

Research Article

STUDIES ON THE OCCURANCE OF DIAZOTROPHS FROM THE RHIZOSPHERE OF LOW LAND RICE GROWN REGIONS OF TAMIL NADU

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ABSTRACT

A survey was conducted for the occurrence of community diazotrophic populations, namely, *Azospirillum*, *Pseudomonas* sp. and *Bacillus* sp. from the rhizosphere of lowland rice was conducted at different locations of Tamil Nadu. The survey revealed the ubiquitous occurrence of diazotrophs in rice rhizosphere where the occurrence *Azospirillum brasilense* was found to be at higher level followed by *Pseudomonas* sp. and *Bacillus* sp. A total number 84 diazotrophic strains, namely 28 no. of *Azospirillum*, 28 no. of *Pseudomonas* sp. and 28 no. of *Bacillus* sp. were isolated. The results of the present study also revealed a marked variation in the community population of *Azospirillum*, *Pseudomonas* and *Bacillus* in the locations of Tamilnadu, observed. A range of 0.89 per cent to 1.41 per cent of *Azospirillum*, 1.12 per cent to 2.50 per cent of *Pseudomonas* and 0.51 per cent to 0.97 per cent of *Bacillus* to the total bacterial population was observed in the survey.

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INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food crop for over two billion people in Asia and four hundreds of millions in Africa and Latin America. To feed the ever-increasing population of these regions, the world's annual rice production must be increased from the present 460 to 760 million tone by the year 2020 (IRRI, 1993). Rhizosphere bacteria that favorably affect plant growth and yield of commercially important crops are now denominated as "plant growth promoting rhizobacteria" (PGPR). They can cause plant growth promotion directly by producing and secreting plant growth regulators or by eliciting root metabolic activities by supplying biologically fixed nitrogen. The well known PGPR includes bacteria belonging to the genera, namely, *Azotobacter*, *Azospirillum*, *Azoarcus*, *Klebsiella*, *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Enterobacter*, *Serratia* and *Rhizobium* on non legumes (Burdman *et al.*, 2000). The Survey was conducted 28 locations of Cauvery delta region of Tamil Nadu where rice is a predominant cereal food crop grown under lowland condition. Random selections of locations were made so that each and every sector of experimental area would get a representation in the survey.

MATERIALS AND METHODS

1. Survey for *Azospirillum*, *Pseudomonas* sp. and *Bacillus* sp. occurrence from the rhizosphere of rice

The survey was conducted at twenty-eight locations of Cauvery deltaic region of Tamilnadu, where rice is a predominant cereal food crop grown under lowland condition. Random selection of locations was made so that each and every sector of the experimental area would get a representation in the survey.

2. Details of locations

The name of twenty-eight locations selected for the survey of *Azospirillum*, *Pseudomonas* sp. and *Bacillus* sp. Occurrence from the rhizosphere of rice (cv. BBT- 5804) are given in Table-1.

3. Collection of rhizosphere soil sample from each location

In each and every location of the survey area, a field which has been under a long rice monoculture practice was selected. The collection of rhizosphere soil sample was made in the field having rice (cv- BBT-5804), as standing crop and tillering stage of crop growth. A total number of 10 rice plants were selected randomly at various places in the field and considered as representative of that location. The selected rice plants were uprooted with entire root system and with the soil adhered to the roots were aseptically packed up in polythene bags and transferred to the laboratory for the isolation and enumeration of the diazotrophs.

3.1. Enumeration of *Azospirillum* population from the rhizosphere of rice (MPN method)

The adhered soil of rice roots, collected from 10 rice plants of a particular location, were pooled and 10 g of soil sample was transferred to 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm for 30 min at ambient temperature. The well mixed suspension of each soil sample was subjected to tenfold serial dilution ranging from 10^{-1} to 10^{-9} , In mineral salts solution of Day and Dobereiner (1976), without malate, as detailed elsewhere in the text. One ml of each dilution was inoculated in a set of five tubes containing 9 ml of nitrogen free semisolid malate medium (Day and Dobereiner, 1976). At least three consecutive dilutions were inoculated and the tubes were incubated for three days at $30 \pm 2^\circ\text{C}$. Tubes showing subsurface, thin pellicle were identified as positive tubes for dinitrogen fixing spirilla and were subjected to acetylene reduction assay (ARA) (≤ 10 n moles / h / tubes of C_2H_4) for confirmation. The MPN counts of *Azospirillum* were calculated on the basis of positive tubes using table provided by Cochran (1950).

