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Characterization of Native Fluorescent Pseudomonads against Sclerotinia Blight of Brinjal (*Solanum melongena* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Brinjal (*Solanum melongena* L.) is one of the most common and known rich source of vitamin B1 (Thiamin), dietary fibre, manganese, niacin (vitamin B3), copper, vitamin B6 (pyridoxine), folate (vitamin B9), potassium, Vitamin K. Brinjal cultivation is affected by several pests and diseases. Sclerotinia blight is one of the most destructive diseases of brinjal caused by *Sclerotinia sclerotiorum* (Lib) de Bary. Under the present investigation two isolates of Fluorescent Pseudomonads (FLPs) *viz*., FLP-Brinjal 2020-1 and FLP-Brinjal 2020-2, were evaluated against

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Sclerotinia sclerotiorum (SCL)under *in vivo* and *in vitro* conditions using dual culture, inverted plate assay. FLP-Brinjal 2020-2 gave maximum inhibition over the control followed by FLP-Brinjal 2020-1 against the pathogen in *in vitro* condition. Experiments conducted in pluck trays indicated that FLP-Brinjal 2020-2 was superior with least sclerotinia blight incidence (20.53%).The identity and diversity among the FLP isolates were examined by sequence analysis of 16s rRNA gene. The gene was amplified by PCR using primers pair, 27 F/1492 R. Amplified DNA products were sequenced and analysed by BLAST (BLASTn) programme at NCBI database for species identification. Among the 10 FLPs isolates assessed 9 were found to have maximum similarity with *Pseudomonas aeruginosa.* FLP 2020-2 and FLP 2020-1 were found to be *Pseudomonas fluorescence* which is in confirmation with the morphological observations. Significant diversity among the isolates was observed when dendrogram of the sequence was plotted using CLUSTAL W.

Keywords: Antagonist; brinjal; biological control; Fluorescent pseudomonads; Sclerotinia sclerotiorum.

1. INTRODUCTION

The population of India is growing day by day and is expected to reach up to 9.7 billion by 2050 [1, 2]. Therefore, a significant food supply increase is required to meet the demand of the population. Brinjal is considered as the king of vegetables. It is one of the most common vegetable crops grown in tropical and subtropical type of climatic conditions. Brinjal (*S. melongena*) belongs to the family of Solanaceae. The Brinjal contributes 9% of the total vegetable production of the country [3]. Brinjal is rich in dietary fiber, Vitamin B1 and copper. Other nutrients like manganese, vitamin B6, niacin, potassium, folate and Vitamin K are also found in good quantities. It also plays an important role in combating malnutrition in under nourished regions.

According to the Food and Agriculture Organization of the United Nations (FAO, UN), a minimum raise of 50% in agricultural food production is required in a scenario of modest economic growth. So, a focus on increasing the yield and decreasing the yield losses is highly needed [4, 5, 6]. Insect pests and diseases cause severe losses to the overall agricultural production. Therefore, reducing the yield losses due to diseases is important for sustainable food production and nutritional security [7, 8]. Various chemicals are used for meeting this objective, they not only control the diseases and insect pests but also increase crop yield, quality and shelf life (Cooper and Dobson, 2007) [9]. Despite their usefulness in disease and insect pest management, continued use of chemicals are a cause of concern because of their negative impact on human health and environment including toxicity in soil and water [10, 11, 12, 13]. Brinjal is affected by several diseases and insect pests which are a major limiting factor for decreasing total brinjal production. They mainly affect the roots, leaves, stems and fruits. Sclerotinia Blight is one of the most destructive disease of brinjal. Yield losses in susceptible crops vary and can be as high as 100% [14].

