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EVALUATION OF ETHIOPIAN ISOLATES OF *Pseudomonas fluorescens* AS BIOCONTROL AGENT AGAINST POTATO BACTERIAL WILT CAUSED BY *Ralstonia (Pseudomonas) solanacearum*

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ABSTRACT

A total of 50 fluorescent pseudomonas were collected from different potato growing areas in Ethiopia isolated and characterized, and evaluated on king's B medium for their antibiosis towards *Ralstonia solanacearum* the pathogen of bacterial wilt of potato. Out of the 50 isolates only three i.e., Pf S2, Pf Wt3 and PfW1 showed inhibition against the growth of the pathogen. To test their antagonistic effect under greenhouse condition an experiment was conducted using sterilized soil. Tubers of bacterial wilt susceptible potato clone CIP 383031.15 were used. The potato tubers were dipped into 48 hrs old culture suspension of the three isolates i.e., Pf S2, Pf Wt3, PfW1 and Pfri (Indian reference strain) for 1 hr and planted in pots containing sterilized soil. Bacterization of tubers with isolates Pf S2, Pf Wt3, and PfW1, significantly reduced by 59.83% the incidence of bacterial wilt compared to the pathogen-inoculated control and increased plant growth (plant height and dry weight) by 59.83%, 76.89% and 28.44%, respectively. This suggests the importance of the studied isolates as plant growth-promoting rhizobacteria.

Key words: microbiology / biocontrol / *Pseudomonas fluorescens* / *Ralstonia solanacearum* / potatoes / potato bacterial wilt

OCENA ETIOPSKIH IZOLATOV *Pseudomonas fluorescens* KOT SREDSTVA ZA BIOLOŠKO ZATIRANJE KROMPIRJEVE OVELOSTI, KI JO POVZROČA *Ralstonia* (*Pseudomonas*) *solanacearum*

IZVLEČEK

Zbrali in opisali smo 50 fluorescentnih pseudomonad z različnih področij v Etiopiji, kjer pridelujejo krompir in na KB gojišču ocenili njihov antibiotični učinek na povzročitelja krompirjeve ovelosti, *Ralstonio solanaceum*. Od 50 izolatov so samo trije, Pf S2, Pf Wt3 in PfW1 inhibirali rast patogena. Da bi ocenili njihovo agonistično delovanje pod pogoji v rastlinjaku, smo naredili poskus s sterilizirano zemljo. Uporabili smo gomolje na ovelost občutljivega klona krompirja CIP 383031.15. Krompirjeve gomolje smo potopili v 48 ur staro kulturo treh izolatov Pf S2, Pf Wt3, PfW1 in Pfri (indijski referenčni sev) za eno uro in posadili v lončke s sterilno zemljo. Okužba gomoljev z izolati Pf S2, Pf Wt3 in PfW1 je značilno znižala pogostnost baketrijske ovelosti za 59,83 % v primerjavi s kontrolno okuženimi rastlinami in izboljšala rast rastlin (višina rastlin in suha snov) za 59,83 %, 76,89 % oziroma 28,44 %. To nakazuje pomen proučevanih izolatov kot rast spodbujajočih rizobakterij.

Ključne besede: mikrobiologija / biološka kontrola / *Pseudomonas fluorescens* / *Ralstonia solanacearum* / krompir / krompirjeva ovelost

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth major crop of the world (CIP, 1984) after rice, wheat and maize. It is an excellent food source in which the tuber provides high energy and quality protein as well as substantial amount of vitamins and minerals.

Potato was first introduced to Ethiopia in 1858 (Pankhurst, 1964) since its introduction, potato production has increased faster than any other food crops covering 50,000 ha (Berga, 1986). However, the yield per unit area is very low compared to those of other countries like Rwanda, Egypt and Kenya. There are many factors that reduce the yield of the crop among which the diseases like late blight (*Phytopthora infestans*), bacterial wilt and viruses play an important role (Yaynu, 1989; Ketema, 1999). Stewart (1956) reported bacterial wilt in areas around Jimma in the western part of the country.

