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Identification and characterization of *Pseudomonas fluorescens* strains effective against *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight of rice in Punjab, India

Mandeep Singh Hunjan*, Anjali Thakur and Pushpinder Paul Singh

Department of Plant Pathology, Punjab Agricultural University, Ludhiana-141004 (Punjab), INDIA
*Corresponding author E-mail: mandeep.hunjan@pau.edu

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Abstract: For the control of bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae,* sixty four *Pseudomonas fluorescens* strains were recovered from rice and wheat rhizosphere. These strains were identified on the basis of internal transcribed spacer (ITS) region. It was observed that the strains showing fluorescence in the selective media showed the amplification of the targeted *P. fluorescens* specific ITS region. The strains were also characterized for the production of the antibiotic 2, 4-diacetylphloroglucinol (DAPG) using *phlD* locus. The characteristic 750bp region was amplified in all the DAPG producing strains. These strains were evaluated against *X. oryzae in vitro* by dual culture method. The *P. fluorescens* strains found effective *in vitro* were further tested in field for their antagonistic potentiality and disease suppression ability. *P. fluorescens* strain number *Pf-4*-R showed maximum inhibition i.e. of 5.5 mm against the test pathogen *X. oryzae* pv. *oryzae*. Talc based powder formulation of the effective strain *Pf*-4-R used for field evaluation, showed that pre-inoculation foliar sprays were effective in controlling bacterial blight of rice with disease suppression efficiency ranging from 29.6 to 65.6 per cent in different treatments.

Keywords: Bacterial blight, *phl*, *Pseudomonas fluorescens; Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

India is a leading country having highest area under rice cultivation, however, the yield per unit area (22.07 q/ha) in the country is among the lowest in the world (http://faostat.fao.org). One of the major limiting factors for lower grain yields are the occurrence of a number of diseases and insect pests. Rice crop is attacked by more than 60 diseases around the world which cause 50-70 per cent loss in rice yield (Jeung *et al.,* 2006). Bacterial blight disease, caused by *Xanthomonas oryzae* pv. *oryzae* is one of the oldest known disease with worldwide incidence reported from different parts of Asia, Northern Australia, Africa and USA (Ghasemiet *et al.,* 2008). Bacterial blight disease reduces yields and yield stability and some management strategies, such as chemicals, are harmful to the environment and in the tropical monsoon climate of Asia; no truly effective bactericide is commercially available for its control (Ou, 1973; Lee *et al.,* 2003). The Punjab state, which is a major contributor to central food pool of India produces about 11.24 million tonnes with productivity of approximately 6033 kg/ha (Anonymous, 2011). In Punjab, although resistant varieties have effectively tackled this pathogen (Bharaj *et al.,* 2006), but resistance in released commercial cultivars is often broken down by the new pathotypes of *X. oryzae* pv. *oryzae* (Lore *et al.,* 2011). Biological control, therefore, assumes special significance in being an eco-friendly, cost-effective alternative strategy for bacterial blight management. This can also be used in integration with other strategies to attain greater levels of protection and rice yields. Antagonistic bacteria are considered ideal biological control agents because they have rapid growth, easy to handle and by virtue of their aggressive colonization of the rhizosphere (Weller, 1983).

Pseudomonas spp. have been studied as biocontrol agent mainly because of their widespread distribution in the soil, their ability to colonize the rhizosphere of host plant and ability to produce a wide range of compounds inhibitory to a wide array of plant pathogens (Thomashow *et al.,* 1995; Rodriguez and Pfender, 1997; Anjaiah *et al.,* 1998; Rangarajan *et al.,* 2003). It has been reported that *P. fluorescens* dominates the group of plant growth promoting rhizobacteria (PGPR) which constitutes about 2-5 per cent of the bacteria adapted to rhizosphere (Landa *et al.,* 2003). Fluorescent pseudomonads are among the most effective rhizobacteria that control plant pathogens through various mechanisms such as producing siderophores inhibiting plant pathogens through competition for iron, producing antibiotics or chitinase and glucanases lysing microbial cells. In addition to disease management, the application of antagonists also enhances the growth and vigor of

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plant in terms of root length or tillering (Manav and Thind, 2002).

