Annals of Agri-Bio Research **28** (2): 197-208, 2023 **Impact Score : 0.28 (Scopus)**

Phosphate Solubilizing Rhizobacteria of Rice: Analysis of Plant Growth Promoting Activity and Environmental Stress Tolerance

TASNIMUL BASHARAT[1], FERDAUSI ALI[1], TUHIN DAS[1], TAKIA BINTE BAKAR[1], NOWSHIN TARANNUM MISHI[1], JANNATUL FERDOUSE[1], MOHAMMAD SERAJ UDDIN[1] AND TANIM JABID HOSSAIN*

Department of Biochemistry and Molecular Biology, University of Chittagong, Chattogram 4331, Bangladesh
**(e-mail: tanim.bmb@gmail.com; Phone : 880 18120 92020 and seema@cu.ac.bd; Phone : 880 18120 92020)*

(Received: November 8, 2022; Accepted: December 27, 2022)

ABSTRACT

Since phosphorus remains mostly in an un-utilizable form in soil, the phosphate solubilizing bacteria (PSB) can be employed to increase availability of soluble usable phosphorus in the rhizosphere. Three highly efficient phosphate solubilizing strains were screened out from the rhizospheric soil of BRRI-28 rice variety and characterized for plant growth promoting and abiotic stress tolerance properties. 16S rRNA gene sequence analysis identified the three isolates as *Enterobacter* and *Klebsiella* strains. They exhibited multiple plant growth promoting traits including auxin secretion, zinc solubilization, or ammonia production. The phosphate solubilizing and zinc solubilizing indices of the isolates were determined. Lipolytic activity was found to be the most common hydrolytic activity detected in all of the isolates. The PSB were further evaluated for their tolerance to different degrees of salinity (3 to 11% NaCl), drought (10 to 50% PEG-6000) and temperatures (20, 30 and 37°C). The isolates tolerated salinity stress up to 7% NaCl, drought stress up to 30% PEG-6000, and grew at all the tested temperatures with maximum growth detected at 30 or 37°C. Therefore, the phosphate solubilizing isolates can be considered candidates as microbial inoculants for plant growth enhancement and agricultural productivity under stress conditions.

Key words: Phosphate solubilizing bacteria, rice PGPR, abiotic stress tolerance, zinc solubilizing bacteria, auxin production, nitrogen-fixing bacteria

INTRODUCTION

Phosphorus is a macronutrient essential for the synthesis of vital cellular structures and catalysis of many biochemical reactions in plants (Dandessa and Bacha, 2018). It is required for the development of roots, strengthening of stalks and stems, formation of flowers and seeds, nitrogen-fixation in the legumes, disease resistance, plant maturity and quality of production (Alori *et al.*, 2017). Particularly noteworthy is its contribution in the absorption and transformation of solar energy into beneficial plant chemicals (Johan *et al.*, 2021). Despite being one of the most essential nutrients, phosphorus is not amply available to plants like oxygen and hydrogen which can be obtained from water, or nitrogen which is fixated from atmosphere by nitrogen-fixing bacteria. Phosphate solubilizing bacteria (PSB) can play a major role in this regard since they solubilize organic and inorganic phosphates and make them available to plants. The evidence for the natural solubilization of phosphates by rhizospheric microorganisms was reported back in 1903 (Awais *et al.*, 2017). Among the rhizospheric microbes, bacteria are known to be more efficient in phosphate solubilization than their fungal counterparts (Tariq *et al.*, 2022). The PSB includes both Gram-positive and Gram-negative organisms and belong to a variety of species particularly within the genera *Acinetobacter, Bacillus, Pseudomonas, Massilia, Arthrobacter, Stenotrophomonas, Ochrobactrum, Cupriavidus, Burkholderia, Pseudomonas, Enterobacter, Pantoea*, *Paraburkholderia, Cronobacter, Ralstonia, Curtobacterium* and *Massilia* (Wan *et al.*, 2020; Kirui *et al.*, 2022). Their phosphorus solubilizing activity depends mainly on their secretion of (i) organic acids such as citric acid, gluconic acid, glyconic acid, lactic acid, oxalic

[1]Department of Microbiology, University of Chittagong, Chattogram-4331, Bangladesh.

acid, malic acid, acetic acid, butyric acid, glyoxalic acid, tartaric acid, etc. which chelate cations bound to phosphates thus converting them into soluble forms and (ii) phosphatases that mineralize and immobilize organic phosphates in soil (Kalayu, 2019). Moreover, microbes produce H_2S as a metabolic intermediate in the process of organic decomposition or sulfate reduction which reacts with ferric phosphate to form ferrous sulfate with concomitant release of the bound phosphorus (Florentino *et al.*, 2016). In addition, the phosphorus solubilizing microorganisms also assimilate ammonium to synthesize amino acids, releasing the excess proton into the surroundings which also assists in dissolving the insoluble phosphates (Gaind, 2016).

In addition to making phosphorus available to plants, the PSB may also provide additional plant growth promoting activities (Chowdhury *et al.*, 2022). For example, many PSB produce a variety of phyto-hormones such as auxins, gibberellins, cytokinins, or polyamides to stimulate plant growth (Santana *et al.*, 2016); protect plants from phyto-pathogens by the secretion of antibiotics and antifungal metabolites (Jog *et al.*, 2014; Paul and Sinha, 2017a), enhance seed germination by inhibiting seed mycoflora that are harmful for plants (Islam *et al.*, 2016); increase accessibility of other trace elements by siderophore production; solubilize the fixed form of zinc making it available to plants (Alori *et al.*, 2017) and so on. Using the above mechanisms, PSB not only promote plant growth, they can also reduce the use of chemical fertilizers which are relatively expensive and less eco-friendly.

In the present work, rhizo-bacteria were isolated from the roots of BRRI-28 rice variety to obtain the PSB strains. The BRRI-28 rice was developed by Bangladesh Rice Research Institute in 1994. Since its release, BRRI-28 has been a very popular and high yielding variety for the Boro (dry) season which stretches from November to April. The grain of this rice variety is medium, slender and white and the average plant height is 90 cm. The yield of BRRI-28 varies by district and is 7.5 tonnes per hectare in average (Mainuddin *et al.*, 2021). The PSB isolated from BRRI-28 in the study, were taxonomically characterized by both molecular and biochemical analysis and examined for their various plant growth-promoting effects such as auxin production, nitrogen fixation, zinc solubilization, ammonia production and secretion of hydrolytic exoenzymes. Moreover, the abiotic stress tolerance of the isolates was assessed in different stress conditions such as drought, high salinity and temperature.

MATERIALS AND METHODS

Soil closely adjoining to the roots of BRRI-28 rice plants was collected from rice fields near Chittagong University. The soil samples were taken in sterile polythene bags, brought to the laboratory and sstored at 4°C. They were then thoroughly mixed and used for subsequent bacterial isolation.

Bacterial isolation was carried out using the conventional spread plate technique. 10 g of sample was dissolved in 90 ml sterile water, shaken for 30 min on a rotary shaker and 10^{-1} serial dilutions were prepared. 100 µl suspension from each dilution was spread on nutrient agar, incubated for 24 h and colonies with dissimilar characteristics were picked. Each colony was repeatedly streaked on fresh media until the colony seems homogenous and preserved at -20°C.

