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Enhancing resistance of tomato and hot pepper to Pythium diseases by seed treatment with fluorescent pseudomonads

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Abstract

Twenty isolates of fluorescent pseudomonads were evaluated for their ability to control damping-off in tomato (*Lycopersicon esculentum*) and hot pepper (*Capsicum annuum*). These isolates were characterized as *Pseudomonas fluorescens* and *Pseudomonas putida*. Two isolates, PFATR and KKM 1 belonged to *P. putida* and the remaining 18 isolates belonged to *P. fluorescens*. Among these isolates, *P. fluorescens* isolate Pf1 showed the maximum inhibition of mycelial growth of *Pythium aphanidermatum* and increased plant growth promotion in tomato and hot pepper. *P. fluorescens* isolate Pf1 was effective in reducing the damping-off incidence in tomato and hot pepper in greenhouse and field conditions. Isolate Pf1 was further tested for its ability to induce production of defense-related enzymes and chemicals in plants. Earlier and increased activities of phenylalanine ammonia lyase (PAL), peroxidase (PO) and polyphenol oxidase (PPO) were observed in *P. fluorescens* Pf1 pretreated tomato and hot pepper plants challenged with *Pythium aphanidermatum*. Moreover, higher accumulation of phenolics was noticed in plants pretreated with *P. fluorescens* isolate Pf1 challenged with *Pythium aphanidermatum*. Thus, the present study shows that in addition to direct antagonism and plant growth-promotion, induction of defense-related enzymes involved in the phenyl propanoid pathway collectively contributed to enhance resistance against invasion of *Pythium* in tomato and hot pepper.

Abbreviations: ISR – induced systemic resistance; KMB – King's medium B; PAGE – polyacrylamide gel electrophoresis; PGPR – plant growth-promoting rhizobacteria; PAL – phenylalanine ammonia-lyase; PO – peroxidase; PPO – polyphenol oxidase; PR – proteins-pathogenesis-related proteins.

Introduction

Pre- and post-emergence damping-off disease caused by *Pythium* spp. in vegetable crops is economically very important worldwide (Whipps and Lumsden, 1991). Rapid germination of sporangia of *Pythium* in 1.5–2.5 h after exposure to exudates or volatiles from seeds or roots (Nelson, 1987; Osburn et al., 1989) followed by immediate infection makes management of the pathogen very difficult (Whipps and Lumsden, 1991). Although fungicides have shown promising results in controlling the damping-off disease, phytotoxicity and fungicide residues are major problems leading to environmental pollution and human health hazards. Development of fungicide resistance by *Pythium* spp. further discourages its use for disease control (Whipps and Lumsden, 1991). Sanitation using sterile or clean water supplies, application of organic compost and regulation of watering and temperature during seedling growth contributed to the management of the disease to some extent. Though soil solarization has been successful under

hot climatic conditions (Katan, 1987), it is impractical during the winter season. Thus, existing control measures are not effective for the control of damping-off disease. Biological control is an alternative approach to the chemical fungicides and it may be a safe, effective and ecofriendly method for plant disease management.

Soil has enormous untapped potential antagonistic microbes i.e. *Trichoderma* spp. and fluorescent pseudomonads which show antagonistic effects against soil-borne plant pathogenic organisms. The saprophytic pseudomonads associated with plants include *P. fluorescens, P. putida* and *P. aeruginosa*. The use of fluorescent pseudomonads is gaining importance for plant growth-promotion and biological control.

Fluorescent pseudomonads control *Pythium* spp. through different modes of action such as competition for nutrients and space (Elad and Chet, 1987), antibiosis (Howie and Suslow, 1991), production of siderophores (Loper, 1988) and lytic enzymes (Frindlender et al., 1993). In addition, induction of resistance by fluorescent pseudomonads is an additional mechanism by which these bacteria protect several crop plants against pests and diseases (Zhou and Paulitz, 1994; Chen et al., 1998; Pieterse et al., 2001; Ramamoorthy et al., 2001b; Zehnder et al., 2001). In tomato, seed treatment with *P. fluorescens* strain 63–28 resulted in induced resistance against *Fusarium oxysporum* f. sp. *lycopersici* by triggering the host to synthesize more phenolic substances (M'Piga et al., 1997). Earlier, we reported that seed treatment and soil application of *P. fluorescens* isolate Pf1 increased the accumulation of enzymes involved in phenyl propanoid pathway and pathogenesis-related proteins (PR-proteins) in response to infection by *F. oxysporum* f. sp. *lycopersici* causing wilt and *Colletotrichum capsici* causing fruit rot in tomato and hot pepper respectively (Ramamoorthy et al., 2001a; Ramamoorthy and Samiyappan, 2001). The objectives of the present study are

(i) to characterize and evaluate the fluorescent pseudomonads isolated from different crops grown in different regions of South India against damping-off in tomato and hot pepper under greenhouse and field conditions.
(ii) to study the induction of various defense-related genes encoding proteins implicated in strengthening of plant cell walls by *P. fluorescens* in response to infection by *Pythium aphanidermatum*.

Materials and methods

Isolation and characterization of microorganisms

Twenty isolates of fluorescent pseudomonads were isolated from rhizosphere soil of different crops grown in different parts of South India (Table 1) using King's medium B (KMB) (King et al., 1954). The isolates were characterized based on standard biochemical tests (Hildebrand et al., 1992). The following tests were carried out: production of fluorescent pigment (King et al., 1954), gelatin liquefaction, nitrate reduction, arginine dihydrolase, levan formation, growth at 4 and 41 °C and different carbon sources utilization (Hildebrand et al., 1992). Results of these tests were scored either as positive or as negative. Grouping was made with the aid of a determinative scheme developed by earlier workers (Champion et al., 1980; Barrett et al., 1986; Hildebrand et al., 1992).

Pythium aphanidermatum was isolated from naturally infected tomato plants using potato dextrose agar (PDA) medium and mass-multiplied in sand–maize medium at 19 : 1 w/w (sand : maize). Pathogenicity was tested in tomato and hot pepper seedlings and the pathogen was maintained in sand–maize medium.

For *in vitro* screening of fluorescent pseudomonads against *Pythium aphanidermatum,* the bacterial isolates were streaked on one side of a Petri dish (1 cm from the edge of the plate) with PDA medium and a mycelial disc (8 mm diameter) of seven-day-old culture of *Pythium aphanidermatum* was placed on the opposite side of the Petri dish perpendicular to the bacterial streak (Vidhyasekaran et al., 1997). The plates were incubated at room temperature (28 ± 2 °C) for 4 days and the zone of inhibition was measured.

Efficacy of fluorescent pseudomonads on plant growth-promotion under laboratory conditions

Fluorescent pseudomonads were grown in Erlenmeyer flasks (250 ml) containing 100 ml of KMB broth for 48 h on a rotary shaker at 28 ± 2 °C. Cells were removed by centrifugation at 8000 *g* for 10 min at 4 °C and washed in sterile water. The pellet was resuspended in a small amount of sterile distilled water and then diluted with an adequate amount of sterile distilled water to obtain a bacterial suspension of 10^8 cfu ml^{-1} (Thompson, 1996).

