RESEARCH ARTICLE

Isolation, Screening, and Molecular Characterization of Rhizosphere Derived Potential Biofertilizer from Different Crops Land for Sustainable Agriculture and Environment

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Abstract: Overpopulation in world has created immense pressure on agricultural land to increase crops production by using excess chemical fertilizers, pesticides, and plant growth regulators. Nowadays, biofertilizer is one of the best concerns of research interest for sustainable development in agriculture and environment. About 30 soil bacteria were isolated from three different locations in Bangladesh by growing on nitrogen-free selective media and primarily had been categorized based on colony features. However, to short out the biofertilizer and biocontrol potential bacteria several assay had been conducted including nitrogen-fixing assay, ammonia production assay, phosphate solubilizing assay, IAA production assay, siderophores production assay, amylase production assay, biocontrol assay, and seed germination bioassay. Different species of *Rhizobium*, *Bacillus*, *Paraburkholderia*, *Priestia*, *Arthrobacter*, *Pseudarthrobacter*, *Rhodanobacter*, *Flavobacterium*, *Mucilaginibacter*, and *Sphingomonas* were identified by analyzing 16S rRNA gene partial sequence analysis by BLASTn that revealed many of these act as biofertilizer. Moreover, the antibiotic resistance tests of biofertilizer potential isolates and all others intensive data analysis in respect of biofertilizer functionality revealed that *Paraburkholderia sacchari* is the best while *Priestia megaterium*, *Bacillus zanthoxyli*, *Arthrobacter globiformis*, and *Bacillus wiedmannii* have also the potentiality to be biofertilizer. The outcome will help the researchers and industries to develop biofertilizer for crops plant.
Keywords: Rhizosphere, PGPB Screening, 16s rRNA gene Sequence, *Paraburkholderia sacchari*, sustainable Agriculture

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1 Introduction

Sustainable agriculture with higher yielding of crop is now one of the most crucial global issues. Increasing the world population creates destructive effect on agricultural land due to uncontrolled application of chemical fertilizers, pesticides, herbicides, and even plant growth regulators for higher yields of crop (Oo et al., 2020). However, the excess and long-term use of chemically synthesized fertilizer and other chemicals for agriculture may deposit on the land that are altering the soil's physical, chemical, and biological properties (Lin et al., 2019). Chemical fertilizer is pricy, nowadays hard to find in developing and least developing countries, emits greenhouse gases, contaminates surface and groundwater, ruins the microbial ecosystem, and can even make people sick when consumed (Bisht and Chauhan, 2020). On the other hand, the Food and Agriculture Organization of UN declared (FAO report., 2019) the world population would reach 9.1 billion by 2050 and the cereal crops demand will have increased by about 70% (Godfray et al., 2010). For future, a safe environment and feeding overpopulation in the world will be challenging because the agriculture and forestry sector directly and indirectly emitted about one fourth of global greenhouse gases in according 2010 through the cultivation of crops and livestock (EPA, USA., 2022).

Microorganisms from natural resources including soil, rhizosphere, water, and waste can be a probable source of plant growth-promoting bacteria (PGPB) that have played a vital role in agriculture by supplying necessary plant nutrients. Among the sources, soil and rhizosphere naturally enrich with PGPBs but about 1-2% of them only can promote plant growth (Backer et al., 2018). However, many of the phytomicrobiome both free-living and symbiotic are not capable to grow on the classical microbial media and the diversity of the PGPB are affected by environmental factors (Hirsch and Mauchline, 2012). So, the nutrients in the distinct soil

are fully unpredictable and changeable. Plants need different types of macronutrients and micronutrients that are absorbed as dissolved minerals through plant roots (Freitas et al., 1997). However, soil-plant-microbe interactions are complicated processes, and extensive explanations of how they affect plant health and productivity have been varied based on species of plants, cultivars, soils, and genotype of PGPB (Mehta and Monteiro, 2014; Lucy et al., 2004). Moreover, the usage of chemical fertilizers and pesticides has been minimized by the biofertilizer potential bacteria that will protect Soil quality, human health, biodiversity, and environmental pollution (Zahid et al., 2015).

Biofertilizer is the natural isolated microbial formulation that has the ability to supply nutrients and/or control pests to plants without toxic formation and eco-friendly manner. Nowadays, biofertilizers are widely used in developed countries Canada, China, USA, Germany, Brazil, and India to build up handsome soil health with natural microflora that ensure higher production through the environmentally friendly approach (Kumar et al., 2017). The sharp growth of the biofertilizer market will be 3 billion by 2024 that indicates, it will be disseminated in all developing countries like Bangladesh. However, the commercial production of biofertilizer data revealed that nitrogen-fixing and phosphate solubilizing PGPB are globally used widely to ensure food safety and security (Soumare et al., 2020). Different microbial strain has been used to mitigate the different necessity of plants while some have multifunctional plant-growth-promoting and biocontrol ability. For example, some can fix nitrogen from the atmosphere (Lin et al., 2012), some can solubilize and mobilize phosphorus (Richardson, 2001), some may synthesize auxin and siderophores, and some can synthesize lytic enzymes (Pieterse et al., 2014). This study's target is to find out multifunctional indigenous plant growth-inducing bacteria and their biofertilizer potentiality has been observed through *in vitro* experiments. However, about 30 types of bacteria were isolated by growing on selective media from rhizospheric and non-rhizospheric soil of three different regions of diverse agricultural land in Bangladesh. The native PGPB should be easily acclimatized to the natural conditions and also ensure strong bonding among soil-microbes-plants interaction. Finally we were find out the desirable biofertilizer potential bacteria and the best one was confirmed *Paraburkholderia sacchari* by 16s rRNA gene sequence analysis.

2 Materials and Methods

2.1 Sample collection

The soil samples were collected from three different districts (Dhaka, Jashore, and Gazipur) of Bangladesh (Figure 1) that fields are cultivated by using the modern agriculture approaches. The soil samples were collected from rice, wheat, bean, tomato, maize, lentil, and pea cultivated land. The samples were collected in sterilized polythene bags and kept at 4°C temperature before the isolation of PGPB. However, we have collected both the rhizospheric and surface soil for diverse PGPB and the P^H of the soils were recorded.

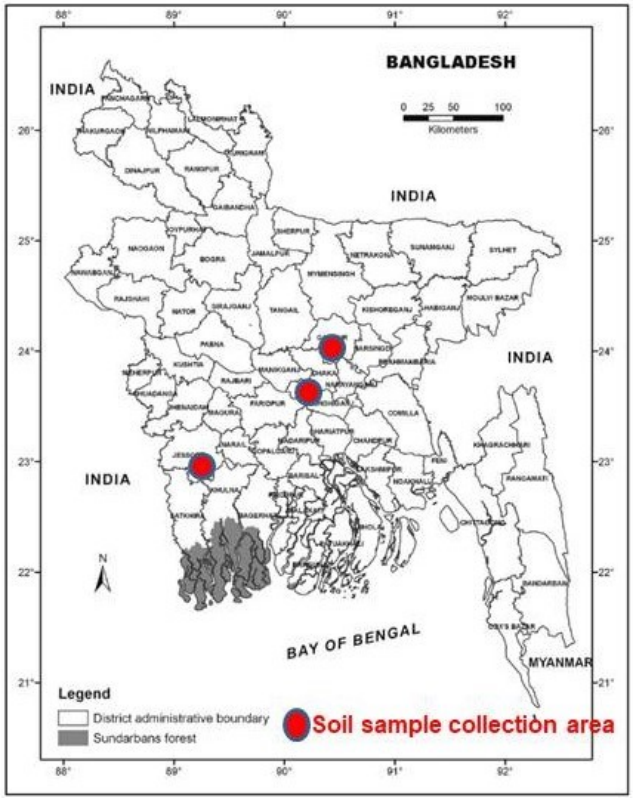

Figure 1. Sample collection area of three different districts.

2.2 Desirable bacteria isolation

Firstly the soil suspension was prepared by adding 1 gm of soil sample into 9 ml deionized autoclaved (Taisite) water on a 30 ml glass test tube. Then the soil suspension was serially diluted i.e. in each case 1 ml of soil suspension was added to 9 ml of deionized autoclaved water and vortexing the mixture was properly to generate a homogenized mixture of soil. Burk's media (BM) (HIMEDIA) was utilized to find out nitrogen fixing PGPB, which is a nitrogen free medium. The serial dilution took place on a laminar air flow hood (ESCO) in which the soil sample had been diluted 10^1 to 10^8 and then spreading 100 μl of each dilution was through the sterile glass rod. The culture plate was incubated (BINDER) at (31 $\pm$ 1)°C (Celsius) temperature for 48 to 72 hrs (hours) for finding diverse nitrogen fixing bacteria. The bacteria pure culture was established by subculture on the same medium through the streaking method. The pure culture had been analyzed by the gram staining to know either the bacteria gram-positive or gram negative.

2.3 Preservation of the isolates

After establishment of pure culture of nitrogen fixing bacteria, the autoclaved nutrient broth (NB) medium (HIMEDIA) was

prepared for liquid culture. The inoculum was shaking (Stuart, Germany) at 32°C temperature at 140 RPM (Round per Minute) for 48-72 hrs. Then equal volume of liquid culture and equal volume of 20%, 40% and 50% glycerol (HIMEDIA) solution were mixed gently by pipetting and preserved these at -20 to -40°C (LIEBHERR) as stock. However, the pure culture plate was preserved around one month at 4°C in a refrigerator (Sharp, Japan) and most of the experiments were conducted by using pure mother culture. On the other hand, the glycerol stock had been tested every two months interval to ensure the viability of the preserved bacteria.

2.4 Determination of nitrogen fixing activity

Bacterial isolates were grown on BM for the detection of nitrogen fixing activity. The Norris Nitrogen Free Mineral Medium (NFMM) (HIMEDIA) used with 1% Glucose (HIMEDIA) and a trace amount (0.05%, W/V) of Bromothymol Blue (BTB) (HIMEDIA) for visual detection of nitrogen fixation by the bacteria. However, the bacterial isolates were grown on BM liquid medium for 48 hrs for spot inoculation on NFMM. After 3-10 days, the nitrogen fixing bacteria start to show a color change from green to blue whereas some bacteria may not show color change or grow yellow colonies that indicates no nitrogen fixation.

