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Biological Control of Tomato Bacterial Wilt Disease by Endophytic *Pseudomonas fluorescens* and *Bacillus subtilis*

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BACTERIAL wilt disease caused by *Ralstonia solanacearum* is a serious threat for agricultural production of tomato. In this study, 80 endophytic bacterial isolates were isolated from healthy tomato plants in *R. solanacearum*-infested fields. Two endophytic antagonists designated HRA32 and HRA69 showing the highest antagonistic activity via *in vitro* screening were identified as *Pseudomonas fluorescens* and *Bacillus subtilis* using 16S rRNA analysis. The selected isolates were evaluated *in vitro* for their activities related to plant nutrition and plant growth regulation. Both of the assessed endophytes were found to exhibit capabilities in ammonia, indole acetic acid (IAA) and siderophore production as well as phosphate solubilization. Pot experiments were adopted to test the control efficiency against tomato bacterial wilt. Results revealed that HRA32 and HRA69 significantly reduced disease incidence when applied as separate treatments. The clear synergetic effect was observed in tomato plants treated with a mixture of the two antagonists reducing disease incidence significantly from 87.22% in the control to 16.66% with biological control efficacy of 80.23%. It is concluded that application of *P. fluorescens* HRA32 and *B. subtilis* HRA69 may be a promising approach for biological control of the tomato bacterial wilt and may play an important role in sustainable agriculture.

Keywords: Biological control, Bacterial Wilt, Endophyte, *Pseudomonas*, *Bacillus*, *Ralstonia solanacearum* .

In Egypt, tomato (*Solanum lycopersicum*) is considered one of the most important vegetable crops. The cultivated area is 216, 385 ha producing 8, 544, 990 tons with a productivity of 394, 898 kg/ha (FAO, 2010). Bacterial wilt of tomato caused by *Ralstonia solanacearum*, causes a considerable amount of damage to tomatoes and many other crops in tropical, subtropical and warm regions of the world and limits the production of many crops *e.g.* potato, tomato, eggplant and pepper. Approximately 450 plant species have been reported as hosts of this pathogen (Grimault *et al.*, 1994; Williamson *et al.*, 2002; Ji *et al.*, 2005; Swanson *et al.*, 2005; Seleim *et al.*, 2014). During infection, the pathogen can invade plant roots through wounds, and multiply in the cortical tissue before invading the xylem elements. In a matter of hours, the bacteria can spread into

the crown and stem through the plant's vascular system (Vasse *et al*., 1995). As the cell concentration number increases, virulence genes are expressed and cells become nonmotile and secrete exopolysaccharide and pectin-degrading enzymes, leading to the death of the plant (Saile *et al*., 1997).

To date, no effective control method has been developed for this wilt disease. Plant breeding, field sanitation, crop rotation, and use of bactericides have met induce with only limited success due to the high variability of the pathogen, high capacity of the pathogen to survive in diverse environments and its extremely wide host range (Lemessa and Zeller, 2007; Nguyen and Ranamukhaarachchi, 2010). Therefore, developing effective biological control agents is very important for the control of tomato bacterial wilt. Biological control not only increases crop yield and suppresses disease but also avoids environmental pollution. Rhizobacteria have been applied to various crops to enhance growth, seed emergence, crop yield and disease control (Wang *et al*., 2010; Babu *et al*., 2015 and Mansotra *et al*., 2015). Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly.

The mechanisms by which PGPR can influence plant growth may differ from species to species as well as from strain to strain. Growth promotion mechanisms may be direct, *i.e*., production of growth hormones, phosphate solubilization, nitrogen fixation or indirect, such as suppression of deleterious microorganisms by siderophore production or secretion of antimicrobial metabolites (Jha *et al*., 2012 and Thokchom *et al*., 2014). PGPR can protect plants against pathogens by various mechanisms such as antibiosis, nutrient competition or niche exclusion (Lugtenberg and Kamilova, 2009).

The objective of this research was to isolate and evaluate potential edophytic antagonists for their ability to suppress the growth of *R. solanacearum in vitro* and *in vivo* conditions in attempt to control bacterial wilt disease in tomato.