Pseudomonads are soil borne, unicellular, rod shaped, obligate aerobic bacteria, with the long axis straight or curved but not helical, motile by means of one or more flagella, gram negative, do not form spore, stalks or sheaths. Fluorescent Pseudomonads (FLPs) belong to the genus Pseudomonas, family Pseudomonadaceae, order Gamma proteobacteria and phylum Proteobacteria. There are 191 species in this genus and Pseudomonads are the most diverse and ecologically significant group of bacteria which are able to survive harsh conditions, ubiquitous and form association with both plants and animals [15]. Three species of Pseudomonas are Fluorescent namely *Pseudomonas aeruginosa*, *P. fluorescence* and *P. putida* [16]. *P. aeruginosa* is the type species of genus Pseudomonas and is more uniform as it has less number of biotypes as compared to other Fluorescent Pseudomonads.

Hence, keeping in mind the exponential need to discover PGPR that are suitable for the crops and climate, following research has been taken up to identify the potential indigenous FLPs of local areas.

It aimed to compare and find potential strains that can be used in disease reduction as an alternative to chemicals with the following objectives. *In vitro* and *in vivo* evaluation of bio-control potential of Fluorescent Pseudomonads against sclerotinia blight of brinjal as well as molecular characterization of native Fluorescent Pseudomonads isolates.

2. MATERIALS AND METHODS

2.1 Antagonistic Bacteria

Ten isolates of Fluorescent Pseudomonads were isolated from the rhizosphere of different plants at the farm of Bihar Agricultural University, Sabour. In this study, the best two performing isolates among them were chosen *viz*., FLP 2020-1 and FLP 2020-2 both of which are indigenous and originated from brinjal rhizosphere.

2.2 Isolation and Identification of the Test Pathogens

Parts of brinjal infected with *Sclerotinia sclerotiorum*(SCL)were removed and surface sterilized using 70% ethanol. They were further cut into small pieces (2.0 × 2.0 × 2.0 cm) using a sterilized scalpel and washed with sterilized distilled water. Then the infected pieces were soaked into 1% sodium hypochlorite for 3 min, then rinsed three times for 1 min in several changes of distilled water and dried between two layers of sterilized filter paper to remove excess water. Finally, the surface-sterilised pieces were plated onto potato dextrose agar (PDA) media prepared by the procedure of Tuite [17] in Petri-plate (70mm) under laminar airflow cabinet and incubated at 25±2°C for 5 days.

After the complete fungal growth was confirmed by microscopic examination, the confirmation was done through Koch Postulate followed by stem cutting method [18] for inoculating plant.

2.3 Dual Culture Technique

Bioagent isolates were streaked in a single straight line 1 cm away from the edge of one side of the Petri-plate (70mm) containing potato dextrose agar (PDA) media. Fungus (4mm agar plug) were placed on opposites side of the Petri-plate, about 1cm away from the rim of the Petri-plate (70mm).A plate without any bioagent inoculation was carried out as negative control [19].

2.4 Inverted Plate Technique

A plate (70mm) containing PDA was inoculated with a 4mm agar plug of active fungal test pathogen using a cork borer and a loop full of bioagent by a streaking loop separately in an individual plate. Half plate with the fungal test pathogen was inverted over the half plate containing the bioagent and in case of negative control half plate with the fungal test pathogen was inverted over a half plate containing only PDA, without any bioagent. Seven replications were maintained for each case [20]. The plate was allowed to incubate until complete growth was reported on control plate and all the experiments were maintained at 25±2 °C.

2.5 Preparation of Talc-Based Formulation of Fluorescent Pseudomonads and Seed Treatment

The talc-based formulation of Fluorescent Pseudomonads was prepared with some modifications to the method described by Vidhyasekaran and Muthamilan [21]. A loopful of Fluorescent Pseudomonads strain was inoculated into the nutrient broth Media and incubated in an incubator cum shaker at 150 rpm for 48hours at 28±2°C. 1kg of talc powder was taken in a sterilized tray and its pH was adjusted to 7 by mixing $CaCO_3$ at the rate of 15 gm kg^{-1}. 10 gm of carboxymethyl cellulose (CMC). The mixture was autoclaved for 30 minutes and two consecutive days. 400 ml of 48hour old bacterial suspension containing 9 X 10^8cfu ml^{-1} (adjusted by Spectrophotometer) was added to the carrier-cellulose mixture under aseptic conditions. After drying (approximately to 30% moisture content) overnight, the formulation was packed in a polypropylene bag, sealed and stored at room temperature (25±2 °C). This *Pseudomonas* formulation powder @ 10g/kg of seeds was mixed in 1 lit of water and the brinjal seeds were soaked in this mixture for 30 minutes before sowing.

