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Evaluation of antagonistic activity of *Pseudomonas* spp. against *Sarocladium oryzae* causing sheath rot disease in rice (*Oryza sativa* L.)

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Abstract
Sheath rot disease of rice (*Sarocladium oryzae)* has become a consequential production constraint in all rice growing countries. The pathogen was isolated from the sheath rot infected rice plants. In this study twenty isolates of *Pseudomonas* spp. were isolated from rhizosphere region of rice plants cultivated in southern districts of Tamil Nadu, India. These bacterial isolates were identified as *Pseudomonas* spp. by morphological and biochemical characterization. The isolates showed positive response for KOH test, oxidase, pigment production, siderophore and HCN production. The antagonistic potential of the twenty isolates of *Pseudomonas* spp. were tested against *S. oryzae* under laboratory condition. The results revealed that the *Pseudomonas* spp. isolate, PTN_4 exhibited maximum inhibitory effect of 82.22 % and 78.89 % in dual culture and volatile compounds assay respectively over the control. Thus, the *Pseudomonas* spp. identified in this study can be utilized for the biological management of sheath rot disease in rice.

Keywords: Rice sheath rot, *S. oryzae*, *Pseudomonas* spp., antagonists, volatile organic compounds

Introduction
Rice sheath rot disease incited by *Sarocladium oryzae* (Sawada) is occurs in all rice growing countries worldwide. The disease is heinously devastative in Tamil Nadu and other rice growing states. In India, the disease caused yield losses of 3–85% depending upon the disease severity (Chakravarty and Biswas 1978) [3].

The sheath rot pathogen solely infects the uppermost leaf sheath and retards translocation of nutrients from the foliage to the panicle. Enormous whitish powdery growth was found inside the affected sheaths and the infected young panicles are rotten (Ou 1985) [17] and remained within the leaf sheath or emerged partially and produced chaffy, discolored and shriveled grains (Gopalakrishnan *et al.*, 2010) [5]. Effective fungicides are available for the management of sheath rot disease which otherwise hold some negative impacts, such as residual toxicity, development of resistance in pathogen population, environmental pollution, health hazards to humans, animals and steeply increases the expenditure for plant protection. In connection with the above mentioned ill effects, plant pathologists have focused their attention to develop environmentally safe, long-lasting, cheap and effective method for the management of plant diseases which is denoted as biological control.

Pseudomonas spp. is the most common bacterial antagonist has been deployed throughout the world due to its production of secondary metabolites like siderophores, antibiotics, volatile compounds and phytohormones (Nagarajkumar *et al.*, 2004) [14] including several enzymes like protease, glucanase and lipases which played a vital role in antagonistic action against the vast range of fungal pathogens (Hamamoto *et al.*, 1994) [6]. In this paper, we have tested the efficacy of bacterial antagonists (*Pseudomonas* spp.) isolated from rhizosphere of rice plants against sheath rot pathogen under laboratory condition.

Materials and Methods
Isolation of the pathogen
The pathogen was isolated from the infected leaf sheath of rice by tissue segment method on potato dextrose agar (PDA) medium. The infected portions were cut into small bits with the help of sterilized scalpel and surface sterilized with 1 per cent sodium hypochlorite. The surface sterilized tissue bits were transferred aseptically to Petri dishes containing sterilized PDA medium. These plates were incubated at room temperature (28± 2 ºC) and observed for the fungus growth. Pure culture of the pathogen was sub cultured and maintained in PDA

slants at 4 ºC and used for further studies. The pathogen was identified based on pathogenicity, cultural and spore characters.

Isolation of *Pseudomonas* spp. from rhizosphere region of rice plants

Antagonistic bacteria were isolated from different rice growing areas of southern Tamil Nadu. The plants were pulled out gently with intact roots and the excess soil adhering on roots were removed gently. Ten gram of rhizosphere soil was transferred to a 250 ml conical flask containing 100 ml of sterile water. After thorough shaking, the antagonist in the suspension was isolated by serial dilution plate method (Pramer and Schmidt 1956) [19]. From the final dilutions of 10^{-5} and 10^{-6} one ml of each aliquot was pipetted out, poured into the sterilized Petri plate containing King's B Medium. The plates were gently rotated clock wise and anti clockwise for uniform distribution and incubated at room temperature (28±2 °C) for 24 hours. The colonies were viewed under UV light at 366 nm. Colonies with characteristics of *Pseudomonas* spp. were isolated individually and purified by streak plate method on King's B medium. The pure cultures were maintained in King's B slants.

