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EFFECT OF SALINITY ON THE GROWTH AND NITROGEN FIXATION OF *AZOTOBACTER VINELANDII*

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The present paper discusses the influence of salinity on the growth and nitrogen-fixing capacity of *Azotobacter vinelandii* in prawn-cum-paddy culture systems. The study on nine strains of *A. vinelandii* revealed that the salinity levels above 40 and below 10‰, in general, reduced the growth. Nitrogen fixation was observed in all the strains at salinity levels from 15 to 50‰.

INTRODUCTION

Free-living *Azotobacter* spp. are responsible for the biological fixation of nitrogen in brackishwater fields and ponds. Fluctuations in salinity during different seasons influence the bacterial activity in biological transformation of the nutrient elements, thus affecting the pond productivity. The influence of salinity on nitrogen-fixing microbes was first worked out by Beijerinck (1901) who noted the resistance of *Azotobacter* spp. to high salt concentrations. Though Lipman (1912) observed that NaCl concentrations of more than 0.5-0.6% was toxic to *Azotobacter* spp., others found that *Azotobacter* spp. from saline soils of Central Asia grew in the presence of higher concentrations of NaCl (Babak, 1965; Lakshmanaperumalsamy *et al.*, 1975). Excess concentrations of salts in soil may render *Azotobacter* spp. inactive, thereby depressing the nitrogen-fixing ability (Iswaran and Sen, 1958). This paper discusses the influence of salinity on the growth and nitrogen-fixing activity of *A. vinelandii*.

MATERIAL AND METHODS

The present investigation was carried out at Narakkal (long. 76° 14' E; lat. 10° 03' N), a fishing hamlet in the Vypeen Island, 15 km north-west of Cochin on the south-west coast of India. Four ponds were selected for regular sampling of which two were perennial prawn culture systems and the other two seasonal prawn culture systems where prawn and paddy were cultivated during the inter-monsoon (October-May) and monsoon (June-September) periods, respectively.

Isolates of *A. vinelandii* were purified by repeated streaking on nitrogen-free agar medium and maintained at room temperature in the same medium. The identification of the isolates was based on Buchanan and Gibbons (1974). The identity was confirmed by morphological and physiological characteristics suggested by Gibbs and Shapton (1968).

Studies were carried out to elucidate the effect of salinity on the growth and nitrogen fixation by growing them in Jensen's medium. Salinity levels used for the experiment ranged from 0 to 60‰. Different levels of salinity were obtained by diluting standard stocks of artificial sea water made in the laboratory. The flasks were incubated for a period of 15 days in dark. After incubation, the growth was measured as absorbance at 420 nm and the amount of fixed nitrogen was estimated using modified Kjeldahl micromethod (Rodina, 1972). The amount of nitrogen fixed was measured as milligramme NH_3-N/100 ml of the medium. The experiment was repeated twice. The growth and nigrogen-fixation data were analysed using the second degree polynomial equation $Y = a+bx+cx^2$.

RESULTS AND DISCUSSION

All the 30 isolates were collected from the water and sediment of the perennial and seasonal ponds. Nine isolates were gram-negative, oval cells occurring individually, in pairs or sometimes in groups. The size ranged from 2.8 to 4.5 µm in diameter and were motile with peritrichous flagella. They were cyst-forming and capsule-producing types, and were capable of producing greenish-yellow water soluble pigments. They utilised mannitol and rhamnose as carbon source, but were unable to utilise starch.

With the increasing salinity level of the medium, all the nine strains showed a steady increase in growth up to the optimum salinity levels, with slight differences. In most of the strains, the maximum growth occurred in the salinity range of 15-53‰, the exceptions being strains AzV2, AzV6 and AzV9 in which the maximum growth was recorded within the salinity range of 5-25‰. In general, the salinity levels above 30‰ and below 5‰ were found non-conducive for the growth of most of the strains. Strains AzV1 and AzV4 were found to be more tolerant to salinities above 25‰, whereas in strain AzV7, growth declined gradually above 25‰ salinity. In contrast, strain AzV5 showed relatively better growth in higher salinity levels.

Nitrogen fixation by all the nine strains also showed the maximum values at optimum salinity levels (Fig. 1) of 25-30‰. Nitrogen fixation was found to be relatively less, in most of the strains, in salinities less than 15 and above 50‰ with slight variations. Differences were also noted in salinity levels at which maximum growth and maximum nitrogen fixation occurred. For instance, in strain AzV1, maximum growth occurred at 30‰ and maximum nitrogen fixation at