The present study focuses on the use of *P. fluorescens* strains that produce 2,4-diacetylphloroglucinol (DAPG) to suppress bacterial blight in rice. Among a variety of metabolites produced by antagonistic fluorescent pseudomonads, diacetyl phluouroglucinol (DAPG) is among the most studied for their role in biocontrol of plant pathogens (Dowling and O'Gara, 1994; Keel *et al.,* 1996; Sharifi-Tehrani *et al.,* 1998; Raaijmakers *et al.,* 1999; Notz *et al.,* 2001, Velusamy *et al.,* 2006, Jambhulkar and Sharma, 2014). In this study, different strains of *P. fluorescens* were isolated from wheat and rice plots amended with different organic amendments in a long term experiment, were characterized and tested for their biocontrol potentiality in a separate experiment at a parallel experimental site.

MATERIALS AND METHODS

Bacterial strains and media: Soil samples were collected from rhizosphere of rice and wheat crops grown on soil amended with different organic amendments in a long term experiment. *Pseudomonas* spp. strains were isolated from the soil suspensions of these rhizosphere samples that were serially diluted and plated onto King's B agar medium (HiMedia Laboratories). Single colonies exhibiting bluish green fluorescence were picked and further purified on the *P. fluorescens* selective media, *Pseudomonas* HiVeg^{TM} Agar (HiMedia Laboratories). The purified cultures were stored at -20^{0}C in silica gel containing vials. Bacterial strains were identified further by using specific primers with respect to 16SrDNA region using primer set 16SF (5'-AGAGTTTGATCCTGGCTCAG-3'); 16SR (5'-CTACGGCTACCTTGTTACGA-3'). The culture of *Xanthomonas oryzae* pv. *oryzae*, PbXo-7 was provided by Rice Pathology Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

***In vitro* antibiosis:** Screening of the bacterial antagonists to *X. oryzae pv. oryzae* was carried out in Bacteriology Laboratory, Department of Plant Pathology by dual culture method. The potent antagonistic bacterial strains were selected on the basis of their ability to produce a zone of inhibition around *X. oryzae pv. oryzae* growth. The culture of *P. fluorescens* was streaked at right angle to the streak of 24 hour old growth the *X. oryzae* pv. *oryzae* on both sides forming an 'H' shape. The inhibition zones were measured after 48 hour of incubation. The dual culture method led to the identification of efficient antagonistic strains which were selected for further studies.

PCR-based screening for *phl* locus: Effective and non effective strains found after dual culture were further identified by PCR based method on the basis of presence of *phl* gene which characterizes the production of antibiotic compound 2,4- DAPG. Antagonistic fluorescent *Pseudomonas* strains were screened for DAPG production by a PCR-based method developed and described by Raaijmakers *et al.* (1997).

The oligonucleotide primer pair PhlD.3F and PhlD.3R listed in Table 1 was developed from sequences within the biosynthetic loci for *phlD* gene of *P. fluorescens* (GenBank accession no. AB125213.1). All the primer sequences were synthesized by Integrated DNA Technologies (Coralville, USA). PCR amplification was carried out in a 25-ml reaction mixture that contained approximately 25 ng of DNA 1X Green GoTaq Flexi Buffer (Promega Inc., USA), 200 mM each dATP, dTTP, dGTP, and dCTP (Promega Inc., USA), 20 pmol of each primer, and 2.0 U of GoTaq DNA polymerase (Promega Inc., USA). PCR amplifications were performed in Eppendorf Mastercycler ProS. The PCR program consisted of an initial denaturation at 94^{0}C for 2 min followed by 30 cycles of 94^{0}C for 60 s, 67^{0}C for 45 s, and 72^{0}C for 60 s. The amplified products (8 ml) were separated on a 1.0 per cent agarose gel in 0.5X TBE buffer at 75 V for 3 h. The gel was stained with ethidium bromide for 30 min and the PCR products were visualized with a UV transilluminator (Syngene, UK).