2.5 Evaluation of phosphate solubilization activity

All bacterial isolates were evaluated for phosphate solubilization by spot inoculation on the center of semi-solid Pikovskaya's agar medium (HIMEDIA). However, the isolates were cultured on nutrient broth for 48-72 hrs and the inoculum were incubated at 32 $\pm$ 1°C for 7 days. The phosphate solubilizing activity was calculated by observing the halo zone diameter around the colony which is measured by the P-solubility Index (PSI). The assay has been conducted in triplicate for the more accurate result that was calculated as PSI = (Colony diameter + Halo zone diameter)/Colony diameter.

2.6 Ammonia production ability

Peptone water (HIMEDIA) is prepared and distributed as 10 ml in each test tube for testing the ammonia production ability of the isolated bacteria. Add freshly grown single colony of the test tubes and shake these at 140 rpm at 30°C for 96 hrs. After that, 0.5 ml of Nesseler's reagent (RESEARCH-LAB) was added each to incubate liquid culture and vortex gently. The color change observes within 10 mins where the faint yellow color illustrates small amount of ammonia production, and the deep yellow/brownish color reveals the production of large amount of ammonia.

2.7 Detection and measurement of Indole Acetic Acid (IAA)

PGPB can produce plant growth hormones e.g. IAA that had been detected and measured by *in vitro* experiments. In the nutrient broth 0.1% tryptophan (HIMEDIA) was added as the precursor of IAA. After autoclaving the liquid medium was added the fresh and unique colony through a sterile toothpick/culture loop. The cultures incubated on a rotary shaker for more than 96 hrs at 30°C and centrifuge (HIMAC) the culture at 10,000 rpm for 10 mins and collect the supernatant. A fresh autoclaved test tube has been selected to conduct the further assay in which freshly prepared Salkowshki's reagent (0.5M $FeCl_3$+$HClO_4$) was taken 4 ml and added 1 ml supernatant from individual bacterial isolates and vortex gently. After 30 mins incubation, the pink/pink-red color indicates the presence of IAA. However, the IAA produced by bacteria can be quantified *in vitro* through the generation of a standard curve of pure IAA. The absorbance of culture and standard were measured by 530 nm wavelength through UV-Spectrophotometry (HACH) where the bacterial liquid medium was considered as blank and the data analysis was done by Microsoft excel. The IAA concentration of the respective bacterial isolate was calculated by the standard curve.

2.8 Measurement of siderophores production

All bacterial isolates were treated for identification of siderophores production by double-layered chrome azurol S agar (HIMEDIA) plate assay (Hu et al., 2011). The 100 mm diameter petri plate was used where 10 ml of CAS-blue agar was primarily added. Then, 6 ml of the appropriate growth medium have been poured on the solidified CAS-agar plate to identify the siderophores. The bacterial isolates were previously cultured on Burk's liquid medium at 32°C for 72 hrs. The liquid culture was added about 10-20 μl volume on the petri plate by using 5-6 mm autoclaved filter paper disk and the plates were incubated at 28°C for 4-7 days. The orange color around the colony was created by siderophores producing bacteria.

2.9 Detection of amylase enzyme production

Amylase, the hydrolytic enzyme breaks the carbohydrate into smaller units. To detect the production of amylase enzyme by the bacterial isolates, we prepared a starch agar medium (HIMEDIA) where 72 hrs liquid bacterial cultures had been introduced through the spot inoculation method. The bacterial cultures were incubated at 28°C for about 7 days and the growing colonies were flooded by using the iodine solution (HIMEDIA). After 5-10 mins, the iodine solution was discarded and observed the halo zone around the colonies in which the clear halo zone indicates the production of amylase enzyme.

2.10 *In vitro* antifungal and antibacterial assay

The best nitrogen fixing and phosphate solubilizing bacterial isolates were selected for *in vitro* antifungal test that had conducted through co-culture method by using *Aspergillus niger*. The fungus culture was established on the Potato Dextrose Agar (PDA) medium (HIMEDIA). The bacterial isolates were initially streaking on fresh PDA medium and then (1×1) cm^2 cubic fungus lumen were aseptically excised and inoculated on the center of the previously stricken bacterial culture plate. However, the co-culture petri plates were incubated at 32°C for about 5-7 days where the clear zone between bacterial isolates and fungi indicated the antagonistic activity of corresponding PGPB. Similarly, the antibacterial assay was done by adding the paper disk with growing liquid culture on the Nutrient Agar (NA) culture plate in which the bacterium *Agrobacterium fabrum* was used as the plant pathogen. However, the culture was incubated at 32°C for 48 hrs and observed the clear zone around the paper disk.

2.11 *In vitro* seed germination bioassay

The viable tomato seeds were collected from the hydroponic laboratory of the Institute of Technology Transfer and Innovation (ITTI), BCSIR. All these seeds' surfaces were sterilized by shocking on freshly prepared 0.1% Mercuric chloride ($HgCl_2$) for 1.5-2.0 mins and then rinsed the seeds with 70% ethanol only for 30 seconds. Before inoculation of best nitrogen fixing and phosphate solubilizing bacterial isolates, the seeds were rinsed five times with autoclaved deionized water and air dried for about 2-3 hrs on laminar air flow hood. On the contrary, the selected bacterial colony was inoculated on the NB medium and incubated at 30°C for 72 hrs by using the rotary shaker. Before inoculation of the PGPB, the NB cultures were centrifuged at 6000 rpm and washed the pellet two times with autoclaved deionized water in which the pellets were finally dissolved in 1 ml water. However, the seeds were introduced to concentrated bacterial isolates and kept it's for overnight to adhere the bacteria on the seed coat and finally dried the seeds for 2 hrs on the laminar hood. The *in vitro* seed germination was conducted by inoculation on sterilized filter paper through the 250 ml conical flask and maintained watering. Seed germination was completed within 4-7 days and compares to the control. The autoclaved distilled water is used as control in the lieu of bacterial isolate. Finally, the growing roots as well as shoots data were recorded and compare to the control. Moreover, the average seedling length of each plant can be determined by Vigor Index (VI) (Razmi et al., 2013).

2.12 Screening of *in vitro* Salt Tolerance

The selected PGPB isolates were screened for salt tolerance due to prove these as abiotic stress tolerant. However, the 72 hrs NB bacterial cultures were spot inoculated on the suitable semisolid bacterial culture where the different concentrations of sodium chloride (NaCl) including 2%, 4%, 6%, 8%, 10%, 12%, and 15% were used respectively. The culture plates were incubated at 32°C for 3-5 days and recorded the data.

2.13 Detection of antibiotic resistance

To ensure environmental safety, the best bacterial isolates for biofertilizer were tested for antibiotic resistance. The bacterial isolates were cultured on NA medium by streaking and immediately spot inoculated the antibiotic disk and observed after 48 hrs. The clear zone around the antibiotic disk suggests susceptibility for the corresponding bacteria and the bacterial growth close to the antibiotic disk indicates resistance to the corresponding bacteria.

2.14 Molecular characterization of the bacteria

The best bacterial isolates were processed for molecular identification by 16S rRNA (Ribosomal RNA) gene sequence analysis. To conduct this experiment bacterial genomic DNA was extracted (TIANGEN Bio tech) from the best biofertilizer bacterial isolates and quantifies the DNA concentration by UV-spectroscopy (EPPENDORF). The universal primer pair 27F (Forward Primer) and 1492R (Reverse Primer) (Macrogen) were used to amplify about 1500 bp of 16S rRNA (ribosomal RNA) gene in which the reaction volume was set at 25 μl for 32 cycles. However, the reaction's initial denaturation was conducted at 94°C for 2 mins and then the total 32 cycles were set as denaturation at 94°C for 1 min, primer annealing at 52°C for 1.5 mins, primer extension at 72°C for 2 mins and final extension at 72°C for 10 mins in a thermal cycler (Bio Red). The PCR products were separated by using 1% agarose gel in 0.5X Tris-EDTA buffer with 2 μl ethidium bromide (20 mg/ml) in which a ladder containing 1500 bp marker DNA was used (Takara) to compare the PCR products. However, the desired bands were cut aseptically from the gel after investigation through the gel documentation system (Cleaver). The gel purification kit (Favorgen) was used to purify the full-length amplified 16S rRNA gene and it was sent to sequencing company (Apical Scientific SDN. BH, Malaysia) to know the full-length sequence of the gene. After 1 month the sequencing result was received and visualized by FinchTV software. To identify the bacterial isolates through its 16S rRNA gene sequence data, the Nucleotide BLAST program (BLASTn) was used and the bacterial isolates were identified through the rRNA/ITS databases of NCBI (https://www.ncbi.nlm.nih.gov/).

2.15 Correlation between isolates and existing data

Finally, the sequences analysis output were cross-checked in combination with our experimental data and the recent

published manuscripts report of the distinct bacterial isolate.

2.16 Data analysis

Most of the experiments have been performed in a triplicate manner. Each assay or experiment was designed either considering positive or negative control or both. The data collection was conducted using the randomized design of petri plate and test tube experiment in which the data analysis had done by Microsoft Excel-2010.

3 Results

3.1 Sample and bacterial isolates description

The PGPB from rhizospheric soil of three different region in Bangladesh have been isolated to establish potential biofertilizer that ensures the sustainability of agriculture and environment. However, it is a tedious and sensitive task to find out the best desirable PGPB from millions of bacteria. We collected 17 soil samples (Table 1) of different crops field (Figure 2A) and preserved it's at 4°C temperature for isolating bacteria. Before isolation of desirable bacteria, the P^H of the soil had been measured in which the soil of Dhaka, Jashore, and Gazipur was revealed acidic, basic, and neutral P^H respectively. The nitrogen-fixing selective media primarily was used to isolate the bacteria.