Bacterial wilt also known as brown rot is caused by *Ralstonia (Pseudomonas) solanacearum E.F Smith,* a soil-borne bacterial species. It is one of the most destructive plant diseases, which is predominantly distributed in the tropical, subtropical and warm temperate regions of the world (Hayward, 1995). It affects as many as 200 plant species representing more than 50 families of particularly members of solanaeceous plants such as potato, tomato, eggplant, pepper and tobacco. For example, it is responsible for yield loss of potato to the extent of 50–80% in Kenya, Burundi and Uganda (Ajanga, 1993) and also up to 70% in India (Sinha, 1986). In Ethiopia, the yield loss caused by the disease is not yet determined, but it occurs in potato growing areas of the country at higher incidences and studies regarding the diversity of the pathogen showed that the strains belong to race 3 of biovar 2 of *R. solanacearum* (Yaynu, 1989; Ketema, 1999).

The common control measures employed in other countries include the use of resistant variety, crop sanitation, crop rotation, selection of disease free planting material and other cultural practices as single or integrated disease management. However, control through the use of resistant varieties alone has showed little success. This is because such kind of resistance is strain specific and liable to break down by virulent and highly polymorphic strains of *R. solanacearum* at an ambient temperature and in nematode infested soil (Prior *et al*., 1994). Successful control of the pathogen through crop rotation is also not always effective since rotation practices recommended for one area may not perform well at other locations in addition to differences in the strains involved (Prior *et al*., 1994).

The use of rhizosphere resident microbial antagonist specifically the fluorescent pseudomonas is noted as a promising control method. The rhizosphere is a habitat in which several biologically important processes and interactions takes place which is primarily due to the influx of mineral nutrients from accumulation of plant roots exudates from plant roots through mass flow and diffusion (Sorensen, 1997; Bias, 2004). Among the rhizosphere organisms fluorescent pseudomonas strains are often selected for biological control strategies because of their ability to utilize varied substrates under different conditions, short generation time and motility that assist colonization of roots. Moreover, they produce active extracellular compounds such as siderophores responsible for the biological suppression of several soil borne plant pathogens (Bagnasco *et al.*, 1998).

Sunaina *et al*., (1997) reported that fluorescent pseudomonas strains when applied to potato seed tubers were found to reduce the population of *Eriwinia cartovora* on roots and tubers by 95–100% and 28–95%, respectively. In related study, Gamliel and Katan (1993) found that inoculation of fluorescent pseudomonas decreased the incidence disease caused by *Sclerotium rolfsii* in bean and *Fusarium* wilt in cotton and tomato. Therefore, this study was initiated to screen or evaluate the antagonistic effect of isolates of *P.fluoresecens* against *R.solanacearum* under laboratory and greenhouse condition.

MATERIAL AND METHODS

Collection of *P. fluoresecens*

Soil samples were collected from potato growing areas around Wolyata, Shahamane and Wonodogenet and then *P.fluoresecens* were isolated following the method of Vlassak, *et al.*, (1992). 1 g of each soil sample was mixed by shaking for 2 h on a rotary shaker at 200 rpm in 100 ml of phosphate buffered saline (PBS). PBS diluted extracts were then plated on King's B (KB) medium which were made selective for isolation of *P. fluorescens* by adding cyclohexamide (100 μg ml^{-1}), chloroamphenicol (13 μg ml^{-1}) and ampicillin (50 μg ml^{-1}) (Simon and Ridge, 1974). After incubation at 28 °C for 24 hr representative types of colonies were further purified on KB agar medium and pure isolates will be preserved on KB slant and stored at 4 °C.

Morphological and biochemical characterization of isolates

The following morphological features: colony type, bacterial shape and gram reaction of the isolates were determined using King's B agar medium. Oxidase, catalase; and starch hydrolysis and levan formation test were tested on media supplemented with 0.2% starch and 5% sucrose was done following the method of Goszczynska *et al.* (2000).

