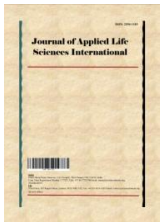

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Salt Tolerant Rhizobacteria from Coastal Region of Bangladesh Portrayed the Potential for Plant Growth Promotion

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Authors' contributions

This work was carried out in collaboration among all authors. Author QFQ designed the study, performed the statistical analysis, and wrote the protocol. Author MSA collected the samples and conducted the experiments. Author MSIR wrote the first draft of the manuscript and managed the literature searches. Authors AR and HMZ managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Plant growth-promoting rhizobacteria can effectively reduce the severity of different abiotic stresses like water stress, temperature stress, salt stress, etc. on plant growth and development. The study aimed at isolating salt-tolerant rhizobacteria followed by their morphological, biochemical and plant growth promotion traits evaluation. Sixteen root samples of nine different plant species were collected from two locations of Patuakhali, a coastal southern district of Bangladesh. Thirty rhizobacteria were isolated, fifteen from each location, to assess their halotolerance and plant growth promoting potential. The isolated rhizobacteria were subjected to morphological (viz. shape, colour and elevation), biochemical (viz. Gram reaction, catalase test and HCN production) and growth-promoting traits [viz. phosphate solubilizing ability, salt tolerance, indole-3-acetic acid (IAA) production, and N_2-fixation] characterization. Twenty-eight isolates were Gram positive, 27 were catalase positive, and nine showed varying degrees of phosphate solubilization on National Botanical Research Institute of Phosphate (NBRIP) medium. Isolate PWB5 showed the highest

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phosphate solubilizing index (PSI = 3.83±0.098) on the 6th day. To screen salt-tolerant rhizobacteria, the isolates were cultured in NBA media containing different (0%, 2.5%, 5%, 7.5%, 10%, 12%, 15%) NaCl concentrations. Isolate PWB12 and PWB13 grew at 15% NaCl concentration. Eleven isolates exhibited IAA producing ability on Winogradsky medium amended with L-tryptophan among which four (PMB13, PMB14, PMB15 and PWB6) were strong IAA producers. Twenty-seven isolates were potential N_2-fixer and among them, 20 were highly efficient, but none of the isolates was HCN producer. The rhizobacteria isolated in the current research work showed some potential plant growth-promoting traits which seem applicable for crop production, especially, under salt stress condition.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR); salt tolerance; phosphorus solubilisation; Indole-3-acetic acid; nitrogen fixation.

1. INTRODUCTION

Soil salinity is the salt content in soil, whereas the progression of salt content is soil body is known as soil salinization. It is one of the major hitches that is challenging the agricultural sustainability in the 21st century. It causes substantial reductions in the amount of cultivable land area and crop productivity, low economic return, and enhanced sand soil erosions [1-3]. Both natural phenomenon and human practices may result in soil salinization. Due to global warming, the sea level will rise and more and more cultivable land will experience increased soil salinity. National Adaptation Program of Action (NAPA) for Bangladesh projected probable sea level rises of 32 cm by 2050, and 88 cm by the end of current century [4]. The conversion of arable lands for shrimp culture in coastal areas and sea level rise along with natural calamities are the roots of soil salinization in this country. This abiotic stress hinders N_2-uptake, reduces growth and reproduction of crop plants. Plant morphological, physiological, and biochemical processes, such as, seed germination, growth, water and nutrient uptake, etc. get interrupted as a result of enhanced salt content [5-6]. Higher Na^+ and Cl^- concentration in the soil negatively affect soil vital elements and lower the essential nutrient uptake ability of plants [7]. Salinity substantially impairs phosphorus (P) uptake in plant as phosphate ions (PO_4^{3-}) gets precipitated with calcium ions [8]. Rice, wheat, barley, cotton, and bean experience significantly low yield under saline stress [9]. Ali [10] reported 69% lower rice production in 2013 compared to that in 1985 in a village of Satkhira, a coastal district of Bangladesh. Alam et al. [11] studied the effect of salinity in the Kalapara coastal belt of Bangladesh and reported the 92% areas of current 36 cropping pattern were salinity affected. They also mentioned that about 200 ha of fodder crops are getting affected by salinity each year.

