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Isolation and *in vitro* screening of native fluorescent pseudomonads against *Sclerotium rolfsii* Sacc

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Abstract
In the present study, 125 fluorescent pseudomonads were isolated from rhizosphere soils of brinjal grown in 10 different locations of Gadag and Haveri districts of Karnataka, India. Out of 125 fluorescent pseudomonad isolates, only seven isolates significantly inhibited the mycelial growth of the *S. rolfsii* Sacc. Among which, APF110 recorded significantly higher inhibition of 51.08 per cent followed by APF91 and APF121. The efficient isolates were further evaluated for beneficial traits and the results were found to be positive for IAA and siderophore production; P, K and Zn solubilization. The IAR screening indicated that all the isolates showed resistance to chloramphenicol, nalidixic acid and ampicillin whereas, found sensitive to kanamycin. The study of antifungal activity of the pseudomonads in association with plant growth promotion focuses significant assessment on the behavior, which probably brings out a competent biocontrol agent in a sustainable agriculture.

Keywords: IAA, Siderophores, IAR, Plant growth promotion, antifungal activity etc.

Introduction
Sclerotium rolfsii is a destructive soil inhabitant of worldwide significance which has a host range of over 500 species of plants (Desai and Schlosser, 1999) [6]. Management of *S. rolfsii* through soil application of fungicides is difficult because of its broad host range as well as its worldwide distribution which precludes such strategy. Once the pathogen is established in soil, it is difficult to eliminate. Thus, management of this soil-borne pathogen has been a major concern in agriculture. Although seed treatment with fungicides is recommended to minimize the infection at early stages, it does not ensure prolonged protection. Moreover, usage of chemical methods leads to ill effects like residual toxicity, environmental pollution and fungicide resistance. Biological control has been proved to be a promising disease management technology especially against soil-borne plant pathogens. Among bacteria with potential utility in bio-control, fluorescent pseudomonads are promising due to their ability to survive in the rhizosphere and inhibit numerous plant pathogens in diverse environments (Johri *et al*., 1997) [10]. Antagonism of fluorescent pseudomonads against *S. rolfsii* has also been reported by several workers (Biswas and Chitreswar, 2000; Muthamilan and Jeyarajan, 1996) [3, 14].

Recently, considerable attention has been paid to plant growth-promoting fluorescent pseudomonads, such as *Pseudomonas fluorescens* and *Pseudomonas putida*. Both these species are aggressive root colonizers and potential biocontrol agents (Johri *et al.*, 1997) [10]. The fluorescent pseudomonads produce fluorescent colonies and form a considerable part of the rhizospheric microbial community (Selvakumar *et al*., 2011) [24]. They form a major group within the rRNA homology group I of *Pseudomonas* '*sensu lato*' and are characterized by abundant production of fluorescent pyoverdin pigments in medium containing low Fe (Palleroni, 1984) [17]. The genus *Pseudomonas* consists of a large group of active bio-control bacterial strains with the general ability to produce antifungal metabolites such as siderophores, hydrogen cyanide (HCN) and proteases. Additionally, *P. fluorescens* is known to produce antibiotics such as phenazine and antimicrobial metabolites such as 2, 4-diacetylphloroglucinol (DAPG), pyoluteorin and salicylic acid (SA), capable of inhibiting a broad spectrum of plant pathogenic fungi, bacteria, and nematodes (Boruah and Kumar, 2002) [4]. Production of IAA is one of the mechanisms through which the they magnify the growth and development plants. Bacterial IAA increases root surface area and length and thereby provides the plant greater access to soil nutrients (Glick, 2012) [18].

It is well known that all the isolates of fluorescent pseudomonads are not equally antagonistic towards a species of pathogen (Neal *et al*., 2012) [16]. This emphasizes the need for identifying isolates specifically effective against *S. rolfsii*, for a planned regional deployment. The aim of the study is to isolate the native fluorescent pseudomonads, screen for the antagonistic action against *S. rolfsii* and analyze the plant growth promoting characteristics.

Material and methods

Foot rot disease-infected brinjal plants were collected from farmer's fields surrounding Dharwad. The causal organism was isolated from the collected specimen using standardized tissue isolation method. The fungal growth that arose through the infected tissue was taken up and transferred aseptically to the sterile media plates. After 4 days, the growth of each isolate was recorded. The fastest-growing isolate producing numerous sclerotia was identified as virulent isolate and used in this study.

