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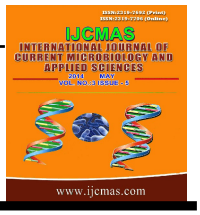

Original Research Article

Screening and characterization of GA_3 producing *Pseudomonas monteilii* and its impact on plant growth promotion

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A B S T R A C T

A total of 59 bacterial isolates were isolated from different rhizospheric soil of four local crops in the vicinity of Surat city, India. These isolates were tested for the gibberellic acid (GA_3) production in a nutrient medium by spectrophotometric method. Out of all these isolates NPB20 produce more gibberellic acid as compared with the others and therefore it was selected for further investigation. The culture filtrate of this bacterium was bioassayed on wheat and chana bean crops and found that it significantly promote the growth in both plants. The isolate NPB20 identified as a *Pseudomonas monteilii* through phylogenetic analysis based on 16s rDNA sequence.

Introduction

Bacteria are abundantly present in the soil, interact with plant roots in the rhizosphere and enhance plant growth and development in certain instances. The plant growth promoting rhizobacteria (PGPR) develop a mutualistic relationship with the host plants and gives a benefit to them through N_2 fixation by nitrogenase, nitrate reductase activity, siderophore production, and phytohormone secretion in the rhizosphere (Fulchieri *et al*., 1993; Cassán *et al*., 2001a, 2001b). Gibberellins production by PGPR promote the growth and yield of many crop plants, deconjugation of gibberellin-glucosyl conjugates exuded by the roots, or in the plant (Piccoli *et al*., 1997), and 3β-hydroxylation by bacterial enzymes of inactive 3-deoxy gibberellins present in roots, to active forms such as GA_1, GA_3, and GA_4 (Piccoli *et al*., 1996; Cassán *et al*., 2001a, 2001b). GAs have been identified and isolated from higher plants, fungi and bacteria. It was reported that 136 GAs from higher plants (128 species), 28 GAs from fungi (7 species), and only 4 GAs (GA_1, GA_3, GA_4, and GA_{20}) from bacteria (7 species) have been identified

till recently (MacMillan, 2002). The current study was carried out to find the gibberellin producing capacity of rhizospheric soil bacteria.

Materials and Methods

Isolation of Rhizobacteria

For isolation of rhizobacteria, plants were carefully dug out without damaging the roots along with the adherent soil and were brought to the laboratory in polythene bags. The soil particles loosely adhering to the roots were gently teased out and used for isolation of rhizobacteria. The samples were inoculated within 6 h of collection. Soil samples (1 g) as described above were mixed in 100 ml sterile distilled water and shaken for 20 min to get the rhizosphere suspension. Similarly, roots with tightly adhering soil particles were cut into small pieces and 1 g of these root pieces were vigorously mixed in sterile 100 ml distilled water and shaken for 20 min to get the rhizoplane suspension. Rhizosphere and rhizoplane suspensions thus obtained were serially diluted up to 10^{-3}. For the isolation of rhizobacteria, 0.1 ml from each dilution was plated on nutrient agar plate supplemented with 70 µg/ml of Clotrimazole to inhibit fungal growth. The plates were incubated at 30°C for 24 h for isolation of rhizobacteria. Morphologically distinct bacterial colonies from each plate were purified by repeated sub - culturing and maintained on Nutrient agar media and stored at 4˚C until used.

Screening of the isolates (Rhizobacteria) for gibberellin production

A total of 59 rhizobacteria obtained as above were screened for their ability to produce gibberellins. 100 ml nutrient medium was dispensed in 250 ml conical flasks and inoculated with rhizobacteria. The culture flasks were incubated at 35˚C for 48 h. 48 h old growth of bacterial culture was centrifuged at 10,000 rpm for 15 - 20 min. The pH value of culture supernatants were adjusted to 2.5 using stock 3.75 N HCl. The culture supernatants were extracted using liquid-liquid (ethyl acetate/$NaHCO_3$) extraction method. The amount of gibberellic acid in the ethyl acetate phase was measured by the UV spectrophotometer at 254 nm against control blank.

Identification of microorganism

Identification of potential gibberellin producing bacterial isolate was carried out by molecular identification based on 16S rDNA sequencing technology.

The bacterial isolate NPB20 was identified as a *Pseudomonas monteilii*, on the basis of partial 16S ribosomal DNA (rDNA) sequence. The chromosomal DNA was isolated through standard procedures (Sambrook and Russel, 2001).

The almost complete 16S rDNAs were PCR amplified using the universal primers which were complementary to the 5' end and 3' end of the prokaryotic 16S rDNA, respectively. The amplification reaction was performed as previously described (Adachi *et al*., 1996). The BLAST search program (http://www.ncbi.nlm.nih. gov/BLAST/) was used to look for nucleotide sequence homology of this bacterial isolate. The closely related sequences were aligned by CLUSTAL W using MEGA version 4.0 software, and the neighbor-joining tree was generated using same software.

Plant growth promoting capacity of microbial isolate

Seeds of wheat *(Triticum aestivum* L.) and Chana bean (*Cicer arietinum*) were surface sterilized with 0.1% aqueous solution of mercuric chloride (Mineo 1990) and treated with 100 ppm uniconazol (Khan *et al.* 2008). Seeds were germinated in sterilized Petri plates lined with absorbent cotton moistened with double distilled water. A 1ml (aliquot) of bacterial CF suspension was applied to the seedlings. The wheat and Chana bean plants were grown in a controlled environment chamber. Germination (%), length of root and length of shoot parameters were observed after 7 days of CF treatment and compared with controls (Distilled water). Various calculations viz. % seed germination, % root growth, germination index were carried out using these data (Tam & Tiquia, 1994).

