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EFFECT OF *PSEUDOMONAS FLUORESCENS* EXTRACELLULAR ENZYMES AND SECONDARY METABOLITES ON *RHIZOCTONIA SOLANI*, THE CAUSAL OF SUGAR BEET DAMPING-OFF DISEASE

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ABSTRACT: Twenty isolates of *Pseudomonas fluorescens*, isolated from sugar beet rhizosphere to determine their antagonistic effect towards *Rhizoctonia solani, in vitro* and *in vivo*. Production of chitinase, β-1,3-glucanase, siderophores and salicylic acid (SA) by *P. fluorescens* strains were evaluated. The isolate Pfkfr2 produced the highest significant inhibition zone of 19.3 mm, followed by the isolates PfSh8 and PfKfr1 which produced inhibition zones of 15.3 and 14.3 mm, respectively. The results obtained from the *in vivo* experiment showed that the isolates Pfkfr12, Pfkfr 11 and Pfkfr 10 showed the highest significant damping off suppression which reflected in the mean number of healthy survival plants (9.33, 9.0 and 9.0 respectively) compared to the infected untreated control (4.0). The isolates Pfkfr2 and Pfkfr8 recorded the highest chitinase activity (83.33 and 82.67 units respectively) while the highest significant β-1,3-glucanase activity recorded by *P. fluorescens* isolate PfKfr2 (277 units) followed by PfSh8 and PfKfr1 (228 and 217 units respectively). The maximum siderophore production was recorded in *P. fluorescens* isolate PfSh8 (16.29 mmol/ml culture filtrate) while the maximum Salicylic acid (SA) production was recorded in PfKfr2 (21.67 mg/ml culture filtrate) followed by the isolate (PfSh3 and PfKfr1) (19.34 and 19.2 mg/ml culture filtrate). A remarkable relationship between the potential antagonistic activity of *P. fluorescens* isolates and their level of production of β -1,3-glucanase and SA have been recorded.

Key words: *Pseudomonas fluorescens*, sugar beet, daming off, lytic enzymes, siderophores.

INTRODUCTION

Damping-off disease in sugar beet seedlings caused by the fungus *Rhizoctonia solani* Kühn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is the most common and most serious fungal root disease of sugar beet in Egypt and world-wide (Abada, 1994, Rush *et al.* 1994 as well as Esh *et al.,* 2004). The disease is endemic in beet producing areas in Egypt. If the infection is light, the fungus may cause crown rot or dry rot canker on maturing roots later in the season. Thus control of this fungus in the seedling stage may reduce the disease later in the season, as well as improving crop stands. To protect crops against soil-borne diseases in general, seeds are commonly treated with fungicides. Since fungicides may affect human health and the environment and since pathogens can develop resistance to fungicides, bacterial inoculants which exhibiting antagonism against plant pathogenic micro-organisms are receiving increased attention as environmentally friendly alternatives to the use of chemical pesticides. Biological control of damping-off in crops caused by *Rhizoctonia solani* has been reported all over the world using antagonistic fungi and bacteria isolated from soils (Ordentlich *et al.,* 1988 and Tweddell *et al.,* 1994). One group of bacteria that show great promise with respect to protecting plant roots from fungal-induced diseases is that containing the fluorescent *Pseudomonas* spp. This group is of particular interest in terms of exploitation since it contains a high proportion of isolates demonstrated to be effective at suppressing soil-borne plant diseases and the degradation of xenobiotics in the rhizosphere (Crowley *et al.,* 1996; Weller, 1988).

The biocontrol activity of these strains is usually caused by the synthesis of one or more antifungal factors, which include such diverse compounds as hydrogen cyanide (Voisard *et al.,* 1989), siderophores, pterines, pyrroles (Shanahan *et al.,* 1992), phenazines (Thomashow and Weller, 1988), phloroglucinols (Shanahan *et al.,* 1992), peptides (Thrane *et al.,* 2000), proteases and chitinases (Nielsen *et al.,* 1998). In fluorescent *Pseudomonas* strains, the biosynthesis of antifungal compounds is regulated by a cascade of endogenous signals, which is channelled through a sensor-kinase and response

regulator encoded by gacAS (Gaffney *et al.*, 1994 and Corbell and Loper, 1995), sigma factors encoded by rpoS (Sarniguet *et al.*, 1995) and rpoD (Schnider *et al.*, 1995), and quorum-sensing systems (Pierson *et al.*, 1998). Several isolates of *Pseudomonas fluorescens* have been successfully used for the biological control of Damping off of sugar beet (Faltin *et al.*, 2004).