Soil sampling

The rhizosphere soil samples for isolation of fluorescent pseudomonads were collected from brinjal growing areas of Haveri and Gadag districts. After removing the top litter layer (2 cm) the soil samples were collected, packed in polythene bags, and were stored at 4 ºC to sustain the viability of organisms. The samples were analyzed for their chemical properties like pH and EC by following standard procedures.

Isolation of fluorescent pseudomonads

The fluorescent pseudomonads were isolated from rhizosphere soils by serial dilution and agar plating technique. The collected bulk soil samples from the rhizosphere were homogenized and 1 g of each sample was suspended in 9 ml sterilized distilled water blanks to dilute them to 10^{-1}. They were serially diluted to 10^{-4}. The soil suspension (0.1 ml) from 10^{-4} dilution was pipetted out and spread onto King's B media plates under aseptic conditions. The plates were incubated at 28 ± 2 ºC for 48 hours. After incubation, plates were observed for bacterial colonies showing the fluorescence under UV trans illuminator. Such colonies were picked and purified by repeated four-way streaking and maintained for further experiments.

Antagonistic activity of fluorescent pseudomonads

The fluorescent pseudomonad isolates were tested for their antagonistic potentiality against *Sclerotium rolfsii* by following the dual culture technique (Dennis and Webster, 1971) [5]. The fluorescent pseudomonad isolates were streaked on potato dextrose agar plates at one end (3 cm away) and the mycelial disc (5 mm) of *Sclerotium rolfsii* was placed on the other side just opposite to the bacterium. The plates were incubated at 27 ± 2 °C until they were completely covered by the *S. rolfsii* in control (85 mm diameter). Each treatment was replicated thrice and the zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic bacterium. The colony diameter of the pathogen in the control plate was also recorded. The per cent inhibition of the growth of the pathogen was calculated by the formula given by Vincent (1947) [28].

$$\text{Per cent inhibition of mycelium} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Evaluation of efficient isolates for N_2 fixation, IAA and siderophore production

The fluorescent pseudomonad isolates were examined for other beneficial characteristics *viz*., *In vitro* N_2 fixation; production of indole acetic acid and siderophores; solubilization of mineral phosphate, potassium and zinc.

In vitro N_2 fixation

The nitrogen fixation by the test isolates was tested on solid Norris N-free agar medium. Ten microliters of overnight grown cultures of the isolates were spotted and the plates were incubated at 28 ± 2 °C for 48 h. The observations on ability of the isolates to grow on N-free medium were recorded.

Indole Acetic Acid (IAA) production

Indole acetic acid production by fluorescent pseudomonad was estimated according to Gorden and Weber (1951). The active culture of each test isolate was grown in 5 ml King's B media broth tubes containing 0.005 M of L-tryptophan and incubated at 28 ± 2 ºC and for 3 days. After incubation, the cultures were centrifuged at 5,000 rpm for 10 minutes. Two drops of O-phosphoric acid along with 4 ml Salkowski reagent (0.5 M $FeCl_3$ in 35 per cent perchloric acid) were added to 2 ml of supernatant and incubated for 30 min under dark conditions. Development of pink colour considered as positive for IAA production.

Siderophore assay

CAS agar plates were prepared by mixing 20 ml CAS reagent in 180 ml sterilized King's B agar. Selected bacterial isolates were spot inoculated (10 µl of 10^6 CFU/ml) at the center of each plate. An un-inoculated plate was maintained as control. After inoculation, plates were incubated at 28 ± 2 ºC. Later, plates were observed for the development of yellow-orange halo zone around the bacterial growth after 72 h of incubation.

Elucidation of P, K and Zn solubilization

Pikovskaya's medium amended with tricalcium phosphate (TCP) was used for qualitative assessment of phosphate solubilization (Pikovskaya, 1948) [19]. Petri plates containing sterilized medium were spot-inoculated with 10 µl of overnight grown cultures of fluorescent pseudomonads and the plates were incubated at 28 ± 2 °C. The colony diameter and solubilization zone were measured 120 h after inoculation. The solubilization efficiency and solubilization index (SI) were calculated by using the following formulae (Nautiyal *et al*., 1999) [15]. Similarly, K solubilization efficiency of bacterial isolates was assayed using Aleksandrow's agar medium amended with 0.1 per cent potassium aluminium silicate (Prajapati and Modi, 2012) [20] and the ability to solubilize Zn was tested on TRIS mineral agar medium amended with 0.1 per cent of insoluble ZnO (Sadiq *et al*., 2014) [22].

