RESEARCH PAPER

Asian Journal of Bio Science, Vol. 4 Issue 1 : 20-23 (April to September, 2009)

Extracellular polysaccharide production by *Rhizobium* sp. nodulating *Macrotyloma uniflorum* (Lam.) Verdc

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(Accepted : January, 2009)

The ability of thirty two *Rhizobium* strains, isolated from root nodules of *Macrotyloma uniflorum* (Lam.) Verdc. were tested for their production of extracellular polysaccharides (EPS) in Yeast Extract Mannitol (YEM) medium. Among the thirty two isolates, maximum amount of EPS was produced by the isolate HGR12 (4690 µg mL^{-1}). Both growth and EPS production started simultaneously. The production of EPS was maximum in the stationary phase of growth (72 hr) when the medium was supplemented with mannitol (1%). The EPS contained glucose, galactose, xylose, rhamnose and raffinose, which were identified by paper chromatography.

Key words : Extracellular polysaccharides, *Rhizobium* sp., *Macrotyloma uniflorum*, Legume – *Rhizobium* association.

INTRODUCTION

The production of rhizobial extracellular polysaccharides (EPS) has created great interest among scientists for a long period of time. Rhizobial EPS act as determinants of host plant specificities and play a role in the initial step of root hair infection (Olivares *et al.*, 1984). EPS protects the organism from the adverse environmental condition(s).

Macrotyloma uniflorum (Lam.) Verdc. is an important pulse and green manure crop. Thirty two *Rhizobium* strains were isolated from the root nodules of *M. uniflorum* plants growing in thirty two soil samples collected from various parts in Andhra Pradesh. Very little information is available on cultural characters of rhizobia associated with this pulse crop. Hence the present work was taken up to study the factors effecting EPS production by the *Rhizobium* sp. isolated from *M. uniflorum*.

MATERIALS AND METHODS

Medium and growth conditions:

The basal medium for the bacterial growth and EPS production was the yeast extract mineral medium (Skernan, 1959) with 1% mannitol. The strains were incubated in 25 ml of the medium in 100 ml conical flasks in three replicates at 30 ± 2°C for 72 h (optimum time for maximum EPS production). The growth was measured spectrophotometrically at 610 nm.

Production of EPS on different sources:

Different carbon sources were added separately to the basal medium replacing mannitol. Individual effect of different chemicals with most suitable carbon source on EPS production was tested. For maximum EPS production by the isolate, the medium was enriched with different supplements which individually increase the EPS production to maximum level. All the supplements added to the medium were filter sterilized.

Isolation of EPS:

Isolation of EPS was done by following the method described by Dudman (1976) and collected by centrifugation, dissolved in minimum volume of distilled water re-precipitated with 3 volumes of acetone, centrifuged, dialyzed and lyophilized. For identification of sugar monomers, dry EPS was hydrolyzed in a sealed tube with 0.5M $BaCO_3$ and concentrated at 45°C under reduced pressure EPS was chromatographed on Whatman No.1 paper using butanol : acetic acid : water (4:3:1) as solvent system. Spraying reagent used for identification of sugar components was aniline pthalate (Patridge, 1949). The sugar derivatives were identified by comparison of their retention times with those of authentic standards.

Estimation of EPS:

The dialyzed cell free supernatant was used for EPS estimation by phenol-sulphuric acid method following Dubois *et al.* (1956). Uronic acid estimation in the EPS was performed by Carbazole reaction (Dische, 1947). The data were statistically analyzed using correlation coefficient between growth and EPS production.

RESULTS AND DISCUSSION

The *Rhizobium* isolates nodulating *M. uniflorum* were