The aim of the present study was to examine the possible role of *in vitro* production of chitinase, β-1,3-glucanase, siderophores, and salicylic acid (SA) by *P. fluorescens* in suppression of *R. solani*.

MATERIALS AND METHODS

Pathogen

A virulent *Rhizoctonia solani* Kühn isolate of the anastmosis group AG4 were obtained from the collection of *R. solani* in the Department of Pests and Diseases, Sugar Crops Research Institute-Agriculture Research Centre, Giza, and maintained on potato dextrose agar (PDA) medium.

Isolation and Identification of *P. fluorescens*

Fluorescent pseudomonads were isolated from the soil collected from the rhizosphere of sugar beet from Kafr El-Shaikh and Sharkia governorates during the season 2003 – 2004 as described by Goddard *et al.*, (2001) on *Pseudomonas* selective agar (PSA Oxoid, UK, SR103E), which is a general selective medium for pseudomonad isolation and identfication. These bacterial colonies were tested for their antagonistic activity against *R. solani* by dual culture technique as described below.

In Vitro Screening of *P. fluorescens*

P. fluorescens isolates were streaked at one side of 9 cm diameter Petri dishs (1 cm from the edge) containing PDA medium. On the opposite side of the Petri dish A 5-mm disc obtained from a 7-day-old *R. solani* culture on PDA was placed (Vidhyasekaran *et al.*, 1997) then the plates were incubated at 28°C till the growth of the fungal in the control treatment reach 9 cm diameter, approx. 6 days. At the end of incubation period, the distance (mm) between the edges of the fungal mycelium and the antagonistic bacterium was recorded. Five replications were used for each isolate.

In Vivo Screening of *P. fluorescens*

To investigate the effect of *P. fluorescens* isolates on damping-off suppression 250 ml

polypropylene tissue culture magnate (Sigma) filled with 50 cm^3 moisten mixture of peatmoss:sand:clay (1:1:1) as shown in fig. 1 then autoclaved at 110 °C for one hour. Two milliliters of a blinded *R. solani* seven days old grown on Czapek-Dox liquid medium were mixed with the sterilized mixture under aseptic condition and left for 5 days under room temperature for fungal dispersal. *P. fluorescens* grown on King medium (King *et al.* 1948) for 48 hours in a shaker incubator at 26 °C and 100 rpm shaking speed then centrifuged at 10000 rpm for 10 minutes. The bacterial cells re-suspended in sterilized distilled water to reach $3x\ 10^6$ cells/ml. each magenta were inoculated with two milliliters of the bacterial suspension and left in room temperature for two more days. Sugar beet seeds variety Sofi, were socked overnight in running tap water, and then surface sterilized with 1% NaOCl for 2-3 minutes. Ten seeds were placed on the surface of the soil mixture in each magenta then covered with 50 cm^3 of moisten autoclaved mixture as previously mentioned. Two controls were used in this experiment; the first one soil was not infested neither with any of the pathogen nor the *P. fluorescens* isolate, while the second one was infested only with the *R. solani*. Three replicates were used for each tested isolate in complete randomized design, and incubated in growth chamber at 27°C for two weeks with 12 hours day and night intervals (fluorescent day light was 3000 lux). Number of pre-,post-emergence damping-off and standing plants were recorded.

