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Characterization of fluorescent pseudomonads isolates and their efficiency on the growth promotion of tomato plant

Fekria M.A. Saber [a,*], Ahmed A. Abdelhafez [b], Enas A. Hassan [b], Elshahat M. Ramadan [b]

[a] *Heliopolis University, Biotechnology Dept., Egypt*
[b] *Dept. of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Egypt*

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Abstract Soil samples were collected from different rhizosphere plants grown in SEKEM farm, Bilbis, El-Sharkyia governorate, Egypt. Four fluorescent pseudomonads isolates, out of seventy one, were selected according to their efficiency to produce IAA, cyanide, antagonistic effect and high ability to solubilize potassium and phosphorus. These isolates were identified using 16S rRNA gene sequencing technique to be different strains of *Pseudomonas otitidis*. Growth curve and growth kinetics were determined on king's broth medium. *Ps. otitidis* SE8 gave the highest specific growth rate, multiplication rate and number of generation being 0.287 h^{-1}, 0.4147 h^{-1} and 4.98 h^{-1}, respectively. This strain also showed the lowest of doubling time. *Ps. otitidis* SE8 and OL2 had higher activity to solubilize phosphorus than other tested strains. Bacterial strain SE8 also gave a considerable amount of soluble potassium as compared with other strains being 80.7 ppm. The maximum IAA and gibberellic acid production was also recorded by *Pseudomonas* SE8 and OL2. A remarkable quantity of siderophores was detected in the case of *Ps. otitidis* SE8 being 28.20 mM DFOM. Application of these isolates as inoculants for tomato plants in green house was performed. The results showed that inoculation of tomato seedling with *Pseudomonas* strains led to suppress the soil-borne pathogen, increased of NPK uptake and supported tomato plant growth.

Introduction

Fluorescent Pseudomonads are considered to be one of the most promising groups of plant growth promoting rhizobacteria involved in biocontrol of plant diseases (Moeinzadeh et al., 2010; Bhattacharyya and Jha, 2012). They produce secondary

* Corresponding author.
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metabolites such as, phytohormones (Keel et al., 1992), volatile compound hydrogen cyanide (HCN) (Defago and Haas, 1990), and siderophores (Neiland, 1995). Plant growth-promoting ability of these bacteria is mainly due to the production of indole-3-acetic acid (IAA) (Patten and Glick, 2002), siderophores (Schippers et al., 1987) and antibiotics (Sunish Kumar et al., 2005; D'aes et al., 2011).

Pseudomonas is an aerobic gram negative, rod-shaped, non-spore former, fast growing, competitive root colonizing bacteria, and commonly found in the rhizosphere of various plants, the largest of the plant growth promoter bacterial groups that includes both fluorescent and non-fluorescent species (Weller, 2007). The most important fluorescent species are *Ps. aeruginosa, Ps. fluorescens, Ps. putida* and the plant pathogen species is *Ps. syringae* (Scarpellini et al., 2004). Several species of rRNA group I pseudomonads have the ability to produce and excrete, under iron limitation condition, soluble yellow green pigments that fluorescence under UV light (Bultreys et al., 2003), named pyoverdines (PVDs) or pseudobactins, which act as siderophores for these bacteria (Meyer, 2000). These molecules are thought to be associated with biocontrol of fungal pathogens in the biosphere (Fuchs et al., 2001). Fluorescent pseudomonads have frequently been considered as effective biological control agents against soil-borne plant pathogens due to their rapid and aggressive colonization of plant roots. Other mechanisms include competition for nutrients in the rhizosphere at preferred colonization sites and production of metabolites, such as antibiotics, siderophores and hydrogen cyanide (Lugtenberg et al., 2001). The abundance of literature on genus *Pseudomonas* is due to their elevated metabolic versatility capable of utilizing a wide range of simple and complex organic compounds and holding an important position in biosphere ecology (Scarpellini et al., 2004).

Mezaache-Aichour et al. (2012) isolated fluorescent Pseudomonads bacteria from rhizosphere of potato plants in Algeria and identified it as *Ps. chlororaphis* which capable of inhibiting the growth of phytopathogenic fungi *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *albedinis*, *F. solani*, *Rhizoctonia solani* and the oomycete *Pythium ultimume*. The role of *Pseudomonas* species to solubilize fixed phosphorus to available phosphorus has also been observed (Castro et al., 2009).

The main objective of this study was to select the most active and beneficial ecofriendly strains of fluorescent pseudomonads which have a broad spectrum of plant-promoting capabilities and antagonistic potential against phytopathogenic fungi that could be used as safe alternative for harmful pesticide agrochemicals. The efficiency of this group on the growth promotion of tomato was also elucidated in pot experiments.