$$\text{Solubilization Index (SI)} = \frac{\text{Colony diameter} + \text{Diameter of halo zone}}{\text{Colony diameter}}$$

$$\text{Solubilization efficiency (SE \%)} = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \times 100$$

Intrinsic antibiotic resistance

The efficient fluorescent pseudomonads were examined for

the intrinsic antibiotic resistance. King's B agar media plates amended with six antibiotics were prepared and 10 µl of overnight cultures were spotted and incubated for 48h at 28 ± 2 °C. The growth on these plates were compared with growth on media plates without antibiotics and scored accordingly.

Results

Morphological characterization of *Sclerotium rolfsii* Sacc. Isolated from infected brinjal plants

The morphological characteristics of the fungus with respect to mycelium and sclerotial bodies were studied. The colonies formed were white cottony with a colour varying from dull white to pure white on potato dextrose agar (PDA) media. Sclerotial bodies produced after eight days of incubation were brown in color having a shape of mustard seeds. Based on their morphological and cultural characters of the mycelium and sclerotia, the fungus was identified as *Sclerotium rolfsii*.

Characterization of soil samples for chemical properties

All the rhizospheric soil samples collected from 10 different locations were analyzed for their chemical properties like pH, EC and organic carbon by following standard procedures.

The variability in pH was studied for all the 20 soil samples and it was in the range of 6.68-7.59. It was further observed that 2 soil samples possessed slightly acidic pH (<7) and all other soil samples exhibited pH of more than 7.0 (Table 1).The electrical conductivity was found to range from 0.26 dSm^{-1} to 0.78 dS m^{-1}. The EC values of all 20 soil samples indicate the status a normal soil. The organic carbon content the soil samples was in the range from 3.29 g kg^{-1} to 6.94 g kg^{-1}. According to manual on methods for physical and chemical analysis of soils (2007), the data obtained in this study shows that the soil organic carbon content is medium.

Isolation and purification of fluorescent pseudomonad isolates: A total of 125 isolates fluorescing on King's B (KB) medium were selected, sub-cultured and maintained on KB medium for further studies. The bacterial colonies that secreted bluish green to yellowish green pigment exhibited fluorescence activity when exposed to UV light.

Screening of fluorescent pseudomonads

The native fluorescent pseudomonads were subjected to *in vitro* screening by dual culture plate technique for their antagonistic efficacy against *S. rolfsii.* Out of 125, only seven isolates *viz.,* APF110, APF91, APF121, APF44, APF16, APF32 and APF17 inhibited the mycelial growth of the *S. rolfsii* to an extent from 27.79 to 51.08 per cent. Among these, APF110 recorded significantly higher inhibition of 51.08 per cent followed by APF91 (46.33 per cent) and APF121 (42.07 per cent). The growth of *S. rolfsii* in dual culture with APF110 was significantly lesser (41.58 mm) compared to the monoculture (85 mm). Comparatively lower inhibition was observed with the isolates APF16 (27.79 per cent), APF17 (31.29 per cent), APF32 (32.45 per cent) and APF44 (28.95 per cent). Based on the inhibitory efficacy against *S. rolfsii*, top three isolates showing maximum zone of inhibition *viz,* APF110, APF91 and APF121 were considered potent thus, selected for the further studies.

In vitro evaluation of fluorescent pseudomonad isolates for their functional characters

The efficient fluorescent pseudomonad isolates *viz.,* APF91, APF110 and APF121 were examined for *in vitro* nitrogen fixation, production of indole acetic acid and siderophores; solubilization of mineral phosphate, potassium and zinc.

All the isolates of fluorescent pseudomonads spotted on the Norris N-free agar medium did not show any growth that indicates none of the isolates could fix the atmospheric nitrogen.But the isolates produced IAA when they were grown in tryptophan supplemented culture medium, as detected by the Salkowaski's reagent. Similarly, all the three isolates formed an orange-colored halo zone around the colony on dark blue colored CAS agar plates indicating the production of siderophores.

