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Endophytic Bacteria Suppress Bacterial Wilt of Tomato Caused by *Ralstonia solanacearum* and Activate Defense-related Metabolites

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Abstract

Introduction: Phytopathogenic microorganisms affect plant health and burden a major threat to food production and ecosystem stability. Increasing the use of chemical pesticides for plant diseases control causes several negative effects on human and environment health. Furthermore, increasing public awareness about the side effects of them led to a research to find alternatives for these products. One of the alternative methods is bio-control utilizing plant associated antagonistic microorganisms.

Materials and methods**:** In this study, 80 endophytic bacteria were isolated from tomato tissues. Their antagonistic activity screened based on agar diffusion test, against tomato bacterial wilt disease (*Ralstonia solanacearum*)**.** They were identified based on the morphological, biochemical properties and 16s rRNA sequence analyses. These strains were evaluated in greenhouse and tested for their ability to induce the production of defense-related enzymes in plants e.g. Peroxidase (PO), polyphenoloxidase (PPO) and phenolics based on spectrophotometer method.

Results: Results showed FS67, FS167 and FS184 strains had maximum inhibition zone forming. They identified as *Pseudomonas mossellii*, *P. fuorescence* and *P. brassicacearum* respectively. FS67 and FS167 strains significantly reduced disease in greenhouse. There was a significant increase in the activity of PO, PPO and phenolics in tomato plants treated with FS67, FS167 and pathogen.

Discussion and conclusion: The present study has shown that *P. mosselli* and *P. fuorescence* might have the potential to control *R. solanacearum.* However, the good results obtained *in vitro* cannot be gained the same as those in greenhouse or field conditions. So, further experiments are needed to determine the effectiveness of these isolates under field conditions.This work support the view that increased defense enzymes activities could be involved, at least in part, in the beneficial effects of endophytic bacteria on plants growth in interaction of pathogens. This is the first report of antagonistic activity of *Pseudomonas mossellii* from Iran.

Key words: Bio-control, Polyphenol oxidase, Phenolic compounds, Peroxidase

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Introduction

Ralstonia solanacearum Smith (1) is an important soil borne bacterial plant pathogen with a worldwide distribution and a wide host range. Some of its economically important plant hosts include tomato, potato, eggplant, pepper, tobacco, banana, chili and peanut (2). However, different strategies for the management of bacterial wilt have been used in the world, to date no effective control methods exist for bacterial wilt disease. Plant breeding, field sanitation, crop rotation and use of bactericides have had only limited success (3). Hence, biological control method has been studied for more than 60 years. Various recent studies have indicated that biological control of bacterial wilt disease could be achieved by using antagonistic bacteria (4, 5). One important group of antagonistic bacteria is bacterial endophytes that offer great potential for protection and have beneficial effects on plants. Bacterial endophytes, as the name indicates, are defined as microorganisms that colonize living internal tissues of plants without causing signs of plant diseases. (6, 7). Nawangsih *et al*. (8) isolated 49 endophytic bacteria, from healthy tomato stems and *Staphylococcus epidermidis* and *Bacillus amyloliquefaciens* were able to control the *R. solanacearum*. Results of other researchers indicated that potential bio-control agents against *R. solanacearum* are mostly species of *Pseudomonas* (9, 10, 11). Maji and Chakrabartty (11) showed that combination of *Pseudomonas* sp. with the pathogen, control the disease and also improved the fresh weight, dry weight and vigor index of the tomato seedlings.Growth promotion of plants by endophytic bacteria may be achieved by production of phytohormones, nitrogen fixation and phosphate solubilization which also protect host plants by the synthesis of antimicrobial substances, production of siderophores and by induction of plant defense mechanisms (12). Induction of plant's defense genes by prior application of inducing agents is called induced resistance. The defense gene products include peroxidase (PO), polyphenol oxidase (PPO) that catalyze the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexins and phenolics synthesis (13). Abo-Elyousr*et al.* (14) showed that application of acibenzolar-S-methyl (ASM) and *Pseudomonas fluorescens* (Pf2) alone or in combination could control the tomato bacterial wilt disease and significant changes in the activities of polyphenol oxidase, glucosidase and peroxidase in tomato after the application of ASM and Pf2 and inoculation with *R. solanacearum*. Bacterial wilt caused *by R. solanacearum* is one of the important diseases in field-grown potato and tomato production in Hamedan province. Considering the limitations of the different strategies for the management of bacterial wilt and due to recent awareness about human health and environment, biological method is preferred for management of *R. solanacearum*. Hence, we studied the effect of native endophytic bacteria against *R. solanacearum* and followed the changes in PO, PPO and total phenolic compounds as biochemical markers after treatment with certain bio-control agents, in order to correlate the levels of these enzymes to the level of tomato bacterial wilt.

