

# Plant Growth Promotion and Suppression of Damping Off in Tomato by Plant Growth Promoting Rhizobacterium *Bacillus amyloliquifaciens*

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## ABSTRACT

Plant growth promoting rhizobacterium (PGPR) is a kind of rhizosphere bacterium that increases plant growth and yield by intimately colonizing plant roots. This study was planned to examine the effect of a PGPR strain namely *Bacillus amyloliquifaciens* PPB10 on growth of tomato plants and suppression of damping off disease caused by *Sclerotium rolfsii*. In this study tomato seeds were treated with PPB10 and sown in sterilized soil. The PGPR isolate PPB10 significantly improved seed germination and seedling vigor of tomato plants compared to non-treated control. Three weeks after sowing, plants treated with the PGPR strain displayed significantly enhanced levels of growth in terms of length and yield of shoot and root biomass compared to non-treated control plants. Seed bacterization with the PGPR significantly increased total chlorophyll contents in the shoot. Moreover, the isolate significantly antagonized the mycelial growth of the pathogen *Sclerotium rolfsii*, while treating tomato seeds with the bacterium significantly suppressed damping off disease. The isolate was found to be an efficient colonizer of the tomato roots. Since the PGPR *B. amyloliquifaciens* PPB10 displayed traits beneficial to the plants, it has the potential to be utilized in the development of an effective seed treatment.

**Keywords:** PGPR, Seed germination, Vigor, Shoot growth, Root growth, Leaf number, Chlorophyll, Root colonization, Antagonist, Seedling mortality.

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### Highlights of this paper

- Plant growth promoting rhizobacterium (PGPR) is a kind of rhizosphere bacterium that dynamically colonize the plant roots and improve plant growth and yield.
- The PGPR strain namely *Bacillus amyloliquifaciens* PPB10 significantly augmented growth of tomato plants and controlled damping off disease caused by *Sclerotium rolfsii*.
- Deployment of PGPR strain PPB10 for improvement of plant growth and suppression of diseases is considered as a key alternative to synthetic fertilizer and pesticides.

## 1. INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most common vegetables in Bangladesh and also a major crop in almost all countries in the world. It is rich in Vitamin A and Vitamin C, and serves as a potent source of antioxidant response compound such as lycopene which prevents carcinogenesis [1]. Tomato is principally well-known for its adaptive capacity to a wider range of soil and climatic conditions, and thus it is grown in almost all home gardens and fields in Bangladesh. Globally, tomato ranks third in respect of production next to potato and sweet potato [2] while in Bangladesh, it positions after potato and brinjal [3]. It is predominantly cultivated in winter season in Bangladesh as the prevailing temperature during this period of time is congenial for its optimum growth and yield. However, due to its palatability, demand of consumption is rising steadily throughout the year. Recent introduction of summer tomato has the potential to overcome the seasonality of the tomato production in Bangladesh. From farmer's point of view, tomato is known as profitable, less risky and high selling price recipient, compared with many field crops [4]. Because of these factors, farmers are bringing more land under tomato production, although the domestic production is still far from meeting the national demands. The imbalance in demand and production has led to excessive use of fertilizer and pesticides in the tomato field. Although, these agrochemicals directly benefit crop plants, they also impose negative effects on the environment and human health. Therefore, green strategies are needed to increase tomato production in Bangladesh. Utilization of beneficial microorganisms seems to be one of the prime substitutes of agrochemicals in agricultural field. Rhizosphere is a natural habitat of diverse groups of beneficial microbes. Plant growth promoting rhizobacterium (PGPR) is a heterogeneous group of rhizosphere bacteria that actively colonize plant roots and improve plant growth and yield. Among the PGPR, various strains of *Bacillus* have been effectively used in efforts to increase plant growth and control plant pathogens [5-9]. The commonly known mechanisms of plant growth enhancement by PGPR may include phytohormone production, nitrogen fixation, phosphate solubilization and harmful pathogen suppression. Presently, there is very limited evidence on the exploitation of PGPR in stimulating tomato growth in Bangladesh. Information on the efficacy of native PGPR population in stimulating tomato growth is necessary to develop eco-friendly formulation of the microbes for the field application. By keeping this in mind objectives were set to evaluate the indigenous PGPR isolate as a root colonizer for inducing growth traits and disease suppression capacity in tomato.

