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Molecular Characterization of Plant Growth Promoting *Pseudomonas* from Rhizosphere of *Euphorbia caducifolia* Haines

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ABSTRACT

A study was conducted during *kharif* 2020 at the Department of Microbiology, Chimanbhai Patel College of Agriculture, S. D. Agricultural University for molecular characterization of plant growth promoting *Pseudomonas* isolates from rhizosphere of *Euphorbia caducifolia*. Twenty *Pseudomonas* isolates (designated as PsEc1 to PsEc20) were axenically isolated from rhizospheric soil sample of *Euphorbia caducifolia* using *Pseudomonas* Isolation Agar Base (a selective medium). These isolates were tested gram negative, rod shaped, and oxidase positive. All 20 isolates were positive for utilization of maltose, esculin, citrate and malonate whereas these isolates showed varied results for utilization of xylose, fructose, galactose, sucrose and sorbitol. 16S*r* DNA sequencing data revealed similarity of the isolates to different species of *Pseudomonas*, i.e., *P. fluroscens* (11), *P. aeruginosa* (3), *P. putida* (3), *P. stutzeri* (2) and *P. syringe* (1). These nucleotide sequences were submitted to the NCBI GenBank database with accession numbers respectively MT775484 to MT775503. The 16S *r*DNA sequences were aligned and used to reconstruct a phylogenetic tree with bootstrap values using software MEGA 6.06. The phylogenetic tree comprised of three clades. Clade I comprised of sixteen isolates, clade II four isolates and clade III only one isolate. The members of clade I belonged to *P. fluorescens*, *P. putida* and *P. stutzeri* whereas members of clade II comprised of *P. aeruginosa*. However, clade III consisted of *P. syringae*. In vitro test showed PsEc17 (*P. fluorescens,* MT775500) as most efficient in solubilizing tricalcium phosphate (78.53 µg ml^{-1}) and IAA production (78.53 µg ml^{-1}). PsEc17 also proved most efficient and showed 69.5% inhibition of *Fusarium oxysporum* f. sp. *cumini.*

KEYWORDS: *Euphorbia caducifolia*, *Pseudomonas*, *Fusarium oxysporum* f. sp. cumini, PGPR

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1. INTRODUCTION

Indescriminate use of chemical to fortify nutrient status of soil has severe environmental implications (Garrido–Sanz et al., 2017). This necessitates use of microbial inoculants benefitting plant growth (Jorquera, 2012; Pattnaik et al., 2019). *Pseudomonas* play an exceptionally significant role in the carbon and nitrogen cycles (Zhang et al., 2020, Zhuang et al., 2021). Microorganisms inhabiting rhizosphere may promote plant growth by contributing to the host plant's endogenous pool of bioactive compounds (Olanrewaju et al., 2019). These bacterial groups are well–known as Plant Growth Promoting Rhizobacteria (Louca et al., 2018, Pattnaik et al., 2019, Singh, 2013). Direct effects are dependent on production of plant growth regulators or improvements in plant nutrients uptake (Zia et al., 2020) whereas indirect effects include suppression of soil borne pathogens through production of antibiotics, HCN, siderophores and other metabolites in the rhizosphere (Goswami et al., 2016).

Pseudomonas is a genus with over a hundard different species that houses the most diverse group of bacteria on the earth (Hesse et al., 2018). PGPR activity of some of these strains has been known for many years (Uppal et al., 2017). *Pseudomonas* is a common bacterium found all over the world in soil, water, and plants and are known for its biofertilizer, phytostimulator and phytopathogen biocontrol activities (Costa–Gutierrez et al., 2020, Saati–Santamaría et al., 2021). They were first identified at the end of 19th century as rod–shaped and polar–flagellated bacteria (Migula, 1984, Pattnaik et al., 2019) and are regarded as progressive (Godinho and Bhosle, 2013). The members of this genus demonstrate a great deal of metabolic diversity and consequently can colonize a wide range of niches (George et al., 2016, Kaminski et al., 2018). These beneficial bacteria colonize rhizosphere or roots and produces secondary metabolites which include antibiotics, extracellular enzymes, hydrogen cyanide (HCN), siderophores and phytohormones (Sun et al., 2021, Kour et al., 2020, Mhlongo et al., 2018). Direct plant growth activities of *Pseudomonas* sp include the production of Indole Acetic Acid (IAA) (Sah and Singh, 2016, Matsuda et al., 2018) and siderophore (Rathore, 2015), phosphate solubilization (Li et al., 2017), ACC deaminase (Khan et al., 2013), root elongation, degradation of toxic compound (Bano and Musarrat, 2003) and as phytopathogens such as *Aspergillus niger* and *A. flavus* (Shah and Daverey, 2020). Several chemically mediated feedback mechanisms are also involved in proper growth regulation of plant by *Pseudomonas* (Rodríguez et al., 2020).