Materials and methods

Isolation of endophytic bacteria: Samples of plant were randomly collected from tomato fields that had been cultivated with tomato for at least two consecutive years, at different villages of Hamedan province, Iran. The selected plants were healthy and their age was fewer than sixty days. Endophytic bacteria were isolated from the

internal tissues of roots, leaf and stems. Samples were briefly washed under running tap water and cut in to 0.5-1 cm long pieces. These pieces were disinfected by soaking each in 3% sodium hypochlorite and then adding 1 ml of Tween 20 (polyoxy ethylene esorbitanmonolaurate) for 3–6 min, depending on the tissue then transferred to 70% ethanol for 1–2 min and finally rinsed three times in sterile distilled water. To check sterility, we plated aliquots of the sterile water which was used in the final rinse in 10% TSA (1.5 g/L of triptone, 0.5 g/L of soy peptone, 1.5 g/L of NaCl, 15 g/L of agar, pH 7.3) at 28 ºC for 15 days and checked the plates for microbial growth colony. The tissues were macerated with 6 mL of aqueous solution (0.9 % NaCl) by a sterile mortar and pestle. To allow the complete release of endophytic microorganisms from the host tissue, macerate was incubated at 28 ºC for 3 hours. A dilution serial was made and was placed on five 10% TSA plates for each dilution (10^{-1} and 10^{-2}). The plates were incubated at 28ºC. Colonies were selected according to their growing time and morphology (color, size, shape) on 2, 5, 10, and 15 days post incubation. All bacterial colonies purified and pure isolates were maintained in sterile water at 4°C for further investigation (15).

***In vitro* antagonistic activity:** Antagonism test was done on nutrient agar medium by using agar-diffusion method (16). One-hundred microliters of *R. solanacearum* suspension containing 10^8 CFU/ml was spread on NA plates and four holes of 7 mm diameter punched into the agar. In these holes 40 µl supernatant, obtained after centrifugation and filtration by 0.22 lm filter (Millipore) of a 48-h-grown culture of each test antagonist (10^9 CFU/ml) was added and the plates incubated at 28 C° for 48 h. The effectiveness of strains was evaluated by measuring the radius of inhibition zone around antagonist bacteria. The experiment was performed with a completely randomized design with three replications and repeated twice.

Identification of potentialant agonistic bacteria: The selected antagonists were differentiated based on their reactions to standard biochemical tests from *Bergey's Manual of Systematic Bacteriology* (17) and Schaad *et al.*, (18). The antagonists were finally characterized by their 16S rDNA sequencing by Macrogen Inc. Korea. Universal primers 27(8F) and 1492R (19) were used for amplification of 16S rDNA region. The final reaction mixture for polymerase chain reaction (PCR) was 25 µl, contained of 2 µl of magnesium chloride [$MgCl_2$] (50 mM), 2.5 µl PCR buffer (10 X), 0.5 µl of dNTPs (10 mM), 1 µl of each forward and reverse primers (10 pmol /µl),15.5 µl sterile deionized water, 1 µl template DNA and 0. 4µl of Taq DNA Polymerase 5unit/µl (CinnaGen, Iran). Amplifications were carried out in an automated thermal cycler (T Gradient-Biometra, Germany) with following conditions: an initial denaturation in 94℃ for 5 min, 35 cycles of denaturation (94℃ for 45s), annealing (60℃ for 50s), extension 72℃ for 2 min and final extension in 72℃ for 10 min. PCR product size was confirmed on 1% agarose gel electrophoresis and photographed under UV light**.** DNA sequences of antagonist strains were compared with sequences deposited at the National Center for Biotechnology Information (NCBI) using BLAST.The phylogenetic and molecular analyses were performed with all the closely related taxa according to procedure as described previously, using MEGA version 5.1 (20). The stability of the relationship was assessed by bootstrap analysis by performing 1000 re-samplings for the tree topology of the neighbor-joining method.

Greenhouse evaluation of antagonist isolates for vascular wilt management: Greenhouse evaluation was carried out by using the root soaking method. Three strains of bacteria with the greatest inhibition in *in vitro* test were tested in the greenhouse to evaluate their ability to control bacterial wilt *in planta*. A potting medium that contains quartz sand (1:3) was sterilized at 121 °C for 20 min. Tomato seeds (Cv "CALL j N 3") were grown in this mixture soil in a greenhouse at 25-30 °C, watered daily with 1/2 Hoagland's solution. One-month-old tomato seedlings, which had 5-6 leaves, were selected for experiments. Prior to planting, seedlings roots were dipped in a suspension (10^9 CFU / ml) of endophytic bacteria for 60 min and transplanted immediately. The non-treated control plants were dipped in distilled water. One week after transplanting, treatments were drenched with pathogen at 10^7 CFU/g soil. Infected control treatments were only inoculated with *R. solanacearum*. Distilled water inoculated plants served as control 2. Three replicates of each treatment were performed in a completely randomized design. The experiments were conducted under greenhouse conditions at 24–28 °C and 75–90% relative humidity in 12 h light and 12 h dark conditions. Disease index percentage was recorded using the following formula (21):

Disease index (%)=[Σ(ni×vi)÷(V×N)]×100

Where, ni= number of plants with respective disease rating; vi= disease rating; V= the highest disease rating (5); and N= the numberof plants observed.

