

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.603.204>

## Atmospheric Nitrogen Fixing Capacity of *Azotobacter* Isolate from Cooch Behar and Jalpaiguri Districts Soil of West Bengal, India

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### ABSTRACT

#### Keywords

Terai Zone,  
Azotobacter,  
Nitrogen fixing  
microorganisms,  
Shape and Size.

#### Article Info

Accepted:  
24 February 2017  
Available Online:  
10 March 2017

Terai Zone of West Bengal is considered as a fragile ecological region because of high rainfall and prolongs winter. Plants cannot derive nutrients effectively from its organic source because of slow mineralization. Soils are acidic and light textured. High rainfall coupled with low pH lead to deficiency of several macro and micro-nutrients and at the same time toxicity of some other elements. A significant amount of applied chemical fertilizers is lost and find its way to pollute environment because of high rainfall. Natural resource management specially with biological nitrogen fixing microorganisms may be a good option for this zone. In the present investigation putative *Azotobacter* species were isolated from soils collected from Cooch Behar and Jalpaiguri districts of West Bengal. Isolate Az-12 was found to have fixed maximum amount of nitrogen (12.66 mg) at the cost of one gram of sugar consumed. It is revealed from the results that among the isolates, Az-3, Az-5, Az-12, Az-14 and Az-19 are ovoid to rod shaped. While the shape was rod with rounded end in isolates of Az-8, Az-11, Az-16 and Az-20. The size of the isolates varied widely. Thus the smallest size was found in Az-8 (2.2-3.5 x 1.2-1.8  $\mu$ m) while the biggest size was found in isolates of Az-14 (3.0-3.2 x 2.0-2.2  $\mu$ m).

### Introduction

The element nitrogen is highly abundant in Earth's atmosphere and is a major component of dietary proteins (as incorporated in amino acids). Plant growth is directly influenced by the availability of reduced nitrogen, leading to the long accepted practice of manuring, fertilizer application, or rotational crop practices (Gresshoff and Rao, 1986). However nitrogen, the most abundant element in the atmosphere is the limiting element for the growth of most organisms (Davey and Wollum, 1984).

Biological nitrogen fixation, which is the reduction of atmospheric nitrogen ( $N_2$ ) to two molecules of ammonia, is the second most important biological process on earth after photosynthesis. In this process  $N_2$  gas is cleaved by the metallo-enzyme nitrogenase (itself made up of two components, the iron protein and the molybdenum-iron protein), linked to hydrogen atoms to yield ammonia ( $NH_3$ ). This in turn is easily assimilated to form the building blocks for intermediate metabolism (purine, pyrimidine, alkaloid, and

amino acid biosynthesis). Nitrogen fixation is mediated exclusively by prokaryotes, including many genera of bacteria, cyanobacteria, and the actinomycete, *Frankia* (Alexander, 1977; Ravikumar *et al.*, 2007). The reaction, in general, requires an anaerobic (lacking O<sub>2</sub> or micro-aerobic (low in O<sub>2</sub> concentration) environment, although exceptions to this rule are found in bacteria that have evolved special O<sub>2</sub> protection mechanisms. Nitrogen-fixing microbes can exist as independent, free-living organisms or in associations of differing degrees of complexity with other microbes and plants (Sylvia *et al.*, 2005). Biological nitrogen fixation (BNF) accounts for about 139-170 million tons of nitrogen fixed annually. This figure is almost double the input of nitrogen from nitrogenous fertilizers (Peoples and Craswell, 1992) thus demonstrating the significance of biological nitrogen fixation in agriculture and natural nitrogen cycle.

*Azotobacter* spp. is free-living aerobic bacteria dominantly found in soils. They are non-symbiotic heterotrophic bacteria capable of fixing an average 20 kg N/ha/per year. Besides, it also produces growth promoting substances and is shown to be antagonistic to pathogens. *Azotobacter* spp. are found in the soil and rhizosphere of many plants and their population ranges from negligible to 10<sup>4</sup> g<sup>-1</sup> of soil depending upon the physico-chemical and microbiological (microbial interactions) properties. *Azotobacter chroococcum* is the most prevalent species found but other species described include *A. agilis*, *A. vinelandii*, *A. beijerinckii*, *A. insignis*, *A. macrocytogenes* and *A. paspali* (FAO, 1982). In soils, *Azotobacter* spp. populations are affected by soil physico-chemical (e.g. organic matter, pH, temperature, soil depth, soil moisture) and microbiological (e.g. microbial interactions) properties. As far as physico-chemical soil properties are concerned, numerous studies have focused on

the nutrients (i.e. P, K and Ca) and organic matter content and their positive impact on *Azotobacter* spp. populations in soils (Pramanix and Misra, 1955; Bescking, 1961; Jensen, 1965; Burris, 1969).

The imported N<sub>2</sub>-fixing strains are not adaptive enough in entirely different ecological condition of this region. Low soil pH, deficiency of some micro-nutrients, aluminium toxicity and low soil temperature set a problem in survivality efficiency of nitrogen fixing bacterial strain collected from other sources. On the other hand location specific/indigenous inoculant strain has better capacity to establish in the rhizosphere and have greater agronomic importance.

