Int.J.Curr.Microbiol.App.Sci (2017) *6*(12): 3883-3898

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 12 (2017) pp. 3883-3898**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.612.449

Isolation, Characterization and Antagonistic Activity of Fluorescent Pseudomonads

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Keywords

Fluorescent pseudomonads, *Sclerotium rolfsii*, *Colletotricum truncatum*, PGPR.

Article Info

Accepted:
28 October 2017
Available Online:
10 December 2017

A B S T R A C T

An attempt was made to isolate 62 strains of fluorescent pseudomonads from rhizosphere of soybean collected from two soybean growing areas *viz*., Belagavi and Dharwad districts of Karnataka. These isolates were subjected for morphological, biochemical and functional characterization. All the isolates were gram negative rods. Colour of the colony varied from light green, green, dark green to light orange. The isolates showed fluorescens under UV light. Out of 62 isolates 36, 24, 52, 36, 39 isolates were positive for starch hydrolysis, arginine hydrolysis, oxidase test, denitrification and gelatin liquification respectively. These isolates exhibited wide variations for their ability to utilize different carbon sources. With respect to MPS, 52 isolates showed MPS activity. Forty seven isolates were positive for HCN production, in which 10 of the isolates were strong HCN producers. All the isolates were positive for siderophore production, which ranged between 6.00 to 36.37mm. Whereas, IAA and GA production ranged from 39 to 28.03 and 0.5 to 18.52 µg /25 ml of broth respectively. All the 62 isolates were assayed for their antagonistic activity against *Sclerotium rolfsii* and *Colletotricum truncatum* using dual plate technique. Out of 62 isolates, 51 and 38 were inhibitory to *S. rolfsii* and *C. truncatum* respectively.

Introduction

Rhizobacteria that exert beneficial effects on plant growth and development are referred to as plant growth promoting rhizobacteria (PGPR) (Ashrafuzzaman *et al.,* 2009). PGPR promote plant growth by various factors like ability to produce plant growth regulators, asymbiotic N_2 fixation, antagonism against phytopathogenic microorganisms by production of siderophores, antibiotics and cyanide, solubilization of mineral phosphates and other nutrients (Sarvanakumar *et al.,* 2007). PGPR may use more than one of above mechanisms to enhance plant growth as experimental evidence suggests that the plant growth stimulation is the net result of multiple mechanisms that may be activated simultaneously (Martinez *et al.,* 2010). *Pseudomonas* is diverse genus that occupies many different niches and exhibits versatile metabolic capacity (Clarke, 1982).

Fluorescent pseudomonads (FP's) a group of PGPR, have frequently been considered as effective biological control agents against soil-borne plant pathogens due to their rapid and aggressive colonization of plant roots.

They produce secondary metabolites with antibiotic properties such as phytohormones, volatile compounds, hydrogen cyanide (HCN) and siderophores. Plant growth-promoting ability of these bacteria is mainly due to the production of indole-3-acetic acid (IAA), siderophores and antibiotics (Lautenberg *et al.,* 2001). Such strains can protect plants from various soil borne pathogens and/or stimulate plant growth (Haas and Defago, 2005). The abundance of literature on genus *Pseudomonas* is due to their elevated metabolic versatility capable of utilizing a wide range of simple and complex organic compounds and holding an important position in biosphere ecology (Scarpellini *et al.,* 2004).

Antagonistic activity of *Pseudomonas fluorescens* and *Pseudomonas putida* in the rhizosphere has been recognized as major factor in the suppression of many phytopathogens. Bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites.

Strains of *Pseudomonas fluorescens* are known to show biological control activity against certain soil-borne phytopathogenic fungi *viz., Macrophomina phaseolina, Rhizoctonia solani, Phytophthora nicotianae* var. *parasitica*, *Pythium* sp. and *Fusarium* sp. (Anand *et al.,* 2010).

Materials and Methods

Isolation of fluorescent pseudomonads

A total of 37 soybean rhizosphere soils were collected from major soybean growing areas of Dharwad (15) and Belagavi (22) districts of Karnataka at crop maturity stage and used for the isolation of fluorescent pseudomonads on King's B medium using serial dilution and spread plate technique.

Characterization of FP isolates

The colonies showing greenish to yellowish pigmentation on King's B medium were picked up and observed for fluorescence under UV light. The isolates were also studied for their colony morphology, cell shape and gram reaction as per the standard procedure given by Barthalomew and Mittewar (1950) and Anonymous (1957). The isolates were subjected to biochemical characterization by employing the standard procedures given by Cappuccino and Sherman (1992). Different biochemical tests performed were denitrification, starch hydrolysis, arginine hydrolysis, gelatin liquification and Oxidase test.

Functional Characterization of FP isolates

The isolates were tested for their ability to solubilise P, HCN production, Siderophore production and ability to produce plant growth promoting substances like IAA and GA.

In vitro screening of fluorescent pseudomonad isolates against fungal pathogens of soybean

All the 62 FP isolates were studied for their antagonistic activity against two fungal pathogens *viz., Sclerotium rolfsii* Sacc., causing collor or root rot (soil borne) and *Colletotricum truncatum* causing anthracnose (foliar pathogen) in soybean plant under *in vitro* condition, these pathogens also causes variety of diseases in many crops.

The pure cultures of *S.rolfsii* and *C. truncatum* were obtained from the Department of Plant Pathology, University of Agricultural Sciences, Dharwad. The dual inoculation technique of Sakthivel and Gnanamanickam (1987) were followed to study the antagonistic activity of the

fluorescent pseudomonads against soil borne and foliar plant pathogens.