For bacterization of seed, seeds of pepper (cv. Co1) and tomato (cv. Co3) were surface sterilized with

2% sodium hypochlorite for 30 s and rinsed in sterile distilled water and dried overnight under a sterile air stream. Ten millilitre of bacterial inoculum (10^8 cfu ml^{-1}) was put in a Petri dish. To this, 100 mg of carboxymethylcellulose was added as adhesive material. One gram of seeds was soaked in 10 ml of bacterial suspension for 12 h and dried overnight in sterile Petri dish.

Plant growth-promoting activity of fluorescent pseudomonads was assessed based on the seedling vigour index by the standard roll towel method (ISTA, 1993). The vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973): Vigour Index = (Mean root length + Mean shoot length) × Germination (%).

Efficacy of fluorescent pseudomonads against damping-off disease under greenhouse conditions

Potting medium (red soil : sand : cowdung manure at 1 : 1 : 1 w/w/w) was autoclaved for 1 h on two consecutive days. The virulent strain of *Pythium aphanidermatum*, mass multiplied in the sand–maize medium, was mixed with the sterilized potting medium at the ratio of 19 : 1 w/w. The soil was placed in pots (15 cm diameter; 30 cm height). Bacterized seeds of tomato and pepper were thickly sown in the pots at 25 seeds per pot. Ridomil as seed treatment at 6 g kg^{-1} of seed was included as a standard treatment for comparison. Pathogen inoculated and pathogen un-inoculated control (healthy) were maintained. Watering was done regularly and damping-off disease incidence was recorded at 25 days after sowing. Plant growth was measured by randomly selecting five plants in each pot. Four pots per replication were maintained. There were three replications and the pots were arranged in a randomized manner.

Efficacy of formulation of P. fluorescens *isolate Pf1 against damping-off disease*

For the development of formulation of *P. fluorescens*, a talc-based formulation was developed (Vidhyasekaran and Muthamilan, 1995). Carboxymethylcellulose (10 g) was mixed with talc powder (1 kg) and the pH was adjusted to 7.0 with calcium carbonate. The mixture was autoclaved for 30 min in two consecutive days. Four-hundred millilitre of bacterial inoculum containing 9×10^9 cfu ml^{-1} was added to the talc mixture and mixed well. The formulation was packed in polythene bags, sealed and kept under room temperature. The population of *P. fluorescens* in the formulation was 2.5×10^8 cfu g^{-1}.

Two field experiments were conducted, one at Agricultural Research Station, Aliyar Nagar and another at Tamil Nadu Agricultural University, Coimbatore, India where the damping-off disease is endemic. Physiochemical properties of soils of both the fields were analyzed [Aliyar Nagar: soil texture – red loam (sand – 43%, silt – 31.5% and clay – 25.5 %) available N, P, K, Ca and Mg were 188, 69, 700, 0.63, and 0.14 kg ha^{-1}, respectively. Coimbatore: soil texture – red sandy loam (sand – 67.3%, silt – 8.2% and clay – 24.5%) available N, P, K, Ca and Mg were 89, 22, 50, 0.26 and 0.12 kg ha^{-1}, respectively]. Seeds treated with formulation at 10 g kg^{-1} were sown (1000 seeds per bed) thickly in rows in the flat nursery bed. As a standard treatment, seeds were treated with ridomil at 6 g kg^{-1} seed. Seeds treated with the talc powder alone were also included. Seeds treated with distilled water served as control. The experiment was laid out with randomized block design with four replications. The damping-off incidence and plant height were recorded at 25 days after sowing as described already.

Induction of defense mechanisms and experimental design

P. fluorescens, isolate Pf1, was used in the induction of defense reactions in tomato and pepper. The following treatments were included in the experiment: (1) seeds treated with *P. fluorescens* isolate Pf1; (2) seeds treated with *P. fluorescens* isolate Pf1 and challenge inoculated with *Pythium aphanidermatum* 15 days after planting (50 g sand–maize medium containing 10^3 cfu g^{-1} medium in each pot); (3) plants inoculated with the pathogen 15 days after sowing; and (4) non-treated plants.

Seeds were sown in earthen pots filled with sterilized potting soil at 25 seeds per pot. Three replications were maintained in each treatment; each replicate consisted of eight pots. The experiments were conducted using randomized block design on a greenhouse bench. The humidity in the greenhouse was maintained at around RH 80%. The temperature was adjusted to 26 °C (day)/20 °C (night).

Plants were carefully uprooted without causing any damage to root tissues at different time intervals (0, 1, 2, 3, 4, 5, 7 and 10 days after the pathogen inoculation).

Four plants were sampled from each replication of the treatment separately (treatments were mentioned in the experimental design) and were maintained separately for biochemical analysis. Fresh roots were washed in running tap water and homogenized with liquid nitrogen in a pre-chilled mortar and pestle. The homogenized root tissues were stored at −70 °C.

Estimation of phenylalanine ammonia lyase (PAL) activity

Root samples (1 g) were homogenized in 3 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvenylpyrrolidone. The extract was filtered through cheesecloth and the filtrate was centrifuged at 16,000 *g* for 15 min. The supernatant was used as enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm (Dickerson et al., 1984). Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. The amount of *trans*-cinnamic acid synthesized was calculated (Dickerson et al., 1984). Enzyme activity was expressed as nmol *trans*-cinnamic acid min^{-1} mg $protein^{-1}$.

Assay of peroxidase (PO)

Root samples (1 g) were homogenized in 2 ml of 0.1 M phosphate buffer, pH 7.0 at 4 °C. The homogenate was centrifuged at 16,000 *g* at 4 °C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% H_2O_2. The reaction mixture was incubated at room temperature (28 ± 2 °C). The changes in absorbance at 420 nm were recorded at 30 s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min^{-1} mg $protein^{-1}$ (Hammerschmidt et al., 1982).

Assay of polyphenol oxidase (PPO)

Root samples (1 g) were homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16,000 *g* for 15 min at 4 °C. The supernatant was used as enzyme source. The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 nm min^{-1} mg $protein^{-1}$ (Mayer et al., 1965).

Estimation of phenol

Root samples (1 g) were homogenized in 10 ml of 80% methanol and agitated for 15 min at 70 °C (Zieslin and Ben-Zaken, 1993). One millilitre of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin–Ciocalteau reagent (1 N) and the solution was kept at 25 °C. The absorbance of the developed blue colour was measured using a spectrophotometer at 725 nm. Catechol was used as the standard. The amount of phenolics was expressed as µg catechol mg $protein^{-1}$.

