Ethiop. J. Biol. Sci. **22(1): 41–60, 2023**
 ISSN: 1819-8678

RESEARCH ARTICLE

PHYTOBENEFICIAL PROPERTIES OF TOMATO (*LYCOPERSICON ESCULENTUM* L.) RHIZOSPHERIC BACTERIA AGAINST BACTERIAL WILT PATHOGEN (*RALSTONIA SOLANACEARUM*) UNDER GREENHOUSE CONDITIONS

Ayele Gucho[1]* and Fassil Assefa[1]

ABSTRACT: Bacterial wilt caused by *Ralstonia solanacearum* is the second most important pathogen causing bacterial wilt diseases in horticultural plants. In Ethiopia, the pathogen is widely distributed, affecting the production of tomatoes. This study was designed to isolate and characterize the pathogenicity of the local strains and study the potential antagonistic rhizobacteria (biological control agents) for potential control of the homologous pathogen using standard methods. All isolates displayed fluid and irregular colonies with pink or light red colours at the centre on the TTC medium typical features of *R. solanacearum* and showed a 60 to 96% wilting disease index percentage after 4 weeks of inoculation. The tomato varieties showed variation in disease resistance to the pathogen, with disease index ranging from 0.34 to 0.86, of which 30% of the tested tomato varieties such as the local Awash, Cochoro, Melkashola, and the commercial variety (Venise) were moderately resistant. Out of the 120 rhizobacteria isolates, 27 (23%) were antagonistic to the test pathogen and classified into their respective taxa based on their cultural and biochemical characteristics in relation to standard reference strains. Thus, the isolates were tentatively identified as *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, and *Bacillus thuringiensis*; *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas aeruginosa*. The isolates were further characterized for additional plant growth promoting properties (PGPP). Most isolates were capable of producing hydrogen cyanide (88%), indole-3-acetic acid (IAA) (83%) (147–645 ug/ml), ammonia (76%); whereas 53% of the isolates solubilized inorganic calcium phosphate (280–471 ug/ml). The isolates *Bacillus subtilis* AAUB14, *Bacillus pumilus* AAUB13 and *Bacillus pumilus* AAUB11 showed the highest number of PGPP as potential candidates for plant protection and health promotion provided that they are validated under greenhouse and field conditions.

Key words/phrases: Antagonism, Disease index, IAA, Moderately resistant, Phosphate solubilization.

[1]Department of Microbial, Cellular, and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. E-mail: ayu22g@gmail.com; asefafasil2013@gmail.com
*Author to whom all correspondence should be addressed

INTRODUCTION

Ralstonia solanacearum, formerly known as *Pseudomonas solanacearum* is a bacterial pathogen that causes bacterial wilt disease in solanaceous species such as tomato, chili, eggplant, and is widely distributed in tropical, and subtropical regions (Yabuuchi *et al*., 1995). Mansfied *et al*. (2012) reported the pathogen is ranked, next to *Pseudomonas syringae* pathovars, as the second most common of the top ten bacterial species causing plant wilt diseases. The severity of the disease is influenced by the host, cultivar, climate, soil type, cropping system, and strain of the pathogen. The pathogen is widely distributed in Ethiopia, with an incidence as high as 45% (Yaynu and Korobko, 1986; Henok Kurabachew and Getachew Ayana, 2016).

The tomato (*Lycopersicum esculentum*) is one of the economically important vegetables severely affected by bacterial wilt infection contributing up to 55% of crop loss in the country (EARO, 2002). Because of its high survival capacity in a variety of environments and wide host range, tomato infection by *R. solanacearum* is extremely difficult to control using chemical pesticides (Nguyen and Ranamukhaarachchi, 2010). The continuous use of agro-chemicals is hazardous to human health and natural ecosystems which necessitates interest in using biological control agents (BCAs) as part of integrated pest management (IPM) to control bacterial wilt disease.

Plant growth-promoting rhizobacteria (PGPR) are one of the important BCA agents that suppress plant pathogens through a diverse set of mechanisms (Saxena *et al*., 2020). They are known to have multiple beneficial traits in the rhizosphere that help the plants directly or indirectly through the acquisition of nutrients, an overall improvement in growth by the production of phytohormones, protection from pathogens and other abiotic stressors. They produce lytic enzymes such as cellulase, chitinases, lipase, protease, and induce systemic resistance against phytopathogens (Saxena *et al*., 2020). They also solubilize inorganic phosphate and produce phytohormones such as indole-3-acetic acid (IAA) to promote plant growth.

The authors indicated that the genera *Bacillus* and *Pseudomonas* are the most ecologically and functionally versatile rhizobacteria. They are widely distributed in nature due to their resistance to various stresses and their long-term survival under unfavourable conditions. It is estimated that *Bacillus* species comprise up to 95% of the Gram-positive bacterial populations (Prashar *et al*., 2013).

They are extensively studied and commercially exploited in the agro-biotechnology industry. However, their potential has not been fully realized sufficiently and requires a continuous search of these bacteria to translate their PGPR function into relevant technologies from laboratory to field for the benefit of mankind. To this end, isolation and characterization based on the cultural, biochemical and genetic features of these microorganisms is essential.

Although it is difficult to easily distinguish these bacteria at the species level, a large number of routine phenotypic tests are used to tentatively distinguish them for biotechnological applications (Drobniewski, 1993). Most often, *Bacillus* spp. are detected and isolated based on the resistance of their spores to heating or ethanol and *Pseudomonas* spp. can impart different pigments on selective growth media that can tentatively differentiate them into their respective taxa (Prashar *et al*., 2013).

Pseudomonas spp. and *Bacillus* spp., alone or in combination with combinations of resistant vegetable and crop varieties, have been shown to be effective in controlling bacterial wilt caused by *Ralstonia solanacearum* on agriculturally important crops and vegetables (Yuliar *et al*., 2015; Ojesola *et al*., 2020). In Ethiopia, studies on the screening of *Pseudomonas* spp., *Serratia marcescens*, and *Bacillus cereus* (Henok Kurabachew and Wydra, 2013) and *Bacillus* spp. (Tsigie Gashaw *et al*., 2022) were undertaken to identify effective strains to suppress *Ralstonia solanacearum* pathogens and reduce bacterial wilt of tomato under greenhouse conditions.

As a result, the purpose of this study was to isolate and characterize *Ralstonia solanacearum* together with PGPR isolates from *Pseudomonas* and *Bacillus* spp. isolated from tomato plants, as well as to assess them *in vitro* biological control against selected pathogen biovars.

