Bangladesh J Microbiol, Volume 40, Number 2, December 2023, pp 66-74

Original Article

https://doi.org/10.3329/bjm.v40i2.73794

Diversity of Plant Growth Promoters (PGP) Isolated from Agricultural Fields of Bangladesh

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With an emphasis on leguminous plants like *Glycine max* (soybean), *Clitoria ternatea*, and non-leguminous plants like *Phaseolus lunatus*, this study investigates the diversity of Plant Growth-Promoting Rhizobacteria (PGPR) isolated from the root nodules and rhizospheres of agricultural soil. Seventeen of the 42 isolates were chosen for further analysis. Isolates were screened through phenotypic characterization, presumptive identification, and evaluation of their plant growth-promoting (PGP) qualities. Amplification of the nitrogen-fixing *nif*H gene, the nodulation-inducing *nod*C gene, and the 16S rRNA were among the molecular investigations carried out. Three examined PGP features were present in 35% of the isolates, according to the results. From pH levels of 4.5 to 9, temperatures of 37ºC to 40ºC and salt concentrations of 1% or less, the isolates showed versatility in a variety of environmental circumstances. Resistant to high temperatures (40°C), slow-growing rhizobia (22%) were shown to be sensitive to high pH (9). Tilt and Tafgor pesticides had a more severe influence on PGP traits and development than Shadhin G and Semcup. The design and efficacy of biofertilizers, agro-economic progress, and sustainable agriculture are among the topics on which this qualitative study sheds important light.

Keywords: PGPR, leguminous plants, nitrogen-fixation

Introduction

Bangladesh is an agricultural nation, with most of its population employed in some capacity. However, in recent times, factors such as population growth, rising food prices, overuse of agricultural land, improper disposal of industrial waste close to farms, changing climate patterns, and the use of various chemicals (pesticides and fertilizers) have reduced the amount of fertile land[1]. To counteract this, numerous studies have been conducted to develop a biofertilizer that would be easily accessible to farmers, affordable, simple to use, and capable of enhancing soil quality and stimulating plant growth without endangering the environment[2-3]. Symbiotic bacteria such as *Rhizobium, Bradyrhizobium* present in root nodule and some free-living bacteria have some innovative properties and thus studied widely all around the world along with in Bangladesh. *Rhizobium* or *Bradyrhizobium* producing root nodules in legumes are very efficient nitrogen fixers and contribute about 500 kg N/ha/year. A recent study included large-scale production (25 m tons /year) of *Rhizobium* or *Bradyrhizobium* inoculants has been successful at BANGLADESH INSTITUTE OF NUCLEAR AGRICULTURE, Mymemsingh. About 1.5 to 2.0 kg inoculant/ha used with seeds may result increased production of pulses by 20 to 40% (National Encyclopedia of Bangladesh).

The group of bacteria known as Plant Growth Promoting Rhizobacteria (PGPR) have garnered interest because they have certain strong plant growth-promoting properties. Three main mechanisms allow PGPR to improve the nutrient status of host plants: (1) biological nitrogen fixation; (2) increasing nutrient availability in the rhizosphere; and (3) phytohormone production, which increases the amount of root surface area available for nutrient absorption. The benefits of microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), emphasize the need for increased study and use of these organisms in modern agriculture for sustainability. Through nitrogen fixation, phosphate solubilization, siderophore formation, and phytohormone production, these PGPRs contribute to soil nutrient enrichment, which in turn supports plant development [3-5]. These bacteria can survive abiotic difficulties such as heavy metal and pesticide contamination, as well as changes in salinity, pH and temperature[4,5]. Finding such resilient PGP bacteria is expected to enhance plant growth and yield even in the face of various stresses. As members of the families *Rhizobiaceae, Phyllobacteraceae,* and *Bradyrhizobiaceae*, the rhizobacteria that can act as PGPR for a long-term sustainable agricultural system were the focus of the present research[4,5]. *Bradyrhizobium*, a slow-growing bacterium that can tolerate high temperatures, salt concentrations, and several pesticides at different concentrations, may be present in *Glycine max* root nodules[6]. Although it is known that *Clitoria ternatea* harbors rhizobia that can withstand high pH levels, fix significant amounts of nitrogen, and develop swiftly[7]. In agriculture, the connection between rhizobia and legumes is particularly important. Pesticides are frequently used in agricultural production nowadays to protect crop productivity and quality. On the other hand, the soil microbiota and leguminous rhizobia may suffer adverse impacts due to the presence of these chemicals. Herbicides can affect nodules by slowing down the rate of nitrogen fixation after nodulation[8]. Considering all these the aim of the present study

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was to isolate bacterial species from agricultural fields and pinpoint PGPs' effectiveness under stressed circumstances. Furthermore, the research would help to identify a decision regarding the study for further examination after appropriate identification with an aim for sustainable agriculture.

