FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (35), No. 1, pp. 177-194, 2015

PHYLOGENETIC RELATIONSHIP, GROWTH PROMOTION AND ANTI-PHYTOPATHOGENIC ACTIVITY OF ENDOPHYTIC BACTERIA ISOLATED FROM TOMATO

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Received:18 October (2015) Accepted: 9 November (2015)

ABSTRACT

The phylogenetic tree based on 16 s rRNA gene sequencing of the previously isolated endophytic bacterial isolates indicated that isolates HMS1, HMS7, HMS8 and HMS9 which have the same branch were belonged to bacterial phylum α- Proteobacteria. However, endophytic bacteria isolates HMS2, HMS3, HMS4, HMS5 and HMS6 have the same branch and belonged to bacterial phylum γ-Proteobacteria. Furthermore, endophytic bacterial isolates HMS10, HMS11 and HMS12 were belonged to bacterial phylum Firmicutes. The present study was designed to evaluate the potential role of these endophytic bacterial isolates in promoting growth of tomato (*Lycopersicon esculentum*) plants. The anti phytopathogenic effect of three of these endophytic bacterial isolates (*Bacillus subtilis* HMS10, *Bacillus subtilis* HMS11 and *Bacillus malacitensis* HMS12) against three of damping off phytopathogenic fungi (*Rhizoctonia solani, Fusarium solani and Macrophomina phaseolina*) under greenhouse conditions were also determined. The results of inoculated plants with endophytic bacterial isolates showed significant differences in all

examined vegetative parameters (root length, shoot length, root fresh and dry weight and shoot fresh and dry weight) as compared to control. Significant differences in photosynthetic pigments and N, P, and K concentrations were also observed. The five isolates which were identified as (*Bacillus subtilis* HMS10, *Bacillus subtilis* HMS11, *Bacillus malacitensis* HMS12, *Rhizobium sp.* HMS1 and *Enterobacter cloacae* HMS2) exhibited the highest values of all growth parameter as compared to control. Generally, inoculation with all three tested endophytic bacterial isolates seem to be effective in reduction of damping off disease incident of tomato seedlings, when applied in mix with all tested pathogens compared to soil infected only with the pathogens.

Keywords: Endophytic bacteria- IAA- Tomato- Plant growth promotion- Bacillus- Enterobacter

INTRODUCTION

Plants are generally associated with diverse microorganisms. Of these microorganisms, endophytic bacteria which are defined as bacteria that their colonies are systemically found in the internal tissues of a plant, showing no external signs of infection or negative effects on their host (Schulz and Boyle 2006). There is a growing international interest in the beneficial role of endophytic microorganisms in plant health and development (Backman and Sikora 2008). Plant growth promotion (PGP) has been documented for many endophytic bacteria (Zachow *et al.,* 2010; Gasser *et al.,* 2011; Malfanova *et al.,* 2011). Endophytes can be beneficial to their host by promoting plant growth and also acting as biocontrol agents (Mercado-Blanco and Bakker 2007; Ryan *et al.,* 2008).

Despite their different ecological niches, free-living rhizobacteria and endophytic bacteria use the same mechanisms to promote plant growth and control phytopathogens (Compant *et al.,* 2005). For example, they can affect plant growth by producing auxins such as indole-3-acetic acid (IAA), or cytokinins; or by degrading the ethylene precursor ACC by ACC deaminase (Long *et al.,* 2008; Ryan *et al.,* 2008). Several studies have been revealed the positive effects of endophytic bacteria inoculation in plants. The results of Barreti *et al.,* (2008) revealed the positive effect of inoculating tomato (*Lycopersicum esculentum L.*) with endophytic bacteria on plant height, leaf area, leaf number, as well as fresh and dry plant weight. Inoculation of sugarcane (*Saccharum spp.*), increased contribution of biological nitrogen fixation, promotion of root development, increased biomass and productivity (Oliveira *et al.,* 2003). Likewise, inoculation of soybean plants (*Glycine max* (L.) Merr.), with endophytic bacteria increased their ability to inhibit growth and

sporulation of pathogenic fungi (Assumpção *et al.,* 2009).