Results and Discussion

Isolation of fluorescent pseudomonads from rhizosphere soil was carried out by employing serial dilution and plating on King's B selective media. It may be attributed to specific choice of media employed for isolation *viz.,* Nutrient agar and King's B. Several others hence supported to use King's B medium for isolation of fluorescent *Pseudomonas* sp. (King *et al.,* 1954). For the isolation of pseudomonads and fluorescent *Pseudomonas* sp., TSA and King's B agar medium were also used (Raaijmakers and Weller, 1998). Total of 62 FP isolates were obtained from the 37 rhizosphere soil samples collected from the major soybean growing areas of Dharwad and Belagavi district of Karnataka (Table 1).

All 62 isolates were used to study morphological traits. The colony morphology of isolates was found to be round to irregular shape and the irregular shaped colonies were found to be spreading type. All the 62 isolates exhibited fluorescence under UV light. However variations with respect to intensity of fluorescence and colony morphology were observed. Based on intensity of fluorescence under UV light, the isolates were classified as very good fluorescence (+++), medium fluorescence (++) and low fluorescence (+). Out of 62 isolates, 27 showed very good fluorescence (+++) and 32 isolated showed medium fluorescence (++) under UV light.

Fluorescence is an important trait for identification, characterization and grouping of fluorescent pseudomonads (Brown and Lowbury, 1968). Among the 62 isolates majority of the isolates produced light green to green pigmented colonies, while some of the isolates appeared to have yellowish orange colonies. Colonies showing fluorescence under transilluminator were marked and a loopful of growth was picked in the laminar air flow for further purification. This could be a useful practice to obtain fluorescent pseudomonads from plate containing several isolated colonies from soil or root samples. It is reported from previous studies that some pigments such as carotenoids produced by pseudomonads species do not diffuse in to the medium and such colonies were found to have yellowish green colour which could resemble other fluorescing pigments (Indi, 2010). The cell morphological studies and the Gram reaction test revealed that all the 62 isolates were rod shaped and displayed a negative reaction for the Gram staining.

The biochemical characterization indicated that out of 62 isolates, 36 were positive for starch hydrolysis and 24 for arginine hydrolysis, 52 for oxidase test, 39 were positive for gelatin liquefaction and 36 exhibited the denitrification ability (Table 2). These results are in conformity with the work of Singh *et al.,* (2007), who reported that out of seventeen isolates, seven isolates were identified to be *Pseudomonas* sp. on the basis of their cultural, morphological and biochemical characters. Similarly, Thirty five isolates of *Pseudomonas fluorescens* were isolated from the rhizosphere of rice fields by Meera and Balabaskar (2012). Among these, seven isolates which showed bright fluorescence under UV light were confirmed to be *P. fluorescens* after cultural and biochemical studies.

All the 62 isolates were also exercised to study the ability of the isolates to grow at 4^0C and 41^0C temperature. It was observed that there is an existence of variation among the isolates. Fifty two of the isolates were able to grow at 4 ^{0}C temperature and 10 isolates showed growth at 41 ^{0}C (Table 3). The

findings are in conformity with the work of Suman *et al.,* (2016) who revealed that, among nineteen isolates, eight isolates showed growth at 4^0C and eleven isolates at 42 ^{0}C.

The isolates were also tested for their ability to utilize different carbon sources *viz.,* glucose, lactose, sucrose, xylose, ribose, β-alanine, meso-inositol, mannitol, maltose, trehalose, L- valine and geraniol. The results revealed that among 62 isolates, 60, 56, 28, 29, 35, 52, 52, 25, 54, 52, 19 and 35 isolates were able to utilize glucose, lactose, sucrose, xylose, ribose, β-alanine, meso-inositol, mannitol, maltose, trehalose, L- valine and geraniol carbon sources respectively. None of the isolates showed growth on rhamnose (Table 3).

By considering the morphological, biochemical characteristics and ability of the isolates to grow at 4^oC and 41^oC and utilize different carbon sources for their growth, 50 isolates were tentatively identified as *P. fluorescens,* 10 as *P. aeruginosa* and 2 isolates as *P. aureofaciens* strains based on Bergey's manual of Bacteriology. The results pertaining to characterization in this study are in line with the observation made by Paramageetham and Prasada Babu, 2012; Suman *et al.,* 2016.

All the isolates were also examined for their functional properties like, P-solubilization, production of plant growth promoting substances and their biocontrol potential (Table 4). Out of 62 isolates, 52 were able to produce clear zone of P- solubilization (TCP) on Pikovskaya's agar medium. These isolates displayed wide variations in the diameter of the zone of solubilization, which varied from 5.31- 21.71mm. The extent of zone of solubilization may or may not correlate with the amount of P solubilized (Rashid *et al.,* 2004). Isolates of *Pseudomonas fluorescens* species differ in the ability to produce phosphatase enzyme and production of organic acids and hence showed different solubilization efficiency.

Fluorescent pseudomonads offer an interesting biological system with their ability to promote plant growth directly through production of plant growth promoting substances (IAA and GA) and indirectly through control of plant pathogens and deleterious organisms or both (Bakthavatchalu *et al.,* 2012).