Euphorbia caducifolia Haines, a member of the plant family Euphorbiaceae (Thakur and Patil, 2011), is sparsely spiny columnar branching plant which is also known as leafless milk hedge, leafless euphorbia and desi thor. It grows on barren coastal plains and in the hilly tracts of the Indian deserts as well on sandy soils (Khan et al., 2013). It is commercially farmed for the latex and other chemicals (Cai et al., 2009). These plants are found around the year lush green and fresh even under harsh climatic conditions of arid and semi–arid regions (Escamilla–Trevino, 2012). One reason for its survival in harsh arid climatic conditions is the physiology of this plant (Lukovic et al., 2009), the other is that there may be some beneficial bacteria in its rhizosphere which act as PGPRs and support the plant growth; and *Pseudomonas* is one of the most predominant PGPR. In this background, the rhizospheric soil of *Euphorbia caducifolia* was taken as a source material for isolation of *Pseudomonas* isolates with a view to develop a suitable bioinoculants for arid and semi arid regions.

2. MATERIALS AND METHODS

2.1. Isolation of Pseudomonas from rhizospheric soil of Euphorbia caducifolia

Soil adhered with the roots of *Euphorbia caducifolia* were collected from the nursery of the Department of Horticulture (latitude and longitude 24°36'17" N and 72°35'94" E), C. P. College of Agriculture, SDAU, Sardarkrushinagar in the month of *kharif* 2020 (June–October). Microbial strains were isolated from the soil using serial dilution and standard plating method (Cappucino and Sherman, 2002) in petriplates having selective medium *Pseudomonas* Isolation Agar Base (M085, HiMedia Laboratories, Mumbai) and incubated at 37°C for 48 h. Colonies with smooth edges and convex surface were isolated. These isolated cultures were purified and axenised by repeated subculturing and isolation of single colony. These cultures were maintained on nutrient agar slants for further experimentation.

The cultures obtained were screened for Gram's reaction and cell shape and arrangement under compound microscope (Nikon N200, Japan). Isolates were tested for oxidase reaction using Oxidase Discs (DD018, HiMedia Laboratories, Mumbai) following manufacturer's protocol. Finally twenty gram negative, rod shaped, oxidase positive isolates (PsEc1 to PsEc20) showing luxuriant growth on *Pseudomonas* Isolation Agar Base were retained for further experimentation.

2.2. Molecular characterization of the Pseudomonas isolates

2.2.1. Isolation of genomic DNA

Single colony of the isolates was inoculated into freshly prepared Luria Bertani broth medium. After an 24 h of incubation, DNA isolation form bacteria was done using Thermo Scientific GeneJET Genomic DNA Purification Kit (K0721, Thermo Fisher Scientific, Waltham, US) following manufacturers protocol.

2.2.2. Amplification of 16S rDNA

Isolated and purified bacterial genomic DNA were subjected to PCR (Polymerase chain reaction) using forward (5'–AGAGTTTGATCCTGGCTCA G–3') and reverse (5'–AAGGAGGTGATCCAGCC–3') primers (Weisberg et al., 1991), dNTPs, Taq polymerase, $MgCl_2$, buffers *etc.* The reaction mixture for PCR contained 50 ng (2 µl) of extracted DNA, 5U Taq polymerase, 10X buffer [10mM Tris HCl, pH 9.0, 1.5 mM $MgCl_2$, 500 mM KCl], 2.5 mM dNTPs and 10 pmol of each forward and reverse primer (Merck, India). PCR amplification was carried out in a thermal cycler and consisted of steps as initial DNA denaturation at 94°C for 5 m., followed by 35 cycles of denaturation at 94°C for 50 seconds, annealing at 55°C for 1.45 m., extension at 72°C for 2 m and a final extension of 72°C for 10 m. The presence and yield of specific PCR product of the 16S rDNA was monitored on 1.5 % agarose gel stained with ethidium bromide.

2.2.3. 16S rDNA sequencing and phylogenetic analysis

The PCR amplified 16S *r*DNA products were sequenced using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequenced products were resolved on an Applied Biosystems model 3500XL automated DNA sequencing system (Applied BioSystems, USA) for all the isolates. The gene sequences obtained with the forward and reverse primers were aligned together using online software Bioedit (www. BioEdit.exe). The 16S *r*DNA sequences of the isolates were compared with sequences already deposited in the databases of National Center for Biotechnology Information (NCBI) GenBank using mega BLAST (Basic Local Alignment Search Tool) and their genetic identity was ascertained. The sequences of all the isolates were aligned using ClustalW algorithm and the evolutionary history was interred using the unweighted pair group method with arithmetic mean (UPGMA) method and the evolutionary distances were computed using pair wise distance method including bootstrap analysis using MEGA software version 6.06 (Tamura et al., 2011). The 16S *r*RNA gene sequences of 20 *Pseudomonas* isolates were deposited in the National Center for Biotechnology Information (NCBI) nucleotide sequence database with the accession numbers MT775484 to MT775503, respectively.

