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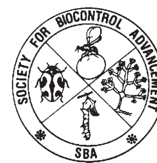

Role of chitinolytic enzymes and volatile compounds produced by endophytic bacteria in the inhibition of mango (*Mangifera indica* L.) root rot pathogens

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ABSTRACT: Endophytic and rhizobacteria isolated from different crops of Goa were studied for the chitinolytic activity and inhibition of fungal pathogens. Fifty–eight isolates were screened *in vitro* against three fungal pathogens, *viz*., *Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotium rolfsii* which cause seedling and graft mortality in mango nurseries. Results indicated that 40% of the isolates effectively inhibited the growth of all the three pathogens and 34% inhibited the growth of *M. phaseolina* and *S. rolfsii* under *in vitro* conditions. Chitinolytic ability of the isolates was tested by growing them in minimal synthetic medium with 0.2% colloidal chitin. Twelve isolates effectively utilized colloidal chitin as a sole carbon source for their growth. Quantitative chitinase assay revealed that the isolates produced significant amounts of chitinase in the minimal medium and the crude chitinase extract of nine endophytic (EB65, EB69, EBh11, EC2, EC2a, EC14, EC18, ESC4, ET14) and three rhizobacteria (IISR6, PDBC-AB2, RBh42) inhibited the mycelial growth as well as sclerotial germination of *R. solani* and *S. rolfsii*. Quantitative assay of chitinase showed that three endophytic bacteria (EBh11, EC18 and EC14) showed maximum chitinase activity. Chitinolytic bacteria reduced the population of all the three fungi when introduced into the soil. Further, most of the isolates produced IAA and solubilized inorganic phosphate in the medium. This study reveals that chitinolytic bacteria, especially endophytes with growth promoting mechanisms, could be better biocontrol agents in the suppression of soil borne phytopathogens.

KEY WORDS: Chitinolytic enzymes, volatile compounds, mango, root rot, *Macrophomina phaseolina, Rhizoctonia solani, Sclerotium rolfsii,* antagonism

INTRODUCTION

Mango seedlings and grafts are severely affected due to root rot disease in the nurseries of Goa. Different fungi, *viz*., *Macrophomina phaseolina* and *Rhizoctonia solani* cause seedling rot in mango grafts and *Sclerotium rolfsii* causes seedling rot in seedlings (root stocks) (Ramesh, 2009). All the pathogens are soil borne and none of the commercially available fungicides are effective in managing the mortality in the nursery. Biological control is a potential alternative to the use of chemical pesticides in plant disease management especially against soil borne pathogens (Chet and Inbar, 1994). There is a growing acceptance that biological control can be successfully exploited as an agricultural control method for soil-borne pathogens (Papavizas and Lumsden, 1980). Potential use of naturally occurring bacteria, actinomycetes and fungi as replacement or supplement for chemical pesticides have been addressed in many studies (Berg *et al.,* 1996; Moricca *et al.,* 2001).

Chitinases are glycosyl hydrolases, which catalyze the degradation of chitin. These enzymes are present in a wide range of organisms such as bacteria, fungi, insects, plants and animals. Chitinases occupy a unique position in agricultural biotechnology as it inhibits fungal development by degrading chitin components of cell wall. Detection of chitin-degrading bacteria from natural sources such as rhizosphere soil is useful in the isolation of bacteria that produce antifungal or other novel compounds. A high correlation between chitinolysis and production of bioactive compounds has been reported (Pisano *et al.,* 1992). Microorganisms, which secrete a complex of mycolytic

enzymes, are considered to be possible biological control agents of plant diseases (Helisto *et al.,* 2001; Chang *et al.,* 2003). The microbial world synthesizes and emits many volatile compounds (Fernando *et al*., 2005). At present the biologically active volatiles causing the inhibitions are not known because many volatiles remained undetected or unidentified (Kai *et al.,* 2006).

The present study aims to screen the bacterial isolates for antagonistic activity against the fungal pathogens of mango root rot by the production of chitinolytic enzymes, volatile antifungal compounds besides screening them for the production of plant growth promoting substances like IAA and potential to solubilize inorganic phosphate.

MATERIALS AND METHODS

Bacterial isolates and fungal phytopathogens

Fifty eight bacterial isolates used in the study were obtained from culture collection of Plant Pathology laboratory, ICAR Research Complex for Goa, Old Goa. The isolates selected were with proven antagonistic activity against one or more bacterial and fungal phytopathogens in the earlier studies. The causal agents of mango graft mortality namely *Macrophomina phaseolina*, *Rhizoctonia solani* and causal agent for mango seedling mortality, *Sclerotium rolfsii*, were isolated from the infected grafts and seedlings, purified and maintained on PDA medium. Pathogenicity of the fungi was confirmed on the mango seedlings.

