Pigment diverse mutants of *Pseudomonas* sp.: inhibition of fungal growth and stimulation of growth of *Cicer arietinum*

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Abstract

A *Pseudomonas* strain MRS16 inhibited growth of different pathogenic fungi (*Aspergillus* sp., *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*) *in vitro*. Larger inhibition zones were obtained on nutrient agar and King's B media compared to potato dextrose agar and pigment production media. Mutants altered in production of fluorescent pigment were derived by nitrosoguanidine mutagenesis. The pigment overproducer mutant MRS16M-1 was more inhibitory whereas nonproducer mutant MRS16M-5 was less inhibitory than parent strain on nutrient agar medium. Addition of iron (100 µM $FeCl_3$) in the medium decreased inhibition of fungal growth, suggesting the involvement of siderophores and other antifungal secondary metabolites. Seed bacterization of two cultivars of chickpea (*Cicer arietinum* cvs. H8618 and C235) differing in susceptibility to wilt caused initial root and shoot stunting at 5 d of growth followed by proliferation of secondary root growth at 10 d. Coinoculation of chickpea with *Pseudomonas* strain MRS16 or mutants and *Rhizobium* sp. *Cicer* strain Ca181 enhanced nodulation, nitrogen fixation and plant dry mass as compared to single inoculation with *Rhizobium* strain under sterile conditions.

Additional key words: antifungal activity, chickpea, fluorescent pigment, nodulation, seed bacterization.

Introduction

The rhizosphere around growing plant roots is colonized by a variety of microorganisms, which interact with each other either in associative, symbiotic, neutralist or antagonistic relationships. Microbial interactions with plants have been found to influence plant growth in ways that are beneficial, neutral or detrimental (Astrom *et al.* 1993, Bolton *et al.* 1990). Fluorescent pseudomonads are abundant in the rhizosphere of various crops and they suppress various plant diseases caused by soilborne pathogenic microorganisms. The disease suppressive effect or biological control of pathogens by the rhizosphere microorganisms may involve mechanisms such as production of antibiotics (Gurusiddaiah *et al.* 1986, Hebbar *et al.* 1992), siderophores (Leong 1986, Loper and Buyer 1991), hydrolytic enzymes (Fridlender *et al.* 1993), other secondary metabolites (Voisard *et al.* 1989) and competition for nutrients (Elad and Baker

1985) or for infection sites on root surfaces (Mandeel and Baker 1991). A few studies have also reported the accumulation of phytoalexins at the site of infection leading to induction of systemic resistance for the biological control of soilborne pathogens (Goel *et al.* 2000).

Seed inoculation with beneficial pseudomonads have been found to stimulate plant growth and yield of different crops and these are referred to as plant growth-promoting rhizobacteria (PGPR) or plant health-promoting rhizobacteria (Kloepper *et al.* 1980). Application of PGPR has been shown to increase the rate of seedling emergence (De Freitas and Germida 1990, Kloepper *et al.* 1986) and plant growth (Sakthivel *et al.* 1986, Zhang *et al.* 1997). The mechanisms of growth promotion by PGPR include increased mobilization of insoluble nutrients and subsequently enhanced plant

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uptake (Lifshitz *et al.* 1987), increased iron availability in the rhizosphere by producing siderophores (Kloepper *et al.* 1980), production of plant growth regulators (Dubeikovsky *et al.* 1993) and increased photosynthetic rate (Zhang *et al.* 1997). Coinoculation of *Pseudomonas* spp. with *Rhizobium* spp. has been reported to enhance nodulation and nitrogen fixation, plant biomass and grain yield in alfalfa (Knight and Langston-Unkefer 1988), pea (Bolton *et al.* 1990), soybean (Dashti *et al.* 1997) and green gram (Sindhu *et al.* 1999).

