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# Development of a Promising Saline Tolerant Plant Growth Promoting Microbial Consortium from Acid-Saline *Pokkali* Soils of Kerala, India

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***Pokkali* land represents the lowlands, often below sea level, in the coastal regions of Ernakulam, Thrissur and Alappuzha Districts of Kerala in South India, where rotational rice-prawn farming is practised organically. The study aimed to isolate and screen salt-tolerant beneficial microbes to develop a microbial consortium with enhanced salt tolerance and plant growth promotion. Ten Pokkali soil samples were collected from Ernakulam District, Kerala, India and enumerated using serial dilution. The microbes were screened for salinity tolerance at electric conductivity (EC) of 0, 2, 4, 6, 8, 10 and 12 dS $m^{-1}$. The highest population of Nitrogen fixers, Phosphate solubilisers, Zinc solubilisers and fluorescent *Pseudomonas* were $7.3\times10^5$, $12\times10^5$, $17\times10^5$ and $1\times10^4$ CFU $g^{-1}$, respectively. Potassium solubilisers, *Azotobacter* and *Azospirillium,* were absent in the soil. Altogether, 29 predominant isolates were obtained, and among these isolates, 17 belonged to Zinc solubilisers, six were Phosphate solubilisers, and five were Nitrogen fixers. The remaining isolate was fluorescent *Pseudomonas*. Novel isolates were evaluated for their plant growth-promoting activities, such as the production of indole acetic acid (IAA), ammonia, hydrogen cyanide (HCN) and siderophore. Among these 29 isolates, 16 isolates showed weakly positive results in siderophore production and 11 isolates appeared strong (red +++) in ammonia production. None indicated positive results in IAA and HCN production. The *in vitro* compatibility among the isolates was tested using standard protocols,and all the isolates were compatible. Ten promising isolates were selected from 29 predominant isolates to develop three talc-based microbial consortia by mixing culture broth media with talc in a 1:3 ratio. The selected isolates included three nitrogen-fixing bacteria ($S_3B_2N$, $S_5B_3N$, and $S_5B_2N$), three phosphate solubilizers ($S_6B_2P$, $S_5B_2P$, and $S_9B_1P$), one fluorescent *Pseudomonas* ($S_8PF$), and three zinc solubilizers ($S_{10}F_1Zn$, $S_7F_1Zn$, and $S_{10}F_2Zn$). The best four promising isolates ($S_9B_1P$, $S_5B_3N$, $S_7F_1Zn$, and $S_8PF$) in the study were selected for molecular characterization and identification.**

(***Key words:*** *Pokkali, Rhizobacteria, Acid saline, Saline tolerant , PGPR)*

*Pokkali* cultivation in the coastal saline lands of Kerala, India, is an unique way of recovering and managing soil salinity. It includes coastal paddy fields in Ernakulum, Thrissur, Alleppey, and Kottayam districts of Kerala, South India. *Pokkali* soils are Kerala's tidal wetlands, characterised by salinity accumulation by tidal action (Sah *et al.,* 2014). The twice-daily tides are vital for plant fertility and production because they contain significant concentrations of sodium, potassium, calcium, and magnesium, all necessary for physiological activities in plant cells. (Kramer, 1984). The soil is acid saline in nature, with a pH ranging from 3.0 to 6.8. The electric conductivity of soil ranges from 12 to 24 dS $m^{-1}$ during the high saline phase (November to May) and from 0.01 to 7.8 dS $m^{-1}$ during the low saline phase (June to October) (Sreelatha and Shylaraj, 2017).

Rice, one of the most important food crops in the world, is highly sensitive to soil salinity at the seedling stage (Mishra *et al.,* 2021). As a suitable alternative to chemical fertilisers, the use of plant growth-promoting microorganisms has increased in recent years due to their potential as biofertilisers. The plant growth promoting rhizobacteria (PGPR) helps in plant growth promotion by two mechanisms, *viz.,* direct supply of ions to plants (Glick *et al.,* 1999) and indirectly depriving ionstoplant pathogens (Ahmad *et al.,* 2008). Nitrogen is one of the primary macronutrients, and nitrogen fertilisers contribute to groundwater contamination with nitrates. An alternative option for this situation is to use nitrogen-fixing bacterial inoculumto increase plant

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growth (Wickramasinghe *et al.,* 2021). Phosphorus is the second most important macronutrient for plant growth. Plants cannot absorb phosphorus as such because it occurs mainly in insoluble forms. Phosphate-solubilizing microorganisms (PSM) have been used as biofertilisers. Zinc deficiency is a significant problem in plant growth. Zinc is abundantly present in the earth's crustandis commonly found in the soil as zinc silicate, zinc oxide, zinc phosphate, and zinc carbonate. The main reasons for zinc deficiency are higher use of chemical fertilisers, intensive farming, and inadequate irrigation systems. Its deficiency mainly affects photosynthesis, reduces flowering and fruit development, and delays crop maturity (Senthilkumar *et al.,* 2021).

