
Potential of Consortia of Halotolerant Plant Growth Promoting Rhizobacteria in Sustainable Agriculture, isolated from saline soil of Sundarban Biosphere Reserve

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Salinity in soil is one of the major abiotic stresses faced by crop plants. It affects the growth and also the yield of crops and eventually makes the land unfit for cultivation. Low-lying areas such as Sunderban in West Bengal is vulnerable to cyclones and floods which results in increased salinity of the soil due to the contamination with saline sea water. In this study potential halotolerant plant-growth promoting rhizobacteria were isolated from soil samples from different areas in Sunderban to use them as inoculants to improve rice growth under salinity stress conditions. The isolates were initially screened for the production of Indole acetic acid (IAA), one of the most important growth hormones. The IAA content was in the range of 4.611µg/ml- 31.581µg/ml. 4 strains producing significantly higher amount of IAA were selected for further studies. Assay was done for Gibberellic acid (GA), another important growth hormone using 4 isolates along with a consortium of the isolates. GA was present in the range of 192.189µg/ml-207.604µg/ml. Seed germination study of *Oryza sativa* were performed by inoculating the seeds with the isolates and germination was studied under saline stress of 2% NaCl. The control was kept as seed germination under normal conditions. Germination of the rice seed was observed after 6 days of incubation treated with 2 isolates and the consortia of the microorganisms. IAA production was optimized with all 4 isolates and the consortia as well. Selected isolates were also screened for siderophore production and phosphate solubilization ability. To evaluate compatibility between strains that present in consortia, dual culture assay was done and none of the strains were found antagonistic to each other. The preliminary study suggests that rhizobacteria isolated from salt stress exposed soil may have the potential to support the seed germination in crop plants and may be used in sustainable agriculture.

Keywords : Consortia, growth promotion, rhizobacteria, seed germination

INTRODUCTION

The Sundarbans Biosphere Reserve, a unique coastal ecosystem in India is a part of the delta formed by Ganges and Brahmaputra rivers and their tributaries and is located between latitude 21°31'-22°40' North and longitude 88°05'-89°06' East (Ghosh *et al.* 2015). It faces considerable challenges due to the high salinity of its soils, not only that low lying areas is affected by cyclones and flood which is also major causes of salinity due to contamination with saline sea water which adversely affects the growth of crops, especially rice, not only that altered composition of rhizospheric bacteria, increase CO₂ level due to global warming also affected C3 crop

cultivation by changing root architecture and soil chemistry in rhizosphere (Tripathi *et al.* 2022). Rice (*Oryza sativa*) crop is staple food for millions of people and very sensitive to saline soil (Fairhurst *et al.* 2002). Rice cultivation is restricted mainly in Monsoon season due to increase in soil salinity than another season. Soil salinity results in poor germination, stunted growth, and reduced yields as reported in many journals (Ke *et al.* 2020). Huge portions of rice-cultivated land are situated in risky regions, mostly in the coastline areas. Climate change leads to the rise of seawater level, which causes flood and triggers the intrusion of saltwater into the inland areas. It is reported that more than 50% of arable land will be threatened by 2050 due to the effect of soil salinization which is the consequence of climate change, improper irrigation practices, and lack proper drainage systems (Masutomi *et al.* 2009).