Antagonism of bacterial isolates against the fungal pathogens under *in vitro*

Antagonistic properties of selected isolates were tested on PDA plates using dual culture technique (Skidmore and Dickinson, 1976). Five day old mycelial discs of the pathogens (7 mm diameter) were placed on two opposite sides of the Petri plate. A loopful of 24 h old grown bacteria from the selected isolates was streaked two cm away from the fungal disc on two opposite sides and incubated at 28±2ºC for 4 days. The diameter of fungal growth was recorded after 5 days.

Assay of antifungal volatile compounds and production HCN

The fungal pathogens were cultured on PDA while the bacterial antagonists were multiplied using King's B broth (King *et al.,* 1954). 40 µl of the 12 h old grown bacterial culture (2 x 10^9 cfu ml^{-1}) was added to 20 ml of the King's B medium in a test tube and poured into a Petri plate (either bottom plate/ lid). Another plate of equal size (either bottom plate / lid) containing the PDA medium inoculated with the fungal disc was placed in the inverted position over the plate containing the bacterial inoculum to avoid any physical contact between both the organisms and sealed with parafilm. The plates were incubated at 28±2ºC for four days. The radial growth of the pathogen in each treatment was measured. Production of HCN was determined by modified method of Miller and Higgins (1970).

Growth of antagonistic bacteria in chitin amended medium and chitinase assay

Colloidal chitin was prepared as described by Hsu and Lockwood (1975). To assess the chitinase producing bacteria, the selected isolates were grown in 5 ml Minimal Synthetic Medium (MSM) (Twedell *et al*., 1994) with 0.2% colloidal chitin, for 48 h at 28 ±2°C on a shaker at 150 rpm. 50 µl from the 48 h old growth was transferred again into 5ml of MSM broth with colloidal chitin. The isolates grown after 2 subsequent transfers were then transferred to solid MSM medium amended with 0.2% colloidal chitin. Qualitative growth of bacteria in the broth was recorded as visual turbidity and on solid medium as the appearance of the colonies in every step. The isolates which grew in MSM with colloidal chitin were inoculated into MSM media amended with 0.2% N-acetyl glucosamine (Hi Media Laboratories, Mumbai) in an another experiment and incubated for 3 days. Bacterial growth was recorded by checking the visual turbidity. Chitinase activity was assayed as described by Twedell *et al.* (1994) by measuring the release of *N*- acetyl-D-glucosamine (NAGA) from colloidal chitin. The concentration of NAGA in the supernatant was determined as described by Reissig *et al.* (1995).

Inhibitory activity of crude chitinase on fungal pathogens

Antifungal activity of bacterial chitinase

A 7mm disc of four days old grown fungal isolate was placed on PDA medium. After 24h of incubation at 28±2ºC, the sterile filter paper discs of 4mm diameter were placed about 2cm away from the inoculation site. Crude chitinase extract was obtained by filter sterilizing the supernatants of 24 h old bacteria grown in MSM broth with 0.2% colloidal chitin. 20µl of the crude enzyme extract was added on the sterile filter paper disc. The plates were incubated at 28±2ºC for 2-3 days and inhibition around the discs was recorded.

Inhibition of sclerotial germination

Crude chitinase extract was prepared as described above and 1ml was dispensed into microcentrifuge tubes. Freshly formed sclerotia (30 no.) of *S. rolfsii* and *R. solani* were immersed in the crude enzyme extract. Ten soaked sclerotia were placed on the PDA medium after 1, 3 and 8 days of incubation. Sclerotia soaked in sterile water, 0.2% bavistin (Carbendazin) served as negative and positive

controls. Germination of sclerotia was recorded in each case.

