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Studies on the occurrence of *Azospirillum* in sesame fields of Cuddalore and Villupuram districts of Tamil Nadu

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ABSTRACT

A survey was conducted at twenty different locations in Cuddalore and Villupuram districts of Tamil Nadu State, India, in selected sesame fields to study the occurrence of indigenous Azospirillum populations. The Azospirillum isolates were isolated and characterized. Two species of Azospirillum, Azospirillum brasilense and Azospirillum lipoferum were isolated. In general, the native Azospirillum population was less in soils with pH 8.0 and above and the Azospirillum rhizobiocoenosis is predominantly by A. brasilense.

Key Words: sesame fields, Azospirillum rhizosphere.

INTRODUCTION

Azospirillum species are widespread and common inhabitants of tropical, subtropical and temperate soils. They are important members of the rhizosphere microflora.and their occurrence is ubiquitous. The rhizobiocoenosis of *Azospirillum* with the roots of *Digitaria decumbens* cv. Transvala [1] and in different plant species like grass, rice, maize, wheat, barley, rye, oats, etc [2] have been reported.

MATERIALS AND METHODS

Azospirillum brasilense Sp7 (ATCC: 29145) and *A. lipoferum* Sp59b (ATCC 29707) were maintained as reference strains.

The survey was conducted at following twenty different locations in Cuddalore and Villupuram districts of Tamil Nadu state, India, where sesame (*Sesamum indicum* L.) is grown as a summer oilseed crop.

Cuddalore district
1. Sivapuri
2. Vallampadugai
3. M. Pudhur
4. Pudhuchattiram
5. C. Mutlur
6. Sethiathope
7. Alapakkam
8. Vadalur
9. Panruti
10. Vridhachalam

Villupuram district
11. Rasapalayam
12. Thirasu
13. Koliyanoor
14. Kidelam
15. Thaduthandur
16. Sithalingamadam
17. Tiruvennainallur
18. Gingee
19. Melmalayanoor
20. Tindivanam

In each location the rhizosphere soil and roots of sesame plants were collected from a selected field and transported to the laboratory under aseptic conditions. The enrichment culture technique as described by Day and Dobereiner [3] was followed for the isolation of *Azospirillum.* Semisolid Nfb medium is used for this.

The native *Azospirillum* population of soil was determined by most probable number (MPN) technique [4] Soil samples from the locations were analyzed for soil textural class (Hygrometer method) and pH (pH meter).

Phase contrast microscopic observations of 72 h old culture revealed plump rods about 1.0 µm in diameter and 2 to 4 µm in length. Intracellular granules of poly β-hydroxybutyrate (PHB) were present [5] and the isolates exhibited a very characteristic vibrioid movement in broth cultures and Gram staining was performed [6].The cultures were examined by phase contrast microscopy *Azospirillum brasilense* was observed as vibrioid (cell width 1.0-1.2 µm) and motile, even when the cultures become alkaline. *A. lipoferum* appeared wider and longer (width 1.0-1.5 µm length 5-30 µm) and nonmotile. These cells fragmented into shorter ovoid cells and became larger and pleomorphic filled with refracticle granules.

All the twenty *Azospirillum* isolates were subjected to various morphological and biochemical tests for species identification and the results presented in Table 3.

Species were identified based on glucose as sole carbon source for growth in semisolid nitrogen free medium, biotin requirement, production of acid from glucose [7], acidification of carbohydrates by *Azospirillum* [8] and auxangraphic method for sole carbon sources

The data were subjected to analysis of variance and differences among the means were compared with DMRT USING SPSS PC+ SOFTWARE [9] at P = 0.05% level.

RESULTS AND DISCUSSION

Strains of *Azospirillum*, were isolated from each of the 20 locations from the root or rhizosphere soil of sesame and designated AS 1 to AS 20.

All the *Azospirillum* isolates produced characteristic dense, white subsurface pellicle in Nfb semisolid medium. The colour of the medium also changed from yellowish green to brilliant blue. Microscopic examination of the 72 h old cultures revealed curved plump rods and the presence of intracellular fat droplets. All the isolates were gram negative in reaction. The results of various biochemical tests and growth characteristics as listed in Table 2 confirmed that all 20 isolates belong to the genus *Azospirillum*.

Among the twenty isolates seven isolates were identified as *A. lipoferum*. They exhibited pleomorphism in alkaline Nfb media, required biotin for growth, able to use glucose as sole carbon source, and produced acid from glucose. Strains AS 3, AS 8, AS 10, AS 11, AS 13, AS 17 and AS 20 were identified as *A. lipoferum*.

The rest of 13 isolates were identified as *A. brasilense* based on their ability to grow without biotin and inability to use glucose as sole carbon source under nitrogen fixing condition. Strains AS 1, AS 2, AS 4, AS 5, AS 6, AS 7, AS 9, AS 12, AS 14, AS 15, AS 16, AS 18 and AS 19 were identified as *A. brasilense.* Seventy per cent of 20 isolates belong to *A. brasilense* and 30 per cent to *A. lipoferum*. The results of the present study clearly revealed that the *Azospirillum* rhizobiocoenosis is predominantly by *A. brasilense*.