3.2. Enumeration of *Pseudomonas* sp. population from the rhizosphere of rice

The rice root system of a particular location, after removing large clumps of soil by gentle shaking, were collected and the soil adhering to the roots were used to determine the population of *Pseudomonas* sp. The plant count method was adopted for the determination of *Pseudomonas* sp. population. Ten grams of shade dried, homogenized and sieved soil was transferred to 90 ml sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm) for 30 min at ambient temperature. The well mixed suspension of each soil sample was subjected to tenfold dilutions upto 10^{-7} dilution. 1 ml of this diluted suspension was transferred aseptically to petridishes and melted Kings' B' agar medium was poured in each petridish. Then, they were rotated in clockwise and anticlockwise direction for uniform distribution and incubated at $30 \pm 2^\circ\text{C}$ for 5-7 days. After the incubation period, the *Pseudomonas* sp. colonies developed in each Petridishes were counted using Arnold colony counter. Three replications were maintained for each soil sample.

3.3. Enumeration of *Bacillus* populations from the rhizosphere of rice

The adhered soil of rice roots, collected from 10 rice plants of a particular location, were pooled and 10g of soil sample was transferred to 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm) for 30 min at ambient temperature. The well mixed suspension of each soil sample was subjected to tenfold serial dilution ranging from 10^{-1} to 10^{-9} and enumeration of *Bacillus* sp. from the rhizosphere soil sample of rice cv. BBT-5804 was performed by the five tube most probable number (MPN)- Plant infection test using *Vigna mungo* as

the legume trap host as described by Somasegaran and Hoben, (1985).

3.4. Enumeration of total heterotrophic population from the rhizosphere of rice

The enumeration of total heterotrophic population from the rhizosphere of rice cv. BBT-5804 was carried out on nutrient agar medium as described by Malik *et al.*, 1997.

4. Isolation of diazotrophic isolates from the rhizosphere of rice

4.1. Isolation of *Azospirillum* from rhizosphere soil sample

10 g of air-dried soil sample of rice rhizosphere (cv. BBT-5804), collected from each location, was transferred to 90 ml sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm) for 30 min at ambient temperature. The well mixed suspension was then diluted appropriately and 0.1 ml of the suspension was aseptically transferred into test tubes containing 10 ml of semisolid malate medium (Nfb) (Day and Dobereiner, 1976) and incubated at 35°C for 36 - 48 hrs to allow subsurface pellicle formation. After the incubation period, the cotton plugs were replaced with acetylene gas. Nitrogenase assay (C_2H_2 reduction) was checked and quantified by gas chromatograph (Chemito, India) fitted with Flame Ionization Detector (FID), as detailed elsewhere in the text. *Azospirillum* culture was isolate from the tubes which showed characteristic pellicle formation and nitrogenase activity.

4.2 Composition of media

4.2.1. Nitrogen free malate medium (NFB) (Day and Dobereiner, 1976).

L-Malic acid	5.0g
Dipotassium hydrogen orthophosphate	0.5g
Magnesium sulphate	0.2g
Sodium chloride	0.1g
Calcium chloride	0.02g
Trace element solution	2.0 ml
Fe EDTA (1.64% w/v), aqueous	1.0 ml
Bromothymol blue	2 ml
(0.5% aqueous solution (dissolve in 0.2N KOH)	
Vitamin solution	1.0 ml
Potassium hydroxide	4.0g
Distilled water	1000 ml
Trace element solution	
Sodium molybdate	0.200g
Magnesium sulphate	0.234g
Boric acid	0.280g
Copper sulphate	0.008g
Zinc sulphate	0.024g
Distilled water	1000 ml
Vitamin solution	
Biotin	0.01g
Pyridoxine	0.02g
Distilled water	1000 ml

4.2.2. RC medium (Rodriguez - Caceres, 1982)

L-Malic acid	5.0g
Dipotassium hydrogen Orthophosphate	0.2g
Sodium chloride	0.1g
Yeast extract	0.05g
Ferric chloride	0.015g
Potassium hydroxide	4.0g
Agar	15.0g
Distilled water	1000 ml
Ph	7.0g

After autoclaving 15 ml of 1:400 aqueous solution of sterilized Congored was added, aseptically.