Plant Growth-Promoting Rhizobacteria (PGPR) are nonpathogenic, highly root colonizingbacteria found on the surface of plant roots that boost plant growth. Plant-bacterial interactions in the rhizosphere influence plant health and soil fertility. Free-living soil bacteria called "plant growth-promoting rhizobacteria" can directly or indirectly help roots. When a putative PGPR can create a favourable effect on the plant after inoculation, it has good competitive abilities over existing rhizosphere communities (Jha and Saraf, 2015).

*Pokkali* cultivation is carried out in naturally organic manure without adding any inputs like manures, fertilisers and plant protection chemicals since these chemicals harm the succeeding prawn farming. The Pokkali soils are highly fertile due to high organic matter content and tidal action. However, the increasing challenges posed by climate change, such as floods, saline water intrusion, and prolonged dry spells, have significantly impacted the soil fertility and nutrient balance of these unique ecosystems.

*Pokkali* soils are highly fertile due to high organic matter content and tidal ingression. However, due to climate change and changes in land use patterns, soil fertility is declining, and toxicities/deficiencies of nutrients are emerging, resulting in decreasing yield. The drought and flood occurring in the same season results in changes in nutrient flux in the soil and the plants cannot recover without the addition of nutrients. Since *Pokkali* cultivation is organic, inorganic fertilizers cannot be introduced into this system. *Pokkali* farming avoids the use of chemical inputs to maintain its organic integrity and to prevent adverse effects on succeeding prawn farming, there is an urgent need to explore eco-friendly alternatives for restoring and enhancing soil fertility. The use of plant growth-promoting rhizobacteria offers a promising solution, as these microbes can improve nutrient availability, counteract the effects of salinity, and boost plant growth naturally. Developing saline-tolerant microbial consortia specific to *Pokkali* soils can help mitigate the adverse effects of salinity while promoting sustainable agricultural practices in these regions. Hence, the present study was carried out with an objective to isolate, screen, and develop a saline-tolerant microbial consortium with plant growth-promoting properties specifically suited for *Pokkali* soils.

## MATERIALS AND METHODS

### Soil sampling and enumeration of micro organisms

Ten soil samples were collected from *Pokkali* paddy fields *viz.* Chellanam, Kumbalangi, Ezhikkara, Kottuvally, Varappuzha, Chittatukkara, Poyya, Puthenvelikkara, Chedamangalam, and Vadakkekara in Ernakulam District, Kerala, India in 2021 from a depth of 0-15 cm. The samples were sieved through a 2 mm mesh and stored at room temperature for soil chemical analysis (Pattnaik *et al.,* 2000). Soil water suspension in the ratio 1:2 was centrifuged and the electrical conductivity ($EC_2$) was measured in the supernatant liquid (Jackson, 1973). Field moisture soil was used for the microbiological studies. Soil microorganisms were enumerated using the serial dilution technique on specific agar media. A suspension was prepared by adding 10 g of soil sample to 90 mL of sterile distilled water, mixing well, and allowing it to stand for 15 minutes. The suspension was serially diluted from $10^{-1}$ to $10^{-5}$, and 1 mL aliquots were plated and incubated. Pikovskaya agar, Jensen's agar, and Zinc Solubilizing Agar were used for isolating phosphate solubilizers, nitrogen fixers, and zinc solubilizers, respectively. Colony characteristics such as size, surface texture, margin, elevation, and color were recorded. Novel isolates were subjected to Gram staining to observe their Gram reaction and cell morphology. Fungal isolates were stained and examined using the Lacto Phenol Cotton Blue method. Pure cultures of the isolates were prepared and stored in glycerol stocks at - 80° C for further analysis.

**Screening of microorganisms for salinity tolerance**

To screen microbial salinity tolerance, growth media was amended with NaCl to achieve EC levels of 0, 2, 4, 6, 8, 10, and 12 dS $m^{-1}$. Microbial isolates were inoculated into the salinity-amended media and incubated at optimal conditions, with growth monitored via $OD_{600}$ measurements over 72 hours. The maximum EC level supporting microbial growth was recorded to determine salinity tolerance by spot inoculation in respective media. The presence of the solubilisation zone was measured, and the degradation index was calculated as a percentage.