HPTLC based quantification: *P. fluorescens* isolates showing the presence of *phl* gene were further evaluated by High Performance Thin Layer Chromatography (HPTLC) method. The bacterial culture broth was prepared on King's B medium on an incubator-shaker for 72 hours. The fermentation broth was then centrifuged at 3500 rpm for five minutes in a centrifuge. The supernatant containing cell free metabolites was collected and acidified to pH 2.0 with 1N HCl and extracted with equal volume of ethyl acetate. The compound is soluble in organic solvent ethyl acetate as it mobilizes from broth to the solvent layer which was collected and kept at 4^{0}C for further process. Production of DAPG was further quantified in each of the antibiotic producing strain by the protocol as given by Kar *et al.* (2013). Each band was quantified in a single beam, single wavelength reflectance mode, and relative front was taken into consideration for confirmation of the test chemical.

Preparation of Talc formulation: *P. fluorescens* strain, Pf-4-R which was found to be effective under *in vitro* evaluation was used for preparation of powder formulation. About 600ml of the 72 hrs old liquid culture of Pf-4-R containing approximately 9×10^{8} cfu/ml, was added to 1 kg of talc powder. The broth was mixed well with talc under sterile conditions. The mixture was then dried under aseptic conditions. The dried inoculated granular talc was grounded to fine powder forming the final product. The formulated product was packed in polythene bags, sealed and stored at 4±2°C.

Table 1. List of primers used in the study.

	Sequence (5'->3')	Expected product length	Reference
Phl2a	GAGGACGTCGAAGAC-CACCA	750	Raaijmakers *et al.* (1997)
Phl2b	ACCGCAGCATCGTG-TATGAG		
Phld.3F	CCG-GATGGTCGCCGTGACTC	746bp	This study
Phld.3R	ACTCGCCCAC-GGCCATTTCG		

Table 2. Treatments with antagonist with different delivery modules used in the study.

Seed Treatment (ST)	Wet seeds of basmati rice cultivar Pusa 1121 treated with the powder formulation at the rate of 10gm/kg seed
Seed Treatment and Seedling Root Dip (ST + RD)	Seed treatment was given in the same manner as described in T_1. Thirty day old seedlings were uprooted and washed thoroughly. The roots were dipped in suspension of antagonist formulation at the rate of 10gm/liter for 12 hours prior to transplanting.
Pre-Inoculation Foliar Spray (Pre-IFS)	Forty five days after transplanting, the plots were sprayed with product formulation at the rate of 10gm/l uniformly covering all the leaves. Inoculation of pathogen was done after 24 hrs
Post-Inoculation Foliar Spray (Post-IFS)	Forty five days after transplanting, the plots were sprayed with product formulation at the rate of 10gm/l uniformly covering all the leaves. Inoculation of pathogen was done 24 hrs prior to spray.
Seed Treatment, Seedling Root Dip and Pre-Inoculation Foliar Spray (ST + RD + Pre-IFS)	As above, in T_1, T_2 and T_3
Seed Treatment, Seedling Root Dip and Post-Inoculation Foliar Spray (ST + RD + Post-IFS)	As above, in T_1, T_2 and T_4
Chemical Seed Treatment	The wet seeds were treated with Emisan 6 and Streptocycline at the rate of 5g and 1g/ kg of seed respectively and were sown in nursery beds. Thirty days old seedlings were transplanted in the plots without any treatment
Untreated Control	Seed and seedlings treated with water only

***In-vivo* evaluation:** The antagonistic strain, Pf-4-R was evaluated in the field during rice seasons of 2011, 2012 and 2013 in the experimental area of Department of Plant Pathology, Punjab Agricultural University, Ludhiana. The experiment was laid out in randomized block design with three replications for each treatment. Thirty days old seedling of basmati rice cultivar Pusa 1121 were transplanted using two seedlings per hill in a plot size of 2×2 m^2 and spacing of 15cm×20cm Agronomic practices recommended by the Punjab Agricultural University, Ludhiana were followed for raising the crop. The efficacy of the antagonists was evaluated by the treatment sets given in Table 2.

Disease inoculation: The concentrations of 1×10^9 CFU/ml of *X. oryzae* pv. *oryzae* was clip inoculated (Kaufmann *et al.* 1973) after 45 days of transplanting. A focus of infection was created by clip inoculating two plants at the center of each plot. Then spread of disease was allowed on its own under natural conditions. The observations on disease intensity were recorded 30 days after inoculation. Sixteen plants around the focus in all directions were selected i.e. four plants in each direction. Leaves from each selected plant were categorized using 0-9 scale.

