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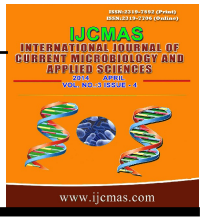

Original Research Article

Plant growth promoting rhizobacterial strains from rice rhizospheric soil

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A B S T R A C T

Rhizospheric microorganisms promoting growth of rice are well known Plant growth promoting rhizobacteria. 10 zones of district Durg of Chhattisgarh state were selected for present study. Among 140 sites only 10 Strains were isolated and characterized on the basis of their morphology, biochemical tests. Strains were isolated on LB agar media. The spread plate technique was used to isolate and purify all the isolates. The characteristics of the bacterial strains were determined using the colony morphology, gram staining as well as biochemical properties. All isolates were screened for plant growth promoting activities such as siderophore production, indole-3-acetic acid production and phosphate solubilizaton

Keywords

Biochemical test,
IAA,
Temperature.

Introduction

Rice (*Oryza sativa*) is a cereal foodstuff which forms an important part of the diet of many people worldwide. Rice, which is being cultivated for several years in the Indian sub continent, is just not a grain, it is life line. Rice rhizosphere contains a high diversity of plant growth promoting bacteria. Microbial diversity in soil is considered important for maintaining the sustainability of agriculture production systems. However, the links between microbial diversity and ecosystem processes is not well understood. The rhizosphere is a region of intense microbial activity where root exudates allow the development of many rhizosphere communities.

Assessing both structural and functional diversity is fundamental in order to fully understand the dynamics of the rhizosphere microbial communities. Functional diversity refer to the number of functional groups in a community Specially, the distinct processes of functions that can potentially be performed by the microbial communities describe the functional diversity, whereas, functional redundancy is the measure of the number of different species within functional group. Several microorganisms are able to promote the plant growth.

Materials and Methods

Sample collection and isolation of bacteria

10 distantly located zones or villages of rice fields were selected from Durg districts of Chhattisgarh state India, 14 locations were marked for this study. rhizospheric soil were collected in a sterilized polythene bags and brought immediately to laboratory for isolation of PGPR.

Isolation enumeration of Rhizobacteria from rice plant root

Bacterial isolates were isolated from the rhizosphere soil of rice filed of district Durg. To estimate the no. of soil microflora using the pour plate methods and triplicate samples of 1gm. Soil, and an appropriate dilutions.

Growth under different temperature conditions

The culture of 10 isolates were streaked on LB agar plates and incubated at 10, 20, 28, 37 and 45°C. The change in growth and colour was observed and recorded after 3 days of incubation.

Biochemical characterization

The biochemical characterization of all the 10 isolates of PGPR were essentially done as per the procedure outlined in Dubey R.C. and Maheshwari D.K.(2006). The test conducted is detailed below.

(i) **Amylase test**
(ii) **Catalase test**
(iii) **Urease test**

Plant growth promoting mechanism Siderophore assay

Siderophore was detected by the formation of orange halos surrounding bacterial colonies on CAS agar plates after 48 hour at 28.c

Phosphate solubilisation

Phosphate solubilisation detected by formation of transparent halos surrounding bacterial colonies on the Pikovskaya agar after 72 hours incubation at 28.c

IAA

Bacterial cultures were incubated in Luria Bertani broth at 28°C. The bacterial cells were removed from the culture medium by centrifugation at 8000xRPM for 10min. 1ml of supernatant was mixed vigorously with 2ml. of salkowaski's regent (4.5 gm. Of Fecl3 per liter in 10.8 M H2So4) and incubated at room temperature in the dark for 30 min. and take absorbance at 530nm.

Results and Discussion

Out of 140 collected samples from ten sites at total Ten bacterial isolates were successfully isolated from the rhizosphere soils of rice field (Table 1). They were designated as As shown in Table 2, the morphological characteristics of PGPR isolates widely varied.. All the isolates produced round shaped and raised colonies having smooth shiny surface with smooth margin. They differed in colour but all were odour less. No pigmentation was observed in the colonies of LB agar plates. Diameter of the colonies of isolates varied from 0.2 to 2 mm.

Table.1 Location of rhizospheric soil

S.No.	Location of rhizospheric soil	pH of the rice field water	Soil
1	Utai	5.7	Loam +clay
2	Machandur	5.9	Loam +clay
3	Khopli	6.1	Loam +clay
4	Selud	6.4	Loam +clay
5	Patan	6.2	Loam +clay
6	Dhamdha	5.6	Loam +clay
7	Dhaur	6.9	Loam +clay
8	Jamgaon	6.4	Loam +clay
9	Nagpura	5.4	Loam +clay
10	Kumhari	5.8	Loam +clay

