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Bio-control potential of fluorescent pseudomonads against sheath blight of rice

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Abstract
Aim: The present study was conducted to investigate the biocontrol potential of fluorescent pseudomonads *in- vitro* and *in-vivo* responsible for causing sheath blight of rice.
Methodology: Indigenous isolates (20 nos.) of fluorescent pseudomonads, collected from the rhizosphere of basmati paddy fields from different locations of Jammu province, which were further characterized for their bio-control activity against *Rhizoctonia solani* causing sheath blight of rice under laboratory and glasshouse conditions at Division of Plant Pathology, Faculty of Agriculture, SKUAST-Jammu, Chatha.
Results: On the basis of biochemical tests, isolate Fp1, Fp2, Fp4, Fp5, Fp6, Fp7, Fp9, Fp11, Fp17 and Fp18 were able to grow at 4°C and were identified as *Pseudomonas fluorescens*, whereas, isolate Fp3, Fp8, Fp10, Fp12, Fp13, Fp14, Fp15, Fp16, Fp19 and Fp20 which grew at 41°C and utilized the acetamide were identified as *Pseudomonas aeruginosa*. Under *in vitro* conditions, out of ten isolates (Fp1- Fp10), Fp6 showed maximum per cent inhibition (84.09 and 86.66) of mycelial growth of *R. solani*, followed by Fp1 (81.81and 84.89), whereas, minimum inhibition was observed by Fp8 (43.40 and 64.00) on PDA and NA media, respectively, using dual culture assay. All the isolates reduced the germination of *R. solani* sclerotia with range of 49.05-90.87% and also enhanced the vigour index of the rice seedlings ranging from 595.05 to 1683.27. In detached leaf assay rice leaves treated with suspension of Fp6 isolate significantly showed minimum lesion length (12.66 mm) with maximum per cent reduction of lesion (81.91%) as compared to untreated control (70.00 mm). The treatment T4 (*P. fluorescens*) and T8 (*P. aeruginosa*) (seed soaking + seedling dip +foliar spray) showed disease intensity of 10.94 and 14.06 per cent with 42.19 and 39.07 per cent disease reduction, respectively, as compared to untreated control.
Interpretation: Due to dominance of fluorescent pseudomonads (*P. fluorescens* and *P. aeruginosa*) in the rice rhizosphere and diverse mechanism of action, it had the potential for reducing sheath blight incidence of rice caused by *R. solani*.

Keywords: Bio-control, fluorescent, pseudomonads, sheath blight

Introduction
Sheath blight of rice, caused by *Rhizoctonia solani* Kühn [Teleomorph: *Thanatephorus cucumeris* (Frank.) Donk.] was first reported in Gurdaspur, India (Pracer and Chahal, 1963) [38], is responsible for causing yield loss of 4-50 per cent (Bhunkal *et al.*, 2015) [9]. The pathogen has a very wide host range *viz*., Maize, Wheat, Jowar, Bajra, Ragi, Torpedo grass, Crowfoot grass, Swollen fingergrass, Jungle rice, Jointed goatgrass, Viper grass, Knot grass, Bermuda grass and Hairy crab (Chahal *et al.* 2003; Nagaraj *et al*. 2017) [12, 30]. Genetic sources of resistance against this disease are not adequate and current management strategies mostly involve application of chemical fungicides (Bhuvaneswari and Raju, 2012) [11]. The application of fungicides is expensive and has adverse effect on the environment as well as human health. In order to reduce the application of chemical pesticide for sustainable agriculture, use of plant growth promoting rhizobacteria (PGPR) has gained momentum in recent decades.
Fluorescent pseudomonads are one of the most promising groups of PGPR, which can control various plant pathogenic micro-organisms (O'Sullivan and O'Gara, 1992) [35]. The fluorescent pseudomonads are motile (one or several polar flagella), non-sporulating rods with Gram negative reaction having 58-69 per cent GC content included all *Pseudomonas* species with the ability to produce fluorescent pigment *viz*., *P. aeruginosa*, *P. syringae*, *P. putida and P. fluorescens* (Bossis *et al.* 2000) [10]. Because of their diverse mechanisms of action, high level of genetic variability and competitiveness in soil, they emerged as effective and economical bio-inoculants for use in integrated disease management. The bio-control mechanism of