Seed bacterization with such organisms has emerged as a powerful technology to enhance plant growth and yield, besides providing protection against diseases. Earlier, Suneesh (2004) and Megha *et al.,* (2007a) made an attempt to characterize PGPR isolates of Western Ghats and studied their functional diversity. Their efforts helped in identifying several PGPR with novel traits useful in agriculture. The present study is complimented with the previous work done. All 62 isolates were screened for their ability to produce IAA and GA. All the fluorescent pseudomonads in the present study produced significantly varying quantities of IAA (3.90 µg to 28.89 µg IAA/25 ml of broth) (Table 4). Among the isolates, DFP48 recorded highest IAA production of 28.89 µg/25ml.

Isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1992) and IAA production by PGPR can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mirza *et al.,* 2001). The results obtained in this study are in line with the observation made by Khakipour *et al.,* (2008), who reported that the IAA produced by *P. fluorescens* and *P. putida* strains varied from 0 to 31.6 mg/l and 0 to 24.08 mg/l, respectively.

Table.1 Morphological characterization of native fluorescent pseudomonad isolates

Sl. NO	Isolate code	Colony morphology	Pigmentation (Under UV light)	Cell shape	Gram Reaction
1	BFP1	Round	Dull green	Rod	Gram-ve
2	BFP2	Irregular	Dull green	Rod	Gram –ve
3	BFP3	Irregular	Dull green	Rod	Gram –ve
4	BFP4	Irregular	Dull green	Rod	Gram –ve
5	BFP5	Round	Dull green	Rod	Gram-ve
6	BFP6	Round	Dull green	Rod	Gram -ve
7	BFP7	Irregular	Yellowish orange	Rod	Gram -ve
8	BFP8	Irregular	Yellowish orange	Rod	Gram -ve
9	BFP9	Round	Dark green	Rod	Gram -ve
10	BFP10	Round	Dark green	Rod	Gram -ve
11	BFP11	Round	Green	Rod	Gram -ve
12	BFP12	Round	Green	Rod	Gram -ve
13	BFP13	Irregular	Light orange	Rod	Gram -ve
14	BFP14	Irregular	Light orange	Rod	Gram -ve
15	BFP15	Round	Light orange	Rod	Gram -ve
16	BFP16	Irregular	Green	Rod	Gram -ve
17	BFP17	Irregular	Green	Rod	Gram -ve
18	BFP18	Round	Green	Rod	Gram -ve
19	BFP19	Round	Dull orange	Rod	Gram -ve
20	BFP20	Round	Dull orange	Rod	Gram -ve
21	BFP21	Round	Dull green	Rod	Gram -ve
22	BFP22	Round	Yellowish orange	Rod	Gram -ve
23	BFP23	Round	Dull green	Rod	Gram -ve
24	BFP24	Round	Dull green	Rod	Gram -ve
25	BFP25	Round	Green	Rod	Gram -ve
26	BFP26	Irregular	Dull green	Rod	Gram -ve
27	BFP27	Irregular	Dull green	Rod	Gram -ve
28	BFP28	Irregular	Dull green	Rod	Gram -ve
29	BFP29	Irregular	Dull green	Rod	Gram -ve
30	BFP30	Round	Dull green	Rod	Gram -ve
31	BFP31	Round	Dull green	Rod	Gram -ve
32	BFP32	Round	Dull green	Rod	Gram -ve
33	BFP33	Round	Dull green	Rod	Gram -ve
34	BFP34	Irregular	Dull green	Rod	Gram –ve
35	BFP35	Irregular	Dull green	Rod	Gram –ve
36	BFP36	Irregular	Dull green	Rod	Gram –ve
37	BFP37	Round	Green	Rod	Gram –ve
38	BFP38	Irregular	Green	Rod	Gram -ve
39	BFP39	Irregular	Dull green	Rod	Gram –ve
40	BFP40	Irregular	Yellowish orange	Rod	Gram –ve
41	BFP41	Round	Yellowish orange	Rod	Gram –ve
42	BFP42	Round	Dark green	Rod	Gram –ve
43	BFP43	Irregular	Dull green	Rod	Gram –ve
44	BFP44	Irregular	Slight orange	Rod	Gram –ve
45	BFP45	Irregular	Slight orange	Rod	Gram –ve
46	DFP46	Round	Green	Rod	Gram –ve
47	DFP47	Round	Green	Rod	Gram –ve
48	DFP48	Round	Yellowish orange	Rod	Gram –ve
49	DFP49	Round	Yellowish orange	Rod	Gram –ve
50	DFP50	Round	Dull green	Rod	Gram –ve
51	DFP51	Round	Dull green	Rod	Gram –ve
52	DFP52	Irregular	Dull green	Rod	Gram –ve
53	DFP53	Irregular	Green	Rod	Gram –ve
54	DFP54	Irregular	Green	Rod	Gram –ve
55	DFP55	Irregular	Green	Rod	Gram –ve
56	DFP56	Round	Yellowish orange	Rod	Gram –ve
57	DFP57	Round	Yellowish orange	Rod	Gram –ve
58	DFP58	Irregular	Dull green	Rod	Gram –ve
59	DFP59	Irregular	Dull green	Rod	Gram –ve
60	DFP60	Irregular	Dull green	Rod	Gram –ve
61	DFP61	Irregular	Yellowish orange	Rod	Gram –ve
62	DFP62	Round	Green	Rod	Gram –ve