**Qualitative estimation of nitrogen, phosphorous and zinc production**

The quantification of nitrogen fixation was carried out using the microkjeldahl method. The total nitrogen content of the sample was expressed as mg of nitrogen fixed per gram of carbon source utilised (Bremner,1960). The quantitative assay for phosphate solubilisation was carried out with the phospho-molybdic blue colour method. The standard phosphate curve was plotted by measuring the colour intensity in a spectrophotometer at 660 nm (Olsen *et al.,*1954). The available zinc content was measured using atomic absorption spectrophotometry. Insoluble ZnO was amended in sterile broth (0.1%) (Sharma *et al.,* 2014).

**Screening for growth promotion traits of promising saline tolerant micro organisms**

Novel isolates were further evaluated for the production of Indole Acetic Acid, Ammonia, Hydrogen Cyanide and Siderpophore by using standard methods. Production of IAA was detected using Salkowski's method. The development of the pink colour qualitatively suggested IAA production; values were determined by spectrophotometry and represented in μg $ml^{-1}$ using a standard graph of IAA production (Bric *et al.,* 1991). Ammonia production was detected by adding the Nessler reagent, and the intensity of colour was classified as follows: yellow - weak (+), orange - medium (++) and reddish brown - (+++). The siderophore production was screened using Chrome Azurol Sulfonate (CAS) agar medium. Isolates which produced a yellow - orange halo zone around the colony were positive for siderophore (Schwyn and Neilands, 1987). The hydrogen cyanide production ability of isolates was evaluated by observing the colour change of the filter paper from yellow to reddish-brown (Bakker and Schippers, 1987).

**Evaluation of compatibility among the promising saline tolerant micro organisms**

To evaluate compatibility, dual culture assays were performed on salinity-amended media. For bacteria-bacteria compatibility, isolates were streaked 2 cm apart on Nutrient Agar Media observing growth and interaction. For bacteria - fungus compatibility, a 5 mm fungal disc was placed in the center of a salinity - amended Potato Dextrose Agar plate, with bacteria streaked 2 cm away. Growth patterns, and inhibition zones were assessed to determine compatibility (Anith *et al.,* 2021).

**Molecular characterisation of promising saline tolerant micro organisms**

The most efficient compatible isolates were identified by 16S rDNA (bacteria) and 18S rDNA (fungi) gene sequencing. For bacteria, the 16S rDNA region was amplified by Polymerase Chain Reaction (PCR) using universal primers 16s-RS-F: forward (5′-CAGGCCTAACACATGCAAGTC-3′) and 16s-RS-R: reverse (5′-GGGCGGWGTGTACAAGGC-3′). For fungi, the 18S rDNA region was amplified using universal primers NS1: 5′-GTAGTCATATGCTTGTCTC-3′ as forward and NS4: 5′-CTTCCGTCAATTCCTTTAAG-3′ as reverse. The PCR products were purified and sequenced for identification. Nucleotide BLAST analysis was performed with the con-sensus sequence obtained after alignment using the Bi-oEdit program. The identity of the organism was con-firmed by analyzing the BLAST output.

**Statistical analysis**

The data were analyzed statistically using the Analysis of Variance (ANOVA) technique, and mean values were compared using the Kerala Agricultural University General R-Shiny-based Analysis Platform Empowered by Statistics (KAU - GRAPES) software (Gopinath *et al.,* 2020), at a 5% probability level.

## RESULTS AND DISCUSSION

In this study, we examined the plant growth-promoting microbial assemblage of salt-adapted Pokkali landraces, evaluated their salinity tolerance, and developed microbial consortia comprising nitrogen-fixing bacteria, phosphate-

solubilizing bacteria, zinc-solubilizing bacteria, and fluorescent *Pseudomonas*. There is significant potential for isolating acid and saline-tolerant strains of plant growth-promoting bacteria specific to the *Pokkali* region, which holds immense promise for mass production and application in organic agriculture.

Research on plant growth-promoting rhizosphere microbes, particularly bacteria, is gaining importance due to their role in increasing crop production and mitigating yield losses by enhancing tolerance to biotic and abiotic stress. Beneficial microbial consortia with multiple plant growth-promoting (PGP) traits are instrumental in regulating essential environmental processes, such as the mineralization of complex organic matter into simpler nitrogen forms and the solubilization of phosphorus and potassium, thereby improving plant growth and productivity. The increasing demand for environmentally friendly agricultural practices has spurred the adoption of microbial fertilizers. Given the negative environmental impacts and rising costs of artificial fertilizers, the use of beneficial soil microorganisms for sustainable and safe agriculture has increased globally over the last few decades.

