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Screening of rhizobacterial isolates against soft rot disease of ginger (*Zingiber officinale* Rosc.)

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Abstract

Twenty rhizobacterial cultures including *Pseudomonas fluorescens*, *Enterobacter agglomerans* and *Bacillus* sp. were tested under *in vitro* and *in vivo* conditions to evaluate their efficacy in inhibiting *Pythium myriotylum* and reducing the incidence of soft rot disease in ginger (*Zingiber officinale*). Under *in vitro* conditions, 19 of the tested rhizobacterial cultures significantly inhibited *P. myriotylum.* Evaluation of these cultures under *in vivo* conditions indicated that seven of the isolates were effective in suppressing the pathogen. Five of the isolates when inoculated along with *Glomus* sp. (vesicular arbuscular mycorrhizae) also enhanced root development without disease incidence.

Key words: ginger, *Glomus* sp., *Pseudomonas fluorescens*, *Pythium myriotylum,* rhizobacteria, soft rot.

Introduction

Soft rot incited by *P. aphanidermatum (*Edson) Fitzp*, P. vexans* de Bary and *P. myriotylum* Drechsler is a serious disease of ginger (*Zingiber officinale* Rosc.) in India resulting in more than 80% crop loss (Joshi & Sharma 1982; Sarma 1994). In Kerala, losses as high as 90% during years of heavy incidence was also reported (Rajan & Agnihotri 1989). The disease is manifested initially by foliar yellowing and later water soaked lesions appear on the collar of the pseudostem which extend to rhizomes and leaves resulting in rotting of the entire plant. The disease being both seed and soil-borne, disease management involves measures that effectively suppress the pathogen through cultural, biological and chemical methods (Dake & Edison 1988; Sarma 1994). Use of Plant Growth Promoting Rhizobacteria (PGPR) and Vesicular Arbuscular Mycorrhizae (VAM) are some of the effective methods which can be exploited for disease control. Rhizobacteria are ideal for use as biocontrol agents as they can provide the frontline defence for plant roots against attack by various pathogens (Pal & Jalali 1998). Among the rhizobacteria, *Pseudomonas fluorescens* Migula and *Bacillus* sp. are effective candidates for biocontrol owing to their rhizosphere competance (Ahmad & Baker 1987). These organisms are reported to be very useful as biocontrol agents against several soil-borne pathogens such as *Sclerotinia sclerotiorum* (Lib.) de Bary, *Fusarium oxysporum* Schl., *Rizoctonia solani* Kuhn., *Macrophomina phaseolina* Tassi, *Pythium vexans* de Bary and *Ralstonia solanacearum* Yabuuchi (Singh *et al.* 2003). The inhibitory effect of *P. fluorescens* against *Phytophthora capsici* Leonian causing foot rot

disease in black pepper has been studied in detail (Paul *et al.* 2001). In ginger, attempts have already been made to manage the disease using antagonistic microorganisms and resident and non-resident isolates of *Trichoderma* sp. (Shanmugam *et al.* 1999; Ram *et al.* 2000). The objective of the present study was to screen rhizobacteria for developing an efficient disease management strategy for the management of soft rot disease of ginger.

Materials and methods

Source of rhizobacteria

The PGPR isolates maintained in the Biocontrol Repository of Indian Institute of Spices Research, Calicut, were used for the experiment (Table 1). The isolates were selected based on their inhibitory potential against pathogens such as *P. capsici, P. vexans*, *P. aphanidermatum* and nematodes. Among the 20 isolates selected, three isolates (IISR-6, 13 and 51) had inhibitory effect on *P. capsici* both under *in vitro* and *in vivo* conditions and two isolates (IISR-859 and 853) had nematicidal action on *Radopholus similis* Cobb and *Meloidogyne incognita* Kofoid & Chitwood, the major nematode parasites of ginger (Anandaraj & Sarma 2003; Beena *et al.* 2003).

Table 1. Rhizobacterial isolates used in the experiments

Isolate No.	Species
IISR-6	*Pseudomonas fluorescens*
IISR-13	*P. fluorescens*
IISR-51	*P. fluorescens*
IISR-147	*Bacillus* sp.
IISR-148	*Bacillus* sp.
IISR-149	*Bacillus* sp.
IISR-150	*Bacillus* sp.
IISR-151	*Bacillus* sp.
IISR-152	*Bacillus* sp.
IISR-153	*Bacillus* sp.
IISR-853	*P. fluorescens*
IISR-859	*P. fluorescens*
IISR-906	*B. lentus*
IISR-907	*Bacillus* sp.
IISR-909	*B. polymixa*
IISR-910	*Bacillus* sp.
IISR-912	*Enterobacter agglomerans*
IISR-913	*Bacillus* sp.
IISR-914	*Bacillus* sp.
IISR-915	*Bacillus* sp.

