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Short Communication

Distribution and Abundance of *Azotobacter* in Wheat Fields of Bangladesh

Muhammad Ali Akond, Sanzida Mubassara and M Motiur Rahman*

Department of Botany, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh

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The distribution and abundance of *Azotobacter* as well as heterotrophic bacteria in root, rhizosphere soil and non-rhizosphere soil samples from various wheat fields of four different areas under three districts were investigated in this study. The potential for nitrogen-fixation of five *Azotobacter* isolates was also detected. All samples tested were positive in their capacity to harbouring A*zotobacter* with a range of 26-100%. The population of heterotrophic bacteria ranged from 2.1 x 10^7 to 1.2 x 10^8 cfu/g sample. Ranges of total number of *Azotobacter* in different samples were 5.2 x 10^4 to 7.2 x 10^4 cfu/g, 17.2 x 10^4 to 25.5 x 10^4 cfu/g, and 12.4 x 10^4 to 16.7 x 10^4 cfu/g respectively for root, rhizosphere soil and non-rhizosphere soil. A positive correlation was found in *Azotobacter* colonization between root and rhizosphere, but it was negative in case of the population between heterotrophic bacteria and *Azotobacter* in rhizosphere. The highest amount of N was found to be fixed by the isolate M_1 and the lowest by the isolate M_4 and it was respectively 9.26 and 5.45 mg N/g substrate. In terms of the capacity to fix nitrogen in laboratory condition the five isolates of *Azotobacter* could be arranged as $M_1 > M_3 > M_5 > M_4 > M_2$.

Keywords: *Azotobacter*, Wheat field, Nitrogen fixing potential

The study of important nitrogen-fixing bacteria is very essential with a view to finding efficient strains to develop biofertilizer for crops like wheat. Biological nitrogen fixation is important in non-leguminous crop, *e.g.*, rice and wheat farming systems because it is an inexpensive source of nitrogen for higher yields. This process diminishes the need for expensive chemical fertilizers, which have been associated with numerous health and environmental problems. Biological nitrogen fixation in agriculture usually refers to nodule forming dicotyledonous leguminous plants. Monocotyledonous plants like rice and wheat lacking genes for nodulation must depend upon nitrogen from chemical fertilizers and various sources of biological nitrogen fixation in the ecosystem by free-living or associative organisms.

Azotobacter is a free-living nitrogen-fixing soil bacterium. This organism was first isolated and described by Beijerinck in 1901[1]. Besides nitrogen fixation, *Azotobacter* has been found to synthesize growth promoting substances and antibiotics[2]. By virtue of these attributes, *Azotobacter* can play nutritional and stimulatory roles and can benefit the plants with its manifold actions. Further, *Azotobacter* inoculation has been found to increase the growth and yield of a wide variety of cereals, pulses, vegetable crops, fruit crops and cash crops[3]. It is indeed interesting to note that *Azotobacter* is among the first organisms to develop in a newly formed soil, and the number of this organism in soil runs parallel with its fertility[4]. Though the occurrence and distribution of *Azotobacter* in the soils of different parts of the world have been well studied[5], such occurrence and distribution in the soils of Bangladesh have so far received very little attention[6]. The present work therefore was undertaken to study the distribution and abundance of *Azotobacter* in some wheat field soils of Manikganj, Dhamrai, Gajipur and Savar, Bangladesh.

Samples of non-rhizosphere soil, rhizosphere soil and wheat plant were collected from ten various wheat fields of four different collection areas such as Manikganj, Dhamrai, Gajipur and Savar. Rhizosphere soil was collected from the rhizosphere region of the plants at the depth of 2-3 cm and non-rhizosphere soil was also collected from a nearby place of the field at the same depth. The soil pH was determined by an electric pH meter. For this purpose a suspension with a soil:water ratio of 1:2.5 (w/v) of each collected sample was used. One gram of each soil and root sample was used for the purpose of isolation of *Azotobacter*. Roots were washed first with tap water and then with distilled water twice. The pieces of roots were taken in a sterile mortar and macerated with the help of a sterile pestle. Then 10 ml of sterile distilled water was added to prepare a suspension of root sample. Suspension for soil sample was made using the serial dilution method. Plates of LG agar medium[7] were inoculated with the suspension of root and soil samples by streaking method. Plates were incubated at 30ºC for 5 days. Development of flat, soft, milky and mucoid colonies was the indication for the growth of *Azotobacter*. For enumeration of *Azotobacter* suspension of both root and soil samples were prepared using serial dilution method and plated on LG agar medium. After incubation 5 days at 30ºC, the colonies appeared on the agar plates were counted. Nitrogen fixation was determined in terms of the quantity of nitrogen gained in 5-days-old culture of each isolate developed in 50 ml LG broth medium. Nitrogen in culture was estimated by micro-Kjeldahl method.