4.3. Isolation of *Pseudomonas* sp. from the rhizosphere of rice

10 g of air-dried soil sample of rice rhizosphere (cv. BBT-5804), collected from each location, was transferred to 90 ml sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm) for 30 min at ambient temperature. They well mixed suspension was then diluted appropriately and 0.1 ml of the dilution was aseptically transferred into petridishes and melted Kings 'B' agar medium was poured into each petridish, mixed uniformly and incubated at 30 ± 2 °C for 5 to 7 days. After the incubation period, the developed *Pseudomonas* sp. colonies in the petridishes were picked up and sub cultured for further study.

4.4. Isolation of *Bacillus* from the rhizosphere of rice

10 g of air-dried soil sample of rice rhizosphere (cv. BBT-5804), collected from each location, was transferred to 90 ml sterile distilled water in a 250 ml Erlenmeyer flask and incubated on a rotary shaker (100 rpm) for 30 min at ambient temperature. They well mixed suspension of each soil sample was subjected to serial dilution ranging from 10^{-1} to 10^{-9} . 1 ml of the suspension, from dilutions 10^{-6} to 10^{-7} aseptically transferred to petridishes and TSAB medium was poured, rotated in clockwise and anticlockwise direction for uniform distribution and incubated at 30 ± 2 °C for 5 to 6 days. At the end of incubation period, *Bacillus* colonies appeared as elevated, translucent, white mucoid one with entire margin. The isolated *Bacillus* cultures were maintained in TSAB slants (Allen *et al.*, 1953).

4.5. Purification of *Azospirillum* isolates

A loopfull of the culture was streaked on RC medium (Rodriguez-Caceres, 1982). Light pink colonies which became scarlet upon storage were picked out and streaked on solidified potato infusion agar (BMS) plates (Baldani and Dobereiner, 1980). Typical pink often wringled colonies were picked out and maintained in nutrient agar slants for further study.

4.5.1. Potato infusion agar (BMS) (Baldani and Dobereiner, 1980)

Washed, peeled, sliced potato	200.0g
L. Malic acid	2.5g
Potassium hydroxide	2.0g
Vitamin solution	1.0 ml

Distilled water	1000 ml
Agar	15.0g

*(Biotin 0.01 g; Pyridoxin, 0.2g; Distilled water, 1000 ml)

Washed potatoes were boiled for 30 min and the solution was filtered through layers of cheese cloth. Malic acid was dissolved in 50 ml of water, 2 drops of bromothymol blue (0.5 percent solution in ethanol) added and pH adjusted to 7.0 as indicated by the solution turning green with KOH. This solution together with sucrose, agar and vitamins are added to the filtrate, made upto 1000 ml with distilled water and sterilized by autoclaving.

4.6. Purification of *Pseudomonas* sp isolates

A portion of the single cell colony formed on the Kings 'B' medium transferred a new Kings 'B' liquid medium. After 24 hours, a loopfull of culture was streaked on the Kings 'B' solid medium. Then the plates were incubated for 3 to 4 days at 32 °C. After the appearance of characteristic *Pseudomonas* sp. colonies, a single colony was transferred to liquid medium again. After streaking on solid agar medium. The typical colonies were examined microscopically and transferred to Kings 'B' agar slants for further study.

4.7. Purification of *Bacillus* isolates

A loopfull of each *Bacillus* isolate was streaked on TSAB agar medium and the plates were incubated for a week. *Bacillus* colonies out as white, translucent absorbed.

4.7. Genus and species characterization of diazotrophic isolates

All the eighty-four diazotrophic isolates, collected from the rice rhizosphere, were subjected to genus and species characterization according to Beijerinck (1922), Tarrand *et al.*, (1978) and Yanni *et al.* (1997) for *Azospirillum*, *Pseudomonas* sp. and *Bacillus* sp. respectively.