In recent years, an increasing number of studies have focused on the role of endophytic as well as rhizospheric microbes in alleviating abiotic stress in plants ( Sah *et al.,* 2014). They constitute an important component of the plant and soil microbiome comprising both bacteria and fungi. Two bacterial endophytes, *Bacillus subtilis* and *Mesorhizobiumciceri,* confer salt tolerance to chickpea by decreasing $H_2O_2$ concentrations and increasing proline content. *Pseudomonas fluorescens* and *P. migulae* ameliorate salinity stress in tomato plants by increasing the 1-aminocyclopropane-1-carboxylate deaminase activity, the key enzyme for ethylene biosynthesis.

**Isolation and preliminary screening of microbial isolates**

A total of 117 isolates were obtained from ten *Pokkali* paddy fields in the Ernakulam District of Kerala, India (Table 1). From these, 29 predominant novel isolates were selected based on preliminary screening. The highest populations of nitrogen fixers, phosphate solubilizers, zinc solubilizers, and fluorescent *Pseudomonas* were $7.3 \times 10^5$, $12 \times 10^5$, $17 \times 10^5$, and $1 \times 10^4$, CFU $g^{-1}$, respectively. Among these, 17 were zinc solubilizers, six were phosphate solubilizers, five were nitrogen fixers, and one was fluorescent *Pseudomonas*. Potassium solubilizers, *Azotobacter*, and *Azospirillum* were absent in the soil samples. The pH of the soil samples ranged from acidic to neutral.

**Screening for salinity tolerance**

The spot assay method was applied to all dominant isolates, except *fluorescent Pseudomonas,* to determine their degrading capacity at various $EC_2$ values (0, 2, 4,

***Table 1.*** *Details of soil samples collected from Pokkali paddy fields in Ernakulam*

| Sample name (Code) | Sample Location | GPS Coordinates | pH | Electrical conductivity (dS $m^{-1}$) |
|---|---|---|---|---|
| $S_1$ | Chellanam | 9.81796°N 76.27897°E | 6.03 | 2.80 |
| $S_2$ | Kumbalangi | 9.85642°N 76.27145°E | 6.15 | 3.10 |
| $S_3$ | Ezhikkara | 10.11325°N 76.23509°E | 6.60 | 2.20 |
| $S_4$ | Kottuvally | 10.12675°N 76.26692°E | 6.49 | 2.20 |
| $S_5$ | Varappuzha | 10.06598°N 76.25999°E | 5.02 | 1.40 |
| $S_6$ | Chittattukara | 10.14898°N 76.20325°E | 5.34 | 3.80 |
| $S_7$ | Poyya | 10.21318°N 76.22973°E | 4.37 | 0.14 |
| $S_8$ | Puthenvelikkara | 10.17322°N 76.2638°E | 4.90 | 3.40 |
| $S_9$ | Chendamangalam | 10.16022°N 76.25368°E | 5.69 | 0.30 |
| $S_{10}$ | Vadakkekkara | 10.15184°N 76.20522°E | 6.26 | 2.80 |

S-Location

6, 8, 10, and 12 dS $m^{-1}$). Five dominant nitrogen-fixing isolates - $S_1B_3N$, $S_5B_3N$, $S_3B_2N$, $S_5B_4N$, and $S_5B_2N$ showed growth at all EC values. The control plate was the only one to display a zone of clearance for isolates $S_1B_4P$, $S_2B_3P$, $S_2B_2P$, and $S_1B_2P$.

The isolates were screened for the extent of zinc solubilization on zinc-solubilizing agar medium, which was amended with zinc oxide as an inorganic source of zinc (Table 2). Twenty-three predominant zinc solubilizers were selected based on their ability to solubilize insoluble zinc. The isolates produced a halo zone around the colony, indicating solubilization of the zinc source in the agar medium. The percentage of degradation values for different treatments ranged from 37.00% to 139.84%, with significant differences observed at $P \leq 0.05$. The highest value was recorded by $S_{10}F_2Zn$ (160.86%) from Vadakkekkara, and the lowest value was recorded by $S_1F_1Zn$ (33%) from Chellanam. The sample from Poyya showed a solubilization percentage above 100% (101.13%).