2.6 Artificial Inoculation of Test Pathogens

For inoculation of *Sclerotinia sclerotiorum* the main stems of 5-week-old brinjal plants (fifth to sixth trifoliolate leaf fully expanded) were horizontally severed with a sterile razor blade at 0.5 cm above either the fourth or fifth node. A single mycelial plug (3 mm^2) was placed mycelial-side down on the cut stem [18]. Observations on no. of diseased plants and healthy plants were recorded.

For this experiment, atrial (with SCL) was conducted in a completely randomized design (CRD) with four replicates (four random blocks) with variety *Muktakeshi* of brinjal. The studies are on six treatments per block as described below (Table 1). All statistical analyses were performed with OPSTAT software.

Table 1. Experimental design

T1	: Only seed
T2	: Bioagent 1 (FLP 2020-1)
T3	: Bioagent 2 (FLP 2020-2)
T4	: Bioagent 1 (FLP 2020-1) + Pathogen
T5	: Bioagent 2 (FLP 2020-2) + Pathogen
T6	: Pathogen

2.7 DNA Extraction and PCR Amplification of Specific Gene of Fluorescent Pseudomonads Strains

Extraction of genomic DNA from all the selected bioagent strains was performed using a DNA isolation kit (Nucleo-pore gDNA Fungal Bacterial Mini Kit (50). After extraction, the quantity, integrity, and quality of the DNA obtained was checked by agarose gel electrophoresis, followed by staining in ethidium bromide, and visualization under UV light. To amplify the 16S rRNA gene, polymerase chain reaction (PCR – Eppendorf Master cycler) was performed from the genomic DNA of strains using a 16S rRNA gene specific primers - 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) as forward and reverse primer respectively, which was set at the amplification conditions (Initial denaturation at 94 °C for 3 minutes, denaturation at 94 °C for 30 seconds, annealing at 50 °C for 45 seconds, extension at 72 °C for 1:50 minutes and final extension at 72 °C for 10 minutes). PCR Products were checked by agarose gel electrophoresis, followed by staining in ethidium bromide, and visualization under UV light, following this, a ladder will be used as size markers. Then sequencing was done to check the diversity [22].

2.8 Identification of Fluorescent Pseudomonads and Sequence Analysis

The amplified 16s rRNA gene region, Pseudomonas specific gene region of the Fluorescent Pseudomonads isolates were amplified with same primer pair in same procedure as above mentioned and sequenced directly by primer walking technique. Then the sequences were compared with those extracted from GenBank using BLASTN programme in NCBI (National Centre for Biotechnology Information) website and aligned using CLUSTAL W programme using MEGA 11 software.

3. RESULTS

3.1 Isolation of Test Pathogen

The infected brinjal plant samples of fusarium wilt, sclerotinia blight and bacterial wilt were collected in polythene bags from vegetable farm of Bihar Agricultural University. The collected samples were processed at PG Lab-3, Department of Plant Pathology. The samples with a fluffy or cottony, white moldy growth (mycelium) around of lower stems were used for *Sclerotinia sclerotiorum* isolation. The surface sterilisation of diseased samples was done with 1% sodium hypochlorite. Standard isolation method was followed for isolation of fungal pathogens. The pure culture of each pathogen was observed and identified based on their colony characteristics and spore morphology.