Figure 2B and 2C in which, the best colonies were isolated from the soil of bean field and the CFU number was

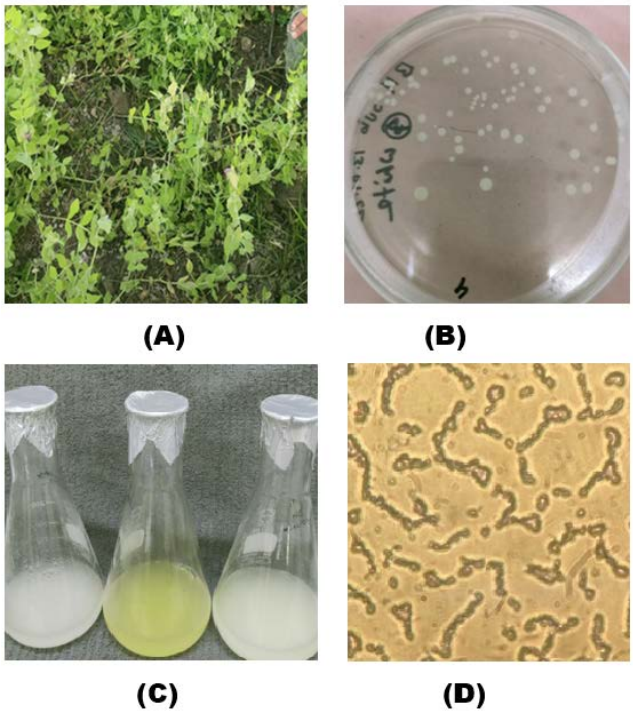

Figure 2. (A) Sample Collection Field; (B) Isolation of Bacteria by Serial Dilution; (C) Liquid Culture and (D) Microscopic Study.

Table 1. Basic Information about samples and bacterial isolates

No. of sample	Location of Sample	Crops Field	Soil P^H	Colony types	Colony shape	Bacterial Diversity	Gram Staining
1	Savar, Dhaka	Rice	4.73	Not Found	--	--	
2	Savar, Dhaka	Rice	4.84	WC, YC	R	2	(+), (-)
3	Savar, Dhaka	Bean	4.16	YC	DL	1	(-)
4	Savar, Dhaka	Bean	4.5	WC, YC	R, DL	2	(+), (-)
5	Savar, Dhaka	Bean	4.56	WC	R	1	(-)
6	Savar, Dhaka	Tomato	4.33	WC, PC, WatC	DL, R, R	3	(-)
7	Savar, Dhaka	Tomato	4.38	WC	R	1	(-)
8	Savar, Dhaka	Maize	4.11	WC	R	1	(+)
9	Savar, Dhaka	Maize	4.98	WC	O	1	(-)
10	Manirumpur, Jashore	Lentil	7.9	WC, YC	R	2	(-)
11	Manirumpur, Jashore	Lentil	7.56	WC	R	1	(-)
12	Manirumpur, Jashore	Pea	8.25	YC, MC	R	2	(-)
13	Manirumpur, Jashore	Pea	8.08	WC, MC	R	2	(+), (-)
14	Manirumpur, Jashore	Pea	8.12	WC, YC, WatC	R	3	(-)
15	Joydebpur, Gazipur	Wheat	6.24	WC, YC, MC	R, R, O	3	(+), (-)
16	Joydebpur, Gazipur	Wheat	6.31	WC, YC	R	2	(+)
17	Joydebpur, Gazipur	Oil seed	6.08	WC,YC	R	2	(+)

Note: Here, (WC = White Colony; YC= Yellow Colony; WatC= Watery Colony; PC= Pink Colony and MC= Milky Colony) (R= Round; DL= Dot Like; O= Oval) (+ = Gram Positive; - = Gram Negative).

2.6×10^6. The isolated PGPB were categorized according to their colony color, shape, size, and gram staining data. The isolated PGPB formed round, oval, and dot-like colony shapes and the colony's color varied from white, yellow, pink, watery, yellowish, and milky. Bean (Dhaka), Pea (Jashore), and Wheat (Gazipur) field rhizospheric soil showed the highest diversity of three bacteria according to the colony color and molecular data (Table 1). The total number of PGPB diversity was thirty (30) that were isolated through Burk's nitrogen-free medium from 17 different samples. However, the bacterial isolates were cultured three or more times on Burk's and NA media according to colony color where the pure culture of each isolate had been developed in the same way. The pure cultures were examined by microscope through gram staining (Figure 2D).

3.2 Plant growth promoting properties

Plant growth-promoting properties of PGPB are directly or indirectly induced the growth and development of plants. In this study, N-fixation, P-solubilization, Ammonia production, and IAA production have been denoted as growth-promoting properties (Table 2) of PGPB because these can directly induce plant growth and development.

Table 2. Plant growth promoting properties of bacterial isolates

Sample ID	N-Fixation	P-Solubalization (Mean±SE) cm	Ammonia Production	IAA Production (Mean±SE) µg/ml
2WC	---	2.24±0.05	+++	8.2±0.62
3YC	---	---	+++	29.5±0.87
4WC	+++	2.52±0.20	+++	4.63±0.46
5WC	++	2.22±0.06	+++	17.86±0.73
6PC	++	---	---	7.36±0.63
8WC	+++	3.62±0.13	++	7.93±0.81
10WC	++	---	++	11.5±0.29
10YC	---	2.32±0.05	---	15.43±0.81
13WC	++	---	+++	2.03±0.15
14YC	---	---	++	1.63±0.24
15WC	++	2.13±0.5	---	4.70±0.42
17WC	---	---	++	8.36±0.60
(-) Control	---	---	---	---

Note: +++ = Highly Positive; ++ = Moderate Positive; — = Negative; SE = Standard Error.

Nitrogen fixing is the most desirable criterion of biofertilizer because nitrogen is one of the most crucial molecules for plant. As the 30 isolates grown on nitrogen-free media, these had been further confirmed by the experiments on nitrogen-free mineral media (NFMM). After cross-checking all the 30 isolates, 7 of them were provided nitrogen fixation activity and the bacteria 4WC as well as 8WC were shown rapid and highest color changed from green to blue i.e. they represented as the best N-fixing bacteria among 30 isolates (Figure 3A). The rest of the 5 isolates were shown moderate N-fixation. However, the molecular data revealed that the 5WC and 8WC bacterial isolates are the same bacteria; *Paraburkholderia sacchari*. On the other hand, ammonia is another pivotal compound for plant that can also be produced by PGPB. In our study, most of the isolated bacteria produced ammonia (Table 2) in which 2WC, 3YC, 4WC, 5WC, and 13MC were the best producer of ammonia (Figure 3C).

Phosphate-solubilization is another key parameter for developing biofertilizer. Among the 30 isolates from three different regions in Bangladesh, only 6 bacteria have the ability to solubilize phosphate at a different level that is calculated by PSI. The 8WC bacterial isolates from the maize field have been shown the highest PSI score followed by the 4WC bacterial isolate from the bean field (Figure 3B). Besides these two bacteria, 2WC, 5WC, 10YC, and 15WC had also significant level of phosphate solubilization activity that would be potential phosphate solubilizing biofertilizer.

Several rhizospheric bacteria had the ability to the production of IAA (Figure 3D). Among the 30 isolates, IAA production ability was examined by biochemical test as well as spectroscopic analysis. Maximum bacterial isolates were synthesized IAA at the presence of 0.1% tryptophan but, we represented the significant IAA production data (Table 2) of biofertilizer potential bacteria. The data represented that 3YC had produced the highest amount of IAA and the 5WC, 10YC, 10WC, and 2YC synthesized higher amounts of IAA and very few bacteria had not produced IAA.

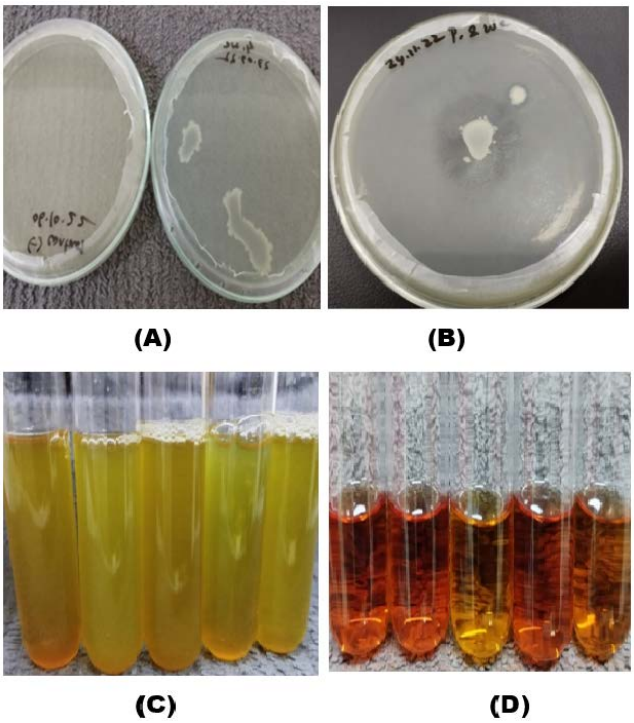

Figure 3. (A) Nitrogen Fixing Activity; (B) Phosphate Solubilizing Activity; (C) Ammonia Production Assay and (D) IAA Production Assay.

3.3 Biocontrol properties

Biofertilizer efficiency has been increased when it shows biocontrol properties. In our study, the siderophores production, enzyme (amylase) production, antifungal and antibacterial

assays have been considered as biocontrol properties of the biofertilizer potential bacteria.

Siderophores are iron (Fe^{3+}) binding important chelating agents that have potential to compete with plant pathogens i.e. inhibit the growth of plant pathogens. To find out the siderophores producing bacterial isolates, we used a double-layered chrome azurol S agar with suitable media plate assay that was observed after one week and recorded the data after 3 days as well as 5 days (Table 3). After 3 days of observation, we found only 6 bacterial isolates formed the orange color zone and after 5 days about 10 bacterial isolates formed the orange color zone (Figure 4A) i.e. indicating the production of siderophores. However, 2WC, 3YC, 7WC, 10WC, 12MC and 14YC were produced siderophores after 3 days while the sample 4WC, 6PC, 8WC and 17WC were synthesized siderophores after 5 days. The early color changes by bacterial isolates were produced higher siderophores compare to the late producing bacterial isolates.