fluorescent pseudomonads normally involves the production of antibiotics (Nagarajkumar *et al*. 2004) [31] lipopeptide, antibiotic viscosinamide, 2,4-diacetylphloroglucinol (Nielsen *et al.* 1998) [34], secondary metabolites and phytohormones (Keel *et al*. 1992) [22], volatile compounds such as HCN (Kulkanisky-sobral *et al*. 2004) [24] siderophores (Neiland, 1995; Rachid and Ahmed, 2005) [33, 39] and also cell wall hydrolysing enzymes such as protease, chitinase and glucanase (Kumar *et al.* 2013) [25]. Plant growth-promoting ability of these bacteria is mainly because of the root colonization, production of indole-3-acetic acid (Patten and Glick, 2002) [37], cytokinins, gibberellins and phosphate solubilising activity (Kapoor *et al.* 2012) [44]. Besides these, PGPR stimulate plant growth through transforming, mobilizing and solubilising the nutrients (Hayat *et al.* 2010) [19]. Diverse population of fluorescent pseudomonads provide better resources for the improvement of plant growth promotion and biocontrol ability, as different strains possess varied modes of action and survival in diverse environmental conditions (Kumar *et al.* 2002) [26].

Therefore, the current study was conducted to evaluate the potential of fluorescent pseudomonads for its bio-control potential against sheath blight of rice.

Material and Methods

Isolation, identification and characterization of fluorescent pseudomonads: Thirty soils samples were collected from the rhizosphere of basmati rice crop from different locations of basmati rice growing regions of Jammu (Chatha, R.S. Pura, Kathua and Vijaypur). Each soil sample was performed for serial dilution up to 10^{-9} dilution to isolate fluorescent pseudomonads on Luria-Bertani (LB) agar medium (Manivannan *et al.* 2012) [27]. Colonies showing fluorescence under UV light were picked up and streaked on King's B agar medium for purification and subjected for bio-chemical test for further identification. Further, the molecular characterization of *P. fluorescens* isolates were confirmed by using species specific primers (16SPSEfluF 5'-TGCATTCAAAACTGACTG- 3' 16SPSSER 5'-AATCACACCGTGGTAACCG-3'),

Screening of fluorescent pseudomonads against *Rhizoctonia solani*: The antagonistic activity of fluorescent pseudomonads isolates against *Rhizoctonia solani* was conducted by dual culture technique (Dennis and Webster, 1971) [15] and per cent inhibition of pathogen growth was recorded (Vincent, 1947) [49].

Evaluation of fluorescent pseudomonads on the germination sclerotia of *Rhizoctonia solani*: Sterilized sclerotia (2.0 per cent sodium hypocholrite) of *R. solani* were inoculated into flasks containing 50 ml King's B broth of 24 hr old culture of fluorescent pseudomonads (2×10^9cfuml^{-1}) isolates and kept on a rotary shaker at 175 rpm at 26±2 °C for 24 hrs. The sclerotia were later gently removed with sterile forceps, dried on a blotter paper, placed onto pre-poured PDA plates and incubated for 5 days in B.O.D incubator at 25 °C. Germination of sclerotia and inhibition of mycelial growth from germinated sclerotia were measured (Kazempour, 2004) [21]. Sclerotia incubated in sterile nutrient broth served as control.

Effect of fluorescent pseudomonads on the germination and vigour index of rice seedlings: Surface sterilized (2.0 per cent sodium hypochlorite) rice seeds (cv. Basmati 370) were soaked in the suspension of fluorescent pseudomonad (2×10^9cfu/ml) isolates for 24 hours (Meera and Balabaskar, 2012) and then placed on moist sterilized filter paper in petri plates (14 cm). Seeds were later incubated in B.O.D incubator at 26 °C for 7 days. Seeds soaked in sterile distilled water served as control. Per cent germination of seeds, roots and shoot length of seedlings were measured to record the vigour index (Ameer Basha *et al*. 2013) [2].

Effect of fluorescent pseudomonads on sheath blight of rice (detached leaf-assay): A loopful of 24 hr old culture of selected fluorescent pseudomonads was inoculated into 250 ml flasks containing 50 ml of nutrient broth and incubated for 24 hr on a rotary shaker at 175 rpm maintained at 26 °C. The leaves of 60- day old rice plant (cv. Basmati 370) were cut into 10 cm long pieces and dipped into the suspension (2×10^9cfu ml^{-1}) of fluorescent pseudomonads. The inoculated leaves supported by clean glass slides were placed in plastic tray (20 cm×20 cm) containing moistened filter papers. Week old mycelial bits of *R. solani* were placed on the surface of leaves (Dath, 1987). For control, the leaves were suspended in sterile distilled water and the mycelial bits of *R. solani* were placed. The trays were covered with plastic sheet and placed in BOD incubator at 25±2 °C. The observations on lesion length around the mycelial bits were recorded after 6 days of inoculation. The disease intensity was calculated as Highest Relative Lesion Length (HRLH) = highest lesion length/ leaf length × 100