4.7.1. Designation of diazotrophic isolates

After the characterization, all the twenty-eight *Azospirillum* isolates were designated as AZS-1 to AZS-28 while the twenty-eight *Pseudomonas* sp isolates were designated as PSE- 1 to PSE- 28 and it was BAC-1 to BAC-28 for *Bacillus* isolates, as detailed elsewhere in the text.

RESULTS AND DISCUSSION**5. Survey for the occurrence of diazotrophicus from the rhizosphere of lowland rice: details of locations in cauvery delataic regions in Tamilnadu Twenty-eight locations, namely,**

Adalaiyur, Alangudi, Ervadi, Iravancherry, Karaiyur, Kayattur, Kottamangalam, Sengamangalam, Sikkal, Tenkarai, Tenpidagai, Themangalam, Theti, Achampatti, Budalur, Indalur, Kadambangudi, Kattur, Kulichapattu, Kullangarai, Kurungalur, Kuruvadipatti, Manangorai, Maraneri, Mathur, Nallicheri, Pinnai Nallur, Pudukudi were selected at Cauvery deltaic region of Tamilnadu in a random manner so that each and every sector of the experimental area would get a representation in the survey. Rice is grown continuously under lowland condition in all the twenty-eight location of the survey area. The field under

cultivation with rice cv. BPT -5804, as standing crop, were selected for the survey. The details of the locations selected for the survey in Cauvery deltaic region of Tamilnadu are given in Table-1.

Table 1. Survey for the occurrence of diazotrophicus from the rhizosphere of lowland rice: details of locations in cauvery deltaic regions in Tamilnadu

S. No.	Name of the location
1.	Adalaiyur
2.	Alangudi
3.	Ervadi
4.	Iravancherry
5.	Karaiyur
6.	Kayattur
7.	Kottamangalam
8.	Sengamangalam
9.	Sikkal
10.	Tenkarai
11.	Tenpidagai
12.	Themangalam
13.	Theti
14.	Achampatti
15.	Budalur
16.	Indalur
17.	Kadambangudi
18.	Kattur
19.	Kulichapattu
20.	Kullangarai
21.	Kurungalur
22.	Kuruvadipatti
23.	Manangorai
24.	Maraneri
25.	Mathur
26.	Nallicheri
27.	Pinnai Nallur
28.	Pudukudi

5.1. Occurrence of *Azospirillum*, *Pseudomonas* sp. and *Bacillus* genera from the rhizosphere of rice grown at Cauvery deltaic regions in Tamilnadu

When the enumeration of diazotrophs were made, it was found that all the diazotrophs viz., *Azospirillum*, *Pseudomonas* sp. and *Bacillus* sp. were found to be widely distributed in the rhizosphere of low land rice grown at Cauvery deltaic region of Tamilnadu but with variation in their level of incidence. The genus *Azospirillum* was found

to occur at high level followed by *Pseudomonas* sp. and *Bacillus* sp. A maximum number of *Azospirillum* cells (7.45 log₁₀ CFU/g dry soil), *Pseudomonas* sp. cells (8.15 log₁₀ CFU/g dry soil) and *Bacillus* cells (7.32 log₁₀ CFU/g dry soil) was recorded from the rhizosphere sample collected Adalaiyur of Tamilnadu. The lowest population of *Azospirillum* cells (6.15 log₁₀ CFU/g dry soil), *Pseudomonas* sp. cells (6.25 log₁₀ CFU/g dry soil) and *Bacillus* cells (5.91 log₁₀ CFU/g dry soil) was recorded with rhizosphere sample collected at Nallicheri of Tamilnadu. In general rhizosphere sample collected from the different locations of at Cauvery deltaic region of Tamilnadu recorded the *Azospirillum* in range of 6.15 to 7.45 log₁₀ CFU/g dry soil and *Bacillus* population in a range of 5.91 to 7.032 log₁₀ CFU/g dry soil.

5.2. Designation of *Azospirillum*, *Azotobacter* and *Rhizobium* isolates

A total number of eighty-four diazotrophic isolates, namely 28 isolates of *Azospirillum*, 28 isolates of *Azotobacter*, and 28 isolates of *Rhizobium* were isolated from 28 rhizosphere soil samples of lowland rice, collected from 28 different location of Cauvery deltaic region of Tamilnadu, as described in "Materials and Methods". The *Azospirillum* isolates were designated as 'AZS' whereas the *Pseudomonas* sp. and *Bacillus* were designated as 'PSE' and 'BAC' respectively, and all the isolates numbered randomly. The designation details the isolates; their site of collection is presented in Table-3.