Material and Methods

Isolation of Ralstonia solanacearum from diseased plants

Tomato plants showing bacterial wilt symptoms were collected from local fields in Giza governerate, Egypt and washed under running tap water to remove sand and soil. The stems of the plants were surface sterilized with 70% alcohol, flamed, chopped using a sterile scalpel and immersed in sterile water to allow the bacteria to ooze out from the infected stem. After 30 min. a loopful of the suspension was streaked onto triphenyl tetrazolium chloride (TTC) agar (0.5% glucose, 1.0% peptone, 0.1% casamino acid and 1.8% agar with 5 ml of a 1% stock solution of 2,3,5-triphenyl tetrazolium chloride) as described by Kelman (1954). After 48 hr incubation at 30^{o}C, colonies showing irregular viscous appearance with pink centre and white border were selected, purified and tested

for its solubility in 3% KOH to eliminate any possible confusion of the organism with other wilt causing pathogen of tomato (Maji and Chakrabartty, 2014). KOH soluble colony designated TW15 was selected as a putative *R. solanacearum.*

Pathogen identification by 16S ribosomal RNA

The isolated phytopathogen was identified by sequencing of 16S rRNA gene. In brief, genomic DNA was extracted using GeneJET™ Genomic DNA Purification Kit (Thermo Scientific, USA) according to manufacturer's protocol and the 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). PCR was performed in a total volume of 50 µl containing 2.5 µl 10X DreamTaq buffer, 50 ng genomic DNA template, 0.4 µM of each primer, 0.2 mM of each dNTP, one unit DreamTaq DNA polymerase (Thermo Scientific, USA) and finally water was added to make volume up to 50 µl. The amplification reaction was done in GeneAmp 9700 thermal cycler (Applied Biosystems, USA) with the following program: initial denaturation at 94°C for 4 min, denaturation at 94°C for 40 s, annealing at 55°C for 40 s, extension at 72°C for 1.5 min for 30 cycles, and a final extension at 72°C for 8 min. The amplified PCR product was purified using the QIAquick PCR Purification kit (Qiagen, Germany) according to the supplier's instructions. The purified DNA was sequenced with 27F and 1492R primers at GATC Biotech (Konstanz, Germany) using ABI 3730xl sequence analyzer (Applied Biosystems, USA). The forward and reverse DNA sequence reads were assembled to obtain the consensus sequence by using DNA Baser Sequence Assembler software v.3.5.3. Bacterial identification was conducted by comparing the obtained sequence against the BLAST server (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzTaxon-e server database (Kim *et al*., 2012).

Biovar determination

Biovar of *R. solanacearum* TW15 was determined according to method described by Hayward (1964) with some modifications. Lactose, maltose, cellobiose, mannitol, sorbitol and dulcitol solutions were filter-sterilized and supplemented to basal medium ($NH_4H_2PO_4$ 1.0 g, KCl 0.2 g, $MgSO_4.7H_2O$ 0.2g, Difco bacto peptone 1.0 g, Agar 3.0 g and Bromothymol blue 80.0 mg per litre). For control glucose water instead of sugar solution was used into the basal medium. 200 µl quantities were dispensed into sterile tissue culture plates (96 wells). 20 µl of bacterial suspension (10^8CFU/ml) was inoculated into each well containing sugar solutions. The plates were then examined after 3 days of inoculation for changing pH which was indicated by the change of colour (Schaad, 1988).

Isolation of endophytic bacterial antagonists

Healthy tomato plants were gently uprooted and brought to laboratory. Root sections were made using a sterile scalpel. The root samples of 2 –3 cm long were excised and surface sterilized by sequential immersion in 70% alcohol for 1 min. 2.5% sodium hypochlorite for 2 min. and 0.1% $HgCl_2$ for 1 min. and

washed in four changes of sterile phosphate buffered saline (PBS). Aliquots from the final buffer wash were checked for sterility. Selected samples were triturated in 10.0 ml of PBS in sterile pestle and mortar. The triturate was serially diluted and used for isolation of potential antagonists to *R. solanacearum* by modified triple layer agar technique (Herr, 1959).