Isolation of pathogen

Pure culture of *P. myriotylum* was isolated from soft rot affected rhizomes of ginger collected from IISR Farm, Peruvannamuzhi, during the monsoon period of 2002 and maintained in Potato Dextrose Agar (PDA) tubes for further use. It was further sub-cultured in PDA for *in vitro* and *in vivo* evaluation.

In vitro *screening*

The isolates selected were screened in initial laboratory tests for their *in vitro* inhibition against *P. myriotylum.* The pathogen was inoculated in the centre of 90 mm petri dish and was streaked with the respective rhizobacterial cultures on either side, 3 cm apart. The plates were incubated under laboratory conditions. The linear growth of the pathogen towards the bacterial growth was measured after 72 h and the percentage inhibition was compared with control using the formula, I= C–T/C x 100, where, I is the per cent inhibition and C and T are the radial growth of the pathogen in control and treatment, respectively.

In vivo *screening-Experiment I*

The efficacy of rhizobacterial cultures was compared with conventionally used fungicide namely, metalaxyl–mancozeb in pot culture experiments in the greenhouse. The study comprised of 22 treatments with three replications in a Randomized Block Design.

Rhizobacterial inoculum preparation

The rhizobacterial cultures were initiated by streaking the stock cultures into nutrient agar plates and incubating at 24°C for 24 h. A loop full of these cultures were transferred to 10 ml sterile distilled water and distributed thoroughly by shaking. One ml of this culture suspension was inoculated to 300 ml of nutrient broth and incubated by shake culture for 48 h. The 300 ml solution was diluted to 1 l and used as the rhizobacterial inoculum for treating the seed as well as for

drenching the soil. These broth cultures had a population of approximately 10^8 ml^{-1}.

Potting mixture preparation

Potting mixture containing sand, soil and farmyard manure in the ratio 1:1:1 was prepared and sterilized by formaldehyde fumigation. A bed of 3 m x 1 m size was made using the potting mixture and drenched with 4% formaldehyde @ 20 l bed^{-1}. The bed was covered with a transparent polythene sheet and the ends were sealed with moistened soil. After 5 days, the polythene sheet was removed and raked to remove excess fumes of formaldehyde. This potting mixture was used for filling the pots (30 cm dia) @ 10 kg pot $^{-1}$ of 30 cm dia pot.

Seed treatment

Rhizomes of required quantity were soaked in respective rhizobacterial suspension/ metalaxyl-mancozeb 0.2% for 1 h and air dried and planted @ 40 g pot^{-1}. These pots were also drenched with the respective rhizobacterial and fungicidal suspensions @ 300 ml pot^{-1} at the time of planting the rhizomes and a repetition of the same treatments was given after 40 days.

Inoculation of pathogen

The pathogen was inoculated to the pots after 1 month of planting of rhizomes. The inoculum was prepared from 72 h old agar culture of *P. myriotylum* grown in 90 mm petri dishes containing 15 ml of PDA macerated with water using a mixer grinder @ 250 ml of water plate^{-1}. This culture fluid was added to the pots @ 100 ml pot^{-1}. The pots inoculated with pathogen alone served as control. An absolute control devoid of any other microbial treatments was also maintained.

Observations

The number of pseudostems infected was recorded at fortnightly intervals and expressed as disease incidence. Roots and soil from the respective treatments were collected and processed for mycorrhizal evaluation (Phillips & Haymann 1970).

In vivo *Experiment II*

The rhizobacterial cultures found effective in *in vivo* Experiment I and the native VAM isolated from the corresponding rhizobacterial treatments of the above were used in *in vivo* Experiment II to study the combined effect of both rhizobacteria and VAM on disease incidence. The experiment consisted of 8 treatments with 3 replications. Rhizobacterial cultures were prepared as described earlier and applied to the sterile potting mixture in polybags of 22 cm x 14 cm size. Spores of VAM were sieved from the respective rhizobacterial treatments and inoculated to the polybag @ 100 spores bag^{-1}. These bags were planted with 20 g rhizomes pre-soaked in the bacterial suspension for 1 h. One week after planting, each bag was inoculated with macerated *P. myriotylum* culture @ of 25 ml bag^{-1}. Observations on disease incidence were recorded from 1 week of inoculation, and after 45 days the plants were uprooted and evaluated for root development.

Results and discussion

In the *in vitro* screening experiment, the percentage inhibition ranged from 0% to 85% in various isolates. Maximum inhibition (84.7%) over control was achieved in IISR-909 (*B. polymixa)* (Table 2).

In the *in vivo* Experiment I (rhizome bacterization followed by soil drenching twice) seven of the isolates were effective in suppressing the pathogen and were on par with each other (Table 3).