5.3. Characterization of diazotrophic isolates

All the eighty-four isolates were subjected to genus and species characterization according to Tarrand *et al.* (1978). Beijernick (1922) and Yanni *et al.* (1977) for *Azospirillum*, *Pseudomonas* sp. and *Bacillus*, respectively. Interestingly all the twenty-eight *Azospirillum* isolates were found to belong *Azospirillum brasilense*, all the twenty-eight *Pseudomonas* sp. isolates found to belong *Pseudomonas* sp. while all other isolates were found to belong to *Bacillus* miscellany group and the results are presented in Table – 4. The occurrence of *Azospirillum* in the rhizosphere of rice has been reported by Lakshmi *et al.* (1977). Rao and Rajaram Mohan Rao (1983); Nayak *et al.* (1986) and latha *et al.* (1977). The occurrence of *Azotobacter* in rice rhizosphere has been reported by many workers (Rangaswami and Venkatesan, 1964; Neelakandan and Rangaswami, 1965). In this present study, the community population of *Azospirillum*, *Azotobacter* and *Rhizobium* genera from the rhizosphere of rice was studied in twenty-eight different locations of Cauvery deltaic region, Tamilnadu. Tamilnadu where rice is grown as monocrop and under lowland condition. The present study revealed the ubiquitous occurrence of *Azospirillum*, *Azotobacter*, and *Rhizobium* in the rhizosphere of rice grown at Cauvery deltaic region, Tamilnadu. Balandreau *et al.* (1975) and Baldani and Dobereiner (1980) reported the ubiquitous occurrence of *Azospirillum* in the rhizosphere of rice

Subramanian (1981) and Yanni et al., (1977) reported the occurrence of *Azotobacter* and *Rhizobium* in the rhizosphere of rice, respectively. The results of the present study are in conformity with findings of Balandreau *et al.* (1975) and

Baldani and Dobereiner (1980) for *Azospirillum* and Subramanian (1981) and Yanni *et al.* (1997) for *Azotobacter* and *Rhizobium*, respectively.

Table 2. Occurrence of community population of diazotrophs in twenty-eight locations of Cauvery deltaic region, Tamilnadu

Location for soil sample collection	Log ₁₀ of CFU/g of dry soil						
	Total bacterial population	<i>Azospirillum</i> population	% of <i>Azospirillum</i> to total bacterial population	<i>Pseudomonas</i> sp. population	% of <i>Pseudomonas</i> sp. population to total bacterial population	<i>Bacillus</i> population	% of <i>Bacillus</i> population to total bacterial population
Adalaiyur	8.20	7.45	0.92	8.15	0.94	7.32	0.94
Alangudi	8.62	6.25	0.90	8.12	0.95	6.15	0.91
Ervadi	9.14	6.00	0.90	7.20	0.94	6.32	0.92
Iravancherry	9.20	7.12	0.93	7.13	0.93	6.61	0.94
Karaiyur	9.23	6.23	0.91	7.18	0.94	7.11	0.93
Kayattur	8.23	6.34	0.88	6.61	0.89	6.64	0.90
Kottamangalam	9.11	6.50	0.91	6.55	0.92	6.26	0.93
Sengamangalam	8.21	6.21	0.87	7.28	0.93	5.98	0.89
Sikkal	9.22	6.31	0.91	7.63	0.96	6.10	0.92
Tenkarai	9.27	7.44	0.94	7.11	0.94	7.12	0.94
Tenpidagai	8.42	7.24	0.92	6.74	0.92	6.16	0.90
Themangalam	9.17	6.41	0.91	6.65	0.93	6.96	0.95
Theti	8.26	6.39	0.88	7.10	0.91	6.81	0.92
Achampatti	9.15	6.71	0.93	8.10	0.98	6.55	0.93
Budalur	8.68	6.45	0.92	7.19	0.94	6.21	0.91
Indalur	8.51	7.12	0.91	6.34	0.90	7.00	0.94
Kadambangudi	8.88	6.95	0.93	6.29	0.92	6.78	0.93
Kattur	9.10	7.29	0.94	6.27	0.91	5.98	0.92
Kulichapattu	9.21	6.71	0.93	7.34	0.95	6.47	0.93
Kullangarai	8.33	6.39	0.90	7.18	0.94	6.63	0.91
Kurungalur	9.42	7.44	0.96	8.12	0.98	7.29	0.95
Kuruvadipatti	9.18	7.39	0.95	7.77	0.97	6.41	0.93
Manangorai	8.89	7.12	0.93	7.14	0.93	6.17	0.92
Maraneri	8.78	7.28	0.94	6.66	0.92	7.15	0.94
Mathur	9.12	6.90	0.93	6.96	0.95	6.95	0.93
Nallicheri	8.20	6.15	0.89	6.25	0.90	5.91	0.88
Pinnai Nallur	9.40	7.35	0.95	6.29	0.93	6.53	0.93
Pudukudi	9.25	7.00	0.94	6.33	0.92	6.84	0.94

