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ISOLATION AND CHARACTERIZATION OF PGPR RICE RHIZOSPHERE FOR PHOSPHATE SOLUBILIZATION

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ABSTRACT

Plant growth promoting rhizobacteria(PGPR) are the bacteria that colonize rhizosphere and plant root and enhance growth of plant by variety of mechanism. Certain PGPR used as biofertilizers might be beneficial for rice cultivation as they promot root and shoot growth thus improving seedling vigour which significantly increases the yield of grain. Pertaining to these facts in the present study plant growth promoting rhizobacteria which are compatible to rice plant root are isolated, characterized and screened for their phosphate solubilizing property. Total 18 rhizobacterial isolates were isolated and out of which 9 isolates were found to be phosphate solubilizers and considered as PGPR. These isolstes must be used as biofertilizers to attain significant productivity and soil fertility.

INTRODUCTION

Rice is the most important staple staple food of Chhattisgarh. Application of chemical fertilizers along with organic manure and inorganic fertilizers is common practice for rice cultivation in this area. A wide variation in productivity on Chhattisgarh at the district level is due to varied agroclimatic condition in which rice is grown and also the level of inputs used. Among various inputs chemical fertilizers are critical one and have direct influence on the productivity of rice. In order to sustain production to meet the requirement of increasing population productivity per unit area is either increased or there must be increase in hybrid rice cultivation or high yielding rice variety (Jena). But the high yielding rice variety requires large amount of chemical fertilizers. These probably leads to environmental pollution and also harmful for human health. So, for sustainable rice cultivation and to lower the input of chemical fertilizers will be beneficial and eco-friendly(Ashrafuzzaman et al, 2009). Now a days application of microbial biofertilizer in agriculture is getting wide attention which helps to enhance the growth of plants and also involve in cleaning up the environment(Trivedi *et al*, 2003). These bacteria fix nitrogen biologically, solublize phosphorous and induce some plant growth hormones that contribute to the improvement of rice growth (Karnwal, 2009; Muhammad *et al*., 2012: Binod *et al*., 2012). Thus the application of plant growth promoting rhizobacteria as inoculants for biofertilization will be a better alternative to decrease the chemical fertilizers input and protect environment(Ali *et al.*, 2010). The main objective of the present study is to isolate, char-

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acterize and screen the plant growth promoting rhizobacteria for its growth promoting activities and to develop them as a biofertilizers.

MATERIALS AND METHOD

Isolation of rhizobacteria

Rhizospheric soil sample of two months old rice plant were collected from two different rice field of Durg district in a sterile plastic bag. A composit sample of rhizospheric soil is used for isolation of rhizobacteria. 10 g of soil sample is suspended in 90 mL of sterile distilled water. Serial dilution technique was performed upto 10-7 dilution. Nutrient Agar Medium was used for isolation of rhizobacteria in sterile petriplates. The petriplates were incubated in bacteriological incubator for 24-48 hrs at 37 °C (Aneja, 2001). Isolated colonies were steaked to obtain single isolation and transferred to nutrient agar slants to make pure culture.

Characterization of isolates

Total number of 18 rhizobacterial isolates were obtained by pure culture and designated as RB1, RB2, RB3, RB4, RB5, RB6, RB7, RB8, RB9, RB10, RB11, RB12, RB13, RB14, RB15, RB16, RB17 and RB18. All these were prilimnary identified on the basis of their morphological characterization such as shape, margin, elevation, colour and surface texture. All the rhizobacterial isolates were biochemically characterized for gram's reaction, catalase test, starch hydrolysis, urease hydrolysis and imvic test as per the standard method (Dubey *et al.,* 2006).

Screening of rhizobacterial isolates for phosphate solubilization

The rhizobacterial isolates were spot inoculated in petriplates containing Pikovskya's medium (Pikovskaya, 1948) in bacteriological incubator for 7 days at 28°C. The isolates forming a clear halo zone were considered as phosphate solubilizers.

RESULT AND DISCUSSION

The study has been undertaken to select the efficient rhizobacterial isolates for plant growth promotion of rice plant. Total eighteen rhizobacterial isolates were isolated and differentiated by their morphological and biochemical characters. The result shows different colony morphologies. Some bacteria are irregular and some are round in shape. Surface texture is generally found rough and few smooth, margin is entire, wavy and undulate. Color of isolates are offwhite, whitish cream and yellow. Biochemical characters of all the rhizobacterial isolates also varies. Out of 18 isolates 9 isolates shows gram positive and 9 shows gram negative reaction. 6 rhizobacterial isolates shows catalase positive and 12 isolate shows catalase negative test. The rhizobacterial isolates RB1, RB2, RB3, RB$, RB5, RB12, RB14, RB17, RB18 hydrolyse starch and RB1, RB2, RB3, RB4, RB5, RB7, RB8, RB11, RB12, RB14, RB15, RB16, RB17, RB18 hydrolyses urea. The biochemical characteristics of rhizobacterial isolates is summarized in Table 2.