MATERIALS AND METHODS

Collection of soil and tomato plant samples

Tomato stem and root adherent soil samples were collected from infected standing tomatoes from farmer's fields in Meki (8°9′ N, 38°49′ E), Zeway (7°54′ N, 38°43′ E), Hawassa (7°3′ N, 38°28′ E), and Bako (9°08′ N, 37°03′ E) districts during the 2018/19 growing seasons. In addition, 120 samples of healthy tomato rhizosphere soil were collected from farmers' fields in four different districts. The soil samples were collected in triplicates, in alcohol-surface sterilized polyethylene bags and brought to the Applied Microbiology Laboratory, College of Natural and Computational Sciences,

Addis Ababa University for isolation of *Ralstonia* spp. and rhizosphere bacteria. This experiment was conducted in the Department of Microbial, Cellular, and Molecular Biology, College of Natural and Computational Science, at Addis Ababa University.

Source of tomato varieties and reference strains

Fourteen local and hybrid varieties of tomato were obtained from the Melkassa Agricultural Research Centre, Ethiopia. Reference strains of *Pseudomonas* and *Bacillus* spp., as well as *Ralstonia solanacearum* APPRCRS, were obtained from the Ethiopian Biodiversity Institute's (EBI) database, the culture collection of Addis Ababa University's (AAU) College of Natural Sciences, and the Ambo Plant Protection Research Centre in Ethiopia (Table 1). This reference strains were used for comparison and check this study isolate similarity based on morphological and biochemical tests result.

Table 1. Source of the reference strains used for comparative studies.

Code of reference strain (RFS)	Reference strain	Isolation host	Institute
RFS1	*P. fluorescens* biotype G	Teff (*Eragrostis teff*)	EBI
RFS2	*P. aeruginosa*	Teff	EBI
RFS3	*P. putida* biotype B	Teff	EBI
RFS4	*P. mendocina*	Teff	EBI
RFS5	*B. cereus*/ *pseudomycoides*	Teff	EBI
RFS6	*B. subtilis* AAU 17	Soybean	AAU
RFS7	*B. pumilus* ANT7	Tomato	AAU
RFS8	*B. megaterium* ANP	Pepper	AAU
RFS9	*Ralstonia solanacearum*	Tomato	Phytopathology Institute

Isolation and characterization of *Ralstonia solanacearum*

Infected tomato plant stems were washed with tap water and surface sterilized with a 1% sodium hypochlorite solution, rinsed three times in sterilized distilled water and air dried. Then, 0.5–1 cm of stems were plated onto Kelman's 2, 3, 5 triphenyl tetrazolium chloride (TTC) agar medium (Kelman, 1954) and incubated in triplicates at 28 ± 2°C for 48 hours. Typical colonies developing a creamy white colour with a pink centre were picked as virulent strains of *Ralstonia* spp. They were gram-stained and observed for cultural characteristics, i.e., colony colour, and shape (Hayward, 1964). The ability of *R. solanacearum* isolates to oxidize disaccharides (maltose, lactose, and cellobiose) and hexose alcohols (mannitol, sorbitol, and dulcitol) were tested to differentiate them into biovars (Hayward, 1964).

Isolation and characterization of rhizosphere bacteria

Rhizosphere bacteria were isolated according to Kumar *et al*. (2012). Soil and root samples from tomato plants were prepared to appropriate dilutions (10^{-1} to 10^{-4}) with sterile distilled water and were spread on nutrient agar plates. The inoculated plates were incubated for 48 hours at 28 ± 2°C. Single colonies of different morphological characteristics, such as size, shape, colour, elevation, and margin were identified from different plates streaked with diluted samples. Representative colonies were repeatedly sub-cultured to obtain pure colonies and preserved at −20°C for further studies.

Selection of *Bacillus* and *Pseudomonas* species

The isolates were regrown on nutrient broth for 24 hours after which 100 l of each sample was transferred to nutrient agar medium, the King B (King *et al*., 1954), to detect *Pseudomonas* spp.; whereas the same culture cohort samples were heated at 80°C for 15 minutes to enrich *Bacillus* spp. All plates were incubated for 24–72 hours at 28 ± 2°C.

Identification of bacteria isolates

Rhizosphere bacteria were identified based on colony morphology, Gram staining, and biochemical tests, including the oxidase, MRVP, starch hydrolysis, casein hydrolysis, gelatin liquefaction, and nitrate reduction tests (Cappuccino and Sherman, 1992). They were also tested for utilization of selected carbohydrates, and their ability to grow at 4 and 50°C on nutrient medium. Plates were examined for fluorescent production at 24, 48, and 72 h after incubation under UV light at 360 nm (Sylvania Blacklite Blue Tubes F 15T8-BLB). The morphological and biochemical characteristics were examined according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Biochemical tests for *P. fluorescens*

Certain biochemical tests were carried out to identify *P. fluorescens* in accordance with Bergey's Manual for Determinative Bacteriology.

Starch hydrolysis

Filter paper was dipped in a dry old culture suspension and incubated for two days in Petri dishes containing starch-agar medium. The plates were then flooded with a one-percent iodine solution. A colourless halo around the grown and blue colour in the rest of the plates indicated that the microorganism was utilizing starch.

Gelatin liquefaction

Filter paper discs were dipped in a day-old culture suspension and were placed on Petri dishes containing gelatin nutrient agar medium. The Petri dishes were incubated at 30°C for two days and then flooded with a 12.5% HgCl solution. The development of a yellow halo around the growth indicates the utilization of gelatin.

Fluorescent pigment

The test tubes containing sterilized Kings B medium were inoculated with the isolate of *Pseudomonas* spp., incubated for five days, and observed. Yellowish-green fluorescent pigment observed under UV light (365 nm) indicated positive results.

Lecithinase production

Lecithinase production was determined on plate count agar containing 10% egg yolk emulsion (egg yolk agar; Difco). After incubation at 30°C for up to 5 days, plates were observed for the presence of colonies surrounded by opaque zones.