## **Materials and Methods**

### **Collection of soil samples**

Soil samples were collected from different locations of Cooch Behar and Jalpaiguri districts of West Bengal from cultivated land. Samples were withdrawn at a depth of 0-10 cm, collected into polyethylene packets, sieved through a 4.0 mm sieve and stored at field moisture content at 4°C.

### **Isolation**

The standard isolation procedure was followed for the isolation of organisms from soils. Serial dilutions of the soil sample was prepared by taking 1.0 gm of soil into 9.0 ml of sterile distilled water and mixed well to get uniform soil suspension. Assuming the low population of *Azotobacter* in soil serial dilution of soil samples were made up to 10<sup>-3</sup> dilution. Jensen's Media (Jensen, 1951) was used for the isolation of *Azotobacter*. For this, diluted soil samples were used for spread plate method and inoculated plates were incubated at 28°C ±1°C. Visible growth of bacterial colonies was observed after 48-72

hour of incubation. *Azotobacter* like organisms were detected by their character colony morphology and picked for further studies.

### **Screening of *Azotobacter* isolates**

All the purified cultures were pester for N<sub>2</sub> – fixing capacity by difference method. Cultures were inoculated into Jensen’s media (a N-free broth media). All the cultures were inoculated into 25 ml liquid medium. The inoculated flasks were then incubated at 28° C for two week with periodic shaking. N<sub>2</sub> -fixed was estimated (by Kjeldahl method of digestion and distillation in Bremner’s apparatus. Three replications were maintained for each isolates and fixation of atmospheric nitrogen was calculated by subtracting the total nitrogen of control flask (uninoculated) from that of inoculated flasks. Results were expressed as mg N<sub>2</sub> fixed per g of sugar consumed considering the quantity of respective sugar present in that particular medium.

### **Morphological and biochemical characterization**

Forty eight hour grown cultures were used to study the bacterial cellular morphology. Bacterial smear was stained with nigrosine (negative staining) and observed using oil immersion lens of a light microscope. Length/diameter of bacterial cells was measured with ocular micrometer. Among other tests, Gram’s staining, spore staining, and capsule staining were implemented along with bacterial motility test.

Biochemical tests were performed to determine citrate utilization (Simmons, 1926), hydrolysis of starch (Blazevic *et al.*, 1975), hydrolysis of casein (Harry and Paul 1962), hydrolysis of gelatin (Frazier, 1926), methyl red test (Clark and Lubs, 1915), Voges-

Proskauer test (Barritt, 1936), Indole test (Kovacs, 1928), nitrate reduction (Blazevic *et al.*, 1973) and presence of enzymes like oxidase (Kovacs, 1956) and catalase (Taylor *et al.*, 1972).

### **Growth performance of *Azotobacter* at different levels of Desiccation**

For this experiment nitrogen free Jensen’s Media was modified by omitting CaCO<sub>3</sub>. Medium was amended with Poly Ethylene Glycol (PEG) @ 0%, 20%, 30%, 40% and 50%. Liquid culture of *Azotobacter* was used for inoculation and each conical flask contains 50.0 ml of amended media. Media with various treatments were inoculated with 1.0 ml (10<sup>7</sup>cells/ml) liquid culture of test isolates. All the inoculated flasks were incubated at 30° C in an incubator with periodic shaking. Three replications were maintained for each treatment. Growth of the bacterium was compared by analyzing the cellular protein by Lawry method. Considering the fast growing nature of the isolates reading was taken up to five days.

**Protein estimation:** bacterial cell protein was estimated by the method developed by Lowry *et al.*, (1951).

### **Growth performance of *Azotobacter* at different levels of aluminum concentration**

For this experiment nitrogen free Jensen’s Media was modified by omitting CaCO<sub>3</sub>. Medium was amended with Al<sup>+3</sup>. @ 0, 2.5, 5, 10, 25 mM. Liquid culture of *Azotobacter* was used for inoculation and each conical flask contains 50.0 ml of amended media. Media with various treatments were inoculated with 1.0 ml (10<sup>7</sup>cells/ml) liquid culture of test isolates. All the inoculated flasks were incubated at 30° C in an incubator with periodic shaking. Three replications were maintained for each treatment. Growth of the

bacterium was compared by analyzing the cellular protein by Lawry method. Considering the fast growing nature of the isolates reading was taken up to five days.

**Protein estimation:** bacterial cell protein was estimated by the method developed by Lowry *et al.*, (1951).

## Results and Discussion

### Isolation of putative *Azotobacter* species

In the present investigation putative *Azotobacter* species were isolated from soils collected from Cooch Behar and Jalpaiguri districts of West Bengal. Isolation was performed on Jensen's medium and initial selection of isolates was done on the basis of their colony morphology and pigment production. Altogether 20 (twenty) bacteria were isolated from various locations- out of which twelve (12) were from Cooch Behar and eight (8) were from Jalpaiguri (Table 1) districts.

