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Research Article

Evaluation of Rhizosphere Bacterial Antagonists against *Ralstonia solanacearum* Causing Tomato (*Lycopersicon esculentum*) Wilt in Central Ethiopia

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Tomato (*Lycopersicon esculentum*) is one of the most commonly grown vegetables in Ethiopia. However, diseases such as bacterial wilt caused by *Ralstonia solanacearum* have been limiting the production. The rhizosphere is an important source of antagonistic bacteria against soilborne pathogens. This study aimed to investigate the antagonistic potential of rhizosphere bacteria against *R. solanacearum* in vitro. The pathogen was isolated from wilted tomato plants and tested for hypersensitivity reactions to ascertain the virulent *R. solanacearum.* Antagonistic rhizobacteria were also isolated from the rhizosphere of healthy tomatoes. Isolates were identified based on cultural characteristics and biochemical tests. The antagonistic effect of rhizobacteria against *R. solanacearum* was tested in vitro. In addition, the growth of rhizobacterial isolates was determined at different levels of temperature, pH, and NaCl. Of the 36 randomly collected colonies, 7 isolates were identified as *Ralstonia* spp., all of which were grouped under *R. solanacearum* biovar III. Similarly, 57 rhizobacteria were isolated, and only 14 had shown antagonistic effects against *R. solanacearum.* The antagonistic rhizobacteria were identified as *Pseudomonas* or *Bacillus* species. Significantly higher ($p \leq 0.05$) antagonistic activity (14.66 mm inhibition zone) was recorded by *Pseudomonas* isolate (P6) than recorded by the rest of the isolates and the positive control. Nine rhizobacterial isolates (out of 14) demonstrated higher or equal inhibition zones recorded by the positive controls. All isolates grew at temperatures ranging 15–45°C, pH 5–9, and 2–5% NaCl. The *Bacillus* spp. grew at all conditions except at pH 3, showing that they can tolerate wide range of growth conditions. The results of this study showed the presence of potential antagonistic bacteria against *R. solanacearum* in the study area, which can be used for the control of bacterial wilt of tomato as an alternative management option. Further study is required to determine the efficacy at greenhouse and field conditions.

1. Introduction

Tomato (*Lycopersicon esculentum*) belongs to the Solanaceae family, which contains several valuable agricultural food crops. It is one of the most commonly grown vegetables with a global production of over 5 million hectares [1]. In 2019 alone, about 180.48 million tons of tomatoes were produced in the world [2]. Tomato grows at altitudes ranging from 700 to 2000 meters above the sea level; agroecological zones that are characterized by warm and dry days are favorable for the optimum growth and development of tomatoes.

Tomato is grown in many parts of Ethiopia and is among the most commonly cultivated vegetable crops. Small-scale farmers, commercial growers, and state farm enterprises grow the crop for its fruits in different regions of Ethiopia. Most intensive production is found in the rift valley, mainly along Awash River valley and the lakes region. It is grown under supplemental irrigation, particularly during dry seasons [3]. Its production has shown a marked increase as it is among the most profitable vegetable crops and generates higher incomes for small-scale farmers compared to other vegetable crops. However, Ethiopia's national average tomato fruit yield was yet low compared with the world average productivity and even when compared with neighboring African countries such as Kenya [4].

The tomato fruit contains abundant and well-balanced nutrition consisting of minerals (calcium, iron, and phosphorus), vitamins (vitamin A and vitamin C), various micronutrients, antioxidants, protein (essential amino acids), sugar, dietary fiber (pectin), and citric acid [1]. Furthermore, the tomato's red pigment, lycopene, has a high antioxidant capacity against oxygen radicals, which are thought to cause cancer, aging, arteriosclerosis, and other diseases [5].

Vegetable crops including tomatoes are extremely prone to soilborne pathogens which result in significant yield and quality deterioration. Bacterial wilt caused by *R. solanacearum* is one of the most devastating plant pathogens [6]. This bacterium affects economically important crops, mainly solanaceous family such as tomato, potato, pepper, and eggplant [7]. *R. solanacearum* possesses a high broad host range globally; it has over 450 host species representing 54 plant families [8, 9], and this pathogen is responsible for severe crop losses worldwide. This pathogen ranked as the second most important bacterial pathogen among the top ten economically important soilborne pathogens that cause severe yield losses on different solanaceous crops in various parts of the world [10]. *R. solanacearum* causes tomato bacterial wilt, which causes major damage to tomato crops in tropical, subtropical, and temperate climates [11, 12].

