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## Efficacy of salt tolerant microbial consortium for sustainable agriculture

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Ghoramara Island, Sunderban is part of the largest mangrove ecosystem in the world. Also known as the 'Sinking Island,' Ghoramara faces significant challenges today, due to the rising sea levels and climate change. The island is particularly vulnerable to the impacts of soil salinization, which aggravates soil erosion and degradation of cropland, thus threatening agricultural productivity. High saline stress reduces crop yield, limits the variety of cultivable crops and compromises food security. In this investigation, attempt was made to enhance plant growth and crop productivity under saline conditions through microbial intervention. The soil samples were collected from the Island and their physicochemical parameters were analysed accordingly. Multiple rhizobacterial samples were isolated from the soil and selected based on their plant growth promoting capabilities. The PGPR flora was further assayed and were found to enhance plant growth by different mechanisms such as nitrogen fixation, phosphate solubilization, phytohormone synthesis, and siderophore production. The bacterial isolates, mostly of species *Bacillus*, exhibited high salt tolerance up to a concentration of 16%, in coherence with its geographical location and current environmental scenario. Based on the results, attempts have been made to develop a unique biofertilizer through administration of a consortia of these bacterial isolates with specific nutrient solution which can improve crop productivity in this saline infested region of India.

**Keywords** : Biofertilizer , Ghoramara, PGPR, salt tolerance

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### INTRODUCTION

Ghoramara island is a remarkable landmass, located within the Sundarbans delta region in South 24 Parganas, West Bengal, India. It stretches between latitudes 21°53'56" N to 21°55'37" N and longitudes 88°06'59" E to 88°08'35" E. This area is renowned for being part of one of the world's largest mangrove ecosystems. This island has gained notoriety as a "vanishing island" since portions of the island have vanished off the map due to certain environmental challenges such as climate change, rising sea levels and coastal erosions which threaten not only the island's existence but also the livelihood of its inhabitants (Chakraborty *et al.*2019).

An abiotic stress that primarily affected the yield of cereals was salt, among many abiotic stresses. When the EC was above 4 dS/m (pH 7–8.5), the soil was regarded as saline. Normal seashore salinity was caused by seawater inundation, but irrigation water and land clearing can also contribute to the salt of agricultural soil. Salinity primarily affected three key aspects of plants, including osmotic imbalance, nutrient absorption challenges, and toxic stressors. When exposed to salinity, the majority of cultivated crops, primarily rice, experience a collapse in growth, development, and yield (Kamila *et al.*2022).

Tropical and subtropical locations grow the annual vegetable crop known as Okra (*Abelmoschus esculentus*). Due to its high quantities of vitamins, minerals, carbs, and lipids, it is regarded as a high-value vegetable crop. *Abelmoschus esculentus* has a high consumer demand and good nutritional value, but its production per

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hectare in saline areas is very low, as salinity may reduce crop yield by 12% to 100%. The main cause of this decreased productivity is the salinity of the soil (Habib *et al.* 2015).

Global food security has been seriously impacted by soil salinity. Crop yields were immediately impacted when an excessive amount of soluble salt was deposited on arable land. High salt intake prevented several physiological and metabolic functions of plants, potentially threatening their existence. Recent studies on the potential of PGPR in boosting the productivity of plants under salt stress have shown that salt-tolerant PGPR could be used to restore saline agroecosystems. Important soil processes like respiration, residue breakdown, nitrification, and denitrification are negatively impacted by the high salt concentration. Therefore, ensuring a viable agricultural yield in saline soils is urgently needed. Recent studies have demonstrated that saline agriculture can benefit from the application of salt-tolerant plant growth-promoting rhizobacteria (ST-PGPR) to increase production and boost soil fertility (Egamberdieva *et al.* 2019).

Therefore, this investigation, was aimed towards analysing the physico-chemical characteristics of saline soil and isolating saline tolerant bacteria from the soils of Ghoramara island of Sundarbans. Further the research was focussed on evaluating the key plant growth promoting properties of bacteria like nitrogen fixation, phosphate and potassium solubilization, followed by siderophore production and IAA production. Based on their performance and compatibility, the efficient bacterial strains were developed into a consortium and subsequently were used to grow *Abelmoschus esculentus* plants under saline stressed conditions. The ability of the consortium to be used as a biofertilizer for enhancing crop productivity under saline stressed conditions, and thereby support sustainable agriculture in saline affected coastal regions was also assessed.

