Biotechnol Lett (2011) 33:2287–2295
DOI 10.1007/s10529-011-0699-0

ORIGINAL RESEARCH PAPER

Plant growth-promotion (PGP) activities and molecular characterization of rhizobacterial strains isolated from soybean (*Glycine max* L. Merril) plants against charcoal rot pathogen, *Macrophomina phaseolina*

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Received: 7 June 2011 / Accepted: 5 July 2011 / Published online: 16 July 2011

Abstract Charcoal rot disease, caused by the fungus *Macrophomina phaseolina*, leads to significant yield losses of soybean crops. One strategy to control charcoal rot is the use of antagonistic, root-colonizing bacteria. Rhizobacteria A_5F and FPT_721 and *Pseudomonas* sp. strain GRP_3 were characterized for their plant growth-promotion activities against the pathogen. *Rhizobacterium* FPT_721 exhibited higher antagonistic activity against the pathogen on dual plate assay compared to strain A_5F and GRP_3. FPT_721 and GRP_3 gave decreased disease intensity in terms of average number of pathogen-infested plants. Lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL), and peroxidase (POD) activities were estimated in extracts of plants grown from seeds that were treated with rhizobacteria, and inoculated with spore suspension of *M. phaseolina*. The activity of these enzymes after challenge with the test pathogen increased. Strains FPT_721 and GRP_3 exhibited maximum increases in LOX, PAL and POD activity (U mg^{-1} fresh leaf wt) compared to strain A_5F.

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Keywords Biocontrol · Induction of systemic resistance determinants · *Macrophomina phaseolina* · Plant stress enzymes · Soybean

Introduction

Charcoal rot disease, caused by the fungus *Macrophomina phaseolina* (Tassi) Goidanich, is the most common root disease of soybeans (Wrather et al. 1997). It was previously considered to be a secondary pathogen since its effects were mainly observed at the final stages of the crop; however the disease can appear at any stage of plant growth (Machado 1987). Soybean [*Glycine max* L. Merril] is a significant source of income for farmers in many countries. The major soybean-producing states in India are Madhya Pradesh, Maharastra, Rajasthan, Gujarat, and Uttar Pradesh. About 90% of India's soybean crop is planted during July, and the remainder is planted during the spring only in Madhya Pradesh. Factors that reduce soybean production, such as diseases, insects, weeds, and weather, can influence the economic or general welfare of many countries and individuals. The fungus is considered to be a soil-borne pathogen and is able to infect several plants viz., cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), sunflower (*Helianthus annuus* L.), and sorghum [*Sorghum bicolor* (L.) Moench]. Because of the wide host range of *M. phaseolina* and the long survival times of the microsclerotia, crop rotation would

probably have little benefit in reducing charcoal rot (Almeida et al. 2003).

Despite inconsistency in field performance, biological control is considered as an alternative or a supplemental way of reducing root diseases in agroecosystem (Sharma and Johri 2003). The widely recognized mechanism of biocontrol mediated by plant growth-promoting rhizobacteria (PGPR) is competition for an ecological niche/substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens (Hass et al. 2002). Induced resistance is a state of enhanced defensive capacity developed by a plant when appropriately stimulated (van Loon et al. 1998). A large number of enzymes have been associated with induced resistance that includes peroxidase (EC 1.11.1.7), phenylalanine ammonia-lyase (EC 4.3.1.5), lipoxygenase (EC 1.13.11.12), β-1,3-glucanase (EC 3.2.1.6), and chitinase (EC 3.2.1.14) (Yan et al. 2002).

The objectives of this study were to evaluate plant growth-promotion activities of rhizobacterial strains for soybean plant against charcoal rot pathogen and their molecular characterization by using Amplified rDNA restriction analysis.

