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RESEARCH ARTICLE

Biological control of sheath blight of rice with fluoresecent pseudomonads

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SUMMARY: In this experiment, a few carefully selected strains of *Pseudomonas fluorescens* particularly those from Karnataka state were evaluated for the suppression of sheath blight of rice. Thirty eight strains of fluorescent *Pseudomonads* were isolated from Karnataka state and twenty five strains were obtained from Tamil Nadu. Total of 63 bacterial strains were used in this study. Eleven strains were screened in the laboratory for their antibiosis towards 3 rice fungal pathogens namely *Rhizoctonia solani, Sarocladium oryzae* and *Pyriclaria oryzae*. All eleven strains also were screened for chitinase production by using a rapid chitinase assay. Very few strains had broad spectrum activity against all 3 rice fungal pathogens. The ones that showed activity against all 3 fungi were strains, T 11, K3, K8,K11 and K13a (5 strains) only 9 of the 63 strains showed activity against any 2 fungal pathogens tested. Citinase production did not always correlate with *in vitro* inhibition of fungi. Eleven of the 63 strains (17.5%) were positive for chitinase production and among the chitinase producers 7 strains did not inhibit any of the 3 fungal pathogen tested. Four other chitinase producers, strains K6, K8, K11 and T14 inhibited at least one of the 3 pathogens. The results from the present study serve to suggest that bacterial strain selection procedures used alone or in combination may be useful in locating strains with superior capabilities for suppressing sheath blight of rice and perhaps will benefit the resource poor rice farmers of the tropics. The present study revealed the probable influence of antagonism, plant growth promotion and induced systemic resistance (ISR) by the mixture of *Pseudomonas* bioformulations in enhancing the disease resistance in rice plants against sheath blight.

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Key Words :
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Rice or paddy (*Oryza sativa*) is the important staple food of the people in the Eastern, Southern and South Eastern part of India. India is the largest rice growing country in the world. The other important rice growing countries are China, Indonesia, Japan, Thailand, Philippines, Pakistan, Brazil, Vietnam, Cambodia, Korea and Taiwan. Over 3000 varieties are under cultivation in different parts of India.

The sheath blight of rice is incited by *Rhizoctonia solani*. Sheath blight has also been reported from Sri Lanka, China, Brazil, Surinam, Venezuela, Madagascar, Philippines, U.S.A. and it has been observed in most rice growing countries (Ou, 1985). In the Philippines it has been estimated that the damage caused by this disease may amount to 25-50 per cent of rice production. In Japan about 120,000 to 1,90,000 hectares have been reported to be infected and a loss of 24,000 to 38,000 tones of rice each year has been lost due (Mizuta, 1956).Subsequent to sheath blight subsequent years, annual crop losses of 20-25 per cent were observed in rice due to *R. solani* (Hori,1960) in Japan. In the U.S., rice sheath blight caused an estimated losses worth 67$ million during 1988 (Anonymous, 1988).

The sheath blight disease development in relation to the physical, environment and the chemical control of the disease has been studied by Rao *et al*. (1979). The disease can be controlled by fungicides or by growing resistant cultivars. However, there are no resistant varieties found till date. Since 1973 commercial stone fruit and rose growers in Australia have protected their crops from crown gall dipping their planting material in a suspension of bacterial cells,

achieving nearly complete control of the disease. It is the first commercial application of a bacterium to control any plant disease (Kerr, 1980).

Sheath blight is one of the most widespread diseases of rice was caused by *Rhizoctonia solani*, *R. solani* causes serious yield loses (*Prasanakumar Reddy et al*., 2009).

EXPERIMENTAL METHODOLOGY

Fungal cultures :

Rice fungal pathogens, *Rhizoctonia solani* (Sheath blight), *Sarocladium oryzae* (Sheath rot) and *Pyricularia oryzae* (blast) were obtained from cultures already available in the laboratory. These pathogens were tested for their pathogenicity in IR50 rice variety raised in the greenhouse.

Inoculum preparation :

Mycelial bits removed from PDA grown plates were inoculated aseptically on to autoclaved mixture of rice hull and rice grain mixed in a 3:1 ratio and contained inoculum of *Rhizoctonia solani* in conical flasks was prepared by growing the fungus in such a rice hull, rice grain mixture for 2 weeks.

