.19 ±(0.09)^{lm}
18	1.36 ± (0.06)^{tu}	1.83 ±(0.03)^{s}	2.99 ±(0.09)^{i}
19	2.20 ± (0.09)^{op}	1.80 ±(0.05)^{s}	1.80 ±(0.01)^{n}
20	2.61 ± (0.08)^{lm}	1.98 ±(0.07)^{rs}	3.65 ±(0.07)^{h}
21	3.35 ± (0.15)^{h}	2.29 ±(0.26)^{q}	2.72 ±(0.08)^{j}
22	1.70 ± (0.06)^{rs}	2.89 ±(0.07)^{no}	2.55±(0.07)^{jk}
23	2.85 ± (0.06)^{k}	2.69 ±(0.08)^{op}	3.96 ±(0.02)^{g}
24	4.47 ± (0.06)^{c}	6.86 ±(0.08)^{c}	4.15 ±(0.08)^{fg}
25	2.78 ± (0.12)^{kl}	8.09 ±(0.07)^{b}	2.38 ±(0.07)^{kl}
26	2.15 ± (0.06)^{op}	8.57 ±(0.08)^{a}	2.48 ±(0.06)^{jk}
27	2.11 ± (0.06)^{p}	5.84 ±(0.07)^{e}	6.05 ±(0.09)^{c}
28	3.10 ± (0.04)^{ij}	5.09 ±(0.12)^{i}	1.98 ±(0.07)^{mn}
29	3.20 ± (0.02)^{i}	6.28 ±(0.08)^{d}	5.26 ±(0.08)^{d}
30	2.34 ± (0.04)^{no}	7.98 ±(0.04)^{b}	7.63 ±(0.05)^{a}
31	4.57 ± (0.03)^{bc}	5.40 ±(0.04)^{fgh}	2.38 ±(0.08)^{kl}
32	1.40 ± (0.04)^{tu}	3.41 ±(0.07)^{m}	1.87 ±(0.06)^{n}
33	4.83 ± (0.06)^{a}	5.15 ±(0.08)^{gi}	1.92 ±(0.05)^{n}

3.5 Plant Growth Promoting Traits

3.5.1 Phosphate solubilization

The ability of isolates to solublize insoluble form of phosphate (tricalcium phosphate) was tested. In general, the phosphate solubilizing potential of screened isolates increased with sodium chloride concentration. Among the 18 cultures tested, 14 showed phosphate solubilizing ability. In the absence of sodium chloride, eight isolates (ST7, ST12, ST13, ST17, ST27, ST30, ST32, ST34, and ST40) showed phosphate solubilization ability. At 5% NaCl, 5 isolates, namely ST1, ST7, ST9, ST30, and ST40, showed positive results for phosphate solubilization. However, at a higher concentration of 10% NaCl, seven isolates (ST1, ST2, ST7, ST8, ST9, ST11, and ST17) solubilized an insoluble form of phosphate. Isolate ST7 solubilized phosphate in all three concentrations of NaCl. Similarly, reports of [38,39] revealed predominance of phosphate solubilizing microorganisms in the Mangrove forest soils of Sourashtra and Sundarbans of India respectively.

3.5.2 Potassium release

Among 18 cultures, only 9 showed potassium releasing ability at 0% NaCl. No potassium releasing activity was observed at 10% NaCl. At 5% NaCl, only three cultures, namely ST17, ST20, and ST32, indicated potassium releasing ability. Prominence of potassium releasing bacteria in mangroves soils was reported [38,39].

3.5.3 Zinc solubilization

The bacterial isolates were able to solubilize zinc only under normal conditions. Among 18 isolates, 9 isolates (ST3, ST7, ST12, ST17, ST18, ST27, ST30, ST34, and ST40) could solubilize zinc at normal conditions. None of the isolates were able to solubilize zinc at 5% and 10% NaCl concentrations. Although, zinc is a micronutrient, its deficiency seriously affects crop growth as well as health of human beings. Paucity or fixation of zinc is noticed in saline soils. Predominance of zinc solubilizing microorganisms in the samples of current study indicates its potential in improving nutrient availability [38 and 39].

3.5.4 HCN production by bacterial isolates

None of the isolates were able to produce HCN at normal (0%) as well as high salt (5% & 10%) concentrations.

Table 5. Mineral solubilization assay

Isolates	P			K			Zn			HCN		
	0%	5%	10%	0%	5%	10%	0%	5%	10%	0%	5%	10%
ST1	-	+	+ + +	-	-	-	-	-	-	-	-	-
ST2	-	-	+ + +	-	-	-	-	-	-	-	-	-
ST3	-	-	-	-	-	-	+	-	-	-	-	-
ST7	+ + +	+	+ + +	+ + +	-	-	+ + +	-	-	-	-	-
ST8	-	-	+ + +	-	-	-	-	-	-	-	-	-
ST9	-	+ +	+ + +	+ + +	-	-	-	-	-	-	-	-
ST11	-	-	+ + +	+ + +	-	-	-	-	-	-	-	-
ST12	+	-	-	+ + +	-	-	+	-	-	-	-	-
ST13	+	-	+	+ + +	-	-	-	-	-	-	-	-
ST17	+	-	+ + +	+ + +	+ +	-	+	-	-	-	-	-
ST18	-	-	-	-	-	-	+	-	-	-	-	-
ST20	-	-	-	-	+	-	-	-	-	-	-	-
ST27	+ +	-	-	+ +	-	-	+ + +	-	-	-	-	-
ST30	+ + +	+ + +	-	+ + +	-	-	+ + +	-	-	-	-	-
ST32	+ + +	-	-	+	+	-	-	-	-	-	-	-
ST34	+	-	-	-	-	-	+	-	-	-	-	-
ST39	-	-	-	-	-	-	-	-	-	-	-	-
ST40	+	+ + +	-	-	-	-	+	-	-	-	-	-