Management of sheath blight of rice by fluorescent pseudomonads under glass house conditions: Sowing of rice (cv. Basmati-370) was done in pots on second fortnight of June, 2013. The treatment group was as in, first and second treatment prior to sowing, the seeds were dipped into the suspension ($2X10^9$ cfu ml^{-1}) of *Pseudomonas fluorescens* (Fp6) and *P. aeruginosa* (Fp10) isolates. The third and fourth treatments involve seedling treatment by dipping the roots into the suspension of *P. fluorescens*, Fp6 and *P. aeruginosa* isolates Fp10. In the fifth and sixth treatments foliar application of *P. fluorescens* Fp6 and *P. aeruginosa* Fp10 isolates were conducted. Combination of treatments (seed treatment followed by seedling root dip and foliar application by *P. fluorescens* isolate Fp6 and seed treatment followed by seedling root dip and foliar application *P. aeruginosa* isolate Fp10) were also maintained. A seed treatment with carbendazim 50% WP @ 2g kg^{-1} seed and foliar application with carbendazim 50% WP @ 0.1 per cent were also maintained along with control. The experiment was conducted in completely randomized block design (CRBD) with three replications for each treatment. The disease intensity was recorded on 0-4 scale (Tiwari and Trimurty, 2009) [48] and the experimental data were statistical analysed for analysis of variance (Gomez and Gomez, 1984) [17].

Result and Discussion

Out of the total fifty isolates, twenty isolates (67%) were identified as fluorescent pseudomonads on the basis of morphological and biochemical characteristics such as fluorescent pigment production under UV light on King's B agar medium, Gram negative reaction, growth at different temperatures (4 and 41 ºC) and utilization of acetamide. Growth at 4 ºC confirmed that 50 per cent isolates (Fp1, Fp2, Fp4, Fp5, Fp6, Fp7, Fp9, Fp11, Fp17 and Fp18) as

Pseudomonas fluorescens. whereas growth at 41ºC along with utilization of acetamide by changing the colour of the medium from yellow to pink identified the isolates Fp3, Fp8, Fp10, Fp12, Fp13, Fp14, Fp15, Fp16, Fp19 and Fp20 as *P. aeruginosa* (Table 1). Production of fluorescent pigment on King's B agar medium, Gram negative reaction and growth at 4ºC are the ideal characteristics for the identification of *P. fluorescens* (Soesanto *et al.*, 2011; Belkar and Gade, 2012) [47, 8]. Species-specific primers, 16SPSEfluF and 16SPSER amplified the DNA fragment of 850 bp in ten isolates, which confirmed that the isolates belong to *P. fluorescens* (Scarpellini *et al.*, 2004) [45]. Therefore, the present findings are in agreement with the previous findings where population of fluorescent pseudomonads were found to be dominant in the rhizosphere of rice crop (Rangarajan *et al.* 2002; Raimam *et al*. 2007, Kapase and Sawant, 2015) [40, 20]. Torres-Rubio *et al.* (2000) reported that the rhizosphere of rice cultivars show the presence of beneficial microbes, among which 51 per cent were *Pseudomonas* spp., such as *P. putida, P. aeruginosa, P. fluorescens* and *P. citchori*.

Under laboratory conditions, out of twenty isolates of fluorescent pseudomonads, ten isolates (Fp1-Fp10) showed antagonistic activity against *R. solani*. The isolate Fp6 was most effective in reducing the mycelial growth to14.00 and 10.00 mm of *R. solani* on PDA and NA media, respectively, with per cent inhibition of 84.09 and 86.66 per cent, as compared to control (Table 2). The results of the present study in agreement with Anand *et al.* (2010) [3] who reported that *P. fluorescens* isolate Pf4 showed maximum inhibition (84.37%) of mycelial growth of *R. solani*. Reddy *et al.* (2010) [43] also reported that the mycelial growth of *R. solani* in PGPR challenged plates ranged from 2.0 to 8.1cm with maximum inhibition of 77.80 per cent by Pf003 isolate. Biocontrol activity of fluorescent pseudomonads are due to the production of different types of cell wall degrading enzymes like chitinase, protease and β-1,3glucanase, which degrade cell wall of various bacterial and fungal plant pathogens (Ruchi *et al*. 2012) [44].

The germination of *R. solani* sclerotia was significantly inhibited (49.05-90.87%) by all the tested isolates (Fp1-Fp10) (Table 2). Isolates Fp6 and Fp1 showed the maximum inhibition of 90.87 and 88.21 per cent over control. Pandey and Pudhir (2013) [36] observed that the invasion of sclerotia by the cells of antagonistic bacteria had resulted in the disintegration of outer and inner layers which ultimately reduced the viability of sclerotia.

