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RESEARCH ARTICLE

Biological control of rhizobacteria against rice diseases caused by *Rhizoctonia solani* (Sheath blight) and *Sarocladium oryzae* (Sheath rot)

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Abstract

Sheath blight and Sheath rot of rice caused by *Rhizoctonia solani* and *Sarocladium oryzae* is an economically important disease causing rigorous yield losses. Biological control of the disease using plant growth promoting rhizobacteria (PGPR) is a potential substitute to the presently available chemical control methods. The present investigation was aimed to study the plant growth promoting (PGP) activity and antagonistic activity of two rhizobacteria *Pseudomonas fluorescence* and *Rhizobium* sp., isolated from rhizosphere area of rice, against two major rice pathogens. In the present work IAA (Indole acetic acid production) was found maximum amount in *Pseudomonas fluorescence* (30 mg / ml) and trace amount in *Rhizobium* sp. Maximum amount of Siderophore production was noticed in both isolates. *Pseudomonas fluorescence* showed positive result for the HCN production but *Rhizobium* sp., was found to be negative and both bacterial isolates solubilized phosphate in very effective manner. Both rhizobacteria were found to produce ammonia and catalase. The cross streak studies revealed that the complete inhibition of mycelia growth of *Rhizotonia solani* (85%) and partial inhibition of *Sarocladium oryzae* (45%) against two rhizobacteria. Antifungal compound extracted from both rhizobacteria were found to exhibit maximum antagonism against rice pathogens. This result concluded that both PGPR's can be used as best bio-control agent against rice pathogens instead of hazards chemicals.

INTRODUCTION

Rice (Oryza sativa) is the most vital food of the people in the eastern, southern and south eastern parts of India. Various diseases in rice causes noticeably affect of crop production. Rice sheath blight caused by *Rhizoctonia solani* is a global production constraint incurring heavy economic losses. Grain losses to an extent of 40% are reported annually with the disease (Tan Wan Zhong et al., 2007). Various review suggest that the sheath rot of rice caused by *Sarocladium oryzae* has been reported to cause up to 1.7 percent loss in grain yield with one percent disease intensity in uplands. The disease affects the quality of the rice grains and severe infection by this disease reduced the yield. To control these severe diseases problems farmers apply various hazardous chemicals. These lead to dramatic environmental problems. The use of microbes as biocontrol agents, get very significant place because of hazardous effects of chemicals. Several bacterial strains were found to possess the ability to protect rice plants from diseases such as blast, sheath blight, sheath rot and stem rot diseases (Vasantha Devi et al., 1989). Krishnamurthy et al., 1998 and Pathak et al., 2004, reported that the PGPR strains are known to colonize and survive both in the rhizosphere and on the phyllosphere. Besides, these PGPR also contribute to enhanced growth of the seedlings, induction of systemic resistance against diseases and thereby in yield increase. For a PGPR to be effective under field conditions, the key is to characterize the strain for plant growth promoting activity and disease suppressing features. Moreover, knowledge on the exact mode of action is essential for devising effective disease management strategies (Renwick et al., 1991). The present study is aimed at *in-vitro* screening of PGPR strains for their plant

growth promoting activity, and its antagonism against *Rhizoctonia solani* and *Sarocladium oryzae*, and to identify superior strains for further use in field conditions for sheath blight and sheath rot management as an alternate or supplement to presently available chemical control methods.

Materials and Methods

Organisms used

Rice pathogens, *Rhiozctonia solani* causal agent of sheath blight and *Sarocladium oryzae* causal agent of sheath rot were obtained from Plant Pathology Laboratory of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The cultures were grown on PDA at 28±1ºC for 7 days and maintained in PDA slants. The bio-control agents used in the study were isolated from rhizospheric soil of rice and characterized by standard procedure. Two isolates used for present study were *Pseudomonas fluorescence* and *Rhizobium* sp, it was maintained on King's B agar and Yeast extract mannitol agar (YEMA) media, and maintained pure culture in Nutrient agar slants at 4^0 c.

Study on Plant growth promoting activity

Indole acetic acid (IAA) production

As per the method described by Patten and Glick., 1996, the bacterial cultures *Pseudomonas fluorescent* and *Rhizobium* sp, were grown in Trypticase soy broth and YEMB with tryptophan (100 microgram per ml) and incubated at 30^0c for 3 days. These cultures were centrifuged and to the supernatant add 2ml of salkowsky reagent and incubated for 30 minutes at room temperature. The presence of Pink color indicates the production of IAA, colorimetric reading of absorbance were taken at 530nm.

Hydrogen Cyanide (HCN) production

Cyanide production was detected as described by Bakker et al., 1987. A change in colour from yellow to orange- brown on the filter paper indicated cyanide production.