Amylase has the potentiality to control some plant pathogens. The amylase production assay revealed (Table 3) that 12 bacterial isolates had generated the enzyme (Figure 4B). The data represented that 2YC, 5WC, 6PC, 8WC, 13MC, and 15WC were the best producer of amylase enzyme. On the other hand, *Aspergillus niger* was used for the anti-fungal test by co-culture method and observed after 5-7 days (Table 3). A significant correlation has been found that those bacterial isolates produced either siderophores or amylase or both had been shown the best anti-fungal properties (Table 3; Figure 4C). The result of the experiments demonstrated that 2WC, 10WC, 10YC, 12MC, 14YC, and 15WC showed the antifungal activities and other isolates had not revealed antifungal activities against the fungi. For the anti-bacterial

Table 3. Screening of biocontrol properties of bacterial isolates

Sample ID	Siderophores Production		Amylase Production	Anti-Fungal Assay Zone (Mean±SE) mm	Anti-bacterial Activity
	After 3 days	After 5 days			
2WC	+++	+++	++	1.83±0.90	+++
2YC	---	---	+++	---	+++
3YC	++	+++	++	---	---
4WC	---	++	++	---	+++
5WC	---	---	+++	---	---
6PC	---	++	+++	---	---
7WC	+++	+++	++	---	++
8WC	---	---	+++	---	+++
10WC	+++	+++	---	2.10±0.06	+++
10YC	---	---	++	2.40±0.12	++
12MC	+++	+++	---	1.72±0.06	---
13WC	---	---	+++	---	++
14YC	+++	+++	---	1.36±0.09	+++
15WC	---	---	+++	2.20±0.12	++
17WC	---	++	---	---	+++
(-) Control	---	---	---	---	+++

Note: Here, +++ = Highly Positive; ++ = Moderate Positive; — = Negative; SE = Standard Error.

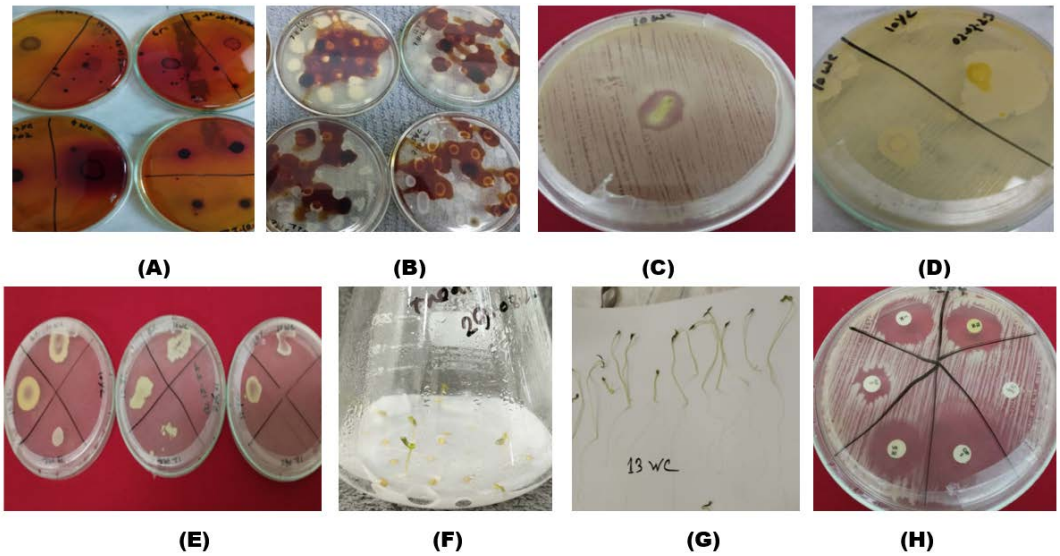

Figure 4. (A) Siderophores Production by Bacteria; (B) Amylase Production; (C) Antifungal Assay; (D) Antibacterial Assay; (E) Salt Tolerance Assay; (F) *In vitro* Seed Germination; (G) Plantlets after 21 Days and (H) Antibiotics Resistance Test.

assay, *Agrobacterium fabrum* was used as a lawn (Figure 4D). Among the 15 tested bacterial isolates, about 10 isolates revealed antibacterial activity but it's not significant while 3YC, 5WC, 6PC, and 12MC did not show antibacterial activity (Table 3).

3.4 Salt tolerance activity of bacterial isolates

Salinity is one of the major threats to agriculture in many countries in which Bangladesh faces high salinity in all the coastal districts. The salt-tolerant PGPB have resisted high salinity and induced plant growth. Among 30 bacterial isolates, 26 can survive more than 2% salt (NaCl) at *in vitro* condition (Table 4) and more than 50% have the ability to resist 8% NaCl (Figure 4E). On the other hand, 7 bacterial isolates had survived more than 10% salinity and only 3 isolates were grown moderately on 15% NaCl. Unfortunately in our study, the best biofertilizer potential bacteria (4WC and 8WC) were not grown at high salinity.

Table 4. Salt tolerance activity of best biofertilizer potential bacteria

Sample ID	Salt Concentration (% of NaCl)						
	2%	4%	6%	8%	10%	12%	15%
2WC	+++	+++	+++	+++	+++	++	---
2YC	+++	+++	+++	+++	---	---	---
7WC	+++	+++	+++	+++	---	---	---
9WC	+++	+++	+++	+++	++	++	---
10WC	+++	+++	+++	+++	+++	+++	++
12YC	+++	+++	+++	+++	+++	++	---
12MC	+++	+++	+++	++	---	---	---
13WC	+++	+++	++	++	---	---	---
14WC	+++	+++	+++	++	++	---	---
14YC	+++	+++	+++	+++	+++	+++	++
15WC	+++	+++	+++	+++	+++	+++	---
16WC	+++	+++	+++	+++	+++	+++	++
(-) Control	+++	+++	+++	+++	+++	+++	+++

Note: Here, +++ = Strong growth; ++ = Weak growth and — = No growth.

3.5 *In vitro* seed germination bioassay

Seed is the vital part of plants for reproduction but some plants' seeds may exist in the dormant stage even in a favorable environment. As a result, we were trying to find out the best PGPB among 30 bacterial isolates based on the seed germination percentage of tomato seed (BARRI Tomato-20) (Figure 4F) and biomass weight of 21 days aged plantlets (Table 5). The data revealed that the negative control inoculated seeds were germinated about 70% that's why; we selected the bacterial isolates those were germinated more than 70% (Table 5) treated seeds. The *in vitro* seed germination assay data revealed that 4WC, 10WC, 13WC, and 17WC can germinate more than 90% of tomato seeds whereas the 13WC bacterial isolates germinated 100% of seeds. The highest length of both shoots (5.20 $\pm$ 0.03 cm) and roots (9.44 $\pm$ 0.03 cm) of 21 days (Figure 4G) seedlings were showed in case of 6WC treated tomato seed.

On the other hand, the 13WC treated 21 days aged plantlets represented the highest weight of net biomass (225.53 $\pm$ 0.75 mg/10 plantlets) as well as dry biomass (24.00 $\pm$ 0.35 mg/10 plantlets) among all isolates of PGPB. However, the 10 isolated PGPB had revealed the highest percentage of seed germination and healthy plantlets in comparison to the control.

3.6 Antibiotic resistance test of biofertilizer potential bacteria

The application site of a biofertilizer is the agricultural land and to sustain the technology must check its environmental safety. Antibiotic resistance among bacteria is now a burning question all over the world. However, to evaluate the antibiotic resistance we applied six antibiotics at different concentrations and observed its result (Table 6) after 48 hrs of incubation at 32°C. In our study 3YC, 4WC, 8WC, and 15WC showed the highest antibiotic resistance (against 2 an-

Table 5. *In vitro* seed (BARI Tomato-20) germination bioassay and effect of PGPB on 21 days plantlets

Sample ID	% of Germination after 7 Days	After 21 Days of *In Vitro* Culture			
		Length of Shoot (Mean±S.E) cm	Length of Root (Mean±S.E) cm	Weight of Biomass/10 plantlets (Mean±S.E) mg	Dry Weight of Biomass/10 plantlets (Mean±S.E) mg
2WC	80%	4.33±0.12	7.54±0.02	87.98±0.90	12.3±0.40
3YC	80%	4.31±0.04	5.90±0.06	82.58±0.05	9.36±0.30
4WC	90%	3.18±0.04	5.24±0.03	68.23±0.13	14.70±0.62
5WC	80%	3.84±0.03	5.51±0.05	48.36±0.06	7.63±0.19
6WC	80%	5.20±0.03	9.44±0.03	127.58±0.04	17.73±0.18
6PC	80%	4.72±0.04	7.76±0.02	137.58±0.04	20.10±0.21
7WC	80%	4.31±0.04	6.82±0.04	106.65±0.08	15.53±0.22
8WC	80%	4.56±0.04	5.82±0.04	93.33±0.07	10.43±0.23
10WC	90%	4.77±0.01	7.65±0.06	136.83±0.72	16.57±0.23
10YC	80%	3.66±0.04	6.76±0.02	109.73±0.69	14.83±0.20
13WC	100%	4.86±0.05	7.69±0.10	225.53±0.75	24.00±0.35
14YC	80%	4.77±0.02	6.42±0.05	156.93±0.75	14.56±0.26
17WC	90%	4.60±0.02	7.35±0.09	123.56±0.34	13.20±0.15
(-) Control	70%	3.78±0.14	6.47±0.06	133.39±0.49	14.80±0.12

Note: Here, SE = Standard Error; (-) Control = Negative Control.