Methodology

Sample collection and processing

Legume and non-legume plants were collected from garden soil of different area of Dhaka. *Phaseolus lunatus* and *Phaseolus valguris* samples were collected from Savar. *Oxalis* was collected from Botanical Garden, Dhaka, and *Mimosa pudica* was collected from Shatchori National Park, Sylhet. Besides these, – *Glycine max* (Soybean) from Hatia and *Clitoria ternatea* (Asian pigeonwings) were collected from National Botanical Garden, Dhaka.

Usually, light brown or pinkish nodules were considered fresh and healthy. It indicates an active nitrogen fixation between legume plants and nitrogen-fixing bacteria.

Rhizobia with the ability to nodulate leguminous spp. were recovered from root nodules. Sample processing was done by surface sterilizing of root nodules as reported by Somasegaran *et al*. (1994)[9]. Under tap water, nodules were completely cleaned of debris and surface microorganisms. Intact nodules were immersed in 95% ethanol for five minutes and were then placed in a 3% H_2O_2 solution for two to three minutes, washed off by rinsing them five to six times with distilled water. On a sterile petri plate with a drop of sterile saline, sterilized nodules were crushed with a glass rod.

Isolation using selective media

Extracts from crushed nodules were streaked on yeast extract-mannitol agar (YMA-g/liter: 0,5 yeast extract, 5 manitol, 0.5 K_2HPO_4, 0.2 MgSO4·7 H2O, 0.1 NaCl, pH 7) plates and incubated at 28–30°C for 24-36 hours[5,10-11]. For pure culture, well-isolated colonies were streaked onto fresh YMA plates.

Morphological Observation

Colony morphology was observed on Yeast Mannitol Agar (YMA) plates after incubation at 28ºC for 24-72 hours. The morphological characteristics (colony color, shape, margin, elevation, texture, opacity etc.) on YMA plate were recorded.

Culture and Metabolic Characteristics

Presumptive Tests

To ascertain the purity of the *Rhizobium*, presumptive tests growth on the Congo Red YMA Plate (CRYMA) with 0.025g YMA + dye was utilized. *Rhizobium* grows slowly and appears as white colonies whereas pollutants grow quickly. BTB was added to 1L of YMA medium (0.5% in ethanol). Based on a blue/yellow color shift over 2–10 days of incubation, *Rhizobium* isolates were categorized into fast/slow groups[11].

Starch hydrolysis

For the amylase test, starch agar was employed; organisms were streaked on plates and cultured for 48 hours at 30°C. Iodine-stained clear tissue surrounding the growth line signifies successful starch digestion, while blue, purple, or black coloration indicates failure.

Biochemical tests

For presumptive identification, different biochemical tests were performed. MR-VP, MIU, oxidase, catalase, nitrate, KIA, citrate utilization, indole test, and starch hydrolysis were done for all the suspected *Rhizobium* samples[11-12].

Test for PGPP (Plant Growth Promoting Properties)

Phosphate solubilization

Placing organisms on *Pikovskaya's* agar and incubating them for 48 hours at 30°C[10,13-14]. phosphate digestion was confirmed by a clear zone surrounding the growth line; failure was shown by a blue, purple, or black zone.

Indole acetic acid production

Tryptic soy agar with inoculum was incubated at 30°C to observe *isolates* that promotes plant growth. Salkowski reagent was used to measure IAA generation following incubation[10,15].

Study of Various Stress Conditions

Isolates were grown on YMA media at varying pH (3.5, 4.5, 7.0, 9.0, 11.0),salt concentrations (0.05%, 0.1%, 1%, 3%, and 5%), and growth was observed after 24 hours of incubation at 30°C [16]. Growth was also observed at different temperatures (4°, 20°, 28°, 37°, 40°, 50°) C on YMA media.

Growth of isolates in presence of different pesticides and recheck of their PGP properties

To observe the pesticidal effects on bacterial development, various pesticides namely Shadhin G, Tilt, Toughgor, and Semcap (commercially known) were utilized. Growth was observed after 24 hours of incubation with the isolates. The freshly made agar plates were individually reviewed with increasing pesticide concentrations (0.025%, 0.05%, 0.075%, 0.1%, and 0.2%). Plates were then incubated for 7 days at 30° C, and the outcome was observed. Phosphate solubilization, IAA production tests were performed again by taking bacterial colony form pesticide containing plates.

