A COMPARATIVE STUDY OF *Azotobacter spp.* FROM DIFFERENT SOIL SAMPLES

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ABSTRACT

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Azotobacter spp. were isolated from different soil samples and analyzed for number of *Azotobacter* per gram of soil, saline tolerant and heat resistant. The maximum number (CFU) of *Azotobacter* in legume crop field soil (sample No. 1) was $11x10^5$ / gm of soil. The lowest number in grain crop field soil (Sample no. 3) was $3x10^5$ / gm of soil. There was not any *Azotobacter* observed in river water sediment. To determine the prominence of *Azotobacter* in the soil sample other determinants such as pH and moisture. All isolate show maximum growth at 0% NaCl, only two isolate 1 and 4 show growth at 0.8 % NaCl. All isolate content was studied which showed maximum growth at 30ºC temperature but isolate 2, 5 and 6 showed optimum growth 20 ºC whereas isolate 1, 3 and 4 showed optimum growth at 40 ºC and no isolate survive at 50 ºC. The result revealed that legume crop field soil contained the high varieties of *Azotobacter spp*. in terms of temperature and salt tolerance, which became a promising source for further study.

Keywords: *Azotobacter,* water sediment, legume crops

INTRODUCTION

Azotobacter is commonly found in the soil and is very effective for the improvement of soil fertility and crop productivity. It can fix nitrogen directly from the atmosphere that helps the plants for better grain production. We use chemical fertilizers in our land ignoring its adverse effects. In Bangladesh, a few numbers of companies commercially produce bio-fertilizer. The bio-fertilizer is economically cheap and it has no harmful effect to the soil and environment.

Many N_2 sources are available for use in supplying N_2 to crops. In addition to inorganic fertilizer, Organic N_2 from animal manure and other waste products and from N_2 fixation by leguminous crops can supply sufficient N_2 for optimum crop production (Rao, 1982)

The family of *Azotobacter* is *Azotobacteriaceae (*Jensen, 1954) comprises a physiological rather homogeneous group than other. *Azotobacter* including *Azomonas* and *Derxia* and relative fragile microbes and some strains require special diluents for their enumirat ion (Billson *et al*., 1970); NaCl (0.9%) is lethal diluents but the salt components of standard media for *Azotobacter* are satisfactory. *Azotobacter* tends to be sensitive to acid pH values, high phosphate concentrations and temperature above 35ºC. *Azotobacter* is found in the rhiosphares of some plants and can produce hormone like growth stimulants (Postage, 1974).

Azotobacter naturally fixed atmospheric nitrogen in the rhizosphare. There are different strains of *Azotobacter* each has varied chemical, biological and other characters. However, some strains have higher nitrogen fixing ability than others.

Besides, nitrogen fixation, *Azotobacter* also produces Thiamine, Riboflavin, Nicotine, Indole acetic acid and gibberellins. When *Azotobacter* is applied to seeds, seed germination is improved to a considerable extent, so also it controls plant diseases due to above substance produced by *Azotobacter.*

Bo-fertilizars are natural fertilizers, which are microbial inoculants of bacteria, algae or fungi individually or in combination, which augment the availability of nutrients for plants. *Rhizobium* is the best known bio-fertilizers. It fixes atmospheric nitrogen symbiotically with legumes. Other boi-fertilizers are *Azotobacter, Azospirillium, Blue green algae* (BGA) and other *Azolla*. Now a day the *Azotobacter* which is non-symbiotic bacteria is coming in the front line of bio-fertilizer.

Azotobacter is being used as bio-fertilizers in several countries. In Bangladesh, exploitation of biological nitrogen fixation is necessary to supply nitrogen from the renewable source to the crops. In this present investigation, we are highly engaged to isolate effective strains of *Azotobacter* from the different soil could be used as bio-fertilizer in future.

MATERIALS AND METHODS

Collection of soil samples and preservation

In this study, isolation of *Azotobacter* from different soil samples was investigated in the Microbilology laboratory of Biotechnology and Genetic Engineering Discipline of Knulna University.

Soil samples were collected from seven different places, which are as follows:

1. Legumes crop field.
2. Non legume (Vegetable) crop field
3. Grain (rice) crop field
4. Grass land
5. Forest and wood land
6. Unused land
7. River water sediment.