### Atmospheric nitrogen fixing capacity of bacterial isolate

The estimations of atmospheric nitrogen fixed by the putative *Azotobacter* are furnished in Table 2. Isolate Az-12 was found to have fixed maximum amount of nitrogen (12.66 mg) at the cost of one gram of sugar consumed. Five isolates viz., Az-8, Az-11, Az-14, Az-16 and Az-20 fixed higher proportion of nitrogen and the values were 8.14, 8.28, 8.41, 8.16 and 8.46 mg/g respectively, while isolate Az-7 was found to have fixed lowest amount of nitrogen (3.16 mg). Among the other isolates, notably Az-5 and Az-19 fixed 7.88 and 7.18 mg/g nitrogen respectively.

Gupta *et al.*, (1992) showed that *Azotobacter* can fix atmospheric nitrogen @ 1.47 to 1.50

(Average, 1.49) mg N per g of carbon source, whereas, Gondotra, *et al.*, (1998) found the range as 13.3 to 21.6 mg N g<sup>-1</sup> glucose. Veena (1999) compared the nitrogen fixing ability of four diazotrophs viz., *Azospirillum*, *Acetobacter*, *Azotobacter* and *Beijerinckia* and found *Azospirillum* to fix highest amount of N (12.56 to 20.96 mg of N/g of malate added) followed by *Acetobacter* (9.13 to 12.6 mg N/g sucrose) and *Azotobacter* (9.06 to 10.46 mg N/g glucose). In the present study the activity is 3.16 to 12.66 mg N g<sup>-1</sup> glucose. Thus the wide variation in nitrogen fixing capacity of different isolates could be attributed to strain variation (Gupta and Tripathi, 1986).

### Characterization and identification of bacterial isolate

#### Morphological and cultural characteristics

The morphological and cultural traits of the putative *Azotobacter* isolates are furnished in Table 3. It is revealed from the results that among the isolates, Az-3, Az-5, Az-12, Az-14 and Az-19 are ovoid to rod shaped. While the shape was rod with rounded end in isolate of Az-8, Az-11, Az-16 and Az-20. The size of the isolates varied widely. Thus the smallest size was found in Az-8 (2.2-3.5 x 1.2-1.8 µm) while the biggest size was found in isolates of Az-14 (3.0-3.2 x 2.0-2.2 µm). The results show that all the isolates were negative (-ve) in Gram staining reaction. None of the isolates were found to produce endospore. It was observed that three isolates viz. Az-5, Az-11 and Az-19 produced capsules. Among the isolates Az-3, Az-12, Az-16 and Az-20 were found to be motile.

In regard to colony morphology, round to irregular in shape was found in isolates of Az-3, Az5, Az8 Az14 and Az-16; while round in shape was found in case of Az-11, Az-12 Az-19 and Az-20. Az-3 was found to have

smooth surface with big colonies. While the colony was raised glistening in case of Az-5, Az-11 Az-16 and Az-19. The isolate Az-20 had flat slimy smooth colony.

Among the isolates, it was found that Az-3, Az-5, Az-14 produced dark brown to black pigment. In case of Az-8, Az-11 and Az-19 the colour of the pigment was brown. Isolates Az-12 and Az-16 produced pale yellow to light brown pigment, while Az-20 produced light brown pigment.

### **Physiological Characteristics**

The physiological characteristics of the putative *Azotobacter* isolates are furnished in Table 4. All the isolates were positive in oxidase and catalase reaction. Isolates Az-3, Az-16 and Az-20 were negative in Methyl Red and Voges Proskauer test while in all other isolates these were positive. Except Az-3, Az-11 and Az-16 all other isolates were positive in hydrolysis of starch reaction, while in the former cases these were negative. None of the isolates showed positive for casein and gelatine hydrolysis. Except Az-11 and Az-12, all the other isolates showed positive reaction in the conversion of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup>. All isolates were found to have positive reaction in case of indole production and citrate utilization.

Observation on shape, size, capsule, colony morphology and pigment production on Jensen's media and the various physiological characters of all the nine strains resembles the genus *Azotobacter* according to the Berge's Manual of Systematic Bacteriology (Krieg and Dobereiner, 1984).

### **Effects of desiccation on *Azotobacter***

An effect of various levels of desiccations on 9 *Azotobacter* species has been studied and the results are furnished in Figure 1. The

desiccation has been created by Poly Ethylene Glycol which reduces the water activity of Jensen's broth medium Growth of all the *Azotobacter* was inhibited by the addition of desiccating agent @ 20% and more than that. Results show that nine strains of *Azotobacter* react differently to the addition of Poly Ethylene Glycol. When the growth is expressed as µg protein per ml of broth it is observed that growth attained by different *Azotobacter* isolates were in the decreasing order of Az-20, Az-3, Az-8, Az-4, Az-14, Az-11, Az-5, Az-19 and Az-16. When the growth of isolated bacteria are expressed as the percentage growth reduction, it is observed that Az-16 and Az-19 show the maximum growth inhibition (Fig 2)

Eaglesham and Ayanaba (1984) observed that during desiccation, rhizobial populations are reduced in size accompanied with two distinct phases of decline i.e. during the initial loss of water from the soil, population numbers fell at an exponential rate, to a much decreased level. Kieft *et al.*, (1987) also observed that changes in soil water potential could cause the death of some portion of the microbial population and that may cause a shift in the active microbial population.

