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Effectiveness of indigenous fluorescent pseudomonads in suppressing *Rhizoctonia solani* root rot disease and promoting plant growth in chilli seedlings

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ABSTRACT

Aims: This study aimed to isolate and evaluate the indigenous fluorescent *Pseudomonas* spp. with bio-control potential against *Rhizoctonia solani* and promoting growth in chilli seedlings.
Methodology: A total of 120 fluorescent bacterial were isolated from the healthy chilli rhizosphere soil from the seven major chilli cultivation localities in Terengganu, Malaysia. Only 115 Gram negative fluorescent isolates were further *in-vitro* screened for antagonistic activities against *R. solani* and plant growth-promoting properties. The 50 most effective fluorescent *Pseudomonads* antagonist against *R. solani* with minimum percentage inhibition of radial growth (PIRG) of 65% were selected. Hierarchical cluster analysis was further conducted with two dendrograms derived from SPSS Statistic 20 to facilitate the comparison between these 50 isolates for antagonistic and growth-promoting properties. A total of 40 fluorescent isolates within the most potential cluster were further selected and identified using 16S rRNA sequencing. Thirty four fluorescent isolates were identified as *Pseudomonas* spp. and six isolates as *Burkholderia* spp. The top 13 ranked fluorescent *Pseudomonas* spp. from the scoring index were evaluated for seed germination and vigor index in chilli seedlings. There was no significant difference in germination rate between fluorescent *Pseudomonas* inoculated with control. However, vigor index of chilli seeds pre-inoculated with fluorescent *P. putida* (B5C1), *P. aeruginosa* (B3C56) and *P. putida* (B5C7) were significantly increased with 4684.9, 4657.3 and 4401.0 over control ($P \leq 0.05$).
Conclusion, significance and impact of study: These selected fluorescent isolates: *P. putida* (B5C1), *P. aeruginosa* (B3C56) and *P. putida* (B5C7) have the potential to be developed as biofungicide against *R. solani* and as growth-promoter in chilli production system.

Keywords: Biocontrol, fluorescent pseudomonads, *Rhizoctonia solani*, *Rhizoctonia* root rot, chilli

INTRODUCTION

Chilli (*Capsicum* spp.) is one of the most important vegetable crops. The annual chilli production in Malaysia (14.18 tons/ha) was reported below the production potential of 15 tons/ha (Department of Agriculture, 2007) due to the pest and diseases problems (Awang *et al*., 2013). Rhizoctonia root rot disease caused by *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (A. B. Frank Donk) was reported as a major pathogen in chilli especially at the early growth stage (Rini and Sulochana, 2006).

Rhizoctonia solani is a widespread and an ecologically diverse soil-borne plant pathogenic fungus in various crops (Guleria *et al.*, 2007). Chemical control was reported as the most common practice in controlling of *Rhizoctonia* root rot disease. However, chemical control of disease in vegetable crops is not advisable in many countries due to residue problems (Rini and Sulochana, 2006) and excessive application of fungicides lead to environmental and food hazardous. Therefore, the application of biological control by using plant growth-promoting rhizobacteria (PGPR) and biological extract (Wong *et al*., 2020) are gaining huge attention as an alternative in plant disease management (Bhattacharyya and Jha, 2012).

Fluorescent *Pseudomonads* are ubiquitous as PGPR that colonize roots in the rhizosphere (Maroniche *et al*.,

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2018). This group of bacteria are well known to suppress fungal root diseases of agronomic crops through the production of antifungal metabolites such as hydrogen cyanide, phloroglucinol, pyoluteorin and pyrrolnitrin (Ahmadzadeh and Tehrani, 2009). Few species of *Pseudomonas* were reported as plant growth promoter such as: *P. fluorescens*, *P. putida*, *P. chlororaphis/aureofaciens*, *P. aeruginosa* and *P. syringae* (Janisiewicz and Marchi, 1992; Anjaiah *et al*., 1998; Audenaert *et al*., 2002). The *Pseudomonas* spp. with the plant growth-promoting traits could be useful in formulation of new fungicides, to improve the cropping systems for more profitably applied (Yadav *et al*., 2014).

Until date, the exploration on the phytobeneficial traits of fluorescent *Pseudomonads* in controlling of *Rhizoctonia* root rot disease in chilli is still insufficient. Hence, this study aimed to isolate and evaluate the fluorescent *Pseudomonas* spp. isolated from the healthy chilli rhizosphere soil with plant growth-promoting and antagonistic effects against *R. solani* in chilli. The application of the selected fluorescent *Pseudomonas* is a potential alternative approach in *Rhizoctonia* root rot disease management toward better chilli productivity and food safety.

MATERIALS AND METHODS

Planting materials and pathogen preparation

Chilli seeds (Kulai variety) were obtained from the Commodity Development Station, Horticulture Division, Department of Agriculture, Terengganu, Malaysia. The chilli seeds were surface sterilized in 70% ethanol, followed by 5% sodium hypochlorite before rinsed with sterilise water. The surface sterilized seeds were air-dried before use.

The pure culture of *Rhizoctonia solani* was obtained from the Culture Collection Unit of Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. The fungus was then maintained on the potato dextrose agar (PDA) and preserved in potato dextrose broth (PDB) containing 40% glycerol at –40 °C.

