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Screening of *Azotobacter strains* from rice plant rhizosphere for phosphate solubilizing properties

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ABSTRACT

Azotobacter is a plant growth promoting bacteria which solubilize phosphorous and make it available for plants. In the present study the efforts have been taken to fulfill the aim to isolate, characterize and screen *Azotobacter* for phosphate solubilization from the rice rhizosphere of Durg district. Total 8 strains of *Azotobacter* were isolated from different rice fields. It was observed that all the strains were found to be phosphate solubilizing but in different quantity. RAZB6 is highest phosphate solubilizer (234.4±1mg/L) and RAZB4 shows minimum (82.09±1mg/L) quantity of phosphate solubilization. Since phosphorous is necessary for the plants proper development and it increases the growth of rice plant and make soil more fertile for other plants growth also so they must be commercialized and used more and more in the crop fields other than the agrochemicals.

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KEY WORDS : Azotobacter, Rice, Phosphate, Soil fertility.

Introduction

India is rich in various natural resources; land resource is one among them in which various agricultural activities are based. With the increase in human population there occurs increase in the need for agricultural products, due to which the modern agriculture practices such as use of agrochemicals for better productivity also increased. But excessive use of agrochemicals caused negative effect on environment, plant as well as human health. It also causes soil infertility, pesticide contamination and decreases the structure quality of soil. It also affects soil microorganism which plays an important role in plant growth. Soil contains natural reserves of plants nutrients but these reserves are largely present in different forms & Phosphorous is one of the major plant growth nutrients after nitrogen. Although it is present abundantly in soil in organic as well as inorganic form yet it is not available to plants until it is present in the free forms. Some groups of microorganism play an important role to make available the soil phosphorous to plants.

The application of biofertilizers in agriculture is serving as the alternate source of nutrient for plant and leads to sustainable agriculture. But still many farmers are not aware of its use and benefits as they only rely on the use of chemical fertilizers. The presence of plant growth promoting N_2 fixing bacteria and the possibilities of a significant increase in plant performance and yield under nutrient limiting conditions by root-associated bacteria have been discussed for many years. Especially rice as important food source for billions of people in the centre of interest. The estimation for biological nitrogen fixation in rice is quite divergent but with that phosphorus solubilization has the same importance it directly affects the plant growth[15]. In present study *Azotobacter* was isolated and studied for its phosphate solubilizing property. *Azotobacter* a gram negative oval or spherical shaped free living soil bacteria. It is mostly found in neutral and alkaline soils[3,17]. In microscope the cells of *Azotobacter* can be seen in the form of irregular culture or chain of various lengths. Cells are motile due to the presence of flagella[20]. But later become non-motile and produce a thick mucus layer forming the cell capsule[21].

Phosphorous is second essential element required by plants for their growth after nitrogen. It is important constituent of nucleic acid, energy rice components such as ADP, ATP, phosphorous, sugar phosphates *etc.* in plants. Deficiency of phosphorous in plants results in the stunted growth, premature leaf fall, necrosis, slow protein synthesis *etc.* Therefore phosphorous present in soil must

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TABLE-1: Morphological Characterization of *Azotobacter* isolates.

S. No.	*Azotobacter* isolates	Shape	Color	Margin	Elevation	Odor	Pigmentation
1.	**RAZB1**	Irregular	Pale white	Entire	Convex	Odorless	No Pigment
2.	**RAZB2**	Irregular	Pale white	Entire	Convex	Odorless	No Pigment
3.	**RAZB3**	Irregular	Pale Yellow	Undulate	Convex	Odorless	No Pigment
4.	**RAZB4**	Irregular	Pale white	Entire	Convex	Odorless	No Pigment
5.	**RAZB5**	Irregular	Pale white	Entire	Convex	Odorless	Brown Pigment
6.	**RAZB6**	Irregular	Pale white	Entire	Convex	Odorless	No Pigment
7.	**RAZB7**	Irregular	Pale white	Entire	Convex	Odorless	Brown Pigment
8.	**RAZB8**	Irregular	Pale white	Entire	Convex	Odorless	No Pigment

be naturally available to plants, which can be achieved by some plant growth promoting microorganisms present in soil and colonize plant roots. *Azotobacter* is one among them. It is considered as plant growth promoting *rhizobacteria* as it promotes the growth of plant by number of mechanisms and can be utilized as a Biofertilizer which will be low cost, renewable source of plant growth Thus biofertilizer is ecofriendly which can be used as the best alternative for better crop yield[18]. In present times Biofertilizers are gaining momentum recently due to increasing emphasis on maintenance of soil health, minimize environmental pollution and cut down on the use of the chemicals in agriculture[16]. *Azotobacter* is found to produce plant growth hormone auxin[30] and solubilize phosphorous[2,5]. It is non symbiotic nitrogen fixing bacteria[32]. Today also various farmers are not aware of its uses and benefits and thus they believe in agrochemical fertilizers only. Therefore more research in the field of agriculture is needed. *Azotobacter* was found to increase plant biomass, height *etc.*[1]. Thus considered as important microorganism in the present study.