Antibiogram

Sensitivity of the isolates were checked for 10 different antibiotics including Trimethoprim (25 µg), Nitrofurantoin (300 µg), Metronidazole (50 µg), Meropenem (10 µg), Rifampin (5 µg), Ceftazidime (30 µg), Azithromycin (15 µg), Novobiocin (30 µg), Imipenem (10 µg), Chloramphenicol (30 µg) by disc diffusion method more commonly known as "The Kirby Bauer method"[17].

DNA extraction and PCR amplification

DNA extraction was done precisely by conventional boiling method of DNA extraction[18]. At OD 260 nm, the Colibari Micro volume Spectrometer was used to measure DNA concentrations. The TE buffer served as the blank. The purity was determined by the absorbance ratio between 260 and 280 nanometers. A ratio of 1.8 typically denotes pure DNA[18].

A set of primers were designed for amplification of approximately 781bp region of *nif*H gene mentioned in the following Table-1[19-20]. Each PCR tube where amplification reactions were performed have a final volume of 14.5 ìl by mixing 2.5 ìl of extracted template DNA solution with 5.25ìl PCR grade water, 0.25 ìl of each primer (30 pmole ìl "1) and 6.25 ìl of 2X master mix (Thermo Scientific™ DreamTaq Green PCR Master Mix (2X), USA).

Table 1: *List of primers used for nif H and nod C gene*

Gene	Direction	Primer sequence	Amplicon Size	Reference
nif H	Forward	CGTTTTACGGCAAG GGCGGTATCGGCA	781bp	19
	Reverse	TCCTCCAGCTCCTCCA TGGTGATCGG		
nod C	Forward	GCCATAGTGGCAACC GTCGT	500bp	20
	Reverse	CTCGCCGCTGCAAGT		

The Veriti™ 96-well thermal cycler (Thermo Fisher Scientific, USA) was used for the PCR which was carried out in a program as follows: initial denaturation at 95°C for 5 min, 35 cycles of 1 min at 94°C, 45s at 68°C, 1 min at 72 °C, followed by a final extension set at 72°C[19]. Gel electrophoresis of the resulting PCR amplicons was carried on 1.5% agarose gels in 1X TE Buffer and Gel was stained with Ethidium Bromide before visualization under UltraViolet gel documentation system (Vilber Lourmat, France).

Similarly using another set of primers mentioned in Table-1 was used to amplify 500 bp region of *nodC* gene where the PCR condition was: initial denaturation at 95°C for 5 min, 35 cycles of 1 min at 94°C, 45s at 56°C, 1 min at 72 °C, followed by a final extension set at 72°C [20].

Results

Presumptive identification test for fast grower and slow grower

The isolates' colonies displayed the same morphology on the YMA medium: circular, convex, smooth, translucent, creamy to transparent texture (Table 2). The isolates were classified as fast acid producers because they changed the bromothymol blue indicator from deep green to yellow in the YMA-BTB. The isolates were identified as Gram-negative bacilli that were unable to absorb congo red present on YMA medium. 90% of the studied isolates had a mucoid texture, indicating the production of exopolysaccharides (Table 2). All the isolates were gram negative except OP2 (*Clitoria ternatea)*, B3 (*Phaseolus lunatus)*, MP1 (*Mimosa pudica)*, MP2 (*Mimosa pudica)*.

Table 2: *Colony Morphology of isolates on YMA plate and Identification of slow grower on CRYMA plate and BTB agar plate*

Sample	Colony characteristics on YMA Plate (After 24 hours)	CRYMA (colony appearance)	BTB (media color) Yellowish colony,
SB1	Medium, gummy, translucent, yellowish color, round colony	Light pinkish colony	media color-change to yellow
SB2	Medium, gummy, yellowish, translucent colony, with polysaccharide	Light pinkish colony	Yellowish colony, media remain unchanged
SB3	Small, gummy, white, translucent colony, with polysaccharide	White colony	Yellowish colony, media - unchanged
SB4	Small, gummy, white, translucent colony, with polysaccharide	White colony	Yellowish colony, media remain unchanged
SB5	Small, translucent white colony	translucent white colony	Pinpoint, translucent colony, media changed to blue
SB6	Small, translucent white colony	translucent white colony	Pinpoint, translucent colony, media changed to blue
OP1	Medium, gummy, white, translucent colony, with polysaccharide	White, small colony	White colony, media color– changed to yellow
OP2	Medium, round, opaque, yellowish colony	Pink colony	Yellowish colony, media color-change to yellow
OA1	Pinpoint, Round, Convex, Irregular Yellowish	White colony	Yellowish colony, media - unchanged
OA2	Super pinpoint, Round, Convex, Irregular, Off white	White colony	White colony, media color– changed to yellow
OA3	Pinpoint, Round, Convex, Regular, Yellowish	pinkish colony	Yellowish colony, media remain unchanged
OA4	Pinpoint, Round, Convex, Regular, white	pinkish colony	Yellowish colony, media remain unchanged
B1	Small, gummy, white, translucent colony, with polysaccharide	White colony	White colony, media color– changed to yellow
B2	Small, gummy, white, translucent colony, with polysaccharide	White colony	White colony, media color– changed to yellow
B3	Small, Round, Umbonate, Entire, White	Pink colony	Yellowish colony, media color-change to yellow
MP1	Pinpoint, Round, Convex, Irregular, Off white	Pink colony	Yellowish colony, media color remains unchanged
MP2	Pinpoint, Round, Flat, Wavy, Off white	Pink colony	Yellowish colony, media color remains unchanged