To detect the presence of phosphate solubilizing ability, isolates were grown on Pikovskaya's agar medium (PVK) containing insoluble tricalcium phosphate and incubated at 30°C for seven days. Formation clear zone around bacterial growth was used as an indicator of phosphate solubilization. Further, the diameter of the clear zone and that of the colony was measured and phosphate solubilization index (PSI) was calculated following Paul and Sinha, 2017 as:

$$PSI = \frac{\text{Diameter of the zone of clearance including colony}}{\text{Diameter of colony only}}$$

16S rRNA genes of selected isolates were amplified by PCR with the genomic DNA used as template using the universal primers 27F (5´- AGAGTTTGATCNTGGCTCAG-3´) and 1492R (5´- GCTTACCTTGTTACGACTT- 3´) as described previously (Hossain *et al.*, 2020). Subsequently, the PCR products were sequenced using the Applied Biosystems

BigDye™ Terminator v3.1 (Thermo Fisher Scientific K. K.) according to the manufacturer's protocol. The sequences were deposited in the GenBank database under the accession numbers OP467564-OP467566.

Sequence similarity analysis was carried out by comparing the 16S rDNA sequences with those in the GenBank database using the blast suite optimized for highly similar sequences (mega blast). The taxonomic assignment was based on per cent identity, query coverage and the number of hits obtained against each taxon (Ali *et al.*, 2021; Hossain *et al.*, 2022).

Morphological and biochemical properties of the isolates such as colony characteristics, cell morphology, catalase, oxidase, indole and sugar fermentation tests were carried out according to conventional techniques.

Fresh colonies of the isolates were streaked on Norris Nitrogen free medium with glucose as the carbon source and incubated for five days at 30°C (Mei *et al.*, 2021). Isolates that grew were streaked again on fresh Norris Nitrogen free medium for two successive times and examined for growth.

For zinc solubilization assay, spot inoculation was performed with freshly grown bacterial colonies on Tris-minimal salt agar (Tris–HCl 6.06 g; NaCl, 4.68 g; KCl, 1.49 g; NH_4Cl, 1.07 g; Na_2SO_4, 0.43 g; $MgCl_2 \cdot 2H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 30 mg (supplemented with 0.1% zinc in the form of zinc oxide) agar plates and incubated for 14 days at 30°C. The formation of clear zone around colonies indicated a positive result. After 14 days of incubation, diameter of clear zone and colonies was measured and zinc solubilization index was calculated following the equation used for PSI determination as described above (Kamran *et al.*, 2017a).

Overnight bacterial colonies were cultured in 3 ml of LB broth supplemented with 1 g/l tryptophan and incubated at 28°C for 24 h. One ml of the culture was transferred to eppendorf tubes and centrifuged at 10000 rpm for 5 min. Subsequently, 0.1 ml of supernatant and 0.1 ml of Salkowski's reagent (2 ml of 0.5 M $FeCl_3$ in 98 ml of 35% perchloric acid) were vigorously mixed and kept in dark for 30 min. Appearance of pink to reddish pink colour indicated a positive result for IAA production.

Freshly grown bacterial colonies were cultured in peptone water at 37°C for 48-72 h and 0.5 ml of Nessler's reagent was added. Development of brown to yellow colour indicated a positive reaction as per standard test protocol.

The selected isolates were tested for the production of protease, lipase, pectinase, cellulase and amylase enzymes as previously described (Hossain *et al.*, 2020, 2021; Uddin *et al.*, 2021). For each enzyme assay, fresh, exponentially grown bacterial culture was streaked on agar media containing the appropriate substrate and incubated for two days at 30°C (Hossain *et al.*, 2020). To detect protease activity, isolates were grown on gelatin agar medium and protease activity was indicated by clear zone developed around the colonies after staining with 15% mercuric chloride solution (Hossain *et al.*, 2021). To detect lipolytic activity, isolates were grown on Tween 80-agar plates stained with methyl red solution and examined for the appearance of clear halo zone around bacterial colonies (Hossain *et al.*, 2020). Pectin-agar media were used to determine the pectinolytic activity. Plates were flooded with potassium iodide solution and clear zones surrounding the colonies confirmed pectinase production (Hossain *et al.*, 2020). Isolates were screened for cellulolytic activity by growing them onto carboxymethyl cellulose media. After 48 h incubation at 30°C, the plates were flooded with Congo red solution for 10 min and de-stained with 1 M NaCl for 15 min. The cellulolytic activity was indicated by yellow zone around colonies (Uddin *et al.*, 2021). Amylolytic activity was examined on starch-agar plates flooded with potassium iodide solution (Hossain *et al.*, 2021). Clear zone around the colonies indicated amylase activity.

To evaluate NaCl tolerance efficiency, 1% of fresh exponentially grown bacterial culture was inoculated in Luria Bertani broth supplemented with 3, 5, 7, 9 or 11% of NaCl and incubated at 30°C for 48 h. Growth was determined by measuring the optical density (OD) in a spectrophotometer (Thermo Fisher Scientific, USA) at 600 nm after 24 and 48 h (Sharma *et al.*, 2016). Osmotic stress was tested by inoculating the isolates in Luria Bertani broth supplemented with 10-50% PEG-6000, followed by incubation at 30°C for 24 to 48 h. Growth was estimated after 24 to 48 h spectrophotometrically at 600 nm.

The optimal temperature for rice cultivation is between 25 and 35°C, and rice growth is favoured in an area with a moderate

temperature (Nishad *et al.*, 2018). Therefore, the temperature tolerance of the isolates was examined at 20, 30 and 37°C. 1% bacterial culture was inoculated in Luria Bertani (LB) broth medium and incubated at the above temperatures for 24 h. Cell growth was estimated after 24-48 h by measuring OD at 600 nm.

RESULTS AND DISCUSSION

Sixty-two rhizobacteria were isolated in total from the root associated soil of BRRI-28 rice variety. Upon their screening for phosphate solubilization on Pikovskaya's medium, seven isolates were found producing clear zones by the degradation of tricalcium phosphate in media. The seven phosphate-solubilizing rhizobacteria were taxonomically characterized by both 16S rRNA gene sequence analysis and biochemical tests which suggested that some of the isolates represented the same strain and there were three dissimilar species among the isolates. Similarity search by NCBI BLAST showed over 99% identity of their 16S rRNA genes to the species of *Klebsiella* (strain PSB7) and *Enterobacter* (strains PSB9 and PSB28). Their morphological, biochemical and growth properties which suggested that the isolates were Gram-negative, indole and oxidase negative, and catalase, citrate and VP positive. The two *Enterobacter* isolates were found to be motile, while the *Klebsiella* was non-motile. The isolates were able to ferment glucose, galactose, mannose, maltose, raffinose and glycerol (Table 1).