2.3. Characterization of the isolates for plant growth promotion traits

The twenty isolates were screened for plant growth promotion activities by assaying them for seed germination, production of ammonia, production of IAA, phosphate solubilization, nitrogen fixation abilities, HCN production and antagonism against fungal plant pathogen (Kaushik et al., 2004).

2.3.1. Seed germination test

The *Pseudomonas* isolates were tested for their influence on seed germination of green gram. Green gram seeds (cultivar GM–4) were surface sterilized with 0.1 % $HgCl_2$ for 3 m, followed by successive washing with sterile distilled water. Treated the seeds with cultures (10^8 cfu ml^{-1}) in triplicates for 10 m and decanted the medium thereafter. Placed whatman No.1 qualitative filter papers in the bottom lid of petriplates and moisturized it with distilled water. The seeds were kept on the surface of filter paper in petriplates. In control plates, the seeds treated with sterilized medium alone were placed. After 7 days, recorded root and shoot length of green gram seedlings.

2.3.2. Production of ammonia

Grown the *Pseudomonas* isolates in peptone water (Dye, 1962) in test tubes in triplicates. Incubated the tubes at 30°C for 4 days. Added 1 ml Nessler's reagent in each tube. Presence of a faint yellow colour indicated small amount of ammonia and deep yellow and brownish color indicated medium and high production of ammonia, respectively.

2.3.3. IAA production

Inoculated loopful of *Pseudomonas* culture in 25 ml LB (Luria–Bertani) broth medium amended with 50 µg ml^{-1} tryptophan in triplicates in test tubes. Incubated them for 24 h at 28°C on rotary shaker. The contents of the tubes were centrifuged at 10,000 g for 15 m and took 2 ml of supernatant and added to it 2 to 3 drops of O–phosphoric acid and 4 ml of reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35 % $HClO_{4-}$) to the aliquot and incubated for 25 m at room temperature and recorded absorbance at 530 nm (Gordon and Weber, 1951). Quantification of IAA produced by the isolates was done by preparing calibration curve made by using IAA (indole acetic acid) as standard (10–100 µg ml^{-1}).

2.3.4. Phosphate solubilization

The *Pseudomonas* cultures were inoculated in Pikovskaya broth medium and incubated the test tubes at 28±2°C on shaker incubator for 3 days (Verma et al., 2004). The bacterial suspension was centrifuged at 10,000 g and took aliquot (0.1 ml–1.0 ml) from the supernatant/filtrate and added to it 10 ml of ammonium molybdate solution (15.0 g of Ammonium molybdate was dissolved in 400 ml of warm distilled water). Added 342 ml of 12N HCl and cooled and made–up the volume to 1 liter with distilled water. The contents were shaked properly and subsequently diluted to 45 ml. Added 0.25 ml of chlorostannous acid [2.5 g of $SnCl_2.2H_2O$ was dissolved in 10 ml of concentrated HCl (heat) and made the volume to 100 ml with distilled water] and immediately made up the volume to 50 ml. Measured the intensity of the developed blue colour solution at 600 nm and recorded the corresponding amount of soluble

phosphorus from the standard curve prepared by plotting absorbance at 430 nm versus known concentration of KH_2PO_4.

2.3.5. Nitrogen fixation abilities

The isolates were inoculated into nitrogen free semi solid medium and incubated at 30°C for 7 days. The isolates capable of growing and forming a visible film beneath the surface of the medium are supposed to do nitrogen fixation (Dobereiner et al., 1995). The isolates capable of developing pellicle in the subsurface were subsequently sub–cultured five times on the N–free semi solid medium. The isolates capable of retaining the pellicle formation nature in the subsurface were considered positive for nitrogen fixation.

2.3.6. Hydrogen cyanide (HCN) production

King's B medium was prepared and amended with 4.4 g l^{-1} glycine. The mixture was autoclaved and 25 ml of this medium was poured in each petriplate. After solidification, streaked the test bacterial isolates in petriplates in triplicates. Single isolate was streaked on each petri plate then soaked Whatman No. 1 filter paper disc (9 cm in diameter) in 0.5 % picric acid in 2 % sodium carbonate and placed this soaked disc in the lid of each petriplate. Sealed the petriplate with parafilm and incubated at 28 ± 2°C for 4 days. Uninoculated control was maintained for comparison of the results.

2.3.7. Antagonism against plant pathogenic fungi

The *Pseudomonas* isolates were assayed for their antagonism against *Fusarium oxysporum* f. sp. *cumini* using half concentration of nutrient agar and potato dextrose agar (PDA) medium. *Fusarium oxysporum* f. sp. *cumini* is one of the most important plant pathogen and is the causal organism of the wilt disease in cumin. *Pseudomonas* isolates were streaked on PDA medium at a distance of 3.0 cm at four places (one each in the four directions) and at the centre of the petriplate the actively growing fungal culture disc was placed aseptically. In this way twenty petriplates were prepared for testing the antagonistic activity of each *Pseudomonas* isolates against the *F. oxysporum* f. sp. *cumini*. One petriplate was maintained as control having only test fungus and no bacterial culture. Mycelial growth was recorded as mean of fungal growth in all the four directions. Antagonist activity was investigated for 7 days after incubation at room temperature. % inhibition of fungus was estimated using the formula given by Vincent (1927).

$$I=(C-T)/C\times100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where, I=% inhibition of mycelia growth., C=Mycelial growth in control., T=Mycelial growth in treatment.