For preparing the triple layer agar medium, 1 ml triturated root suspension was placed in a Petri dish to which 10 ml of 10% trypticase soy agar (TSA) (4 g of TSA in 1 liter DW) was added and stirred well. Petri dishes were allowed to stand for 1 hr to for solidification of the medium. A second layer of agar (5-7 ml of 0.1g agar in 100 ml DW) was then poured on top of the first layer. Plates were incubated for 2 days at 30°C. Afterwards, 5 ml of molten TTC agar seeded with *R. solanacearum* TW15 was overlain on the second layer using a 5 ml pipette as the third layer. Plates were kept at 30°C for 2 days. Thereafter, bacterial colonies showing inhibition zones were purified on TSA medium as suspected antagonists.

Screening of antagonists for effective suppression of R. solanacearum TW15

Eighty isolates showing antagonistic activity in the three-layer cultures were screened for effective suppression of the pathogen using perforated agar plate method. Candidate antagonistic bacteria and *R. solanacearum* TW15 were cultivated in King's B broth for 48 hr at 28°C. One-hundred microliters of *R. solanacearum* TW15 suspension containing 10^8CFU/ml was spread on King's B plates and holes of 9 mm diameter punched into the agar. In these holes 50 µl suspension of each test antagonist (10^8CFU/ml) was added and the plates incubated at 28°C for 48 hr. Inhibition of *R. solanacearum* TW15 growth was assessed by measuring the diameter of inhibition zone (mm) after incubation for 48 hr at 28°C. The most active antagonistic bacteria designated HRA32 and HRA69 were selected and identified by 16S rRNA sequencing as described above.

Characterization of antagonists for plant growth promotion (PGP) traits

Siderophore production

Siderophore production was detected by the universal method of Schwyn and Neilands (1987) using blue agar plates containing the dye chrome azurol S (CAS). Orange halos around the colonies on blue were indicative for siderophore production.

Phosphate Solubilization

To determine phosphate solubilization qualitatively, Pikovaskya (PKV) agar plates were inoculated with the selected bacterial antagonists. After incubation at 28±1ºC for 5 days, the formation of a clear zone around bacterial growth indicating phosphate solubilization capacity as described by Gaur (1990).

NH_3 production

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48–72 hr at 28 ± 2 ºC. Nessler's reagent (0.5 ml) was

added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

Detection of HCN production

Production of HCN by bacterial antagonists was observed according to the method of Lorck (1948). Freshly grown cells were spread on King's B or nutrient agar medium containing glycine (4.5 g/l). A sterilized filter paper saturated with 1% solution of picric acid and 2% sodium carbonate was placed in the upper lid of a petri dish. The petri dish was then sealed with parafilm and incubated at 30°C for 4 days. A change in color of the filter paper from yellow to reddish brown indicated HCN production.

Determination of indole acetic acid (IAA) production

Quantitative analysis of IAA was performed using the method of Loper and Scroth (1986). Bacterial culture was inoculated in King's B or nutrient broth medium with tryptophan (1, 2, and 5 mg/ml) or without tryptophan incubated at 28 ± 2 °C for one week. Cultures were centrifuged at 3000 rpm for 30 min. Two milliliters of the supernatant was mixed with 2 drops of orthophosphoric acid and 4 ml of Solawaski's reagent (50 ml of 35% perchloric acid and 1 ml of 0.5 M $FeCl_3$). Development of pink color indicates IAA production. Optical density was measured at 530nm using Jasco spectrophotometer. Concentration of IAA produced by cultures was measured with the help of standard graph of IAA.

Biocontrol activity of the endophytic antagonists against bacterial wilt

Pot Experiments were conducted at Botany and Microbiology Department, Faculty of Science, Cairo University. The two endophytic antagonists HRA23 and HRA69 were evaluated for control of tomato bacterial wilt according method described by Li *et al*. (2008). Antagonist *P. fluorescens* HRA32 was cultivated in King's B broth at 28°C for 24 hr while *B. subtilis* strain HRA69 was cultured in LB broth (trypton 10 g, yeast extract 5 g, NaCl 10 g, pH 7.0 ± 0.2) at 28°C for 24 hr. The cells were harvested by centrifugation (4000*x g*, 10 min), washed, and resuspended with sterilised saline (0.85% sodium chloride) to the final concentration of 10^8 CFU/ml. Pathogen *R. solanacearum* TW15 was grown in casamino acids peptone glucose (CPG) broth (1.0% peptone, 0.1% casamino acids, 0.5% glucose) (Smith et al. 1995) at 28°C and 150 rpm on rotary shaker for 48 hr; the cells were harvested by centrifuge (4000 *x g*, 10 min), washed and resuspended with sterilised saline until the final concentration of bacteria got 10^8 CFU/ml.