3.2 *Sclerotinia sclerotiorum* (SCL)

The colony of fungi on petri dish was white and the layer of mycelia was of irregular pattern, sclerodes were also seen which was rounded and black. The width of hyphae was 2.5-9 µm, microconidia were of 2-3 µm in size, width of 2-6.5 µm and length of 2.5-14µm for sclerotia, width of 4-6 µm and length of 6-12 µm for ascospore which was hyaline.

3.3 *In vitro* evaluation of FLPs against *Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum showed the full growth within 5 days of inoculation into the petri-dish. The recorded data indicates that two FLPs isolates showed their potentiality to control the growth of *Sclerotinia sclerotiorum* in *in vitro.* The maximum radial growth of fungus was observed in FLP 2020-1 (1.71 mm) and minimum radial growth of fungus was reported in FLP 2020-2 (0.96 mm) in case of dual culture method. Similarly, maximum mycelium inhibition compare to negative control of the fungus was reported under FLP 2020-2 (98.59%) which is at per with FLP 2020-1 (97.56%). In case of inverted plate

technique the maximum radial growth of fungus was observed in FLP 2020-1 (3.07 mm) and minimum radial growth of fungus was reported in FLP 2020-2 (1.36 mm). Similarly, maximum mycelium inhibition compare to negative control of the fungus was reported under FLP 2020-2 (98.06%) followed by FLP 2020-1 (95.61%). (Table 2, Figs. 1 and 2).

3.4 *In vivo* evaluation of FLPs against *Sclerotinia sclerotiorum* (SCL)

Brinjal seeds (Var. *Muktakeshi*) were bacterized with FLPs isolates by talc-based formulation containing $1x10^9$ CFU/gm in case of treatments with FLPs isolates (FLP 2020-1, FLP 2020-2, FLP 2020-1+SCL, FLP 2020-2+SCL). The seedlings of FLPs treated seeds were challenged with SCL through stem cutting method. One negative control without FLPs treatment was also carried out by same procedure. Disease Incidence (%), biocontrol efficiency and no. of diseased and healthy plants were recorded under these various treatments. No disease incidence was observed under the treatments without any pathogen inoculation. Least disease incidence was reported under FLP 2020-2 (20.53%) treatment (FLP 2020-2+SCL) followed by FLP 2020-1 (23.47%) treatment (FLP 2020-1+SCL) compare to negative control (90.60%) treatment (Treatment with only pathogen). Maximum disease control was reported by FLP 2020-2 (77.33%) followed by FLP 2020-1 (74.09%) compare to negative control treatment (0%). So, FLP 2020-2 has more biocontrol efficiency than FLP 2020-1 against *Sclerotinia sclerotiorum*. No diseased plant was observed under the treatments which are without pathogen inoculation. Maximum healthy plant was observed with FLP 2020-2 treatment (around 23 plants), which was at per with FLP 2020-1 treatment (22 plants) compare to negative control (around 3 plants). Least no. of diseased plant was reported with FLP 2020-2 (6 plants) followed by FLP 2020-1 (around 7 plants) compare to negative control (around 27 plants). (Table 3; Fig 3)

3.5 Molecular Characterization of FLP Strains

Bacterial genomic DNA was isolated from fifteen different FLP strains and isolated genomic DNA was analysed by electrophoresis on 1 % agarose gel. PCR was carried out for the amplification of 16S rDNA region by using 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) as forward and reverse primers respectively. Amplified PCR products were also analysed by electrophoresis on 1 % agarose gel. The DNA bands were visualized under UV trans-illuminator. Using this pair of primers, 1500 bp amplified band was obtained which was specific for 16S rDNA gene in *Pseudomonas spp*. isolates. Further PCR products were sequenced and diversity among strains was reported.

3.6 Construction and Analysis of Phylogenetic Structure Deduced from Nucleotide Sequences of the 16s rRNA

The nucleotide sequences of the 16S rRNA, Pseudomonas specific genes from ten Fluorescent Pseudomonads isolates were determined and phylogenetic tree based on this data showing evolutionary relatedness of the isolates with known *Pseudomonas* spp., which were obtained by BLAST of the nucleotide sequences of our investigating isolates in NCBI website, was constructed by the Neighbour-Joining (NJ) method with Bootstrap value 1000.