Fluorescin production, gelatin liquefaction, salt tolerance, and siderophore detection and carbohydrate utilization tests were performed following the methods of Goszczynska *et al.* (2000), Arnow (1937) and Pickett *et al*. (1991).

In vitro inhibition test

All isolates of *P. fluorescens* were first screened for their toxicity toward the pathogen on KB agar plates in dual culture assays (Ganesan and Gnanamanickam, 1987). KB plates were prepared by mixing suspension of cells scraped from 48–72 hours old culture of the pathogen with cooled and molten KB agar (42 °C). The agar suspension was then dispensed into Petri dishes and was spot inoculated with the test strain from a 24 hours old culture (Skathivel and Gnanamanickam, 1987). Like wise, KB agar plate spot inoculated with sterile water were used as a control. Assay plates were maintained at 28 °C and observed for inhibition zones after 2 to 3 days.

The bacterial designation of isolates of *P.fluoresecens* used in this study is described as follows

Pfs: refers to isolates of *P. fluorescens* from Shashamane area
Pfwt: refers to isolates of *P. fluorescens* from Wondogenet area
Pfw: refers to isolates of *P. fluorescens* from Wolayta area
Pfb: refers to isolates of *P. fluorescens* from Bako area
Pfa: refers to isolates of *P. fluorescens* from Ambo area
Pfri: refers to reference strain of *P. fluorescens* from India
Rs262-b: refers to *R. solanacearum* (pathogenic isolate) kindly provided by Ambo Plant Protection Research Center.

Tobacco hypersensitivity reaction (hr)

Since there is a possibility that *R. solanacearum* may lose its virulence and become avirulent upon storage, a preliminary pathogencity and hypersensitivity test was done to confirm its virulence and race 3 strain of *R. solanacearum*. The pathogenic isolate Rs 262-b obtained from

Ambo Plant Protection Research Center was injected on tobacco leaves following the method of Lazano and Sequeria (1970).

Pathogencity test

Potato tubers of the susceptible cv, CIP 383032.15 obtained from Holleta Agricultural Research Center (HARC), were planted in 20 cm diameter plastic pots (one plant per pot) filled with sterilized soil. At three-leaf stage potato plants were inoculated following stem puncture method of Winsted and Kelman (1952). Control plants were injected with sterile distilled water. After inoculation the plants were put in an incubator at a temperature of 28 °C for 48 hours and then transferred to green house where the minimum and maximum temperature was 14.8 °C and 35.2 °C, respectively.

Greenhouse experiment

The greenhouse experiment was conducted at the National Soil Research Center, in Addis Ababa with a minimum and maximum temperature of 19–25 °C and 25–31 °C, respectively. Based on the efficiency of *in vitro* antibiosis result, three isolates of *P. fluorescens* were chosen for this experiment. The three selected isolates Pfwt 3, Pfw 1, Pfs 2 and Pfri (one reference strain introduced from India) were grown on KB agar medium for 24 hours and diluted to give a suspension, which was adjusted optically to concentration of 10^9 cfu ml^{-1} (OD_{600} = 1.0) (Mulya *et al*., 1996). Potato tuber (CV CIP 383032.15) which was highly susceptible to bacterial wilt, was surface sterilized with disinfectants before treatment. The soil was autoclaved at 121 °C for one hour and amended with fertilizer (N : P : K = 2 : 1 : 1) (Mulya *et al*., 1996) at a rate of 10 g per pot as a solution. Three kilogram sterilized soil were filled into the plastic pots with a diameter of 20 cm, which were surface sterilized with alcohol into which two potato tubers were planted with the following treatment.