***Table 2.*** *Zinc solubilisation capacity (%) of saline tolerant microbes from Pokkali soil*

| Sample | Mean value of Zinc solubilisation (%) |
|---|---|
| $S_1$ | 37.00d |
| $S_2$ | 82.13bc |
| $S_5$ | 79.35bc |
| $S_6$ | 59.10cd |
| $S_7$ | 101.13b |
| $S_8$ | 75.60bc |
| $S_9$ | 56.93cd |
| $S_{10}$ | 139.84a |

S-Location; Values followed by similar letters are not significantly different at 5 % level ($P \leq 0.05$)

The plate assay may fail in cases where no halo is present. This could be due to the different organic acids released by organisms, which have variable diffusion speeds. The quantitative solubilization method in broth tests produced more reliable results (Nautiyal, 1999). Salt-tolerant indigenous zinc solubilizing bacteria such as *Klebsiella pneumonia*, *Acinetobacter pittii, Acinetobacter calcoaceticus,* and *Pantoea agglomerans,* isolated from forest organic soils, promote yield and root growth in *Oryza sativa* grown in zinc-deficient alluvial soils (Prajapathi *et al.,* 2002).

A qualitative assay was performed to screen 15 novel strains for their ability to solubilize inorganic phosphate. Data were recorded by measuring the clear zone around the colony. In the present study, the phosphate solubilizing capacity of bacterial and fungal strains decreased as the electrical conductivity increased. The percentage of degradation values for different treatments ranged from 35.16% to 160.20%, with significant differences at $P \leq 0.05$ (Table 3). The phosphate solubilization potential (halo/clearing zone) of the isolate from Chittattukara, $S_6B_2P$, exhibited the highest P solubilization capacity (178.9%), followed by $S_1B_2P$ (138.4%) and $S_1B_1P$ (94.73%). The lowest value was recorded by $S_5B_2P$ (14.20%), followed by $S_9B_1P$ (22.20%) and $S_1F_1P$ (33.30%). A diverse range of salt-tolerant phosphate-solubilizing bacterial strains belonging to various genera (*e.g., Pantoea, Burkholderia, Aerococcus, Pseudomonas, Bacillus, Ensifer, Gordonia, Acinetobacter, Arthrobacter, Providencia, Serratia, Alcaligenes, Cobetia, Microbacterium, Agrobacterium, Acromobacter, Tetrathiobacter, Aphanothece*) can tolerate elevated salinity concentrations (Dey, 2021).

The genus *Alcaligenes* has been previously reported to exhibit salt tolerance. Behera *et al.* (2015) described a halophilic strain, *A. faecalis* D-TSB-1, capable of tolerating 15% NaCl. Similarly, Egamberdieva *et al.* (2019) reported salt-tolerant *A. faecalis* TSAU3 from the rhizosphere of wheat growing in saline soil with an EC

***Table 3.*** *Phosphorous degradation capacity (%) of saline tolerant microbes from Pokkali soil*

| Sample | Mean value of Phosphate solubilisation (%) |
|---|---|
| $S_1$ | 78.46b |
| $S_2$ | 35.16b |
| $S_5$ | 84.36b |
| $S_6$ | 160.20a |
| $S_9$ | 96.61ab |

S-Location; Values followed by similar letters are not significantly different at 5 % level (P≤0.05)

value of 560 ± 61 mS $m^{-1}$. Bric *et al.* (2013) also identified a halotolerant Alcaligenes sp. strain from coastal rice fields with a soil EC of 6.88 dS $m^{-1}$. However, previous studies have not investigated the mechanisms behind salt tolerance and growth promotion in Alcaligenes strains.

Quantification of nitrogen fixation, phosphorous and zinc solublization by saline-tolerant microorganisms.

Following the spot assay, five nitrogen fixers, six phosphate solubilizers, and sixteen zinc solubilizers were selected for further evaluation. The nitrogen-fixing ability of bacteria is crucial for plants, offering a potential alternative to reduce chemical fertilizer application. Nitrogen-fixing bacteria from various locations were tested for their nitrogen fixation ability in Jenson's broth media using the Micro-Kjeldahl method. The amount of nitrogen fixation ranged from 0.2 mg N fixed $g^{-1}$ of sucrose to 14.3 mg N fixed $g^{-1}$ of sucrose, with significant variation at different electrical conductivities (EC). The isolate $S_5B_3N$ from Varappuzha showed the highest nitrogen-fixing ability across different EC values. The nitrogen fixation by $S_5B_3N$ was 13.454 mg N fixed $g^{-1}$ of sucrose (EC 2), 13.313 mg N fixed $g^{-1}$ of sucrose (EC 6), 12.608 mg N fixed $g^{-1}$ of sucrose (EC 8), and 14.3 mg N fixed $g^{-1}$ of sucrose (EC 10). $S_3B_2N$ exhibited high nitrogen-fixing ability at EC 2 (6.545 mg N fixed $g^{-1}$ of sucrose) but lower values at EC 6 (3.443 mg N fixed $g^{-1}$ of sucrose). Among the five nitrogen-fixing isolates, $S_5B_2N$ recorded the lowest nitrogen fixation (0.2 mg N fixed $g^{-1}$ of sucrose). The maximum nitrogen fixation occurred between EC 4 and EC 10. Based on the nitrogen fixation values across various EC values, $S_5B_3N$, $S_5B_2N$, and $S_3B_2N$ were considered the most promising nitrogen fixers (Table 4).

