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Antagonistic potentiality of native rhizobacterial isolates against root rot disease of okra, incited by *Rhizoctonia solani*

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Among seventy isolates, antagonistic twenty one representing biovars of *Pseudomonas fluorescens* (biovars I, II, III, and V) were collected from the rhizosphere of okra, chilli, ground nut, brinjal, cabbage and tomato from different agro-ecological regions of West Bengal and were subjected to evaluate for their antifungal activity under *in vitro* condition against *Rhizoctonia solani*, the most important soil-borne plant pathogen two isolates, PF-8 and PF-7 effectively inhibited the mycelial growth of *Rhizoctonia solani* (72.05 and 68.25%, respectively) in dual culture method. The vigour index of okra was also recorded maximum for the isolate PF-8 (2415.7) followed by PF-7 (2063.25). Mechanisms of fungal inhibition were elucidated by tracing secondary-metabolite production of the rhizobacterial isolates. Most of the isolates expressed a specific mechanism of antagonism, as represented by production of pigments, Siderophores, cyanide (HCN), Indole Acetic acid (IAA) and Salicylic Acid (SA) production, and Phosphate solubilization. Rhizobacterial isolate PF-8 was found to be effective as seed and soil treatment for management of root rot disease of okra. The antagonistic nature of fluorescent pseudomonads against fungal pathogens can be assigned to design a potential candidate for development of agriculture sector to be used in biological control of soil borne plant pathogens.

Key words: Fluorescent pseudomonads, secondary metabolites, *Rhizoctonia solani*, okra root rot.

INTRODUCTION

Okra, which is also known as "*Bhindi*", is one of the important vegetables of India in general and West Bengal in particular with production of 0.662 million tones from 58,400 ha with an average productivity of 11.4 t.ha⁻¹. A number of fungi, bacteria, viruses, nematodes and insects attack this crop. The total loss of vegetable on this account has been estimated up to 20 to 30% but if the pathogens are allowed to develop, this loss may increase up to 80 to 90% (Hamer and Thompson, 1957). Among the diseases, okra root rot incited by *Rhizoctonia solani* is the most serious problems for okra cultivation,

especially in the *post-kharif* under Gangetic Alluvial Region of West Bengal. The biological control of plant pathogenic fungi has received considerable attention as an alternative strategy through the use of saprophytic micro flora.

Pseudomonades have been found in large populations, which inhabit in the rhizosphere of several agriculture crops. For the survival of plant growth promoting rhizobacteria, the production of plant growth promoting substances is an important trait. PGPR (Plant Growth Promoting Rhizobacteria) enhance plant productivity by a range of mechanisms. The plant growth promoted by PGPR is still now pandemonium mechanism and not clearly understood, but is thought to include some beneficial direct and indirect effects resulting to several

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hypothesis such as production of antibiotics, siderophores, or cyanhidric acid (HCN), that decrease the growth of phytopathogens and other deleterious microorganisms, production of plant growth regulators, or improvements in plant nutrient uptake (Glick, 1995; Myoungsu et al., 2005) and production of phytohormones such as auxins (Asghar et al., 2002), cytokinins (Arhipova et al., 2005) and gibberellins (Gutierrez-Manero et al., 2001; Joo et al., 2004) as well as through the solubilization of phosphate minerals (Freitas et al., 1997) are usually believed to be involved. Root colonization by PGPR may induce the production of plant growth substances by the plant (Patten and Glick, 2002).

This present investigation was undertaken to find out and characterize the effective isolates of fluorescent pseudomonads and possible mechanisms for antagonism elucidated by determining secondary-metabolites production and its application for management of root rot disease of okra.