Tomato seedlings (*Solanum lycopersicum*) cultivar Castlerock of 21 days old and plastic pots containing a mixture of clay and sand (1:1 w/w. Pots were sterilized by autoclaving for 30 min. and each pot was transplanted with one seedling. The experiment included five treatments: (I) HRA32 treatment, plants were treated with antagonistic endophyte *P. fluorescens* HRA32 and pathogen *R. solanacearum* TW15; (II) treatment HRA69, plants were treated with antagonistic endophyte *B. subtilis* HR69 and pathogen *R. solanacearum* TW15;

(III) treatment HRA32/HR69, plants were treated with a mixture of *P. fluorescens* HRA32 and *B. subtilis* as well as pathogen *R. solanacearum* TW15; (IV) Control 2, Plants were only inoculated with *R. solanacearum* TW15; and (V) Control 1, plants were treated with the same volume of sterilised saline.

Cultures of antagonistic endophytes were diluted 100 times with sterilised saline to 10^8 CFU/ml. Plant roots were soaked in HRA32, HRA69 or mixture of their suspension for 20 min. and transplanted immediately; sterilised saline was applied into each control plant and 60 pots were used as replication. At 5 day after transplanting, treatments HRA23, HRA69, HRA32/HR69 and Control 2 were drenched with pathogen *R. solanacearum* TW15 at 10^8 CFU/g soil. The number of wilted plants was recorded in 30 days after transplanting. Disease incidence (DI) was calculated according to Li *et al*. (2008) with the following formula:

$$\text{DI (\%)} = \frac{\text{Number of wilted plants in each treatment}}{\text{Total number of plants in each treatment}} \; x \; 100$$

Biological control efficacy (BCE) was calculated according to Guo *et al*. (2004) as :

BCE (%) = $[(D_C \;\; D_T)D_C]$, where D_C is disease incidence of control 2 and D_T is disease incidence of the treatment group.

Statistical analysis

The measured data were subjected to the analysis of variance (ANOVA) appropriate to the design. The significant differences between treatments were compared with the critical difference at 5% level of probability by the Duncan's test using PASW 17.0 statistics software (SPSS Inc).

Results and Discussion

Isolation and identification of Ralstonia solanacearum

It was found that stem pieces of wilted tomato plants when dipped in water showed a continuous white streaming of bacterial ooze. From this suspension bacterial colonies were isolated on TTC agar. After 48 hr of incubation at 30°C, viscous colonies with pink centre and white border developed on the plate were picked. KOH soluble colony designated TW15 was selected and its 16S rRNA gene was amplified by PCR. The amplicon was purified and sequenced and the obtained sequence (1, 401 bp) was submitted to the GenBank database under the accession number KU132386. BLASTn analysis of the 16S rRNA sequence revealed that it was phylogenetically clustered with *R. solanacearum* showing 100% similarity to *R. solanacearum* UQRS 652 (accession number KC757033.1) and *R. solanacearum* ISPaVe 1322 (accession number AM690478.1). These results are in close agreement with the EzTaxon results. The results of biovar test revealed that *R. solanacearum* TW15 had positive response for lactose, maltose and cellobiose and negative results were observed for mannitol, sorbitol and dulcitol. Based on these results, *R. solanacearum* TW15 was classified as biovar 2.

Isolation, screening and identification of antagonistic endophytes

For isolation of potential antagonistic endophytes that suppress *R. solanacearum* TW15, modified triple layer agar technique and perforated agar method were used. Of 80 screened potential antagonistic isolates, two isolates designated HRA32 and HRA69 showing the largest inhibition zones were selected for identification and further studies (Fig. 1).