Soil samplers were collected from seven different places. For the purpose of soil sample collection several plastic bags, a marking pen, spatula, alcohol and knives were taken. At first we selected a field for sampling, then selected four or five points in that field and collected soil and mixed the four or five point soil samples. Sufficient amount of soil was collected from each site; kept in a polythene bag and tagged. Soil sample were collected from top 4 cm of the soil profile, as this is where most of microbial activities takes place, and thus where most of the bacterial population is concentrated.

Determination of field soil moisture

50 gm fresh samples were taken separately in a clean 150ml beaker, weight of the beaker was taken before pouring soil sample. It was then kept in the over at 105ºC ± 3ºC for 24 hours, and then again weight of soil sample and beaker was taken combinedly. Difference of moisture content of the soil was recorded and calculated for the moisture content.

Determination of field soil pH

25 gm (field moist) was taken in a clean dry 150 ml beaker and 50 ml distilled water was added. The contents were thoroughly stirred with vortex machine. pH of the suspension was measured with a digital pH meter.

Media Preparation

To prepare of one liter of culture media Ashby's media or Nutrient agar media or Jensen's media) , the following steps were followed.

- According to media composition, the reagents were weighted by electronic balance
- 1000 ml distilled water was measuring by volumetric flask and taken in a conical flask.
- The reagents (except agar) were mixed with the distilled water.
- After mixing reagents, pH was adjusted by adding HCl or NaoH solution if required.
- After pH adjustment, agar was mixed into the solution.
- After mixing the agar, the media was autoclaved by autoclave machine.
- Finally the media was poured in sterile Petri Plates.

Isolation

10 gm collected sample was added to 90 ml of sterile distilled water in a sterile conical flask (250 ml), shaken well by vortex machine than allowed to stand for 30 minutes. 1 ml of sample suspension was then transferred to sterile 9 ml distilled water containing McCarty bottle and shaken well by hand and again allowed to stand for 30 minutes. In this way, samples were diluted up to 10^5 dilution fraction. One ml of sample suspension (from 10^1 to 10^5 fraction) were taken in a sterilized Petri Plates contain approximately 15 ml melted (45ºC), Ashby's medium, and then incubated at 28±2ºC temperature for about 2 – 3 days. After incubation the individual colony was appeared on the medium. The number of *Azotobacter* per gram of soil was then calculated.

Purification

The isolated organisms were purified through repeated plating. Media used for the purpose were Ashby's media, Jensen's media and nutrient agar media.

Preservation

The purified isolated was then transferred to the slants of Nutrient agar media. The 1 dram vial was kept in the polyethylene bags, properly tied and preserved as stock culture.

Physiological studies of selected Azotobacter

The following studied were made on the physiological activities of the organisms.

a. Salt tolerance

Nutrient agar slant containing different concentration of Sodium chloride (viz. 0%, 0.2%, 0.4%, 0.6% and 0.8%) were inoculated and incubated at 28ºC for 48 hours. The growth of different concentrations of NaCl was then compared with the control.

b. Temperature tolerance

To find out the optimum temperature for growth, Nutrient agar slants were inoculated and were allowed to grow at different temperature (Viz. 10ºC, 20ºC, 30ºC, 40ºC, 50ºC).

RESULTS AND DISCUSSION

In the present study on *Azotobacter* 7 (seven) soil samples were collected from different places (Table 1). The soil samples were brought to the laboratory, and then pH (Table 1) and moisture content (Table 1) were determined. The pH and soil moisture ranges from 4.2 to 5.6 and 17.09% to 87.31% respectively. River water sediment was high pH (5.6) and lowest pH (4.2) of soil sample was collected from non legume (vegetable) crop field. Highest percentage of moisture (87.31%) in river water sediment and unused land soil sample contained low moisture content (17.09%).

Samples were studied for the determination of total number of Bacteria (Table 1) The highest number of bacteria 11x 10^5 / gm of soil was observed in the soil collected from the legume crop field (sample no. 1) and the lowest count 3 x 10^5 / gm of soil was in the soil of grain (rice) crop field (sample No. 3) There was not any *Azotobacter* colony observed in river water sediment.

