

Isolation of Phosphate solubilizing Bacteria from agriculture soil collected from of Saharsa district.

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ABSTRACT

In the present study isolates of phosphate solubilizing bacteria were isolated from agriculture soil of Bathnaha Village under Saharsa district. Altogether six isolates were isolated from soil out of which two belong to *Azotobacter chroococcum*, one belongs to *Pseudomonas fragi* and three belong to *Bacillus subtilis*. Isolates were cultured in PVK medium and Solubilizing efficiency (SE) and Solubilizing Index (SI) were calculated on the basis of Halozone and colony diameter. SE varied from 10.49 to 12.29 and SI varied from 4.309 to 4.598. Maximum value at SE and SI was recorded for isolate no. 6 at *Bacillus subtilis*.

Key Words - Phosphate solubilizing bacteria, Solubilizing Efficiency, Solubilizing Index

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INTRODUCTION

Phosphate is one of the most important macro nutrients required by plants after nitrogen (Anand *et al.*, 2016). Phosphorus is the important component of several metabolic pathways of plants including photosynthesis, respiration, signal transduction (Sharma *et al.*, 2013). The concentration of soluble phosphorus in soil is usually very low ranging from 400-1260 mg/kg (Fernandez 2014). Soil contains sufficient quantity of phosphorus in insoluble form to which plant cannot take up. Plant can take up soluble form of phosphorus to overcome this difficulty phosphate fertilizers are used in agriculture system. Their demand is increasing up to 2.5% to 3.0% per year (Brindraban *et al.*, 2020). Chemical fertilizer has a drawback that its soluble form immobilizes in soil and became unavailable to plants (Kumar *et al.*, 2017). A large number of microbes including Bacteria and Fungi are able to soluble form to soluble form. This property of microbes motivated for the study of their phosphate solubilizing

capacity (Wendimu *et al.*, 2023). Phosphate solubilizing bacteria are more efficient in phosphate solubilization in phosphate solubilizing fungi (Paul *et al.*, 2013). There are several soil bacteria capable of phosphate solubilization including species of *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Burkholderia* etc. These bacteria colonize in rhizosphere of growing plants. These bacteria are termed as plant growth promoting rhizobacteria (PGPB).

MATERIAL & METHODS

Soil samples were collected from agriculture fields of Bathnaha Village and brought to laboratory in polythene bags. Samples were diluted up to 10⁻⁵ dilution in sterilized distilled water. Diluted samples were inoculated in PKV medium and incubated at 30°C for 24hrs. colonies exhibiting clear zones were pick up and incubated in nutrient agar medium to get pure culture cell and colony morphology were studied: - Gram staining, Biochemical test and Sugar fermentation test were performed for each

isolate. Isolates were identified on the basis of above characters with the help of standard monograph.

Determination of Solubilizing Efficiency (SE) and Solubilizing Index (SI): -

For the determination at SE and SI pure culture at isolates were inoculated in PKV medium and inoculated at 30! for 24 Hrs. Diameter of colonies and clear zone (Halozone) were measured with calipers. SE and SI were calculated by following formula:

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

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

RESULT & DISCUSSION: -

Although 6 isolates were isolated out of which two belong to *Azotobacter chroococcum*, one belongs to *Pseudomonas fragi* and three belong to *Bacillus subtilis*.

Isolate no. 1 and 2 belong to *Azotobacter chroococcum*, isolate no.3 belong to *Pseudomonas fragi* isolate no.4,5 and 6 belong to *Bacillus subtilis*. Isolate of *Azotobacter* and *Pseudomonas* were gram -ve while isolates of *Bacillus* were gram +ve. Morphology and gram reaction of isolates are mentioned in Table 1, Biochemical test in Table 2, and sugar fermentation test in Table 03. All 6 isolates were able to solubilize phosphate. Hilda and Fraga (1999) also reported the isolates of *Pseudomonas* are able to solubilize phosphate. In present study isolate no.03 of *Pseudomonas fragi* showed phosphate solubilization. Solubility efficiency of this bacterium was observed as 12.16 and solubility index as 4.374. Solubility efficiency and Solubility index was highest in isolate no.6 of *Bacillus subtilis*. It was observed that during phosphate solubilization pH of medium decreased. This drop of pH is due to production of organic acid by phosphate solubilizing bacteria (Rasid *et al.*, 2004).