Biochemical characterization of *Pseudomonas* spp. isolates

The bacterial antagonists were identified and characterized based on the diagnostic test detailed in the Bergey's manual for determinative bacteriology (Bergey *et al.,* 1939) [2]. Biochemical test *viz.,* Gram's reaction, oxidase, KOH, pigment production, starch hydrolysis, anaerobic growth, siderophore production, HCN production and growth at 4 °C and 45 °C were carried out for confirmation of *Pseudomonas* spp.

Evaluation of different isolates of *Pseudomonas* spp. on the growth of *S. oryzae* under *in vitro*

The antagonistic activity of *Pseudomonas* spp. against *S. oryzae* was tested by dual culture technique (Dennis and Webster 1971) [4]. Twenty isolates of *Pseudomonas* spp. were streaked for four cm length (one cm away from the edge of the plate) on each PDA medium. A nine mm mycelial disc of the pathogen was placed to the opposite side of the same Petri plate perpendicular to the bacterial streak. Three replications were maintained for each treatment. The plates were incubated at room temperature (28±2 °C) for 15 days. The medium inoculated with the pathogen was served as control. When the control plate reached full growth, the radial growth of the pathogen was measured in the other treatments.

The results were expressed as per cent inhibition (PI) over control by using the following formula.

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Where,

Dc = average diameter of fungal growth (mm) in control

Dt = average diameter of fungal growth (mm) in treatment.

Efficacy of volatile organic compounds produced by *Pseudomonas* spp. against *S. oryzae*

Effects of volatile compounds produced by effective *Pseudomonas* spp. on mycelial growth of *S. oryzae* were studied by paired dish technique (Laha *et al.,* 1996) [12]. *Pseudomonas* spp. was uniformly spread onto KB agar plates (90 mm). Subsequently, a plug (9mm) from the agar of each of the fungi, which were incubated for 7 days, was punched and placed onto the center of a fresh PDA plate. A sandwich was made with the PDA medium with the fungi on the bottom and the *Pseudomonas* spp. coated KB on the top. A set of two plates was sealed with parafilm and incubated at 28 °C.

Statistical analysis

Experimental data were statistically analyzed using analysis of variance (ANOVA) and the SPSS version 16.0. The treatment means were separated at 5% significant level using Duncan's Multiple Range Test (DMRT).

Results

Isolation of pathogen

Colony appeared as white, compact or cottony and reverse exhibited whitish orange. The mycelium was septate, hyaline and branched. The conidiophores were branched and hyaline. The conidia were hyaline, single celled, smooth and cylindrical. The mycelial characters and conidia of *S. oryzae* were viewed through compound microscope.

Isolation of *Pseudomonas* spp. from rhizosphere region of rice plants

In the present study, twenty isolates of *Pseudomonas* spp. were isolated from the rhizhosphere soil of different locations of southern Tamil Nadu *viz*; Madurai, Theni, Thirunelveli, Thenkasi, Kanyakumari, Virudhunagar and Ramanathapuram. These isolates were subcultured, maintained as pure culture and used for further studies (Table 1).

Biochemical characterization of *Pseudomonas* spp.

The isolates of rhizobacteria obtained were evaluated in detail for their morphological and biochemical characteristics as given in Bergey's manual of systematic bacteriology. All the isolates of *Pseudomonas* spp. showed transparent, smooth and small colonies with diffusible yellow green pigment on King's B medium and also resulted positive to KOH test, anaerobic growth, oxidase test, siderophore production, HCN production and growth at 4 °C. These isolates showed negative result to Gram's reaction, starch hydrolysis and growth at 45 °C (Table 2).

