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Antagonistic activity of plant growth promoting rhizobacteria isolated from Ground nut (*Arachis hypogea*) and Sorghum (*Sorghum bicolor*) against *Sclerotium rolfsii*

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ABSTRACT

Sixty four (64) isolates of plant growth promoting rhizobacteria from Ground nut (*Arachis hypogea*) and *Sorghum (Sorghum bicolor)* belonging to *Bacillus* (20), *Pseudomonas* (20), *Rhizobium* (12) and *Azotobacter* (12) were isolated and identified based on their morphological, physiological and biochemical characteristics using standard methods. These test isolates were screened in vitro for the antagonistic activity and the mechanism involved for their antagonism that is either HCN production or siderophore production against Sclerotium rolfsii

Key words : *Bacillus, Pseudomonas, Rhizobium, Azotobacter and Sclerotium rolfsii.*

Introduction

Plant Growth Promoting Rhizobacteria (PGPR) are reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen, production of siderophores that chelate iron and make it available to the plant root, solubilization of minerals such as phosphorus, synthesis of phytohormones and through biocontrol activity. *Sclerotium rolfsii* Sacc. [*Athelia rolfsii* (Curzi) Tu & Kimbrough] causes the disease known as southern blight in a wide variety of crops. *Sclerotium rolfsii* forms brownish sclerotia that can survive in soil for long periods, frequently tolerating biological and chemical degradation due to the presence of melanin in the outer membrane (Chet, 1975). Among the methods employed to manage *S. rolfsii* are the following: fungicide applications, solarization, use of antagonistic microorganisms, deep plowing, crop rotation, and incorporation of organic and inorganic residues (Punja, 1985). It was attempted in the present study to isolate them from the rhizospheres of ground nut and sorghum and screened *in vitro* for the antagonistic activity against *Sclerotium rolfsii*. The mode of action of PGPR with biocontrol activity is studied with reference to the production of HCN and siderophores (Labuschagne *et al.*, 2011).

Material and Methods

The rhizospheric soil samples (twenty) were collected from fields growing ground nut from different villages of Mahaboobnagar district, Andhra Pradesh, India. Different bacteria were isolated using their respective media; *Rhizobium* was isolated on yeast extract mannitol agar, *Azotobacter* on

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Jensen's medium, *Pseudomonas* on King's B medium and *Bacillus* on nutrient agar. Bacterial cultures were maintained as slant cultures. Isolates of *Bacillus* (20), *Pseudomonas* (20), *Azotobacter* (12) and *Rhizobium*(12) were biochemically characterized by Gram's reaction, carbohydrate fermentation, oxidase test, H_2S production, IMViC tests, NO_2 reduction, starch and gelatin hydrolysis as per the standard methods (Cappuccino and Sherman, 1992).

Antagonistic Activity

Pure isolates of common disease causing soil phytopathogen *Sclerotium rolfsii*, was obtained from the Dept. of Plant Pathology, College of Agriculture, Rajendranagar.

Antagonistc activity was verified by following dual culture technique (Skidmore and Dickinson, 1976). First, the bacterial isolates were streaked on respective media plates and incubated at respective temperature and time. Loopful of each bacterial isolate was streaked on the potato dextrose agar plate at one end, which was pre-inoculated with 5 days old, 5mm mycelial disc of test pathogen at the other end. Control plate was maintained by placing only pathogen mycelial disc in the centre without bacteria. The assay plates were incubated at 28 ± 1°C for 5 days and observations were made on inhibition of mycelial growth of the test pathogens. For each bacterial isolate three replications were maintained with suitable controls.

The per cent growth inhibition over control was calculated by using the formula:

Percent Inhibition =

$$\frac{\text{Growth of Pathogen in control (mm)} - \text{Growth of Pathogen in treatment (mm)}}{\text{Growth of Pathogen in control (mm)}} \times 100$$

Note: In this the percent inhibition in control is taken as zero percent.

Mechanisms Involved For Biocontrol Activity

Siderophore Production

Siderophore production was estimated qualitatively. 0.5% of cell free culture supernatant was added to 0.5mL of 0.2% aqueous Ferric chloride solution. Appearance of orange or reddish brown colour indicated the presence of siderophore (Yeole and Dube 2000).