Several agricultural scientists and research organizations in Bangladesh are trying to develop salt tolerant crop varieties. However, variety development costs years of hard work and resources before reaching farmer's field. Plant growth-promoting rhizobacteria (PGPR) are gaining increasing attention because of their growth stimulating traits, even under different biotic and abiotic stress conditions. PGPR are bacteria that selectively colonize in plant root and live as symbiotic or asymbiotic association with many plants. These special group of bacteria contributes in plant growth stimulation through a number of primary and secondary mechanisms [12-13]. Augmented mineral nutrient mobilization, N_2-fixation, suppression of soil-borne phytopathogens, improved plant stress tolerance, and phytohormone production are some of these mechanisms [13]. Bacteria from *Rhizobium, Bacillus, Pseudomonas, Azospirillum, Microbacterium, Methylobacterium,* etc. have been reported to improve numerous abiotic stress tolerance (e.g., salinity) in host plants [14]. The swath of coastal belt of Bangladesh may harbour such halotolerant PGPR which unfortunately remained rarely explored. Thus, once these extremely important bacterial community isolate and identified, could be used in crop productivity enhancement and quality improvement under salt stressed conditions. Therefore, this study was designed to isolate salt tolerant rhizobacteria from coastal regions of Bangladesh and evaluate their plant growth stimulating potential through biochemical approaches.

2. MATERIALS AND METHODS

2.1 Site Selection for Sample Collection

Maitbhanga and West-Veribadh, the two salt-affected village of Kalapara Upazila of Patuakhali

district, were selected for plant sample collection upon consultation with the SRDI regional officer, local NGO personnel and farmers. The soil salinity and pH data of the sampling locations are presented in Table 1.

2.2 Plant Samples Collection

In order to isolate rhizobacteria, plant samples with their roots were collected from different portions of the field of each sampling sites. Roots were washed after collection to get rid of the soils as much as possible. In total 16 plant samples of nine plant species Table 2 were collected from the sampling sites. Immediately after collection, each sample was kept in a labelled air tight plastic zipper lock bag and stored at 4°C inside an ice box and immediately brought to the laboratory.

2.3 Isolation of the Rhizobacteria

The isolation was done using nutrient agar medium (sucrose 10g/L, nutrient broth 10 g/L, agar 15 g/L; pH 6.5) [15]. The pH of the solution was adjusted to 6.5 with 1% NaOH and 1 mM HCl before autoclaving. All plant roots were washed with sterilized distilled water in a test tube to isolate bacterial strains from each plant root. Then series dilutions (10^{-1}, 10^{-2} and 10^{-3}) were made to reduce the density of the bacterial population. Each diluted sample then cultured separately on petri dishes using a solid nutrient rich medium. Each medium was sterilized by autoclaving (JSAC-80 JSR) (121°C, 15 psi, 20 minutes) prior to inoculation. To inoculate inside bio-safety cabinet (JSCB-900SB JSR) a sterile glass spreader was used. The incubation was done in microbial incubator (EN-120 Nuve) at 28±2°C for two days. The bacterial isolates were selected on the basis of distinct morphological features like size, shape and colour for sub culturing. The pure colonies were isolated and maintained on nutrient broth agar (NBA) plates at 4°C for regular use. Pure isolates were preserved in Eppendorf tube containing 30% glycerol solution in low temperature refrigerator (-20°C) for longer period.

2.4 Morphological Characterization

For the morphological characterization (colour, shape and edge shape) were recorded by growing the pure cultures on nutrient agar media, the colony of the pure cultured bacterial isolate was determined. After 24 hours of incubation, the bacterial colonies were observed with the help of a hand magnifying glass to identify their colony colour, shape and edge shape.

