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Research Article

Interstrain differences of Chilli *Azospirillum* isolates on their plant growth promoting traits under *in vitro* conditions

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Abstract

In the present study, an attempt was made to screen the efficiency of *Azospirillum* isolates obtained from the chilli rhizosphere soils of Cuddalore district in terms of their ability in production of certain plant growth promoting traits (indole acetic acid production, gibberellic acid production and siderophore production) under *in vitro* conditions. The results clearly revealed that the isolate Azo - 2 (*Azospirillum brasilense*) obtained from Palur location recorded maximum production of the above traits with (IAA); (GA_3); and of catechol and salicylate types of siderophores respectively.

Keywords: *Azospirillum*, IAA, GA_3 and Siderophore

Introduction

Among the spice crops of India, Chilli (*Capsicum annuum* L.), a member of Solanaceae family is an important one. It is grown over an area of about 758 thousand ha with a production of 1234.10 thousand tones and productivity of 1628 kg ha^{-1}. It is an indispensible condiment of every home as used in the daily diet. Being introduced by Portuguese in 17^{th}century, now the crop is grown all over India, especially in Andhra Pradesh, Karnataka, Tamilnadu and Maharashtra, account for 3/4 of the total area besides Madhya Pradesh, West Bengal, Punjab, Bihar and Rajasthan. Mineral nutrition is one of the main factors, which influences on growth, yield of chilli to a great extent. A group of microorganisms are known to significantly contributing the nutritional requirements in many crops including chilli. The genus *Azospirillum* is known for their importance plant growth through different mechanisms *viz.*, biological nitrogen fixation, production of plant growth promoting hormones, improving root development and mineral uptake etc (Kanchana *et al*., 2013).

Among the nitrogen fixing bacteria, *Azospirillum* is consider to be an associate symbiotic bacterium in root system and help fixing substantial amounts of nitrogen and several soil bacteria and fungi (Gaur and Ostwal, 1972; Sivasakthi *et al*., 2013). The occurrence of *Azospirillum* in the rhizosphere varied from 1 to 10 per cent of total rhizosphere population (Okon, 1985; Sivasakthivelan and Saranraj, 2013). The rhizosphere contains 100 times more Azospirilla than in the non-rhizosphere soils (De Coninck *et al.*, 1988; Kanchana *et al*., 2013).

Azospirillum has been isolated from different agro climatic zones and in crops all over the world (Michiels *et al.*, 1989; Sureshkumar *et al*., 2011; Saranraj *et al*., 2013). Kavitha (2000) isolated *Azospirillum* species from wetland rice and reported that *Azospirillum* accounts for 18 per cent of the total heterotrophic population. The association of *Azospirillum* with cashew and its possible role in improving crop growth and yield was reported by Purushothaman (2002).

The efficiency of biofertilizers like *Azospirillum* is often studied with their ability under *in vitro* conditions prior to field level inoculations. Among the several traits nitrogen fixing ability in terms of nitrogenase activity, cell N content, production of growth promoting substances like IAA, GA_3, siderophores production are commonly practiced. Production of IAA, GA_3 and siderophores by *Azospirillum* were previously reported by many researchers (Thuler *et al.,* 2003; Tiwari *et al*., 2003; Pedraza *et al*., 2004; Bashan *et al.,* 2004; Ashrafuzzaman *et al.,* 2009; Andres Naiman *et al.,* 2009; Baset Mia *et al.,* 2010; Vinithadali, 2013). Similarly, the nitrogenase activity and cell N content of *Azospirillum* isolates were reported earlier (Mallik *et al.,* 1997; Dobbelaere *et al.,* 2003; Hesham and El-Komy, 2005; Vinithadali, 2013). Hence, in the present study, an attempt was made to screen the superior *Azospirillum* isolate in terms of above said parameters.

Materials and Methods

The Chilli *Azospirillum* isolates obtained previously from ten different locations of Cuddalore district were designated as Azo - 1 to Azo - 10 were utilized for the present study. The interstrain different of *Azospirillum* isolates for their growth promoting traits by various growth promoting traits by using the standard protocols as described below.

Acetylene reduction assay : Bergersen (1980)

Cell nitrogen content : Humphries (1956)

Indole acetic acid production:Gorden and Paleg (1957)

Gibberellic acid Production : Borrow *et al.,* (1955)

Siderophore production : Reeves *et al.,* (1983)

The *in vitro* experiments were conducted as triplicates. Statistical analysis of all the parameters was carried out through analysis of variance (ANOVA) (Gomez and Gomez, 1984).