Results and Discussion

Isolation of rhizobacteria

A total of 59 morphologically distinct rhizobacteria designated as NPB 1-59 had been isolated from rhizosphere and rhizoplane samples of Rice (*Oryza sativa*). Rhizosphere and rhizoplane support greater microbial community as compared to the non rhizosphere soil (bulk soil) because plant roots secrete various nutrient-rich compounds (e.g., sugars, amino acids, vitamins, organic acids) into the surrounding by "rhizodeposition", which create nutritional enrichment around roots creating unique environment for soil microorganisms (Compant *et al.*, 2005).

Screening of the isolates (rhizobacteria) for gibberellin production

Many of the isolates produce gibberellic acid and the production of GA_3 was in the range of 7.50 μg/ml to 93.93 μg/ml. Out of them 18 isolates produced less than 25 μg/ml, 13 isolates produced in between 25 to 50 μg/ml and 10 isolates produced more than 50 μg/ml amount of Gibberellic acid. The minimum potential was shown by NPB 38 (7.50μg/ml) whereas isolate NPB 20 produced 93.93μg/ml, which was significantly more than the other isolates. Gibberellins producing ability is inherent in all groups of microorganisms including epiphytic and rhizospheric bacteria. (Bastián *et al*., 1998; Gutierrez-Manero *et al*., 2001; Cassan *et al.* 2001, Mitter *et al.*, 2002). GA analysis of the culture filtrates of microbial isolates showed that the bioactive GA_3 production capacity of NPB 20 was higher than others, which narrates the significance of this isolated microbial strain. On the basis of these results, isolate NPB 20 was selected for further study.

Identification of GA_3 producing bacteria

Microorganisms comprise a diverse group of living organisms and due to the possible existence of different morpho/biotypes of microbes within single species, traditional morphological and biochemical methods are not considered reliable for identification.

On the other hand, DNA sequence analysis methods are objective, reproducible and provide rapid identification, and thus gaining importance. Many rDNA genes are highly conserved for the members of the same taxonomic group, and therefore are used extensively for identification. (Kim and Lee, 2000; Lee *et al.*, 2001; Sugita and Nishikawa, 2003). Molecular identification carried out on the base of 16S rDNA gene for bacteria.

Figure.1 Identification of bacterial isolate NPB 20 by phylogenetic analysis.

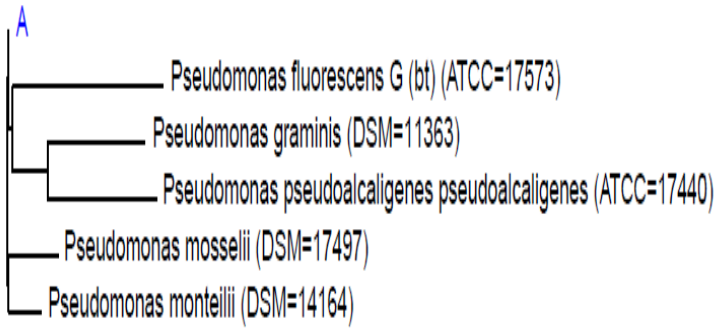

Table.1 Effect of microbial extracts on growth attributes of wheat and chana bean plants

Crops	Days→ Isolates↓	Shoot Length (cm)	Root Length (mm)	Germination index
Wheat	Control	22.42	43.33	100
	Pseudomonas monteilii	22.37	43.67	
Chana	Control	19.33	112.67	97.72
	Pseudomonas monteilii	21.37	111.67	

On the basis of 16S rDNA sequences comparison with the available sequences at GenBank, EMBL sequences, NPB 20 shows higher homology (98%) to *Pseudomonas monteilii*. (Figure 1) The 16S rDNA sequence was submitted to NCBI GenBank and was given accession no. KF719177.

Plant growth promoting capacity of microbial isolate

The microbial isolate was bioassayed on wheat and chana for its growth promoting capacity in terms of seed germination, shoot length, and root length of crop plants. Wheat *(Triticum aestivum* L.) and Chana bean (*Cicer arietinum*) were chosen for bioassay experiment as they lack seed dormancy, show high germination rate and are easily available.

Seed germination rate of Wheat, and Chana seed was recorded in the form of germination index which depends upon seed germination% and root growth % presented in table 1. There was no germination observed in negative control as in that seeds were treated with uniconazol and irrigated with distilled water. Test samples in which seeds were uniconazol and irrigated with culture filtrate of isolate showed the significant germination. Since uniconazol blocks gibberellin synthesis during seed germination, the shoot length and root length of seedlings is associated with microbial metabolite activity (Choi *et al*. 2005).

The microbial isolate was bioassayed on wheat and chana for its growth promoting capacity. The microbial broth suspension of NPB20 significantly promoted growth of wheat and chana seedlings. In both crops, seed germination, the root length, and shoot length parameters significantly promoted compared to positive control. Current results confirm previous reports of shoot length promotion through microbial culture filtrate treatment (Choi *et al*. 2005).

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