Materials and methods

Soil, compost and rhizosphere plant samples

Twelve soil rhizosphere samples were collected from some economical plants e.g. Mugwort, Gazania, Hypericum, Goldenrod, Sugar bush, Cotton, Corn, Tomato, Cucumber, Bean, Sesame, Olive. All plants were grown in SEKEM farm, Bilbis, Elsharkyia governorate, Egypt. Two soil types (sandy and sandy loam soils) and Compost were used throughout the cultivation experiment. Sandy soil (Sand 90%; Selt 4.5% and Clay 5.5% Bulk density = 1.7 g/cm^3) and compost sample were obtained from SEKEM farm, while Sandy loam soil (Sand, 78.2%; Selt, 13.5% and Clay, 8.3% Bulk density = 1.61 g/cm^3) was obtained from Qanater city, El-Kalupia governorate. Their physicochemical analyses were carried out in the lab of Soil and Water Dept., Heliopolis University, and are presented in Table 1.

Tomato seedlings and phytopathogenic fungi used

F. oxysporum F5 and *R. solani* F6 were obtained from Desert Research Center, El-Mataria, Cairo, Egypt. Seedlings of tomato *Lycopersicun esculentum*, variety fayrouz were obtained from El-Mizan company, SEKEM farm, Bilbis, Elsharkyia governorate, Egypt.

Isolation and screening of fluorescent pseudomonads

Soil rhizosphere samples were serially diluted and the suspensions were used to isolate fluorescent pseudomonads bacteria (FPB) on plates containing King's agar medium (King et al., 1954) at 30 °C for 48 h. The distinct single colonies of FPB that illuminate fluorescence under UV light (Woomer et al., 1990) were used in the next experiments.

Assessment of biological activities of fluorescent pseudomonads isolates

Phosphate and potassium solubilizing assay

FPB isolates were tested for their ability to solubilize phosphate and potassium on Pikovskaya's (SubbaRao, 1982) and modified Aleksandrov's (Sindhu et al., 1999) media, for 5 and 12 days respectively at 30 °C and the results were expressed as solubilization index according to the method of Edi-Premono et al. (1996). Isolates that gave the highest values in this measurement and the all following analyses were identified (described later) and quantitatively assayed for available

Table 1 Physicochemical analysis of soil and compost.

Items	$pH_{(1:2.5)}$	$EC_{(1:2.5)}$	O.M	O.C	T.N	T.P	T.K	C:N	K^+	Na^+	Mg^{++}	Ca^{++}	$SO_4^=$	Cl^-	HCO_3^-	CO^3
Unit	–	dS/m	%	%	%	%	%	Ratio	Meq/L	Meq/L	Meq/L	Meq/L	Meq/L	Meq/L	Meq/L	Meq/L
Sand	8.3	1.6	0.53	0.31	0.042	0.3	0.025	7.4:1	1.88	6.13	4.3	4.7	1.7	13.9	1.52	nd
Sandy loam	7.9	1.4	0.62	0.36	0.12	0.33	0.102	3:1	1.67	5.64	3.8	2.3	1.84	10.4	1.34	nd
Compost	7.3	2.3	22	12.7	0.8	0.35	0.15	15.8:1	17.3	4.1	13.2	16.3	123.24	8.6	2.4	1.04

(O.M) Organic Matter, (O.C) Organic Carbon, (T.N) Total Nitrogen, (T.P) Total Phosphorus, (T.K) Total Potassium, (C:N) Carbon/nitrogen ratio, (nd) not detected.

phosphorus and potassium in Pikovskaya's (amended with 5 g of tricalcium phosphate/l) and Aleksandrov's (contains 1 g feldspare/l) broth after 15 days, respectively. The available phosphorous and potassium were determined in the supernatant according to Jackson (1958).

Detection of cyanide and indole acetic acid (IAA) production

Hydrogen cyanide (HCN) production by all collected FPB isolates was assessed by the propagation of these isolates on 5 ml of King's broth medium in test tube containing sterilized filter paper strip (Whatman No. 1) saturated with cyanide reagent (2% sodium carbonate + 0.5% picric acid), inoculated with loop of the tested isolate and incubated at 30 °C for 1–2 days. Positive results were recorded when the paper strip turned from yellow to orange brown color according to Lorck (1948). For the detection of IAA and/or IAA analog production, King's medium (DSM 125), amended with 1 mM/l tryptophan, was overlaid with a nitrocellulose membranes disk (82 mm-diameter) saturated with Salkowski's reagent (Loper and Scroth, 1986).

HPLC analysis of microbial hormones

The amount of IAA and Gibberellic acid (GA_3) produced in liquid culture by bacterial strains were determined quantitatively by High performance liquid chromatography (HPLC). The most efficient bacterial isolates were grown in King's B broth (supplemented with 1 mM tryptophan for indols production) and incubated at 30 °C under shaking culture (150 rpm) for 5 days. Extraction, purification and quantification of IAA and gibberellins were applied according to the method described by Tien et al. (1979) using HPLC (Hewlett Packard series 1050) equipped with variable UV detector and BDS-HYPESIL C_{18} column (Dim 250 × 4.6 mm for Particle size (μ)). The growth hormones were identified on the basis of retention time of phytohormone standards (commercially grade, Sigma Chemical USA Company).

