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Research Article

STUDY OF *AZOTOBACTER SPECIES* ISOLATED FROM DIFFERENT SOIL SAMPLES OF TARAI REGION OF UTTARAKHAND

Tarun Sharma, Nishant Rai*
Department of Biotechnology, Graphic Era University, Dehradun -248001 (Uttarakhand), India.
Corresponding Author: nishantrai1@gmail.com

ABSTRACT

***Azotobacter* species 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 was $11x10^5$/gm soil. The lowest number in grain crop field soil was $3x10^5$/gm soil. There was no Azotobacter observed in river water sediment. All isolates showed maximum growth at 0% NaCl except two isolates numbers 1 and 4 which showed growth at 0.8% NaCl. All the isolates studied showed maximum growth at 30ºC temperature but isolate number 2, 5 and 6 showed optimum growth at 20ºC whereas isolate numbers 1, 3 and 4 showed optimum growth at 40ºC and no isolate survived at 50ºC. The results revealed that legume crop field soil contained high varieties of *Azotobacter* species in terms of temperature and salt tolerance, which became a promising source for further study.**

Key words: *Azotobacter*, water sediment, legume crops, soil.

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 enumeration (Billson *et al.,* 1970); NaCl (0.9%) is lethal diluent but the salt components of standard media for *Azotobacter* are satisfactory. *Azotobacter* tends to be sensitive to acidic pH values, high phosphate concentrations and temperature above 35ºC. *Azotobacter* is found in the rhizospheres of some plants and can produce hormone like growth stimulants (Postage, 1974). *Azotobacter* naturally fixed atmospheric nitrogen in the rhizosphere. 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*. Bio-fertilizers 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 bio-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 that could be used as bio-fertilizer in future.

MATERIALS AND METHODS

Collection of soil samples and preservation

Soil samples were collected from seven different places of Uttarakhand - Legumes crop field, Vegetable crop field, Rice crop field, Grass land, Forest and wood land, Unused land, River water sediment.

For soil sample collection several plastic bags, a marking pen, spatula, alcohol and knives were taken. At first, fields for sampling were selected, and then four to five points in that field were selected for soil collection and they were mixed. Sufficient amount of soil was collected from each site; kept in a sterile

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 150 ml beaker, weight of the beaker was taken before pouring soil sample. It was then kept in the oven at 105ºC ± 3ºC for 24 hours and then again total weight of soil sample with beaker was taken. Difference of moisture content of the soil was recorded and calculated for the moisture content.

Determination of field soil pH

Twenty five gram (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

Ashby's media, Nutrient agar media and Jensen's media were used.

Isolation

Ten gram 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 in test tube 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 isolates were then transferred to the slants of Nutrient agar media. The 1 gram 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.

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.

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, soil samples were collected from different places of Uttarakhand. The soil samples were brought to the laboratory, and then pH and moisture content were determined (Table 1). The pH and soil moisture ranged from 4.2 to 5.6 and 17.09% to 87.31% respectively. River water sediment showed high pH (5.6) and lowest pH (4.2) of soil sample was from vegetable crop field. Highest percentage of moisture (87.31%) was observed in river water sediment and low moisture content (17.09%) in unused

land soil sample. Samples were studied for the determination of total number of Bacteria (Table 1). The highest number of bacteria 11x 10^{-5}/gm of soil were 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 rice crop field (sample No. 3) There was not any *Azotobacter* colony observed in river water sediment. Salinity test was done for obtaining saline tolerant *Azotobacter* isolates (Table 2). It was found that no isolate survived in 1.0% NaCl concentration. Isolate 1, 2, 3, 4, 5 and 6 showed maximum growth in 0% NaCl while isolates no. 1, 3, 4 showed equal growths both at 0% and 0.2% and isolate no. 1 and 4 showed good growth at 0.4% and 0.6% NaCl concentration. Only isolates no. 1 and 4 grew in 0.8% NaCl concentration. So *Azotobacter* isolates no. 1 and 4 were salt resistant bacteria that can be used for production of bio-fertilizer in future. According to Bergey's manual of Systematic Bacteriology (Krieg, 1984) at more than 1% NaCl concentration only *A. Chrococcum, A. Vinelandii* and *A. armeniacus* can survive.

Temperature test was done for obtaining the heat tolerant *Azotobacter* isolate (Table 3). All isolates showed maximum growth at 30ºC. These three isolates no. 1, 3 and 4 showed maximum growth both at 30ºC and 40ºC. No isolate survived at 50ºC. Only isolate no. 4 showed growths at 10ºC. The findings 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 1. pH, moisture and number of CFU of different soil samples

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**

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.	++++	+++	+	-	-	-

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	DW 1000ml
$CaCO_3$	5.0 g/L	$CaCO_3$	2.0 g/L	
Agar	15.0 g/L	Agar	15.0 g/L	
DW 1000 ml		DW 1000 ml		

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 taken and it has more potential than other isolate. This isolate can be used as suitable substrate for production of bio-fertilizer.

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