Isolation and Gram staining of fluorescent bacteria

The fluorescent bacteria were isolated from disease-free chilli farms from seven localities at Terengganu, Malaysia. Ten plants were randomly sampled from each field, the soil adhering to the root zone were collected and defined as rhizospheric soil. Fluorescent bacterial were isolated following methods by Zhou *et al.* (2012). Five grams of soil samples was added into a conical flask containing 100 mL sterilized distilled water and shaked using an orbital shaker for 20 min. Serial dilution of the soil suspension was then filled up to 10^3 and 0.1 mL of the aliquot was spread onto King's B agar. After 48 h of incubation at 28 ± 2 °C, the colonies grown on the agar were observed under Fluorescence Analysis Cabinet (model CM-10A, Spectronics, USA) at 365 nm wavelength for the fluorescent characteristic. All fluorescent colonies were selected, purified and maintained onto King's B agar.

All the obtained fluorescent bacterial isolates were tested for Gram-positive (GP) or Gram-negative (GN) reaction using 3% potassium hydroxide (KOH) (Trigiano *et al*., 2004). In this test, the bacterium that produced sticky structures when in contact with 3% of the KOH were considered as GN.

In vitro testing for antagonistic activities against *R. solani*

The dual culture (DC) assay was conducted as described by Dennis and Webster (1971). A 5 mm of *R. solani* plug was placed on the one side of potato dextrose agar (PDA). On the same PDA plate, fluorescent bacteria was streaked in a straight line at a distance of 1.5 cm from the *R. solani* plug. Plate without the fluorescent bacteria (*R. solani* alone) was served as a control. After incubated at 28 ± 2 °C for 3 days, the inhibition activity of fluorescent bacteria against *R. solani* on the plate was recorded using the following PIRG formula (1) (Vincent, 1927; Maurya *et al*., 2014).

$$\text{PIRG (\%)} = \frac{\text{C-T}}{\text{C}} \text{ X } 100 \qquad (1)$$

PIRG = Percentage inhibition radial growth
C = Mycelial radius of *Rizoctonia solani* in control plate
T = Mycelial radius of *Rizoctonia solani* in dual culture plate

This experiment was repeated twice. The scoring index for dual culture assay based on PIRG was denoted by figure 0-4, where 0 = no antagonistic effect detected, 1 = 65-84.99%, 2 = 75–84.99%, 3 = 85-94.99% and 4 = ≥ 95%.

The effect of volatile compounds (VOC) produced by the fluorescent bacteria towards the growth of *R. solani* was measured using paired Petri dish technique as described by Gagne *et al.* (1991). The nutrient agar (NA) plate containing fluorescent bacteria and the PDA plate with *R. solani* were paired together without lids and sealed tightly with parafilm. NA plate without fluorescent bacteria was prepared and served as control. Three days after incubation at 28 ± 2 °C, the inhibition activity was recorded using the PIRG formula (1). The scoring index for volatile compounds production based on PIRG was denoted by figure 0-4, where 0 = no antagonistic effect detected, 1 = 1- 4.99%, 2 = 5 – 9.99%, 3 = 10-14.99% and 4 = ≥ 15%.

The efficiency of fluorescent bacteria in hydrogen cyanide (HCN) production was conducted by spreading the respective fluorescent bacteria on tryptic soy agar (TSA) supplemented with 4.4 g/L glycine as described by Donate-Correa *et al*. (2004). Filter paper disc, *Whatman* no. 93 was then soaked in picric acid solution (0.5% picric acid and 2% Na_2CO_3 in 100 mL of distilled water) and

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placed in the lid of the TSA plate that containing the fluorescent bacteria. The plate was then sealed tightly and incubated at 28 ± 2 °C for three days. The plate without the fluorescent bacteria was served as a control. The colour changes on the filter paper disc was observed and recorded based on the scale developed by Donate-Correa *et al*. (2004). The colour density of HCN produced was then read at 625 nm wavelength using UV Spectrophotometer (model UV-1800, Shimadzu, Malaysia). For scoring index assessment, the density of colour was denoted to figure 0-3. Where, 0 = no HCN detected, 1 = 0.001-0.005, 2 = 0.006–0.010 and 3 = ≥ 0.010.

In vitro testing for plant growth-promoting property

All fluorescent bacterial isolates were evaluated for phosphate solubilizing ability as described by Pikovskaya (1948). The single colony of the respective fluorescent bacterial was spot-inoculated on Pikovskaya's agar (PVK) and incubated at 28 ± 2 °C for 3 days. The formation of halo zone around the fluorescent bacterial colony were indicated positive in phosphate solubilisation. The diameter of the halo zone formed around the fluorescent bacterial colony was measured and transformed to phosphate solubilization index as followed (2) (Mujahid *et al*., 2014):

$$\text{Phosphate solubilization index} = \frac{\text{Total diameter of clear zone (with bacterial colony)}}{\text{Diameter of bacteria colony}} \text{x } 100 \qquad (2)$$

Phosphate solubilisation index obtained was further categorised into four groups denoted by figure 0-3, where 0 = no phosphate solubilizing activity, 1= 105-109.99%, 2 = 110-114.99% and 3 = ≥150%.

The fluorescent bacteria was evaluated for indole acetic acid (IAA) production by growing in nutrient broth (NB) containing 0.2% L-tryptophan and incubated at 28 ± 2 °C for 48 h. The liquid culture of the fluorescent bacteria was then centrifuged at 5,000 rpm for 20 min. Two mL of the supernatant were mixed with 80 µL of orthophosphoric acid and 4 mL of Salkowski's reagent (50 mL of 35% perchloric acid + 1 mL of 0.5 M $FeCl_3$). The tubes containing the reagent were kept in the dark at 28 ± 2 °C for 20 min to allow the reaction to occur. The presence of pink/red colour in the test tubes after 20 min of incubation indicated that the production of IAA. A colorimetric technique (Ehmann, 1977) was performed to quantify (µg/mL) the produced IAA. The colour density was read at 530 nm using UV Spectrophotometer (Model UV-1800, Shimadzu, Malaysia) and denoted with the Figures 1-4, where <4.99 µg/mL as 1, 5-9.99 µg/mL as 2, 10-14.99 µg/mL as 3 and ≥ 15 µg/mL as 4.

