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

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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

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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.