The relative phosphate solubilization capacity was estimated based on the diameter of phosphate solubilization zone presented as PSI (Fig. 1a). The *Enterobacter* sp. strain PSB28 showed the highest phosphate solubilization capacity (PSI 5.60) followed by *Klebsiella* sp. strain PSB7 (PSI 4.70) and *Enterobacter* sp. strain PSB9 (PSI 2.41).

The PSB isolates were further examined for additional traits of plant growth enhancement such as nitrogen fixation, zinc solubilization, auxin production, and ammonia production. Only PSB9 showed all the above plant growth promoting traits, whereas PSB7 and PSB28 had ammonia production and zinc solubilization activity but lacked the nitrogen fixation and auxin production ability (Table 2). The extent

Table 1. Morphological and biochemical properties of the PSB

Features	PSB7	PSB9	PSB28
Colony properties			
Colour	Off white	Off white	Creamy white
Texture	Transparent	Opaque	Opaque
Margin	Entire	Entire	Entire
Shape	Circular	Circular	Circular
Elevation	Convex	Raised	Pulvinate
Diameter	0.4 cm	0.3 cm	0.3 cm
Cellular properties			
Gram staining	-	-	-
Cell shape	Short rod	Short rod	Cocci
Cell arrangement	Single	Pair	Pair
Growth on media			
Slant growth	Spreading	Filiform	Filiform
Growth in broth	Sediment	Sediment	Turbid
Deep glucose agar	Growth thoughout the media	Growth thoughout the media	Growth thoughout the media
Growth	Facultative	Facultative	Facultative
Condition	Anaerobic	Anaerobic	Anaerobic
Biochemical test			
Catalase	+	+	+
Oxidase	-	-	-
Indole	-	-	-
Citrate	+	+	+
Motility	-	+	+
Methyl red	-	-	-
Voges-proskauer	+	+	+
Sugar fermentation			
Glucose	+,	+,	+
Galactose	+,	+,	+
Mannose	+	+	+
Maltose	+,	+,	-
Raffinose	+	+	+
Glycerol	+	+	+

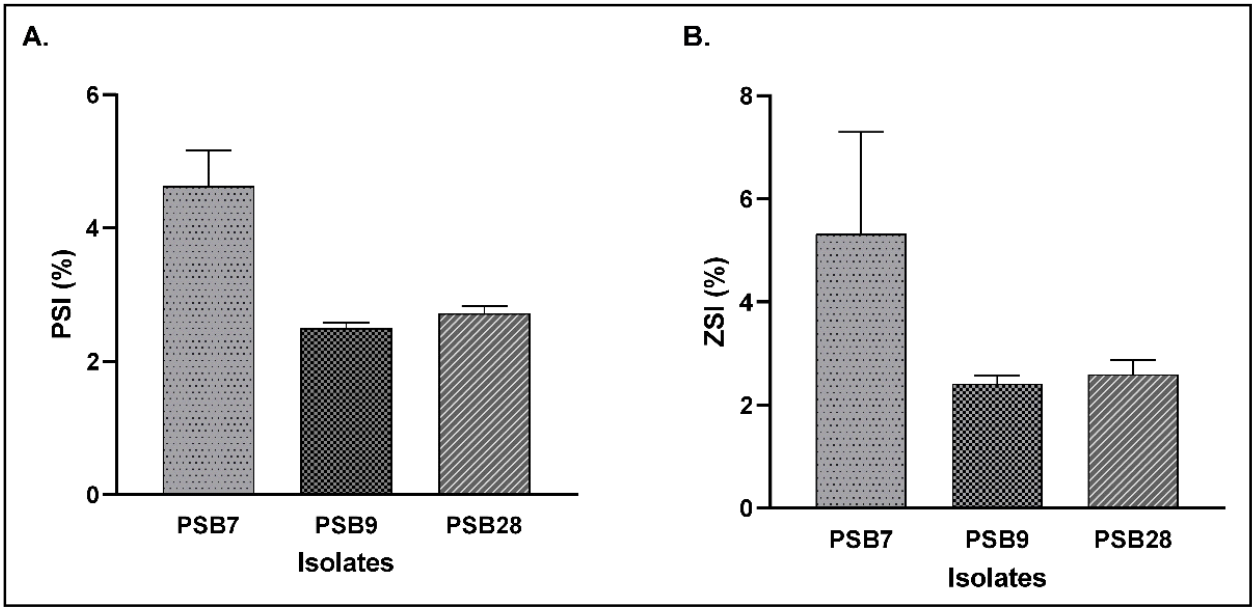

Fig. 1. Phosphate solubilization index (PSI) of rhizobacterial isolates (A) after seven days of incubation on Pikovskaya's agar plate supplemented with tricalcium phosphate at 30°C and zinc solubilization index (ZSI) (B) after 14 days of incubation at 30°C on zinc oxide-containing agar media. The results are the mean value of three replicates; error bars represent standard deviation.

of zinc solubilization as indicated by zinc solubilization index (ZSI) suggested that PSB7 and PSB28 both possessed relatively high capacity for zinc solubilization. The three PSB were further investigated for the production of extracellular hydrolytic enzymes including protease, lipase, pectinase, cellulase and amylase in which the isolates showed production of relatively limited number of enzyme (Table 3). Only lipase was produced by all the three PSB strains. PSB9 could produce pectinase also, but protease, cellulase and amylase activities were not detected in any of them.

Table 2. Additional plant growth promoting activity of the PSB

Isolates	Nitrogen fixation	Ammonia production	IAA production	Zinc solubilization
PSB7	-	++	-	+++
PSB9	+++	+++	++	++
PSB28	-	+++	-	+++

- = no production, + = low production, ++ = moderate production and +++ = high production.

Table 3. Hydrolytic enzyme secreted by the PSB

Isolates	Lipase	Pectinase	Protease	Cellulase	Amylase
PSB7	+	-	-	-	-
PSB9	+	+	-	-	-
PSB28	+	-	-	-	-

-= no hydrolytic activity and + = hydrolytic activity detected.

Whether the isolates can counteract environmental stress while enhancing plant growth was assessed at 20, 30 and 37°C temperature, 3 to 11% NaCl, and 10 to 50% PEG. The PSB could grow well at all temperatures tested with the maximum growth usually observed at 30°C and reduced growth at 20°C. Moderate to good growth was observed at most of the drought and salinity conditions (Fig. 2). The isolates showed good tolerance up to 7% of NaCl and 30% of PEG 6000. Expectedly, growth was reduced at the extreme drought and salinity stresses.