Inhibition of fungi in soil by the bacterial isolates

Two isolates (EB65 and EB69) selected on the basis of their chitinolytic activity and one isolate (EC21) (all are species of *Pseudomonas*) selected based on strong HCN production were tested *in vivo* for their inhibitory activity in soil against the three fungal pathogens. 250 gm of sterile soil was taken in pots and 4 days old fungal (mycelium) pathogen from one fully grown plate was added and mixed well. Initial population of the fungus was estimated by serial dilution. The soil containing fungal inoculum was further distributed into five small pots. 5ml of 48h old grown cultures of the above bacterial isolates was added into the inoculated soil. The experiment was conducted twice for each of the pathogen. Soil inoculated only with fungus, soil inoculated with fungus and treated with 0.2% bavistin were kept as the controls. Another set of three containers with 50g soil each was inoculated only with 5ml of 48h old cultures (mycelium) of the three isolates to assess the initial population of antagonistic bacteria. Soil samples from each of these pots were taken at 7 and 14 days after the treatment to assess the population of bacteria and fungi and expressed as CFU g^{-1} of soil.

Indole acetic acid (IAA) production and phosphate solubilization

Exponentially grown culture of selected isolates were inoculated in 5ml Tryptic soya broth amended with 100µg/ml of tryptophan and incubated at 28±2ºC on shaker at 150rpm for 48h. The presence of IAA in the culture filtrate was quantified (Gorden and Paleg, 1957) and expressed as µg/ml. Pikovskaya (PKV) agar plates with 0.5% tricalcium phosphate as the sole source of phosphorus (Pikovskaya,1948) were spot inoculated with a loopful of culture of selected isolates. After incubation at 28±2ºC for 5 days, formation of a clear zone around the spot was recorded.

Data on fungal inhibition was analyzed using ANOVA and the mean comparison were made using Least Significance Difference (LSD) test ($P<0.05$).

RESULTS AND DISCUSSION

Inhibition of *M. phaseolina*, *R. solani* and *S. rolfsii* by antagonistic bacteria

Fifty eight bacterial isolates used in this study include both endophytic and rhizobacteria from different crop plants. 33 isolates were Gram Positive and 25 were Gram negative. Out of these ESC4, ET14 and RBa9 effectively inhibited the growth of all the three fungi. 12% of them inhibited all the three fungi by 80% and above (data not shown). The antagonism may be due to several mechanisms like production of lytic enzymes, antibiotics and siderophore production. The antagonistic activity has often been associated with production of secondary metabolites (Silva *et al*., 2001). Results of only 12 isolates which are chitinolytic in nature are presented and discussed in this paper. Interestingly out of 31 endophytes and 27 rhizobacteria screened, majority (9 out of 12) of the chitinolytic bacteria belonged to endophytic group. Morphological and biochemical characterization of the isolates indicated that ten of the chitinolytic isolates are species of *Pseudomonas* (EB65, EB69, EC2, EC2a, EC14, EC18, ET14, IISR6, PDBC-AB2 and RBh 42a) and two are species of *Bacillus* (EBh11 and ESC4). Ramesh *et al*. (2009) reported that species of *Pseudomonas* are the major antagonistic endophytic bacteria possessing different mechanisms of antagonism. All the 12 chitinolytic bacteria except IISR6, PDBC-AB2, and RBh42a (which are rhizobacteria) inhibited all the pathogens under *in vitro* and the per cent inhibition was more than 60% (Table 1).

In the volatile antifungal compound assay, EC18 inhibited the mycelial growth of all the three pathogens. EB65, ESC4, EC2, and RBh42 inhibited the mycelial growth of *R. solani* and *S. rolfsii.* All the isolates inhibited the mycelial growth of *S. rolfsii*. HCN production was observed in EB65, EB69, EC2, EC14, EC18 and IISR6 (Table 2). Howell *et al*. (1988) reported that volatile compounds such as ammonia produced by *Enterobacter cloacae* were involved in the suppression of *Pythium ultimum* induced damping-off of cotton. Volatiles can be an advantageous tool for rhizobacteria because they are small molecules that can easily diffuse through the porous structure of the soil and over great distances in the atmosphere. Volatile compounds produced by antagonistic bacteria are known to diffuse in the soil and bring about control of fungal pathogens. The findings clearly demonstrate that bacterial volatiles add another vital component to the action profile of significant growth inhibiting strategies of endophytic/ rhizobacteria and have important commercial applications.

Utilization of chitin by antagonistic bacteria

To study the ability of the bacterial isolates to utilize chitin as a sole carbon source, the bacterial isolates were inoculated into minimal media with colloidal chitin and pure N-acetyl glucosamine. Results of the primary screening revealed that 12 isolates out of 58 isolates were effective chitinolytic strains. All the chitinolytic isolates except EBh11, ESC4 and ET14 had grown in the MSM medium, with colloidal chitin in the initial transfer. However, all the bacterial isolates showed growth when transferred again to MSM medium with colloidal chitin.