2.5 Biochemical Characterization

The Gram test and catalase test of the isolated rhizobacteria were done according to the method mentioned by Ahmed [16] and Wheelis [17] respectively. To determine the production of HCN, bacteria were streaked onto nutrient broth (NB) agar plates supplemented with glycine (4.4 g/L). The petri dishes were inverted and piece of filter paper was impregnated with 0.5% picric acid and 2% sodium carbonate were placed on the upper lid and the petri dish was sealed with parafilm and incubated at 28°C for 7 days. Discoloration of filter paper color from yellow to orange brown was considered as the indication of HCN production. To screen catalase producing isolate, bacterial colonies were picked with the help of a sterile tooth pick and mashed up in 30% H_2O_2 solution on a glass slide. Formation of gas bubbles were taken as positive indication for catalase production.

2.6 Screening of Salt Tolerant Rhizobacteria

Salt tolerance of the bacterial Isolates were studied on nutrient agar medium amended with different amount of NaCl. Briefly, isolates were inoculated on nutrient agar medium (pH 6.5) containing different concentration of NaCl (0%, 2.5%, 5%, 7.5%, 10%, 12%, 15%) and incubated at 28±2°C for 24 hours. Then all bacterial isolates were observed to identify their growth condition. Only the surviving strains were selected for the next trail with higher salt concentration.

Table 1. Soil salinity and pH data of sample collection locations (here, n = number of soil samples)

Locations	Parameters	Soil depths			n
		0-3 (cm)	3-6 (cm)	6-9 (cm)	
Maitbhanga	Salinity (ds)	7.3	5.4	5.1	9
	pH	5.5	6.1	6.1	
West-Veribadh	Salinity (ds)	21.8	8.6	6.9	9
	pH	4.6	4.8	4.9	

2.7 Screening of Phosphate Solubilizing (PSB), Indole-3 Acetic Acid (IAA) Producing, HCN Producing, and N_2-Fixing Bacteria

Screening of phosphate solubilizing, IAA producing and N_2-fixing bacteria were done following the procedures mentioned in Khatun et al. [18] Asha et al. [19] and Rahman et al. [20]. The phosphate solubilizing indices (PSI) were calculated based on the equation suggested by Premono et al. [21]. Modified Winogradsky's mineral solution was used as a media for screening of IAA producing rhizobacteria and the media was prepared as described in Rahman et al. [20]. The medium was supplemented with 100 mg/L L-tryptophan and the pH of the solution was adjusted to 6.0-6.2 with 0.1M HCL and 0.1M NaOH. Thirty milliliters of liquid medium were inoculated with overnight grown bacteria and incubated at room temperature in a horizontal shaker for 72 hours under dark condition. After 3 days, the culture media were centrifuged at 10,000 rpm for 10 minutes to obtain cell free supernatant. After centrifugation, the supernatant was decanted and pH was adjusted to 2.5 to 3.0 with 2 M HCL. Then 2 mL of supernatant and 2 mL of Salkowski's reagent (2% of 0.5 M $FeCl_3$ solution in 35% of $HClO_4$) were taken in the test tube and kept in dark condition for 30 minutes. Development of reddish pink, pink, light pink and yellow colour indicated strong, medium, slight, and no IAA producing isolates, respectively. Modified Winogradsky's N-free mineral medium was used to identify potential nitrogen fixing bacteria. The ability to grow in N-free medium was taken as the positive indication of nitrogen fixing ability of the isolated rhizobacteria and the growth of bacterial colony in N-free medium was considered to measure their N_2-fixing ability.

2.8 Determination of P in PSB Grown Liquid Media

Phosphorus solubilization was quantified using the procedure followed by Khatun et al. [18] Briefly, each isolate was inoculated in separate bottles containing Pikovskaya's [22] mineral medium supplemented with tricalcium phosphate and placed in horizontal shaker (JSOS-500 JSR) at 28±2°C at 100 rpm. After 72 and 144 hrs, culture samples were collected for the determination of phosphorus released in the medium and the pH of the medium were measured. Solubilized P were determined using the method mentioned by Olsen and Sommers [23] with the help of a spectrophotometer (TG-60, Korea).

3. RESULTS

3.1 Morphological Characteristics of the Isolated Bacterial Strains

A total of 30 bacteria were isolated from 16 plant samples. The isolated bacteria along with origins are presented in Table 2 and the pure cultures of the strains are shown in Plate 1. Code names to the isolated bacteria were given according to the sampling location/origin (Table 3).

