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Research Article

Plant Growth Promoting Rhizobacteria for Biocontrol of Tomato Bacterial Wilt Caused by *Ralstonia solanacearum*

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Bacterial wilt induced by *Ralstonia solanacearum* is one of the most damaging and widespread diseases of tomatoes in the world. Biological control with rhizobacteria is one of the efficient components of integrated pest management methods used to control the disease and enhance production. To this end, plant growth-promoting rhizobacteria (*Bacillus* isolate BDUA1, and *Pseudomonas* isolates BBDUA2 and BDUA3) isolated from the tomato rhizosphere were evaluated for their plant growth-promoting traits using standard methods, and selected isolates were also tested for their biocontrol efficacy on tomato bacterial wilt disease under greenhouse conditions. All isolates produced cellulase and lipase, and only BDUA1 and BDUA3 produced protease and amylase. Besides, BDUA1 and BDUA2 showed phosphate solubilization and production of indole-3-acetic acid, HCN, and siderophore, while BDUA3 solubilized phosphate and produced HCN and siderophore. Our results showed that BDUA1 and BDUA2 reduced bacterial wilt incidence on the Maya variety by 51.9% and 48.5%, respectively, and on the Melkesalsa variety by 51.8% and 48.5%, respectively. Treatment of the Melkesalsa variety with BDUA1 displayed the highest height (36.91 cm), followed by treatment with BDUA2 (31.74 cm) on the same variety. BDUA1 induced the highest effect on increasing the dry weight of shoots and roots by 4.16 g and 0.59 g in the Maya variety and in the Melkesalsa variety by 3.63 g and 0.48 g, respectively. Similarly, BDUA2 had the greatest effect on increasing the dry weight of shoots and roots by 3.8 g and 0.54 g of the Maya variety and on the Melkesalsa variety by 3.12 g and 0.41 g, respectively. The overall result showed that BDUA1 and BDUA2 could be used as promising plant growth promotion and biocontrol agents for the management of tomato bacterial wilt disease provided they were validated under field conditions.

1. Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is one of the major problems in the production of commercially important crops. *Ralstonia solanacearum* has a wide host range of approximately 450 species from at least 54 families [1]. Depending on the *R. solanacearum* strains, soil type, host cultivars, cropping pattern, and environment, it results in substantial output losses ranging from 10% to 100% [2–5]. Moreover, it has been found to cause yield losses of tomatoes of up to 91% in tropical, subtropical, and temperate regions of the world [6].

Tomatoes (*Solanum lycopersicum* L.) are the second-most important vegetable produced globally, next to potatoes [7]. It is widely grown throughout the tropics and subtropics with an annual production of approximately 186.8 million tons in 2020 worldwide [8]. In Ethiopia, the total annual production of tomatoes was about 41,948 tons from 6,434 hectares of land [9]. Tomato juice, tomato paste, tomato catch-up, and whole peeled tomatoes are processed in Ethiopia for local consumption and exported to Somalia, Djibouti, and Saudi Arabia [10,11]. However, tomato bacterial wilt is one of the most damaging and widespread tomato diseases in Ethiopia, and its incidence is up to 55% in major tomato-producing regions of the country [12]. It is one of the major constraints on tomato production, which causes significant yield losses in different parts of the country [13]. Tomato bacterial wilt management is complicated by the ability of the pathogen to survive in a variety

of environments; the lack of chemical control; the high variability; and the extremely wide host range [14, 15]. Besides, traditional agricultural practices are often based on synthetic fertilizers and pesticides, which reduce the organic properties of vegetables and adversely affect human and environmental health [16]. This prompts the need for other eco-friendly strategies to control the disease and maintain environmental safety. Under such circumstances, the use of plant growth-promoting rhizobacteria (PGPR) plays an important role in promoting plant growth and antagonistic effects against phytopathogens [17].

Numerous bacterial species, including *Bacillus subtilis*, *Bacillus velezensis*, *Bacillus amyloliquefaciens*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas brassicacearum*, *Paenibacillus polymyxa*, and *Streptomyces* strain UT4A49, are potential biocontrol agents against tomato bacterial wilt disease [18–22]. There are also some promising studies in Ethiopia using antagonistic rhizobacteria such as *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Pseudomonas putida* to promote plant promotion and biocontrol of tomato bacterial wilt [13, 23]. However, the response of PGPR to plant growth is influenced by the number of bacteria, plant-bacteria combinations, the genotype of the plant, plant features, soil organic matter, soil type, and ecological conditions [24]. Hence, this study is mainly aimed at further evaluating the potential of plant growth-promoting rhizobacteria as biocontrol agents against tomato bacterial wilt disease.

2. Materials and Methods

2.1. Bacterial Strain and Culture Condition. The pathogen *R. solanacearum* and three PGPR isolates (BDUA1, BDUA2, and BDUA3) previously isolated from the tomato rhizosphere in our laboratory have multitrait plant growth promotion (PGP) and high inhibition activity against *R. solanacearum* were used for greenhouse experiments. BDUA1 was a *Bacillus* sp., whereas BDUA2 and BDUA3 were *Pseudomonas* spp. The bacterial isolates were characterized based on morphological and biochemical characterization. All isolates were stored at −80°C in 20% glycerol before being refreshed on Nutrient Agar (NA) (Oxoid, England) and Casamino Acid Peptone Glucose (CPG) Agar (Difco, England) plates. One colony of *R. solanacearum* was streaked and incubated in CPG plates for 48 hours at 28 ± 2°C, while the PGPR isolates were incubated in NA plates for 48 hours at 28 ± 2°C.

2.2. Characterization of Plant Growth-Promoting Traits

2.2.1. Phosphate Solubilization. For phosphate solubilization, the bacterial isolates were grown in a glucose yeast medium (2 g of yeast extract, 10 g of glucose, and 15 g of agar) per liter [25]. Two other solutions were prepared separately, one containing 10 g of $CaCl_2$ in 100 mL of distilled water and the other containing 5 g of K_2HPO_4 in 50 mL of distilled water. These solutions were then added to 1 L of glucose yeast (GY) medium just before pouring onto a Petri plate, forming insoluble $Ca_3(PO_4)_2$. Bacterial isolates that had been previously cultured into GY medium were inoculated into GY Petri plates and incubated at 28 ± 2°C for 7 days. The halos visible around their colonies are considered phosphate solubilizers.

2.2.2. Ammonia Production. The production of ammonia was tested by inoculating 10^6/ml of 48 hours grown culture into 10 mL of peptone broth (4%) and incubating it at 25°C for 72 hours. Then, 0.5 mL of Nessler's reagent was added to the culture broth. The color change from yellow to brown indicated the production of NH_3 [26]. The isolates were grown for 48 hours, adjusted to an inoculum size of 10^6/mL, inoculated into 10 mL of peptone broth (4%), and incubated for 72 hours at 25°C.