The aim of the present study was to evaluate the potential role of the isolated and identified endophytic bacterial isolates in promoting growth of tomato (*Lycopersicon esculentum*) plants and to determine the ability of three of them (*Bacillus subtilis* HMS10, *Bacillus subtilis* HMS11 and *Bacillus malacitensis* HMS12) to protect tomato plants against the artificial inoculation with three of the soil-borne phytopathogens (*M. phasolina, R. solani and F. solani*) of damping-off under greenhouse conditions.

MATERIALS AND METHODS

The present experiments were carried out in green house belongs to Central Lab. of Organic Agriculture, (ARC), and Genetics Dep. Faculty of Agriculture, Minia University.

Endophytic bacterial strains

The 12 endophytic bacterial strains which have been used in the present work were previously isolated from tomato plants and identified by microbiological, physiological and molecular techniques by Mahmoud *et al.,* (2015). Phylogenic tree based on 16 s rDNA gene sequencing was constructed and analyzed according to (Wu *et al.,* 2012**).**

Surface sterilization of tomato seeds

Seeds of tomato (cv. Castle Rock) were obtained from the Ministry of Agriculture, Egypt. For comprehensive elimination of epiphytic microorganisms existing in tomato seeds, they were surface sterilized with 70 % ethanol for 1 min, 3 % Sodium hypochlorite for 3 min followed by 70 % ethanol wash for 1 min. Then, seeds were rinsed in sterile distilled water three times and blot dried.

Preparation of bacterial inoculum

The twelve endophytic bacterial isolates were grown on NA broth with constant shaking at 150 rpm for 48h at 30°C to approximately 10^6 cfu/ml. The bacterial cells were harvested by centrifugation at 13,000 rpm for 10 min, re-suspended in sterile distilled water and used for inoculation according to methods of Thompson, (1996).

Seed bacterization

Required quantity of seeds were soaked in ten milliliters of bacterial suspension containing 10^6 cfu/ml for 3h and dried under shade. The seeds soaked in sterile distilled water were maintained as control.

Pots experiment

For determining the effectiveness of the endophytic bacterial isolates on vegetative growth parameter of tomato seeds, plastic pots (12 cm width) under greenhouse conditions were filled with 3 kg of sterilized soil/sand in 1:1 ratio. 100 g of sterilized vermiculite and 4 g of rock phosphate were added to sterilized mixture of soil. Three replicate pots were specified for each treatment in completely randomized experimental design. Ten Coated seeds of tomato (Castle rock) were planted in each pot and irrigated weakly.

After 45 days of planting, tomato seedling were collected and the

percentage of seed germination was calculated. Different growth parameters included shoot and root length, fresh and dry weight for shoot and root were measured. The vigor index (mean root length + mean shoot length × % germination) was determined as described by Abdul Baki and Anderson, (1973). Chlorophyll a, chlorophyll b, and carotenoids were determined using spectrophotometer at the wavelengths of 440, 644, and 662 nm **(**Fadeel, 1962**)**. Nitrogen, phosphorus, and potassium contents were determined according to the methods described by (Dawwam *et al.,* 2013).

Preparation of pathogenic inocula

Three fungal strains (*Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina*) were tested under greenhouse conditions for their pathogenicity using susceptible tomato cultivar (Castle Rock). Inocula of these fungal isolates were prepared by growing each pathogen on corn sand meal medium supplemented with 0.2 % peptone solution (Abd El-Moity, 1985). Flasks containing the medium were inoculated with equal disks (0.5 cm in diameter) of five days old cultures. Inoculated flasks were then incubated at 25º C for 15 days. All inoculum were adjusted to contain $5x10^6$ cfu/gm by adding sterilized media and mixing thoroughly.

Soil infestation

Inocula of fungal strains ($5x10^6$ cfu/gm for each) were added to soil at the rate of 10 gm/kg soil. Plastic pots (15 cm – diameter) with infested soil were planted using 30 days old tomato cv. (Castle Rock) seedling. Plastic pots contain non-infested soil, supplied with the same amount of autoclaved sand corn meal were served as control. Three replicates were used for each treatment and each replicate containing 6 pots.

Preparation of endophytic bacteria inocula.

The endophytic bacterial isolates (HMS10, HMS11 and HMS12), were grown on liquid NA medium for 2 days, at 28ºC. After centrifugation, the pelleted cells were re-suspended in sterilized distilled water and adjusted to contain $3x10^6$ cfu/ml. At the age of 30 days, tomato seedlings (cv. Castle Rock) were treated with antagonistic endophytic bacterial isolates by the root-dipping method (Xue *et al.,* 2009) before transplantation into plastic pots.