Treating rice seeds (Basmati-370) with fluorescent pseudomonads significantly induced enhancement in per cent germination, shoot length, root length and vigour index in 10-day-old rice seedlings (Table 3). Highest germination (83.33%), shoot length (11.10cm), root length (10.0cm) and vigour index (1683.27) was recorded by Fp6 isolate, followed by Fp1 and Fp10 isolates. Adhikari *et al.* (2013) [1] also observed that vigour index based on the germination percentage, root and shoot length was maximum by *P. fluorescens* isolates. *P. fluorescens* has been shown to enhance seed germination, root and shoot length and vigour in several crops (Ramamoorthy *et al.*, 2001; Khalid *et al* 2004; Egamberdiev, 2008) [41, 23, 16].

The data in Table 4 reveal that the rice leaves treated with the suspension (2×10^9cfu/ml) of Fp6 isolate significantly lowered the lesion length (12.66 mm) with the per cent lesion reduction of 81.91, as compared to untreated control (70.00 mm). Choi *et al.* (2006) [13] observed that successful colonization of the bacterial cells and production of antifungal compounds on the hydrophobic rice sheath surface was one of the important traits of fluorescent pseudomonads for the management of sheath blight of rice due to exo-polysaccharids production, biofilm formation and swarming motility.

The integrated application of *P. fluorescens*, Fp6 and *P. aeruginosa* Fp10 as seed + seedling dip + foliar application and foliar application of carbendazim 50% WP (0.1%) significantly reduced the intensity of sheath blight of rice (Table 5). The maximum reduction in disease intensity (42.19%) was observed in integrated application of isolate Fp6 of *P. fluorescens* as seed dressing, seedling dipping and foliar application, followed by 39.07 per cent by combined application of *P.aeruginosa* (Fp10) as seed dressing, seedling dipping and foliar application, and 36.57 per cent in foliar application of carbendazim (0.1%). Tiwary and Trimurty (2009) [48] also reported that under field conditions, seed treatment along with two foliar sprays of *P. fluorescens* resulted in minimum disease severity (23.10%), followed by seed treatment with *P. fluorescens* (23.50%), foliar application of carbendazim 50% WP (37.10) as compared to 49.90 per cent in untreated control. Neha *et al.* (2016) [32] also recorded that seed treatment with *P. fluorescens* @ 10 ml kg^{-1} along with seedling dipping of roots @ 3.0 l ha^{-1} significantly enhanced the germination (75.82%), plant height (25.52), number of tillers (16.99) and grain yield (48.23 g pot^{-1}). Due to the production of secondary metabolites such as antibiotics, soil has untapped potential of antagonistic microbes, which are helpful in reducing pathogen population through different modes of action such as competition for nutrients and space, antibiosis, mycoparasitism, production of siderophore, volatile metabolites (HCN), extracellular lytic enzymes, phytoharmones and induced systemic resistance (Gupta *et al.*, 2002; Ashofteh *et al.*, 2009) [18, 7].

Table 1: Description of fluorescent pseudomonads isolates collected form rice rhizosphere in rice growing regions of Jammu

S. No.	Isolates	Place of collection	Geographic location	Fluorescence	Gram reaction	Growth of fluorescent pseudomonads		Acetamide Agar
						4 °C	41 °C	
1	FP-1	Chatha	32.58N/75.00'E	+	-	+	_	_
2	Fp-2	Chatha	32.58N/75.00'E	+	-	+	_	_
3	Fp-3	Chatha	32.58N/75.00'E	+	-	_	+	+
4	Fp-4	R. S. Pura	32.63'N /74.73'E	+	-	+	_	_
5	Fp-5	R. S. Pura	32.63'N /74.73'E	+	-	+	_	_
6	Fp-6	R. S. Pura	32.63'N /74.73'E	+	-	+	_	_
7	Fp-7	R. S. Pura	32.63'N /74.73'E	+	-	+	_	_
8	Fp-8	Kathua	32.17N /32.55'E	+	-	_	+	+
9	Fp-9	Kathua	32.17N /32.55'E	+	-	+	_	_
10	Fp-10	Kathua	32.17N /32.55'E	+	-	_	+	+

11	Fp-11	Kathua	32.17N /32.55'E	+	-	+	_	_
12	Fp-12	Kathua	32.17N /32.55'E	+	-	_	+	+
13	Fp-13	Kathua	32.17N /32.55'E	+	-	_	+	+
14	Fp-14	Vijaypur	32.58N/75.00'E	+	-	_	+	+
15	Fp-15	Vijaypur	32.58N/75.00'E	+	-	_	+	+
16	Fp-16	Vijaypur	32.58N/75.00'E	+	-	_	+	+
17	Fp-17	Vijaypur	32.58N/75.00'E	+	-	+	_	_
18	Fp-18	Vijaypur	32.58N/75.00'E	+	-	+	_	_
19	Fp-19	Vijaypur	32.58N/75.00'E	+	-	_	+	+
20	Fp-20	Vijaypur	32.58N/75.00'E	+	-	_	+	+

