19 September 1988; Revised 14 January 1989

1. Anonymous, *The Wealth of India*, CSIR, New Delhi, 1962.
2. Belikov, V. V. and Tochkova, T. V., *Farm Zh. (Kiev)*, 1973, **28**, 40.
3. Kaushal, G. P., Sekhon, B. S. and Bhatia, I. S., *Mikrochim Acta*, 1979, **1**, 365.
4. Rybachenko, A. I., Georgieuskii, V. P., Piotrovskaya, A. G. and Bogaevskaya, T. A., *Otkryt. Izobret.*, 1981, **47**, 209.
5. Khabarov, A. A., Morozov, A. V. and Khabarova, L. P., *Otkryt. Izobret.*, 1986, **25**, 33.
6. Volkova, A. I., Vasilchak, T. A., Maksyutina, N. P. and Gritsenko, E. N., *Farm. Zh. (Kiev)*, 1984, **4**, 44.
7. Haug, M., Brigitte Goldbach, M. H. and Gierschnor, K., *Ind. Obst. Gemueseverwert.*, 1984, 69012, 567.
8. Lei, J. Z., Zhachui, Y. J. and Zhongyao Tongba, *China*, 1982, **7**, 27.
9. Mabrey, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*, Springer–Verlag, Berlin, Heidelberg, New York, 1970.
10. Wagner, H., Bladt, S. and Zgainski, E. M., *Plant Drug Analysis*, Springer–Verlag, Berlin, Heidelberg, New York, Tokyo, 1984.
11. Stahl, E., *Thin Layer Chromatography, A Laboratory Handbook*, Springer–Verlag, Heidelberg, New York, 1969.
12. Prabhu, K. R. and Venkateswarlu, V., *Curr. Sci.*, 1971. **40**, 320.

## CATION EXCHANGE CAPACITY OF SOIL AND ADSORPTION OF *AZOSPIRILLUM* TO SOIL PARTICLES

K. GOVINDARAJAN and
D. PURUSHOTHAMAN
*Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore 641 003, India*

*AZOSPIRILLUM* occurs as a predominant diazotroph in the rhizosphere of grasses and cereals[1]. The population of *Azospirillum* in soil and the rhizosphere zone of plants is influenced by many factors, of which soil is an important one. Microorganisms in soil mostly occur adsorbed to soil particles and rarely as unadsorbed cells[2]. The influence of the cation exchange capacity (CEC) of soil on the adsorption of *Azospirillum* and other heterotrophic bacteria on soil particles is reported.

Three different soil types were used in these studies. Soil samples were collected from the top 0–10 cm, air-dried, and pounded and sieved through a 50 mesh sieve. The total heterotrophic bacteria in the soils were enumerated by the dilution plate technique on yeast extract glucose agar[3]. CEC of the soils was determined as described earlier[4]. One hundred grams of each soil was taken in a beaker and leached thrice with 1 N ammonium acetate, pH 7.0. A standardized cell suspension of *Azospirillum brasilense* strain S.3, which is resistant to both streptomycin and chloramphenicol at 100 $\mu g\ ml^{-1}$ of each and contains a pink chromogen (obtained from D. Hubbell, University of Florida, USA), was added to the soil samples and mixed well. Three replications were maintained in each treatment.

After overnight incubation, the populations of the inoculated *Azospirillum* and the total heterotrophic bacteria were estimated using yeast extract glucose agar. The soil samples were then leached thrice with sterile distilled water with minimum disturbance to the soil, after which the populations of the inoculated *Azospirillum* and total heterotrophs in the soil samples were again determined. As strain S.3 was pink the colonies of *Azospirillum* on the agar medium were easily counted. The percentages of inoculated *Azospirillum* and heterotrophs retained in the soil samples were calculated.

Some physical properties of the three soil types are presented in table 1. Adsorption of *Azospirillum* and heterotrophic bacteria on soil particles is given in table 2. The study revealed that the CEC of soil is closely associated with the adsorption of not only cells of *Azospirillum* but also of the heterotrophic bacteria of the soil. The higher the soil CEC, higher was the percentage of adsorbed cells. Alluvial (clay) soil with a CEC of 33.9 m.e. retained 82.8% of *Azospirillum* in adsorbed state. The cations present in the soils might have contributed to the increase in the adsorption of *Azospirillum* cells to soil particles. Calcium ion-mediated adsorption has been suggested for root nodule bacteria[5]. It is believed that when microorganisms are adsorbed to solid surfaces they enjoy nutritional advantages. Cells adsorbed to surfaces in water take up substances in solution more easily than unadsorbed cells which are likely to be carried away. Other advantages to the bacteria