### **Effects of Aluminium on *Azotobacter***

The results of *Azotobacter* growth as influenced by various levels of aluminium concentration is presented in graph 3. Results show that the growths of all the nine isolates were drastically reduced even at 2.5 mM aluminium concentration. When the results are expressed as percentage reduction of growth (Graph 4) it is observed that 75 to 87% growths were inhibited by isolated species. The least growth inhibition was observed after 6 days of growth by Az-14 and the maximum growth inhibition was observed by Az-16.

The amount of inorganic monomeric Al, instead of total Al, is taken as a better indicator for Al toxicity (Bruce *et al.*, 1988). The enumeration of acid- and Al-tolerant microorganisms in acidic soils by Kanazawa and Kunito (1996) also indicated that fungi accounted for most of the highly Al resistant microorganisms. This is reasonable because fungi and yeasts are generally more tolerant to acidity than bacteria (Myrold and Nason, 1992). This may be because of some organic acids which are involved in the detoxifying of inorganic monomeric Al in the GM medium. Acid tolerant strains *Beijerinckia dextralis* reacts differently to aluminium. Barbosa *et al.*, (2002) observed that a decline in the number of CFU was observed immediately after the end of the exponential phase.

In our experiment we also observed variation in tolerance levels among the *Azotobacter* Sp isolated from acid soil of north Bengal.

Woods *et al.*, (1987) used liquid culture to study the acidity and Al tolerance of *Rhizobium*. Measurement of the multiplication in liquid culture indicated that fast growing rhizobia (*R. loti*) were tolerant of acidity and aluminum (at least 50  $\mu\text{M}$  Al at pH 4.5). Slow growing Lotus rhizobia (*Bradyrhizobium* sp.) were less tolerant to acidity but equally tolerant of Al. Both genera were able to nodulate *Lotus pedunculata* in acid soils (pH 4.1 in 0.01M $\text{CaCl}_2$ ) and the slow growing strain were more effective than the fast growing strain in these soils over 30 days.

In conclusion, terai Zone of West Bengal is considered as a fragile ecological region because of high rainfall and prolongs winter. Plants cannot derive nutrients effectively from its organic source because of slow mineralization. Soils are acidic and light textured. High rainfall coupled with low pH

lead to deficiency of several macro and micro-nutrients and at the same time toxicity of some other elements. A significant amount of applied chemical fertilizers is lost and find its way to pollute environment because of high rainfall. Natural resource management specially with biological nitrogen fixing microorganisms may be a good option for this zone.

*Azotobacter* spp. are free-living aerobic bacteria dominantly found in soils. They are non-symbiotic heterotrophic bacteria capable of fixing an average 20 kg N/ha/per year. Besides, it also produces growth promoting substances and is shown to be antagonistic to pathogens. *Azotobacter* spp. are found in the soil and rhizosphere of many plants and their population ranges from negligible to  $10^4$  g<sup>-1</sup> of soil depending upon the physico-chemical and microbiological (microbial interactions) properties.

An important aspect of research on *Azotobacter* is to select highly efficient strain of rhizobia for a particular host plant. The imported N<sub>2</sub>-fixing strains are not adaptive enough in entirely different ecological condition of this region. Low soil pH, deficiency of some micro-nutrients, occasional draught spell and low soil temperature set a problem in survivality, nitrogen fixing capacity of nitrogen of *Azotobacter* collected from other sources. On the other hand location specific indigenous inoculant strain has better adaptability in the rhizosphere and has greater agronomic importance. Hence, indigenous N<sub>2</sub>-fixing *Azotobacter* strains should be collected from local microbial resources and should be screened for their N<sub>2</sub>-fixing ability as well as for their adaptability in the soil of this region. Altogether 20 putative *Azotobacter* sp were isolated from nine different places of Cooch Behar and Jalpaiguri district.