Materials and methods

Fungal culture and spore suspension

An isolated culture of anamorphic ascomycetous pathogen, *M. phaseolina*, was maintained on potato/dextrogen agar. A suspension of 10^8 microsclerotia per ml in 10 mM $MgSO_4$ was prepared from a culture grown for 7 days at 25°C on PDA. This sclerotial suspension (100 µl/g) was added to soil and allowed to germinate and grow for 2 days at 25°C. The final density of colony-forming units (cfu) in soil was determined by plating dilutions of the soil suspension on PDA: 1.2×10^6 microsclerotia/ml was used for inoculation.

Bacterial cultures

The two isolates employed in this study were selected among 189 bacterial isolates screened on the basis of in vitro antibiosis dual plate assay against test pathogen (*M. phaseolina*) together with important plant growth promoting properties viz., P-solubilization, IAA, secretion of hydrolytic enzymes and siderophore production. In this study an ISR producing strain (Pathak et al. 2004; Sharma et al. 2007b), *Pseudomonas* sp. strain GRP3, which was recovered from the rhizosphere of soybean that showed plant growth-promoting properties (Sharma et al. 2003, 2007a), was considered as reference strain (Table 1).

Seed microbiolization

Cultures of $FPT_7$21 and A_5F and *Pseudomonas* sp. strain GRP_3, grown for 24 h, were used to treat soybean seeds. Seed 'microbiolization' was performed according to Sharma et al. (2003). Seeds were surface-sterilized with 0.1% $HgCl_2$ (acidified with H_2SO_4) and washed repeatedly with sterile triple-distilled water prior to sowing. Overnight grown bacterial cultures were centrifuged at 11,000×*g* for 20 min and the pellets were washed with 10 mM phosphate buffer saline (PBS, pH 7.2). Pellets were resuspended in PBS and the OD_{540} adjusted to 0.2 (10^8 cfu ml^{-1}). Surface-sterilized seeds were placed in PBS containing 0.1% carboxymethylcellulose (CMC) as a binder for 1 h, followed by drying under air. Sterile conditions were maintained throughout. Seeds immersed only in water (non-microbiolized) treated as control.

Table 1 Plant growth-promotion (PGP) and antagonistic properties of bacterial isolates

Habitat	Isolate	PGP properties								Zone of inhibition for *M. phaseolina* (cm ± sem)
		Sid	*P sol*	*Rhl*	*IAA*	*Pro*	*Xyl*	*Cell*	*Chi*	
Soybean rhizosphere	$FPT_7$21	+	nd	nd	+	+	nd	nd	+	1.8 ± 0.18
	A_5F	+	nd	nd	+	+	nd	nd	+	0.8 ± 0.13
Soybean rhizoplane	GRP_3	+	+	+	+	+	+	nd	+	1.5 ± 0.15

All tests were performed in three replicates

Sid siderophore production, *P sol* Phosphate solubilization, *Rhl* rhamnolipid production, *IAA* indole acetic acid production, *Pro* protease, *Xyl* xylanase, *Cell* cellulose, *Chi* chitinase production, *nd* not detected, *sem* standard error of mean

Effect of bacterial strains on disease reduction

Microbiolized soybean seeds were planted in plastic pots containing unsterilized soil and maintained in the greenhouse (25 ± 2°C). Seven days after planting, the plants were inoculated by spraying with the microsclerotial suspension (1.2×10^6 microsclerotia/ml) in the rhizosphere area. Each treatment was run with five replications and each replication consisted of four plants per pot. The uninoculated plants were sprayed with water. Thus four treatments were evaluated for each bacterial strain ($FPT_7$21 and A_5F and *Pseudomonas* sp. strain GRP_3): inoculated (T1) and uninoculated (T2) plants from microbiolized seeds; inoculated (T3) and uninoculated (T4 control) plants from non-microbiolized seeds. Disease severity was assessed and analyzed statistically at the time of nodulation (on 45th day). The root rot severity index was recorded at the end of the experiments from two independent assessment and the scores were averaged for statistical analysis.

