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Research Article

IN VITRO EFFICACY OF INDIGENOUS *PSEUDOMONAS FLUORESCENS ISOLATES* AGAINST *RHIZOCTONIA SOLANI*

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ABSTRACT

Five different isolates of *Pseudomonas fluorescens* were isolated from rhizosphere soil of rice plant collected from different localities of Assam *viz.*, Darrang, Jorhat, Biswanath Chariali, Titabar and Dibrugarh. These isolates were designated as Pf1 (Darrang), Pf2 (Jorhat), Pf3 (Biswanath Chariali), Pf4 (Titabar) and Pf5 (Dibrugarh) based on the place of collection. The antagonistic potential and other characteristics of the five isolates of *P. fluorescens* were studied in detail for colony characters, colony colour, growth type, morphology of cell, fluorescence and biochemical tests. In biochemical tests it was observed that all the isolates of *P. fluorescens* produced similar results with regard to oxidase test (positive), gelatin liquefaction (positive), catalase test (positive), nitrate reduction test (positive) and starch hydrolysis (negative). The fluorescent pigments produced by the isolates colleted from Dibrugarh and Jorhat were much higher than the isolates collected from other three localities. The isolates were tested for their *in vitro* antagonistic activity against *R. solani*. The isolate Pf 2 isolated from Jorhat have shown 40.66 per cent inhibition compared to control. The isolate Pf2 was found to be highly effective in reducing the sclerotia production in 88.64 per cent and the dry weight reduced in 82.72 per cent in 4 per cent MSM as compared to control may be due to the more antagonistic potential due to production of higher amount of antibiotic compounds *viz.,* lytic enzymes, such as chitinase and β-1,3-glucanase in culture, and induced chitinase. The results offer a scope for utilizing the antagonist in organic farming also in an IDM module which managed the rice sheath blight disease.

INTRODUCTION

Sheath blight caused by Rhizoctonia solani Kuehn [Syn. Corticium sasaki (shira)] Matsomoto = Thanatephorus cucumeris (Frank) Donk] occurs in almost all rice growing tracks of the world (Ou, 1972). It is a serious disease of rice in the eastern and south eastern countries of the world (Dasgupta, 1992). Introduction of high yielding, high fertilizer responsive varieties and changed cultural practices have contributed to wide spread occurrence with higher severity of the disease in India and all the states of North East India. It is now considered as a major disease of rice in India (Roy, 1987). Yield loss estimated to be in around 5.2 to 50 per cent (Roy, 1979) due to this disease where the loss varies based on degree of susceptibility of the particular variety and crop stages at which disease appears (Ali and Hussain, 1997). Currently, the disease is managed mostly by application of systemic fungicides and antibiotics (Dev and Mary, 1986). No genetic resistance has been reported for this disease and all the rice cultivars are susceptible to the pathogen. Indiscriminate use of chemical is also not environmentally safe to beneficial soil organism and human being besides inducing resistance in pathogen towards chemical. The bacterial antagonists have the twin advantage of faster multiplication and higher rhizosphere competence hence, *P. fluorescens* have been successfully used for biological control of several plant pathogens (Ramamoorthy *et al*., 2002) and biological control using PGPR strains especially from the genus Pseudomonas is an effective substitute for chemical pesticides to suppress plant diseases (Compant *et al*., 2005).

The present study was aimed at isolation, characterization, *in-vitro* screening of several rhizosphere isolates of P. *fluorescens* from rice seedlings against *R. solani.* Also studies were conducted to determine the sensitivity of sheath blight pathogen to fungicides as well as to the crude metabolites of *P. fluorescens* in order to devise strategies for rice sheath blight disease management.

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MATERIALS AND METHODS

Isolation and characterization of Pseudomonas fluorescens

Five isolates of *P. fluorescens* were isolated from rhizosphere of rice plants grown in different localities of Assam on King's B medium (King *et al*., 1954). Isolation of *P. fluorescens* was made by following the serial dilutions and pour plate method using the specific King's B medium. Bacterial colonies that have exhibited fluorescence at 365 nm were selected and purified for further studies.