**Table 1** *Some physical and chemical properties of the soils examined in this study*

| Soil type | pH | Total nitrogen (%) | Organic carbon (%) | Electrical conductivity (mmho × cm$^{-1}$) | CEC (m.e./100 g/ of soil) |
|---|---|---|---|---|---|
| Alluvial soil (clay) | 8 5 | 0.12 | 0 68 | 0.37 | 33.9 |
| Laterite soil | 6 0 | 0 10 | 0 60 | 0 09 | 19 3 |
| Red loam | 7 1 | 0 08 | 0 84 | 0 10 | 15 4 |

**Table 2** *Adsorption of Azospirillum and heterotrophic bacteria to soil particles*

| Soil type | *Azospirillum* ( × 10$^6$) | | | Heterotrophic bacteria ( × 10$^6$) | | |
|---|---|---|---|---|---|---|
| | Initial | Final | Adsorbed cells (%) | Initial | Final | Adsorbed cells (%) |
| Alluvial soil (clay) | 21 | 17.4 | 82 8 | 36.0 | 29 6 | 82.2 |
| Laterite soil | 21 | 12 0 | 57 1 | 28 8 | 18 4 | 63 8 |
| Red loam | 21 | 8 2 | 39.0 | 17.0 | 6.8 | 40 0 |

Bacterial numbers are per gram of soil on oven dry weight basis. Each figure is mean of three estimations.

are protection and proximity to a suitable environment[6].

28 July 1988


1. Wani, S. P., In: *Biological Nitrogen Fixation*, (ed.) N. S. Subba Rao, Oxford and IBH, New Delhi, 1988, p. 124.
2. Hattori, T., *Microbial Life in the Soil*, Marcel Dekker, New York, 1973, pp. 427.
3. Allen, O. N., *Experiments in Soil Bacteriology*, Burgess Publ. Co., Minneapolis, USA, 1953, p. 69.
4. Jackson, M. L., *Soil Chemical Analysis*, Prentice-Hall, New Delhi, 1973, pp. 485.
5. Dazzo, F. B., In: *Adsorption of Microorganisms to Surfaces*, (eds) G. Bitton and K. C. Marshall, John Wiley and Sons, New York, 1980, p. 253.
6. Okon, Y. and Kaupulkik, Y., *Plant Soil*, 1986, **90**, 3.


## MULTIPLE XANTHINE DEHYDROGENASE ISOZYMES IN THE EXOTIC CARP *CTENOPHARYNGODON IDELLA* (CYPRINIDAE: PISCES)

B. K. PADHI and A. R. KHUDA-BUKHSH
*Department of Zoology, University of Kalyani, Kalyani 741 235, India*

XANTHINE dehydrogenase (XDH; E.C. 1.2.3.2) is a polymorphic enzyme involved in purine metabolism[1]. From the available data on XDH isozymes in fish[2–3], two codominant alleles at an autosomal locus are believed to be involved. XDH activity is known to respond to nutritional status of the organism[1] and is independent of age and sex[3]. Usually, homozygotes show one XDH band on electrophoresis, and heterozygotes three bands, suggesting dimeric structure of the enzyme[3]. So far as the authors are aware, XDH isozyme patterns in fish have not yet been reported from India. During our studies on the XDH isozyme patterns of some fishes, we came across multiple XDH isozymes in the exotic carp, *Ctenopharyngodon idella*.

Ten adult living specimens of *C. idella*, collected from the local fish farm (Ganga Matsya Utpadan Kendra, Rathtala), served as the material for the present study. Muscle, heart, liver, eye, kidney and brain were dissected out quickly and homogenized separately in cold (4°C) distilled water. The homogenate was centrifuged at 11,000 *g* at 4°C for 30 min. Known amount of supernatant was immediately subjected to polyacrylamide slab gel electrophoresis[4,5] at a constant current of 3 mA/slot and 200 V in the cold (6–7°C) using Tris–glycine (pH 8.6) as the running buffer. Staining for detection of XDH activity was done following Nakano and Whiteley[6].

Four or five XDH bands were observed in the zymograms (figure 1A, B) of all tissues examined, except liver, which showed two bands. Except for the slowly anodal band in kidney which showed intense