3.2 Morphological Characteristics

The morphological characteristics of isolated bacteria were diverse. All the isolates produced colony of different shape, elevation, and colour (Table 4). Most of the isolate are cream in colour, round in shape and elevated.

3.3 Biochemical Characteristics

Among the isolates, 28 were Gram positive and 2 were Gram negative. Again, 27 isolates were catalase positive and 3 were catalase negative (Table 5). None of the isolated bacteria showed HCN production on plate assay.

3.4 Salt Tolerant Bacterial Isolates

Though all isolated rhizobacteria were collected from two saline locations, they showed varying level of salt tolerance under different concentrations of NaCl solution. Out of the thirty tested isolates, only two (PWB12 and PWB13) isolates survived at NaCl concentration as high as 15%. Twelve isolates showed tolerance up to 12% NaCl, 1 isolate up to 10% NaCl, 7 isolates up to 7.5% NaCl; and the rest 8 isolates survived in 5% NaCl (Table 6).

3.5 Phosphate Solubilizing Capacity

Nine of the isolated (PMB2, PMB8, PMB9, PMB13, PWB4, PWB5, PWB8, PWB10 and PWB11) rhizobacteria exhibited phosphate solubilizing ability by producing clear halo zones surrounding the colony on Pikovskya's agar medium. The isolate PWB5 showed the highest phosphate solubilizing potential (PSI = 3.83) after six days of incubation (Fig. 1). The results of quantitative determination of phosphorus revealed that PWB5 and PMB1 solubilized the highest (0.320 ppm) and the lowest (0.179 ppm)

amount of P in Pikovskya's mineral broth medium (Table 5 and Fig. 2b). All the isolates reduced the medium pH from the neutral pH after six days of incubation. The highest average pH reduction was observed in PWB11 (pH 2.94) followed by PWB8 (pH 2.97) (Fig 2a). Phosphorous solubilization by the isolates and change in medium pH had negative correlation; i.e., with the reduction in medium pH, P solubilization increased (Fig. 2c).

Table 2. From two locations of Patuakhali district, a total of sixteen plant sample of nine different species were collected for the screening, and isolation of salt tolerant rhizobacteria

Local name	Scientific name	No. of sample
Shama	*Echinochola crusgalli*	1
Rice	*Oryza sativa*	3
Angta	*Paspalum distichum*	2
Helencha	*Solanum melongena*	1
Bishkatali	*Polygonus hydropiper*	2
Chanchi	*Altrnanthera sessilis*	1
Premkata	*Chrysopogon aciculatus*	2
Datura	*Datura starmonium*	2
Pani chesra	*Scirpus juncoides*	2

Table 3. Name of rhizobacterial isolates isolated from roots of sixteen plant samples collected from two villages of Patuakhali district of Bangladesh

Upazila	Union	Village	Bacterial isolates
Kalapara, Patuakhali	Latachapli	Maitbhanga	PMB1, PMB2, PMB3, PMB4, PMB5, PMB6, PMB7, PMB8, PMB9, PMB10, PMB11, PMB12, PMB13, PMB14, and PMB15
		West-Veribadh	PWB1, PWB2, PWB3, PWB4, PWB5, PWB6, PWB7, PWB8, PWB9, PWB10, PWB11, PWB12, PWB13, PWB14, and PWB15

Table 4. Morphological characteristics of rhizobacteria isolated from nine plant samples of two coastal villages of Patuakhali district

Colony Shape	Colony Elevation					
	Raised			Non-raised		
	Colony Colour					
	Whitish	Cream	Pink	Whitish	Cream	Pink
Round	PMB2, PMB9, PMB11, PMB14	PMB1, PMB3, PMB5, PMB6, PMB10, PMB15, PWB1, PWB3, PWB4, PWB5, PWB6	PMB4, PWB8		PWB11, PWB12, PWB14, PWB15	PWB10
Oval		PMB7, PMB12, PWB2, PWB7			PWB13	
Irregular	PMB13	PMB8			PWB9	