Two control treatments were considered; the first (C1) was treated only with pathogen while the second control (C2) was not treated either with pathogen or tested isolates. Pots of one tomato seedlings were arranged in completely randomized block design with three replicates for each treatment and 6 pots for each replicates. The pots were placed in a greenhouse maintained at 28 ^oC ±2 with relative humidity of 30%, and a 12 h/12 h photoperiod. All pots were received the same treatment of irrigation and nutrition regime. The percentage of disease incidence in each treatment was calculated using the formula of Haggag and El- Gamal (2012) (Total number of diseased plants/Total number of plants) ×100%.

RESULTS AND DISSCUTION

Phylogenetic of the previously isolated endophytic bacteria

Twelve endophytic bacterial strains were previously isolated from roots, stems and leaves of tomato plant on PDA medium (Mahmoud *et al.*, 2015). Microbiological, physiological and biochemical characterization of these endophytic bacterial isolates were also conducted. Sequence analysis of the 16S rRNA gene indicates that endophytic bacterial isolates have homology with different bacterial strains. According to the percentages of homology with the Closest NCBI strain, the twelve isolates were recognized to different strains (Table 1).

Phylogenetic tree based on 16S rRNA sequences from twelve endophytic bacterial isolates, which was constructed using the neighbor-joining (NJ) method, as shown in Figure (1). The phylogenetic tree indicated that endophytic bacterial isolates (HMS1, HMS7, HMS8 and HMS9) which grouped together to the same cluster were belonged to bacterial phylum *α*- Proteobacteria. However, endophytic bacterial isolates (HMS10, HMS11 and HMS12) were belonged to bacterial phylum Firmicutes. Furthermore, endophytic bacteria isolates (HMS2, HMS3, HMS4, HMS and HMS6) have the same cluster and belonged to bacterial phylum *γ*- Proteobacteria. These results are in accordance with the findings of Malfanova *et al.*, (2011) who reported that the most predominant and studied endophytes belong to two major phyla (Proteobacteria and Firmicutes) and include members of *Bacillus* (Deng *et al.*, 2011), *Enterobacter* (Taghavi *et al.*, 2010), *Serratia* (Taghavi *et al.*, 2009). Species of these genera are ubiquitous in the soil/rhizosphere which represents the main source of endophytic colonizers (Hallmann and Berg, 2006).

The potential role of endophytic bacterial isolates in growth promotion of tomato plants

Effect of endophytic bacterial isolates on seedling growth of tomato

Different growth parameters (i.e. shoot and root length; fresh and dry weight of shoot and root) were measured after 45 days of sowing. Data in Table (2) indicated a significant and highly significant increase in root length due to treatments with all endophytic bacterial isolates when compared to control. The highest root length was observed in the treatment with isolates HMS10, HMS11 and HMS1 (10.97, 10.67 and 8.48cm, respectively) which significantly differed from all other treatments. Concerning the shoot length, all isolates exhibited significant increase in shoot length except isolate HMS4 which displayed negative effect on the shoot length of tomato plants (7.88 cm) as compared to control (8.10 cm). Treatment with isolates (HMS10, HMS1 and HMS12) showed the highest values of shoot length (14.35, 13.00 and 12.87cm, respectively).

The highest root fresh weight was observed in the case of inoculation

with isolates HMS10, HMS12, HMS1 and HMS2 (0.27, 0.24, 0.21 and 0.2g/plant respectively) which was significantly higher than all other treatments. The lowest root fresh weight (0.09 g/plant) was obtained when isolate HMS5 was used for treatment. On the other hand, treatment with all isolates except isolate HMS4 showed a significant increase in shoot fresh weight as compared to control. The maximum level of shoot fresh weight was achieved when isolates HMS10 (0.64 g/plant) and HMS1 (0.47 g/plant), were used for inoculation. Adversely, the lowest values of root fresh weight (0.17g/plant) were obtained by treatment with isolate HMS4.