MATERIALS AND METHODS

Collection of soil samples and isolation, characterization and identification of rhizobacterial isolates

Fluorescent pseudomonads were isolated from the soil collected from the rhizosphere of different crops with King's medium B (KMB) (King et al., 1954). The antagonistic rhizobacterial isolates were characterized on the basis of their morphological (cell shape, cell arrangement, gram reaction), cultural (colony type, pigment production) and biochemical identification keys of Bossis (1995) for *Pseudomonas* sp. Pseudomonads were identified to species and biovars on the basis of oxidase, arginine dihydrolase, denitrification, gelatine hydrolysis, levan production, and acid production from trehalose, utilization of tryptophane and L-tartrate (Bossis et al., 2000). The rhizobacterial isolates were bioassayed for their ability to promote/inhibit seedling growth on okra using the method as described by ISTA (1966). These bacterial colonies were tested for their antagonistic activity against *R. solani* by dual culture technique.

In vitro antagonistic activity against soil borne fungal pathogen

For each treatment three replications were used. Percent inhibition over control was calculated by using the formula as follows:

$$I = \frac{C - T}{C} \times 100$$

I = percent inhibition of mycelium; C = growth of mycelium in control, and T = growth of mycelium in treatment.

Secondary metabolites production by bacterial isolates

Bacterial strains producing an antagonism towards pathogen (*R. solani*), were tested for the production of secondary metabolites like production of IAA (Gordon and Weber, 1951), Siderophore (Reeves et al., 1983), HCN (Lorck, 1948; modified by Alstrom, 1989), Salicylic acid (Meyer et al., 1992) and Phosphate solubilization (Ammonium phosphomolybdate method).

Preparation of mass inoculums

250 ml of nutrient sucrose broth (0.1% sucrose) was inoculated with PF-8 isolates and mass culturing was done in rotary shaker incubator for 60 h at $28 \pm 2^\circ\text{C}$ and the bacterial cultures were mixed in sterilized talc containing 1% CMC. Talc mixed culture containing (3.5×10^8 cfu / g of talc mixed product) was used for seed treatment as well as soil treatment.

Effect of rhizobacterial isolates on management of root rot disease of okra

The experiment was conducted in C-Block farm, BCKV, Kalyani during pre-kharif season 2011. Seven treatments (Table 4) were used in three replications in RBD in 9 m² plot size. Standard package of practices were maintained except for disease management measures. Disease incidence (%) was calculated based on the following formula:

$$(\text{Number of infected plants} / \text{Total number of plants}) \times 100$$

Fruits picking was done in seven days intervals and total plot yield was obtained (kg/ 9 m² plot).

Statistical analysis

Correlation and regression were calculated based on the dependable (percentage of growth inhibition of pathogen) and independent variables (IAA, HCN, SA, Siderophore production and phosphate solubilization). A Hierarchical cluster analysis with the UPGMA algorithm was performed with the SPSS 10.0 analysis system.

RESULTS AND DISCUSSION

Twenty one out of the seventy isolates isolated from different crops of different regions (Table 1) exhibited antagonistic activity towards *R. solani*. Using the phenotypical criteria (Bossis et al., 2000) seven, ten and three isolates were identified as *P. fluorescens* biovar I, V, III respectively. Only one isolate of PF- 23 identified as *P. fluorescens* biovar II (Table 2). Most of the antagonistic bacteria (*P. fluorescens* biovar V) were isolated from new gangetic alluvial region and remaining (*P. fluorescens* biovar I) from terai, old alluvial and red & lateritic region of West Bengal (Table 1).

In vitro antagonism

The demonstration of higher inhibition level was elucidated by restriction of growth toward *R. solani* by fluorescent pseudomonads. Antifungal activity shown by these isolates is given in the Table 4 which reveals that there is variation in the antifungal activity among the isolates. The effective antagonistic rhizobacterial isolates exhibited antagonistic activity in the range of 42 to 72% and significantly highest antagonistic activity against *R. solani* was observed by PF-8 isolates (72.05% inhibition) in dual culture method. The data depicted in the Table 3

Table 1. List of location, rhizosphere of rhizobacterial isolates and antagonistic isolates.