2.2.3. Indole-3-Acetic Acid (IAA) Production. For the production of IAA, the isolates were inoculated by adding 50 μL of the cell suspension to 5 mL of sterile peptone yeast extract broth (3 g of beef extract, 10 g of peptone, 0.204 g of L-tryptophan, 5 g of NaCl per liter of distilled water; pH of 7) in 15 mL culture tubes and incubated at 28 ± 2°C for 72 hours in the dark [27]. Then, 1.5 mL of the broth was centrifuged at 10,000 rpm for 10 min, followed by pipetting 1 mL of the supernatant and mixed with 2 mL of Salkowski reagent (1 mL of 0.5 M $FeCl_3$, 50 mL of 35% $HClO_4$ solution) in test tubes. Then, the culture tubes were incubated at 37°C for 1 hour in the dark. The appearance of pink color in the test tubes is indicative of IAA production.

2.2.4. Production of HCN. Production of HCN was determined using the method described in Dinesh et al. [28] by growing on Nutrient Glucose Agar (NGA) plates (2.5 g of glucose, 5 g of peptone, 3 g of beef extract, and 15 g of agar per liter) with 4.4 g L^{-1} glycine. A Whatman No. 1 filter paper soaked in 2% Na_2CO_3 in a 0.5% picric acid solution was placed on the top of the Petri plate. Petri plates were sealed with parafilm and incubated at 28 ± 2°C for 72 hours. The HCN production was indicated by the color change of Whatman No. 1 filter paper from orange to red.

2.2.5. Siderophore Production. The production of siderophore was performed according to the method described by Kheirandish and Harighi [29]. Bacterial isolates were spotted on King *B* medium with 1000 M FeCl3 and incubated at 28 ± 2°C for 48 hours. The bacterial colonies were then wiped off the plates with a sterile cotton swab. The *R. solanacearum* suspension was then spread on a medium and incubated at 28 ± 2°C for 24 hours. Inhibition of *R. solanacearum* around spots indicates the production of siderophore.

2.2.6. Detection of Amylase, Protease, Lipase, and Cellulose Production. The production of amylase, protease, and cellulase was measured using the method described by Cappuccino and Sherman [26], and lipase production was determined using the protocol described by Dastager et al.

[30]. The protease activity was detected by spot-inoculating the isolates on skimmed milk agar plates and incubating them at 28 ± 2°C for 48 hours. Clear zone formation around colonies was an indicator of activity. To determine cellulase production, the bacterial isolates were inoculated on basal medium containing 1% cellulose. The basal medium was made of K_2HPO_4–1 g, $NaNO_3$–1 g, $MgSO_4$–0.5 g, KCl–1 g, yeast extract–0.5 g, glucose–1 g, Agar–15 g, and distilled water–1000 mL. The plates were incubated at 28 ± 2°C for 48 hours and submerged in 0.01% Congo red solution for 15 minutes, and the plates were decolorized with 1% NaCl solution for 5 minutes. Bright areas on a red background indicate positive results for cellulase production.

Lipase production was determined by agar diffusion, i.e., inoculating the isolates into an NA medium supplemented with 0.01% of $CaCl_2$. H_2O mixed with Tween 80 was incubated at 37°C for 2–4 days [30]. The formation of an opaque white area around the growing colony is considered a lipase producer. In order to detect amylase production, the bacterial isolates were on spot inoculated onto sterile starch agar (beef extract–3 g, starch, soluble–2 g, peptone–5 g, agar–15 g per liter of distilled water) plates. The agar plates were incubated for 48 hours at 28 ± 2°C. The plates were then swamped with iodine solution (0.3% I_2–0.6 KI%), kept for 1 minute, and then poured off. Hence, the blue-black color surrounding the colony indicates amylase production.

2.3. Biocontrol Experiments of the Isolates against Bacterial Wilt under Greenhouse Conditions

2.3.1. Preparation of Bacterial Inoculum. *Ralstonia solanacearum* was streaked on NA and incubated at 28 ± 2°C for 48 hours. Colonies were harvested using distilled water, and the inoculum was made by adjusting the cell suspension to an optical density (OD) of 0.06 at 620 nm, corresponding to approximately 7.8×10^7 CFU/mL. The PGPR isolates (BDUBA1, BDUBA2, and BDUBA3) were streaked on NA and incubated for 48 hours at 28 ± 2°C. The cell suspensions were adjusted to an OD of 0.2 at 620 nm, corresponding to about 2.6×10^8 CFU/mL [31].

2.4. Plant Growth Conditions and Inoculation. Seeds of the Maya variety (TC1) and Melkesalsa variety (TC2), susceptible to bacterial wilt, were obtained from the Melkassa Agricultural Research Center (MARC). Seeds of the two tomato varieties were surface disinfected by sequential immersions in 70% (*v/v*) ethanol for 3 minutes and 1% sodium hypochlorite solution for 3 minutes. They were then rinsed three times with sterile distilled water under aseptic conditions [32]. The potting mix (loam soil: sand to 2 : 1 *w/w*) was prepared and filled into the disinfected plastic pots with 1% sodium hypochlorite containing approximately 3 kg of potting soil. The loam soil used for the greenhouse experiment contained approximately equal amounts of silt, clay, and a relatively small proportion of sand. Disinfected tomato seeds were sown into pots in a greenhouse. The 4-week-old tomato seedlings of each tomato variety were uprooted and soaked in each selected suspension of PGPR isolate for 60 minutes before being transplanted into plastic pots containing approximately 3 kg of potting soil in a greenhouse. Thirty mL of each PGPR suspension was poured into each plastic pot immediately. Control seedlings were soaked in distilled water. The roots of each tomato plant were artificially injured and infected with *R. solanacearum* by pouring 35 mL of the bacterial suspension into each plastic pot at the base of the plant after 2 days of transplanting.

Each tomato variety was treated with only *R. solanacearum* (positive control), and distilled water was used as a negative control. The greenhouse experiments were performed in a completely randomized design with three replications. Plants were grown in a greenhouse at 25–29°C, 75–85% relative humidity, and 12 hours of light and 12 hours of dark conditions. The seedlings were watered with distilled water when necessary. The treatments were T1: BDUA1 + RS, T2: BDUA2 + RS, T3: BDUA3 + RS, T4: BDUA1 + BDUA2 + RS, T5: BDUA1 + BDUA3 + RS, T6: BDUA2 + BDUA3 + RS, T7: RS (positive control treated with *R. solanacearum*), and T8 : HC (negative control).