Table 2. *In vitro* evaluation of Fluorescent Pseudomonads isolates against *Sclerotinia sclerotiorum* of brinjal

	Sclerotinia sclerotiorum			
	Dual culture method		**Inverted plate technique**	
Treatments	**Radial growth (mm)**	**Inhibition over control (%)**	**Radial growth (mm)**	**Inhibition over control (%)**
FLP 2020-1	1.71[b]	97.56[a]	3.07[b]	95.61[b]
FLP 2020-2	0.96[a]	98.59[a]	1.36[a]	98.06[a]
Control	70[c]	0[b]	70[c]	0[c]
SeM (±)	0.24	0.35	0.28	0.39
CD (0.01)	0.71	1.04	0.82	1.17
CV (%)	2.61	1.42	2.94	1.61

Table 3. *In vivo* evaluation of Fluorescent Pseudomonads isolates against *Sclerotinia sclerotiorum* of brinjal

Treatments	*Sclerotinia sclerotiorum*			
	Disease incidence (%)	Disease control (%)	No. of healthy plant	No. of diseased plant
Control	0[a]	-	29[a]	0[a]
FLP 2020-1	0[a]	100[a]	29[a]	0[a]
FLP 2020-2	0[a]	100[a]	29[a]	0[a]
FLP 2020-1+SCL	23.47[c]	74.09[c]	22[b]	7[c]
FLP 2020-2+SCL	20.53[b]	77.33[b]	23[b]	6[b]
SCL	90.60[d]	0[d]	3[c]	27[d]
SeM (±)	0.75	0.32	0.44	0.28
CD (0.01)	2.23	0.96	1.32	0.84
CV (%)	6.69	0.86	29.25	8.64

Fig 1: *In vitro* antagonistic potential of FluorescentPseudomonads isolates against *Sclerotinia sclerotiorum* of brinjal under dual culture technique

Fig 2: *In vitro* antagonistic potential of Fluorescent Pseudomonads isolates against *Sclerotinia sclerotiorum* of brinjal under Inverted plate technique

Table 4. Fluorescent Pseudomonads isolates, used in phylogenetic analysis

Sl. No.	Fluorescent Pseudomonads isolates	Origin	Code	Similarity	Sequenced product length
1.	*Pseudomonas spp.*	Brinjal Rhizosphere	PF	*Pseudomonas aeruginosa* (90-95%)	1215 bp
2.	*Pseudomonas spp.*	Brinjal Rhizosphere	Br2 (FLP 2020-1)	*Pseudomonas fluorescence* (98.5%)	1239 bp
3.	*Pseudomonas spp.*	Brinjal Rhizosphere	Br4 (FLP 2020-2)	*Pseudomonas aeruginosa* (98.6%)	946 bp
4.	*Pseudomonas spp.*	Brinjal Rhizosphere	Pt	*Pseudomonas aeruginosa* (99%)	1274 bp
5.	*Pseudomonas spp.*	Rice Rhizosphere	Ri	*Pseudomonas aeruginosa* (97.5%)	1101 bp
6.	*Pseudomonas spp.*	Wheat Rhizosphere	Wh	*Pseudomonas aeruginosa* (98.6%)	1224 bp
7.	*Pseudomonas spp.*	Barley Rhizosphere	B	*Pseudomonas aeruginosa* (98.3%)	1276 bp
8.	*Pseudomonas spp.*	Mustard Rhizosphere	Mu2	*Pseudomonas aeruginosa* (98%)	1285 bp
9.	*Pseudomonas spp.*	Mango Rhizosphere	Ma	*Pseudomonas aeruginosa* (98.7%)	1154 bp
10.	*Pseudomonas spp.*	Turmeric Rhizosphere	Tu	*Pseudomonas aeruginosa* (98.6%)	1239 bp