Location	Lat and long	Soil Rhizosphere	No. of isolates	Antagonistic isolates
Purulia (Hura)	23.32208° N Lat; 86.65498° E Long	Okra	5	PF2
Daspur (W. Midnapore)	22.60464° N Lat; 87.72351° E Long	Chilli	6	PF3
N. Dinajpur (Chopra)	26.42204° N Lat; 88.28401° E Long	Brinjal	7	PF4,PF5
Kalyani (Nadia)	22.98515° N Lat; 88.43335° E Long	Okra	8	PF7,PF8,PF23
Beharampore (Murshidabad)	24.16912° N Lat; 88.32502° E Long	Okra	4	PF9
Hoogly (polba)	22.96489° N Lat; 88.31884° E Long	Potato	5	PF10
Malda	25.06209° N Lat; 88.14974° E Long	Bean	3	PF11
Purulia (Hura)	23.32208° N Lat; 86.65498° E Long	Citrus	4	PF13,14
Kalyani (Nadia)	22.98515° N Lat; 88.43335° E Long	Potato	4	PF15
Kalyani (Nadia)	22.98515° N Lat; 88.43335° E Long	Cabbage	3	PF16
Hoogly (polba)	22.96489° N Lat; 88.31884° E Long	Brinjal	3	PF17
Daspur (W. Midnapore)	22.60464° N Lat; 87.72351° E Long	Ground nut	3	PF18
Burdwan	23.2500° N Lat; 87.8500° E Long	Tomato	3	PF19
Kalyani (Nadia)	22.98515° N Lat; 88.43335° E Long	Brinjal	4	PF20
Panskura (East Midnapore)	22.42841° N Lat; 87.72411° E Longitude	Groundnut	3	PF21
Kalyani (Nadia)	22.98515° N Lat; 88.43335° E Long	Chilli	3	PF22
Purulia (Baghmundi)	23.12° N Lat; 86.3° E Long	Okra	2	PF24

Table 2. Biochemical characteristics for the identification of isolates belonging to *P. fluorescens* and *P. putida* (Bossis et al., 2000).

Isolate	Fluorescence	Arginine	Oxidase	Tabac	Gelatine	Trehalose	Lev	Deni	L-ara	L(+)tart +
PF2	+	+	+	-	+	+	+	-	NA	NA
PF3	+	+	+	-	+	-	-	-	NA	NA
PF4	+	+	+	-	+	+	+	-	NA	NA
PF5	+	+	+	-	+	+	+	-	NA	NA
PF7	+	+	+	-	+	-	-	+	NA	NA
PF8	+	+	+	-	+	-	-	-	NA	NA
PF9	+	+	+	-	+	-	-	-	NA	NA
PF10	+	+	+	-	+	-	-	-	NA	NA
PF11	+	+	+	-	+	-	-	-	NA	NA
PF13	+	+	+	-	+	+	+	-	NA	NA
PF14	+	+	+	-	+	-	-	+	NA	NA
PF15	+	+	+	-	+	-	-	+	NA	NA
PF16	+	+	+	-	+	-	-	-	NA	NA
PF17	+	+	+	-	+	-	-	-	NA	NA
PF18	+	+	+	-	+	-	-	-	NA	NA
PF19	+	+	+	-	+	-	-	-	NA	NA
PF20	+	+	+	-	+	+	+	-	NA	NA
PF21	+	+	+	-	+	-	-	-	NA	NA
PF22	+	+	+	-	+	-	-	-	NA	NA
PF23	+	+	+	-	+	+	+	+	+	+
PF24	+	+	+	-	+	+	+	+	NA	NA

L-ara, L-arabinose; Den., Denitrification; Lev., Levan; L(+) tart., L(+) tartrate; +, positive result; -, negative result; NA, not applicable.

indicated that the vigour index based on germination percentage, root length and shoot length was also recorded maximum for the isolate PF-8 (2415.7) followed by PF-7 (2063.25). Variation in the antifungal activity of

the Fluorescent pseudomonads isolates was also been observed by other workers. Tripathi and Johri (2002) observed *in vitro* inhibition of *Colletotrichum dermatium*, *R. solani* and *Sclerotium rolfisii* by fluorescent

Table 3. Effect of seed bacterization with native rhizobacterial isolates and their combinations on seed germination, root length, shoot length and vigour index.