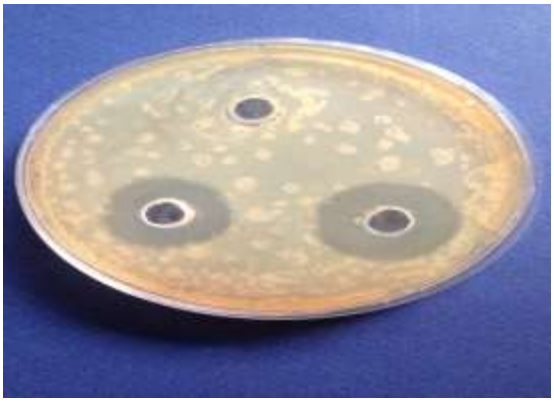

Fig. 1. Screening antagonistic activity of endophytic isolates against *R. solanacearum* by perforated agar method.

The most efficient antagonistic endophytes HRA32 and HRA69 were subjected to identification by amplification and sequencing their 16S rRNA gene. Based on BLASTn analysis and EzTaxon-e server database results, HRA32 and HRA69 were identified as *Pseudomonas fluorescens* and *Bacillus subtilis*, respectively. 16S rRNA sequence of *P. fluorescens* HRA32 and *B. subtilis* HRA69 were submitted to GenBank database under the accession numbers KU132384 and KU132385, respectively.

Characterization of antagonistic endophytes for PGP traits

Siderophore production

Siderophores production assay was conducted on the Chrome azurol S agar medium. The two bacterial species (*P. fluorescens* HRA32 and *B. subtilis* HRA69) produced siderophore that indicated by formation of yellow–orange halo around the bacterial growth due to iron chelation by siderophore production as shown in Fig. 2. These results were agreed with several authors who documented siderophore production by *Pseudomonas* spp (Hammami et al. 2013; Dharni *et al*., 2014 and Gusain *et al*., 2015). In addition, siderophore production by *Bacillus* spp was described as an important trait in growth promotion and suppression of phytopathogens (Colo *et al*., 2014 and Das *et al*., 2014; Haiyambo *et al*., 2015 and Sunar *et al*., 2015). Siderophores are low-molecular-weight molecules that are secreted by many microorganisms in iron limiting conditions (Crowley *et al*., 1991). There are several reports that showed microbial siderophores have a positive correlation with plant growth promotion

and biocontrol efficacy; therfore production of siderophores is one of the key factors that should be considered in PGPR screening programs (Chaiharn *et al*., 2009 and Sulochana *et al*., 2014).

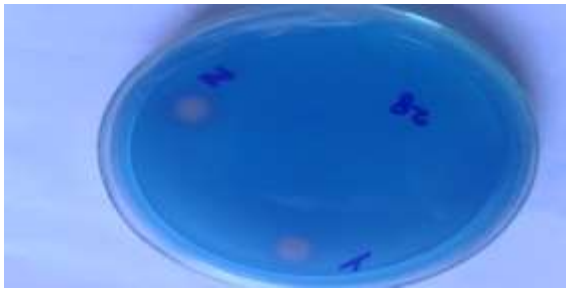

Fig. 2. Siderophore production by endophytic antagonists.

Phosphate solubilization

The ability of *P. fluorescens* HRA32 and *B. subtilis* HRA69 to solubilize inorganic phosphate was performed on Pikovaskya (PKV) agar plates qualitatively. Results revealed that both investigated isolates had the ability to solubilize inorganic phosphate efficiently. However *B. subtilis* HRA69 was more active in phosphate solubilization compared to *P. fluorescens* HRA32 (Fig. 3). Phosphate solubilizing bacteria increase availability of phosphorus to plants by solubilization of insoluble phosphorus in soil into soluble forms available for plant growth hence they act as biofertilizers. Phosphate is often the limiting nutrient for microbial and plant growth in soil and it is believed that solubilization of insoluble P from rock phosphate is due to the excretion of microbial metabolites such as organic acids (Gyaneshwar *et al*., 1998; Carrillo *et al*., 2002 and Rodriguez *et al*., 2004). Many reports recorded plant yield increases due to phosphate solubilizing bacteria in different crops (Afzal *et al*., 2005; Naik *et al*., 2008; Saïdi *et al*., 2013 and Shi *et al*., 2014).