Fig 3: Healthy and SCL infected plant under challenge inoculation condiitons

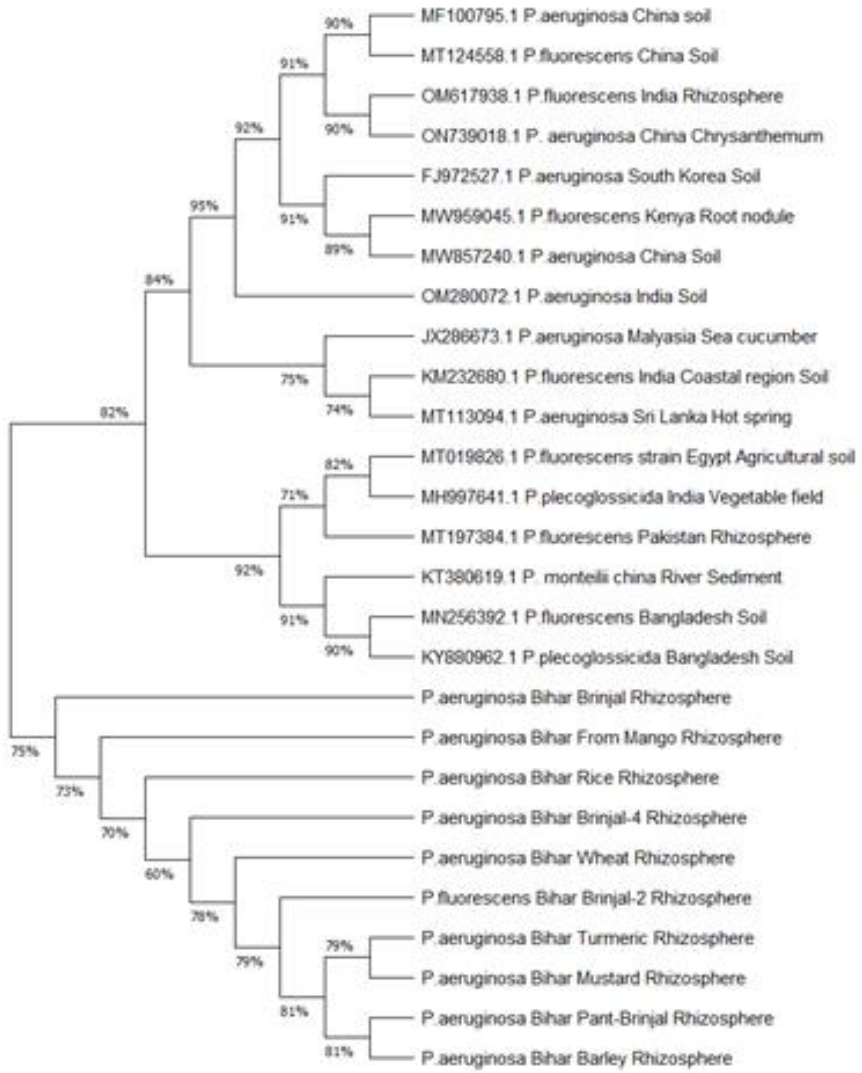

Fig. 4. Phylogenetic tree based on 16S rRNA gene sequences

In the constructed phylogenetic tree (Fig 4), our ten investigating Fluorescent Pseudomonad isolates grouped into a single cluster and known *Pseudomonas* spp.(Fig 4) from NCBI (National Centre for Biotechnology Information) website into another cluster. These two clusters showed 82% identity with each other. Among the 10 FLP isolates assessed 9 were found to have maximum similarity with *Pseudomonas aeruginosa* including FLP 2020-2 and FLP 2020-1 was found to be *Pseudomonas fluorescence* which is in confirmation with the morphological observations (Table 4).