Disease rating was calculated as following scale:

1=no symptoms. 2=one leaf wilted. 3=two to three leaves wilted. 4=four or more leaves wilted. 5=whole plant wilted.

Induction of defense mechanisms and experimental design: According to the greenhouse results, best antagonistic bacteria were selected for evaluation of induction of defense enzymes activity. Green house experimental design was performed as above-mentioned. In this test after pathogen inoculation, top leaves of tomatoes were harvested at different time intervals: 0, 18, 24, 48, 72 and 96 h after pathogen inoculation for determination of total phenolic compounds, guaiacol peroxidase(PO) and polyphenol oxidase (PPO) activities. Treatments were replicated three times and the pots were arranged in a completely randomized design.

Preparation of crude enzyme extracts: Enzyme extract: Leaf tissue from treated and pathogen- inoculated tomato plants was immediately homogenized with liquid nitrogen and mechanically homogenized using a mortar in 4 ml of 50 mM potassium phosphate buffer (pH 7.0) with 0.1 mM EDTA and (4 % (w/v) PVP (poly-vinyl-pyrrolidone). The homogenate was centrifuged at 15,000 g for 30 min at 4 °C. The supernatants were stored at −80 °C or immediately used for determination enzymes activities and total protein. For all enzymes under investigation, each treatment consisted in four replications (plants) and two spectrophotometric readings were taken per replication using a spectrophotometer (WARIAN® CARY 100Conc).

Protein contents of the extracts were determined according to standard procedure of Bradford, (22).To each 50 µl of supernatant, 2.5 ml of Bradford reagent was added and mixed by vortex. After 5 min, readings were performed at 595 nm using a spectrophotometer. Protein concentration, expressed in mg per ml of sample (mg protein ml^{-1}), was determined using Bovine serum albumin (BSA) as the standard curve.

Enzyme activity assays Peroxidase assay: Guaiacol peroxidase (GPOX) activity was determined at 30 °C by measuring the conversion of guaiacol to tetraguaiacol at 470 nm. The reaction mixture consisted of 2.5 ml of a mixture containing 0.25 percent (v/v) guaiacol in 10mM, 0.1 M phosphate buffer (pH 7.0), and 0.1M hydrogen peroxide H2O2 (2 mM). Enzyme extract (50 µl) was added to initiate the reaction, which was followed colorimetrically at 470 nm at 30s intervals up to 3 min in a spectrophotometer. Activity was expressed as the increase in absorbance at 470 nm (min^{-1} mg^{-1} of protein). The boiled enzyme extract was used as blank (23).

Polyphenol oxidase assay (PPO): Polyphenol oxidase activity was determined as per the procedure given by Mayer *et al*. (24). The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm at 30s intervals for 3 min (min^{-1} mg^{-1} of protein).

Extraction and Determination of total phenolic compounds: The amount of total phenolics in extracts was determined with the Folin- Ciocalteu reagent.

0.5 g sample was grinded in 80% ethanol containing HCL 10%. The homogenate was centrifuged at 10000 rpm for 20 minutes and the supernatant was saved. The residue was re-extracted in 80% ethanol, centrifuged and the supernatant was evaporated to dryness using water bath set at 60°C. To determine total phenolic compounds, one ml of the ethanolsupernatant, prepared as described above, was added to 5 ml distilled water and 250 µl Folin-Ciocalteu reagent (Merck) and the solution was incubated at room temperature. After 3 min, 1 ml of a saturated solution of Na_2CO_3 and 1 ml distilled water was added and the reaction mixture was incubated for 1 h. The absorbance of the developed blue color was measured at 725 nm using a blank of water and reagent only. Gallic acid was used as a standard and the total phenolic compounds were expressed as milligram gallic acid per g of leaf fresh weight (25).

Statistical analysis: The data obtained in this study was subjected to the analysis of variance (ANOVA), using the SAS 9.1.3 statistical software, for a completely randomized design and completely randomized block design. The means were separated by Duncan's multiple range tests with $P< 0.05$ being accepted as significant.

Results

***In vitro* antagonistic activity assays:** All bacteria were screened against *R. solanacearum* by agar-diffusion method. The results of test indicated FS67, FS167 and FS184 strains had maximum inhibition zone and they were selected and used for further studies (Table 1).

Table1- *In vitro* inhibition of growth of *Ralstonia solanacearum* by antagonist isolates (FS67, FS167 and FS184) in agar diffusion method.

Antagonistic strain	Inhibition zone (mm)
FS167	*17.9± 1 [a]
FS67	15.3 ± 0.9 [a]
FS184	13.4 ± 1 [a]
	CV = 18.2 %

*The values given are mean (n= 9) with standard deviation. Values with different letter indicate significant differences ($P< 0.05$) according to Duncan's multiple range tests.