Chlorophyll estimation

Chlorophyll estimation was performed according to Hiscox and Israelstam (1979). Fresh leaves were removed and chopped into small pieces and 7 ml dimethylsulfoxide (DMSO) added to 100 mg tissue. The tubes containing leaf pieces and DMSO were incubated at 65°C for 3 h; extract was made up to 10 ml with DMSO and the A_{645} and A_{663} were measured employing DMSO as blank.

Enzyme activity detection bioassay

Enzyme assays were performed on leaf extracts. Soybean leaves were collected seven days after pathogen inoculation and stored at 4°C until plant extracts were prepared according to Lanna et al. (1996). Leaves were collected from three plants for each repetition in each treatment. Three repetitions per treatments were employed for each enzyme analyzed. Leaf tissue extracts of about 1 g fresh wt were ground in a mortar and pestle containing liquid N_2. The powder was macerated for 3 ml 50 mM sodium phosphate buffer, pH 6.5, containing 1% polyvinylpyrrolidone and 1 mM PMSF (extraction buffer), and then centrifuged at 15,000×*g* for 30 min at 4°C. The supernatants were kept on ice and used for determination of total protein, lipoxygenase (LOX), peroxidase (POD), and phenylalanine ammonia-lyase (PAL) activities. One unit represents the amount of enzyme that produces one µmol product per min.

Protein concentration

Total soluble protein content of the samples was estimated by using the Lowry method with BSA as standard.

LOX, PAL and POD activities

LOX activity was detected employing method described by Axelrod et al. (1981), which is based on the increase in A_{234} that result from the conjugated double bond system in the hydroperoxide produced from the substrate, linoleic acid. The reaction mixture consisted of 3 ml 50 mM sodium phosphate buffer, pH 6.0, 60 µl linoleic acid (10 mM), and 30 µl plant extract. Absorbance readings were made every 30 s for 10.5 min at 25°C. A value of 25,000 M^{-1} cm^{-1} was employed for the molar extinction coefficient (ε) of linoleic acid. LOX activity was expressed as µmol product formed min^{-1} mg $protein^{-1}$.

PAL activity was determined according to Pascholati et al. (1986). Conversion of L-phenylalanine to cinnamic acid at 37°C was followed at 290 nm reading every 30 s for 5.5 min after the start of the reaction. The reaction mixture contained 30 µl leaf extract and 3 ml 0.2% phenylalanine; the reference used 30 µl extraction buffer. PAL activity was expressed as µmol cinnamic acid min^{-1} mg $protein^{-1}$.

POD activity was determined (Allain et al. 1974) in the clear extract by monitoring the increase in A_{436} at 30°C. The reaction mixture consisted of 3 µl leaf extract and 3057 µl 20.1 mM guaiacol, 12.3 mM H_2O_2 and 10 mM sodium phosphate buffer pH 6). Absorbance readings were taken every 30 s for 15.5 min. Enzyme activity was calculated employing molar extinction coefficient (ε) of 2.47 mM^{-1} cm^{-1}. POD activity was expressed as µmol H_2O_2 min^{-1} mg $protein^{-1}$.

Statistical analysis

The effects of the treatments on plant growth and disease suppression was analyzed using multiple comparisons of means according to Duncan's

multiple range tests (DMRT) at the 5% level. Critical deviation (CD) and standard error of mean (SEM) for each treatment was calculated.

Recovery of genomic DNA and PCR amplification of 16S rDNA

Total DNA from bacterial isolates was prepared according to the procedure of Bazzicalupo and Fani (1994). The amplified 16S rRNA gene was obtained from each bacterial isolate by PCR amplification employing the eubacterial universal primers (Weisburg et al. 1991); fDI (5′-AGAGTTTGATCCTGG-3′) and rP2 (5′-TACCTTGTTACGACTT-3′) which was targeted at universally conserved regions and permitted amplification of approximately 1,500-bp fragment. PCR amplification was carried out in a thermocycler. Reaction tubes contained 25 ng (5 µl) DNA extract, 1 U *Taq* polymerase (Genei, India), 1 X buffer (10 mM Tris/HCl [pH 9.0], 1.5 mM MgC^{l2}, 500 mM KCl), 10 mM dNTPs and 0.25 mM of each primer. Initial DNA denaturation and enzyme activation steps were performed at 95°C for 7 min, followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 51°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The presence and yield of specific PCR product (16S rRNA gene) was monitored on 0.8% agarose (wt/vol.); gel electrophoresis was carried out at 100 V for 30 min in 1 × Tris–acetate–EDTA buffer and visualization by ethidium bromide staining and viewing on a UV transilluminator (GelDoc, Vilber Lourmat, CN08, France).

