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Original Article

Isolation and characterization of phosphate solubilizing bacteria from paddy field of Bhilai region

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Abstract

A study was conducted the microorganism is isolated from paddy field site for phosphate solubilizing ability. In the initial investigation bacterial isolate has shown clear zone in phosphate solubilizing medium. The isolate was characterized for gram's staining and biochemical tests based on Bergey's Manual. In microscopic examine we have obtain gram positive rod like colony that characterized by biochemical activities like- Motility, Methyl Read, VogesProskaur (VP), Catalase, Starch Hydrolysis, Gelatin hydrolysis, Urea hydrolysis, Citrate utilization, Indole production, Carbohydrate fermentation test. The isolate was screened for their phosphate solubilizing capacity in PSB medium. Result indicates that *bacillus* species is the good source of insoluble phosphate to soluble phosphate in soil.

Keywords: Paddy soil, *Bacillus* spp., PSB medium.

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INTRODUCTION

The concentration of soluble phosphate in soil is usually very low which leads to deficiency of soluble phosphate and make it a limiting factor in plant nutrient (Forster, 1995). Lower the quantity of phosphate solubilizing microorganisms (PSM) play an important role in supplementing phosphorus to the plant, allowing a sustainable use of phosphate fertilizer (Gaur, 1990). A Sufficient supply of phosphorus in the early stages of plant growth promotes physiological functions including early root formation, and is important for laying reproductive parts of plants. It is vital to seed formation and its content is higher in seeds than in any other part of the plant. It helps plants to survive winter rigors and also contributes to disease resistance in some plants. Assimilation of phosphate from organic compounds by plants and microorganisms take place through the enzyme "phosphatase" which is produced by in microorganisms. Phosphate can be absorbed by plants only in soluble form which is available in very less amount in soil. The transformation of insoluble phosphate into soluble form is done by several microbes present in the soil. A large fraction of soil microbes can dissolve insoluble inorganic phosphates present in the soil and make them available to the plants. There is high population of microorganisms are present in fertile soil and they are ubiquitous and at the same time highly proliferating. A single plant growth promoting phosphate solubilising bacterium may possess one or more than one of these plant beneficial traits (Bhattacharyya and Jha, 2011).

MATERIAL AND METHODS

Sample Collection: Soil samples were collected from Paddy fields at Bhilai, District-Durg, and Chhattisgarh, India. The composite soil samples were taken to the laboratory in sterile polythene bags and analyzed within 10-12 hr.

Processing of Sample: 1.0 gm of soil was weighed and mixed with 10 ml sterile distilled water in test tube. Test tube was shaken vigorously for 5 to 10 min to form homogenous suspension. The soil solution than was allowed to settle down for 10-15 min before further processing.

Isolation of Phosphate Solubilizing Bacteria (PSB): 1.0 gm of fine powdered soil sample was dissolved in 10 ml of sterilized distilled water and rinsed thoroughly for 5mins. From this first dilution 1 ml was transferred to 9ml of sterile distilled water to form 10^{-1} dilution. Similarly 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions were made for each soil sample. 0.1 ml from 10^{-3}, 10^{-4} and 10^{-5} dilutions was taken by sterile pipette and transferred to in Petri-dishes containing sterilized and cooled medium (containing glucose 10.0 g, yeast extract 0.5 g, $Ca_3(PO_4)_2$ 2.5 g, $CaCl_2$ 0.1 g, $MgSO_4$. $7H_2O$ 0.25 g and agar 18.0 g in one litre of distilled water with pH of 7.0) and incubated at 37°C for 2 days. (A. Gandhi *et al.*, 2014) Colonies showing halo zones were picked and purified further using same medium and maintained in nutrient agar (Lapage *et al.*, 1970) (containing Beef extract 3.0 g, Peptone 5.0 g, NaCl 5.0 g, Agar 18.0 g in 1000 ml distilled water with pH of 7.2) slants and preserved under refrigerated (4°C) condition for further study.