**Table.1** Isolates of putative *Azotobacter* and their places of isolation

Sl. No.	Isolates	Places of isolation
1	Az-1	Cooch Behar Seed Farm, Govt. of West Bengal, Cooch Behar.
2	Az-2	UBKV Farm, Pundibari, Cooch Behar-2, Cooch Behar
3	Az-3	UBKV Farm, Pundibari, Cooch Behar-2, Cooch Behar
4	Az-4	UBKV Farm, Pundibari, Cooch Behar-2, Cooch Behar
5	Az-5	Chhoto Rang Rash, Cooch Behar-2, Cooch Behar.
6	Az-6	Chhoto Rang Rash, Cooch Behar-2, Cooch Behar.
7	Az-7	Gitaldah, Dinhata 1, Cooch Behar.
8	Az-8	Gosanimari, Dinhata-1, Cooch Behar.
9	Az-9	Gosanimari, Dinhata-1, Cooch Behar.
10	Az-10	Gosanimari, Dinhata-1, Cooch Behar.
11	Az-11	Maruganj, Tufanganj-1, Cooch Behar.
12	Az-12	Maruganj, Tufanganj-1, Cooch Behar.
13	Az-13	Damanpur, Alipurduar 1, Jalpaiguri
14	Az-14	Damanpur, Alipurduar 1, Jalpaiguri
15	Az-15	Salsalabari, Alipurduar 2, Jalpaiguri.
16	Az-16	Salsalabari, Alipurduar 2, Jalpaiguri.
17	Az-17	Palash Bari, Falakata, Jalpaiguri.
18	Az-18	Palash Bari, Falakata, Jalpaiguri.
19	Az-19	Palash Bari, Falakata, Jalpaiguri.
20	Az-20	Mohitnagar, Block-Jalpaiguri, Jalpaiguri.

**Table.2** Atmospheric nitrogen fixed (mg N per gram of sugar consumed) by putative *Azotobacter* isolates

Sl.No.	Isolates No.	Mg N-fixed/gm of Sugar consumed
1	Az-1	4.78
2	Az-2	6.45
3	Az-3	7.88
4	Az-4	5.75
5	Az-5	7.98
6	Az-6	5.38
7	Az-7	3.16
8	Az-8	8.14
9	Az-9	5.25
10	Az-10	5.16
11	Az-11	8.28
12	Az-12	12.66
13	Az-13	3.92
14	Az-14	8.41
15	Az-15	5.27
16	Az-16	8.16
17	Az-17	5.67
18	Az-18	5.28
19	Az-19	7.18
20	Az-20	8.46

Average of three replications

**Table.3** Morphological and cultural characteristics of putative *Azotobacter* isolates

Characters	Putative <i>Azotobacter</i>								
	Az-3	Az-5	Az-8	Az-11	Az-12	Az-14	Az-16	Az-19	Az-20
Shape	Ovoid to rod shaped	Ovoid to rod shaped	Rod with rounded end	Rod with rounded end	Ovoid to rod shaped	Ovoid to rod shaped	Rod with rounded end	Ovoid to rod shaped	Rod with rounded end
Size (µm)	2.5-3.0 x 1.8	2.8 x 1.8-2.0	2.2-3.5 x 1.2-1.8	2.5-3.5 x 1.2-2.0	2.5-2.8 x 1.8-2.0	3.0-3.2 x 2.0-2.2	2.0-2.8 x 1.8-2.2	2.5-3.5 x 1.2-1.8	2.0-3.0 x 1.2-1.4
Gram staining	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Endospore	-	-	-	-	-	-	-	-	-
Capsules	-	+	-	+	-	-	-	+	-
Motility	+	-	-	-	+	-	+	-	+
Colony morphology	Round to irregular in shape, smooth surface big colonies	Round to irregular in shape, raised glistening raised colony	Round to irregular in shape, slightly raised smooth colony	Round in shape, raised glistening slimy colony	Round in shape, raised smooth gummy colony	Round to irregular in shape, raised slimy colony	Round to irregular in shape, raised gummy glistening colony	Round in shape, raised gummy glistening colony	Round in shape, flat slimy smooth colony
Pigment production	Dark brown to black	Dark brown to black	Brown	Brown	Pale yellow to light brown	Dark brown to black	Pale yellow to light brown	Brown	Light Brown

**Symbols: ‘+’, Positive; ‘-’, Negative.**



**Table.4** Physiological characteristics of putative *Azotobacter* isolates

Characters	Putative <i>Azotobacter</i>								
	Az-3	Az-5	Az-8	Az-11	Az-12	Az-14	Az-16	Az-19	Az-20
Oxidase	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
MR	-	+	+	+	+	+	-	+	-
VP	-	+	+	+	+	+	-	+	-
Hydrolysis of starch	-	+	+	-	+	+	-	+	+
Casein	-	-	-	-	-	-	-	-	-
Gelatin	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	-	-	+	+	+	+
Indole	+	+	+	+	+	+	+	+	+
Utilization of Citrate	+	+	+	+	+	+	+	+	+