Table 3: *Collected plants along with their chosen isolates*

Root nodule collected from plants	Chosen isolates annotation
1. *Glycine max*	SB1, SB2, SB3, SB4, SB5, SB6
2. *Clitoria ternatea*	OP1, OP2
3. *Oxalis* spp.	OA1, OA2, OA3, OA4
4. *Phaseolus lunatus*	B1, B2, B3
5. *Mimosa pudica*	MP1, MP2

Among the 17 isolates including 2 slow growers were analyzed thoroughly (Table 2). Morphological and microscopic analysis were performed. About 76% of isolates were gram-negative rod-shaped bacteria. Biochemically they have shown huge diversity. On a CRYMA plate and BTM plates, 53% of the isolates had similar growth characteristics for *Rhizobium*. Among them 22% of the isolates displaying characteristics similar to slow-growing rhizobia and the remainder being fast-growing. All of the isolates showed diverse biochemical profiles (Figure 1).

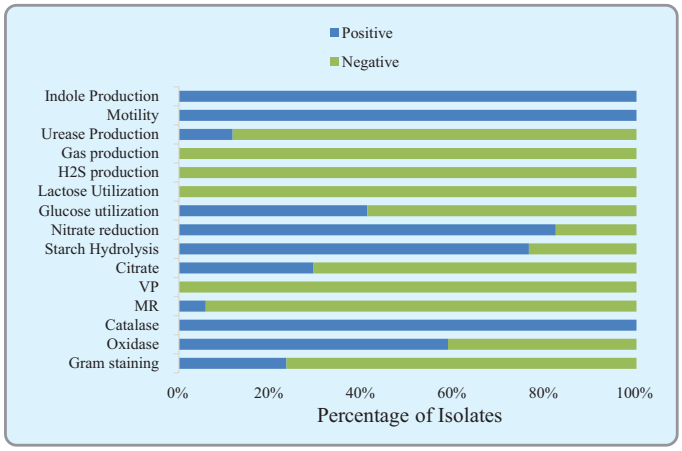

Figure 1: *Biochemical profiling of different isolates*

Biochemical profiling

Seventeen isolates were chosen for further studies. Except four, all 13 isolates were found to be Gram-negative The isolates showed similar results for all the biochemical test performed. Not much diversity was observed among the fast or slow grower isolates although the sampling sites were different.

Effect of different stress conditions on growth

Effects of different stress conditions such as different pH, temperature, salt concentration and different pesticides on growth were observed. The growth evaluation at different pH values showed optimum growth of all isolates at neural and slightly acidic pH (4.5 to 7). Most of the isolates were able to grow in a broad pH range (4.8 to 11.0) whereas slow grower SB5 and SB6 isolates did not grow at higher pH (Fig. 1). The ability to survive at temperatures between 14 and 28°C was observed in all tested isolates. The highest growth was observed at 20ºC - 28ºC. While most of the isolates could grow up to 37ºC. At 40ºC most isolates were unable to grow except SB5 and SB6 that showed moderate growth (Fig.2). All the isolates grew well at salt concentration up to 1% but at 3% most of isolates could not grow well or could not grow at all. Whereas OP2 and MP2 from non-legumes could tolerate up to 5% salt concentration (Fig. 3).

