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Plant growth promoting rhizobacteria mediated biological control of *Sclerotium oryzae* (Cattanoe)

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ABSTRACT

Twenty bacterial isolates were evaluated for their antagonistic activity against Sclerotium oryzae (Cattanoe). The antagonists were selected based on their ability to inhibit the external growth of S. oryzae from infected rice plants. Three bacterial isolates viz., SRR-1, SRR-3 and SRR-6 were identified as potent antagonists of S. oryyzae by dual culture technique. Both SRR-1 and SRR-3 inhibited the mycelial growth of S. oryzae upto the extent of 88% followed by SRR-6 (86.66%). All the fungicides tested showed 100 per cent inhibition of mycelial growth of the pathogen except validamycin which inhibited only 88.88% when compared to control. The potential bacterial bioagent SRR-1 was found to be most compatible with the systemic fungicide thiophanate methyl (95.70%) and also with insecticide cartap hydrochloride (91.69%). Among the three antagonists, the bioagent SRR-1 produced diffusible volatile metabolites which inhibited the pathogen growth upto 100% under in vitro.

Key words: *rice, Sclerotium oryzae, stem rot, fungicides, antagonists*

In India, Andhra Pradesh is one of the important rice producing states in the country and ranks fourth in area (3.94 m.ha) and second in production (19.84 mt) next to West Bengal (www.indiastat.com, 2007-2008). Stem rot of rice, once a minor disease has now become one of the major diseases inflicting heavy losses in most of the Asian countries caused by *S. oryzae* affecting rice production. The rice stem rot fungus was first described from Italy in their sclerotial stage and was named *Sclerotium oryzae* Catt. (Cattaneo, 1876). In India, this disease was reported for the first time and the infected plants produced excessive number of tillers from the base of the stem (Shaw, 1913). In Andhra Pradesh, the stem rot incidence has been increasing from 2005 onwards and it is spreading widely under Godavari Delta, where rice-rice system is followed and the disease causes 18 to 56% yield loss (Naipictuasdharwad, 2009). The management of stem rot of rice by the use of resistant cultivars has not been successful because of lack of an adequate level of host resistance and due to its soil born nature. In the absence of desired host resistance, the disease is currently managed mostly by application of systemic fungicides and insecticides. Conventional management strategies like crop rotation, adjusting the date of planting, cultural methods and soil treatment are not effective (Chellemi *et al.,* 1997). Resistant cultivars are either location-specific or generally not preferred by growers due to low consumer preference. Thus, control of *S. oryzae* seems to be difficult due to lack of universal control treatments.

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that are used for enhancing crop growth and suppressing plant diseases and the native biocontrol agents are a group of PGPR that can promote growth and suppress plant pathogens and their applicability as biocontrol agents has drawn wide attention because of production of secondary metabolites such as siderophores, antibiotics, volatile compounds, HCN, enzymes and phytohormones. Moreover, bio-control agents are not phytotoxic, it may have the growth promoting effect and it causes little disturbance in ecological balance.As most PGPRs show inconsistent performance in the field conditions, there is urgent need for survey of indigenous strains suited

to local conditions. To address this question, the present study was aimed at identification of fungicidal tolerant potential biocontrol agents from rice fields against *S. oryzae* in order to devise strategies for rice stem rot disease management.

MATERIALS AND METHODS

Infected samples and rhizospheric soils were collected from rice fields of Agricultural Research Station, Nellore and Chittoor districts of Andhra Pradesh where the disease incidence was high. The fungal pathogen *S. oryzae* was isolated from the infected leaf sheath by tissue segment method on potato dextrose agar medium (Rangaswamy and Mahadevan, 1999). Composite soil samples were collected from rhizosphere of healthy plants in rice fields infected with stem rot under flooding condition were shade dried and serial dilution technique was followed to isolate the bacterial antagonists (Johnson and Curl, 1977). Dual culture technique was employed to evaluate the antagonistic activity of bacterial isolates against *S. oryzae* under *in vitro* (Morton and Stroube, 1995). Three replications were maintained along with suitable controls. Per cent inhibition of mycelial growth of test pathogen was calculated:

$$I = \frac{C - T}{C} \times 100$$

where,
I = Per cent reduction in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatments

Six fungicides *viz.,* propiconazole (0.10%), hexaconazole (0.20%), validamycin (0.20%), carbendazim (0.10%), thiophanate-methyl (0.10%) and copper oxychloride (0.25%) were evaluated by poisoned food technique (Nene and Thapliyal, 1993) against the pathogen. Six insecticides monocrotophos (0.22%), acephate (0.15%), cartap hydrochloride (0.15%), chlorpyriphos (0.25%), flubendiamide (0.01%) and profenophos (0.2%) were determined by measuring optical density (OD) at 600 nm after 24 hours of incubation with three replications and the nutrient broth without bacteria served as control (Kishore *et al*., 2005).