The disease severity was calculated using the following formula-

Disease severity, DS (%) = Sum of individual scores × 100 / (Total leaves observed × maximum score)

The per cent disease suppression efficiency was calculated using the formula:

Disease suppression efficiency (%) = (DS of control – DS of treated group) × 100 / DS of control

RESULTS AND DISCUSSION

Isolation of *Pseudomonas fluorescens:* Out of 64 samples collected from rice and wheat rhizosphere, *P. fluorescens* was isolated from as many samples;

however their number differed due to the sampling experimental site. Among these strains, four strains (*Pf-2*-R, *Pf-4*-R, *Pf*-9-R and *Pf*-31-R) isolated from rice crop and three strains (*Pf* -43-W, *Pf* -48-W and *Pf* -64-W) from wheat crop formed dark bluish green colored colonies with good growth rates (Thakur *et al.*, 2011). Out of these, the strain number *Pf-4*-R and *Pf-48*-W exhibited dark green colored colonies with maximum growth rate.

PCR based identification: The strains showing good growth and fluorescence characters were further identified by the 16srDNA region amplification. It was found that all the strains showed the amplification of the targeted ITS (Internal transcribed spacer) region of the DNA of 850 base pair (Fig. 1), specific for the *P. fluorescens.* So, it was confirmed that all the strains of bioagent showing fluorescent characteristics in the selective medium were of *P. fluorescens.*

Phl based molecular identification of DAPG producing strains: DNA for all the strains was isolated and amplified using *phlD* based primer sets. Perusal to Figure 2, amplification of typical 750 base pair band was observed in all the strains that produced bluish green colonies. However in one strain i.e. *Pf-39* -W isolated from wheat rhizosphere which showed light yellow pigmentation of the media showed no amplification at this locus suggesting that this strain of *P. fluorescens* does not produce any DAPG. It is also evident from Table 3 that this strain showed no inhibition zone when co-cultured with *X. oryzae* pv. *oryzae*, however it showed moderate growth rate. Another set of primers which were designed in-house i.e. phld.3, showed approximately 700bp fragment along with another 200 bp amplicons from both wheat and rice strains. However, wheat isolated showed a third amplicon of approximately 300bp suggesting diversity of *phlD* locus in *P. fluorescens* strains recovered from wheat rhizosphere (Fig. 3).

***In vitro* efficacy of *P. fluorescens* against *X. oryzae* pv. *oryzae* :** All the strains of *P. fluorescens* recovered from wheat and rice rhizosphere were evaluated *in vitro* against *X. oryzae* pv. *oryzae.* The inhibition zones were measured for each strain. It is evident from Table 1 that out of 32 rice rhizosphere strains, 17 were inhibitory to pathogen. Among these 17 strains, eight strains formed inhibition zone of 1.5 mm to 3 mm and three strains showed inhibition zone of more than 3 mm. Strain number *Pf-4*-R exhibited high colony growth rate and also showed maximum inhibition i.e. of 5.5 mm. Similarly, out of 32 wheat rhizosphere