Determination of Chitinases

Isolates of *P. fluorescens* were cultured in 250 ml conical flasks containing 50 ml of chitin–peptone medium (glucose 0.5%, peptone 0.2%, colloidal chitin 0.2%, K_2HPO_4 0.1%, $MgSO_4$ _ $7H_2O$ 0.05% and NaCl 0.05%, pH 6.8) (Lim *et al.*, 1991) at 28°C for 96 h in a rotary shaker incubator. After the incubation period the cultures were centrifuged at 12,000 rpm. for 20 min at 4°C and the supernatant was used as enzyme source. Colloidal chitin was prepared from crab shell chitin (Sigma) according to Berger and Reynolds (1958). The reaction mixture contained 0.25 ml of enzyme solution, 0.3 ml of 1M sodium acetate buffer (pH 5.3) and 0.5 ml of colloidal chitin (0.1%) and incubated in a water bath at 50°C for 4 hours. Chitinase activity was determined by measuring the release of reducing sugars by the method of Nelson (1944). One unit of chitinase was determined as 1 nmol of reducing

sugar released per minute per mg of protein. Protein content in all the samples was determined as described by Bradford (1976) using bovine serum albumin as the standard.

Determination of β-1,3-glucanase

P. fluorecens isolates were grown in 250 ml conical flasks containing 50 ml of peptone medium containing laminarin (0.2%) (from Laminaria digitata; Sigma) (Lim *et al.*, 1991) at 28°C for 96 h on a rotary shaker incubator. The cultures then centrifuged as previously mentioned and the resulted supernatant used as enzyme source. The reaction mixture contained 0.25 ml of enzyme solution, 0.3 ml of 0.1M phosphate buffer (pH 5.5) and 0.5 ml of laminarin (0.2%) (Lim *et al.*, 1991) then incubated at 40°C for 2 h in a water bath. β-1,3-glucanase activity was determined as 1 nmol of glucose released per minute per mg of protein. Protein content in all the samples was determined as described by Bradford (1976) using bovine serum albumin as the standard.

Determination of Siderophore Production

P. fluorescens strains were grown in KB broth (King *et al.* 1948) for 3 days at 28°C and centrifuged at 3000g for 10 min and the supernatants were collected. The pH of the supernatant was adjusted to 2.0 with diluted HCl and equal quantity of ethyl acetate was added in a separating funnel, mixed well and ethyl acetate fraction was collected. Five milliliters of ethyl acetate fraction was mixed with 5ml of Hathway's reagent (1.0 ml of 0.1M $FeCl_3$ in 0.1 N HCl to 100 ml distilled water1.0 ml of potassium ferricyanide). The absorbance for dihydroxy phenols was read at 700nm in a Spectrophotometer (Reeves *et al.*, 1983). A standard curve was prepared using dihydroxy benzoic acid. The quantity of siderophore synthesized was expressed as mmol benzoic acid/ml of culture filtrate.

Determination of Salicylic Acid Production

P. fluorescens isolates were grown at 28°C for 48h on a rotary shaker incubator in 250 ml conical flasks containing 50 ml of the succinate medium (succinic acid,4.0 g; K_2HPO_4,6.0 g; KH_2PO_4,3.0 g; (NH_2) SO_4, 1.0 g; $MgSO_4$-$7H_2O$ 0.2 g; distilled water. 1000ml; pH 7.0) (Meyer and Abdallah, 1978). The cultures then centrifuged as previously

mentioned and 4ml of the filtrate was acidified with 1 N HCl to pH 2.0 and SA was extracted in $CHCl_3$ (2x2ml). To the pooled $CHCl_3$ phases, 4ml of distilled water and 5ml of 2M FeCl3 were added. The absorbance of the purple iron–SA complex, which was developed in the aqueous phase was read at 527nm in a Spectrophotometer. A standard curve was prepared with SA dissolved in succinate medium. The quantity of SA in the culture filtrate was expressed as mg/ml (Meyer *et al.*, 1992).

Statistical Analysis

Data were statistically analyzed by analysis of variance according to Snedecor and Cochron, 1982 using SPSS system version 8, (1997).

RESULTS AND DISCUSSION

Soil-borne fluorescent pseudomonades have been widely used for the control of seedling and root diseases as they can enhance plant growth and yield apart from suppressing the growth of pathogen (Rabindran and Vidhyasekaran, 1996; Vidhyasekaran and Muthamilan, 1999).