Tomato is one of the most susceptible crops, and *R. solanacearum* can completely destroy tomato harvest [13, 14]. The presence of *R. solanacearum* in small-holder farms can discourage farmers from planting vegetables and may result in a significant reduction in food supply [13]. This pathogen causes substantial yield losses depending on cultivar, climate, soil type, cropping practices, and pathogen strain [14].

The bacterial wilt of tomato is generally difficult to manage due to several reasons [15, 16]. Chemical soil treatment, commonly applied management options, presents the risk of soil and water contamination. Bactericide residues are toxic to human, plant, and soil health as well as contribute to the emergence of pesticide-resistant varieties [17]. Particularly, applying the chemical method in the field is difficult because the bacterium is located inside the plant xylem and at depth in the soil [18]. In addition, there is no effective chemical product available for *Ralstonia*-induced wilt. A comprehensive review by Sharma et al. [19] outlined multiple advantages of biocontrol methods. Biological control agents are thus known to be effective alternatives to control this pathogen as well and are increasingly being applied in the field.

Rhizosphere soils of the host plant are an important source of plant growth-promoting rhizobacteria (PGPR), such as antagonistic bacteria against *R. solanacearum* [20]. PGPR are potential biological control agents, as they are known for growth promotion as well as disease reduction in crops [20]. Rhizobacteria use a wide range of mechanisms involved in the suppression of plant pathogens. For example, PGPR benefit the host plant through the expression of multiple activities that act directly and indirectly to inhibit the activities of pathogens and promote plant health [21].

The occurrence of bacterial wilt disease in Ethiopia was reported in 1956 for the first time and has been known to cause significant yield loss on tomatoes [22]. Since then, it has been one of the most important and widespread bacterial plant pathogens in Ethiopia, mainly in the off-cropping season. Disease incidences of *R. solanacearum* have reached as high as 55% on tomatoes in major tomato-producing areas of Ethiopia [23].

Biological control of bacterial wilt could be achieved using various species of antagonistic rhizobacteria, such as *Bacillus* and *Pseudomonas* species, which have been frequently isolated from potato and tomato rhizosphere samples in Ethiopia [24]. However, limited studies have been conducted in Ethiopia regarding the exploration of the biological control potential against tomato wilt disease. Therefore, this study was initiated to screen and evaluate the antagonistic potential of rhizosphere bacteria against tomato bacterial wilt causing *R. solanacearum* in vitro.

2. Materials and Methods

2.1. Description of Study Area. Tomato fields that are heavily infected with bacterial wilt disease were identified from Toke Kutaye district (Guder) area of farmer's field in the Oromia region where it is located 126 km from Addis Ababa and 12 km west of Ambo. This area has a latitude and longitude of 8°58′N and 37°46′E, respectively, with an elevation of 2101 meters above the sea level. It receives an annual mean rainfall of 812–1699 mm and the average temperature is 16.22°C. It is famous for its Guder River Falls and year-round fruit production, using plentiful water resources in the surrounding area where it promotes tomato production using irrigation activities [25].

2.2. Study Design and Period. The study was experimental aiming at evaluating the antagonistic efficacy of rhizosphere bacteria against tomato disease causing *R. solanacearum* in vitro. The study was conducted from November 2020 to June 2021.

2.3. Sample Size and Sampling Techniques. Thirty infected tomato plants and other 30 rhizosphere soil samples (from healthy tomato rhizosphere) were collected from farmers' field. The tomato samples were selected purposively by observing the visible symptoms of infected tomato plants with wilt for *R. solanacearum* and healthy tomato plant rhizosphere for rhizosphere bacteria.

Furthermore. field diagnosis was done by critically observing the visible symptoms of bacterial wilt, presence of adventitious roots that were starting to appear on the stem, the collapse of the stem and milky-white, and slimy ooze exudates from the stem [26]. Tomato plant samples that showed wilt symptoms were collected from five farmers' fields and brought to the laboratory using polyethylene bags, and isolation of the target pathogen was performed within 72 h.

2.4. Streaming Test. Streaming tests were performed on the infected tomato plant to diagnose the presence of *R. solanacearum* ooze. Stems of infected tomato plants were

cut above the soil level; the cut surfaces were suspended in a test tube containing clean water, and it appeared as cloudy streaming down [16].