Table 5. Bacterial isolates classified according to their response in Gram test and catalase test

Catalase test	Gram test	
	Gram (+ve)	Gram (-ve)
Catalase (+ve)	PMB1, PMB2, PMB3, PMB4, PMB5, PMB6, PMB7, PMB9, PMB10, PMB11, PMB12, PWB1, PWB2, PWB3, PWB4, PWB5, PWB6, PWB7, PWB8, PWB9, PWB10, PWB11, PWB12, PWB13, PWB15	PMB15, PWB14
Catalase (-ve)	PMB8, PMB13, PMB14	

Table 6. The table showing salt tolerance, N_2-fixing ability and IAA production potential of rhizobacteria isolated from the coastal villages. Isolates were cultured in salt containing nutrient broth medium and incubated at 28°C for 24 hours to see their response in salt condition. The isolates are listed according to the highest concentration of NaCl in which they survived. Bacterial isolates were grown in N-free Winogradsky medium for 48 hours at 28°C to identify their ability of N_2-fixation. Ability of the isolates to grow in N-free medium indicates the potential N-fixers. The isolates were also grown in modified Winogradsky medium containing L-tryptophan after 72 hours at 28°C for evaluating their IAA production potential

NaCl tolerance	N_2- fixation				IAA production			
	No	Little	Medium	High	No	Slight	Medium	Strong
0%	-	-	-	-	-	-	-	-
2.5%	-	-	-	-	-	-	-	-
5%	PMB12	PMB5, PMB7, PWB1, PWB4	PMB14, PMB15	PMB10	PMB5, PMB12	PMB7, PWB1	PMB10, PWB4	PMB14, PMB15
7.5%	PMB3	PMB2, PMB4, PWB14, PWB15	PMB8	PMB9	PMB2, PMB3, PMB4, PMB9, PWB14, PWB15	PMB8		
10%			PWB6					PWB6
12%		PMB6, PMB11, PWB3, PWB5, PWB7, PWB8, PWB9, PWB10, PWB11	PMB1, PWB2	PMB13	PMB1, PMB6, PMB11, PWB2, PWB3, PWB5, PWB7, PWB8, PWB9, PWB10		PWB11	PMB13
15%	PWB12		PWB13		PWB12		PWB13	

Table S1. pH change and amount of solubilized phosphorus (ppm) by the selected bacterial isolates in liquid media after day 3 and day 6

Name of the isolates	pH change		Solubilized P (ppm)	
	At day 3	At day 6	At day 3	At day 6
PMB2	3.41	3.82	0.204	0.189
PMB8	4.1	5.38	0.213	0.255
PMB9	3.65	3.61	0.196	0.209
PMB13	3.88	3.76	0.193	0.198
PWB4	3.86	3.91	0.209	0.191
PWB5	3.78	3.71	0.238	0.32
PWB8	3	2.94	0.19	0.231
PWB10	3.9	4.01	0.157	0.223
PWB11	2.98	2.91	0.178	0.233