Treatments applied were:

T1. Rs 262-b (pathogen inoculated)
T2. Pfw 1 treated (Wolayta isolate)
T3. Pfs 2 treated (Shashamane isolate)
T4. Pfwt 3 treated (Wondogenet isolate)
T5. Pfri treated (Indian reference strain)
T6. Pfw 1 treated and Rs 262-b inoculated
T7. Pfs 2 treated and Rs 262-b inoculated
T8. Pfwt 3 treated and Rs 262-b inoculated
T9. Pfri treated and Rs 262-b inoculated
T10. Control (Neither treated nor inoculated)

Potato tuber in T1 was planted in soil infested with 100 ml of 10^9cfu ml^{-1} suspension of the pathogen (Sunaina *et al*., 1997), while in T2 to T5 each tuber was dipped in the suspension of each antagonist isolates for 60 minutes and planted in sterilized soil. But in T6 to T9 after dipping the potato tuber in each suspension for 60 minutes they were planted in sterilized but pathogen inoculated (infested) soil. And potato tuber in T10 was used as control.

Plants were watered regularly with deionized water and treatments were arranged in a completely randomized design with three replication. Observation on percent survival and plant height was taken. Then, the plants were cut at the soil level and dry weights of the shoot were determined after oven drying at 60 °C for 72 hrs.

The experimental data was analyzed using one-way analysis of variance and comparison of means at 5% level was made by Tukey's test. Statistical analysis was done using SPSS v12.0 (SPSS Inc., Chichago, IL. USA).

RESULTS

Isolation and characterization of isolates

A total of 50 fluorescent bacteria isolates were isolated from soil samples collected from different potato growing regions of the country. All isolates produced yellow-green diffusible pigment of variable intensities on King's B medium. Morphologically, they were short rods and form levan on NA with 5% sucrose, which was white, doomed, and mucoid shining colony (Table 1).

Table 1. Distribution of isolates of *P. fluorescens* in different potato growing areas and their cultural characteristics
Preglednica 1. Geografska distribucija izolatov *P. fluorescens* in njihove značilnosti

Origin	No. of isolates	Characteristics			
		Morphology	Colony appearance	Fluorescein production	Levan formation
Shashamanae	10	short rods	small shiny	+	+
Wondogenet	10	short rods	small shiny	+	+
Wolayta	15	short rods	small shiny	+	+
Ambo	8	short rods	small shiny	+	±
Bako	7	short rods	small shiny	+	±

Key: + =Positive reaction; ±=Intermediate reaction

Table 2. Carbohydrate utilization by selected isolates of *P. fluorescens*
Preglednica 2. Izkoriščanje ogljikovih hidratov različnih izolatov *P. fluorescens*

	Carbon source utilization				
	Pfa	Pfb	Pfs	Pfws	Pfw
Arabinose	+	±	+	+	+
Galactose	±	+	+	±	+
Glucose	+	+	+	+	+
Fructose	+	+	+	+	+
Mannose	+	±	+	±	+
Trehalose	+	+	+	+	+

Key: + =positive reaction; – = negative reaction; ±=Intermediate reaction

Table 3. Distinctive feature of *R. solanacearum* and *P. fluorescens*
Preglednica 3. Značilne razlike med *R. solanacearum* in *P. fluorescens*

Microorganism	Hypersensitivity reaction	Pathogencity test	Siderophore production	Antibiotic production
R. solanacearum	+	+	–	–
P. fluorescens	ND	ND	+	+(–)

Key: + = positive reaction; – = negative reaction; ND = Not Done

Biochemical properties of the isolates are presented in Table 3 All the isolates were found to be gram negative, oxidase and catalase positive. They were able to grow at 4 °C but not at 41 °C and tolerated NaCl concentration from 1 to 2% but not at 5%. They utilized the tested carbohydrates and produced yellow color on the medium, which was an indication of the utilization of each carbohydrate. In addition they liquefied gelatine but failed to hydrolyze starch. They also produced catechol type siderophore.