Pathogenicity test of isolates

A virulence study of selected isolates was carried out according to Artal *et al*. (2012). Seeds of the cultivar Moneymaker were surface sterilized as before and sown on trays filled with sterile soil. Bacterial isolates (*R. solanacearum*) were cultured in nutrient broth medium for two days at 28 ± 2°C and adjusted to 10^8 cfu/ml (an optical density of 0.7) using a spectrophotometer model 6405UV/VIS at 600 nm. Twenty-one day-old tomato seedlings were individually transplanted into plastic pots filled with 2 kg of sterilized loam soil, with ten replicates for each treatment. The roots of each tomato plant were wounded with a sharp, sterilized needle, inoculated with 20 ml of inoculum containing 10^8 cfu/ml of the pathogen (*R. solanacearum*), and left for 21 days. The infected plant roots were cut to re-isolate the pathogen, confirm Koch's postulates, and compute the bacterial wilt index using the following formula described by Winstead and Kelman (1952).

$$\text{Disease index (\%)} = [\sum (ni \times vi) \div (V \times N)] \times 100$$

Where, ni = the number of plants with the respective infected rating; vi = the infected rating; V = the highest infected rating (5); and N = the number of plants observed. The infected rating was determined as follows: 1 = no symptoms; 2 = one leaf wilted; 3 = two to three leaves wilted; 4 = four or

more leaves wilted; and 5 = the whole plant wilted.

Race determination and hypersensitive reaction tests

The isolates were tested for their hypersensitive reaction and race differentiation of bacterial wilt (*R. solanacearum*) on tobacco leaf according to the leaf infiltration method of Lozano and Sequeira (1970). Tobacco (*Nicotiana tabacum* L.) seeds were surface sterilized as before and sown on plastic trays filled with sterilized loamy sandy soil and grown in a greenhouse with 12/12-hour photoperiod for 30 days. Each seedling was transplanted into 2 kg sterilized soil in plastic pots, and the soil mixture of sand: loam soil: compost (1:3:1). After 45 days of post-transplanting, the leaves were injected with a suspension of 10^8 cfu/ml bacterial isolates using a syringe and leaves injected with distilled water were used as a negative control (Klement *et al*., 1964). The plants were observed daily (from 24 h to two weeks) to check for necrotic reaction or yellowing regions in the vicinity surrounding an infection point.

Screening of the isolates for antagonism against *R. solanacearum*

The isolates were screened for antagonistic properties against *R. solanacearum* pathogens (test organisms) using the dual culture (cross-culture) method according to Dhingra and Sinclair (1995). Thus, each isolate was streaked on Petri plates containing nutrient agar and incubated at 30°C, for 48 h, after which a 24 hour. *R. solanacearum* culture was streaked perpendicular to the isolate. They were incubated at 30°C for 24 to 48 h and observed for the presence of growth inhibition between the antagonist and the test organism.

Selection of antagonistic rhizosphere bacteria against *R. solanacearum*

The antagonistic properties of the rhizosphere bacteria against *R. solanacearum* were evaluated using the disc diffusion method (Hudzicki, 2009). The rhizosphere bacterial isolates were grown on 100 ml of nutrient broth (Oxoid) in 250 ml conical flasks on a shaker (ZJZD-III, Shanghai, China) at 30°C for three days. The culture was then centrifuged (Wagtech international, United Kingdom) at 10,000 rpm for 15 minutes to recover the culture filtrates. Then, a 24 h old culture of the test pathogen (*R. solanacearum)* was spread in triplicate onto nutrient agar medium (Oxoid) and left for hours on which 6 mm filter paper discs impregnated with each culture filtrate were placed in the centre and incubated at 30°C for 48 h to detect inhibition zones around the discs.

Pattern of resistance of different tomato varieties for resistance to selected *R. solanacearum* isolates RS2

Seeds of fourteen tomato varieties (Bishola, Chali, Cochoro, Fetan, Galilama, Melkasalsa, Metadel, Miya, Melkashola, Arp tomato, Moneymaker, Venise, Awash, and Roma VF) were collected from the Melkasa Agricultural Research Centre, seed agencies and used for evaluation of resistance against the pathogens. Seeds were surface-sterilized as before and sown separately in sterilized soil trays in the greenhouse. The three-week-old tomato seedlings were transplanted into two-kilogram plastic pots (three pots per cultivar) filled with loam soil in a greenhouse. Forty-eight-hour cultures of *R. solanacearum* RS2 isolated in this study were prepared on tetrazolium medium (Kelman, 1954) and adjusted to a concentration of 10^8 from which 20 ml was injected with a hypodermic syringe into the stems slightly below the petiole inoculations were made one week after transplanting. Non-inoculated pots were also included as a control and all pots were arranged in a completely randomized design (CRD) with three replications under greenhouse condition. The wilt symptoms and wilted tomato plants were recorded and graded with a disease rating scale of 0–5 after 21 days of inoculation. Each variety was computed using the disease index of bacterial wilt using the following formula. After that, the tomato cultivars were classified as resistant, moderately resistant or susceptible according to Aslam *et al*. (2017).

Disease severity index = $\sum (m \times n) \div (M \times N)$

Where m = disease severity grade, n = number of plants at the grade, M = the highest disease severity grade, and N= number of total test plant.

Other plant growth-promoting characteristics of the antagonistic rhizobacteria

Production of ammonia

The qualitative assay of ammonia production by bacteria was done according to Cappuccino and Sherman (1992). The isolates were grown in test tubes with 5 ml peptone water for 4 days at 28 ± 2°C. The development of a yellow colour after adding 1 ml of Nessler's reagent indicated the production of ammonia.

Hydrogen cyanide production

Hydrogen cyanide (HCN) production was detected according to Bakker and Schippers (1987). Bacterial isolates of 100 µl (1×10^6 CFU ml^{-1}) were

incubated at 28 ± 2°C for 2–5 days in HCN induction medium (5 g/l tryptic soy broth, 0.88 g/l glycine, and 3 g/l Agar). Whatman filter paper strips were impregnated with picric acid solution (2.5 g picric acid and 12.5 g Na_2CO_3) and placed on the lids of the plates, sealed with parafilm, and incubated at 28 ± 2°C for 48–72 hours. A change in the colour of the filter paper strip from yellow to orange-brown was considered positive for HCN production.

Qualitative and quantitative determination of phosphate solubilization

The isolates were evaluated *in vitro* for their phosphate solubilization activity on Pikovskaya's (PVK) agar medium following the procedures described by Donate-Correa *et al*. (2004). The isolates were grown for 24 h and spot inoculated (10^6 CFU/ml) on the medium containing (g/l), glucose (10.0), $Ca_3(PO_4)_2$ (5.0), $MgCl_2.6H_2O$ (5.0), $MgSO_4.7H_2O$ (0.25), KCl (0.2) and $(NH)_2SO_4$ (0.1) and incubated at 30 ± 2°C for 4–7 days. The plates were observed for clear zone formation around colonies, and the phosphate solubilization index (PSI) was calculated according to the formula:

$$PSI = \frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}}$$

Following qualitative observations, the purified isolates were quantified using National Botanical Research Institute Phosphate (NBRIP) medium with tricalcium phosphate as the sole phosphate source (Nautiyal, 1999). The amount of phosphate released was measured using a known standard curve made from potassium dihydrogen orthophosphate, KH_2PO_4 (µg /ml).

