

## Production of hydroxamate-type of siderophores by *Rhizobium* sp. isolated from root nodules of *Macrotyloma uniflorum* (Lam.) Verdc

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### ABSTRACT

Thirty two *Rhizobium* strains isolated from root nodules of *Macrotyloma uniflorum* (Lam.) Verdc were used to study their ability to produce siderophores on Chrome-Azurol S agar medium. The symbiont was able to produce hydroxamate type of siderophores in culture after 4h of incubation. Maximum siderophore production was observed after 24h. Carbon and nitrogen sources greatly influence the siderophore production by all the strains tested. Among the carbon, nitrogen sources and cell wall affecting agents lactose, glutamic acid and SDS were found to increase siderophore production. Paper Electrophoresis of the siderophore extract showed the presence of trihydroxamate type of siderophores.

**Key words :** Siderophore production, salt concentration, *Rhizobium* species, *Macrotyloma uniflorum*.

Siderophores are low molecular weight iron chelating compounds produced by microorganisms under iron stress conditions (Lankford, 1973 and Neilands, 1981). The role of these compounds is to scavenge iron from the environment. They make the mineral, which is almost essential, available to the microbial cell (Neilands, 1995). Most aerobic and facultative anaerobic microorganisms synthesize atleast one siderophore. Apart from their role in transport of Fe (III), the siderophores may act as growth factors and some are potent antibiotics. In general, siderophores are grouped as hydroxamates, catecholates and carboxylates (Neilands, 1981). Hydroxamate type of siderophores mobilize iron in neutral and alkaline soils in which other naturally occurring compounds are ineffective as iron chelators because of competition from other metal ions (Cline *et al.*, 1982). Hydromamate siderophores of the ferrichrome type are of special ecological interest because of their production by many soil organisms. Ferric hydroxamate complex is more stable and predominant in rhizosphere (O' Sullivan and Gara Feryal, 1992). Siderophore production by *Rhizobium* has been of special interest in view of the prominent role of iron enzyme at several stages of nitrogen fixation and assimilation process (Neilands, 1986). Rhizobia enhance plant growth and affect deleterious microorganisms by producing siderophores.

*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 of Andhra Pradesh. They were identified as *Rhizobium* sp. by morphological, cultural and biochemical characteristics. The aim of this study was to investigate hydroxamate type of siderophore production in culture of *Rhizobium* sp. isolated from root nodules of *M. uniflorum*, and the effect of various factors such as incubation time, carbon, nitrogen and cell wall affecting agents on siderophore production.

### MATERIALS AND METHODS

#### *Chrome- Azurol S (CAS) agar medium for detection of siderophores:*

For siderophore detection Chrome-Azurol S (CAS) agar medium (Schwyn and Neilands, 1987) devoid of iron was used. The isolate was grown on synthetic medium (with 10 mM iron and without iron) of known composition (Jadhav and Desai, 1992) for 24 h on a rotary shaker at 30±2°C. After 24h, the culture was centrifuged and the cell free supernatant was applied to CAS plates containing wells made with cork borer.

#### *Arnou's assay for detection and estimation of catechol-type of siderophores:*

Catechol- type of siderophores in the culture supernatant was detected by Arnou's assay (Arnou, 1937).