The spoonful of the inoculum was placed in the centre of the each hull or the rice plants, 25-30 days near the soil level. Appearance of sheath blight symptoms and disease development were observed. Sheath blight lesions were measured after 7 days or dead seedlings killed by *R . solani* were counted after 7 days.

Collection of samples in rice rhizospheres :

Thirty eight root samples were collected from three locations flooded rice fields. Samples were transported from field on ice and were refrigerated until isolations were made to obtain bacterial isolates.

Isolation of fluorescent *Pseudomonas* strains :

A soil suspension was obtained from each rhizosphere sample by shaking 1 g of rice roots plus tightly adhering soil in 9 ml of 0.1m. A loopful of suspension was streaked on to King's medium B (KB) agar plates and incubated for 2 days at 27^0C. Fluorescent colonies were identified by observing the plates under UV light (366 nm) and single colonies were re-streaked on KB for obtaining pure cultures. The cultures were stored in 5 ml of sterile distilled water in caped test tubes and transfers were made once in 8 weeks. These samples yielded 38 fluorescent *Pseudomonas* strains.

Twenty five other fluorescent strains were also obtained from collections already available in the laboratory and were included in this study. These strains were from Tamil Nadu.

Characterization of the fluorescent bacteria (Buchanan and Gibbons, 1974) :

The strains of fluorescent bacteria were characterized by using microbiological tests as listed in Bergeys manual of determinative bacteriology (Buchanan and Gibbons, 1974).

EXPERIMENTAL FINDINGS AND DISCUSSION

The results obtained from the present investigation have been discussed under following heads:

Pathogenicity of *Rhizoctonia solani* to rice :

Inoculated IR 50 rice plants showed characteristic sheath blight lesions on leaf sheath within 7days under green house conditions. Lesions started to appear in 3-4 days. The leaf sheath which had dark brown lesions became soft and water-soaked. Finally as the disease progressed, the whole plant became yellowish, dry and was killed.

Isolation of fluorescent *Pseudomonas* from rhizosphere sample of rice :

Thirty eight strains of fluorescent *Pseudomonas* were isolated from the 38 rhizosphere sample collected from rice growing areas of Karnataka. In addition, 25 strains obtained from Tamil Nadu were also included. Thus, a total of 63 strains were used in this study.

The distribution of strains obtained were from the following locations :

In Tamil Nadu : Alapakkam (4 strains) Ramapuram (1 strain), Maduravoyal (20 strains).

In Karnataka: Hebbal (16 strains) Maddur (15 strains) Mandya (7 strains).

All these 63 strains were grouped as fluorescent *Pseudomonads* on the basis of production of a fluorescent pigment evident under UV light (366 nm) in the minimal iron containing King's medium B (KB).

Characterization of fluorescent Pseudomonads by siderophore production :

The green fluorescent pigment of the *Pseudomonads* was readily seen on King's B medium (low iron containing medium) but not in $FeCl_3$ - amended KB medium, Fe-KB medium (0.1 µm $FeCl_3$) totally suppressed the fluorescent pigment production (Table 2). Therefore these bacterial strains had the properties of the siderophore producing rhizobacteria.

Results from test for primary characters (Table 1). were formed to characterize 11 efficient strains: 1.Gram reaction: The cells of 11 fluorescent Pseudomonad strains were Gram – negative and were small rods.

Oxidase test :

All strains proved to be positive for oxidase production .They gave a purple colour within a second when the cells were rubbed on a filter paper impregnated with aqueous tetra –methyl-para – phenylene diamine dihydrochloride solution (Table 2).

Table 1 : Determinative scheme for the identification of biovars of *Pseudomonas fluorescens*

Biovar.	Fluorescens on KB	Oxidase	Arginine dihydrolase	Gelatin hydrolysis	Levan formation	Nitrate reduction
I	+	+	+	+	+	-
II	+	+	+	+	+	+
III	+	+	+	+	-	+
IV	+	+	+	+	+	+
V	+	+	+	+	-	-

Symbol : + denotes a positive result , - denotes a negative result

Levan formation :

11 efficient strains were tested for this test. 3 strains were positive for levan formation while 8 strains were negative for levan formation (Table 2).

Gelatin liquefaction :

Of the 11 strains, 8 strains liquefied the gelatin medium and 3 strains of the bacteria did not liquefy the gelatin medium after 5 days incubation. Incomplete liquefaction observed after 7days (Table 2).