Amplified rDNA restriction analysis (ARDRA)

16S rRNA amplification product of bacterial genomic DNA was digested with *Hae*III and *Taq*I. 15 µl of (120 µg) 16S rDNA amplification product was digested with restriction endonuclease. For 30 µl reaction mixture, added the following: restriction enzyme (10 U), 1 U (Genei, India); restriction buffer (10 X), 1 X (Genei, India); 16S rDNA amplicon 120 µg, and Milli-Q water. The reaction mixture was incubated at 37°C for *Hae*III, and at 65°C for *Taq*I for 4 h. Restriction product was resolved on 2.5% agarose gel in 1 × TAE at 60 V for 5 h and visualized on a UV transilluminator.

Phylogenetic analysis

Phylogenetic tree was constructed on the basis of restriction fragment pattern of the amplicon of 16S rDNA using the software NTSYS pc version 2.02i. The clustering was done using Jaccard's similarity coefficient based on presence and absence of band ignoring their intensities. The dendrogram was constructed employing the unweighted pair group means average (UPGMA) methods according to Sneath and Sokal (1973).

Results

Plant growth promotion in greenhouse bioassay

Plant–microbe interactions play an important role in soil and plant health. In the artificial (greenhouse) trial, strain $FPT_7$21 showed the largest increase in shoot length and total chlorophyll content for treatment T2 compared to other treatments. A similar result was obtained for strain GRP_3 compared to strain A_5F. The plants which were inoculated with pathogen alone (T3) showed reduction in shoot length and total chlorophyll content compared to control (T4) (Table 2). In addition, strain $FPT_7$21 afforded the greatest protection against pathogen compared to strain A_5F and GRP_3 (Table 2). Maximum root-rot severity index was observed for the strain A_5F (Table 2).

Bacterial treatments change in LOX, PAL and POD activity

Higher LOX, PAL and POD activity was observed in soybean plants inoculated with pathogen (treatments T1 and T3) as seen in Table 3. Plants grown from microbiolized seeds presented the highest LOX activity (T1) compared to control treatment (T4). The presence of the pathogen/rhizobacterium alone resulted in similar increase in enzymes activity. Strain $FPT_7$21 and GRP_3 exhibited maximum increase in LOX, PAL and POD activity compared to strain A_5F (Table 3).

Amplified rRNA restriction analysis

The amplification of 16S rRNA of the two selected isolates and reference strain GRP_3 along with

Table 2 Effect of promising PGP and antagonistic isolate on root-rot index, shoot length and chlorophyll content of soybean under artificial (greenhouse) infestation, on 45 days after sowing

Bacterial isolate*	Root-rot index				Shoot length (means, cm)				Total chlorophyll (mg g^{-1} fresh weight of leaf)			
	T1	T2	T3	T4 (control)	T1	T2	T3	T4 (control)	T1	T2	T3	T4 (control)
$FPT_7$21	2.82^a	−(nd)	+(d)	−(nd)	28.8^a	34.8^a	18.7^a	29.8^a	4.16^a	4.98^a	1.13^a	2.89^a
A_5F	8.44^b	−(nd)	+(d)	−(nd)	25.7^b	32.2^b	18.2^a	30.9^a	2.89^b	3.71^b	1.09^a	2.74^a
GRP_3	5.88^c	−(nd)	+(d)	−(nd)	27.1^{ac}	33.2^c	18.5^a	30.6^a	3.68^{ac}	4.32^{ac}	1.12^a	2.87^a
CD at 5%	0.92	–	–	–	0.81	0.64	1.1	0.98	0.49	0.54	1.06	1.22
SEM	0.34	–	–	–	0.27	0.21	0.32	0.26	0.28	0.33	0.18	0.14