Table 4. Morphological and biochemical characteristics of *P. fluorescens* isolates
Preglednica 4 Morfološke in biokemijske lastnosti izolatov *P. fluorescens*

	Characteristics of isolates				
	Pfa	Pfb	Pfs	Pfws	Pfw
Gram reaction	–	–	–	–	–
Colony morphology	S& R	S &R	S&R	S&R	S&R
Pigment production	+	+	+	+	+
Bacterial shape	RS	RS	RS	RS	RS
Oxidase	+	+	+	+	+
Catalase	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Starch hydrolysis	–	–	–	–	–
Gelatin liquefaction	±	±	+	+	+
Salt Tolerance					
1% NaCl	+	+	+	+	+
2% NaCl	+	+	+	+	+
3% NaCl	–	–	–	–	–
Growth at 4OC	+	+	+	+	+
Growth at 41OC	–	–	–	–	–
Siderophore detection	+	+	+	+	+
Levan formation	+	+	+	±	±

Key: + = positive reaction; – = negative reaction; ± = Intermediate reaction, S R = Smooth & round, RS = Rod shaped

Hypersensitivity reaction

Injection of bacterial suspension of Rs 262-b isolate into tobacco leaves caused yellow necrosis with in 24 to 48 hours. There was no progress of necrosis to the adjoining tissues and wilting of the plant even after some weeks showing hypersensitive reaction. In addition, the control plant injected with distilled water remained symptom less.

Pathogencity test

The result of the pathogencity test showed that, the bacterial isolate was pathogenic to potato. Development of wilt on the potato plant was rapid and complete wilting of the plant occurred within 8 to 13 days. Infected potato plants exhibited epinasty, stunting and browning of stems upon dissection.

In vitro antibiosis

Out of the 50 fluorescent isolates screened against *P. solanacearum* on KB medium only three local isolates i.e., Pfwt 3 (Wondogenet), Pfw1 (Wolayta), Pfs1 (Shashamane) and Pfri (reference strain) were capable of inhibiting the growth of the pathogen while others did not produce any antibiosis. The diameter of inhibition zones ranged from 1.2 to 2.4 cm where the minimum and maximum inhibition caused by *Pfri* and *Pfw1,* respectively table 1.The isolate PfW1 from Wolayta and PfS2 from Shashamane produced higher zones of inhibition than the rest including the reference strain from India.

Table 5. *In vitro* antibiosis and zones of inhibition caused by *P. fluorescens* isolates on KB medium with *R. solanacearum*

Preglednica 5. *In vitro* antibiotsko delovanje in cone inhibicije, ki jih povzročijo izolati *P. fluorescens* na KB gojišču z *R. solanacearum*

Origin	No.of isolates tested	No.isolates with antibiosis	Name of isolates	Zone of inhibition, cm
Shashamanae	10	1	PfS 2	1.5
Wondogenet	10	1	PfWt 3	1.32
Wolayta	15	1	PfW 1	2.4
Ambo	8	–	–	–
Bako	7	–	–	–
India	1	1	Pf ri	1.2

Key: + = positive reaction; – = negative reaction

Greenhouse experiment

Bacterial wilt suppression by *P. fluorescens*

Data from the greenhouse experiment showed that only 40% of plants survived in T1 (where potato tuber planted in pathogen infested soil without bacterization). On the other hand, the potato tubers treated with the selected antagonistic isolates prior to planting in pathogen infested soil significantly suppressed the incidence of bacterial wilt and also increased its survival rate by 59.83% as compared to (T1). The control treatment did not show any wilt symptoms and had 100% survival.

Table 6. Suppression of bacterial wilt caused by the pathogen in plant treated with P. fluorescens

Preglednica 6. Zatiranje s patogenom povzročene bakterijske ovelosti z obdelavo s *P. fluorescens*

Treatment	Percent survival
T1. Rs262-b ino.	$40.0^{b} \pm 2.34$
T2. Pfw 1 + Rs 262-b	$99.89^{a} \pm 0.85$
T3. Pfs 2 + Rs262-b	$99.8^{a} \pm 0.84$
T4. Pfwt 3 + Rs262-b	$99.83^{a} \pm 1.09$
T5. Pfri + Rs262-b	$99.8^{a} \pm 1.31$
T6. Control	$100^{a} \pm 1.00$

· Numbers are mean and SD of three replicate (two plant in each pot)

* Means followed by the same letter with in a column are not significantly different at $\alpha = 0.05$ by Tukey's test.