Screening of antagonistic activity of *Pseudomonas* spp. against *S. oryzae* under *in vitro*

The results of the dual culture technique signaled that all the isolates inhibited the growth of test fungus significantly. Twenty isolates of *Pseudomonas* spp. were tested against *S. oryzae* under *in vitro*. Among the isolates, PTN_4 drastically inhibited the mycelial growth of *S. oryzae* (16.00mm) with 82.22 per cent growth reduction followed by PTN_2 and PTK_1 were the next effective, which recorded 20.00 and 28.00 mm growth of the pathogen with 77.78 and 68.89 per cent growth reduction over control respectively (Table 3).

Efficacy of volatile organic compounds produced by *Pseudomonas* spp. against *S. oryzae*

The volatile organic compounds of the effective *Pseudomonas* spp. isolates from the dual culture were further tested against *S. oryzae*. Among all the isolates, PTN_4, PTN_2 and PTK_1 isolates produced volatile compounds which strongly inhibited the growth of the pathogen upto 78.89 %, 71.11% and 64.44% respectively when compared to control (Table 4; Fig.1).

Discussion

Pseudomonas is a remarkable biological control agent against several phytopathogens. These beneficial bacteria harbour in the rhizosphere soil of different crops. *Pseudomonas fluorescens* was successfully isolated from the rhizosphere soil on King's B medium (King *et al.*, 1954) [10]. This study was carried out to evaluate the potential of rhizobacterial inoculum to control a sheath rot disease in rice under laboratory condition.

The biochemical tests *i.e* gelatin liquefaction, starch hydrolysis, catalase test, oxidase test, IAA production, siderophore production and hydrogen cyanide production further validated the isolates to be *P. fluorescens* as reported by earlier workers (Nathan *et al.*, 2011) [15]. Siderophore production was determined by the colour change of Fe-CAS dye in agar medium. It was reported that *P. fluorescens* secreted fluorescent, yellow green water soluble siderophores under iron-limiting conditions (O'sullivan and O'Gara 1992) [16]. The production of HCN by *Pseudomonas* spp. and their antifungal activity was observed by Paramageetham and Prasada Babu (2012) [18]. According to Kumar *et al.*, (2012) [11] the production of HCN was the principle mechanism for biocontrol in many bacteria.

In the present investigation, the antagonistic activity of twenty isolates of *Pseudomonas* spp. against *S. oryzae* was found to be significantly different with each other. Isolate PTN_4 was found to effectively reduce the mycelial growth of *S. oryzae* by 82.22 per cent and proved its efficiency as compared to other isolates over control. Isolates PTN_2 and PTK_1 were recorded the next most effective antagonists against the pathogen. Similarly, Reddy *et al.*, (2007) [20] demonstrated that fluorescent Pseudomonads isolates effectively inhibited the mycelial growth of *S. oryzae*. Saravanakumar *et al.*, (2007) [21] tested the antagonistic activity of *P. fluorescens* strains against *S. oryzae* under *in vitro* conditions which revealed the significant performance of the strains Pf1, TDK1 and PY15. Meera and Balabaskar (2012) [13] reported the maximum inhibition of 93.3 per cent by *P. fluorescens* (Pf 013) and minimum of 68.2 per cent by the isolate Pf 03. Karthikeyan *et al.*, (2013) [8] reported that *P. fluorescens* actively inhibited *S. oryzae* upto 68.9 per cent. The above findings are in concordance with the results of the present studies.

Volatile organic compounds from *Pseudomonas* spp. were also found to play an important part in biological control. In our study, *Pseudomonas* strain PTN_4 and PTN_2 produced VOCs which effectively inhibited the mycelial growth of *S. oryzae* on PDA medium compared with other isolates. In accordance with Kavitha *et al.*, (2012) [9] reported that volatile antibiotics produced by *P. fluorescens* isolate KPf1 and *P. chlororaphis* isolate PA2 inhibited the profuse growth of the pathogen *P. aphanidermatum*. *P. fluorescens* suppressed the mycelial growth of *R. bataticola* in agar plate due to the production of volatile antifungal compounds (Abou-Aiy *et al.*, 2015) [1].