Materials and Methods

All the laboratory experiments were conducted in Department of Botany, Swami Shri Swaroopanand Saraswati Mahavidyalaya, Hudco Bhilai (C.G.). The chemicals used for present experiments were purchased from Himedia laboratories Pvt. Ltd. and Loba Chemie Laboratory reagents and fine chemicals. All the glasswares used were Borosil made.

Collection of soil sample: The soil samples from Rhizosphere region of soil were collected from different rice fields of ten different areas of Durg district and they were collected in a sterile polythene bags and brought to laboratory for the isolation of *Azotobacter*.

Isolation of *Azotobacter*: The *Azotobacter* was isolated from Rhizosphere soil from rice fields of 10 different areas of Durg district. *Azotobacter* from the rhizosphere soil was isolated by making a series of dilution of soil from 10^{-1} to 10^{-7} [10]. Then grown on Ashby's Agar medium (Composition*- Ingredients in Gms / Litre, Mannitol 20.000, Dipotassium hydrogen phosphate 0.200, Magnesium sulphate 0.200, Sodium chloride 0.200, Potassium sulphate 0.100, Calcium carbonate 5.000 , Agar 15.000 Final pH at 25°C 7.4±0.2) and incubation for 48 h at 30ºC.Bacterial culture was repeated for three times to obtaining the purity of the cultured isolate of bacteria[10].

Culture maintenance: To maintain the culture the bacteria were regularly sub-cultured in Ashby's agar slants.

Characterization Study:

1. **Morphological characterization:** All the Phosphate solubilizing bacterial isolates were checked for their purity and then studied for the colony morphology. The cell shape and gram reaction was also recorded as per the standard procedures. Different characteristics of colonies such as shape, size, elevation, surface, margin, color, odor,pigmentation,

TABLE-2 : Biochemical characterization of Azotobacter isolates.

S. No.	*Azotobacter* isolates	Gram's reaction	Catalase test	Oxidase test	Urease Test	Citrate test	Indole test	Starch hydrolysis	MR test	VP test	Gelatin test
1.	RAZB1	_	+	+	+	+	+	+	+	_	_
2.	RAZB2	_	+	+	+	+	+	+	+	_	_
3.	RAZB3	_	+	+	+	+	+	+	+	_	_
4.	RAZB4	_	+	+	+	+	+	+	+	_	_
5.	RAZB5	_	+	+	+	+	+	+	+	_	_
6.	RAZB6	_	+	+	+	+	+	+	+	_	_
7.	RAZB7	_	+	+	+	+	+	+	+	_	_
8.	RAZB8	_	+	+	+	+	+	+	+	_	_

+ Indicates Presence, - Indicates Absence

TABLE-3: Solubilization Efficiency of phosphate by *Azotobacter* isolates

S. No.	*Azotobacter* isolates	Growth	Colony Diameter in cm	Clearance Zone in cm	Solubilization Index	Solubilization Efficiency
1.	**RAZB1**	+	0.6	0.9	1.05	150
2.	**RAZB2**	++	0.8	1.0	1.25	125
3.	**RAZB3**	+++	1.0	1.6	1.6	160
4.	**RAZB4**	+	0.3	0.6	2.0	200
5.	**RAZB5**	+	0.6	1.0	1.67	166
6.	**RAZB6**	+++	1.0	1.9	19	190
7.	**RAZB7**	+	0.6	0.8	1.34	133
8.	**RAZB8**	++	0.8	1.0	1.25	250
9.	**Control**	-	-	-	-	-

etc. were recorded.

2. **Biochemical characterization of *Azotobacter*:** All the strains of *Azotobacter* were biochemically characterized for (a) Catalase, (b) Oxidase test, (c) Urease, (d) Starch hydrolysis, (e) Gelatin test (f) carbohydrate fermentation & (g) Indole test[12].