Table.2 Morphological characteristics of PGPR isolate

Isolate	Shape	Size (mm)	Elevation	Surface	Margin	Colour	Odour	Pigmentation
AK1	Round	**0.2-0.9**	Raised	Smooth shiny	Smooth	Off whitish	odorless	none
AK2	Irregular	**0.8-1.0**	depressed	Smooth shiny	Smooth	Off whitish	odorless	none
AK3	Round	**0.8-1.2**	Round	Smooth shiny	Smooth	Brownish	odorless	none
AK4	Round	**0.9-1.4**	Round	Smooth shiny	Smooth	Yolk Brown	odorless	none
AK5	Round	**1.2-1.7**	Round	Smooth shiny	Smooth	Brownish	odorless	none
AK6	Round	**1.6-1.8**	Round	Smooth shiny	Smooth	Yellowish	odorless	none
AK7	Round	**1.5-1.7**	Round	Smooth shiny	Smooth	Yolk yellowish	odorless	none
AK8	Round	**1.7-2.0**	Round	Smooth shiny	Smooth	Whitish	odorless	none
AK9	Round	**1.4-1.7**	Round	Smooth shiny	Smooth	yellowish	odorless	none
AK10	Round	**1.3-1.8**	Round	Smooth shiny	Smooth	Off white	odorless	none

Table.3 Characteristics of bacteria

Isolate	Cell shape	MOTALITY	Gram Stain
AK1	ROD	Motile	Gram (-)ve
AK2	ELLIPSOIDAL	Motile	Gram (-) ve
AK3	ROD	Motile	Gram (-) ve
AK4	ROD	Motile	Gram (-) ve
AK5	ELLIPSOIDAL	Motile	

Table.4 Growth on different temperature

Isolate	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C	45°C	50°C
AK1	-	-	-	+	+	+	-	-	-	-
AK2	-	-	-	+	+	+	+	-	-	-
AK3	-	-	-	+	+	+	+	+	+	+
AK4	-	-	-	+	+	+	+	+	+	+
AK5	-	-	-	+	+	+	+	+	+	-
AK6	-	-	+	+	+	+	+	-	-	-
AK7	-	-	-	+	+	+	-	-	-	-
AK8	+	+	+	+	+	+	-	-	-	-
AK9	-	-	-	+	+	+	-	-	-	-
Ak10	-	+	+	+	+	+	-	-	-	-

Table.5 Characteristics of PGPR isolates

Isolate	IAA Production	Phosphate solubilization	Siderophore Production
AK1	-	-	-
AK2	-	-	+
AK3	-	-	+
AK4	+	-	-
AK5	-	-	-
AK6	+	-	-
AK7	+	-	+
AK8	+	+	+
AK9	+	-	-
AK10	+	-	-

Microscopic observations were performed to investigate the some characteristics of PGPR isolates such as shape, gram reaction and motility (Table 3). Eight isolates were rod shaped while AK2 and AK5 showed ellipsoidal shape. All the isolates were motile and gram negative in reaction. It was also noted that the growth of isolates on LB agar plates varied in temperature (Table 4). The growth of all isolates was good in the temperature ranges of 20 to 30°C. In addition, AK3

and AK4 isolates were found to grow at 50°C.

We investigated the IAA production, phosphorus solubilisation and siderophore production of PGPR isolates. Isolates AK4, AK6, AK7, AK8, AK9 and AK10 induced the production of IAA. Isolates AK8 was found to be good producers of IAA. On the contrary, AK1, AK2, AK3 was found to be weak producer. On the other hand, only AK8 isolate had ability to solubilize the phosphorus and AK2, AK3, AK7 and AK8 can produce Siderophore (Table 5).

PGPR colonize plant roots and exert beneficial effects on plant growth and development by a wide variety of mechanisms. To be an effective PGPR, bacteria must be able to colonize roots because bacteria need to establish itself in the rhizosphere at population densities sufficient to produce the beneficial effects. IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. IAA may function as important signal molecule in the regulation of plant development. Ten isolates, six isolates are positive for IAA production viz. AK4, AK6, AK7, AK8, AK9 and AK10.

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. Most of phosphorus in soil is present in the form of insoluble phosphates and cannot be utilized by the plants. The ability of bacteria to solubilize mineral phosphates has been of interest to agricultural microbiologists as it can enhance the availability of phosphorus and iron for plant growth. PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to rice that represent a possible mechanism of plant growth promotion under field conditions. In our experiments, only AK8 isolate was able to solubilize phosphate in the rhizosphere soil. Furthermore, this isolate was found to be medium producer of IAA. It is important to note that several phosphate-solubilizing bacilli occur in soil. But their numbers are not usually high enough to compete with other bacteria commonly established in the rhizosphere. A large body of evidence suggests that PGPR enhance the growth, seed emergence and crop yield, and contribute to the protection of plants against certain pathogens and pests. Ten isolates, two isolatesAK8 and AK5 showed better performances in aspects of seed germination and growth of seedlings with IAA production (Table 5). In addition to increment of seed germination and growth of seedlings by isolate AK8 , it was positive for both phosphorus solubilization and IAA production. These results suggest that the increased growth of rice seedlings by application of PGPR is probably due to induction of IAA production and phosphorus solubilization. Taken together, results suggest that PGPR are able to induce the production of IAA, solubilization of phosphorus, and resistance to pathogens and pests, thereby improving growth of plants.

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