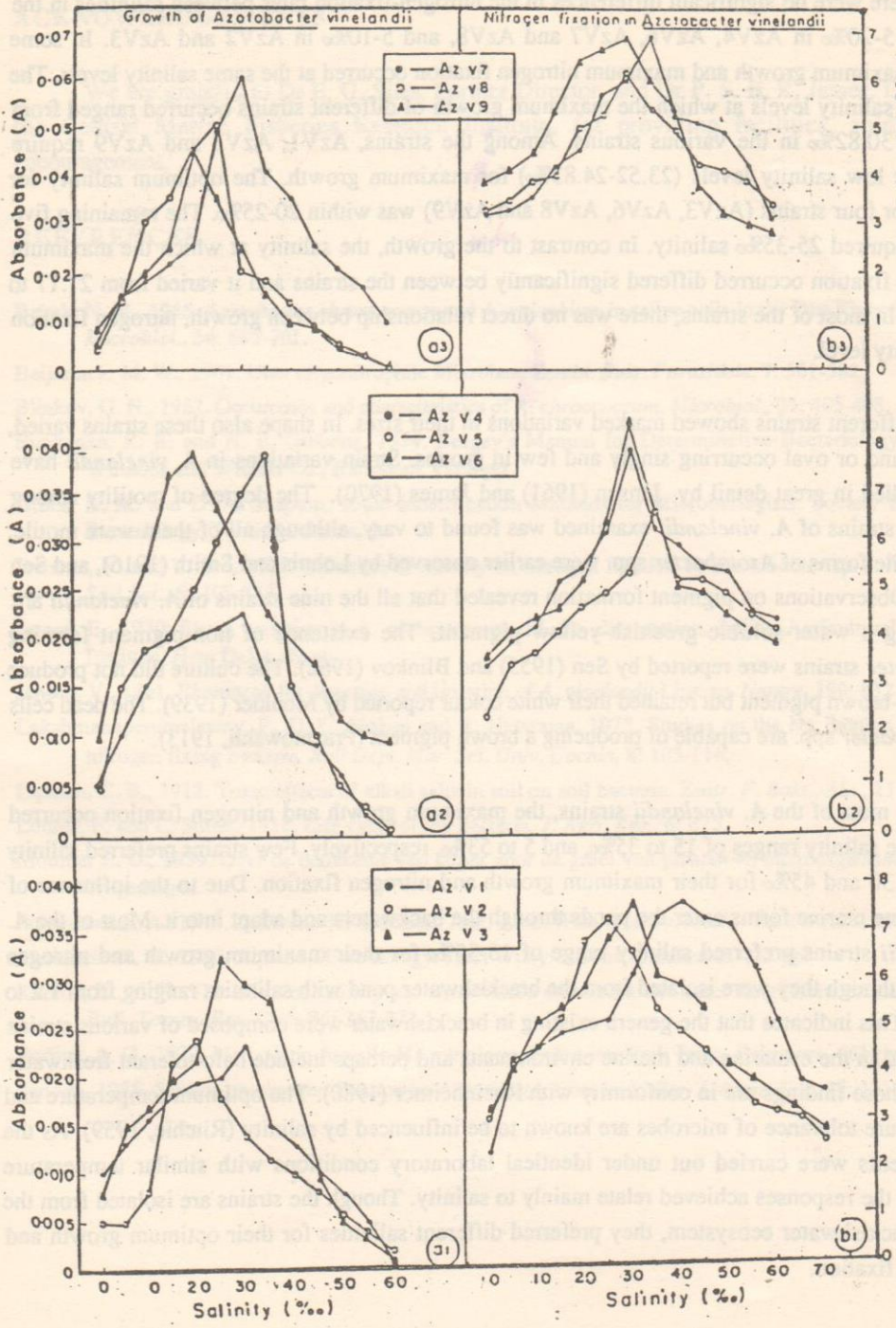

Fig 1. Growth and N_2 fixation in *A. vinelandii*

40‰. There were no significant differences in the nitrogen-fixation rates between salinities in the range of 5-20‰ in AzV4, AzV6, AzV7 and AzV8, and 5-10‰ in AzV2 and AzV3. In some strains, maximum growth and maximum nitrogen fixation occurred at the same salinity levels. The optimum salinity levels at which the maximum growth of different strains occurred ranged from 29.52 to 30.82‰ in the various strains. Among the strains, AzV1, AzV3 and AzV9 require relatively low salinity levels (23.52-24.83‰) for maximum growth. The optimum salinity for growth for four strains (AzV3, AzV6, AzV8 and AzV9) was within 20-25‰. The remaining five strains required 25-35‰ salinity. In contrast to the growth, the salinity at which the maximum nitrogen fixation occurred differed significantly between the strains and it varied from 27.17 to 40.83‰. In most of the strains, there was no direct relationship between growth, nitrogen fixation and salinity level.

Different strains showed marked variations in their sizes. In shape also these strains varied, being round or oval occurring singly and few in groups. Strain variations in *A. vinelandii* have been studied in great detail by Jensen (1961) and James (1970). The degree of motility among different strains of *A. vinelandii* examined was found to vary, although all of them were motile. Non-motile forms of *Azotobacter* spp. were earlier observed by Lohnis and Smith (1916), and Sen (1955). Observations on pigment formation revealed that all the nine strains of *A. vinelandii* are producing a water-soluble greenish-yellow pigment. The existence of non-pigment forming *Azotobacter* strains were reported by Sen (1955) and Blinkov (1962). The culture did not produce the black-brown pigment but retained their white colour reported by Moulder (1939). The dead cells of *Azotobacter* spp. are capable of producing a brown pigment (Prazmowskii, 1913).

In most of the *A. vinelandii* strains, the maximum growth and nitrogen fixation occurred within the salinity ranges of 15 to 35‰, and 5 to 53‰, respectively. Few strains preferred salinity between 30 and 45‰ for their maximum growth and nitrogen fixation. Due to the influence of tides, some marine forms enter the ponds through the backwaters and adapt into it. Most of the *A. vinelandii* strains preferred salinity range of 15-35‰ for their maximum growth and nitrogen fixation, though they were isolated from the brackishwater pond with salinities ranging from 1.2 to 36.4‰. This indicates that the genera existing in brackishwater were composed of various strains originated in the estuarine and marine environments and perhaps include halo-tolerant freshwater forms. These findings are in conformity with Rheinheimer (1980). The optimum temperature and temperature tolerance of microbes are known to be influenced by salinity (Ritchie, 1959). As the experiments were carried out under identical laboratory conditions with similar temperature regimes, the responses achieved relate mainly to salinity. Though the strains are isolated from the same brackishwater ecosystem, they preferred different salinities for their optimum growth and nitrogen fixation.

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