**('+++' confluent growth,'++' moderate growth,'+' feable growth,'-'no growth)*

Table 6. Growth of bacterial isolates at different pH concentrations

Isolates	0%			5%			10%		
	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9
ST1	+	+ + +	+ +	+ + +	+ +	+ + +	+	+ +	+ + +
ST2	+ +	+	+	+	+	+ + +	+	+ +	+ +
ST3	+	+ +	+	+ + +	+ +	+ + +	+ +	+	+ + +
ST7	+	+	+	+ +	+	+	-	-	-
ST8	+	+	+	+ + +	+ +	+ + +	+	+ +	+ + +
ST9	+	+	+ +	-	+	+ + +	-	+	+ +
ST11	+	+ +	+ +	+	+ +	+ + +	-	-	+
ST12	+	+ +	+ +	+	-	+ + +	+	+ +	+ + +
ST13	+ + +	+ +	+ +	+ +	+ +	+ + +	+	+ +	+ + +
ST17	-	-	+	-	-	+	-	-	-
ST18	+ + +	+ +	+ +	+ + +	+ +	+ +	+ +	+ +	-
ST20	+	+ +	+ +	+	+	+ + +	-	-	+
ST27	+ + +	+	+ +	+ + +	+ +	+ +	-	+	-
ST30	+	+	+	-	-	+	-	-	-
ST32	+ + +	+ +	+	+ +	-	+	+	-	-
ST34	+ +	+ +	+ +	-	+	+	-	-	-
ST39	+ +	+	+	+ +	+	+ + +	+	+ +	+ + +
ST40	+ + +	+	+ +	+	+	+ + +	-	+	+ + +

**('+++' confluent growth,'++' moderate growth,'+' feable growth,'-' no growth)*

Table 7. Growth of bacterial isolates at different temperatures

Isolates	0%			5%			10%		
	20°C	30°C	40°C	20°C	30°C	40°C	20°C	30°C	40°C
ST1	+	+ + +	+ +	+	+ + +	+ +	-	+	+
ST2	+	+ + +	+ +	+	+ + +	+ +	-	+ +	-
ST3	-	+ + +	-	-	+ + +	+	-	+ +	-
ST7	+	+ + +	+ + +	-	+ +	-	-	+	-
ST8	-	+ + +	-	-	+ + +	+ + +	-	+ +	+ +
ST9	+	+ + +	-	+	+ + +	-	-	+ +	-
ST11	+	+ + +	+ + +	+	+ +	-	-	+	-
ST12	+ +	+ + +	-	+	+ + +	+ + +	-	+	-
ST13	+	+ + +	+	-	+ + +	+ +	-	+ +	-
ST17	-	+ + +	-	-	+	-	-	+	-
ST18	+	+ + +	+ + +	+	+ + +	+ + +	-	+ +	-
ST20	-	+ + +	-	+	+ + +	-	-	+	-
ST27	+	+ + +	+ +	-	+ + +	-	-	+	-
ST30	-	++	+ +	-	+	-	-	+	-
ST32	+	+ + +	+	+	+ +	+ +	-	+	-
ST34	-	+ + +	-	-	+ +	-	-	+	-
ST39	+	+ + +	+ + +	+	+ + +	+ + +	-	+ +	+ +
ST40	-	+ + +	+ + +	+	+	+ +	-	+ +	-

**('+++' confluent growth,'++' moderate growth,'+' feable growth,'-' no growth)*

3.6 Growth of Isolates at Different pH, and Temperatures

Among the eighteen isolates, all except bacterial isolate ST17 could survive at pH 4, 7 and 9 under normal conditions. At 5% and 10% NaCl concentrations, the growth was higher at pH 9, whereas moderate at pH 4.

At different salt concentrations, all 18 isolates showed maximum activity at 30°C. Only 4 selected isolates behaved normally at 40°C in 0% NaCl (ST7, ST11, ST18, ST39, and ST40) and 5% NaCl (ST8, ST12, ST18, and ST39), respectively. Poor growth was observed at 20°C in 0 and 5% NaCl. There was a complete absence of growth and 10% NaCl. This study supported the findings of [40], who isolated

halotolerant bacteria from Weston Park pond and Dew pond in Derbyshire Peak District in Sheffield and found their growth at temperatures ranging from 35°C to 37° C on medium supplemented with 2-3.5% NaCl.

4. CONCLUSION

It is evident that the sampling region has a wide variety of moderately halo-tolerant bacteria. The present research has identified several important characteristics of the salt-tolerant PGPR, including phosphate and zinc solubilization, potassium release, HCN production, and other essential traits. Finally, it is evident that the bacterial isolates from the mangrove area are not only halotolerant but also have characteristics that promote plant growth. These plant-growth promoting bacteria can be utilized to develop plant probiotic formulations to promote crop growth and sustain agriculture in abiotically stressed soils such as saline, sodic, and saline sodic soils.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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