Arginine dihydrolase production :

The bacterial strains were positive for arginine dehydrolase and the medium turned pink colour. All 11 strains were positive for the test (Table 2).

Nitrate reduction :

Ten bacterial strains did not reduce nitrate and one bacterial strain reduced nitrate. Nitrite was formed 5 days after inoculation when the medium developed a characteristic pink or red colour, which is an indication for the presence of nitrate (Table 2).

Identifcation :

On the basis of the results from these tests for primary characters and in comparing the results with those published earlier, these 63 fluorescent strains which were gram- negative, oxidase positive, arginine dihydrolase positive and gelatin liquefying were identified as strains of *Pseudomonas* fluorescens. The 3 strains which did not liquefy gelatin belonged to the *P. putida* type (Table 2).

The non-producers of leaven can be grouped into biovar III or V of *P. fluorescens* and those that were negative for nitrate reduction will belong to biovar 1 or V of *P. fluorescens*. Other strains belonged to either biovar II or IV.

Results from the laboratory tests in which all 63 bacterial strains were screened against the rice pathogens, *R solani, S. oryzae* and *P.oryzae* are presented in Table 3. Of the 63 strains

Table 2 : Characterization of *Pseudomonas flourescens* strains from rice rhizosphere

Sr. No.	Bacterial strain	Fluorescens	Oxidase	Argining dihydrolysis	Gelatin hydrolysis	Levan formation	Nitrate reducation	Source (rice cultivar)	Stage of the rice crop	Location
1.	T9	+	+	+	+	+	+	IR - 50	Booting	Tamil Nadu Maduravoyal
2.	T11	+	+	+	+	_	_	IR - 50	Booting	Madhuravoyal
3.	T25	+	+	+	+	_	_	CO.31	Booting	Madhuravoyal
4.	K3	+	+	+	_	_	_	Jaya 4	Booting	Karnataka Maddur
5.	K8	+	+	+	+	_	_	IR 64	Booting	Hebbal
6.	K10	+	+	+	+	_	_	IR 36	Booting	Hebbal
7.	K11	+	+	+	+	_	_	IMA/IR 54	Booting	Hebbal
8 .	K12	+	+	+	_	_	_	Jaya 2	Booting	Maddur
9.	K13a	+	+	+	+	+	_	P/IR 21178/79	Booting	Hebbal
10.	K15a	+	+	+	_	_	_	IMA/IR 46	Booting	Hebbal
11.	K25	+	+	+	+	+	_	Mandya 1	Booting	Mandya

Table 3 : Tests for antibiosis *in vitro* and chitinase production by fluorescent *Pseudomonas* strains isolated from rice rhizosphere

Sr. No.	Bacterial strains No.	Dia. of *R. solani*	Inhibition of *S. oryzae*	Zone (mm) *P. oryzae*	Chitinase
1.	T1	0	62	20	0
2.	T2	0	0	0	0
3.	T3	0	0	0	0
4.	T4	0	0	0	0
5.	T5	0	0	0	0
6.	T6	0	0	0	0
7.	T7	0	0	0	0
8.	T8	0	0	0	0
9.	T9	11	0	15	0
10.	T10	0	0	0	0
11.	T11	3	22	12	0
12.	T12	0	0	0	0
13.	T13	0	0	0	0
14.	T14	0	10	0	+ *
15.	T15	0	0	0	0
16.	T16	0	0	0	++ *
17.	T17	0	0	0	0
18.	T18	0	0	27	0
19.	T19	0	0	0	+ *
20.	T20	0	0	0	0
21.	T21	0	0	0	+ *
22.	T22	0	0	0	0
23.	T23	0	0	0	0
24.	T24	0	0	0	0
25.	T25	9	0	28	0
26.	K1	0	20	0	0
27.	K2	0	0	17	0
28.	K3	3	58	17	0
29.	K4	0	0	0	0
30.	K5	0	0	0	0
31.	K6	0	0	12	+ *
32.	K7	0	0	0	0
33.	K8	17	17	26	++ *
34.	K9	0	0	0	+ *
35.	K10	5	0	0	0
36.	K11	20	5	13	++ *
37.	K12	8	0	0	0
38.	K13	0	0	18	0
39.	K13a	7	12	18	0
40.	K14b	0	0	0	0
41.	K15a	5	0	0	0
42.	K15b	0	0	0	0
43.	K16a	0	0	0	0
44.	K16b	0	0	0	0
45.	K17b	0	55	0	0
46.	K17c	0	0	0	0
47.	K17d	0	30	10	0
48.	K18	0	0	0	0
49.	K18b	0	0	0	+ *
50.	K18c	0	0	0	+ + *
51.	K18d	0	0	0	++ *
52.	K19a	0	0	0	0
53.	K19b	0	0	0	0
54.	K19d	0	20	0	0
55.	K22	0	0	0	0
56.	K24	0	24	0	0
57.	K25	6	30	0	0
58.	K26	6	0	0	0
59.	K27	0	20	0	0
60.	K28	0	68	0	0
61.	K29	0	0	0	0
62.	K30	0	0	0	0
63.	K31	0	13	0	0