IISR6, EC2 and EC2a showed comparatively better growth than other isolates. All the chitinolytic isolates except ET14 produced colonies when streaked on solid MSM medium with colloidal chitin (Table 3). Chitinolytic properties of these isolates were confirmed by their growth in pure form N-acetyl glucosamine, the monomers of chitin. All of them grew in medium with NAGA as the sole source of carbon thus confirming their ability in metabolizing chitin as the sole source of carbon.

Table 1. *In vitro* inhibition of fungal growth by antagonistic bacteria

Isolates	*M. phaseolina*		*R. solani*		*S. rolfsii*	
	Fungal growth (cm)	Per cent Inhibition	Fungal growth (cm)	Per cent Inhibition	Fungal growth (cm)	Per cent Inhibition
EB65	1.45^{d}	83.89	1.80^{f}	80.00	0.35^{ef}	96.11
EB69	1.80^{d}	80.00	1.45^{fg}	83.89	0.35^{ef}	96.11
EBh11	2.30^{bcd}	74.44	2.75^{d}	69.44	0.40^{ef}	95.56
EC2	2.00^{cd}	77.78	2.35^{de}	73.89	0.65^{de}	92.78
EC2a	1.85^{d}	87.94	1.80^{f}	88.00	0.45^{def}	95.00
EC14	3.30^{b}	63.33	1.55^{fg}	82.78	0.55^{def}	93.89
EC18	3.15^{bc}	65.00	1.85^{ef}	79.44	0.35^{ef}	96.11
ESC4	1.20^{d}	86.67	1.60^{fg}	82.22	0.85^{cd}	90.56
ET14	1.35^{d}	85.00	1.25^{g}	86.11	0.20^{f}	97.78
IISR6	1.90^{cd}	78.89	6.75^{c}	25.00	0.85^{c}	90.56
PDBC-AB2	2.10^{d}	76.67	6.00^{b}	33.33	1.25^{cd}	86.11
RBh42	2.20^{bcd}	75.56	7.15^{b}	20.56	5.90^{b}	34.44
Control	9.00^{a}	0.00	9.00^{a}	0.00	9.00^{a}	0.00
LSD ($P<0.05$)	1.17		0.51		0.41	

*Mean of two replications and diameter of fungal growth after 4 days; experiment was repeated twice; in a column, means followed by the same letter are not significantly different at 5% level determined by LSD ($p<0.05$)

Study was carried out to quantify the amount of chitin released which indirectly indicates the ability of isolates to produce chitinase. Our results showed that the maximum release of chitin was recorded in EBh11 (0.36 µmol NAGA/h) followed by EC18 (0.32 µmol NAGA/h) (Table 3). *Pseudomonas* species are known to produce one or an array of antifungal metabolites, and lytic enzymes such as chitinase and glucanase in culture (Fridlender *et al.,* 1993; Neilsen *et al.,* 1998; Viswanathan and Samiyappan, 2001). Studies on chitinolytic microorganisms have yielded a large increase in knowledge regarding their role in inhibition of growth of fungal plant pathogens. Additionally, the production of chitinase may be part of a lytic system that enables the bacteria to use living hyphae rather than chitin as the actual growth substrate (De Boer *et al.,* 1999).

Inhibition of mycelial growth and sclerotial germination by crude chitinase

Crude chitinase extract from EB65, EB69, EBh11, EC2, EC18, ESC4 and ET14 inhibited mycelial growth of *R. solani* and *S. rolfsii.* Crude chitinase extract from EC14, PDBC-AB2 and RBh42 inhibited the mycelial growth of only *S. rolfsii*. Crude chitinase extract of none of the isolates could effectively lyse the mycelia of *M. phaseolina* which may be due to fast growing strain of fungus coupled with meager chitinase concentration in the crude extract.

Another study was carried out to determine the effectiveness of crude enzyme extract on the germination of sclerotia of two fungi, *viz., R. solani* and *S. rolfsii*. Results revealed that after an incubation period of three days, almost all the isolates were effective against *S. rolfsii* but their effectiveness against *R. solani* was limited. After an incubation of 8 days, crude chitinase extract from EB69 and EC18 recorded maximum inhibition (75.0%, 66.67% respectively) of sclerotia from *R. solani*. Except from EBh11, crude chitinase extract from all other bacterial isolates inhibited sclerotial germination of *S. rolfsii*. Maximum inhibition was recorded by IISR6 (81.82%) and RBh42 (75.0%) (Figure 1 and 2). This indicates the potential of these strains in inhibiting the germination of resting spore. Savithri and Gnanamanickam (1987)