The ability of the fluorescent pseudomonads to solubilize mineral phosphate was ascertained by spotting cultures on Pikovskaya's agar and observing for formation of clear zone of solubilization around the colony. All the three isolates formed clear halo zone on the medium ranging from 16.42 mm to 19.71 mm in diameter (Table 4). The highest phosphate solubilization was exhibited by APF110 with an index of 2.94 and 194.37 per cent efficiency followed by APF121 with a solubilization index of 2.82 and efficiency of 182.44 per cent. APF91 recorded a solubilization index of 2.53 with an efficiency of 153.19 per cent.

The K solubilization was tested by spotting cultures on Aleksandrow's medium. The results depicted that all the three isolates were able to form clear halo zone ranging from 17.42 to 18.28 mm in diameter. The highest potassium solubilization was exhibited by APF121 with an index of 2.91 and 190.84 per cent efficiency followed by APF91 with an index of 2.85 and efficiency of 184.93 per cent. APF110 recorded a solubilization index of 2.83 with an efficiency of 182.80 per cent (Table5).

Similarly, the potentiality of the isolates to solubilize ZnO was evaluated by spotting cultures on TRIS minimal agar and the results indicated that all the three isolates formed zone of solubilization ranging from 19.57 mm to 24.14 mm in diameter. The highest zinc solubilization was shown by APF110 with an index of 3.32 and 231.67 per cent efficiency followed by APF121 with an index of 3.08 and efficiency of 208.37 per cent. APF91 recorded a solubilization index of 3.05 and efficiency of 204.71 per cent (Table6).

Screening intrinsic antibiotic resistance

The ability of the isolates to resist to the antibiotics was ascertained and results are presented in Table 13. A total of six antibiotics at two concentrations each were added to the media and the growth pattern of isolates was examined after two days of incubation. All the isolates showed resistance to chloramphenicol (10 and 20 ppm), nalidixic acid (5 and 10 ppm) and ampicillin (100 and 150 ppm) whereas, found sensitive to kanamycin. APF 110 was found to be sensitive and remaining two were resistant to tetracycline (10 and 20 ppm). Streptomycin inhibited the growth of all the isolates at 150 ppm whereas, at 100 ppm, APF110 and APF121 were found resistant (Table 7).

Discussion

Certain strains of rhizosphere colonizing pseudomonads have gained much attention worldwide recently due to their potentiality in promoting plant growth. Therefore, in this study, we isolated native fluorescent pseudomonads from the rhizosphere of brinjal that may broadly be used at as potential inoculants. The isolation of fluorescent pseudomonads from

rhizosphere soil yielded 125 isolates with various morphological appearances. All those isolates were primarily screened for antagonistic activity against *Sclerotium rolfsii* and the efficient isolates subsequently analyzed for plant growth promoting attributes.

Out of 125 isolates screened, only seven isolates inhibited the mycelial growth of the *S. rolfsii*. Among these, APF110 recorded significantly higher inhibition followed by APF91 and APF121. The fluorescent pseudomonads produce a variety of metabolites which are detrimental to many other microbes and some of which are implicated in biological control of plant pathogens. Many workers have reported the antagonistic property of fluorescent pseudomonads against numerous plant pathogens (Manivannan *et al*., 2012; Arunasri *et al.,* 2013; Aly *et al*., 2015) [13, 2]. Similarly, Singh *et al.* (2003) [25] also screened 186 bacterial strains for their biocontrol activity against *S. rolfsii* and observed inhibition zones formed by fluorescent pseudomonads ranging from 4-15 mm in diameter.

All the isolates produced IAA as indicated by development of pink colour following the addition of Salkowaski's reagent. 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) [18].

In this study, all the three isolates formed orange colored halo zone around the colony indicating the production of siderophores. The results indicated that the highest production of siderophore was recorded byAPF110 followed by APF121 and APF91. A similar work has been documented by Sreedevi *et al*. (2014) [26] who isolated 10 *Pseudomonas* spp. from paddy soil among which, three isolates showed positive results for siderophore production on CAS agar medium.