## 2. MATERIALS AND METHODS

### 2.1. Location

The Experiment was conducted at the Agriculture Field Laboratory of International University of Business Agriculture and Technology (IUBAT), Dhaka, Bangladesh and Microbiology Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

### 2.2. Origin of Seeds and Plant Growth Promoting Rhizobacterium

The tomato plant Minto F1 cv. Year Round (Lal Teer Seed Company, Dhaka, Bangladesh) was used as host throughout the study. The PGPR strain *Bacillus amyloliquifaciens* PPB10 and the damping off pathogen *Sclerotium*

*rolfsii* SR-1 were obtained from the microbial stock culture of Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. The rhizobacterial strain was previously isolated from the roots of cucumber (*Cucumis sativus* L. cv. Baromashi) plants. This strain had the ability to produce phytohormone IAA (Indole-3-Acetic Acid), fix N<sub>2</sub> (nitrogen) and showed antagonistic activity against various pathogens [10].

### 2.3. Culture and Preparation of Bacterial Inocula for Tomato Seed Treatment

The bacterium was placed in a 300 ml Erlenmeyer flask containing 100 ml Yeast Peptone (YP) broth medium and cultured on a shaker at 120 rpm (revolutions per minute) for 48 h at 28±2°C. Bacterial cells were collected from the broth by centrifugation at 15000 rpm for 1 min at 4°C followed by washing the bacterial pellet twice with sterile distilled water. The bacterial pellet was suspended in 0.5 ml sterile distilled water, vortexed and used for seed treatment. Tomato seeds were surface sterilized in 5% NaOCl for 1 min, washed thrice in sterile distilled water and blotted dry. The dried seeds were then placed in bacterial suspension and the preparation was mixed thoroughly with frequent stirring for 5 min. The treated seeds were spread out on a Petri plate and air dried overnight inside a clean bench at room temperature. The number of bacterium on the seed was approximately 10<sup>8</sup> CFU/seed.

### 2.4. Effect of Bacterial Seed Treatment on Germination and Vigor Index in Tomato

With the aim of determining the impact of the PGPR treatment on seed germination and seedling vigor, 100 seeds were treated with the bacterium and incubated in ten 9-cm petri plates on two layers of sterile moistened filter paper. Seeds treated with water in place of bacterial suspensions were considered as a control treatment. Seeds were incubated at 28±2°C in a light incubator. Sufficient moisture was maintained for germination by adding 5 ml distilled water to the petri plates every other day. If the radicles were half of the seed length, germination was estimated to have occurred. The germination percentage was recorded after 7 days. Plant length was measured from the root tip to the shoot tip. Vigor Index was estimated from the following formula:

Vigor Index = % Germination × total plant length

### 2.5. Effect of Seed Treatment with Bacterium on Growth of Tomato Plant

The rhizobacterial isolate was tested for its ability to stimulate growth in tomato plants. Surface-sterilized tomato seeds were treated with the rhizobacterial isolate as we described above. Surface-sterilized seeds treated with water were considered as control. Field soil containing 1.87% organic matter, 1.08% organic carbon, 0.27% nitrogen (N), 0.09% phosphorus (P) and 0.87% potassium (K) with pH 6.38 was used as potting medium. The soil was autoclaved twice at 24 h intervals at 121°C and 15 psi (pounds per square inch) for 20 minutes. About 190 g of the sterilized soils were placed in each sterilized pot (9.5 cm × 7.0 cm size). Treated tomato seeds were sown in each pot. Plants were cultivated in vinyl house and were watered on alternate days. After growing for three weeks, tomato plants were harvested and observation was made on fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, shoot and root length, leaf number and leaf chlorophyll content. Number of leaves per plant was determined by counting the leaves from the base to the tip of the shoot.

### 2.6. Determination of Photosynthetic Pigments

Fresh leaves were harvested for extracting chlorophyll using the methods as described by Arnon [11]. Fresh leaf samples (0.5 g) was chopped and placed in a shaker with 80% acetone in dark until the leaves were entirely

bleached. The acetone extract was centrifuged at 13,000 rpm for 10 min, and the supernatant was collected to measure chlorophyll a (Chl a) and chlorophyll b (Chl b) at 663 and 645 nm absorbance, respectively, using a spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll were calculated by using following formulas:

$$\begin{aligned}\text{Chlorophyll a } (\mu\text{g/ml}) &= 12.7 (A663) - 2.69 (A645) \\ \text{Chlorophyll b } (\mu\text{g/ml}) &= 22.9 (A645) - 4.68 (A663) \\ \text{Total chlorophyll } (\mu\text{g/ml}) &= 20.2 (A645) + 8.02 (A663)\end{aligned}$$