Siderophore Production

Bacterial cultures, *Pseudomonas fluorescence* and *Rhizobium* sp., were grown on King's B broth and YEMB, incubated at 30^0c. The 3 day old cultures were centrifuged at 9000 rpm for 20 minutes, supernatant was collected and added equal amount of 70% acetone and again centrifuged at 9000 rpm for 20 minutes. The supernatant was taken and equal amount of diethyl ether was added and centrifuged at 9000rpm for 20 minutes. The resulting supernatant contains Siderophore. It was confirmed by performing Thin layer chromatography (TLC) along with proper standard.

Phosphate solubilization

The isolates were screened for phosphate solubilization as per methodology described by Gupta et al., 1994. Pikovskaya agar medium was used for point inoculation and incubates it at 30±0.1 °C for 3-4 day. Zone around the colony indicate the phosphate solubilizing capacity of isolates. Solubilization efficiency was calculated by using standard formula (Nguyen et al., 1992).

$$\text{Solubilization efficiency} = \frac{\text{Diameter of zone of inhibition}}{\text{Growth diameter}} \text{ x } 100$$

Ammonia production

Both the bacterial isolates were tested for the production of ammonia as described by Cappuccino and Sherman 1992. Overnight grown bacterial cultures were inoculated in 10 ml peptone broth and incubated at 30±0.1°C for 48 h in incubator shaker. After incubation 0.5 ml of Nessler's reagent was added. The development of faint yellow to dark brown color indicated the production of ammonia.

Catalase activity

Catalase test was performed by 3% hydrogen peroxide, it was added to 48 hr old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The effervescence indicated catalase activity (Cappuccino and Sherman 1992).

Antifungal assay

Cross streak method

The antagonistic properties of PGPR strains was tested against *Rhizoctonia solani* and *Sarocladium oryzae* on Tryptic Soy Agar (TSA) plates by adopting little modified method of Gupta et al., 2001. An agar block of 5 day old culture of test pathogen was placed in the centre of plates containing TSA. A loopful of 24 h old culture of

PGPR strain was then streak inoculated at either sides of pathogen disc at a distance of 2 cm apart. The fungal pathogen culture inoculated centrally on TSA plates, and plates devoid of PGPR's, served as control and the plates were incubated at 25±1ºC for 5 days and inhibition of the colony growth of the test pathogen was measured. Percentage inhibition rate of mycelial growth was determined by using the following formula

$$\%\ \text{of inhibition} = (C - T)/C \times 100$$

Where, C is the growth of fungus in control plate and T is the growth of fungus in treatment.

Extraction of antifungal compound

Antifungal compound was extracted by modified method of Selvaraj et al., 2011**.** A loopful of actively growing *Pseudomonas fluorescence* and *Rhizobium* sp were inoculated into pigment production broth and after 5 days the broth was centrifuged at 5000 rpm for 30 minutes. The supernatant was adjusted to pH 2.0 with concentrated hydrochloric acid and extracted with equal volume of benzene. The benzene layer was evaporated in a water bath and the residence was re-suspended in 0.1N sodium chloride and used as curd antifungal extract.

Effect of antifungal extract on fungal biomass

100 ml potato dextrose broth (PDB) was inoculated with 5 day old culture of pathogens separately and treated four flask each with 3ml of antifungal compound extracted from *Pseudomonas fluorescence* and *Rhizobium* sp, remaining two flask without antifungal compound act as control and incubated at 28±2ºC for 4- 5 day and took dry weight of treated and control fungal matt with whatman no.1 filter paper and its percentage of inhibition was calculated using standard formula.

$$\%\text{of inhibition} = \frac{C - T}{C} X100$$

Where, C is the growth of fungi in control plate and T is the growth of fungi in treatment.

Result and Discussion

The control of soil- borne pathogens is difficult because of their ecological behaviour, their extremely broad host range and the high survival rate of resistant forms such as chlamydospores and sclerotia under different environmental conditions. Many researchers have mentioned that biological control offers an environmentally friendly alternative to protect plants from soil borne pathogens (Whipps, 2001). Present study describes the isolation of antagonistic bacteria, *Pseudomonas fluorescence* and *Rhizobium* sp., against rice pathogen and presents a preliminary characterization of the mechanism of antagonism, by *in-vitro* techniques.

Plant growth promoting characteristic of both the rhizobacteria used in study was presented in Table 1. In current study *Pseudomonas fluorescence* was found to be positive for HCN production so it act as inducer of plant resistance and *Rhizobium* sp, was found negative for HCN production even after 72 hours of incubation. The IAA production was maximum in *Pseudomonas fluorescens* (30.00 mg/ml) compared to *Rhizobium* sp., (7.00 mg/ml), it produce only trace amount. In the present work, *Pseudomonas fluorescence* produced high level of siderophore compared to *Rhizobium* sp., siderophore producing bacteria are good candidates for plant growth promotion, especially in neutral to alkaline soil. A specific spot from the extracted siderophore preparation was found corresponding with the standard spot with the same Rf value. *Pseudomonas fluorescens* showed maximum phosphate solubilization zone (10mm) in Pikovsakaya medium and *Rhizobium* sp. showed (7 mm) zone diameter. Both isolates were found to produce ammonia and catalase.