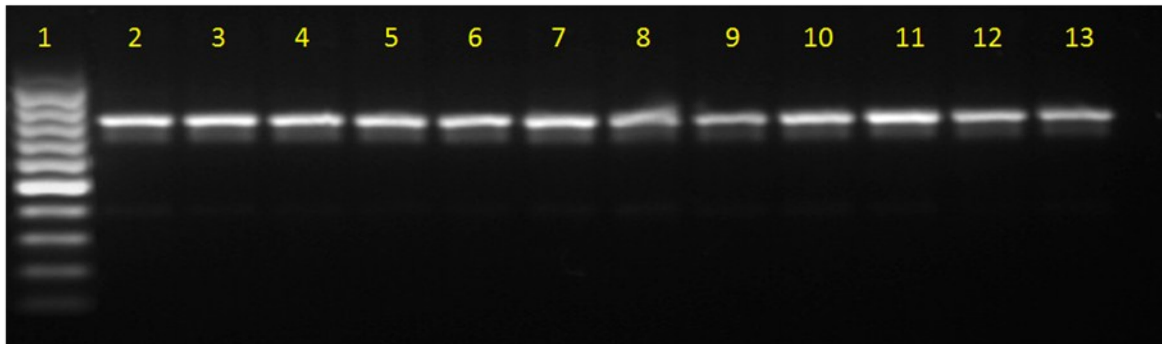

Fig. 1. *The amplification products from twelve strains of P. fluorescens isolated from rice and wheat rhizosphere showing 850 bp amplicon specific to 16srDNA. Lane 1 contains 100bp DNA ladder (Promega Inc.), Lanes 2 to 7 contain Pf-2-R, Pf-4-R, Pf-9-R, Pf-18-R, Pf-31-R, Pf-32-R; Lanes 8-13 contain amplified DNA from the strains Pf-34-W, Pf-39-W, Pf-48-W, Pf-49-W, Pf-61-W, Pf-64-W.*

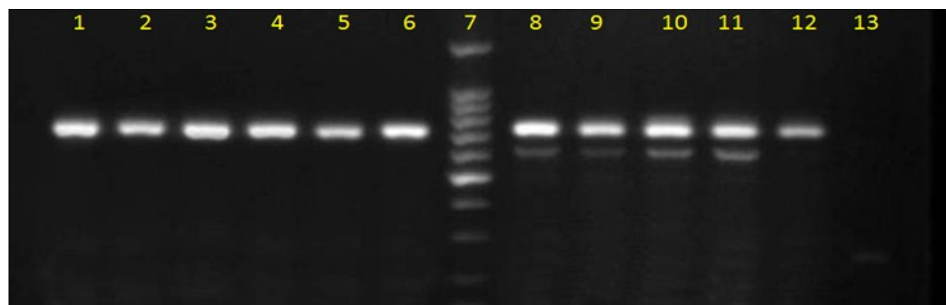

Fig. 2. *The 750 bp amplicon specific to phld gene amplified from six rice rhizosphere strains and six wheat rhizosphere strains with phlD primers. Lanes 1 to 6 contain amplified DNA from the strains Pf-2-R, Pf-4-R, Pf-9-R, Pf-18-R, Pf-31-R, Pf-32-R; Lane 7 contains 100bp DNA ladder (Promega Inc.); Lanes 8-12 contain amplified DNA from the strains Pf-34-W, Pf-48-W, Pf -49-W, Pf-61-W, Pf-64-W. No amplification in lane 13 (Pf-39-W).*

Table 3. *In-vitro* evaluation of potent strains of *P. fluorescens* against *X. oryzae* pv. *oryzae* by dual culture method.

Isolated from	*Pf* strains	Color	Growth	Inhibition zone (mm)
Rice rhizophere	*Pf*-2-R	Green	++++	3.5
	Pf-4-R	Dark green	+++++	5.5
	Pf-9-R	Green	+++	3.0
	Pf-18-R	Green	+++	3.0
	Pf-31-R	Dark green	++++	5.0
	Pf-32-R	Green	+++	3.0
Wheat rhizosphere	*Pf-34*-W	Green	+++	3.0
	Pf-39-W	Light Yellow	+++	0
	Pf-48-W	Green	+++	3.0
	Pf-49-W	Green	+++	2.5
	Pf-61-W	Green	+++	3.0
	Pf-64-W	Green	+++	3.0

+++ Bacterial colonies appearing within 48 hours; with good growth
++++ Bacterial colonies appearing after 24 hours; with good growth
+++++ Bacterial colonies appearing within 24 hours; with very good growth