Hydrogen Cyanide Production

The HCN production was tested by the method of Castric and Castric (1983). First respective media plates i.e., YEMA (*Rhizobium*), Nutrient agar (*Bacillus*), Succinate agar (*Pseudomonas*), Azotobacter medium (*Azotobacter*) were prepared separately and incubated for 24h. After that, 1ml of culture of each test isolate was inoculated on respective media plates separately. A disc of whatman filter paper No.1 of the diameter equal to the petri plate size, impregnated with alkaline picric acid solution (0.5% picric acid (w/v) in 1% sodium carbonate) was placed in the upper lid of the inoculated petri plates under aseptic condition. The control plate did not receive the inoculum. The plates were incubated-upside up at 28±2°C for 48-72h. Change in colour from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

Results and Discussion

On the basis of cultural, morphological and biochemical characteristics a total of 64 soil isolates were grouped into *Bacillus*, *Pseudomonas*, *Azotobacter*, and *Rhizobium* as described by Preeti *et al.* (2011) Characterized 28 bacterial cultures by microscopic and cultural examinations and out of which four isolates were identified as *Pseudomonas spp.* and others were *Bacillus subtilis*. Production of HCN and siderophore are responsible for the biocontrol activity of the isolates. Rakh *et al.* (2011) also reported that *Pseudomonas cf. monteilii 9*, showed highest antagonistic activity against *Sclerotium rolfsii* through the production of diffusible antibiotics, volatile metabolites, hydrogen cyanide and siderophores which affected *Sclerotium rolfsii* growth *in vitro*.

All the 64 plant growth promoting rhizobacterial isolates were examined for the potential to inhibit fungal pathogen *Sclerotium rolfsii* under *in vitro* conditions. Based on both per cent inhibition and inhibition zone out of 64 isolates, 21 isolates exhibited inhibition potential against phytopathogen, while the remaining 43 isolates did not show inhibitory activity against pathogen tested. Each isolate having some per cent inhibition, but some isolates only showed per cent inhibition with inhibition zone.

Antagonistic Activity of *Bacillus* Isolates

Out of 20 *Bacillus* isolates five isolates showed inhibition potential against *Sclerotium rolfsii*, viz. GMdB (37.2%), SMdB (50.12%), GKsB (40.01%), SKsB (38.75%) and GMhB (45.12%). The maximum per

cent inhibition against *Sclerotium rolfsii* was showed by SMdB (50.12%) with inhibition zone 11 mm . The isolates SMdB and GMhB showed significant difference in their inhibition ability. The isolate SMdB (50.12%) showed significantly higher per cent inhibition followed by GMhB (45.12%) against *Sclerotium rolfsii*. The data on both inhibition zone and per cent inhibition of *Sclerotium rolfsii* by *Bacillus* isolates were shown in (Table 1).

Similar results reported by Singh *et al.* (2008) that biocontrol agents *Trichoderma viride, T. harzianum, Coniothyrum minitans, Pseudomonas fluorescens and Bacillus subtilis* used for the control of soil borne pathogens (*Pythium aphanidermatum, Rhizoctonia solani, Sclerotinia sclerotiorum and Sclerotium rolfsii [Corticium rolfsii]*) in Sunflower.

Antagonistic Activity of *Pseudomonas* Isolates

Out of 20 *Pseudomonas* isolates, six isolates showed inhibition potential against *Sclerotium rolfsii*, viz. GMdP (35.67%), GKsP (41.26%), GMrP (43.56%), GMhP (28.00%), GLP (29.12%) and SKcP (28.19%). The maximum per cent inhibition against *Sclerotium rolfsii* was showed by GMrP (43.56%) with inhibition zone 11 mm [Plate 1 (b)]. The isolates GMrP and GKsP showed significant difference in their inhibition ability. The isolate GMrP (43.56%) showed significantly higher per cent inhibition followed by GKsP (41.26%) against *Sclerotium rolfsii*. The data on both inhibition zone and per cent inhibition of *Sclerotium rolfsii* by *Pseudomonas* isolates were shown in (Table 2).