Table 2. Inhibition of fungi by production of volatile antifungal compounds and production of HCN in culture by antagonistic bacteria

Isolates	Growth of fungi* (diameter in cm)			HCN Production after 48 h
	M. phaseolina	*R. solani*	*S. rolfsii*	
EB65	8.8^{a}	0.7^{f}	0.3^{de}	+
EB69	8.5^{ab}	9.0^{a}	0.2^{de}	+
EBh11	8.5^{ab}	9.0^{a}	0.4^{d}	-
EC2	8.5^{ab}	5.3^{d}	0.1^{e}	+
EC2a	8.2^{bc}	3.2^{e}	0.2^{de}	-
EC14	9.0^{a}	9.0^{a}	0.3^{de}	+
EC18	3.1^{e}	1.1^{f}	0.1^{e}	+
ESC4	8.0^{bcd}	3.6^{e}	1.2^{c}	-
ET14	8.0^{bcd}	8.0^{b}	2.7^{b}	-
IISR6	7.5^{d}	8.2^{b}	0.1^{e}	+
PDBC-AB2	7.7^{cd}	7.0^{c}	1.3^{c}	-
RBh42	8.5^{ab}	5.1^{d}	0.1^{e}	-
Control	9.0^{a}	9.0^{a}	9.0^{a}	-
LSD (P<0.05)	0.55	0.42	0.26	

*Mean of two replications and diameter of fungal growth in cm after 4 days; experiment was repeated twice; + Positive for HCN production; - Negative for HCN production; In a column, means followed by the same letter are not significantly different at 5% level determined by LSD (p<0.05)

Table 3. Growth of antagonistic bacteria in MSM media amended with colloidal chitin, pure NAGA (N-acetyl glucosamine) and quantification of chitinase produced by antagonistic bacteria

	MSM with colloidal chitin			MSM with NAGA		Quantity of chitin released
Isolates	Second transfer			First transfer		NAGA (ìmol/h)
	24h	48h	48h*	24h	48h	
EB65	+	+	+	++++	++++	0.07
EB69	+	+	+	++++	++++	0.08
EBh11	+	+	+	++	++	0.36
EC2	+	++	+	++++	++++	0.05
EC2a	+	++	+	++++	++++	0.05
EC14	+	+	+	++++	++++	0.18
EC18	+	+	+	++++	++++	0.32
ESC4	+	+	+	+	++	0.13
ET14	–	+	–	++++	++++	0.10
IISR6	+	+++	+	++++	++++	0.07
PDBC-AB2	–	+	+	++++	++++	0.05
RBh42	+	++	+	++++	++++	0.07
Control	-	-	-	-	-	0.00

+ indicates the degree of growth; - indicates absence of growth; * growth on solid medium MSM-CC; the experiment was repeated twice and the results were consistent

found that soaking sclerotia in a bacterial cell suspension reduced sclerotial germination by 47%. In an another study, sclerotial germination decreased with prolonged incubation in bacterial suspension, with most bacterial strains causing 50–100% inhibition of sclerotial germination after 4 weeks (Vasanthadevi *et al.,* 1989).

Suppression of fungal population in the soil by antagonistic bacteria

In case of EB65 treatment, reduction *M. phaseolina* population and bacterial population was observed after 7 and after 14days. However *R. solani* and *S. rolfsii* population increased at 7th day and again reduced to the initial level after 14 days. Though population of antagonistic bacteria reduced, it was observed that the population was at around 2-7 x 10^6 after 14 days of treatment. Population of all the three fungi increased after 7 days when treated with EB69. After 14 days it was observed that the population of *M. phaseolina* reduced below the initial level. Population of *R. solani* and *S. rolfsii* started declining compared to the population at 7th day. A similar trend in the population of fungi was noticed in treatment with EC21, both after 7 days and after 14 days of experiment. However, population of fungal pathogens increased consistently from the beginning and up to 14 days in control. Treatment with Bavistin provided maximum reduction of population in all the three fungal pathogens (Table 5). In all the treatments, it was observed that the population of the fungi in the antagonist treated soil was lesser than that in the control, both after 7 and 14 days. In some cases the population reduction of fungi is at par with fungicidal treatment, thus proving the efficacy of the antagonists in controlling the fungal population under natural conditions.