Table 2: *In vitro* screening of fluorescent pseudomonads isolates against *Rhizoctonia solani* and effect on germination of sclerotia of *Rhizoctonia solani*

Isolate	Potato Dextrose Agar medium		Nutrient Agar medium		Radial growth (mm)	Per cent Inhibition
	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)		
Fp-1	16.00	81.81	11.33	84.89	10.33	88.21
Fp-2	32.00	63.63	19.67	73.77	31.00	64.64
Fp-3	38.00	56.81	22.67	69.77	32.67	62.74
Fp-4	41.66	52.65	24.33	67.56	41.00	53.23
Fp-5	43.66	50.38	33.00	56.00	44.67	49.05
Fp-6	14.00	84.09	10.00	86.66	8.00	90.87
Fp-7	21.00	76.13	14.67	80.44	24.67	71.86
Fp-8	49.80	43.40	27.00	64.00	40.33	53.99
Fp-9	43.80	50.22	28.33	62.22	32.67	62.74
Fp-10	17.00	80.68	12.00	84.00	13.67	84.41
Control	88.00		75.00		87.67	
CD (P=0.05)	4.238		3.126		2.392	
SE± (m)	1.436		1.059		0.810	

Table 3: Effect of fluorescent pseudomonads on germination and vigour index of rice seedlings

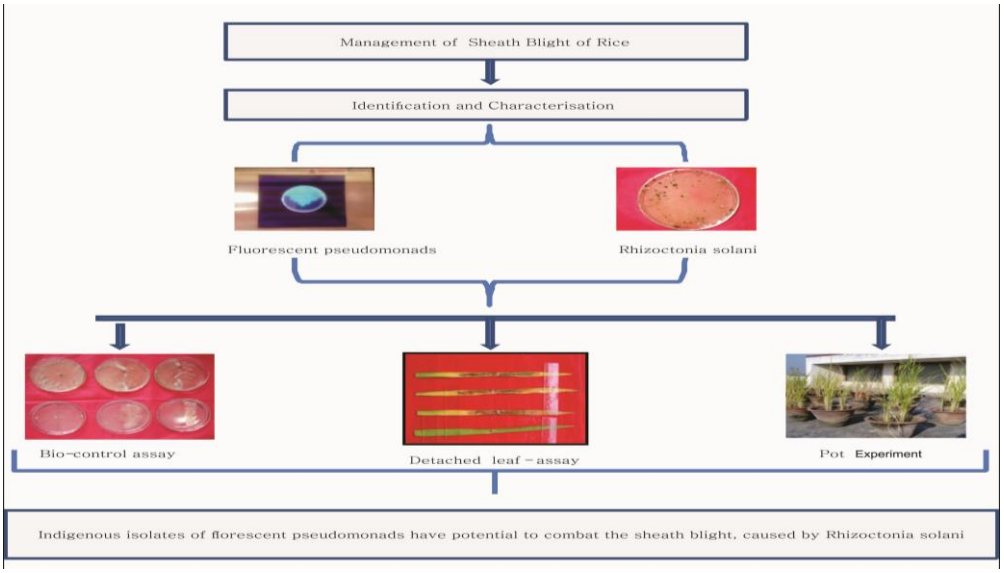
Isolate	Germination (%)	Mean shoot length (cm)	Mean root length (cm)	Vigour index
Fp-1	80.56	10.20	8.50	1466.19
Fp-2	61.11	7.00	6.10	996.09
Fp-3	55.56	6.30	6.50	927.85
Fp-4	44.44	5.50	5.10	679.93
Fp-5	38.39	5.20	5.30	595.05
Fp-6	83.33	11.10	10.0	1683.27
Fp-7	63.89	7.50	6.50	1066.96
Fp-8	47.22	5.60	5.50	741.35
Fp-9	58.33	6.20	6.70	985.78
Fp-10	77.78	8.90	8.00	1454.49
Control	36.11	4.70	5.00	548.87
CD (P=0.05)	1.805	2.243	1.902	323.542
SE± (m)	0.661	0.760	0.644	109.607

Table 4: Effect of fluorescent pseudomonads on sheath blight of rice (detached leaf-assay)