Materials and methods

Bacterial cultures and host seeds: *Pseudomonas* sp. strain MRS16 (Sindhu *et al.* 1999) was maintained on Luria Bertani (LB) medium (Sambrook *et al.* 1989). *Rhizobium* sp. strain Ca181 was maintained on yeast extract mannitol agar (YEMA) medium (Somasegaran and Hoben 1985). Pure cultures of *Aspergillus* sp., *Fusarium oxysporum* f. sp. *ciceri*, *Pythium aphanidermatum* and *Rhizoctonia solani* were obtained from the Department of Plant Pathology, CCS Haryana Agricultural University, Hisar and maintained on potato dextrose agar (PDA) medium. Seeds of chickpea cvs. H8618 (tolerant to blight and wilt) and C235 (tolerant to blight and susceptible to wilt) were obtained from Pulses Section, Department of Plant Breeding, CCS Haryana Agricultural University, Hisar.

Derivation of mutants: *Pseudomonas* sp. strain MRS16 was grown in 50-cm^3 flasks containing 20 cm^3 LB broth for 18 h on a rotary shaker at 28 ± 1 °C. Cells were centrifuged at 12 000 *g* for 10 min and washed twice with 0.1 M citrate buffer (pH 5.5). N-methyl-N′-nitro-N-nitroso-guanidine (NTG) was added to a final concentration of 50 µg cm^{-3} (dissolved in acetone) to cells resuspended in citrate buffer. After incubation for 1 h at 28 ± 1 °C, cells were spun down, washed with sterilized H_2O and resuspended in LB broth. After incubation of 2 h at 28 ± 1 °C, serial dilutions were made and 0.1 cm^3 aliquots were spread over King's B (KB) medium plates. Colonies were selected on the basis of differences in fluorescent pigment production.

Antifungal activity test: Antifungal activity of *Pseudomonas* sp. MRS16 was tested against *Aspergillus* sp., *F. oxysporum*, *P. aphanidermatum* and *R. solani* in four different growth media viz. KB, nutrient agar (NA), potato dextrose agar (PDA) and pigment production medium (PPM) - 20 g of Difco peptone, 20 g of glycerol, 5 g of NaCl, 1 g of KNO_3, 15 g of agar, 7.2 pH, 1 dm^3 of distilled water (Rosales *et al.* 1995). Spore suspensions of different fungi were prepared in 0.85 % sterilised saline from 5-d-old fungus on PDA plates and 0.1 cm^3 of this spore suspension was spread over the media plates.

Chickpea (*Cicer arietinum* L.) is a major legume crop grown in arid and semi-arid zones of Northern and Central India. A variety of plant pathogenic fungi has been reported to cause wilt and blight diseases on this legume crop. In this study, mutants of *Pseudomonas* sp. strain MRS16 altered in production of fluorescent pigment were derived and the effect of mutation was examined on growth inhibition of different pathogenic fungi in culture. The effect of seed bacterization was also studied on chickpea seedling growth and nodulation.

Pseudomonas sp. strain MRS16 or its mutants were spot inoculated onto various media plates and zones of inhibition of fungal growth around the spotted growth of *Pseudomonas* were recorded after incubation for 4 d at 28 ± 1 °C. The antifungal activity of the mutants was also tested in NA medium plates, supplemented with 100 µM $FeCl_3$.

Siderophore production: The universal chemical assay method described by Schwyn and Neilands (1987) was used for qualitative detection of siderophore production by *Pseudomonas* sp. and its mutants. A loopful growth of 5-d-old cultures of *Pseudomonas* sp. from LB slopes was spot inoculated on to medium plates containing chrome azurol S dye (CAS). The formation of orange zones around the colonies was recorded after incubation at 28 ± 1 °C for 3 - 4 d.

Seed treatment: Chickpea healthy seeds were surface sterilised with acid-alcohol (H_2SO_4:ethanol, 7:3, v/v) for 5 min and thoroughly washed with sterilized H_2O six times (Dadarwal *et al.* 1985). The surface sterilized seeds were inoculated with LB broth culture of *Pseudomonas* strain or its mutants for 30 min. The inoculated seeds were germinated in 0.8 % water agar at 28 ± 1 °C under controlled conditions. Uninoculated seeds treated with LB broth were sown in plates as control. Effect of inoculation on tap root and shoot length was measured at 5 and 10 d of growth.