### 2.7. In Vitro Screening of Bacterial Isolate for Antagonism

Antagonistic activities of rhizobacterial strain against *Sclerotium rolsii* strain SR-1 was performed in dual culture assay. In this assay, both pathogen and bacterium were inoculated on the same potato dextrose agar (PDA) plate at 3 cm apart. The radial growth of the pathogen was measured after incubation at 25° C for 5 days. The percentage inhibition of pathogen radial growth was calculated by the following equation.

$$\% \text{ Inhibition of growth} = \frac{X - Y}{X} \times 100$$

Where,

X = Mycelial growth of pathogen in the absence of antagonist.

Y = Mycelial growth of pathogen in the presence of antagonist.

### 2.8. Inoculum Preparation of *Sclerotium rolsii*

Wheat grain (100 g) was taken in 100 ml of distilled water in a 500 ml Erlenmeyer flask and autoclaved at 121°C for 1 h. The autoclaved wheat grains were inoculated with 10–15 disks (5 mm) obtained from the actively growing margin of 5-day-old PDA cultures of *S. rolsii* SR-1. After two weeks of incubation at 25°C in the dark, the fully colonized wheat grains were air-dried at laboratory temperature (25±1°C). The dried wheat grain inocula were ground to fine particles (1–2 mm) and stored at 4°C until use.

### 2.9. Effect of Rhizobacterial Treatment on Damping Off of Tomato

Field soil was autoclaved twice at 1 day interval at 121°C for 1 h. Powdered wheat grain inoculum was mixed with the potting soil to a concentration of 1.0% (w/w) before the tomato seeds were sown. Soil mixed with an equal amount of autoclaved wheat grain was used as a negative control. Plastic pots were filled with about 140 g of the potting medium and inoculum mixtures. Tomato seeds of PGPR and control treatment were prepared as above and were sown into the potting medium (10 seeds/pot). Each treatment consists of three replicates. Seedlings were grown in a growth room under the same conditions as described above. After 3 weeks of growth, number of survived seedlings were counted.

### 2.10. Root Colonization

Root colonization by the bacterium was determined following the protocol of Hossain, et al. [12]. Briefly, roots were collected from plants at one week, two weeks and three weeks after seed sowing. Roots were washed carefully with running tap water to eliminate adhering soil particles. The collected root samples were then rinsed thrice with sterile distilled water and finally blotted to dryness. Roots were segmented into top, middle and bottom regions. Each of root segments was weighed and homogenized in sterilized distilled water with mortar and pestle. Subsequently, appropriate dilutions were made and an aliquot of 100 µl suspension from each dilution was plated

onto KB media. After 24 h of incubation at  $28\pm 2^{\circ}\text{C}$ , the number of bacterial colony was counted. Colony-forming units (CFU) per gram of root tissue was calculated for each sample by using the mean value of colony counts obtained from the triplicates.

### 2.11. Statistical Analysis

A completely randomized design was followed for each experiment, with three replications for each treatment. Each of the experiments were repeated at least twice and the data were shown from representative experiment with similar results. The data were statistically analyzed using SPSS (Version 17) and Microsoft Office Excel 2007. Significant difference between treatments were compared by using paired t test ( $P=0.05$ ).

## 3. RESULTS AND DISCUSSION

### 3.1. Germination and Vigor Index Improvement in Tomato

The bacterial treatment showed a significant effect on the germination rate and Vigor Index (VI) of tomato seedlings compared to the control. The PPB10 increased the germination rate of tomato seeds by 14.37% over the control, while the VI of seedlings was increased by 26.93% compared to the control Table 1. These findings indicate that treatment with PPB10 can increase both the germination rate of seeds and vigor of seedlings in tomato.

**Table-1.** Improvement of germination and vigor index of tomato by *Bacillus amyloliquifaciens* PPB10.

Treatment	% Germination	% Increase in germination over control	Vigor index (VI)	% Increase in VI over control
Control	75.56 $\pm$ 2.21	-	619.59 $\pm$ 31.14	-
PPB 10	86.42 $\pm$ 2.78*	14.37	786.42 $\pm$ 38.45*	26.93

Note: \*Indicates significant difference by t-test ( $p < 0.05$ ).