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To reduce salt stress in crop many salts tolerant transgenic crop varieties are available but these transgenesis technologies are quite expensive and time consuming. In order overcome this problem, one of the alternative strategies for sustainable agricultural practice is to use halotolerant PGPR (plant growth promoting rhizobacteria) (Kumar *et al.*2020). Many areas of Sundarbans have been reported to harbour halotolerant eco-friendly rhizobacteria (Pallavi *et al.*2023). This group of rhizospheric bacteria are mainly free living, could effectively colonize with plant roots. PGPR improves plant growth by direct and indirect mechanisms. In direct mechanism they can fix atmospheric nitrogen, make insoluble minerals like potassium, phosphorus in soluble form, production of siderophores (Barbaccia *et al.* 2022) and by indirect mechanism PGPR can produce plant growth regulator like IAA (indole-3-acetic acid), GA (gibberellic acid) (Patel *et al.* 2017). Positive impacts of IAA in crops are improvement of root morphology, root initiation, cell division, root surface area. The effectiveness of PGPR is to increase the growth of various crops under salt stress conditions have been reported previously (Pallavi *et al.*2023). The primary selection of locally isolated salt-tolerant PGPR for salinity removal is important to ensure the effectiveness, and it has been reported that the indigenous strains are more effective in augmenting plant resistance to salinity stress compared to PGPR originated from the non-saline ecosystem (Cardinale *et al.*2015; Egamberdieva *et al.* 2009). It has been reported that halotolerant PGPR reduce salinity stress by numerous different mechanisms like osmoregulatory component (proline, glycine betaine, soluble sugars) secretion, increase water uptake capacity, production of antioxidant against different ROS as well as production of phytohormones, secretion of exopolysaccharide (Ha-Tran *et al.*2021).

In this study potential halotolerant plant growth promoting rhizobacteria were isolated from soil samples from different areas of Sundarbans to use them as inoculants to improve rice growth. Some of them produce potentially important phytohormones like IAA, GA which improve rice seed germination as well as increase seedling vigor, improve nutrient uptake, and reduce the

detrimental effects of salinity on plant development. As the population continues to rise, it is important to develop enduring agricultural practices that can mitigate the negative effects of salinity on crop production and many previous studies reported on Indian saline ecosystem contain many eco-friendly halotolerant PGPR which could be use in sustainable agricultural practice. This study focuses to isolate halotolerant PGPR from saline soils of the Sundarbans Biosphere Reserve and evaluate their potential in IAA and GA production that could promote rice seed germination. By isolating the most effective strains, this research aspires to contribute to the development of sustainable agricultural practices in Sundarbans.

MATERIALS AND METHODS

Isolation of Rhizobacteria

Soil samples were collected from 5 different sites (Canning, Dobanki, Frazerganj, Gadkhali, Kumirmari) of Sundarban, West Bengal. For isolation of microorganism, 1g of each soil sample was dissolved in 10ml of 0.9% saline water. 100 μ l of suspensions from serial dilutions (10^{-1} , 10^{-2} , 10^{-3}) were spread on Nutrient agar media containing petri plates. The nutrient agar media was supplemented with various concentrations of NaCl at 1%, 2%, 4%. The plates were incubated at 37°C for 24hrs.

Screening of isolates for IAA production

IAA assay was done following the colorimetric protocol given by Gordon and Weber (1951). 24hr grown 100 μ l bacterial culture inoculated in nutrient broth supplemented with 0.1% L-Tryptophan and incubated in a shaker at 120 rpm for 48 hrs. Culture was harvested by centrifugation at 10,000 rpm (Remi Cooling Centrifuge, C-248L, India) for 10 min at 4°C. 2ml of Salkowski reagent (0.5M FeCl₃ in 35% Perchloric acid) added to 1ml of supernatant (ratio-2:1) and incubated for 30mins. Absorbance was measured at 530 nm in UV-Vis spectrophotometer (Shimadzu, Japan). The standard plot of IAA was used to calculate the concentration of IAA.

Selection of Consortia

After screening and selection of high IAA producing isolates, consortia of the isolates were

prepared by adding 25µl culture of each isolate (DS2408, DS2411, DS2413, and DS2415) into 5ml of nutrient broth and incubated at 37°C.

Optimisation of IAA

IAA was optimized by using different concentration of L-Tryptophan (0.1%-0.5%) and incubation time (Day1-Day7) at 37°C. The cultures were incubated in a shaker at 120 rpm for 7 days and measured production of IAA at every 24-hr interval for 7 days.