Quantitative determination of siderophores

A hundred ml Erlenmeyer flasks containing 25 ml of sterilized King's medium was inoculated with 2 ml of 10^7 cell/ml of the identified isolates and incubated at 30 °C on a rotary shaker (160 rpm) for stationary phase of growth (36 h). Quantitative assessment of produced siderophore was determined in supernatants by modified CAS assay method (Alexander and Zuberer, 1991).

Analysis of the antagonistic potential of isolated bacteria

Fluorescent pseudomonads were tested in vitro for antagonism toward fungal pathogens; *F. oxysporum* F5 and *R. solani* F6. They were tested on PDA plates using a dual culture technique (Skidmore and Dickinson, 1976). Observations of width of inhibition zone and mycelia growth of tested pathogens were recorded and inhibition percentage of pathogen growth was calculated using the following formula (Vincent, 1927):

Inhibition percentage $(I) = C - T/C \times 100$, where C = diameter of pathogen mycelial growth (mm) in control and T = diameter of pathogen mycelial growth in dual plate.

Growth curves and growth kinetics of Pseudomonas strains

In this experiment, selected identified isolates were grown on King's broth medium as shake batch cultures using rotary shaker (180 rpm) at 30 °C for 96 h. During the bacterial growth optical density at 650 nm was followed to draw the growth curve. The parameters of growth kinetics were calculated as follows: Specific growth rate (μ), Doubling time $(t_d) = \ln 2.\mu^{-1}$, according to Painter and Marr (1963) Multiplication rate (MR) = μ/ln 2, Number of generation $(N) = t.t_d^{-1}$ according to Stanier et al. (1970) where t is the time of exponential phase.

Molecular identification of antagonistic fluorescent pseudomonads by rpob primer

The bacterial isolates were identified by partial 16S rRNA gene sequence analysis in the laboratory of Environmental Biotechnology of Graz Univ., Graz, Austria according to Berg et al. (2002). Bacterial 16S rRNA gene sequences were amplified by PCR using the eubacterial primer pair 27f (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492r (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) (Lane, 1991). PCR product was sequenced with the Applied Biosystems 3130l Genetic Analyser sequencer, Data Collection v3.0, Sequencing Analysis v5.2 (Foster City, USA) at the sequencing core facility ZMF, Medical University of Graz, Austria. Obtained sequences were aligned with reference RNA sequences from NCBI (National Center for Biotechnology Information) database (http://blast.ncbi.nlm.nih.gov) using the nucleotide basic local alignment and search tool (BLAST).

Evaluation of the selected isolates as promoting and antagonistic bacteria

Inoculant preparation

For fungal inoculants, 500 ml glass bottles were 2/3 filled with sorghum seed and 150 ml tap water were added then autoclaved for 1 h at 120 °C. Each bottle was inoculated with a 4 mm diameter disk of one of the tested pathogenic fungi, and incubated at 25 ± 2 °C for 3 weeks. After incubation, the bottles were emptied, and the growth paste was slowly dried under room temperature to allow for maturation (Kazempour, 2004; Al-Juboory and Juber, 2013). For bacterial inoculants, bacterial strains were grown in King's broth medium for 48 h (up to stationary phase) at 28 ± 2 °C on rotary shaker (150 rpm) and cell counts were determined on King's broth medium by MPN technique. Inoculation was carried out by pipetting 30 ml of the mixed bacterial culture (contained about 10^9 cells/ml)/pot.

Pot experiment

Experiment was carried out under greenhouse conditions using two soil types and tomato seedlings (*L. esculentum,* variety fayrouz) at green house of Heliopolis University, 3 Cairo-Bilbis Desert Road, in June 2013. Sand and Sandy loam soils were previously autoclaved for one hour at 121 °C. Sterilized soil amended with 0.5% matured compost, free from soil-borne pathogens and weeds, was transferred to plastic pots (30 cm diam.), each containing 7.5 kg soil/pot. Pots experiment was

arranged as completely randomized design with 5 replicates per treatment (i.e. 12 treatments) as shown in Table 6.

Inoculation of infested soil was carried out with addition of 10 g of each fungal inoculum per pot and mixed thoroughly with soil. Thereafter, tomato seedlings were transplanted on 15th of June 2013. After three months of transplanting, growth parameters e.g. shoot and root dry weight and NPK contents. Disease symptoms caused by both pathogenic fungi (root rot) were expressed by the percentage of post emergence damping-off up to 45 days from transplanting, as described by Abd-Elmotelep (1996), according to the following equation:

% Post-emergence damping off = (No. of dead seedlings/total no. of emerged seedlings) × 100.