Identification of potentialantagonistbacteria to *R. solanacearum*: Based on standard biochemical methods (Table 2) and molecular identification (BLAST search), antagonist bacteriaFS67, FS167 and FS184 were identified as *Pseudomonas mosselii*, *P. fluorescens* and *P. brassicacearum* respectively. The sequences of the amplified products (universal primers 8F and 1492R for 16srRNA) then, were deposited in the GenBnk database and

assigned accession numbers: FS67:KY231157, FS167: KY231156 and FS184: KY231158. The Phylogenetic tree showing inter-relationship of these strains with closely related species of the genus *Pseudomonas* inferred from aligned unambiguous sequences of 16S rRNA gene. FS67, FS167 and FS184 clustered with and belonged to the genus *Pseudomonas* (Figure 1). Because the present work is the first report of isolation and antagonistic activity of *P. mosselii* from tomato plants from Iran, we indicated some important biochemical characteristics of it in Table 2.

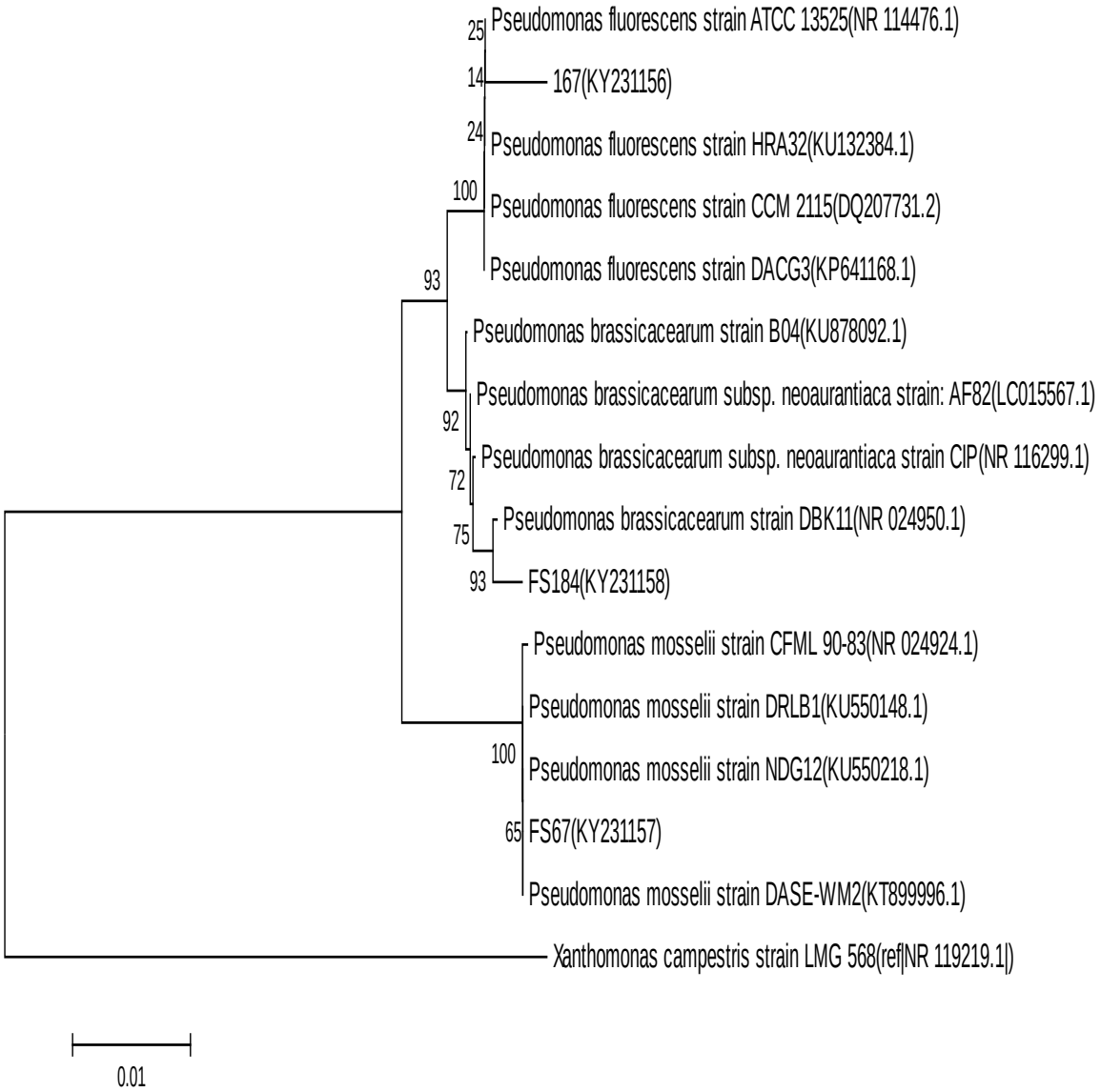

Fig. 1- Phylogenetic tree analysis of antagonist strains, FS67, FS167 and FS184 based on the nucleotide sequence of 16S rRNA gene. The branching pattern was generated by the neighbor joining (NJ) method. Bootstrap probability values are indicated at branch-points (tree re-sampled for 1,000 times). Accession numbers are indicated in parentheses.