3. RESULTS AND DISCUSSION

3.1. Isolation of pseudomonas

Total 50 bacterial cultures were isolated from the rhizospheric soil of desi thor plant (*Euphorbia caducifolia* Haines) using *Pseudomonas* Agar base (M085, Himedia, Mumbai). These isolates were further screened for oxidase test using oxidase discs (DD018, Himedia, Mumbai). This screening was important because *Pseudomonas* is generally oxidase positive (Gordon and Mcleod, 1928). Thus, oxidase positive 20 isolates were retained and maintained on *Pseudomonas* agar base in slants. All the isolates (PsEc1 to PsEc20) were rod shaped and gram negative. *Pseudomonas* Isolation Agar Base is a selective medium for isolation of *Pseudomonas*, and is a modification of medium originally formulated by King et al. (1954). The medium contains pigment–enhancing components and the selective agents, triclosan (Collee et al., 1996) which selectively inhibits non–pseudomonads. The biochemical diversity of these *Pseudomonas* isolates was determined based on carbohydrate utilization pattern consisting of 9 various carbon sources. All the isolate were positive for utilization of maltose, esculin hydrolysis, citrate utilization and malonate utilization. However, these isolates showed a varying degree for utilization of xylose, fructose, galactose, sucrose and sorbitol.

3.2. Molecular characterization of the Pseudomonas isolates

3.2.1. Isolation of genomic DNA and PCR amplification of 16S rDNA

Isolated DNA from the *Pseudomonas* isolates were of good quality and showed single band upon electrophoresis on 0.8 % agarose gel. The average concentration of genomic DNA of the 20 isolates of *Pseudomonas* was 42.17 ng μl^{-1}. However, for further experimentation, the DNA concentration of all the isolates were maintained at 25 ng μl^{-1}. The 16S rDNA gene amplification by polymerase chain reaction with the universal forward and reverse primers revealed a band size of about 1.5 kb in all the bacterial isolates (Figure 1).

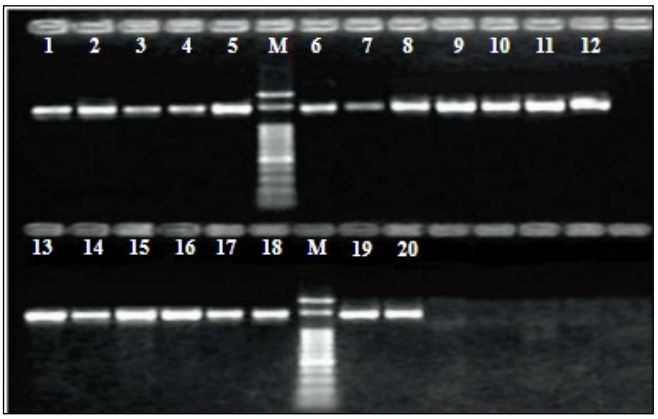

Figure 1: PCR amplified 16S rDNA of 20 *Pseudomonas* isolates

Analysis of 16S *r*DNA gene fragments produced by digestion of PCR amplified 16S *r*DNA gene with restriction enzymes were also done for assessing genetic diversity among PGPR isolates of *Pseudomonas* obtained from the

rhizosphere of *Mentha piperita* (Santoro et al., 2016). This technique was used successfully for genetic diversity analysis of fluorescent *Pseudomonas* communities isolated from rhizospheres of various crop plants under a variety of environmental and climatic conditions (Mehri et al., 2011, Nievas et al., 2012). Polymerase chain reaction can be used to study variation of *Pseudomonas* communities as a function of crop management strategies (Achouak et al., 2000) and application of pesticides or other chemical compounds (Viti and Giovannetti, 2005, Braun et al., 2006, Wang et al., 2008, Wu et al., 2009).

3.2.2. Phylogenetic analysis

Phylogenetic authentification of the 20 isolates were done based on 16S rDNA sequences of the *Pseudomonas* isolates. The gene sequences obtained were compared using the BlastN tool of NCBI GenBank database nucleotide sequences with similarity values ≥99%. The results suggested that all the isolates belonged to different species of *Pseudomonas*, *i.e., P. fluorescens* (11), *P. aeruginosa* (3), *P. putida* (3), *P. stutzeri* (2) and *P. syringae* (1). The nucleotide sequence data were submitted to the NCBI GenBank database with unique accession numbers (Table 1). These DNA sequences were aligned and used to construct a phylogenetic tree with bootstrap values (Figure 2).

The phylogenetic tree comprised of three clades. Clade I comprised of 16 isolates, clade II 4 isolates and clade III only 1 isolate. The members of clade I belonged to *P. fluorescens*, *P. putida* and *P. stutzeri* whereas members of clade II comprised of *P. aeruginosa*. However, clade III consisted of *P. syringae*. The overlapping of some species in 2 clades may be due to horizontal gene transfer which may let to stable integration of genetic material following transfer between individuals (Figure 2).

