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Identification and Evaluation of Antagonistic Potential of Different Fluorescent Pseudomonads in Northern Plains of West Bengal

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ABSTRACT

To achieve future food security, reliable crop protection will be critically important. Development of biological control for soil borne disease is accepted as a safe, durable and ecofriendly alternative for chemical management strategy. *Pseudomonads* possess many traits like production antibiotics, lytic enzymes, siderophores, hydrogen cyanide (HCN) that make them well suited as biocontrol and growth-promoting agents. An attempt was taken to isolate and identify different fluorescent Pseudomonads and to evaluate their antagonistic ability. Twelve fluorescent Pseudomonads isolates were collected from different crop rhizosphere from different locations of northern parts of West Bengal. Isolates characterized morphologically and biochemically. All the isolates were gram negative rods with smooth and glossy colony on kings B and nutrient agar medium with convex elevation but variation was found in respect to pigment production. A huge variation was found in respect to biochemical characterization. From the result of biochemical analysis, the isolated sp. can be identified as *Pseudomonas fluorescens* or *Pseudomonas putida* or *Pseudomonas aeruginosa.* Some of the isolate produce green, some isolates produce yellowish green and rest of them produce red/pink pigments. Isolates showed a huge variation in respect to pathogenicity against soil borne pathogen *Slerotium rolfsii.* Percent inhibition varied from 31 to 74 %. Isolate 3 and 8 can be efficient biocontrol agent as highest growth inhibition of the pathogen *Sclerotium rolfsii* was obtained by the isolate 8 (74.37%) followed by isolate 3 (63.15%). In respect to growth parameter isolate 8, 9 and 10 were found better among the isolates where as in respect to disease reduction isolate 3, 8, 9 and 11 were promising under *in vivo* study.

Keywords Fluorescent, Pseudomonads, *Slerotium rolfsii*, Antagonistic, Characterization.

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INTRODUCTION

As the population of India is increasing the estimated total food requirement will increase in future. The indiscriminate use of pesticide had led to the change in the biological balance of the microbes via the suppression of native antagonistic flora; development of fungicide resistant strain of pathogens, ground water and stuff population, health and environmental haz-

ards, residue persistence, development of resistance to pesticide and ecogenic risk. Development of biological control for soil borne disease is accepted as a safe, durable and ecofriendly alternative for agrochemicals. A microorganism can exert antagonism towards a plant pathogen directly by producing substances (Cook and Baker 1983) or indirectly by activating mechanisms of host resistance towards the pathogen (Van Loon *et al.* 1998). *Pseudomonads* possess many traits that make them well suited as biocontrol and growth-promoting agents. These include the ability to (i) grow rapidly *in vitro* and to be mass produced (ii) rapidly utilize seed and root exudates (iii) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant (iv) produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth-promoting substances) (v) compete aggressively with other microorganisms and (vi) adapt to environmental stresses. The most effective strains of *Pseudomonas* have been Fluorescent pseudomonas which are characterized by their production of yellow green pigments, termed pyoverdines or pseudobactins, that fluorescence under UV irradiation and function as siderophore (Abdalla 1991). It has also been seen that the siderophores enantio-pyochelin and pyoverdine, and the biosurfactant viscosin—the siderophores are mainly responsible for the antagonistic activity of the *Pseudomonas fluorescens* under iron-limited conditions (Deveau *et al,* 2016). *Pseudomonas* sp, has shown activity against a wide range of Basidiomycetes, Deuteromycetes and Ascomycetes, including several economically important pathogens like *Rhizoctonia solani, Botrytis cinerea, Verticillium dahliae* and *Sclerotinia* sp. (Ligon *et al.* 2000). The antifungal metabolites and lytic enzymes produced by *Pseudomonas fluorescens* inhibited the mycelium growth of certain fungi (Radhajeyalakshmi *et al.* 2000). Treatment of the plant growth promoting rhizobacteria increased in the germination percentage, emergence, seedling vigor, plant stand, root and shoot growth, total biomass of the plants, seed weight, early flowering, increased grain, fodder, fruit yields (Van Loon *et al.* 1998 and Ramamoorthy and Samiyappan 2001). The plants plant growth promoting ability and reduction of disease incidence is due to increase in the nitrogen fixation, the production of growth hormones, the solubilization of phosphorus and oxidation of sulfur, increase in nitrate availability, extracellular production of antibiotics, lytic enzymes, hydrocyanic acid, and increases in root permeability (Enebak and Carey 2000). The induction of systemic resistance in plants by *Pseuodomonas fluorescens* through production of several defense related enzymes like phenylalanine ammonia-lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), B-1,3-glucanases, chitinases have been evidenced by many workers (Van Loon 1997, Prabhukarthikeyan *et al.* 2018).

