

# **Halotolerant plant probiotic bacterial isolates of mangrove soils of Chidambaram and Thanjavur**

## **ABSTRACT**

Halotolerant plant growth-promoting Rhizobacteria (HTPGPR) are beneficial microbes that can be exploited to mitigate abiotic stresses on plants. In the current investigation, 48 rhizobacterial isolates from eight saline soil samples from 2 mangrove ecosystems in Tamil Nadu (Chidambaram and Thanjavur) were obtained. They found that they tolerated 15% NaCl under in vitro conditions. On the basis of intracellular sodium accumulating capacity, polysaccharide production, and biofilm formation, 18 isolates were screened and named (ST1, ST2, ST3, ST7, ST8, ST9, ST11, ST12, ST13, ST17, ST18, ST20, ST27, ST30, ST32, ST34, ST39, and ST40). These 18 strains were further characterized for their ability to mineralize phosphate, potassium, and zinc, HCN production, pH, and temperature tolerance. Among the 18 isolates, ST7, ST17, and ST30 were found to be multimicrobial solubilizers. Bacterial isolate ST17 was found to grow only in alkaline (pH 9.0) and at mesophilic temperature of 35°C at 0 and 5% NaCl.

*Keywords: Halotolerant, Salinity, Plant growth promoting bacteria, Mangrove ecosystem*

## **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 rhizospheric 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<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> [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), 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**

Halotolerant bacteria were isolated from mangrove soil using soil dilution and spread plate through the enrichment culture method 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<sup>-5</sup> and 10<sup>-6</sup>) were applied, spread using a glass spreader, and incubated at 28°C for two days. After the incubation period, colonies with different morphologies were chosen, purified, and kept as glycerol stocks at -20°C.

### **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).

### **2.4. Plant growth promoting traits**

#### **2.4.1. 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 [25].

#### **2.4.2. Potassium solubilization**

The efficiency of selected eighteen bacterial isolates to solubilize potassium was assessed using Alexandrov's agar medium with 0.3% potassium aluminium 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 solubilization.

#### **2.4.3. 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 [26].

#### **2.4.4. Qualitative assay of HCN production**

Using the [27] 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.

## 2.5. 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 [28] [29]. At various temperatures, including 20, 35, and 45 °C, these plates were incubated, and the growth pattern was monitored.

## 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. Physio-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 %).

**Table 1. Physio-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 | 3.49      | 0.32   | 4              |
| Thanjavur non Rhizosphere -2   | 7.69 | 3.90      | 0.34   | 6              |
| Thanjavur Rhizosphere -1       | 7.61 | 3.19      | 0.46   | 13             |
| Thanjavur Rhizosphere -2       | 7.52 | 3.51      | 0.49   | 8              |
| Chidambaram non Rhizosphere -1 | 7.77 | 2.52      | 0.52   | 4              |
| Chidambaram non Rhizosphere -2 | 7.74 | 2.59      | 0.55   | 1              |
| Chidambaram Rhizosphere -1     | 7.89 | 2.65      | 0.63   | 7              |
| Chidambaram Rhizosphere -2     | 7.81 | 2.19      | 0.69   | 5              |

### 3.2. Isolation of halotolerant bacterial isolates

To enrich saline tolerant microorganisms in the soil samples, pH (8.5) and EC (4 ds/m) were adjusted and incubated for 15 days at room temperature. After the incubation period, a 1 gram soil sample was serially diluted to  $10^{-6}$ . Dilutents from  $10^{-3}$  and  $10^{-4}$  were plated on 5 different media, as mentioned in Section 2.0. A total of forty eight bacterial isolates (ST1 to ST48) were chosen from rhizosphere and non rhizosphere soil 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 isolates from starch casein agar media at  $10^{-3}$  and  $10^{-4}$  dilution. Our findings parallel those of [30], who identified bacteria from soil with varying morphological characteristics, indicating the presence of a diverse bacterial community in soil.

### 3.3. Screening salt tolerance potential of bacterial isolates

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% and thirty seven isolates in 10% NaCl and only five isolates in 15% NaCl concentration. This implies that the isolates were halotolerant organisms, which is consistent with the findings of [31], who discovered that halotolerant bacteria can grow in a wide range of salt concentrations. 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.