The ability of several isolates to solubilize tricalcium phosphate *in vitro* suggested the application of those isolates in crop fields. Rodriguez and Fraga (1999) [21] had studied that *Pseudomonas* and other phosphate solubilizing bacteria (PSB) like *Bacillus* are capable to increase the availability of phosphorus in soil. All the isolates might be potential inoculants for alkaline soil based on the ability to solubilize phosphate bounded by calcium which mostly exists in alkaline soils, whereas in the acidic soil, P are mostly fixed by Fe or Al (Goldstein, 1995) [8]. Siderophore is one of the biocontrol mechanisms belonging to PGPR groups, including *Pseudomonas* sp. under iron limiting condition, PGPR produce a range of siderophore which have a very high affinity for iron. Therefore, the low availability of iron in the environment would suppress the growth of pathogenic organisms including plant pathogenic fungi (Whipps, 2001) [29]. Brindavathy and Gopalaswamy (2014) reported that, release of potassium by the formation of clearing zone around the colonies was due to production of exopolysaccharides. Intrinsic antibiotic resistance (IAR) screening was carried to assess the tolerance of the isolates towards six antibiotics. The IAR analysis depicted that all the isolates showed resistance to chloramphenicol, nalidixic acid and ampicillin whereas, found sensitive to kanamycin. Suneesh (2004) [27] also documented that the most of the fluorescent pseudomonad isolates were found resistant to spectinomycin, ampicillin, nalidixic acid, chloramphenicol and kanamycin. Djuric *et al.* (2011) [7] reported that the isolated fluorescent pseudomonad isolates were resistant to ampicillin and chloramphenicol while most of them were sensitive to tetracycline. Based on splendid biocontrol activities and plant promoting attributes, we propose three isolates as potential inoculants.

Table 1: Physico-chemical properties of the collected soil samples

Sl. No.	Sample code	Soil type	pH	EC (dS m^{-1})	OC (g kg^{-1})
1	RBNR1	Black soil	7.54	0.48	5.75
2	RBNR2	Black soil	7.04	0.37	5.56
3	RBNR3	Black soil	7.26	0.31	5.65
4	SDPR	Black soil	6.69	0.29	4.78
5	HNST	Black soil	6.68	0.26	4.65
6	HLGR	Black soil	7.12	0.43	6.24
7	HLHL1	Black soil	7.22	0.44	4.78
8	HLHL2	Black soil	7.33	0.52	6.94
9	KDMD1	Black soil	7.35	0.55	4.12
10	KDMD2	Black soil	7.59	0.76	3.85
11	KDMD3	Black soil	7.56	0.69	3.43
12	KRPR1	Black soil	7.44	0.52	4.80
13	KRPR2	Black soil	7.62	0.78	4.95
14	VDKP1	Black soil	7.13	0.42	3.43
15	VDKP2	Black soil	7.41	0.55	4.67
16	NYKR1	Black soil	7.52	0.65	4.83
17	NYKR2	Black soil	7.43	0.66	3.91
18	RMGR1	Black soil	7.34	0.54	3.38
19	RMGR2	Black soil	7.26	0.43	3.29
20	RMGR3	Black soil	7.29	0.50	3.69

Table 2: Antagonistic activity of fluorescent pseudomonads against *Sclerotium rolfsii* Sacc. using dual culture plate technique

Sl. No.	Isolate	Percent inhibition (%)	Sl. No.	Isolate	Percent inhibition (%)
1	APF1	10.99	64	APF64	07.44
2	APF2	4.32	65	APF65	04.31
3	APF3	8.62	66	APF66	0.78
4	APF4	4.71	67	APF67	09.80
5	APF5	1.18	68	APF68	00.78
6	APF6	1.56	69	APF69	06.67
7	APF7	5.09	70	APF70	03.91
8	APF8	6.67	71	APF71	02.74
9	APF9	0.39	72	APF72	09.80
10	APF10	7.45	73	APF73	07.05
11	APF11	3.53	74	APF74	01.17
12	APF12	4.71	75	APF75	02.35
13	APF13	1.96	76	APF76	05.09
14	APF14	7.06	77	APF77	10.58
15	APF15	8.24	78	APF78	11.37