Table 1: *Pseudomonas* spp. isolated from southern districts of Tamil Nadu

S. No	Isolates	Place of collection	District	Latitude(ºN)	Longitude(ºE)
1	PMD_1	AC & RI, Madurai	Madurai	9.93	78.12
2	PMD_2	Alanganallur	Madurai	10.05	78.09
3	PMD_3	Sholavandan	Madurai	10.02	77.96
4	PMD_4	Usilampatti	Madurai	9.97	77.79
5	PMD_5	Melur	Madurai	10.03	78.34
6	PMD_6	Chellampatti	Madurai	9.94	77.90
7	PMD_7	Sekkanurani	Madurai	9.94	77.97
8	PTN_1	Chinnamanur	Theni	9.84	77.38
9	PTN_2	Uthamapalayam	Theni	9.81	77.33
10	PTN_3	Veerapandi	Theni	9.96	77.45
11	PTN_4	Cumbum	Theni	9.73	77.28
12	PTN_5	Periyakulam	Theni	10.12	77.55
13	PTV_1	Ambasamuthirum	Thirunelveli	8.71	77.45
14	PTK_1	Thenkasi	Thenkasi	8.96	77.31
15	PKK_1	Thirupathisaram	Kanyakumari	8.20	77.45
16	PKK_1	Nagarkovil	Kanyakumari	8.18	77.41
17	PRP_1	Kamudhi	Ramanathapuram	9.41	78.36
18	PRP_2	Paramakudi	Ramanathapuram	9.55	78.59
19	PSG_1	Manamadurai	Sivagangai	9.69	78.46
20	PVN_1	Kovilpatti	Virudhunagar	9.17	77.88

Table 2: Biochemical characterization of different isolates of *Pseudomonas* spp.

Isolates	Colony colour	Biochemical test									
		Gram's reaction	Pigment production in KB medium	Oxidase test	Starch hydrolysis	Growth at		KOH test	Anaerobic growth	HCN production	Siderophore production
						4 °C	45 °C				
PMD_1	Light yellow	-	+	+	-	+	-	+	+	+	+
PMD_2	Light yellow	-	+	+	-	+	-	+	+	+	+
PMD_3	Pale green	-	+	+	-	+	-	+	+	+	+
PMD_4	Light yellow	-	+	+	-	+	-	+	+	+	+
PMD_5	Light yellow	-	+	+	-	+	-	+	+	+	+
PMD_6	Pale green	-	+	+	-	+	-	+	+	+	+
PMD_7	Light yellow	-	+	+	-	+	-	+	+	+	+
PTN_1	Light yellow	-	+	+	-	+	-	+	+	+	+
PTN_2	Light yellow	-	+	+	-	+	-	+	+	+	+
PTN_3	Pale green	-	+	+	-	+	-	+	+	+	+

PTN_4	Pale green	-	+	+	-	+	-	+	+	+	+
PTN_5	Light yellow	-	+	+	-	+	-	+	+	+	+
PTV_1	Light yellow	-	+	+	-	+	-	+	+	+	+
PTK_1	Pale green	-	+	+	-	+	-	+	+	+	+
PKK_1	Light yellow	-	+	+	-	+	-	+	+	+	+
PKK_2	Pale green	-	+	+	-	+	-	+	+	+	+
PRP_1	Light yellow	-	+	+	-	+	-	+	+	+	+
PRP_2	Light yellow	-	+	+	-	+	-	+	+	+	+
PSG_1	Light yellow	-	+	+	-	+	-	+	+	+	+
PVN_1	Light yellow	-	+	+	-	+	-	+	+	+	+

+ Positive Reaction; - Negative Reaction

Table 3: Effect of *Pseudomonas* spp. on the mycelial growth of *S. oryzae in vitro*