Microscopic Study of Bacteria: Size, shape, arrangement and gram's nature of the isolates were studied. For grams staining smear was prepared from the isolated culture on clean slide and stained. The stained smear was observed under microscope (Amit Sagervanshi *et al.*, 2012).

Identification of Bacterial isolates through Biochemical tests: Biochemical test were performed as suggested by microbiology practical books, which include following tests Gram staining, IMViC reaction, Catalase test, Starch hydrolysis test, Gelatin hydrolysis test, Urea hydrolysis test, Carbohydrate fermentation test (Glucose, Fructose, Lactose, and Sucrose)**.**

DISCUSSION AND RESULT: Concentration of soluble phosphate in soil is usually very low which leads to deficiency of soluble phosphate and make it a limiting factor in plant nutrient. In the present investigation soil sample was collected from specific area (Paddy field) because of the possibility of occurrence of phosphate solubilizing microbes. For initial growth of micro flora PSB medium was used. Serial dilution was performed to isolate the single colonies. In present investigation the organism is capable of doing phosphate solubilization; it is given clear zone around the colony which can be reported that is phosphate solubilizing microorganisms. As per their morphological and staining and biochemical methods the colony represents *Bacillus* sps.

Table 1: Colony characterization of the isolate

Test	Appearance
Size	Small
Shape	Round
Margin	Entire
Elevation	Raised
Surface texture	Smooth
Consistency	Moist
Optical Character	Translucent
Pigmentation	White

Identification of Bacterial isolates through Biochemical tests: Strain was subjected to the biochemical test for their identification.

CONCLUSION

Phosphorus is the essential macro nutrient source for plant growth promotion. Our present study follows the isolation and identification of phosphate solubilizing bacteria from paddy soil. Sample was collected from paddy field of Bhilai at Chhattisgarh state. These were brought to then the laboratory and isolation were done on the basis of gram's staining. Biochemical characterizations were performed with this isolate. The isolate was screened for their phosphate solubilizing capacity in PSB medium. The result shows the isolate is *Bacillus* spp.

Table 2: Biochemical characterization of the isolate

Biochemical Test	**Strain**
Gram's Staining	+ (Rod)
Motility	-
Methyl Read Test	+
VogesProskaur (VP) Test	+
Catalase Test	-
Starch Hydrolysis Test	-
Gelatin hydrolysis test	-
Urea hydrolysis test	+
Citrate utilization test	+
Indole production test	-
Carbohydrate fermentation test Glucose Fructose Sucrose Lactose	 + + + -
Identification:	***Bacillus* sp.**

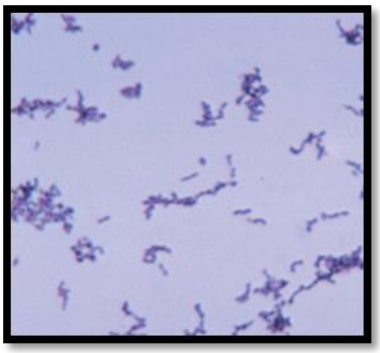

Fig 1: Gram's staining- *Positive rod*

Fig 2: Methyl Red Test- *Positive*

Fig 3: VP Test- *Positive*

Fig 4: Urea Utilization Test- *Positive*

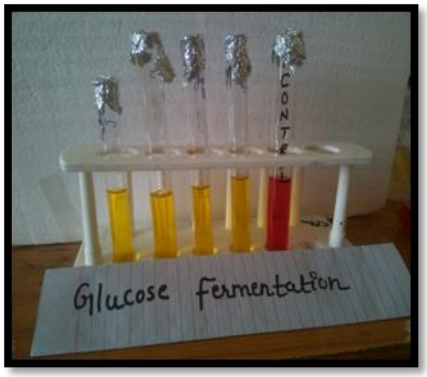

Fig 5: Glucose Fermentation Test- *Positive*

Fig 6: Citrate Utilization Test- *Positive*

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