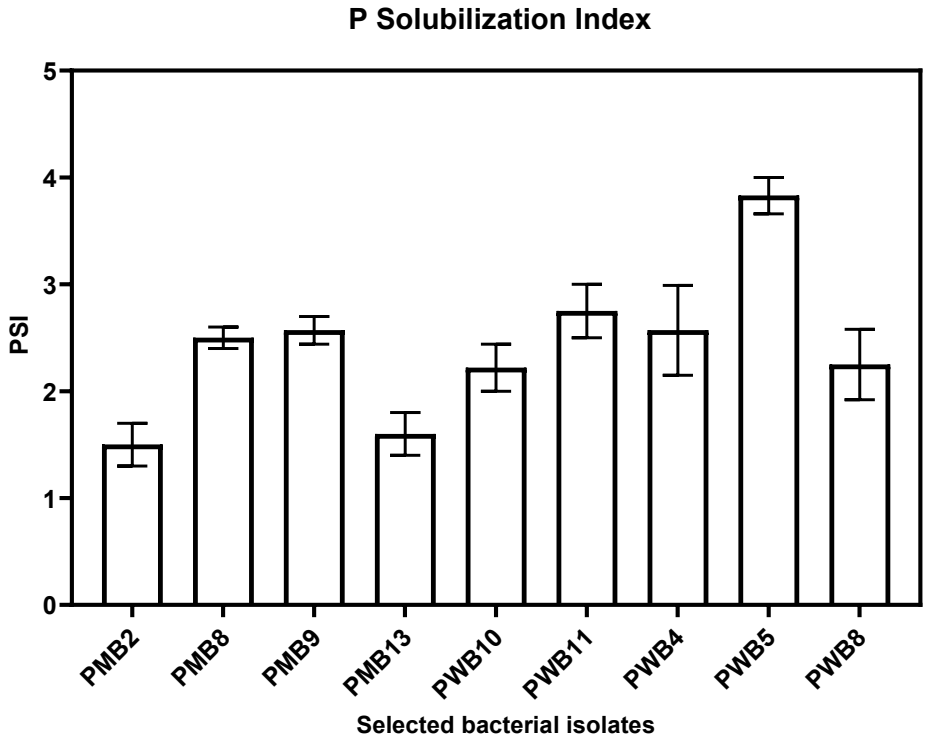

Fig. 1. Phosphate solubilizing index (PSI) of the nine-salt tolerant bacterial isolates, rest twenty-one showed no such capability, collected from the coastal districts of Bangladesh, when grown at 28°C for six days in $Ca_3(PO_4)_2$ containing Pikovskaya agar medium

3.6 IAA Producing Rhizobacteria

The production of reddish pink, pink, light pink and yellow colour in L-tryptophan test denoted strong, medium, slight and no IAA producers. Among the isolated rhizobacteria, four (PMB13, PMB14, PMB15, and PWB6) strong, four (PMB10, PWB4, PWB11, and PWB13) medium, and three (PMB7, PMB8, and PWB1) slight IAA producers found (Table 6).

3.7 N_2-Fixing Rhizobacteria

The bacteria that had grown in N-free Winogradsky's medium were potentially identified as N_2-fixing bacteria. Among the 30 rhizobacteria, only 3 (PMB3, PMB12, and PWB12) did not grow in N-free medium. Among the isolates that were able to grow in N-free medium, 5 isolates (PMB2, PMB4, PMB9, PMB10 and PMB13) showed vigorous growth

and 3 (PWB2, PWB6 and PWB13) showed medium growth (Table 6).

4. DISCUSSION

Solubilization of mineral phosphate, production of IAA and nitrogen fixation are the primary mechanism of plant growth promotion by rhizobacteria as considered as the preliminary selection criteria of PGPR. Clear halo zones on $Ca_3(PO_4)_2$ containing Pikovoskaya agar media revealed qualitative P-solubilization potential of 9 isolates from both sampling location. The isolates exhibited variation in their phosphate solubilizing capacity (PSI range: 1.50 - 3.83) after 6th day (Fig. 1) of incubation. The difference in P-solubilization might be due to variations in their ability to produce organic acids or other methods employed to mineralize $Ca_3(PO_4)_2$. These nine bacterial isolates lowered the growth medium pH to different extent (Table S1) and except for PMB 8 and PWB 11. The correlation study revealed that P solubilization is highly correlated with lowering culture medium pH (Fig. 2c). Chen et al. [24] and Rashid et al. [25] also observed such phosphate solubilizing difference among the isolated bacteria and confirmed the presence of different organic acids through HPLC analysis. The release of phosphorus from tricalcium phosphate by the capable isolates indicate they might be useful in mobilizing fixed pool of soil phosphate when applied in crop fields. Rodríguez and Fraga [26] studied *Pseudomonas* and other PSBs like *Bacillus* and *Rhizobium* found them to be proficient in increasing the phosphorous availability in soil. Nautiyal and Mehta [27] also documented higher crop yield due to improved P solubilization in soil and uptake by plants primed with PGPR.