MATERIALS AND METHODS

Isolation of rhizobacteria and plant pathogens collection of soil sample

For isolation of fluorescent Pseudomonads, soil samples were collected from different locations of northern area of West Bengal like Pundibari, Hoglabari and Khagribari. Soil sample were collected from the rhizosphere of different crops like wheat, rice, potato, brinjal, ginger, turmeric, bhindi, lentil, mung bean along with the root.

Isolation of biocontrol agents fluorescent Pseudomonads (Enrichment process)

Kings B medium was used for the isolation of fluorescent Pseudomonads. One gram of soil along with root was allowed to grow in kings B broth at 28± 1°C. After bacterial growth *Pseudomonads* were purified by streaked plate method and kept at 4°C for further use.

Isolation of fungi

The pathogenic fungi *Sclerotium rolfsii* isolated from infected potato plant part. Infected roots and stems were cut into small pieces, sterilized with 1% sodium hypochlorite (NaOCl) solution for 10 to 20 sec and placed on PDA media. The plates were incubated at 28±1°C in BOD incubator for 5 to 6 days. Fungal hyphal tips growing from infected part were transferred to PDA slant (Rangaswami 1972). Pure culture tubes were preserved in refrigerator at 4°C used for further studies.

Morphological studies

The confirmation of the fluorescent Pseudomonads isolates was performed with the following studies (Buchanan and Gibbson 1974)

Colony morphology

Pure cultures of the selected isolates were streaked on KMB agar and nutrient agar media separately for colony development. The individual colonies were examined for shape, size, structure of colonies and pigmentation.

Biochemical characterization gram differentiation (Gram 1884)

Take a loopful of test organism and spread on a clean slide with the help on loop and allowed it to dry and gently heat to fix the smear. Then cover the smear with crystal violet for one minute after that wash in running water for few second and rinse the smear with iodine and cover it for one minute then apply alcohol decolorizer (95%) drop wise till the dye does not run off the smear. Rinse with water and cover the smear with safranin for 30 seconds. Rinse with water and blot dry the smear. Observe under oil immersion lens, gram positive bacteria will stain blue / violet and gram-negative bacteria will give red color.

KOH solubility test

One loopful of bacterial suspension was mixed with 1 drop of 3% KOH on a clean glass slide. Gram negative bacteria will form gummy substance after mixing with KOH while gram positive will not form gummy substance.

Oxidase test: (Suman *et al.* 2016)

To the 24 h old bacterial culture oxidase discs are placed on them. The isolates showing blue coloration of discs were taken as positive

Biochemical characterization using biochemical test kit

Biochemical test of isolates of bacteria from different root rhizosphere were done using by KB002 and KB 009 biochemical test kit (for gram negative rod) manufactured by Himedia Laboratories Pvt, limited. On incubation, organisms undergo metabolic changes which are indicated by color change in media that can be either interpreted visually or after addition of reagent.

Preparation of inoculum

The *Pseudomonus fluorescent* isolates were inoculated in King's B broth and allowed to grow in at 28±1°C for 72 hs.

Inoculation of the strip

For biochemical analysis and carbohydrate utilization kits were inoculated with the broth culture of different isolates of fluorescent Pseudomonads. Kits were opened aseptically under, sealing taps was peeled off, each culture was taken with the help of micropipette for inoculation of each well following surface inoculation method. Inoculated kits were incubated at 28±1°C in for 24 hs. The result was interpreted as per the standards given in the result interpretation chart. Addition of reagents in the well no. 5 and 6 were done at end of incubation period.