Table 1- Morphology and Gram Staining of isolates

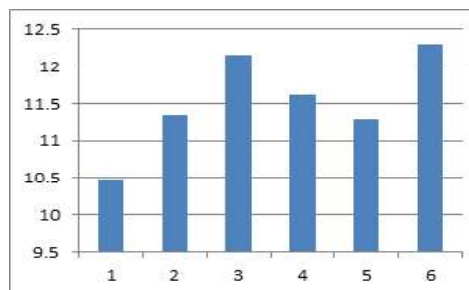
Isolate	Cell Shape	Colony Morphology	Gram Staining
1	Oval	Circular and white	-ve
2	Oval	Elevated	-ve
3	Spherical	Circular	-ve
4	Rod	Irregular	+ve
5	Rod	Irregular	+ve
6	Rod	Irregular	+ve

Table 2- Biochemical test of isolates

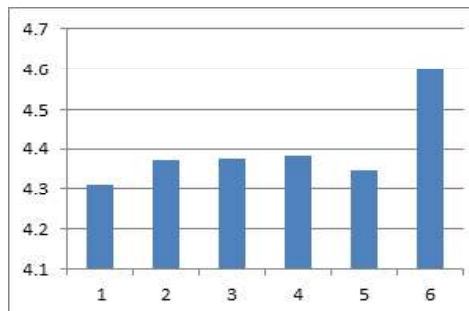
Isolate	Cation	Oxidation	Indole	Citrate	MR (Methyl Red)
1	+ve	+ve	-ve	+ve	-ve
2	+ve	+ve	-ve	+ve	-ve
3	+ve	+ve	-ve	+ve	-ve
4	+ve	-ve	-ve	+ve	-ve
5	+ve	-ve	-ve	+ve	-ve
6	+ve	-ve	-ve	+ve	-ve

Table 3- Sugar fragmentation test of isolates

Isolate	Glucose	Lactose	Sucrose	Mannitol	Arabinose
1	+ve	-ve	+ve	+ve	-ve
2	+ve	-ve	+ve	+ve	-ve
3	-ve	-ve	+ve	+ve	-ve
4	+ve	-ve	-	-ve	-ve
5	+ve	-ve	-	-ve	-ve
6	+ve	-ve	-	-ve	-ve



Graph 1-Solubilizing efficiency at isolates



Graph 2-Solubilizing index at isolates.

Phosphate solubilizing bacteria are isolated on PVK medium on the basis of Halozone formation (Ahmad *et al.*, 2008). In present study also phosphate solubilizing bacteria were isolated on PVK medium.

CONCLUSION

Phosphorus is an important macronutrient for plants. Plants can take up soluble phosphorus from soil which is in very low quantity in soil. Soil has abundant insoluble phosphorus which is not available to plants. Phosphate solubilizing bacteria solubilize insoluble phosphorus and make it available to plants. In the present study six isolates of bacteria were isolated from agriculture soil of Bathnaha village which were able to solubilize phosphate. Out of six isolates two belong to *Azotobacter chroococcum*, one belongs to *Pseudomonas fragi* and 3 belongs to *Bacillus subtilis*. Solubilizing Efficiency (SE) and Solubilizing Index (SI) of all isolates were calculated. SE varied from 10.49 to 12.29. Maximum SE recorded for isolate no. 6 of *Bacillus subtilis*. SI varied from 4.309 to 4.598. Isolate no 1 to 3 were gram negative 4 to 6 were gram positive. Biochemical test and sugar fermentation test were performed for all isolates and identified on the basis of these characters.

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