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Screening of Phosphate solubilizing bacteria from rhizosphere of Maize plant

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ABSTRACT

Some soil bacteria like *Azotobacter, Azospirillum, Pseudomonas* are capable of solubilizing phosphorus. Plant cannot uptake insoluble phosphate. They require inorganic phosphate in soluble form. Solubilization of phosphorus is the basic need for plant growth. In the present study, phosphorus solubilizing efficiency and phosphorus solubilization index of an isolate of soil bacteria was estimated. The isolate was isolated from rhizosphere of Maize plant from a local agriculture field. Morphological, Gram staining, Biochemical and Sugar fermentation study of isolate was performed. The isolate was oval shaped forming circular pigmented colony on Ashby's medium. It was Gram negative. Catalase, Oxidase, VP and NR positive, Indole and MR negative. Sugar fermentation tests revealed that the isolate was Glucose, Mannitol and Arabinose positive, Fructose, Sucrose and Raffinose negative. The phosphorus solubilizing efficiency observed as 11.33 and the solubilizing index was 4.58.

Key Words - Solubilizing efficiency, Solubilization index, Biochemical test, Gram staining

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INTRODUCTION

Plant requires Nitrogen, Phosphorus and Potash as macronutrients. These are the major requirements of plant. Phosphorus is the major component of DNA, RNA, ATP, Phospholipids and several enzymes needed for the plant life. Phosphorus remain in inorganic form or organic form in the soil but plant cannot absorb Phosphorus from these compounds. Plant absorb Phosphorus in soluble inorganic form. Some soil bacteria are able to solubilize Phosphorus such as *Azotobacter, Azospirillum, Pseudomonas, Pantoea, Enterobacter, Bacillus, Rhizobium*, etc.. The concentration of Phosphorus in soil is low. To overcome it, phosphorus fertilizer are added in the field. The need to phosphorus fertilizer is increasing. Chemical fertilizer is not eco-friendly. They gradually deteriorate soil fertility and causes pollution. This problem may be solved by the application of phosphate solubilizing bacteria. These microorganisms solubilize inorganic phosphate by secreting low molecular weight organic acid in which phosphate salt dissolve (Goldstein, 1995).

Microbes like *Azotobacter, Azospirillum, Rhizobium, Bacillus, Pseudomonas*, etc. are known as Plant growth promoting rhizobacteria (PGPR). Several of these bacteria produces antimicrobial substance, fixes atmospheric Nitrogen, solubilize Phosphorus, produces phytohormones and thus maintain plant health.

MATERIALS & METHODS

Soil samples were collected from rhizosphere of Maize plants. Samples were diluted up to the 10^{-5} dilution and inoculated in Ashby's medium, PKV medium, NFB medium and Aleksandrow medium. It was incubated at 30°C for 24 hours. Colonies and cell morphology were studied. By repeated culture, pure isolate was obtained and tested for Gram staining, Biochemical test and Sugar fermentation test. On the basis of these tests, isolate was identified as described in Bergey's manual of Systematic bacteriology (2012).

Isolation of Phosphorus solubilizing bacteria:

For isolation of Phosphorus solubilizing bacteria, 1gm soil sample was mixed thoroughly in 10m autoclaved water and serial dilution was made up to 10^{-5} dilution. Diluted soil sample was inoculated in Pikovskaya medium and incubated at 30°C for 3 days. Colonies exhibiting clear zones were selected as phosphorus solubilizer. Selected colonies were plated on Nutrient agar for obtaining pure culture. The pure culture was again inoculated in PKV medium and its solubilizing efficiency was confirmed.

Estimation of Solubilizing Index and Solubilizing efficiency:

Qualitative analysis of Phosphorus solubilizing activity of isolate was calculated by measuring Halozone and Colony diameter on PKV medium. Halozone and Colony diameter were measured using Calipers. Solubilizing efficiency (SE) and solubilization index (SI) were calculated by the formula:-

$$\mathrm{SE} = \frac{\text{Halozone} - \text{Colony diameter}}{2}$$

$$\mathrm{SI} = \frac{\text{Halozone}}{\text{Colony diameter}}$$

RESULT

The cells of isolate were oval, motile and colonies were circular, pigmented. The isolate was Gram negative. Catalase, Oxidase, VP and NR positive, Indole and MR negative. Sugar fermentation tests revealed that the isolate was Glucose, Mannitol and Arabinose positive, Fructose, Sucrose and Raffinose negative. The result is mentioned in Table No. 01.

Table 1- Gram staining, Cell and colony morphology, Biochemical test and Sugar fermentation test of isolates

Cell Morphology	Colony Morphology	Gram Staining	Biochemical test		Sugar Fermentation test	
Oval, Motile	Large, Circular, Pigmented	Negative	Catalase	+ve	Glucose	+ve
			Oxidase	+ve	Fructose	-ve
			Indole	-ve	Sucrose	-ve
			Methyl Red	-ve	Lactose	-ve
			Urease	-	Mannitol	+ve
			Nitrate Reductase	+ve	Raffinose	-ve
			VP	+ve	Arabinose	+ve

Solubilization index and solubilization efficiency were calculated by the formula given in Materials and Methods. The solubilization efficiency was measured as 11.33mm and solubilization index was 4.58 mm. The result is mentioned in Table No. 02.

Table 2- P-Solubilization efficiency and P-solubilization index of isolate

Colony Size (mm)	Solubilization zone (mm)	Efficiency (mm)	Solubilization Index
6.33	29.00	11.33	4.58

CONCLUSION

Soil sample was collected from rhizosphere of Maize plant from a local agriculture field and cultured in Ashby's medium. By repeated culture, pure culture was obtained and its morphological, gram staining, biochemical test and sugar fermentation tests were performed. It was tested for phosphorus solubilizing efficiency. The isolate was Gram negative. Catalase, Oxidase, VP and NR positive, Indole and MR negative. Sugar fermentation tests revealed that the isolate was Glucose, Mannitol and Arabinose positive, Fructose, Sucrose and Raffinose negative.

Solubilization index and solubilization efficiency were calculated by the formula given in Materials and Methods. The solubilization efficiency was measured -11.33mm and solubilization index was 4.58 mm.

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