2.5. Evaluation of Disease Symptoms. Wilt incidence was assessed based on a disease rating scale (0 to 5), where 0 = no wilt symptoms, 1 = one wilted leaf, 2 = two wilted leaves, 3 = three wilted leaves, 4 = wilting of all leaves without an apex, and 5 = wilting of the entire plant, death of the plant, as described by Winstead [33]. Symptoms of tomato bacterial wilt were evaluated within four weeks of the first appearance of symptoms. Wilt incidence (WI) was calculated using the following formula [34]:

$$\text{WI\%} = \frac{T}{N} \times 100. \quad (1)$$

WI = Wilt incidence,

T = Total number of plants wilted in each category,

N = Total number of plants tested.

The biocontrol efficacy was calculated using the following formula [35].

$$\text{BE\%} = \frac{(\text{DIC} - \text{DIAT})}{\text{DIC}} \times 100, \quad (2)$$

where BE = biocontrol efficacy, DIC = disease incidence of the control, and DIAT = disease incidence of antagonist treated.

Plant growth parameters were measured at the end of the experiment (one month after transplanting). Before being removed from the greenhouse, the height of tomato plants was measured, and the uprooted tomato plants were rinsed with tap water to remove adhering soil, blotted dry, and the fresh weight of shoots and roots was weighed and recorded. Besides, after drying at 80°C for 48 hours using oven-dry, the dry weights of shoots and roots were measured and noted.

2.6. Data Analysis. The data from the greenhouse experiment was analyzed using the IBM SPSS statistical software version 25. The data were checked for homogeneity using

Kolmogorov–Smirnov and $P > 0.05$ was considered as the data were normally distributed. One-way analysis of variance (ANOVA) was used to compare parameters across at least three independent groups. Tukey's test was used to compare mean outcomes between treatments of the same tomato variety, with a significance threshold of $\alpha = 0.05$. For parameters with two independent groups, the Independent Sample Test was performed to compare the mean value between cultivars with the same treatment.

3. Results

3.1. Characterization of the PGPR for Growth Promoting and Enzyme Production. The plant growth promotion traits and production of hydrolytic enzymes by PGPR *in vitro* are presented in Table 1. The data showed that the isolate BDUA1 was positive for all PGP characteristics, followed by BDUA2 and BDUA3, which were endowed each with six PGP characteristics. All of the isolates produced cellulase, lipase, HCN, siderophore, as well as solubilized inorganic phosphate. All but BDUA2 and BDUA3 produced amylase and IAA, and only the isolate BDUA1 produced protease and ammonia.

3.2. Tomato Bacterial Wilt Incidence and Progress. In the greenhouse experiment, there was no significant difference between tomato bacterial wilt incidences on the tested tomato varieties in the same treatment (Figure 1). The data showed that the Maya variety (TC1) and Melkesalsa variety (TC2) treated with BDUA1 showed the lowest tomato bacterial wilt incidence of 38.6% and 41.4%, respectively. Among all treatments, the Maya variety (TC1) treated with the combination of BDUA2 and BDUA3 displayed the highest wilt incidence of 61%, followed by a 55.6% wilt incidence treated with the combination of BDUA1 and BDUA3 in the same cultivars compared to the control treatment (80.5%).

Tomato bacterial wilt symptoms appeared from week one and progressed up to the fourth week in both varieties (Figure 2). Inoculation of PGPRs reduced bacterial wilt incidence in both the tomato varieties from week one to week four compared to the positive control. Besides, the lowest disease incidence was recorded on the Melkesalsa variety treated with BDUA1 at week three. In week four, a lower incidence of tomato bacterial wilt was observed in the treatment of BDUA1 in both tomato varieties, and the bacterial wilt incidence was almost similar in the treatment of BDUA3 of the Melkesalsa variety.

3.3. Effect of PGPR on Tomato Plant Biomass. The plant growth promotion efficiency of PGPR isolates was monitored based on the tomato height as shown in Table 2. The results demonstrated that treatments of BDUA1 and BDUA2 isolate induced a significant variation in the height of both tomato varieties compared to the positive control ($P = 0.05$). The BDUA1 inoculated plants showed the highest height in both tomato varieties compared to the positive control. The highest plant height was recorded with BDUA1 (42.25 cm) inoculation in the Maya variety, followed by BDUA2 (38.65 cm) inoculation. Moreover, inoculation of the Melkesalsa variety with BDUA1 displayed the highest height (36.91 cm), followed by BDUA2 (31.74 cm) inoculation. The consortium treatments for BDUA1 and BDUA3 had the least impact on plant height in both tomato varieties.

Table 1: Production of hydrolytic enzymes and plant promoting properties of potential plant growth promoting rhizobacteria antagonists.

Characteristic	BDUA1	BDUA2	BDUA3
Amylase production	+	−	+
Protease production	+	−	−
Cellulase production	+	+	+
Lipase production	+	+	+
Phosphate solubilization	+	+	+
IAA production	+	+	−
Ammonia production	+	−	−
HCN production	+	+	+
Siderophore production	+	+	+
Multiple PGP characters	9	6	6

+indicates positive reaction, −indicates negative reaction, and PGP is plant growth promotion.

The effects of potential PGPR antagonists on the fresh weight of shoots and roots are indicated in Table 3. The data clearly indicated that inoculation of BDUA1 and BDUA2 antagonists significantly increased the fresh weight of shoots and roots compared to the positive control. However, there was no significant difference between these two treatments, showing the highest fresh weight of shoot and roots on both tomato cultivars compared to the negative control (HC) ($P = 0.05$). In general, BDUA1 inoculation showed a maximum effect on the shoot and root fresh weight of 19.8 g and 2.2 g in the Maya variety and 17.3 g and 2.0 g in the Melkesalsa variety. It is interesting to note that the consortium treatments of BDUA1 and BDUA3 were the least effect on the fresh shoot and root weight of tomato cultivars compared to positive control.

The effectiveness of PGPR antagonists to enhance the growth of roots and shoots is shown in Table 4. The BDUA1 and BDUA2 inoculations showed significant variations in the dry weight of shoots and roots compared to the diseased control (RS) ($\alpha = 0.05$). Thus, inoculation of BDUA1 and BDUA2 displayed the highest shoot and root dry weights in both tomato varieties. BDUA1 induced the highest effect on increasing the dry weight of shoots and roots by 4.16 g and 0.59 g in the Maya variety and in the Melkesalsa variety by 3.63 g and 0.48 g, respectively. Similarly, BDUA2 had the greatest effect on increasing the dry weight of shoots and roots by 3.8 g and 0.54 g of the Maya variety and on the Melkesalsa variety by 3.12 g and 0.41 g, respectively. Consortium treatments of BDUA1 and BDUA3 had the least impact on the dry weight of shoots and roots in both tomato varieties.