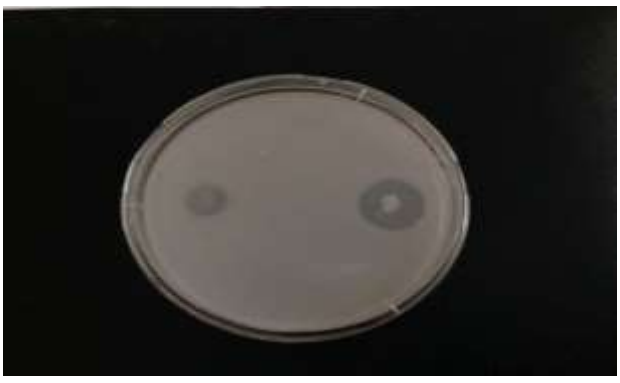

Fig. 3. Phosphate solubilization by endophytic antagonists.

NH_3 production

The production of ammonia was investigated in peptone water. Both *P. fluorescens* HRA32 and *B. subtilis* HRA69 were positive for ammonia production (Table 1). It was suggested that produced ammonia increases available nitrogen in soil and may has a positive effect on plant growth. In accordance with these findings Ahmed *et al*. (2008) screened 72 isolates of plant growth promoting rhizobacteria and found that all assessed fluorescent pseudomonads isolates were able to produce ammonia. Similar results were obtained by Anitha and Kumudini (2014).

TABLE 1. Characterization of selected bacterial isolates for plant growth promoting traits

Characteristics	Bacterial isolate	
	***P. fluorescens* HRA32**	***B. subtilis* HRA69**
Siderophore production	+	+
Phosphate Solubilization	+	+
NH_3 production	+	+
HCN production	+	-

Detection of HCN production : Production of HCN by bacterial isolates was tested. For the two bacterial isolates, only *P. fluorescens* HRA32 showed a positive result that observed by changing the color of the filter paper from yellow to reddish brown indicating HCN production (Table 1). The cyanide ion derived from HCN is a potent inhibitor of many metalloenzymes, especially copper containing cytochrome *c* oxidases (Blumer and Haas, 2000). In a previous study, HCN production by *Pseudomonas* sp was reported as important factor for control of bacterial canker disease of tomato caused by *Clavibacter michiganensis* subsp. *Michiganensis* (Lanteigne *et al*., 2012).

Determination of IAA production : Quantitative analysis of IAA was performed using King's B broth and nutrient broth medium with tryptophan (1, 2 and 5 mg/ml) or without tryptophan. Results revealed that *P. fluorescens* HRA32 was able to produce IAA without tryptophan, while IAA production by *B. subtilis* HRA69 was tryptophan-dependent. A significant increase in the production of IAA was observed in the presence of increased concentrations of tryptophan (1, 2 and 5 mg/ml) (Table 2). These findings were in close agreement with those reported in various studies dealing with IAA production by rhizobacteria showing significant enhancement of IAA by corporation of tryptophan into culture medium (Baldan *et al*., 2015 ;Sauvêtre and Schröder, 2015). Tryptophan and indole-3-acetamides are the key intermediates in the IAA biosynthesis pathways (Barbieri and Galli, 1993; Patten and Glick, 2002). Auxin may increase plant growth through cell enlargement, cell division, root initiation, increased growth rate and apical dominance (Frankenberger and Arshad 1995).

TABLE 2. Production of IAA by the two antagonistic endophytes.

Tryptophan concentration (mg/ml)	Procuction of IAA (µg/ml)	
	***P. fluorescens* HRA32**	***B. subtilis* HRA69**
0	3.12	-
1	12.56	23.17
2	28.46	33.22
5	48.39	59.66