Coinoculation of *Pseudomonas* cultures with *Rhizobium* sp. *Cicer* strain Ca181 on chickpea: Surface sterilized seeds of chickpea cv. C235 were inoculated with YEMA broth culture of *Rhizobium* sp. alone or coinoculated with *Pseudomonas* strain or mutants by mixing broth of the inoculants in 1:1 ratio (v/v). The viable counts in broth were 5.4 ×10^8 cells cm^{-3} and 10 g seeds were inoculated with 1 cm^3 of the broth. The inoculated seeds were sown in sterilized chillum jar assemblies containing washed river sand in upper jar and Sloger's nitrogen free salt solution (Sloger 1969) in the lower assembly. Uninoculated seeds were sown as

control. Three healthy seedlings were kept in each chillum jar and three replicates were used for each treatment. The jars were kept in a net house under natural light conditions (daily maximum irradiance of about 950 $\mu mol\ m^{-2}\ s^{-1}$, day/night temperature of about 22/10 °C and relative humidity of about 65 %) and watered with quarter strength Sloger's medium periodically. Observations for nodulation, nitrogenase activity and symbiotic effectiveness were taken at 60, 80 and 100 d of plant growth.

Nitrogenase activity was determined by measuring acetylene reduction activity (ARA) in whole root system with intact nodules in 250-cm^{-3} flasks. One tenth volume of air was removed with a sterile syringe and an equal amount of freshly prepared acetylene was injected into these flasks. After incubation for 1 h at 28 ± 1 °C, gas samples were assayed for acetylene reduction using a gas liquid chromatograph (*Nucon Aimil 5500*, New Delhi, India) fitted with a dual flame ionization detector and *Porapak N* columns (2 mm × 2 m). The oven temperature was kept at 110 °C. Nitrogen as carrier gas, and H_2 as fuel gas, were used at 30 - 35 and 20 - 25 $cm^3\ min^{-1}$ respectively. A 0.5 cm^3 gas sample from each flask was injected to determine the amount of ethylene produced as described by Sindhu and Dadarwal (1986). After the nitrogenase assay, nodules were detached and the fresh masses of nodules determined. The shoot portions were dried in an oven at 90 °C for 24 h and the dry masses measured.

Results

Isolation of mutants varying in pigment production: Twenty seven mutants of *Pseudomonas* sp. strain MRS16 were obtained by treatment with NTG and screened for fluorescent pigment production in KB medium. Two mutants, one pigment overproducer, MRS16M-1 and one non-producer, MRS16M-5, were selected for further studies.

Antifungal activity *in vitro*: *Pseudomonas* sp. MRS16 showed varying diameters of inhibition zones (devoid of mycelial growth) of the four pathogenic fungi, *Aspergillus* sp., *F. oxysporum*, *P. aphanidermatum* and *R. solani*. However, the levels of growth inhibition varied in all the four plating media tested (Table 1). Larger inhibition zones (from 8 to 25 mm) were observed on NA and KB media followed by PPM and PDA media.

The antifungal activity of the mutants along with the parent strain was studied on NA medium plates. Mutant MRS16M-1 showed wider zone of fungal growth inhibition whereas, mutant MRS16M-5 showed reduced inhibition zones on NA medium against all the four fungi tested. Growth inhibition zones of different fungi by *Pseudomonas* sp. MRS16 and the mutant MRS16M-1 decreased in iron amended medium (Table 2), suggesting that fungal inhibition by the parent strain and its mutant could be due to production of siderophore as well as some other antifungal compounds. The mutant MRS16M-5 lacking pigment production showed comparatively less effect of iron addition in the NA medium on fungal growth inhibition indicating that this

Table 1. Antagonistic interactions of *Pseudomonas* strain MRS16 with various pathogenic fungi. Data are diameters of inhibition zones [mm] across the spotted growth of *Pseudomonas* strain on different growth media. Means of three replications ± SE.

Fungal pathogens	Growth media			
	KB	NA	PDA	PPM
Aspergillus sp.	10.2 ± 0.21	11.0 ± 0.26	5.1 ± 0.34	1.3 ± 0.24
F. oxysporum	8.3 ± 0.16	8.2 ± 0.31	4.6 ± 0.36	6.1 ± 0.26
P. aphanidermatum	21.5 ± 0.94	25.2 ± 0.62	12.4 ± 0.29	24.0 ± 0.39
R. solani	17.3 ± 0.37	21.0 ± 0.71	15.3 ± 0.41	20.1 ± 0.47

Table 2. *In vitro* antifungal activity of *Pseudomonas* strain/mutants in NA medium with and without iron. Data are diameters of inhibition zones [mm] across the spotted growth of *Pseudomonas* strain. Means of three replications ± SE.