Corresponding author:
M Motiur Rahman, Department of Botany, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh
Cell: 01818 137949; E-mail: motiur_bot@yahoo.com

Table 1 shows the pH values of different soil samples. All the soil samples were found to be slightly acidic to near the neutral ranging from 6.45 to 6.90.

Table 1. *pH values of soil samples collected from different wheat fields*

Collection place	pH value of soil sample	
	Rhizosphere soil	Non-rhizosphere soil
L_1	6.65	6.45
L_2	6.80	6.65
L_3	6.75	6.90
L_4	6.60	6.55

L_1 = Wheat fields of Manikganj; L_2 = Wheat fields of dhamrai; L_3 = Wheat fields of Gajipur; L_4 = Wheat fields of Savar.

Table 2 shows the occurrence of *Azotobacter* in different soil and root samples collected from various wheat fields of four different areas. The percentage of samples tested positive for *Azotobacter* ranged from 26-45% for root, 98-100% for rhizosphere soil and 84-99% for non-rhizosphere soil. Usually *Azotobacter* does not prefer rhizoplane for colonization probably due to acidity of the concentrated root exudates of plants[8]. This study reflects also the findings of Shilpi *et al*.[9]. *Azotobacter* is widely distributed and a considerable number of surveys in all the continents have detected *Azotobacter* in 30-80% soil samples.

Table 2. *Occurrence of Azotobacter in different wheat fields of four collection areas*

Sample type	% positive sample			
	L_1	L_2	L_3	L_4
Wheat root	45	38	32	26
Rhizosphere soil	99	100	98	100
Non-rhizosphere soil	87	97	99	84

L_1 = Wheat fields of Manikganj; L_2 = Wheat fields of dhamrai; L_3 = Wheat fields of Gajipur; L_4 = Wheat fields of Savar.

Table 3 shows that rhizosphere soil harbours the nitrogen-fixing *Azotobacter* in higher numbers than the non-rhizosphere soil does. This finding is in agreement with the study of Bhat[4], Kavimandan *et al.*[10], Fuller and Hanks[11], Subba Rao[1] and Shilpi *et al*.[9] though in 1988 Sattar and Solaiman[12] reported higher *Azotobacter* population in non-rhizosphere soil compared to rhizosphere ones of rice fields. In general, the population of the organism ranged from 2.6×10^5 to 4.5×10^5 cfu/g, 1.7×10^5 to 2.5×10^5 cfu/g and 1.2×10^5 to 1.7×10^5 cfu/g of root samples, rhizosphere and non-rhizosphere soils respectively.

Azotobacter is rarely found in the soil more acidic than the pH value 6.0[5]. This investigation shows that the pH values within the range of 6.45-6.90 contains a considerable quantity of *Azotobacter*. The highest percentage for *Azotobacter* positive samples tested was 100% in case of rhizosphere soil and the lowest was found in wheat root samples and it was only 26%. The percentage ranges of the samples tested positive were 26-45%, 98-100% and 84-99% respectively for root, rhizosphere and non-rhizosphere soil. Shilpi *et al.*[9] also reported almost similar result in case of rice field ecosystem.

Population of *Azotobacter* in various samples is presented in the Table 3. *Azotobacter* population was found to be the lowest in the rhizoplane and highest in the rhizosphere soil samples, which ranged respectively from 5.2×10^4 to 7.2×10^4 cfu/g and 17.2×10^4 to 2.5×10^5 cfu/g (Table 3). The average population size of the *Azotobacter* in case of non-rhizosphere soil is 1.5×10^5 cfu/g. This study reflects much better finding of *Azotobacter* population than in the report made by Shilpi *et al.*[9], and was in agreement with the findings of Vancura *et al.*[13], Abd-el-Malek[14], Subba Rao[1] and Rao and Venkateswarlu[15].