Table.2 Biochemical characterization of native fluorescent pseudomonads

Sl.NO	Isolates	Denitrification	Starch hydrolysis	Arginine hydrolysis	Gelatin liquification	Oxidase test	Tentaive Identification
1	BFP1	-	-	-	+	+	*P. fluorescens*
2	BFP2	-	+	-	+	+	*P. fluorescens*
3	BFP3	-	+	-	-	+	*Pseudomomas spp*
4	BFP4	-	-	-	-	+	*P. fluorescens*
5	BFP5	-	+	-	-	+	*P. aeruginosa*
6	BFP6	-	+	+	-	-	*Pseudomomas spp*
7	BFP7	-	+	-	+	+	*P. fluorescens*
8	BFP8	+	-	-	-	+	*P. fluorescens*
9	BFP9	+	-	-	-	+	*P. fluorescens*
10	BFP10	-	-	-	+	+	*P. fluorescens*
11	BFP11	-	+	+	+	+	*P. fluorescens*
12	BFP12	-	+	-	+	+	*P. fluorescens*
13	BFP13	+	-	-	+	+	*P. fluorescens*
14	BFP14	+	-	-	-	+	*P. fluorescens*
15	BFP15	-	+	+	+	+	*P. fluorescens*
16	BFP16	-	+	-	+	+	*P. fluorescens*
17	BFP17	+	-	+	-	+	*P. fluorescens*
18	BFP18	-	-	-	+	-	*P. aeruginosa*
19	BFP19	+	-	-	-	+	*P. fluorescens*
20	BFP20	-	+	-	+	+	*P. fluorescens*
21	BFP21	+	+	+	-	-	*P. aeruginosa*
22	BFP22	-	-	-	+	-	*P. fluorescens*
23	BFP23	-	-	+	-	-	*P. aeruginosa*
24	BFP24	-	-	-	-	+	*P. aeruginosa*
25	BFP25	-	-	-	-	+	*P. fluorescens*
26	BFP26	-	-	-	+	-	*P. aeruginosa*
27	BFP27	-	+	-	+	+	*P. fluorescens*
28	BFP28	+	+	-	-	+	*P. fluorescens*
29	BFP29	+	-	-	-	+	*P. fluorescens*
30	BFP30	+	+	-	+	-	*P. aeruginosa*
31	BFP31	+	+	+	+	+	*P. fluorescens*
32	BFP32	-	+	+	+	+	*P. fluorescens*
33	BFP33	+	+	+	+	+	*P. fluorescens*
34	BFP34	+	+	-	-	+	*P. fluorescens*
35	BFP35	+	+	-	-	+	*P. fluorescens*
36	BFP36	+	+	+	+	+	*P. fluorescens*
37	BFP37	+	+	+	-	+	*P. fluorescens*
38	BFP38	+	+	+	+	+	*P. fluorescens*
39	BFP39	-	+	+	-	+	*P. fluorescens*
40	BFP40	+	+	+	+	+	*P. fluorescens*
41	BFP41	+	+	-	+	-	*P. aeruginosa*
42	BFP42	+	+	-	-	+	*P. fluorescens*
43	BFP43	+	+	+	-	+	*P. fluorescens*
44	BFP44	+	+	-	+	+	*P. fluorescens*
45	DFP45	+	+	+	+	+	*P. fluorescens*
46	DFP46	+	+	+	+	+	*P. fluorescens*
47	DFP47	+	-	-	+	+	*P. fluorescens*
48	DFP48	+	-	-	-	+	*P. fluorescens*
49	DFP49	-	+	+	+	+	*P. fluorescens*
50	DFP50	+	-	-	+	+	*P. aeruginosa*
51	DFP51	+	-	+	+	+	*P. fluorescens*
52	DFP52	+	-	-	+	+	*P. fluorescens*
53	DFP53	-	-	-	-	+	*P. fluorescens*
54	DFP54	+	-	-	-	-	*P. fluorescens*
55	DFP55	+	-	-	+	+	*P. fluorescens*
56	DFP56	+	+	-	+	+	*P. fluorescens*
57	DFP57	+	+	+	+	-	*P. aeruginosa*
58	DFP58	+	-	+	+	+	*P. fluorescens*
59	DFP59	-	+	-	+	+	*P. fluorescens*
60	DFP60	+	+	+	+	+	*P. fluorescens*
61	DFP61	+	+	+	+	+	*P. fluorescens*
62	DFP62	+	+	+	+	+	*P. fluorescens*

Note: (+) positive reaction for the test, (-) negative reaction for the test

Table.3 Ability of fluorescent pseudomonad isolates to utilize different carbon sources and their ability to grow at 4 ^{0}C and 41 ^{0} C temperature