Plant growth–promoting isolates were screened and identified as *Pseudomonas plecoglossicida, P. fluorescens, P. libaniensis,* and *P. aeruginosa* based on 16S *r*DNA sequence analysis (Rameshkumar et al., 2011). *Pseudomonas fluorescens* was identified earlier as one of the most efficient PGPR strains and was characterized based on 16S *r*RNA gene sequencing (Hesse et al., 2018). Similarly, IAA producing isolate from rhizospheric soil of alfalfa (*Medicago sativa*) field was identified as *Pseudomonas putida* UB1 by 16S *r*RNA

Table 1: 16S rDNA sequence based validation of the 20 *Pseudomonas* isolates

Isolates	16S rRNA gene	% Similarity	Accession number match with NCBI database	Nucleotide no. of isolates used in present study	Accession numbers of isolates used in present study
PsEc1	*Pseudomonas fluorescens*	99.79	MN099299	1431	MT775484
PsEc2	*Pseudomonas fluorescens*	99.86	MH282433	1432	MT775485
PsEc3	*Pseudomonas stutzeri*	99.70	U58660	1349	MT775486
PsEc4	*Pseudomonas fluorescens*	99.37	MK920194	1432	MT775487
PsEc5	*Pseudomonas aeruginosa*	99.80	MN647070	1467	MT775488
PsEc6	*Pseudomonas aeruginosa*	99.87	AM419153	1522	MT775489
PsEc7	*Pseudomonas putida*	99.81	LN610443	1029	MT775490
PsEc8	*Pseudomonas fluorescens*	99.51	FJ972536	1427	MT775491
PsEc9	*Pseudomonas putida*	99.87	FM163468	1528	MT775492
PsEc10	*Pseudomonas fluorescens*	99.86	LC507956	1457	MT775493
PsEc11	*Pseudomonas syringae*	99.78	AY699269	896	MT775494
PsEc12	*Pseudomonas stutzeri*	99.19	KU921576	1351	MT775495
PsEc13	*Pseudomonas fluorescens*	99.86	AB266613	1426	MT775496
PsEc14	*Pseudomonas fluorescens*	99.87	HQ876463	1488	MT775497
PsEc15	*Pseudomonas fluorescens*	99.87	HG796166	1510	MT775498
PsEc16	*Pseudomonas fluorescens*	99.93	AM229082	1499	MT775499
PsEc17	*Pseudomonas fluorescens*	99.93	DQ536515	1489	MT775500
PsEc18	*Pseudomonas fluorescens*	99.87	EU373313	1498	MT775501
PsEc19	*Pseudomonas aeruginosa*	99.85	LT844657	1369	MT775502
PsEc20	*Pseudomonas putida*	99.86	HF545846	1383	MT775503