Total nitrogen, phosphorus and potassium

Total nitrogen, phosphorus and potassium were determined in tomato plant samples using Kjeldahl digestion method as described by Jackson (1973), spectrophotometer method (Olsen et al., 1954) and flame photometric method (Chapman and Pratt, 1961) respectively.

Statistical analysis

Statistical analysis of the data was treated by one way analysis of variance (ANOVA) as described by Snedecor and Cochran (1980). The mean values were compared by LSD at 5% using SPSS software, Ver. 19. Regression analyses were also carried out for the selected strain from the exponential growth phase.

Results and discussion

Screening for FPB isolates

It is well known that the root microbiome contains several rhizobacterial species. Out of these groups, fluorescent pseudomonads actively promote plant growth, suppress diseases, and consequently influence plant metabolism and reduce the detrimental effects of various stresses. Therefore, it was found valuable to isolate these bacteria from the rhizosphere of different plants which include some conventional and medicinal plants.

Seventy-one isolates of fluorescents pseudomonads were isolated from the rhizosphere of different economic plants being mugwort, gazania, hypericum, goldenrod, sugar bush, cotton, corn, tomato, cucumber, bean, sesame and olive. The qualitative assessment of these isolates for their ability to produce cyanide, indole acetic acid and solubilization of phosphate and potassium were done to select the most efficient isolates along with their activity against *F. oxysporum* F5 or *R. solani* F6 by dual culture technique.

With respect to potassium and phosphorus solubilization by tested isolates of fluorescent pseudomonads, results indicated that isolates OL4 gave the highest values of potassium solubilization index and SE8 showed high potentiality of phosphorus solubilization as phosphate solubilization index. All fluorescent pseudomonads gave positive results for their ability to produce indole acetic acid, the highest indication (+ + +) was detected in the case of bacterial isolates SE8 and AR10. Additionally, isolates OL2, OL4 and AR10 showed high activity to produce hydrogen cyanide (Table 2). The tested isolates highly varied in their efficiency as biocontrol agents. High antagonistic potential were elucidated for isolates OL2 and AR10 which were isolated from the rhizosphere of olive and mugwort plants, respectively, being 34.09% of reduction in mycelial growth of *F. oxysporum* F5 whereas the reduction percentages of the mycelial growth of *R. solani* were 45.45% and 32.72%, in that order (Table 2).

Identification of the most active fluorescent Pseudomonads by gene sequencing

Fluorescent pseudomonads isolates, which showed high activity in two or three of tested biological characters, being OL2, SE8, OL4 and AR10, were identified by 16S rRNA gene sequencing. Identification procedure revealed that the four isolates were *Ps. otitidis* OL2, *Ps. otitidis* SE8, *Ps. otitidis* OL4 and *Ps. otitidis* AR10, with 99% similarity. It means that all selected fluorescent pseudomonads belong to the same species (Fig 1).

Growth curve and growth kinetics of Pseudomonas strains

Results in Fig. 2 and Tables 3 and 4 showed that all tested *Pseudomonas* strains grew exponentially during the first 24 h. To differentiate between the four strains, regression analyses (straight line equation) and growth kinetics were estimated at the first 10 h of growth from the exponential growth phase. *Ps. otitidas* SE8 gave the highest specific growth rate, multiplication rate and number of generation on king's medium being 0.287 h^{-1}, 0.4147 h^{-1} and 4.98 h respectively. This strain exhibited the lowest value of doubling time being 2.41 h as compared with other strains on king's medium. It is expected due to the highest specific growth rate leads to decrease the

Table 2 Qualitative assessment of cyanide, indole acetic acid, phosphate and potassium solubilization activity (expressed by solubilization index) and antagonistic effect produced by fluorescent pseudomonads isolates.

Isolate's code	Source (plant)	Cyanide	IAA	Phosphate solubilization index[a]	Potassium solubilization index[a]	% of reduction in mycelial growth of fungal pathogens	
						Fusarium oxysporum	*Rhizoctonia solani*
OL2	Olive	+ + +	+ +	1.55 ± 0.05	2.35 ± 0.65	34.09	45.45
SE8	Sesame	–	+ + +	3.45 ± 0.05	2.37 ± 0.13	9.09	10
OL4	Olive	+ + +	+ +	1.35 ± 0.15	2.60 ± 0.00	15.9	29.09
AR10	Mugwort	+ + +	+ + +	2.25 ± 0.00	2.00 ± 0.20	34.09	32.72

[a] Solubilization index = (colony diameter + halozone diameter)/colony diameter.