The highest dry weight of roots (0.13 and 0.11 g/Plant) was achieved by treatment with isolates HMS10 and HMS12 while inoculation with isolates HMS4, HMS5 and HMS8 showed the lowest value of root dry weight with the same value (0.03g/plant) as compared with the control treatment (0.03g/plant). Regarding shoots dry weight, the maximum level was revealed when isolates HMS10, HMS12 and HMS1 (0.38, 0.27 and 0.26g/plant, respectively) were used for inoculation. However, the lowest values were obtained by the treatment with isolates HMS3, HMS4 and HMS5 with the same value (0.08g/plant).

Table (1): Identification of endophytic bacteria isolated from tomato (*Lycopresicum esculentum*) based on 16S rDNA sequence

Isolates	accession No.	% Similarity	Closest NCBI strain and accession No.	Phylum
HMS1	KT587347	99	*Rhizobium sp.* HJX3 KP979534	α-Proteobacteria
HMS2	KT587348	100	*Enterobacter cloacae* strain VITDAJ KP305912	γ-Proteobacteria
HMS3	KT750022	97	*Pantoea sp*. GrF KC311261	γ-Proteobacteria
HMS4	KT750023	99	*Pantoea ananatis* ITCC<IND>:B0055 JF756691	γ-Proteobacteria
HMS5	KT750024	92	*Serratia marcescens* strain RS8101 HQ123487	γ-Proteobacteria
HMS6	KT750025.	99	*Enterobacter sp.* UIWRF0482 KR190045	γ-Proteobacteria
HMS7	KT750026	99	*Agrobacterium tumefaciens* strain R6-364 JQ659820	α-Proteobacteria
HMS8	KT750027	99	*Agrobacterium sp.* HJX27 KP979558	α-Proteobacteria
HMS9	KT750028	99	*Ensifer adhaerens* KT321683	α-Proteobacteria
HMS10	KT750029	100	*Bacillus subtilis* strain MSEB 67 KP261080	Firmicutes
HMS11	KT750030	99	*Bacillus subtilis* strain JPS1-2 JQ308564	Firmicutes
HMS12	KT750031	99	*Bacillus malacitensis* strain F-61 KT027712	Firmicutes

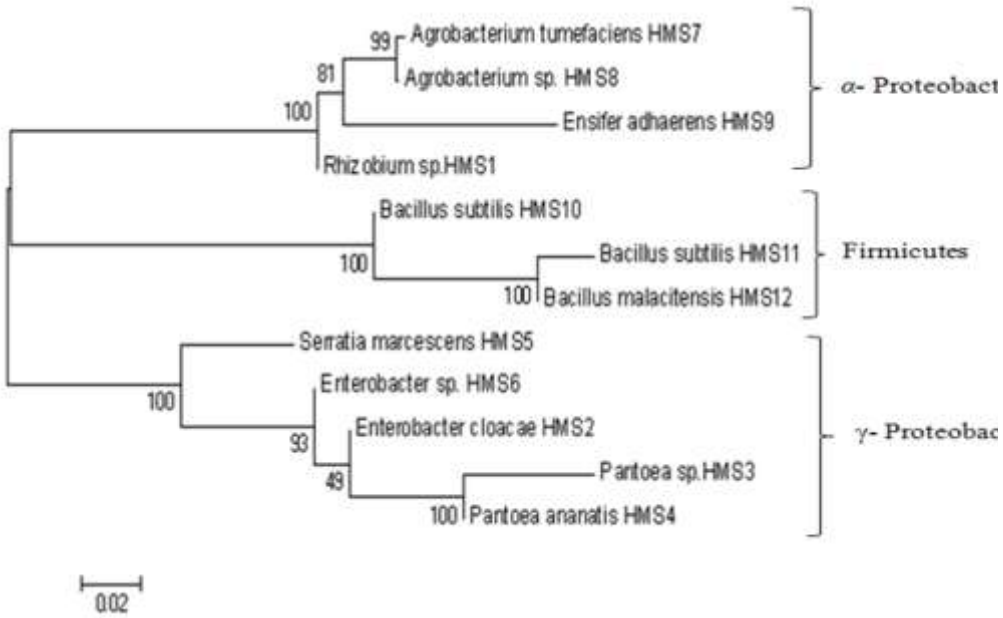

Fig. (1): Phylogenetic tree of the 16S rRNA genes showing the relationships of the endophytic bacteria associated with different parts of tomato plant. The tree was constructed using neighbor-joining method. Scale bar indicates 2% substitution of nucleotide.