Table 1. pH, moisture and number of CFU of different soil sample

Sample No.	Place of Collection	pH	Moisture %	Number of CFU (x10^5)
1	Legumes crop field.	4.5	21.65	11
2	Non legume (Vegetable) crop field	4.2	30.89	4
3	Grain (rice) crop field	4.4	81.15	3
4	Grass land	4.5	18.76	9
5	Forest and wood land	4.3	44.92	7
6	Unused land	4.7	17.09	7
7	River water sediment.	5.6	87.31	0

Salinity test was done for obtaining saline tolerant *Azotobacter* isolates (Table 2). Saline Tolerant capacity is one of the most important attribute of *Azotobacter* for salinity region of western part of Bangladesh. It was found that no isolate survived in 1.0% NaCl concentration. Isolate 1,2,3,4,5 and 6 were showed maximum growth in 0% NaCl while isolates no. 1,3,4 were showed equal growth both at 0% and 0.2% and isolate no. 1 and 4 were well growth at 0.4% and 0.6% NaCl concentration. Only isolates no. 1 and 4 also grown in 0.8% NaCl concentration. So *Azotobacter* isolates no. 1 and 4 were salt resistance bacteria can be used for production of bio-fertilizer in future. According to Bergey's manual of Systematic Bacteriology (Krieg, 1984) more than 1% NaCl concentration only A. *Chrococcum, A. Vinelandii & A. armeniacus* can survive.

Table 2. Growth of isolated *Azotobacter* in different salt (NaCl) concentration

Sample No.	0.0% Salt	0.2% Salt	0.4% Salt	0.6 % Salt	0.8 % salt	1.0 % salt
1	++++	++++	+++	+++	+	-
2	++++	++	-	-	-	-
3	++++	++++	++	-	-	-
4	++++	++++	+++	++	+	-
5	++++	++	+	-	-	-
6	++++	+++	+	-	-	-

Temperature test was done for obtaining the heat tolerant *Azotobacter* isolate (Table 3). All isolates were showed maximum growth at 30ºC. These three isolates no. 1, 3 and 4 showed maximum growths both at 30ºC and 40ºC. No isolate survived at 50ºC. Only isolate no. 4 showed growths at 10ºC. The finding suggested that the incubation temperature should be 30ºC for obtaining the maximum growth of the isolates. According to Rao (1982) only *Azotobacter* and *Azospirillium* can survive up to 37ºC.

Table 3. Heat tolerant test of isolate *Azotobacter*

Sample No.	10ºC Temp	20ºC Temp	30ºC Temp	40ºC Temp	50ºC Temp
1	-	++	++++	+++	-
2	-	+++	++++	+	-
3	-	++	++++	++++	-
4	+	+	++++	++++	-
5	-	+++	++++	+	-
6	-	+++	++++	++	-

Table 4. Elements of Ashby's Media, Jensen's Media and Nutrient Agar media

Ashby's Media		Jensen's Media		Nutrient Agar media	
Manitol	20. g/L	Sucrose	20. g/L	Beef Extract	10. g/L
K_2HPO_4	0.2 g/L	K_2HPO_4	1.0 g/L	Peptone	5.0 g/L
$MgSO_4.7H_2O$	0.2 g/L	$MgSO_4.7H_2O$	0.5 g/L	NaCl	1.0 g/L
NaCl	0.2 g/L	NaCl	0.5 g/L	Agar	15.0 g/L
K_2SO_4	0.1 g/L	$FeSO_4$	0.1 g/L	Distill Water	1000ml
$CaCO_3$	5.0 g/L	$CaCO_3$	2.0 g/L		
Agar	15.0 g/L	Agar	15.0 g/L		
Distill Water	1000 ml	Distill Water	1000ml		

CONCLUSION

This study revealed that *Azotobacter* contained in different soil sample was varied. The legume crop field soil sample contains highest number of *Azotobacter* than other soil sample and it's more potential than other isolate. This isolate can be used as suitable substrate for production of bio-fertilizer. Bangladesh has cheap manpower and other facilities to production of *Azotobacter* bio-fertilizer at low cost from this isolate.

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