## **MATERIALS AND METHODS**

### ***Physico-chemical characterization of soil samples***

Five soil samples were collected from Ghoramara island (21.914° N, 88.129° E), situated

in the South 24 Parganas district of West Bengal. The soil was further characterized for various physico-chemical properties such as pH, organic carbon content. The quantity of various macronutrients and micronutrients was also measured using standard methods (Dandwate, 2020).

### ***Isolation of bacteria from soil sample***

Bacteria were isolated from soil using serial dilution technique wherein dilutions upto  $10^{-4}$  were made and incubated for 24 hours at 37°C (Goswami *et al.* 2014).

### ***Characterization of bacterial isolates***

Gram staining was carried out for analysis of gram nature of the isolated bacterial samples followed by growth on HiChrome<sup>®</sup> specific media (HiMedia Laboratories).

### ***Antibiotic sensitivity***

The test was carried out using Disc Diffusion method in agar plates streaked with the bacterial isolate broth. Four antibiotic discs, namely, MRP 10, PB 300, COT 25 and CN 30, were placed in the plates using flamed forceps were incubated for 24 hours (Khan *et al.* 2019).

### ***Toxicity test***

All the bacteria isolates were streaked on Blood agar plates and incubated for 24 hours to test for  $\alpha$ -haemolysis (Buxton, 2005).

### ***Interaction Study***

An interaction study of the bacterial strains SG1, SG2, SG3, SG4, and SG5 was done to examine their relationships, cooperative (mutualistic) or competitive (antagonistic) and influence on growth, survival, and molecule production. (Sadiq *et al.* 2023).

### ***Test for plant growth-promoting properties of bacterial isolates***

The bacterial isolates were tested for their ability to fix nitrogen solubilize potassium and

phosphorous using Jensen, Pikovskaya and Aleksandrow media, that was modified using a pH indicator bromothymol blue (Marakana *et al.* 2018) (Amri *et al.* 2023) (Etesami *et al.* 2017). Phosphate quantification of the bacterial isolates was done using Barton's reagent (Pingale *et al.* 2013). The bacterial isolates were tested for their ability to produce siderophores using O-CAS assay method. (Perez-Miranda *et al.* 2007). The ability of bacterial isolates to synthesize IAA, was tested by growing them in Tryptic Soya Broth (TSB), prepared with tryptophan and without tryptophan for both 24 hours and 48 hours. OD was measured at 530nm values were recorded (Marakana *et al.* 2018).

### Salt tolerance test

The five bacterial samples were tested for salt tolerance. Nutrient broths with varying levels of sodium chloride (NaCl) concentrations (2%, 4%, 6%, 8%, 10%, 12%, 14% and 16%) were prepared. Each of the bacterial samples were inoculated in different concentrations of saline NB and incubated for 24 hours at 37° C in a shaker and their growth was observed (Sharma *et al.* 2021)

### Preparation of consortium

A consortium was prepared, primarily comprising of SG3, SG4 and SG5 isolates, which was then applied to *Abelmoschus esculentus* (Bhindi or Okra) plants, grown under experimental conditions for pot-culture analysis. After allowing the plants to grow naturally for four weeks, the consortium was added. These plants were maintained simultaneously with controls set up.

### Statistical Analysis

One way ANOVA of the plant data was performed to test the significance of the data.

## RESULTS AND DISCUSSION

Physico-chemical properties of the soil sample were characterized such as pH, organic carbon content, etc. Quantitative measurement of various macronutrients and micronutrients was done using standard methods.