SEM standard error of mean, *−(nd)* not detected i.e. all plants were healthy, *+(d)* detected, i.e., all plants were dead, *CD* critical difference

For columns, different *superscript letters* indicate a statistically difference ($P < 0.05$) according to Duncan multiple range test (DMRT)

* Four treatments were evaluated for each bacterial strain (FPT_7 21, A_5F and *Pseudomonas* sp. strain GRP_3): inoculated (T1) and uninoculated (T2) plants from microbiolized seeds; inoculated (T3) and uninoculated (T4 control) plants from non-microbiolized seeds

Table 3 Effect of promising PGP and antagonistic isolate on LOX, PAL and POD activity in soybean under artificial (greenhouse) infestation, on 45 days of sowing

Bacterial isolate*	LOX (U mg^{-1} fresh leaf weight)				PAL (U mg^{-1} fresh leaf weight)				POD (U mg^{-1} fresh leaf weight)			
	T1	T2	T3	T4 (control)	T1	T2	T3	T4 (control)	T1	T2	T3	T4 (control)
$FPT_7$21	8.89^a	4.22^a	7.41^a	2.76^a	4.43^a	2.18^a	3.21^a	1.06^a	4.86^a	2.95^a	4.16^a	2.11^a
A_5F	6.24^b	3.26^b	6.01^b	2.68^a	3.38^b	1.42^b	2.67^b	0.97^a	3.62^b	2.14^b	3.98^a	1.96^a
GRP_3	7.71^c	3.81^{ac}	7.03^{ac}	2.81^a	4.01^{ac}	1.86^{ac}	2.98^{ac}	1.02^a	4.14^c	2.68^{ac}	4.02^a	2.01^a
CD at 5%	0.28	0.32	0.46	0.19	0.36	0.22	0.18	0.23	0.34	0.26	0.48	0.38
SEM	0.08	0.24	0.16	0.04	0.18	0.12	0.06	0.19	0.09	0.11	0.19	0.14

CD critical difference, *SEM* standard error of mean

For columns, different *superscript letters* indicate a statistically difference ($P < 0.05$) according to Duncan multiple range test (DMRT)

* Four treatments were evaluated for each bacterial strain (FPT_7 21, A_5F and *Pseudomonas* sp. strain GRP_3): inoculated (T1) and uninoculated (T2) plants from microbiolized seeds; inoculated (T3) and uninoculated (T4 control) plants from non-microbiolized seeds

standard *Pseudomonas fluorescens* biovar. I, III, and IV (ATCC 13525, ATCC 17400 and ATCC 12983, respectively), *Sinorhizobium meliloti* 102F34, *Mesorhizobium ciceri* G-3-97, *Bradyrhizobium japonicum* SB102 and *Rhizobium gallicum* R602 SPT, each generated a band of 1 kb. Restriction with *Hae*III yielded a banding pattern which was comparable to that obtained with *Taq*1. Restriction digestion of the amplicons with *Hae*III and *Taq*1 resulted in generation of fragment of size, 400-1000 bp, varying from two to six in number (Fig. 1a, b). A composite ARDRA profile generated with two restriction endonucleases (*Hae*III and *Taq*I) indicated that the three isolates and standard reference bacteria grouped into three clusters (cluster 1 contains isolate A_5F; cluster 2 contain *P. fluorescens* biovars I, III, and IV, and strain GRP_3; cluster 3 contain *Sino*-, *Meso*-, *Brady*-, and *Rhizobium* along with strain $FPT_7$21) and indicated strains A_5F, $FPT_7$21 and strain GRP_3 made distinct group with standards. Strain GRP_3 showed 58% similarity with *P. fluorescens* biovars I, III, and IV whereas strain $FPT_7$21 exhibited 45% similarity with *Sinorhizobium* and *Mesorhizobium*. Strain A_5F was least similar (24%) to other two clusters and was present on an entirely separate lineage (Fig. 2).