Modified version of sealed petri plate technique described by Dennis and Webster (1971) was followed to study the influence of volatile substances on the growth of pathogen. The effect of culture filtrate of potential bacterial antagonists on the growth of virulent pathogen was studied for non-volatile compounds as per the method described by Dennis and Webster (1971). Results are expressed as means of inhibition (%) of the growth of fungal pathogens in the presence and absence of any bacterial isolate, and the percent inhibition was calculated.

Talc based formulation of potential biocontrol agent was developed as described by Vidhyasekharan and Muthamilan (1995) and applied to soil by multiplying on FYM @ 100 g pot^{-1} before sowing. For mass multiplication of pathogen, shoots of water sedge (*Typha angustata*) were cut into pieces of 5 cm large were washed and dipped in a solution containing peptone 10g, K_2HPO_4 0.1g, Sucrose 20g and $MgSO_4.7H_2O$ 0.2g per litre and sterilized. The sterilized *Typha* bits were inoculated aseptically with the pathogen and incubated at 28±2°C for colonization and sclerotial formation (Sudhakar, 1996). The potential bacterial antagonist and compatible fungicide was evaluated under glass house on rice cv. NLR 34242 following complete randomized design (CRD). Three replications were maintained for each treatment. The plant yield parameters were recorded. The per cent disease incidence (PDI) was also calculated:

$$PDI = \frac{\text{Number of infected tillers plant}^{-1}}{\text{Total number of tillers hill}^{-1}} \times 100$$

Completely randomized design (CRD) was used for radial growth, per cent disease incidence, poisoned food technique and dual cultural technique. Two-way CRD was used for spectrophotometric method (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

During the survey in the rice fields of Nellore district of Andhra Pradesh where the stem rot incidence high, the symptoms observed after the mid tillering stage were small, blackish, irregular lesions on the outer leaf sheath near the water logged areas. On splitting the infected culm, dark greyish mycelium was found within the hollow stem and numerous small brown sclerotia were seen dotted all over the inner surface. The pathogen *S. oryzae* isolated from infected leaf sheath and was purified by single hyphal tip method and

maintained on PDA. Mycelium was hyaline and irregular and branching occurred near the distal spectrum of cell, approximately at right angle. Constriction took place at the point of origin and formation of septum in the branched hyphae, which are characteristic feature of this fungus. Similar methodology was followed by Tullis (1953), Ali and Singh (1993) for the purification of the pathogen. The fungus perpetuates by producing hard sclerotial bodies and under favorable conditions, the sclerotial bodies germinate and colonize the plant tissue. Twenty native bacterial antagonists were isolated from rhizospheric soils of healthy rice plants and tentatively designated as SRR-1 to SRR-20 and maintained on nutrient agar slants.

All the bacteria tested in this study exhibited antagonistic activities against stem rot fungi *S. oryzae*. Among the 20 bacterial isolates, the antagonists SRR-1 and SRR-3 were superior in suppressing the pathogen in dual culture technique followed by SRR-6 and SRR-2 isolates. The least inhibition was recorded in case of SRR-7 (44.44%) (Table 1 and Fig 1). Our results were in agreement with Elangovan and Gnanamanikum (1992) who evaluated the biocontrol potential of *P. fluorescens* strains against *S. oryzae* in the Chengalpet district of north Tamil Nadu under *in vitro* conditions producing inhibition zones ranging between 93.33% to 66.66%. The bacterial antagonists SRR-1 and SRR-3 suited to native conditions are of prime importance. Moreover, the presence of inhibition prior to any mycelia contact indicates may be due to the release of diffusible components into the medium by antagonistic bacterium.

Application of chemicals to crops in order to prevent or inhibit disease development is a fundamental means of managing diseases caused by fungi. The *in vitro* screening for effective fungicide against pathogen is a simplistic approach to understand the current status

Table 1. Evaluation of the antagonistic activity of bacteria against *Sclerotium oryzae* by dual culture technique under *in vitro*

Isolate	Linear growth of *Sclerotium oryzae* (mm)	Per cent inhibition of mycelia growth of *S. oryzae* over control
SRR-1	10	88.88
SRR-2	13	85.55
SRR-3	10	88.88
SRR-4	45	50.00
SRR-5	14	84.44
SRR-6	12	86.66
SRR-7	50	44.44
SRR-8	45	50.00
SRR-9	45	50.00
SRR-10	35	61.11
SRR-11	40	55.55
SRR-12	25	72.22
SRR-13	15	83.33
SRR-14	30	66.66
SRR-15	40	55.55
SRR-16	35	61.11
SRR-17	40	55.55
SRR-18	40	55.55
SRR-19	35	61.11
SRR-20	30	66.66
Control	90	100
CD (0.05)	5.45	

