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Production of indole acetic acid by *Rhizobium* sp. nodulating *Macrotyloma uniflorum* (Lam.) Verdc

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ABSTRACT

Cultural and nutritional conditions were optimized for indole acetic acid production by *Rhizobium* sp. isolated from root nodules of *Macrotyloma uniflorum* plants. The isolates produced maximum (92 and 90 mg/ml) of IAA after 144 h of incubation. The effect of different carbon and nitrogen sources on IAA production was also studied and revealed that the preferred carbon and nitrogen sources were glucose for HGR3 and xylose for HGR8 and casamino acid for these two strains. Addition of cell wall affecting agents increased the IAA production over control by the strain HGR3. Manganese sulphate, Zinc sulphate and Mercuric chloride were found to increase IAA production. In these two *Rhizobium* isolates, maximum amount of IAA was produced by HGR3. The compound from the strain HGR3 was extracted, purified and confirmed as IAA.

Key words : *Macrotyloma uniflorum, Rhizobium* species, Indole acetic acid.

Rhizobia are symbiotic partners forming nitrogen fixing nodules on legumes. These bacteria share characteristics with plant growth promoting rhizobacteria (PGPR). Nodule inducing bacteria, like other PGPR, are capable of colonizing the roots of non-legumes and produce phytohormones, siderophores and HCN. Rhizobia are the first group of bacteria, which are attributed to the ability of PGPR to release IAA that can help to promote the growth and pathogenesis in plant (Mandal *et al.*, 2007).

Macrotyloma uniflorum (Lam.) Verdc. is an important pulse crop of South India. It derives its importance for its adaptability to severe drought and environmental conditions. Very little is known about the *Rhizobium* sp. associated with root nodules of this host. Thirty two *Rhizobium* strains were isolated from the fresh healthy root nodules of *M. uniflorum* plants grown in thirty two soil samples collected from various parts in Andhra Pradesh. They were identified as *Rhizobium* sp. by morphological, cultural and biochemical characteristics. Very few studies have been carried out on IAA production by root nodule symbionts associated with this species. Two strains HGR3 and HGR8 produced high amount of IAA in YEM broth containing 0.1% L-tryptophan after 144 h. The objective of this study was, therefore, to investigate the IAA production by *Rhizobium* isolates HGR3 and HGR8 and regulation of various factors such as incubation time, along with carbon sources (0.1%), nitrogen sources (6.1%), cell wall affecting (0.1%) agents and the effect of metal chlorides and sulphates (10 mg).

MATERIALS AND METHODS

Estimation of IAA production:

The IAA in cell free supernatant was estimated by the method adapted from Gordon and Weber (1951).

Effect of incubation period was studied by inoculating *Rhizobium* isolates separately into L-tryptophan supplemented medium and incubated for 210 h at 30 ± 2^0C. The samples were withdrawn every 24 h and the growth and IAA were estimated.

Different carbon sources were also added to the tryptophan supplemented basal medium omitting mannitol. Then the different chemicals were added individually to the tryptophan supplemented basal medium having most suitable carbon and nitrogen sources. The individual effect of the chemicals on IAA production was also measured.

The *Rhizobium* sp. isolated from *M. uniflorum* was inoculated into 200 ml of YEM medium with most suitable substance and incubated at 28 ± 2^0 C for 3 days on rotary shaker. After incubation, the IAA was extracted according to the method described by Sinha and Basu (1981).

Purification and detection of IAA:

Partial purification of IAA from crude extract was done by using silica gel column chromatography (22 x 5 cm) and fractions were collected with solvent system using ethyl acetate and hexane (20 : 80 v/v). Each fraction (10-20 ml) was tested on Thin Layer Chromatography (TLC) with solvent system (ethyl acetate and hexane, 2 : 8) and then developed with Salkowski reagent (Morales *et al*., 2003).