2.5. Media Preparation. The media were prepared as described by Schaad et al. [27]. Nutrient agar (NA), King' B media (KB), and triphenyl tetrazolium chloride (TZC) agar were prepared for culturing of *Bacillus*, *Pseudomonas*, and *R. solanacearum,* respectively. TZC media contain (g/L) casamino acid 1, peptone 10, and glucose 5, and for solid media, agar 17 was used for culturing of *R. solanacearum*. The media pH was adjusted to 6.5–7.0 using NaOH and HCl and sterilized at 121°C for 15 minutes. The media was cooled to 55°C; 5 mL of 0.5% filter sterilized TZC stock solution was added and poured into Petri plates to solidify.

2.6. Isolation of Ralstonia solanacearum. Isolation of *R. solanacearum* was performed as described by Kelman [28]. One gram of infected tomato root and stem samples was washed with tap water, sequentially surface sterilized using 2% sodium hypochlorite (NaOCl), 70% ethanol, and rinsed repeatedly in sterile distilled water. Samples were then aseptically crushed using a mortar and pestle and suspended in 10 mL of sterile distilled water for 30 minutes. Then, 0.1 mL of the suspension was spread onto the TZC agar and incubated at 30°C for 36 h. Typical colonies of *R. solanacearum* were isolated and purified on NA and preserved in 20% glycerol for the subsequent tests. Identification of *R. solanacearum* was done based on colony characteristics on TZC agar plate, Gram reaction, motility tests, cytochrome oxidase test, and other several biochemical tests [28, 29]. Moreover, virulent *R. solanacearum* colonies were differentiated from avirulent strains based on colony characteristics.

2.6.1. Hypersensitivity Test. The hypersensitivity test was conducted to determine the ability of the isolates to induce a hypersensitivity reaction. A single colony of *R. solanacearum* showing virulent characteristics was cultured in the NA medium. Approximately, 10^8 CFU/mL of freshly cultured bacterial suspensions was prepared using 0.5 McFarland turbidity standards. The suspension of *R. solanacearum* was injected into the interveinal or leaf node areas of *Pelargonium zonale* leaves and placed under greenhouse conditions at 26°C and relative humidity of 60% [30]. The collapse of the tissue after 48–72 h was recorded as a positive result. Groups of tomato plants that did not receive the bacterial suspension were used as negative controls.

2.6.2. Biovar Determination. Categorization of the *R. solanacearum* isolates into biovars levels was done based on the degradation of disaccharides (sucrose, lactose, and maltose) and hexose alcohols (mannitol, sorbitol, and dulcitol) [29, 31, 32]. Standard biovar test medium (basal medium) was prepared by adding (g/L) 1 $NH_4H_2PO_4$, 0.2 KCl, 0.2 $MgSO_4.7H_2O$, 1.0 bactopeptone, 3.0 agar, and 0.03 bromothymol blue into a final volume of 1 L of distilled water [27]. 10 mL of each of 10% solutions of dulcitol, mannitol, and sorbitol was separately added to conical flasks containing basal media. The mix was boiled, and the pH was adjusted to 7.0. Five mL of each mix was poured into test tubes and autoclaved at 121°C for 15 minutes. Similarly, 10 mL of each of the 10% filter sterilized lactose, maltose, and cellobiose solutions was separately added to sterilize and unsolidified basal media aseptically and mixed gently. Five mL solution of this mix was added into sterilized test tubes and allowed to solidify at room temperature. Fresh cultures of *R. solanacearum* isolates were inoculated and incubated at 30°C and for 2–5 days. Control groups were kept without bacterial inoculation. After incubation, the color change from olive green to orange (yellow) color was observed and recorded as a positive result.

2.7. Isolation of Rhizosphere Bacteria. Rhizosphere soil samples were collected from healthy tomato plants at a depth of 1–5 cm, as well as from highly tomato wilt infected farms. The samples were placed in polyethylene bags, maintained at 4°C, and transported to the Ambo Agricultural Research Center (AARC) bacteriology laboratory. One gram of sieved soil sample was added into test tubes containing 9 mL sterile distilled water and shaken with a vortex mixer for 5 minutes at 120 rpm. Then, ten-fold serial dilutions (from 10^{-1} to 10^{-9}) were prepared. A 0.1 mL of the suspension from 10^{-6} dilution (for *Pseudomonas* spp.) and 10^{-8} dilution (for *Bacillus* spp.) were spread on to King' B and nutrient agar, respectively [33]. To be able to isolate spore-forming *Bacillus* spp., diluted samples were heat-treated (at 80°C) for 10 minutes to eliminate vegetative cells, nonspore-forming bacterial and fungal spores before dispensing them onto nutrient agar [34].