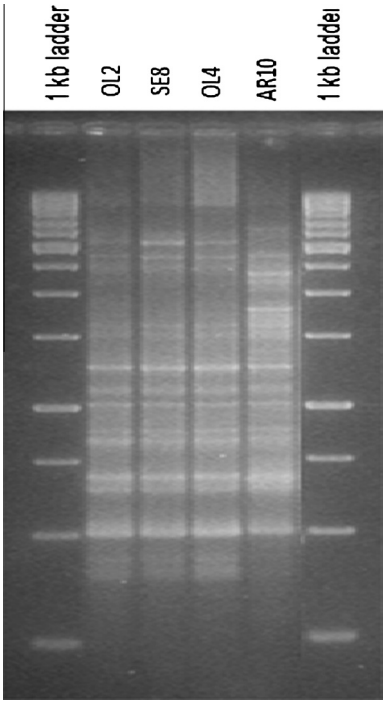

Fig. 1 PCR Fingerprints of four fluorescent pseudomonads. 1 kb ladder band (the primer), the identified bands are OL2 for *Ps. otitidis*, SE8 for *Ps. otitidis*, OL4 for *Ps. otitidis* and AR10 for *Ps. otitidis*.

doubling time. Beyenal et al. (2003) observed that the maximum specific growth rate of *Ps. aerugenosa* was 0.29 h^{-1} (the obtained results showed that the specific growth rate of fluorescent pseudomonads strains ranged from 0.2173 to 0.2874 h^{-1}). Results of this study also are in line with Fallahzadeh et al. (2010) who observed that the maximum specific growth rate of *Ps. aerugenosa* on king's medium was 0.269 h^{-1} (while the growth rate of *Pseudomonas* SE8, in this study was 0.2874 h^{-1}).

Biological activities of the selected Pseudomonas strains

In this experiment the most active *Pseudomonas* strains were selected according to their biochemical activities, being *Ps. otitidis* OL2 and *Ps. otitidis* SE8. Both isolates liberated soluble phosphorus in the Pikoviskaya's broth medium which was

Table 3 Regression analysis of the exponential growth of *Pseudomonas* strain.

Strains	Exponential growth equation (straight line equations)
Ps. otitidis OL2	$y = 0.0427e^{0.2677x}$
Ps. otitidis SE8	$y = 0.0386e^{0.2874x}$
Ps. otitidis OL4	$y = 0.0932e^{0.2173x}$
Ps. otitidis AR10	$y = 0.0547e^{0.2427x}$

Table 4 Growth kinetics of *Ps. otitidis* strains growing on King's medium at 30 °C under shake condition.

Ps. otitidis strains	μ (h^{-1})	td (h)	MR	*N*
OL2	0.2677	2.59	0.3862	4.63
SE8	0.2874	2.41	0.4147	4.98
OL4	0.2173	3.19	0.3135	3.76
AR10	0.2427	2.86	0.3502	4.20

determined as available phosphorus. It was found that *Ps. otitidis* SE8 had higher activity to solubilize phosphorus than other strain (Table 5). This may be attributed to the efficiency of *Ps. otitidis* SE8 to produce higher amount of acid which led to increase the available phosphorus compared with other tested bacterial strain. This result is in agreement with Verma et al., 2001; Damayanti et al., 2007 and Castro et al., 2009 who observed the role of *Pseudomonas* species to solubilize fixed phosphorus to available phosphorus. *Ps. otitidis* SE8 also produced slightly higher amount of soluble K after 15 days of inoculation (80.7 ppm) than other tested strain. It means that *Pseudomonas* strains had the ability to dissolve potassium from insoluble feldspar in modified Alexandrov's medium. Parmer and Sindhu (2013) observed that twenty bacterial strains among 137 tested cultures showed significant potassium solubilization on mica powder and the amount of K released by different strains varied from 15 to 48 mg/l, these values were lower than observed by *Pseudomonas* cultures in this investigation (80.67 mg/l).

The maximum IAA production was recorded by *Ps. otitidis* SE8 (27.67 μg/ml) grown on King's medium supplement with tryptophan, whereas it was 13.76 μg/ml with *Ps. otitidis* OL2. Gibberellic acid produced by both tested strains were 2.65 and 2.77 μg/ml for *Ps. otitidis* OL2 and *Ps. otitidis* SE8,

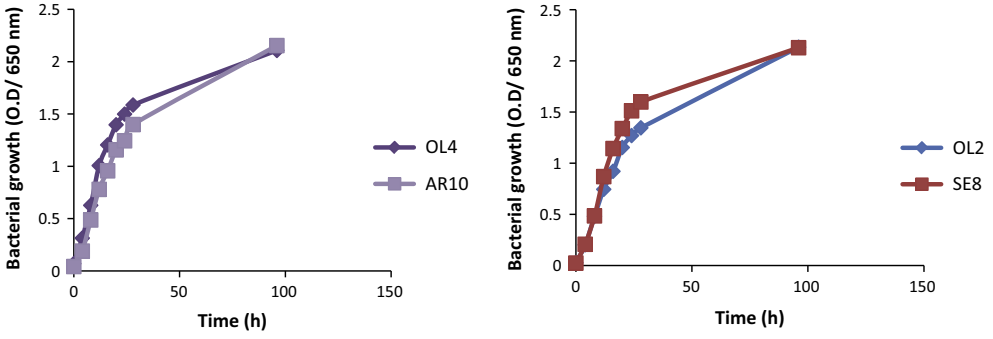

Fig. 2 Growth curve of the four tested strains of *Ps. otitidis*.