Soil analysis tests of Ghoramara showed that the rhizospheric environment was mildly acidic (pH 6.8), EC value 4.5ds/m, with micronutrients namely Iron (4.6 mg/kg), Magnesium (71 mg/kg) Manganese (3.81 mg/kg) and Zinc (7 mg/kg) and macronutrients, especially nitrogen (211 kg/ha), phosphorus (160 kg/ha), and potassium (450 kg/ha). These results can be comparatively analysed with the study of the rhizosphere of halophilic bacteria isolated from agricultural soils of coastal regions. In a similar study from Saurashtra region

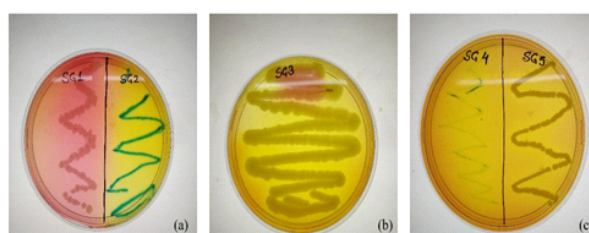


Fig 1: HI Chrome<sup>®</sup> Bacillus (a) SG1, SG2 (b) SG3 (c) SG4, SG5 of Gujarat, it was reported that the physico-chemical characteristics of the soil samples such as pH, E.C., organic carbon content, and available phosphorus and potash ranged from 7.4–8.1, 0.76–1.59 dS/ m, 4.03–7.47 g/kg, 29.57–54.33 and 166.70–248.33 kg/ ha respectively (Reang *et al.* 2022).

The five bacterial strains isolated from the soil sample of Ghoramara, were gram-positive in nature. The colour on HiChromeBacillus<sup>™</sup> agar media was used to identify the different *Bacillus* species among the isolates as represented in Fig. 1. The probable identification of the bacterial isolates showed that only SG2 could be *Enterococcus* sp, and SG1, SG3, SG4 and SG5 were *Bacillus* sp.

Upon analysis of antibiotic sensitivity of bacterial isolates, against antibiotics like Meropenem (10µg), MRP 10, PB 300, COT 25, and CN 30 and Co-trimoxazole (25µg), it was revealed that all the bacterial isolates tested to be sensitive to the antibiotics. A toxicity test was performed on Blood Agar plates to assess the haemolysis pattern of the bacterial isolates. Out of all the isolates, only SG1 showed a Haemolysis while the rest showed negative results when cultured on the Blood Agar medium.

An interaction study of the bacterial strains SG1, SG2, SG3, SG4, and SG5 was conducted.

Positive results amongst all the bacterial strains were seen, thereby indicating absence of antagonistic behaviour (Fig. 2).

The ability of bacterial isolates to fix nitrogen and solubilize phosphorus and potassium were tested. All the bacterial isolates namely SG1, SG2, SG3, SG4 and SG5 showed positive results in modified Jensen, Aleksandrow and Pikovskaya media, indicating that all the bacterial isolates were able to fix nitrogen and solubilize phosphorous and potassium respectively.

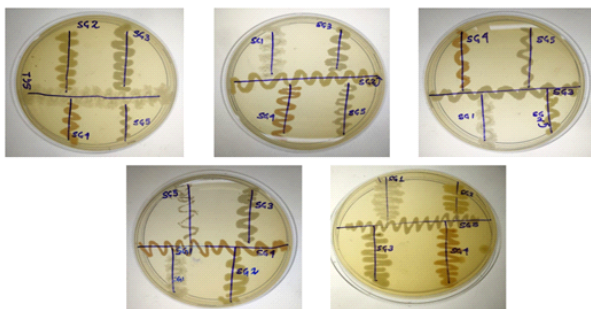


Fig 2: Interaction in solid media amongst the bacterial isolates

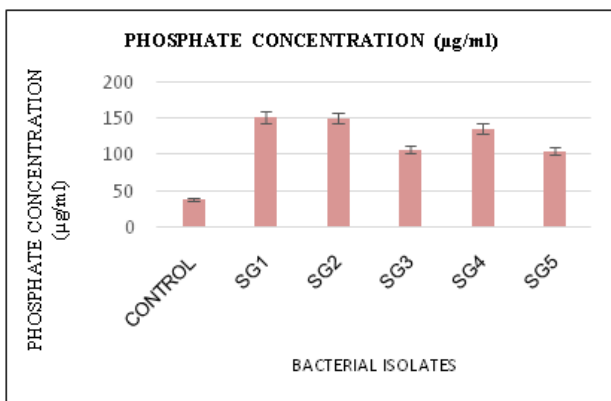


Fig 3 : Quantification of phosphate solubilizing ability of bacterial isolates

Further, the bacterial isolates were quantified for their ability to solubilize phosphate and results are presented in Fig. 3. These observations concurred with the study of (Reang *et al.* 2022) where the solubilization of phosphates were confirmed from the formation of clear zones in Aleksandrow agar plates. (Reang *et al.* 2022)