Finally, the plates were incubated at 30°C for 36 h. Distinct bacterial colonies were then transferred to nutrient broth (NB); repeated subculturing was done to obtain pure cultures, and the pure isolates were preserved at −20°C in 20% glycerol.

2.7.1. Identification of Rhizosphere Bacteria. Rhizosphere bacteria were identified based on colony morphology, Gram test, motility test, oxidase test, and other several biochemical tests as described in the literature [35–38].

2.8. Screening Antagonistic Potential of Rhizosphere Bacteria against R. solanacearum. Preliminary screening of the antagonistic effect of rhizosphere bacteria against *R. solanacearum* was done using the dual culture (cross-culture) method as described by Ganesan and Gnanamanickam [39]. In this method, the rhizosphere bacteria isolates were streaked across the Petri plates containing nutrient agar and incubated at 30°C for 4 days. Then, *R. solanacearum* cultures were streaked perpendicular to the rhizosphere bacteria isolates. The Petri dish inoculated with pathogen alone in the absence of the antagonist was serving as a control, and the experiment was done in triplicate. Isolates showing antagonistic effects (inhibition zones)

against the tested pathogens were observed and selected for further experiments [40].

2.9. Evaluation of the Antagonistic Effects of Selected Rhizosphere Bacteria against R. solanacearum. Efficacy of the potential isolates against *R. solanacearum* was done by using the disc diffusion method [41]. The rhizosphere bacteria isolates were cultured on nutrient broth in conical flasks on a shaker and incubated at room temperature for 5 days. The culture was then centrifuged at 10000 rpm for 10 min, and the supernatant was used to test the efficacy. *R. solanacearum* suspension of about 10^8 CFU/mL was prepared using 0.5 McFarland turbidity standard and spread onto Mueller-Hinton agar using a cotton swap. Sterile 6 mm standardized paper disks were loaded with the supernatant and allowed to stay for 30 minutes. The discs were then placed on *R. solanacearum* cultured plates. The Petri dishes inoculated with the pathogen alone in the absence of the antagonist were used as negative controls, while cultures of *B. subtilis* were used as positive controls. Cultures were incubated at 30°C for 36 h, and the experiment was done in triplicate. After incubation, the radial growth inhibition of *R. solanacearum* was measured using a digital caliper.

2.10. Effect of Temperature, pH, and Salt on the Growth of Rhizosphere Bacteria Isolates. The effects of pH, NaCl, and temperature levels on the growth of *Pseudomonas* and *Bacillus* spp. were determined on basal nutrient broth media [42]. In the basal media, the desired NaCl concentrations and pH values were adjusted prior to autoclaving. The pH values were adjusted at 3, 5, 7, and 9; the NaCl concentrations at 2, 5, and 10%; and the temperature at 15, 25, 35, 45, and 80°C. An equal volume (0.1 mL) of the stock cultures of selected isolates was inoculated into each of the desired pH, NaCl, and temperature levels and incubated for 1–3 days. Before and after incubation, ten-fold serial dilutions (from 10^{-5}–10^{-9}) depending on the bacterial growth (cell turbidity) were prepared and bacterial colony counts were made on nutrient agar. The experiments were done in triplicate.

2.11. Data Analysis. The data were analyzed using SPSS software version 20. Descriptive statistics (mean and standard deviation) were used to present the inhibition zone due to each rhizosphere bacterial isolate against *R. solanacearum*. One-way analysis of variance (ANOVA) with Duncan's multiple range test was used to compare the means of the three replications of inhibition zones of rhizosphere bacteria with a level of significance considered as $P \leq 0.05$. The results are given in tables.

3. Results

3.1. Identification of Virulent Strains of R. solanacearum. A total of 36 bacterial colonies were isolated from the samples collected from wilted tomato plants, out of which 7 isolates exhibited the characteristic feature of *R. solanacearum* colony (Table 1, Figure 1). Virulent

Table 1: Hypersensitivity reaction of *Pelargonium zonale* leaves node to infiltration with isolates of *R. solanacearum*.