Seed germination and seedling vigor index

The inoculum of fluorescent bacterial for seed germination test was prepared by serial dilution technique and was adjusted to a final concentration of 10^9 CFU/mL. Chilli seeds were sterilised using 1% sodium hypochlorite ($NaClO_3$) for 1 min and rinsed thoroughly in sterilized distilled water before inoculated with fluorescent bacterial inoculum (10^9 CFU/mL) for 24 h at 28 ± 2 °C. Sterile distilled water was used to replace the bacterial suspension for the control. After inoculation, the chilli seeds were placed in Petri dish containing moisten filter papers and incubated in growth chamber for 10 days with the photoperiod of 12/12-h light (32 °C) /dark (24 °C). Each plate contained 20 seeds with 3 replications of each treatment and arranged in Completely Randomized Design (CRD). The number of germinated seeds were determined based on formula (3). Plumule and radical lengths of each germinated seed were measured in order to determine the vigor index (Abdul-Baki and Anderson, 1973) using the following formula (4):

$$\text{Seed gemination rate} = \frac{(\text{number of total seed tested} - \text{number of germinated seed})}{\text{number of total seed tested}} \text{x } 100\% \qquad (3)$$

$$\text{Vigor index} = (\text{mean of root length} + \text{mean of shoot length}) \text{ x seed germination rate } (\%) \qquad (4)$$

Identification of fluorescent Pseudomonads using 16S ribosomal RNA sequencing

One millimetre of 24 h fluorescent bacterial culture was pipetted into 1.5 mL micro centrifuge tube and centrifuged at 14,000 rpm for 2 min to collect the pellet. The DNA from the pellet was extracted using Wizard® Genomic DNA Purification Kit, PROMEGA by following the manufacturer's protocol. Bacterial 16S rRNA gene sequences were then amplified by PCR technique using the universal primer pair 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') (Lane, 1991).

The PCR reaction mixture (25 µL) were then amplified using MJ Mini thermal cycler, model PTC-1148, BIO RAD, Singapore by following cycling conditions: 1 cycle of initial denaturation at 95 °C for 2 min, followed by 33 cycles of denaturation at 95 °C for 30 sec, annealing at 52.6 °C for 50 sec and extension at 72 °C for 1 min and the final extension at 72 °C for 5 min. DNA of the amplified PCR products were separated by gel electrophoresis on 2% agarose gel at 80 V for 60 min in 1 × TAE buffer. The gel was then stained using Diamond™ Nucleic Acid Dye, PROMEGA. The DNA fragment was observed using E-Gel Imager, Life Technologies, Fisher Scientific, USA.

Finally, the PCR product was purified using Wizard® SV Gel and PCR Clean-Up System, PROMEGA. The purified PCR products were then sent to the First BASE Laboratories for gene sequencing. The sequences obtained were aligned using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 and blasted in NCBI (National Centre for Biotechnology Information) database website using the nucleotide basic local alignment for identification.

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Data analysis

All data obtained for seed germination were evaluated for significance by an analysis of variance (ANOVA) followed by Duncan test at $p < 0.05$ level. Hierarchical clusters analysis were used to group the fluorescent bacterial into the same cluster with the *in vitro* antagonistic and plant growth-promoting properties. All statistical analyses were conducted using IBM SPSS Statistic 20.

RESULTS

In vitro* antagonistic activity of fluorescent *Pseudomonads* against *R. solani

A total of 120 fluorescent isolates were successfully isolated from the seven sampling localities. Gram stain of all the 120 fluorescent isolates was conducted and only 115 isolates were classified as Gram negative or potential fluorescent *Pseudomonads* isolates. Based on the dual culture testing, 50 (43.5%) out of 115 fluorescent isolates were successfully inhibited *R. solani* with minimum PIRG of 65%. Detail results of dual culture (DC), volatile compounds (VOC) and hydrogen cyanide (HCN) production of the 50 isolates were tabulated in Table 1. Based on DC testing, B3C44 was exhibited as the strongest antagonist against *R. solani* with 96.86% of PIRG (Table 1 and Figure 1). Whereas, isolate B1C3 was recorded with the highest PIRG (19.51%) in VOC testing (Table 1 and Figure 2). Besides, only 10 out of the 50 fluorescent isolates were positive in hydrogen cyanide production with the highest optical density value of 0.028 nm recorded by isolate B4C4.

Plant growth-promoting property of the fluorescent isolates

The phosphate solubilization assay exhibited that the five fluorescent bacterial isolates (B2C3, B6C4, B6C7, B6C9, B6C10) were capable to solubilize phosphate in PVK agar with the formation of halo zone around the colony. For IAA production, 17 fluorescent bacterial isolates were successfully produced IAA. Three fluorescent bacterial isolates (B4C1, B4C5 and B5C7) were exhibited the highest concentration of IAA (+++).

Hierarchical cluster analysis

The efficiency of the 50 fluorescent bacterial to promote plant growth and antagonist against *R. solani* were analysed to produce two dendrograms derived from SPSS Statistic 20 to facilitate their comparison. The clusters were designated as a dendrogram A for antagonistic activity against *R. solani* (Figure 3) and dendrogram B for plant growth-promoting activity (Figure 4). Dendrograms with three clusters were produced for antagonistic activity against *R. solani* (dendrogram A) and plant growth-promoting activity (dendragram B), respectively.