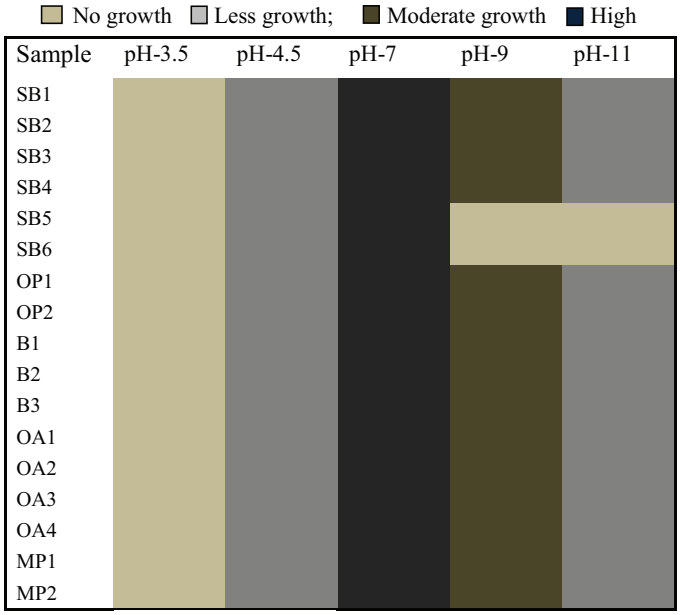

Figure 2: *Effect of different pH*

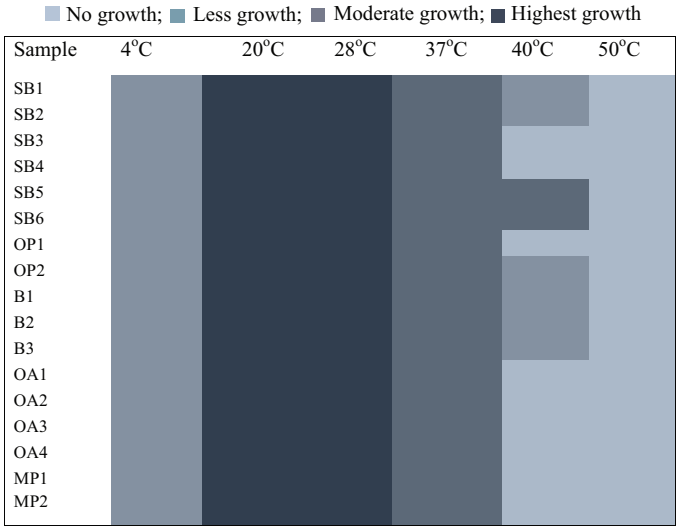

Figure 3: *Effect of different growth temperatures on isolates*

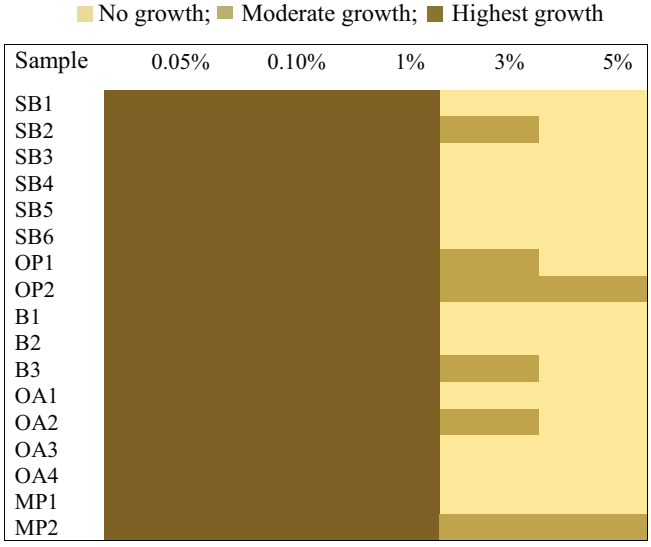

Figure 4: *Effect of different salt concentration on growth*

Growth in different pesticide

Almost all the isolates could grow in presence of up to 0.1% pesticides but growth decreased with higher concentration.

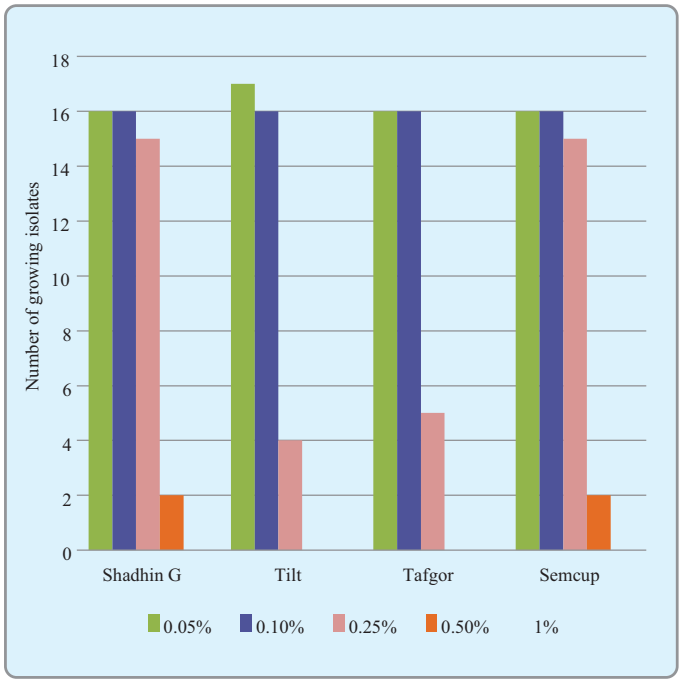

Figure 5: *Effect of different pesticides at different concentrations on bacterial growth*

Antibiogram

Antibiograms were conducted because antibiotics are widely used in modern medicine and are, in one way or another, contaminating soil. They also impact the rhizosphere, the living environment that grows in the soil, and they may pose a threat to human health as well as other living beings in the future.