Native-PAGE analysis

The isoform profiles of PO and PPO were examined by discontinuous native polyacrylamide gel electrophoresis (native-PAGE) (Laemmli, 1970). Root samples were collected on the third day after pathogen challenge, at which time the activity of PO and PPO was maximum. The protein extract was prepared by homogenizing 1 g of root samples in 2 ml of 0.1 M sodium phosphate buffer pH 7.0 and centrifuged at 16,000 *g* for 20 min at 4 °C. The protein content of the sample was determined (Bradford, 1976). Samples (50 µg protein) were loaded onto 8% polyacrylamide gels (Sigma, USA). After electrophoresis, PO isoforms were visualized by soaking the gels in staining solution containing 0.05% benzidine (Sigma, USA) and 0.03% H_2O_2 in acetate buffer (20 mM, pH 4.2) (Nadlony and Sequira, 1980). For assessing PPO isoforms profile, the gels were equilibrated for 30 min in 0.1% p-phenylene diamine followed by addition of 10 mM catechol in the same buffer (Jayaraman et al., 1987).

Statistical analysis

All the experiments were repeated once with similar results. The data were statistically analyzed (Gomez and Gomez, 1984) and treatment means were compared by Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines.

Results

Characterization of fluorescent pseudomonads

Twenty isolates of fluorescent pseudomonads isolated from rhizosphere soil of different crops were found to be fluorescent on KMB and all the tested isolates did not grow at 41 °C indicating that they did not belong to *P. aeruginosa*. Most of the bacterial isolates showed a positive response for gelatin liquefaction and trehalose utilization tests except two isolates i.e. PFATR and KKM1 (Table 1). Hence, these two isolates were grouped under *P. putida* group. The remaining 18 isolates were classified as *P. fluorescens.* The isolates were grouped into different biovars based on other primary characters of fluorescent pseudomonads and utilization of various carbon sources. Based on that, isolate KKM1 was grouped into the *P. putida* biovar I and the isolate PFATR was grouped into the *P. putida* biovar II. Among the 18 isolates of *P. fluorescens,* 16 isolates showed positive results to both gelatin and trehalose utilization tests. The two isolates i.e. ARR2 and VPT 10 showed positive results to either one of these two tests. ARR2 showed positive results to gelatin liquefaction and negative to trehalose utilization test whereas the isolate VPT10 showed negative results to gelatin liquefaction and positive to trehalose utilization test. Although these two isolates showed positive results to one test and negative results to other test, they were tentatively grouped in *P. fluorescens*. Based on the similarity in the biochemical tests, the isolates i.e. Pf1, PB2, ARR1 and PFNL were grouped into *P. fluorescens* biovar I; FP7, PFAR, ARR1G, PSV, VPT4 and VPT10 were grouped under *P. fluorescens* biovar II; PFKO belonged to *P. fluorescens* biovar III; PFSA was classified under *P. fluorescens* biovar IV and ARR2, PFNA, EP1, PCU, PFCOT and PFCOP were grouped under *P. fluorescens* biovar V (Table 1).

Effect of fluorescent pseudomonads on growth of Pythium aphanidermatum

Among the twenty fluorescent pseudomonads isolates tested for their efficacy in inhibiting the mycelial growth, five isolates, *P. fluorescens* Pf1, PFCOT, FP7, PFCOP and PB2 showed higher inhibitory effect on mycelial growth of *Pythium aphanidermatum* when compared to other tested isolates. Among these five isolates, *P. fluorescens* isolate Pf1 biovar I exhibited the maximum inhibition of mycelial growth *in vitro* by recording a inhibition zone of 13.7 mm (Table 1).

P. fluorescens isolates Pf1 and FP7 and *P. putida* isolate PFATR showed an increased vigour index of tomato and pepper in the roll towel assay (Table 1).

Based on the *in vitro* inhibition of mycelial growth against *Pythium aphanidermatum* and plant growth promotion, seven isolates of *P. fluorescens,* Pf1, FP7, PSV, PB2, PFCOT and PFCOP and *P. putida* isolate PFATR were screened for further studies of assessing their efficacy against damping-off disease in tomato and hot pepper. *P. fluorescens* isolate PSV was included in this study as this isolate effectively increased the vigour index of brinjal (Ramamoorthy and Samiyappan personal communication).

Of the seven fluorescent pseudomonads tested under greenhouse conditions for their efficacy in controlling damping-off, all were effective in tomato and most of them in pepper (Table 2). *P. fluorescens* isolate Pf1 showed the lowest disease incidence of 35.6% and 31.1% in tomato and pepper respectively, and increased the plant growth. The efficacy of *P. fluorescens* isolate Pf1 was comparable with that of ridomil (Table 2).

The efficacy of formulations of *P. fluorescens* isolate Pf1 against damping-off was tested under field conditions. Formulations of *P. fluorescens* isolate Pf1 was effective for the control of damping-off incidence and increased plant growth under field conditions in two locations. The efficacy was comparable with chemical treatments. Seed treatment with talc powder alone had no effect on disease incidence and plant growth (Table 3).

Induction of defense-related enzymes and phenolic compounds

In tomato and pepper, seed treatment with *P. fluorescens* isolate Pf1 induced the plants to synthesize PAL, whereas an additional increase in the synthesis was observed in *P. fluorescens* isolate Pf1-pretreated plants challenge inoculated with *Pythium aphanidermatum*. The activity reached the maximum level on the third day after pathogen challenge and thereafter the activity remained at higher levels throughout the experimental period of 10 days. In plants treated with the pathogen alone, increased activity of PAL was observed for a period of 2–4 days and thereafter declined drastically in both tomato and hot pepper (Figure 1A,B). Earlier and increased activities of PO (Figure 1C,D) and PPO (Figure 1E,F)

Table 1. Characterization of fluorescent pseudomonads and their efficacy on inhibition of *Pythium aphanidermatum* and vigour index of vegetable crops

