International Journal of Botany

ISSN: 1811-9700

science alert

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International Journal of Botany 6 (2): 117-121, 2010
ISSN 1811-9700

Quantification of Heterotrophic Bacteria and *Azospirillum* from the Rhizosphere of Taro (*Colocasia esculenta* L. Schott.) and the Nitrogen Fixing Potential of Isolated *Azospirillum*

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Abstract: Occurrence and distribution of heterotrophic soil bacteria and an important associative microaerophilic nitrogen fixing *Azospirillum* from the rhizosphere of Taro (*Colocasia esculenta* L. Schott.) was studied. Samples of Taro root and rhizosphere soil were collected from ten different sites of Jahangirnagar University campus. Organisms were found to occur in root and rhizosphere soil of Taro. The quantity of both total heterotrophic bacteria and *Azospirillum* spp. in two different samples varied significantly with the range of 7.55×10^6 to 97.66×10^7 and 0.1×10^6 to 11×10^6 MPN g^{-1} dry wt., respectively. Rhizosphere soil was found to harbor the highest population of both types of organisms. The nitrogen fixing potential of ten isolates of *Azospirillum* spp. was also determined in an enrich medium. As per the efficiency of nitrogen fixation (2 to 6.16 mg N g^{-1} substrate) the isolated *Azospirillum* strains could be arranged as J-4>J-6>J-3>J-9>J-5>J-7>J-2>J-10>J-8>J-1.

Key words: Heterotrophic bacteria, *Azospirillum*, nitrogen fixation, Taro rhizosphere

INTRODUCTION

Nitrogen is a very important plant nutrient whose limitation can affect yield considerably. Production of chemical nitrogen fertilizers besides being costly depletes non-renewable sources and poses human and environmental hazards. To complement and eventually substitute mineral fertilizers with biologically fixed nitrogen would represent an economically beneficial and ecologically sound alternative (Glick *et al*., 1999). Inorganic or mineral fertilizer N is the rich and readily available source of N as plant nutrient contributing to environmental hazards in a variety of way.

Soil microorganisms are the biological components of soil that constitute less than 0.5% (w/w) of the soil mass (Tate, 1995). They play an important role in soil process that determines plant productivity. Heterotrophic bacteria are one of the main components of microbial community. The population of these bacteria gives an indication about the soil sustainability for agriculture. In our country, studies on these organisms especially on associative and free-living diazotrophs have yet not been done at the level of mentionable magnitude.

Azospirillum is an important free-living, microaerophillic, nitrogen-fixing bacteria which make a symbiotic association on roots of different crops. Nitrogen fixation may significantly contribute to plant nutrition in gramineous system (Lima *et al*., 1987). The contribution of *Azospirillum* to the increase in the yield of crops is not only for its N_2 fixation, but also for its other activities like the production of growth promoting substances and enhancing the uptake of nutrition by root. Therefore, *Azospirillum* species are considered to be plant growth-promoting rhizobacteria (Lucy *et al*., 2004) able to produce hormone like substances (Bottini *et al*., 1989) and fix atmospheric nitrogen in association with grasses (Elmerich *et al*., 1992). A significant activity of these bacteria is the production of auxin-type phytohormone that affects root morphology and thereby, improve nutrient uptake from soil. This may be more important than their N_2-fixing activity (Dobbelaere *et al*., 1999). *Azospirillum* obtained from the roots of a crop can usually colonize on the roots of other crops and can also enhance the growth. The significant effect of *Azospirillum* inoculation on the grain yield of rice, wheat, oat, barley and sorghum have been reported Subba Rao (1979).

This organism has wide adaptability to different environmental conditions. This organism has been found to occur in soil and in root of different plants in various environments including desert. The nitrogen fixing bacteria *Azospirillum* has been found in temperate and tropical regions associated with a wide variety of grasses and cereal crops (Neyra and Döbereiner, 1977; Berkum

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and Bohlool, 1980). *Azospirillum* has also been found to occur in the saline non-rhizosphere soil (Rahman *et al*., 2007) and in the coastal region of Bangladesh (Khan *et al*., 2003).