4. DISCUSSION

4.1 *In vitro* Evaluation of Fluorescent Pseudomonads Isolates against *Sclerotinia sclerotiorum*

In our investigation *Sclerotinia sclerotiorum* showed complete growth within 5 days of inoculation into the Petri-plate. Growth inhibition of the fungus was reported by all the isolates - FLP 2020-1 and FLP 2020-2 in dual culture method as well as inverted plate technique in *in vitro* condition.

The radial growth of the pathogen varies from 1 to 2 mm and 1 to 3 mm respectively in case of dual culture method and inverted plate technique against the bio-agents. The strains of FLPs shows inhibition against the pathogen at the range of 98% to 99% and 96% to 98% respectively in case of dual culture method and inverted plate technique. The maximum radial growth of fungus was observed in FLP 2020-1 (1.71 mm) and minimum radial growth of fungus was reported in FLP 2020-2 (0.96 mm) in case of dual culture method. Similarly, maximum mycelium inhibition compare to negative control of the fungus was reported under FLP 2020-2 (98.59%) which is at per with FLP 2020-1 (97.56%) in case of dual culture method. The maximum radial growth of fungus was observed in FLP 2020-1 (3.07 mm) and minimum radial growth of fungus was reported in FLP 2020-2 (1.36 mm) in case of inverted plate technique. Similarly, maximum mycelium inhibition compare to negative control of the fungus was reported under FLP 2020-2 (98.06%) followed by FLP 2020-1 (95.61%) in case of inverted plate technique.

Three Pseudomonads strains was examined by Mokrani et al., [23]. These Pseudomonads strains showed antagonistic effect against *Fusarium oxysporum* f.sp *phaseoli* and *Sclerotinia sclerotiorum* in dual culture. They showed inhibition ranging from 47.78 % to 100 per cent. The possible reasons for mycelial inhibition in dual culture method are the antimicrobial substances like - Phenazine-1-carboxylic acid, acetamido anthranilic phenol, HCN etc. and in inverted plate technique are the volatile compounds like – dimethyl disulphide, nepthalene, 1-methyl, benzaldehyde etc. which are produced by *fluorescent pseudomonads.* So, from our present study we can confirm that FLP 2020-2 produces more antimicrobial substances and volatile compounds than FLP 2020-1 [24-27].

4.2 *In vivo* Evaluation of Fluorescent Pseudomonads Isolates against *Sclerotinia sclerotiorum*

Seed bacterization with Fluorescent Pseudomonads enhances the plant growth as well as resistance against plant pathogenic fungi under controlled and field conditions [28]. Bacterization of peanut seeds with GRC1 strain of *Pseudomonas spp.* resulted in reduced stem-rot of peanut in *Sclerotium sclerotiorum* infested soil by 87%.

Fernando et al., [29] worked on biological control of *Sclerotinia sclerotiorum* by *Pseudomonas* and *Bacillus spp*. on canola petals and reported disease incidence of 18.8 to 27.9% in *Pseudomonas spp.* treated plot. Singh et al., [30] observed *in vivo* efficacy of *Pseudomonas* against *Sclerotinia sclerotiorum* of rajmash by pot experiment and they found 36% disease intensity in *Pseudomonas* treated pot which is less than untreated pot. Biocontrol of *Sclerotinia sclerotiorum* in oilseeds were also reported by Kamal et al., [31].

In present study brinjal seeds (Var. *Muktakeshi*) were bacterized with FLPs isolates by FLPs formulation powder in case of treatments with FLPs isolates (FLP 2020-1, FLP 2020-2, FLP 2020-1+SCL, FLP 2020-2+SCL) and stem cutting method was carried out to inoculate the pathogen in the respective treatments with the test pathogen (Pathogen, FLP 2020-1+SCL, FLP 2020-2+SCL). No disease incidence was observed under the treatments without any pathogen inoculation.