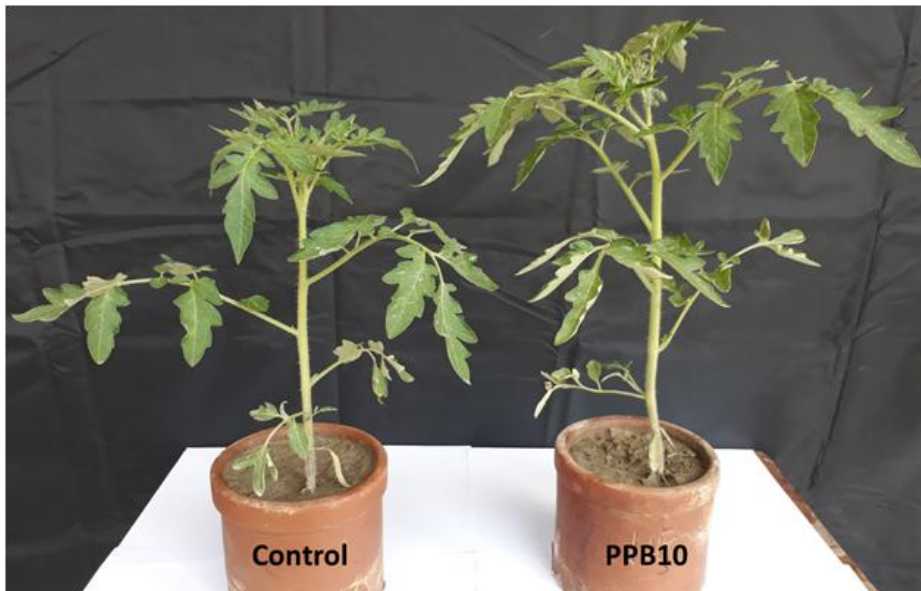
### 3.2. Plant Growth Promotion in Tomato Plants

Plant growth enhancement has significantly been observed with the rhizobacterial treatment in tomato plants compared to non-inoculated control Figure 1. The mean shoot length of tomato plantlets ranged from 25.57 cm to 31.2 cm. The maximum shoot length of 31.2 cm was found in PPB10 treated plants, while the lowest was in control-treated plants. The increase in shoot length by PPB10, was 22.02% Table 2. Plants grown with the bacterial isolate showed longer primary roots and more secondary and lateral roots than the plants in control pots Figure 3. The maximum root length of 19.37 cm was found in treatment with isolate PPB10, which were 26.02% higher than control treatment Table 2. The PGPR pretreatment of tomato seed stimulated significantly higher fresh and dry shoot weight per plants as compared to untreated plants. The percent increase in shoot fresh and dry weight by PPB10 over control was 57.42% and 86.42%, respectively. Tomato plants treated with the rhizobacterial strain had shown significantly higher fresh and dry root weight compared to those of untreated plants Table 2. The highest fresh root weight was obtained from PPB10 treated plants and the percent increment in root fresh weight by PPB10 over control was 80.16%. Similarly, the highest and the lowest root dry weight were observed in PPB10 and control-treated plants, respectively. The leaf number was increased in PGPR-inoculated plants compared to those treated with control. The highest leaf number of 6.85 (7) plant<sup>-1</sup> was observed in plants treated with PPB10 Table 2 which was 41.23% higher than control. These data indicate that the PPB10 is a PGPR that positively stimulates shoot and root growth in tomato plants.

**Table-2.** Effect of seed treatment with *Bacillus amyloliquifaciens* PPB10 on shoot, root and leaf growth of tomato plants.

Treatment	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)	Leaf number /plant
Control	25.57±0.74	15.37±0.56	8.76±0.53	0.81±0.04	1.26±0.11	0.16±0.01	4.85±0.44
PPB10	31.20±.88*	19.37±0.58*	13.79±0.55*	1.51±0.09*	2.27±0.21	0.26±0.01*	6.85±0.46*
% Increase over control	22.02	26.02	57.42	86.42	80.16	62.50	41.24

Note: \*Indicates significant difference by t-test ( $p < 0.05$ ).