Isolates	Host plant	Primary character							Carbon source utilization										Species and biovar	Inhibition zone (mm)	Vigour index	
		Fluorescent on K.B. medium	Gelatin Liquefaction	Arginine dihydrolase	Levan formation	Nitrate reduction	Growth at 4°C	Growth at 41°C	Glucose	Sorbitol	Ethanol	Trehalose	Phenyl acetate	Glycine	Nicotinate	Adonitol	Erythritol	Mannitol			Tomato	Hot pepper
Pf1	Urdbean	+	+	+	+	−	+	−	+	−	+	+	−	+	−	+	+	+	Pf biovar I	13.7^{h}	1506.2^{kl}	1464.5^{j}
FP7	Rice	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	−	Pf biovar II	11.7^{fg}	1420.8^{i}	1109.4^{fg}
PFNL	Pepper	+	+	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	Pf biovar I	0.0^{a}	1333.2^{h}	1063.4^{de}
PFKO	Pepper	+	+	+	−	−	+	−	+	−	−	+	−	−	−	−	−	+	Pf biovar III	5.7^{c}	1032.2^{b}	1086.0^{ef}
PFATR	Tapioca	+	−	+	+	−	+	−	+	+	−	−	−	−	−	+	+	+	Pp biovar II	0.0^{a}	1504.2^{kl}	1371.8^{i}
PFNA	Banana	+	+	+	−	−	−	−	+	+	−	+	−	−	−	−	−	−	Pf biovar V	4.3^{b}	1186.2^{e}	1203.2^{h}
EP1	Sugarcane	+	+	+	−	−	+	−	+	+	+	+	−	−	−	+	+	+	Pf biovar V	3.3^{b}	1233.2^{f}	1131.8^{g}
PCU	Banana	+	+	+	−	−	+	−	+	−	+	+	−	−	−	−	+	+	Pf biovar V	0.0^{a}	1180.2^{de}	986.3^{b}
PFSA	Maize	+	+	+	+	−	−	−	+	+	+	+	−	−	−	−	+	+	Pf biovar IV	3.3^{b}	1229.7^{f}	1008.6^{bc}
PFAR	Groundnut	+	+	+	+	+	+	−	+	+	+	−	−	−	+	−	+	+	Pf biovar II	6.7^{cd}	1081.2^{c}	1142.4^{g}
PSV	Cotton	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	+	Pf biovar II	0.0^{a}	1149.3^{d}	1136.1^{g}
VPT4	Sugarcane	+	+	+	+	+	+	−	+	+	−	+	+	−	−	−	+	−	Pf biovar II	4.3^{b}	1297.0^{g}	1138.8^{g}
KKM1	Sugarcane	+	−	+	+	−	+	−	+	−	+	−	−	+	−	+	+	−	Pp biovar I	7.3^{d}	1230.5^{f}	1046.2^{d}
ARR1	Sugarcane	+	+	+	+	−	+	−	+	+	+	+	−	−	+	+	+	+	Pf biovar I	0.0^{a}	1361.5^{h}	1137.0^{g}
VPT10	Sugarcane	+	−	+	+	+	+	−	+	+	+	+	−	−	−	−	+	+	Pf biovar II	7.3^{d}	1360.5^{h}	1128.0^{g}
PB2	Rice	+	+	+	+	−	+	−	+	+	+	+	−	−	−	+	+	+	Pf biovar I	10.3^{e}	1506.2^{l}	1186.2^{h}
ARR1G	Sugarcane	+	+	+	+	+	+	−	+	−	−	+	+	−	+	−	+	−	Pf biovar II	7.7^{d}	1237.3^{f}	1090.8^{ef}
ARR2	Sugarcane	+	+	+	+	−	−	−	+	+	−	−	−	−	−	−	+	−	Pf biovar V	0.0^{a}	1258.2^{f}	1026.5^{cd}
PFCOP	Rice	+	+	+	+	−	−	−	+	+	+	+	−	−	−	+	−	+	Pf biovar V	12.0^{g}	1448.2^{ij}	1148.8^{g}
PFCOT	Tomato	+	+	+	−	−	+	−	+	−	+	+	−	−	−	+	−	+	Pf biovar V	10.7^{ef}	1473.0^{jk}	1146.1^{g}
Control																				0.0^{a}	753.0^{a}	783.6^{a}

Symbol '+' denotes a positive result, symbol '−' denotes negative result Pf – *P. fluorescens*; Pp – *P. putida*. Means followed by a common letter within a column are not significantly different ($P = 0.05$) by DMRT. The data are mean of three replications.

Table 2. Efficacy of fluorescent pseudomonads isolates against damping-off disease in solanaceous vegetable crops under green house conditions

Isolates of fluorescent pseudomonads	Tomato		Hot pepper	
	DI* (%)	Plant height (cm)	DI* (%)	Plant height (cm)
Pf1	35.6[b]	16.5[f]	31.1[b]	15.1[f]
FP7	53.3[cd]	15.3[e]	55.6[d]	14.5[ef]
PFATR	62.2[d]	14.6[cd]	42.2[c]	13.6[bc]
PSV	57.8[d]	15.1[de]	37.8[bc]	14.9[f]
PB2	48.9[c]	14.9[de]	69.0[e]	13.9[cd]
PFCOT	51.2[c]	15.0[de]	57.8[d]	14.1[d]
PFCOP	55.5[cd]	14.3[bc]	68.9[e]	14.1[d]
Ridomil	31.1[b]	14.1[b]	28.89[b]	13.2[b]
Control (pathogen-inoculated)	71.11[e]	8.1[a]	75.6[e]	7.7[a]
Control (pathogen-uninoculated)	0.00[a]	14.1[b]	2.3[a]	14.3[de]

DI – Disease incidence (%).
*The percentage data were arcsine transformed prior to the analysis.
Means followed by a common letter within a column are not significantly different ($P = 0.05$) by DMRT.

Table 3. Efficacy of talc-based formulation of *P. fluorescens* Pf1 on the management of damping off disease in solanaceous vegetable crops under field conditions