Name of the antagonists	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
PF2	58.3	11.5	4.6	938.63
PF3	66.7	10.3	3.9	947.14
PF4	58.3	10.1	2.5	734.58
PF5	66.7	11.8	2.9	980.49
PF7	91.7	16.4	6.1	2063.25
PF8	83.3	21.5	7.5	2415.70
PF9	66.7	13.6	5.4	1267.30
PF10	75.0	12.9	3.1	1200.00
PF11	66.7	14.2	3.3	1167.25
PF13	66.7	10.7	2.4	873.77
PF14	58.3	11.2	2.9	822.03
PF15	66.7	12.1	3.7	1053.86
PF16	66.7	12.3	3.1	1027.18
PF17	66.7	13.1	5.4	1233.95
PF18	66.7	15.1	3.3	1227.28
PF19	58.3	8.4	2.8	652.96
PF20	75.0	11.1	3.2	1072.50
PF21	58.3	8.8	4	746.24
PF22	66.7	9.3	3.7	867.10
PF23	58.3	9.4	2.4	687.94
PF24	58.3	10.1	2.5	734.58
Control	58.3	8.4	2.1	612.15

pseudomonads. The data depicted in the Table 3 indicated maximum vigour index based on germination percentage, root length and shoot length for the isolate PF-8 (2415.7) followed by PF-7 (2063.25). The effective rhizobacterial antagonist isolates were selected for further characterization of secondary metabolites traits (Table 4).

Secondary metabolites traits

Twenty one rhizobacterial isolates were selected for the secondary metabolites trait analysis. They were tested for their ability to produce antifungal metabolites, Phytohormones- IAA, HCN, Siderophores, SA and Phosphate solubilization activity. Significantly higher amount of secondary metabolites were produced by PF-8 and PF-7 isolates as compared with others. The production of IAA was in the range of 19.76 to 137.19 µg/ml. Thirteen isolates produced IAA in between 19 and 50 µg/ml and eight isolates produced IAA more than 50 µg/ml amount. Ahamad et al. (2005) also reported the production of IAA by 11 isolates of pseudomonads from different crop plants in the range of 5.34 to 22.4 mg/ml. Similarly Karnwal (2009) also reported the varying amounts of IAA production by fluorescent pseudomonads. Anandaraj and Sarma (2003) reported that growth-promoting strains of fluorescent Pseudomonads were found to synthesize

phytohormones viz IAA and Gibberellic Acid (GA). The production of significant levels of IAA and cause of shoot and root elongation in soybeans are nothing but the application of plant growth promoting rhizobacteria, Pseudomonas sp (Gupta et al., 2002). Thus, this present findings indicated that the eight rhizobacterial isolates having the potentiality of higher production of IAA may be exploited in future for enhancement of plant growth. It was noteworthy that, production of HCN was important secondary metabolite in terms of pathogen inhibition. All the rhizobacterial isolates of this present study were cyanogenic but the isolates PF- 8 and PF-7 were capable of production of significantly higher amount of HCN. PF- 8 and PF-7 could be characterized as potential antagonistic isolates in terms of production of higher amount of secondary metabolites traits like HCN, siderophore, SA, IAA and phosphate solubilization activity. In order to predict the antagonistic activity of rhizobacterial isolates with biochemical parameters a linear multiple regression model was developed using different biochemical parameters through stepwise method.

$$Y = 23.957 - 0.0574 X_2(\text{IAA}) + 3.66.57 X_3(\text{HCN}); R^2 = 0.890; R^2_{\text{adj}} = 0.878 \quad (1)$$

$$Y = 0.0026X - 0.0497; R^2 = 0.8479 \quad (2)$$

HCN production, the single secondary metabolite

Table 4. Production of secondary metabolites.

