Interactions between the root exudates of pearl millet and *Azospirillum brasilense*

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Abstract. Root exudates of different pearl millet varieties showed quantitative differences in organic carbon, reducing sugars, total and amino nitrogen. The growth and nitrogenase activity of *Azospirillum* were stimulated by the addition of root exudates in the culture medium. Root exudates were also found to support the growth of *Azospirillum* in the rhizosphere. Inoculation with *Azospirillum* significantly enhanced the root exudation in axenically grown pearl millet plants accompanied by an increase in the permeability of roots. N\textsubscript{2}-ase activity of the inoculated plants differed among the varieties and was related to the amount of organic carbon released in the exudates. Addition of extraneous carbon source significantly increased the nitrogenase activity of the roots as the carbon compounds provided by the root exudates appear to be inadequate for the optimum expression of nitrogenase activity. The implications of these results in the pearl millet-*Azospirillum* association were discussed.

Keywords. Pearl millet; root exudates; *Azospirillum* inoculation; nitrogenase activity.

1. Introduction

The associative symbiotic bacterium *Azospirillum* has been receiving increasing attention in view of its nitrogen fixing association with the roots of a number of cereal plants (Dobereiner and Day 1975; Subba Rao 1980; Kapulnik *et al* 1981). This organism has been reported to increase the plant growth and yields through a number of processes like nitrogen fixation (Von Bulow and Dobereiner 1975; Kapulnik *et al* 1981), phytohormone synthesis (Tien *et al* 1979; Venkateswarlu and Rao 1983) and increasing the nutrient uptake (Barea *et al* 1983; Lin *et al* 1983). However the various physiological interactions between the host plant and the bacterium leading to the formation of the symbiosis are not yet fully understood. For example, not much information is available on the interactions between root exudates and the microsymbiont although the activity of the bacterium in the root zone is greatly influenced by these compounds as a source of energy and nutrients (Boddey and Dobereiner 1982). The present investigation therefore was conducted to study the interactions between root exudates of pearl millet and *Azospirillum*, such as the biochemical characterization of the root exudates, effect of root exudates on growth and nitrogenase activity of the bacterium, and the influence of inoculation on the exudation pattern and permeability of the roots.
2. Materials and methods

2.1 Collection and purification of root exudates

Four varieties of pearl millet (BJ-104, MP-15, MHB-110 and MHB-118) were grown axenically in glass tubes of 200 × 25 mm size containing acid washed and sterilized quartz sand. Surface sterilized seeds were pre-germinated on plain agar plates and 4 seedlings each were transferred into the tubes which were then kept in an artificially illuminated growth room (14 hr photo period with 30°C day and 25°C night temperatures, light intensity, 12,000 lux). The plants were maintained with Hoagland’s half strength mineral nutrient solution containing 50 ppm nitrogen as ammonium nitrate. The moisture level in the tubes was maintained in such a way as to provide adequate water to the plants without causing any water-logging condition around the roots. Plants grew well and a normal root system developed.

Root exudates were collected by removing 12 day old plants from the tubes and rinsing the roots and sand from each tube separately in distilled water. The exudates from all plants of one variety were pooled and centrifuged to remove the suspended matter. The supernatant was concentrated by vacuum evaporation to 1/3 of the original volume and desalted by passing through ion exchange columns (Hussain and McKeen 1963; Rai and Strobel 1966). The crude exudates were passed through Dowex-50 (H+ form) and Dowex-1 (formate form) columns to separate it into cationic, anionic and neutral fractions. The individual fractions were evaporated to dryness at 60°C and dissolved in distilled water. For quantitative and thin-layer chromatography (TLC) analysis the different fractions were used directly i.e. cationic for amino acids, anionic for organic acids and neutral for sugars. Alternately all the fractions were pooled together, concentrated and adjusted to a final volume of 1.0 ml representing 10 seedlings. This exudate solution was used to study the effects on the bacterial growth etc.

To study the effect of inoculation with *A. brasilense* on the pattern of exudation in pearl millet (var. BJ-104) 1.0 ml cell suspension of 4 day old *A. brasilense* strain isolated from the roots of pearl millet was added to each tube when the plants were 3 days old. The control tubes received the same amount of autoclaved cells. Root exudates at 8, 12 and 15 days after planting were collected from each set and purified as above for biochemical analysis.

2.2 Biochemical analysis of the exudates

The organic carbon and total nitrogen contents from the crude exudates were estimated by Walkley and Black rapid titration and micro kjeldahl methods respectively (Jackson 1958). The reducing sugars and amino nitrogen were estimated from the respective fractions by Nelson’s arsenomolybdate method (Nelson 1944) and ninhydrin method of Moore and Stein (1948) respectively.

2.3 Effect of root exudates on *A. brasilense*

For growth studies a malate liquid medium containing only 50 % of the malic acid (2.5 g/l), but supplemented with 50 ppm ammonium sulphate was used. Filter sterilized root exudates were added in 3 replicate tubes containing 4 ml of the above medium so