Microbial hormone production in soil can promote plant growth specially under abiotic stresses. Among the phytohormones produced, the auxins (IAA), at low concentrations, are known to participate in major development process like, root development, cell elongation and cell division stimulation, etc. Various rhizobacterial species possess the ability to produce IAA with or without L-tryptophan supplementation in culture medium or rhizospheric soil [28] Rahman et al. [20] Asha et al. [19] [29]. Production of IAA has been shown in *Bacillus*, *Pseudomonas*, *Azotobacter*, *Azospirillium*, *Phosphobacteria*, *Glucanoacetobacter*, *Aspergillus niger,* and *Penicillium*. Different degree of IAA production observed among the isolates of this study, where four (PMB13, PMB14, PMB15 and PWB6) isolates converted maximum L-tryptophan into IAA. Culture conditions, strains of rhizobacteria, growth stage and availability of substrates are responsible for the variation of IAA production level in different bacteria [20] Yousef [30] documented that bacteria can produce IAA in a wide range of pH (pH 5-9) and also in the presence of 0.5% and 1% NaCl. Bacteria use IAA as a tool for building symbiotic relationship with host plants [31] Patel et al. [32] Samuel and Muthukkaruppan [33] and fighting abiotic stresses which enable plant better adaptation in those environments. Metoui Ben Mahmoud et al. [34] also demonstrated that PGPR inoculation

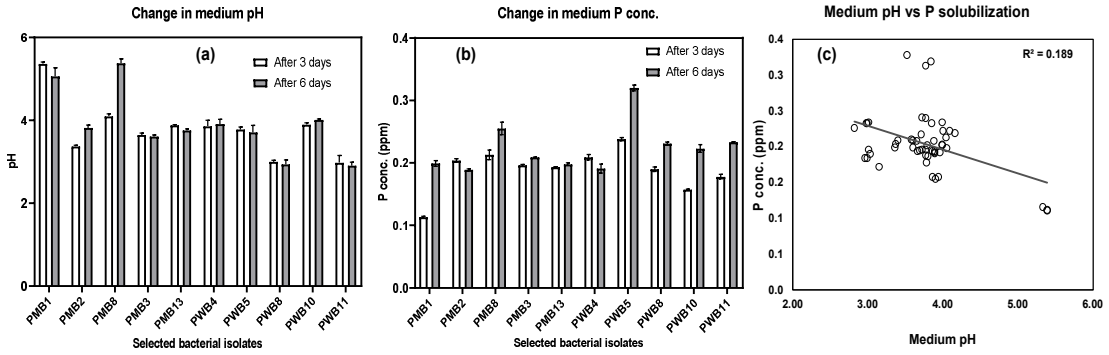

Fig. 2. The isolates were cultured in the agar free Pikovskaya liquid medium for observing the phosphorus solubilizing and medium pH altering capability of the selected isolates. Here, (a) showing the medium pH change by the isolates after 6 days of incubation in liquid medium, (b) denoting the change in the medium P concentration (ppm) after day 6, and (c) showing the correlation between medium pH change and P solubilization, which is a strong indication that the isolates produced organic acids to solubilize insoluble P