Table 1. Details of *Azospirillum* isolates from the rhizosphere of sesame

S. No.	Place	Isolate No.	Soil textural class	pH	Indigenous *Azospirillum* population in soil*
Cuddalore District					
1.	Sivapuri	AS 1	Clay loam	7.3	1.6
2.	Vallampadugai	AS 2	Clay	7.8	1.1
3.	M. Pudhur	AS 3	Clay	7.9	3.8
4.	Pudhuchattiram	AS 4	Clay loam	7.2	1.7
5.	C. Mutlur	AS 5	Sandy clay loam	7.6	1.3
6.	Sethiathope	AS 6	Clay loam	7.8	1.2
7.	Alapakkam	AS 7	Sandy clay loam	7.3	4.8
8.	Vadalur	AS 8	Clay loam	7.0	1.9
9.	Panruti	AS 9	Sandy clay loam	7.1	2.3
10.	Vridhachalam	AS 10	Clay loam	7.9	1.6
Villupuram District					
11.	Rasapalayam	AS 11	Sandy clay loam	7.0	1.8
12.	Thirasu	AS 12	Clay	7.6	3.2
13.	Koliyanoor	AS 13	Clay loam	7.3	1.7
14.	Kidelam	AS 14	Clay loam	8.1	0.8
15.	Thaduthandur	AS 15	Clay	7.8	1.2
16.	Sithalingamadam	AS 16	Clay loam	8.0	0.4
17.	Tiruvennainallur	AS 17	Clay loam	7.6	1.2
18.	Gingee	AS 18	Clay	7.8	4.6
19.	Melmalayanoor	AS 19	Clay loam	8.0	0.8
20.	Tindivanam	AS 20	Clay loam	8.3	0.3

** $\times 10^6$/g of oven dry soiL*

Table 2. Characterization of *Azospirillum* spp.

S. No.	Characters	Response of the isolates of *Azospirillum* spp.
1.	ARA (Acetylene reduction assay)	+
2.	Cell shape	Curved plump rod
3.	Gram reaction	Negative
4.	Pleomorphism	±
5.	Biotin requirement	±
6.	Glucose as sole carbon source in semisolid nitrogen free medium	±
7.	Acidification of glucose, fructose, mannitol	±
8.	Acidification of sucrose, lactose, cellulose, starch and maltose	–
9.	Sole carbon source in +N medium glucose, fructose, mannitol, succinate, lactate, malate, pectin	+
	Cellulose, sucrose, maltose, starch	–
10.	Nitrate dependant anaerobic growth	+
	Dissimilation of NO_3^- to gas	±

Table 3. Species characterization of *Azospirillum* isolates

S. No.	Isolate	Pleo-morphism	Biotin-requirement	Glucose as sole C source	Acid from glucose	Nitrite reductase	Identified as *Azospirillum* species
1.	AS 1	-	-	-	-	+	*A. brasilense*
2.	AS 2	-	-	-	-	+	*A. brasilense*
3.	AS 3	+	+	+	+	+	*A. lipoferum*
4.	AS 4	+	+	+	+	-	*A. brasilense*
5.	AS 5	-	-	-	-	-	*A. brasilense*
6.	AS 6	-	-	-	-	+	*A. brasilense*
7.	AS 7	-	-	-	-	-	*A. brasilense*
8.	AS 8	-	-	-	-	-	*A. lipoferum*
9.	AS 9	-	-	-	-	-	*A. brasilense*
10.	AS 10	+	+	+	+	+	*A. lipoferum*
11.	AS 11	-	-	-	-	-	*A. lipoferum*
12.	AS 12	-	-	-	-	-	*A. brasilense*
13.	AS 13	+	+	+	+	+	*A. lipoferum*
14.	AS 14	-	-	-	-	-	*A. brasilense*
15.	AS 15	-	-	-	-	-	*A. brasilense*
16.	AS 16	-	-	-	-	-	*A. brasilense*
17.	AS 17	+	+	+	+	+	*A. lipoferum*
18.	AS 18	-	-	-	-	-	*A. brasilense*
19.	AS 19	-	-	-	-	-	*A. brasilense*
20.	AS 20	-	-	-	-	-	*A. lipoferum*

The results of the present survey revealed the ubiquitous nature of *Azospirillum* in sesame soils. The ubiquitous occurrence of *Azospirillum* in all the sesame field soils surveyed may be attributed to the requirement for high temperature

prevalent in tropical conditions. The occurrence of *Azospirillum* in soils has been reported from different countries (9, 10, 11, 12, 13, 14, and 15]. The optimum temperature for nitrogen dependant growth by *Azospirillum* has been found to be between 32 and 36°C and this is similar to the temperature optimum reported for other nitrogen fixing bacteria from tropical environment [16]. In the present study, the community population of *Azospirillum* was estimated in the above mentioned twenty locations in two districts of Tamil Nadu State. The results revealed a marked variation in the community population of *Azospirillum* in the 20 locations observed. The population ranged from 0.3 to 4.8×10^6 CFU g^{-1} of soil. The occurrence of very low population of $< 10^3$ CFU g^{-1} in Belgian soils [17] and only 10^3-10^6 of *Azospirillum* population per g of soil in wheat soils [18] ,the occurrence of only $10^5 – 10^6$ viable cells of *Azospirillum* per g soil in India[19] maximum population in sandy loam and minimum population in loamy soil (20) widespread distribution in saline soils (21) have been reported. The present study clearly revealed the populations of *Azospirillum* was above 3×10^5 g^{-1} of soil in all the locations surveyed and hence can be considered high. The *Azospirillum* population was relatively less ($< 0.9 \times 10^6$ CFU) in soils with pH 8.0 and above. Interestingly, the four locations (M. Pudhur, Alapakkam, Thirasu and Gingee), which recorded high populations of 3.0×10^6 and above CFU g^{-1} of soil had pH ranging from 7.3 to 7.9.

CONCLUSION

The present study clearly indicates the ubiquitous nature of Azospirillum.Anyhow it predominates in soils with pH less than 8.

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