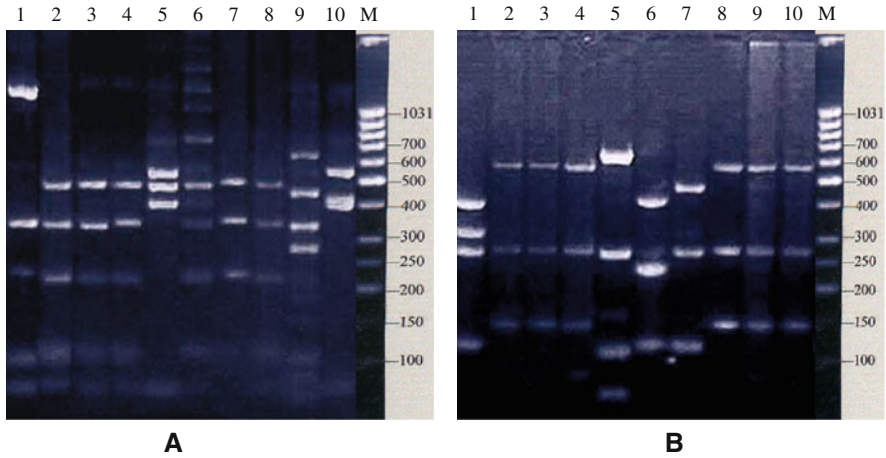

Fig. 1 Molecular fingerprinting analysis of bacterial isolates. **a**, **b** demonstrate ARDRA pattern of PCR amplified 16S rDNA with restriction endonucleases i.e., *Hae*III and *Taq*1. *Lane M* Marker; *Lane 1* A5F; *Lane 2 P. fluorescens* bv. IV; *Lane 3 P. fluorescens* bv. I; *Lane 4 P. fluorescens* bv. III; *Lane 5* GRP_3; *Lane 6* $FPT_7$21; *Lane 7 Bradyrhizobium* strain SB102; *Lane 8 Rhizobium* strain R602SPT; *Lane 9 Mesorhizobium* strain G-3-97; *Lane 10 Sinorhizobium* strain 102F34

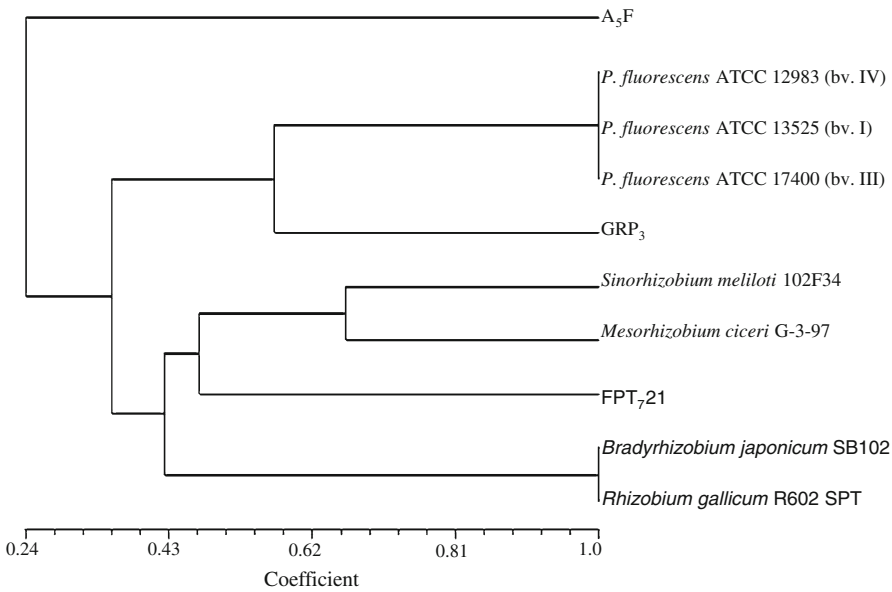

Fig. 2 Phylogenetic tree based on ARDRA pattern of PCR amplified 16S rDNA with two restriction endonucleases i.e., *Hae*III and *Taq*1 showing relatedness among the isolates