Phosphorus is one of the most critical nutrients for plant growth, and the lack of phosphorus, therefore, results in the plants' growth disturbance (Mohamed *et al.*, 2018). Although when chemical fertilizers are added to the soil, plants can only utilize low amounts of phosphatic fertilizers (Bindraban *et al.*, 2020). In this consideration, choosing a highly effective PSB can essentially increase the amount of phosphorus in the plant rhizosphere. Additionally, it contributes to the growth of plants by participating in processes like photosynthesis, energy transmission, signalling, macromolecular biosynthesis and respiration (Wu *et al.*, 2019). In the present study, three of the rice rhizobacterial isolates exhibited phosphate solubilizing efficacy. Their genetic identification revealed that two

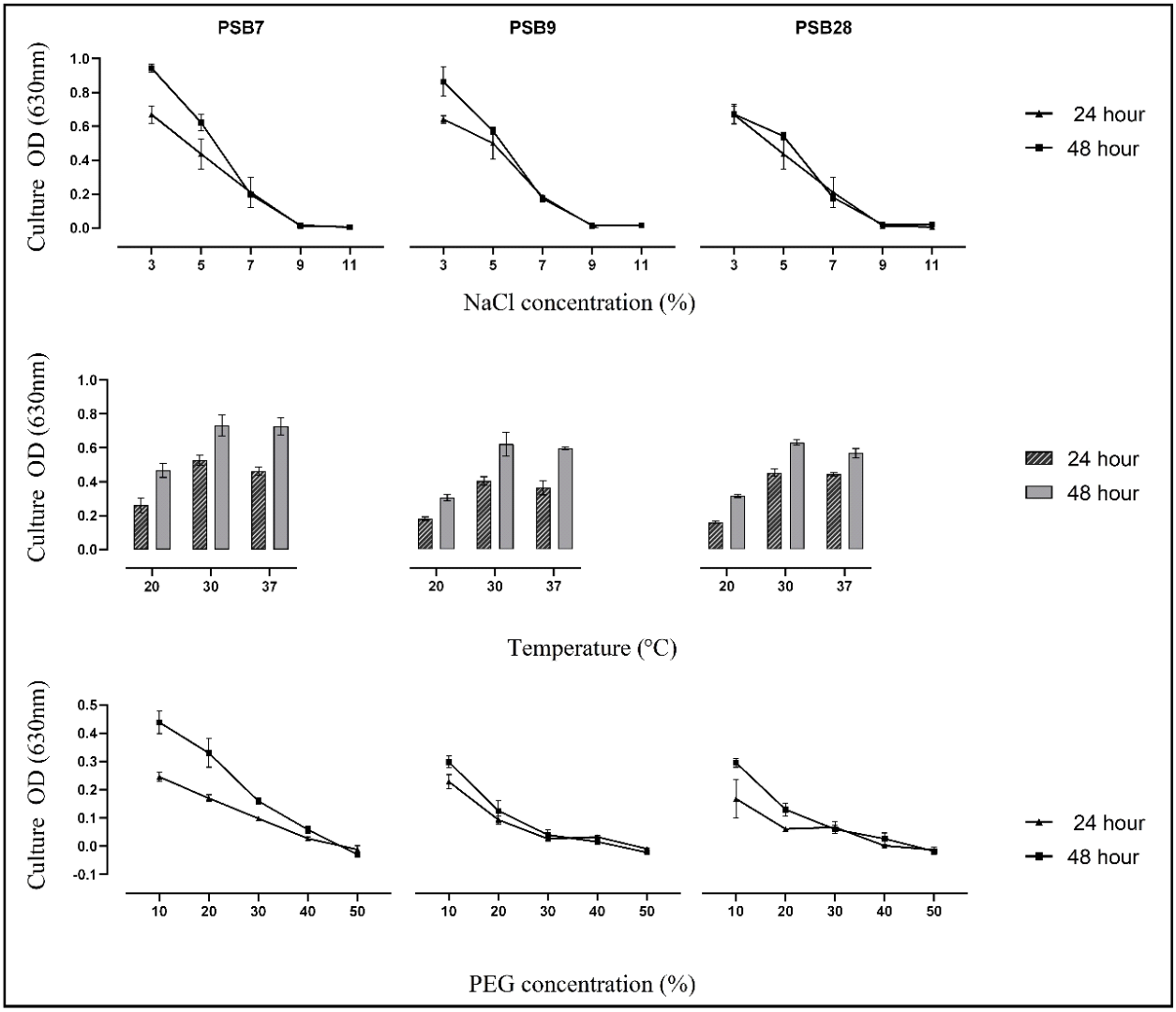

Fig. 2. Tolerance of the isolates to temperature (A), salt (B) and drought (C) stresses. The values given are the mean of three replicates and error bars indicate the standard deviation.

of them belonged to *Enterobacter* and one was *Klebsiella*. Gram staining confirmed the three strains as gram-negative bacteria. Indeed, previous research suggested that the rhizosphere of some plants preferred Gram-negative to the Gram-positive rhizobacteria (Zapata *et al.*, 2021). For example, a number of studies reported several efficient gram-negative PSB from sugarcane and rice rhizosphere including - *Klebsiella* sp., *Pseudomonas* sp., *Enterobacter* sp. and *Rhizobium* sp. which were able to release high amount of phosphorus (Rfaki *et al.*, 2015; Awais *et al.*, 2017). It was proposed that Gram-negative bacteria dissolve mineral phosphates more efficiently than Gram-positive strains due to the production of a significant number of organic acids into the extracellular media by the metabolism of carbohydrates, primarily glucose (Chakdar *et al.*, 2018; Kalayu, 2019). However, phosphate solubilization has been detected in both Gram-positive and Gram-negative bacteria species belonging to the genera *Acinetobacter, Arthrobacter, Bacillus, Burkholderia, Cronobacter, Cupriavidus, Curtobacterium, Enterobacter, Massilia, Ochrobactrum, Pantoea, Paraburkholderia, Pseudomonas, Ralstonia, Stenotrophomonas,* etc. (Wan *et al.*, 2020; Kirui *et al.*, 2022).

The motility test showed that the two *Enterobacter* strains were motile and the *Klebsiella* strain was non-motile while all were able to utilize citrate as a carbon source. The flagellar motility and citrate utilization are both thought to play a significant role in the competitive colonization of bacteria in the roots

and their maintenance (Liu *et al.*, 2017; O'Neal *et al.*, 2020). Moreover, it is believed that bacterial motility is crucial for phosphorus transfer in the soil (Shahid *et al.*, 2015). Chemotaxis allows motile bacteria as easier access to root exudates in the rhizospheric zone (Shahid *et al.*, 2015; Feng *et al.*, 2021). Furthermore, it was also suggested that motility might improve rhizoplane competency with regards to bacterial migration from bulk soil to roots and along roots, enabling the PGPR to colonize plant roots in a variety of ways (Liu *et al.*, 2017; Vandana *et al.*, 2021). Following root surface colonization, PGPR can promote plant growth by influencing plant nutrition and, in some situations, enhancing the plant's disease resistance (Pérez-Montaño *et al.*, 2014). Our results also demonstrated that the three PSB were capable of producing catalase which is also important since it is related to the ability of bacteria to protect plants against the oxidative stress (Santos *et al.*, 2018).