Evaluation of endophytic antagonists for bacterial wilt biocontrol

In vivo evaluations were conducted to assess the screened antagonists using tomato seedlings. All tested bio-control agents caused a significant reduction of wilt disease compared to the control (Table 3 & Fig. 4). Results indicated that *P. fluorescens* HRA32 was more efficient in controlling bacterial wilt disease compared to *B. subtilis* HRA69. Trials with *P. fluorescens* HRA32 and *B. subtilis* HRA69 as separate treatments had disease incidence of 30.83% and 43.13%, respectively. Clear synergetic effect was observed when *P. fluorescens* HRA32 and *B. subtilis* HRA69 were applied together as a mixture causing the lowest disease incidence (16.66%). Biological control efficacy of *P. fluorescens* HRA32 and *B. subtilis* HRA69 was 63.24% and 48.41%, respectively, whereas its maximum value (80.23%) was achieved by using a mixture of them. These results agreed with those obtained by Li *et al*. (2008) who reported the use of efficient biocontrol agent *B. subtilis* strain AR12 that showed high biocontrol efficiency (90.18%) against bacteria wilt of tomato. In a similar study, *Pseudomonas aeruginosa* T1, *Pseudomonas sp*. BH25, *Pseudomonas* sp. AM12, *Pseudomonas* sp. AM13 and *Pseudomonas putida* R6 were reported as potent agents in control bacterial wilt in tomato (Maji and Chakrabartty, 2014).

TABLE 3. Efficacy of antagonistic endophytes in controlling tomato bacterial wilt.

Treatment	Disease incidence (%)	Biological control efficacy (%)
Control 1	0	---------
Control 2	$87.22 \pm 4.19^{(a)}$	---------
HRA32	$30.83 \pm 3.53^{(c)}$	$63.24 \pm 5.74^{(b)}$
HRA69	$43.133 \pm 2.30^{(b)}$	$48.41 \pm 4.96^{(c)}$
HRA32/HRA69	$16.66 \pm 2.35^{(d)}$	$80.23 \pm 1.97^{(a)}$

The same letter in each column indicates no significant difference according to Duncan's multiple range test ($p < 0.05$).

(±) represent standard deviations (SD).

Fig. 4. Efficacy of antagonistic endophytes in controlling tomato bacterial wilt.
HRA32: plants were treated with antagonistic endophyte *P. fluorescens* HRA32 and pathogen *R. solanacearum* TW15; HRA69: plants were treated with antagonistic endophyte *B. subtilis* HR69 and pathogen *R. solanacearum* TW15; HRA32/HR69: plants were treated with a mixture of *P. fluorescens* HRA32 and *B. subtilis* as well as pathogen *R. solanacearum* TW15; Control 2: Plants were only inoculated with *R. solanacearum* TW15; Control 1: plants were treated with the same volume of sterilised saline.

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السيطرة البيولوجية لمرض الذبول البكتيري في الطماطم بواسطة بعض أنواع البكتريا الداخلية الجذرية

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يعتبر مرض الذبول البكتيري الناجم عن الرالستونيا سولاناسيرم واحدا من أهم الامراض المهددة للإنتاج الزراعي للطماطم. في هذه الدراسة ، تم فحص ٨٠ عزلة بكتيرية داخلية من جذور نباتات الطماطم السليمة في الحقول المنتشر فيها مرض الذبول البكتيري و تم إختيار أكثر عزلتين نشاطا ضديا للرالستونيا و تم تعريفهما على أنهما سودوموناس فلورسينس و باسيلس ساتيليس. و تم تقييم نشاط العزلتين معمليا لانشطتهما المتعلقة بتغذية و تنظيم نمو النبات فأظهرت كلتا العزلتان القدرة على إنتاج الامونيا و حمض الإندول الخلِي و حامله الحديد بالإضافة إلى القدرة على إذابة الفوسفات. و بإختبار كفاءة العزلتين في السيطرة على مرض الذبول البكتيري كشفت النتائج إنخفاضا كبيرا في حدوث المرض عند معالجة النباتات بأي من العزلتين منفردا. وقد لوحظ تأثير التآزر واضحا في نباتات الطماطم المعامله بمزيج من العزلتين في الحد من الإصابة بالمرض بشكل ملحوظ من ٨٧.٢٢٪ (في النباتات الغير معاملة) إلي ١٦.٦٦٪ مع فعالية المكافحة البيولوجية تقدر بـ ٨٠.٢٣٪ . و خلصت الدراسة إلى أن تطبيق عزلتين سودوموناس فلورسينس و باسيلس ساتيليس قد يكون نهجا واعدا للمكافحة البيولوجية لمرض الذبول البكتيري في الطماطم ويمكن أن تلعب دورا هاما في مجال الزراعة المستدامة.