Table (2): The vegitative growth parameters of seedlings recorded after seed fortification with the endophytic bacterial isolates by pre-sowing soaking inoculation.

Isolates	Root length (cm)	Shoot length (Cm)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
Control	**5.58**	**8.10**	**0.08**	**0.03**	**0.15**	**0.06**
HMS1	8.48	13.00	0.21	0.09	0.47	0.26
HMS2	8.18	12.67	0.20	0.08	0.31	0.18
HMS3	6.00	8.33	0.11	0.05	0.18	0.08
HMS4	6.42	7.88	0.10	0.03	0.17	0.08
HMS5	6.24	8.40	0.09	0.03	0.18	0.08
HMS6	6.67	10.73	0.14	0.07	0.35	0.19
HMS7	7.67	8.37	0.12	0.04	0.21	0.18
HMS8	6.67	8.55	0.11	0.03	0.23	0.18
HMS9	6.02	8.33	0.12	0.04	0.24	0.19
HMS10	10.97	14.35	0.27	0.13	0.64	0.38
HMS11	8.33	12.55	0.18	0.08	0.42	0.23
HMS12	10.67	12.87	0.24	0.11	0.44	0.27
LSD0.05	0.053	0.075	0.017	0.024	0.02	0.005

Effect of endophytic bacteria on germination percentage and seedling length

After 45 days of planting in pots under greenhouse conditions, the seedling growth parameters like, seedling length and vigor index in addition to germination percentage was recorded and the results are presented in Table (3). Data showed that all the treatments of endophytic bacterial isolates increased the percentage of seed germination of tomato. Seed bacterisation with isolate HMS10 showed the highest percentage of seed germination (100%) followed by treatments with the three isolates HMS1, HMS8 and HMS12 which have the same percentage of seed germination (96.67%) compared to the control (80%).

Table (3). Effect of endophytic bacterial isoletes on the seedling growth indicators of tomato.

Isolates	% Germination	RL+SL	Vigor index
Control	80.00	13.68	1095.10
HMS1	96.67	21.48	2076.73
HMS2	90.00	20.85	1876.43
HMS3	83.33	14.33	1195.07
HMS4	86.67	14.30	1239.27
HMS5	90.00	14.64	1317.67
HMS6	83.33	17.40	1450.00
HMS7	90.00	16.04	1443.67
HMS8	96.67	15.22	1471.20
HMS9	83.33	14.35	1195.83
HMS10	100.00	25.02	2501.67
HMS11	93.33	21.20	1978.67
HMS12	96.67	23.52	2273.60
LSD0.05	14.26	0.092	247.7

Concerning the seedling length, treatments with all isolates showed significant increase of seedling length as compared to the control treatment. The highest value of seedling length (25.02 cm) was observed when seeds were bacterized with isolate HMS10. The untreated control seedlings had the lowest vigor index (1095.10) as shown in (Table 3). However, all the treatments with endophytic bacterial isolates significantly increase the vigor index of tomato germinated seeds as compared to control treatment. Treatment with isolate HMS10 showed the highest value of vigor index (2501.67) followed by isolate HMS12 (2273.60) and HMS1 (2076.73).

Generally, data in Table (2) indicated that some endophytic bacterial isolates (HMS10, HMS12, HMS1, HMS11 and HMS2) revealed significant increase in all vegetative parameters of seedling growth (root length, shoot length, root fresh and dry

weight and shoot fresh and dry weight) as compared to control. In addition, data in Table (3) showed that inoculation with endophytic bacterial isolates increased the percentage of seed germination as well as seedling length. Based on traditional and 16s rRNA identification methods some of these isolates were identified as HMS1 (*Rhizobium sp.*), HMS2 (*Enterobacter cloacae*), HMS10 and HMS11 (*Bacillus subtilis*) and HMS12 (*Bacillus malacitensis*). These results are in accordance with the findings of Amaresan *et al.,* (2012) who stated that treatment with different isolates of endophytic bacteria related to genus *Bacillus* significantly increased all vegetative parameter of tomato and chilli plants. Likewise, the results of Garcı´a-Fraile *et al.,* (2012) indicated that rhizobium strains colonize the roots of tomato and pepper plants promoting their growth in different production stages and increasing yield and quality of seedlings and fruits.