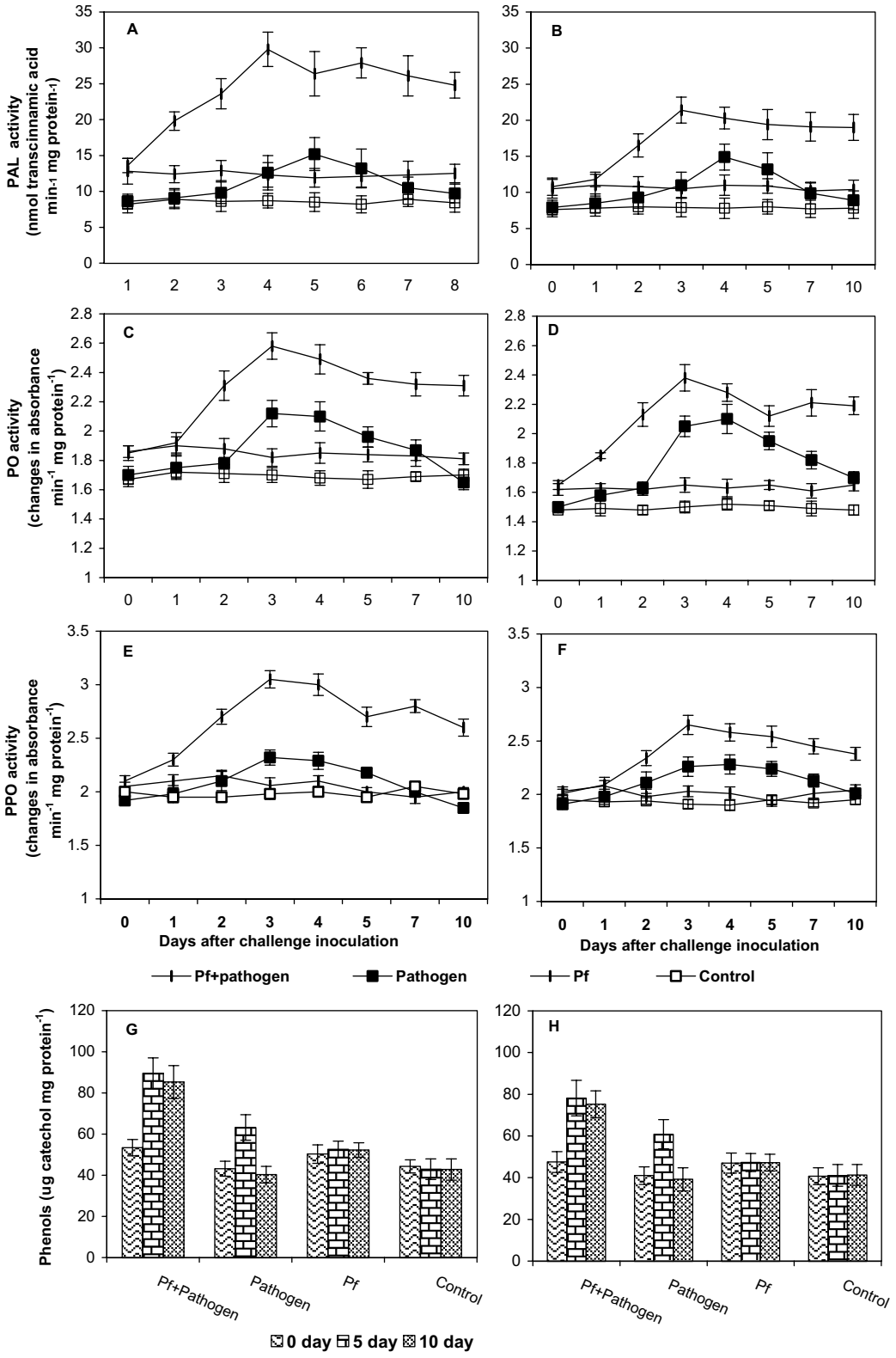
Treatments	Trial-I				Trial-II			
	Tomato		Hot pepper		Tomato		Hot pepper	
	DI* (%)	Plant growth (cm)	DI* (%)	Plant growth (cm)	DI* (%)	Plant growth (cm)	DI* (%)	Plant growth (cm)
Pf formulation	34.3[b]	16.2[b]	39.0[b]	14.8[b]	35.5[b]	15.8[c]	34.6[a]	14.1[b]
Talc	65.3[c]	11.7[a]	62.3[c]	8.7[a]	68.0[c]	11.2[a]	43.7[b]	9.5[a]
Ridomil	27.5[a]	15.6[b]	30.5[a]	14.2[b]	28.5[a]	13.0[b]	32.7[a]	13.3[b]
Control	63.0[c]	11.9[a]	62.0[c]	9.7[a]	67.8[c]	11.0[a]	45.7[c]	9.3[a]

DI – Disease incidence (%).
*The Percentage data were arcsine transformed prior to the analysis.
Means followed by a common letter within a column are not significantly different ($P = 0.05$) by DMRT.
Trial I was conducted at Agricultural Research Station, Aliyar Nagar. Trial II was conducted at Tamil Nadu Agricultural University, Coimbatore.

were observed in *Pseudomonas*-pretreated tomato and pepper plants challenge inoculated with the pathogen and remained at higher levels throughout the experimental period. The activity reached maximum levels on the third day after challenge inoculation.

The maximum phenolic content was observed in *P. fluorescens*-pretreated plants challenge inoculated with the pathogen and the higher amounts of phenolics were noticed even on the 10th day after the pathogen challenge. In plants inoculated with the pathogen alone the phenolic content declined to the initial level on the 10th day after inoculation. Plants treated with *P. fluorescens* alone also had increased content of phenolics compared to untreated plants (Figure 1G,H).

In tomato plants, the expression of PO isoforms, PO1, PO2, PO3, PO4 and PO5 was greater in *P. fluorescens* pretreated plants challenged with the pathogen and also plants inoculated with the pathogen alone than in untreated plants or plants treated with *P. fluorescens* alone (Figure 2A). Similarly, PPO isoforms PPO2 and PPO3 were prominent in tomato plants treated with *P. fluorescens* and challenge inoculated with the pathogen (Figure 2B). In pepper plants, PO isoforms PO1 and PO2 were noticed in all induction treatments except untreated plants in which the expression of PO1 and PO2 was very weak (Figure 2C). Similarly, PPO1 isoform was expressed at higher levels in all the induction treatments. In untreated plants,


A
B
C
D
E
F
G
H
PAL activity (nmol transcinnamic acid min-1 mg protein-1)
PO activity (changes in absorbance min-1 mg protein-1)
PPO activity (changes in absorbance min-1 mg protein-1)
Days after challenge inoculation
Pf+pathogen
Pathogen
Pf
Control
Phenols (ug catechol mg protein-1)
Pf+Pathogen
Pathogen
Pf
Control
0 day 5 day 10 day

this constitutive PPO1 was not expressed prominently (Figure 2D).

Discussion

Many of the fluorescent pseudomonads, predominantly *P. fluorescens*, were isolated from suppressive soil for the management of soil-borne diseases (Mills et al., 1989; Ongena et al., 1999; Stephens et al., 1993; Weller and Cook, 1986). The present study indicates that the majority of the strains isolated from rhizhosphere of different crops belonged to *P. fluorescens*. The majority of the fluorescent pseudomonads were found to be *P. fluorescens* biovar V in rhizosphere soil of wheat in Australia (Sands and Rovira, 1971). The present study also indicates that majority of the fluorescent pseudomonads isolated from different crops cultivated in India belonged to *P. fluorescens*. Rosales et al. (1993) reported that some of the isolates within each genus of bacteria isolated from rice rhizosphere could be differentiated phenotypically and through protein profile studies. Variation was observed in each dominant group of fluorescent pseudomonads. Such variation could be influenced by soil type, cropping patterns, soil moisture and other environmental factors.

After isolating and identifying the fluorescent pseudomonads, selecting an effective isolate is the first and foremost important step in biological control. *P. fluorescens* isolate Pf1 showed the maximum inhibitory effect on mycelial growth. In addition, *P. fluorescens* isolate Pf1 increased the vigour index. As certain strains improve plant growth in addition to biological control, these strains are collectively called plant growth-promoting rhizobacteria designated as PGPR (Kloepper et al., 1980). The use of fluorescent pseudomonads for increasing the yield and crop protection is an attractive approach in the modern system of sustainable agriculture. Fluorescent pseudomonads having antagonistic activity and increasing the plant growth would certainly be promising in evaluating suitable isolates in biological control (Viswanathan and Samiyappan, 1999).

The present study also indicates that application of talc-based formulation of *P. fluorescens* isolate Pf1 increases plant growth in the both field experiments and reduced disease incidence. The increase in plant growth might be associated with secretion of auxins, gibberellins and cytokinins (Dubeikovsky et al., 1993; Ramamoorthy and Samiyappan, 2001) and suppression of deleterious microorganisms in the rhizosphere (Gamliel and Katan, 1993).