3.4. Effectiveness of PGPR Isolates as a Biological Control against Tomato Bacterial Wilt. The potential of PGPR isolates to reduce the incidence of bacterial wilt on tomato

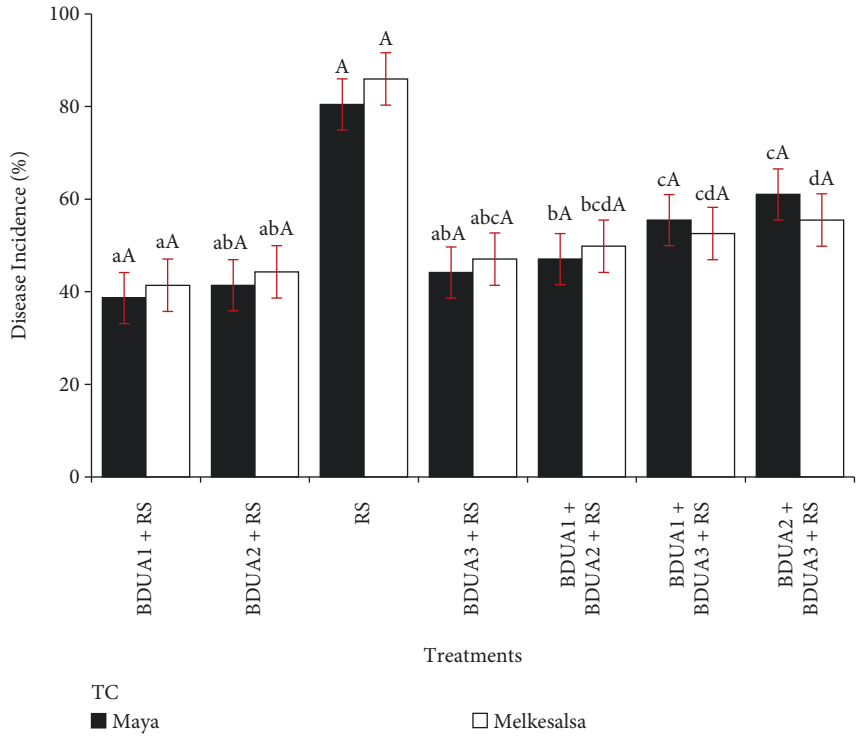

Figure 1: Effect of plant growth-promoting rhizobacteria antagonists on the disease incidence of tomato varieties under greenhouse conditions. Bars with similar lower or uppercase letter(s) on top are not significantly different according to Tukey's test ($\alpha = 0.05$). Lowercase and uppercase letters refer to the comparison between treatments with the same tomato variety and varieties with the same treatment, respectively. TC: tomato cultivar, RS refers to *Ralstonia solanacearum* (positive control).

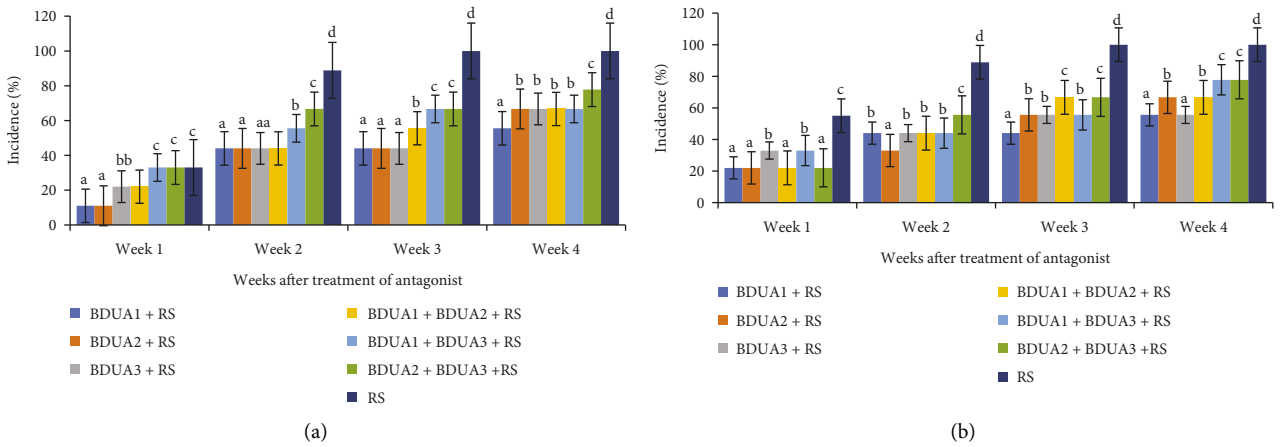

Figure 2: Effect of plant growth promoting rhizobacteria antagonists on the progress of tomato bacterial wilt incidence on Maya (a) and Melkesalsa (b) varieties after inoculation of *Ralstonia solanacearum* (RS) under greenhouse conditions. Bars with similar small letters on top are not significantly different within the weeks according to Tukey's test ($\alpha = 0.05$).

Table 2: Effect of plant growth promoting rhizobacteria antagonists on the height tomato plants under greenhouse conditions.

Treatment	Plant height (cm)	
	Maya variety (mean ± SE)	Melkesalsa variety (mean ± SE)
BDUA1 + RS	42.25 ± 1.64^{c}	36.91 ± 0.19^{bc}
BDUA2 + RS	38.65 ± 2.48^{bc}	31.74 ± 2.71^{bc}
BDUA3 + RS	26.74 ± 2.19^{ab}	28.93 ± 2.68^{abc}
BDUA1 + BDUA2 + RS	24.10 ± 2.16^{a}	25.68 ± 0.75^{abc}
BDUA1 + BDUA3 + RS	18.39 ± 0.85^{a}	19.63 ± 0.73^{ab}
BDUA2 + BDUA3 + RS	19.97 ± 0.93^{a}	21.39 ± 2.92^{abc}
RS	14.77 ± 0.47^{a}	12.65 ± 2.90^{a}
HC	43.17 ± 0.69^{c}	38.34 ± 2.03^{c}

Mean followed similar superscripts in a column are not significantly different in Tukey's test ($\alpha = 0.05$). RS refers to *Ralstonia solanacearum* (positive control), HC, negative control, and SE, standard Error.

Table 3: Effect of bacterial antagonists on the fresh weight of tomato plants under greenhouse conditions.