Ability of fluorescent pseudomonad isolates to utilize different carbon sources															Growth at	
Sl. No	**Isolates**	**Glucose**	**Lactose**	**Sucrose**	**Xylose**	**Ribose**	**B-alanine**	**Rhamose**	**Meso-Inositol**	**Mannitol**	**Maltose**	**Trehalose**	**L-valine**	**Geraniol**	**4 ^{0}C**	**41 ^{0}C**
															+	-
2	BFP2	+	-	-	-	-	+	-	-	-	-	-	-	-	+	-
3	BFP3	+	+	-	-	+	+	-	-	-	+	+	-	+	+	-
4	BFP4	+	-	-	-	+	+	-	-	-	-	+	-	+	+	-
5	BFP5	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-
6	BFP6	+	+	-	-	+	+	-	+	-	+	-	-	+	-	+
7	BFP7	+	+	-	-	+	+	-	+	-	+	-	-	+	+	-
8	BFP8	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
9	BFP9	+	+	-	+	+	+	-	-	-	+	+	-	+	+	-
10	BFP10	+	+	-	-	-	+	-	-	-	+	+	-	-	+	-
11	BFP11	+	+	-	-	-	+	-	-	-	+	+	-	-	+	-
12	BFP12	+	+	-	-	-	+	-	+	-	-	+	-	-	+	-
13	BFP13	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-
14	FBP14	+	-	-	-	-	+	-	+	-	+	+	-	-	+	-
15	BFP15	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-
16	BFP16	+	+	-	-	+	+	-	+	-	+	+	-	+	+	-
17	BFP17	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-
18	BFP18	+	+	-	-	-	+	-	-		+	+	-	-	-	+
19	BFP19	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-
20	BFP20	+	+	-	+	+	+	-	+	-	+	+	-	+	+	-
21	BFP21	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-
22	BFP22	+	-	+	+	-	+	-	+	-	-	+	+	-	-	+
23	BFP23	+	-	-	-	-	+	-	+	-	+	+	-	-	-	+
24	BFP24	+	+	-	-	-	+	-	-	-	+	+	-	-	-	+
25	BFP25	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-
26	BFP26	+	+	-	-	-	+	-	+	-	+	+	-	-	-	+
27	BFP27	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-
28	BFP28	+	+	-	-	+	+	-	+	-	+	-	+	+	+	-
29	BFP29	+	+	-	-	+	+	-	+	-	+	+	-	+	+	-
30	BFP30	+	+	+	+	+	+	-	+	-	+	-	-	+	-	+
31	BFP31	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-
32	BFP32	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-
33	BFP33	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
34	BFP34	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
35	BFP35	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
36	BFP36	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-

37	BFP37	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-
38	BFP38	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
39	BFP39	+	+	+	+	-	+	-	+	-	-	-	+	-	+	-
40	BFP40	-	+	-	-	-	-	-	+	-	+	-	-	-	+	-
41	BFP41	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+
42	BFP42	+	+	-	-	-	+	-	+	-	+	-	-	-	+	-
43	BFP43	+	+	-	-	+	+	-	+	-	+	+	-	+	+	-
44	BFP44	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
45	BFP45	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-
46	DFP46	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
47	DFP47	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
48	DFP48	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-
49	DFP49	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-
50	DFP50	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+
51	DFP51	+	+	+	-	+	+	-	+	+	+	+	-	+	+	-
52	DFP52	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
53	DFP53	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-
54	DFP54	+	+	+	+	-	+	-	+	+	+	+	-	-	+	-
55	DFP55	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
56	DFP56	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-
57	DFP57	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+
58	DFP58	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
59	DFP59	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
60	DFP60	+	+	+	+	+	+	-	+	+	+	+	-	+	+	-
61	DFP61	+	-	-	-	-	+	-	+	-	-	+	-	-	+	-
62	DFP62	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-

Note: (+) growth on respective carbon source (-) no growth

Table.4 Functional characterization of native fluorescent pseudomonad isolates

Sl. No.	Isolates	P- solubilization (mm) (Qualitative)	IAA (µg/25 ml)	GA (µg/25 ml)	HCN production	Siderophore Production (mm)
1	BFP1	-	9.28	0.56	-	8.90
2	BFP2	-	9.17	0.77	+	7.90
3	BFP3	5.31	9.43	1.43	-	6.00
4	BFP4	6.54	5.53	1.27	-	7.90
5	BFP5	-	7.15	0.98	-	8.97
6	BFP6	-	5.08	1.89	-	6.03
7	BFP7	-	3.90	1.37	++	10.40
8	BFP8	7.08	4.83	2.14	++	11.00
9	BFP9	5.86	7.86	1.42	++	8.97
10	BFP10	8.92	12.35	1.49	+	10.90
11	BFP11	7.40	7.43	2.13	+	10.00
12	BFP12	-	12.16	0.94	++	8.10
13	BFP13	11.34	7.41	1.12	-	8.90
14	BFP14	9.66	10.28	2.23	-	6.90
15	BFP15	16.67	8.57	3.15	-	8.97
16	BFP16	17.65	8.73	3.70	+	9.47
17	BFP17	10.08	9.64	1.16	-	12.40
18	BFP18	13.21	6.00	2.13	++	19.30
19	BFP19	-	9.08	3.14	++	22.20
20	BFP20	18.09	14.26	4.14	++	21.18
21	BFP21	17.18	8.83	3.17	++	14.90
22	BFP22	20.00	19.97	12.19	+++	21.97
23	BFP23	19.94	13.14	4.48	-	10.90
24	BFP24	14.58	10.10	2.26	++	7.90
25	BFP25	18.80	14.57	2.63	++	9.90
26	BFP26	7.07	20.39	1.20	+++	10.40
27	BFP27	16.00	18.71	2.22	++	8.00
28	BFP28	15.62	8.78	2.87	++	9.37
29	BFP29	-	10.82	3.06	-	13.20
30	BFP30	19.00	16.88	2.85	++	8.00
31	BFP31	-	6.00	4.03	+	21.40
32	BFP32	19.04	8.74	3.36	-	9.40
33	BFP33	16.74	17.35	2.52	+	15.70
34	BFP34	20.07	13.14	4.14	+	13.80
35	BFP35	17.15	19.34	2.19	++	20.10
36	BFP36	10.00	12.72	4.55	++	7.80
37	BFP37	7.31	11.89	3.19	++	9.00
38	BFP38	21.02	24.00	16.29	**+++**	25.00
39	BFP39	18.80	12.18	9.96	+++	21.17
40	BFP40	14.21	4.93	9.88	-	13.10
41	BFP41	14.27	19.21	7.91	++	21.10
42	BFP42	21.11	23.74	13.12	+++	23.60
43	BFP43	-	11.29	10.16	-	15.30
44	BFP44	15.73	13.55	9.89	++	18.10
45	BFP45	20.12	19.37	8.97	-	10.10
46	DFP46	15.32	19.94	10.18	++	12.90
47	DFP47	20.96	22.55	12.85	+++	23.47
48	DFP48	21.71	28.89	13.15	+++	29.23
49	DFP49	17.55	5.88	8.91	++	19.83
50	DFP50	15.52	9.83	11.49	++	21.90
51	DFP51	18.65	12.88	4.91	+	11.30
52	DFP52	10.69	8.82	9.77	+	13.40
53	DFP53	16.13	16.77	0.91	+	21.00
54	DFP54	21.65	28.03	18.52	+++	36.37
55	DFP55	19.82	19.00	10.24	++	17.87
56	DFP56	20.32	24.81	12.20	+++	24.00
57	DFP57	11.00	19.39	6.89	++	22.83
58	DFP58	17.23	8.94	9.98	++	22.32
59	DFP59	20.94	10.00	10.12	+	14.40
60	DFP60	13.06	17.18	1.92	++	22.90
61	DFP61	20.58	20.06	10.25	++	23.10
62	DFP62	20.34	26.41	12.99	+++	26.70
	S.Em. ±	**0.68**	**0.19**	**0.05**	**-**	**0.05**
	C.D. @ 1 %	**2.52**	**0.71**	**0.18**	**-**	**0.19**