Qualitative and quantitative production of indole-3-acetic acid (IAA)

The ability of bacterial isolates to produce IAA was checked qualitatively as described by Hartmann *et al*. (1983). For this, 200 µl of each bacterial culture (1×10^8 CFU ml^{-1}) was grown in 50 ml of nutrient broth amended with tryptophan (1g l^{-1}). The bacterial cultures were incubated at 30°C on an orbital shaker for 72 h. The cultures were centrifuged at 3000 rpm for 30 min, from which 2 ml of each supernatant was mixed with two drops of orthophosphoric acid and 4 ml of Salkowaski's reagent (50 ml of 70% per chloric acid and 1 ml of 0.5 M$FeCl_3$ and 49 ml sterilized distilled water), and incubated at room temperature in dark for 30 min. The development of pink colour was visually checked as an indicator of IAA production. One ml of each supernatant was quantified spectrophotometrically (6405 UV/Vis., Jenway, England) at 530 nm against a standard curve constructed with known concentrations of IAA.

Statistical analysis

Statistical analysis was done using SAS software version 9.4, with the ANOVA procedure, and the level of significance for Duncan's multiple range test (DMRT) was set at $p<0.05$.

RESULTS AND DISCUSSION

Isolation of *Ralstonia solanacearum*

All isolates were characterized by whitish-edge colonies with pink centres, which are typical features of *R. solanacearum* isolates (Kelman, 1954). They also showed positive reaction to tests for motility, oxidase, catalase, and H_2S production; growth, at 37°C; and NaCl tolerance at 1.5% (data not shown).

In the present study, the results of the biovar test showed that nineteen isolates both oxidized three disaccharides (maltose, lactose, and cellobiose) and three sugar alcohols (mannitol, sorbitol, and dulcitol) while other two isolates only utilized the disaccharides. The data showed that nineteen isolates were categorized as Biovar III, whereas the two isolates were classified into Biovar II, based on utilization of disaccharides and sugar alcohols tests according to Hayward (1964) and Shahbaz *et al*. (2015).

Pathogenicity and race determination of isolates

The pathogenicity study showed that all isolates were capable of causing wilt symptoms on tomato seedlings (Table 2). Thus, isolates RS2, RS5, and RS3 displayed the highest disease index percentages of 96%, 94%, and 90 % wilting, respectively, followed by isolates RS1, RS7-8 RS10-14, RS16, RS19, and APPRCRS with 69–85% wilting after four weeks of infection. All isolates displayed a hypersensitivity response with brown necrosis with a yellow halo on tobacco leaves after 36 hours of infection indicating that they belonged to Race 1 (Table 2). Nevertheless, isolates RS-17 and RS-20 showed yellow colour within 48 h, suggesting they belonged to Race 3 (Table 2). Both races developed rapid necrosis and complete wilting on the susceptible moneymaker tomato variety. The results generally indicated that the 21 bacterial isolates were pathogenic and could infect tomato plants, cause wilt symptoms and varied in their pathogenicity under greenhouse conditions. The pattern of wilt infection (pathogenicity) in this study (60–96%) was slightly higher than the 52% to 97% wilting observed in tomato under greenhouse experiments (Seleim *et al*., 2014) after four weeks of inoculation.

Table 2. Pathogenicity test and hypersensitivity of bacterial wilt for *Ralstonia solanacearum* isolates on moneymaker seedlings and tobacco leaves under greenhouse condition.

Isolates	Disease index after inoculation (%)		Reaction of tobacco	Race	Biovar	Pathogenicity test on money maker variety
	3 weeks	4 weeks				
RS-1	68	84	HR	1	III	+
RS-2	76	96	HR	1	III	+
RS-3	70	90	HR	1	III	+
RS-4	64	82	HR	1	III	+
RS-5	74	94	HR	1	III	+
RS-6	50	60	HR	1	III	+
RS-7	60	74	HR	1	III	+
RS-8	70	82	HR	1	III	+
RS-9	50	68	HR	1	III	+
RS-10	62	74	HR	1	III	+
RS-11	70	80	HR	1	III	+
RS-12	62	78	HR	1	III	+
RS-13	56	74	HR	1	III	+
RS-14	66	70	HR	1	III	+
RS-15	54	68	HR	1	III	+
RS-16	68	74	HR	1	III	+
RS-17	52	66	C	3	II	+
RS-18	54	62	HR	1	III	+
RS-19	60	70	HR	1	III	+
RS-20	50	64	C	3	II	+
APPRCRS	68	82	HR	1	III	+

N = no reaction, HR = hypersensitivity reaction, and C = collapse of infiltrating area. Ambo Plant Protection Research Centre *Ralstonia solanacearum* (APPRCRS)

Isolation and characterization of *Pseudomonas* and *Bacillus* spp.

In this study, a total of 27 antagonistic rhizobacteria were isolated from the rhizosphere of healthy and diseased tomato plants from the central and western parts of Ethiopia, of which 15 were *Bacillus* spp. and 12 were categorized under *Pseudomonas* spp. They were tentatively identified into their respective species based on their cultural, microscopic, and biochemical characteristics in comparison to the standard reference strains (Table 3).

The *Bacillus* spp. were spore-forming gram-positive rods with gray-white, opaque colonies. They were classified as *B. pumilus* (five strains), *B. subtilis* (five strains), *B. cereus* (three strains), and *B. thuringiensis* (two strains), with *B. subtilis* and *B. pumilus* dominating in terms of the number of isolates and substrates used, but differing in lactose utilization, starch utilization, and gelatin hydrolysis (Table 3).

Table 3. Cultural and biochemical characteristics of the dominant rhizobacteria isolated from the tomato rhizosphere.