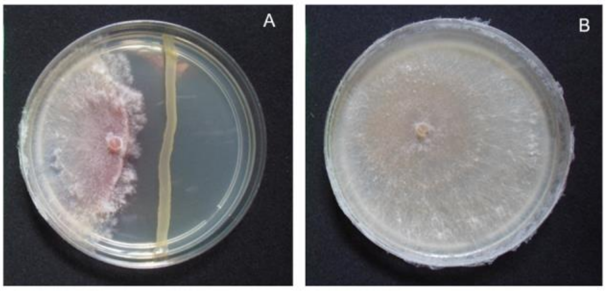

Figure 1: The inhibition of *Rhizoctonia solani* by fluorescent *Pseudomonas* isolate B3C44 (A) and control (*R. solani* alone) (B), in dual culture (DC) assay on PDA after 3 days of incubation at 28 ± 2 °C.

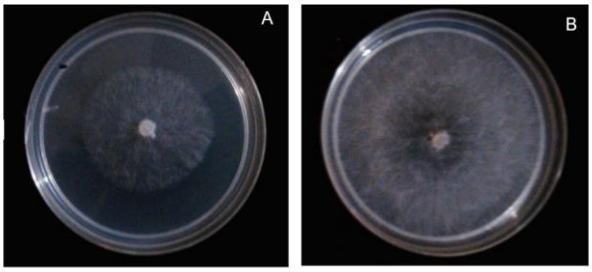

Figure 2: The inhibition of *Rhizoctonia solani* by fluorescent *Pseudomonas* isolate B1C3 (A) and control (B), in volatile organic compounds production (VOC) assay on PDA after 3 days of incubation at 28 ± 2 °C.

A total of 34 isolates in clusters 1 and 2 were found to have high degree of antagonistic effects as compared to cluster 3. All the 34 isolates were selected for molecular identification. In addition, those isolates with high degree of IAA concentration and phosphate solubilization index grouped in cluster 1 and 2 in dendrogram B were also selected for identification. From these two dendrograms, a total of 40 fluorescent isolates were selected for identification by using 16S rRNA sequencing.

Identification of the fluorescent isolates using 16S ribosomal RNA sequencing

Single band with 1500 bp of PCR product was successfully visualized on 2% agarose gel under UV transilluminator for 40 selected fluorescent bacterial isolates. The nucleotide sequences were identified as *Pseudomonas aeruginosa*, *P. alcaligenes*, *P. balearica*, *P. monteilii*, *P. mosselii*, *P. putida*, *P. stutzeri*, *Burkholderia* sp., *B. ambifaria*, *B. anthina*, *B. cepacia*, and *B. metallica* (Table 2) with similarity ranging from 84-100%. Overall results indicated that the 34 fluorescent bacterial isolates were *Pseudomonas* spp. and six isolates were *Burkholderia* spp. *Pseudomonas aeruginosa* was the most abundant species with 21 isolates were identified.

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Table 1: *In vitro* screening of antagonistic activities and plant growth promoting performances of 50 fluorescent bacterial isolates.

No	Isolate	Dual culture assay PIRG (%)	Volatile compounds production PIRG (%)	HCN production (nm)	Phosphate solubilization activity	Indole acetic acid (IAA) production
1	B1C3	73.73	19.51	0.011	-	+
2	B2C3	73.73	0.00	0.000	+	-
3	B2C13	67.45	13.66	0.000	-	-
4	B3C4	93.33	5.85	0.000	-	+
5	B3C19	84.71	0.00	0.000	-	++
6	B3C28	95.29	0.00	0.000	-	-
7	B3C36	91.76	1.46	0.000	-	-
8	B3C37	94.90	6.34	0.000	-	-
9	B3C38	94.90	0.00	0.000	-	-
10	B3C39	95.69	0.00	0.000	-	-
11	B3C40	94.51	2.44	0.000	-	-
12	B3C41	94.12	0.00	0.000	-	-
13	B3C42	94.90	3.90	0.000	-	-
14	B3C43	93.73	10.73	0.000	-	-
15	B3C44	96.86	0.00	0.000	-	-
16	B3C45	93.73	0.00	0.000	-	-
17	B3C46	96.47	0.00	0.000	-	-
18	B3C47	92.55	3.41	0.000	-	-
19	B3C48	89.02	9.76	0.000	-	-
20	B3C49	89.41	0.00	0.000	-	-
21	B3C50	93.33	2.44	0.000	-	-
22	B3C51	89.41	0.00	0.000	-	-
23	B3C52	89.41	0.00	0.000	-	-
24	B3C53	90.98	0.00	0.000	-	-
25	B3C54	90.20	0.00	0.000	-	-
26	B3C56	93.46	15.12	0.000	-	-
27	B3C59	84.31	6.83	0.000	-	-
28	B3C60	87.06	0.00	0.000	-	-
29	B3C61	85.49	7.80	0.000	-	-
30	B3C63	85.10	0.00	0.000	-	+
31	B3C65	73.73	0.00	0.000	-	+
32	B4C1	70.20	4.39	0.008	-	+++
33	B4C2	69.41	2.44	0.000	-	+
34	B4C3	70.59	14.15	0.006	-	++
35	B4C4	69.80	0.00	0.028	-	+
36	B4C5	70.98	0.00	0.003	-	+++
37	B4C6	69.02	0.00	0.009	-	++
38	B4C7	69.80	0.00	0.002	-	++
39	B4C8	70.20	0.00	0.017	-	-
40	B4C9	70.59	0.00	0.016	-	++
41	B4C10	69.41	0.00	0.000	-	-
42	B4C11	69.80	2.93	0.006	-	-
43	B5C1	86.27	0.00	0.000	-	++
44	B5C4	67.84	0.00	0.000	-	+
45	B5C7	83.53	0.00	0.000	-	+++
46	B6C2	81.96	0.00	0.000	-	-
47	B6C4	70.98	1.95	0.000	+	+
48	B6C7	68.63	0.00	0.000	+	-
49	B6C9	65.49	0.00	0.000	+	-
50	B6C10	80.39	0.00	0.000	+	-