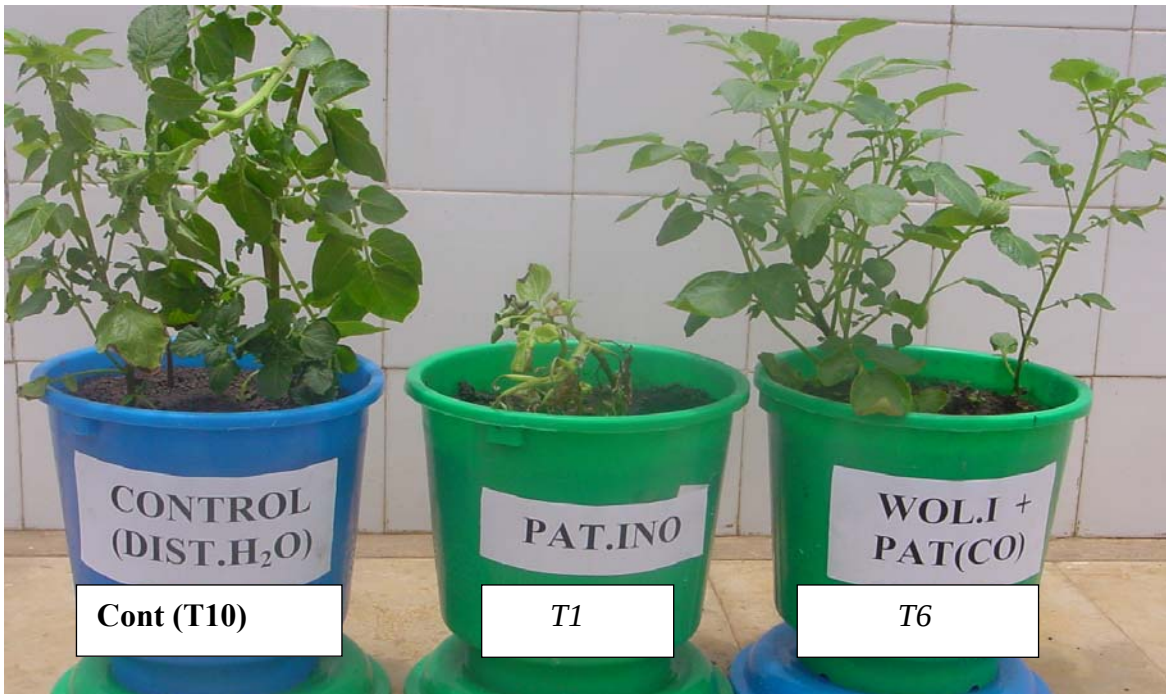

Figure 1. Potato plant showing difference between T1 (pathogen inoculated), T6 (Pfw1 treated and Rs 262-b inoculated) and T10 (non-treated control).
Slika 1. Rastline krompirja, ki kažejo razlike med T1 (inokulirana s patogenom), T6 (tretirana s Pfw1 in okulirana s Rs 262-b) in T10 (netretirana kontrola).

Effect of bacteria treatment on plant height and biomass

Data on plant height and dry weight of potato were recorded in the Table 4. Plant height and biomass were significantly lower in the treatment of pathogen-infested soil (T1) than the rest of all treatment (T2–T10). Potato tuber treated with *P. fluorescens* isolates prior planting in pathogen free (T2–T5) and infested soil increased plant height and dry weight compared to T1. However, *P. fluorescens* treated and pathogen inoculated treatment (T6–T9) do not show an increased plant height and biomass better than the control (T10).