Results and Discussion

Screening for nitrogenase activity and cell nitrogen content

The *in vitro* nitrogenase activity and cell nitrogen content of *Azospirillum* isolates obtained from Chilli rhizosphere soils of Cuddalore district are presented in Table - 1. Among the ten isolates, Azo - 2 obtained from rhizosphere soil of Palur recorded the highernitrogenase activity of 376.21 n moles of C_2H_4 produced mg^{-1} of protein h^{-1} and cell nitrogen of 42.28 mg g^{-1} of cell weight followed by Azo - 10 with 362.30 and 38.92 as respective values. Whereas, the reference strain *Azospirillum brasilense* MTCC - 125 produced 355.65 n moles of C_2H_4 produced mg^{-1} of protein h^{-1} and cell nitrogen of 37.51 mg g^{-1} of cell weight. The minimum nitrogenase activity of 195.00 n moles of C_2H_4 mg^{-1} of protein h^{-1} and cell nitrogen content of 27.00 mg $g^{-1}$was recorded in Azo - 8. Based on the nitrogenase activity and cell nitrogen content of the isolates Azo - 2 was selected for further studies.

Siripin *et al.* (2000) screened 35 isolates of N_2-fixing bacteria to assess the production of plant growth promoting substances. Each strain has different potential in N_2-fixing ability and has difference in physiology and morphology of the colonies and the cells. N_2-fixing bacterial inoculation increased vetiver growth and development. Patiyuth *et al.,* (2000) revealed that the N_2-fixing bacteria (*Azospirillum*) produced plant growth hormone, Indole-3-acetic acid (IAA) at 30-40 μg/ml in the broth media (Sivasakthi *et al*., 2014; Usharani *et al*., 2014).

Screening for phytohormones production

The indole acetic acid (IAA) and gibberellic acid (GA_3) producing potential of *Azospirillum* isolates obtained from the Chilli rhizosphere soil samples of Cuddalore district were investigated and the results were furnished in Table - 2. It was evidenced that all the ten isolates of *Azospirillum* (Azo-1 to Azo-10) were able to produce both phytohormones *viz.,* IAA and GA_3 with varying quantities between them. The phytohormones production ranged from 29.83 to 79.85 g 25 ml^{-1} broth for IAA and from 2.76 to 8.75 g 25 ml^{-1}broth for GA_3 was recorded.

Table – 1 Screening of *Azospirillum* isolates for nitrogenase activity and cell nitrogen content under *in vitro* conditions

Name of the isolate	Nitrogenase activity*	Cell nitrogen content**
Azo-1	343.20	37.58
Azo-2	376.21	42.28
Azo-3	352.00	37.94
Azo-4	296.38	36.02
Azo-5	325.00	36.82
Azo-6	273.54	31.25
Azo-7	217.12	28.62
Azo-8	195.00	27.00
Azo-9	248.03	29.92
Azo-10	362.30	38.92
MTCC 125	355.65	37.51
S.E.	4.20	0.54
C.D.(P = 0.05)	8.65	1.12

* n moles of C_2H_4 produced mg^{-1} of cell protein h^{-1}
**mg g^{-1} cell weight

Table – 2 Screening of *Azospirillum* isolates for indole acetic acid and gibberellic acid production

Name of the isolate	IAA (µg 25 ml^{-1} of broth)	GA_3 (µg 25 ml^{-1} of broth)
Azo-1	61.51	6.25
Azo-2	79.85	8.75
Azo-3	66.05	6.92
Azo-4	52.38	4.70
Azo-5	56.92	5.14
Azo-6	47.66	4.14
Azo-7	36.51	3.18
Azo-8	29.83	2.76
Azo-9	43.10	3.58
Azo-10	70.67	7.48
MTTC 125	75.21	8.09
S.E.	1.45	0.27
C.D.(P = 0.05)	2.99	0.54

The *Azospirillum* isolate Azo-2 produced the maximum amount of 79.85 g of IAA 25ml^{-1}and 8.75 g of GA_3 25 ml^{-1}of nitrogen free malate broth. It was followed by the reference strain MTCC - 125 with 75.21 g of IAA and 8.09 g of GA_3 25 ml^{-1}. The results of the present study was similar with the findings of Usharani *et al*. (2013) and Kanchana *et al*. (2014).

Screening for siderophore production

All the *Azospirillum* isolates produced both catechol and salicylate type of siderophore with varying quantities. The catechol type of siderophore produced by *Azospirillum* isolates ranged from 1.32 to 4.96 g ml^{-1}and salicylate type ranged from 2.56 to 8.54 g ml^{-1}of culture broth. The isolate Azo-2 produced higher quantity of 4.96 and 8.54 g ml^{-1}of catechol type and salicylate type of siderophore respectively followed by the reference strain MTCC - 125 with 4.61 and 7.87 g ml^{-1}of catechol and salicylate type of siderophore. The minimum amount 1.32 and 2.56 g of catechol and salicylate type of siderophore ml^{-1}was respectively produced by the isolate Azo-8 in Table - 3. From the above studies, it was observed that the results of the present study are in accordance to the results of earlier works of Usharani *et al*. (2014).