In the present study twenty isolates of *P. fluorescens* were isolated from the rhizosphere of sugar beet with *Pseudomonas* selective agar (Oxoid), eight isolates from El-Sharkiah governorate (PfSh) and twelve isolates from Kafr-Elshaikh (PfKfr) as shown in Table 1.

In Vitro and *Iin Vivo* Screening of *P. fluorescens*

These bacterial colonies were tested for their ability to inhibit the mycelial growth of *R. solani in vitro* by dual culture technique. Among them, Pfkfr2 was the most effective one in inhibiting the mycelial growth of *R. solani*. The isolate Pfkfr2 produced the highest significant inhibition zone of 19.3 mm, followed by the isolates PfSh8 and PfKfr1, which produced inhibition zones of 15.3 and 14.3 mm, respectively (Table 1). The isolate PfSh1 recorded the lowest inhibition zone 4.3 mm while, the other tested isolates differed significantly in the produced inhibition zones.

On the other hand the *in vivo* experiment results (Table 2 and fig. 1 & 2) showed that all the tested isolates were able to suppress damping off in varied degrees. Among the 20 tested isolates Kafr El-Shaikh isolates showed the highest significant

Table1: *In vitro* inhibition of *Rhizoctonia. solani* mycelial growth by various isolates of *Pseudomonas. fluorescens* isolated from sugar beet rhizosphere obtained from El-Sharkia and Kafr El-Shaikh governorates

P. fluorescens isolates	Inhibition zone (mm)	Location	Governorate
PfSh1	4.3 K	El-Hosaneia	El-Sharkeia
PfSh2	6.3 HIJ		
PfSh3	5.7 IJK		
PfSh4	10.7 E	Saan El-Hagar	
PfSh5	9.0 FG		
PfSh6	10.7 E		
PfSh7	6.7 HI	Kafr-Saqr	
PfSh8	15.3 B		
PfKfr1	14.3 BC	Sakha	Kafr El-Shaikh
PfKfr2	19.3 A		
PfKfr3	13.3 CD		
PfKfr4	9.3 EF		
PfKfr5	9.0 FG	Al-Hamoul	
PfKfr6	5.0 JK		
PfKfr7	5.3 IJK		
PfKfr8	7.7 GH		
PfKfr9	6.0 IJ		
PfKfr10	12.3 D	Al- Riyadh	
PfKfr11	12.0 D		
PfKfr12	12.7 D		

Data are mean of five replications. LS.D at (0.05) = 1.311
Means followed by the same letter in a column are not significantly different according to Duncan's multiple range test.

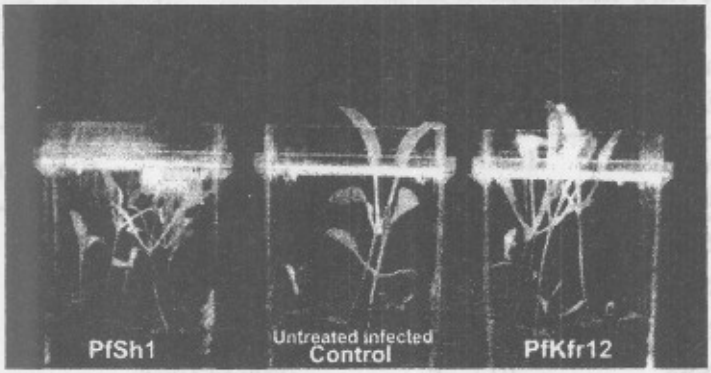

Fig.1: effect of *Pseudomonas. fluorescens* isolates Pfkfr12 and PfSh1 on suppressing *Rhizoctonia solani* damping off on sugar beet seedlings