Antagonistic effect

The bacterial isolates were screened by dual culture test. A loopful of fresh bacterial culture was streaked in both side of plate containing twenty ml of PDA (without antibiotic) leaving 1 cm from the margin and then 5 mm disc of pathogenic cultures were placed at the center and after that petri plates were incubated at 28±1°C for 3 to 4 days. The percentage of inhibition of the test fungus with each bacterial isolate were calculated. The plates which were streaked with sterilized water in place of bacterial isolate kept as control. Percent inhibition over control was calculated by using the formula of Vincent (1947) as follows:

$$I=(C-T) / C \times 100$$

I=percent inhibition of mycelium
C= growth of mycelium in control
T=growth of mycelium in treatment

Plant growth promotion and biocontrol efficacy preparation of talc-based formulation

Four hundred ml of bacterial suspension containing 9×10^8 cfu/ ml was mixed with 1 kg of purified talc powder (sterilized at 105 °C for 12 h), 15 g calcium carbonate (to adjust the pH to neutral) and 10 g of carboxy methyl cellulose (CMC) as an adhesive under aseptic conditions following the method described by Vidhyasekaran and Muthamilan (1995).

Seed bacterization

The rhizobacterial isolates were bio assayed for their ability to promote or inhibit seedling growth used the method as described by Shende *et al.* (1977) with few modifications. French bean seeds were surfaced sterilized with 1% sodium hypochlorite for 3 minutes. After that seed was prepared by mixing with talc formulation.

Pot experiment

Another set of experiment were taken on portrays to record the effect of seed bacterization on root length, shoot length and germination for which vigor index was calculated.

Preparation of pathogen inoculums

Soil was fumigated by formalin for 3 day and covered it by transparent polythene. After 3 days the polythene sheet was removed and kept the soil for 7 days. Then the pro trays were filled with the soil. In each pro tray, 3 sclerotia of *Sclerotium rolfsii* were inoculated. After the growth of pathogen in soil the seeds of French been treated with talc formulation of the isolates of fluorescent Pseudomonads was sown in the pro trays containing the inoculum of pathogen.

Data recorded

The results of germination, shoot length, root length, fresh weight of root and shoot, dry weight of root and shoot at 15 DAS and disease incidence (% of seeds rotted) were taken. Vigor index was calculated by this formula (Prathibha and Siddalingeshwara 2013)

Seedling vigor index = (mean root length + mean shoot length) × germination percent.

RESULTS

Isolation of the fluorescent Pseudomonads

Different fluorescent Pseudomonads were isolated from different parts of North Bengal like parts of Cooch Behar and Dinhata subdivisions having diverse edapho-climatic situation from the rhizoplane of different crops like wheat, potato, mustard, maize, ginger, lentil and different vegetables. The inventory of the isolate is given in the Table 1.

All the isolates showed greenish type of pigmentation under UV light. When streaked on Kings B medium the colony appearance showed a wide variation. Twelve isolates based on pigmentation and colony morphology were purified for further work.

All the 12 isolates were characterized morphologically and presented in the Table 2. All the isolates are gram negative in gram reaction. The margins of the bacterial colony were irregular. Isolates 1, 3, 4, 5, 10 and 12 showed yellowish green color. Red color was found in Pf isolate no. 2, 9, 11 and 13. Isolate 6 and 8 produced green color. On King's B medium all the isolates give smooth colony whereas on Nutrient Agar, they give a white glossy type of colony. All the isolates were very fast in growth and covers the plate within 24 hrs of incubation. The surface of the bacterial colonies was smooth shiny and convex for all the

Table 1. Isolates of fluorescent Pseudomonads from different locations of Northern Parts of West Bengal.

Sl. No.	Isolates	Source	Location
1	Pf1	Rhizosphere of wheat	Pundibari, Coochbehar
2	Pf2	Rhizosphere of maize	Hoglabari, Coochbehar
3	Pf3	Rhizosphere of potato	Khairabari, Coochbehar
4	Pf4	Rhizosphere of mung bean	Madhupur. Coochbehar
5	Pf5	Rhizosphere of tea	Pundibari, Coochbehar
6	Pf6	Rhizosphere of ginger	Pundibari, Coochbehar
7	Pf8	Rhizosphere of brinjal	Dinhata, Coochbehar
8	Pf9	Rhizosphere of rice	Pundibari, Coochbehar
9	Pf10	Rhizosphere of ladies' finger	Pundibari, Coochbehar
10	Pf11	Rhizosphere mustard	Pundibari, Coochbehar
11	Pf12	Rhizosphere of lentil	Pundibari, Coochbehar
12	Pf13	Rhizosphere of wheat	Pundibari, Coochbehar

Table 2. Morphological character of different fluorescent Pseudomonads isolates.