The antagonistic activity of certain *Pseudomonas* isolates against *Aspergillus niger, Sclerotium rolfsii* and *Fusarium udum* was also reported by Satyavani *et al.* (2009). Rakh *et al.* (2011) reported that *Pseudomonas cf. monteilii 9*, showed highest antagonistic activity against *Sclerotium rolfsii*. In dual cultures, the *Pseudomonas cf. monteilii 9* inhibited the *Sclerotium rolfsii* up to 94 % in terms of dry weight through the production of diffusible antibiotics, volatile metabolites, hydrogen cyanide and

Table 1. Antagonistic potential of *Bacillus* isolates on the radial growth of *Sclerotium rolfsii* under *in vitro* conditions

Sl. No.	Isolate	*Inhibition zone (mm)	* Per cent inhibition	
1	GMdB	4.00	37.2	(38.20)
2	SMdB	11.00	50.12	(45.80)
3	GKsB	7.00	40.01	(39.58)
4	SKsB	6.00	38.75	(38.29)
5	GMrB	00	9.25	(17.58)
6	SMrB	00	16.78	(24.16)
7	GMhB	9.00	45.12	(42.63)
8	SMhB	00	17.12	(24.75)
9	GNB	00	25.35	(29.95)
10	SNB	00	8.12	(16.97)
11	GLB	00	10.12	(18.17)
12	SLB	00	20.17	(26.93)
13	GIB	00	19.87	(25.99)
14	SIB	00	17.12	(24.63)
15	GKcB	00	25.00	(30.02)
16	SKcB	00	19.91	(22.39)
17	GPB	00	25.59	(30.05)
18	SPB	00	20.12	(27.02)
19	GSB	00	21.24	(28.22)
20	SSB	00	24.87	(29.57)
21	Control	00	00	(0.00)
	Standard error of mean (SEM)			0.98
	CD @ 0.05 probability			2.93

* Mean of three replications
Figures in the parenthesis are angular transformed values

Table 2. Antagonistic potential of *Pseudomonas* isolates on the radial growth of *Sclerotium rolfsii* under *in vitro* conditions

Sl. No.	Isolate	*Inhibition zone (mm)	*Per cent inhibition	
1	GMdP	8.00	35.67	(36.63)
2	SMdP	00	19.81	(26.08)
3	GKsP	10.00	41.26	(39.98)
4	SKsP	00	9.12	(17.93)
5	GMrP	11.00	43.56	(41.25)
6	SMrP	00	2.12	(8.61)
7	GMhP	6.00	28.00	(27.08)
8	SMhP	00	19.12	(26.19)
9	GNP	00	3.57	(11.09)
10	SNP	00	2.19	(8.66)
11	GLP	7.00	29.12	(32.87)
12	SLP	00	3.56	(10.87)
13	GIP	00	4.21	(12.27)
14	SIP	00	16.81	(24.19)
15	GKcP	00	11.34	(19.68)
16	SKcP	7.00	28.19	(27.06)
17	GPP	00	9.15	(17.66)
18	SPP	00	27.35	(31.58)
19	GSP	00	10.12	(18.65)
20	SSP	00	11.45	(19.73)
21	Control	00	00	(0.00)
	Standard error of mean (SEM)		0.22	
	CD @ 0.05 probability		0.68	

* Mean of three replications
Figures in the parenthesis are angular transformed value

siderophores which affect *Sclerotium rolfsii* growth *in vitro*.

Antagonistic Activity of *Rhizobium* Isolates

Out of 12 *Rhizobium* isolates eight isolates showed inhibition potential against *Sclerotium rolfsii*, viz. GMdR (41.23%), SMdR (95.37%), GKsR (39.12%), GMrR (40.12%), SMrR (80.15%), GMhR (41.3%), GNR (39.45%) and GLR (40.15%) . The maximum per cent inhibition against *Sclerotium rolfsii* was showed by SMdR (95.37%) with inhibition zone 36 mm [Plate 1(c)]. The data on both inhibition zone and per cent inhibition of *Sclerotium rolfsii* by *Rhizobium* isolates were shown in (Table 3). The isolates SMdR and SMrR showed showed significant difference in their inhibition ability. The isolate SMdR (95.37%) showed significantly higher per cent inhibition followed by SMrR (80.15%) against *Sclerotium rolfsii*.