Isolate	Highest lesion length (mm)	Reduction in lesion length (%)
Fp-1	13.33	80.95
Fp-2	24.33	65.24
Fp-3	32.00	54.28
Fp-4	41.00	41.42
Fp-5	42.33	42.38
Fp-6	12.66	81.91
Fp-7	21.00	70.00
Fp-8	44.30	36.71
Fp-9	33.30	52.42
Fp-10	14.66	79.05
Control	70.00	
CD (P=0.05)	3.857	
SE± (m)	1.307	

Table 5: Management of sheath blight of rice

Treatment	Per cent Disease intensity	Reduction in disease intensity
T_0 = Control	53.13	
T1= Seed treatment of Fp6 (*P. fluorescens*)	31.06	22.07
T_2 = Seedling treatment of Fp6 (*P. fluorescens*)	30.13	23.00
T_3 = Foliar application of Fp6 (*P. fluorescens*)	25.81	27.32
$T_4 = T_1 + T_2 + T_3$ (Fp6)	10.94	42.19
T_5 = Seed treatment of Fp10 (*P. aeruginosa*)	34.25	18.94
T_6 = Seedling treatment of Fp10 (*P. aeruginosa*)	31.19	21.94
T_7 = Foliar application of Fp10 (*P. aeruginosa*)	26.19	26.94
$T_8 = T_5 + T_6 + T_7$ (Fp10)	14.06	39.07
T_9 = (Seed treatment with carbendazim @ 2g/kg of seed)	27.32	25.82
T_{10} = (Foliar application with carbendazim @ 0.1%))	16.56	36.57
CD(P=0.05)	8.747	
SE± (m)	3.01	



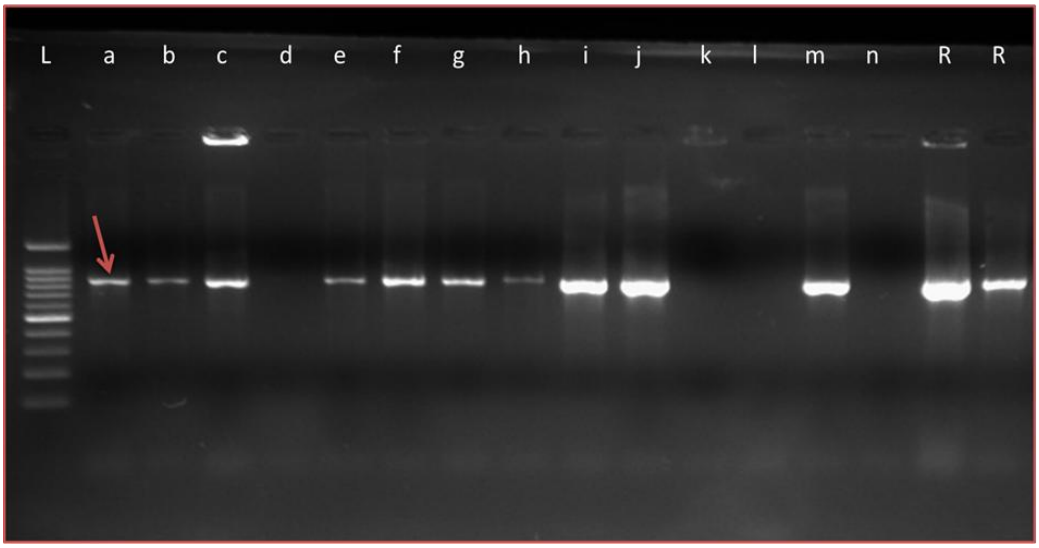


Fig 1: Molecular characterization of fluorescent pseudomonads isolates (850bp). L= ladder, Fp1(a), Fp2(b), Fp4(c), Fp5(e), Fp6(f), Fp7(g), Fp9(h), Fp11(i), Fp17(j), Fp18(m), R, (ATCC), R.(ITCC).

Conclusion

The study conclusively proves that if the fluorescent pseudomonads are introduced for the management of sheath blight they will not only manage the disease successfully, but will be also a step towards reducing the undue dependence on chemical fungicides.

References

1. Adhikari A, Dutta S, Nandi S, Bhattacharya I, De Roy M, Sarkar G *et al*. Antagonistic potential of native rhizobacterial isolates against root rot disease of okra, incited by *Rhizoctonia solani*. Afr. J. Agri. Research. 2013; 8:405- 412.