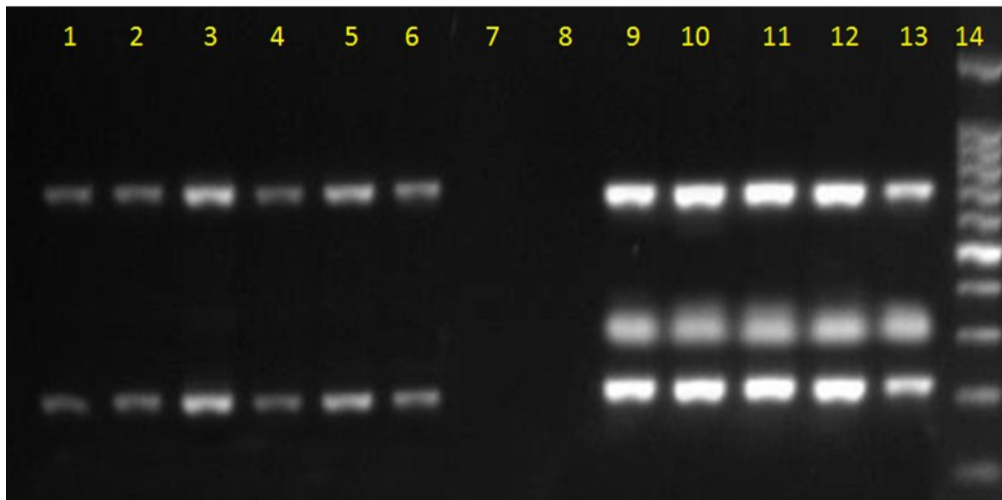

Fig. 3. *The ~750 bp amplicon specific to phld gene amplified from six rice rhizosphere strains and six wheat rhizosphere strains with phlD.3 primers. Lanes 1 to 6 contain amplified DNA from the strains Pf-2-R, Pf-4-R, Pf-9-R, Pf-18-R, Pf-31-R, Pf-32-R; Lane 7 is negative control; Lane 8 contains amplified DNA from Pf-39-W; Lanes 9-13 contain amplified DNA from the strains Pf-34-W, Pf-48-W, Pf-49-W, Pf-61-W, Pf-64-W; Lane14 contains 100bp DNA ladder (Promega Inc.).*

strains, 13 were found to be inhibitory to the pathogen. Among these 13 strains, three formed inhibition zone of 1.0 to 2 mm and five strains showed inhibition zone in the range of 2 mm to 3 mm. The strains *Pf-34*-W, *Pf* -48-W, *Pf-61*-W and *Pf-64*-W recovered from wheat rhizosphere also showed good colony growth and inhibition zone of 3 mm.

From both the crops, eleven strains showing inhibition zone of more than 2.5 mm were selected and re-evaluated following same procedure. In the second experiment too, strain number *Pf -4*-R isolated from rice rhizosphere showed the maximum inhibition zone of 5.5 mm with dark green colored colonies and maximum growth rate. The data is presented in Table 3.

The strains of the bioagent found effective under *in vitro* evaluation by dual culture and showing the presence of *phl* gene were further evaluated for the production of DAPG antibiotic by HPTLC method. It was found that all the effective strains (*Pf*-2-R, *Pf*-4-R, *Pf*-18-R, *Pf*-48-W and *Pf*-64-W) produced antibiotic

Table 4. Evaluation of different delivery methods of *P. fluorescens* formulation against bacterial blight under field conditions.

Treatments	Mean disease Severity (%)			
	2011*	2012*	2013**	Mean
Seed treatment	23.3	26.4	18.2	22.6[a]
S T + R D	20.9	21.2	17.1	19.7[b]
Pre-I F S	16.6	10.1	12.6	13.1[c]
Post- I F S	23.7	14.7	14.8	17.7[d]
ST + RD + Pre-IFS	13.5	10.9	11.9	12.1[c]
ST + RD + Post-IF S	20.3	18.2	13.2	17.2[d]
Chemical seed treatment	27.9	24.1	20.2	23.9[a]
Untreated control	30.2	28.7	19.7	26.4[e]
P>F (0.01)	Years <0.001 Treatments <0.001 Years X treatment 0.06			

Mean figures followed by same letter do not differ significantly; * The disease severity data obtained in years 2011 and 2012 do not significantly differ from each other; **The disease severity data obtained in year 2013 differed significantly from disease severity data in 2011 and 2012 both.