- = absent; + = present (low); ++ = present (moderate); +++ = present (high)

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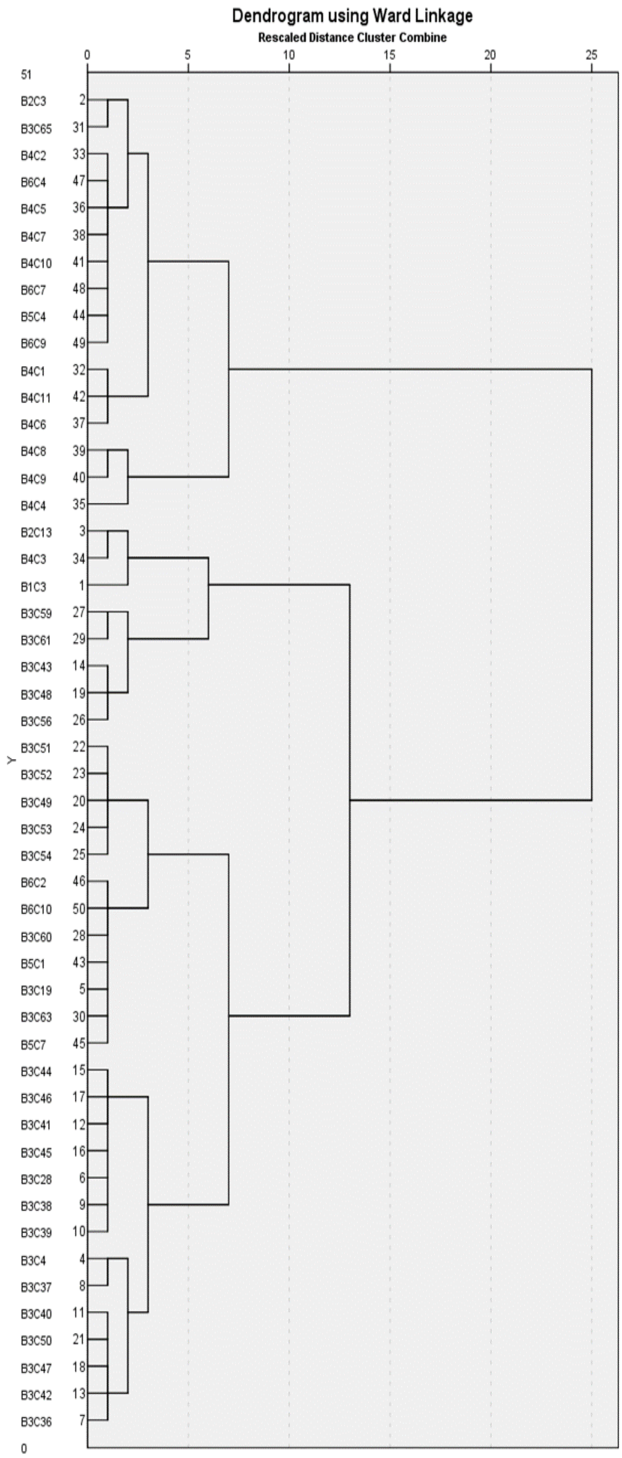

Figure 3: Clustering of 50 fluorescent bacterial isolates based on antagonistic activities (DC + VOC + HCN) in dendrogram A.

