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Microbiology

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HARNESSING POTENTIAL PGPR FROM RHIZOSPHERIC SOILS OF OCIMUM SP.

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ABSTRACT

P.fluorescens DDI(I)1 is a gram negative, rod shaped and mesophilic bacterium. The isolate was obtained from the rhizospheric soil of *Ocimum* sp. in Delhi(India). In the present investigation, we found that *Pseudomonas* DDI(I)1 exhibited numerous plant growth promoting properties. These properties include production of ammonia, production of hydrogen cyanide, production of auxin (IAA) and solubilization of phosphorus. These multiple PGP traits of this isolate render it as a potential candidate to be used as a bio-inoculant for crops in sustainable agriculture.

INTRODUCTION

Sustainable agriculture is vitally important as it offers the potential to meet our future agricultural needs. This is something that we could not achieve through conventional agricultural practices. Soil microorganisms capable of enhancing plant growth and health offer a potential way to replace the conventional agricultural practice. Plant root exudates attract microbes and feed them and, in turn, the plants often benefits from the microbes. Plant Growth-Promoting Rhizobacteria (PGPR) are naturally occurring soil bacteria that actively colonizes plant roots and imparts beneficial effects on plant's growth and controlling plant diseases by releasing chemicals in the rhizospheric zone (Tsegaye et al., 2017). PGPR are better than chemical fertilizers as they do not cause pollution (maintaining soil health) as well as pose no threat to human or animal health (conserving biodiversity) (Vandana et al., 2018).

Ocimum belongs to family Lamiaceae. It is widely grown and has high medicinal value. It is also known as tulsi or basil. *Ocimum sanctum* (Linn.) is the most prominent species amongst the genera. Plants have square stems, fragrant opposite leaves and whorled flower on spiked inflorescence. It is native to India and parts of Taiwan, China, North and Eastern Africa. Tulsi is popularly known as "the queen of herbs" or "the elixir of life" and promotes longevity (Cohen et al., 2014). Due to its numerous medicinal properties, we have tried to evaluate the plant growth promoting abilities of rhizospheric bacteria isolated from its rhizosphere.

MATERIALS AND METHODS

SAMPLE COLLECTION AND ISOLATION OF RHIZOS PHERIC BACTERIA

The *Ocimum* plant rhizosphere soil samples were collected from different localities of Delhi, Kurukshetra and Haridwar. The samples were stored in sterile polythene bags and then used within 24 hours for the isolation of rhizospheric bacterial community. The chosen bacterial culture DDI(I)1 was isolated on Kings B medium by serial dilution technique by incubating plates at 28 °C for 3 days. Successive dilutions were made up to 10^{-7} and 0.1 ml aliquot of this diluted suspension was spread on the plates of Kings B agar medium and incubated. Well isolated colonies were re-streaked to make pure culture (Ashrafuzzaman et al., 2009).

MORPHOLOGICAL AND BIOCHEMICAL CHARAC TERIZATION

The bacterial isolate was examined for morphology by visualising the colony characteristics including colony colour, shape, surface, elevation, margin and pigmentation. Gram staining procedure and biochemical tests *i.e.* IMVIC, catalase, sugar fermentation, gelatin liquefaction, urease test and nitrate reduction were performed using standard available protocols (Cappuccino and Sherman, 2010).

SCREENING FOR PLANT GROWTH PROMOTING TRAITS

Ammonia production and Phosphate solubilization test. The bacterial culture was inoculated in 10ml peptone water and incubated for 48 h at 36 ±2 °C. After incubation, nesseler's reagent was added in the sample. Development of brown to yellow color indicated ammonia production (Cappuccino and Sherman, 2010). For qualitative assay of phosphate solubilization, bacterial culture was inoculated on the plates containing Pikovaskaya medium incorporated with tri-calcium phosphate. Formation of clear zone around bacterial growth after 7 days of incubation indicated solubilization of phosphate (Pikovaskaya et al., 1948).

Production of Indole acetic acid. Indole acetic acid (IAA) production by the isolate was assayed colorimetrically (Okon et al., 1977). Bacterial culture was grown in LB medium amended with 100 mgL^{-1} tryptophan by incubating in a shaker at 250 rpm at 28 ± 2 C for 7 days. After centrifugation, 2ml of supernatant was mixed with 4ml Salkowski reagent and absorbance of the resultant pink color was read after 30 min at 535nm in colorimeter.

HCN production. To determine hydrogen cyanide production, bacterial culture was streaked on modified agar plates (nutrient broth ammeded with 4.4g glycine/l). A whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed on top of the plate. Plates were sealed with parafilm and incubated at 36±2 °C for 4 days. Development of orange to red color indicated HCN production (Lorck, 1948).

RESULTS AND DISCUSSION

ISOLATION OF PGPR ISOLATES

Among the 266 isolates obtained from 24 different rhizospheric soil samples of *Ocimum* sp., one of the most promising isolate DDI(I)1 was isolated from the rhizospheric soil sample from *Ocimum* sp. obtained from Delhi(India).

