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RESEARCH ARTICLE

PREVALENCE OF AZOSPIRILLUM ISOLATES IN PADDY RHIZOSPHERE SOILS OF DIFFERENT LOCATIONS IN CUDDALORE DISTRICT, TAMIL NADU

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ABSTRACT

In the present study, rhizosphere soil samples from paddy fields were collected from twenty different locations of Cuddalore District, Tamil Nadu. The occurrence of *Azospirillum* population in the rhizosphere soils of paddy plant grown at nineteen selected locations was studied. The location, namely Pinnalur recorded the maximum community population of *Azospirillum* (8.98 cfu × 10^5 g-1). Twenty different *Azospirillum* isolates were isolated and identified in the present study. The *Azospirillum* isolates were designated as "AZO" (AZO - 1 to AZO - 19). The maximum IAA and Gibberellic acid production by *Azospirillum* was recorded by the isolate AZO - 7 (88.72 µg 25 ml-1 of broth and 9.72 µg 25 ml-1 of broth).

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are free - living, soil - borne bacteria, which enhance the growth of the plant either directly or indirectly (Kloepper *et al.,* 1980; Glick and Ibid, 1995). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, and HCN production, production of phytohormones such as auxins, cytokinins, and gibberellins, and lowering of ethylene concentration (Glick and Ibid, 1995; Glick *et al.,* 1999).

Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* as well as *Alcaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter diazotrophicus* and *Bradyrhizobium japonicum* have been shown to produce auxins which help in stimulating plant growth (Patten and Glick, 2002). Plant growth promoting rhizobacteria (PGPR) may promote growth directly by atmospheric nitrogen fixation, solubilization of minerals, production of siderophore or production of plant growth regulators (Kloepper, 1997). Some bacteria support plant growth indirectly, by production of antagonistic substances or by inducing host resistance towards plant pathogens. The use of PGPR bioinoculants form one of the vital components for a long term sustainable agriculture system (Tilak *et al.,* 2005). *Azospirillum* is a free living N_2 fixing bacterium, it can successfully grow in the rhizospheric zone of wheat, maize, rice, cotton, tomato, bhendi and many other and fix 20 - 30 kg ha-1 per cropping season.

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But no work has been done on its property to mitigate the damages induced by silk dye waste on histopathology of lung *Azospirillum* synthesis and secretes considerable amount of biologically active substance like vitamin B, nicotinic acid, pentothenic acid, biotin, heteroauxins, and gibberellins etc., which enhanced the root of growing plants. *Azospirillum* has the ability to produce antifungal antibiotics and fungi static compounds against pathogens like *Fusarium, Alternaria, Trichoderma* (Waain *et al.,* 1988).

MATERIALS AND METHODS

Details of the locations

The survey was conducted at nineteen locations in Cuddalore District of Tamil Nadu comprising of Naanjalur, Siluvaipuram, Vayalur, Kerapalayam, Vallampadugai, Silambimangalam, Pinnalur, Periyapattu, Mullipallam, Gopalapuram, Kaayalpattu, Aandikoozhi, Aalapakkam, C.Mutlur, Thillaividankan, Sinkarakuppam, Adoor, Kodipallam, Manampadi and Poondiyankuppam.

Collection of paddy rhizosphere from different locations

The rhizosphere samples were collected at different locations of the paddy plant. The collected soil samples were brought to laboratory for isolation of *Azospirillum*.

Isolation and enumeration of Azospirillum population

Ten gram of air dried rhizosphere soil samples was transferred to 100 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker for 30 minutes. The well-mixed suspension was diluted appropriately up to 10^{-6} dilution. One ml of the suspension from 10^{-5} and 10^{-6} dilution was aseptically transferred to sterile Petriplates and 15 - 20 ml

of selective NFB medium was added to each petriplates mixed well with agar by rotating at clockwise and anticlockwise and allowed to set. Then, the plates were incubated at 28°C for 48 hours. After the incubation period, the colonies were developed and transferred to NFB agar slants and maintained at 4°C for further study. The *Azospirillum* colonies were enumerated after incubation using colony counter.

Designation of Azospirillum isolates

The *Azospirillum* isolates obtained from the rhizosphere of paddy plant grown at nineteen different location of Cuddalore District were designated as *AZO* and numbered randomly.

Characterization of Azospirillum isolates

All the nineteen *Azospirillum* isolates, obtained from rhizosphere of paddy grown at Cuddalore District were characterized based on LOPAT test (Lelliott *et al.,* 1966).

Estimation of Indole acetic acid (IAA)

The IAA present in the methanol extract was determined using the method of Gorden and Paleg (1957).

Estimation of Gibberellic acid (GA3)

The gibberellic acid production by plant growth promoting rhizobacteria was determined by following the method of Borrow *et al.* (1955).

Estimation of Siderophore Production

Siderophore production by the plant growth promoting rhizobacteria was estimated by the method described by Reeves *et al.* (1983)

RESULTS AND DISCUSSION

The occurrence of *Azospirillum* population in the rhizosphere soils of Paddy plant grown at nineteen selected locations was studied and the results were showed in Table - 1.