Table 2-Some important biochemical characteristics of *Pseudomonas mosselii* strains.

Biochemical tests	
Gram reaction	-*
Pigment production	+
Catalase test	+
Oxidase test	+
Citrate utilization	+
Arginine decarboxylase	+
Gelatin liquefication	+
Growth at 4°C	+
Growth at 42°C	-
Glucose fermentation	-
Lactose fermentation	-
Levan test	-
Indole production	-
Assimilation of:	
Adonitol	+
Erythritol	d
Mannitol	+
Sorbitol	+
sucrose	+
Galactose	+
Lecithinase	+
Gelatinase	d
Tryptophan	+
Inositol	+
Pectolytic activity	-
antibiotic sensitivity:	
Ampicillin	+
Tetracyclin	+
Chloramphenicol	-

*(+) 90% or more of the strains are positive; (-) negativereaction; (d) 11±89% of the strains are positive.

Greenhouse evaluation of antagonist isolates for bacterial-wilt management: In greenhouse evaluation, FS67and FS167 isolates reduced disease severity of bacterial-wiltwith compared pathogen inoculated treatment significantly ($P < 0.05$). Disease index (%) was 40, 57.5 and 75.6%in antagonist treatments, FS167, FS67 and FS184 respectively. Disease severity in FS184 treatment was not significantly different from infected control (81.5%, Figure 4).

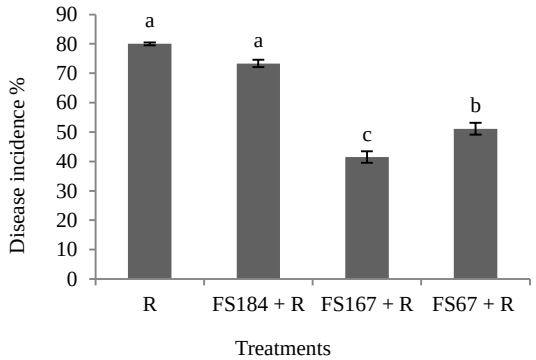

Fig. 2- Effect of antagonist strains FS67, FS167 and FS184 on disease incidence of *Ralstonia solanacearum* in greenhouse. R: of *Ralstonia solanacearum.* Bars represent the mean ± standard error values.

Assay of defense related enzymes

PPO, PO and total phenolic compounds activities analysis in tomato leaves: All antagonistic bacteria influenced the changes in defensive related enzymes and phenolic compounds activities, and the maximum activity of these enzymes occurred in different stages. Data in Figure 3 showed that all treatments indicated significant increases in the activity of PPO when compared to control plants. The activity of PPO were higher than non-inoculated control and reached maximum at 48 h after pathogen inoculation (Figure 3), but it started to decrease only in pathogen-inoculated treatment at 48 h. All antagonistic treatment started decreasing after 72 h. The PO activities of tomato treated with antagonistic bacteria were increased rapidly at 18 h after pathogen inoculation. It declined in all treatments 24 h after pathogen inoculation, except for *P.fluorescens* treatment; however, it was high level in all antagonistic treatment compared to only pathogen-inoculated control. Maximum PO activity was noticed in the *P.fluorescens* and *R. solancearum* inoculated plants compared to all treatments and kept higher level from 18 to 72 h and got to maximum (1.6U/mg protein $ml^{-1)}$) at 72 h(Figure 4).

There was a progressive and significant increase in total phenols activity in plants treated with antagonistic bacteria and pathogen. Its activity dropped at 18 until 72 h after pathogen challenge and then declined at 96 h. Maximum level of total phenols in only pathogen-inoculated plants was at 24 h but its decline was at 48 h after the pathogen inoculation (Figure 5). Total phenols activity was significantly higher in plants treated with *P. fluorescens*, resulted in the maximum accumulation of phenol (7.16 (mg/gFW)) when compared to the pathogen inoculated control (1.46 (mg/gFW)).