**Figure-1.** Enhancement of growth of tomato plants by treatment with *Bacillus amyloliquifaciens* PPB10.

### 3.3. Improvement of Photosynthetic Compounds in Tomato Leaves

It is clear from Table 3 that PPB10 significantly improved the photosynthetic pigments such as chlorophyll a and chlorophyll b in leaves of tomato plants when compared with the non-treated control plants. Such improvement was highly remarked in chlorophyll a contents. An increase of 32.20% leaf chlorophyll “a” content in plants grown in the presence of PPB10 was observed compared to the control. Similarly, the chlorophyll b and total chlorophyll content were increased by 57.59% and 48.74%, respectively in PPB10 treated plants compared to untreated control plants.

**Table-3.** Effect of seed treatment with *Bacillus amyloliquifaciens* PPB10 on photosynthetic pigment content in leaves of tomato plants.

Treatment	Photosynthetic pigments (µg/g fresh wt.)		
	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Control	15.56±0.50	6.06±0.44	20.23±0.53
PPB10	20.57±0.51*	9.55±0.47*	30.09±0.78*

Note: \*Indicates significant difference by t-test ( $p < 0.05$ ).

### 3.4. In Vitro Antagonism of *Sclerotium rolfisii*

The rhizobacterial isolate demonstrated substantial antagonistic activity towards *S. rolfisii* on PDA. In dual culture assay, mycelial growth of *S. rolfisii* was significantly inhibited by the presence of PPB10. The observed inhibition of mycelial growth of *S. rolfisii* was 62.90% by PPB10 compared to those in control plates Table 4.

**Table-4.** Inhibition of mycelial growth of *Sclerotium rolfisii* and suppression of damping off in tomato by *Bacillus amyloliquifaciens* PPB10.

Treatment	Diameter of mycelial growth of <i>S. rolfisii</i> (cm)	% Inhibition of mycelial growth of <i>S. rolfisii</i>	% Seedling mortality	% Protection
Control	43.83±0.74	-	76.67±2.40	-
PPB10	16.26±0.45	62.90±1.60	43.29±1.17*	43.54±2.36

Note: \*Indicates significant difference by t-test ( $p < 0.05$ ).

### 3.5. Suppression of Damping Off in Tomato

Treatment with the PGPR strain PPB10 showed consistent suppression of damping off in the greenhouse experiments. The percent seedling mortality in control and PPB10-treated pots was recorded to be 76.67% and 43.29%, respectively Table 4. Compared with the control, the average disease protection by PPB10 was 43.54% against damping off in tomato.

### 3.6. Root Colonization

The root colonization ability of rhizobacterium is considered an essential factor to be effective plant growth promoters. In this study, the root colonization assays showed that the tested isolate vigorously colonized the roots of tomato plants. Nevertheless, the root population densities increased progressively with growth stages Figure 2, where the lowest root population ( $173 \times 10^7$  cfu) was observed at the first week of growth and the largest root population ( $322 \times 10^7$  cfu) was found at the third week of growth.

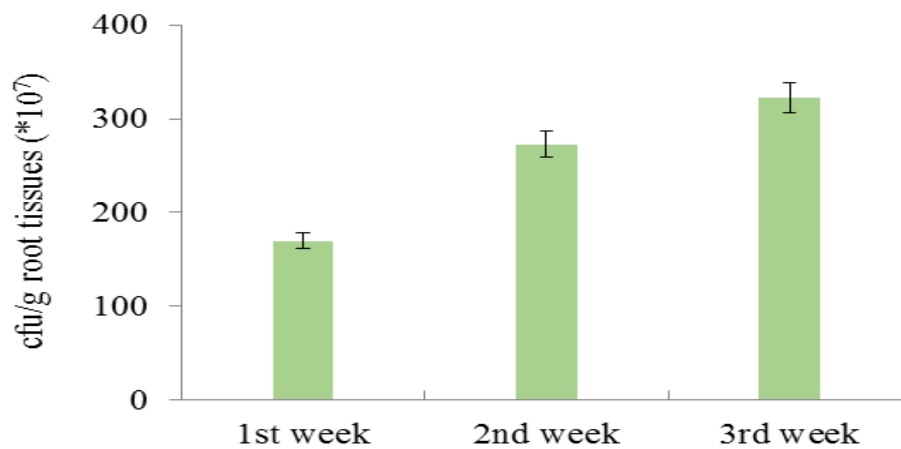


Figure-2. Population density (CFU) of *Bacillus amyloliquifaciens* PPB10 in the root tissues of tomato plants at different stages of growth.



Figure-3. Plants grown with *Bacillus amyloliquifaciens* PPB10 showed longer primary roots and more secondary and lateral roots.