Note: (+) slight production, (++) moderate production, (+++) maximum production (-) no production

Table.5 Antagonistic activity of fluorescent pseudomonad isolates against *Sclerotium rolfsii* (soil borne pathogen) under *in vitro* condition

Sl. NO.	Isolates	Zone of inhibition (cm)	Per cent inhibition (%)
1	BFP1	-	-
2	BFP2	3.73 (11.13)*	41.48 (39.28)*
3	BFP3	-	-
4	BFP4	3.12 (10.16)	34.63 (36.04)
5	BFP5	2.85 (9.72)	31.70 (34.53)
6	BFP6	2.98 (9.94)	33.15 (35.68)
7	BFP7	2.82 (9.66)	31.29 (34.27)
8	BFP8	3.78 (11.21)	42.04 (39.88)
9	BFP9	3.00 (9.97)	33.33 (35.41)
10	BFP10	-	-
11	BFP11	2.60 (9.27)	28.89 (32.86)
12	BFP12	3.50 (10.78)	38.89 (38.37)
13	BFP13	-	-
14	BFP14	-	-
15	BFP15	2.03 (8.19)	22.59 (29.24)
16	BFP16	2.52 (9.12)	27.96 (32.05)
17	BFP17	-	-
18	BFP18	-	-
19	BFP19	-	-
20	BFP20	-	-
21	BFP21	3.03 (10.02)	33.70 (35.50)
22	BFP22	6.33 (14.57)	70.37 (54.00)
23	BFP23	3.13 (10.18)	34.74 (36.30)
24	BFP24	3.25 (10.38)	36.11 (36.84)
25	BFP25	3.32 (10.49)	36.85 (37.06)
26	BFP26	2.42 (8.94)	26.85 (31.81)
27	BFP27	2.45 (9.00)	27.22 (32.05)
28	BFP28	-	-
29	BFP29	-	-
30	BFP30	3.37 (10.57)	37.41 (37.59)
31	BFP31	3.08 (10.11)	34.26 (35.90)
32	BFP32	3.32 (10.49)	36.85 (36.84)
33	BFP33	3.37 (10.57)	37.41 (37.59)
34	BFP34	3.20 (10.30)	35.56 (36.66)
35	BFP35	3.50 (10.78)	38.89 (38.37)
36	BFP36	3.35 (10.54)	37.22 (37.41)
37	BFP37	3.25 (10.38)	36.11 (36.84)
38	BFP38	4.10 (11.68)	45.56 (41.71)
39	BFP39	3.38 (10.59)	37.59 (37.76)
40	BFP40	3.12 (10.16)	34.63 (36.04)
41	BFP41	3.15 (10.22)	35.00 (36.62)
42	BFP42	4.47(12.20)	49.63 (43.57)
43	BFP43	2.53 (9.15)	28.15 (32.63)
44	BFP44	3.32 (10.49)	36.89 (37.41)
45	DFP45	2.60 (9.27)	28.89 (32.87)
46	DFP46	3.20 (10.30)	35.56 (36.44)
47	DFP47	4.10 (11.68)	45.56 (41.50)
48	DFP48	5.30 (13.30)	58.89 (48.17)
49	DFP49	3.77 (11.19)	41.85 (39.84)
50	DFP50	3.82 (11.26)	42.41 (60.01)
51	DFP51	3.42 (10.65)	37.96 (56.97)
52	DFP52	3.32 (10.49)	36.85 (55.79)
53	DFP53	3.40 (10.62)	37.78 (56.38)
54	DFP54	3.37 (10.57)	37.41 (56.38)
55	DFP55	3.25 (10.38)	36.11 (55.26)
56	DFP56	3.32 (10.49)	36.85 (55.79)
57	DFP57	3.53 (10.83)	39.26 (57.88)
58	DFP58	3.58 (10.91)	39.81 (58.01)
59	DFP59	3.22 (10.33)	35.74 (54.93)
60	DFP60	3.42 (10.65)	37.96 (56.64)
61	DFP61	3.35 (10.54)	37.22 (56.31)
62	DFP62	5.48 (13.54)	60.93 (73.52)
63	Control	0.00 (1.00)	0.00 (1.00)
	S.m. ±	**0.094**	**0.752**
	C.D.@ 1 %	**0.350**	**2.791**