Discussion

We selected two rhizobacterial isolates along with reference strain *Pseudomonas* sp. strain GRP_3, which were screened on the basis of in vitro antibiosis assays against the test pathogen *M. phaseolina*, together with other important plant microbe interaction properties such as P-solubilization and siderophore production. Kloepper (1991) has also suggested the use of rapid screening techniques for the selection of effective isolates. In the bacterial selection process, a similar emphasis was also given (Table 1). The production of siderophores occurs only under iron-limited conditions. Such conditions are likely to prevail in the rhizosphere, and competition for iron through the production of siderophore is one of the mechanisms of bacterial antagonism against soil-borne pathogens. Thus, siderophore production by specific ISR-eliciting rhizobacteria can play a dual role in disease suppression by depriving resident pathogens from iron locally and by inducing resistance in the plant systemically. High-affinity iron

uptake systems of the PGPRs that have promiscuous siderophores may interfere with iron nutrition of many pathogenic bacteria and fungi leading to their suppression (Sharma et al. 2003). Sharma et al. (2003) described the role of siderophores which is one of the determinants of ISR in effecting plant nutrition wherein they overcome problem of iron non availability in calcareous soils by incorporation of siderophore producing strains of fluorescent pseudomonads (FLPs). The sidrophore-producing bacterium, *Pseudomonas* strain GRP3, was employed in a pot experiment to assess the role of microbial siderophores in the iron nutrition of mung bean employing Fe-citrate, Fe-EDTA, and $Fe(OH)_3$ in different concentrations. The plant showed a reduction of chlorotic symptoms and enhanced chlorophyll level in bacterized plant. Bacterization with GRP_3 increased peroxidase activity and lowered catalase activity in roots. There was also a significant increase in total and physiologically available iron. Such siderophores producing systems have the potential of improving iron availability to plants and a reduction of fertilizer usage. Sharma et al. (2007c) have described molecular characterization of rhamnolipid that recovered from *Pseudomonas* sp. strain GRP3, considered as an inducing determinant of biocontrol activity. Since rhamnolipids are involved in the lysis of the plasma membrane of zoosporic fungi, *Pythium* and *Phytophthora* strains, application of such rhamnolipid producing rhizobacteria could facilitate control of damping-off of plant pathogens.

Genetic profiles generated by ARDRA, strains GRP_3 and FPT_721 were predictive of genus *Pseudomonas* and *Rhizobium,* respectively, whereas strain A_5F was different. Because ARDRA detects interspecies and inter strain as well as interoperon variability and enables a relatively fast multiple strain analysis per taxon, this technique is appropriate to obtain indicative phylogenetic and taxonomic information. This information can be used to select strains for further detailed taxonomic studies. ARDRA fingerprinting also allows the construction of a database for identification purposes (Sneath and Sokal 1973).

PGP bacteria-triggered ISR fortifies plant cell wall strength and alters host physiology and metabolic responses, leading to an enhanced synthesis of plant defense chemicals upon challenge by pathogens and/or abiotic stress factors (Broetto et al. 2005). In the present study, an enhanced level in the amount of JA/ET-dependent enzymes was observed. In seeds that were treated with rhizobacterial strains together with spore suspension showed significant increase in activity of LOX, PAL, and POD. The observed increases in LOX activity in plant tissue expressing resistance in diverse pathosystems have been reported (Croft et al. 1990). The increase in LOX activity over the control observed in treatment T2 (uninoculated microbiolized seeds) may be explained by the recognized growth promoting effect of rhizobacteria (Gutierrez-Manero et al. 2001). The difference in LOX activities between treatments T3 and T4 was due to enzyme induction in the plant caused by the pathogen that, together with constitutive production (see treatment T4), apparently afforded an increase in enzyme activity in treatment T3.