DAPG in varying quantities. However, *Pf-4*-R produced maximum amount of antibiotic (2113.7 AU), which was closely followed by strain *Pf-64*-W (2030.8 AU). However, other strains viz. *Pf-48*-W (1247.0 AU), *Pf*-2-R (809.5 AU) and *Pf-18*-R (762.5 AU) produced significantly lesser amount of the DAPG. The results observed were in conformation with dual culture findings, as measure of inhibition zone produced was found to be directly correlated with the amount of antibiotic produced. Based on these results, the strain *Pf-4-R* was selected for evaluation under field conditions.

***In-vivo* evaluation:** Significant reduction in disease severity was observed in the treatments with pre-inoculation foliar spray (Fig. 4), either alone (50.4%) or in combination with seed treatment and seedling root dip treatment (54.2%) over the control plots. As is evident from Table 4, highest mean disease severity was observed in the untreated control treatment (DS, 36.4%) followed by chemical control (DS, 23.9%). Similarly, post-inoculation foliar sprays were also effective in managing bacterial blight disease using *Pf-4*-R formulation (Table 4). In this three year experiment, results were consistent for the first two experimental seasons and did not differ significantly, however we obtained significant variation during the third year. The mean disease severity values for 2011 and 2012 seasons were 22.1 and 19.3 per cent respectively while during the third year, it was observed to be 16 per cent. It may be attributed to the prevailing relatively dry conditions during the plant inoculations and the subsequent pathogenesis period. Initial seed priming with *Pf-4*-R as seed treatment and seedling root dip treatment improved the overall plant vigour, but that did not contribute much towards reducing disease severity at later stages of crop growth. However, with pre-inoculation foliar sprays of *Pf-4*-R formulations (alone or in combination), significant reduction in disease development was noticed (Fig. 4). It was also observed that following the pre-inoculation foliar spray treatment, the lesion lengths on the clipped leaves developed partially. So, under field conditions pre-inoculation foliar spray treatment proves to be effective treatment in disease control showing maximum disease suppression efficiency followed by post-inoculation foliar spray.

Soil rhizobacteria are often difficult to identify and characterize on simple isolation media or on the basis of their colony structure and morphology. However, molecular tools have provided markers for accurate identification of these bacteria and in the present study *P. fluorescens* isolated from the rhizosphere of rice and wheat were identified on the basis of molecular markers based on ITS (Internal transcribed spacer) region. Molecular identification was performed by using the primer set 16SF–16SR and it was observed that all the strains showed the amplification of the targeted ITS region of the DNA of 850 base pair, specific for the *P. fluorescens.* It was confirmed that all the strains of bioagent showing fluorescent characteristics in the selective medium were *P. fluorescens.*

The findings were in conformation with Scarpellini *et al.* (2004), who also used the polymerase chain reaction to amplify a specific portion of the 16S gene, allowing the recognition of *P. fluorescens* from other group of *Pseudomonas.* Eleven strains in this study were found to be effective against *X. oryzae* pv. *oryzae*. Native strains of rhizobacteria *P. fluorescens* have been reported for antagonist activity against *X. oryzae* pv. *oryzae* by Sakthivel *et al.* (1988) and Ramanamma *et al.* (1994) who also reported formation of large inhibition zones by *P. fluorescens* against *X. oryzae* pv. *oryzae* in synthetic medium. Similarly, Battu and Reddy (2009) isolated 20 *P. fluorescens*