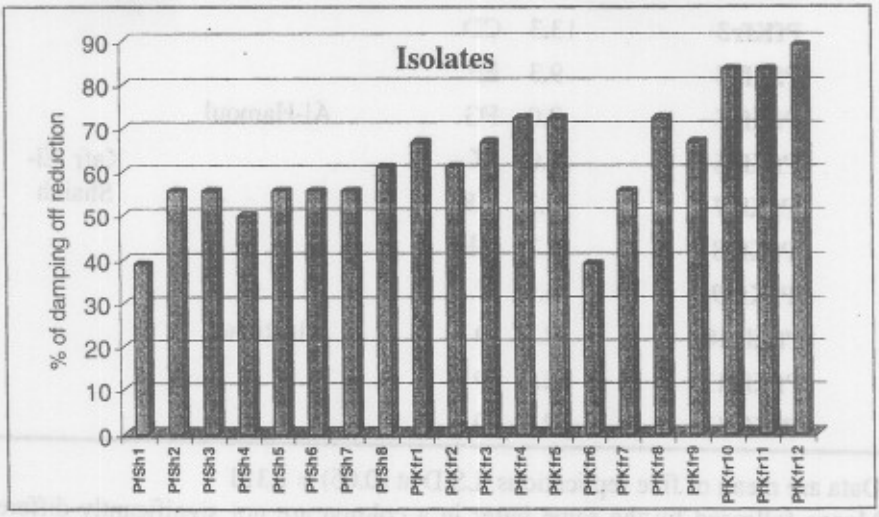

Fig.2: Percentage of sugar beet damping off reduction resulted by *Pseudomonas fluorescens*

Table 2: Effect of different *Pseudomonas. fluorescens* isolates in controlling sugar beet damping off disease in greenhouse

P. fluorescens isolates	Pre-emergence		Post-emergence		Survival	
PfSh1	2.00	BC	1.67	AB	6.33	E
PfSh2	1.33	CDE	1.33	BC	7.33	DE
PfSh3	1.67	E	1.00	BCD	7.33	DE
PfSh4	1.67	BCD	1.33	BC	7.00	DE
PfSh5	1.67	BCD	1.00	BCD	7.33	DE
PfSh6	1.67	BCD	1.00	BCD	7.33	DE
PfSh7	1.33	CDE	1.33	BC	7.33	DE
PfSh8	1.00	CDE	1.33	BC	7.67	CDE
PfKfr1	0.67	DE	1.33	BC	8.00	BCD
PfKfr2	1.00	CDE	1.33	BC	7.67	CDE
PfKfr3	1.00	CDE	1.00	BCD	8.00	BCD
PfKfr4	0.67	DE	1.00	BCD	8.33	ABCD
PfKfr5	0.67	DE	1.00	BCD	8.3	ABCD
PfKfr6	2.67	AB	1.00	BCD	6.33	E
PfKfr7	2.00	BC	0.67	CDE	7.33	DE
PfKfr8	1.33	CDE	0.33	DE	8.33	ABCD
PfKfr9	1.67	BCD	0.33	DE	8.00	BCD
PfKfr10	0.33	E	0.67	CDE	9.00	ABC
PfKfr11	0.67	DE	0.33	E	9.00	ABC
PfKfr12	0.67	DE	0.00	E	9.33	AB
***R. solani* (+ control)**	3.67	A	2.33	A	4.00	F
Water (- control)	0.33	BCD	0.00	DE	9.67	A

Data are mean of three replications. LSD at (0.05) for:
Pre-emergence = 1.069; Post-emergence = 0.7106 ; Survival = 1.267

efficiency in suppressing the occurrence of damping off than El-Sharkiah isolates. The isolates Pfkfr12, Pfkfr 11 and Pfkfr 10 showed the highest significant damping off suppression which reflected in the mean number of healthy survival plants (9.33, 9.0 and 9.0, respectively) compared to the infected untreated control (4.0) as shown in table 2 and fig. 1. On the other hand, El Sharkia isolates showed a moderate significant damping off suppression ranged from38.88% to 55.55% as shown in fig.1. also there was no correlation between the fungal growth inhibition level in the *in vitro* (Table 1) experiment and the *in vivo* experiment Table (2). This suggested that various isolates of *P. fluorescens* produce varying amounts of antifungal compounds and also has different mechanisms of pathogen and disease suppression (Rabindran and Vidhyasekaran, 1996; Vidhyasekaran and Muthamilan, 1999).

