International Journal of Microbiological Research 4 (3): 296-299, 2013
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2013.4.3.81151

Prevelence of *Azotobacter* sp. in Chilli (*Capsicum annuum* L.) Rhizosphere Soil of Cuddalore District, Tamil Nadu, India

D. Kanchana, M. Jayanthi, P. Saranraj and D. Sujitha

Department of Microbiology, Annamalai University, Annamalai Nagar,
Chidambaram-608 002, Tamil Nadu, India

Abstract: Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, which enhance the growth of the plant either directly or indirectly. The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production, production of phytohormones such as auxins, cytokinins and gibberellins and lowering of ethylene concentration. There are many reports on plant growth promotion and yield enhancement by plant growth-promoting rhizobacteria (PGPR). *Azotobacter* is a one of the PGPR bacteria used to enhance the growth of plants. The mechanisms of plant growth promotion by *Azotobacter* include: the ability to produce phytohormones, N_2 fixation. In this present study, ten *Azotobacter* isolates were isolated from the chilli rhizosphere soil in Cuddalore district, Tamil Nadu, India and designated as Azo-1 to Azo-10. The highest IAA production was recorded by the isolate Azo-4 obtained from Kothattai soil compared to other isolates. Maximum nitrogen fixation 16.9 ± 0.5 kg of N g^{-1} of malate was also recorded by the isolate Azo-4.

Key words: PGPR · *Azotobacter* sp. · Chilli · Rhizosphere Soil and Indole Acetic Acid

INTRODUCTION

Chilli (*Capsicum annuum* L.) is an important commercial spice cum vegetable crop of India. Chilli belongs to the family Solanaceae, genus *Capsicum* and the two important cultivated species are *Capsicum annum* and *Capsicum frutescens*. Chilli forms an essential ingredient of Indian curry. There is no spice probably as popular as chilli and no other spice has become such an indispensable ingredient of the daily food of majority people of the world [1]. Chilli is a rich source of vitamin 'C' and 'A' with plenty of minerals. The colour plays an important role in assessing the quality of chillies. The principal colouring matter is capsanthin, the carotenoid pigment which contributes about 35 per cent to the total pigments. India is a major producer, consumer and exporter of chilli contributing 25 per cent to total world production. India exported 1.69 lakh t of dry chilli in 2007-2008 and the value of the export was Rs.906.44 crore [2].

Azotobacter is motile coccal shaped, Gram negative bacterium an Gram negative and coccal shaped and motile bacterium. *Azotobacter* is highly versatile in utilizing carbon sources, therefore application of organic carbon containing sources to the soil to improve nitrogen fixation capacity of diazotrophs [3]. The family Azotobacteriaceae comprises of two genera namely, *Azomonas* (non-cyst forming) with three species (*Azomonas agilis, Azomonas insignis* and *Azomonas macrocytogenes*) *and Azotobacter* (cyst forming) comprising of 6 species, namely, *Azotobacter chroococcum, Azotobacter vinelandii, Azotobacter beijerinckii, Azotobacter nigricans, Azotobacter armeniacus* and *Azotobacter paspali. Azotobacter* is generally regarded as a free-living aerobic nitrogen-fixer.

MATERIALS AND METHODS

Isolation and Enumeration of *Azotobacter* from the rhizosphere soils of Chilli: The *Azotobacter* strains were isolated from different rhizosphere soils following the method of Allen [4]. Ten gram of representative soil samples were suspended in 90 ml sterilized distilled water in 250 ml Erlenmeyer flasks thoroughly by shaking which will provide 10^{-1} dilution of samples are made with limited

Corresponding Author: D. Kanchana, Department of Microbiology, Annamalai University, Annamalai Nagar, Chidambaram-608 002, Tamil Nadu, India.

water blanks because of this bacteria are very low in soils. The dilutions are restricted to 10^{-3} or 10^{-4} using sterile pipettes. One ml of the each dilution transferred aseptically to the sterile petriplates. Three petriplates were used for each dilution. To each petriplates, 15-20 ml of selective media Waksman 77 medium are added and incubated at room temperature (30 ± 2°C). Colonies of these bacteria would appear after 3-5 days if necessary the plates are to be incubated for 7 days, as these bacteria are slow growers. *Azotobacter* cells grow as varied slimy colonies on agar surface and aged cultures showed brown black colouration due to pigment production. The smooth colonies with glistening appearance were picked up and purified by streak plate technique and the single colonies were subculture in Waksman base medium no. 77 strains were maintained in (4°C) refrigerator after significant growth. The isolated *Azotobacter* isolates were counted in Qubec colony counter.