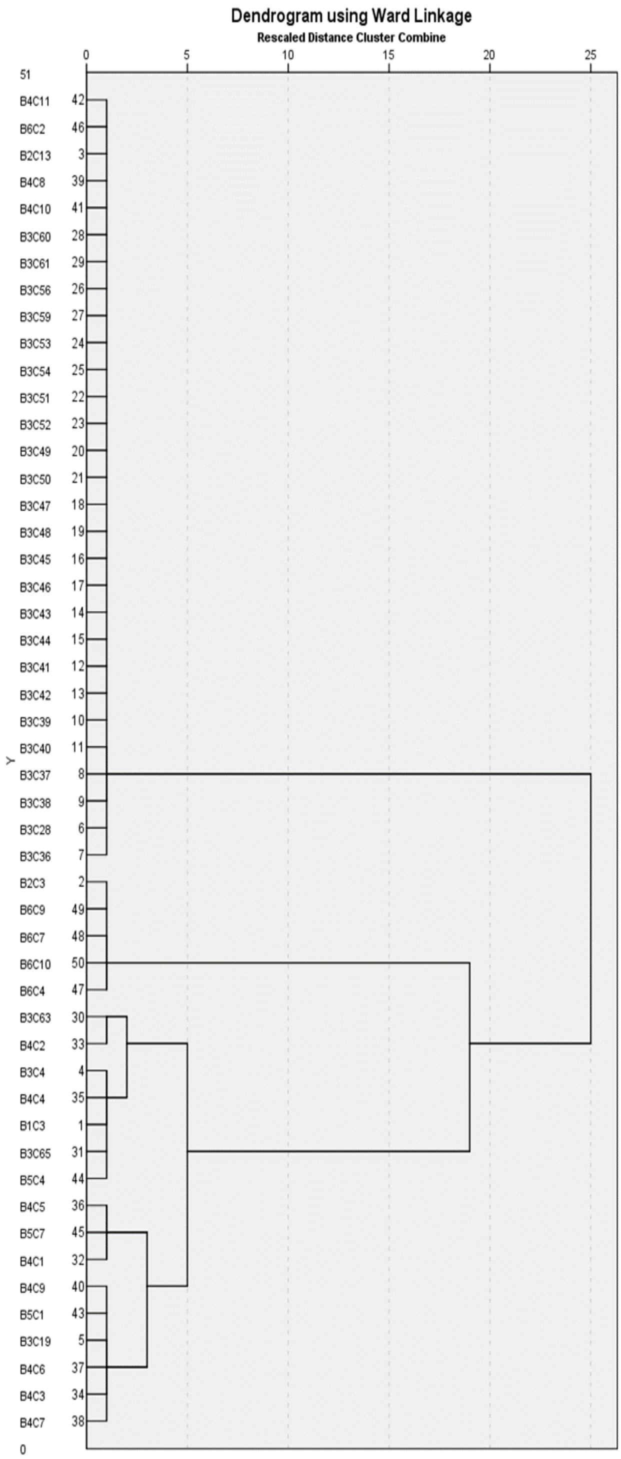

Figure 4: Clustering of 50 fluorescent bacterial isolates based on plant-growth promoting activities (phosphate + IAA) in dendrogram B.

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Table 2: Identification of the selected fluorescent bacterial isolates using 16S rRNA sequencing.

No.	Isolate	ID from NCBI
1	B1C3	*Pseudomonas mosselii*
2	B2C3	*Burkholderia cepacia*
3	B2C13	*Pseudomonas mosselii*
4	B3C4	*Pseudomonas stutzeri*
5	B3C19	*Pseudomonas mosselii*
6	B3C28	*Pseudomonas aeruginosa*
7	B3C36	*Pseudomonas aeruginosa*
8	B3C37	*Pseudomonas aeruginosa*
9	B3C38	*Pseudomonas aeruginosa*
10	B3C39	*Pseudomonas stutzeri*
11	B3C40	*Pseudomonas alcaligenes*
12	B3C41	*Pseudomonas aeruginosa*
13	B3C42	*Burkholderia* sp.
14	B3C43	*Pseudomonas aeruginosa*
15	B3C44	*Pseudomonas balearica*
16	B3C45	*Pseudomonas aeruginosa*
17	B3C46	*Pseudomonas aeruginosa*
18	B3C47	*Pseudomonas aeruginosa*
19	B3C48	*Pseudomonas aeruginosa*
20	B3C49	*Pseudomonas aeruginosa*
21	B3C50	*Pseudomonas aeruginosa*
22	B3C51	*Pseudomonas aeruginosa*
23	B3C52	*Pseudomonas aeruginosa*
24	B3C53	*Pseudomonas aeruginosa*
25	B3C54	*Pseudomonas aeruginosa*
26	B3C56	*Pseudomonas aeruginosa*
27	B3C59	*Pseudomonas aeruginosa*
28	B3C60	*Pseudomonas aeruginosa*
29	B3C61	*Pseudomonas aeruginosa*
30	B3C63	*Pseudomonas aeruginosa*
31	B4C1	*Pseudomonas monteilii*
32	B4C3	*Pseudomonas putida*
33	B4C5	*Pseudomonas putida*
34	B5C1	*Pseudomonas putida*
35	B5C7	*Pseudomonas putida*
36	B6C2	*Burkholderia ambifaria*
37	B6C4	*Burkholderia cepacia*
38	B6C7	*Burkholderia metallica*
39	B6C9	*Burkholderia anthina*
40	B6C10	*Burkholderia cepacia*

Selection of the most potential fluorescent *Pseudomonas* spp.

The fluorescent *Pseudomonas* spp. with the highest antagonistic and plant-growth promoting activities were selected based on the minimum total score of 5 using the scoring index as described by El-Sayed *et al.* (2014) (Table 3). The top 13 isolates of fluorescent *Pseudomonas* spp. which scored with the total index with minimum of score 5 were selected as the most potential PGPR from the total 40 fluorescent *Pseudomonas* isolates evaluated.

Seed germination and seedling vigor index

All selected 13 fluorescent *Pseudomonas* isolates were further tested for seed germination and vigor index. The plumule and radical growth of chilli seedlings pre-inoculated with the respective fluorescent *P. putida* (B5C1), *P. aeruginosa* (B3C56) and *P. putida* (B5C7) were significantly promoted compared to the uninoculated chilli seedlings (control) (Table 4 and Figure 5). However, there was no significant increase in chilli seed germination rate between pre-inoculated with the respective fluorescent *Pseudomonas* isolates and control. Interestingly, chilli seeds pre-inoculated with fluorescent *P. putida* (B5C1), *P. aeruginosa* (B3C56) and *P. putida* (B5C7) significantly increased seedling vigor index with 4684.9%, 4657.3% and 4401.0% over control (Table 4). In contrast, some fluorescent *Pseudomonas* isolates such as *P. aeruginosa* (B3C48, B3C43, B3C37, B3C61), *P. putida* (B4C3), *P. mosselii* (B3C19, B4C1) and *P. stutzeri* (B3C4) showed inhibitory effects on chilli seedling growth with significant decreases in vigor index.

Figure 5: The early growth performance of chilli seedlings inoculated with the respective fluorescent *Pseudomonas* isolates after 10 days of incubation in growth chamber at 32 °C in light for 12 h and interval with 24 °C in dark for 12 h.

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Table 3: Scoring index assessment of potential fluorescent Pseudomonads spp. with biocontrol agents and plant-growth promotion properties.