Phosphate solubilization

Further result shows 9 rhizobacterial isolates produce halo zone around it in Pikovskya's agar medium which are considered as phosphate solubilizers.

The outcome of present study shows the existence of phosphate solubilizing bacteria in the rhizospheric soil of rice pla t. Similar to our finding phosphate solubilizing bacteria were reported by many other workers from rice rhizosphere and also from the rhizospheric soil of other plants. Rhizobacteria Kurthia is isolated and characterized for phosphate solubilizing activity from the rhizospheric soil of tea plant from Darjaleeng Hills(Sharma *et al*., 2012). Phospate solubilizers were also isolated from the rhizospheric soil of mash bean(Qureshi *et al*., 2012), tall fescus (Monk *et al*., 2009), rice grown in acidic soil(Thakuria et al 2004), rice crop of eastern Uttar Pradesh (Shahi *et al*., 2009) and legume plants(Khan *et al*., 2010).

CONCLUSION

Present study on the rhizospheric bacterial isolates of rice plants indicate that isolates are plant growth promoting rhizobacteria which plays an important role in phosphate solubilization and thus enhance the growth of plants. So the rhizospheric soil enriched with these microbial inoculants will probably increase the soil fertility and replace chemical fertilizers for sustainable farming of rice. In conclusion it is suggested that the rhizobacterial isolates having best characteristics of plant growth promotion must be commercially identified since identification of theserhizobacterial isolate may lead to the introduction of new organism that can be applied as a biofertilizer. Further studies are required to investi-

Table 1. Morphological characterization of rhizobacterial isolates

S. No.	Rhizobacterial Isolates	Shape	Color	Margin	Elevation
1.	RB1	Rod	Whitish cream	Entire	Raised
2.	RB2	Rod	Whitish cream	Entire	Flat
3.	RB3	Rod	Whitish cream	Entire	Raised
4.	RB4	Rod	Pale white	Irregular	Raised
5.	RB5	Cocci	White	Irregular	Convex
6.	RB6	Rod	Creamy white	Entire	Raised
7.	RB7	Rod	Brown	Lobed	Convex
8.	RB8	Rod	Whitish cream	Entire	Flat
9.	RB9	Cocci	Creamy white	Filamentous	Raised
10.	RB10	Rod	Pale white	Irregular	Flat
11.	RB11	Rod	Pale white	Irregular	Raised
12.	RB12	Cocci	Creamy white	Filamentous	Convex
13.	RB13	Cocci	Pale white	Lobed	Convex
14.	RB14	Cocci	Pale white	Lobed	Flat
15.	RB15	Rod	Yellow	Entire	Flat
16.	RB16	Rod	Whitish cream	Irregular	Raised
17.	RB17	Rod	Pale white	Entire	Flat
18.	RB18	Rod	Creamy white	Entire	Flat

Table 2. Biochemical characterization of rhizobacterial isolates

S. No.	Rhizo-bacterial Isolates	Gram's reaction	Catalase test	Amylase test	Urease test
1.	RB1	+	+	+	+
2.	RB2	+	+	+	+
3.	RB3	+	-	+	+
4.	RB4	+	+	+	+
5.	RB5	+	-	+	+
6.	RB6	-	+	-	-
7.	RB7	-	-	-	+
8.	RB8	-	-	-	+
9.	RB9	-	+	-	-
10.	RB10	-	+	-	-
11.	RB11	-	-	-	+
12.	RB12	+	-	+	+
13.	RB13	-	-	-	-
14.	RB14	+	-	+	+
15.	RB15	-	-	-	+
16.	RB16	-	-	-	+
17.	RB17	+	-	+	+
18.	RB18	+	-	+	+

Table 3. Phosphate soubilization shown by rhizobacterial isolates

S. No.	Rhizobacterial Isolates	Gram's reaction
1.	RB1	-
2.	RB2	+
3.	RB3	-
4.	RB4	+
5.	RB5	-
6.	RB6	-
7.	RB7	-
8.	RB8	-
9.	RB9	+
10.	RB10	+
11.	RB11	+
12.	RB12	-
13	RB13	-
14	RB14	+
15	RB15	+
16	RB16	+
17	RB17	+
18	RB18	-

gate the phosphate solubilizing efficiency of these rhizobacterial isolates in the fieild condition.

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