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Nitrogen Fixation by *Azotobacter* in Rice Rhizosphere

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ABSTRACT

A microaerophilic culture of *Azotobacter chroococcum* when introduced through seeds of rice varieties survived and multiplied well in the rhizosphere throughout the crop growth under sterile and unsterile conditions. The rate of multiplication of *A. chroococcum* was more in the sterile soil than under unsterile soil. The colonization of *Azotobacter* in the rhizosphere was more pronounced during the boot-leaf and flowering stages than the initial stages. Although the varieties considerably differed in their abilities to harbour *Azotobacter* in the root region, this inference does not bear any statistical significance. There was more nitrogen fixation in the rhizosphere soil than in the uncropped soil. The study further revealed that in the water - logged rice field soils, apart from *Azotobacter* several other photosynthetic microorganisms play a subtle role in nitrogen fixation.

INTRODUCTION

In many parts of India fertilizers are inadequately used and rice production depends chiefly on the natural fertility of soil. A continuing supply of nitrogen over the years despite removal of this element by rice crop is claimed to result from the fixation of atmospheric nitrogen by microorganisms in the rice fields (Yoshida *et al.*, 1973 Yoshida, 1975). Of the several kinds of microorganisms recognised to fix atmospheric nitrogen, *Azotobacter* has been drawing the attention of scientists for quite a long time (Rangaswami and Subba Raja, 1962; Mehrotra and Lehri, 1971). Recently, it has been claimed that apart from *Azotobacter*, several species of photosyntheic bacteria and blue green algae are associated with dinitrogen fixation, particularly in the water-logged soils of rice fields (Yoshida *et al.*, 1973; Koch and Oya, 1974). In the present communication the survival and activities of *Azotobacter chroococcum* in the rhizosphere of several varieties of rice are reported.

MATERIALS AND METHODS

The strains of *Azotobacter chroococcum*, used in these investigations were isolated from the rhizosphere of the rice variety, Bhavani grown under submerged soil conditions. To isolate microaerophilic culture of *Azotobacter* to suit to the specific requirements of waterlogged conditions, anaerobic jar technique of isolation was followed (Harrigan and Mc Cance, 1966). Several isolates of *Azotobacter chroococcum* obtained from the rhizosphere of the rice variety, Bha-

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vani were screened for nitrogen fixation (Rangaswami and Subba Raja, 1962) and the efficient AB- 2 strain was used in the further studies. Seeds of the rice varieties were obtained from the Paddy Breeding Station of the Tamil Nadu Agricultural University, Coimbatore and they were surface-sterilized with 0.1 per cent mercuric chloride solution. They were sown to 5 kg of paddy field soil (N = 0.126 per cent, organic carbon = 0.92 per cent, pH 8.0) taken in circular earthen pots of 30 cm diameter. While one set of pots carried sterilized soil the other set was filled with unsterile soil. For making comparisons, soil without crop was also maintained under sterile and unsterile conditions.

Before sowing the seeds, they were treated with the peat soil based culture of *A. chroococcum* (Ca. 14 x 10^4 cells / seed), shade dried and sown (Brown, 1974). The soils received fertilizers at the rate of 60 kg of potash (K_2O) and 60 Kg of phosphorus (P_2O_5) per hectare. Adequate number of replications were maintained under each treatment. Throughout the experimental period, the soils were maintained under submerged conditions. Sterilized tap water was used to irrigate the crop under sterile soil.

The enumeration of *Azotobacter* in the rhizosphere of the rice varieties was done following the standard serial dilution plate technique (Pramer and Schmidt, 1966) using Waksman No. 77 agar medium. The counts were made at four distinct growth phases of the crop, *viz.*, seedling, tillering, boot-leaf and flowering stages. The rhizosphere soil samples of the cultivars collected at the last stage of crop growth were analysed for total nitrogen content (Bremner, 1960).

To find out the subtle role of *Azotobacter* and other photosynthetic nitrogen fixing microorganisms in rice field soils in the process of N fixation another experiment was conducted. One g soil samples obtained from the rhizosphere of the variety of IR 8 raised under sterilized and unsterilized conditions were inoculated to 50 ml of Ashby's mannitol broth. One set of flasks was incubated under darkness while the other set maintained under sunlight (Ca. 4 hr sunshine/day) for a period of 15 days with ocassional shaking. At the end of the incubation period an aliquot of the broth was withdrawn and total nitrogen analysed.

RESULTS AND DISCUSSION

The efficiency of nitrogen fixation by various *Azotobacter chroococcum* isolates from the rhizosphere of rice varieties indicated that the strain AB2 fixed the maximum quantity of nitrogen under *in vitro* conditions (Table I).