Table 7. Effect of bacterization with isolates of *P. fluorescens* on plant height and biomass
Preglednica 7. Učinek bakterizacije z izolati *P. fluorescens* na višino rastlin in biomaso

Treatment	Height, cm	Dry weight, gm
T1. Rs262-b ino	$20.83^{c} \pm 2.1$	$1.9^{c} \pm 1.51$
T2. Pfw1	$32.13^{a} \pm 1.1$	$11.97^{a} \pm 2.52$
T3. Pfs2	$32.87^{a} \pm 0.76$	$11.83^{a} \pm 1.19$
T4. Pfwt3	$32.83^{a} \pm 1.1$	$11.7^{a} \pm 2.41$
T5. Pfir	$32.53^{a} \pm 0.92$	$11.53^{a} \pm 0.95$
T6. Pfw1 + Rs262-b	$27.9^{b} \pm 1.8$	$6.77^{b} \pm 1.42$
T7. Pfs2 + Rs262-b	$26.77^{b} \pm 1.3$	$6.67^{b} \pm 1.12$
T8. Pfwt3 + Rs262-b	$27.17^{b} \pm 1.5$	$6.67^{b} \pm 1.12$
T9. Pfri + Rs262-b	$26.5^{b} \pm 0.87$	$6.63^{b} \pm 1.24$
T10. Control	$27.4^{b} \pm 3.9$	$6.63^{b} \pm 1.5$

▪ = Numbers are mean and S.D of three replicates (two plant in each pot)
* Means followed by the same letter with in a column are not significantly different by Tukey's test at $\alpha = 0.05\%$.

DISCUSSION

The genus *Pseudomonas* is a very large and important group of non-fermenting, gram negative bacteria, living as saprophytes in soils, sediments and fresh water (Bossis *et al*., 2000). Some of the species in this genus are already known to improve plant growth and health and are implicated in the natural suppression of certain soils to various soil borne diseases like bacterial wilt of potato, fusarium and verticilium wilt of vegetable crops whereas others are involved in the biodegradation of natural or man made toxic chemical compounds (Bossis *et al*., 2000)

Biochemical reactions such as fluorescien production, levan formation, certain carbohydrate utilizations and morphological features of the isolates obtained in this study were similar to the result reported by (Palleroni, 1993). The result of oxidase, catalase, starch hydrolysis, gelatine liquefaction, growth temperature, salt tolerance and carbohydrate utilization test were also similar with the characteristics described for the species *P. fluorescens* (Stainer *et al*., 1966; Bossis *et al*., 2000). Although the characteristics of *P. fluorescens* were similar with that of *P.putida,* the isolates were further differentiated on the basis of gelatine liquefaction (gelatinase) test, where all isolates of *P. fluorescens* were positive and all isolates of *P. putida* were negative according to Pickett *et al*. (1991).

Similarly, the result of siderophore detection test agreed with result reported by Arnow (1937). The siderophore production is the main property of certain species in the genus *Pseudomonas* that is known to chelate and thereby scavenge the ferric iron. The low ferric iron availability significantly reduces the pathogenesis of organisms in the rhizosphere, especially in iron deficient environment. In this respect this is an adopted and very important biological control strategy (Glick, 1995).

The utilization of different carbohydrate sources except starch by the isolates agreed with result by Palleroni (1993) and revealed the metabolic and ecological diversity of *P. fluorescens*, which presuppose its success of survival and fitness in a new environment and give them a competitive advantage than any other pathogenic organism in a specific niche where it could be applied or treated as biocontrol agent. In addition avariety of microorganisms produce polysaccharide levan which is a homopolysaccahride consisting of 90–100 levan when they grow on sucrose medium. This polysaccharide is known to improve the soil structure i.e., soil aggregation via promotion of porous structure to improve aeration. It also increase soil adhesion to plant root playing a major role in the root colonization stage and provide protection from colonization of the root zone by pathogenic organism which increases its success in ecological competition (Bossis *et al*., 2000).

