

Original Research Article

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Siderophore Production by Rhizobia Isolated from Cluster Bean [*Cyamopsis tetragonoloba* (L.) Taub.] Growing in Semi-Arid Regions of Haryana, India

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ABSTRACT

Keywords

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A total of 13 *Rhizobium* strains were isolated from the root nodules of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] plant which was collected from 10 different villages of three districts (Bhiwani, Hisar, and Mahendergarh) of Haryana state. On the basis of morphological analysis, they were recognized as rhizobia. All the *Rhizobium* strains isolated from root nodules of cluster bean plant were studied for its ability to produce chelating molecule. On Chrome-Azyrol S agar medium *Rhizobium* is able to produce siderophore after 5 days of incubation. Maximum siderophore production observed after 7 days of incubation.

Introduction

Iron is a crucial nutrient required at a number of stages in rhizobial and plant growth, including biological nitrogen fixation (Robson and Postgate, 1980; Souza *et al.*, 2015). It exists in aerobic soil and water environment in the Fe³⁺ state, mostly insoluble at neutral pH (Neilands, 1981; Chaiharn *et al.*, 2008). To solubilize and confiscate iron, most of the microorganisms produce extracellular, low molar-mass iron transport compounds called siderophores (Neilands, 1981). Siderophores are broadly grouped into two main categories, viz. phenolates and hydroxamates. Rhizobia

infecting chickpea, mungbean, pigeonpea, clusterbean, peanut and Acacia are known to produce species-specific siderophores of hydroxamate, catechols and organic acid type as well as some unknown types of siderophores (Dudeja *et al.*, 1997a, b). Biological control of plant pathogens and deleterious microbes through production of antibiotics and siderophores or through competition for nutrients and space can significantly improve nutrient uptake and promote growth by increasing seedling emergence, vigor and yield (Chaiharn *et al.*, 2008). Symbiotic relationship undergoes in leguminous plant with root nodule bacteria. The relationship is iron dependent, nodule

formation require iron as well as nitrogenase system and leghaemoglobin for nitrogen fixation (Raychaudhuri *et al.*, 2005). The aerobic atmosphere caused surface iron to oxidize to insoluble oxyhydroxide. Polymer and reduced the level of free iron, hence rhizobia choose the way for iron uptake by producing iron chelating molecule known as siderophore. The compound is secreted by rhizobia solubilize and bind iron and transport back into microbial cell (Payne, 1994). Deficiency of iron has been reported to limit nodule development, leghemoglobin contents, nodule biomass and nitrogenase activity (O'Hara *et al.*, 1988) indicating relationship between siderophore production by rhizobia and effectiveness of N₂ fixation. Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) is an economically important *kharif* grain legume crop of Indian subcontinent but it nodulates poorly in the Northern region of India. The efficient siderophore producing rhizobia that nodulating cluster bean is yet an area of research. Therefore, different *Rhizobium* strains infecting cluster bean were screened for the presence of iron chelator siderophores.

Materials and Methods

The present study was conducted at the Department of Microbiology, CCS Haryana Agricultural University, Haryana, India. A total 10 soil samples were collected from Bhiwani, Hisar and Mahendergarh districts of Haryana. Cluster bean plants were carefully uprooted and the root system was rinsed in running water to remove adhering soil particles. The healthy, unbroken, firm and preferably pink nodules were selected and washed in water several times They were then surface sterilized by using 0.1% HgCl₂ followed by 70% ethanol for 30 second. Thereafter nodules were crushed in a sterilized petri dish and a loopful of nodule sap was streaked on yeast extract mannitol agar (YEMA) medium plates containing congo-red dye.

The plates were incubated at 28±2°C for 2-3days. The colorless/whitish gummy colonies were picked up from the plates and were streaked for purification purpose. Single rhizobial clones were picked up from the plates and maintained on YEMA slants. The identity of the cultures was established by morphologically.

Detection of rhizobial isolates for siderophore production

Siderophore production was detected by CAS (Chrome Azurol S) assay (Modified method of Schwyn and Neilands, 1987). Five µl of each log phase grown cluster bean rhizobial culture was spotted on the CAS plates and incubated at 30°C for 5-7 days. The presence of iron chelator (siderophore) is indicated by the decolourization of the blue-coloured ferric dye complex, resulting in yellow halo zones around the colonies.