In addition to direct antagonism and plant growth promotion, *P. fluorescens* Pf1 increased the activities of various defense-related enzymes and chemicals in response to infection by the pathogen. It is well known that all plants are endowed with defense genes which are quiescent in nature and appropriate stimuli or signals are needed to activate them. It has been reported that application of *P. fluorescens* triggers/activates plants' latent defense mechanisms in response to infection by pathogen. Inducing a plant's own defense mechanism by prior application of a biological agent is a novel strategy in plant disease management. In the present study, it has been observed that seeds treated with *P. fluorescens* isolate Pf1 increased the activities of various defense-related enzymes which lead to the synthesis of defense chemicals in the plants.

PAL plays an important role in the biosynthesis of phenolic phytoalexins (Daayf et al., 1997). When groundnut plants were sprayed with *P. fluorescens,* increase in activity of PAL was observed (Meena et al., 2000). Cucumber plants treated with *Pseudomonas corrugata* had initially higher levels of PAL and levels were lower after challenging the plant with *Pythium aphanidermatum* (Chen et al., 2000). Increase in mRNAs encoding PAL and chalcone synthase were recorded in the early stages of the interaction between bean roots and various rhizobacteria (Zdor and Anderson, 1992). De Meyer et al. (1999) reported that

Figure 1. (A) Influence of seed treatment with *P. fluorecens* Pf1 on PAL activity in tomato challenged with or without *Pythium aphanidermatum*. (B) Influence of seed treatment with *P. fluorecens* Pf1 on PAL activity in pepper challenged with or without *Pythium aphanidermatum*. (C) Changes in PO activity by seed treatment with *P. fluorecens* Pf1 in tomato challenged with or without *Pythium aphanidermatum*. (D) Changes in PO activity by seed treatment with *P. fluorecens* Pf1 in pepper challenged with or without *Pythium aphanidermatum*. (E) Changes in PPO activity by seed treatment with *P. fluorecens* Pf1 in tomato challenged with or without *Pythium aphanidermatum*. (F) Changes in PPO activity by seed treatment with *P. fluorecens* Pf1 in pepper challenged with or without *Pythium aphanidermatum*. (G) Accumulation of phenolics by seed treatment with *P. fluorecens* Pf1 in tomato challenged with or without *Pythium aphanidermatum*. (H) Accumulation of phenolics by seed treatment with *P. fluorecens* Pf1 in pepper challenged with or without *Pythium aphanidermatum*. Vertical bars indicate standard deviations of three replications.

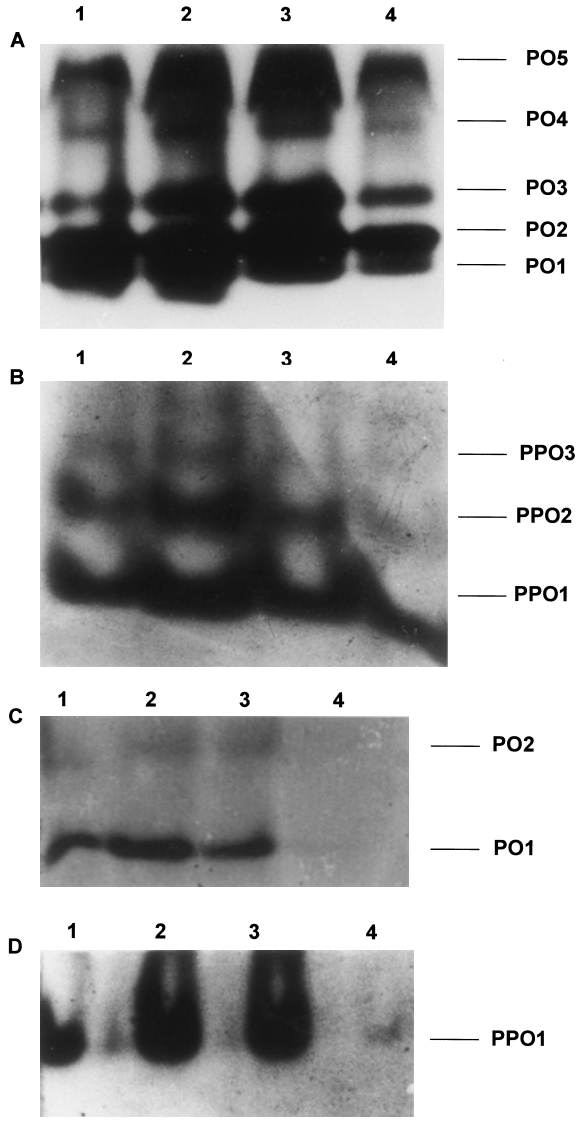


Figure 2. (A) PO isoforms profile in tomato induced by *P. fluorescens* Pf1 challenged with or without *Pythium aphanidermatum*. (B) PPO isoforms profile in tomato induced by *P. fluorescens* Pf1 challenged with or without *Pythium aphanidermatum*. (A) PO isoforms profile in pepper induced by *P. fluorescens* Pf1 challenged with or without *Pythium aphanidermatum*. (B) PPO isoforms profile in pepper induced by *P. fluorescens* Pf1 challenged with or without *Pythium aphanidermatum*. Lane 1: Seed treatment with *P. fluorescens* Pf1 alone; Lane 2: *P. fluorescens* Pf1-treated plants challenged with *Pythium aphanidermatum*; Lane 3: Plants inoculated with *Pythium aphanidermatum*; Lane 4: Untreated plants.

rhizosphere colonization by *P. aeruginosa* 7NSK2 activated PAL in bean roots and increased the salicylic acid levels in leaves. In our previous study, increased activity of PAL was observed in *P. fluorescens*-treated tomato and pepper plants (during flowering stage) in response to infection by *F. oxysporum* f. sp. *lycopersici* and *C. capsici* (Ramamoorthy et al., 2001a; Ramamoorthy and Samiyappan, 2001). In the present study, increased activity of PAL was recorded in *P. fluorescens* isolate Pf1-treated tomato and pepper seedlings challenged with the pathogen, reached maximum on the third day after challenge inoculation and was maintained at higher levels throughout the experimental period. In plants inoculated with the pathogen alone the activity declined greatly on the fourth day after challenge inoculation. Invasion of root tissues by the pathogen might have resulted in decreased activity of PAL whereas earlier and increased activity of PAL due to *P. fluorescens* isolate Pf1 treatment might have prevented fungal invasion and thus the activity was maintained at the higher levels.