The PSB varied in their solubilization indices (SI) was found between 5.60 and 2.50 which is similar to those reported by Elias *et al.* (2016) wherein the phosphate solubilizing activity varied in the range of 4.50 to 2.56. Paul and Sinha (2017a) reporteds SI of 2.85 for the strain *Pseudomonas aeroginosa* KUPSB12. The *Enterobacter* sp. strain PSB9 of the present study had highest SI, while PSB28 and the *Klebsiella* sp. strain PSB7 had a better activity. Similar to their phosphate solubilization index, PSB7 and PSB28 also showed a relatively high activity in their solubilization of zinc, another essential nutrient for optimal plant growth. The deficiency of zinc causes yield loss of crops around the world. PGPR which are capable of zinc solubilization transforms the inorganic zinc into available forms. Bacterial strains that have shown zinc solubilization on a lab scale include *Pseudomonas fragi, Pantoea dispersa, Pantoea agglomerans, Enterobacter cloacae, Rhizobium* sp. (Kamran *et al.*, 2017a) *Bacillus* sp. (Zaheer *et al.*, 2019) *Bacillus* sp., *Pseudomonas striata*, *Pseudomonas fluorescence*, *Burkholderia cenocepacia,* etc. (Abaid Ullah *et al.*, 2015). Previous research showed that *Enterobacter cloacae* isolated from rice roots could solubilize the insoluble Zn compounds such as ZnO, $ZnCO_3$ and $Zn_3(PO_4)_2$ (Kamran *et al.*, 2017). Vaid *et al.* (2014) showed an increase of rice growth by 42.7% when inoculated with zinc-solubilizing bacteria. Growth enhancement by the zinc solubilizing PGPR was also demonstrated in *Zea mays* L., soybean and wheat (Khande *et al.*, 2017). The Zn-solubilizing bacterial strains solubilize the unavailable form of zinc by producing chelating ligands; secreting organic acids, vitamins and phytohormones; and through oxidoreductase systems and proton extrusion. Organic acid production by bacterial strains is a major mechanism used for Zn solubilization. Among organic acids, 2-ketogluconic acid and gluconic acid production by PGPR is responsible for the solubilization of Zn (Kushwaha *et al.*, 2021; Li *et al.*, 2021).

The three phosphate solubilizing rhizobacteria showed additional growth promoting activities including IAA production, nitrogen fixation, or ammonia production. PSB9 had all the above traits but PSB7 and PSB28 had only ammonia production activity. IAA, the most well-known phytohormone, is essential for root, flower and leaf cell division as well as senescence and initiation (Meng *et al.*, 2019). IAA is also considered to assist rice plants to have deep root systems, which enable the plants to survive in arid environments. In soil microorganisms, IAA biosynthesis can be triggered by the presence of L-tryptophan derived from root exudates (Susilowati *et al.*, 2018). Bacterial species of the genera *Aerobacter, Pseudomonas, Bacillus* and *Klebsiella* were reported to have the potential to produce IAA. Moreover, several *Enterobacter* species were previously demonstrated to have a capacity of releasing high levels of IAA (Ghosh *et al.*, 2015; Nutaratat *et al.*, 2017). Nitrogen fixation is also an important growth promoting quality. By producing the nitrogenase enzyme, bacteria convert nitrogen gas to ammonia that plants can use (Rapson *et al.*, 2020; Watanabe *et al.*, 2021). In the present study, only one of the *Enterobacter* strains showed nitrogen fixing activity. *Enterobacter* species were already described previously having potential to fix atmospheric nitrogen (Ji *et al.*, 2020; Panneerselvam *et al.*, 2021). Although previous research also reported such activity in some *Klebsiella* species (Macedo-Raygoza *et al.*, 2019), the other *Klebsiella* strain of this study didn't show nitrogen assimilation from atmosphere. All the three PSB isolates, however, could produce ammonia. Hence, they might be able to provide ammonia to the plants as a nitrogen source for their growth. (Banik

et al., 2019). Ammonia production by the *Enterobacter* and *Klebsiella* species is also supported by previous studies (Kusale *et al.*, 2021) which helps plants in root and shoot elongation and increases their biomass. In the screening of extracellular hydrolytic enzyme production, all isolates showed lipolytic activity by which they might lyse fungal cells and help plants in the biocontrol of pathogenic fungi (Figueira *et al.*, 2019). The lipase activity might also provide them with higher root colonizing ability (Lelapalli *et al.*, 2021). The *Enterobacter* sp. strain PSB9 additionally had pectinase activity which can facilitate its invasion and colonization in the plant root tissue (Adeleke *et al.*, 2021).

The PSB isolates exhibited tolerance to temperature, salt and drought up to a certain level. Growth was not hampered at any of the temperatures examined i.e. 20, 30 and 37°C, while the optimal temperature for rice cultivation is between 25 and 35°C. Salt tolerance towards 3 to 7% NaCl was found in all isolates. Similar findings were also reported in other studies (Sharma *et al.*, 2015; Abdelmoteleb and Gonzalez-Mendoza, 2020). Tolerance of drought equivalent to at least 30% PEG 6000 was also detected in all PSB isolates. In separate studies, Ahmed *et al.* (2021) and Chen *et al.* (2021) reported *Enterobacter* and other species tolerating an osmotic potential up to –0.3 MPa (15% PEG). At higher concentrations of PEG, however, bacterial growth gradually decreased. In another study, tolerance to as much as 40% PEG by some bacterial isolates was reported (Getahun *et al.*, 2020). It has been suggested that the PGPR strains that are capable of enduring the harsh environments can also help plants survive environmental stresses (Mohanty *et al.*, 2021). Plants are exposed to a variety of abiotic stimuli during ontogenesis including the salinity, temperature and drought, which can cause severe reduction in crop yield and compromise with their survival. Stress adapting phosphate solubilizing microbes play important functions in promoting plant growth under the abiotic stress conditions (Kour and Yadav, 2022). Studies have shown that rhizobacteria help improve stress tolerance by enhancing plant growth, stimulating production of phytohormones, solubilizing phosphates, lowering ethylene levels, and upregulating the expression of dehydration response and antioxidant genes (Gupta *et al.*, 2022).The PSB of this study, having the excellent *in vitro* abilities for both plant growth promotion and abiotic stress tolerance should, therefore, be tested for their applicability in further field studies. Their successful inoculation can impart a significant boost in plant growth and agricultural yield.

FUNDING

This study was funded by University of Chittagong, Bangladesh.

ACKNOWLEDGEMENTS

The authors would like to thank Maisha Samio, Department of Microbiology, University of Chittagong for her support in this project. The authors also thank members of the Biochemistry and Pathogenesis of Microbes–BPM Research Group, Chattogram for their help.

REFERENCES

Abaid Ullah, M., Hassan, M., Jamil, M., Brader, G., Shah, M., Sessitsch, A. and Hafeez, F. (2015). Plant growth promoting rhizobacteria: An alternate way to improve yield and quality of wheat (*Triticum aestivum*). *Int. J. Agric. Biol.* **17**: 51-60.

Abdelmoteleb, A. and Gonzalez-Mendoza, D. (2020). Isolation and identification of phosphate solubilizing *Bacillus* spp. from *Tamarix ramosissima* rhizosphere and their effect on growth of *Phaseolus vulgaris* under salinity stress. *Geomicrobiology J.* **37**: 901-908.