The predominant mycorrhizae associated with ginger was *Glomus* sp. (Figs. 1 & 2). Studies on mycorrhizal colonization in root and soil revealed that the isolates IISR-51, 907, 910, 912, 913, 914, 915 and 152 were more effective in enhancing the VAM spore load in the soil indicating enhanced colonization (Table 4).

In the *in vivo* Experiment II, where the selected isolates namely IISR-51, 151, 859, 914, 906 and 915 along with VAM (*Glomus* sp.) were inoculated, a well developed root system without disease incidence was observed

Table 2. *In vitro* inhibition of *Pythium myriotylum* by rhizobacterial cultures

Treatment	*In vitro* inhibition (%)
IISR-6	38.8 b
IISR-13	74.1 hi
IISR-51	73.4 ghi
IISR-853	70.6 fgh
IISR-859	69.9 efg
IISR-906	75.3 i
IISR-907	74.6 i
IISR-909	84.7 j
IISR-910	40.0 b
IISR-912	67.5 def
IISR-913	74.1 hi
IISR-914	0.0 a
IISR-915	73.4 ghi
IISR-147	65.2 d
IISR-149	66.4 de
IISR-150	72.9 ghi
IISR-148	71.8 ghi
IISR-151	75.3 i
IISR-152	47.1 c
IISR-153	71.8 ghi
Control	0.0 a

Values followed by the same letters are not significantly different in DMRT test at P=0.05

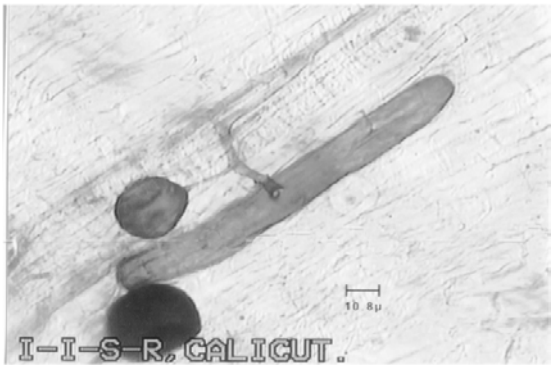

Fig. 1. Vesicular arbuscular mycorrhizae associated with ginger roots

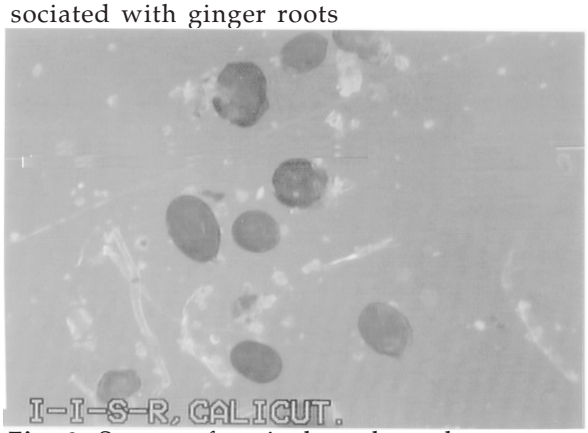

Fig. 2. Spores of vesicular arbuscular mycorrhizae in soil

Table 3. Efficacy of rhizobacterial cultures against *Pythium myriotylum* (*In vivo* Experiment I)

Treatment	Disease incidence (%)
IISR-6	32.2 abcdefg
IISR-13	26.8 abc
IISR-51	4.2 a
IISR-853	33.3 abcd
IISR-859	14.2 ab
IISR-906	3.3 a
IISR-907	45.7 abcd
IISR-909	75.0 d
IISR-910	55.6 bcd
IISR-912	41.7 abcd
IISR-913	20.0 ab
IISR-914	16.4 ab
IISR-915	14.8 ab
IISR-147	33.6 abcd
IISR-149	41.9 abcd
IISR-150	36.2 abcd
IISR-148	33.1 abcd
IISR-151	6.7 a
IISR-152	34.2 abcd
IISR-153	21.4 abc
Metalaxyl-Mancozeb	36.7 abcd
Pythium myriotylum alone	67.1 cd
Absolute control	0.0 a

Values followed by the same letters are not significantly different in DMRT test at P=0.05

Table 4. VAM spore load in the soil in rhizobacterial treatments

Treatment	Spore load (cc^{-1})
IISR-6	32.5 cdef
IISR-13	16.0 fg
IISR-51	61.5 a
IISR-853	21.0 efg
IISR-859	34.0 cde
IISR-906	32.0 cdef
IISR-907	27.0 abc
IISR-909	12.5 bcd
IISR-910	49.0 abc
IISR-912	55.0 ab
IISR-913	53.5 ab
IISR-914	61.0 a
IISR-915	60.0 a
IISR-147	16.5 fg
IISR-149	41.0 bcd
IISR-150	17.5 efg
IISR-148	10.0 g
IISR-151	31.0 def
IISR-152	55.0 ab
IISR-153	18.0 efg
Metalaxyl-Mancozeb	11.0 g
Pythium myriotylum alone	31.0 def
Absolute control	11.0 g