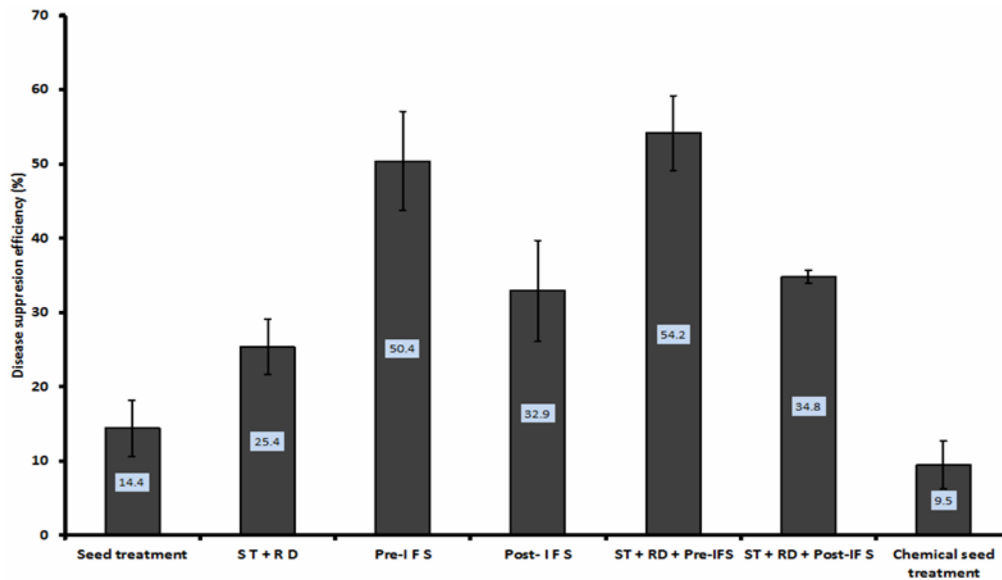

Fig. 4. *Disease suppression efficiency of P. fluorescens strain Pf-4R against bacterial blight of rice using different delivery methods.*

strains from rice growing soil samples and characterized them by dual culture test against rice fungal pathogens. The potent strains were further characterized by molecular markers based on *phl* gene. They concluded that antagonism of the bioagent is dependent on the presence *Phl* gene which signifies the production of antibiotic like substance, DAPG inhibitory to phytopathogens as evident from our studies as well. Root-associated fluorescent *Pseudomonas* spp. producing the antibiotic 2, 4-diacetylphloroglucinol (*Phl* gene) is a key component of the natural biological control. Velusamy *et al.* (2004) characterized plant associated bacteria from different states of India and provided positive conformation to the role of 2, 4-Diacetylphloroglucinol (DAPG) as an antibacterial compound in suppression of bacterial blight of rice. It was also noted in our studies that strain *Pf-4-R*, that produces maximum DAPG, also showed maximum antagonism towards *X. oryzae* pv. *oryzae.* Raaijmakers *et al.* (1999) also reported that fluorescent *Pseudomonas* spp. that have *Phl* gene play an important role in the natural suppressiveness of these soils to take-all disease of wheat.

In the course of isolations of fluorescent pseudomonads, the strains showing fluorescent colored colonies with good growth rates were selected. The *P. fluorescens* strains show wide spectrum of inhibitory activity, mostly due to production of fluorescent siderophores. Santhi *et al.* (1987) had reported the role of fluorescent pigments in antagonism exhibited by *P. fluorescens.* Similarly in this study also, eleven strains were found to highly inhibitory to the pathogen and produce inhibition zones of more than 2.5 mm. Though some researchers have found no correlation between *in-vitro* antagonism exhibited by the bacteria and their ability to suppress disease under greenhouse or field conditions (Papavizas and Lewis, 1983), there are other reports suggesting it is possible to identify efficient biocontrol agent based on their inhibition in dual culture method in the laboratory (Gnanamanickam and Mew, 1992; Mew and Rosales, 1986). To control various plant disease in other crops, fluorescent Pseudomonads have been routinely employed as seed treatment (Niranjana et al., 2009), seedling root dip (Verma, 2009) or soil drenching (Jayalakshmi et al., 2010). These delivery systems have been used individually or in combinations to achieve different levels of disease control. In this study, it was found that strain which showed *in-vitro* antibiosis could also suppress disease effectively in the field experiments. Pre-inoculation foliar sprays either alone or in combination of seed treatment and seedling root dip provided maximum disease control. Similar results were reported by other workers also (Rishnamurthy and Gnanamanickam, 1998), wherein they have found that bioagent when applied as seed treatment + root dip + two foliar spray gave better disease control of other rice diseases such as sheath blight.

Conclusion

It is concluded from the present studies that different strains of *Pseudomonas fluorescens* can be very effectively pre-screened prior to *in vitro* testing against *Xanthomonas oryzae* pv. *oryzae* using molecular screens; particularly amplifying the DAPG producing loci. It is further concluded that the Pf-4 strain selected on the above said basis when used as pre inoculation foliar spray as antagonist to *X. oryzae pv. oryzae* suppressed the bacterial blight disease effectively under field conditions and can be incorporated in integrated disease management practices.

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