	GM+				**GM−**		
Group	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**GP7**
	***Bacillus* spp.**				***Pseudomonas* spp.**		
Species	**AAUB *cereus* 4, 6, 36, RFS5**	**AAUB *pumilus* 11, 13, 38, RFS7**	**AAUB *subtilis*14, 16, 37, RFS6**	**AAUB *thuringiensis* 44**	**AAUP *aeruginosa* 2, 42, 90, RFS2**	**AAUP *fluorescens* 17, 38, 58, 63, RFS1**	**AAUP *putida* 9, 35, 45, 55 RFS3**
Colony colour/ Texture	Gray white/ opaque	Gray white/opaque	Gray white/opaque	Gray white/opaque	Blue green	Yellow green	Yellow green
Florescent	−	−	−	−	+	+	+
Lecithinase	+	−	−	+	+	−	−
Spore	+	+	+	+	−	−	−
Cry proteins	−	−	−	+	NA	NA	NA
VP	+	+	+	+	−	−	−
MR	−	+	+	−	−	−	−
Indole	−	−	−	−	−	−	−
Citrate	+	+	+	+	+	+	+
NR	−	−	+	−	+	−	−
Arabinose	−	+	+	−	−	+	−
Xylose	−	+	+	−	+	+	−
Glucose	+	+	+	+	+	+	+
Mannose	−	+	+	−	−	+	+
Mannitol	−	+	+	−	+	+	−
Trehalose	+	−	−	+	−	+	−
Maltose	−	+	+	+	−	+	+
Fructose	+	+	+	+	−	−	+
Sucrose	+	−	+	−	−	+	−
Lactose	−	+	−	−	−	−	−
Starch	+	−	+	+	−	−	−
Gelatin	−	−	+	+	+	+	−
Growth at 4°C	NA	NA	NA	NA	−	+	+
Growth at 42°C	NA	NA	NA	NA	+	−	−

GM+ = Gram-positive; GM− = Gram-negative; VP = Voges-proskaver test; MR = Methyl red test; AAUB = Addis Ababa University *Bacillus*; AAUP = Addis Ababa University *Pseudomonas*; RFS = Reference strains

The other two species, *Bacillus cereus* and *Bacillus thuringiensis*, were less diverse in their biochemical reactions and showed similar patterns, i.e., they ferment glucose but not lactose, maltose, mannitol, or trehalose, and are able to grow on egg-yolk agar. This group is easily distinguished from other members of the aerobic endospore-forming bacteria for its inability to produce acid from mannitol and its capacity to produce lecithinase, which forms halo zones on plate count agar containing 10% egg yolk emulsion (Logan and De Vos, 2009).

Although differentiation among *Bacillus* species can be difficult and a large number of phenotypic tests are used to distinguish them, sometimes only a single feature separates species. Thus, *Bacillus thuringiensis* can only be distinguished from *B. cereus* by the production of cry genes proteins that inhibit the growth of some groups of insects (Table 3). Drobniewski (1993) also established that *Bacillus thuringiensis* can be distinguished from other closely related *Bacillus* species (i.e., *B. cereus*) by its ability to synthesize these parasporal crystal proteins during the sporulation process.

The other antagonistic strains belong to the species *Pseudomonas fluorescens* (five strains), *P. putida* (five strains), and *P. aeruginosa* (two strains). Non-sporing gram-negative rods of *Pseudomonas* spp. had a yellow or greenish blue colour (due to pigment production) with a translucent or opaque texture on a nutrient agar medium surface. Although *Pseudomonas* spp. share many phenotypic traits, blue pigment production can be tentatively used to distinguish *P. aeruginosa* from *P. fluorescens* complex (Blazevic *et al*., 1973). They were MR, VP, and indole negative in general. These characters are the ones that differentiate this group of gram-negative bacteria from other members of the gram-negative coliforms (Holt *et al*., 1994).

The data also showed that the nitrate test showed that *P. aeruginosa* strains were positive as opposed to *P. fluorescens* and *P. putida* (both negative). Likewise, the gelatin test differentiated *P. fluorescens* (positive) from *P. putida* (negative) (Blazevic *et al*., 1973). These species are also widely distributed in agricultural soil (Saxena *et al*., 2020) and marine environments (Parvathi *et al*., 2009). These isolates were identified as *Pseudomonas fluorescens, P. putida* and *P. aeruginosa* based on cultural and biochemical characters (Table 3). They are the most abundant microorganisms in the rhizosphere (area around the roots). Botelho and Mendonça-Hagler (2006) estimated that 65 percent of the non-phytopathogenic florescent pseudomonas were *P. fluorescens, P. putida,*

and *P. chlororaphis*.

The data showed that *P. fluorescens* and *P. putida* complex can be identified based on trehalose utilization and gelatin liquefaction (Sheath *et al*., 1981). Thus, *P. fluorescens* exhibited a positive result for both the tests, whereas *P. putida* strains showed a negative response, and both species were negative to nitrate reduction, which differentiate them from *P. aeruginosa* (Table 3). Unlike the other species, *P. putida* failed to hydrolyze gelatin, which is one of the important characteristics routinely used to distinguish *P. putida* from *P. aeruginosa* (Sapkota, 2022).

Evaluation of different tomato varieties for resistance to bacterial wilt pathogen (*R. solanacearum*)

The disease index showed that the tomato varieties showed variation in disease resistance in which the four cultivars; Awash, Cochoro, Melkashola, and Venise displayed moderate resistance (MR), with DI ranging from 0.3 to 0.4 (Table 4) while two cultivars (moneymaker and Roma VF) were highly susceptible (HS) (Table 4) recording DI ranging from 0.76 to 0.86. This result was significantly different from the work of In *et al*. (1996), who showed that only three tomato varieties (10%) were moderately resistant to *R. solanacearum*. However, Aslam *et al*. (2017) screened more resistant and moderately resistant (57%) tomato varieties against *R. solanacearum*. These differences might be attributed to the severity of the virulence of the pathogen, genetic make-up of the varieties, and ecological conditions.

Table 4. The disease index of different tomato varieties and their reaction to bacterial wilt pathogen (*R. solanacearum*).

Tomato variety	Origin	Disease severity index	Resistance/susceptibility
Awash	Local	0.34	Moderately resistant
Venise	Exotic	0.36	Moderately resistant
Cochoro	Local	0.36	Moderately resistant
Melkashola	Local	0.4	Moderately resistant
Bishola	Local	0.44	Moderately susceptible
Chali	Local	0.42	Moderately susceptible
Fetan	Local	0.44	Moderately susceptible
Metadel	Local	0.46	Moderately susceptible
Arp tomato	Local	0.56	Susceptible
Galilama	Local	0.58	Susceptible
Melkasalsa	Local	0.58	Susceptible
Miya	Local	0.58	Susceptible
Moneymaker	Exotic	0.76	Highly susceptible
Roma VF	Exotic	0.86	Highly susceptible

Plant growth-promoting characteristics of the rhizosphere bacteria

In this study, the antagonistic rhizobacteria to *Ralstonia solanacearum* were tested for additional PGP properties, i.e., production of IAA, ammonia, hydrogen cyanide, and ability to solubilize inorganic phosphate enhance plant production (Table 5). Among these, 76% (13) of the bacterial isolates were able to produce ammonia, 88% (15) hydrogen cyanide, 53% (9) phosphate solubilization, and 83% (14) IAA (Table 5).