Values followed by the same letters are not significantly different in DMRT test at P=0.05

Table 5. Effect of rhizobacterial isolates and VAM on growth promotion and incidence of soft rot disease (*In vivo* Experiment II)

Treatment	Root length (cm)	Biomass (g)	Disease incidence (%)
IISR-51 + VAM + *Pythium myriotylum*	20.8 c	6.0 a	0.0
IISR-151 + VAM + *Pythium myriotylum*	21.0 c	4.9 b	0.0
IISR-859 + VAM + *Pythium myriotylum*	23.7 b	2.2 d	0.0
IISR-906 + VAM + *Pythium myriotylum*	25.0 a	3.0 c	0.0
IISR-913 + VAM + *Pythium myriotylum*	23.8 d	1.5 e	0.0
IISR-915 + VAM + *Pythium myriotylum*	20.0 b	6.0 a	0.0
Pythium myriotylum	3.9 f	0.2 f	78.8
Absolute control	14.2 e	1.2 e	0.0

Values followed by the same letters are not significantly different in DMRT test at P=0.05

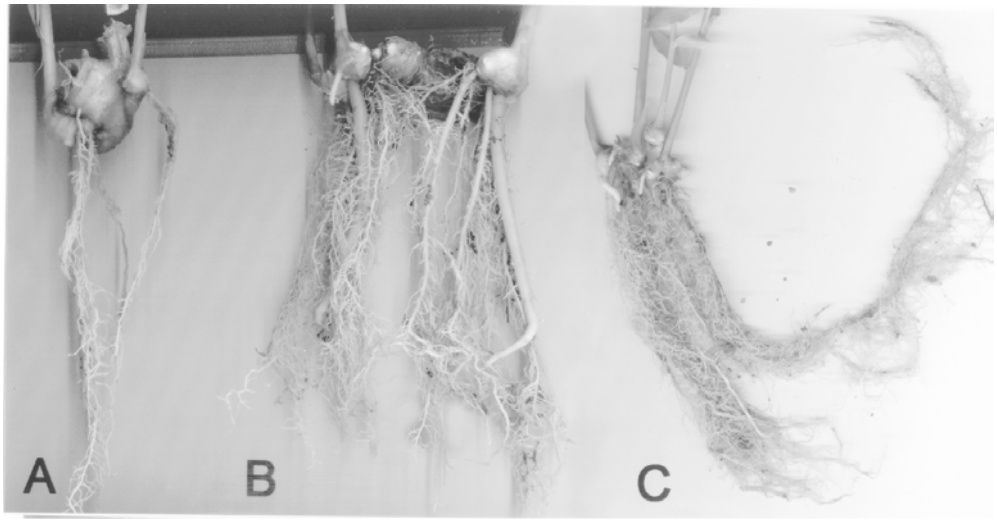

Fig. 3. Enhanced root development in ginger in response to combined application of vesicular arbuscular mycorrhizae and rhizobacteria
(A) = Control; (B) = IISR-51+VAM; (C) = IISR-915+VAM

when compared to control which had poor root development (Table 5 and Fig. 3).

The results of the present investigation indicated the potential of rhizobacteria in suppressing soft rot disease of ginger. The disease suppression may be through direct or indirect interaction. Direct suppression by rhizobacteria could be attributed to the production of iron sequestering siderophores which inhibit the growth of *Pythium* spp. by reducing the availability of iron (Whipps *et al*. 1991) or through antibiosis and HCN production (Paul & Sarma 2003; Sikora *et al.* 1990). Indirectly, rhizobacteria enhance VAM colonization which in turn improve the plant's resistance through enhanced root development and also by way of nematode suppression. Moreover one of the *Pseudomonas* isolates (IISR-859) found effective in suppressing the disease was antagonistic to both *M. incognita* and *R. similis* (Anandaraj & Sarma 2003; Beena *et al.* 2003). The efficacy of fluorescent *Pseudomonas* against *Pythium* sp. have been proved by many workers (Ongena *et al.* 1999; Vogt & Buchenauer 1997) and similar results have been obtained in rice (Sulochana *et al.* 2003), wheat (Mukerjee & Rai 2000) and chillies (Srinivasa & Krishnaraj 1992). In chillies, simultaneous inoculation resulted in increased shoot dry mass, fruit yield, shoot phosphorus content and micronutrient content. The present study indicates the potential of rhizobacteria as a plant growth promoter as well as a bioprotectant in combating soft rot disease of ginger.

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