Treatments	Maya variety		Melkesalsa variety	
	Shoot fresh weight (g) (Mean ± SE)	Root fresh weight (g) (Mean ± SE)	Shoot fresh weight (g) (Mean ± SE)	Root fresh weight (g) (Mean ± SE)
BDUA1 + RS	19.81 ± 0.77^{c}	2.23 ± 0.08^{c}	17.30 ± 0.08^{bc}	2.08 ± 0.01^{bc}
BDUA2 + RS	18.12 ± 1.17^{bc}	2.04 ± 0.13^{bc}	14.88 ± 3.61^{bc}	1.78 ± 0.43^{bc}
BDUA3 + RS	12.54 ± 2.44^{ab}	1.42 ± 0.27^{ab}	13.56 ± 2.66^{abc}	1.62 ± 0.3^{abc} 2
BDUA1 + BDUA2 + RS	11.29 ± 1.48^{a}	1.27 ± 0.1^{a} 7	12.04 ± 0.35^{abc}	1.44 ± 0.04^{abc}
BDUA1 + BDUA3 + RS	8.62 ± 0.40^{a}	0.97 ± 0.04^{a}	9.20 ± 0.34^{ab}	1.00 ± 0.04^{ab}
BDUA2 + BDUA3 + RS	9.36 ± 0.44^{a}	1.06 ± 0.05^{a}	10.03 ± 1.36^{abc}	1.20 ± 0.16^{abc}
RS	6.93 ± 0.22^{a}	0.78 ± 0.02^{a}	5.92 ± 1.35^{a}	0.71 ± 0.16^{a}
HC	20.94 ± 0.33^{c}	2.37 ± 0.03^{c}	17.97 ± 0.95^{c}	2.16 ± 0.11^{c}

Means value followed by similar superscript letters in a column are not significantly different in the Tukey's test ($\alpha = 0.05$). RS, *Ralstonia solanacearum* (positive control), HC, negative control, and SE, standard error.

Table 4: Effect of plant growth promoting rhizobacteria antagonists on dry weight of tomato plants under greenhouse conditions.

Treatment	Maya variety		Melkesalsa variety	
	Shoot dry weight (g) (Mean ± SE)	Root dry weight (g) (Mean ± SE)	Shoot dry weight (g) (Mean ± SE)	Root dry weight (g) (Mean ± SE)
BDUA1 + RS	4.16 ± 0.16^{c}	0.59 ± 0.02^{b}	3.63 ± 0.01^{bc}	0.48 ± 0.01^{bc}
BDUA2 + RS	3.80 ± 0.24^{bc}	0.54 ± 0.03^{b}	3.12 ± 0.75^{bc}	0.41 ± 0.10^{bc}
BDUA3 + RS	2.63 ± 0.51^{ab}	0.37 ± 0.07^{a}	2.85 ± 0.56^{abc}	0.37 ± 0.07^{abc}
BDUA1 + BDUA2 + RS	2.37 ± 0.31^{a}	0.34 ± 0.04^{a}	2.52 ± 0.07^{abc}	0.33 ± 0.01^{abc}
BDUA1 + BDUA3 + RS	1.81 ± 0.08^{a}	0.25 ± 0.01^{a}	1.93 ± 0.07^{ab}	0.25 ± 0.00^{ab}
BDUA2 + BDUA3 + RS	1.96 ± 0.09^{a}	0.27 ± 0.01^{a}	2.10 ± 0.28^{abc}	0.27 ± 0.03^{abc}
RS	1.45 ± 0.04^{a}	0.21 ± 0.01^{a}	1.24 ± 0.28^{a}	0.16 ± 0.03^{a}
HC	4.40 ± 0.06^{c}	0.62 ± 0.01^{b}	3.77 ± 0.20^{c}	0.49 ± 0.02^{c}

Mean followed by similar superscript letters column are not significantly different in the Tukey's test ($\alpha = 0.05$). RS: *Ralstonia solanacearum* (positive control), HC, negative control, and SE, standard error.

varieties is shown in Table 5. These PGPR antagonists reduced the incidence of tomato bacterial wilt, ranging from 24.2% to 51.9%. Thus, BDUA1 and BDUA2 significantly reduced tomato bacterial wilt incidence from the Maya variety (TC1) by 52% and 48.5%, respectively, and in the Melkesalsa variety (TC2) by 51.8% and 48.5%, respectively, without showing significance between the two inoculants. Similarly, BDUA3 reduced bacterial wilt incidence in the Maya and the Melkesalsa varieties by 45.1% and 45.3%, respectively, but was not significantly different from the combined treatment of BDUA1 and BDUA2, with a decrease of 41.5% from the Maya and 42% from the Melkesalsa varieties.

4. Discussion

Crop disease biocontrol is gaining popularity as an environmentally safe alternative to chemical pesticides. However, a shortage of studies on understanding the mechanism of PGPR makes it challenging to design a strategy for agricultural sustainability in the near future [36]. This study showed that all potential PGPR-produced siderophores, HCN, cellulase, lipase, solubilized phosphate, and BDUA1 also produced IAA and ammonia. This finding clearly indicates that *Bacillus* isolates and *Pseudomonas* isolates showed multiple plant growth-promoting traits and produced hydrolytic enzymes. Similarly, Kheirandish and Harighi [29] reported that strains such as *Pseudomonas putida* Pp17, *Pseudomonas fluorescens* Pf11, and *Pseudomonas fluorescens* Pf16 produced siderophore, HCN, and protease. Multiple modes of action have been reported to be the main reasons for PGPR's plant growth promotion and disease suppressing ability [37].

Pseudomonas fluorescens strains are reported to be effective biocontrol agents due to their ability to use different substrates under different conditions, short growth times, and motility, which facilitate root colonization [38]. Zhou et al. [39] reported that *Pseudomonas brassicacearum* J12 significantly reduced tomato bacteria wilt incidence. Similarly, Kurabachew and Wydra [31] reported that *Bacillus cereus* BC1AW and *Pseudomonas putida* PP3WT significantly reduced bacterial wilt incidence by 46.8% and 44.7% in tomato genotypes King Kong 2 and L390 by 33.6% and 30%, respectively, under greenhouse experiments. This study demonstrated that treatment of BDUA1 and BDUA2 significantly reduced bacterial wilt incidence in both tomato varieties. Similar studies have revealed that evaluating *Pseudomonas fluorescens* VSMKU3054 and *Pseudomonas protegens* RS9 in greenhouses significantly reduced bacterial wilt disease in tomatoes by 59.5% and 65.6%, respectively [40, 41]. In another study, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* effectively reduced bacterial wilt on tomatoes by 85% and 100% in greenhouse conditions, respectively [42]. *Bacillus velezensis* GL3, *Bacillus pumilus* WP8, and *Bacillus methylotrophicus* DR-08 are the most effective biocontrols of tomato bacterial wilt by 77.67%, 90%,

Table 5: Effectiveness of potential plant growth promoting rhizobacteria isolates as a biological control against tomato bacterial wilt.