Biochemical examination (Mahmoud *et al.,* 2015) showed that the isolates HMS10, HMS12, HMS1, HMS11 and HMS2 were positive for indole formation demonstrating production of a significant amount of IAA production. Thus, the plant growth promoting phenomenon observed in the present work could be attributed to the ability of the isolate to produce IAA, as IAA positively influences root growth and development, thereby enhancing nutrient uptake (Khalid *et al.,* 2004). In the same way, Spaepen *et al.,* (2007) and Taghavi *et al.,* (2009) reported that the production of plant growth-promoting molecules like IAA is an important contribution of endophytic microorganisms which can stimulate both rapid responses such as cell elongation and long-term responses such as cell division and differentiation of plants tissues. Investigations on the associated PGP characteristics with the targeted objective of seedling growth promotion indicated that most of the organisms possessed the capability for ammonia and indole production, the latter forming the precursor for the phytohormone IAA.

Effect of endophytic bacteria on photosynthetic pigments

Generally, data presented in Table (4) revealed that inoculation with all studied endophytic bacterial isolates significantly increased the photosynthetic pigments (chlorophyll a, b and carotenoids) of tomato leaves as compared to control treatment. Treatments with isolates HMS10, HMS1, HMS12 and HMS2 showed higher records of photosynthetic pigments rather than the control. The increase in total chlorophyll content recorded in the study reflected the increased rate of chlorophyll synthesis which enhanced photosynthesis and resulted in better plant growth.

These results are in accordance with the findings of Deivanai *et al.,* (2014) which indicated that inoculation of rice seeds with endopytic bacterial isolates significantly increased the chlorophyll a, chlorophyll b, and carotenoids as compared with uninoculated treatment.

Table (4): Effect of inoculation with endophytic bacterial isolates on photosynthetic pigments (Chlorophyll A, B and Carotenoids) of tomato shoots after 45 days of sowing

Isolates	Chlorophyll A (mg/g FW)	Chlorophyll B (mg/g FW)	Carotenoids (mg/g FW)
Control	3.11	1.67	2.00
HMS1	5.22	3.13	3.27
HMS2	4.07	2.72	2.83
HMS3	3.30	1.95	2.25
HMS4	3.41	2.05	2.18
HMS5	3.51	2.11	2.24
HMS6	4.16	2.89	3.04
HMS7	3.60	2.16	2.30
HMS8	3.71	2.23	2.36
HMS9	3.74	2.25	2.39
HMS10	5.49	3.30	3.43
HMS11	4.50	2.72	2.83
HMS12	5.34	3.00	3.13
LSD0.05	0.729	0.177	0.168

Effect of endophytic bacteria on NPK content of tomato

The results for plant nutrient uptake treated with bacterial isolates are given in Table (5). Generally it was observed that NPK contents of whole shoot of treated seedlings were significantly higher than that of untreated seedlings. Tomato seedling exhibited maximum percentages of N content when inoculated with isolates HMS10 (2.31%), HMS1 (2.22%) and HMS12 (2.08%) and higher than that of control plants. Regarding, Phosphorus and Potassium, shoots revealed a significant higher level of Phosphorus (0.23%) and Potassium (1.72%) content in seedlings inoculated with HMS10 bacterial isolate as compared to the control.

In the present study, it was found that inoculation with bacterial isolates not only improved the growth of seedlings but also increased the uptake of shoot NPK contents which were significantly higher in inoculated seedlings than control (Table 5). This increase might be due to high nitrogen fixation and phosphate solubilisation ability of endophytic bacteria. Increased nutrient uptake associated with seed treated plants may be the result of more root-shoot ratio resulting in enhanced nutrition because of seed treatment with bacteria (Kumar *et al.*, 2013). The increased nutrient uptake parameters could be attributed to the enhancement of root growth and development. The differences in plant growth promotion among the isolates are attributed to their individual competencies. Several bacteria,

particularly those belonging to the genus *Bacillus* spp., convert insoluble phosphate into soluble forms by secreting organic acids such as formic acid, acetic acid, propionic acid, citric acid, fumaric acid, gluconic acid, glyoxylic acid, ketobutyric acid, malonic acid, succinic acid, and tartaric acid. These acids make lower pH and bring about the dissolution of bound forms of phosphate. Some of the hydroxyl acids may chelate with calcium and iron resulting in effective solubilisation and utilization of phosphates (Paul and Sundara Rao 1971).