Adeleke, B. S., Babalola, O. O. and Glick, B. R. (2021). Plant growth-promoting root-colonizing bacterial endophytes. *Rhizosphere* **20**: 100433. *https://doi.org/10.1016/j.rhisph.2021.100433.*

Ahmed, B., Shahid, M., Syed, A., Rajput, V. D., Elgorban, A. M., Minkina, T., Bahkali, A. H. and Lee, J. (2021). Drought tolerant Enterobacter sp./Leclerciaadecarboxylata secretes indole-3-acetic acid and other biomolecules and enhances the biological attributes of *Vigna radiata* (L.) R. Wilczek in water deficit conditions. *Biology* **10**: 1149.

Ali, F., Sharup Das, Hossain, T. J., Chowdhury, S. I., Zedny, S. A., Das, T., Chowdhury, M. N. A. and Uddin, M. S. (2021). Production optimization, stability and oil emulsifying potential of biosurfactants from selected

bacteria isolated from oil contaminated sites. *Royal Soc. Open Sci.* **8**: 211003. *https://doi.org/10.1098/rsos.211003.*

Alori, E. T., Glick, B. R. and Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Front. Microbiol.* **8**. *https://www.frontiersin.org/articles/10.3389/fmicb.2017.00971.*

Awais, M., Tariq, M., Ali, A., Ali, Q., Khan, A., Tabassum, B., Nasir, I. A. and Husnain, T. (2017). Isolation, characterization and inter-relationship of phosphate solubilizing bacteria from the rhizosphere of sugarcane and rice. *Biocat. Agric. Biotech.* **11**: 312-321.

Banik, A., Dash, G. K., Swain, P., Kumar, U., Mukhopadhyay, S. K. and Dangar, T. K. (2019). Application of rice (*Oryza sativa* L.) root endophytic diazotrophic Azotobacter sp. Strain Avi2 (MCC 3432) can increase rice yield under green house and field condition. *Microbiol. Res.* **219**: 56-65.

Bindraban, P. S., Dimkpa, C. O. and Pandey, R. (2020). Exploring phosphorus fertilizers and fertilization strategies for improved human and environmental health. *Biol. Fertility of Soils* **56**: 299317.

Chakdar, H., Dastager, S. G., Khire, J. M., Rane, D. and Dharne, M. S. (2018). Characterization of mineral phosphate solubilizing and plant growth promoting bacteria from termite soil of arid region. *3 Biotech.* **8**: 463. *https://doi.org/10.1007/s13205-018-1488-4.*

Chen, J., Sharifi, R., Khan, M. S. S., Islam, F., Bhat, J. A., Kui, L. and Majeed, A. (2021). Wheat microbiome: Structure, dynamics and role in improving performance under stress environments. *Front. Microbiol.* **12**: *https://doi.org/10.3389/fmicb.2021.821546.*

Chowdhury, M. N. A., Hossain, T. J., Zedny, S. A., Akther, K., Rahman, M. and Islam, S. (2022). *Plant growth promoting and abiotic stress tolerance traits of chrysanthemum-associated rhizobacteria* [SSRN Scholarly Paper]. *https://doi.org/10.2139/ssrn.4306331.*

Dandessa, C. and Bacha, K. (2018). Review on role of phosphate solubilizing microorganisms in sustainable agriculture. *Int. J. Curr. Res. Acad. Rev.* **6**: 48-55.

Elias, F., Woyessa, D. and Muleta, D. (2016). Phosphate solubilization potential of rhizosphere fungi isolated from plants in Jimma Zone, Southwest Ethiopia. *Int. J. Microbiol.* **2016**. *https://doi.org/10.1155/2016/5472601.*

Feng, H., Fu, R., Hou, X., Lv. Y., Zhang, N., Liu, Y., Xu, Z., Miao, Y., Krell, T., Shen, Q. and Zhang, R. (2021). Chemotaxis of beneficial rhizobacteria to root exudates: The first step towards root-microbe rhizosphere interactions. *Int. J. Mol. Sci.* **22**: 6655. *https://doi.org/10.3390/ijms22136655.*

Figueira, C., Ferreira, M. J., Silva, H. and Cunha, A. (2019). Improved germination efficiency of *Salicornia ramosissima* seeds inoculated with *Bacillus aryabhattai* SP1016-20. *Ann. Appl. Biol.* **174**: 319-328.

Florentino, A. P., Weijma, J., Stams, A. J. M. and Sánchez-Andrea, I. (2016). Ecophysiology and application of acidophilic sulfur-reducing microorganisms. In: *Biotechnology of Extremophiles:* **Vol. 1**: 141-175. P. H. Rampelotto (ed.). Springer International Publishing.

Gaind, S. (2016). Phosphate dissolving fungi: Mechanism and application in alleviation of salt stress in wheat. *Microbiol. Res.* **193**: 094-102.

Getahun, A., Muleta, D., Assefa, F. and Kiros, S. (2020). Plant growth-promoting rhizobacteria isolated from degraded habitat enhance drought tolerance of acacia (*Acacia abyssinica* Hochst. Ex Benth.) seedlings. *Int. J. Microbiol.* **2020**. *https://doi.org/10.1155/2020/8897998.*

Ghosh, P. K., Sen, S. K. and Maiti, T. K. (2015). Production and metabolism of IAA by Enterobacter spp. (Gammaproteobacteria) isolated from root nodules of a legume *Abrus precatorius* L. *Biocatalysis Agric. Biotech.* **4**: 296-303.

Gupta, A., Mishra, R., Rai, S., Bano, A., Pathak, N., Fujita, M., Kumar, M. and Hasanuzzaman, M. (2022). Mechanistic insights of plant growth promoting bacteria mediated drought and salt stress tolerance in plants for sustainable agriculture. *Int. J. Mol. Sci.* **23.** *https://doi.org/10.3390/ijms23073741.*

Hossain, T. J., Chowdhury, S. I., Mozumder, H. A., Chowdhury, M. N., Ali, F., Rahman, N. and Dey, S. (2020). Hydrolytic exoenzymes produced by bacteria isolated and identified from the gastrointestinal tract of Bombay duck. *Front. Microbiol.* **11**: 2097. *https://doi.org/10.3389/fmicb.2020.02097.*

Hossain, T. J., Das, M., Ali, F., Chowdhury, S. I. and Zedny, S. A. (2021). Substrate preferences, phylogenetic and biochemical properties of proteolytic bacteria present in the digestive tract of Nile tilapia (*Oreochromis niloticus*). *AIMS Microbiol.* **7**. *https://doi.org/10.3934/microbiol.2021032.*

Hossain, T. J., Nafiz, I. H., Mozumder, H. A., Ali, F., Rahman, N. and Khan, M. S. (2022). *Antipathogenic action and antibiotic sensitivity*

*pattern of the borhani-associated lactic acid bacterium Weissella confusa **LAB**-11* (SSRN Scholarly Paper No. 4161742). *https://papers.ssrn.com/abstract=4161742.*

Islam, S., Akanda, A. M., Prova, A., Islam, Md. T. and Hossain, Md. M. (2016). Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Front. Microbiol.* **6**: *https://doi.org/10.3389/fmicb.2015.01360.*

Ji, C., Liu, Z., Hao, L., Song, X., Wang, C., Liu, Y., Li, H., Li, C., Gao, Q. and Liu, X. (2020). Effects of *Enterobacter cloacae* HG-1 on the nitrogen-fixing community structure of wheat rhizosphere soil and on salt tolerance. *Front. Plant Sci.* **11**: 1094. *https://doi.org/10.3389/fpls.2020.01094.*

Jog, R., Pandya, M., Nareshkumar, G. and Rajkumar, S. (2014). Mechanism of phosphate solubilization and antifungal activity of Streptomyces spp. Isolated from wheat roots and rhizosphere and their application in improving plant growth. *Microbiol. (Reading, England)* **160**: 778-788.