Among the isolates, *Bacillus pumilus* AAUB11, *Bacillus pumilus* AAUB13, *Bacillus subtilis* AAUB14, *Pseudomonas putida* AAUP58, and *Pseudomonas fluorescens* AAUP63 were endowed with all four PGP characters tested (Table 5).

Table 5. Plant growth promoting properties of *Bacillus* and *Pseudomonas* isolates collected from tomato plants from central parts of Ethiopia.

Taxonomic group	PSB (PSI)	P (µg mL^{-1})	IAA (µg mL^{-1})	HCN	NH_3	Multiple PGP
B. cereus AAUB4	–	–	197.04^{g}	+	–	2
B. pumilus AAUB11	2.17^{bc}	414.80^{d}	311.82^{b}	+	+	4
B. pumilus AAUB13	2.22^{bc}	432.42^{c}	299.51^{c}	+	+	4
B. subtilis AAUB14	3.30^{a}	471.41^{a}	645.18^{a}	+	+	4
B. cereus AAUB16	1.51^{c}	280.23^{e}	–	+	+	3
B. subtilis AAUB36	–	–	283.46^{d}	–	+	2
B. subtilis AAUB43	–	–	225.43^{f}	+	–	2
B. thuringiensis AAUB44	–	–	295.8^{c}	+	+	3
Pseudomonas aeruginosa AAUP2	2.03^{bc}	453.17^{b}	–	+	+	2
Pseudomonas putida AAUP9	2.66^{ab}	451.28^{b}	197.04^{g}	–	+	3
Pseudomonas florescens AAUP17	2.39^{b}	450.02^{b}	–	+	–	2
Pseudomonas fluorescens AAUP 38	–	–	190.86^{g}	+	+	3
Pseudomonas aeruginosa AAUP 42	–	–	163.7^{h}	+	–	3
Pseudomonas putida AAUP45	–	–	224.19^{f}	+	+	3
Pseudomonas putida AAUP 58	2.67^{ab}	452.44^{b}	273.58^{d}	+	+	4
Pseudomonas fluorescens AAUP63	2.17^{bc}	433.05^{c}	147.65^{i}	+	+	4
Pseudomonas aeruginosa AAUP 90	–	–	257.53^{e}	+	+	3

The isolates had phosphate solubilization indices (1.5–3.30) and amounts of phosphate solubilized 280–471P (µg ml^{-1}) ($p \leq 0.05$), indicating significant phosphate solubilization variability among isolates. The majority of them produced IAA in concentrations ranging from 147 to 645 µg ml^{-1}. *Bacillus subtilis* AAUB14 showed the highest activity in phosphate solubilization and IAA production, followed by *Pseudomonas putida* AAUP 58 (Table 5). Similarly, *B. pumilus* produced 445.5 µg ml^{-1} IAA (Singh *et al.*, 2011). In

this study, the phosphate solubilization ability of *Bacillus* spp. and *Pseudomonas* spp. far exceeded that of other microorganisms such as *Pseudomonas putida* (247 mg l^{-1}) (Pandey *et al.*, 2006), and *Bacillus megaterium* (140 mg l^{-1}) (El-Komy, 2005).

The performance of the isolates in IAA production (147–645 µg ml^{-1}) was much higher than that of the *Bacillus aryabhattai* strain that produced 248 µg ml^{-1}) (Nadieline *et al*., 2019) and *B. licheniformis* and *Bacillus* spp. with IAA producing abilities of 78 and 101 µg ml^{-1}, respectively (Nabti *et al*., 2013). The data also showed that *Bacillus* sp. produced more IAA (322 µg ml^{-1} on average) than *Pseudomonas* isolates (207 µg ml^{-1}). According to Blumer and Hass (2000), IAA production promotes plant growth.

Most of the isolates (>80%) were positive for the production of NH_3 and HCN which are implicated in defense regulation against phytopathogens (Table 5). The two plant growth properties were distributed equally among the different groups irrespective of their species. These metabolites are part of the volatile organic compounds (VOCs) that are widely distributed among florescent pseudomonads and aerobic spore-forming bacteria and are the major factors in antagonizing different root pathogens of vegetables and other crops (Kremer and Souissi, 2001). The authors estimated that about 50% of the rhizobacteria collected from potato roots are cyanogenic.

In vitro evaluation of selected rhizosphere bacterial isolates against *R. solanacearum*

The *in vitro* screening of the antagonistic effect of the isolates showed variation on the selected strains of *Ralstonia solanacearum* (RS2, RS3, and RS5) and the reference strain (Table 6). The isolates suppressed the test strains with an inhibition zone of 8–16 mm after two days of inhibition, irrespective of the test pathogen. Based on these findings, the rhizobacterial isolates *Bacillus subtilis* AAUB14, *Bacillus pumilus* 13, and *Bacillus pumilus* AAUB11 displayed the largest inhibition zones, showing the greatest potential as *in vitro* antagonists against *R. solanacearum* (Table 6). The data also showed most of the *Bacillus* species showed significantly higher antagonistic abilities than the *Pseudomonas* spp. (Table 6). The pattern of inhibition on the test pathogens did not differ significantly, indicating that isolates had similar levels of resistance or sensitivity to the antagonists.

Table 6. *In vitro* evaluation of antagonistic effects of the rhizosphere bacteria isolates against the selected strains of *Ralstonia solanacearum* on a modified medium.