Isolates	Gram reaction	Margin	Color on Kings B	Colony type Kings B	Colony type N A	Growth type	Surface	Elevation
Pf 1	-ve	irregular	Greenish yellow	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 2	-ve	irregular	Red	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 3	-ve	irregular	Greenish yellow	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 4	-ve	irregular	Greenish yellow	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 5	-ve	irregular	Greenish yellow	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 6	-ve	irregular	Green	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 8	-ve	irregular	Green	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 9	-ve	irregular	Red	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 10	-ve	irregular	Greenish yellow	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 11	-ve	irregular	Red	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 12	-ve	irregular	Greenish yellow	smoothy	glossy	fast	Smooth, shiny	Convex
Pf 13	-ve	irregular	Red	smoothy	glossy	fast	Smooth, shiny	Convex

Table 3. Biochemical characterization of isolates.

Carbohydrates	Isolates											
	Pf1	Pf2	Pf3	Pf4	Pf5	Pf6	Pf8	Pf9	Pf10	Pf11	Pf12	Pf13
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	+	+	-	-	-	-	-	-
Xylose	+	+	+	-	-	-	-	-	-	+	+	+
Maltose	-	-	-	+	-	-	+	+	-	-	-	-
Fructose	-	-	-	-	-	-	-	-	-	+	-	-
Dextrose	+	+	+	+	+	-	+	-	-	+	-	+
Galactose	+	+	+	+	-	-	-	-	-	+	-	-
Rraffinose	-	-	-	-	+	-	-	-	-	+	-	-
Trehalose	-	-	+	-	+	-	+	-	-	+	-	-
Melibiose	+	-	+	-	-		+	-	-	+	-	+
Sucrose	-	-	+	-	-	-	-	-	-	+	-	+
l-arabinose	+	-	+	+	-	+	-	+	-	+	-	+
Mannose	+	+	+	+	-	-	+	+	-	+	-	+
Inulin	-	-	+	+	+	+	-	+	-	+	-	+
Sodium gluconate	-	-	+	+	+	+	+	+	-	+	-	+
Glycerol	+	+	+	-	+	+	-	+	-	+	-	+
Salicin	-	-	-	-	+	-	+	+	-	+	-	+
Duleitol	-	+	-	-	-	-	+	+	-	+	-	+
Inositol	-	+	-	-	-	+	-	+	-	+	-	+
Sorbitol	-	-	-	-	+	+	-	+	-	+	-	+
Mannitol	-	+	+	-	-	+	-	+	-	+	-	+
Adonitol	-	-	-	-	-	+	-	+	-	+	-	+
Arabitol	-	-	-	-	-	+	-	+	-	+	-	+
Erythritol	+	+	-	-	-	+	+	+	-	+	-	+
Alfa methyl d mannoside	-	-	-	-	-	+	-	+	-	+	-	+
Rhamnose	-	-	-	-	-	-	-	+	-	-	-	-
Cellobiose	-	-	-	-	-	-	-	-	-		+	-
Melezitose	-	-	+	-	-	+	-	-	-	-	+	-
Alfa methyl d mannoside	-	-	-	+	-	+	-	-	-	+	-	-
Xylitol	-	-	-	-	+	-	-	-	-	-	-	+
onpg	-	-	-	-	-	-	-	-	-	-	-	-
Esculin hydrolysis	-	-	-	-	-	-	-	+	-	-	-	-
D-arabinose	+	-	-	+	-	-	-	-	-	-	-	-
Citrate utilization	-	+	+	-	-	-	-	-	-	-	-	-
Malonate utilization	-	-	-	-	-	-	-	-	-	-	-	-
Sarbose	-	-	-	-	-	-	+	-	-	-	-	-

12 isolates. The red colour may be due to production of pyorubin produced by *Pseudomonas aeruginosa. Pseudomonas aeruginosa* is the main pathogenic species in the family Pseudomonaceae. It is known for its ability to produce a variety of pigments when grown in culture, such as pyocyanin (blue), pyorubin (red), pyomelanin (black), and pyoverdin (yellow-green to yellow-brown and fluorescent), and for its characteristic "corn taco" or "grape"-like odor. On both the medium (Nutrient Agar and King's B) all the isolates showed convex type of colony.