2. Ameer Basha S, Raghavendra G, Nagesh Kumar MV, Dharma Reddy K, Sudhakar R. Performance of native fluorescent Pseudomonas on *in vitro* seed germination and seedling vigour of *Sorghum bicolor* (L.) Moench. Intern. J Bio-resource Stress Manag. 2013; 4:487-491.
3. Anand M, Naik MK, Ramegowda G, Rani GSD. Bio-control and plant growth promotion activity of indigenous isolates of *Pseudomonas fluorescens.* J. Mycopatho. Research. 2010; 48:45-50.
4. Aneja KR. Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International (P) Ltd., New Delhi, 2003.
5. Anonymous. Standard Evaluation System for Rice. International Rice Research Institute, Manila Philippines, 1996.
6. Anonymous. International Seed Testing Association. International Rules for Seed Testing. Seed Science and Technology, 1996.
7. Ashofteh F, Ahmadzadeh M, Fallahzadeh-Mamaghani V. Effect of mineral components of the medium used to grow biocontrol strain UTPF61 of *Pseudomonas fluorescens* on its antagonistic activity against *Sclerotium* wilt of sunflower and its survival during and after the formulation process. J. Plant Patho. 2009; 91:669-675.
8. Belkar YK, Gade RM. Biochemical characterization and growth promotion activities of *Pseudomonas fluorescens.* J. Plant Dis. Sci. 2012; **7**:170-174.
9. Bhunkal N, Singh, Ram and Mehta N. Assessment of losses and identification of slow blighting genotypes against sheath blight of rice. J Mycol. Pl. Pathol. 2015; 45:285-292.
10. Bossis E, Lemanceau P, Latour X, Gardan L. The taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida*: Current status and need for revision. Agronomie. 2000; 20:51-63.
11. Bhuvaneswari V, Krishnam Raju S. Efficacy of new combination fungicide against rice sheath blight caused by *Rhizoctonia solani* (Kuhn*).* J Rice Res. 2012; 5:57-61
12. Chahal KS, Sokhi SS, Rattan GS. Investigations on sheath blight of rice in Punjab. Indian Phytopath. 2003; 56:22-26.
13. Choi GJ, Kim JC, Park EJ, Choi YH, Jang KS, Lim HK *et al*. Biological control activity of two isolates of *Pseudomonas fluorescens* against rice sheath blight. Plant Patho. J. 2006; 22:289-294.
14. Dath AP. A modified multipurpose detached leaf technique for rice sheath blight investigations. Curr. Sci. 1987; 56:269-270.
15. Dennis C, Webster J. Antagonistic species groups of *Trichoderma* I. Production of non-volatile antibiotics. *Transac.* Bri. Myco. Soci. 1971; 57:25-39.
16. Egamberdieva D. Plant growth promoting properties of rhizobacteria isolated from wheat and pea grown in loomy sand soil. Turk. J Bio. 2008; 24:9-15.
17. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research John Wiley & Sons, New York, USA, 1984.
18. Gupta A, Meyer JM, Goel R. Development of heavy metal resistant mutants of phosphate solubilising *Pseudomonas* sp. NBRI4014 and their characterization. Cur. Microbio. 2002; 45:323-332.
19. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in in plant growth promotin: A review. Ann. Microbio. 2010; 60:385-388.
20. Kapase VU, Sawant T. Isolation and characterization of fluoresecent Pseudomonads from rice and carrot rizospheric soil. J Microbio. 2015; 4:8-13.
21. Kazempour KN. Biological control of *Rhizoctonia solani*, the causal agent of rice sheath blight by antagonistic bacteria in greenhouse and field conditions. Pl. Path. J. 2004; 3:88-96.
22. Keel C, Schnider U, Maurhofer M, Voisard C, Laville J, Burger U *et al*. Suppression of root diseases by *Pseudomonas fluorescens* CHAO: Importance of bacterial secondary metabolite, 2,4diacetylphoroglucinol. Mol. Pl. Microbe Interact. 1992; 5:4-13.
23. Khalid A, Arshad M, Zahir ZA. Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. J App. Microbio. 2004; 96:473-480.
24. Kulkinsky-Sobral J, Araujo WL, Mendes R, Geraldi O, Pizzirani- Kleiner A, Azevedo JL. Isolation and characterization of soybean associated bacteria and their potential for plant growth promotion. Environ. Microbio. 2004; 6:1244-1251.
25. Kumar A, Kumar A, Pratush A. Phosphate solubilizing bacterial diversity from the rhizospheric soils of sub-temperate region of Himachal Pradesh. Vegetos. 2013; 26:143-150.
26. Kumar RN, Thirumalai AV, Gunasekaran P. Genotyping of antifungal compounds producing plant growth promoting rhizobacteria, *Pseudomonas fluorescens.* Curr. Sci. 2002; 82:1463-1466.
27. Manivannan M, Ganesh P, Suresh Kumar R, Tharmaraj K, Shiney Ramya B. Isolation, screening, characterization, and antagonism assay of PGPR isolates from the rhizosphere of rice plant in Cuddalore district. Int. J. Pharma. Bio. Arch. 2012; 3:179-185.
28. Meera TW, Balabaskar P. Isolation and characterization of *Pseudomonas fluorescens* from the rice fields. Int. J of Food, Agricul. Vete. Sci, 2012; 2:1260- 1264.
29. Montealegre JR, Reyes P, Perez LM, Herrera R, Silva P, Besoain X. Selection of bio antagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Electro. J. Biotech. 2003; 6:115-127.
30. Nagaraj BT, Sunkad G, Pramesh D, Naik MK, Patil MB. Host range studies of rice sheath blight fungus *Rhizoctonia solani* (Kuhn*).* Int. J. Curr. Microbiol. App. Sci. 2017; 6: 3856-3864.
31. 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. *Microbial Research*, 2004; 159:73-81.
32. Neha KV, Balabaskar P, Naveenkumar R. Survey and occurrence of *Rhizoctonia solani* (Kuhn) causing sheath blight of rice and *in vitro* efficacy of bacterial antagonists against *Rhizoctonia solani* (Kuhn). J Envir. Bio. 2016; 37:1421-1427.
33. Neiland JB. Siderophore: structure and function of microbial iron transport compounds. Bio. Chemist. 1995; 270:26723-26726.
34. Nielsen MN, Sorensen J, Fels J, Pedersen HC. Secondary metabolites and endochitinase-dependent antagonism toward plant - pathogenic microfungi of *Pseudomonas fluorescens* isolates under field condition in Uttrranchal hills. Ind. Phyto. 1998; 64:161-167.
35. O' Sullivan DJ, Gara FO'. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root

