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 brassicaeearum, 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 BDU3) 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 BDU3 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 CaCl₂ in 100 mL of distilled water and the other containing 5 g of K₂HPO₄ 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 Ca₃(PO₄)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 10⁶/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 NH₃ [26]. The isolates were grown for 48 hours, adjusted to an inoculum size of 10⁶/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 FeCl₃, 50 mL of 35% HClO₄ 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% Na₂CO₃ 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.
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 K₂HPO₄–1 g, NaNO₃–1 g, MgSO₄–0.5 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. Clear zone formation around the growing colony indicates amylase production.

Lipase production was determined by agar diffusion, i.e., inoculating the isolates into an NA medium supplemented with 0.01% of CaCl₂. H₂O 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 and incubated at 28°C for 48 hours. Colonies were harvested using distilled water, and the inoculum was made by adjusting the cells suspension to approximately 7.8 × 10⁷ CFU/mL. The PGPR isolates (BDUA1, BDUA2, and BDUA3) were streaked on NA and incubated at 28 ± 2°C. The cell suspensions were adjusted to an OD of 0.2 at 620 nm, corresponding to about 2.6 × 10⁹ CFU/mL [31].

### 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 × 10⁷ CFU/mL. The PGPR isolates (BDUA1, BDUA2, and BDUA3) 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 × 10⁹ 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/v) 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]:

\[
\text{WI}\% = \frac{T}{N} \times 100.
\]

where 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].

\[
\text{BE}\% = \left(\frac{\text{DIC} - \text{DIAT}}{\text{DIC}}\right) \times 100,
\]

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 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

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

| Treatment               | Maya variety (mean ± SE) | Melkesalsa variety (mean ± SE) |
|-------------------------|--------------------------|-------------------------------|
| BDUA1 + RS              | 42.25 ± 1.64<sup>c</sup> | 36.91 ± 0.19<sup>bc</sup>    |
| BDUA2 + RS              | 38.65 ± 2.48<sup>bc</sup>| 31.74 ± 2.71<sup>bc</sup>    |
| BDUA3 + RS              | 26.74 ± 2.19<sup>ab</sup>| 28.93 ± 2.68<sup>bc</sup>    |
| BDUA1 + BDUA2 + RS      | 24.10 ± 2.16<sup>a</sup>| 25.68 ± 0.75<sup>bc</sup>    |
| BDUA1 + BDUA3 + RS      | 18.39 ± 0.85<sup>a</sup>| 19.63 ± 0.73<sup>b</sup>     |
| BDUA2 + BDUA3 + RS      | 19.97 ± 0.93<sup>a</sup>| 21.39 ± 2.92<sup>bc</sup>    |
| RS                      | 14.77 ± 0.47<sup>a</sup>| 12.65 ± 2.90<sup>a</sup>     |
| HC                      | 43.17 ± 0.69<sup>a</sup>| 38.34 ± 2.03<sup>c</sup>     |

Mean followed similar superscripts in a column are not significantly different in Tukey’s test (α = 0.05). RS refers to <em>Ralstonia solanacearum</em> (positive control), HC, negative control, and SE, standard Error.
also produced IAA and ammonia. This finding clearly showed that all potential PGPR-produced siderophores, which is important for agricultural sustainability in the near future [36]. This study of PGPR makes it challenging to design a strategy for environmentally safe alternative to chemical pesticides. How-

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 incidence 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 methylolitrophos DR-08 are the most effective biocontrols of tomato bacterial wilt by 77.67%, 90%,

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

| Treatments              | Maya variety (Mean ± SE) | Melkesalsa variety (Mean ± SE) |
|-------------------------|--------------------------|---------------------------------|
|                         | Shoot fresh weight (g)   | Root fresh weight (g)           | Shoot fresh weight (g)   | Root fresh weight (g)           |
| BDUA1 + RS              | 19.81 ± 0.77             | 2.23 ± 0.08                     | 17.30 ± 0.08bc           | 2.08 ± 0.01bc                   |
| BDUA2 + RS              | 18.12 ± 1.17             | 2.04 ± 0.13                     | 14.88 ± 0.61bc           | 1.78 ± 0.43bc                   |
| BDUA3 + RS              | 12.54 ± 2.44ab           | 1.42 ± 0.27ab                   | 13.56 ± 2.66abc          | 1.62 ± 0.3abc                   |
| BDUA1 + BDUA2 + RS      | 11.29 ± 1.48a            | 1.27 ± 0.14                     | 12.04 ± 0.35abc          | 1.44 ± 0.04abc                  |
| BDUA1 + BDUA3 + RS      | 8.62 ± 0.40              | 0.97 ± 0.04                     | 9.20 ± 0.34abc           | 1.00 ± 0.04abc                  |
| BDUA2 + BDUA3 + RS      | 9.36 ± 0.44a             | 1.06 ± 0.05                     | 10.03 ± 1.36abc          | 1.20 ± 0.16abc                  |
| RS                      | 6.93 ± 0.22a             | 0.78 ± 0.02                     | 5.92 ± 1.35a             | 0.71 ± 0.16a                    |
| HC                      | 20.94 ± 0.33c            | 2.37 ± 0.03                     | 17.97 ± 0.95c            | 2.16 ± 0.11c                    |

Means value followed by similar superscript letters in a column are not significantly different in the Tukey’s test (α = 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 (Mean ± SE) | Melkesalsa variety (Mean ± SE) |
|------------------------|--------------------------|---------------------------------|
|                        | Shoot dry weight (g)     | Root dry weight (g)             | Shoot dry weight (g)     | Root dry weight (g)             |
| BDUA1 + RS             | 4.16 ± 0.16              | 0.59 ± 0.02                     | 3.63 ± 0.01bc            | 0.48 ± 0.01bc                   |
| BDUA2 + RS             | 3.80 ± 0.24              | 0.54 ± 0.03                     | 3.12 ± 0.75bc            | 0.41 ± 0.10bc                   |
| BDUA3 + RS             | 2.63 ± 0.51              | 0.37 ± 0.07                     | 2.85 ± 0.56abc           | 0.37 ± 0.07abc                  |
| BDUA1 + BDUA2 + RS     | 2.37 ± 0.31              | 0.34 ± 0.04                     | 2.52 ± 0.07abc           | 0.33 ± 0.01abc                  |
| BDUA1 + BDUA3 + RS     | 1.81 ± 0.08              | 0.25 ± 0.01                     | 1.93 ± 0.07ab            | 0.25 ± 0.00ab                   |
| BDUA2 + BDUA3 + RS     | 1.96 ± 0.09             | 0.27 ± 0.01                     | 2.10 ± 0.28abc           | 0.27 ± 0.03abc                  |
| RS                     | 1.45 ± 0.04              | 0.21 ± 0.01                     | 1.24 ± 0.28a             | 0.16 ± 0.03                     |
| HC                     | 4.40 ± 0.06              | 0.62 ± 0.01                     | 3.77 ± 0.20              | 0.49 ± 0.02                     |

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

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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 incidence 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 methylolitrophos DR-08 are the most effective biocontrols of tomato bacterial wilt by 77.67%, 90%.
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* B5n5 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 incompatibility of the bacterial isolates. Growth-promoting traits and produced hydrolytic enzymes. *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.

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

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

The authors declare that they have no conflicts of interest.

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