SCREENING FOR PLANT GROWTH PROMOTING TRAITS

Phosphate solubilization and production of ammonia. A very common way to increase the availability of nutrients in the soil is solubilization of minerals. In this study, isolate DDI(I)1 showed clear visible halo around the colony on

Pikovskaya agar media plates (Fig. 1, Table 1). The zone of phosphate solubilization was 22 mm and the solubilization index was found to be 4.4. This is in co-relation with study conducted by Kannahi and Kowsalya (2013). They reported phosphate solubilization by *Pseudomonas fluorescens* and the zone of solubilization was found to be 34.6 mm. *Pseudomonas* sp. PMP and PVP2 showed great P solubilisation efficiency (Biyyani et al., 2016). Another important PGP trait is ammonia production which helps in the growth enhancement of plant. The isolate DDI(I)1 changed the colour to orange in peptone broth which shows significant ammonia production (Fig. 2; Table 1). This is in accordance with the studies conducted by Biyyani et al. (2016) where strong ammonia production was shown by two *Pseudomonas* isolates PLP and DMP1.

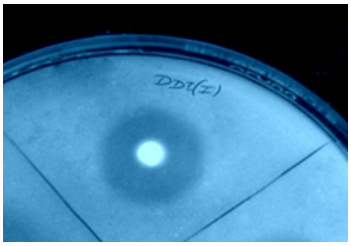

Fig.1 Zone of phosphate solubilization formed around the colony

Fig.2 Ammonia production indicated by orange colour formation in Peptone Broth

Table 1
Plant growth promoting traits of the isolate DDI(I)1.

PGP traits	Response	Observation/justification
Ammonia production	+	Appearance of orange colour
Phosphate solubilisation	+	Zone size = 22 ± 2.64 (mm) Solubilization index = 4.4
HCN production	+	Filter paper turns to orange colour
IAA production	+	22 ± 1.12 µg/ml

Production of Indole acetic acid. Indole acetic acid (IAA) is an important native auxin (Ashrafuzzaman et al., 2009). It plays a major role in seed germination, seedling growth, development of vascular system, *etc*. (Zhao, 2010). Indole acetic acid production was exhibited by isolate DDI(I)1 (Fig. 3;Table 1) .The test was confirmed by the appearance of deep pink colour in the transparent LB broth amended with L-tryptophan amino acid. The isolate produced 22 ± 1.12 g/ml of IAA. Similar findings were reported by Goswami et al. (2013). They recorded that 29µg/mL of IAA was produced by *Pseudomonas* sp. isolated from Olive green. Also, Saranraj *et al.* (2013) recorded maximum IAA production of 28µg/mL by *Pseudomonas fluorescens* PF-8 isolated from paddy rhizosphere. In another study, *Pseudomonas* isolate DMP2 produced 23.4µg/mL IAA (Biyyani et al., 2016). These studies are in co-relation with our findings.

HCN Production. Production of HCN can be considered as a defence regulator against plant pathogens. The isolate showed good production of hydrogen cyanide by changing the colour of the filter paper to orange (Fig. 4; Table 1). HCN producing *Pseudomonas* exhibited increased plant biomass and uptake of nutrients (Selvakumar et al., 2009). Singh et al. (2013) confirmed production of hydrogen cyanide by *P. aeruginosa* and *P. fluorescens*. In a study conducted by Tsegaye et al. (2019), bacterial isolates P. fluorescent biotype G and P. aeruginosa, were positive for HCN production. This also favours our study on production of HCN.

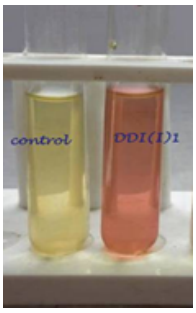

Fig.3 IAA Production shown by isolate DDI(I)1

Fig.4 HCN Production indicated by change of colour of filter paper to orange colour

Morphological and biochemical characterization. On the basis of morphological and biochemical characterization, it was found that the isolate DDI(I)1 belongs to *Pseudomonas* sp. (Table 2).

Table 2
Morphological, Cultural and Biochemical Characteristics of isolate DDI(I)1

Characteristics	Result	Characteristics DDI(I)1	Result
Colony	Yellowish green, small, round, transparent	VP	-
Gram reaction	-ve	Citrate	+
Shape	rods	Catalase	+
Pigmentation	Yellowish green	Oxidase	+
Indole	-	Hydrogen sulphide production	-
Methyl-red	-	Nitrate reduction	-

CONCLUSION

This present study suggested that the *P. fluorescens* DDI(I)1 is able to exhibit multiple plant growth promoting traits such as production of ammonia, hydrogen cyanide production, photohormone synthesis and solubilization of phosphorus. Due to the multiple PGP traits exhibited by our rhizospheric isolate, it may be useful as a bio-inoculant in sustainable agriculture.

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