The result of the tobacco leaf infiltration test showed that, the pathogen induced chlorosis of the infiltrated tobacco leaf which is the characteristics of race 3 (Marin and El - Nashaar, 1993) but do not cause wilting of the plant. In this respect, the tobacco was shown not to be the host of the isolate belonging to race 3 strain of *R. solanacearum*. On the other hand, the pathogen caused wilting of the potato plant with in 3 to 6 days after inoculation showing the virulent nature of isolate Rs 262-b.

In the *In vitro antibiosis* the smallest and largest inhibition zone of 1.2 and 2.4 cm diameter were caused by Pfri (Indian isolates) and Pfw1 (Wolayta isolates), respectively. Pfw1 have 200% efficiency being the most efficient isolates followed by Pfs2 and Pfwt3 with 125% and 111% efficiency, respectively. Similarly, (Savithiry and Gnanamanickam, 1987) and (Anuratha and Gnanamanickam, 1990) obtained 2.5 to 4cm and 1.0 to 2.8 cm inhibition zones on KB agar medium by *P. fluorescens* against *R. solani* and *R. solanacearum* respectively. Therefore, these three isolates along with the Indian reference strain were used as a candidate antagonist for the greenhouse experiment.

The result of greenhouse experiment showed that treatment of potato tuber with selected isolates of fluorescent pseudomonas increased mean survival of potato plant by 59.83%

compared to T1 (Table 6). This suggests a higher level of protection of potato from bacterial wilt and thus the most efficient isolates could be used in biological control of the disease. Similarly, Aspiras and Cruz (1985); Anuratha and Gnanamanikam (1990) and Gamliel and Katan (1993) reported that utilization of antagonistic rhizosphere bacteria such as *Bacillus spp*., *P. fluorescens* and *P. putida* significantly increased the survival rate of potato, tomato, eggplant and cotton by 60–90%, 90% and 84–90%, respectively against bacterial and fusarium wilt disease.

In the same way, bacterization of tuber with selected efficient isolates (T6–T9) significantly increased the plant height and dry weight by 76.89% and 28.44%, respectively as compared to T1. This result agreed with Sivamari and Gnanamanickam (1988) where they found an increased plant height and biomass of banana seedling by 62.17% and 61.54%, respectively due to bacterization with the suspension of *P. fluorescens* prior to planting in *F. oxysporium f.sp cubense* infested soil. This is due to the fast growth rate followed by their aggressive root colonization nature that results in displacement of the pathogen and also high competitive and wide metabolic capability of the fluorescent pseudomonas isolates. In addition to siderophore produced by the isolates scavenging Fe (III) and induced systemic resistance, also the specificity between fluorescent pseudomonads and the potato variety may also play a vital role in the wilt suppression and growth promotion of potato (Whips, 2001).

Potato tuber planted after bacterization in treatments T2–T5 showed the most significant growth enhancement (Plant height and dry weight) when compared to potato plant in other treatments. This could be explained on the basis of the possibility of production of growth stimulating substance (hormone), increased nutrient availability in presence of isolates. The results presented in this study suggest the plant growth promoting nature of the bacteria isolated from the soils of Ethiopia according to Glick (1995). Therefore, the evidence presented here is suggestive of the potential of the Ethiopian isolates as biological control agent for bacterial wilt of potato by exploiting the interaction between rhizosphere microorganisms. Plant protection rendered this way can be maximized by combining different methods in an integrated disease management such as resistant variety and biocontrol. Besides the use of specific substrate that enhance selective growth and multiplication of the antagonist, use of multiple microbial inoculant rather than a single species alone, genetic manipulation of the desired isolate (the promising one), improving delivery of the formulation of the biocontrol agent etc, can be considered as untapped potential of *P. fluorescens* in the biological control of potato bacterial wilt in the country.

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