Table 3. Designation of diazotrophic isolate obtained from the rhizosphere of lowland rice twenty-eight location in Cauvery deltaic region, Tamilnadu

Location	Designation of the isolate		
	<i>Azospirillum</i>	<i>Azotobacter</i>	<i>Rhizobium</i>
Adalaiyur	AZS-1	PSE-1	BAC-1
Alangudi	AZS-2	PSE-2	BAC-2
Ervadi	AZS-3	PSE-3	BAC-3
Iravancherry	AZS-4	PSE-4	BAC-4
Karaiyur	AZS-5	PSE-5	BAC-5
Kayattur	AZS-6	PSE-6	BAC-6
Kottamangalam	AZS-7	PSE-7	BAC-7
Sengamangalam	AZS-8	PSE-8	BAC-8
Sikkal	AZS-9	PSE-9	BAC-9
Tenkarai	AZS-10	PSE-10	BAC-10
Tenpidagai	AZS-11	PSE-11	BAC-11
Themangalam	AZS-12	PSE-12	BAC-12
Theti	AZS-13	PSE-13	BAC-13
Achampatti	AZS-14	PSE-14	BAC-14
Budalur	AZS-15	PSE-15	BAC-15
Indalur	AZS-16	PSE-16	BAC-16
Kadambangudi	AZS-17	PSE-17	BAC-17
Kattur	AZS-18	PSE-18	BAC-18
Kulichapattu	AZS-19	PSE-19	BAC-19
Kullangarai	AZS-20	PSE-20	BAC-20
Kurungalur	AZS-21	PSE-21	BAC-21
Kuruvadipatti	AZS-22	PSE-22	BAC-22
Manangorai	AZS-23	PSE-23	BAC-23
Maraneri	AZS-24	PSE-24	BAC-24
Mathur	AZS-25	PSE-25	BAC-25
Chidambaram	AZS-26	PSE-26	BAC-26
Pinnai Nallur	AZS-27	PSE-27	BAC-27
Pudukudi	AZS-28	PSE-28	BAC-28

Table 4. Genus and speciation of diazotrophic isolates obtained at rhizosphere lowland rice

Place of collection	Designation	Genus and Specification
Adalaiyur	AZS-1	<i>Azospirillum brasilense</i>
Alangudi	AZS-2	<i>Azospirillum brasilense</i>
Ervadi	AZS-3	<i>Azospirillum brasilense</i>
Iravancherry	AZS-4	<i>Azospirillum brasilense</i>
Karaiyur	AZS-5	<i>Azospirillum brasilense</i>
Kayattur	AZS-6	<i>Azospirillum brasilense</i>
Kottamangalam	AZS-7	<i>Azospirillum brasilense</i>