**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**

| Isolate | 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                           |



|      |    |    |    |    |    |   |    |   |   |   |   |   |
|------|----|----|----|----|----|---|----|---|---|---|---|---|
| ST11 | -  | -  | ++ | ++ | -  | - | -  | - | - | - | - | - |
|      |    |    | +  | +  |    |   |    |   |   |   |   |   |
| ST12 | +  | -  | -  | ++ | -  | - | +  | - | - | - | - | - |
|      |    |    |    | +  |    |   |    |   |   |   |   |   |
| ST13 | +  | -  | +  | ++ | -  | - | -  | - | - | - | - | - |
|      |    |    |    | +  |    |   |    |   |   |   |   |   |
| ST17 | +  | -  | ++ | ++ | ++ | - | +  | - | - | - | - | - |
|      |    |    | +  | +  |    |   |    |   |   |   |   |   |
| ST18 | -  | -  | -  | -  | -  | - | +  | - | - | - | - | - |
| ST20 | -  | -  | -  | -  | +  | - | -  | - | - | - | - | - |
| ST27 | ++ | -  | -  | ++ | -  | - | ++ | - | - | - | - | - |
|      |    |    |    |    |    |   | +  |   |   |   |   |   |
| ST30 | ++ | ++ | -  | ++ | -  | - | ++ | - | - | - | - | - |
|      | +  | +  |    | +  |    |   | +  |   |   |   |   |   |
| ST32 | ++ | -  | -  | +  | +  | - | -  | - | - | - | - | - |
|      | +  |    |    |    |    |   |    |   |   |   |   |   |
| ST34 | +  | -  | -  | -  | -  | - | +  | - | - | - | - | - |
| ST39 | -  | -  | -  | -  | -  | - | -  | - | - | - | - | - |
| ST40 | +  | ++ | -  | -  | -  | - | +  | - | - | - | - | - |
|      |    | +  |    |    |    |   |    |   |   |   |   |   |

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

### 3.5. Growth of isolates at different pH, and temperatures

Among the eighteen isolates, all except 10 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.

**Table 5. 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)

At different salt concentrations, all 18 isolates showed maximum activity at 35°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 [33], 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.

**Table 6. Growth of bacterial isolates at different temperatures**

| Isolates | 0%    |       |       | 5%    |       |       | 10%   |       |       |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|          | 20° C | 35° C | 40° C | 20° C | 35° C | 40° C | 20° C | 35° C | 40° C |
| ST1      | +     | +++   | ++    | +     | +++   | ++    | -     | +     | +     |
| ST2      | +     | +++   | ++    | +     | +++   | ++    | -     | ++    | -     |
| ST3      | -     | +++   | -     | -     | +++   | +     | -     | ++    | -     |
| ST7      | +     | +++   | +++   | -     | ++    | -     | -     | +     | -     |
| ST8      | -     | +++   | -     | -     | +++   | +++   | -     | ++    | ++    |
| ST9      | +     | +++   | -     | +     | +++   | -     | -     | ++    | -     |
| ST11     | +     | +++   | +++   | +     | ++    | -     | -     | +     | -     |
| ST12     | +     | +++   | +++   | +     | +++   | +++   | -     | +     | -     |
| ST18     | +     | +++   | +     | -     | +++   | ++    | -     | ++    | -     |
| ST39     | -     | +++   | -     | -     | +     | -     | -     | +     | -     |
| ST40     | +     | +++   | +++   | +     | +++   | +++   | -     | ++    | -     |
| ST2      | -     | +++   | -     | +     | +++   | -     | -     | +     | -     |
| ST7      | +     | +++   | +++   | +     | +++   | +++   | -     | ++    | -     |
| ST2      | +     | +++   | ++    | -     | +++   | -     | -     | +     | -     |
| ST3      | -     | ++    | ++    | -     | +     | -     | -     | +     | -     |
| ST3      | +     | +++   | +     | +     | ++    | ++    | -     | +     | -     |
| ST3      | -     | +++   | -     | -     | ++    | -     | -     | +     | -     |
| ST3      | +     | +++   | +++   | +     | +++   | +++   | -     | ++    | ++    |

ST4 -     + + +     + + +     +     +     + +     -     + +     -  
0

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

#### 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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