Pour plate method

King's B medium, a selective one (Kings *et al*., 1954) was used for the isolation of *P. fluorescens*. One ml of soil suspension from aliquot dilutions (10^5 to 10^8) was aseptically added to sterile Petri plates containing twenty ml of sterile medium and incubated at 28 ± 2^0 C for 48 h. After incubation well separated individual colonies with yellow green and blue white pigments were marked and detected by viewing under UV light. The individual colonies were picked up with sterile loop and transferred to fresh King's B slants and the pure cultures so obtained were stored in refrigerator at 4°C for further use.

Characterization of P. fluorescens isolates

Morphological characterization

Pure cultures of the selected isolates were streaked on King's B agar Petri plates separately for colony development. The individual colonies were examined for shape, size, structure of colonies and pigmentation.

Fluorescent pigment

The test tubes containing sterilized Kings B medium were inoculated with the isolate of *Pseudomonas* sp. incubated for five days and observed. Yellowish green fluorescent pigment observed under UV light (365 nm) indicated positive results.

Physiological characterization

Growth at 4°C

Twenty four hours old culture of the test organisms were spotted on the King's B plates and incubated for 24 to 48 hours at 4°C and the observation on growth was recorded at the end of the incubation period

Growth at 42°C

Twenty four hours old culture of the test organisms were spotted on the King's B plates and incubated for 24 to 48 hours at 42°C and the observation on growth was recorded at the end of the incubation period.

Biochemical tests for P. fluorescens

For the identification of *P. fluorescens*, certain biochemical tests were conducted according to Bergey's Manual for Determinative Bacteriology (Breed et al., 1989).
Gram staining, Starch hydrolysis, Oxidase test, Catalase test, Denitrification, Gelatin liquefaction were done against all the five isolates by using standard procedure.

In vitro evaluation of P. fluorescens isolates against R. solani

Isolates of *P. fluorescens* were isolated using King's B medium. Colonies that have shown fluorescence at 365 nm were selected, purified and used for our studies. These isolates were tested for their *in vitro* antagonistic ability against rice sheath blight pathogen *R. solani* by dual culture technique (Rabindran and Vidyasekaran, 1996). Bacterial isolate was streaked at one side of petridish (1 cm away from the edge) containing PDA. Five mm mycelial plug from seven-day-old PDA cultures of rice pathogens were placed at the opposite side of petridishes (90 mm) perpendicular to the bacterial streak. Petri dishes were then incubated at 28±2ºC for 5 days. Petridishes inoculated with fungal discs alone were served as control. The petriplates were arranged in a completely randomized block design and each treatment was replicated five times. Observations on percent inhibition and mycelial growth of test pathogens were recorded and percent inhibition of pathogen growth was calculated.

In vitro antagonistic effect of different isolates of P. fluorescens on sclerotia production of R. solani in 4% MSM (W/W)

Three numbers of 5 mm mycelia disc from seven-day-old PDA cultures of *R. solani* were placed in the Erlenmeyer's flask required size of 500 ml and 1 ml suspension *of P. fluorescens* (10^{-6}) dilution was added to the Erlenmeyer's flask. The flask were arranged in a completely randomized block design and each treatment was replicated five times. The flask were kept in an incubator at 28±2ºC for 30 days. Observations were recorded on number of sclerotia produced in each flask and their dry weight.

Effect of volatile compound of P. fluorescens on growth of R. solani

P. fluorescens was streaking in the petri dish containing King's B media and *R. solani* was inoculated in PDA media, then both the plates were joined together inverted to each other and incubated at 28±2ºC for 5 days. Petridishes inoculated with fungal discs alone were served as control. Observation of the growth of *R. solani* was recorded (Claydon *et al*; 1987).

RESULTS AND DISCUSSION

On the antagonistic potential and other characteristics, five isolates of *P. fluorescens* were studied in detail for colony, colour, growth type, fluorescence, and cell shape. All the five isolates were designated as Pf1(Darrang), Pf2(Jorhat), Pf3(Biswanath Chariali), Pf4(Titabar) and Pf5(Dibrugarh) which produced different colonies and rod shaped cell. In physiological test it was found that all the five isolates were able to grow at 4°C but not able to grow at 42°C. In biochemical tests it was observed that all the isolates of *P. fluorescens* produced similar results with regard to oxidase test (positive), gelatin liquefaction (positive), catalase test (positive), nitrate reduction test (positive) and starch hydrolysis (negative).