Place of collection	Designation	Genus and Specification
Sengamangalam	AZS-8	<i>Azospirillum brasilense</i>
Sikkal	AZS-9	<i>Azospirillum brasilense</i>
Tenkarai	AZS-10	<i>Azospirillum brasilense</i>
Tenpidagai	AZS-11	<i>Azospirillum brasilense</i>
Themangalam	AZS-12	<i>Azospirillum brasilense</i>
Theti	AZS-13	<i>Azospirillum brasilense</i>
Achampatti	AZS-14	<i>Azospirillum brasilense</i>
Budalur	AZS-15	<i>Azospirillum brasilense</i>
Indalur	AZS-16	<i>Azospirillum brasilense</i>
Kadambangudi	AZS-17	<i>Azospirillum brasilense</i>
Kattur	AZS-18	<i>Azospirillum brasilense</i>
Kulichapattu	AZS-19	<i>Azospirillum brasilense</i>
Kullangarai	AZS-20	<i>Azospirillum brasilense</i>
Kurungalur	AZS-21	<i>Azospirillum brasilense</i>
Kuruvadipatti	AZS-22	<i>Azospirillum brasilense</i>
Manangorai	AZS-23	<i>Azospirillum brasilense</i>
Maraneri	AZS-24	<i>Azospirillum brasilense</i>
Mathur	AZS-25	<i>Azospirillum brasilense</i>
Nallichery	AZS-26	<i>Azospirillum brasilense</i>
Pinnai Nallur	AZS-27	<i>Azospirillum brasilense</i>
Pudukudi	AZS-28	<i>Azospirillum brasilense</i>
Adalaiyur	PSE-1	<i>Pseudomonas</i> sp.
Alangudi	PSE-2	<i>Pseudomonas</i> sp.
Ervadi	PSE-3	<i>Pseudomonas</i> sp.
Iravancherry	PSE-4	<i>Pseudomonas</i> sp.
Karaiyur	PSE-5	<i>Pseudomonas</i> sp.
Kayattur	PSE-6	<i>Pseudomonas</i> sp.
Kottamangalam	PSE-7	<i>Pseudomonas</i> sp.
Sengamangalam	PSE-8	<i>Pseudomonas</i> sp.
Sikkal	PSE-9	<i>Pseudomonas</i> sp.
Tenkarai	PSE-10	<i>Pseudomonas</i> sp.
Tenpidagai	PSE-11	<i>Pseudomonas</i> sp.
Themangalam	PSE-12	<i>Pseudomonas</i> sp.
Theti	PSE-13	<i>Pseudomonas</i> sp.
Achampatti	PSE-14	<i>Pseudomonas</i> sp.
Budalur	PSE-15	<i>Pseudomonas</i> sp.
Indalur	PSE-16	<i>Pseudomonas</i> sp.
Kadambangudi	PSE-17	<i>Pseudomonas</i> sp.
Kattur	PSE-18	<i>Pseudomonas</i> sp.
Kulichapattu	PSE-19	<i>Pseudomonas</i> sp.
Kullangarai	PSE-20	<i>Pseudomonas</i> sp.
Kurungalur	PSE-21	<i>Pseudomonas</i> sp.
Kuruvadipatti	PSE-22	<i>Pseudomonas</i> sp.

Place of collection	Designation	Genus and Specification
Manangorai	PSE-23	<i>Pseudomonas</i> sp.
Maraneri	PSE-24	<i>Pseudomonas</i> sp.
Mathur	PSE-25	<i>Pseudomonas</i> sp.
Nallichery	PSE-26	<i>Pseudomonas</i> sp.
Pinnai Nallur	PSE-27	<i>Pseudomonas</i> sp.
Pudukudi	PSE-28	<i>Pseudomonas</i> sp.
Adalaiyur	BAC-1	<i>Bacillus</i> sp.
Alangudi	BAC-2	<i>Bacillus</i> sp.
Ervadi	BAC-3	<i>Bacillus</i> sp.
Iravancherry	BAC-4	<i>Bacillus</i> sp.
Karaiyur	BAC-5	<i>Bacillus</i> sp.
Kayattur	BAC-6	<i>Bacillus</i> sp.
Kottamangalam	BAC-7	<i>Bacillus</i> sp.
Sengamangalam	BAC-8	<i>Bacillus</i> sp.
Sikkal	BAC-9	<i>Bacillus</i> sp.
Tenkarai	BAC-10	<i>Bacillus</i> sp.
Tenpidagai	BAC-11	<i>Bacillus</i> sp.
Themangalam	BAC-12	<i>Bacillus</i> sp.
Theti	BAC-13	<i>Bacillus</i> sp.
Achampatti	BAC-14	<i>Bacillus</i> sp.
Budalur	BAC-15	<i>Bacillus</i> sp.
Indalur	BAC-16	<i>Bacillus</i> sp.
Kadambangudi	BAC-17	<i>Bacillus</i> sp.
Kattur	BAC-18	<i>Bacillus</i> sp.
Kulichapattu	BAC-19	<i>Bacillus</i> sp.
Kullangarai	BAC-20	<i>Bacillus</i> sp.
Kurungalur	BAC-21	<i>Bacillus</i> sp.
Kuruvadipatti	BAC-22	<i>Bacillus</i> sp.
Manangorai	BAC-23	<i>Bacillus</i> sp.
Maraneri	BAC-24	<i>Bacillus</i> sp.
Mathur	BAC-25	<i>Bacillus</i> sp.
Nallichery	BAC-26	<i>Bacillus</i> sp.
Pinnai Nallur	BAC-27	<i>Bacillus</i> sp.
Pudukudi	BAC-28	<i>Bacillus</i> sp.