PO and PPO catalyze the last step in the biosynthesis of lignin and other oxidative phenols. In the present study, seed treatment with *P. fluorescens* induced the activities of PO and PPO. In bean, rhizosphere colonization of various bacteria induced PO activity (Zdor and Anderson, 1992). The higher PO activity was noticed in cucumber roots treated with *P. corrugata* challenged with *Pythium aphanidermatum*. (Chen et al., 2000). The native-PAGE study indicates that PO and PPO isoforms were prominently expressed in *P. fluorescens* isolate Pf1-treated roots in response to infection by the pathogen. PPO transcript levels increased in young leaves of tomato when mature leaflets were injured (Thipyapong and Steffens, 1997). Our earlier studies also indicated that induction of a new and unique PPO1 isoform and higher level expression of PPO2 were noticed in *P. fluorescens*-treated tomato plants in response to infection by *F. oxysporum* f. sp. *lycopersici* (Ramamoorthy et al., 2001a).

Phenolic compounds may be fungitoxic in nature and may increase the mechanical strength of the host cell wall. In the present study, seed treatment with *P. fluorescens* isolate Pf1 resulted in increased accumulation of phenolic substances in response to infection by the pathogen. M'Piga et al. (1997) reported that *P. fluorescens* isolate 63–28 induced the accumulation of phenolics in tomato root tissues. The hyphae of the pathogen surrounded by phenolic substances exhibited considerable morphological

changes including cytoplasmic disorganization and loss of protoplasmic content. Accumulation of phenolics by prior application of *P. fluorescens* in pea has been reported against *P. ultimum* and *F. oxysporum* f. sp. *pisi* (Benhamou et al., 1996). Benhamou et al. (2000) reported that an endophytic bacterium, *Serratia plymuthica* induced the accumulation of phenolics in cucumber roots following infection by *P. ultimum. P. fluorescens* Pf1 isolate also induced the accumulation of phenolic substances and PR-proteins in response to infection by *F. oxysporum* f. sp. *lycopersici* in tomato (Ramamoorthy et al., 2001a) and *C. capsici* in pepper (Ramamoorthy and Samiyappan, 2001). Since several defense-related genes encoding proteins are synthesized in ISR by fluorescent pseudomonads, it has been hypothesized that induced resistance by *P. fluorescens* isolate Pf1 is related to multigenic/polygenic (horizontal) resistance in plants which is effective against multiple pathogens/races of pathogens. Tuzun (2001) described that constitutive accumulation of defense-related gene products was an integral part of both multigenic resistance and ISR. In cucumber, rhizobacteria induced resistance against cucumber mosaic virus (CMV) and tomato mottle virus (ToMoV) (Zehnder et al., 2001). Induced resistance by fluorescent pseudomonads has broad spectrum activity against several fungal, bacterial and viral diseases (Hoffland et al., 1996;1997; Maurhofer et al., 1994; Wei et al., 1991; 1996; Zehnder et al., 2001).

In conclusion, fluorescent pseudomonads isolated from soils from southern parts of India mostly belonged to *P. fluorescens* group. Application of talc-based formulation of *P. fluorescens* consistently reduced the incidence of damping-off in tomato and pepper. Prior treatment of tomato and pepper with *P. fluorescens* triggered the plant-mediated defense mechanism in response to infection by *Pythium aphanidermatum*. Earlier studies revealed that *P. fluorescens* isolate Pf1 is effective against Fusarium wilt of tomato and fruit rot of pepper. Thus, it has been found that *P. fluorescens* isolate Pf1 shows broad-spectrum protection against different pathogens attacking the same crops.

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References

Abdul Baki AA and Anderson JD (1973) Vigour determination in soybean seed by multiple criteria. Crop Science 13: 630–633

Barrett EL, Solanes RE, Tang JS and Palleroni NJ (1986) *Pseudomonas fluorescens* biovar V its resolution into distinct component groups and relationship of these to other *P. fluorescens* biovars to *P. putida* and to psychrophilic pseudomonads associated with food spoilage. Journal of General Microbiology 132: 2709–2721

Benhamou N, Bélanger RR and Paulitz TC (1996) Induction of differential host responses by *Pseudomonas fluorescens* in Ri T-DNA-transformed pea roots after challenge with *Fusarium oxysporum* f. sp. *pisi* and *Pythium ultimum*. Phytopathology 86: 114–118

Benhamou N, Gagné S, Quere DL and Dehbi L (2000) Bacterial-mediated induced resistance in cucumber: Beneficial effect of the endophytic bacterium *Serratia plymuthica* on the protection against infection by *Pythium ultimum.* Phytopathology 90: 45–56

Bradford MM (1976) A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248–254

Champion AB, Barrett EL, Palleroni NJ, Soderberg KL, Kunisawa R, Contopoulou R, Wilson AC and Doudoroff M (1980) Evolution in *Pseudomonas putida,* a biological control agent in soil. Phytopathology 70: 195–200

Chen C, Belanger RR, Benhamou N and Paulitz TC (1998) Induced systemic resistance (ISR) impairs pre-and post-infection development of *Pythium aphanidermatum* on cucumber roots. European Journal of Plant Pathology 104: 877–886.

Chen C, Belanger RR, Benhamou N and Paulitz TC (2000) Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiological and Molecular Plant Pathology 56: 13–23

Daayf F, Bel-Rhlid R and Bélanger RR (1997) Methyl ester of *p*-coumaric acid: A phytoalexin-like compound from long English cucumber leaves. Journal of Chemical Ecology 23: 1517–1526

De Meyer G, Capieau K, Audenaert K, Buchala A, Metraux JP and Höfte M (1999) Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. Molecular Plant-Microbe Interactions 12: 450–458

Dickerson DP, Pascholati SF, Hagerman AE, Butler LG and Nicholson RL (1984) Phenylalanine ammonia-lyase and hydroxy cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum.* Physiological Plant Pathology 25: 111–123

Dubeikovsky AN, Mordukhova EA, Kochethov VV, Polikarpova FY and Boronin AM (1993) Growth promotion of black currant soft wood cuttings by recombinant strain *Pseudomonas fluorescens* BSP53a synthesizing an increased amount of indole-3-acetic acid. Soil Biology and Biochemistry 25: 1277–1281

Elad Y and Chet I (1987) Possible role of competition for nutrient in biocontrol of Pythium damping-off by bacteria. Phytopathology 77: 190–195

Frindlender M, Inbar J and Chet I (1993) Biological control of soil-borne plant pathogens by a β-1,3-glucanase producing *Pseudomonas cepacia*. Soil Biology Biochemistry 25: 1211–1221

Gamliel A and Katan J (1993) Suppression of major and minor pathogens by fluorescent pseudomonads in solarized soil and non-solarized soil. Phytopathology 83: 68–75

Gomez KA and Gomez AA (1984) Statistical procedures for agricultural research. John Wiley and Sons, New York, USA

Hammerschmidt R, Nuckles EM and Kuć J (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiological Plant Pathology 20: 73–82

Hildebrand DC, Schroth MN and Sands DC (1992) *Pseudomonas.* In: Schaad NW (ed) Laboratory Guide for Identification of Plant Pathogenic Bacteria, 2nd edition, American Phytopathological Society. St. Paul, MN,

Hoffland E, Hakulinem J and Van Pelt JA (1996) Comparison of systemic resistance induced by avirulent and nonpathogenic *Pseudomonas* species. Phytopathology 86: 757–762