Biochemical test of the Pseudomonads isolates was done by using KB002 and KB 009 Biochemical test kit and oxidase test was done using disc, results of the biochemical tests of 12 Pseudomonads isolates were presented in the Tables 3 and 4. All the isolates showed positive result in oxidase test. From the other results it was found that in respect to maltose utilization, isolate 1, 2, 3, 5, 6, 10, 11, 12 and13 shows negative result so those isolates may be identified as the *Pseudomonas fluorescens* or *Pseudomonas putida* or *Pseudomonas aeruginosa.* Isolate 2, 3, 6, 9, 11 and13 utilized mannitol, so they can be identified as *Pseudomonas fluorescens* and isolate 1, 4, 5, 8, 10 and 12 can be identified as *Pseudomonas putida*. In case of nitrate reduction isolate 2, 3, 4, 5, 6 and 13 showed positive result so they can be identified as *Pseudomonas fluorescens* or *Pseudomonas aeruginosa* and isolate 1, 8, 9, 10 and 11 showed negative result so they can be identified as *Pseudomonas putida.* This result may support the findings of Prabhukarthikeyan *et al.* (2015) who characterized different fluorescent Pseudomonas strains biochemically using KB002 test kit and based on the result of biochemical kit (KB002), ten strains showed positive to more than ten tests and interpreted as *P. fluorescens.*

In pot experiment with French bean isolate 3, 8, 9, 10 and 11 showed better result in respect to vigor index and disease reduction capacity. Isolate 3 showed positive result in oxidase test, galactose mannitol utilization nitrate reduction and negative result in maltose utilization. So, isolate 3 may be identified as *Pseudomonas fluorescens.* Isolate 11 showed positive result in respect to oxidase test, galactose, mannitol utilization, negative in nitrate reduction and maltose utilization. Isolate 11 can be identified as *Pseudomonas putida*. Isolate 8 showed positive result for oxidase test and maltose utilization and negative result in galactose, mannitol utilization and nitrate reduction. Isolate 9 showed positive result in oxidase test, maltose and mannitol utilization but negative in galactose utilization and nitrate reduction. Isolate 10 showed positive result in oxidase test and negative in galactose, mannitol, maltose utilization, nitrate reduction. So, isolate 8, 9 and 10 may be *Pseudomonas fluorescen*s or *Pseudomonas putida* or *Pseudomonas aeruginosa*. In accordance with Suman *et al.* (2016) isolates under study may be *Pseudomonas fluorescens* or *Pseudomonas putida* or *Pseudomonas aeruginosa.* For more appropriate identification molecular identification of the isolates should be done. For field level application molecular identification should be done because *Pseudomonas aeruginosa* cannot be use in the field as it may be human pathogen.

Table 4. Biochemical characterization of isolates.

Character	Isolates											
	Pf1	Pf2	Pf3	Pf4	Pf5	Pf6	Pf8	Pf9	Pf10	Pf11	Pf12	Pf13
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+
Lysine utilization	+	+	+	+	+	+	+	+	+	+	+	+
Ornithine utilization	+	+	+	+	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	+	+	+	+	+
Phenylaline deamination	-	-	-	-	-	-	-	-	-	+	-	-
Nitrate reduction	-	+	+	+	+	+	-	-	-	-	-	+
Hidrrogen sulphide production	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Adonitol	-	-	-	-	-	+	-	+	-	+	-	+
Lactose	-	-	-	-	+	+	-	-	-	-	-	-
Arabinose	+	-	-	+	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	+	+	-	+	-	+	-	+

Table 5. Percent growth inhibition of the test fungi *Sclerotium rolfsii* by different *Pseudomonads* isolates.

Name of the isolates	% inhibition
Pf1	35.56 (36.60)
Pf2	46.85 (43.20)
Pf3	63.15 (52.62)
Pf4	41.78 (40.27)
Pf5	38.47 (38.33)
Pf6	38.11 (38.12)
Pf8	74.37 (59.59)
Pf9	31.11 (33.90)
Pf10	50.00 (45.00)
Pf11	53.33 (46.91)
Pf12	52.96 (46.70)
Pf13	32.22 (34.59)
CD at 5%	3.45
SEM ±	1.17
CV	4.71

Evaluation of the fluorescent Pseudomonads isolates

The *Pseudomonas* isolates were selected on the basis of percent growth inhibition of the pathogen *Sclerotium rolfsii* in dual culture, plant growth promotion and % disease reduction.