Symbols: '+', Positive; '-', Negative

**Fig.1** Graph showing the growth of *Azotobacter* as influenced by various levels of desiccation

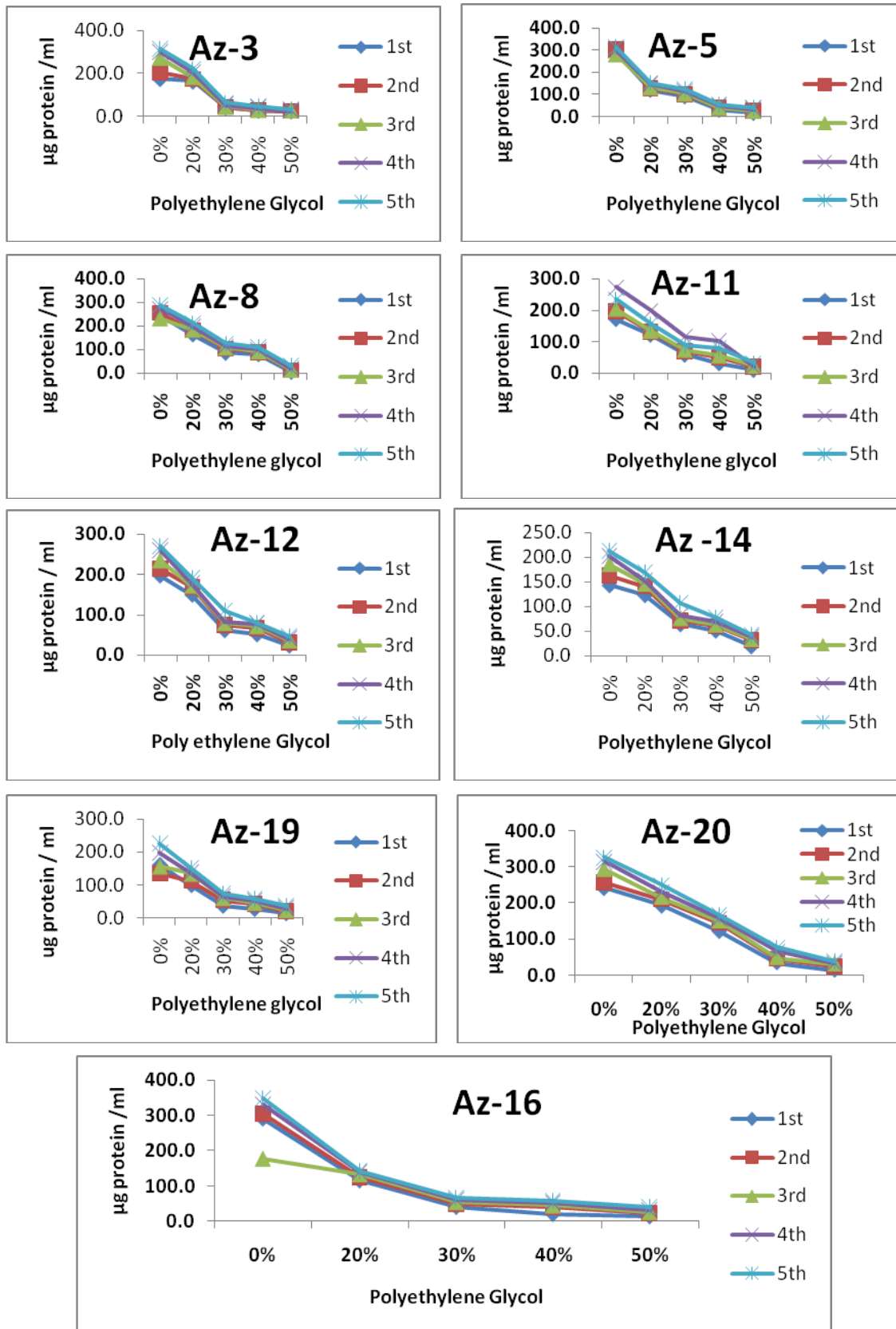


Fig.2 Comparison of growth of *Azotobacter* under different levels of desiccation