Screening of *Azotobacter* isolates for phosphate solubilization:All the isolates of *Azotobacter* were screened for phosphate solubilization. The test *Azotobacter* cultures were inoculated in Pikovaskaya's medium composition in petriplates. The plates were then incubated at 37 ºC for 14 days in an incubator. A plate without inoculation of *Azotobacter* was kept as control. *Azotobacter* showing white turbid zone around them *i.e.* phosphate solubilizing zone is selected for further studies. The diameter of zone with colony was measured by using a scale.

The quantitative estimation or abilities of the isolated phosphate solubilizing bacterium to solubilizer TCP on Pikovskaya's agar media Composition - Ingredients in Gms / Litre, Yeast extract 0.500, Dextrose 10.000, Calcium phosphate 5.000, Ammonium sulphate 0.500, Potassium chloride 0.200, Magnesium sulphate 0.100, Manganese sulphate 0.0001, Ferrous sulphate 0.0001, Agar 15.000) was determined in terms of solubilization index (SI). Phosphate solubilization index was calculated by measuring the colony diameter and the halo zone diameter and the colony diameter, using the formula[31].

Phosphate Solubilization Index (SI) = Colony diameter + Halo zone diameter/Colony diameter.

Quantitative estimation of phosphate solubilization: The phosphate solubilization potential of the *Azotobacter* strain was estimated in Pikovaskayas broth media with 5gm/ml amount of tricalcium phosphate. All the *Azotobacter* were cultured in broth Pikovaskayas medium for 14 days at 37ºC. A test tube without bacterial strain was also kept as control. After 14 days the media was centrifuged at 10,000 rpm for 10 min. in a cooling centrifuge. The quantity of phosphorous was determined in supernatant using UV Visible spectrophotometer following the procedure of [13]. A change in pH of medium due to the growth of *Azotobacter* was recorded.

Observation and Result

Total 8 strains of *Azotobacter* were isolated and named RAZB1, RAZB2, RAZB3, RAZB4, RAZB5, RAZB6, RAZB7 and RAZB8. The *Azotobacter* isolated was maintained by their regular subculture in agar slants of Ashby's medium. Except one i.e. RAZB3 which was pale yellow in colour all other isolates were recorded pale white