Note: BFP isolates obtained from Belagavi soil sample and DFP isolates from Dharwad soil sample; (+) low inhibition of pathogen, (++) moderate inhibition, (+++) good inhibition and (-) no inhibition.
* Arcsine values

Table.6 Antagonistic activity of fluorescent pseudomonad isolates against *Colletotricum truncatum* (foliar pathogen) under *in vitro* condition

Sl. NO	Isolates	Zone of inhibition (cm)	Per cent inhibition
1	BFP1	-	-
2	BFP2	-	-
3	BFP3	-	-
4	BFP4	-	-
5	BFP5	-	-
6	BFP6	-	-
7	BFP7	2.90 (9.80)*	32.22 (34.57)*
8	BFP8	3.73 (11.14)	40.81 (39.69)
9	BFP9	3.60 (10.93)	39.66 (39.02)
10	BFP10	-	-
11	BFP11	-	-
12	BFP12	-	-
13	BFP13	-	-
14	BFP14	-	-
15	BFP15	-	-
16	BFP16	-	-
17	BFP17	-	-
18	BFP18	-	-
19	BFP19	-	-
20	BFP20	3.92 (11.41)	42.70 (40.79)
21	BFP21	-	-
22	BFP22	6.60 (14.88)	74.36 (59.56)
23	BFP23	-	-
24	BFP24	-	-
25	BFP25	-	-
26	BFP26	2.55 (9.18)	28.33 (32.15)
27	BFP27	2.52 (9.12)	27.96 (31.91)
28	BFP28	-	-
29	BFP29	-	-
30	BFP30	2.92 (9.83)	32.41 (34.68)
31	BFP31	-	-
32	BFP32	3.30 (10.46)	36.67 (37.25)
33	BFP33	3.62 (10.96)	40.19 (39.32)
34	BFP34	3.33 (10.52)	37.04 (37.47)
35	BFP35	3.52 (10.80)	38.40 (38.28)
36	BFP36	3.98 (11.51)	44.40 (41.77)
37	BFP37	3.63 (10.98)	40.94 (39.76)
38	BFP38	3.23 (10.35)	36.77 (37.31)
39	BFP39	2.88 (9.77)	32.49 (34.73)
40	BFP40	-	-
41	BFP41	3.48 (10.75)	38.70 (38.45)
42	BFP42	5.54 (13.08)	63.22 (52.64)
43	BFP43	3.70 (11.08)	41.18 (39.90)
44	BFP44	2.20 (8.52)	24.44 (29.62)
45	BFP45	2.30 (8.72)	25.56 (30.34)
46	DFP46	3.48 (10.75)	38.70 (38.45)
47	DFP47	3.67 (11.03)	40.74 (39.65)
48	DFP48	3.37 (10.57)	37.41 (37.69)
49	DFP49	2.93 (9.86)	32.59 (34.80)
50	DFP50	3.40 (10.62)	37.78 (37.91)
51	DFP51	3.08 (10.11)	34.26 (35.81)
52	DFP52	3.75 (11.16)	41.67 (40.19)
53	DFP53	3.60 (10.93)	40.00 (39.21)
54	DFP54	5.02 (12.95)	56.55 (48.74)
55	DFP55	3.57 (10.88)	39.63 (39.00)
56	DFP56	6.10 (14.29)	67.78 (55.39)
57	DFP57	3.53 (10.83)	39.26(38.78)
58	DFP58	4.07 (11.63)	45.57 (42.44)
59	DFP59	2.87 (9.74)	31.85 (34.34)
60	DFP60	4.10 (11.68)	45.56 (42.43)
61	DFP61	3.02 (10.00)	33.52 (35.36)
62	DFP62	6.35 (14.59)	70.84 (57.30)
63	Control	0.00 (1.00)	0.00 (1.00)
	S.Em. ±	**0.110**	**0.421**
	C.D. @ 1 %	**0.420**	**1.582**

Note: * Arcsine formed values

The variations in IAA production could be an inherent metabolic variability among the isolates (Leinhos and Vacek, 1994). The level of expression of IAA depended on the biosynthetic pathway, the location of genes involved and the presence of enzymes that could convert active free IAA into an inactive conjugated form (Patten and Glick, 1996).

Gibberellic acid is a class of phytohormone most commonly associated with modifying plant morphology by the extension of plant tissue, particularly the stem tissue (Salisbury, 1994). The amount of GA production by different FP isolates ranged from 0.56 to 18.52 µg/25ml of broth. The isolate DFP54 was found to produce maximum amount of GA (18.52 µg per 25 ml broth) (Table 4). These results could be compared with those reported earlier by Lenin and Jayanti (2012), who observed the production of GA_3 by isolates of *Pseudomonas* ranged from 6.21 to 6.80 µg per 25 ml broth. Similarly, Suneesh (2004) reported that all the 48 fluorescent Pseudomonads isolated from the moist deciduous forests produced GA in the range of 0.72 to 5.27 µg per 25 ml of broth.