The population of the heterotrophic bacteria was also determined in the studied samples (Table 4). The highest mean population size for heterotrophic bacteria (1.2×10^8 cfu/g sample) was observed in rhizosphere soil samples and the lowest one was in case of root samples (2.1×10^7 cfu/g sample). The population of heterotrophic bacteria in wheat root samples varied from 2.1×10^7 to 3.3×10^7 cfu/g, and it ranged from 9.9×10^7 cfu/g to 1.2×10^8 cfu/g and from 3.2×10^7 to 4.3×10^7 cfu/g respectively in rhizosphere and non-rhizosphere soils which is slightly smaller than that of wheat reported by Khan *et al.*[16]. According to Alexander[17], plate counts of heterotrophic bacteria ranges usually from several hundred to up to 200 million cfu/g dry soil.

Linear correlation analysis reveals that the population size of rhizospheric *Azotobacter* is positively correlated with that of root (r = 0.96, p = <0.1), *i.e*., higher number of *Azotobacter* in rhizosphere increases the probability of root colonization by it. But at the same time there was found a negative correlation (r = -81) in between the colonization of heterotrophic bacteria and *Azotobacter* in wheat rhizosphere which reflects an adverse effect of heterotrophic bacteria on *Azotobacter* colonization in rhizosphere soils.

Table 3. *Population of Azotobacter in various samples collected from different wheat fields of four collection areas*

Sample type	Number of organism per gram sample				Mean
	L_1	L_2	L_3	L_4	
Wheat root	6.8×10^4	5.5×10^4	5.2×10^4	7.2×10^4	6.2×10^4
Rhizosphere soil	2.5×10^5	1.7×10^5	2.0×10^5	1.8×10^5	2.0×10^5
Non-rhizosphere soil	1.7×10^5	1.5×10^5	1.5×10^5	1.2×10^5	1.5×10^5

L_1 = Wheat fields of Manikganj; L_2 = Wheat fields of dhamrai; L_3 = Wheat fields of Gajipur; L_4 = Wheat fields of Savar.

Table 4. *Population of total heterotrophic bacteria in various samples collected from wheat fields of four collection sites*

Sample type	Number of organism per gram sample ($x10^6$)				Mean
	L_1	L_2	L_3	L_4	
Wheat root	3.3×10^7	2.5×10^7	2.1×10^7	2.8×10^7	2.7×10^7
Rhizosphere soil	1.2×10^8	1.2×10^8	9.9×10^7	1.1×10^8	1.1×10^8
Non-rhizosphere soil	4.3×10^7	3.2×10^7	3.6×10^7	4.0×10^7	3.8×10^7

L_1 = Wheat fields of Manikganj; L_2 = Wheat fields of dhamrai; L_3 = Wheat fields of Gajipur; L_4 = Wheat fields of Savar.

Among various colonies grown on the LG agar plates five were selected based on their growth performances to be studied for their nitrogen fixing potential which might led to any effective application of this organism through studying other attributes in the near future. Information about five selected strains are given in the Table 5. Species of *Azotobacter* was first studied by Beijerinck[17] showing the capacity to fix atmospheric nitrogen. The nitrogen fixing potential of the selected five strains of *Azotobacter* in this study was determined (Table 5).

Table 5. *Nitrogen fixing potential of the selected Azotobacter isolates recover from wheat fields*

Selected isolate	Source	Collection place	Nitrogen fixing potential (mg N/g substrate)
M_1	Rhizosphere soil of wheat	L_1	9.26
M_2	Histosphere of wheat root	L_3	5.72
M_3	Rhizosphere soil of wheat	L_4	7.52
M_4	Non-rhizosphere soil	L_2	5.45
M_5	Histosphere of wheat root	L_2	6.93

L_1 = Wheat fields of Manikganj; L_2 = Wheat fields of dhamrai; L_3 = Wheat fields of Gajipur; L_4 = Wheat fields of Savar.

It was found that the selected strains could fix nitrogen ranging from 5.45 to 9.26 mg N/g substrate by 5-days-old 25 ml culture. The strain M_1 could fix the highest amount of nitrogen (9.26 mg N/g substrate) and the strain M_4 fixed the least amount (5.45 mg N/g substrate). Shilpi *et al.*[18] reported nitrogen fixation by *Azotobacter* within the range of 0.69-1.18 mg N/25 ml culture. According to Hamdi[19] the normal capacity range for nitrogen fixation by *Azotobacter* is 10 mg N/g of carbohydrate consumed. Jensen[20] reported a range of nitrogen fixation by *Azotobacter* was 10-15 mg N/g of agar consumed. So, the present study shows that the isolates recovered from the wheat fields are of average standard in terms of their nitrogen fixing potential in the laboratory condition.

