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# Production of catechol-type of siderophores by *Rhizobium* strains from *Sesbania sesban* (L.) Merr.

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Twenty six Rhizobium strains isolated from root nodules of Sesbania sesban (L.) Merr. were studied for their ability to produce siderophores. Of them, only nine strains have the ability to produce catechol-type of siderophores in culture after 4 h of incubation and at neutral pH. Mannitol and sucrose at the concentration 2% stimulated growth and siderophore production. Among nitrogen sources, arginine and proline stimulated growth and siderophore production. Maximum amount of siderophores was produced by SSR 19. 2, 3- dihydroxy benzoic acid (DHBA) was present in siderophore extract of this strain. Arginine and proline were identified as conjugated amino acids of the siderophore. The outer membrane protein profiles of the SSR 19 grown in iron containing medium revealed the presence of single protein band of molecular mass 40 K Da.

Key words: Siderophore production, 2,3-DHBA, *Sesbania sesban*, *Rhizobium* species.

## INTRODUCTION

Siderophores are low molecular weight high-affinity ferric iron chelators, synthesized and secreted by many microorganisms in iron deprivation. The compounds solubilize and bind iron and transport it back into the microbial cell, usually through specific membrane receptors (Payne, 1994). Besides microbial iron nutrition, many siderophores also play a very important role in microbial infection and the antagonism of Plant Growth Promoting Rhizobacteria (PGPR) against plant pathogens (Franza *et al.*, 2005). Basically siderophores are considered to be two types, viz., secondary hydroxamic acids and catechol type (Neilands, 1981). Further, most of the catecholates are derivatives of 2,3-dihydroxy benzoic acid (2,3-DHBA) and consists of 2,3-DHBA and one or more amino acid residues (Xie *et al*., 2006). Siderophore production by rhizobia has been studied only in a limited number of strains (Dudeja *et al.*, 1997). Hence, the present work was taken up to study the siderophore synthesizing capacity of twenty six Rhizobium strains isolated from root nodules of Sesbania sesban (L.) Merr., as it is an important character of PGPR.

## MATERIALS AND METHODS

Microorganisms and growth conditions: *Rhizobium strains* from *S. sesban* were isolated from root nodules of *Sesbania sesban*, collected from different regions in Andhra Pradesh, India. The identity of the strains as *Rhizobium* was confirmed by plant infection test (Vincent, 1970). The isolated strains were designated as SSR 1 to SSR 26 (SSR for *S. sesban Rhizobium*). A representative isolate from *S. sesban* was identified as *Rhizobium radiobacter* MTCC 8917 (=*Agrobacterium radiobacter*). Since *Agrobacterium* and *Rhizobium* are still treated as separate genera in Bergey's Manual of Systematic Bacteriology, we used the term *Rhizobium* sp. with strain numbers as 1 to 26.

For siderophore production, a synthetic medium of known composition as given by Jadhav and Desai (1992) was used.

*Arnow's assay:*

Catechol-type of siderophores was detected and estimated in culture supernatant by Arnow's assay(Arnow, 1937).

*Atkin's assay:*

Hydroxamate-type of siderophores were detected and estimated in culture supernatant by Atkin's assay (Atkin *et al.*, 1970).

*Optimization of cultural conditions*

*Siderophore production as a function of time:*

The *Rhizobium* strains were separately grown in a basal medium with constant shaking on rotary shaker (120 rpm)

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