Screening of bacteria for the production of growth promoting substances

The amount of IAA produced by the isolates ranged from 0.02µg/ml to 49.51µg/mI which indicates these microbes could be used as plant growth promoters. The capacity to synthesize IAA is widespread among soil and plant associated bacteria. By and large, microorganisms isolated from the rhizosphere and rhizoplane of various crops are more active in producing auxins than those from root free soil because of rich supplies of substrates exuded from roots compared with non-rhizosphere soil (Strzelczyk and Pokojska-Burdzeij, 1984). It has been estimated that 80% of bacteria isolated from the rhizosphere can produce IAA (Patten and Glick 1996; Ahmad *et al*., 2006). All the 58 isolates were screened for their ability to solubilise phosphate. RBa9 had the largest clearing zone of 2.9 cm. It is well known that phosphate solubilising microorganisms

Table 4. Inhibition of mycelial growth by crude chitinase obtained from MSM medium

	Inhibition of mycelial growth (after 2 days)		
Isolates	*M. phaseolina* MF-1a	*R. solani* MF-3b	*S. rolfsii*
EB65	–	+	+
EB69	–	+	+
EBh11	–	+	+
EC2	–	+	+
EC2a	–	–	–
EC14	–	–	+
EC18	–	+	+
ESC4	–	+	+
ET14	–	+	+
IISR6	–	–	–
PDBC-AB2	–	–	+
RBh42	–	–	+
Bavistin (0.2%)			
Control	–	–	–

+ Presence of inhibition zone; - Absence of inhibition zone; each isolate was replicated thrice

Table 5. Population of antagonistic bacteria and fungi (CFU g^{-1} of soil) when introduced into the soil

Antagonists	Pathogens	Fungal population (x 10^3)			Bacterial population (x 10^5)		
		Initial	7 DAT	14 DAT	Initial	7 DAT	14 DAT
EB65	*M. phaseolina*	2.0	1.0	0.8	9000.0	0.6	0.28
	R. solani	1.0	2.1	1.0	9000.0	92.0	27.6
	S. rolfsii	1.0	2.1	1.0	9000.0	10.0	73.6
EB69	*M. phaseolina*	2.0	2.3	1.8	3100.0	120.0	13.2
	R. solani	1.0	6.0	4.5	3100.0	1.0	9.0
	S. rolfsii	1.0	9.2	8.0	3100.0	70.0	73.6
EC21	*M. phaseolina*	2.0	2.2	1.0	100.0	120.0	10.0
	R. solani	1.0	3.1	2.0	100.0	0.4	0.7
	S. rolfsii	1.0	9.0	7.5	100.0	80.0	60.0
Bavistin	*M. phaseolina*	2.0	1.4	1.0	-	-	-
	R. solani	1.0	1.0	0.1	-	-	-
	S. rolfsii	1.0	0.3	0.1	-	-	-
Control	*M. phaseolina*	2.0	7.8	8.5	-	-	-
	R. solani	1.0	9.8	12.0	-	-	-
	S. rolfsii	1.0	10.0	23.0	-	-	-

Values are means of two experiments

in soil solubilize insoluble phosphates mainly by secreting acids into the medium (Dave and Patel, 2003; Chung *et al.,* 2005).

Results of this study indicate that the mechanism of pathogen inhibition by antagonistic bacteria may be through secretion of chitinase and production of volatile antifungal compounds including HCN. All the chitinolytic strains and all the HCN producers except EB113 were very effective against the three fungal pathogens in the bioassay indicating their role in the control of fungal pathogens. Compatible biocontrol agents with different mechanism of action like production of antibiotics, siderophores could be combined with chitinolytic isolates to maximize the biocontrol efficacy. Application of a consortium of PGPR strains would more closely mimic the natural situation and might broaden the spectrum of action to enhance the efficacy and reliability of control (Duffy, 2001). Still, most of the focus has been on free-living rhizobacterial strains, especially to *Pseudomonas* and *Bacillus*. Much remains to be learned from non-symbiotic endophytic bacteria that have unique associations and apparently a more pronounced growth-enhancing effect on host plants (Bais *et al.,* 2004; Chanway *et al.,* 2000). In this direction our study provides a valuable piece of information that endophytic bacteria from various plants could be used as potential biocontrol agent against fungal phytopathogens as well as PGPR as they possess multiple mechanisms of action. Our study concludes that bacteria from endophytic tissues are potential source of chitinolytic bacteria which were promising antagonists against *M. phaseolina*, *R. solani* and *S. rolfsii.* Further, volatile antifungal compounds including HCN contribute to the antagonistic potential of the endophytic bacteria.

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