Characterization of *Azotobacter* Isolates: The morphological characteristics of *Azotobacter* cells were studied by following the production of acid from glucose [5] and utilization of different carbon sources

Quantitative Estimation of Indole Acetic Acid by *Azospirillum* Isolates: The dinitrogen fixation of the *Azospirillum* isolates was determined by employing the Salper's reagent proposed by Gorden and Paleg [6].

Determination of Dinitrogen Fixation of the *Azospirillum* Isolates: The dinitrogen fixation of the *Azospirillum* isolates was determined by Microkjeldhal assay described by Humphries [7].

RESULTS AND DISCUSSION

Ten different places namely Keezhamanakudi, Sivapuri, Kavarapattu, Kothattai, Karikuppam, Pudhupettai, Periyapattu, Pudhuchathiram, Mutlur and Vallam were selected from Chidambaram taluk, Cuddalore district for the collection of rhizosphere soil samples of Chili. The rhizosphere soil samples were analyzed for the physico-chemical properties, the occurrence of different PGPR and total microbial population. The survey was conducted at ten locations in Cuddalore district of Tamil Nadu comprising Keezhamanakudi, Sivapuri, Kavarapattu, Kothattai, Karikuppam, Pudhupettai, Periyapattu, Pudhuchathiram, Mutlur, Vallam (Table 1). The population of *Azotobacter* ranged from 1.3 to 3.2 $\times 10^5$ cfu g^{-1} of soil and the soil samples collected from Keezhamanakudi recorded the maximum occurrence. The lowest occurrence was o bserved with *Azotobacter* which ranged from 1.3 $\times 10^5$ cfu g^{-1} of soil in Periyapattu.

Table 1: Occurrence of PGPR organisms in the rhizosphere soil of chili

Name of the location	*Azotobacter* × 10^5 cfu g^{-1} soil
Keezhamanakudi	3.2
Sivapuri	3.1
Kavarapattu	2.3
Kothattai	3.1
Karikuppam	2.0
Pudhupettai	2.2
Periyapattu	1.3
Pudhuchathiram	2.0
Mutlur	3.1
Vallam	3.0

Table 2: Designation of the *Azotobacter* organisms in the rhizosphere soil samples

Name of the location	Azotobacter
Keezhamanakudi	Azo-1
Sivapuri	Azo-2
Kavarapattu	Azo-3
Kothattai	Azo-4
Karikuppam	Azo-5
Pudhupettai	Azo-6
Periyapattu	Azo-7
Pudhuchathiram	Azo-8
Mutlur	Azo-9
Vallam	Azo-10

The *Azotobacter* isolates *viz*., Azo-1 to 10 was characterized for their morphological and physiological characters and the results are presented in Table 3. The morphological characters *viz*., cell shape, motility, gram reaction were observed. All the ten isolates were coccal shaped, motile and Gram negative. It was also observed that, all the ten isolates could utilize various carbon sources such as citrate, starch, mannitol and sodium benzoate at 0.5% concentration for their growth. But the isolates could not utilize sodium benzoate at 1% concentration and exhibited no growth. All the isolates could not utilize rhamnose for growth. Based on the above characteristics, the isolates were tentatively identified as *Azotobacter chroococcum.* The finding of the present study was in line with Saranraj *et al*. [8].

The *Azotobacter* isolates were screened for their efficiency to produced Indole acetic acid and nitrogen fixing efficiency. All the isolates produced IAA under *in vitro* condition and the results were furnished in Figure 1.

Table 3: Characterization and identification of *Azotobacter* isolates

	Reaction of Isolates									
Character studies	Azo-1	Azo-2	Azo-3	Azo-4	Azo-5	Azo-6	Azo-7	Azo-8	Azo-9	Azo-10
Cell shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Gram reaction	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Utilization of citrate	+	+	+	+	+	+	+	+	+	+
Utilization of starch	+	+	+	+	+	+	+	+	+	+
Utilization of mannitol	+	+	+	+	+	+	+	+	+	+
Utilization of sodium benzoate (0.5%)	+	+	+	+	+	+	+	+	+	+
Growth in mannitol media with 1.0% benzoate	-	-	-	-	-	-	-	-	-	-
Utilization of rhamnose	-	-	-	-	-	-	-	-	-	-