Table 3 : Conted....

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+ * = Poor chitinase producers ; Grade 1-2 (on a scale of 0-5).
++ * = High chitinase producers; Grade 3-5 (on a scale of 0-5).

11 strains, inhibited *R.solani* and the inhibition zones ranged from 3 to 20 mm diameter (Table 3). These strains were considered as " efficient strains " against *R. solani* and were evaluated further in greenhouse experiment for suppression of rice sheath blight and were characterized in to biovars of *P. fluorescens* .

In laboratory tests, 16 bacterial strains also inhibited *S. oryzae*, the sheath rot pathogen. Inhibitation zones had 5 to 62 mm diameter. Thirteen of the 63 strains also inhibited the blast pathogen. *P. oryzae*. The inhibition zones ranged from 10 to 28 mm diameter (Table 3).

Chitinase activity :

Of total of 63 bacterial strains that were screened for chitinase production, 52 per cent strains (Table 3) were negative for chitinase activity.

These strains did not show any fluorescens (score = 0) just like the checks. Among the remaining number of strains, 5 strains (%) showed strong chitinase activity (intense fluorescens of score ++) and 6 strains showed weak chitinase activity (mild/ weak fluorescens of score +) (Table 3).

Table 4 : Greenhouse evaluation for suppression of sheath blight by strains of pseudomonas fluorescens

Sr. No.	Bacterial strain	Percentage of germination	Plant height (cm)	Per cent sheath blight suppression
1.	T9	88.50%	13.10%	74.20%
2.	T11	100.00%	15.40%	57.20%
3.	T25	97.00%	18.50%	79.50%
4.	K3	85.70%	11.50%	16.70%
5.	K8	100.00%	19.20%	74.30%
6.	K10	97.10%	18.60%	73.60%
7.	K11	100.00%	15.50%	31.50%
8.	K12	100.00%	17.40%	85.80%
9.	K13a	100.00%	17.70%	65.80%
10.	K15a	100.00%	16.60%	74.30%
11.	K25	100.00%	17.40%	65.80%
12.	control	91.40%	6.40%	0

Two of the large chitinase producers were strains, K8 and K11 inhibited all three pathogens, they caused 17-20 mm Diameter inhibition zones in *R.solani,* 5-17 mm diameter inhibition zone in *S .oryzae* and 13-26 mm diameter inhibition zones in *P. oryzae*.

Greenhouse evaluation :

Results for 11 strains of bacteria that were evaluated on IR 50 seedling for sheath blight suppression are presented.

Of the 11 strains evaluated, 6 strains, KB,K10,K12, K15a, T9 and T25, induced more than 70 per cent suppression of sheath blight others induced sheath suppression that ranged from 16.7 to 65.8 per cent .Strains K12 gave the highest sheath blight suppression of 85.8 per cent .

This study is part of an ongoing research effort to suppress rice sheath blight with bacterial antagonists. In this particular study, the antagonists, used were strains of *Pseudomonas fluorescence* isolated from the rice rhizosphere of parts of Tamil Nadu and Karnataka.

From 38 rice rhizosphere samples, 38 strains of fluorescent Pseudomonads were isolated. This once again goes to prove that strains of *P. fluorescence* are commonly present in the rice rhizosphere also in Karnataka. Earlier, Sakthivel and Gnanamanickam (1987) reported on the incidence of *P. fluorescens* in southern India and the results on isolation in this study agree with the reported results.

Suppression belonged to biovar V. In the earlier studies (Sakthivel *et al.*, 1986) biovar III was dominant and strains of this group suppressed sheath rot well (Sakthivel and Gnanamanickam, 1987).