Fungal pathogens	MRS16		MRS16M-1		MRS16-M5	
	NA	NA+Fe^{3+}	NA	NA+Fe^{3+}	NA	NA+Fe^{3+}
Aspergillus sp.	11.0 ± 0.26	6.3 ± 0.37	10.2 ± 0.57	2.4 ± 0.31	7.0 ± 0.51	6.1 ± 0.54
F. oxysporum	8.2 ± 0.31	2.1 ± 0.26	16.1 ± 0.49	4.6 ± 0.54	4.6 ± 0.43	2.2 ± 0.31
P. aphanidermatum	25.2 ± 0.62	10.6 ± 0.46	38.3 ± 0.54	12.5 ± 0.91	20.1 ± 1.21	10.3 ± 0.81
R. solani	21.0 ± 0.71	15.5 ± 0.42	36.0 ± 0.71	18.1 ± 0.78	12.1 ± 0.92	10.0 ± 0.76

mutant probably lacks siderophore production and other antifungal metabolites responsible for growth inhibition of fungi.

Siderophore production: *Pseudomonas* sp. strain MRS16 and its mutant MRS16M-1 showed siderophore production in CAS plates by forming the orange halo zones around the colonies. However, the size of halo zones varied. The mutant MRS16M-5 showed no halo zone formation suggesting that the mutation responsible for eliminating the synthesis of pigment has probably decreased the production or export of the siderophore.

Seed bacterization effect: Effect of seed bacterization with *Pseudomonas* sp. strain MRS16 and its mutants MRS16M-1 and MRS16M-5 on seedling growth was studied in two cultivars of chickpea (H8618 and C235) grown in water agar. *Pseudomonas* sp. strain MRS16 and both of its mutants showed root stunting effect at 5 d growth in both the cultivars followed by development of numerous secondary roots and rootlets at 10 d (Fig 1). Cultivar H8618 responded more significantly to

inoculations compared to the other cultivar C235. Although the non-pigment producer mutant MRS16M-5 also enhanced growth of secondary roots in comparison to uninoculated seedlings but enhancement of root growth was comparatively less than the parent strain MRS16.
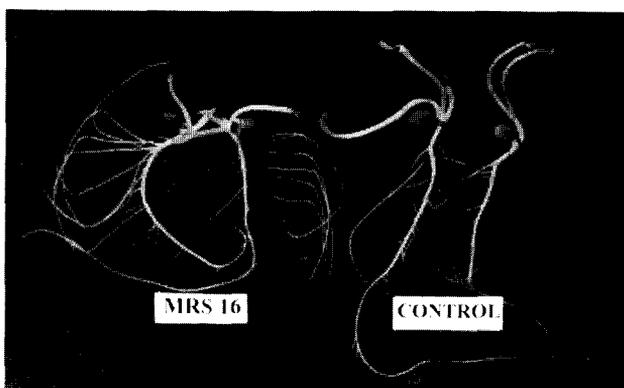

Fig 1. Effect of *Pseudomonas* strain MRS16 on seedling growth of chickpea cv. H8618.

Table 3. Effect of *Pseudomonas* strain/mutants on seedling growth [mm] of chickpea cvs. C235 and H8618. (Means ± S.E., *n* = 12).