Percent growth inhibition of the test fungi *Sclerotium rolfsii* by different Pseudomonads isolates are presented in the Table 5. Data presented in the table reveals that isolates varied widely in capacity of growth inhibition. Highest growth inhibition of the pathogen, *Sclerotium rolfsii* was obtained by the isolate 8 (74.37%) followed by isolate 3 (63.15%). In case of isolate 3, growth inhibition of pathogen *Sclerotium rolfsii* was found without physical contact of biocontrol agent and pathogen. Similar finding was also obtained by Priyanka *et al.* (2017) who found that out of 62 isolates of fluorescent Pseudomonas, 51 were inhibitory to *Sclerotium rolfsii.* They also found that the maximum percent inhibition of 70.37 was observed in BFP22 isolate, followed by DFP62 and DFP48 isolate with percent inhibition of 60.93 and 58.89 respectively. which was significantly superior over all other isolates. This may be due to production of antibiotic or defense enzymes by fluorescent pseudomonads. Prabhukarthikeyan *et al.* (2018) reported that Strain FP7 of fluorescent pseudomonads recorded the maximum percent inhibition of *P. aphanidermatum* under *in vitro* conditions. Strains FP7 and TPF54 both increased plant growth in turmeric plants in vitro. Strain FP7 alone contained all the evaluated antibiotic biosynthetic genes. They also found that application of FP7 bioformulations significantly increased the activities of defense enzymes viz., PO, PPO, PAL, SOD and CAT against the rhizome rot pathogen in turmeric.

The result of *in vivo* bio control and growth promoting efficacy of different fluorescent pseudomonas isolates on french bean seeds is presented in Table 6. Data presented in that table revealed that a marked variation was observed in all the parameters

Table 6. *In vivo* bio-control (against *Sclerotium rolfsii*) and growth promoting efficacy of fluorescens Pseudomonads isolates on French bean seeds.

Isolates	Germination (%)	Increased of germination % over control	Root length (cm)	Increase of root length over control	Shoot length (cm)	Increase of shoot length over control	Vigor index	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	% Disease incidence (% seeds rotted)
Pf1	80	33.33	4.08	36.22	14.00	14.28	1446	1.41	0.43	2.40	0.77	20
Pf2	70	16.66	4.11	18.57	12.67	1.14	1175	1.48	0.30	2.20	0.57	30
Pf3	90	50.00	4.21	42.41	15.67	27.35	1789	1.49	0.43	2.23	0.73	10
Pf4	40	33.33	4.02	19.50	11.33	12.35	614	1.47	0.30	2.00	0.73	60
Pf5	90	50.00	4.08	23.83	15.00	47.57	1717	1.63	0.30	2.27	0.67	20
Pf6	80	33.33	4.07	23.83	15.67	47.57	1579	1.53	0.57	2.00	0.77	20
Pf8	80	33.33	4.32	18.57	16.33	12.35	1652	1.33	0.50	2.10	0.80	10
Pf9	90	50.00	3.98	18.57	14.67	47.57	1679	1.67	0.50	2.30	0.67	10
Pf10	80	33.33	4.47	36.22	17.00	14.28	1718	1.47	0.57	2.20	0.67	20
Pf11	80	33.33	3.90	23.83	16.00	12.35	1592	1.57	0.53	2.17	0.60	10
Pf12	80	33.33	4.33	23.83	14.00	27.35	1466	1.57	0.40	1.97	0.60	20

Table 6. Continued.

Isolates	Germination (%)	Increased of germination % over control	Root length (cm)	Increase of root length over control	Shoot length (cm)	Increase of shoot length over control	Vigor index	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	% Disease incidence (% seeds rotted)
Pf13	80	33.33	3.80	42.41	13.00	27.35	1344	1.50	0.53	2.13	0.60	30
Control	60	---	3.60	---	9.33	---	776	0.80	0.07	1.07	0.20	40
CD at 5%	---	---	N/A	---	4.277	---	---	0.392	0.128	0.408	0.157	
SEm±	---	---	0.205	---	1.457	---	---	0.133	0.044	0.139	0.054	
CV	---	---	8.715	---	17.761	---	---	15.879	18.027	11.587	14.419	