The strains of FLPs shows disease incidence at the range of 20% to 24% and disease control percentage from 74% to 78%. Least disease

incidence was reported under FLP 2020-2 (20.53%) treatment (FLP 2020-2+SCL) followed by FLP 2020-1 (23.47%) treatment (FLP 2020-1+SCL) compare to negative control (90.60%) treatment (Treatment with only pathogen). Maximum disease control was reported by FLP 2020-2 (77.33%) followed by FLP 2020-1 (74.09%) compare to negative control treatment (0%). So, FLP 2020-2 has more biocontrol efficiency than FLP 2020-1 against *Sclerotinia sclerotiorum*.No diseased plant was observed under the treatments which are without pathogen inoculation. Maximum healthy plant was observed with FLP 2020-2 treatment (around 23 plants), which was at per with FLP 2020-1 treatment (22 plants) compare to negative control (around 3 plants). Least no. of diseased plant was reported with FLP 2020-2 (6 plants) followed by FLP 2020-1 (around 7 plants) compare to negative control (around 27 plants).

Fluorescent Pseudomonads produce antimicrobial substances (Phenazine-1-carboxylic acid, acetamido anthranilic phenol etc.) which can inhibit the growth of mycelium and germination of ascospores of the pathogen [29]. Bacterial antagonists induce resistance by enhancement of lignification and stimulation of host defence enzymes and synthesis of pathogenesis-related (PR) proteins. The PR proteins like- chitinase and β-1,3-glucanase inhibits fungal pathogens. The enhanced accumulation of PR proteins and oxidative enzymes including chitinase and b-1,3-glucanase may also be responsible for the reduction of Sclerotinia infection. As, fungi have chitin and glucan as cell wall components, increased activity of chitinase and β-1,3- glucanase may prevent establishment of pathogen. These activities of the bio-agent can be the reason behind the least disease incidence percentage and more disease control percentage in the bio-agent treated plot [32].

4.3 Molecular Characterization of Fluorescent Pseudomonads Isolates

In the present study the nucleotide sequences of the 16s rRNA, Pseudomonas specific genes from ten Fluorescent Pseudomonads isolates were determined and a phylogenetic tree based on this data showing evolutionary relatedness of the isolates with known *Pseudomonas* spp., which were obtained from NCBI database, were constructed by the Neighbour-Joining (NJ) method with Bootstrap value 1000. The Phylogenetic analysis of the Fluorescent Pseudomonad isolates grouped into a single cluster and known *Pseudomonas* spp. (Fig 4) from NCBI (National Centre for Biotechnology Information) website were into another cluster. These two clusters showed 82% identity with each other. Among the 10 FLP isolates assessed, 9 were found to have maximum similarity with *Pseudomonas aeruginosa* including FLP 2020-2 and FLP 2020-1 was found to be *Pseudomonas fluorescence*. So, we can say that our investigating isolates are related to *Pseudomonas aeruginosa* strain and *Pseudomonas* spp. strain. All investigating isolates formed a cluster which may be due to geographical characteristics. Similar observation was recorded by Krishnamoorthi et al., [33] working on molecular characterization of *Pseudomonas* spp. by 16S rRNA primer pair 27F& 1492Rand observed 1500 bp amplified band after PCR and they identified *Pseudomonas aeruginosa* after the analysis of sequence of the strains [34].

5. CONCLUSION

The present experiment was carried out to characterize the two best performing native Fluorescent Pseudomonads isolated and collected from the rhizosphere of Brinjal from agroclimatic zone of Bihar. The isolates were examined against *Sclerotium sclerotiorum* of brinjal under *in vivo* and *in vitro* conditions by dual culture method and inverted plate technique. In both conditions, FLP 2020-2 gave the better result by inhibiting the fungal pathogen. From our study we can suggest the use of the respective strain of Fluorescent Pseudomonads as not only a bio-control measure of diseases but also as plant growth promoting rhizobacteria for achieving sustainable agriculture and our goal should be to make them into reliable, assessable product for farmers at commercial level.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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