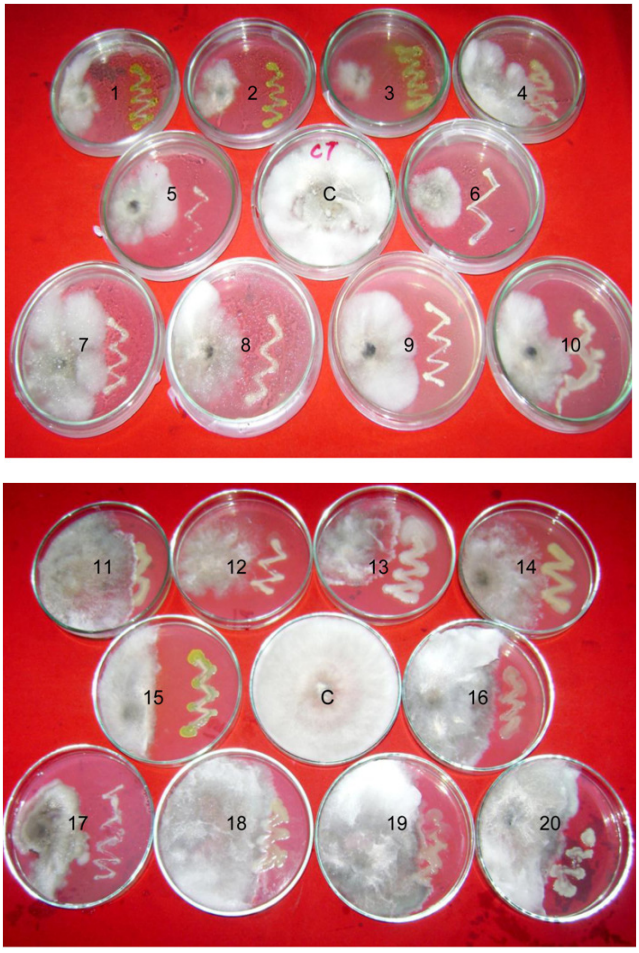

Fig. 1. Evaluation of native potential antagonists by dual culture technique.
The numbers from 1-20 are the antagonistic bacteria SRR-1 to SRR-20.

of the fungicide in particular geographical region to control rice stem rot. Of all the fungicides tested, the systemic fungicides *viz.,* propiconazole, hexaconazole, carbendazim, thiophanate-methyl and the non-systemic fungicide copper oxychloride showed 100 per cent inhibition of mycelial growth of the pathogen except validamycin which inhibited only 88.88% when compared to control (Table 2). Our results were in agreement with that of Ram Singh *et al.* (1988) who reported that carbendazim and thiophanate-methyl effectively inhibited sclerotial germination and mycelial growth of *S. oryzae.*

Table 2. Evaluation of efficacy of fungicides against *S. oryzae* by poisoned food technique

Fungicides	Concentration (%)	Mycelial growth of pathogen(mm)	Per cent inhibition over control
Propiconazole	0.10%	0.0	100
Hexaconazole	0.20%	0.0	100
Validamycin	0.20%	10	88.88
Carbendazim	0.10%	0.0	100
Thiophanate-methyl	0.10%	0.0	100
Copper oxychloride	0.25%	0.0	100
Control	-	90.00	0.0
CD (0.05)	2.70		

The spectrophotometric analysis revealed that the potential antagonist SRR-1 was most compatible with thiophanate-methyl (95.70%) followed by propiconazole (86.86%) and the least compatible with copper oxychloride (67.98%) (Table 3). In the case of insecticides, SRR-1 was more compatible with cartap hydrochloride (91.69%) followed by acephate (83.86%) and least compatible with flubendiamide (59.38%) (Table 4). Similar observations were made by Khan and Gangopadhyay (2008) who reported carbendazim and carboxin were least toxic to *P. fluorescens* strain PFBC-25 whereas captan was most inhibitory to this strain. The biocontrol agent SRR-3 was more compatible with thiophanate-methyl (83.85%) followed by validamycin (81.35%) and less compatible with copper oxychloride (41.24%). In the case of insecticides SRR-3 was more compatible with cartap hydrochloride (84.97%) followed by chlorpyriphos (66.07%) and less compatible with flubendiamide (59.87%) compared to control (100%). In the case of SRR-6 antagonist, higher compatibility was recorded with thiophanate-methyl (91.06%) followed by propiconazole (86.12%) and least compatibility with copper oxychloride (51.13%). In the case of insecticides SRR-6 was more compatible with cartap hydrochloride (89.59%) followed by acephate (79.85%) and less compatible with flubendiamide (61.51%) compared to control (100%). The present findings revealed that the isolate SRR-1 was found to be most compatible antagonistic bacteria with thiophanate-methyl and cartap when compared to SRR-3 and SRR-6.