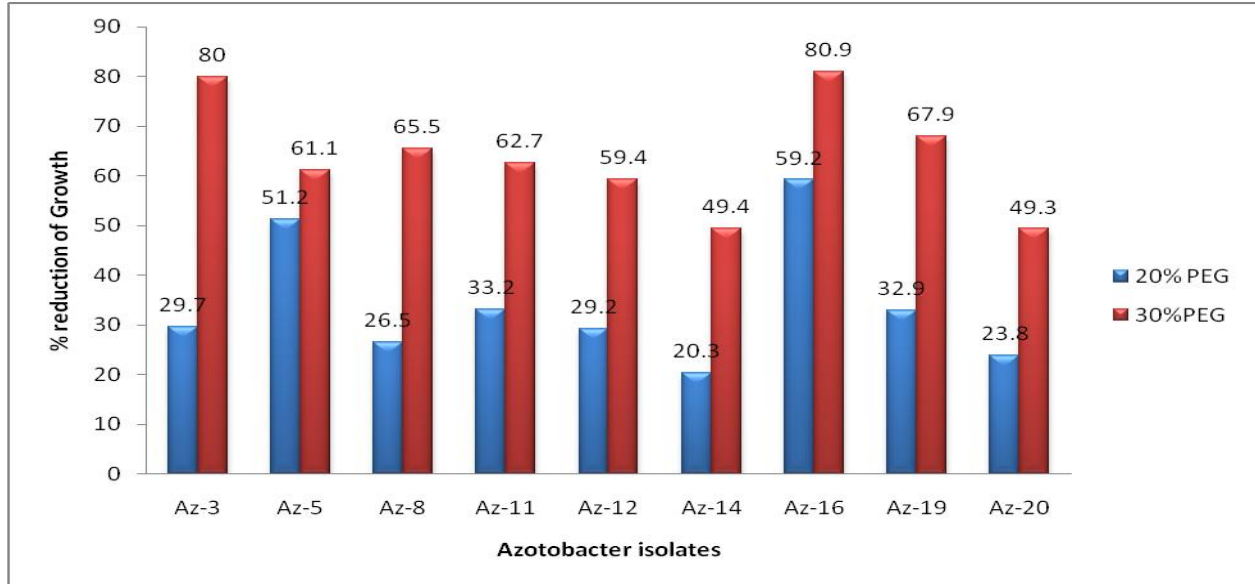
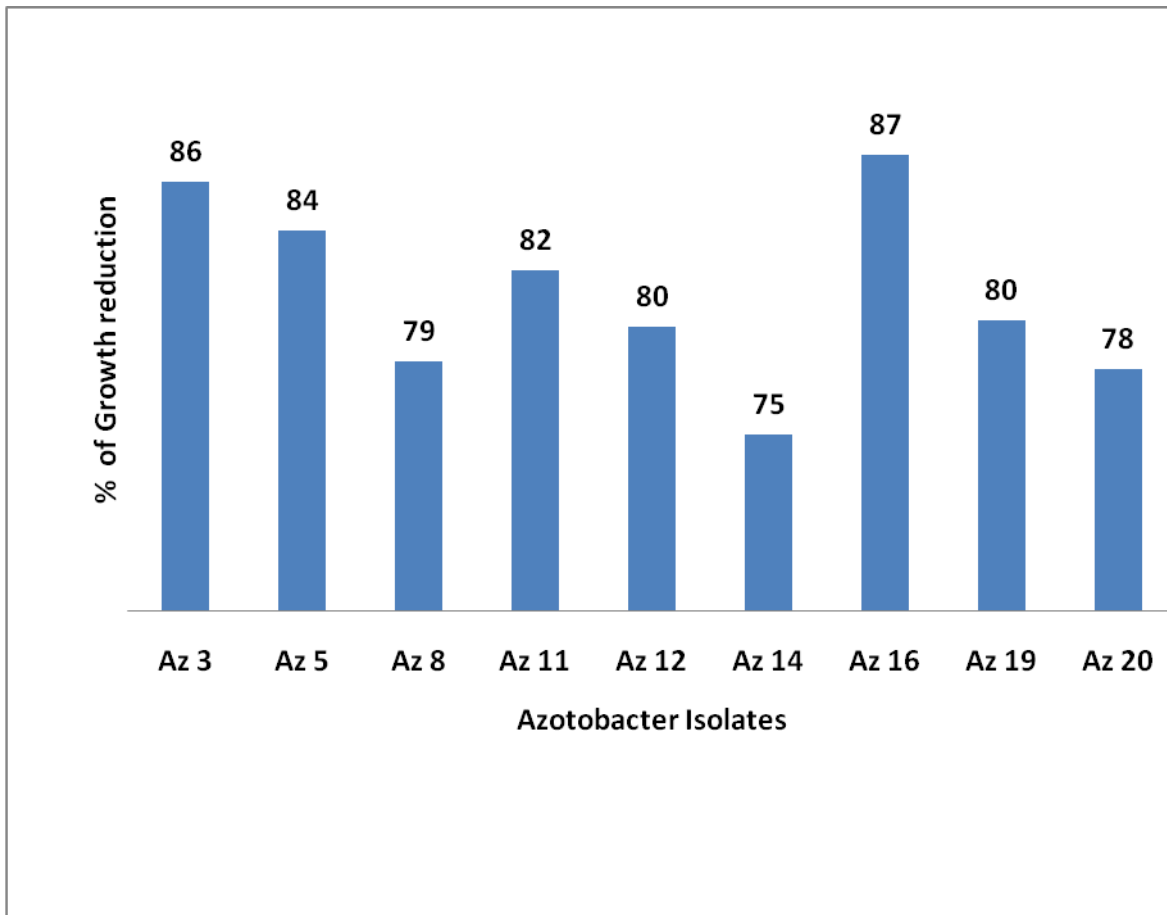
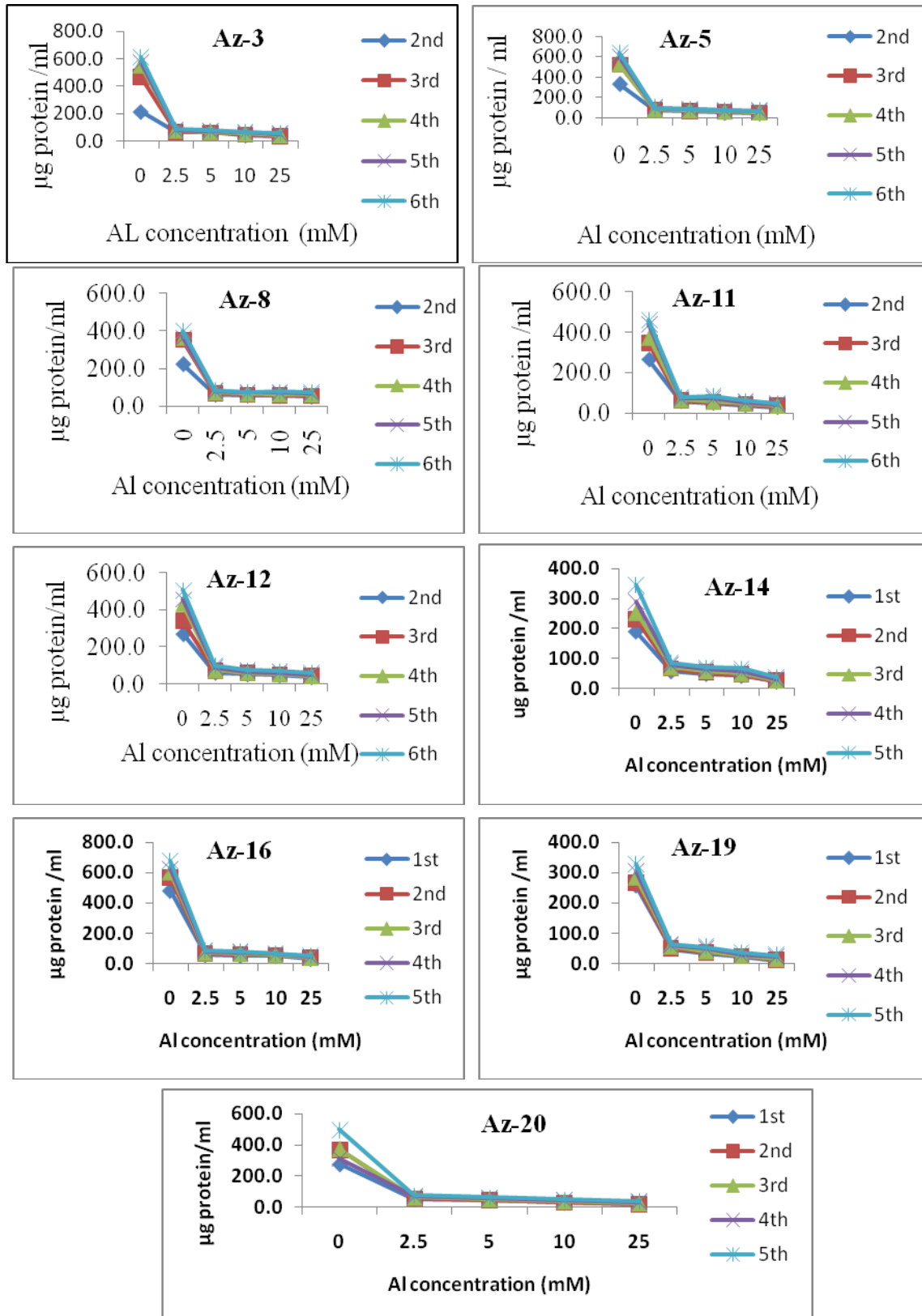