Treatment	cv. C235 root length 5 d	10 d	shoot length 5 d	10 d	cv. H8618 root length 5 d	10 d	shoot length 5 d	10 d
Control	94.4±5.4	122.8±7.7	85.4±6.1	94.3±14.7	95.7±6.1	132.2±8.2	12.1±2.7	57.3±8.7
MRS16	38.7±3.6	54.2±4.2	16.7±1.9	31.2±4.7	34.1±4.2	127.8±6.2	12.2±2.9	34.0±3.2
MRS16M-1	39.4±4.1	58.6±3.9	14.3±1.5	34.3±5.9	32.4±5.1	131.0±7.8	13.2±2.4	42.4±4.6
MRS16M-5	41.5±3.4	62.1±5.2	15.3±1.8	32.4±5.4	32.8±5.4	128.6±6.2	13.1±2.6	44.6±5.8

Table 4. Effect of coinoculation of chickpea with *Pseudomonas* strain/mutants and *Rhizobium* strain Ca181 on nodule number [plant^{-1}], fresh mass [mg plant^{-1}], nitrogenase activity [μmol (C$_2$H$_2$ reduced) plant^{-1} h^{-1}], and root and shoot dry masses [mg plant^{-1}]. Mean ± S.E, *n* = 9. * - values significantly different at $P < 0.05$.

Age	Treatment	Nodule number	Nodule f.m.	Nodule ARA	Root d.m.	Shoot d.m.
60 d	Control	-	-	-	168±9.89	227±14.9
	Ca181	35±1.69	803±29.8	4.51±0.37	432±11.5	668±16.6
	Ca181 + 16	42±3.09*	956±18.4*	4.89±0.31*	548±20.3*	880±21.6*
	Ca181 + 16M-1	49±3.85*	871±24.3	5.24±0.43*	584±11.6*	932±25.4*
	Ca181 + 16M-5	38±3.29	786±39.6	4.68±0.41	490±15.5*	842±19.6*
80 d	Control	5±2.82	82±46.3	-	286±15.2	372± 9.9
	Ca181	44±1.69	974±40.6	6.88±0.31	701±32.3	1025±16.3
	Ca181 + 16	56±2.94*	1058±27.6*	7.56±0.28*	1045±30.6*	1476±12.9*
	Ca181 + 16M-1	74±2.44*	1186±56.6*	8.22±0.36*	1078±23.7*	1588±23.9*
	Ca181 + 16M-5	70±2.16*	1142±33.5*	7.79±0.34*	996±22.9*	1432±14.5*
100 d	Control	12±4.18	138±39.2	-	374±10.1	502±12.9
	Ca181	52±1.69	1124±30.0	7.42±0.22	952±18.1	1634±22.8
	Ca181 + 16	80±5.09*	1376±50.3*	8.84±0.38*	1372±20.6*	2436±32.3*
	Ca181 + 16M-1	88±3.85*	1445±38.1*	9.56±0.44*	1358±35.8*	2240±19.3*
	Ca181 + 16M-5	96±6.48*	1492±44.6*	9.42±0.39*	1242±22.8*	2278±14.9*

Effect of coinoculation of *Pseudomonas* strain/mutants with *Rhizobium* sp. *Cicer* strain Ca181 on chickpea: Seed inoculation of chickpea singly with *Rhizobium* strain Ca181 increased both the root and shoot dry masses of chickpea at all the three stages of plant growth (Table 4). Coinoculation of *Pseudomonas* cultures and *Rhizobium* strain Ca181 further enhanced the nodule number, nodule fresh mass, acetylene reduction activity (ARA) as well as root and shoot dry mass in comparison to inoculation with *Rhizobium* strain alone (Table 4, Fig 2). More growth enhancement was observed with pigment overproducer mutant MRS16M-1 at 60 and 80 d of plant growth while the parent and mutant MRS16M-5 (less pigment producer) performed better at 100-d-old plants. At this stage, *Pseudomonas* mutant MRS16M-5 showed maximum gains in nodule number and nodule mass. The plant dry mass (PD) ratio of coinoculated plants over control or *Rhizobium*-inoculated plants varied

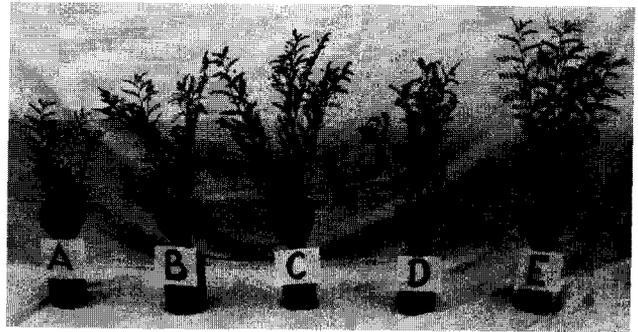from 3.70 to 4.85 and 1.26 to 1.52 respectively at different stages of plant growth.