Treatment	Biocontrol efficacy on maya variety (%) (Mean ± SE)	Biocontrol efficacy on melkesalsa variety (%) (Mean ± SE)
BDUA1 + RS	51.99 ± 1.95^{c}	51.83 ± 1.58^{d}
BDUA2 + RS	48.53 ± 1.87^{bc}	48.46 ± 1.82^{cd}
BDUA3 + RS	45.12 ± 1.64^{bc}	45.27 ± 1.85^{bcd}
BDUA1 + BDUA2 + RS	41.53 ± 1.97^{b}	42.03 ± 1.86^{abc}
BDUA1 + BDUA3 + RS	31.06 ± 1.79^{a}	38.84 ± 1.74^{ab}
BDUA2 + BDUA3 + RS	24.17 ± 1.90^{a}	35.45 ± 1.63^{a}
RS	–	–

Mean values with the same letter superscript in a column are not significantly different in the Tukey's test ($\alpha = 0.05$), RS: *Ralstonia solanacearum* (positive control) and SE: standard error.

and 90% under greenhouse conditions, respectively [43–45]. This tomato bacterial wilt disease reduction by BDUA1 and BDUA2 could be attributed to the production of siderophore, protease, and hydrogen cyanide. According to Zhou et al. [39]; inoculating tomato plants with *Pseudomonas brassicacearum* J12 reduced tomato bacterial wilt by 46% in a greenhouse environment. This could be due to *Pseudomonas brassicacearum* J12 producing siderophore, protease, HCN, and 2, 4-diacetylphloroglucinol. Besides, the suppression of bacterial wilt may be a result of tomato plants developing systemic resistance, which is mediated by the jasmonic acid and ethylene hormones as well as plant defense enzymes like peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, and lipoxygenase [46, 47].

The current study revealed that the combination of BDUA2 and BDUA3 showed the least biocontrol efficacy against bacterial wilt in both tomato cultivars. These results contradict the claim that the consortium treatment of cyanobacteria RZ2AB2.1, *Bacillus subtilis* BSn5 RBI IPBL 2.3, and *Bacillus cereus* strain APSB-03 RBI 2AB 2.2 showed the best ability to reduce the growth of bacterial wilt disease in tomato development [48]. This could be attributed to the incompatibility of the bacterial isolates.

The current findings revealed that inoculation of BDUA1 and BDUA2 significantly increased the plant height, fresh weight, and dry weight of the tomato cultivars as compared to the positive control. The improvement in plant growth induced by the treatments with BDUA1 and BDUA2 might be attributed to their production of plant growth promoters such as IAA and phosphate solubility. Similarly, a study by Kurabachew and Wydra [31] reported that *Bacillus cereus* BC1AW and *Pseudomonas putida* PP3WT increased shoot dry weight by 3.8 g and 3.6 g in KK2 and 2.3 g and 2.2 g in L390, respectively, under greenhouse conditions compared to the control. Likewise, *Pseudomonas protegens* RS-9 significantly enhanced the tomato height to 95.45 cm and dry weight by 44.58% compared to the control [40]. This study is also in line with the recent study showing that the treatment of *Pseudomonas fluorescens* VSMKU3054 significantly promoted tomato plant biomass (fresh and dry weight of shoots and roots) compared with the control [41].

In this study, in the dual inoculation treatments, no significant difference was shown for all evaluated parameters (height, fresh and dry weight) of the two tomato cultivars with respect to a positive control (RS). The results disagree with the study reported previously by Agarwal et al. [43], who showed significant improvement in tomato plant growth parameters such as shoot length and fresh and dry weight by a combination of *Bacillus megaterium* GS2, *Bacillus velezensis* GL3, and *Bacillus atrophaeus* GMC1 in a pot experiment. However, a study that agreed with our findings found that combinations of *Bacillus fortis* IAGS162 and *B. subtilis* IAGS174 failed to exhibit any synergistic effects on plant protection and growth promotion with respect to single inoculation under greenhouse conditions [49].

Rhizobacteria vary in terms of both quantity and quality due to a complex interplay between soil type, plant species and diversity, cultivar type, climate, and agricultural and fertilization practices [50–52]. In order to increase tomato production, it is essential to explore and pinpoint PGPR strains that might be used as potential plant growth promoters and biocontrol agents of tomato bacterial wilt disease under specific ecological and environmental conditions. The current findings demonstrated that *Bacillus* isolate (BDUA1) and *Pseudomonas* isolate (BDUA2) possess desirable plant-promoting effects on the height and dry weight of shoots and roots, as well as significantly reduced tomato bacterial wilt in both tomato varieties under greenhouse conditions.

5. Conclusion

The study confirmed that *Bacillus* isolate (BDUA1) and *Pseudomonas* isolate (BDUA2) showed multiple plant growth-promoting traits and produced hydrolytic enzymes. Besides, these potential isolates possess desirable plant-promoting effects on the height and fresh and dry weight of shoots and roots under greenhouse conditions. *Bacillus* isolate (BDUA1) and *Pseudomonas* isolate (BDUA2) showed significantly reduced tomato bacterial wilt in both tomato varieties under greenhouse conditions. The overall results of this study confirmed that BDUA1 and BDUA2 can be used for plant growth promotion and as a promising biocontrol for the management of bacterial wilt disease of tomatoes provided that their effectiveness is validated under field conditions.

Data Availability

The data can be obtained from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] T. P. Morais, P. A. Zaini, S. Chakraborty et al., "The plant-based chimeric antimicrobial protein SlP14a-PPC20 protects tomato against bacterial wilt disease caused by *Ralstonia solanacearum*," *Plant Science*, vol. 280, pp. 197–205, 2019.

[2] Y. A. Nion, Y. A. Nion, and K. Toyota, "Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*," *Microbes and Environments*, vol. 30, no. 1, pp. 1–11, 2015.

[3] A. Palilo, *Prevalence and Management of Tomato Bacterial Wilt Using Selected Resistant Varieties in Morogoro Region, Tanzania*, Sokoine University of Agriculture, Morogoro, Tanzania, 2019.

[4] M. Z. Radhi, M. B. Adam, H. Saud, M. M. Hamid, P. S. a. Tony, and G. H. Tan, "Efficacy of smart fertilizer for combating bacterial wilt disease in *Solanum lycopersicum*," *Direct Research Journal of Agriculture and Food Science*, vol. 4, no. 7, pp. 137–143, 2016.