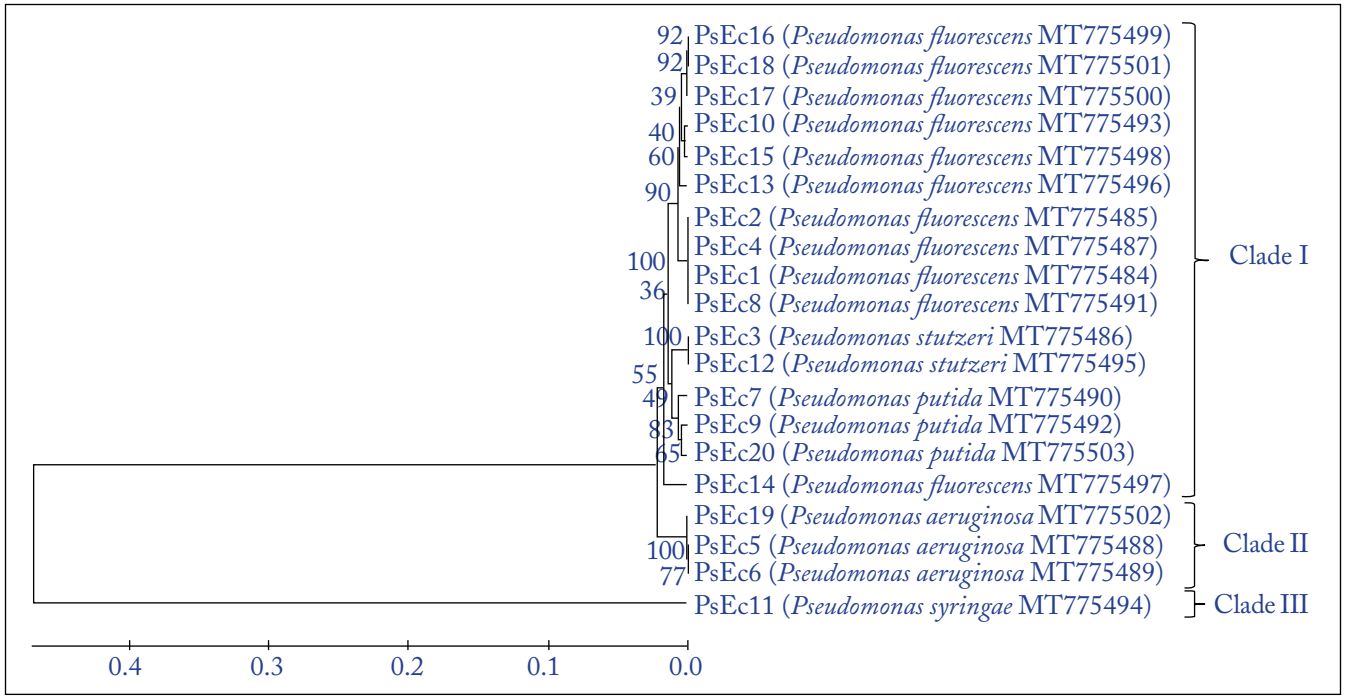

Figure 2: Phylogenetic relationships among *Pseudomonas* isolates based upon Unweighted Pair Group Method with Arithmetic mean (UPGMA) using MEGA 6.06 software

partial gene sequencing (Bharucha et al., 2013). Further, out of twenty Pseudomonads isolated from the rhizosphere soils of paddy areas in Malaysia, 15 strains were identified as *Pseudomonas fluorescens*, 3 as *Pseudomonas luteola*, one as *Pseudomonas aeruginosa* whereas one isolate (TS14) showed doubtful identification (Noori and Saud, 2012). However, Qessaoui et al. (2019) isolated *Pseudomonas* from the rhizospheric soil of tomato nursery under greenhouse condition. They used Newman–Keuls test to ascertain pairwise differences among the isolates and identified them using *rpo*D gene sequencing. Ten isolates of rhizobacteria from the leaves and stems of healthy wild Pistachio trees (*Pistacia atlantica* L.) from various locations of Baneh and Marivan regions of Iran were isolated and each group were further identified by partial sequencing of the 16S *r*RNA gene by Etminani and Harighi (2018).

3.3. Characterization of the Pseudomonas isolates for plant growth promotion activities

The isolate, PsEc13 was most effective in promoting root length of green gram (6.04 cm) however maximum shoot length was observed by inoculation with the isolate PsEc10 (8.36 cm). Regarding promotion of root growth, the isolate PsEc13 was followed respectively by the isolates PsEc20 (5.68 cm), PsEc17 (5.10 cm) and PsEc10 (5.08 cm). However, for shoot growth promotion, the isolate PsEc10 was the best followed by the isolate PsEc13 (7.02 cm), PsEc17 (6.22 cm) and PsEc20 (6.04 cm). Inoculation of green gram seeds with the isolate PsEc13 showed an increase of root and shoot growth respectively by 72.57 and 86.72% over control. However, inoculations of green gram seeds with the isolate PsEc20 resulted in increase of root and shoot growth over control by 45.16 and 122.35% higher over control (Table 2).

Sindhu et al. (2002) observed that coinoculation of fluorescent *Pseudomonas* spp. with an effective *Mesorhizobium* strain from chickpea (Ca181) resulted in significant increase in nodule weight and shoot biomass. They suggested that fluorescent pseudomonads have emerged as the largest and potentially the most promising group of PGPR with their rapid growth, simple nutritional requirements, ability to utilize diverse organic substrates and mobility.

14 isolates (PsEc2, PsEc3, PsEc4, PsEc5, PsEc6, PsEc7, PsEc8, PsEc9, PsEc10, PsEc11, PsEc14, PsEc15, PsEc17 and PsEc20) produced only medium amount of ammonia. The isolates PsEc3 and PsEc12 showed pellicle formation in the sub–surface layer of N–free medium and retained their property of growth and pellicle formation on N–free medium upon subsequent sub–culturing which indicated positive result for N–fixation by the isolates. Phosphate solubilization by the isolates ranged from 25.08 µg ml^{-1} (PsEc1) to 76.48 µg ml^{-1} (PsEc17). Thus, PsEc17 was most capable of solubilizing tricalcium phosphate after an incubation of 48 h. IAA production by the isolates ranged from 18.90 µg ml^{-1} (PsEc1) to 78.53 µg ml^{-1} (PsEc17). Thus, PsEc17 was most efficient in IAA production which was followed closely by PsEc15 (75.35 µg ml^{-1}) and PsEc14

Table 2: Plant growth promotion traits of *Pseudomonas* isolates obtained from the rhizosphere of *Euphorbia caducifolia* Haines