Dual culture studies revealed that the mycelial growth of *R. solani* in plates ranged from 5.34 to 7.67 cm as against control (9.0 cm). Among the bacterial strains, the Pf2 effectively controlled the pathogen with a mycelial growth of 5.34 cm and an inhibition of 40.57% (Table 1).

Table 1 In-vitro antagonistic effect of *P. fluorescens* on mycelial growth of rice sheath blight pathogens.

Pseudomonas fluorescens **(Different district)**	**Mycelia growth (cm)**	**Inhibition over control (%)**
Pf1 (Darrang)	6.81[b]	24.33
Pf2 (Jorhat)	5.34[d]	40.66
Pf3 (B.N.Chariali)	6.58[bc]	26.88
Pf4 (Titabar)	6.50[bc]	27.77
Pf5 (Dibrugarh)	6.40[c]	28.88
Control (*R.solani* alone)	9.00[a]	0.00

Antagonistic behaviour of *Pseudomonas fluorescens* on reducing production and weight of sclerotia of *R. solani* was studied on maize-meal-sand-medium. The production as well as the dry weight of sclerotia of *R. solani* were reduced significantly in presence of the antagonistic bacterial isolates of *Pseudomonas fluorescens* collected from different localities of Assam. However, maximum suppression of sclerotia and their dry weight was observed when isolates Pf2 was applied with *R. solani* as compared to other four isolates of *P. fluorescens*. It may be due to the more antagonistic potential of the isolates as well as produced antibiotic 2,4-diacetyl phloroglucinol, iron chelating siderophore, hydrogen cyanide, lytic enzymes, such as chitinase and β-1,3-glucanase in culture, and induced chitinase in plants (Nandakumar *et al*., 2002) Table 2.

Table 2 Effect of the different isolates of *Pseudomonas fluorescens* in reducing the number of sclerotia production of *Rhizoctonia solani* in 4% MSM

Treatment	**Number of sclerotia**	**Per cent inhibition (%)**	**Dry weight (gm)**	**Per cent inhibition (%)**
T_1 = Pf1 + *R. solani* applied simultaneously	71.6[b]	71.38	2.86[b]	64.95
T_2 = Pf2 + *R. solani* applied simultaneously	28.4[e]	88.64	1.41[d]	82.72
T_3 = Pf3 + *R. solani* applied simultaneously	60.6[c]	75.77	2.51[bc]	69.24
T_4 = Pf4 + *R. solani* applied simultaneously	51.8[cd]	79.29	2.34[bc]	71.32
T_5 = Pf5 + *R. solani* applied simultaneously	46.6[d]	81.37	2.08[c]	74.50
T_6 =*R. solani* alone	250.2[a]	-	8.16[a]	-

Effect of volatile compound of P. fluorescens on growth of R. solani

The isolates of *P. fluorescens* which showed the best result in dual culture was taken subsequently tested to see the effect of volatile compound of *P. fluorescens* by following the inverted plate method and incubated at 28 ± 2ºC for 15 days. It was found that the growth of *R. solani* was restricted and no sclerotia formed when it was treated with the isolate Pf2 as compared to control. In the treated plate *R. solani* produced fluffy mycelium, distended laterally towards periphery of the petriplate and no sclerotia produced. It could be presumed that the metabolic activity of *R. solani* might have changed due to volatile compound released by the *Pseudomonas fluorescens* (Pf2). The release of volatile substances by *Trichoderma spp.* against the fungal pathogens has been reported by many workers. Both hyperparasitism and volatile metabolites may be involved in the inhibition of the pathogen by the antagonist as also observed in the present investigation. Inhibitory volatile substances such as alkyl pyrons may also contribute to the biocontrol activity of some *Trichoderma* strains (Claydon *et al*., 1987). Dennis and Webster (1971) recorded maximum of 49.5% growth inhibition of *R. solani* by volatile antibiotics produced by *T. viride*.

CONCLUSION

From the present investigation it can be concluded that *P. fluorescens* isolated from Jorhat District (Pf2) of Assam was found to be the most inhibitory for the sheath blight pathogen with a percent inhibition of 40.66 per cent substantiated through dual culture *in vitro.* Maximum percent inhibition of sclerotia (88.64 per cent) and their dry weight also reduced to (82.72 per cent) was observed when isolates Pf2 was allowed to grow with *R. solani*.

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