The cultural and morphological characteristics of the selected nitrogen-fixing isolates are presented in Table 7. Three isolates formed medium-sized water droplet colonies with irregular shapes and entire margins. $S_5B_3N$ and $S_5B_2N$ exhibited convex elevations, while $S_3B_2N$ showed a raised elevation with a viscid texture. Gram-positive, rod-shaped cells were observed in three selected isolates.

Phosphate solubilization by microorganisms isolated from various locations was quantified in Pikovskaya broth using the phospho-molybdic blue color method. The phosphate solubilization ranged from 6 to 25 µg of P. There was no significant difference in phosphate solubilization across different EC values. Isolates such as $S_9B_1P$, $S_5B_2P$, and $S_5B_3P$ exhibited high solubilization at EC values greater than 8 d $Sm^{-1}$, while $S_6B_2P$, $S_6B_1P$, and $S_1F_1P$ showed maximum solubilization at EC values

***Table 4.*** *Quantitative esimation of nitrogen fixation (mg N fixed $g^{-1}$) of sucrose by saline tolerant microbes from Pokkali soil*

| Sl. No. | Isolates | Control | Electrical conductivity (EC, dS $m^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 6 | 8 | 10 | 12 |
| 1 | $S_5B_2N$ | 4.007b | 7.109a | 4.571b | 7.955a | 7.39a | 0.482c | 0.2c |
| 2 | $S_1B_3N$ | 4.007b | 0.905c | 4.43b | 0.34c | 0.764c | 6.968a | 6.263a |
| 3 | $S_5B_3N$ | 5.276b | 13.454a | 2.033b | 13.313a | 12.608a | 14.3a | 1.046b |
| 4 | $S_5B_4N$ | 3.443b | 0.905de | 1.469d | 0.482e | 6.636a | 2.174c | 0.764e |
| 5 | $S_3B_2N$ | 3.584c | 6.545a | 4.853abc | 3.443c | 4.289bc | 5.558ab | 3.725bc |

Values followed by similar letters are not significantly different at 5 % level (P≤0.05) S-location, B-Bacteria, N-Nitrogen

below 4 d $Sm^{-1}$. Based on these results, $S_6B_2P$, $S_5B_2P$, and $S_9B_1P$ were considered the most efficient phosphate solubilizers across various EC ranges (Table 5).

The morphological characteristics of the selected phosphate solubilisers are shown in Table 7. Three isolates exhibited medium-sized colonies with an irregular shape and smooth texture. $S_5B_2P$ and $S_6B_2P$ were cream-colored, while $S_9B_1P$ appeared pale yellow. $S_6B_2P$ showed raised elevation with an undulating margin, $S_5B_2P$ had a lobate margin with flat elevation, and $S_9B_1P$ exhibited an undulating border with raised elevation. $S_5B_2P$ and $S_6B_2P$ were gram-positive, rod-shaped cells, while $S_9B_1P$ was gram-negative.

Zinc solubilization by microorganisms isolated from various locations was quantified in zinc solubilizing broth. The amount of Zn solubilization ranged from 144.4

***Table 5.*** *Quantitative estimation of phosphate solubilization (μg $mL^{-1}$) by saline-tolerant microbes from Pokkali soil*

| Sl. No. | Isolates | Control | Electrical conductivity (EC, dS $m^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 6 | 8 | 10 | 12 |
| 2 | $S_5B_2P$ | 9.527b | 6.430d | 8.777c | 11.430b | 4.880b | 12.417b | 14.477a |
| 3 | $S_5B_3P$ | 8.867bc | 6.347d | 6.353d | 7.150d | 7.833c | 8.050c | 6.830b |
| 4 | $S_6B_1P$ | 7.923cd | 9.513c | 8.313cd | 8.640cd | 6.587bc | 6.033c | 6.423b |
| 5 | $S_6B_2P$ | 14.190a | 11.780b | 13.247b | 9.227c | 6.847b | 7.500cc | 8.527b |
| 6 | $S_9B_1P$ | 7.450d | 17.410a | 15.827a | 14.413a | 25.000a | 22.873a | 14.093a |