Isolates	Growth inhibition against RS (%)	IAA production (µg/ml)	HCN production (Absorbance at 625 nm)	Siderophore production (µmole benzoic acid /ml)	Salicylic acid production (µg/ml)	Phosphate solubilization (µg/ ml culture filtrate)
PF2	63.15 ^{cd} (52.62)	24.42 ^{kl}	0.111 ^d	15.0 ^{cde}	3.07 ^{def}	29.53 ^l
PF3	65.1 ^c (53.79)	43.86 ^h	0.121 ^c	19.89 ^b	0.465 ⁱ	67.98 ^b
PF4	56.1 ^{gh} (48.50)	76.36 ^d	0.098 ^{efg}	11.84 ^{def}	1.61 ^h	27.24 ^l
PF5	53.8 ^{hi} (47.18)	52.59 ^g	0.091 ^{fgh}	10.64 ^{ef}	4.33 ^{bc}	35.03 ^k
PF7	68.25 ^b (55.70)	81.49 ^c	0.135 ^b	30.43 ^a	4.99 ^b	105.26 ^a
PF8	72.05 ^a (58.08)	137.19 ^a	0.157 ^a	33.65 ^a	6.0 ^a	108.59 ^a
PF9	56 ^{gh} (48.45)	29.34 ^j	0.09 ^{gh}	12.90 ^{def}	2.76 ^{defg}	53.87 ^{cd}
PF10	58.4 ^{fg} (49.84)	22.67 ^{lm}	0.101 ^{de}	14.84 ^{cde}	1.76 ^{gh}	51.34 ^{de}
PF11	63.7 ^{cd} (52.95)	19.76 ^m	0.110 ^d	16.18 ^{bcd}	2.61 ^{efgh}	50.93 ^{def}
PF13	54.65 ^{hi} (47.65)	74.43 ^{de}	0.092 ^{fgh}	17.73 ^{bc}	2.83 ^{defg}	49.2 ^{defg}
PF14	44.05 ⁱ (41.58)	72.43 ^e	0.073 ^{jk}	10.87 ^{ef}	2.38 ^{fgh}	37.84 ^{lk}
PF15	55.7 ^{ghi} (48.27)	28.25 ^{jk}	0.087 ^{ghi}	13.44 ^{cdef}	3.07 ^{def}	45.67 ^{fgh}
PF16	61.7 ^{de} (51.77)	37.95 ⁱ	0.106 ^{de}	12.43 ^{def}	3.72 ^{cd}	57.33 ^c
PF17	59.55 ^{ef} (50.51)	29.10 ^j	0.091 ^{fgh}	13.16 ^{cdef}	2.64 ^{defgh}	51.49 ^{de}
PF18	52.95 ⁱ (46.69)	27.74 ^{jk}	0.083 ^{hij}	10.71 ^{ef}	2.06 ^{fgh}	45.18 ^{gh}
PF19	58.85 ⁱ (50.10)	37.00 ⁱ	0.081 ^{hij}	13.60 ^{cdef}	1.55 ^h	46.09 ^{efgh}
PF20	47.25 ^k (43.42)	23.74 ^l	0.076 ^{ji}	12.68 ^{def}	3.06 ^{def}	42.08 ^{hij}
PF21	54.75 ^h (47.73)	64.71 ^f	0.091 ^{fgh}	10.40 ^{ef}	2.84 ^{defg}	44.30 ^{ghi}
PF22	50.2 ^j (45.11)	106.04 ^b	0.082 ^{hij}	9.58 ^f	3.61 ^{cde}	39.19 ^{ijk}
PF23	42.05 ^l (40.43)	37.10 ^j	0.065 ^k	9.65 ^f	2.26 ^{fgh}	35.83 ^k
PF24	47.9 ^{jk} (43.80)	24.93 ^{kl}	0.078 ^{ij}	11.98 ^{def}	2.49 ^{fgh}	29.11 ^l
SEM(±)	0.610	0.875	0.0748	0.998	0.2274	1.216
CD (P=0.05)	(1.799)	2.580	0.0224	2.946	0.6707	3.586

Value with the same letters indicates they are not significantly different (p=0.05) by DMRT analysis; all values in parentheses are arc-sine transformed values of percent values.

variables of rhizobacterial isolates can able to explain the variation in antagonistic potentiality of rhizobacterial isolates by 84% (Figure 1). Based on the stepwise regression equation it was observed that HCN production was one of the most important predictors for evaluation of antagonistic activity of rhizobacterial isolates against soil-borne plant pathogens. Similarly, Ramette et al. (2003) reported that microbial production of HCN has been reported as an important antifungal

trait to control root infecting fungi. The collar rot of sunflower disease suppression by fluorescent pseudomonads was promoted by HCN production (Shivani et al., 2005). The increased production of HCN by the efficient strain of *P. fluorescens* contributed to effective inhibition of mycelial growth of *Rhizoctonia solani* *in vitro* (Kuklinsky-sobral et al., 2004) and appears to be a major factor in control of soil-borne disease by *Pseudomonas fluorescens* CHA0 (Jamali et al.,

2009).