Out of the 11 strains characterised (Table 2) there were 3 non-lignefiers of gelatin and they obviously belonged to *P. putida* type.

Summary :

In this study, carefully selected strains of *P. fluorescens* particularly those from Karnataka state were evaluated for the suppression of sheath blight of rice. Thirty eight strains of fluorescent Pseudomonads were isolated from Karnataka state and twenty five strains were obtained from Tamil Nadu. Total of 63 bacterial strains were used in this study.

All the eleven strains were screened in the laboratory for their antibiosis towards 3 rice fungal pathogens namely *R.solani, S. oryzae* and *P. oryzae*. All eleven strains also were screened for chitinase production by using a rapid chitinase assay.

Citinase production did not always correlate with *in vitro* inhibition of fungi. Eleven of the 63 strains were positive for chitinase production and among the chitinase producers, 7 strains did not inhibit any of the 3 fungal pathogen tested. Four other chitinase producers, strains K6, K8, K11 and T14 inhibited at least one of the 3 pathogens.

The results of the present study serve to suggest that bacterial strain selection procedures used alone or in combination may be useful in locating strains with superior capabilities for suppressing sheath blight of rice and perhaps will benefit the resource poor rice farmers of the tropics.

The present study revealed the probable influence of antagonism, plant growth promotion and induced systemic resistance (ISR) by the mixture of *Pseudomonas* bioformulations in enhancing the disease resistance in rice plants against sheath blight disease.

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REFERENCES

Anonymous (1988). Sheath blight: Losses cost $67million year**.** *Rice J.,* **41** (4) : 5-8.

Buchanan, R.E. and Gibbons, M.E. (1974). *Bergeys manual of determinative bacteriology* (8th Ed.). Williams and Wilkins Co., Baltimore.

Chen, C.C. and Chu, C.L. (1973). Studies on the control of rice blast and sheath bight with benlate. *J. Taiwan Agric. Res.,* **22**:41-46.

Gupta, C.D., Dubey, R.C., Jang, S.C. and Maheshwari, D.K. (2001). Antibiotic mediated necrotrophic effect of Peudomonas GRC2 againts two fungal plant pathogens. *Curr. Sci.,* **81**:91-94.

Hadwan, H. and Khare, H.S. (1990). *In vitro* interaction of Trichoderma isolate with *Rhizoctonia solani* causing damping off and fruit rot of tomato. *Indian J. Ecol.*, **17**:125-127.

Hori, M. (1986). On forecasting the damage due to sheath blight of rice plants and critical point for judging the necessity of chemical control of disease. *Rev. Pl. Port. Res.,* **2**:70-73.

Kerr, A. (1980). Biological control of crown gall through production of Agrocin. *Plant Dis,* **64**: 25-30.

Mizuta, H. (1956). Studien uber die pilze dor reisoflanze in Japan. *J.Coll. Agric. Tokyo*, **2**: 237-276.

Ou, S.H. (1985). *Rice diseases.* Commonwealth Mycological Institute, Kew Survey, ENGLAND, pp.256-368.

Prasana Kumar Reddy, B., Reddy, K.R.N. and Somasekhhara Rao (2009). Sheath blight disease of *Oryza sativa* and its management by bio control and chemical control *in vitro*. *IEJEAFChe.*, 641-646.

Rao, S.V., Narasimman, V. and Lakshminarayanan, C. (1979). Control measures of sheath blight disease. *J. Plant Pathol.,* **12**: 23:34-39.

Singh, A., Rohila, R., Singh, U.S., Sayary, S., Wilocqut, L. and Duveiller, E. (2002). An improved inoculation technique for sheath blight of rice caused by *Rhizoctoni solani*.*Canadian J. Plant Pathol.*, **24**:65-68.

Sakthivel, N. and Gnanamanickam, S.S. (1987). Evaluation of *Pseudomonas fluorescens* for suppression of sheath rot disease and for enhancement of grain yields in rice, *Oryza sativa* L. *Appl. Environ.,* **53**: 2056-2059.

Sakthivel, N., Sivamani, E., Unnamalai, N. and Ganamanickam, S.S. (1986). Plant growth – promoting rhizobacteria in enhancing plant growth and suppressing plant pathogens. *Curr. Sci.*, **55**: 22-25.

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