The results of the present study also revealed a marked variation in the community population of *Azospirillum*, *Pseudomonas* and *Bacillus* in the locations of Cauvery deltaic region, Tamilnadu, observed. A range of 0.89 per cent to 1.41 per cent of *Azospirillum*, 1.12 per cent to 2.50 per cent of *Pseudomonas* and 0.51 per cent to 0.97 per cent

of *Bacillus* to the total bacterial population was observed in the survey. In the present study, Twenty eighty cultures of *Azospirillum* (AZS-1 to AZS-28), Twenty eight cultures of *Pseudomonas* (PSE-1 to PSE-28) and Twenty eight cultures of *Bacillus* (BAC-1 to BAC-28) were isolated from the rhizosphere of lowland rice grown at 28 locations of Cauvery deltaic region, Tamilnadu and were identified based upon the morphological and physiological characteristics as mentioned in Bergeys's manual of determinative Bacteriology VIIIth edition. Baldani and Dobereiner (1980) reported that 98 per cent of the population which harboured rice roots were *Azospirillum brasilense* was the predominant species associated with C₃ plants in tropical zone (Baldani and Dobereiner, 1986). The results of the present study clearly revealed the predominance of *Azospirillum brasilense* in the rice soils of surveyed area.

Rangaswami and Venkatesan (1964) and (Yanni *et al.*, 1997) reported the predominance of *Pseudomonas* sp. and *Bacillus* sp. in the rhizosphere of lowland rice, respectively. The results of the present study also conformity with the above findings.

REFERENCES

- Allen, E.K., O.N. Allen and A.S. Newman. 1953. Pseudomodulation of leguminous plants induced by 2-bromo-3-5-dichlorobenzoic acid. *Amer. J. Bot.*, 40: 429-435.
- Baldani, V.L. and D. J. Dobereiner. 1980. Host plant specificity in the infection of cereals with *Azospirillum* spp. *Soil Biol. Biochem.*, 12: 433-439.
- Burdman, S., E. Jurkevitch and Y. Okon. 2000. Recent advances in the use of plant growth promoting rhizobacteria (PGPR) in agriculture in microbiol interactions in Agriculture and forestry, vol.2. (Eds) Subba rao, N.S. and Y.R. publishers Science, Inc. Enfield.
- Cochran, W.G. 1950. Estimation of bacterial densities by means of the "Most probable number". *Biometric.*, 6: 105- 116.
- Day, J.M. and Dobereiner. 1976. Physiological aspects of N₂ fixation by a Spirillum from Digitaria roots. *Soil Biol. Biochem.*, 8:45-50.
- Malik, K.A., B. Rackshanda., S. Mehnaz., G. Rasul., M.S. Mirza and S. Ali. 1997. Association of nitrogen-fixing plant growth promoting rhizobacteria (PGPR) with kallar grass and rice. *Plant and soil.*, 194: 37-44.
- Rodriguez - Caceres, E.A. 1982. Improved medium for isolation of *Azospirillum* spp. *Appl. Environ. Microbiol.*, 44: 990-991.

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