Table 5 Quantitative assessments of some biological activities of the most efficient strains in liquid medium.

Bacterial isolates	Available phosphorus (ppm)	Available potassium (ppm)	Siderophore production (mMDFOM)	Indole acetic acid (μg/ml)	Gibberellic acid (μg/ml)
Ps. otitidis OL2	6.55 ± 0.88	57.33 ± 0.54	14.29 ± 4.83	13.76 ± 0.95	2.65 ± 0.49
Ps. otitidis SE8	7.63 ± 0.43	80.67 ± 0.91	28.20 ± 4.81	27.67 ± 9.34	2.77 ± 1.62

respectively. *Ps. otitidis* SE8 produced a considerable amount of siderophores being 28.20 mM DFOM compared with *Ps. otitidis* OL2 being 14.29 mM DFOM. In this respect, it was found that the quantity was as lower as 28.20 mM DFOM than that observed by Ahmed (2014) being 48.0 mM DFOM and Sharma and Johri (2003) being 216.23 mg/ml.

Pot experiment to evaluate the biocontrol agent

Results presented in Table 6 showed that the plant height, number of branches and dry weight of tomato plants were highly affected by different bio-treatments. In general, the highest figures of plant height and number of branches were noticed when seedlings were inoculated with *Pseudomonas* strains and infected with *R. solani* F6 and *F. oxysporum* F5 in sandy or sandy loam soils. It could be noticed that both tested bacterial strains individually or mixed culture gave the highest values of shoot and root dry weight of plant infected by *R. solani* F6 in both tested soils, while mixed culture or *Ps. otitidis* SE8 alone gave the highest values in sandy soil in presence of *F. oxysporum* F5.

Also, data revealed that the highest values of shoot and root dry weights were pronounced with *Ps. otitidis* in sandy loam soil infested with *F. oxysporum* F5. All seedlings in the control treatment of soil inoculated with *R. solani* only pronounced showed 100% and 70% damping off in sandy and sandy loam soils, respectively, whereas soil infested with *F. oxysporum* gave 100% and 60% post damping off, in respective order. Therefore, it could be concluded that biocontrol agents of fluorescent *Pseudomonas* significantly enhanced plant health.

Table 7 demonstrated a better response in nitrogen percentage of roots in both tested soils whereas the highest total nitrogen contents (%) were recorded in the case of both tested *Pseudomonas* strains (OL2 and SE8) in both soil types being 1.28% for SE8 and 1.49% for OL2 in sandy and sandy loam soils infected with *R. solani* F6, respectively. The corresponding figures for *F. oxysporum* F5 were 1.22% and 1.44%. The highest phosphorus contents of root were 0.123 and 0.118 ppm for *Ps. otitidis* OL2 and *Ps. otitidis* SE8, in that order, in sandy soil infected with *R. solani* F6. The corresponding figures for sandy loam soil were 0.117 and 0.117 ppm. The soils infected with *F. oxysporum* F5 exhibited the same trend with lower phosphorus content as compared with soil infected with *R. solani* F6. The highest significant figure of potassium content was noticed in tomato of plant root cultivated in sandy loam soil inoculated with *Ps. otitidis* OL2 and *Ps. otitidis* SE8 and infected with *R. solani* F6 being 0.0879 and 0.0963 ppm, respectively. The same trend was also recorded in the case of *F. oxysporum* F5. Table 8 shows that shoots of tomato

Table 6 Effect of the selected FPB strains on plant height (cm), number of branches, dry weight and disease expression of tomato plants infected with two phytopathogenic fungi grown in sandy soil and sandy loam soil during 75 days under greenhouse condition.