Taro is used to refer to *Colocasia esculenta* L. Scott. a member of Aracaceae family, is an ancient crop grown throughout the human tropics for its edible corms and leaves as well as its nutrient value. As far as its consumer acceptance is concerned, *Colocasia esculenta* commonly known as Taro or cocoyam is an important food of developing countries like Africa, West Indies, the Pacific and Asia territory.

The nitrogen-fixing bacteria *Azospirillum* may play vital role in the nitrogen economy in Taro (*Colocasia esculenta*) rhizosphere. As the roots of this plant also contain Calcium Oxalate, the rhizosphere environment of taro can be considered to be different from that of other plants specially crop plants. Comprehensive and systematic works on *Azospirillum* from the rhizosphere of Taro almost have not been studied. Therefore, the present work was proposed to emphasize on the study of the occurrence, distribution of heterotrophic bacteria and *Azospirillum* and the nitrogen-fixing potential of *Azospirillum* from the rhizosphere of Taro (*Colocasia esculenta* L. Scott).

MATERIALS AND METHODS

Roots and rhizosphere soil of Taro were collected from ten different sites of Jahangirnagar University campus in 2006-2007 (Table 1). The determination of soil pH was done on aqueous soil suspensions (1:2.5) using an electric meter. Collected root samples of Taro were placed under a gentle stream of water. After removing adhering soil, root samples were thoroughly washed for several times with distilled water. These root samples were macerated in mortars and sterile water added to each macerated sample to prepare suspension (1:10). Suspension of soil samples was prepared by mixing soil with sterile water. Dilutions (10^{-1} to 10^{-7}) of the samples were prepared using sterile water. The dilution plate count method using nutrient agar was used to estimate the population of total heterotrophic bacteria in the samples. To determine the population of *Azospirillum* in the both root and soil sample, the samples were processed as described by Watanabe and Brotonegro (1981) and the population in root and soil sample was counted by the Most Probable Number (MPN) method of Alexandra (1965). Five screw capped tubes of semi solid nitrogen free bromothymol blue medium were incubated with each of the selected dilutions of every sample. Then inoculated tubes were incubated at 35° for 72 h. After incubation the growth of *Azospirillum* appeared in the tubes as the characteristic thin, dense, white pellicle, a few mm below the surface of the medium (Döbereiner, 1980). The pellicles were examined microscopically for the presence of gram negative, vibroid and actively motile cells. From the number of tubes showing the characteristic growth, the MPN of *Azospirillum* was determined following the MPN chart given by Alexander (1965).

Nitrogen fixation was determined in terms of the quantity of nitrogen gained in the 72 h old culture of each strain developed in 25 mL semi-solid nitrogen free malate medium. The total nitrogen in nitrogen culture was estimated by Micro-Kjeldahl method (Cottenie, 1980).

RESULTS AND DISCUSSION

The initial and one of the most important factor is pH for the growth of *Azospirillum*. Earlier observation indicated that *Azospirillum* requires near neutral pH for abundant occurrence (Döbereiner and Day, 1976; Döbereiner *et al*., 1976). In this study, the pH value of soil and root of ten samples ranged from 5.05 to 7.82 and 6.15 to 6.98, respectively (Table 1). Most of the organisms could grow in the pH ranging from 6.0 to 8.0. The optimum pH value for the growth of the strains was found to 7.0 or very close to 7.0.

Table 1: pH values of the rhizosphere soil and roots of *Colocasia esculenta* samples collected from 10 different locations of the Jahangirnagar University, Dhaka, Bangladesh

		pH values of samples	
Location	Description	Rhizosphere soil	Root
L_1	Eastern side of Biological Science Faculty, JU	7.82	6.62
L_2	Northern side of Gymnasium, JU	6.62	6.89
L_3	North Eastern side of Physics Department, JU	6.56	6.56
L_4	Front side of Kamal Uddin Hall Area, JU	7.03	6.35
L_5	Old Arts Faculty Area, JU	6.51	6.26
L_6	Western side of Nawab Faijunnesa Hall, JU	5.05	6.78
L_7	Jahanara Imam Hall Area, JU	6.95	6.34
L_8	Botanical Garden Area, JU	7.48	6.54
L_9	Southern side of Transport Office, JU	6.91	6.15
L_{10}	Western side of Mir Mosharraf Hossain Hall, JU	6.98	6.98