		Antagonistic activities						Plant growth-promoting performances					
		Dual culture assay		Volatile compounds production		Hydrogen cyanide (HCN) production		Phosphate solubilization activity		Indole acetic acid (IAA) production			
Bacterial isolates	Bacterial name	PIRG (%)	Score	PIRG (%)	Score	(nm)	Score	Index (%)	Score	(µg/mL)	Score	Total scores	Rank
B1C3	*Pseudomonas mosselii*	73.73 (±2.24)	1	19.51 (±3.15)	4	0.011 (±0.0081)	3	-	0	7.50 (±0.25)	2	10	1
B4C3	*Pseudomonas putida*	70.59 (0)	1	14.15 (±4.66)	3	0.006 (±0.0038)	2	-	0	11.37 (±0.45)	3	9	2
B4C1	*Pseudomonas monteilii*	70.20 (±0.88)	1	4.39 (±2.89)	1	0.008 (±0.0048)	2	-	0	16.30 (±7.54)	4	8	3
B3C4	*Pseudomonas stutzeri*	93.33 (±2.24)	3	5.85 (±1.59)	2	-	0	-	0	8.88 (±0.42)	2	7	4
B3C56	*Pseudomonas aeruginosa*	93.46 (±1.13)	3	15.12 (±5.01)	4	-	0	-	0	-	0	7	4
B4C5	*Pseudomonas putida*	70.98 (±2.15)	1	-	0	0.003 (±0.0015)	1	-	0	15.49 (±0.21)	4	6	5
B5C1	*Pseudomonas putida*	86.27 (±6.50)	3	-	0	-	0	-	0	10.74 (±4.36)	3	6	5
B5C7	*Pseudomonas putida*	83.53 (±1.75)	2	-	0	-	0	-	0	15.74 (±0.21)	4	6	5
B3C43	*Pseudomonas aeruginosa*	93.73 (±2.15)	3	10.73 (±2.12)	3	-	0	-	0	-	0	6	5
B3C19	*Pseudomonas mosselii*	84.71 (±2.15)	2	-	0	-	0	-	0	11.45 (±0.31)	3	5	6
B3C37	*Pseudomonas mosselii*	94.90 (±3.28)	3	6.34 (±2.55)	2	-	0	-	0	-	0	5	6
B3C61	*Pseudomonas aeruginosa*	85.49 (±2.24)	3	7.80 (±1.31)	2	-	0	-	0	-	0	5	6
B3C48	*Pseudomonas aeruginosa*	89.02 (±3.28)	3	9.76 (±4.49)	2	-	0	-	0	-	0	5	6

Scoring index:

Dual culture assay;	Hydrogen cyanide (HCN);	Volatile compound production;	Phosphate solubilization;	Indole acetic acid (IAA);
0 : 0	0 : 0	0 : 0	0 : 0	0 : 0
1 : 65 - 74.99	1 : 0.001 - 0.005	1 : 1 - 4.99	1 : 105 - 109.99	1 : 1 - 4.99
2 : 75 - 84.99	2 : 0.006 - 0.010	2 : 5 - 9.99	2 : 110 - 114.99	2 : 5 - 9.99
3 : 85 - 94.99	3 : > 0.010	3 : 10 - 14.99	3 : ≥ 150	3 : 10 - 14.99
4 : ≥95		4 : ≥ 15		4 : ≥ 15

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Table 4: The early growth performance of chilli inoculated with the selected fluorescent *Pseudomonas* spp. isolates and *in vitro* screenings of the bacteria.

Bacterial code	Bacterial ID	Plumule (mm)	Radicle (mm)	Germination rate (%)	Vigor index
Control		10.80 (±2.00) ^{cd}	16.00 (±5.08) ^{bcd}	87.10 (±3.15) ^{ab}	2328.7 (±613.8) ^{bcd}
B5C1	*Pseudomonas putida*	20.30 (±0.15) ^{a}	30.50 (±2.40) ^{a}	92.70 (±7.64) ^{ab}	4684.9 (±547.9) ^{a}
B3C56	*Pseudomonas aeruginosa*	18.10 (±1.78) ^{ab}	32.70 (±5.32)^{a}	91.70 (±10.00) ^{ab}	4657.3 (±737.5) ^{a}
B5C7	*Pseudomonas putida*	18.30 (±1.78) ^{ab}	30.70 (±5.32) ^{a}	90.00 (±10.00) ^{ab}	4401.0 (±737.5) ^{a}
B4C5	*Pseudomonas putida*	13.20 (±2.91) ^{bc}	20.10 (±6.47) ^{b}	98.30 (±2.89) ^{a}	3292.5 (±1001.5) ^{ab}
B3C19	*Pseudomonas mosselii*	13.20 (±4.18) ^{bc}	17.40 (±8.69) ^{bc}	86.70 (±14.43) ^{ab}	2726.2 (±1422.2) ^{bc}
B4C1	*Pseudomonas monteilii*	12.50 (±1.83) ^{c}	17.70 (±4.11) ^{bc}	85.0 (±8.66) ^{ab}	2587.5 (±685.7) ^{bc}
B1C3	*Pseudomonas mosselii*	10.27 (±4.00) ^{cd}	15.57 (±7.56) ^{bcd}	93.33 (±7.64) ^{ab}	2464.5 (±1260.8) ^{bcd}
B4C3	*Pseudomonas putida*	8.70 (±5.95) ^{cde}	13.70 (±9.37) ^{bcde}	76.70 (±15.28) ^{ab}	1843.0 (±731.4) ^{bcde}
B3C61	*Pseudomonas aeruginosa*	6.50 (±0.26) ^{def}	12.10 (±0.88) ^{bcde}	88.30 (±5.77) ^{ab}	1630.0 (±19.5) ^{bcde}
B3C4	*Pseudomonas stutzeri*	6.60 (±4.41) ^{def}	9.40 (±5.06) ^{cde}	76.70 (±27.54) ^{ab}	1389.2 (±1045.8) ^{cde}
B3C37	*Pseudomonas aeruginosa*	3.80 (±1.62) ^{ef}	6.70 (±4.05) ^{de}	66.70 (±27.54) ^{b}	839.5 (±217.7) ^{de}
B3C48	*Pseudomonas aeruginosa*	1.50 (±0.40) ^{f}	4.40 (±1.40) ^{e}	81.70 (±17.56)^{ab}	507.7 (±88.3)^{e}
B3C43	*Pseudomonas aeruginosa*	1.60 (±0.46) ^{f}	4.20 (±2.11) ^{e}	76.70 (±14.43) ^{ab}	460.5 (±246.2)^{e}