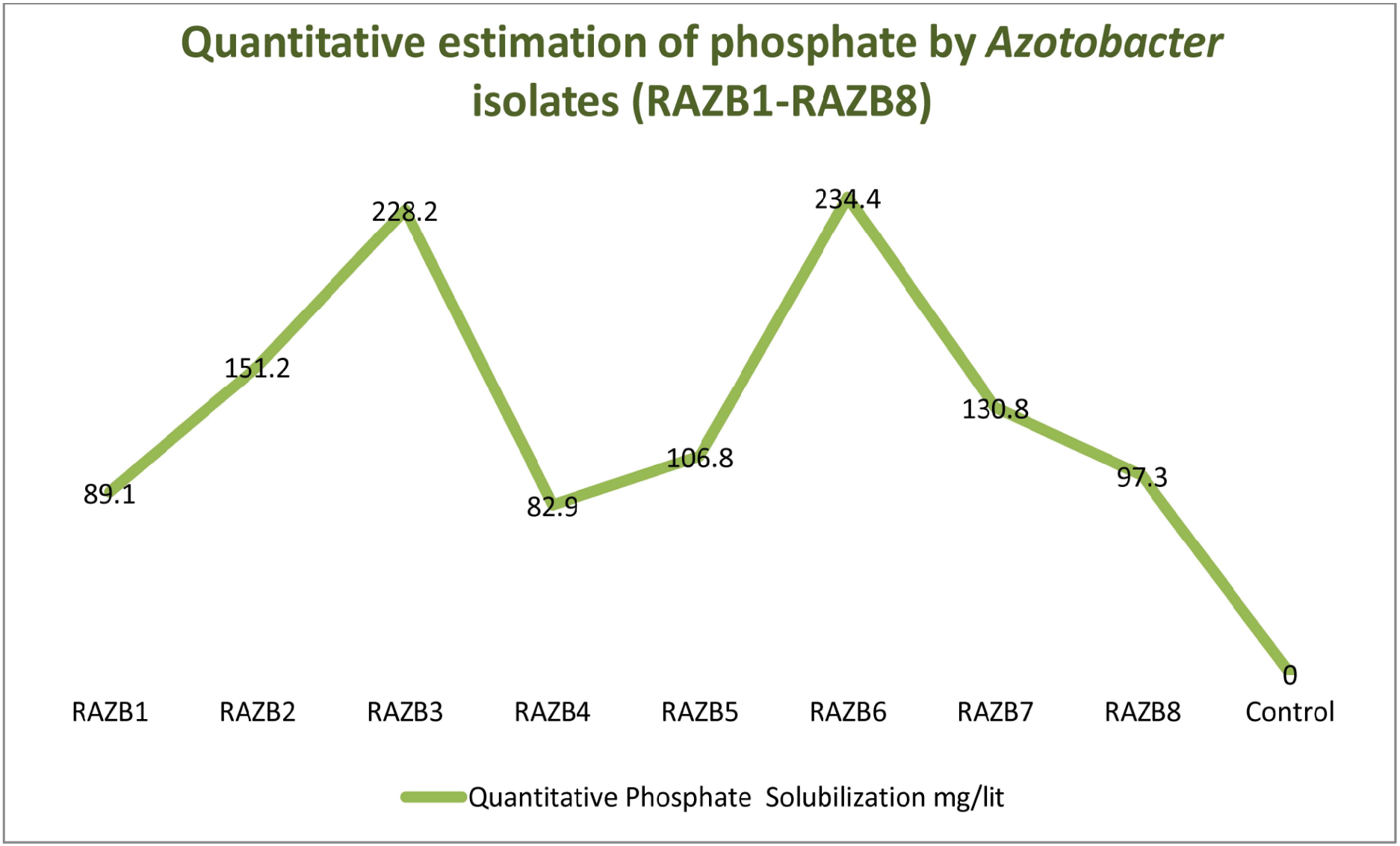

Fig. 1 : Graphical representation of Quantitative estimation of phosphate by Azotobacter isolates

TABLE-4: Quantitative estimation of phosphate by *Azotobacter* isolates

S. No.	*Azotobacter* isolates	Quantitative Phosphate Solubilization mg/lit
1.	**RAZB1**	89.1
2.	**RAZB2**	151.2
3.	**RAZB3**	228.2
4.	**RAZB4**	82.9
5.	**RAZB5**	106.8
6.	**RAZB6**	234.4
7.	**RAZB7**	130.8
8.	**RAZB8**	97.3
9.	**Control**	-

in colour. All were irregular in shape, margin of RAZB3 was undulate and rest show entire margin. Elevation was convex of all the isolates. All were found to be odorless. Brownish pigment was observed after 10 days of growth in RAZB5 and RAZB7. Various biochemical tests of all isolates were performed and all were found to be gram negative. All isolates show positive results for Catalase, oxidase, urease citrate Indole starch hydrolysis and MR test and negative results for gelatin production and VP test.

All the eight isolates screened for phosphate solubilization test show clear zone of phosphate solubilization around them within 15 days. Quantitative determination of all the isolates was performed which gave variable results. All the isolates show phosphate solubilization in Pikovaskaya's medium but in different quantity. Maximum phosphate solubilization was recorded in RAZB6 i.e. 234.4mg/L and minimum in RAZB4 *i.e.* 82.9mg/L. change in pH was also recorded.

Discussion

Total 8 strains of isolated *Azotobacter* (RAZB1, RAZB2, RAZB3, RAZB4, RAZB5, RAZB6, RAZB7 and RAZB8) from different rice field areas of Durg district were tested for phosphate solubilization and were found to be phosphate solubilizer although varied in solubilization in solid and liquid media. Solubilizing index of phosphorus was observed by some workers[11,19]. Also presented almost similar result of *A. chroococcum* which solubilize phosphate ranging from 1.993µg/L to 3.125µg/L[2]. Similarly various species of *Azotobacter* solubilize phosphorous between 19.01µg/L to 93.72µg/L[1]. Combined application of *Azotobacter* and *pseudomonas* increased the growth of rice plant than they were used alone[26]. *Azotobacter* from rice plant soil showed the production of Indole acetic acid required for plant growth[27].

Conclusion and outlook

The present study was aimed to evaluate the *in-vitro* phosphate solubilization activity of *Azotobacter* species and it was observed that *Azotobacter* was phosphate solubilizer as observed by many other researchers also. Thus it was concluded that due to potent phosphate solubilizing activity, it must be exploited as biological fertilizer for sustainable agriculture. Much more attention is required in studies, research and application of *Azotobacter* isolated from different environmental conditions.

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