Table – 3 Screening of *Azospirillum* isolates for siderophores production

Name of the isolate	Siderophore production (µg ml^{-1})	
	Catechol Type	Salicylate Type
Azo-1	3.62	7.11
Azo-2	4.96	8.54
Azo-3	3.94	7.76
Azo-4	2.96	5.60
Azo-5	3.29	6.19
Azo-6	2.74	4.21
Azo-7	2.08	3.23
Azo-8	1.32	2.56
Azo-9	2.41	3.95
Azo-10	4.26	7.85
MTTC 125	4.61	7.87
S.E.	0.10	0.26
C.D.(P = 0.05)	0.22	0.53

Conclusion

The present study clearly concluded that the *Azospirillum* (Azo - 2) isolated from Palur location were found to be efficient strains in terms of exhibiting plant growth promoting traits **(**nitrogenase activity, Phytohormones such as IAA and GA_3 production and Siderophore production).

References

Andres Naiman., Alejandra Latronico and Garcia de Salamone. 2009. Inoculation of wheat with *Azospirillum brasilense* and *Pseudomonas fluorescens* impact on the production and culturable rhizosphere microflora. European Journal of Soil Biology, 45: 44 - 51.

Ashrafuzzaman, M., FaridAkhtarHossen, M. Razi Ismail, M. AnamulHoque, Zahurul Islam, M. Shahidullan and SariahMeon. 2009. Efficiency of plant growth - promoting rhizobacteria (PGPR) for the enhancement of rice growth. Indian journal of Agricultural Sciences, 5 (7): 17 – 22.

Baset Mia, M.A., R. Ashraf Shah and Z. H. Shamsuddin. 2010. Nitrogen Fixation and Transportation by Rhizobacteria: A Scenario of Rice and Banana. International Journal of Botany, 6: 235 - 242.

Bashan, Y., G. Holguin and L.E. De Bashan. 2004. *Azospirillum* plant relationships: Physiological, molecular, agricultural and environmental advances (1997-2003). Canadian Journal of Microbiology, 50: 521 - 577.

Bergersen, F.J. 1980. Methods for Evaluating Biological nitrogen fixation, John Wiley and Sons, New York, p. 702.

Borrow, A., Brain, P.W., Chester, U.E., Curtis, P.J., Hemming, H.G., Jeffereys, E.C., Lloyd, R.B., Nixon, I.S., Norris, J.L.F., Radley, N. 1955. *J. Food Sci.Agr.*, 6: 340 348.

De Coninck, K., S. Horemans, S. Randombage and K. Vlassak. 1988. Occurrence and survival of *Azospirillum* in temperature regions. Plant Soil, 110: 213-218.

Dobbelaere, S., J. Vanderleyden and Y. Okon. 2001. Plant growth promoting of diazotrophs in the rhizosphere. Critical Reviews in Plant Sciences, 22: 107 -149.

Gaur, A. C. and Ostwal, K. P. 1972. Influence of phosphate dissolving bacilli on the yield and phosphate uptake of wheat crop. Indian J. Exp. Biol. 10: 339.

Gomez, K.A., Gomez, A.A. 1984. Statistical procedures for agricultural research, 2nd Ed., Wiley, NY, pp. 680.

Gorden, S.A., Paleg, L.G. 1957. *Physiol. Plantarum*,10: 347 348.

Hesham, M. A and El-komy. 2005. Co-immobilization of *Azospirillum lipoferum* and *Bacillus megaterium* for successful phosphorus and nitrogen nutrition of wheat plants. Food Technology and Biotechnology, 43(1): 19 - 27.

Humphries, E. C. 1956. Mineral components and ash analysis. Modern method of plant analysis. Springer Verlag, Berlin, 1: 468 - 502.

Kanchana, D., M. Jayanthi, D. Kanchana, P. Saranraj and D. Sujitha. 2013. Evaluation of Plant growth promoting substance production by *Azospirillum* sp. isolated from rhizosphere of Chilli (*Capsicum annuum* L.). *International Journal of Microbiological Research*, 4(3): 300 - 304.

Kanchana, D., M. Jayanthi, G. Usharani, P. Saranraj and D. Sujitha. 2013. Prevalence of *Azotobacter* sp. in Chilli (*Capsicum annuum* L.) rhizosphere soil of Cuddalore district, Tamil Nadu, India. *International Journal of Microbiological Research*, 4(3): 296 - 299.