[5] R. Srinivasan, U. Karaoz, M. Volegova et al., "Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens," *PLoS One*, vol. 10, no. 2, Article ID e0117617, 2015.

[6] K. N. Murthy, K. Soumya, A. Udayashankar, C. Srinivas, and S. Jogaiah, "Biocontrol potential of plant growth-promoting rhizobacteria (PGPR) against *Ralstonia solanacearum*: current and future prospects," *Biocontrol Agents and Secondary Metabolites*, Elsevier, Duxford, United Kingdom, 2021.

[7] M. Siddiq and M. A. Uebersax, *Handbook of Vegetables and Vegetable Processing*, Wiley Blackwell, NY, USA, 2018.

[8] Faostat, "Food and agriculture organization of the United Nations," 2021, https://www.fao.org/faostat/en/#data/QCL/visualize.

[9] CSA, "The federal democratic republic of ethiopia central statistical agency agricultural sample survey 2020/21 (2013 E.C.) volume I report on area and production of major crops (private peasant holdings, meher season)," 2021, https://www.instepp.umn.edu/sites/instepp.umn.edu/files/product/downloadable/Ethiopia_2003-4_Vol4.pdf.

[10] Y. Baredo, "Gamo Gofa Zone diagnosis and planning document," *Livestock and Irrigation Value Chains for Ethiopian Smallholders (LIVES) Project*, 2013.

[11] M. Tewodros and K. Asfaw, "Promotion and evaluation of improved technologies through participatory approach in south Ethiopia: experience from hot pepper," *Unique Research Journal of Agricultural Sciences*, vol. 1, no. 4, pp. 57–62, 2013.

[12] K. S. Biratu, T. Selvaraj, and T. Hunduma, "*In vitro* evaluation of actinobacteria against tomato bacterial wilt (*Ralstonia solanacearum* EF Smith) in West Showa, Ethiopia," *Journal of Plant Pathology & Microbiology*, vol. 4, no. 1, pp. 1–9, 2013.

[13] H. Kurabachew and G. Ayana, "Bacterial wilt caused by *Ralstonia solanacearum* in Ethiopia: status and management approaches: a review," *International Journal of Phytopathology*, vol. 5, no. 3, pp. 107–119, 2017.

[14] N. Nasir, A. A. Mairawita, F. Alamsyah, and A. Raju, "Isolation of *Pesudomonas fluorescens* from infected and uninfected soil by *Ralstonia solanacearum* capable of producing siderophore, HCN and soluble phosphate," *Scholars Research Library*, vol. 10, no. 1, pp. 82–90, 2018.

[15] Y. Yanti, W. Warnita, R. Reflin, and C. R. Nasution, "Characterizations of endophytic *Bacillus* strains from tomato roots as growth promoter and biocontrol of *Ralstonia solanacearum*," *Biodiversitas Journal of Biological Diversity*, vol. 19, no. 3, pp. 906–911, 2018.

[16] B. N. Aloo, B. A. Makumba, and E. R. Mbega, "The potential of bacilli rhizobacteria for sustainable crop production and environmental sustainability," *Microbiological Research*, vol. 219, pp. 26–39, 2019.

[17] S. Gouda, R. G. Kerry, G. Das, S. Paramithiotis, H.-S. Shin, and J. K. Patra, "Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture," *Microbiological Research*, vol. 206, pp. 131–140, 2018.

[18] Y. Cao, H. Pi, P. Chandrangsu et al., "Antagonism of two plant growth promoting *Bacillus velezensis* isolates against *Ralstonia solanacearum* and *Fusarium oxysporum*," *Scientific Reports*, vol. 8, no. 1, pp. 4360–4414, 2018.

[19] C. Li, W. Hu, B. Pan et al., "Rhizobacterium *Bacillus amyloliquefaciens* strain SQRT3-mediated induced systemic resistance controls bacterial wilt of tomato," *Pedosphere*, vol. 27, no. 6, pp. 1135–1146, 2017.

[20] M. Kaari, J. Joseph, R. Manikkam, A. Sreenivasan, and G. Venugopal, "Biological control of *Streptomyces* sp. UT4A49 to suppress tomato bacterial wilt disease and its metabolite profiling," *Journal of King Saud University Science*, vol. 34, no. 1, Article ID 101688, 2022.

[21] F. Safdarpour and G. Khodakaramian, "Endophytic bacteria suppress bacterial wilt of tomato caused by *Ralstonia solanacearum* and activate defense-related metabolites," *Biological Journal of Microorganism*, vol. 6, no. 24, pp. 39–52, 2018.

[22] D. Sun, T. Zhuo, X. Hu, X. Fan, and H. Zou, "Identification of a *Pseudomonas putida* as biocontrol agent for tomato bacterial wilt disease," *Biological Control*, vol. 114, pp. 45–50, 2017.

[23] H. Mekonnen and M. Kibret, "The roles of plant growth promoting rhizobacteria in sustainable vegetable production in Ethiopia," *Chemical and Biological Technologies in Agriculture*, vol. 8, no. 1, pp. 15–11, 2021.

[24] B. Yagmur and A. Gunes, "Evaluation of the effects of plant growth promoting rhizobacteria (PGPR) on yield and quality parameters of tomato plants in organic agriculture by principal component analysis (PCA)," *Gesunde Pflanzen*, vol. 73, no. 2, pp. 219–228, 2021.

[25] A. Beneduzi, D. Peres, L. K. Vargas, M. H. Bodanese-Zanettini, and L. M. P. Passaglia, "Evaluation of genetic diversity and plant growth promoting activities of nitrogen-fixing bacilli isolated from rice fields in South Brazil," *Applied Soil Ecology*, vol. 39, no. 3, pp. 311–320, 2008.

[26] J. Cappuccino and N. Sherman, "Biochemical activities of microorganisms," *Microbiology, A Laboratory Manual*, The Benjamin/Cummings Publishing Company, California, USA, 1992.

[27] M. Sarwar and R. Kremer, "Determination of bacterially derived auxins using a microplate method," *Letters in Applied Microbiology*, vol. 20, no. 5, pp. 282–285, 1995.

[28] R. Dinesh, M. Anandaraj, A. Kumar, Y. K. Bini, K. P. Subila, and R. Aravind, "Isolation, characterization, and evaluation of multi-trait plant growth promoting rhizobacteria for their

growth promoting and disease suppressing effects on ginger," *Microbiological Research*, vol. 173, pp. 34–43, 2015.