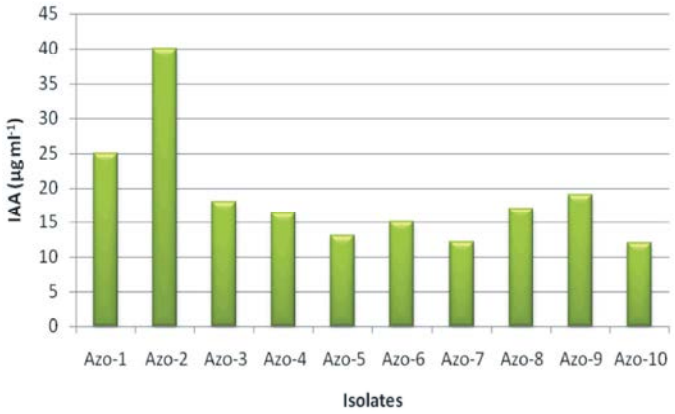

Fig. 1: Indole Acetic Acid (IAA) production by *Azotobacter* isolates

Table 4: Nitrogen fixation by *Azotobacter* isolates

Azotobacter Isolates	Nitrogen fixation (µg of Ng^{-1} of malate)
Azo-1	12.4±0.6
Azo-2	9.5±0.5
Azo-3	10.0±0.3
Azo-4	16.5±0.4
Azo-5	14.8±0.6
Azo-6	8.0±0.5
Azo-7	8.4±0.5
Azo-8	9.6±0.4
Azo-9	9.5±0.5
Azo-10	14.0±0.5

The amount of IAA produced ranged from 15.18 to 24.08 µg ml^{-1} of culture filtrate. The highest IAA production was recorded by the isolate Azo-4 obtained from Kothattai soil (Table 4). The finding of the present study was similar to the results of Sivasakthi *et al*. [9].

CONCLUSION

From the present study, it was concluded that the plant growth promoting rhizobacteria *Azotobacter* the highest IAA production was recorded by the isolate Azo-*4* obtained from Kothattai soil compared to other isolates. Maximum nitrogen fixation 16.9 ± 0.5 kg of N g^{-1} of malate was also recorded by the isolate *Azo-4*. Taken together, these results suggest that *Azotobacter* are able to induce IAA production, N_2 fixing ability improving growth of plants. The use of *Azotobacter* as inoculants biofertilizers is an efficient approach to replace chemical fertilizers and pesticides for sustainable crop cultivation.

REFERENCES

1. Parvathi, S. and A.H.J. Yurnus, 2010. Chilli an economically profitable crop of Malaysia, Spi. India, 13(7): 18-19.
2. Anonymous, 2006. Available technologies to raise yield of spices. Surv. Indian Agric, The Hindu Yearobok, pp: 82-86.
3. Rao, V.R., 1978. Effect of carbon sources on a symbiotic nitrogen fixation a paddy soil. Soil Biol. Biochem., 10: 319-321.
4. Allen, O.N., 1953. Experiments in Soil Bacteriology. Burgess publ. co., Minneapology Minnesota, U.S.A. pp: 69-70.

5. Kreig, N.R. and J.J. Tarrand, 1978. Taxonomy of the root associated nitrogen fixing bacterium Spirillum lipoferum In: Limitations and potentials for Biological Nitrogen fixation in the tropics, Dobereiner, J. (Ed). Plenum press, New York, pp: 317-334.
6. Gorden, S.A. and L.G. Paleg, 1957. Quantitative measurement of Indole acetic acid, Physiol. Plantarum, 10: 347-348.
7. Humphries, E.C., 1956. Mineral Composition and ash analysis: In: Modern methods of plant analysis vol. I. Peach and M.V. Tracy (Eds), Springer-Verlag, Berilin, pp: 468-502.
8. Saranraj, P., P. Sivasakthivelan and S. Siva Sakthi, 2013. Prevalence and production of plant growth promoting substance by *Pseudomonas fluorescens* isolated from paddy rhizosphere soil of Cuddalore district, Tamil Nadu, India. African Journal of Basic and Applied Sciences, 5(2): 95-101.
9. Sivasakthi, S., D. Kanchana, G. Usharani and P. Saranraj, 2013. Production of plant growth promoting substance by *Pseudomonas fluorescens* and *Bacillus subtilis* isolated from paddy rhizosphere soil of Cuddalore district, Tamil Nadu, India. International Journal of Microbiology Research, 4(3): 227-233.