Gibberellic Acid Assay

Gibberellic acid assay was performed for 4 isolates and consortia of the isolates. 4 days grown 2ml culture centrifuged at 10,000 rpm (Remi Cooling Centrifuge, C-248L, India) for 10mins at 4°C. To 1.5ml of supernatant, 0.2ml of zinc acetate and 0.2ml of ferro cyanide added and centrifuged at 2500 rpm for 15mins. 30% (1:1 v/v) added to supernatant and incubated at room temperature for 75 mins. Absorbance measured at 254 nm in UV-Vis spectrophotometer (Shimadzu, Japan) and concentration determined from standard curve (Holbrook *et al.* 1961).

Siderophore production

The siderophore production ability of the strains DS2408, DS2411, DS2413, and DS2415 was evaluated using Chrome Azurol S (CAS) agar plate method (Schwyn *et al.* 1987). When siderophore were present, they chelate Fe (III) from the CAS-Fe (III) complex, causing a color changed from blue to yellow or orange halo zone surrounds the colony after 72 h incubation at 37°C.

Phosphate solubilizing ability

Phosphate solubilizing ability of consortium were tested in presence of Pikovskaya agar media supplemented with bromophenol blue indicator (0.5%) for 72h incubation at 37°C. Phosphate solubilization was indicated by clear halo zones obtained around the colonies and calculated as PSI by using formula (Edi *et al.* 1996).

Phosphate Solubilizing Index (PSI) = (CD + HD) / CD Where CD= Colony Diameter (cm) and HD= Halo zone Diameter (cm).

Dual culture assay

To evaluate antagonistic interaction, if any, between bacterial strains this method was used. Bacterial strains DS2408, DS2411, DS2413, and DS2415 were grown on nutrient agar containing petri plate side by side in any combination. If one strain was inhibited by other strain clear zone was observed. The presence of an inhibition zone indicated antagonism.

Seed Germination

Plant growth promotion ability was tested *in vitro* by modified method of Arora *et al.* (2020). Rice seeds were surface sterilized with 70% alcohol for 1 min and washed with sterile water. Seed germination was conducted for two sets. For the first set, 5ml culture of each isolate and the consortia was taken in 5 different sterile petri plates and rice seeds were soaked in it for 30 mins. For the second set, 5ml of 2% saline solution and 5 ml culture of each isolate and the consortia was taken in petri plates and rice seeds soaked in the culture suspension under saline stress conditions for 30 mins. Rice seeds in sterile water taken as control and all seeds placed in petri plates. After germination, root length, shoot length, dry weight and fresh weight measured.

RESULTS AND DISCUSSION

Isolation of Halotolerant PGPR

A total of 43 morphologically diverse halotolerant PGPR colonies were obtained from the rhizospheric soil samples. The characteristics of isolates varied from small to large-sized, round or irregular-shaped colonies with smooth or entire margins and either opaque or translucent. These isolates were then screened for Indole Acetic Acid (IAA) production.

Indole Acetic Acid Production

20 isolates out of 43 isolates were found to be able to produce IAA in the presence of 0.1% L-Tryptophan. None were able to produce IAA in the absence of L-Tryptophan. IAA was produced in the range of 4.611µg/ml- 31.581µg/ml. Isolate DS2408 produced the highest amount of IAA at 31.581µg/ml (Table 1, Fig.1).

Table 1: IAA production by isolates at 0.1% L-Tryptophan

Isolates	IAA Content (µg/ml)
KS2401	9.309
KS2408	9.483
DS2403	6.96
DS2408	31.581
DS2410	10.788
DS2411	28.275
DS2412	8.091
DS2413	16.704
DS2414	9.309
DS2415	16.53
DS2417	9.048
CS2404	5.394
CS2405	9.22
CS2406	10.179
CS2407	4.611
CS2408	10.353
GS2402	9.048
FS2401	8.004
FS2404	6.873
FS2405	9.048

Table 2: Morphological characteristics of screened isolates

Isolates	Shape	Size	Colour	Margin	Gram Character
DS2408	Round	Tiny	Yellow	Smooth	Gram negative rods
DS2411	Irregular, Floral	Large	Creamy white	Smooth	Gram negative rods
DS2413	Round	Medium	Pale creamy white	Smooth	Gram negative rods
DS2415	Round	Medium	Pale white	Smooth	Gram negative rods

Colony morphology of selected isolates

4 isolates were chosen for further studies based upon their IAA production ability- DS2408,

DS2411, DS2413, and DS2415 and they were diverse in their colony morphology (Table 2) based on colony shape, size, colour and margin.