Fig 2. 60 d-old plants not inoculated (*A*), or inoculated with *Rhizobium* strain Ca181 alone (*B*) or in combination of *Pseudomonas* strains MRS16M-1 + Ca181 (C), MRS16M-5 + Ca181 (D), MRS16 + Ca181 (E).

Discussion

Pseudomonas strain MRS16 used in this study inhibited the growth of four pathogenic fungi, *i.e.*, *Aspergillus* sp., *F. oxysporum*, *P. aphanidermatum* and *R. solani* under cultural conditions (Table 1). Larger inhibition zones of fungal growth were observed on NA and KB media by *Pseudomonas* strain MRS16 as compared to PDA and PPM media (Table 1), suggesting that constituents present or lacking in NA and KB media enhanced the antagonistic activity of *Pseudomonas* strain MRS16. A similar influence of the constituents of medium on antagonistic activity and pigment production have earlier been reported (James and Gutterson 1986, Kanner *et al.* 1978, Rosales *et al.* 1995). The strains of *P. fluorescens* and *P. aeruginosa* have also been reported to produce one pigment (siderophore) or more than one pigment (antibiotics), respectively (Chang and Blackwood 1969, Sakthivel *et al.* 1986). Mutants derived for altered pigment production ability also showed variation in their antifungal activity (Table 2). The pigment overproducer mutant MRS16M-1 showed increased zone of fungal growth inhibition whereas non-producer mutant showed reduced growth inhibition zones. The inhibition of fungal growth in iron added medium suggested that antifungal activity of *Pseudomonas* strain and its mutants MRS16M-1 may be the cumulative effect of both siderophore and antibiotics. However, a little effect of iron addition on the antifungal activity was observed with the pigment non-producer mutant MRS16M-5 (Table 2), suggesting that mutation for pigment synthesis has probably affected siderophore production ability.

Detection of siderophore using CAS agar plate is based on the ability of siderophore to act as chelator with varying affinity for iron. The presence of this iron chelator is indicated by decolorization of blue coloured

ferric-CAS complex, resulting in an orange halo around the colonies on CAS plates. Using this approach, it was observed that the parent strain MRS16 and mutant MRS16M-1 produced halo zones whereas the mutant MRS16M-5 showed no halo zone formation confirming that the mutation has eliminated the siderophore production ability.

Seed bacterization of chickpea with *Pseudomonas* strain and its mutants showed a strong inhibitory effect on root and shoot elongation at 5 d of growth (Table 3). The inhibition of seedling growth could be due to synthesis or secretion of some inhibitory agent when the bacterium was grown in synthetic medium as well as in root exudates of chickpea. Two cultivars differing in their tolerance to wilt showed a cultivar difference in sensitivity to the metabolite(s) formed by the bacterium. Similarly, the production of toxic metabolites with an inhibitory effect on wheat root growth by other non-fluorescent *Pseudomonas* isolates have been reported (Astrom *et al.* 1993, Bolton and Elliott 1989, Fredrickson *et al.* 1987). Loper and Schroth (1986) reported an overall correlation between the bacterial production of indole-acetic acid (IAA) and inhibitory effect on root growth, and suggested that IAA may be a main active agent in plant growth inhibition.

On coinoculation of *Pseudomonas* with *Rhizobium* sp. *Cicer* strain Ca181, nodule number and nodule fresh mass and plant dry mass increased significantly (Table 4). The possible mode of nodule promotion after coinoculation with *Pseudomonas* could be due to the increased surface area of roots due to development of secondary roots (Fig. 1) for attachment of rhizobia or the enhanced production and release of flavonoid-like compounds (Parmar and Dadarwal 1999) that induce the

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transcription of rhizobial nodulation genes. The increased nodulation and nitrogen fixation by rhizobia, more nutrient uptake by enhanced surface area of roots and suppression of growth of deleterious bacteria by *Pseudomonas* spp. are the factors that enhanced root and shoot dry masses of chickpea. The pigment non-producer mutant MRS16M-5 coinoculated with *Rhizobium* sp. *Cicer* strain Ca181 also increased the nodule mass and total plant dry matter of chickpea, suggesting that

fluorescent pigment did not affect the symbiotic parameters of chickpea under sterile conditions. Marek-Kozaczuk *et al.* (1996) have also reported that Tn5-derived mutants of *Pseudomonas* sp. 267 defective in production of fluorescent siderophore did not affect the nodulation and growth of clover under gnotobiotic conditions. However, the exact role of siderophores could only be ascertained under field conditions having variations in iron availability and indigenous microflora.