Taxonomic group	***R. solanacearum* RS5**	***R. solanacearum* RS3**	***R. solanacearum* RS2**
B. cereus AAUB4	9.90 ± 0.18^{c}	7.17 ± 0.16^{c}	11.60 ± 0.43^{b}
B. pumilus AAUB11	15.07 ± 0.1^{a}	15.03 ± 0.16^{a}	14.9 ± 0.10^{a}
*B. pumilus*AAUB13	15.27 ± 0.08^{a}	15.73 ± 0.21^{a}	15.50 ± 0.13^{a}
B. subtilis AAUB14	16.3 ± 0.05^{a}	16.23 ± 0.21^{a}	16.30 ± 0.13^{a}
B. cereus AAUB16	13.07 ± 0.15^{b}	12.17 ± 0.52^{b}	15.17 ± 0.38^{a}
B. subtilis AAUB36	10.53 ± 0.25^{bc}	10.00 ± 0.50^{c}	8.17 ± 0.38^{c}
B. subtilis AAUB43	8.40 ± 0.38^{c}	8.67 ± 0.29^{c}	8.87 ± 0.28^{c}
B. thuringiensis AAUB44	13.53 ± 0.47^{b}	11.47 ± 0.31^{b}	14.87 ± 0.29^{a}
Pseudomonas aeruginosa AAUP2	8.50 ± 0.25^{c}	9.83 ± 0.63^{c}	8.47 ± 0.53^{c}
Pseudomonas putida AAUP9	6.80 ± 0.18^{d}	8.07 ± 0.50^{c}	9.33 ± 0.38^{c}
Pseudomonas fluorescens AAUP17	13.33 ± 0.35^{b}	11.30 ± 0.61^{bc}	13.10 ± 0.43^{b}
Pseudomonas fluorescens AAUP 38	12.43 ± 0.41^{b}	9.00 ± 0.50^{c}	9.50 ± 0.25^{c}
Pseudomonas aeruginosa AAUP 42	8.17 ± 0.38^{c}	10.50 ± 0.25^{bc}	10.17 ± 0.38^{c}
Pseudomonas putida AAUP45	14.07 ± 0.26^{ab}	10.00 ± 0.25^{c}	12.47 ± 0.35^{b}
Pseudomonas putida AAUP 58	10.43 ± 0.26^{bc}	$10.83 \pm 0.14b^{c}$	13.43 ± 0.30^{b}
Pseudomonas fluorescens AAUP63	12.53 ± 0.38^{b}	10.00 ± 0.25^{c}	11.60 ± 0.18^{b}
Pseudomonas aeruginosa AAUP 90	13.33 ± 0.38^{b}	10.00 ± 0.50^{c}	14.37 ± 0.40^{ab}

The tested isolates showed a similar pattern of inhibition zones (7–14.66 mm) in *Bacillus* isolated from the rhizosphere of healthy tomatoes from some parts of the country (Tsigie Gashaw *et al*., 2022). Furthermore, Huang *et al*. (2013) indicated inhibitory zones for bacteria isolated from the tomato rhizosphere ranged from 11.2 to 15.2 mm, which is consistent with the findings of this study.

CONCLUSION

In this study, most of the bacterial wilt pathogen isolates infecting tomato belonged to Race 1 Biovar III, while two strains were categorized into Race 3 and Biovar II. The pathogens varied in their disease index percentage, most of which were moderately resistant, whereas the two exotic varieties, Moneymaker and Roma VF, were highly susceptible to the pathogen.

With regard to PGPR, the isolates were identified in the genera *Bacillus* (four species) and *Pseudomonas* (three species) with the best inhibitory activity against *R. solanacearum* (RS2) and plant growth-promoting traits. *Bacillus pumilus* AAUB 11, *Bacillus pumilus* AAUB13, *Bacillus subtilis* AAUB14, *Pseudomonas putida* AAUP58, and *Pseudomonas fluorescens* AAUP63 performed well in terms of PGPR characteristics. However, the *Bacillus* spp. showed better antagonistic properties against the *R. solanacearum* test strains. Thus, they can be utilized as potential biocontrol agents provided that they are tested and validated under greenhouse and field conditions.

ACKNOWLEDGEMENTS

The authors would like to thank the Ethiopian Biotechnology Institute (EBTI), the Austrian Development Agency (ADA), and the Department of Microbial, Cellular, and Molecular Biology at Addis Ababa University for financial and technical assistance.

REFERENCES

Artal, R.B., Gopalakrishnan, C., and Thippeswamy, B. (2012). An efficient inoculation method to screen tomato, brinjal and chilli entries for bacterial wilt resistance. *Pest Manag. Hort. Ecosyst*. **18**(1): 70–73.

Aslam, M.N., Mukhtar, T., Hussain, M.A., and Muhammad Rahee, M. (2017). Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. *J. Plant Dis. Prot.* **124**: 585–590.

Bakker, A.W. and Schippers, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth-stimulation. *Soil Biol. Biochem*. **19**: 451–457.

Blazevic, D.J., Koepcke, M.H., and Matsen, J.M. (1973). Incidence and identification of *Pseudomonas fluorescens* and *Pseudomonas putida* in the clinical laboratory. *Appl. Microbiol*. **25**(1): 107–110.

Blumer, C. and Hass, D. (2000). Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. *Arch. Microbiol.* **173**: 170–177.

Botelho, G.R. and Mendonça-Hagler, L.C. (2006). Fluorescent *Pseudomonads* associated with the rhizosphere of crops: an overview. *Braz. J. Microbiol.* **37**(4): 401–416.

Cappuccino, J.G. and Sherman, N. (1992). **Microbiology: A Laboratory Manual**. Addison-Wesley, New York.

Dhingra, O.D. and Sinclair, J.B. (1995). **Basic Plant Pathology Methods**. Second edition. CRC Press, Boca Raton, FL.

Donate-Correa, J., Leon-Barrios, M., and Perez-Galdona, R. (2004). Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. *Plant Soil* **266**: 261–272.

Drobniewski, F.A. (1993). *Bacillus cereus* and related species. *Clin. Microbiol. Rev.* **6**: 324–338.

EARO (Ethiopian Agricultural Research Organization) (2002). EARO Annual Report 2000, Addis Ababa, Ethiopia.

El-Komy, H.M.A. (2005). Co-immobilization of *Azo-spirillum lipoferum* and *Bacillus megaterium* for successful phosphorus and nitrogen nutrition of wheat plants. *Food Technol. Biotechnol.* **43**: 19–27.

Hartmann, A., Singh, M., and Klingmuller, W. (1983). Isolation and characterization of *Azospirillum* mutants excreting high amounts of indole acetic acid. *Can. J. Microbiol*. **29**: 916–923.

Hayward, A.C. (1964). Characteristic of *Pseudomonas solanacearum*. *J. Appl. Bact.* **27**: 265–277.

Henok Kurabachew and Getachew Ayana (2016). Bacterial wilt caused by *Ralstonia solanacearum* in Ethiopia: Status and management approaches: A review. *Int. J. Phytopathol*. **5**(3): 107–119.

Henok Kurabachew and Wydra, K. (2013). Characterization of plant growth promoting

rhizobacteria and their potential as bioprotectant against tomato bacterial wilt caused by *Ralstonia solanacearum*. *Biol. Control* **67**(1): 75–83.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Stanley, J.T., and Williams, S.T. (1994). **Bergey's Manual of Determinative Bacteriology**. Ninth edition. Williams and Wilkins. Baltimore, M.D.