Siderophore detection of the bacterial isolates were done to check for the increased solubility of the ferric iron in soil and ultimately better plant utilization. Out of all the isolates only SG1, SG3 and SG5 showed the ability in producing siderophores, by producing clear halo in the

media. Indole Acetic Acid produced by the bacteria were quantified and the results have been represented in Fig. 4.

A salt tolerance test of the bacterial isolates was performed. Isolates SG3, SG4, and SG5 were able to grow in salt concentrations as high as 16%. Conversely, SG1 and SG2 were viable at such saline levels at 8% and 12% salinity respectively. In a study done by Albadaiwi *et al.* (2019), the halotolerant bacterial strains were found to be siderophore producers similar to the 17 siderophore rich bacterial strains. From all the above-mentioned assays and their inferences, SG3, SG4 and SG5 were considered for incorporation into a bacterial consortium for their better ability to promote plant growth on the basis of the results obtained.

Consortium application to the potted plants of *Abelmoschus esculentus* showed considerable visible differences between the treated plant and the non-treated plant (control). The treatment patterns were as follows:

1. Treatment 1- 3.5 ml from SG3, SG4, SG5 each + 39.5 ml water
2. Treatment 2- 3.5 ml from SG3, SG4, SG5 each + 39.5 ml water + 1 g salt
3. Treatment 3- 3.5 ml from SG3, SG4, SG5 each + 39.5 ml water + 2 g salt
4. Control 1- 50 ml water + 1 g salt
5. Control 2- 50 ml water + 2 g salt

Visible differences including height, number of fruits and other plant parameters were observed upon application of consortia (Fig.5).

Soil analysis was done after the application of consortia, whereby the pH was 6.8, EC value was 1.4 dS/m, other macro and micro-elements values of Iron (2.86 mg/kg), Magnesium (125 mg/kg) Manganese (2.88 mg/kg) and Zinc (2.56 mg/kg) and macronutrients, especially nitrogen (198.91 kg/ha), phosphorus (160 kg/ha), and potassium (420.36 kg/ha). The data showed that EC values had decreased, thereby representing a reduction in the salinity of the soil, also the decrease in the values of macro and micronutrients of the soil represented better uptake and utilization of the nutrients by the plants.

**Table 1:** Significance of the data as tested by One way ANOVA

Parameter	Source	SS	df	SE	MS	F-value	p-value
PLANT HEIGHT	Between Groups	20784.6	4		5196.1	13195	<0.001
	Within Groups	3.94	10	0.362	0.394	—	—
	Total	20788.5	14		—	—	—
NUMBER OF LEAVES	Between Groups	58.6	4		14.65	75.5	<0.001
	Within Groups	1.94	10	0.254	0.194	—	—
	Total	60.54	14		—	—	—
NUMBER OF NODES	Between Groups	912.8	4		228.2	6005	<0.001
	Within Groups	0.38	10	0.113	0.038	—	—
	Total	913.18	14		—	—	—
NUMBER OF INTERNODES	Between Groups	845.2	4		211.3	1046	<0.001
	Within Groups	2.02	10	0.259	0.202	—	—
	Total	847.22	14		—	—	—
NUMBER OF BUDS	Between Groups	41.6	4		10.4	29.7	<0.001
	Within Groups	3.5	10	0.342	0.35	—	—
	Total	45.1	14		—	—	—
LEAF LENGTH	Between Groups	72.4	4		18.1	108.4	<0.001
	Within Groups	1.67	10	0.236	0.167	—	—
	Total	74.07	14		—	—	—
LEAF BREADTH	Between Groups	25.3	4		6.33	81.2	<0.001
	Within Groups	0.78	10	0.161	0.078	—	—
	Total	26.08	14		—	—	—
NUMBER OF FRUITS	Between Groups	8.8	4		2.2	44.0	<0.001
	Within Groups	0.5	10	0.129	0.05	—	—
	Total	9.3	14		—	—	—