S. No.	Isolates	Mycelial growth of the pathogen (mm)*	Percent growth reduction over control
1	PMD_1	52.00	42.22 (40.35)**
2	PMD_2	47.00	47.78 (44.41)
3	PMD_3	54.00	40.00 (38.95)
4	PMD_4	34.00	62.22 (53.15)
5	PMD_5	60.00	33.33 (35.20)
6.	PMD_6	56.00	37.78 (38.27)
7.	PMD_7	54.00	40.00 (38.97)
8.	PTN_1	36.00	60.00 (50.61)
9.	PTN_2	20.00	77.78 (62.04)
10.	PTN_3	40.00	55.56 (47.54)
11.	PTN_4	16.00	82.22 (65.49)
12.	PTN_5	39.00	56.67 (47.95)
13.	PTV_1	62.00	31.11 (34.41)
14.	PTK_1	28.00	68.89 (55.64)
15.	PKK_1	58.00	35.56 (36.86)
16.	PKK_2	43.00	52.22 (45.17)
17.	PRP_1	69.00	23.33 (28.69)
18.	PRP_2	65.00	27.78 (31.65)
19.	PSG_1	67.00	25.56 (30.29)
20.	PVN_1	64.00	28.89 (31.91)
21.	Control	90.00	0.00 (00.36)
CD (P=0.05)		2.38	-

*Mean of three Replications

** Values in the parentheses are arc sin transformed values

P-*Pseudomonas* spp. MD-Madurai; TN-Theni; VN-Virudhunagar; TV-Thirunelveli;

TK-Thenkasi; KK-Kanyakumari; RP-Ramanathapuram; SG-Sivagangai.

Table 4: Efficacy of volatile organic compounds of effective *Pseudomonas* spp. against the mycelial growth of *S. oryzae*

S. No.	Isolates	Production of volatile compounds	Mycelial growth of the pathogen (mm)*	Percent growth reduction over control
1	PMD_1	+	58.00	35.56^{h}
2	PMD_2	+	53.00	41.11^{g}
3	PMD_4	+	38.00	57.78^{d}
4	PTN_1	+	40.00	55.56^{d}
5	PTN_2	+	26.00	71.11^{b}
6	PTN_3	+	46.00	48.89^{ef}
7	PTN_4	+	19.00	78.89^{a}
8	PTN_5	+	44.00	51.11^{e}
9	PTK_1	+	32.00	64.44^{c}
10	PKK_2	+	48.00	46.67^{f}
11	Control	-	90.00	0.00^{i}
CD (P=0.05)			1.87	-

*Mean of three Replications

[a]Means with same letter do not have significant difference according to Duncan's multiple range test at $p<0.05$.

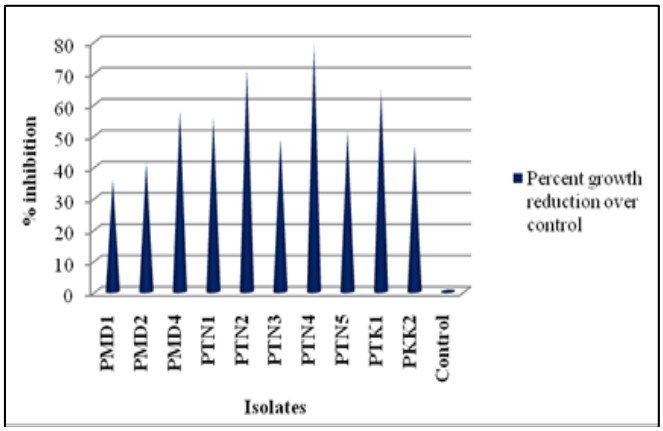

Fig 1: Efficacy of volatile organic compounds of effective *Pseudomonas* spp. against the mycelial growth of *S. oryzae*