The two tailed Pearson's correlation between antagonistic activity of rhizobacterial isolates and their secondary metabolites production revealed that HCN, siderophore production and P-solubilization activity of rhizobacterial isolates were significantly positively correlated with the antagonistic activity of rhizobacterial isolates even at 1 % level (Table 5).

Many *P. fluorescens* isolates are known to

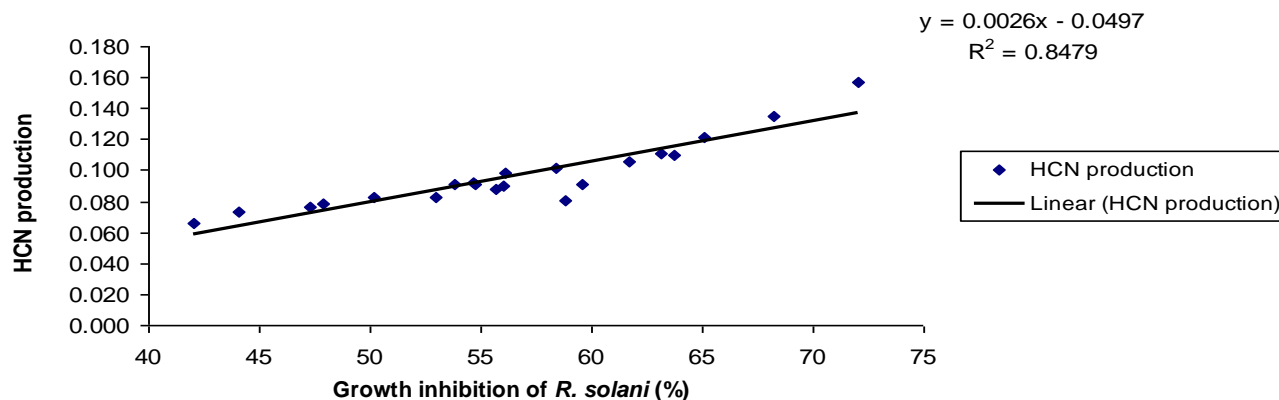


Figure 1. Relationship between HCN production and antagonistic potentiality of rhizobacterial isolates.

Table 5. Correlation matrix including all the 5 independent variables with the dependent variable (% Growth inhibition).

Parameter	Growth inhibition (%)
IAA production	0.229
HCN production	0.920**
Siderophore	0.765**
SA production	0.331
P-solubilization	0.724**
Growth inhibition (%)	1.00

** Significant at 1% level of probability (P=0.01).

secrete fluorescent and yellow-green, water soluble siderophores under iron-limiting conditions (Rosales et al., 1993). Additionally, siderophore mediated competition for iron was shown to be major mechanism of antagonistic activity of fluorescent pseudomonads in the control of some parasitic fungi, limiting iron availability to them (Miethke and Marahiel, 2007). Rassouli et al. (2005) reported that 201 indigenous *Pseudomonas* spp. isolated from Iranian soils were siderophore producers in CAS-agar medium.