Values followed by similar superscripts are not significantly different at 5 % level (P≤0.05) S-location, B-Bacteria, P-phosphorus

***Table 6.*** *Quantitative esimation of zinc solubilization (mg $L^{-1}$) by saline tolerant microbes from Pokkali soil*

| Sl. No. | Isolates | Control | Electrical conductivity (EC, dS $m^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 6 | 8 | 10 | 12 |
| 2 | $S_5B_1Zn$ | 13.72g | 24.04d | 22.56e | 29.87b | 21.84f | 28.99c | 33.1a |
| 3 | $S_{10}F_2Zn$ | 91.23a | 49.83e | 52.30d | 66.48b | 27.90f | 63.50c | 26.50g |
| 4 | $S_8F_2Zn$ | 36.12e | 34.05f | 42.36d | 44.52c | 33.02g | 54.07a | 51.23b |
| 5 | $S_1F_4Zn$ | 44.29b | 33.78c | 24.21d | 60.40a | 21.50f | 23.05e | 19.80g |
| 6 | $S_5F_3Zn$ | 54.97a | 57.23a | 36.69b | 32.01bc | 37.38b | 21.61cd | 13.73d |
| 7 | $S_7F_1Zn$ | 49.11c | 37.67f | 59.95b | 144.4a | 37.67f | 44.83d | 42.21e |
| 8 | $S_{10}F_3Zn$ | 39.6d | 40.5c | 56.16b | 62.4a | 29.94f | 28.97g | 31.87e |
| 9 | $S_8F_1Zn$ | 48.95c | 57.38b | 45.71e | 101.1a | 26.19g | 48.36d | 35.09f |
| 10 | $S_{10}F_1Zn$ | 57.28d | 62.12c | 85.04a | 73.09b | 26.2g | 32.1e | 30.98f |
| 11 | $S_7B_1Zn$ | 36.56a | 25.64c | 33.05b | 21.5e | 23.12d | 15.28f | 13.49g |
| 12 | $S_1F_1Zn$ | 47.42a | 24.55b | 19.33d | 23.5c | 16.74f | 18.14e | 15.16g |
| 13 | $S_1F_2Zn$ | 16.38g | 23.13a | 16.7f | 17.65d | 16.79e | 18.08c | 18.48b |
| 14 | $S_5F_1Zn$ | 41.85a | 17.1g | 22.13d | 22.61c | 17.92f | 20.00e | 26.32b |
| 15 | $S_5F_2Zn$ | 20.43g | 25.63b | 21.86e | 28.6a | 24.3c | 23.59d | 20.97f |
| 16 | $S_{10}F_2Zn$ | 10.29g | 14.17c | 13.61d | 25.25a | 16.76b | 12.81e | 11.35f |

Values followed by similar letters are not significantly different at 5 % level (P≤0.05) S-location, F-Fungus, Zn-Zinc

to 10.29 mg $L^{-1}$, with significant differences observed at various EC values. $S_7F_1Zn$ (144.4 mg $L^{-1}$) and $S_8F_1Zn$ (101.1 mg $L^{-1}$) showed the highest solubilization at EC 6, while $S_{10}F_2Zn$ produced the highest solubilization under control conditions. $S_{10}F_2Zn$ had the lowest solubilization across all EC values compared to the other eleven zinc solubilisers (Table 6).

**Growth promotion by saline-tolerant microorganisms**

In this study, twenty-seven isolates tested negative for IAA production. IAA production by PGPR varies between species and is influenced by factors such as culture conditions, growth stage, and substrate availability. Salt-tolerant PGPRs (ST-PGPRs) produce IAA, which are essential for cell division and elongation, helping plants cope with salt stress. Notable ST-PGPRs known for producing IAA under salt stress include *Azotobacter, Arthrobacter, Azospirillum, Pseudomonas, Stenotrophomonas* and *Rahnella*. Previous studies have shown that the application of phytohormone-producing ST-PGPRs can help mitigate yield loss in crops during salt stress (Egamberdieva *et al.,* 2008, 2018; Piccoli *et al.,* 2011; Abd-Allah *et al.,* 2017). In saline conditions, P. putida modulated IAA synthesis in plant tissues, improving the growth parameters of cotton (Yao *et al.,* 2010). Additionally, the inoculation of ST-PGPRs has been observed to enhance mineral uptake, protect plants from ion toxicity, and promote root and shoot growth under saline conditions (Egamberdieva *et al.,* 2019).

