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Research Article

Rhizomonas: A plant growth promoting bacteria isolated from agricultural soil

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Abstract

In the present study a bacteria was isolated from agricultural soil near Ahmednagar, Mahatrashtra and identified as *Rhizomonas* species by morphological and biochemical tests. It was further tested for plant growth promoting activities like indole acetic acid production, hydrogen cyanide production, ammonia production, nitrogen fixation. The bacteria were able to produce indole acetic acid, hydrogen cyanide and ammonia. It was also able to grow on nitrogen free media, which indicated that it can fix atmospheric nitrogen. The effect isolate on wheat plant was tested by pot assay and data was analyzed by student t test. The P values for mean shoot length and mean root length were 0.00428 and 0.00688 respectively. The isolate significantly increased root and shoot length of wheat plant.

INTRODUCTION

As agricultural production intensified over the past few decades, producers became more and more dependent on agrochemicals as a relatively reliable method of crop protection helping with economic stability of their operations. However, increasing use of chemical inputs causes several negative effects (Gerhardson, 2002). Furthermore, the growing cost of agrochemicals, particularly in less-affluent regions of the world, and consumer demand for pesticide-free food has led to a search for substitutes for these products (Weger *et al.*, 1995). There has been a large body of literature describing potential uses of plant associated bacteria as agents stimulating plant growth and managing soil and plant health (Naphade and Shaikh, 2014).

Plant growth-promoting bacteria (PGPB) are associated with many plant species and are commonly present in the soil. PGPB enhances the plant growth by variety of ways (Hetal *et al.*, 2011). These ways include like nitrogen fixation, phosphate solubilisation, indole acetic acid production, hydrogen cyanide production, ammonia production etc. (Kumar *et al.*, 2012; Thokal *et al.*, 2013).

There are many bacteria which are identified as plant growth promoting bacteria but we have selectd and isolated *Rhizomonas* from agricultural field soil and studied for it plant growth promoting characters.

MATERIALS AND METHODS

Sample collection

Sample was collected from agricultural field: Latitude: 19.1028°N, Altitude: 74.7265°E, Ahmednagar, Mahatrashtra. The sample was collected in sterile vial and stored at 4^0C until use.

Enrichment and isolation

For enrichment of bacteria 0.1 gram of sample was inoculated in 250 ml capacity conical flask containing1 00 ml of sterile nutrient broth. The flask was then incubated at 37ºC for 24h on rotary shaking incubator at 200 rpm. After incubation the bacteria was isolated on nutrient agar plates.

Identification

The isolate was identified by morphological and biochemical characteristics as per the Bergey'sManual of Determinative Bacteriology (Holt *et al*., 1994).

Plant growth promoting activities

After the isolation, isolate was tested for their ability to produce indole acetic acid (IAA), hydrogen cyanide (HCN) production, ammonia production and nitrogen.

Production of indole acetic acid

Bacterial culture was grown for 24h in nutrient media supplemented with typtophan (0.1%) at 37^0C. After incubation cell free culture (2ml) was mixed with 4ml of Salkowski's reagent and incubated for 30 min at room temperature in dark. Development of cherry red colour indicates IAA production (Gordon *et al*., 1951).

Production of ammonia

The isolate was grown in peptone water tube and incubated at 30°C for 4 days. 1 mL Nessler's reagent was added in each tube. Tubes were observed for presence of a yellow to brownish colour for maximum production of ammonia (Cappuccino *et al*., 1992).

Production of HCN

The isolates were screened for the production of hydrogen cyanide by method suggested by Lorck (1948). Nutrient agar was amended with 0.44% glycine. A Whatman filter paper no.1 soaked in 2% sodium carbonate and 0.5% picric acid solution was placed inside the plate without touching to agar surface. Plates were sealed with parafilm and incubated for 24-72 h at 37ºC. After incubation plates were observed for brown colour formation which indicates HCN production Ana Marques *et al*., (2010).

Nitrogen fixation

Nitrogen fixing ability of the isolates was assessed by streaking the isolate on Norris agar plates. The plates were then incubated at 37ºC for 24 h. And after incubation the plates were observed for growth (Kumar *et al.*, 2012).

Effect of isolate on wheat plant

Effect of isolate on growth of wheat plant was tested by pot experiment. Isolate was inoculated in the nutrient broth and incubated at 37ºC for 24 h. Surface sterilized wheatseeds are treated with 24h old bacterial culture for 10 minute. 5 treated seeds were then transferred in separate pot containing sterile soil. Seeds treated with sterile distilled water were also transferred in separate pot and used as control. The pots were watered with sterile distilled water timely. After 20 days root and shoot length of plant was noted and data was analyzed by student t test.

Results and discussion

Isolation and identification

After incubation on nutrient agar plate one dominant colony was selected for further study. The colony was re streaked on nutrient agar plate and colony characters were recorded (Table 1).

Table 1. Colony characteristics of isolate.