Table 6. Antibiotic resistance test of best biofertilizer bacterial isolates

Sample ID	Name of Antibiotics with Concentration					
	Ciprofloxacin (30 mg)	Ampicillin (25 mg)	Tetracycline (30 mg)	Chiplosporin (5 mg)	Rifampicin (5 mg)	Vancomycin (30 mg)
2WC	S	S	S	S	S	S
3YC	S	R	S	S	S	R
4WC	S	R	S	S	S	R
5WC	S	S	S	S	S	S
6PC	S	R	S	S	S	S
7WC	S	R	S	S	S	S
8WC	S	R	S	S	S	R
9WC	S	R	S	S	S	S
10WC	S	S	S	S	S	S
10YC	S	R	S	S	S	S
12MC	S	S	S	S	S	S
13WC	S	R	S	S	S	S
15WC	S	R	S	R	S	S
17WC	S	R	S	S	S	S
(-) Control	+++	+++	+++	+++	+++	+++

Note: Here, S = Susceptible; R =Resistance and +++ = Strong growth.

tibiotics). Among the 14 tested bacterial isolates, 10 bacteria resisted Ampicillin-25mg, 3 bacteria resisted Vancomycin-30mg and 1 bacteria resisted Chiplosporin-5mg. However, all the tested antibiotics (Figure 4H) were shown susceptibility against 2WC, 5WC, 10WC, and 12MC isolates.

3.7 Molecular identification of bacteria and potentiality as biofertilizer

For molecular identification of isolated bacteria 16S rRNA gene sequencing has been done and aligned these data through NCBI existing 16S rRNA gene sequence database (**January 15, 2023**). The direct sequencing of the 16S rRNA gene had been conducted after PCR by 27F and 1492R universal primer pair (Figure 5A), and selected the best sequence similarity (95-100%) after BLASTn analysis. In our study, the sequence data (Figure 5B) revealed that about 15 bacterial isolates have either biofertilizer/biopesticide/abiotic stress tolerant activity (Table 7).

But, only 8 bacterial isolates (*Priestia megaterium*; *Paraburkholderia sacchari*; *Rhizobium* dioscoreae; *Bacillus* tropicus; *Bacillus zanthoxyli*; *Arthrobacter globiformis*; *Bacillus wiedmannii*; and *Bacillus* paramycoides) have biofertilizer potentiality where some were established biofertilizer strain already in different country and some can be the candidate of potential biofertilizer. However, some of these PGPB have both potentialities as biofertilizers and biopesticides (*Paraburkholderia sacchari*; *Bacillus* tropicus; *Bacillus zanthoxyli*; *Arthrobacter globiformis*; *Bacillus wiedmannii*; and *Bacillus* paramycoides). Moreover, the molecular data revealed that the samples 2WC and 7WC; 4WC and 8WC; 12MC and 14WC are similar PGPB. The bacteria 2WC, 4WC, and 14WC were shown the highest similarity to *Priestia megaterium* (96.45%), *Paraburkholderia sacchari* (99.58%), and *Arthrobacter* nitrophenolicus (96.62%) respectively in comparison to the 7WC, 8WC, and 12MC. The molecular data, literature review, and our experimental data illustrate that the bacterial isolates 2WC, 4WC, 7WC, 8WC, 9WC,

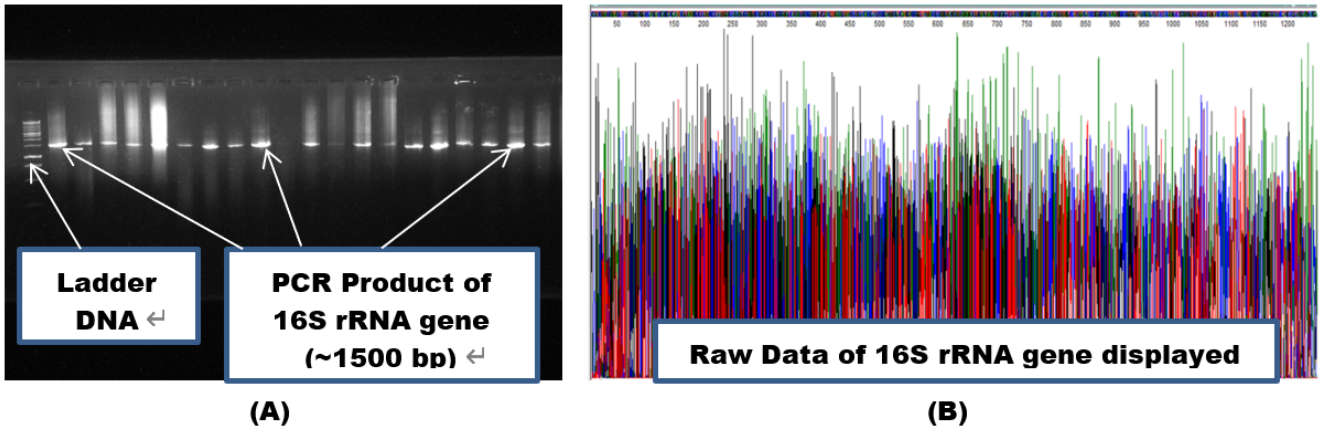

Figure 5. (A) Gel Electrophrosis of 16S rRNA Gene of Isolated Bacteria; (B) Raw sequencing data of 16S rRNA gene.

Table 7. Molecular identification data of isolates and its Potentiality as Biofertilizer

Sample ID	16srRNA gene length in bp (F/R)	Possible Genus/Species by BLAST Analysis	Similarity (%) NCBI database	Accession number	Potentiality as Biofertilizer with References
2WC	1221/1184	*Priestia megaterium*	98.53%	NR_116873.1	Biofertilizer (Santos et al., 2022) and act as endophytic PGPB (Rios-Ruiz et al., 2022)
7WC	949/1106	*Priestia megaterium*	96.45%	NR_116873.1	
3YC	1268/1227	*Rhodanobacter humi*	97.32%	NR_156929.1	Acid and alkali tolerant (Dahal and Kim, 2017)
4WC	1264/1259	*Paraburkholderia sacchari*	99.58%	NR_025097.1	PGPB (Vio et al., 2020; Velázquez et al., 2019; Kaur et al., 2021)
8WC	1254/1281	*Paraburkholderia sacchari*	98.91%	NR_025097.1	
5WC	1290/1163	*Bacillus salipaludis*	98.64%	NR_180481.1	Saline tolerant (Xue et al., 2021)
6WC	1218/1223	*R. endophyticum CCGE 2052*	97.56%	NR_116477.1	Phytate solubilization (López-López et al., 2010)
6WatC	754/962	*Rhizobium dioscoreae*	98.08%	NR_179313.1	Endophytic PGPB (Ouyabe et al., 2020)
9WC	1191/1187	*Bacillus tropicus*	99.20%	NR_157736.1	PGPB (Efe, 2020)
10WC	1228/1169	*Bacillus zanthoxyli*	98.94%	NR_164882.1	Biofertilizer & Biopesticides (Tan et al., 2022)
10YC	1249/1206	*Sphingomonas yabuuc*	99.31%	NR_028634.1	Dibenzofuran degredation (Wijayaratih et al., 2008)
13WC	480/898	*Arthrobacter globiformis*	98.21%	NR_026187.1	PGPB (Stassinosa et al., 2021)
12MC	567 (R)	*Arthrobacter nitrophenolicus*	94.75%	NR_181903.1	Herbicides Degradation (Mulla et al., 2019)
14WC	1204/1231		96.62%		
15WC	1251/1425	*Bacillus wiedmannii*	98.30%	NR_152692.1	Biofertilizer & Biopesticides (Saad et al., 2020)
17WC	1417/1382	*Bacillus paramycoides*	98.66%	NR_157734.1	Biofertilizer (Omeiri et al., 2022)

10WC, 13WC, 15WC, and 17WC had a great potentiality as biofertilizer where *Paraburkholderia sacchari* is the first report from Bangladesh as biofertilizer. The other bacterial isolates were to some extent biofertilizer potentiality but this is not the major function done by these bacteria. The 6PC sample was shown a potential role as biofertilizer in our experiment but no species had been found after BLASTn searching.

4 Discussion

In our study 30 bacteria were isolated from the rhizospheric soil of different crops land. Our aim was to find out multi-functional indigenous PGPB that can be potential biofertilizer and will ensure sustainable development in agriculture and environment by minimizing the use of chemical fertilizers and pesticides.

Nitrogen (N) is the most abundant molecule consumed by plants for its growth, development, and yield. Naturally, some nitrogen-fixing bacteria can fix atmospheric N but this is not enough for crops plant (Thilakarathna et al., 2016). The chemical fertilizers not only increase the cost of crop production but also alter the P^H of soil and induce phyto-pathogens (Halenárov et al., 2016; Ansari et al., 2019). For sustainable development in agriculture and the environment, nitrogen-fixing bacteria can be an alternative to chemically synthesized nitrogen fertilizer. Many researchers (Sagar et al., 2022; Aasfar et al., 2021; Meena et al., 2017) described that *Azospirillum*, *Azotobacter*, *Rhizobium*, *Azorhizobium*, *Sinorhizobium*, *Arthrobacter*, *Acetobacter*, *Clostridium*, *Bacillus*, *Enterobacter*, *Erwinia*, *Corynebacterium*, *Klebsiella*, *Mycobacterium*, *Pseudomonas*, *Xanthobacter* and *Mycobacterium* can fix nitrogen either by free-living or symbiotic condition. We found that *Paraburkholderia sacchari* was the best nitrogen fixer, and the *Bacillus* salipaludis, *Bacillus zanthoxyli*, *Arthrobacter globiformis*, and *Bacillus wiedmannii* represented as moderate nitrogen fixers. However, the molecular data of 16S rRNA sequencing data and literature proved these bacterial isolates can fix atmospheric nitrogen. On the other hand, some isolated bacteria had produced ammonia which will be also a great source of nitrogen for plants through the formation of ammonium (NH_4^+) (Lazarovits, 2001). In our study, *Paraburkholderia sacchari* and *Arthrobacter globiformis* produced the highest amount of ammonia and fix nitrogen.