References

Astrom, B., Gustafsson, A., Gerhardson, B.: Characteristics of a plant deleterious rhizosphere pseudomonad and its inhibitory metabolite(s). - J. appl. Bacteriol. **74**: 20-28, 1993.

Bolton, H., Jr., Elliott, L.F.: Toxin production by a rhizobacterial *Pseudomonas* sp. that inhibits wheat root growth. - Plant Soil **114**: 269-278, 1989.

Bolton, H., Jr., Elliott, L.F., Turco, R.F., Kennedy, A.C.: Rhizoplane colonization of pea seedlings by *Rhizobium leguminosarum* and a deleterious root colonizing *Pseudomonas* sp. and effects on plant growth. - Plant Soil **123**: 121-124, 1990.

Chang, P.C., Blackwood, A.C.: Simultaneous production of three phenazine pigments by *Pseudomonas aeruginosa* Mac 436. - Can. J. Microbiol. **15**: 439-444, 1969.

Dadarwal, K.R., Sindhu, S.S., Batra, R.: Echology of Hup+ *Rhizobium* strains of cowpea miscellany: native frequency and competence. - Arch. Microbiol. **141**: 255-259, 1985.

Dashti, N., Zhang, F., Hynes, R., Smith, D.L. Application of plant growth-promoting rhizobacteria to soybean [*Glycine max* (L.) Merr.] increases protein and dry matter yield under short-season conditions. - Plant Soil **188**: 33-41, 1997.

De Freitas, J.R., Germida, J.J.: Plant growth-promoting rhizobacteria for winter wheat. - Can. J. Microbiol. **36**: 265-272, 1990.

Dubeikovsky, A.N., Mordukhova, E. A., Kochetkov, V.V., Polikarpova, F.Y., Boronin, A.M.: Growth promotion of blackcurrant softwood cuttings by recombinant strain *Pseudomonas fluorescens* BSP53a synthesising an increased amount of indole-3-acetic acid. - Soil Biol. Biochem. **25**: 1277-1281, 1993.

Elad, Y., Baker, R.: The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp. by *Pseudomonas* spp. - Phytopathology **75**: 1053-1059, 1985.

Fredrickson, J.K., Elliott, L.F., Engibous, J.C.: Crop residues as substrate for host-specific pseudomonads. - Soil Biol. Biochem. **19**: 127-134, 1987.

Fridlender, M., Inbar, J., Chet, I.: Biological control of soil borne plant pathogens by a β-1,3-glucanase-producing *Pseudomonas cepacia*. - Soil Biol Biochem. **25**: 1211-1221, 1993.

Goel, A.K., Sindhu, S.S., Dadarwal, K.R.: Application of plant growth-promoting rhizobacteria as inoculants of cereals and legumes. - In: Singh, T., Yadav, A.K., Raychaudhury, S. (ed.): Recent Advances in Biofertilizer Technology. University Publications, New Delhi 2000. (In press).

Gurusiddaiah, S., Weller, D.M., Sarkar, A., Cook, R.J.: Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. - Antimicrob. Agents Chemother. **29**: 488-495, 1986.

Hebbar, K.P., Davey, A.G., Dart, P.J.: Rhizobacteria of maize antagonistic to *Fusarium moniliforme*, a soil-borne fungal pathogen: colonization of rhizosphere and roots. - Soil Biol. Biochem. **24**: 989-997, 1992.

James, D.W., Gutterson, N.I.: Multiple antibiotics produced by *Pseudomonas fluorescens* HV37a and their differential regulation by glucose. - Appl. environ. Microbiol. **52**: 1183-1189, 1986.