Optimization Study of IAA Production

DS2408 isolate was found to produce the maximum amount of IAA at 102.399µg/ml, after 48 hrs of incubation at 37°C in the presence of 0.3% L-Tryptophan. The data of IAA production described above is the maximum amount of IAA produced by a particular isolate at a given Tryptophan concentration and incubation period.

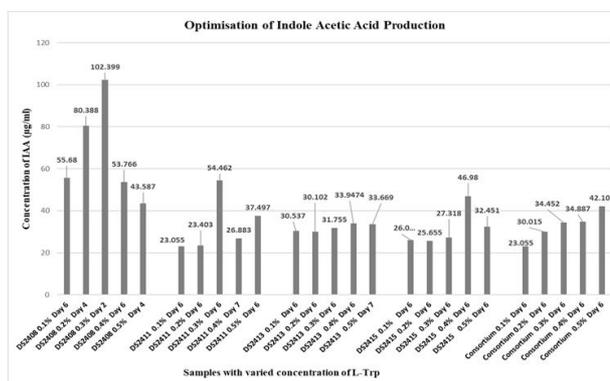


Fig.1: IAA production at different levels of L-Trp over 7 days

Gibberellic Acid Production

3 out of the 4 isolates and the Consortia of isolates were found to produce Gibberellic Acid (GA). DS2411 was found not to produce any gibberellic acid (Table 3). High levels of GA were produced by DS2415.

Estimation of Siderophore

It was observed that all four bacterial strains DS2408, DS2411, DS2413, and DS2415 were

Table 3: Gibberellic acid production by isolates

Isolates	DS2408	DS2411	DS2413	DS2415	Consortia
GA Content ($\mu\text{g/ml}$)	199.4472	--	192.1896	207.6048	194.5008

+ = Present; - = Absent

positive for siderophore production. The production ability of siderophore was roughly estimated based on formation of yellow color zone surrounding the bacterial colonies. DS2415 Showed maximum siderophore production ability (Fig.2).

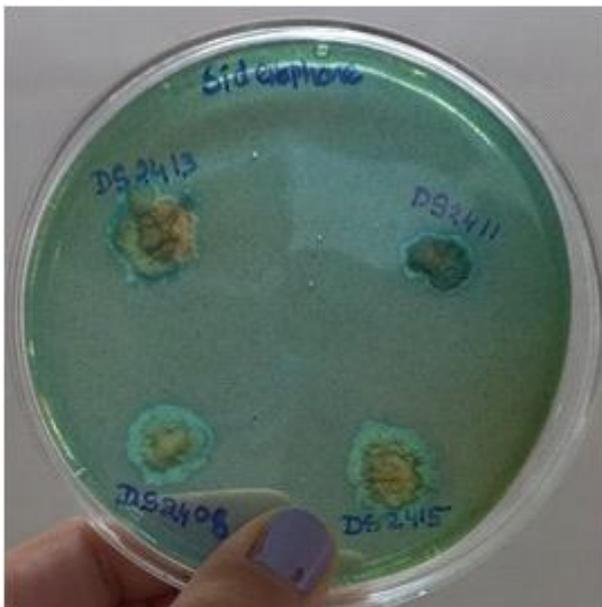


Fig. 2: Yellow colour zone surrounding bacterial colony indicate siderophore production

Phosphate solubilization

None of the strains were able to solubilize inorganic phosphate present in the media (Fig.3).

Assessment of antagonistic interaction between strains

In the present study, none of the strains showed antagonism against each other; they did not produce clear zone of inhibition around each other. The growth of one strain did not suppress growth of other, it proved that all four strains were compatible and could coexist with each other (Fig.4). So, it was feasible to use all four strains for consortium.