Chitinase and β-1,3-glucanase Activity

Among the 20 isolates of *P. fluorescens* tested for production of chitinase, Pfkfr2 and Pfkfr8 recorded the highest chitinase activity (83.33 and 82.67 respectively) followed by Pfkfr12 and Pfkfr9 (76.33 and 72.67 respectively) (Table 3). While, the isolates PfSh5 PfSh2, PfSh7, PfKfr10 and PfKfr4 recorded the lowest chitinase production (41.33, 43.00, 43.33, 44.67 and 45.33 units respectively). No remarkable relationship between the antagonistic potential of *P. fluorescen* isolates and their level of chitinase production was observed. On the other hand, Data presented in Table 4 show that, the highest significant β-1,3-glucanase activity recorded by *P. fluorescens* isolate PfKfr2 (277 units) followed by PfSh8 and PfKfr1 (228 and 217 units respectively). It is worthy to mention that there was a remarkable relationship between the antagonistic activity of *P. fluorescens* isolates and their level of production of β -1,3-glucanase. As shown in tables 1 and 4 it was clear that the isolates, which recorded high inhibition zone, exhibited the highest β -1,3-glucanase activity and vers versa.

Several cell wall degrading enzymes such as chitinase and β-1,3-glucanase are involved in the biological control of plant pathogens by *P. fluorescens*. Many biocontrol agents secrete chitinase and β-1,3-glucanase

capable of degrading chitin and b-1,3-glucan, respectively, which are the major components of fungal cell walls (Velazhahan *et al.*,1999; Zhang and Yuen 2000 and Meena *et al.*, 2001). Several studies indicated that the antagonistic potential of *P. fluorescens* against various soil borne plant pathogens are correlated with production of lytic enzymes (Lim *et al.*, 1991; Velazhahan *et al.*, 1999; Meena *et al.*, 2001).

In the present study, the results indicate that there was a significant relationship between antagonistic activity of *P. fluorescens* and the level of β-1,3- glucanase production suggesting that production of β-1,3- glucanase maybe responsible for the antagonistic activity of *P. fluorescens* against *R. solani* these results are in agree with the results of Fridlender *et al.*, (1993) and Meena *et al.* (2001) and Nagarajkumar *et al.* (2004).

Data in table 5 show that all the isolates produced siderophore with significant differences. Also, Significant maximum levels of siderophore production was recorded in *P. fluorescens* isolate PfSh8 (16.29 mmol/ml culture filtrate) followed by PfKfr6, PfKfr1, PfKfr11, PfKfr3 and PfKfr5 (14.23, 14.16, 13.87, 13.81, and 13.43 mmol/ml culture filtrate, respectively). The lowest siderophore production was recorded in isolate PfKfr8 (2.67 mmol/ml culture filtrate) followed by PfSh6 (4.15 mmol/ml culture filtrate) and PfSh3 (5.73 mmol/ml culture filtrate). No relationship found between the antagonistic potential of *P. fluorescens* isolates and their siderophore production.

Many *P. fluorescens* isolates known to secrete fluorescent water-soluble siderophoresunder ironlimiting conditions (O'Sullivan and O'Gara, 1992).

These fluorescent siderophores, which have very high affinity for ferric iron, will form ferric-siderophore complex and make it unavailable to other organisms, but the producing organisms can utilize these complexes via a specific receptor in their outer cell membrane (Buyer and Leong, 1986).

Due to iron starvation, the growth of pathogenic fungi and bacteria in the rhizosphere will be restricted. Fluorescent pseudomonades produce several siderophores such as pyoverdine (Pseudobactin), pyochelin and SA (Dave and Dube, 2000). Elad and Baker (1985) observed a direct

Table 3: Chitinase activity produced by *Pseudomonas. fluorescens* strains after 3 days incubation in peptone medium containing 0.2%, colloidal chitin

P. fluorescens isolates	Chitinase activity (units/mg protein)	
PfSh1	59.33	F
PfSh2	43.33	H
PfSh3	53.00	G
PfSh4	64.00	DE
PfSh5	41.33	H
PfSh6	64.00	DE
PfSh7	43.00	H
PfSh8	69.00	C
PfKfr1	63.00	DEF
PfKfr2	83.33	A
PfKfr3	62.00	DEF
PfKfr4	45.33	H
PfKfr5	52.00	G
PfKfr6	59.67	F
PfKfr7	65.00	D
PfKfr8	82.67	A
PfKfr9	72.67	BC
PfKfr10	44.67	H
PfKfr11	60.33	EF
PfKfr12	76.33	B

Data are mean of three replications. LSD at (0.05) = 3.815

Means followed by the same letter in a column are not significantly different according to Duncan's multiple range test.