Table (5): Effect of inoculation with endophytic bacterial isolates on N, P and K contents in tomato shoot after 45 days of sowing

Isolates	N%	P%	K%
Control	1.51	0.14	1.12
HMS1	2.22	0.22	1.59
HMS2	2.01	0.21	1.49
HMS3	1.66	0.17	1.25
HMS4	1.71	0.17	1.30
HMS5	1.67	0.17	1.20
HMS6	1.86	0.19	1.42
HMS7	1.80	0.18	1.38
HMS8	1.84	0.18	1.40
HMS9	1.75	0.35	1.36
HMS10	2.31	0.23	1.72
HMS11	1.95	0.20	1.45
HMS12	2.08	0.22	1.69
LSD0.05	0.13	0.02	0.075

Antagonistic *activity* of endophytic bacterial isolates under greenhouse conditions.

The results of the *in vitro* antagonism experiment revealed that three out of the twelve endophytic bacterial isolates could significantly reduce the mycelial growth of five of the major phytopathogenic fungi (*Fusarium solani, Fusarium semitictum, Macrophomina phasolenia, Rhizoctonia solani* and *Aspergillus niger*) by forming an inhibition zone (Mahmoud *et al.*, 2015). These three antagonistic isolates were identified as (*Bacillus subtilis* HMS10, *Bacillus subtilis* HMS11 and *Bacillus malacitensis* HMS12). In the present experiment, the antiphytopathogenic efficiency of these endophytic bacterial isolates were evaluated for their ability to protect tomato plants against the artificial inoculation with three soil-borne phytopathogens (*M. phasolina, R. solani and F. solani*) of damping-off. The commercial tomato cultivar (Castle Rock) was used in this experiment. Data presented in Table (6) revealed that soil infected with the three phytopathogens (*M. phasolina, F. solani and R. solani*) significantly increased damping off of tomato seedlings (87.5, 62.5 and 83.3%, respectively) and reduced survival rate (12.5, 37.5 and 16.7%, respectively) than the control (100%) (Fig. 1).

Fig (2): Effect of inoculation with different damping off configurations of fungi (*M. phasolina, F. solani and R. solani*) on tomato seedling under greenhouse condations.

Table (6): Disease incidence and survival rate of tomato seedlings infected with *M. phasolina, R. solani* or *F. solani* in the presence and/or absance of endopytic bacterial isolates (HMS10, HMS12 and HMS11)

Isolates		% of	% of
Phytopathogen	Endopytic bacterial isolate	Disease incidence	Survival
-	-	0.0	100.0
M. phasolina,	**-**	**87.5**	**12.5**
‘’	HMS10	37.5	62.5
‘’	HMS11	50.0	50.0
‘’	HMS12	50.0	50.0
F. solani	**-**	**62.5**	**37.5**
‘’	HMS10	50.0	50.0
‘’	HMS11	50.0	50.0
‘’	HMS12	50.0	50.0
R. solani	**-**	**83.3**	**16.7**
‘’	HMS10	41.7	58.3
‘’	HMS11	37.5	62.5
‘’	HMS12	29.2	70.8
LSD. 0.05		16.58	

Inoculation of tomato seedlings with the three actively antagonistic endophytic bacterial showed significant decreasing in the percentages of damping off disease incident of tomato seedlings, when applied in mix with all tested pathogens compared to soil infected only with the pathogens. Treatment of tomato seedling with isolates HMS10, HMS11 and HMS12 significantly decreased the percentage of damping off disease incidence (37.5, 50 and 50%, respectively) when compared with the soil infected with *M. phasolina* only (87.5%). Likewise, inoculation with these isolates increased the survival rate of tomato seedling (62.5, 50 and 50%, respectively) compared with the soil infected with *M. phasolina* only (12.5%).

On the other hand, data in Table (6) indicate that all tested isolates have reduced tomato damping off disease incidence caused by *F. solani* from 62.5% to 50%. Moreover, inoculation with these endophytic bacterial isolates increased the survival rate of tomato seedling to 50% in comparison with that infected with *F. solani* (37.5%). Concerning *R. solani*, all isolates (HMS10, HMS12 and HMS11) exhibited significant decrease of damping off disease incidence rate caused by this fungus to be 41.7, 37.5 and 29.2%, respectively instead of 83.3% in the presence of *R. solani* only (Table 6). In addition, survival rate of tomato seedling were significantly increased from 16.7% to 58.3, 62.5 and 70.8 due to inoculation with these strains (HMS10, HMS12 and HMS11), respectively. Of these endophytic bacterial isolates, HMS12 showed the lowest value of damping off disease incidence (29.2%) as well as the highest value of survival rate (70.8%) in the presence of *R. solani*.