taken. Percent germination varied from 40% to 90% showing considerable variation among the isolates in terms of its effect on plant at a very early stage of germination. The plant also showed wide variation in response to different isolates in terms of root and shoot development at an early stage of the seedling growth. The results indicated that isolate 3 has higher vigor index among 12 isolates under study. This result is in accordance with the findings of Priyanka and Goudar (2021) who reported that the growth parameters like plant height, number of branches, total dry matter production, of soybean plants were increased by the application of strains of *Pseudomonas fluorescens* in soybean field where challenge inoculation of *Sclerotium rolfsii* was done. Prabhukarthikeyan *et al.* (2018) reported that application of strain FP7 of fluorescent Pseudomonas in the field increased the defense molecules, maximum plant height, maximum size of stem girth and maximum number of leaves and yield attributing characters like primary rhizomes, secondary rhizomes, fresh weight and dry weight in turmeric plants in turmeric plants thereby reducing the incidence of rhizome rot disease. The vigor index of the isolates 10, 9 and 8 followed closely. Highest root dry weight was recorded for isolate 10 (0.57g) and 5 (0.57g) which is closely followed by isolate 11, 13, 8, 9 and 3. Highest shoot dry weight was recorded for isolate 8 (0.8 g) which is closely followed by 1, 3, 9 and 10. Lowest disease incidence was recorded in isolate 3, 8, 9 and 11 (10% seeds rotted). So isolate 3, 8, 9 and 11 can be used in further studies as they have capacity of biological control and plant growth promotion. Prabhukarthikeyan *et al.* (2018) reported that application of strain FP7 of fluorescent pseudomonas in the field increased the defense molecules, maximum plant height, maximum size of stem girth and maximum number of leaves and yield attributing characters like primary rhizomes, secondary rhizomes, fresh weight and dry weight in turmeric plants in turmeric plants thereby reducing the incidence of rhizome rot disease. Some isolates of *Pseudomonas fluorescens* was found to be potential agents for the biocontrol of different soil borne diseases like stem rot of tuber caused by *Sclerotium rolfsii* (Reddy *et al.* 2021), against *Sclerotium rolfsii* in Soybean (Priyanka and Goudar 2021), against rhizome rot of turmeric caused by Pythium aphanidermatum (Prabhukarthikeyan *et al,* 2018), This result may be due to production of a number of secondary metabolites including siderophores, hydrogen cyanide (Reddy *et al.* 2021, Prabhukarthikeyan *et al,* 2015, Priyanka *et al.* 2017). Agaras *et al.* (2015) reported that *P. fluorescens* produces a wide array of antibiotic metabolites such as pyoluteorin, pyrrolnitrin, phenazine and DAPG which suppress the fungal pathogens.

DISCUSSION

Twelve *Pseudomonas fluorescent* isolates were collected from different crop rhizosphere from different locations of northern parts of West Bengal.

All the isolates were gram negative rods with smooth and glossy colony on Kings B and Nutrient agar medium with convex elevation but variation was found in respect to pigment production. Some of the isolate produce green, some isolates produce yellowish green and rest of them produce red/pink pigments.

Biochemical test was done to identify the isolated *Pseudomonas* sp. A huge variation weas found in respect to biochemical characterization. From the result of biochemical analysis, the isolated sp. can be identified as *Pseudomonas fluorescens* or *Pseu-*

domonas putida or *Pseudomonas aeruginosa*. Before field level application, isolates should be identified by molecular technique.

In dual culture the isolates showed a huge variation in respect to pathogenicity against soil borne pathogen *Slerotium rolfsii.* Percent inhibition varied from 31 to 74 %. Isolate 3 and 8 can be efficient biocontrol agent as highest growth inhibition of the pathogen *Sclerotium rolfsii* was obtained by the isolate 8 (74.37%) followed by isolate 3 (63.15%).

In vivo trial was conducted with *Pseudomonas fluorescens* with French bean as test plant. Reduction in no of seed rotted or pre-emergence damping off was recorded along with vigor index of the seedlings and plant dry matter as growth parameter. In respect to growth parameter isolate 8, 9 and 10 were found better among the isolates under study whereas in respect to disease reduction isolate 3, 8, 9 and 11 were promising.

REFERENCES

Abdalla MA (1991) Pyoverdins and pseudobactins. Handbook of microbial iron chelates. CRC press, Boca Raton FL, pp 139-153.

Agaras BC, Scandiani M, Luque A, Fernandez L, Farina F, Carmona M, Gally M, Romero A, Wall C, Valverde C (2015) Qualification of the potential bio control and direct plant growth promotion abilities based on multiple biological traits distinguish different groups of *Pseudomonas* spp isolates. *Biological Control* 90: 173-186, https://doi.org/10.1016/j.biocontrol.2015.07.003.