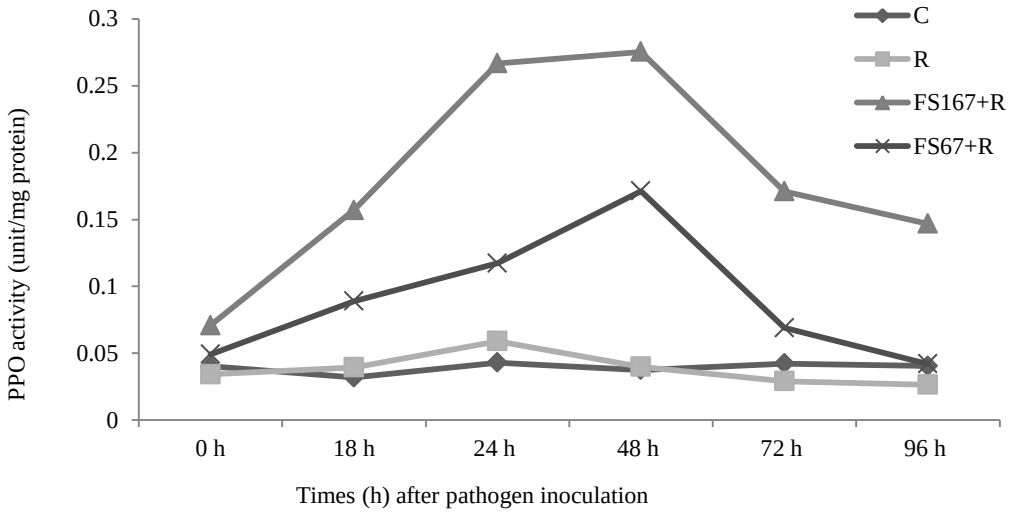

Fig. 3- Induction of activities of polyphenol oxidase in tomato plants treated with bio-control agents (FS167 and FS67) 0-96 h after *R. solancearum* inoculation. C: non-inoculated and R: only pathogen inoculated plants.

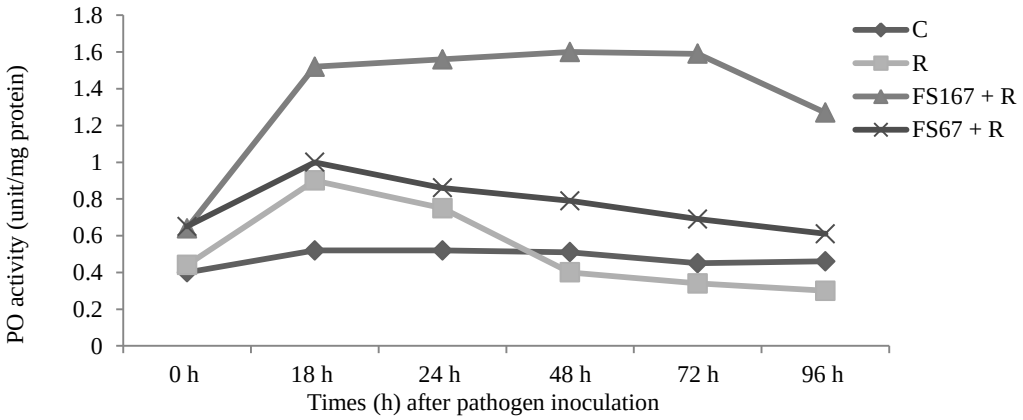

Fig. 4- Induction of activities of peroxidase in tomato plants treated with bio-control agents (FS167 and FS67) 0-96 h after *R. solancearum* inoculation. C: non-inoculated and R: only pathogen inoculated plants.

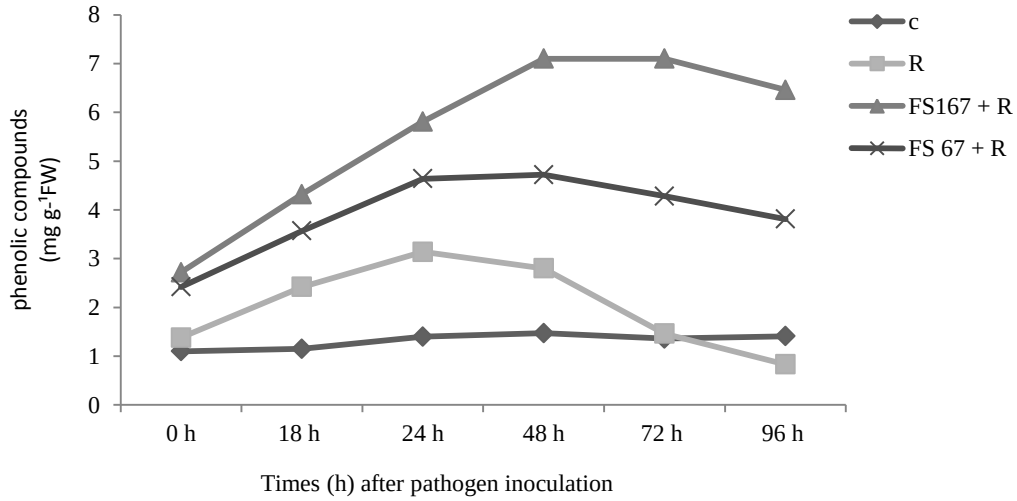

Fig. 5- Induction of activities of total phenols in tomato plants treated with bio-control agents (FS167 and FS67) 0-96 h after *R. solancearum* inoculation. C: non-inoculated and R: only pathogen inoculated plants.