Akhtar *et al.* (2010) studied the effects of *Bacillus pumilus, Pseudomonas alcaligenes,* and *Rhizobium spp.* on wilt disease. They reported that combined application of *B. pumilus and P. alcaligenes* with *Rhizobium spp.* resulted in the greatest increase in plant growth.

Antagonistic Activity of *Azotobacter* Isolates

Out of 12 *Azotobacter* isolates two isolates showed inhibition potential against *Sclerotium rolfsii*.viz. SKcA (35.67%) and GPA (34.12%). The maximum per cent inhibition against *Sclerotium rolfsii* was showed by SKcA (35.67%) with inhibition zone 5 mm [Plate 4.9 (d)]. The data on both inhibition zone and per cent inhibition of *Sclerotium rolfsii* by *Azotobacter* isolates were shown in (Table 4). The isolates SKcA and GPA showed significant difference in their inhibition ability. The isolate SKcA (35.67%) showed significantly higher per cent inhibition followed by GPA (34.12%) against *Sclerotium rolfsii.*

The antagonistic activity of bacteria was attributed mainly due to production of antagonistic compounds, such as antibiotics, siderophores, ammonia, HCN, and hydrolytic enzymes (Baker, 1987). In the present investigation, out of the 21 antagonistic isolates, 18 isolates produced HCN out of which 10, 5 and 3 isolates showed strong, moderate and weak HCN production respectively. The isolates which exhibited strong (+++) HCN production showed very good biocontrol potential against the phytopathogens tested where as the isolates with moderate HCN production showed moderate biocontrol activity. The HCN production by the isolates had a significant positive correlation with the inhibitory activity against *Sclerotium rolfsii*.

Out of the 21 antagonistic isolates, 14 isolates produced siderophores, out of which five isolates produced strongly followed by nine isolates which

Table 3. Antagonistic potential of *Rhizobium* isolates on the radial growth of *Sclerotium rolfsii* under *in vitro* conditions

Sl. No.	Isolate	*Inhibition zone (mm)	*Per cent inhibition
1	GMdR	16.00	41.23 (40.03)
2	SMdR	36.00	95.37 (77.59)
3	GKsR	11.00	39.12 (38.77)
4	GMrR	15.00	40.12 (39.35)
5	SMrR	30.00	80.15 (63.89)
6	GMhR	16.00	41.3 (40.03)
7	GNR	12.00	39.45 (38.98)
8	GLR	15.00	40.15 (39.29)
9	GIR	00	37.8 (37.77)
10	GKcR	00	38.91 (38.60)
11	GPR	00	29.00 (32.91)
12	GSR	00	21.20 (27.50)
13	Control	00	00 (0.00)
	Standard error of mean (SEM)		0.16
	CD @ 0.05 probability		0.68

* Mean of three replications
Figures in the parenthesis are angular transformed value

Table 4. Antagonistic potential of *Azotobacter* isolates on the radial growth of *Sclerotium rolfsii* under *in vitro* conditions

Sl. No.	Isolate	*Inhibition zone (mm)	*Per cent inhibition
1	GMdA	00	26.67 (30.53)
2	SMdA	00	24.25 (27.35)
3	SKsA	00	30.15 (33.24)
4	GMrA	00	29.85 (32.83)
5	SMhA	00	30.25 (33.27)
6	SNA	00	24.35 (27.03)
7	SLA	00	29.90 (26.15)
8	SIA	00	33.56 (34.91)
9	SKcA	5.00	35.67 (36.45)
10	GPA	4.00	34.12 (36.29)
11	SPA	00	32.89 (35.01)
12	SSA	00	30.12 (33.54)
13	Control	00	00 (0.00)
	Standard error of mean (SEM)		0.33
	CD @ 0.05 probability		1.05

* Mean of three replications
Figures in the parenthesis are angular transformed value

showed medium production. The siderophore production by the isolates had a significant positive correlation with the inhibitory activity against *Sclerotium rolfsii.* The role of siderophores production by biological control strains in the antagonism of phyto-pathogens was reviewed by Loper (1988).

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