The activity of plant POD and PAL enzymes are stress indicators. POD participates in the cell wall polysaccharides processes such as oxidation of phenols, suberization, and lignification of host plant cells during the defense reaction against pathogenic agents (Ray et al. 1998). The high peroxidase activities detected in treatments are linked to lignification and generation of peroxides that inhibit pathogens directly or generate other free radicals with antimicrobial effects (Hammerschmidt 1999). The peroxidase activity results reported in this study are in agreement with those of Podile and Lakshmi (1998), who observed an increase in POD activity in pea plants treated with *B. subtilis*, 7 days after inoculation with *Fusarium udum*.

PAL plays a key role in the phenylpropanoid pathway wherein lignin is one of the major products. Deposition of lignin is an inducible defense mechanism employed for protection against pathogen invasion (Liang et al. 1989). The type of bacterized plant response induced after challenge with a pathogen resulted in the formation of structural barriers, such as thickened cell wall because of the deposition of callose and the accumulation of phenolic compounds at the site of pathogen attack (Benhamou et al. 1998). PAL activity was higher in plants grown from microbiolized seeds that were challenge inoculated with charcoal rot pathogen. PAL activity is reflective of disease proliferation and consequent stress. Studies with different plant species showed that PAL activity increases with the biotic and abiotic stresses including bacterial infection (Hammerschmidt 1999; Yan et al. 2002).

To combat invasions by micro-organisms, plants have evolved several lines of defense. Besides pre-existing physical and chemical barriers, inducible resistance mechanisms can be activated upon pathogen infection. The increases in the activities of these enzymes (LOX, PAL, and POD) indicate that they may be important defense responses to pathogen infection. Plants that respond with higher activities of these enzymes may have enhanced resistance to pathogen attack. The data presented in this study described PGPR-phytopathogen-plant interactions in terms of rhizobacteria-mediated ISR. Besides inducing ISR, PGPR can exert a protective action against those soilborne pathogens that are particularly prone to attack emerging seedlings. ISR-eliciting rhizobacteria can be applied on seeds and then will readily colonize emerging plant-roots. Thus, seedlings can be better protected at an earlier growth stage (Kloepper et al. 1989). These properties make ISR-inducing PGPR a useful tool to reduce diseases caused by pathogens that are sensitive to JA- and ET-dependent defenses. Integrating ISR-triggering PGPR into disease management programme in conjunction with other strategies will be a worthwhile approach to explore. Understanding the diversity within populations of biocontrol agents holds the promise of pairing specific genotypes with their most supportive plant hosts or soil environments to maximize root colonization and disease suppression. Finally, establishing the presence and functionality of individual populations within a particular soil is just one first step toward fully understanding the nature of suppressiveness within that soil. The future studies of biologically based soil suppressiveness will present new insights into the microbial ecology of agricultural soils and lay the foundation for the development of creative management strategies for the suppression of soilborne diseases.

Acknowledgement This research was supported by grant No. SR/FT/L-03/2006 to DKC from SERC division of Department of Science & Technology (DST), Govt. of India, New Delhi, India. The fungus, *M. phaseolina* and seeds of soybean were kindly provided by Dr. Mahaveer Sharma, from the National Research Centre for Soybean (NRCS), Indore (M.P.), India. Author is grateful to Prof. B.N. Johri and Dr. Anil Prakash, Department of Biotechnology, BU, Bhopal for their kind support during experiments. Mr. Ankit Kumar is gratefully acknowledged for providing a bacterial culture A_5F for experiments. A kind support of Prof. Shakti Baijal, Dean, FASC, MITS, is gratefully acknowledged.

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