In the present study, most of the isolates displayed resistance to the widely used narrow spectrum antibiotic metronidazole, however all the isolates displayed sensitivity to the broad-spectrum antibiotics like rifampicin , imipenem, meropenem, and novobiocin. Out of four Gram positive isolate only one showed multiple drug resistant. While among Gram negative isolates most of the isolates were multidrug resistant against 3 to 6 drug out of the ten tested. Not much difference against drug resistance was observed among the isolates from leguminous or non-legume plants or among the fast and slow growers. However isolates that showed resistant against 3 or more anibiotics were considered as multiple antibiotic resistant strains that could be considered as harmful if persisted in the environment. PGP properties observed when grown without any pesticide

PGP properties in presence of different pesticides

All the isolates could tolerate Shadhin G and Semcup up to 0.25% concentration except SB5 which was sensitive to these two pesticides even at lowest concentration. All isolates were able to grow up to at 0.1% concentration of Tilt and Tafgor except OP2

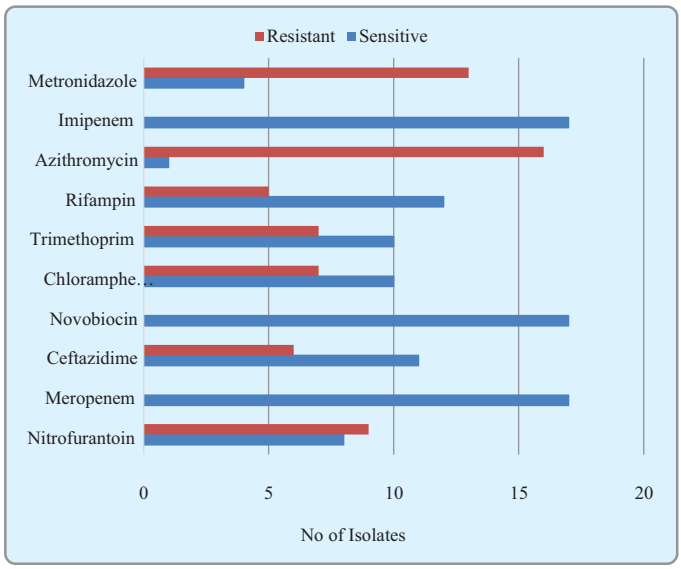

Figure 6: Antibiotics sensitivity test (Disc Diffusion)

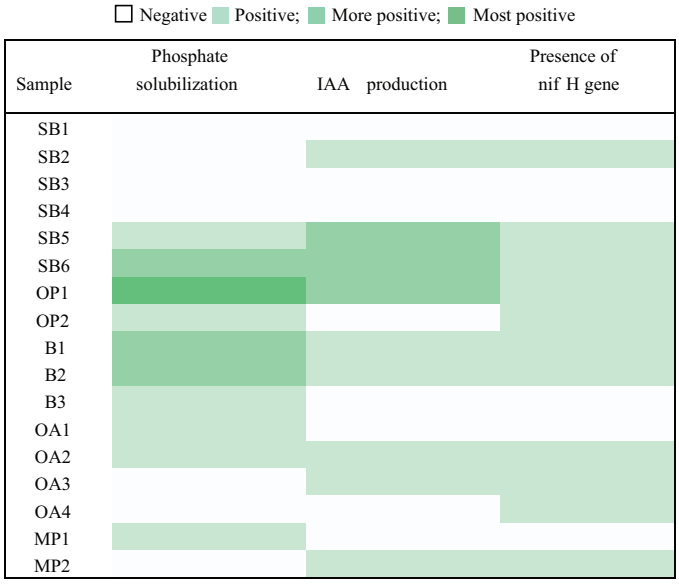

Figure 7: *Chart for 3 PGP (Plant Growth Promoting) Properties of purified bacterial isolates*

which was relatively sensitive. Six isolates (SB5, SB6, OP1, B1, B2, OA2) showed all the three-plant growth promoting properties when grown in absence of pesticides. Among these isolates OP1 showed the highest phosphate solubilization and higher amount of Indole Acetic Acid production. Selected six isolates showed a decrease in Phosphate solubilization and IAA production in presence of pesticides (Table 5).