Means followed by the same letters within column are not significantly different from each other by Duncan test at $P \leqslant 0.05$.

Discussion

Plant growth-promoting rhizobacteria (PGPR) from Pseudomonads are particularly suited as bio-control agent because they are indigenous and abundantly present in the rhizosphere soil, high growth rate and capable to control plant diseases by variety of mechanisms (Höfte and Altier, 2010).

The fluorescent *Pseudomonas* spp. isolated from the rhizosphere soil of the healthy chilli field was found to be effective in inhibiting the growth of *R. solani*. The strongest antagonist (B3C44) was obtained with 96.86% of PIRG against *R. solani* and this high antagonistic activity of fluorescent *Pseudomonas* spp. against *R. solani* with more than 80% of inhibition rate was also reported by Ahmadzadeh and Tehrani (2009). The effectiveness of the selected fluorescent *Pseudomonas* spp. in inhibiting of *R. solani* was also associated to the production of antifungal metabolites such as volatile compounds and hydrogen cyanide (Ahmadzadeh and Tehrani, 2009).

The genera of *Pseudomonas* and *Burkholderia* are often abundantly present in the rhizosphere and surrounding soil of many crop plants. In this study, fluorescent *Pseudomonas* were focused as it help to stimulate plant growth by increasing the availability and uptake of mineral nutrients through various mechanisms like phosphate solubilization or by enhancing root growth through the production of phytohormones such as auxin (Vessey, 2003). The production of antibiotics, hydrogen cyanide, lytic exoenzymes, cyclic lipopeptides, competition for nutrients and niches, competition for iron mediated by siderophores, competition for carbon and induced systemic resistance are the most commonly bio-control mechanisms of fluorescent *Pseudomonas* spp. against pathogens (Höfte and Altier, 2010).

There are numerous soil rhizobacteria have been explored as efficient bio-control against soil borne disease caused by *R. solani* including *Bacillus subtilis* CA32 in eggplants (Abeysinghe, 2009), *Pseudomonas fluorescens* In5 (Michelsen and Stougaard, 2011), *Burkholderia cepacia* T1A-2B and *Pseudomonas sp.* T4B-2A in tomato (Curtis *et al*., 2010), and *Burkholderia cepacia* BC-1 and *Serratia marcescens* N1-14 in cucumber (Roberts *et al.*, 2005).

Besides antagonistic effects against *R. solani*, the isolated fluorescent *Pseudomonas* spp. also able to promote plant growth through IAA production. The application of phosphate-solubilizing microorganism is essential for improve root growth and plant health. Most *Pseudomonas* spp. was reported capable to solubilize of inorganic phosphate (Bhoopander *et al*., 2005). In contrary, in the current study, none of the selected fluorescent *Pseudomonas* spp. showed positive in phosphate solubilization on PVK agar. Nine isolates of fluorescent *Pseudomonas* spp. were able to produce IAA with varying degree in the concentration. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR where high IAA level produced by fluorescent *Pseudomonas* spp. is a general characteristic (Ahmad *et al*., 2005). However, the plant regulators of PGPR also includes gibberellin, ethylene and others (Arshad and Frankenberger, 1992). This may explain to the better performance in the growth of bacterial-treated seedlings rather than control even at no absence of IAA in this study.

In the chilli seed germination and vigor index test, three IAA-producing isolates of fluorescent *Pseudomonas* (*Pseudomonas putida* B5C1, *Pseudomonas aeruginosa* strain B3C56 and *Pseudomonas putida* B5C7) were significantly improve the growth of plumule and radicle over the control. Chilli seed germination rates were slightly improved with pre-inoculated with the three selected fluorescent *Pseudomonas* isolates. The increase in plumule and root length of chilli seedlings treated with *Pseudomonas putida* was associated to the production of IAA (Caron *et al*., 1995). This finding was also supported by Mohite (2013), where the IAA-producing *Pseudomonas putida* improved the length of seedling roots of canola seeds as by inducing the proliferation of lateral roots and root hairs. The plant regulator (IAA) play a critical role in promoting the plant growth. Seed treatment with fluorescent *Pseudomonas sp.* improved seed germination and seedling vigor over the control but varied in the rate of enhancement with different bacterial isolates (Ameer-Basha *et al*., 2013).

CONCLUSION

Chilli seeds pre-inoculated with *Pseudomonas putida* (B5C1), *Pseudomonas aeruginosa* (B3C56) and *Pseudomonas putida* (B5C7) significantly enhanced the vigor index of chilli seedling. Besides, these selected potential isolates also exhibited prominent *in vitro* capability in suppressing the growth of *R. solani.* The significant effects of the selected fluorescent *Pseudomonas* isolates in suppression of *R. solani* and seedling growth-promotion of chilli revealed the potential of bio-fungicide and bio-fertiliser development in chilli production system to reduce the dependency on chemical in controlling *R. solani* root rot disease.

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