Kanchana, D., M. Jayanthi, G. Usharani, P. Saranraj and D. Sujitha. 2014. Interaction effect of combined inoculation of PGPR on growth and yield parameters of Chili var K1 (*Capsicum annuum* L.). *International Journal of Microbiological Research*, 5(3): 144 - 151.

Kavitha, K. 2000. Studies on *Azospirillum* associated with rice (*Oryza sativa* L.).M.Sc. (Ag.) Thesis, TNAU, Coimbatore, pp. 139.

Mallik, K. A., R. Bialal, S. Mehnaz, G. Rasul, M. S. Mirza and S. Ali. 1997. Association of nitrogen fixation, plant growth promoting rhizosphere (PGPR) with kallar grass andd rice. Plant Soil, 194: 37 - 44.

Michiels, K., J. Vanderleyden and A. Van Gool. 1989. *Azospirillum* plant root associations. Biology and Fertility of Soils, 8: 356 - 368.

Okon, Y. 1985. *Azospirillum* as a potential inoculant for agriculture. Trends Biotechnol., 3: 223 - 228.

Patiyuth, S., B. Tangcham and S. Muanjang. 2000. Studies on N_2-fixing bacteria association with Vetiver 1. Biosynthesis of plant growth hormone by *Azospirillum* 2 use of the gus A gene to study *Azospirillum*. In: Abstract on Poster Papers, ICV-2, P. 20.

Pedraza, R.O., A. Ramirez-Mata, M. L. Xiqui and B. E. Baca. 2004. Aromatic amino acid aminotransferase activity and indole-3-acetic acid production by associative nitrogen-fixing bacteria. FEMS Microbiology Letters, 233: 15 -21.

Purushothaman, D., 2002. Biology of *Azospirillum association* with cashew (*Anacardium occidentale* L.) pp: 1. In: National symposium and XII Southern Regional Conference on 'Microbial inoculants'. Annamalai Univ., India, March 2002 (Abstr.)

Reeves, M., Neilands, P.L, Ballows, A., 1983. *J. Bacteriol.*, 154: 324 329.

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.

Siripin, S., A. Thirathorn, A. Pintarake and T. Yathaputanon. 2000. Use of ^{15}N isotope dilution method fixing bacterial inoculation on the growth of vetiver grass. In: Abstract of poster papers, ICV-2, p-30.

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 Microbiological Research*, 4(3): 227 - 233.

Sivasakthi, S., G. Usharani and P. Saranraj. 2014. Biocontrol potentiality of Plant growth promoting rhizobacteria (PGPR) – *P. fluorescens* and *B. subtilis*: A Review. *African Journal of Agricultural Sciences*, 9(16): 1265 – 1277.

Sivasakthivelan, P and P. Saranraj. 2013. *Azospirillum* and its formulations: A Review. *International Journal of Microbiological Research*, 4(3): 275 - 287.

Suresh Kumar, R., P. Ganesh, K. Tharmaraj and P. Saranraj. 2011. Growth and development of black gram (*Vigna mungo*) under foliar application of Panchagavya as organic source of nutrient. *Current Botany*, 2 (3): 9 -11.

Thuler, D. S., E. I. Foh, W. Handro and H. R. Barbosa. 2003. Plant growth regulators and amino acids released by *Azospirillum* sp. in chemically defined media. Letters in Applied Microbiology, 37: 174 - 178.

Tiwari, M., S. Paroda and K. R. Dadarwal. 2003. Associative diazotrophs of pearl millet (*Pennisetumglaucum*) from semi arid region-isolation and characterization. Indian Journal of Experimental Biology, 41: 341 - 345.

Usharani, G., D. Kanchana, M. Jayanthi, P. Saranraj and D. Sujitha. 2013. Evaluation of certain resistance inducing chemicals against Sheath blight incidence in Paddy (*Oryza sativa* L.). *International Journal of Microbiological Research*, 4(3): 333 - 335.

Usharani, G., D. Kanchana, M. Jayanthi, P. Saranraj and D. Sujitha. 2014. Effect of Salicylic acid and *Pseudomonas fluorescence* on the growth and yield of Paddy IR-50. *International Journal of Microbiological Research*, 5(1): 54 - 60.

Usharani, G., D. Sujitha, S. Sivasakthi and P. Saranraj. 2014. Effect of Arbuscular Mycorrhizal (AM) Fungi (*Glomus fasciculatum* L.) for the improvement of growth and yield of Maize (*Zea mays* L.). *Central European Journal of Experimental Biology*, 3(2): 19 – 25.

Vinithadali. K. 2013. Screening of PGPR isolates for plant growth promoting traits from maize rhizosphere soil samples of Perambalur district. Journal of Applicable Chemistry, 2 (6):1600-1608.