References

1. Abou-Aiy HE, Neweigy NA, Zaghloul RA, Ei-Sayed SA, Bahloul AM. Evaluation of some biocontrol agents against soil pathogenic fungi. Research journal of pharmaceutical, biological and chemical sciences. 2015; 6(1):439-448.
2. Bergey, David Hendricks, Robert Stanley Breed, Everitt George Dunne Murray, Parker Hitchens A. Manual of determinative bacteriology. Manual of determinative bacteriology, Fifth Edn, 1939.
3. Chakravarty DK, Biswas S. Estimation of yield loss in rice affected by sheath rot [*Acrocylindrium oryzae*]. Plant Disease Reporter, 1978.
4. Dennis C, Webster J. Antagonistic properties of species-groups of *Trichoderma*: I. Production of non-volatile antibiotics. Transactions of the British Mycological Society 1971; 57(1):25-IN23.
5. Gopalakrishnan C, Kamalakannan A, Valluvaparidasan V. Survey of seed-borne fungi associated with rice seeds in Tamil Nadu, India. Libyan Agric Res Cent J Int. 2010; 1:307-309.
6. Hamamoto, Tetsuo, Motohiro Kaneda, Koki Horikoshi, Toshiaki Kudo. Characterization of a Protease from a Psychrotroph, *Pseudomonas fluorescens* 114. Appl. Environ. Microbiol. 1994; 60(10):3878-3880.
7. Kandoliya UK, Vakharia DN. Antagonistic effect of *Pseudomonas fluorescens* against *Fusarium oxysporum f. sp. ciceri* causing wilt in chickpea. Legume Res. 2013; 36(6):569-575.
8. Karthikeyan, Arumugam, Ramalingam Parthasarathy, Appu Manikandan. Isolation of *Trichoderma viride* and *Pseudomonas fluorescens* organism from soil and their treatment against rice pathogens. Journal of Microbiology and Biotechnology Research. 2013; 3(6):77-81.
9. Kavitha K, Nakkeeran S, Chandrasekar G. Rhizobacterial-mediated induction of defense enzymes to enhance the resistance of turmeric (*Curcuma longa* L) to

Pythium aphanidermatum causing rhizome rot. Archives of Phytopathology and Plant Protection. 2012; 45(2):199-219.
10. King, Elizabeth O, Martha K, Ward, Donald E Raney. Two simple media for the demonstration of pyocyanin and fluorescin. The Journal of laboratory and clinical medicine. 1954; 44(2):301-307.
11. Kumar, Ajay, Amit Kumar, Shikha Devi, Sandip Patil, Chandani Payal *et al*. Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an *in vitro* study. Recent research in science and technology. 2012; 4(1).
12. Laha GS, Verma JP, Singh RP. Effectiveness of fluorescent pseudomonads in the management of sclerotial wilt of cotton. Indian Phytopathology. 1996; 49(1):3-8.
13. Meera T, Balabaskar P. Isolation and characterization of *Pseudomonas fluorescens* from rice fields. International Journal of Food, Agriculture and Veterinary Sciences 2012; 2(1):113-120.
14. Nagarajkumar M, Bhaskaran R, Velazhahan R. Involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiological research. 2004; 159(1):73-81.
15. Nathan, Prakash, Xavier Rathinam, Marimuthu Kasi, Zuraida Abdul Rahman, Sreeramanan Subramaniam. A pilot study on the isolation and biochemical characterization of Pseudomonas from chemical intensive rice ecosystem. African journal of Biotechnology. 2011; 10(59):12653-12656.
16. O'sullivan, Daniel J, Fergal O'Gara. Traits of fluorescent Pseudomonas spp. involved in suppression of plant root pathogens. Microbiology and Molecular Biology Reviews. 1992; 56 (4):662-676.
17. Ou, Shu Huang. Rice diseases: IRRI, 1985.
18. Paramageetham Ch, Prasada Babu G. Antagonistic Activity of Fluorescent Pseudomonads against a Polyphagous Soil Born Plant Pathogen (*Sclerotium rolfsii)*. 2012; 1:436.
19. Pramer D, Schmidt EL. Exp. Soil Micro. Burges Publishing Company, Minneopolis, USA, 1956.
20. Reddy KRN, Choudary KA, Reddy MS. Antifungal metabolites of *Pseudomonas fluorescens* isolated from rhizosphere of rice crop. J Mycol Pl Pathol. 2007; 37(2):1-5.
21. Saravanakumar D, Harish S, Loganathan M, Vivekananthan R, Rajendran L, Raguchander T *et al*. Rhizobacterial bioformulation for the effective management of *Macrophomina* root rot in mungbean. Archives of Phytopathology and Plant Protection. 2007; 40(5):323-337.