The obtained results are in good accordance with previous studies which have been concluded that *Bacillus* spp. Can effectively protect many plant species against damping off diseases (Abdel- Monaim, 2010; Atef, 2008; Hashem and Hamada, 2002; Nourozian *et al.,* 2006; Soleimani *et al.,* 2005). The mechanism of *Bacillus* action on pathogens may be due to its attack and bind the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Soleimani *et al.,* 2005; Zaghloul *et al.,* 2007), producing siderophores which act as chelators for iron element and the toxic agent hydrogen cyanide was also produced (Soleimani *et al.,* 2005).

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درجة القرابة و تشجيع النمو والنشاط المضاد للممرضات النباتية لسلالات بكتيريا الاندوفيتس المعزولة من الطماطم

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لقد تم تصميم هذه التجربة لتقييم الدور المحتمل لبكتيريا الاندوفيتس التي سبق عزلها وتعريفها في تحفيز نمو نبات الطماطم وكذلك تحديد التأثير المضاد للممرضات النباتية لثلاثة عزلات من بكتريا الاندوفيتس (*Bacillus subtilis* HMS10 و *Bacillus subtilis* HMS11 و *Bacillus malacitensis* HMS12) ضد ثلاثة من الفطريات المسببة لمرض موت البادرات (*Rhizoctonia solani, Fusarium* solani *and Macrophomina phaseolina*) تحت ظروف الصوبة. و توضح شجرة القرابة الوراثية والتي تم استنباطها من عمل تتابع 16s rRNA الى ان عزلات بكتريا الاندوفيتس (HMS1, HMS7, HMS8, HMS9) والتي لها نفس الفرع كانت تتبع شعبةProteobacteria- α بينما عزلات بكتريا الاندوفيتس (HMS10, HMS11 , HMS12) تتبع الشعبة البكتيرية Firmicutes وكذلك تتبع العزلات (HMS2, HMS3, HMS4, HMS , HMS6) نفس الفرع والتي تنتمى الى شعبة -γ Proteobacteria. ولقد اظهرت نتائج تلقيح نباتات الطماطم ببكتيريا الاندوفيتس اختلافات معنوية في جميع صفات النمو الخضرية (طول الجذر- طول المجموع الخضرى- الوزن الجاف والرطب للجذر- الوزن الجاف والرطب للمجموع الخضري) وذلك بالمقارنة بالكنترول ولقد لوحظ ان الاختلافات كانت معنوية في صفة تركيز الصبغات الضوئية وكذلك فى محتوى N P K. واظهرت العزلات الخمس (*Bacillus subtilis* HMS10 ، *Bacillus subtilis* HMS11 ، *Bacillus malacitensis* HMS12 ، *Rhizobium sp.* HMS1، *Enterobacter cloacae* HMS2) اعلى قيم لجميع الصفات الخضرية وذلك عند مقارنتها بالكنترول. ويعتبر التلقيح بالثلاثة عزلات من بكتريا الاندوفيتس المختبرة فعال فى الحد من مرض سقوط البادرات الحادث في الطماطم وذلك عند تطبيقها على كل الفطريات الممرضة المختبرة بالمقارنة بتلك المصابة فقط بالممرضات.محتوى N P K. واظهرت العزلات الخمس (*Bacillus subtilis* HMS10 ، *Bacillus subtilis* HMS11 ، *Bacillus malacitensis* HMS12، *Rhizobium sp.* HMS1، *Enterobacter cloacae* HMS2) اعلى قيم لجميع الصفات الخضرية وذلك عند مقارنتها بالكنترول. ويعتبر التلقيح بالثلاثة عزلات من بكتريا الاندوفيتس المختبرة فعال فى الحد من مرض سقوط البادرات الحادث في الطماطم وذلك عند تطبيقها على كل الفطريات الممرضة المختبرة بالمقارنة بتلك المصابة فقط بالممرضات.