Isolate code	Reaction	
	After 48 h injection	After 72 h after injection
RS1	No reaction	No reaction
RS3	Have reaction	Collapse of infiltrating area
RS7	Have reaction	Collapse of infiltrating area
RS8	Have reaction	Have reaction
RS12	No reaction	Have reaction
RS13	No reaction	No reaction
RS27	No reaction	Have reaction
Control group	No reaction	No reaction

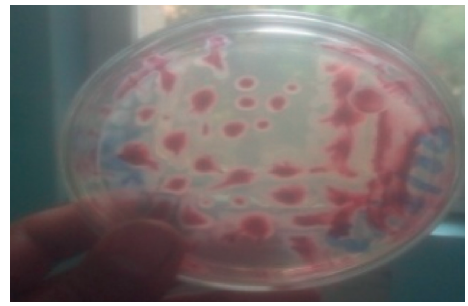

Figure 1: *R. solanacearum* colonies with its characteristic features.

R. solanacearum colonies (large irregular shaped, fluidal and opaque colonies, white to cream color, slimy to pink or red color in the center, and elevated) were then differentiated from the avirulent strains (smaller, off-white, dark red margin, and nonfluidal or less fluidal colonies) on TZC agar media [28]. All of the 7 isolates possessed cultural features of virulent *R. solanacearum* [16, 28, 29, 43] (Additional file 1).

3.2. Hypersensitivity Test of R. solanacearum. Except isolates designated as RS1 and RS 13, all others showed localized cell necrosis and distinct color changes at the spreading edge on the leaves of *Pelargonium zonale* after 48 and 72 h of injection. There was variation in the extent of the reaction (from heavy to collapse of infiltrating areas) between the five isolates (Table 1). Isolates RS3 and RS7 were relatively more virulent and caused collapse after 72 h of inoculation.

3.3. Identification of R. solanacearum Using the Biochemical Test. All the tested isolates had similar test results on the tested parameters except H_2S production, where only two isolates produced H_2S gas (Additional file 1).

3.4. Differentiation of R. solanacearum into Biovars. Change of the initial olive green media to yellow color indicates the ability of inoculated isolates to digest the given substrate. Accordingly, all the tested isolates were set to biovar III (Additional file 1).

3.5. Identification of Rhizosphere Bacteria. A total of 57 bacteria were isolated from healthy tomato rhizosphere soil. Fourteen isolates demonstrated antagonistic activity against

Table 2: Mean radial zone of inhibition produced by *B. subtilis* (reference isolates) and rhizosphere bacteria (*Bacillus* and *Pseudomonas* spp.) against *R. solanacearum*, $n = 3$.

Groups	Isolate code	Inhibition zone in mm (mean ± SD)
AARC isolates	Abac13	12.67 ± 0.57^{bc}
	Abac14	10.3 ± 0.57^{e}
	Abac2	10 ± 1^{ef}
	Abac4	12.3 ± 1.5^{bcd}
Isolated rhizobacteria	B28	13.3 ± 0.57^{ab}
	B1	12.3 ± 1.5^{bcd}
	B12	7 ± 0^{h}
	B13	11.3 ± 0.57^{cde}
	B2	10.67 ± 0.57^{de}
	B7	9.67 ± 0.57^{ef}
	P1	8.67 ± 1.15^{fg}
	P12	8 ± 1^{gh}
	P2	11.3 ± 1.5^{cde}
	P23	12.3 ± 0.57^{bcd}
	P4	10.3 ± 0.57^{e}
	P5	12 ± 0^{bcd}
	P6	14.67 ± 0.57^{a}
	P7	11 ± 1^{cde}

Each data point is the mean of three replicates. The means with different letters are significantly different from each other as evaluated by Duncan's multiple range tests (DMRT) analysis at $p \leq 0.05$. Means with the same latter are not significantly different. AARC, Ambo Agricultural Research Center.

Figure 2: Antagonistic activities (inhibition zones) of the different rhizosphere bacteria against the pathogen (*R. solanacearum*).

R. solanacearum. The 14 isolates were identified based on different colony characteristics on King' B media or nutrient agar and biochemical tests (Additional files 2(a)–2(c)). Eight of the 14 isolates were tentatively identified as *Pseudomonas* spp., while the rest were as *Bacillus* spp. (Additional file 2(b)).