Fig.4 Comparison of growth of *Azotobacter* under different levels of aluminium concentration



**Fig.3** Graphical presentation of growth of *Azotobacter* (cell protein) as influenced by various levels of aluminium concentration



All the isolates were selected on the basis of their colony morphology using N-free Jensen's medium. All the isolates showed positive nitrogen fixing capacity ranging from 3.16 to 12.66 mg N per gram of sucrose consumed.

On the basis of their N<sub>2</sub> fixing capacity 9 (nine) isolates were selected for further studies. Morphological and biochemical characters were studied and all the isolates were identified as *Azotobacter* sp.

Stress tolerance was studied for desiccation and aluminium. It was observed that growth of *Azotobacter* sp was reduced by 50% in presence of 20% polyethylene glycol, a desiccating agent which reduces the water activity of liquid medium. However, it was observed that Al<sup>+3</sup> at the concentration of 2.5 mM affected the growth of *Azotobacter*.

Finally, it can be concluded that the *Azotobacter* sp isolated from the soils of *Terai* zone of West Bengal were highly sensitive to aluminium even at the concentration of 2.5 mM Al<sup>+3</sup>. The isolated *Azotobacter* sp can be used as inoculum for nitrogen nutrition of crop plants but soil should be treated with lime for getting better benefit from inoculated biofertilizer.

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**How to cite this article:**

Puspendu Bikash Bag, Parimal Panda, Bappa Paramanik, Bisweswar Mahato and Ashok Choudhury. 2017. Atmospheric Nitrogen Fixing Capacity of *Azotobacter* Isolate from Cooch Behar and Jalpaiguri Districts Soil of West Bengal, India. *Int.J.Curr.Microbiol.App.Sci*. 6(3): 1775-1788. doi: <https://doi.org/10.20546/ijcmas.2017.603.204>