Detection of nifH gene and nodC gene

Expected bands were found for 11 isolates near the position 700bp (*nifH* gene-780bp). Expected bands were found at position 500bp from the PCR product of *nodC* (500 bp) gene (Fig. 8a and 8b).

Table 5: *Effect of PGP properties in presence of different pesticides.*

Sample	Phosphate solubilization					IAA production					Presence of *nif*H gene				
	w/o	S.G	Sc	Tt	Tg	w/o	S.G	Sc	Tt	Tg	w/o	S.G	Sc	Tt	Tg
SB1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SB2	-	-	-	-	-	+	-	-	++	+	+	+	+	+	+
SB3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SB4	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
SB5	+	+	+	-	-	++	-	-	-	-	+	+	+	+	+
SB6	++	+	+	-	+	++	-	-	-	-	+	+	+	+	+
OP1	+++	+	+	-	-	++	-	-	+	+	+	+	+	+	+
OP2	+	+	+	-	+	-	-	+	-	-	+	+	+	+	+
B1	++	+	+	-	-	+	-	+	-	-	+	+	+	+	+
B2	++	+	+	+	+	+	-	+	++	++	+	+	+	+	+
B3	+	+	+	-	+	-	-	+	-	-	-	-	-	-	-
OA1	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
OA2	+	+	+	-	-	+	-	++	++	++	+	+	+	+	+
OA3	-	-	-	-	-	+	-	++	+++	+++	+	+	+	+	+
OA4	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
MP1	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
MP2	-	-	-	-	-	+	-	++	+++	++	+	+	+	+	+

Table-5. Here, w/o- without pesticide ; S.G- Shadhin G; Sc- Semcup; Tt- Tilt; Tg- Tafgor; Marked isolates had all the mentioned PGP properties and were the expected isolates.

Figure 8: *a: PCR amplified nifH gene, 8b: PCR amplified nodC gene*

Discussion

Abiotic stress is a common occurrence in the legume-*Rhizobium* symbiosis, which significantly affects the nodulation process therefore nitrogen fixation [23] .

The aim of the study was to isolate and analyze PGPR (Plant Growth Promoting Rhizobacteria) from both leguminous (*Glycine max, Clitoria ternatea* and *Phaseolus lunatus)* and non-leguminous plants (*Oxalis* spp., *Mimosa pudica).* It has been proposed that these rhizobacteria would harbor some plant growth promoting properties such as production of plant growth hormones, mineralization of phosphate, environmental nitrogen fixation, chelation of iron by siderophore production, protecting plant from abiotic stress and pest and pathogen attack [5]. Due to climate changes, excessive pressures on agricultural field with the increasing demand of mankind, ultimately results in loss of soil fertility and the outcome is decreasing the agricultural production while increasing pathogen and pest attacks. Information extracted from the study could be a point of interest in combating those problems as well as improving effectivity and economy of the existing bio-fertilizer [3].

The study started by plant sample collection from various Garden soils within Dhaka city. Out of 42 collected, 17 isolates were selected for further screening. Most of the isolates were rod-shaped and dominated by Gram-negative bacteria and six of theses isolates were selected after their Plant growth promoting characteristics. Based on PGP screening about 60% of the isolates were able to solublize inorganic phosphate and between 40 to 60% isolates were able to produce IAA in the presence of different pesticides (Table 5). But as this study's diversity analysis relied solely on some morphological and biochemical tests, it might not accurately depict the richness of rhizosphere bacteria. Rhizobia are highly valuable inoculums for legumes grown in arid and

semiarid areas because of their tolerance to abiotic stresses. Overall, our study showed that the strains under investigation were quite tolerant of salinity *in vitro*, with some strains able to withstand concentrations as high as 1%. These findings supported earlier studies where rhizobia were isolated from woody legumes in general and Dryland acacia tree species in particular were able to tolerate higher salinity [24-25]. The range of salinity tolerance in rhizobia can vary significantly between species, and even within strains of the same species [26]. While Assefa (1993)[27] discovered that some strains of slow-growing rhizobia from woody legumes were more tolerant to NaCl than fast-growing species, Zerhari *et al*.[28] reported that fast-growing rhizobia isolated from some Acacia species were tolerant to high NaCl concentrations compared to slow-growing strains. Other studies have suggested that growth rate and salt tolerance are not correlated with growth rate [29] but rather are for physiological and biochemical mechanisms [30].