[29] Z. Kheirandish and B. Harighi, "Evaluation of bacterial antagonists of *Ralstonia solanacearum*, causal agent of bacterial wilt of potato," *Biological Control*, vol. 86, pp. 14–19, 2015.

[30] S. G. Dastager, A. Pandey, J. C. Lee, W. J. Li, and C. J. Kim, "Polyphasic taxonomy of novel actinobacteria showing macromolecule degradation potentials in Bigeum Island, Korea," *Current Microbiology*, vol. 59, no. 1, pp. 21–29, 2009.

[31] H. Kurabachew and K. Wydra, "Characterization of plant growth promoting rhizobacteria and their potential as bioprotectant against tomato bacterial wilt caused by *Ralstonia solanacearum*," *Biological Control*, vol. 67, no. 1, pp. 75–83, 2013.

[32] K. N. Anith, A. Sreekumar, and J. Sreekumar, "The growth of tomato seedlings inoculated with co-cultivated *Piriformospora indica* and *Bacillus pumilus*," *Symbiosis*, vol. 65, no. 1, pp. 9–16, 2015.

[33] N. Winstead, "Inoculation techniques for evluating resistance to *Pseudomonas solanacearum*," *Phytopathology*, vol. 42, pp. 623–634, 1952.

[34] A. M. Khairy, M. R. Tohamy, M. A. Zayed, and M. A. Ali, "Detecting pathogenic bacterial wilt disease of potato using biochemical markers and evaluate resistant in some cultivars," *Saudi Journal of Biological Sciences*, vol. 28, no. 9, pp. 5193–5203, 2021.

[35] M. Chen, J. Wang, Y. Zhu, B. Liu, W. Yang, and C. Ruan, "Antibacterial activity against *Ralstonia solanacearum* of the lipopeptides secreted from the *Bacillus amyloliquefaciens* strain FJAT-2349," *Journal of Applied Microbiology*, vol. 126, no. 5, pp. 1519–1529, 2019.

[36] H. Chandran, M. Meena, and P. Swapnil, "Plant growth-promoting rhizobacteria as a green alternative for sustainable agriculture," *Sustainability*, vol. 13, no. 19, Article ID 10986, 2021.

[37] Y. Bashan and L. E. De-Bashan, "How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—a critical assessment," *Advances in Agronomy*, vol. 108, pp. 77–136, 2010.

[38] S. Nasir, "Review on major potato disease and their management in Ethiopia," *International Journal of Horticulture*, vol. 4, no. 5, pp. 239–246, 2016.

[39] T. Zhou, D. Chen, C. Li et al., "Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia solanacearum* and identification of its antimicrobial components," *Microbiological Research*, vol. 167, no. 7, pp. 388–394, 2012.

[40] R. Rai, R. Srinivasamurthy, P. K. Dash, and P. Gupta, "Isolation, characterization and evaluation of the biocontrol potential of *Pseudomonas protegens* RS-9 against *Ralstonia solanacearum* in tomato," *Indian Journal of Experimental Biology*, vol. 55, pp. 595–603, 2017.

[41] P. Suresh, S. Vellasamy, K. S. Almaary, T. M. Dawoud, and Y. B. Elbadawi, "*Fluorescent pseudomonads* (FPs) as a potential biocontrol and plant growth promoting agent associated with tomato rhizosphere," *Journal of King Saud University Science*, vol. 33, no. 4, Article ID 101423, 2021.

[42] A. F. Mohammed, A. R. Oloyede, and A. O. Odeseye, "Biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum* using *Pseudomonas* species isolated from the rhizosphere of tomato plants," *Archives of Phytopathology and Plant Protection*, vol. 53, no. 1-2, pp. 1–16, 2020.

[43] H. Agarwal, B. Dowarah, P. M. Baruah, K. S. Bordoloi, D. B. Krishnatreya, and N. Agarwala, "Endophytes from *Gnetum gnemon* L. can protect seedlings against the infection of phytopathogenic bacterium *Ralstonia solanacearum* as well as promote plant growth in tomato," *Microbiological Research*, vol. 238, Article ID 126503, 2020.

[44] S. M. Im, N. H. Yu, H. W. Joen et al., "Biological control of tomato bacterial wilt by oxydifficidin and difficidin-producing *Bacillus methylotrophicus* DR-08," *Pesticide Biochemistry and Physiology*, vol. 163, pp. 130–137, 2020.

[45] M. Shen, D. Xia, Z. Yin, Q. Zhao, and Y. Kang, "*Bacillus pumilus* WP8 exhibits biocontrol efficacy against tomato bacterial wilt via attenuation of the virulence of the pathogenic bacterium," *Acta Agriculturae Scandinavica Section B Soil and Plant Science*, vol. 68, no. 5, pp. 379–387, 2018.

[46] S. Jogaiah, M. Abdelrahman, L. S. P. Tran, and S. I. Ito, "Different mechanisms of trichoderma virens-mediated resistance in tomato against fusarium wilt involve the jasmonic and salicylic acid pathways," *Molecular Plant Pathology*, vol. 19, no. 4, pp. 870–882, 2018.

[47] P. Suresh, V. Shanmugaiah, R. Rajagopal, K. Muthusamy, and V. Ramamoorthy, "*Pseudomonas fluorescens* VSMKU3054 mediated induced systemic resistance in tomato against *Ralstonia solanacearum*," *Physiological and Molecular Plant Pathology*, vol. 119, Article ID 101836, 2022.

[48] Y. Yanti and H. Hamid, "Development of the PGPR and cyanobacteria consortium for growth promotion and control *Ralstonia syzigii* subsp. indonesiensis of tomato," *IOP Conference Series: Earth and Environmental Science*, IOP Publishing, Bristol, UK, 2021.

[49] C. Felici, L. Vettori, E. Giraldi et al., "Single and co-inoculation of *Bacillus subtilis* and *Azospirillum brasilense* on *Lycopersicon esculentum*: effects on plant growth and rhizosphere microbial community," *Applied Soil Ecology*, vol. 40, no. 2, pp. 260–270, 2008.

[50] N. O. Igiehon and O. O. Babalola, "Rhizosphere microbiome modulators: contributions of nitrogen fixing bacteria towards sustainable agriculture," *International Journal of Environmental Research and Public Health*, vol. 15, no. 4, pp. 574–625, 2018.

[51] L. Philippot, J. M. Raaijmakers, P. Lemanceau, and W. H. Van Der Putten, "Going back to the roots: the microbial ecology of the rhizosphere," *Nature Reviews Microbiology*, vol. 11, no. 11, pp. 789–799, 2013.

[52] M. Zahid, M. K. Abbasi, S. Hameed, and N. Rahim, "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*, vol. 6, pp. 207–210, 2015.