To date, studies in biological control have focused mainly on fluorescent pseudomonads, producing fluorescent pigments and siderophores like pyoverdine (Haas and Defago, 2005), because of their metabolic versatility, their excellent root colonization ability and antimicrobial metabolites (O'Sullivan and O'Gara, 1992). Similarly, Kumar et al. (2000) reported that the *P. fluorescens* produced siderophores and antifungal metabolites which are involved in the control of phytopathogenic fungi. Salicylic acid (SA) production has been observed for several bacterial strains, and exogenously applied SA can induce resistance in many plant species. Fluorescent pseudo-omonads strains were known to produce salicylic acid (Dowling and O'Gara, 1994), and salicylic acid was a systemic signal molecule inducing disease resistance (Neuenschwander et al.,

1996). Salicylic acid produced by *P. fluorescens* in the rhizosphere is thought to be involved in ISR (Maurhofer et al., 1994). The results of this present investigation (Table 4) regarding SA production accounted to be ranged between 1.55 and 3.61 $\mu\text{g/ml}$, isolate PF-8 being the significantly highest producer (6.0 $\mu\text{g/ml}$) followed by PF-7 (4.99 $\mu\text{g/ml}$), are in confirmation with the findings of Nagarajkumar et al. (2004) who reported that 14 strains of *Pseudomonads* could produce SA and its amount varied from 2 to 16 $\mu\text{g/ml}$.

Gupta et al. (2002) reported *Pseudomonas* sp. acted as a potent phosphate solubilizer. Das et al. (2003) examined the tricalcium phosphate solubilizing activity of *P. fluorescens* and phosphate solubilizing pseudomonads are also reported to suppress damping-off disease in tomato caused by *Pythium* spp. (Srivastav et al., 2004). Likewise, in this present study, phosphate solubilization activity was noticed to be in the range of 27.24 to 108.59 $\mu\text{g/ml}$ culture filtrate, PF-8 and 7 being the significantly greatest solubilizers. Thus from the above finding, it may be concluded that HCN, siderophore production and phosphate solubilizing activity were the three most important secondary metabolites significantly contributed towards the biocontrol potentialities of rhizobacteria.

Effect of rhizobacterial isolates on management of root rot disease of okra

The data presented in Table 6 revealed that all the treatments were found to be significantly superior over control. Combination of seed treatment and soil application of PF-8 was found to be the most effective methods both in respect to minimizing the root and collar rot disease of okra (52.6% disease control) and obtaining the highest yield (30.8% enhancement of yield) (Table 6). The lowest collar rot disease severity of okra was obtained in case of seed plus soil application with carbendazim, however, it was found statistically at par with seed treatment plus soil application of PF-8. Similar

Table 6. Effect of different treatments on root rots disease and yield of okra.

Treatments	Disease incidence (%)	Disease reduction over control (%)	Yield
T1: Seed treatment with PF-8	19.4 ^c	31.93	3.90 ^{bc}
T2: Soil application with PF-8	17.5 ^d	38.71	4.05 ^{ab}
T3: T1+T2	13.3 ^e	52.63	4.33 ^a
T4: Seed treatment with carbendazim	21.5 ^b	24.68	3.67 ^c
T5: Soil Treatment with carbendazim	17.3 ^d	39.30	3.95 ^{bc}
T6: T4+T5	13.1 ^e	54.03	4.15 ^{ab}
T7: Control	28.5 ^a		3.31 ^d
SEM (±)	0.1054		0.0525
CD at 5%	0.3247		0.161

Value with the same letters indicates they are not significantly different ($p=0.05$) by DMRT analysis.

observation of plant disease management (Verticillium wilt) through the application of *Pseudomonas fluorescens* and *Pseudomonas putida*, was recorded by Mercado-Blanco et al. (2004). Bhatia et al. (2005) also reported the reduction of collar rot incident incited by *Sclerotium rolfsii* with seeds bacterization of fluorescent pseudomonads in sunflower. Several reports evidenced the crucial role of fluorescent Pseudomonads in soil suppressive to fusarium wilt (De Boer et al., 1999) and take-all disease caused by the phytopathogen fungus *Gaeumanomyces graminis* var. *tritici* (Weller, 2007).

Yield data presented in the Table 6, for all the treatments were found to be significantly superior over control. The significantly highest yield of okra was recorded in seed plus soil treatment with the native antagonist rhizobacterial PF-8. Similar observations of enhancement of yield were recorded by several workers. Improvement of yield with fluorescent pseudomonads was reported by Rao et al. (1999) in lentil and Ahmadzadeh et al. (2009) in bean.

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