The fluorescent pseudomonads have been the most widely studied group of PGPR with respect to biocontrol of soil borne plant pathogens. The increased interest in the fluorescent *Pseudomonas* sp. in worldwide as biocontrol agents gained momentum after the initial studies conducted at the University of California, Berkeley, during the late 1970s (Weller *et al.,* 1988). In addition to all the beneficial traits as discussed earlier, 62 isolates were used to study their antagonistic potential against two fungal pathogens (*S. rolfsii* and *C. truncatum*) of soybean under *in vitro* condition using dual plate technique (Vincent, 1947).

Among 62 isolates, 52 isolates showed antagonistic activity against one or the other phytopathogen (Table 5 and 6). Further, out of these antagonistic isolates, 51 isolates were inhibitory to *S. rolfsii*, 38 isolates were inhibitory to *C. truncatum* and 36 isolates showed efficacy against both the pathogen. Against *S. rolfsii*, the zone of inhibition varied from 2.03 to 6.33 cm with percent inhibition of 22.59 to 70.37 (Table 5). The maximum percent inhibition of 70.37 was observed in BFP22, which was significantly superior over all other isolates. The isolates DFP62 and DFP48 were on par with each other with percent inhibition of 60.93 and 58.89 respectively. For *C. truncatum* the zone of inhibition varied from 2.20 to 6.35 cm with per cent inhibition of 24.44 to 74.36 (Table 6). The isolate BFP22 exhibited maximum inhibition of about 74.36 which is followed by DFP62 (70.84), DFP56 (67.78), BFP42 (63.22) and DFP54 (56.55). These observations are in line with the earlier reports on fluorescent pseudomonads against plant pathogenic fungi like *Fusarium, Rhizoctonia, Macrophomina, Pyricularia, Alternaria, Sclerotium, Colletotrichum, Pythium* and *Phytophthora* (Mercado-Blanco *et al.,* 2004; Bhatia *et al.,* 2005; Ahmadzadeh *et al.,* 2006; Rakh *et al.,* 2011; Vishwanath *et al.,* 2012; Manivannan *et al.,* 2012; Prasad *et al.,* 2013). The effectiveness of fluorescent pseudomonads against multiple pathogens is also known (Tripathi and Johri, 2002; Suneesh, 2004; Kandoliya and Vakharia, 2014; Aly *et al.,* 2015; Arif Fouzia *et al.,* 2016 and Megha *et al.,* 2007b).

Important aspect of microbial antagonistic activity is best realized when it is applied for right cause. Therefore, understanding the mechanisms of antagonistic activity could be key to application of strains for specific purposes. In the present investigation, out of the 52 antagonistic isolates, 47 isolates produced HCN (Table 4). Among 47 isolates, 10 isolates were strong (+++), 26 isolates were moderate (++) and 11 isolates were

weak (+) HCN producer. The isolates which exhibited strong (+++) HCN production (BFP22, BFP26, BFP42, DFP47, DFP54, DFP56 and DFP62) showed very good biocontrol potential against the phytopathogens tested whereas the isolates with moderate HCN production showed moderate biocontrol activity. Production of HCN is known to induce systemic resistance in plants (Wei *et al.*, 1991). Voisard *et al.*, (1989) reported HCN production as a mechanism of biocontrol of plant pathogens. Similarly, Ahmadzadeh and Sharifi-Tehrani (2009) detected the production of HCN by six isolates of fluorescent pseudomonads and the strains exhibited good *in vitro* antifungal activity against *Rhizoctonia solani*.

The siderophore production by antagonistic microorganisms is believed to be a mechanism of pathogen suppression. Siderophore production test using CAS agar plate has been used for rapid screening of potential beneficial bacterial isolates (Schwyn and Neiland, 1987). Bacteria with the ability to produce siderophore can enhance plant growth by increasing the availability of iron near the roots for plant uptake (Alexander and Zuberer, 1991). Although, all 52 antagonistic isolates of this study produced siderophores, the zone of clearance on CAS agar ranged from 6 to 36.37 mm (Table 4). Twenty one isolates produced the zone of clearance between 5 to 10 mm, which were significantly superior to the remaining isolates. Similarly, among the isolates tested, 15 isolates recorded 10-15 mm zone of clearance, 3 isolates exhibited the zone of clearance between 25 to 30 mm. The isolates DFP54 recorded highest zone of clearance of 36.36 mm and three isolates did not show any clearance zone.

The siderophores are usually produced by various beneficial soil microbes. Among them fluorescent pseudomonads are also involved in inhibition of *S. rolfsii* which is positively correlated (r = +0.336**) with production of siderophores by fluorescent pseudomonads (Indi, 2010). The concept of disease suppression by siderophores and the role of siderophores produced by fluorescent pseudomonads in plant growth promotion were explained for the first time by Kloepper *et al.,* (1980).

This study resulted in obtaining 62 fluorescent pseudomonads from the soybean rhizosphere samples collected from Belagavi and Dharwad districts of Karnataka. Some of these isolates exhibited MPS activity, PGPR production, HCN production and siderophore production. They also showed antagonistic activity against *S. rolfsii* and *C. truncatum* under *in vitro* condition. Application of these microbes for diseases management and their practical use requires further investigation under field conditions.

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How to cite this article:

Priyanka, Geeta Goudar, P. Jones Nirmal Nath and Patil, P.V. 2017. Isolation, Characterization and Antagonistic Activity of Fluorescent Pseudomonads. *Int.J.Curr.Microbiol.App.Sci.* 6(12): 3883-3898. doi: https://doi.org/10.20546/ijcmas.2017.612.449