Johan, P. D., Ahmed, O. H., Omar, L. and Hasbullah, N. A. (2021). Phosphorus transformation in soils following co-application of charcoal and wood ash. *Agronomy* **11**. *https://doi.org/10.3390/agronomy11102010.*

Kalayu, G. (2019). Phosphate solubilizing microorganisms: Promising approach as biofertilizers. *Int. J. Agron.* **2019** : 01-07.

Kamran, S., Shahid, I., Baig, D. N., Rizwan, M., Malik, K. A. and Mehnaz, S. (2017a). Contribution of zinc solubilizing bacteria in growth promotion and zinc content of wheat. *Front. Microbiol.* **8**: 2593. *https://doi.org/10.3389/fmicb.2017.02593.*

Khande, R., Sharma, S. K., Ramesh, A. and Sharma, M. P. (2017). Zinc solubilizing Bacillus strains that modulate growth, yield and zinc biofortification of soybean and wheat. *Rhizosphere* **4**: 126-138.

Kirui, C. K., Njeru, E. M. and Runo, S. (2022). Diversity and phosphate solubilization efficiency of phosphate solubilizing bacteria isolated from semi-arid agroecosystems of eastern Kenya. *Microbiol.Insights* **15**: *https://doi.org/10.1177/11786361221088991.*

Kour, D. and Yadav, A. N. (2022). Stress adaptive phosphorus solubilizing microbiomes for agricultural sustainability. *J. Appl. Biol. Biotech.* **10**: *https://doi.org/10.7324/JABB.2022.106ed.*

Kusale, S. P., Attar, Y. C., Sayyed, R. Z., Malek, R. A., Ilyas, N., Suriani, N. L., Khan, N. and El Enshasy, H. A. (2021). Production of plant beneficial and antioxidants metabolites by *Klebsiella variicola* under salinity stress. *Molecules* **26**: 1894. *https://doi.org/10.3390/molecules26071894.*

Kushwaha, P., Srivastava, R., Pandiyan, K., Singh, A., Chakdar, H., Kashyap, P. L., Bhardwaj, A. K., Murugan, K., Karthikeyan, N., Bagul, S. Y., Srivastava, A. K. and Saxena, A. K. (2021). Enhancement in plant growth and zinc biofortification of chickpea (*Cicer arietinum* L.) by *Bacillus altitudinis*. *J. Soil Sci. Plant Nut.* **21**: 922-935.

Lelapalli, S., Baskar, S., Jacob, S. M. and Paranthaman, S. (2021). Characterization of phosphate solubilizing plant growth promoting rhizobacterium *Lysinibacillus pakistanensis* strain PCPSMR15 isolated from *Oryza sativa*. *Curr. Res. Microbial Sci.* **2**: 100080. *https://doi.org/10.1016/j.crmicr.2021.100080.*

Li, X. L., Zhao, X. Q., Dong, X. Y., Ma, J. F. and Shen, R. F. (2021). Secretion of gluconic acid from nguyenibacter sp. L1 is responsible for solubilization of aluminum phosphate. *Front. Microbiol.* **12**: 784025. *https://doi.org/10.3389/fmicb.2021.784025.*

Liu, H., Carvalhais, L. C., Crawford, M., Singh, E., Dennis, P. G., Pieterse, C. M. J. and Schenk, P. M. (2017). Inner plant values: Diversity, colonization and benefits from endophytic bacteria. *Front. Microbiol.* **8**: 2552. *https://doi.org/10.3389/fmicb.2017.02552.*

Liu, Y., Jiang, X., Guan, D., Zhou, W., Ma, M., Zhao, B., Cao, F., Li, L. and Li, J. (2017). Transcriptional analysis of genes involved in competitive nodulation in *Bradyrhizobium diazoefficiens* at the presence of soybean root exudates. *Scientific Rep.* **7**: 10946. *https://doi.org/10.1038/s41598-017-11372-0.*

Macedo-Raygoza, G. M., Valdez-Salas, B., Prado, F. M., Prieto, K. R., Yamaguchi, L. F., Kato, M. J., Canto-Canché, B. B., Carrillo-Beltrán, M., Di Mascio, P., White, J. F. and Beltrán-García, M. J. (2019). *Enterobacter cloacae*, an endophyte that establishes a nutrient-transfer symbiosis with banana plants and protects against the black sigatoka pathogen. *Front. Microbiol.* **10**: 804. *https://doi.org/10.3389/fmicb.2019.00804.*

Mainuddin, M., Alam, M. M., Maniruzzaman, M., Kabir, M. J., Mojid, M. A., Hasan, M. M., Schmidt, E. J. and Islam, M. T. (2021). Yield, profitability and prospects of irrigated Boro rice cultivation in the North-West region of Bangladesh. *PLOS ONE* **16**: e0250897.

Mei, C., Chretien, R. L., Amaradasa, B. S., He, Y.,

Turner, A. and Lowman, S. (2021). Characterization of phosphate solubilizing bacterial endophytes and plant growth promotion *in vitro* and in greenhouse. *Microorganisms* **9**: 1935. *https://doi.org/10.3390/microorganisms9091935.*

Meng, X., Wang, Z., He, S., Shi, L., Song, Y., Lou, X. and He, D. (2019). Endogenous hormone levels and activities of IAA-modifying enzymes during adventitious rooting of tree peony cuttings and grafted scions. *Hort. Environ. Biotech.* **60**: 187-197.

Mohamed, E. A. H., Farag, A. G. and Youssef, S. A. (2018). Phosphate solubilization by *Bacillus subtilis* and Serratiamarcescens isolated from tomato plant rhizosphere. *J. Environ. Prot.* **9**: *https://doi.org/10.4236/jep.2018.93018.*

Mohanty, P., Singh, P. K., Chakraborty, D., Mishra, S. and Pattnaik, R. (2021). Insight into the role of PGPR in sustainable agriculture and environment. *Front. Sustain. Food Systems* **5**. *https://www.frontiersin.org/articles/10.3389/fsufs.2021.667150.*

Nishad, A., Mishra, A. N., Chaudhari, R., Aryan, R. K. and Katiyar, P. (2018). Effect of temperature on growth and yield of rice (*Oryza sativa* L.) cultivars. *IJCS* **6**: 1381-1383.