Table 4: β-1,3-glucanase activity produced by *Pseudomonas. fluorescens* strains after 3 days incubation in peptone medium containing 0.2%, laminarin

P. fluorescens isolates	β-1,3-glucanase activity (units/mg protein)	
PfSh1	62.00	P
PfSh2	103.67	JK
PfSh3	84.00	MN
PfSh4	139.00	H
PfSh5	121.33	I
PfSh6	152.67	G
PfSh7	96.33	KL
PfSh8	228.00	B
PfKfr1	217.00	C
PfKfr2	277.00	A
PfKfr3	191.00	D
PfKfr4	138.67	H
PfKfr5	126.33	I
PfKfr6	75.33	O
PfKfr7	77.33	NO
PfKfr8	109.67	J
PfKfr9	88.33	LM
PfKfr10	175.00	EF
PfKfr11	171.67	F
PfKfr12	181.33	E

Data are mean of three replications. LSD at (0.05) = 8.018

Means followed by the same letter in a column are not significantly different according to Duncan's multiple range test.

Table 5: Production of Siderophore (mM/ml culture filtrate) and Salicylic acid (mg/ml culture filtrate) by *Pseudomonas. fluorescens* strains

P. fluorescens isolates	Siderophore production (mmol/ml culture filtrate)		Salicylic acid production (mg/ml culture filtrate)	
PfSh1	11.46	E	3.23	M
PfSh2	11.72	E	6.53	J
PfSh3	5.73	J	4.60	L
PfSh4	12.55	D	13.67	E
PfSh5	6.02	IJ	11.53	FG
PfSh6	4.15	K	13.73	E
PfSh7	10.71	F	7.23	I
PfSh8	16.29	A	19.43	B
PfKfr1	14.16	B	19.20	B
PfKfr2	12.46	D	21.67	A
PfKfr3	13.81	BC	16.33	C
PfKfr4	9.69	G	11.97	F
PfKfr5	13.43	C	11.40	G
PfKfr6	14.23	B	3.67	M
PfKfr7	6.47	I	4.27	L
PfKfr8	2.76	L	9.67	H
PfKfr9	6.49	I	5.80	K
PfKfr10	8.72	H	15.43	D
PfKfr11	13.87	BC	15.43	D
PfKfr12	6.56	I	15.83	D

Data are mean of three replications. LSD at (0.05) for:
Siderophore = 0.5279, Salicylic acid = 0.4733
Means followed by the same letter in a column are not significantly different according to Duncan's multiple range test.

correlation between production of siderophores by fluorescent pseudomonads and their capacity to inhibit germination of chlamydospores of *Fusarium oxysporum*. Paulitz and Loper (1991) also demonstrated that transposon mutants deficient in the production of siderophoresand antifungal metabolites still had biocontrol activity comparable to the wild type isolates that produced these metabolites. Siderophores are also known to induce systemic resistance in plants (Leeman *et al.*, 1996).

The role of SA in induction of systemic acquired resistance was demonstrated using transgenic tobacco engineered to express the SA-hydroxylase gene (nahG) (Gaffney *et al.*, 1993). SA produced by *P. fluorescens* in the rhizosphere is thought to be involved in ISR (Maurhofer *et al.*, 1998). Maurhofer *et al.* (1998) demonstrated that expression of SA biosynthetic genes (pchA and pchB) in *P. fluorescens* isolate P3 significantly improved its ability to induce systemic resistance in tobacco against tobacco necrosis virus. In the present work, the SA production was found to be the maximum inPfKfr2 (21.67 mg/ml culture filtrate) followed by the isolate (PfSh3 and PfKfr1) (19.34 and 19.2 mg/ml culture filtrate) and PfKfr3 (16.33 mg/ml culture filtrate) (table 5). The isolates PfSh1 and PfKfr6 recorded the lowest SA production (3.23 and 3.67 mg/ml culture filtrate respectively). Also, a remarkable relationship between the antagonistic potential of *P. fluorescens* isolates and their level of production of SA was observed.