Phosphorus is an indispensable macronutrient for plants but some soil may not be rich in soluble phosphate. Some PGPB can convert insoluble forms of phosphate to soluble forms that cause of increasing plant growth and development (Kalayu, 2019; Prabhu et al., 2019). Some bacterial strains

have been proved by researchers as phosphate solubilizers e.g. *Sinorhizobium meliloti*, *Bacillus flexus*; *Bacillus megaterium* (Ibal et al., 2019), *Rhizobium*, *Pseudomonas*, and *Enterobacterbacteria* (Anand et al., 2016). The bacterial isolates were shown significant phosphate solubilization activity in this research including *Priestia megaterium*, *Paraburkholderia sacchari*, *Bacillus salipaludis*, *Sphingomonas yabuuc*, and *Bacillus wiedmannii*. Among the 30 isolates, five were phosphate solubilizing PGPB where *Paraburkholderia sacchari* given the highest PSI in Pikovskaya's agar plate assay. However, phosphate solubilization by PGPB may be occurred by secretion of organic acid, mineral dissolving compounds, extracellular enzymes, and release of phosphate during substrate degradation (Choudhary et al., 2018).

IAA is one of the most common and native phytohormones for plants that are widely produced by rhizospheric soil bacteria. About 80% of rhizospheric soil bacteria can produce IAA (Leinhos and Vacek, 1994). However, (Ahmadloo et al., 2015; Joseph et al., 2007) reported the plant roots released sap which acts as a precursor of IAA, and the biosynthesis of IAA is mostly induced by the up taking of plants. In our study, many of the PGPB including *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pantoea*, and *Pseudomonas* have been reported for the production IAA. Among these, the bacteria *Rhodanobacter humi*, *Bacillus salipaludis*, and *Sphingomonas yabuuc* were the best producer of IAA when o.1% L-tryptophan was used as a precursor. However, the bacterial IAA together with plant IAA can regulate some development of plants (Pii et al., 2015) and even affect the synthesis of ACC deaminase as well as stress response (Di Benedetto et al., 2019).

Siderophores are iron-chelating secondary metabolites that can bind to the iron and transport it into the cell. Iron (Fe) is the widely present molecule in the soil (Andrews et al., 2003) where both Fe(II) and Fe(III) are found in the natural environment but Fe(III) is poorly bioavailable due to the low solubility at neutral and basic pH (Soares, 2022). The siderophores not only chelate the Fe(II) and Fe(III) but also form a stable complex with Cu(II) and Zn(II) (Hofmann et al., 2020). Many researchers (Kumar et al., 2017; Baars et al., 2018; Ferreira, 2019; Vindeirinho et al., 2021) reported that many species of *Rhizobium*, *Azotobacter*, *Pseudomonas*, *Escherichia*, and *Pantoea* are the biggest producer of a verity of siderophores. In our research, we found five bacteria produced siderophores after three days of culture including *Rhodanobacter humi*, *Priestia megaterium*, *Bacillus zanthoxyli*, *Arthrobacter nitrophenolicus*, and *Priestia flexa*. Moreover, after five days of culture, two more bacteria also produced siderophores *Paraburkholderia sacchari* and *Bacillus paramycoides* but, these productions of siderophores were lower in compare to first five bacteria.

Amylase enzyme plays a vital role in seed germination in which the bacterial amylase converts the seed starch into glucose and provides the energy for growing roots as well as shoots (Beck and Ziegler, 1989). In our study, almost 80% of bacterial isolates were able to produce amylase in which some producing moderate and some producing higher amounts of amylase. However, the antagonistic activities of our isolated bacteria have been conducted against the plant pathogens *Aspergillus niger* (Bansod and Rai, 2008) and *Agrobacterium fabrum* (Meyer et al., 2018). The *Sphingomonas yabuuc*, *Bacillus zanthoxyli*, and *Priestia megaterium* created the largest zone against the fungus *Aspergillus niger* that was supported by (Jha et al., 2019), the fungal pathogen *Aspergillus niger* were strongly protected by rhizospheric bacteria. On the other hand, the isolated bacteria *Priestia megaterium*, *Pseudarthrobacter defluvii*, *Bacillus tropicus*, *Bacillus zanthoxyli*, and *Priestia flexa* were shown antibacterial properties against the *Agrobacterium fabrum* at minimal level.

Salinity is now one of the major concerns in Bangladesh and all over the world (Dasgupta et al., 2015) because in Bangladesh about 30% of agricultural lands of the coastal region have been affected by high salinity. It causes ion toxicity, nutritional insufficiency, osmotic stress, and oxidative stress in plants which can be reduced the amount of water absorption by plants from the soil. However, some bacteria can resist high salt concentrations and evolve into distinctive properties to promote competition in the soil ecosystem, particularly in the rhizosphere. According to (Zhang et al., 2018) among 162 bacterial strains 67%, 28%, and 9% of bacteria were grown on 5%, 10%, and 15% NaCl respectively. On the other hand we found, among 30 isolates seven, eleven, and ten isolates could grow more than 12%, 6%, and 4% NaCl respectively at *in vitro* conditions. Moreover, two of the bacteria were sensitive to salinity but all the soil samples were collected from the non-salinity region of Bangladesh. The best salinity resistance bacteria were shown in our study *Bacillus zanthoxyli*, *Priestia flexa*, and *Bacillus paramycoides*.

The seed germination rate directly influences the production of crops because the seed is the most common reproductive part of most plants. Many researchers reported (Cendales et al., 2017; Galambos et al., 2020; Singh et al., 2018) that some of the bacteria of *Bacillus*, *Enterobacter*, *Pantoea*, and *Paraburkholderia* genus influenced the seed germination and plant development of tomato. However, the use of bacterial inoculum can improve seedling emergence, protect plants from some diseases, and help them grow faster and withstand environmental stress (Lugtenberg et al., 2002). The PGPB may influence seed germination and plantlets development by producing IAA, extracellular amylase, and siderophores, regulating ACC deaminase, or controlling the biotic and abiotic stress. In our study, the bacteria *Arthrobacter globiformis*, *Rhizobium endophyticum*, *Mucilaginibacter carri*, and *Bacillus zanthoxyli* were shown the best germination of tomato seed as well as the highest fresh and dry weight of germinated plantlets after 21 days. Moreover, the highest length of shoots and roots were found by the inoculum of *Rhizobium endophyticum*, and the best vigority was shown by

the *Arthrobacter globiformis*. We also found *Paraburkholderia* genus had a moderate vigority in case of tomato seed germination bioassay.

As the ultimate goal of the research is to screen best biofertilizer potential bacteria for agricultural land. To save the environment, the best potential bacteria must need to test antibiotic resistance because this is now the alarming issue globally. Many researchers reported about biofertilizer potential bacteria but they did not find out the bacterial resistance against antibiotics. In our research, some potential bacteria were shown one or two antibiotic resistance among the six testing antibiotics. Recent studies on the rhizospharic microbiome suggested that at early stage plants naturally choose antibiotic-resistant bacteria (Yu et al., 2023) while antibiotic resistance may also necessary to compete with the native rhizospheric microbiota.

Initially the 30 isolated bacteria were categorized based on the gram staining assay and finally the 16s rRNA gene sequence was used for molecular identification while some recent manuscripts supported the potentiality of our isolates in correlation with experimental data. The 16s rRNA gene is about 1550 bp long that contain both variable and conserved regions in which, the variable region analysis is the common tool of bacterial taxonomic study (Ibal et al., 2019). In our research both the forward and reverse primer synthesized gene sequences were used where most of the query sequence length was around 1000-1250 bp. After aligning by BLASTn, we considered the coverage similarity and E-value for selecting the bacteria. Many authors (Aloo et al., 2021; Rossmann et al., 2012) reported that some bacteria of *Pseudomonas*, *Bacillus*, *Enterobacteriaceae*, *Serratia*, *Pantoea*, and *Enterobacter* genus were found in the region of the rhizosphere and these are benefical to plants. However, the genus *Bacillus*, *Priestia*, *Paraburkholderia*, *Rhizobium*, *Pseudarthrobacter*, *Arthrobacter*, *Agrobacterium*, *Rhodanobacter*, and *Flavobacterium* were screened by our research. Among them, the experiment and molecular study revealed that the *Paraburkholderia sacchari* and *Arthrobacter globiformis* contain the most biofertilizer potentiality.

5 Conclusion

In summary, among the 30 isolated bacteria the experimental data suggest that 2WC, 4WC, 8WC, 10WC, 13WC, and 15WC (6 isolates) were represented the highest potentiality as biofertilizer. Based on the current molecular study of the 16s rRNA gene sequence, the potential bacterial isolates are *Paraburkholderia sacchari*, *Priestia megaterium*, *Bacillus zanthoxyli*, *Arthrobacter globiformis*, and *Bacillus wiedmannii* where the isolates 4WC and 8WC denoted the best potential biofertilizer and this is the *Paraburkholderia sacchari*. For this reason, *Paraburkholderia sacchari* may be used as microbial fertilizer. However further studies are needed for scale-up process and the field trials of the potential bacteria that will confirm as commercial biofertilizer for various crops.

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Author Contributions

Md. Saddam Hossain and Md. Rezaul Karim have contributed to the study conception and the study design was done by Md. Saddam Hossain. Material preparation, data collection and analysis were performed by Md. Saddam Hossain, Sharmin Akter, Md. Rakibul Hasan, Razia Sultana, Debabrata Karmakar and Nishat Tasnim. However, the molecular characterizations have been conducted only by Md. Saddam Hossain. The first draft of the manuscript was written by Md. Saddam Hossain and all authors commented on the initial draft of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declared that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

References

Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroua, Y., Kadmiri, I.M., 2021. Nitrogen fixing Azotobacter species as potential soil biological enhancers for crop nutrition and yield stability. Frontiers in Microbiology, 12: 628379. https://doi.org/10.3389/fmicb.2021.628379

Ahmadloo, F., Kochaksaraei, M.T., Azadi, P., Hamidi, A., Beiramizadeh, E., 2015. Effects of pectinase, BAP and dry storage on dormancy breaking and emergence rate of Crataegus pseudoheterophylla Pojark. New Forests, 46: 373-386. https://doi.org/10.1007/s11056-014-9466-0

Aloo, B.N., Mbega, E.R, Makumba, B.A., Hertel, R., Daniel, R., 2021. Molecular identification and *in vitro* plant growth- promoting activities of culturable Potato (*Solanum tuberosum L.*) rhizobacteria in Tanzania. Potato Research, 64: 67-95. https://doi.org/10.1007/s11540-020-09465-x

Anand, K.U., Kumari, B.A. and Mallick, M.A., 2016. Phosphate solubilizing microbes: an effective and alternative approach as biofertilizers. International Journal of Pharmacy and Pharmaceutical Sciences, 8(2): 37-40.