References

1. Subba Rao NS. 1982. *Biofertilizers in Agriculture*, pp 77-91. Oxford & IBH Publishing Co Pvt Ltd, New Delhi.
2. Shende ST, Apte RG & Singh T. 1975. Multiple action of *Azotobacter*. *Ind J Genet Plant Breeding*. **35**: 314.
3. Bhandari SC & Somani LL. 1990. *Azotobacter*. In *Biofertilizers* (Somani LL ed), pp 355-361. Scientific Publishers, Jodhpur.
4. Bhat JV. 1961. Bacterization experiment with *Azotobacter*. In *Radioisotopes, Fertilizers and Cow Dung Gas Plant* (Raghvan D, Kachroo P & Doraiswami S eds), p 317. ICAR, New Delhi.
5. Thompson JP & Skermann VBD. 1979. *Azotobacteraceae: The Taxonomy and Ecology of the Aerobic Nitrogen-Fixing Bacteria*. Academic Press, London.
6. Sattar MA. 1991. Rice based BNF research in Bangladesh: Problems and prospects. In *Biological Nitrogen Fixation Associated with Rice Production* (Dutta SK & Charles S eds), pp 211-218. Oxford & IBH Publishing Co Pvt Ltd, New Delhi.
7. Dobereiner J. 1980. Forage grasses and grain crops. In *Methods for Evaluating Biological Nitrogen Fixation* (Bergressen FJ ed), pp 535-555. John Wiley & Sons, New York.
8. Dobereiner J. 1974. Nitrogen fixing bacteria in the rhizosphere. In *The Biology of Nitrogen Fixation* (Quispel A ed), pp 86-120. North Holland, Amsterdam.
9. Shilpi RY, Akond MA & Khan ZUM. 1993. Distribution and abundance of *Azotobacter* in the soils of Jahangirnagar University campus. *Bangladesh J Life Sci.* **5**(1): 19-24.
10. Kavimandan SK, Lakshmi Kumari M & Subba Rao NS. 1978. Non-symbiotic nitrogen-fixing bacteria in the rhizosphere of wheat, maize and sorghum. *Proc Indian Acad Sci*. **87**B: 299-302.
11. Fuller WH & Hanks K. 1982. Distribution of *Azotobacter* in arid soils. *Plant Soil*. **64**(3): 355-361.
12. Sattar MA & Solaiman ARM. 1988. Distribution and abundance of *Azotobacter* spp in rice rhizosphere of some Bangladesh soils. *Bangladesh J Sci Ind Res*. **23**(1-4): 201-203.
13. Vancura V, Abd-el-Malek Y & Zayed MN. 1965. *Azotobacter* and *Beijerinckia* in the soils and rhizosphere of plants in Egypt. *Folia Microbiol*. **10**: 224-229.
14. Abdel-el-Malek Y. 1971. Free-living nitrogen-fixing bacteria in Egyptian soils and their possible contribution to soil fertility. *Plant Soil.* Special Volume: 423-442.
15. Rao AV & Venkateswarlu B. 1982. Occurrence of *Azotobacter* and nitrogen fixation in the desertic soils. *Indian J Microbiol.* **22**(4): 255-257.
16. Khan ZUM, Akond MA & Mubassara S. 2001. Population of heterotrophic bacteria and *Azospirillum* in wheat field soil and nitrogen-fixing potential of the same soil. *Bangladesh J Life Sci.* **13**(1-2): 1-5.
17. Alexander M. 1977. *Introduction to Soil Microbiology.* Wiley Eastern Ltd, New Delhi.
18. Shilpi RY, Akond MA & Khan ZUM. 1998. Growth and nitrogen-fixing potential of *Azotobacter* isolated from the Jahangirnagar University campus. *Bangladesh J Life Sci.* **10**(1-2): 1-5.
19. Hamdi YA. 1982. Application of nitrogen-fixing systems in soil improvement and management. *FAO Soil Bull.* **49**: 45-82.
20. Jensen HL. 1953. *Azotobacter* as a crop inoculant. *Proc Int Congr Microbiol Rome*. **6**: 245.