Table 1 Occurrence of *Azospirillum* sp. in rhizosphere of paddy

SI.NO	Rhizosphere soil sample	*Azospirillum* sp. cfu x 10^5 g-1
1	Naanjalur	8.57
2	Siluvaipuam	8.45
3	Vayalur	8.71
4	keerapalayam	8.60
5	Silambimangalam	8.13
6	Pinnalur	8.98
7	Periyapattu	8.08
8	Mullipallam	7.90
9	Gopalapuram	7.71
10	Kaayalpattu	7.49
11	Aandikoozhi	7.33
12	Aalapakkam	7.15
13	C.Mutlur	6.90
14	Thillaividankan	6.76
15	Sinkarakuppam	6.53
16	Adoor	6.23
17	Kodipallam	5.92
18	Manampadi	5.75
19	Poondiyankuppam	5.60

The location, namely Pinnalur recorded maximum community population of *Azospirillum* (8.98 cfu × 10^5 g-1) and least population of *Azospirillum* was recorded as of 5.60 cfu × 10^5 g-1 in Poondiyankuppam. Nineteen different *Azospirillum* isolates were isolated and identified in the present study. The *Azospirillum* isolates were designated as "AZO" series and numbered randomly (AZO – 1 to AZO – 19). The details of designation of the isolates were shown in Table - 2.

Table 2 Designation of *Azospirillum* isolates

SI NO	Rhizosphere soil sample	*Azospirillum* sp. Designation
1	Naanjalur	AZO – 1
2	Siluvaipuram	AZO–2
3	vayalur	AZO–3
4	Keerapalayam	AZO–4
5	Vallampadugai	AZO –5
6	Silambimangalam	AZO –6
7	Pinnalur	AZO –7
8	Periyapattu	AZO –8
9	Mullipallam	AZO –9
10	Gopalapuram	AZO –10
11	Kayalpattu	AZO –11
12	Aalapakkam	AZO –12
13	C.Mutlur	AZO –13
14	Thillaividankan	AZO –14
15	Sinkarakuppam	AZO –15
16	Adoor	AZO –16
17	Kodipallam	AZO –17
18	Manampadi	AZO –18
19	Poondiyankuppam	AZO –19

IAA (indole-3- acetic acid) is the member of the group of phytohormones and is generally considered the most important native Auxin (Ashrafuzzaman *et al.,* 2009). IAA synthesis is widespread among plant-associated bacteria (Patten and Glick, 1996) and provides bacteria with a mechanism to influence plant growth (Patten and Glick, 2002. It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division, and differentiation, and gene regulation (Ryu and Patten, 2008).

Table 3 Indole Acetic Acid (IAA) production of *Azospirillum* isolates

SI NO	*Azospirillum* isolates	Indole Acetic Acid (IAA) (µg 25 ml-1 of broth)
1	AZ – 1	75.31
2	AZ –2	72.08
3	AZ –3	83.21
4	AZ –4	80.65
5	AZ –5	61.06
6	AZ –6	55.10
7	AZ –7	88.72
8	AZ –8	53.28
9	AZ –9	51.79
10	AZ –10	50.19
11	AZ –11	48.20
12	AZ –12	47.45
13	AZ –13	45.00
14	AZ –14	41.97
15	AZ –15	38.70
16	AZ –16	33.17
17	AZ –17	30.14
18	AZ –18	25.39
19	AZ –19	22.16

The twenty *Azospirillum* isolates obtained from the rhizosphere soils of tomato plant was tested for their efficiency of Indole acetic acid (IAA) production. All the above 20 isolates taken from the study showed positive results producing IAA. The amount of IAA produced expressed in µg/ml of culture filtrate. The maximum IAA production by *Azospirillum* was recorded by the isolate AZO - 7 (88.72 µg 25 ml-1 of broth). The minimum production of IAA was found in AZO-19 (22.16 µg 25 ml-1 of broth) isolates. The Gibberellic acid produced *Azospirillum* was estimated and the results were

showed in Table-4. The isolate *Azospirillum* (AZO-7) showed maximum Gibberellic acid production (9.72 µg 25 ml-1 of broth) and least Gibberellic acid production was showed by AZO - 19 (3.13 µg 25 ml-1 of broth).

Table 4 Gibberellic Acid (GA3) production of *Azospirillum* isolates

SI NO	*Azospirillum* isolates	Gibberellic acid (GA3) (µg 25 ml-1 of broth)
1	AZ – 1	8.80
2	AZ –2	9.06
3	AZ –3	8.53
4	AZ –4	8.88
5	AZ –5	8.31
6	AZ –6	8.02
7	AZ –7	9.72
8	AZ –8	7.93
9	AZ –9	7.32
10	AZ –10	6.77
11	AZ –11	6.12
12	AZ –12	5.73
13	AZ –13	5.55
14	AZ –14	5.03
15	AZ –15	4.73
16	AZ –16	4.32
17	AZ –17	3.88
18	AZ –18	3.57
19	AZ –19	3.13

Saranraj *et al*. (2013) examined the occurrence of *Pseudomonas fluorescens,* and the population ranged between 7.71 × 10^6 cfu g-1 and 7.21 x10^6 cfu g-1 of soil.The *Pseudomonas fluorescens* was isolated and identified. The ten *Pseudomonas fluorescens* isolates obtained from the rhizosphere of paddy were tested for their efficiency of IAA and siderophore production. The maximum IAA production was recorded by the isolate PF-8. The minimum production of IAA was found in PF-4 isolates. The isolate *Pseudomonas fluorescens* (PS-8) showed the maximum siderophore production and the least siderophore production was showed by the *Pseudomonas fluorescens* isolate PS - 4.

CONCLUSION

From the present research, it was concluded that the plant growth promoting rhizobacteria (PGPR) isolates *Azospirillum* have the capacity to produce plant growth promoting substances and the isolates AZO-7 isolated from Pinnalur was highly effective in the production of plant growth promoting substances when compared to other *Azospirillum* isolates. The isolate *Azospirillum* (AZO-7) showed the maximum production of Indole Acetic Acid (IAA) and Gibberellic Acid (GA3) when compared to other *Azospirillum* isolates. In conclusion, application of the PGPR isolate *Azospirillum* will enhance the growth and yield of paddy

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