Ansari, R.A, Sumbul, A., Rizvi, R., Mahmood, I., 2019. Organic soil amendments: potential tool for soil and plant health management. Plant Health Under Biotic Stress: Organic Strategies, 1: 1-35. https://doi.org/10.1007/978-981-13-6043-5_1

Baars, O., Morel, F.M. and Zhang, X., 2018. The purple nonsulfur bacterium Rhodopseudomonas palustris produces novel petrobactinrelated siderophores under aerobic and anaerobic conditions. Environmental microbiology. 20(5): 1667-76. https://doi.org/10.1111/1462-2920.14078

Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D.L., 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Frontiers in plant science, 23(9): 1473.
https://doi.org/10.3389/fpls.2018.01473

Bansod, S. and Rai, M., 2008. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic Aspergillus fumigatus and A. niger. World Journal of Medical Sciences, 3(2): 81-8.

Beck, E. and Ziegler, P., 1989. Biosynthesis and degradation of starch in higher plants. Annual review of plant biology, 40(1): 95-117.
https://doi.org/10.1146/annurev.pp.40.060189.000523

Bisht, N. and Chauhan, P.S., 2020. Excessive and disproportionate use of chemicals cause soil contamination and nutritional stress. Soil Contamination-Threats and Sustainable Solutions, 16: 1.
http://dx.doi.org/10.5772/intechopen.87652

Cendales, T.C., Rodríguez González, C.A., Villota Cusquer, C.P., Tapasco Alzate, O.A., Rodrguez, A.H., 2017. *Bacillus* effect on the germination and growth of tomato seedlings (*Solanum lycopersicum L.*). Acta Biológica Colombiana, 22(1): 37-44.
http://dx.doi.org/10.15446/abc.v22n1.57375

Choudhary, M., Jat, H.S., Datta, A., Yadav, A.K., Sapkota, T.B., Mondal, S., Meena, R.P., Sharma, P.C., Jat, M.L., 2018. Sustainable intensification influences soil quality, biota, and productivity in cereal-based agroecosystems. Applied Soil Ecology, 126: 189-198.
https://doi.org/10.1016/j.apsoil.2018.02.027

Dahal, R.H., Chaudhary, D.K. and Kim, J., 2017. Acinetobacter halotolerans sp. nov., a novel halotolerant, alkalitolerant, and hydrocarbon degrading bacterium, isolated from soil. Archives of microbiology, 199: 701-710.
https://doi.org/10.1007/s00203-017-1349-2

Di Benedetto, N.A., Campaniello, D., Bevilacqua, A., Cataldi, M.P., Sinigaglia, M., Flagella, Z., Corbo, M.R., 2019. Isolation, screening, and characterization of plant-growth-promoting bacteria from durum wheat rhizosphere to improve N and P nutrient use efficiency. Microorganisms, 7(11): 541.
https://doi.org/10.3390/microorganisms7110541

Dasgupta, S., Hossain, M. M., Huq, M., Wheeler, D., 2015. Climate change and soil salinity: The case of coastal Bangladesh. Ambio, 44: 815-826.
https://doi.org/10.1007/s13280-015-0681-5

Efe, D., 2020. Potential plant growth-promoting bacteria with heavy metal resistance. Current Microbiology. 77(12): 3861-8.
https://doi.org/10.1007/s00284-020-02208-8

Fahad, S., Chavan, S.B., Chichaghare, A.R., Uthappa, A.R., Kumar, M., Kakade, V., Pradhan, A., Jinger, D., Rawale, G., Yadav, D.K., Kumar, V., Farooq, T.H., Ali, B., Sawant, A.V., Saud, S., Chen, S., Poczai, P., 2022. Agroforestry Systems for Soil Health Improvement and Maintenance. Sustainability, 14(22): 14877.
https://doi.org/10.3390/su142214877

Ferreira, C., 2019. Vox como representante de la derecha radical en España: un estudio sobre su ideologa. Revista Española de Ciencia Política, 51(51): 73-98.
https://doi.org/10.21308/recp.51.03

Freitas, A., Kaiser, S., Chandler, D.J., Hall, D.C., Kim, J.-W., Hammidi, T., 1997. Appearance Management as Border Construction: Least Favorite Clothing, Group Distancing, and Identity Not! Sociological inquiry, 67(3): 323-335.
https://doi.org/10.1111/j.1475-682X.1997.tb01099.x

Galambos, N., Compant, S., Moretto, M., Sicher, C., Puopolo, G., Wäckers, F., Sessitsch, A., Pertot, I., Perazzolli, M., 2020. Humic acid enhances the growth of tomato promoted by endophytic bacterial strains through the activation of hormone-, growth-, and transcription-related processes. Frontiers in Plant Science, 11: 582267.
https://doi.org/10.3389/fpls.2020.582267

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M., Toulmin, C., 2010. Food security: the challenge of feeding 9 billion people. Science, 327(5967): 812-818.
https://doi.org/10.1126/science.1185383

Halenárová, E., Medo, J., Kovácsová, S., Charousová, I., Maková, J., Elbl, J., Záhora, J., Javoreková, S., 2016. Effect of vermicompost on changes in the bacterial community in maize rhizosphere. Journal of Central European Agriculture, 17(4): 1033-1049.
http://dx.doi.org/10.5513/JCEA01/17.4.1808

Hirsch, P.R. and Mauchline, T.H. 2012. Who's who in the plant root microbiome? Nature biotechnology, 30(10): 961-962.
https://doi.org/10.1038/nbt.2387

Hofmann T., Lowry G.V., Ghoshal S., Tufenkji, N., Brambilla, D., Dutcher, J.R., Gilbertson, L.M., Giraldo, J.P., Kinsella, J.M., Landry, M.P., Lovell, W., Naccache, R., Paret, M., Pedersen, J.A., Unrine, J.M., White, J.C., Wilkinson, K.J., 2020. Technology readiness and overcoming barriers to sustainably implement nanotechnology-enabled plant agriculture. Nature Food, 1(7): 416-425.
https://doi.org/10.1038/s43016-020-0110-1

Hu, Q.-P., and Xu, J.-G., 2011. A simple double-layered chrome azurol S agar (SD-CASA) plate assay to optimize the production of siderophores by a potential biocontrol agent *Bacillus*. African Journal of Microbiology Research, 5(25): 4321-4327.
https://doi.org/10.5897/AJMR11.238

Ibal, J.C., Pham, H.Q., Park, C.E., Shin, J.-H., 2019. Information about variations in multiple copies of bacterial 16S rRNA genes may aid in species identification. PLoS One, 14(2): e0212090.
https://doi.org/10.1371/journal.pone.0212090

Ibarra-Galeana, J.A., Castro-Martnez, C., Fierro-Coronado, R.A., Armenta-Bojrquez, A.D., Maldonado-Mendoza, I.E., 2017. Characterization of phosphate-solubilizing bacteria exhibiting the potential for growth promotion and phosphorus nutrition improvement in maize (Zea mays L.) in calcareous soils of Sinaloa, Mexico. Annals of Microbiology, 67: 801-811.
https://doi.org/10.1007/s13213-017-1308-9

Jha, V., Kumari, N., Prasad, B., Ranjan, T., 2019. Ethanol production by *Aspergillus niger* US4MTCC9931 and Saccharomyces cerevisiae MTCC174 using different lignocellulosic biomass feed stocks. BioResources, 14(4): 8753-8764.

Joseph, B., Patra, R. and Lawrence, R., 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (Cicer arietinum L.). International Journal of Plant Production, 1(2): 141-152.

Kalayu, G., 2019. Phosphate solubilizing microorganisms: promising approach as biofertilizers. International Journal of Agronomy, 2019: 1-7.
https://doi.org/10.1155/2019/4917256

Kaur, C., Selvakumar, G. and Ganeshamurthy, A.N., 2017. Burkholderia to *Paraburkholderia*: the journey of a plant-beneficial-environmental bacterium. Recent advances in applied microbiology, 2017: 213-228.
https://doi.org/10.1007/978-981-10-5275-0_10

Kumar, S., Soukup, M. and Elbaum, R., 2017. Silicification in grasses: variation between different cell types. Frontiers in Plant Science, 8: 438.
https://doi.org/10.3389/fpls.2017.00438

Lazarovits, G., 2001. Management of soil-borne plant pathogens with organic soil amendments: a disease control strategy salvaged from the past. Canadian Journal of Plant Patholog, 23(1): 1-7.
https://doi.org/10.1080/07060660109506901

Leinhos, V. and Vacek, O., 1994. Biosynthesis of auxins by phosphate-solubilizing rhizobacteria from wheat (Triticum aestivum and rye (Secale cereale). Microbiological Research, 149(1): 31-35.
https://doi.org/10.1016/S0944-5013(11)80132-1

Lin, M., Fiore, A.M., Cooper, O.R., Horowitz, L.W., Langford, A.O., Levy, H., Johnson, B.J., Naik, V., Oltmans, S.J., Senff, C.J., 2012. Springtime high surface ozone events over the western United States: Quantifying the role of stratospheric intrusions. Journal of Geophysical Research: Atmospheres, 117(D21).
https://doi.org/10.1029/2012JD018151

Lin, W., Lin, M., Zhou, H., Wu, H., Li, Z., Lin, W., 2019. The effects of chemical and organic fertilizer usage on rhizosphere soil in tea orchards. PloS one, 14(5): e0217018.
https://doi.org/10.1371/journal.pone.0217018

López-López, A., Rogel, M.A., Ormeno-Orrillo, E., Martínez-Romero, J., Martnez-Romero, E., 2010. Phaseolus vulgari seed-borne endophytic community with novel bacterial species such as *Rhizobium* endophyticum sp. Systematic and Applied Microbiology, 33(6): 322-327.
https://doi.org/10.1016/j.syapm.2010.07.005