The potential antagonist SRR-1 produced volatile metabolites which inhibited *S. oryzae* (100%) followed by SRR-3 (86.66%) over control after 7 days of inoculation (Table 5 and Fig 2). The inhibition potential might be due to antibiosis mediated by specific or non-specific metabolites of microbial origin, *viz*., lytic agents, enzymes, volatile compounds or other toxic substances secreted against pathogen. Culture filtrates of the antagonists tested in this study for detection of non-

Table 3. Compatibility of the potential bacterial antagonists SRR-1, SRR-3 and SRR-6 with fungicides

Fungicides	SRR- 1		SRR- 3		SRR-6	
	OD value at 600 nm	Compatibility (%)	OD value at 600 nm	Compatibility (%)	OD value at 600 nm	Compatibility (%)
Validamycin (0.2%)	1.371	75.71	2.085	81.35	2.243	83.95
Copper oxychloride (0.25%)	1.231	67.98	1.057	41.24	1.366	51.13
Carbendazim (0.1%)	1.468	81.07	2.016	78.66	1.924	72.01
Propiconazole (0.1%)	1.573	86.86	1.823	71.13	2.301	86.12
Thiophanate-methyl (0.1%)	1.733	95.70	2.149	83.85	2.433	91.06
Hexaconazole (0.2%)	1.315	72.62	1.807	70.66	2.186	81.82
Control	1.811	100.00	2.563	100.00	2.672	100.00
CD (0.05)	5.93	-	3.45	-	3.35	

Table 4. Compatibility of the potential bacterial antagonists SRR-1, SRR-3 and SRR-6 with insecticides

Insecticides	SRR- 1		SRR- 3		SRR-6	
	OD value at 600 nm	Compatibility (%)	OD value at 600 nm	Compatibility (%)	OD value at 600 nm	Compatibility (%)
Acephate (0.15%)	2.442	83.86	1.924	61.83	2.468	79.85
Cartap Hydrochloride(0.15%)	2.670	91.69	2.644	84.97	2.769	89.59
Flubendiamide (0.01%)	1.729	59.38	1.863	59.87	1.901	61.51
Profenophos (0.2%)	2.220	76.24	2.016	64.79	1.933	62.54
Monocrotophos (0.22%)	1.976	67.86	2.044	65.69	2.225	71.99
Chloropyriphos (0.25%)	2.407	82.66	2.056	66.07	2.080	67.30
Control	2.912	100.00	3.112	100.00	3.091	100.00
CD (0.01)	2.99	-	2.37	-	3.58	
CD (0.05)	2.14	-	1.70	-	2.56	
S. Em±	0.70	-	0.56	-	0.84	

volatile diffusible antibiotic, results were recorded after 7 days of incubation which revealed that none of the bacterial antagonists were able to produce non-volatile metabolites and so unable to inhibit the pathogen.

Soil bacteria are an essential component of the biotic community in rhizosphere and they are largely responsible for ecosystem functioning as they participate in most nutrient transformations (Hackl *et al*., 2004). Although the main diversity of life has been proven to be microbial, the vast majority of soil bacteria still remain unknown due to the fact that only a minor percentage of naturally occurring microorganisms can be cultured (Pace, 1997). From the findings, it is evident that all the treatments were significantly superior over control in reducing the per cent disease incidence. Maximum reduction was observed in the treatment, foliar spray of antagonistic bacteria SRR-1 + foliar spray of fungicide (thiophanate-methyl) at 30 DAT in which PDI of 16.93 per cent was recorded when compared to treatment inoculated control (84.12%). The treatment, foliar spray of antagonistic bacteria SRR-1 + foliar spray of fungicide (thiophanate-methyl) at 30 DAT also recorded maximum grain yield hill^{-1} (29.41 g), straw yield hill^{-1} (36.12 g), panicle weight (12.34 g), panicle length (34 cm), per cent of filled grain (88.57%) and 1000 grain weight (19.09 g) when compared to other treatments (Table 6). Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion (Cleyet-Marcel et al., 2001; Kloepper, 1994; Glick, 1995).