JU: Jahangirnagar University

Alexander (1977) reported that the plate counts of heterotrophic bacteria usually give values ranging from several thousand to 200 million per g dry soil. The greatest population is in the surface horizons. According to Brady (1974) the numbers of bacteria are high, normally ranging from a few hundred million to 3×10^{12}. Shenouda *et al*. (1996) reported the microbial content of sand dunes in Siwa Oasis and Matrough in North Western coast of Egypt. Khan *et al.* (2003) found that heterotrophic bacterial population ranged from 1.33×10^7 to 24.67×10^7 cfu g^{-1} sample and *Azospirillum* population varied from 0.34×10^6 to 27.46×10^6 MPN g^{-1} sample. In this investigation, the soil samples were found to bear more bacteria than root samples of *Colocasia esculenta.* The average population of heterotrophic bacteria in the rhizosphere soil sample ranged from 9.66×10^6 cfu g^{-1} (location-4) to 98×10^6 cfu g^{-1} (location-8), whereas in root sample (endorhizosphere) the range was 7.55×10^6 cfu g^{-1} (location-9) to 97.66×10^6 cfu g^{-1} (location-5) (Table 2). Statistical analysis showed that the variation in the population of heterotrophic bacteria in the collected samples due to variation in location is not significant indicating that the characteristics of the locations has no influence on the population of heterotrophic bacteria.

The occurrence of *Azospirillum* in the roots of rice and grasses (Lakshmi *et al*., 1977) and in the stem of rice (Watanabe and Barraquio, 1979) has been reported. There is report that only 10% of soil and root samples from temperate regions contained *Azospirillum*, whereas more than 50% of tropical samples were found positive (Döbereiner *et al*., 1976; Döbereiner, 1978). Roots of wheat, rice, maize and forage grasses were found to harbor *Azospirillum* (Da-Silva and Döbereiner, 1978). They found that soils under grasses retained more *Azospirillum* than others. In this study, both soil and root samples were found to contain considerable amount of *Azospirillum*. It was found that Taro rhizosphere soil harbored more *Azospirillum* than Taro root. Overall variation of the population (MPN) of *Azospirillum* in the collected samples was from 0.1×10^6 to 11×10^6 MPN g^{-1} dry wt. The highest *Azospirillum* population (9.04×106 MPN g^{-1} dry wt.) was observed in Taro rhizosphere soil sample of location-3 (L-3) and the lowest one (2.15×106 MPN g^{-1} dry wt.) was found in root of location-10 (L-10). Analysis showed that the population of *Azospirillum* varied significantly in different samples but not in different locations (Table 3).

Döbereiner *et al.* (1976) first reported that the bacterium *Azospirillum* is widely distributed in the rhizosphere of several tropical grasses. Ravikumar *et al.* (2002) observed that bacterial density of *Azospirillum* was found higher in the roots of *Avicennia marina*

Table 2: Heterotrophic bacterial count in the rhizosphere soil and root sample of *Colocasia esculenta*

Location	Number of total cfu g^{-1} sample ($\times10^6$) Rhizosphere soil	Root
L_1	27.33	37.88
L_2	10.88	14.55
L_3	18.44	13.22
L_4	9.66	19.66
L_5	66.33	97.66
L_6	38.00	9.88
L_7	31.44	28.89
L_8	98.00	58.00
L_9	57.77	7.55
L_{10}	37.99	30.88
Mean value ($\times10^6$)	39.58	31.81
Sig. At 5%	0.538	

Table 3: Population of total *Azospirillum* in the rhizosphere soil and root of selected *Colocasia esculenta*

	Number of total MPN g^{-1} sample ($\times10^6$)					
Location	Rhizosphere soil			Root		
L_1	8.80	8.79	8.81	1.00	0.95	1.11
L_2	1.98	1.90	2.9	0.39	0.29	0.41
L_3	11	10.26	9.04	1.99	2.01	2.15
L_4	2.50	2.51	2.49	1.26	1.96	2.02
L_5	2.01	2.51	2.23	0.26	0.28	0.29
L_6	4.41	4.64	4.66	0.49	0.41	0.51
L_7	2.75	2.56	2.71	0.31	0.39	0.35
L_8	3.29	3.59	3.8	0.56	0.49	0.55
L_9	2.19	2.59	2.90	1.7	1.46	1.67
L_{10}	3.39	3.35	3.29	0.1	0.25	0.15
Mean value ($\times10^6$)						
CD at 5% level	0.007					