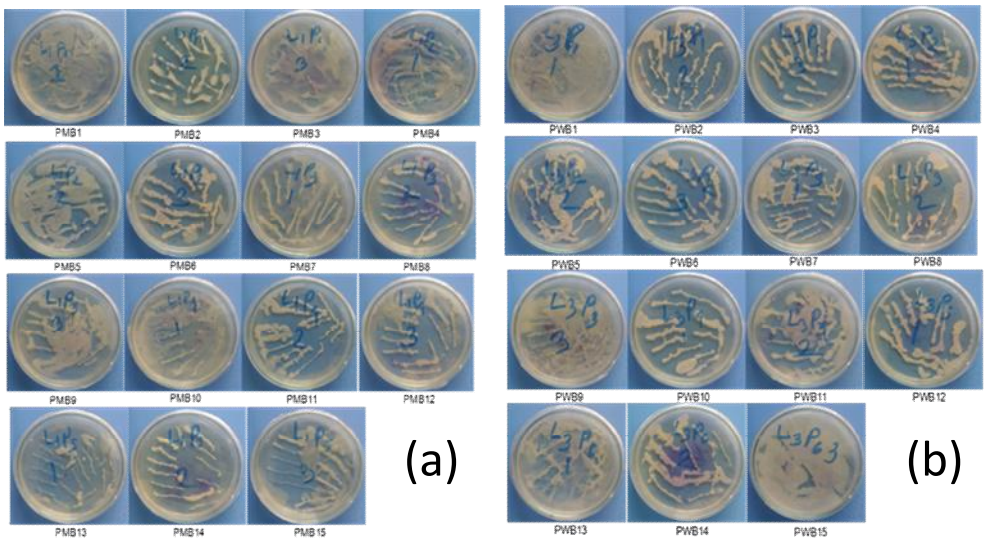

Plate 1. Pure cultures of isolated bacteria from samples collected from (a) Maitbhanga and (b) West-Veribadh areas used in this study

increased IAA and proline production in salt-sensitive barley cultivar Rihane, when cultivated on 100 and 200 mM NaCl supplemented medium. Salt-tolerant PGPR introduction also significantly increased the biomass, root and shoot growth of soybean at 150 and 200 mM NaCl condition compared to control uninoculated plants [35] Kerbab et al. [36] also conferred NaCl stress alleviation in wheat using halotolerant bacteria inoculation.

Biological N_2-fixation is receiving priority as a sustainable supplier of essential nitrogen in rice ecosystem as synthetic nitrogenous fertilizers have raised concerns of environmental pollution over the years. Rhizobacterial isolates grew in N-free medium indicated their atmospheric N_2-fixing traits [19] Not only the symbiotic N_2-fixing rhizobacteria, but also several free-living and non-symbiotic bacterial strains has been reported to have the atmospheric N_2-fixing ability [37]. Xu et al. [38] isolated N_2 fixing bacteria from giant reed and switchgrass. Nitrogenase activity in free-living bacteria facilitates them to fix atmospheric nitrogen [39]. Acetylene reduction assay and NifH gene identification were not performed in this study to affirm their N_2-fixing ability, but are required prior to their intended utilization as N_2-fixing PGPR.

Salt tolerance is considered as an important criterion for root-associated bacteria to be successfully applied in coastal crop fields. In this study, we identified some potential rhizobacteria that were able to grow different salt concentrations. At 10% NaCl, half of the isolates survived and at 12% NaCl, 46.66% isolates survived in current study. Bacteria withstand salinity stress by adopting osmoregulation approach; either through osmotic equilibrium management by creating microenvironment surrounding them or by accumulating similar solutes to balance high osmotic potential in the environment [40-42] Also, IAA plays significant role in salinity stress mitigation and in plant growth promotion [43-44]. PGPR produced phytohormones and other determinants facilitate boosted root length, root surface area and number of root tips, which contribute in additional uptake of nutrients and thus serve plants under stress conditions [45]. Soil bacteria also assist plants to sustain salt tolerance through tissue-specific sodium transporter *HKT1* regulation [46]. Khalid et al. [44] also showed that foliar application of IAA reduced Na^+ accumulation in salt-stressed maize shoots and roots. Yang et al. [47] stated the lowering of salt stress in quinoa upon halotolerant bacteria inoculation. Several researchers reported that PGPR can improve growth of tomato, pepper, canola, bean, and lettuce even when they are experiencing salinity stress [48-50].

All together our study results revealed that the saline soils of southern Bangladesh harbour rhizobacteria that were able to meet some

selection criteria for PGPR. The bacteria isolated from this preliminary study will be used as a base resource for further study involving in vitro and in-vivo plant growth promotion studies.

5. CONCLUSION

To cope up with the increasing salinity in cultivable lands of coastal areas, use of PGPR-based biofertilizer can be a potential tool in establishing and subsequent growth of plant stand under abiotic stress. The present study was fruitful in isolating and characterizing 30 halotolerant rhizobacteria with numbers of plant growth promoting traits, e.g., P solubilization, IAA production, N_2-fixation. These bacteria have the potential to be applied for plant growth improvement under salinity stressed environment, and hence should be studied further involving plant growth experiments.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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