Table 5. Effect of volatile metabolites on the growth of *S. oryzae*

Potential bacterial antagonists	Radial growth of *Sclerotium oryzae* (mm)	Per cent inhibition over control (%)
SRR-1	00	100.00
SRR-2	70	22.22
SRR-3	12	86.66
SRR-6	70	22.22
Control	90	00.00
CD (0.01)	4.87	
CD (0.05)	3.52	
S. Em±	1.17	

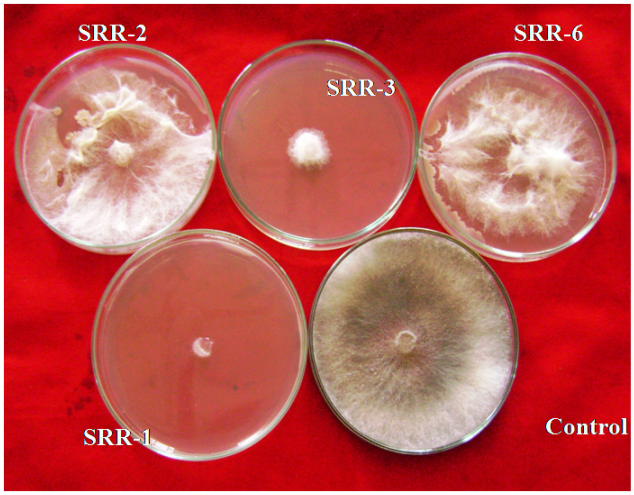

Fig 2. Effect of volatile metabolites by antagonistic bacteria on *S. oryzae*

Inoculation of crop plants with certain strains of PGPR at an early stage of development improves biomass production through direct effects on root and shoot growth. Several reviews discuss specific aspects of growth promotion by PGPR (Cleyet-Marcel et al., 2001; Glick, 1995). This may be due to the fact that crops in complementary rotations can share same or similar populations and the possibility exists of utilizing beneficial relationships between plant and rhizosphere over successive crops to develop a sustainable crop production system. For sustainable crop production the components involved should be eco-friendly so that the beneficial organism would be safe and IDM practices would go a long way helping stabilized crop production (Anahosur, 2001).

From the above investigations, the yield components of rice plant were maximum in the treatment, foliar spray of antagonistic bacteria + foliar spray of fungicide at 30 DAT. It also recorded least PDI, maximum panicle weight, panicle length, per cent of filled grain and 1000 grain weight compared to other treatments.

The present findings are in agreement with the findings of Singh & Sinha (2007) in that the integration of biocontrol agent with compatible fungicide gave significantly higher disease control than obtained by either biocontrol agent (or) fungicide (Singh and Sinha, 2007). Our result explains that significant success in biocontrol is achieved under *in vitro* conditions. The present findings concludes the biological control, integration with fungicidal treatment was found to be a more reliable approach to manage soil borne plant pathogen *S. oryzae* and it is proposed to evaluate the efficacy of fungicidal tolerant potential antagonistic bacteria SRR-1 along with its compatible fungicide thiophanate-methyl under field conditions.

Table 6. Integrated management of *S. oryzae* by potential bacterial antagonist (SRR-1) and compatible fungicide (thiophanate-methyl) under green house conditions

Treatments name	Per cent disease incidence (%)	Grain yield hill^{-1} (g)	Straw yield hill^{-1} (g)	Panicle weight (g)	Panicle length (cm)	Per cent of filled grain (%)	1000 grain weight (g)
Soil application of antagonistic bacteria (SRR-1) at the time of planting	47.61	16.12	20.81	7.31	19.23	58.15	12.51
Soil application of fungicides (thiophanate-methyl) at the time of planting	43.91	17.32	23.12	8.55	22.91	61.25	13.01
Foliar spray of antagonistic bacteria at 30 DAT@4g/L of water	40.20	20.81	28.12	8.59	26.12	70.81	13.98
Foliar spray of fungicides at 30 DAT	35.44	21.15	30.12	9.12	27.31	75.10	14.15
Soil application of antagonistic bacteria (SRR-1) at the time of + Soil application of fungicides (thiophanate-methyl) at the time of planting	28.04	23.51	32.23	10.24	29.98	77.12	16.20
Foliar spray of antagonistic bacteria at 30 DAT@4g/L of water + Foliar spray of fungicides at 30 DAT	16.93	29.41	36.12	12.34	34.00	88.57	19.09
Control(pathogenalone)	84.12	15.28	20.50	6.24	18.00	54.54	11.48
C.D (0.05) 1.67	1.94	2.95	2.03	2.65	3.37	1.52	

*Mean of three replications

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