Huang, J., Wei, Z.S., Tan, S., Mei, X., Yin, S., Shen, Q., and Xu, Y. (2013). The rhizosphere soil of diseased tomato plants as a source for novel microorganisms to control bacterial wilt. *Appl. Soil Ecol.* **72**: 79–84.

Hudzicki, J. (2009). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol, American Society for Microbiology, Washington, DC.

In, M.S., Choi, E.J., and Choi, J.E. (1996). Resistance of tomato cultivars to bacterial wilt caused by *Pseudomonas solanacearum* and the effect of soil sterilization. *RDA J. Agric. Sci*. *Crop Protection* **38**: 473–476.

Kelman, A. (1954). The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology* **44**: 693–695.

King, E.O., Ward, M.K., and Raney, D.E. (1954). Two simple media for the demonstration of pyocyanin and fluorescin. *J. Lab. Clin. Med.* **44**(2): 301–307.

Klement, Z., Farkas, G.L., and Lovrekovich, I. (1964). Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. *Phytopathology* **54**: 474–477.

Kremer, R.J. and Souissi, T. (2001). Cyanaide production by rhizobacteria and potential for suppression of weed seedling growth. *Curr. Microbiol*. **43**(3): 182–186.

Kumar, A., Kumar, A., Devi, S., Patil, S., Payal, C., and Negi, S. (2012). Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: An *in vitro* study. *Recent Res. Sci. Technol.* **4**(1): 1–5.

Logan, N.A. and De Vos, P. (2009). Genus I. *Bacillus*. In: **Bergey's Manual of Systematic Bacteriology**, pp. 21–128 (De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K., and Whitman, W.B., eds.). Springer, New York.

Lozano, J. and Sequeira, L. (1970). Differentiation of races of *Pseudomonas solanacearum* by a leaf infiltration technique. *Phytopathology* **60**: 833–838.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A., Toth, I., Salmond, G., Gary, D., and Foster, G.D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.* **13**(6): 614–629.

Nabti, E.H., Mokrane, N., Ghoul, M., Manyani, H., Dary, M., and Megias, M.G. (2013). Isolation and characterization of two halophilic *Bacillus* (*B. licheniformis* and *Bacillus* sp.) with antifungal activity. *J. Ecol. Health Environ.* **1**(1):13–17.

Nadieline, C.V., Ndiaye, M.F., Fall, S., Krasova, T., Le Quere, A., and Diegane, D.D. (2019). Isolation and characterization of potential phosphate solubilizing bacteria in two regions of Senegal. *Afr. J. Microbiol. Res.* **13**(28): 609–618.

Nautiyal, C.S. (1999). An efficient microbiological growth medium for screening phosphorus solubilizing microorganisms. *FEMS Microbiol. Lett.* **170**(1): 265–270.

Nguyen, M.T. and Ranamukhaarachchi, S.L. (2010). Soil-borne antagonists for biological control of Bacterial Wilt Disease caused by *Ralstonia solanacearum* in tomato and pepper. *J. Plant Pathol.* **92**(2): 395–406.

Ojesola, C.O., Akintokun, A.K., Akintokun, P.O., and Oloyede, A.R. (2020). *In-vitro* antagonistic effect of *Bacillus thuringiensis* on *Ralstonia solanacearum*, the causal

agent of Bacterial Wilt Disease of tomato (*Lycopersicon esculentum* Mill). *Niger. J. Biotechnol.* **37**(2): 177–193.

Pandey, A., Trivedi, P., Kumar, B., and Palni, L.M. (2006). Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (B0) isolated from a sub-Alpine location in the Indian Central Himalaya. *Curr. Microbiol*. **53**: 102–107.

Parvathi, A., Krishna, K., Jose, J., Joseph, N., and Nair, S. (2009). Biochemical and molecular characterization of *Bacillus pumilus* isolated from coastal environment in Cochin, India. *Braz. J. Microbiol.* **40**(2): 269–275.

Prashar, P., Kapoor, N., and Sachdeva, S. (2013). Rhizosphere: Its structure, bacterial diversity and significance. *Rev. Environ. Sci. Biotechnol.* **13**: 63–77.

Sapkota, A. (2022). *Pseudomonas putida* - An overview. Microbe Notes July 3, 2022.

Saxena, A.K., Kumar, M., Chakdar, H., Anuroopa, N., and Bagyaraj, D.J. (2020). *Bacillus* species in soil as a natural resource for plant health and nutrition. *J. Appl. Microbiol.* **128**(6): 1583–1594.

Seleim, M.A.A., Abo-Elyousr, K.A.M., Abd-El-Moneem, K.M., and Saead, F.A. (2014). First report of Bacterial Wilt caused by *Ralstonia solanacearum* Biovar 2 Race1 on tomato in Egypt. *Plant Pathol. J.* **30**(3): 299–303.

Shahbaz, M.U., Mukhtar, T., Ul-Haque, M.I., and Begum, N. (2015). Biochemical and serological characterization of *Ralstonia solanacearum* associated with chilli seeds from Pakistan. *Int. J. Agric. Biol.* **17**: 31–40.

Sheath, P.H.A., Stevens, M., and Sackin, M.J. (1981). Numerical taxonomy of *Pseudomonas* based on published records of substrate utilization. *Antonie Van Leeuwenhoek* **47**: 423–448.

Singh, R.P., Bijo, A., Baghel, R.S., Reddy, C., and Jha, B. (2011). Role of bacterial isolates in enhancing the bud induction in the industrially important red alga *Gracilaria dura*. *FEMS Microbiol. Ecol*. **76**: 381–392.

Tsigie Gashaw, Baye Sitotaw, and Solomon Yilma (2022). Evaluation of rhizosphere bacterial antagonists against *Ralstonia solanacearum* causing tomato (*Lycopersicon esculentum*) wilt in Central Ethiopia. *Int. J. Agron.* 2022: 1–9.

Winstead, N.N. and Kelman, A. (1952). Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum. Phytopathology* **42**: 628–634.

Yabuuchi, E., Kosako, Y.Y., Hotta, H.I., and Nishiuchi, Y. (1995). Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia gen. nov*.: Proposal of *Ralstonia pickettii*, *Ralstonia solanacearum* and *Ralstonia eutropha*. *Microbiol. Immunol*. **39**: 897–904.

Yaynu, H. and Korobko, A.P. (1986). Incidence of bacterial wilt in some potato growing areas of Ethiopia. In: **Proceedings of the 15th annual meeting of the Ethiopian Phytopathological Committee** (EPC), 13–14 March 1990, Addis Ababa, Ethiopia.

Yuliar, Nion, Y.A., and Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes. Environ*. **30**(1): 1–11.