Fig. 3: Growth of bacterial strains on PVK plate, no clear zone observed surrounding the colonies.



Fig 4: Pairing of isolates used within the consortium.



Fig. 5 : Germination in rice seedlings after 16 days of inoculation

Germination of Rice Seeds

Increased root length was observed in case of DS2413 and consortia treated rice seedlings (*O. sativa*). Similarly shoot length was also enhanced in presence of DS2413 and microbial consortia (Fig 5, Table 4), though strain DS2411 had also help to increase the root and shoot length. The

Table 4: Germination characteristics of seedlings

Isolates + Treatment	Root Length (cm) ± SD	Shoot Length (cm) ± SD	Fresh Weight (gm) ± SD	Dry Weight (gm) ± SD
Consortia+ Seeds	4.3±0.02	12.5±0.3	0.27±0.01	0.066±0.03
DS2411+ Seeds	0.5±0.03	3.8±0.1	0.02±0.1	0.010±0.11
DS2413+ Seeds	3±0.02	9.2±0.2	0.02±0.02	0.006±0.1
DS2408 + Seeds	No germination observed	No germination observed	No germination observed	No germination observed
DS2415 + Seeds	No germination observed	No germination observed	No germination observed	No germination observed

consortia had increased the fresh weight (0.27 gm) and dry weight (0.066 gm) of seedlings at 16th day of incubation. Though, DS2411 and DS2413 showed positive impact on these parameters of rice seedlings under saline condition. No germination was observed for the other two strains DS2408 and DS2415 treated rice seeds. Those two strains showed no profound effect on *O. sativa* (Fig.5).

In this study potential halotolerant plant-growth promoting rhizobacteria were isolated from soil samples from different areas in Sunderban to use them as inoculants to improve rice growth under salinity stress conditions. Halotolerant rhizobacteria enable the plant growth in saline soils and are known to exert ameliorating effects in saline soil (Saxena *et al.* 2018; Sagar *et al.* 2020, 2022; Kapadia *et al.* 2021). The rhizobacteria studied in this work have shown the potential to produce important phytohormones like Indole Acetic Acid (IAA) and Gibberellic Acid (GA). IAA is an essential plant hormone that is responsible for signalling pathways in plants, tissue differentiation, apical dominance and growth of roots. Harikrishnan *et al.* (2014) isolated a total of 90 actinomycetes from rice rhizosphere, of rice fields in the southern districts of Tamil Nadu, India. All isolates were screened for antagonism towards phytofungus pathogens such as *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum*, *Fusarium udum* and *Alternaria alternata*. Out of 95 isolates, 65 were found to be producing IAA was confirmed by colorimetric method. In this study, isolate VSMGT1014 produced IAA in the ISP-2

medium supplemented with 0.5% L - tryptophan in the amount of 15.96 µg/ml. Gibberellin helps in seed germination, cell elongation, flowering and delaying of senescence. The optimization study gives an idea of the potential of IAA production of the isolates under different L-Trp concentrations in the minimal amount of time. The isolates also able to produce siderophore which can mitigate iron deficiency in plant as similar was found in previous report (Sayed *et al.* 2012). The germination of seedlings, inoculated with the isolates, under saline conditions provides. Application of PGPR on different crops for increased crop productivity and salt stress alleviation have also been reported in previous studies (Mishra *et al.* 2010; Albadaawi *et al.* 2019; Tripathi *et al.* 2022). Halotolerant plant growth-promoting rhizobacteria isolated from saline soil improve nitrogen fixation and alleviate salt stress in rice plants (Khumairah *et al.* 2022). From this study we observe that rhizobacteria isolated from the saline soils of Sundarbans, have the ability to promote plant germination which can be used for enhanced crop production in salinity affected soils, which would not support agriculture otherwise. This provides a biologically safe and effective method of sustainable agriculture especially for crops such as rice, which is a staple crop for the population.

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DECLARATION

Conflict of interest. Authors declare no conflicts of interest.

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