HCN production by rhizobacteria has been postulated to play an important role in the biological control of pathogens (Voisard et al., 1989). Frankenberger et al., 1989, Glick et al., 1995, and Xie et al., 1996, stated that the high level of IAA production in *Pseudomonas fluorescence*. Thomas et al., 1996, described that IAA have been shown to be critical to biocontrol or growth-promoting activity of numerous PGPR strains. As that of current work Kumar et al., 1992, proved that high amount of siderophore production in Pseudomonadas and its inoculation of chickpea and soybean seed resulted in increased seed germination, growth and yield of plant. Ajay Kumar et al., 2012 stated the result which is similar to present work, the isolate FBJ6 (*Pseudomonas* sp.) showed highest phosphate solubilization zone (20 mm) in PVK agar, ammonia production and catalase activity. He stated that the activities must lead to high resistant to environmental, mechanical and chemical stress.

In cross streak assay, *Pseudomonas fluorescens* was found to be effective in inducing growth retardation of both pathogens, shown in figure 1. The maximum inhibition of *Rhizoctonia solani* (85%) and partial inhibition of

Sarocladium oryzae (45%) was observed in plate cross streaked with *Pseudomonas fluorescens*. In case of *Rhizobium* sp., partially inhibition was found against both the phytopathogen but not that of *Pseudomonas fluorescens*. This result is in agreement with Tjamos et al., 2004. Similar to our work Prasanna Reddy, et al., 2009 reported that *Pseudomonas fluorescens* was found to effectively inhibit (55-85%) the mycelia growth of major fungal pathogens.

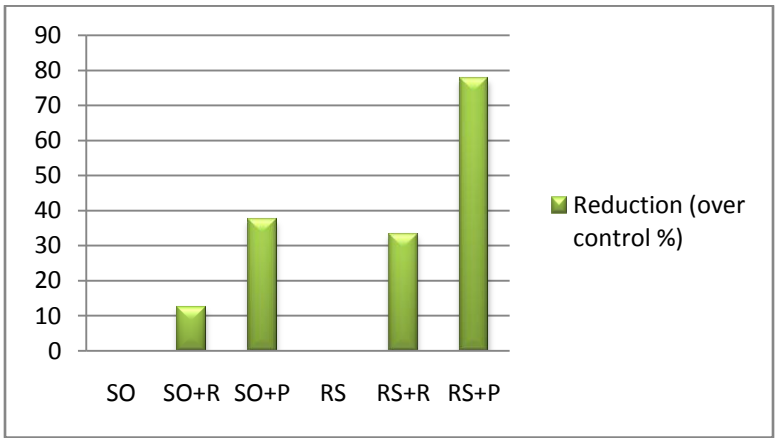
As a part of the study maximum inhibition of fungal biomass was observed with crude antifungal compound from *Pseudomonas fluorescens* against *Rhizoctonia solani* was found to be 78% and in *Sarocladium oryzae* the rate of inhibition was found to be 37.5%, but comparatively *Rhizobium* sp., shows less rate of inhibition on both pathogenic fungi, it was presented in Table 2.

Table 1: Plant growth promoting activity of rhizobacteria

Test	Pseudomonas fluorescencs	Rhizobium sp.
Hydrogen cyanide production	+++	-
Indole acetate acid production	+++ (30 mg/ml)	+ (7mg/ml)
Siderophore production	+++	+
Phosphate solubilization	+++ (10mm)	++ (7mm)
Ammonia production	+++	+++
Catalase production	+++	+++

+++ - maximum production, ++- moderate production, +- less production, - - no production

Figure 1: Mycelial growth reduction of phytopathogens with biocontrol agents in cross streak assay



SO- S. oryzae, R- Rhizobium sp, P- P. fluorescence, RS- R. solani

Table 2: Inhibition rate of antifungal compounds on fungal biomass

S. No	Phytopathogen	Antagonistic bacteria	Dry weight of fungal Mycelium (grams)	% of inhibition
1.	*Sarocladium oryzae*	-	0.8	-

2.	*Sarocladium oryzae*	*Pseudomonas fluorescens*	0.5	37.5%
3.	*Sarocladium oryzae*	*Rhizobium* sp.,	0.7	12.5%
4.	*Rhizoctonia solani*	-	0.9	-
5.	*Rhizoctonia solani*	*Pseudomonas fluorescens*	0.2	78%
6.	*Rhizoctonia solani*	*Rhizobium* sp.,	0.6	33%

Mean of three replicates

Conclusions

The results presented through this manuscript suggested that the utilization of *Pseudomonas fluorescens* and *Rhizobium* sp., for the control of Sheath rot and Sheath blight were a promising strategy for the management of the disease in field conditions. Further investigations on the type of antimicrobial components and field experiments will make both the rhizobacteria as one of the most suitable candidates in suppressing the phytopathogenic fungi and facilitate to replace hazardous chemicals

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