16	APF16	27.79	79	APF79	05.49
17	APF17	0.79	80	APF80	08.23
18	APF18	1.56	81	APF81	11.76
19	APF19	1.18	82	APF82	01.96
20	APF20	0.79	83	APF83	05.88
21	APF21	0.27	84	APF84	0.38
22	APF22	0.79	85	APF85	03.52
23	APF23	1.18	86	APF86	04.70
24	APF24	2.35	87	APF87	08.23
25	APF25	1.56	88	APF88	11.76
26	APF26	0.79	89	APF89	03.92
27	APF27	1.96	90	APF90	01.17
28	APF28	1.18	91	APF91	46.39
29	APF29	2.35	92	APF92	09.80
30	APF30	3.14	93	APF93	08.23
31	APF31	5.49	94	APF94	06.67
32	APF32	32.44	95	APF95	04.31
33	APF33	3.92	96	APF96	03.52
34	APF34	1.17	97	APF97	01.56
35	APF35	1.96	98	APF98	09.80
36	APF36	2.35	99	APF99	05.88
37	APF37	2.74	100	APF100	03.52
38	APF38	3.91	101	APF101	10.58
39	APF39	0.78	102	APF102	06.67
40	APF40	1.17	103	APF103	09.02
41	APF41	1.56	104	APF104	03.91
42	APF42	1.17	105	APF105	03.14
43	APF43	9.41	106	APF106	31.22
44	APF44	28.94	107	APF107	09.41
45	APF45	12.55	108	APF108	11.37
46	APF46	10.97	109	APF109	04.31
47	APF47	13.72	110	APF110	51.08
48	APF48	06.27	111	APF111	12.15
49	APF49	02.74	112	APF112	09.02
50	APF50	09.80	113	APF113	05.88
51	APF51	05.49	114	APF114	11.76
52	APF52	01.56	115	APF115	01.56
53	APF53	09.02	116	APF116	05.49
54	APF54	05.88	117	APF117	12.15
55	APF55	09.80	118	APF118	07.84
56	APF56	13.72	119	APF119	01.17
57	APF57	01.18	120	APF120	05.88
58	APF58	05.49	121	APF121	42.07
59	APF59	11.76	122	APF122	11.76
60	APF60	04.31	123	APF123	07.05
61	APF61	08.62	124	APF124	01.56
62	APF62	0.39	125	APF125	01.17
63	APF63	11.76	126	Control	00
S.Em. (±)		0.71	C.D. @ 1%		0.62

Table 3: Growth promotional activities by the efficient fluorescent pseudomonad isolates

Isolate	*In vitro* N_2 fixation	IAA	Siderophore
APF91	−	+	+
APF110	−	+	+
APF121	−	+	+

Note: + indicates positive, − indicates negative

Table 4: Qualitative analysis of mineral phosphate solubilization by the efficient fluorescent pseudomonad isolates

Isolate	Culture diameter (mm)	Phosphate solubilization		
		Solubilization zone (mm)	Solubilization efficiency (%)	Solubilization index
APF91	11.28	17.28	153.19	2.53
APF110	10.14	19.71	194.37	2.94
APF121	9.00	16.42	182.44	2.82
S.Em. (±)		0.12	1.20	0.01
C.D. @ 1%		0.49	4.90	0.05

Table 5: Qualitative analysis of potassium solubilization by the efficient fluorescent pseudomonad isolates

Isolate	Culture diameter (mm)	Potassium solubilization		
		Solubilization zone (mm)	Solubilization efficiency (%)	Solubilization index
APF91	9.42	17.42	184.93	2.85
APF110	10.00	18.28	182.80	2.83
APF121	9.28	17.71	190.84	2.91
S.Em. (±)		0.12	1.26	0.01
C.D. @ 1%		0.49	5.15	0.05

Table 6: Qualitative analysis of zinc solubilization by the efficient fluorescent pseudomonad isolates

Isolate	Culture diameter (mm)	Zinc solubilization		
		Solubilization zone (mm)	Solubilization efficiency (%)	Solubilization index
APF91	9.56	19.57	204.71	3.05
APF110	10.42	24.14	231.67	3.32
APF121	10.28	21.42	208.37	3.08
S.Em. (±)		0.14	1.39	0.01
C.D. @ 1%		0.57	5.68	0.05

Table 7: Intrinsic antibiotic resistance screening of the efficient fluorescent pseudomonad isolates

Sl. No.	Antibiotic	Conc. (ppm)	Isolate		
			APF91	APF110	APF121
1	Ampicillin	100	++	++	++
		200	+	+	+
2	Tetracycline	10	+	−	+
		20	+	−	+
3	Kanamycin	50	−	−	−
		75	−	−	−
4	Nalidixic acid	5	+	+	+
		10	+	+	+
5	Chloramphenicol	10	++	++	++
		20	++	++	++
6	Streptomycin	100	−	+	+
		150	−	−	−

Note: + indicates fair growth, ++ indicates good growth, − indicates growth is absent

Conclusion

Three isolates of fluorescent pseudomonads *viz.*, APF91, APF110 and APF121 were identified as potential biocontrol agents along with plant growth promotional activities. We consider for further research to determine their activities in promoting plant growth and biological control of pathogens.

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