Lucy, M., Reed, E. and Glick, B.R., 2004. Applications of free living plant growth-promoting rhizobacteria. Antonie van leeuwenhoek, 86: 1-25. https://doi.org/10.1023/B:ANTO.0000024903.10757.6e

Lugtenberg, B.J., Chin-A-Woeng, T.F. and Bloemberg, G.V., 2002. Microbe-plant interactions: principles and mechanisms. Antonie Van Leeuwenhoek, 81: 373-383. https://doi.org/10.1023/A:1020596903142

Meena, S.K., Rakshit, A., Singh, H.B., Meena, V.S., 2017. Effect of nitrogen levels and seed bio-priming on root infection, growth and yield attributes of wheat in varied soil type. Biocatalysis and Agricultural Biotechnology, 12: 172-178. https://doi.org/10.1016/j.bcab.2017.10.006

Mehta, P.K. and Monteiro, P.J., 2014. Concrete: microstructure, properties, and materials. McGraw-Hill Education

Meyer, T., Renoud, S., Vigouroux, A., Miomandre, A., Gaillard, V., Kerzaon, I., Prigent-Combaret, C., Comte, G., Moréra, S., Vial, L., Lavire, C., 2018. Regulation of hydroxycinnamic acid degradation drives Agrobacterium fabru lifestyles. Molecular Plant-Microbe Interactions, 31(8): 814-822. https://doi.org/10.1094/MPMI-10-17-0236-R

Mulla, S.I., Bharagava, R.N., Belhaj, D., Saratale, G.D., Bagewadi, Z.K., Saxena, G., Kumar, A., Mohan, H., Yu, C.-P., Ninnekar, H.Z., 2019. An overview of nitro group-containing compounds and herbicides degradation in microorganisms. Microbial Metabolism of Xenobiotic Compounds, 319-35. https://doi.org/10.1007/978-981-13-7462-3_16

Omeiri, M., Khnayzer, R., Yusef, H., Tokajian, S., Salloum, T., Mokh, S., 2022. *Bacillus* spp. isolated from soil in Lebanon can simultaneously degrade methomyl in contaminated soils and enhance plant growth. Biocatalysis and Agricultural Biotechnology, 39: 102280. https://doi.org/10.1016/j.bcab.2022.102280

Oo, K.T., Win, T.T., Khai, A.A., Fu, P., 2020. Isolation, screening and molecular characterization of multifunctional plant growth promoting rhizobacteria for a sustainable agriculture. American Journal of Plant Sciences, 11(6): 773-792. https://doi.org/10.4236/ajps.2020.116055

Ouyabe, M., Irie, K., Tanaka, N., Kikuno, H., Pachakki, B., Shiwachi, H., 2020. Response of upland rice (Oryza sativa L.) inoculated with non-native plant growth-promoting bacteria. Agronomy, 10(6): 903. https://doi.org/10.3390/agronomy10060903

Pieterse, C.M., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M., Bakker, P.A.H.M., 2014. Induced systemic resistance by beneficial microbes. Annual review of phytopathology, 52: 347-375. https://doi.org/10.1146/annurev-phyto-082712-102340

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., Crecchio, C., 2015. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biology and fertility of soils, 51: 403-415. https://doi.org/10.1007/s00374-015-0996-1

Prabhu, N., Borkar, S. and Garg, S., 2019. Phosphate solubilization by microorganisms: overview, mechanisms, applications and advances. Advances in biological science research, 161-176. https://doi.org/10.1016/B978-0-12-817497-5.00011-2

Razmi, Z., Hamidi, R. and Pirasteh-Anosheh, H., 2013. Seed germination and seedling growth of three sorghum (Sorghum bicolor L.) genotypes as affected by low temperatures. International Journal of Farming and Allied Sciences, 20: 851-856.

Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Functional Plant Biolog, 28(9): 897-906. https://doi.org/10.1071/PP01093

Rios-Ruiz, W.F., Tuanama-Reátegui, C., Huamán-Córdova, G., Valdez-Nuñez, R.A., 2023. Co-Inoculation of Endophytes *Bacillus* siamensis TUR07-02b and *Priestia megaterium* SMBH14-02 Promotes Growth in Rice with Low Doses of Nitrogen Fertilizer. Plants, 12(3): 524. https://doi.org/10.3390/plants12030524

Rodrigues-dos Santos, A.S., Rebelo-Romão I., Zhang H., Vílchez, J.I., 2022. Discerning Transcriptomic and Biochemical Responses of Arabidopsis thaliana Treated with the Biofertilizer Strain *Priestia megaterium* YC4-R4: Boosting Plant Central and Secondary Metabolism. Plants, 11(22): 3039. https://doi.org/10.3390/plants11223039

Rossmann, B., Mller, H., Smalla, K., Mpiira, S., Tumuhairwe, J.B., Staver, C., Berg, G., 2012. Banana-associated microbial communities in Uganda are highly diverse but dominated by Enterobacteriaceae. Applied and environmental microbiology, 78(14): 4933-4941. https://doi.org/10.1128/AEM.00772-12

Saad, M.M., Kandil, M. and Mohammed, Y.M., 2020. Isolation and identification of plant growth-promoting bacteria highly effective in suppressing root rot in fava beans. Current Microbiology, 77: 2155-2165. https://doi.org/10.1007/s00284-020-02015-1

Sagar, A., Yadav, S.S., Sayyed, R.Z., Sharma, S., Ramteke, P.W., 2022. *Bacillus* subtilis: a multifarious plant growth promoter, biocontrol agent, and bioalleviator of abiotic stress. Bacilli in Agrobiotechnology: Plant Stress Tolerance, Bioremediation, and Bioprospecting, 561-580. https://doi.org/10.1007/978-3-030-85465-2_24

Singh, V.K., Singh, A.K., Singh, P.P., Kumar, A., 2018. Interaction of plant growth promoting bacteria with tomato under abiotic stress: a review. Agriculture, Ecosystems & Environment, 267: 129-140. https://doi.org/10.1016/j.agee.2018.08.020

Soares, E.V., 2022. Perspective on the biotechnological production of bacterial siderophores and their use. Applied Microbiology and Biotechnology, 106(11): 3985-4004. https://doi.org/10.1007/s00253-022-11995-y

Soumare, A., Diedhiou, A.G., Thuita, M., Hafidi, M., Ouhdouch, Y., Gopalakrishnan, S., Kouisni, L., 2020. Exploiting biological nitrogen fixation: a route towards a sustainable agriculture. Plants, 9(8): 1011. https://doi.org/10.3390/plants9081011

Stassinos, P.M., Rossi, M., Borromeo, I., Capo, C., Beninati, S., Forni, C., 2022. Amelioration of salt stress tolerance in rapeseed (Brassica napus) cultivars by seed inoculation with *Arthrobacter globiformis*. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 156(2): 370-383. https://doi.org/10.1080/11263504.2020.1857872

Tan, Y., Du, C., Xu, L., Yue, C., Liu, X., Fan, H., 2022. Endophytic bacteria from diseased plant leaves as potential biocontrol agents of cucumber Fusarium wilt. Research Square, 23 November 2022, Preprint (Version 1). https://doi.org/10.21203/rs.3.rs-2290076/v1

Thilakarathna, M.S., McElroy, M.S., Chapagain, T., Papadopoulos, Y.A., Raizada, M.N., 2016. Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. Agronomy for Sustainable Development, 36: 1-6. https://doi.org/10.1007/s13593-016-0396-4

Tsavkelova, E.A., Cherdyntseva, T.A., Botina, S.G., 2007. Bacteria associated with orchid roots and microbial production of auxin. Microbiological research, 162(1): 69-76.

Velázquez, E., Carro, L., Flores-Flix, J.D., Menndez, E., Peix, A., 2019. Bacteria-inducing legume nodules involved in the improvement of plant growth, health and nutrition. Microbiome in Plant Health and Disease: Challenges and Opportunities, 79-104. https://doi.org/10.1007/978-981-13-8495-0_4

Vindeirinho, J.M., Soares, H.M. and Soares, E.V., 2021. Modulation of siderophore production by Pseudomonas fluorescens through the manipulation of the culture medium composition. Applied Biochemistry and Biotechnology, 193: 607-618. https://doi.org/10.1007/s12010-020-03349-z

Vio, S.A., Garca, S.S., Casajus, V., Arango, J.S., Galar, M.L., Bernabeu, P.R., Luna, M.F., 2020. *Paraburkholderia*. Beneficial Microbes in Agro-Ecology, 2022: 271-311. https://doi.org/10.1016/B978-0-12-823414-3.00015-0

Wijayaratih, Y., Radjagukguk, B., Martani, E., Prijambodo, I.D., 2008. Karakteristik Konsorsium Bakteri Perombak Dibenzofuran Dari Sedimen Mangrove (Dibenzofuran-degrading Bacterial Consortium Characteristics From Mangrove Sediments). Jurnal Manusia dan Lingkungan, 15(2): 59-69.

Xu, Y., Zheng, C., Liang, L., Yi, Z., Xue, S., 2021. Quantitative assessment of the potential for soil improvement by planting Miscanthus on saline-alkaline soil and the underlying microbial mechanism. GCB Bioenergy, 13(7): 1191-1205. https://doi.org/10.1111/gcbb.12845

Yu, Y., Zhang, Q., Zhang, Z., Zhou, S., Jin, M., Zhu, D., Yang, X., Qian, H., Lu, T., 2023. Plants select antibiotic resistome in rhizosphere in early stage. Science of the Total Environment, 858: 159847. https://doi.org/10.1016/j.scitotenv.2022.159847

Zahid, M., Abbasi, M.K., Hameed, S., Rahim, N., 2015. Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (Zea mays L.). Frontiers in microbiology, 6: 207. https://doi.org/10.3389/fmicb.2015.00207

Zhang, S., Fan, C., Wang, Y., Xia, Y., Xiao, W., Cui, X., 2018. Salt-tolerant and plant-growth-promoting bacteria isolated from high-yield paddy soil. Canadian journal of microbiology, 64(12): 968-978. https://doi.org/10.1139/cjm-2017-0571