The rhizosphere is a narrow zone around plant roots, which habitats a large and dynamic microbial community [13]. Some of these rhizosphere bacteria have been the focus of many studies because of their beneficial interactions with plants. The PGPR, especially *Bacillus* spp., colonize roots of both monocot and dicot plants, and promote plant

growth. We assessed phenotypic changes of tomato plantlets treated with a previously reported PGPR [10]. At 30 days after inoculation, plants treated with *B. amyloliquifaciens* PPB10 were taller and had healthier appearance than control plants. Treated plants had higher shoot biomass yield, leaf number and leaf chlorophyll content. Root architecture has also been altered after the PGPR inoculation. Plants treated with this PGPR had longer primary roots and possessed more secondary and lateral roots than control plants. Root weight was also increased after treatment with the PGPR, indicating that the PPB10 positively stimulate root biomass production. Based on these results, we conclude that the PGPR strain PPB10 promotes aerial and root growth of tomato plantlets.

Generally, microorganisms within the rhizomicrobiome can play an important role in promoting plant growth and providing better plant protection through several indirect or direct mechanisms [13]. In this study, the effects on plant growth and development associated with this PGPR strain may be mediated by Indole Acetic Acid (IAA) produced by it. Previous research discovered that the tested PGPR produced IAA and promoted cucumber plant growth [10]. In fact, the PGPR isolate PPB10 produced higher IAA than previously reported strains [14]. Production of IAA by the bacterium appears to be a vital mechanism of plant growth promotion because IAA plays an important role in root development and nutrient acquisition [15]. This commemorates with our findings that enhanced root growth was observed in the treated plants. In addition, improved nutrient uptake has been extensively documented in different plant species following inoculation with various PGPR. Acetylene reduction activity was shown by the PPB10, which is a commonly recognized proof for nitrogenase activity and N<sub>2</sub>-fixing ability [10]. Therefore, nitrogen fixation by the bacterium may seem to play an additional role in plant growth promotion of tomato plant. Transport of N from diazotrophic N-fixing rhizobacteria to the roots of several nonleguminous crops has been confirmed [16]. This indicates a complex interplay of multiples mechanisms operated by the PGPR may have role in stimulating plant growth promotion in tomato plants.

In the present study, PGPR strain PPB10 applied as a seed treatment significantly suppressed damping off in tomato. This result indicates that the fungal antagonist *B. amyloliquifaciens* PPB10 is an effective biocontrol agent against tomato damping off pathogen *S. rofsii*. Strains of *B. amyloliquifaciens* has been shown to be effective biocontrol agent against various pathogens in prior studies [10, 17]. The bacterium may produce one or more of antifungal compounds inhibitory to *S. rofsii*. Competitive root colonization by PPB10 might also play an important role in the effectual control of soil-borne diseases. Colonization of plant roots by the bacterium appeared to be heterogeneous along the root system and its competitiveness concerning this process is an essential condition for plant growth promotion [18]. PGPR can interact with a wide range of plant species and establish mutualistic relationship with them. In this study, PPB10 was an efficient root colonizer, since the CFU counts for this strain were more than 10<sup>7</sup> cfu g<sup>-1</sup> root. Previously, this PGPR also colonized the roots of cucumber plants, indicating that this PGPR can colonize host of diverse taxonomic groups. The lateral roots and root hairs are specially colonized by PGPR [19]. These regions have diverse roles for expressing their plant beneficial properties [20].

In this study, PGPR strain PPB10 improved seed germination and vigor of tomato seedlings. A positive effect on aerial and root biomass yields by the PGPR was observed. Seed bacterization with this PGPR also suppressed damping off disease caused by *S. rofsii*. These denote the potential of *B. amyloliquifaciens* strain PPB10 for commercial use as biofertilizer and biocontrol agents in the tomato field.

#### 4. CONCLUSION

The PGPR *B. amyloliquifaciens* PPB10 significantly improved seed germination and seedling vigor of tomato plants compared to non-treated control. Seed bacterization with PPB10 significantly increased plant growth and leaf chlorophyll contents. Moreover, the root colonizing isolate extensively inhibited the radial growth of the



pathogen *Sclerotium rolfsii*, while treating tomato seeds with the bacterium significantly suppressed damping off disease. Since this PGPR inoculant showed several traits beneficial to tomato plants, it may be utilized in the development of an effective seed treatment as a substitute to chemical fungicides.

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