Sr. No.	Colony characters	Results
1	Size	1 mm
2	Shape	Circular
3	Colour	Yellowish
4	Margin	Entire
5	Elevation	Convex
6	Opacity	Opaque
7	Consistency	Sticky
8	Gram nature	Gram negative short rod
9	Motility	Motile

Biochemical characteristics

Isolated *Rhizomonas* shows positive catalase and oxidase test and able to fermented glucose, fructose, sucrose, maltose and ribose with the production of acid but the lactose. *Rhizomonas* also gave a positive nitrate reduction test.

Plant growth promoting traits

Production of Indole acetic acid

When 0.5 mL of cell free broth was mixed with 2ml of salkowaski's reagent and incubated for 30 minute in dark, formation of cherry red colour was observed, it means the isolate was able to produce indole acetic acid. An extensive work is carried out on IAA production by bacteria. IAA production by *Bacillus* and *Pseudomonas* sps. was reported by Minaxi *et al.* (2011); Kumar *et al.*, (2012) and Jangu and Sindhu (2012). Yasmin *et al.* (2009) reported higher IAA production in the presence of precursor L-tryptophan and Joseph *et al.* (2007) studied IAA production by *Bacillus*, *Pseudomonas* and *Azotobacter*, *Rhizobium*. Ashrafuzzaman *et al.* (2009) reported that the IAA production was also influenced by cultural conditions, growth stage and substrate availability.

Production of ammonia

After addition of Nessler's reagent, a dark brown colour was developed in test sample where as a pale yellow colour was observed in control tube.

It indicates isolate was able to produce ammonia.Production of ammonia is the important trait of PGPR, which may indirectly influence the plant growth. Isolate produce ammonia which is important in nitrogen cycle for utilization of atmospheric nitrogen. *Joseph et al.,* (2007) isolated *Pseudomonas* species from soil it produced ammonia as a plant growth promoting trait.

Production of HCN

After 5 days, brown colour was developed on filter paper. It indicated, isolate was able to produce hydrogen cyanide. HCN plays an important role in plant defence against insects and animals by inhibiting cellular respiration and also prevents the ATP production. Selvakumar *et al.,* (2009) isolated *Pseudomonas* fragi CS11RH1from rhizospheric soil formed brown colour which indicates HCN production.

Nitrogen fixing ability

When isolate was streaked on Norris agar plate and incubated for 24h at 37^0C growth was observed. It means that the isolate was able to fix the nitrogen. Riggs *et al.* (2001) isolated number of PGPR strains such as *Azoarcussp.*, *Beijerinckiasp.*, *Klebsiella Pneumoniae*, *Pantoeaagglomerans* and *Rhizobium sp*. which are able to fix atmospheric N_2 in soil and make it available to plants. Parmar and Dadarwal (1999) recorded fluorescent Pseudomonads to promote nodulation in chickpea and latter demonstrated the role of this group of rhizosphere microbiota in N_2 fixation. Biological nitrogen fixation is a component of sustainable agriculture (Gachande and Khansole, 2010)

Fig 1: IAA production by *Rhizomonas*

Fig2: Ammonia production by *Rhizomonas*

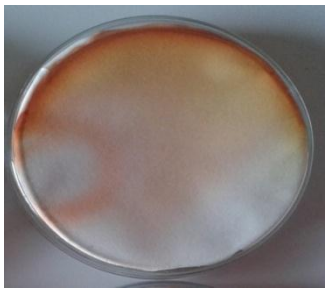

Fig3: HCN production by *Rhizomonas*

Effect of isolate on plant

Mean shoot and root lengths of control and test plants were measured and standard deviation was calculated(https://www.easycalculation.com/statistics/standard-deviation.php). Data was analyzed with the help of student t test. Mean shoot length of test was obtained 19.9 and mean shoot length of control was obtained 9.48. Mean root length of test was 6.99 while mean root length of control was 2.35. The P values for root and shoot length was calculated by using student t test (www.studentttest.com).

Table 2: Root and shoot length of wheat plant

Sr. No.	Parameter / Plant	Shoot length (cm) ± SD					Root length (cm) ± SD				
		1	2	3	4	5	1	2	3	4	5
1	Test	20 ± 4.06	20.8 ± 4.06	16.4 ± 4.06	14 ± 4.06	24.5 ± 4.06	4.73 ± 2.00	5.54 ± 2.00	6.8 ± 2.00	6.9± 2.00	10 ± 2.00
2	Control	11.7 ± 1.34	9.5± 1.34	8.7± 1.34	9.3± 1.34	8.2 ± 1.34	2.06 ± 0.39	2.45 ± 0.39	2.03 ± 0.39	2.23 ± 0.39	3.0 ± 0.39

Table 3: P values for root and shoot length of wheat plant

Sr. No.	Parameter / Plant	Mean value (cm)	
		Shoot length	Root length
1	Test	19.9	6.99
2	Control	9.48	2.35
3	P value	0.00428	0.00688

The data was fond significant a 95 % confidence interval. This indicates the isolate can exert a significant effect on growth of plant. Form the present study it is clear that *Rhizomonas* is a plant growth promoting bacteria.

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