Discussion and conclusion

In the present investigation, functional characteristics of *P. mosselii, P. fluorescens* and *P. brassicacearum* have been determined and *P. mosselii* and *P. fluorescens* could control the bacterial wilt, and *P. fluorescens* was the best among them. Ramesh and Phadke (26) screened endophytic bacteria and rhizobacteria for their antibacterial activity against *R. solanacearum* in eggplant. They reported that *Pseudomonas* spp. and *Bacillus* spp. significantly reduced wilt incidence in the greenhouse condition.The first event leading to activation of plant anti-microbial defenses is the recognition of the pathogen by plant to percept signaling components immediately after invasion of the pathogen. This phenomenon will increase the rate of enzymes production related to plant defense mechanism such as PO and PPO (27, 28). Many researches has shown that inoculation of plants with antagonists, can result enhanced level of these enzymes after pathogen attack (29, 30,31). In the present study, results show that antagonistic treatments can significantly increase activities of PPO, PO and total phenols at some periods after challenge inoculation with *R. solanacearum*. Plants treated with

R. solanacearum alone also, showed increased PPO, PO and total phenols activities but the increase was moderately less. Un-inoculated control plants (C) showed only basal level of PPO, PO, and total phenols activities in leaves and did not show any significant variation in their specific activity in this period. Plants treated with antagonistic bacteria showed high level of activities of PPO, PO and total phenols at 0 h after pathogen inoculation, that is indicated antagonistic bacteria has a role in inducing resistant of tomato plants. However, their activities declined in different times of pathogen inoculation in all treatments; their activities were significantly higher than the pathogen inoculated and un-inoculated controls. Maximum activities of PPO, PO and total phenols (0.27, 1.6 l mol min^{-1} mg^{-1} of protein and 4.72 mg/gFW respectively) were observed in combination of *P. fluorescens* and pathogen treatments. The results obtained are in agreement with those of Murthy *et al*. (32) who screened *P. fluorescens* isolated from rhizosphere of tomato against *R. solanacearum.* They showed the seedling treatment of *P. fluorescens* isolates induced a significant increase in the activities of peroxidase, Polyphenol oxidase , phenylalanine ammonia lyase and *β*-1, 3-glucanase and increase in enzyme activity was observed in *P. fluorescens* treated tomato seedlings challenged with *R. solanacearum*. Seleim *et al.* (33) studied the effect of rhizobacterial strains to control bacterial wilt of tomato under greenhouse and field conditions and the effect of these bio-agents in induction of PO and PPO activity *in planta*. Under greenhouse conditions, *P. putida* and *P. fluorescens* reduced the disease and activity of PO and PPO in tomato plants significantly increased. Inoculation of plants with some antagonistic bacteria elicits induced resistance that resulted to increasing defense-related enzymes activities and allows the plants to react to the pathogen attack strongly (30, 34). This work support the view that increased enzyme activities could be involved, at least in part, in the beneficial effects of endophytic bacteria on tomato plants growth in interaction of pathogens. Our results showed relative correlation among PO, PPO and phelolic compounds activities with the efficacy of test bio-agents and the induction of systemic resistance. It is very important that plant defense mechanism activate in an appropriate time to prevent the pathogen overcome. In infected plants which pathogen overcome, the rate of defense enzymes activities cannot reach to appropriate level at the right time. However, the enhanced signaling capacity in induced plants would facilitate a faster and stronger basal defense reaction (30). In addition, the first hours (especially 72 h) after pathogen invasion it is very critical that the level of component related to plant resistance especially defense enzymes reach to the high level to prevent the pathogen overcome. According to the literatures after this critical time, the level of defense related enzymes decrease in resistance and susceptible plants, however this time can be changed depending on pathogen, bio-control agents and plant host (35, 36). Microbes acting through ISR (such as some strains of *Pseudomonas*) colonize the root where they send signals to the plant which induced directly defense genes or prime for enhanced expression of defense genes upon subsequent pathogen attack (priming phenomenon) (37). The present study indicates that plants treated with antagonistic bacteria quickly, react to the pathogen attack and activation of defense related enzyme occurred earlier and higher than non-induced plants. However, it is difficult to claim that induced disease resistance against *R. solanacearum* is associated with the “priming” effect of the bio-control bacteria. Because priming

shows another layer of complexity of ISR and here the evidence appears insufficient to claim priming, since further studies are required to understand priming effect of these bacteria. Ideally, the presence of defense priming would be supported by analyses of the defensive state of a plant, especially activation of transcription factor (TF) genes before and after challenging with pathogen (30, 36, 38).