Hoffland W, Bakker PAHM and Van Loon LC (1997) Multiple disease protection by rhizobacteria that induce systemic resistance-reply. Phytopathology 87: 138

Howie WJ and Suslow TV (1991) Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. Molecular Plant-Microbe Interactions 4: 393–399

ISTA (1993) Proceedings of International Seed Test Association, International rules for seed testing. Seed Science and Technology 21: 1–152

Jayaraman KS, Ramanuja MN, Vijayaraghavan PK and Vaidyanathan CS (1987) Oxidative enzyme in pearl millet. Food Chemistry 24: 203

Katan J (1987) Soil solarization. In: Chet K (ed) Innovative Approaches to Plant Disease Control (pp 77–105) John Wiley, Chichester

King EO, Ward MK and Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescein. Journal of Laboratory Clinical Medicine 44: 301–307

Kloepper JW, Schroth MN and Miller TD (1980) Effects of rhizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield. Phytopathology 70: 1078–1082

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

Loper JE (1988) Role of fluorescent siderophore production in biological control of *Pythium ultimum* by a *Pseudomonas fluorescens* strain. Phytopathology 78: 166–172

Maurhofer M, Hase C, Meuwly P, Metraux JP and Défago G (1994) Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHAO: influence of the gacA gene and of pyoverdine production. Phytopathology 84: 139–146

Mayer AM, Harel E and Shaul RB (1965) Assay of catechol oxidase a critical comparison of methods. Phytochemistry 5: 783–789

Meena B, Radhajeyalakshmi R, Marimuthu T, Vidhyasekaran P, Doraisamy S and Velazhahan R (2000) Induction of pathogenesis-related proteins, phenolics and phenylalanine ammonia lyase in groundnut by *Pseudomonas fluorescens.* Journal of Plant Disease and Protection 107: 514–527

Mills JL, Longman D and Murray DD (1989) Commercial prospects for biological and biotechnological weed, plant disease and pest control. In: Proceedings of the 1989 British Crop Protection Conference-Weeds, Vol 3 (pp 1005–1012) British Crop Protection Council, Thornton Heath, Surrey.

M'Piga P, Bélanger RR, Paulitz TC and Benhamou N (1997) Increased resistance to *Fusarium oxysporum* f. sp. *radicis lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63–28. Physiological Molecular Plant Pathology 50: 301–320

Nadlony L and Sequira I (1980) Increase in peroxidase activities are not directly involved in induced resistance in tobacco. Physiological Plant Pathology 16: 1–8

Nelson EB (1987) Rapid germination of sporangia of *Pythium* species in response to volatiles from germination seeds. Phytopathology 77: 1108–1112

Ongena M, Daay F, Jacques P, Thonart P, Benhamou N, Paulitz TC, Cornelis P, Koedam NM and Bélanger RR (1999) Protection of cucumber against Pythium root rot by fluorescent pseudomonads: Predominant role of induced resistance over siderophores and antibiosis. Plant Pathology 48: 66–76

Osburn RM, Schroth MN, Hancock JG and Hendson M (1989) Dynamics of sugarbeet colonization by *Pythium ultimum* and *Pseudomonas* species: Effects on seed rot and damping-off. Phytopathology 79: 709–716

Pieterse CMJ, Van Pelt, JA, Van Wees SCM, Ton J, Leon-Kloosteriziel KM, Keurentjes JJB, Verhagen BWM, Knoester M, Van der Sluis I, Bakker PAHM and Van Loon LC (2001) Rhizobacteria-mediated induced systemic resistance: triggering, signalling and expression. European Journal of Plant Pathology 107: 51–61

Ramamoorthy V and Samiyappan R (2001) Induction of defense-related genes in *Pseudomonas fluorescens* treated chilli plants in response to infection by *Colletotrichum capsici*. Journal of Mycology and Plant Pathology 31: 146–155

Ramamoorthy V, Raguchander T and Samiyappan R (2001a) Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f. sp. *lycopersici*. Plant and Soil (in press)

Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V and Samiyappan R (2001b) Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pest and diseases. Crop Protection 20: 1–11

Rosales AM, Vantomme R, Swings J, De Ley J and Mew TW (1993) Identification of some bacteria from paddy antagonistic to several fungal pathogens. Journal of Phytopathology 138: 189–208

Sands DC and Rovira AD (1971) *Pseudomonas fluorescens* biotype G, the dominant fluorescent pseudomonads in south Australian soil and wheat rhizospheres. Journal of Applied Bacteriology 34: 161–175

Stephens PM, Crowley JJ and O'Connell C (1993) Selection of pseudomonad strains inhibiting *Pythium ultimum* on sugarbeet seeds in soil. Soil Biology and Biochemistry 25: 1283–1288

Thipyapong P and Steffens JC (1997) Tomato polyphenol oxidase. Differential response of the polyphenol oxidase F promotor to injuries and wound signals. Plant Physiology 115: 409–418

Thompson DC (1996) Evaluation of bacterial antagonist for reduction of summer patch symptoms in Kentucky blue grass. Plant Disease 80: 856–862

Tuzun S (2001) The relationship between pathogen-induced systemic resistance (ISR) and multigenic (horizontal) resistance in plants. European Journal of Plant Pathology 107: 85–93

Vidhyasekaran P and Muthamilan M (1995) Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. Plant Disease 9: 782–786

Vidhyasekaran P, Sethuraman K, Rajappan K and Vasumathi K (1997) Powder formulations of *Pseudomonas fluorescens* to control pigeon wilt. Biological control 8: 166–171

Viswanathan R and Samiyappan R (1999) Induction of systemic resistance by plant growth promoting rhizobacteria against red rot disease in sugarcane. Sugar Tech 1: 67–76

Wei G, Kloepper JW and Tuzun S (1991) Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth promoting rhizobacteria. Phytopathology 81: 1508–1512

Wei L, Kloepper JW and Tuzun S (1996) Induced systemic resistance to cucumber diseases and increased plant growth by plant growth promoting rhizobacteria under field conditions. Phytopathology 86: 221–224

Weller MD and Cook RJ (1986) Increased growth of wheat by seed treatment with pseudomonads and implications of *Pythium* control. Canadian Journal of Plant Pathology 8: 328–334

Whipps JM and Lumsden DR (1991) Biological control of *Pythium* species. Biocontrol Science and Technology 1: 75–90

Zdor RE and Anderson AJ (1992) Influence of root colonizing bacteria on the defense responses in bean. Plant and Soil 140: 99–107

Zehnder GW, Murphy JF, Sikora EJ and Kloepper JW (2001) Application of rhizobacteria for induced resistance. European Journal of Plant Pathology 107: 39–50

Zhou T and Paulitz JC (1994) Induced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp. on cucumber. Journal of Phytopathology 142: 51–63

Zieslin N and Ben-Zaken R (1993) Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiology and Biochemistry 31: 333–339.