Treatments		Growth parameters								Disease expression	
		Sandy soil				Sandy loam soil				% post-emergence damping off	
Fungal treatments	Bacterial inocula	Height (cm)	No. of branches	Dry weight (g/plant)		Height (cm)	No. of branches	Dry weight (g/plant)		Sandy	Sandy loam
				Shoots	Roots			Shoots	Roots		
Un-treated	Un-inoculated	51.6 ab	11.2 e	8.57 abc	1.04 bcd	46.86 a	20.4 a	7.35 cd	1.23 abc	0	0
	Ps. otitidis OL2	48.52 ab	15.8 d	4.73 cd	0.54 def	52.7 a	16.8 a	8.77 c	1.12 bc	0	0
	Ps. otitidis SE8	45.06 b	15 de	3.56 de	0.27 ef	52.4 a	16.8 a	8.95 c	1.22 abc	0	0
	Ps. otitidis Mixture (OL2 + SE8)	47.5 ab	17.2 cd	7.07 bcd	1.08 bcd	54.4 a	19.8 a	10.23 abc	1.45 ab	0	0
Rhizoctonia solani F6	Un-inoculated	0	0	0	0	56.0 a	20.4 a	10.37 abc	1.55 ab	100	70
	Ps. otitidis OL2	55.2 a	21.8 ab	13.03 a	1.7 ab	55.6 a	22.8 a	14.19 a	2.08 a	0	0
	Ps. otitidis SE8	53.6 a	20.4 bc	12.94 a	1.81 a	55.6 a	22.0 a	13.65 ab	1.85 ab	0	0
	Ps. otitidis Mixture (OL2 + SE8)	51.75 ab	25 a	9.61 ab	1.33 abc	52.2 a	21.0 a	10.42 abc	1.38 abc	20	0
Fusarium oxysporum F5	Un-inoculated	0	0	0	0	22.0 b	6.0 b	3.81 d	0.57 c	100	60
	Ps. otitidis OL2	52.5 ab	19.2 bcd	7.56 bcd	0.75 cde	52.6 a	18.2 a	9.17 bc	1.54 ab	0	0
	Ps. otitidis SE8	53.2 A	23.2 ab	10.69 ab	1.24 abcd	53.0 a	19.8 a	10.83 abc	1.80 ab	0	0
	Ps. otitidis mixture (OL2 + SE8)	49.92 ab	23.2 ab	9.37 abc	1.32 abc	42.4 a	20.0 a	8.66 c	1.23 abc	0	20

Value in the same column sharing the same letter don't differ significantly, according to Duncan's at 5% level.

Table 7 Effect of the selected FPB strains on root content of nitrogen, phosphorus and potassium (ppm) of tomato plants infect with phytopathogenic fungi grown in sandy and sandy loam soils for 75 days under greenhouse condition.

Treatments		Nutrients content											
		Sandy soil						Sandy loam soil					
Fungal treatments	Bacterial inocula	N		P		K		N		P		K	
		%	Uptake (g/plant)	ppm	Uptake (μg/plant)	ppm	Uptake (μg/plant)	%	Uptake (g/plant)	ppm	Uptake (μg/plant)	ppm	Uptake (μg/plant)
Un-treated	Un-inoculated	1.26 ab	0.013	0.115 ab	1.196	0.0709abc	0.73736	1.11 ab	0.014	0.094 abc	1.151	0.0716 abc	0.88068
	Ps. otitidis OL2	0.67 bc	0.004	0.071 bc	0.381	0.0507 bc	0.27378	1.04 abc	0.012	0.094 abc	1.053	0.0675 abc	0.756
	Ps. otitidis SE8	0.68 bc	0.003	0.070 bc	0.285	0.0489 bc	0.20049	1.39 a	0.017	0.116 ab	1.410	0.0970 a	1.1834
	Ps. otitidis Mixture (OL2 + SE8)	0.97 abc	0.01	0.094 abc	1.015	0.0689abc	0.74412	1.33 a	0.019	0.118 ab	1.704	0.0892 ab	1.2934
Rhizoctonia solani F6	Un-inoculated	0	0	0	0	0	0	1.33 a	0.021	0.117 ab	1.812	0.0809 ab	1.25395
	Ps. otitidis OL2	1.12 ab	0.019	0.123 ab	2.096	0.0800 ab	1.36	1.49 a	0.031	0.117 ab	2.432	0.0879 ab	1.82832
	Ps. otitidis SE8	1.28 a	0.023	0.118 ab	2.127	0.0786 ab	1.42266	1.38 a	0.026	0.117 ab	2.166	0.0963 a	1.78155
	Ps. otitidis Mixture (OL2 + SE8)	0.98 abc	0.013	0.091 abc	1.210	0.0644abc	0.85652	1.25 ab	0.017	0.143 a	1.969	0.0886 ab	1.22268
Fusarium oxysporum F5	Un-inoculated	0	0	0	0	0	0	0.49 c	0.003	0.045 c	0.254	0.0355 c	0.20235
	Ps. otitidis OL2	1.02 abc	0.008	0.090 abc	0.677	0.0587abc	0.44025	1.34 a	0.021	0.120 ab	1.842	0.0825 ab	1.2705
	Ps. otitidis SE8	1.22 ab	0.015	0.112 ab	1.384	0.0730abc	0.9052	1.44 a	0.026	0.115 ab	2.068	0.0794 ab	1.4292
	Ps. otitidis Mixture (OL2 + SE8)	1.05 abc	0.014	0.094 abc	1.245	0.0568abc	0.74976	1.07 abc	0.013	0.092 abc	1.130	0.0667 abc	0.82041

Value in the same column sharing the same letter don't differ significantly, according to Duncan's at 5% level.

Table 8 Effect of the most efficient bacterial strains on shoot nitrogen, phosphorus and potassium content (ppm) of tomato plants infect with phytopathogenic fungi grown on sandy and sandy loam soils for 75 days under greenhouse condition.