Nutaratat, P., Monprasit, A. and Srisuk, N. (2017). High-yield production of indole-3-acetic acid by Enterobacter sp. DMKU-RP206, a rice phyllosphere bacterium that possesses plant growth-promoting traits. *3 Biotech.* **7**: 305. *https://doi.org/10.1007/s13205-017-0937-9.*

O'Neal, L., Vo, L. and Alexandre, G. (2020). Specific root exudate compounds sensed by dedicated chemoreceptors shape azospirillumbrasilense chemotaxis in the rhizosphere. *Appl. Environ. Microbiol.* **86**: e01026-20. *https://doi.org/10.1128/AEM.01026-20.*

Panneerselvam, P., Senapati, A., Sharma, L., Nayak, A. K., Kumar, A., Kumar, U., Prabhukarthikeyan, S. R., Mitra, D. and Sagarika, M. S. (2021). Understanding rice growth-promoting potential of *Enterobacter* spp. isolated from long-term organic farming soil in India through a supervised learning approach. *Curr. Res. Microbial Sci.* **2**: 100035. *https://doi.org/10.1016/j.crmicr.2021.100035.*

Paul, D. and Sinha, S. N. (2017a). Isolation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa* KUPSB12 with antibacterial potential from river Ganga, India. *Ann. Agrarian Sci.* **15**: 130-136. *https://doi.org/10.1016/j.aasci.2016.10.001.*

Pérez-Montaño, F., Alías-Villegas, C., Bellogín, R. A., del Cerro, P., Espuny, M. R., Jiménez-Guerrero, I., López-Baena, F. J., Ollero, F. J. and Cubo, T. (2014). Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiological Res.* **169**: 325-336.

Rapson, T. D., Gregg, C. M., Allen, R. S., Ju, H., Doherty, C. M., Mulet, X., Giddey, S. and Wood, C. C. (2020). Insights into nitrogenase bioelectrocatalysis for green ammonia production.*Chem. Sus. Chem.* **13**: 4856-4865.

Rfaki, A., Nassiri, L. and Ibijbijen, J. (2015). Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of faba bean (*Vicia faba* L.) in Meknes Region, Morocco. *British Microbiol. Res. J.* **6**: 247-254.

Santana, E. B., Marques, E. L. S. and Dias, J. C. T. (2016). Effects of phosphate-solubilizing bacteria, native microorganisms and rock dust on *Jatropha curcas* L. growth. *Genet. Mol. Res.* **15**: *https://doi.org/10.4238/gmr.15048729.*

Santos, A. de A., Silveira, J. A. G. da, Bonifacio, A., Rodrigues, A. C. and Figueiredo, M. do V. B. (2018). Antioxidant response of cowpea co-inoculated with plant growth-promoting bacteria under salt stress. *Braz. J. Microbiol.* **49**: 513-521.

Shahid, M., Hameed, S., Tariq, M., Zafar, M., Ali, A. and Ahmad, N. (2015). Characterization of mineral phosphate-solubilizing bacteria for enhanced sunflower growth and yield-attributing traits. *Ann. Microbiol.* **65**: Article **3**. *https://doi.org/10.1007/s13213-014-0991-z.*

Sharma, A., Singh, P., Kumar, S., Kashyap, P. L., Srivastava, A. K., Chakdar, H., Singh, R. N., Kaushik, R., Saxena, A. K. and Sharma, A. K. (2015). Deciphering diversity of salt-tolerant bacilli from saline soils of eastern Indo-gangetic Plains of India. *Geomicrobiology J.* **32**: 170-180.

Sharma, S., Kulkarni, J. and Jha, B. (2016). Halotolerantrhizo bacteria promote growth and enhance salinity tolerance in peanut. *Fron. Microbiol.* **7**: 1600. *https://doi.org/10.3389/fmicb.2016.01600.*

Susilowati, D. N., Riyanti, E. I., Setyowati, M. and Mulya, K. (2018). Indole-3-acetic acid producing bacteria and its application on the growth of rice. *AIP Conference Proceedings 2002* (1) : 020016. *https://doi.org/10.1063/1.5050112.*

Tariq, M. R., Shaheen, F., Mustafa, S., Ali, S., Fatima, A., Shafiq, M., Safdar, W., Sheas,

M. N., Hameed, A. and Nasir, M. A. (2022). Phosphate solubilizing microorganisms isolated from medicinal plants improve growth of mint. *Peer J.* **10**: e13782. *https://doi.org/10.7717/peerj.13782.*

Uddin, M., Lima, S., Hossain, T., Kar, N., Zahan, Y. and Olarewaju Abdulkareem, B. (2021). Evaluation of cellulolytic endo-1,4-β-d-glucanase activity in the digestive fluid of adult phytophagous beetle *Hoplasoma unicolo*r. *Tropical Life Sci. Res.* **32**: 53-68. *https://doi.org/10.21315/tlsr2021.32.3.4.*

Vaid, S. K., Kumar, B., Sharma, A., Shukla, A. K. and Srivastava, P. C. (2014). Effect of zinc solubilizing bacteria on growth promotion and zinc nutrition of rice. *J. Soil Sci. Plant Nut.* **22**: 889-910.

Vandana, U. K., Rajkumari, J., Singha, L. P., Satish, L., Alavilli, H., Sudheer, P. D. V. N., Chauhan, S., Ratnala, R., Satturu, V., Mazumder, P. B. and Pandey, P. (2021). The endophytic microbiome as a hotspot of synergistic interactions, with prospects of plant growth promotion. *Biology* **10**: 101. *https://doi.org/10.3390/biology10020101.*

Wan, W., Qin, Y., Wu, H., Zuo, W., He, H., Tan, J., Wang, Y. and He, D. (2020). Isolation and characterization of phosphorus solubilizing bacteria with multiple phosphorus sources utilizing capability and their potential for lead immobilization in soil. *Front. Microbiol.* **11**: 752. *https://doi.org/10.3389/fmicb.2020.00752.*

Watanabe, Y., Aoki, W. and Ueda, M. (2021). Sustainable biological ammonia production towards a carbon-free society. *Sustainability* **13**: 9496. *https://doi.org/10.3390/su13179496.*

Wu, F., Li, J., Chen, Y., Zhang, L., Zhang, Y., Wang, S., Shi, X., Li, L. and Liang, J. (2019). Effects of phosphate solubilizing bacteria on the growth, photosynthesis and nutrient uptake of *Camellia oleifera* Abel. *Forests* **10**: 348. *https://doi.org/10.3390/f10040348.*

Zaheer, A., Malik, A., Sher, A., Mansoor Qaisrani, M., Mehmood, A., Ullah Khan, S., Ashraf, M., Mirza, Z., Karim, S. and Rasool, M. (2019). Isolation, characterization and effect of phosphate-zinc-solubilizing bacterial strains on chickpea (*Cicer arietinum* L.) growth. *Saudi J. Biol. Sci.* **26**: 1061-1067. *https://doi.org/10.1016/j.sjbs.2019.04.004.*

Zapata, T., Galindo, D. M., Corrales-Ducuara, A. R. and Ocampo-Ibáñez, I. D. (2021). The diversity of culture-dependent gram-negative rhizobacteria associated with *Manihot esculenta* Crantz plants subjected to water-deficit stress. *Diversity* **13**: 366. *https://doi.org/10.3390/d13080366.*