These results showed *P. fluorescens* and *P.mossellii* are promising tools in integrated management of plant diseases. This study is the first report of antagonistic activity of *P. mossellii* from Iran. *P.mossellii* is extensively distributed in the diverse environments of soil and water. It can easily colonize soil and it is able to degrade a wide variety of aromatic chemicals (39,40). Plant growth promoting and antifungal activity of *P. mosselii* were reported in chickpea, groundnut and banana plants (41, 42, 43). Singhai and *et al*. (44) revealed the potential of *P. mosselii* strain R1 in promoting plant growth as well as inducing antimicrobial mechanisms in the potato plants against *Streptomyces* scabies, when applied with vermicompost. In the present work, also some plant growth promoting and bio-control traits of *P. mossellii* were evaluated. *P. mossellii* could solubilize phosphate and produce indole-3-acetic acid (IAA), sidrophore, hydrogen cyanide and antifungal degrading enzymes as well as exhibited antifungal activity (data has been not showed). However, the good results obtained *in vitro* cannot gain the same as those in greenhouse or field conditions. In this study *P. brassicacearum* could control *R. solanacearum* in *in-vitro* conditions but not in greenhouse. There is some evidence which showed that effect of antagonistic agents in crop productivity and disease control varies under laboratory, greenhouse and field conditions. It is because many factors may influence activities of bio-control agents (45), soil is an unpredictable environment, and intended results will be difficult to be achieved (46). In the natural conditions (*in vivo*), bio-control agents have to be rhizosphere competent and effectively colonize the rhizosphere environment of the host plant for successful and effective disease management (47). Plant root exudates and root electrical signals selectively influence bacterial colonization (48). In addition, production of inhibitory substances that are involved in the reduction of *R. solanacearum* and induction of resistance to crop diseases are needed to efficient colonization of bio-control agents (49, 50). Numerous rhizo bacteria is able to trigger ISR appear to be endophytes (51), so these factors can also influence their bio-control capacities. Evidence is accumulating that there is not one definitive resistance pathway to ISR (52), so any activity of bio-control agents and their influence on host and pathogen depends on bio-control agents and the plant–pathogen system used.

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باکتری‌های اندوفیت گوجه فرنگی بیماری پژمردگی باکتریایی ناشی از *Ralstonia solanacearum* را سرکوب کرده و متابولیت‌های دفاعی را فعال می‌کنند

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چکیده

مقدمه: میکروارگانیسم‌های پاتوژن گیاهی بر سلامت گیاهان اثر گذاشته و تهدید اصلی برای تولید غذا و پایداری اکوسیستم هستند. افزایش استفاده از مواد شیمیایی جهت کنترل بیماری‌های گیاهی باعث ایجاد اثرات منفی بر سلامت انسان و محیط زیست شده است. افزایش نگرانی های عمومی درباره عوارض جانبی آنها منجر به تحقیقات برای پیدا کردن محصولات جایگزین برای این مواد شده است. یکی از این روش‌های جایگزین، کنترل زیستی با استفاده از میکروارگانیسم‌های آنتاگونیست مرتبط با گیاه است.

مواد و روش‌ها: در این تحقیق، ۸۰ باکتری اندوفیت از قسمت‌های مختلف گوجه‌فرنگی جداسازی و فعالیت آنتاگونیستی آن‌ها علیه پژمردگی باکتریایی ناشی از *Ralstonia solanacearum* با استفاده از روش نشت در آگار ارزیابی شدند. این استرین‌ها براساس ویژگی‌های مرفولوژیکی، بیو شیمیایی و آنالیز توالی 16s rRNA شناسایی و در گلخانه نیز ارزیابی شدند. توانایی آنها در القا آنزیم‌های دفاعی در گیاه مانند پراکسیداز، پلی فنل اکسیداز و ترکیبات فنلی در گلخانه براساس روش اسپکتروفتومتری بررسی شدند.

نتایج: براساس ارزیابی آزمایشگاهی سه استرین FS67، FS167 و FS184 بیشترین هاله بازدارندگی را ایجاد کردند و به ترتیب بعنوان *Pseudomonas mosselli*، *P. fuorescence* و *P. brassicacearum* شناسایی شدند.استرین‌های FS67 و FS167 در گلخانه باعث کاهش بیماری شدند. فعالیت پراکسیداز، پلی فنل اکسیداز و ترکیبات فنلی در تیمار استرین‌های FS67 و FS167 و پاتوژن بطور معنی داری افزایش یافتند.

بحث و نتیجه‌گیری: مطالعه حاضر نشان داد که *P. fuorescence* و *P. mossellii* میتوانند پتانسیل کنترل ***R. solanacearum*** را داشته باشند. اما از آنجا که نتایج به دست آمده از تحقیقات آزمایشگاهی ممکن است همان نتیجه را در گلخانه و یا مزرعه بوجود نیاورد لذا تحقیقات بیشتر برای تعیین اثرات این استرین‌ها در شرایط مزرعه به نظر ضروری می‌رسد. این مطالعه نشان داد که فعالیت آنزیم‌های آنتی اکسیدان میتواند بخشی از اثرات مفید باکتری‌های اندوفیت بر گیاه در برهمکنش با پاتوژن‌ها باشند. این اولین گزارش از فعالیت آنتاگونیستی *P. mosselii* در ایران است

واژه‌های کلیدی: کنترل زیستی، پلی فنل اکسیداز، ترکیبات فنلی، پراکسیداز

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