pathogens. Microb. Review. 1992; 56:662-676.
36. Panday S, Pudhir VS. Mycoparasitism of potato black scurf pathogen (*Rhizoctonia solani* Khun.) by biological control agents to sustain production. Indian J Horti. 2013; 70:71-75.
37. Patten CL, Glick BR. Role of *Pseudomonas putida* indole acetic acid in development of host plant root system. App. Enviromen. Microbio. 2002; 68:3745-3801.
38. Pracer CS, Chahal DS. Sheath blight of rice caused by *Rhizoctonia solani* Kuhn, new record in India. Curr. Sci. 1963; 32:328-329.
39. Rachid D, Ahmed B. Effect of iron and growth inhibitors on siderophore production by *Pseudomonas fluorescens.* African J. Biotechn. 2005; 4:697-702.
40. Raimam MP, Albino U, Cruz MF, Lovato GM, Spago F, Ferracin TP *et al*. Interaction among free-living N-fixing bacterial isolated from *Drosera villosa* var. Villosa and AM fungi (*Glomus clarum*) in rice (*Oryza sativa*). App. Soil Ecol., 2007; 35:25-34.
41. Ramamoorthy V, Viswanathan R, Rhaguchander T, Prakasam V, Samiyappan R. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pest and diseases. Crop Protec. 2001; 20:1-11.
42. Rangarajan S, Salllena LM, Nair S. Diversity of *Pseudomonas* spp. isolated from the rice rhizosphere population grown along the salinity gradient. Microbio Eco. 2001; 43:280-289.
43. Reddy BP, Rani J, Reddy MS, Kumar VK. Isolation of siderophore producing strains of rhizobacterial fluorescent pseudomonads and their biocontrol against rice fungal pathogens. Int. J. App. Bio. Pharma. Techn. 2010; 1:133-137.
44. Ruchi, Kapoor R, Kumar A, Kumar A, Patil S, Thapa S, Kaur M. Evaluation of plant growth promoting attributes and lytic enzyme production by fluorescent pseudomonas diversity associated with apple and pear. Int. J Sci. Res. *Pub.*, 2012; 2:2250-315.
45. Scarpellini M, Franzetti L, Galli A. Development of PCR assay to identify *Pseudomonas fluorescens* and its biotype. FEMS Microbio. Lett. 2004; 236:257-260.
46. Singh R, Sinha AP. Biological control of sheath blight with antagonistic bacteria. Ann. Plant Prot. Sci. 2009; 17:107-110.
47. Soesanto L, Mugiastuti E, Rahayuniati RF. Biochemical characterization of *Pseudomonas fluorescens* P60. J. Biotechn. Biodiver. 2011; 2:19-26.
48. Tiwari PK, Thrimurty VS. Efficiency of *Pseudomonas fluorescens* isolates for plant growth promotion and disease management in rice. Ann. Plant Protec. Sci., 2009; 17:119-123.
49. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 1947, 159-350.