It is worthy to mention that, there was a significant relationship between inhibitory activity of *P. fluorescens* isolates *in vitro* and their level of SA production. However, several studies indicated that the ability of fluorescent pseudomonads to produce SA might not always be correlated with their ISR activity (Press *et al.*, 1997; Chen *et al.*, 1999). Chen *et al.* (1999) reported that *Pseudomonas corrugata* isolate 13 and *Pseudomonas aureofaciens* isolate 63-28, varied in SA production *in vitro*, induced the same level of resistance in cucumber against Pythium root rot. The present studies suggest that more than one mechanism may be involved in the suppression of *R. solani* by *P. fluorescens*. Involvement of more than one mechanism has been reported in

other systems as well (Belanger *et al.*, 1995; Arras and Arru, 1997; Guetsky *et al.*, 2002).

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تأثير الإنزيمات المفرزة خارجيا والأيضيات الثانوية للبكتيريا *سيدوموناس فلوريسنت* علي فطر *رايزوكتونيا سولاني* المسبب لمرض موت بادرات بنجر السكر

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مركز البحوث الزراعية - الجيزة

تم عزل عشرين عزله *سيدوموناس فلوريسنت* من رايزوسفير جذور بنجر السكر لتقدير قدرتهم التضادية علي الفطر *رايزوكتونيا سولاني* في المعمل و علي النباتات. و قد تم تقييم انتاج البكتيريا لإنزيمات الكايتينيز و بيتا ١-٣ جلوكابيز و كذلك السيدوفورس و حامض السالسيليك. وجد ان العزلة Pfkfr2 احدثت تكون اكبر منطقة تضاد (١٩,٣ ملليمتر) مع نمو الفطر رايزوكتونيا سولاني في الاطباق بشكل معنوي و تبعتها العزلات PfSh8 و PfKfr1 التي احدثت منطقة تضادية (١٥,٣ -١٤,٣ ملليمتر علي الترتيب). و قد أظهرت نتائج اختبارات قدرة العزلات علي تثبيط حدوث مرض موت البادرات ان العزلات Pfkfr12 و Pfkfr 11 و Pfkfr 10 ثبطت حدوث المرض بمعنوية كبيرة و التي انعكست علي شكل زيادة متوسط النباتات الحية (٩,٣٣ و ٩ و ٩ نبات علي الترتيب) مقارنة بتجربة المقارنة المعدية و الغير معاملة بالبكتيريا (٤ نبات). تفوقت العزلات Pfkfr2 و Pfkfr8بشكل معنوي علي باقي العزلات في انتاج انزيم الكايتينيز (٨٣,٣٣ و ٨٢,٦ وحده انزيمية علي الترتيب) في حين سجلت العزلة PfKfr2 اعلي انتاج لانزيم بيتا ١-٣ جلوكانيز (٢٧٧ وحدة انزيم) تبعتها العزلات PfSh8 و PfKfr1 (٢٢٨ و ٢١٧ وحدة انزيم علي الترتيب). كان اعلي انتاج للسيدروفورس بواسطة العزلة PfSh8 و التي انتجت ١٦,٢٩ ميليمول/مللي راشح في حين سجلت العزلة PfKfr2 اعلي انتاج لحامض السالسليك (٢١,٦٧ ميليجرام / ميللي راشح و تبعتها العزلتان PfSh3 وPfKfr1 (١٩,٣٤ و ١٩,٢ ميليجرام / ميللي راشح). و قد وجدت علاقة واضحة بين قوة القدرة التضادية للعزلات المختبرة و قدرتها علي انتاج انزيم بيتا ١-٣ جلوكاتيز و حامض السالسليك.