Isolates	Germination test[a]		Ammonia-produtionb	IAA Production (µg ml^{-1})[c]	Phosphate solubilization (µg ml^{-1})[c]	Nitrogen fixation	HCN productiond	Antibiotic production	Inhibition of *F. oxysporum* f. sp. *cumini* (%)
	Root length (cm)	Shoot length (cm)							
PsEc1	3.78^{f}	3.42^{pq}	Low	18.90^{l}	25.08^{o}	No	Moderate	Low	32.2
PsEc2	3.98^{e}	4.52^{i}	Medium	22.58^{k}	27.80^{mn}	No	Moderate	Moderate	24.0
PsEc3	3.48^{hi}	3.32^{q}	Medium	22.33^{k}	29.08^{l}	Yes	Low	Low	53.6
PsEc4	3.88^{ef}	4.32^{jk}	Medium	19.58^{l}	30.70^{k}	No	High	Moderate	60.8
PsEc5	3.60^{gh}	3.38^{pq}	Medium	30.50^{g}	34.45^{j}	No	High	No	53.6
PsEc6	3.84^{ef}	3.88^{lm}	Medium	40.63^{e}	26.73^{n}	No	Low	No	35.8
PsEc7	3.44^{hi}	5.52^{e}	Medium	24.25^{j}	27.38^{n}	No	High	Low	32.2
PsEc8	3.54^{hi}	5.02^{h}	Medium	27.08^{h}	39.38^{h}	No	Moderate	Moderate	50.0
PsEc9	3.78^{f}	4.46^{i}	Medium	19.92^{l}	30.60^{k}	No	High	Low	42.9
PsEc10	5.08^{c}	8.36^{a}	Medium	25.27^{ij}	62.65^{c}	No	High	No	60.8
PsEc11	3.38^{ij}	3.98^{l}	Medium	19.27^{l}	33.53^{j}	No	Moderate	No	64.3
PsEc12	3.74^{fg}	3.88^{lm}	Low	19.45^{l}	28.75^{lm}	Yes	High	Moderate	28.6
PsEc13	6.04^{a}	7.02^{b}	Low	35.22^{f}	58.10^{d}	No	High	Moderate	25.0
PsEc14	3.86^{ef}	4.40^{ij}	Medium	74.53^{b}	37.28^{i}	No	High	Low	25.0
PsEc15	4.14^{d}	5.22^{g}	Medium	75.35^{b}	47.42^{e}	No	High	Moderate	39.3
PsEc16	3.28^{j}	3.46^{p}	Low	70.47^{c}	46.25^{f}	No	High	Low	67.9
PsEc17	5.10^{c}	6.22^{c}	Medium	78.53^{a}	76.47^{a}	No	High	Moderate	69.5
PsEc18	3.56^{h}	5.38^{f}	Low	26.15^{hi}	33.47^{j}	No	Moderate	Low	25.0
PsEc19	3.48^{hi}	4.22^{k}	Low	29.50^{g}	44.58^{g}	No	High	Low	35.8
PsEc20	5.68^{b}	6.04^{d}	Medium	63.10^{d}	69.48^{b}	No	High	Moderate	28.6
Control	3.50^{hi}	3.76^{mo}	–	–	–	–	–	–	–
SEm±	0.05	0.04	–	0.42	0.39	–	–	–	–
CD (p=0.05)	0.16	0.13	–	1.17	1.13	–	–	–	–

[a]Average of five observations. Seed germination test (Greengram seedlings)a; [b]Low ammonia production → faint yellow colour; Medium ammonia production → deep yellow colour; High ammonia production → brownish colour; [c]Average of four observations; [d]Low HCN production → yellow colour, Moderate HCN production → orange colour, High HCN production → Brown colour; *Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range test at 5 per cent level of significance

(74.53 µg ml^{-1}) (Table 2).

Pseudomonas is most widely reported as PGPR as a potential to produce plant growth promotor, phytohormones, phosphate solublization, hydrogen cyanide, siderophore, antibiotics, hydrolytic enzymes and antimicrobial compounds (Noori and Saud, 2012). *Pseudomonas nitroreducens* strain IHB B 13561 (PnIHB) enhances the growth of *Arabidopsis thaliana* and *Lactuca sativa* via specific pathways involved in the promotion of cell development and enhancement of nitrate uptake (Trinh et al., 2018). They noted high expression levels of nitrate transporter gene, NRT2.1 (responsible for high-affinity nitrate transport in roots) and cyclin–B1 promoter gene in plants inoculated with PnIHB.

Phosphate solubilizing is explained by the production of various organic acids and enzymes (Chen, 2006). These acids (Gluconic, tartaric, and oxalic acids) and enzymes transform insoluble phosphates into forms that that can be easily assimilated by plants. Indole-3-acetic acid is the most important auxin produced by bacteria, plants and fungi (Sadf-Zouaoui et al., 2007). It initiates root, leaf and

flower development. Its importance lies in its central role in cell division, elongation, fruit development and senescence (Duca et al., 2014). The *Pseudomonas* isolates from apple and pear rhizospheric soil showed auxins, gibberellins and cytokinins production in the range of 1.83–21.00 μg ml^{-1}, 116.10–485.80 μg ml^{-1} and 63.60–90.00 μg ml^{-1} respectively (Sharma and Saikia, 2014). These isolates showed phosphate solubilizing activity in the range of 199.50 to 413.40 μg ml^{-1} and siderophore production in the range of 20–21 mm in plate assay. They identified 2 isolates (An–1–kul and An–13–kul) which possessed multiple PGP (Plant growth promoting) traits. *Pseudomonas* is known to prevail in varied ecosystems. Even *Pseudomonas* isolate obtained from marine water showed multiple plant growth promoting traits (Goswami et al., 2013).