The survival of *Azotobacter chroococcum* in the rhizosphere of different rice varieties are presented in Table II. In sterile soil, the seed inoculated *Azotobacter* survived well in the rhizosphere of all the rice cultivars. That the *Azotobacter* counts were more in the last stage of plant growth indicated the multiplication of the organism in the rhizosphere of the varieties. Gopalakrishnamoorthy *et al.* (1967) demonstrated that seed inoculated *A. chroo-*

TABLE I. Nitrogen fixation by different strains *Azotobacter chroococcum*

Strain	Nitrogen fixed* (mg of N/g of mannite)
AB 1	16.80
AB 2	18.00
AB 3	17.60
AB 4	11.50
AB 5	12.30
AB 6	8.60
AB 7	14.70
AB 8	13.00
AB 9	15.60
AB 10	12.80

*Data represent average of three estimations

coccum readily established in the rhizosphere of rice seedling which resulted in better growth. *Azotobacter* survived till the last stage of the crop growth in unsterile soil; but the rate of multiplication was not as much as in the sterile soil. The reason is obvious; *Azotobacter* has to survive in the midst of myriads of microbial communities under unsterile system each one competing with the other for space and nutrition. It is reconciled from this study that in spite of such keen competition for survival in such ecological niches like rhizosphere, *Azotobacter* is able to thrive. Recently, it has been brought out at the International Rice Research Institute, the Philippines, (Yoshida and Ancajas, 1973) that nitrogen fixing activity of the rice soil was maximum only during the last phase of the crop growth. The present data add additional evidence, for only in the flowering stage of the crop, *Azotobacter* was significantly more in number than in other stages. It has been reported that the amount of carbohydrates in rice culture solution was more in the rice rhizosphere only in the later stages of growth than in the earlier stages (Yoshida, 1975). Though it was apparent that the rice cultivars IR 8

TABLE II. Colonization of Azotobacter in the rhizosphere of rice varieties under sterile and unsterile conditions (Azotobacter population X 10^4/g)

Rice variety	Sterile soil				Unsterile soil			
	Seedling stage	Tillering stage	Boot-leaf stage	Flowering stage	Seedling stage	Tillering stage	Boot-leaf stage	Flowering stage
IR 8	180.0	15.0	27.0	200.0	13.0	15.0	12.5	37.5
IR 20	130.0	15.5	20.0	45.0	14.0	22.5	20.0	17.0
Co 36	15.0	10.5	20.0	95.0	4.5	23.0	11.5	9.0
Co 38	35.0	21.0	10.5	75.0	5.5	20.0	21.5	8.0
TKM 6	15.0	18.0	19.0	125.0	6.0	5.5	5.5	5.0
Bhaveni	50.0	25.0	30.5	100.0	6.0	11.0	14.0	18.5
Ponni	20.0	24.0	19.5	55.0	4.5	6.0	15.0	19.0
Vaigai	90.0	16.0	17.5	20.0	4.0	16.0	10.5	11.0
Karikalan	30.0	31.0	22.0	25.0	7.0	13.5	21.0	7.5
Kannaki	60.0	13.0	10.5	70.0	3.0	10.5	6.5	10.0
Uncropped soil (Control)	—	—	—	—	8.0	10.0	8.5	3.0

Soils: C. D. (P = 0.05)=4.10; Steges: C. D. (P=0.05) = 16.86; Stages X Soil: C. D. (P=0.05)=22.42

TABLE III. Per cent nitrogen* in the rhizosphere of different rice varieties grown under sterile and unsterile condition

Rice variety	Unsterile soil	Sterile soil
IR 8	0.1484	0.1078
IR 20	0.1708	0.1438
CO 36	0.1642	0.1428
CO 38	0.1396	0.1434
TKM 6	0.1384	0.1098
Bhavani	0.1456	0.0924
Ponni	0.1596	0.1064
Vaigai	0.1400	0.1310
Karikalan	0.1428	0.1248
Kannaki	0.1064	0.1095
Uncropped soil	0.1120	0.1024

*Data represent average of three estimations.

and CO. 36 harboured more numbers of *Azotobacter* in the rhizosphere, it did not bear any statistical significance. Each variety of rice is considered to be specific in harbouring the nitrogen fixing microflora in the rhizosphere (Yoshida *et al.* 1973) and this statement requires to be critically examined with more number of rice varieties under different ecological conditions.

The data presented in Table III illustrated that nitrogen fixation is more pronounced in the rhizosphere soil than in the uncropped soil. Besides, under unsterile soil there was more nitrogen fixation than in sterile soil which may be due to the activities of other nitrogen fixing microorganisms in the rhizosphere.

The study on the critical role of *Azotobacter* and other photosynthetic microorganisms in respect of nitrogen fixation in the rhizosphere (Table IV)

TABLE IV. Nitrogen fixation in the rhizosphere of IR 8 rice variety.

Soil sample	Nitrogen fixation (mg of N/g of the soil) Light	Dark
Sterile soil inoculated with *Azotobacter chroococcum*	4.9	4.2
Unsterile soil inoculated with *A. chroococcum*	6.38	5.62
Uncropped sterile soil inoculated with *Azotobacter chroococcum*	0 923	0.510
Uncropped unsterile soil inoculated with *Azotobacter chroococcum*	3.28	2.89

Data represent average of three estimations.

indicated that the *Azotobacter* inoculated sterilized soil recorded the least value for nitrogen fixation whereas the same soil under unsterile conditions recorded greater value for nitrogen fixation under illuminated conditions. This difference in the nitrogen fixation may be solely attributable to the photosynthetic organisms in rice soils. Their quantity and distribution in the rhizosphere soil with age of the crop need to be investigated further. Joshi *et al.* (1968) suggested that *Azotobacter chroococcum* and *Rhodopsuedomonas capsulatus* when introduced on the seed coat along with farmyard manure, facilitated the establishment of *A.chroococcum*. The role of the non-symbiotic nitrogen fixers could not be overlooked and it deserved further investigation.

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