Treatments		Nutrients content											
		Sandy soil						Sandy loam soil					
Fungal treatments	Bacterial inocula	N		P		K		N		P		K	
		%	Uptake (g/plant)	ppm	Uptake (μg/plant)	ppm	Uptake (μg/plant)	%	Uptake (g/plant)	ppm	Uptak (μg/plant)	ppm	Uptake (μg/plant)
Un-treated	Un-inoculated	1.88 ab	0.161	0.129 abc	11.021	0.0888 a	7.61016	1.33 ab	0.098	0.110 abc	8.070	0.0679ab	4.99065
	Ps. otitidis OL2	1.19 ab	0.056	0.077 bc	3.619	0.0533ab	2.52109	1.55 ab	0.136	0.097 bc	8.542	0.0682ab	5.98114
	Ps. otitidis SE8	1.14 ab	0.041	0.076 bc	2.713	0.0534ab	1.90104	1.83 a	0.164	0.130 abc	11.590	0.0844 a	7.5538
	Ps. otitidis Mixture (OL2 + SE8)	1.22 ab	0.086	0.099 bc	7.021	0.0708ab	5.00556	1.81 a	0.187	0.125 abc	12.824	0.0839 a	8.6417
Rhizoctonia solani F6	Un-inoculated	0	0	0	0	0	0	1.36 ab	0.141	0.105 abc	10.899	0.0703ab	7.29011
	Ps. otitidis OL2	1.85 a	0.241	0.127 abc	16.561	0.0876 a	11.41428	1.58 ab	0.224	0.140 ab	19.838	0.0871 a	12.35949
	Ps. otitidis SE8	2.00 a	0.279	0.130 abc	18.080	0.0879 a	12.25326	1.47 ab	0.12	0.127 abc	17.336	0.0818 a	11.1657
	Ps. otitidis Mixture (OL2 + SE8)	1.58 ab	0.152	0.144 ab	13.848	0.0708ab	6.80388	1.34 ab	0.14	0.133 ab	13.869	0.0819 a	8.53398
Fusarium oxysporum F5	Un-inoculated	0	0	0	0	0	0	0.65 b	0.025	0.047 c	1.806	0.0339 b	1.29159
	Ps. otitidis OL2	1.76 a	0.133	0.104 abc	7.825	0.0708ab	5.35248	1.48 ab	0.136	0.134 ab	12.279	0.0807 a	7.40019
	Ps. otitidis SE8	2.04 a	0.218	0.186 a	19.859	0.0875 a	9.3625	1.46 ab	0.158	0.126 abc	13.668	0.0838 a	9.07554
	Ps. otitidis Mixture (OL2 + SE8)	1.56 ab	0.146	0.104 Abc	9.773	0.0705ab	6.60585	1.22 ab	0.105	0.101 Bc	8.747	0.0703ab	6.08798

Value in the same column sharing the same letter don't differ significantly, according to Duncan's at 5% level.

seedlings with *Ps. otitidis* OL2 and *Ps. otitidis* SE8 gave positive effects on the nitrogen contents of shoots where the total nitrogen contents were 1.85% and 2.00%, respectively in sandy soil infected with *R. solani* F6 respectively. The corresponding figures for *F. oxysporum* F5 were 1.76% and 2.04%. The highest phosphorus content of tomato shoot were recorded in sandy soil inoculated with biococktail mixture and infested with *R. solani* F6 being 0.144 ppm, whereas the significant value of phosphorus content was noticed in sandy soil inoculated with *Ps. otitidis* SE8 and infested with *F. oxysporum* F5 being 0.186 ppm. The highest significant values were recorded in the case of *Ps. otitidis* SE8 in sandy and sandy loam soils infested with *R. solani* F6 where the values ranged from 0.0818 to 0.0879 ppm. Strains SE8 also showed the significant figures in the case of *F. oxysporum* F5 being 0.0875 and 0.0838 ppm for sandy and sandy loam soils, respectively. Generally it could be stated that NPK are essential nutrients for different crops. The tested fluorescent pseudomonads strains used in the pot experiment revealed that both *Ps. otitidis* OL2 and *Ps. otitidis* SE8 led to microbial colonization in the rhizosphere increasing uptake of nitrogen, phosphorus and potassium (NPK) in all treatments of pot experiments. In general, it is suggested that these bio fertilizer may excrete phytohormons, act as biocontrol agents and therefore resulted in healthy plants that can absorb more nutrients that the un inoculated tomato plants. This increase in nutrients uptake by plants might be explained through organic acid production by both plants and plant growth promoting rhizobacteria, decreasing soil pH (Karlidag et al., 2007). In this respect, Glick (1995) mentioned that solubilization of unavailable forms of nutrients is one of the essential criteria in facilitating the transport of the most of nutrients.

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