

adult moth, it escapes by biting a circular hole at the apex of the gall (figure 1). After the exit of the insect, the gall cavity may be occupied by ants, fungal mycelium, or other insects for shelter.

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OCCURRENCE OF THE NEW SPECIES *AZOSPIRILLUM HALOPRAEFERENS* IN ASSOCIATION WITH RICE ROOTS

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BACTERIA of the genus *Azospirillum* are widely distributed in the rhizosphere of tropical and subtropical grasses¹. Association of *Azospirillum* with the roots of rice plants has been reported². It is generally accepted that *Azospirillum* can enhance the growth of the plant³. Initially two species of *Azospirillum*, viz. *A. lipoferum* and *A. brasilense* were reported⁴, to which a third species, *A. amazonense*, was added⁵. A fourth species, *A. halopraeferens*, which is characteristic for its high salt (3% NaCl) tolerance, has

been reported⁶. The occurrence of a variant of *A. halopraeferens* associated with rice roots is reported here.

Roots of a local rice variety were collected from a rice soil of pH 8.0. Root bits (0.5 cm) from the washed root system were inoculated in nitrogen-free semi-solid malate medium⁷. Undulating subsurface pellicle formed on incubation was purified by three serial transfers into fresh semisolid medium. Each transfer was made at 48 h.

The isolate was gram-negative, highly motile and vibrioid. It can grow and fix nitrogen in presence of 5% NaCl in the N-free semisolid malate medium, but it is not obligately halophilic. The characteristics of *A. halopraeferens* listed by Reinhold *et al.*⁶, except DNA base composition, were compared with our isolate (table 1). Photomicrographs of our isolate, *A. lipoferum* and *A. brasilense* are shown in figure 1.

Based on the observations, it is concluded that our isolate is a nutritional variant of *A. halopraeferens* reported by Reinhold *et al.*⁶ This variant has been deposited in the culture bank of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University.

With the occurrence of large areas of saline soils in the country and saline irrigation water, this highly

Table 1 Characters of *A. halopraeferens* and present isolate

Character	<i>A. halopraeferens</i>	Our isolate
Salt tolerance (NaCl)	3%	5%
Utilization of glucose	-	+
Utilization of sucrose	-	+
Acidification of peptone-based glucose broth	-	+

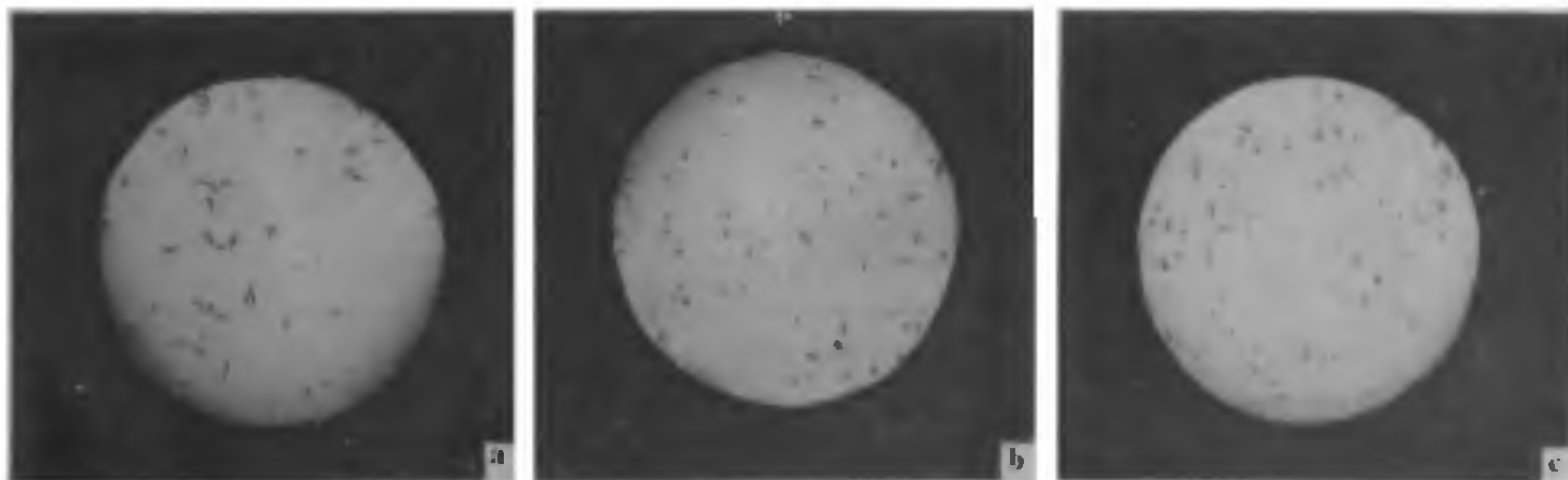


Figure 1. Photomicrographs of; a, *Azospirillum brasilense*; b, *A. lipoferum*; c, *A. halopraeferens*.

saline-tolerant diazotrophic *Azospirillum* might be interesting for practical applications.

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BOVINE MANURE LIQUOR AS A GROWTH MEDIUM FOR *RHIZOBIUM* INOCULANTS

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WE examined if bovine manure liquor could serve as a substitute for yeast extract mannitol broth to support the growth of rhizobia.

Fresh bovine manure collected from Jersey Cross breeds was used. The chemical composition of the oven-dried (45 C) manure was determined by standard procedures¹. The manure contained 1.95% N (micro-Kjeldahl method), 0.71% P (vanadomolybdate yellow colour method), 0.3% each of Na and K (flame photometry), 4.4% Ca and 0.84% Mg (EDTA method), 0.149% of Fe, 0.023% Mn, 0.003% Cu, 0.013% Zn (atomic absorption spectrometry).

A 10% slurry in distilled water was prepared and filtered through muslin. The pH adjusted to 7.0 with 10 N HCl. The slurry was dispensed (100 ml) into 250 ml Erlenmeyer flasks. A set of flasks containing 100 ml of standard yeast mannitol broth² were also prepared. The flasks were autoclaved at 15 psi for 15 min. Another set of flasks containing 100 ml of 10% manure extract were also used without sterilization. All the flasks were inoculated with 1 ml of a 48-h-old *Rhizobium* culture (strain A1, a streptomycin-resistant strain from groundnut) and incubated at 28 ± 2°C for 7 days. Viable cell counts were made by dilution plate on congo-red yeast mannitol agar supplemented with 10 µg/ml streptomycin sulphate every 24 h for 7 days.

The results are presented in table 1. As expected, it was found that multiplication of *Rhizobium* in manure liquor was as good as in the commonly used but expensive yeast mannitol broth. The percentage increase in growth in yeast mannitol broth over that in sterile manure liquor was 6–21%, which is apparently not commensurate with the cost involved. On the other hand bovine manure liquor is absolutely cost-free to the farmer and is available without any seasonal limitations. But it should be sterilized before use since unsterilized manure liquor seems to inhibit introduced bacteria with a weak saprophytic ability either by metabolic inhibition or by exhausting nutrients needed for transient bacteria³.

Table 1 Multiplication of *Rhizobium* sp. in bovine manure liquor

Treatment	Cell number ($\times 10^9$ /ml)					
	Initial ($\times 10^5$ /ml)	24 h	48 h	72 h	144 h	168 h
Yeast mannitol broth	0.39	00.04	00.32	46.70	49.00	43.70
Sterilized manure liquor	0.39	00.04	00.30	39.00	40.50	37.30
Unsterilized manure liquor	0.39	00.01	00.03	00.40	03.60	02.60