Buchanan RE, Gibbson E (1974) Bergeys manual of determinative bacteriology, 8th edition.

Cook RS, Baker KF (1983) The nature and practice of biological control of plant pathogens. *American Phyto Pathological Society, St Paul, Minn* 36: .539.

Deveau A, Gross H, Palin B, Mehnaz S, Schnepf M, Leblond P, Dorrestein PC, Aigle B (2016) Role of secondary metabolites in the interaction between *Pseudomonas fluorescens* and soil microorganisms under iron-limited conditions. *FEMS Microbiology Ecology* 92 (8): 107. https://doi.org/10.1093/femsec/fiw107

Enebak SA, Carey WA (2000) Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. *Plant Dis* 84:306-308.

Gram HC (1884) Uber die isolierte Farbung der schizomyceten in schnitt-und trockenprapaten. *Fortschritte der Medizin* 2: 185-1289

Ligon JM, Hill DS, Hammer PE, Torkewitz NR, Hofmann D, Kempf HJ, van Pee KH (2000) Natural products with antifungal activity from pseudomonas biocontrol bacteria. Pest Management 56 : 688-695.

Prabhukarthikeyan SR, Karthikeyan G, Raguchander T (2015) Biochemical characterization of fluorescent Pseudomonads from turmeric rhizosphere, *Biochem Cell Arch* 15 (1): 299-303

Prabhukarthikeyan SR, Keerthana U, Raguchander T (2018) Antibiotic-producing *Pseudomonas fluorescens* mediates rhizome rot disease resistance and promotes plant growth in turmeric plants *Microbiological Research* 210: 65-73. https://doi.org/10.1016/j.micres.2018.03.009.

Prathibha KS, Siddalingeshwara KG (2013) Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescence* as Rhizobacteria on seed quality of sorghum. *Int J Curr Microbiol App Sci* 2 (3): 11-18.

Priyanka, Goudar G (2021) Screening of Fluorescent Pseudomonad Isolates against *Sclerotium rolfsii* Sacc., of Soybean (*Glycine max*). *Legume Research* 44(6): 652-660. DOI: 10.18805/LR-4145.

Priyanka, Goudar G, Nath PJN, Patil PV (2017) Isolation, Characterization and Antagonistic Activity of Fluorescent Pseu domonads. *International Journal of Current Microbiology and Applied Sciences*. 6 (12): 3883-3898

Radhajeyalakshmi R, Meena B, Thangavelu R, Deborah SD, Vidhyasekaran P, Velazhahan R (2000) A 45-kDa chitinase purified from Pearl Millet (*Pennisetum glaucum* (L.) R. Br.) shows antifungal activity. *Journal of Plant Diseases and Protection* 107 (6): 605–616. http://www.jstor.org/stable/43215366.

Ramamoorthy V, Samiyappan R (2001) Induction of defense related genes in *Pseudomonas fluorescens* treated chilli plants in response to infection by *Colletotrichum capsici*. *Journal of Mycology Plant Pathology* 31(2): 146-155.

Rangaswami G (1972) Diseases of crop plants in India. Prentice Hall of India Pvt Ltd, New Delhi, pp 520.

Reddy NHS, Sivakumar T, Balabaskar P, Kuralarasi K (2021) Bio-control efficiency of *Pseudomonas fluorescens* against stem rot of tuberose caused by *Sclerotium rolfsii*. *Plant Archives* 21: 437-443. DOI Url: https: // doi.org / 10.51470 / PLANT AR CHIVES.2021.v21.no1.061

Shende ST, Apte RG, Singh T (1977) Influence of *Azotobacter* on germination of rice and cotton seed. *Curr Science* 46(19): 675-676.

Suman B, Gopal AV, Reddy RS, Triveni S (2016) Isolation and characterization of *Pseudomonas fluorescens* in the rice rhizospheric soils of Rangareddy district in Telangana state. *International Journal of Microbiology Research and Reviews,* 5 (1): 164-169.

Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis related protein. *European Journal of Plant Patholog,* 103: 753-765.

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria, Annual Review of Phytopathology SYSTEMICISTAN 36: 453-483

Vidhyasekaran P, Muthamilan M (1995) Development of formulation of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Disease* 79(8): 782-786.

Vincent JM (1947) Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 159: 850. https://doi.org/10.1038/159850b0