Kanner, D., Gerber, N.N., Bartha, R.: Pattern of phenazine pigment production by a strain of *Pseudomonas aeruginosa*. - J. Bacteriol. **134**: 690-692, 1978.

Kloepper, J.W., Leong, J., Teintze, M., Schroth, M. N.: Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. - Nature **286**: 883-884, 1980.

Kloepper, J.W., Scher, F.M., Lalibert, E.M., Tipping, B.: Emergence promoting rhizobacteria: description and implications for agriculture. - In: Swinburne, T.R. (ed.): Iron, Siderophores and Plant Diseases. Pp. 155-164. Plenum Press, New York 1986.

Knight, T.J., Langston-Unkefer, P.J.: Enhancement of symbiotic dinitrogen fixation by a toxin-releasing plant pathogen. - Science **241**: 951-994, 1988.

Leong, J.: Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. - Annu. Rev. Phytopathol. **24**: 187-209, 1986.

Lifshitz, R., Kloepper, J.W., Kozlowski, M.: Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. - Can. J. Microbiol. **33**: 390-395, 1987.

Loper, J.E., Buyer, J.S.: Siderophores in microbial interactions on plant surfaces. - Mol. Plant-Microbe Interact. **4**: 5-13, 1991.

Loper, J.E., Schroth, M.N.: Influence of bacterial sources of indole-3-acetic acid on root elongation of sugarbeet. - Phytopathology **76**: 386-389, 1986.

Mandeel, Q., Baker, R.: Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of non-pathogenic *Fusarium oxysporum*. - Phytopathology **81**: 462-469, 1991.

Marek-Kozaczuk, M., Deryto, M., Skorupska, A.: Tn5 insertion mutants of *Pseudomonas* sp. 267 defective in siderophore production and their effect on clover (*Trifolium pratense*)

nodulated with *Rhizobium leguminosarum* bv. *trifolii*. - Plant Soil **179**: 269-274, 1996.

Parmar, N., Dadarwal, K.R.: Stimulation of nitrogen fixation and induction of flavonoid-like compounds by rhizobacteria. - J. appl. Microbiol. **86**: 36-44, 1999.

Rosales, A.M., Thomashow, L., Cook, R.J., Mew, T.W.: Isolation and identification of antifungal metabolites produced by rice-associated antagonistic *Pseudomonas* spp. - Phytopathology **85**: 1028-1032, 1995.

Sakthivel, N., Sivamani, E., Unnamalai, N., Gnanamanickam, S.S.: Plant growth-promoting rhizobacteria in enhancing plant growth and suppressing plant pathogens. - Curr. Sci. **55**: 22-25, 1986.

Sambrook, J., Fritsch, E.F., Maniatis, T.: Molecular Cloning: a Laboratory Manual. - Cold Spring Harbor Laboratory, New York 1989.

Schwyn, B., Neilands, J.B.: Universal chemical assay for the detection and determination of siderophores. - Anal. Chem. **160**: 47-56, 1987.

Sindhu, S.S., Dadarwal, K.R.: *Ex planta* nitrogenase induction and uptake hydrogenase in *Rhizobium* sp. (cowpea miscellany). - Soil Biol. Biochem. **18**: 291-295, 1986.

Sindhu, S.S., Gupta, S.K., Dadarwal, K.R.: Antagonistic effect of *Pseudomonas* spp. on pathogenic fungi and enhancement of plant growth in green gram (*Vigna radiata*). - Biol. Fertil. Soils **29**: 62-68, 1999.

Sloger, C.: Symbiotic effectiveness and nitrogen fixation in nodulated soybean. - Plant Physiol. **44**: 1666-1668, 1969.

Somasegaran, P., Hoben, H.J.: Methods in Legume-*Rhizobium* Technology. - Niftal University of Hawaii Publication, Hawaii 1985.

Voisard, C., Keel, C., Hass, D., Defago, G.: Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. - EMBO J. **8**: 351-358, 1989.

Zhang, F., Dashti, N., Hynes, R.K., Smith, D.L.: Plant growth-promoting rhizobacteria and soybean [*Glycine max* (L.) Merr.] growth and physiology at suboptimal root zone temperature. - Ann. Bot. **79**: 243-249, 1997.