Most of the isolates (PsEc4, PsEc5, PsEc7, PsEc9, PsEc10, PsEc12, PsEc13, PsEc14, PsEc15, PsEc16, PsEc17, PsEc19 and PsEc20) produced high amount of HCN production. However, the isolates PsEc2, PsEc4, PsEc8, PsEc12, PsEc13, PsEc15, PsEc17 and PsEc20 showed moderate antibiotic production whereas other isolates showed low or no antibiotic production. The isolates varied widely in their antifungal activity when tested against the fungus *Fusarium oxysporum* f. sp. *cumini*, the causative agent of wilt disease in cumin. PsEc17 proved most efficient and showed 69.5 % inhibition *Fusarium oxysporum* f. sp. *cumini* whereas, PsEc2 showed the least inhibition (24.0 %) of this fungal pathogen (Table 2).

HCN production was observed in *P. putida* KC010526, *P. putida* KC010527, and *P. putida* KC010528. These bacteria showed phosphate solubilizing capacity and could suppress *Fusarium oxysporum* (Ngoma et al., 2013). One of the important mechanisms of suppression of plant pathogens by PGPR bacteria is through production of siderophore. Siderophores are useful in phytostabilization by facilitation of plant growth and coalescence of metals and reduce metal bioavailability in soil (Chen et al., 2017). Gull and Hafeez (2012) observed that 8 out of 14 *Pseudomonas* isolates showed antagonism against *Rhizoctonia solani* in dual culture assays. Spectrochemical analysis of siderophores indicated that all the bacterial strains produced catecholate siderophores.

Pseudomonas segetis strain P6 proved to act as a potential biocontrol agent due to its plant growth–promoting (PGP) and quorum quenching (QQ) activities (Rodríguez et al., 2020). They detected involvement of QQ activity in enzymatic degradation of signal molecules in quorum sensing communication systems against a broad range of N–acylhomoserine lactones (AHLs). *Pseudomonas fluorescens* was observed to be most effective PGPR strains followed by *Paenibacillus polymyxa*, *Bacillus subtilis* and *Pseudomonas putida,* respectively in inhibited growth of *Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani* in vitro (Sarhan and Shehata, 2014).

Plant Growth Promoting (PGP) 19 *Pseudomonas* isolates increased seed germination and plant growth of tomatoes under organic growing conditions. Growth stimulation mechanisms, including the production of phytohormones, phosphate solubilization, ammonia production and colonization of plant roots, are the most efcacious mechanisms that explain PGPR effects (Suslow and Schroth, 1982, Bruto et al., 2014). Chin-A-Woeng et al. (2000) reported that the ability of *Pseudomonas* isolates to suppress disease relies mainly on their ability to colonize roots. The results revealed a signifcant increase in seed germination due to the mixed bacteria formulation compared to the control. This efect is due to the increased synthesis of hormones linked to growth such as IAA and gibberellins, which triggered the activity of specifc enzymes that promote early germination (Bharathi et al., 2004).

Pseudomonas has a wider capability to adapt to varied climatic conditions. Cold adapted N–fixing *Pseudomonas migulae* S10724 isolated from plant rhizosphere could significantly enhance the growth of native green gram (*Vigna radiata*) at Pantnagar, Indian Shiwalik Himalayas (Suyal et al., 2014). The strain significantly (p\0.05) stimulated the growth of roots (45.3%) and shoots (45.6%) of green gram plants in addition to fresh and dry weight, total chlorophyll and nitrate reductive activity. Walley and Germida (1997) observed enhancement of shoot dry weight from 16 to 48% and root dry weight from 82 to 137% when inoculated with fluorescent pseudomonads. Gupta et al. (2002) reported that peanut seeds bacterized with *Pseudomonas* GRC2 showed a significant increase in germination (83%) under field conditions. *Pseudomonas* do not form a symbiosis similar to that formed by rhizobia with plants, although they are able to penetrate plant tissues and establish themselves as endophytes (MarquezSantacruz et al., 2010).

5. CONCLUSION

Pseudomonas fluroscens, *P. aeruginosa*, *P. putida*, *P. stutzeri* and *P. syringe* were isolated from the rhizospheric soil sample of *Euphorbia caducifolia*. Of these, *Pseudomonas fluorescens* (PsEc17) was most efficient isolate for plant growth promotion activities *in vitro*. PsEc17 produced highest, IAA production, phosphate solubilization, antibiotic production, antifungal activity and HCN production. *Pseudomonas fluorescens* (PsEc17) is proved as best plant growth promoting isolate and can be developed as bioinoculant.

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