Rhizobia are considered mesophilic, with optimal growth temperatures of 28 to 30°C [31]. The maximum temperatures (Tmax) for growth of free-living rhizobia in soil are between 35 and 45°C[32]. Zerhari *et al*[27]. and Assefa[28], also showed the ability of some rhizobia strains isolated from woody legumes to tolerate temperatures ranging from 4 to 43°C. But, even though rhizobia grow at elevated temperatures, this does not indicate that they are efficient N_2 fixers [33].

Rhizobia strains contain variants that can be useful in tolerating abiotic stresses such as temperature extremes, pH, and salinity. The growth of most of our isolates over a wide range of pH, including acidic pH values, suggests that there is a potential to inoculate them under different soil pH conditions if other conditions could not significantly affect their performance. Soil pH is one of the obvious influencing factors of microbial activity and populations, as revealed by several studies, and can therefore ultimately influence plant growth significantly. The evaluated strains' tolerance to different pH values ranging from (4.0–9.0) corroborates the results of previous research showing rhizobia's ability to grow in a diverse range of soil pH. All tested isolates grow best at neutral and slightly acidic pH (4.8–6.8) [33]. The exploration of tolerant trains is predicted to increase plant growth and yield, even under a combined stresses condition.

Each PGP trait of bacteria is the result of sequential metabolic reactions mediated by various specific functional proteins (enzymes) along the defined metabolic pathway. The metabolic pathways for any specific PGP trait may be more than one depending upon the type of the PGP substances and bacterial genera/species. Pesticides adversely affect protein synthesis and the metabolic enzymes[34]. Even though the chemical properties of the pesticides in the current study was not observed but from previous references it could be hypothesized that, it seems probable the pesticides employed in this study might have inhibited the functioning of the enzymes participating in different metabolic pathways of PGP traits (PS, and IAA) in the isolates under study. Therefore, these properties declined under pesticide stress (Table 5 and Fig. 7).

Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of a chemical fertilizer. As a result, a large portion of soluble inorganic phosphate applied to the soil is immobilized rapidly and becomes unavailable to plants[35]. Since microorganisms are involved in a variety of processes that affect the transformation of soil P and are therefore an integral part of the soil P cycle, the present study's findings demonstrated that a diverse group of potential phosphate solubilizing bacteria are associated with rhizosphere and could serve as effective biofertilizer candidates for improving the P-nutrition of crop plants.

The toxicology of pesticides to organisms varies due to their functional groups, and even among pesticides of similar functional groups there is a great deal of variability[36]. In our study, the degree of inhibition of beneficial traits of the strains under pesticide stress varies from one pesticide to another. Furthermore, pesticides damage structural proteins that are essential for the growth of the organism and are also responsible for geno-toxicity[37], which ultimately results in decreased functioning and survival of organisms exposed to high concentrations of pesticides [38].

Around 65% isolates showed positive bands after PCR amplification using the *nifH* specific primers between the position 700bp and 800bp in agarose gel. This indicated those isolates might be capable of nitrogen fixation in plant root association. About 47% isolates gave positive PCR amplification for *nodC* gene and gave band in position at 500bp. This indicated the isolates might have the ability to form nodule.

So based on the qualitative analysis six isolates - SB5, SB6, OP1, B1, B2, OA2 were most convenient for bio-fertilizer designing and production.

This work, which was primarily focused on examining the diversity of rhizobia isolated from root nodules from various leguminous and non-legume plants, enabled us to identify effective rhizobia that could fix nitrogen and withstand a range of environmental constraints. The strains' tolerance to these factors needs to be further assessed in symbiosis with the host plant; from this angle, additional complementary investigations on plants origins would be fascinating to further elucidate the responses of this symbiosis to specific climatic conditions.

Since *in vitro* results cannot always be reliably reproduced under field conditions, more field trials utilizing these bacteria would be required to understand their potential in the agro-ecosystem as PGPRs. Consequently, based on the results obtained during this study, it can be assumed that the selected isolates could be beneficial for their use as potential agents and promoters of plant growth and development.

Conclusion

This study has demonstrated that pesticides have an adverse effect on rhizobia's PGP activities in addition to their effects on the growth of the bacteria; these findings clearly revealed an additional aspect of the toxicological mechanisms of the pesticides through which they decline plant growth; the study demonstrated that pesticides should be carefully screened in a laboratory before being applied in the field; and that additional molecular research on the interaction between pesticides and rhizobacteria is required to determine which genes or enzymes in rhizobacteria are affected by pesticide-stress.

Plant infection tests, field level analysis, and application will be carried out in the future to assess the response of those isolates on plant growth promotion; additionally, synergistic effects of the isolates with other organisms or among them may be checked on plant growth promotion. Future studies for Plant Growth Promoting Properties (test for siderophore production and ACC deaminase production) will be conducted for a comprehensive analysis of PGPR.

Conflict of Interest

All authors have contributed equally.

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