3.6. In Vitro Evaluation of Antagonistic Activity of Rhizosphere Bacteria against R. solanacearum. *R. solanacearum* isolate designated as RS3 was selected for this test. The antagonistic activity of the 14 isolates is given in Table 2 and Figure 2. The observed radial inhibition zone for the isolates ranged 7–14.66 mm, and there were significant variations in the mean diameter of the inhibition zones ($P < 0.05$). Isolates designated as P6 (14.66 mm), B28 (13.33 mm), P23 (12.33 mm), B1 (12.33 mm), P5 (12 mm), P2 (11.33 mm), B13 (11.33 mm), and P7 (11 mm) have shown better performances to inhibit the growth of *R. solanacearum* in vitro. The maximum zone of inhibition (14.66 mm) was recorded by isolate P6, which was significantly higher even from the positive control.

3.7. Effects of Temperature, pH, and NaCl Levels on the Growth of Selected Rhizosphere Bacteria Isolates. The growth of eight isolates showing high antagonistic activity against

Table 3: Growth expressed as CFU/mL ($\times 10^6$) at different temperature, pH, and NaCl levels values.

Inoculum at different conditions		*Pseudomonas* isolates					*Bacillus* isolates		
		P2	P5	P6	P7	P23	B1	B13	B28
Initial/inoculum size		4.6	4.3	4.4	4.7	4.2	4.3	4.5	4.7
Temperature (°C)	80	3.1	4.1	3.6	4	3.8	241000	274000	286000
	45	1020	750	1200	860	1400	280000	273000	251000
	35	278000	221000	266000	220000	256000	288000	293000	281000
	25	292000	220000	278000	222000	284000	294000	270000	288000
	15	297000	220000	236000	220000	259000	273000	290000	262000
pH	3	1120	990	700	4.2	890	4.8	4.4	3.9
	5	20000	261000	277000	229000	291000	860	750	268000
	7	260000	269000	236000	284000	251000	261000	270000	288000
	9	25000	261000	236000	272000	289000	960	840	15000
NaCl concentration (%)	2	241000	262000	277000	253000	269000	286000	260000	274000
	5	910	1060	1190	20000	19100	17300	18900	21000
	10	4.4	4.2	3.6	4	3.8	990	1250	1400

R. solanacearum was observed at different levels of temperature, pH, and NaCl concentration, and all of them grew at temperatures ranging 15–45°C, pH values 5–9, and NaCl concentrations 2–5% (Table 3). The selected three *Bacillus* spp. grew at all conditions except at pH 3. However, the five selected *Pseudomonas* spp. did not grow at the highest values of temperature and pH (80°C and pH 10), but most (4 out of 5) grew at pH 3 as well (Table 3).

4. Discussion

Globally, tomato production provides nutritional, economical, and health benefits to the wider society. However, its productivity has always been challenged by several factors including bacterial wilt, a disease caused by *Ralstonia solanacearum*. This study aims to contribute scientific information to the continued efforts addressing the potential use of indigenous (rhizosphere) bacteria to control bacterial wilt disease of tomatoes. Accordingly, virulent *R. solanacearum* (from wilted tomato plants) and antagonistic bacteria (from the rhizosphere of healthy tomato plants) were isolated from tomato farms in central Ethiopia. The antagonistic activities of the rhizobacteria were tested against the virulent *R. solanacearum* isolated.

All isolated *R. solanacearum* showed more or less similar characteristics, except for some colony features and H_2S production. Variations among strains of *R. solanacearum* in the production of H_2S were also reported [16]. Such physiological differences may have a direct influence on the pathogenicity potential among strains of *R. solanacearum*. In addition, all *R. solanacearum* isolates were found to be biovar III type. In line with this, biovar III types were reported as the major causative agent of tomato wilt [44, 45]. *R. solanacearum* isolated in this study also showed a variable degree of hypersensitivity reaction (Table 1). Pathogenicity variations among virulent strains of *R. solanacearum* were also reported by Popoola et al. [43] and Seleim et al. [46]. Environmental drivers (factors) could be the causes for the emergence of genetically diverse types among strains.