SS- Sum of squares; Df- degree of freedom; MS- mean square; SE- standard error; F-value- F-statistic P-value= Probability value

### **One way ANOVA of the plant data**

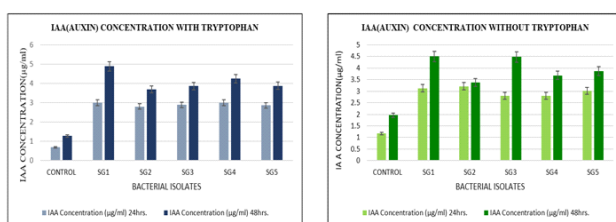
One-way ANOVA revealed highly significant differences ( $p < 0.001$ ) among control and treatment groups for all measured parameters. The treatments contributed strongly to variation

in plant growth and yield, particularly in height, node number, internodal length, and fruit production ( Table 1).

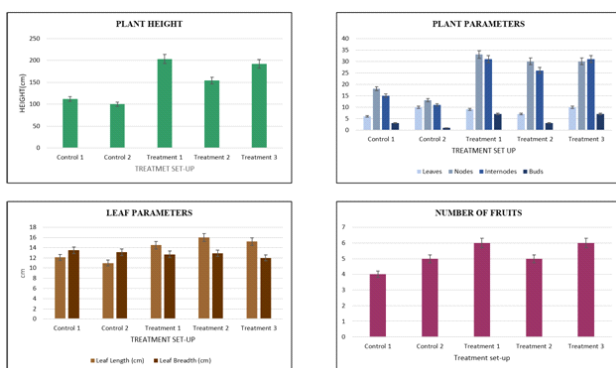
Among all groups, Treatment 1 and Treatment 3 exhibited the highest overall performance, with

superior vegetative growth and reproductive output compared to both controls and Treatment 2. This indicates a strong plant growth-promoting effect of the microbial consortium, even under saline conditions.

A recent study by Redondo-Gómez *et al.* (2021) showed the significance of using a consortium of multiple halophilic strains isolated from halophytes to improve the growth of several crops under saline conditions.



**Fig 4:** Analysis of IAA concentration of bacterial isolates with and without tryptophan



**Fig 5:** Effect of various treatments on plant growth parameters

Under our research, considerable improvement in plant height, strength and leaf surface area was observed in plants supplemented with bacterial consortia in comparison to the control setups, towards the beginning of the sixth week. Hence this ST-PGPR consortium was aimed to develop a unique biofertilizer to promote plant growth, soil fertility and soil binding in saline infested regions of India.

## CONCLUSION

This study demonstrates the ability of saline tolerant plant growth promoting bacteria isolated from the saline soils of Ghoramara island to promote crop productivity under high saline stress. The selected isolates SG3, SG4 and SG5

were found to tolerate up to 16% salt concentration with key PGPR properties of nitrogen fixation, potassium solubilization, phosphate solubilization, IAA synthesis and siderophore production. A consortium of SG3, SG4 and SG5 was prepared and applied on Okra (*Abelmoschus esculentus*) plants, to observe plant growth under experimental setup. Out of all the treatment sets, positive effects on plant height, strength and growth indicated the demand for further studies on the various aspects and characteristics of the ST-PGPR treatment on the Okra plants. This bacterial consortium of bacterial strains isolated from Ghoramara, Sundarbans proved its potential as a unique biofertilizer designed specifically to improve crop productivity in the saline-infested regions of our country. Further, the increased plant growth would reduce the drastic effects of soil erosion, which is a major cause of concern for these coastal and mangrove regions as already discussed. Therefore, this investigation worked towards creating a bacterial consortium biofertilizer for enhancing crop yield, promoting soil binding, and improving soil fertility, and also aimed to create a larger socio-economic impact on society by conserving this island, which would also boost the livelihood of the people of this region.

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## DECLARATION

Conflict of Interest. Authors declare no conflict of interest regarding the publication of this paper.

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