Indole acetic acid (IAA) is a phytohormone that promotes plant growth by enhancing root and stem development. In this study, twenty-seven isolates tested negative for IAA production. None of the isolates produced HCN. Of the sixteen zinc solubilizers, fifteen showed weakly positive results for siderophore production, while five nitrogen fixers and five phosphate solubilizers tested negative for siderophores.

***Table 7.*** *Morphological and cultural characteristics of microbes isolated from Pokkali soils*

| Sample name | Texture | Size | Form | Colour | Elevation | Margin | Gram staining |
|---|---|---|---|---|---|---|---|
| $S_9B_1P$ | Smooth | Small | Irregular | Pale yellow | Raised | Undulate | Gram +ve, cocci |
| $S_5B_2P$ | Smooth | Medium | Irregular | Cream | Flat | Lobate | Gram +ve, rod |
| $S_6B_2P$ | Smooth | Medium | Irregular | Cream | Flat | Undulate | Gram –ve, rod |
| $S_5B_3N$ | Viscid | Medium | Round | Water droplet | Convex | Entire | Gram +ve, rod |
| $S_5B_2N$ | Viscid | Medium | Irregular | Water droplet | Convex | Convex | Gram +ve, rod |
| $S_3B_2N$ | Opaque | Medium | Round | Water droplet | Raised | Entire | Gram +ve, cocci |
| $S_8PF$ | Mucoid | Medium | Round | Fluorescent yellow | Raised | Entire | Gram +ve, cocci |

S-location, B-Bacteria, N-Nitrogen, P-Phosphorus, Zn-Zinc, PF-Fluorescent *Pseudomonas*

***Table 8.*** *16S rDNA (bacteria) and 18S rDNA (fungi) gene sequencing results of promising saline tolerant microbes from Pokkali soil*

| Sl. No. | Isolate | Description | Accession number | Percent similarity (%) |
|---|---|---|---|---|
| 1 | $S_5B_3N$ | *Paenibacillus polymyxa* | KY908483.1 | 99.30 |
| 2 | $S_9B_1P$ | *Priestia megaterium* | ON627838.1 | 99.83 |
| 3 | $S_8PF$ | *Alcaligenes faecalis* | ON626494.1 | 99.80 |
| 4 | $S_7F_1Zn$ | *Talaromyces purpureogenus* | KR809559.1 | 99.74 |

S-location-Bacteria, N-Nitrogen, P-Phosphorus, Zn-Zinc, PF-Fluorescent *Pseudomonas*

Only $S_8B_1Zn$ and $S_1F_1P$ were capable of producing siderophores. Out of the twenty-seven isolates, eleven were high ammonia producers, three were medium producers, nine showed negative results, and five produced weak amounts of ammonia.

**Development of consortia of saline tolerant micro organisms from Pokkali soil**

From these twenty-nine predominant isolates, the ten best isolates were chosen to develop three talc-based microbial consortium by mixing culture broth media with talc in a 1:3 ratio. Three nitrogen fixers ($S_3B_2N$, $S_5B_3N$, and $S_5B_2N$), three phosphate solubilisers ($S_6B_2P$, $S_5B_2P$, and $S_9B_1P$), three zinc solubilisers ($S_8F_1ZN$, $S_7F_1ZN$, and $S_{10}F_2ZN$), and one fluorescent *Pseudomonas* were selected for this depending on their functional efficiency.

**Identification of selected strains**

Sequencing of amplicons resulted in both forward and reverse sequences. Pair-wise alignment of both sequences was conducted using Clustal Omega software. A percent similarity of consensus sequences was analysed using the BLASTn tool on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 8).

## CONCLUSION

The study identified four promising saline-tolerant plant growth-promoting rhizobacteria from acid-saline Pokkali soils of Kerala: *Paenibacillus polymyxa* ($S_5B_3N$), *Priestia megaterium* ($S_9B_1P$), *Talaromyces purpureogenus* ($S_7F_1Zn$), and *Alcaligenes faecalis* ($S_8PF$). Among the 29 predominant isolates, ten were highly promising, including three nitrogen fixers ($S_3B_2N$, $S_5B_3N$, and $S_5B_2N$), three phosphate solubilizers ($S_6B_2P$, $S_5B_2P$, and $S_9B_1P$), one fluorescent *Pseudomonas* ($S_8PF$), and three zinc solubilizers ($S_{10}F_1Zn$, $S_7F_1Zn$, and $S_{10}F_2Zn$). These isolates were selected to develop three distinct microbial consortia, which can serve as sustainable and eco-friendly biofertilizer alternatives to chemical inputs. This approach aligns with the organic nature of *Pokkali* cultivation and requires further validation and testing under pot culture and field conditions.

## CONFLICTS OF 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.

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