The isolated rhizobacteria were closely related to *Pseudomonas* and *Bacillus* species based on biochemical and cultural characteristics (Additional files 2(a)–2(c)). A number of previous studies also documented several members of *Pseudomonas* and *Bacillus* species having antagonistic activities against *R. solanacearum* [21, 47, 48]. *Bacillus* and *Pseudomonas* species are well-known bacterial antagonists that have the ability to suppress the growth of bacterial phytopathogens including *R. solanacearum* [49]. Various species of antagonistic rhizobacteria, such as *Bacillus cereus, B. subtilis, Paenibacillus macerans, Serratia marcescens, B. pumilus, Pseudomonas putida*, and *P. fluorescens* against bacterial wilt, were isolated from rhizosphere soil samples collected from potato and tomato in Ethiopia [21, 23, 24]. Moreover, the potential use of *Pseudomonas* strains to develop active biocontrol against tomato bacterial wilt is documented in several studies. *Pseudomonas* is known to possess many traits that make them well suited as biocontrol and growth-promoting agents [50, 51]. Several *Pseudomonas* species were reported to be effective against a broad spectrum of plant pathogens [52]. A study by Mohammed et al. [53] showed that *Pseudomonas* species, which were isolated from the rhizospheres of tomato plants, had significantly reduced the incidence of bacterial wilt and promoted the growth of tomatoes. Regarding *Bacillus* spp., they have also been frequently isolated from the rhizosphere of tomato plants and found to possess antagonistic activity for several plant pathogens [45, 54]. Cao et al. [55] reported *Bacillus* species isolated from rhizosphere soil of tomatoes, which possessed strong antagonistic ability against *R. solanacearum*. Furthermore, another study in Nigeria by Akintokum et al. [56] demonstrated the effectiveness of *Bacillus* spp. against *R. solanacearum* in a greenhouse condition.

In this study, comparable antagonistic activities to the positive controls were recorded from 5 isolates (3 *Pseudomonas* and 2 *Bacillus* spp.). Even, a higher inhibition zone (14.67 mm) was recorded by a *Pseudomonas* isolate compared to the positive control (12.67 mm). A similar study by Huang et al. [57] reported inhibition zones ranging from 11.2 to 15.2 mm for bacteria isolated from the rhizosphere of healthy tomatoes. A higher inhibition zone (up to 30.5 mm) was also recorded by bacteria isolated from the

rhizosphere of diseased tomatoes further ascertaining that the rhizosphere of healthy as well as wilted tomatoes are important sources of antagonistic bacteria against *R. solanacearum*.

The rhizobacteria isolated in this study exhibited variable optima in terms of temperature, pH, and salt levels. However, all of the isolates could grow in a wide range of temperature (15–45°C), pH values (5–9), and NaCl concentrations (2–5%), which are important features in terms of field application. Particularly, the *Bacillus* spp. grew at all conditions, except at pH 3, showing its high elasticity in terms of the tested environmental parameters. Similarly, Chari et al. [47] documented abiotic stress-tolerant plant growth-promoting *Bacillus* spp. isolated from different rhizospheric soils. The five selected *Pseudomonas* spp. in this study did not grow at the highest values of temperature and pH (80°C and pH 10), but most of them could tolerate pH 3, implying that they can be good candidates of biocontrol agents in acidic soils.

5. Conclusions

The results of this study extend the knowledge that the rhizosphere of the host plant can be an important source of antagonistic microorganisms against pathogenic bacterial plant diseases. Among the rhizobacteria, members of *Bacillus* and *Pseudomonas* species have been potential antagonistic bacteria against plant diseases, such as *R. solanacearum*. In this study, the isolated antagonistic rhizobacteria against *R. solanacearum* were identified as *Bacillus* or *Pseudomonas* species. The isolates demonstrated strong activity against *R. solanacearum* showing their potential use as a biocontrol agent against this tomato bacterial wilt [58]. Studies at greenhouse and field levels and strain-level identification of the isolates are required to get insights into the real application of the isolates in protecting the tomato field from such devastating pathogens.

Data Availability

The data generated or analyzed during this study are included within this article and its additional files.

Disclosure

The funding body does not have any role in the design of the study and collection, analysis, and interpretation of data, and in writing the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

TG and SY conducted the experiment. BS prepared the manuscript. All authors conceived and designed the study and edited, read, and approved the final manuscript.

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Supplementary Materials

Additional file 1: characterization of *R. solanacearum* isolated from the wilted tomato plants, central Ethiopia, 2021. Additional file 2a: morphological characterizations of isolated bacteria from healthy tomato rhizosphere, central Ethiopia, 2021. Additional file 2b: biochemical characterization of the isolated bacteria from healthy tomato rhizosphere, central Ethiopia, 2021. Additional file 2c: characterization of the bacteria isolated from the rhizosphere of healthy tomato plant against the different carbohydrate utilization, central Ethiopia, 2021. (*Supplementary Materials*)

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