(148.88×10^4 g^{-1} dry wt.) and rhizosphere sediment of *Suaeda monoica* (20×10^3 g^{-1} dry wt.) in mangroves of India. Shanta *et al*. (2006) studied that root histosphere of *Colocasia esculenta* (L.) and stem (rhizome) of the same plant were found to be suitable for *Azospirillum* as these samples showed higher population. This report is quite in agreement with the present investigation in case of rhizosphere of *Colocasia esculenta.* Khan *et al.* (2003) studied the population of heterotrophic bacteria and *Azospirillum* in the rhizosphere soil and root histosphere of *Cynodone dactylone*, *Acanthus ilicifolium*, *Ocimum basilicum* and *Amaranthus spinosusus* as well as non rhizosphere soil in the coastal habitat of Patuakhali, Bangladesh. They also found the variation in the population of heterotrophic bacteria and *Azospirillum.*

Khan *et al.* (2001) reported that nitrogen-fixing potentials of *Azospirillum* isolated from wheat field of Dhaka ranged from 15.12 to 22.16 mg N g^{-1} substrate. They also reported that some thermophilic strains of *Azospirillum* isolated from Bangladesh soil could fix nitrogen well at 55°C and the values ranged from 10.08 to 28.00 mg N g^{-1} substrate. The ten selected *Azospirillum* strains examined for their nitrogen fixing potential were

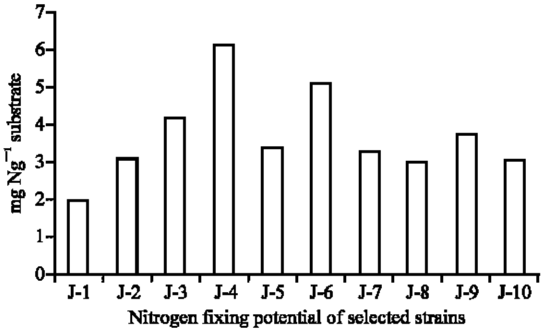

Fig. 1: Nitrogen-fixing potential of the selected strains of *Azospirillum*

J-1, J-2, J-3, J-4, J-5, J-6, J-7, J-8, J-9 and J-10. All the selected strains could fix atmospheric nitrogen in semi-solid nitrogen-free malate medium (without bromothymol blue). The nitrogen fixing potential of the selected strains in the presence of malate as sole carbon source is shown in the Fig. 1. In the present study, the extent of nitrogen fixation in all ten strains ranged from 2.00 mg N/substrate to 6.16 mg N g^{-1} substrate. Among the selected strains J-4 fixed the highest amount of nitrogen followed by J-6 and J-1 fixed the least amount of nitrogen. As per their nitrogen fixing capability, the selected strain could be arranged as J-4>J-6>J-3>J-9>J-5>J-7>J-2>J-10>J-8>J-1.

Döbereiner and Day (1976) reported the fixation of 115 mg N g^{-1} lactate. Such a higher value of nitrogen fixation was not reported in other studies. Lakshmi *et al.* (1977) and Okon *et al.* (1977) reported the nitrogen fixation values of 12 to 36 and 20 to 24 mg N/s substrate respectively. Khan and Akond (1996) reported that the amount of nitrogen fixation by their strains was ranged from 3.6 to 5.26 mg N g^{-1} substrate which is also similar to the present finding.

This study indicates that the rhizosphere of *Colocasia esculenta* harbor a considerable number of *Azospirillum* and the bacterium is also capable to fix atmospheric nitrogen. In future, suitable and efficient strains can be isolated to develop the inoculants of this organism for various crops of Bangladesh.

ACKNOWLEDGMENTS

I want to give thanks to Almighty Allah who gives me the ability to complete my research work soundly. I also wish to give my gratitude to all honorable teachers of my department who provided me academic co-operation and inspiration.

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