Results and Discussion

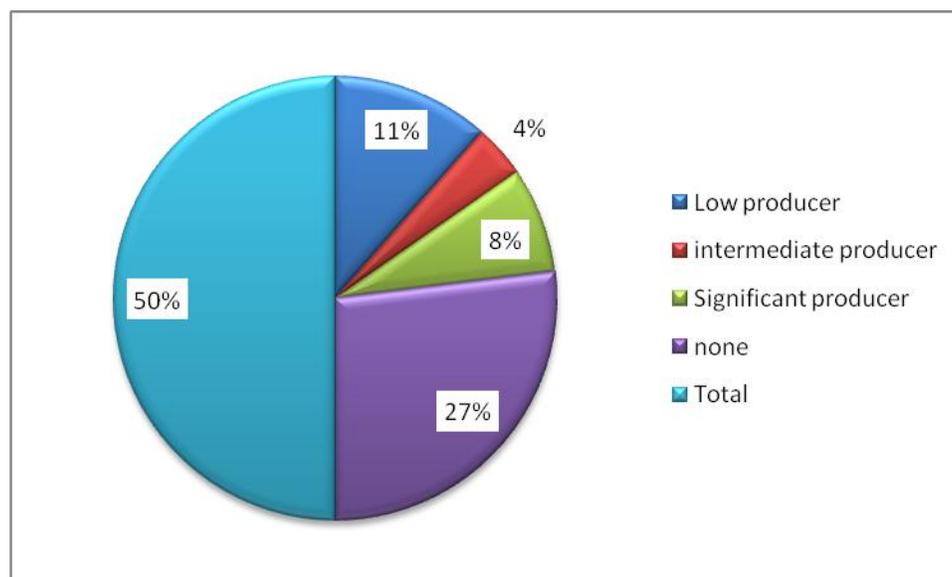
In the present study, a total of 13 *Rhizobium* strains were isolated from mature healthy root nodules of cluster bean crop being grown in different semi-arid parts of Haryana. Microscopically they all were found as Gram -ve small rods and confirmed them as rhizobia. All the 13 *Rhizobium* strains infecting cluster bean were screened for siderophore production using CAS agar plates. The results showed that of 13 strains only 6 showed siderophore production by changing the blue color to orange and formed halos (Table 1). All the studied rhizobia were classified into 3 categories for siderophore production: low producers (+), intermediate producers (++) and significant producers (+++). Twenty three percent of the rhizobial isolates were found to be significant IAA producers, 7% intermediate producers, 23% low producers whereas 53% did not produce siderophore (Fig. 1).

Table.1 Details of soil sample collection sites along with nomenclature of rhizobia and siderophore production by rhizobia

Sr. No.	Rhizobia isolates	Village	Districts	Siderophore production
1	GB-2a	Bhagi	Bhiwani	+++
2	GB-2c	Bhagi	Bhiwani	+
3	GB-8b	Swarh	Bhiwani	-
4	GB-10a	Achina	Bhiwani	-
5	GB-10d	Achina	Bhiwani	+++
6	GB-14b	Morwala	Bhiwani	+
7	GB-16b	Bilawal	Bhiwani	-
8	GH-1b	Dobhi	Hisar	-
9	GH-2a	Siswala	Hisar	+
10	GM-2b	Sighara	Mahendergarh	-
11	GM-3a	Sehalang	Mahendergarh	++
12	GM-14a	Sisod	Mahendergarh	-
13	GM-15a	Sisod	Mahendergarh	-

(+ = Poor, ++ = Moderate, +++ = Good, - = No siderophore production)

Fig.1 Siderophore production by rhizobial isolates



The potential siderophore producers were GB-1a and GB-10d (Table 1). The production amount varied significantly among these rhizobial isolates. The size of halos varied from strain to strain. In one strain no halo formation with change in color was observed. Similarly, using CAS screening method different *Bradyrhizobium* and *Rhizobium*

strains, viz. *R. meliloti* (Schwyn and Neilands 1987); *Rhizobium* sp. infecting mungbean, chickpea, *Medicago* and clusterbean (Suneja *et al.*, 1992, 1994) and *B. japonicum* (Guerinot *et al.*, 1990) were reported to be siderophore-positive. However, using the same CAS assay method *R. meliloti*, *R. leguminosarum* (Reigh and O'Connell, 1988)

and *R. leguminosarum* bv. *trifolii* (Ames-Gottfred *et al.*, 1989; Reigh and O'Connell, 1988) were found to be siderophore-negative. In this study also, there is a significant difference in siderophore production from rhizobia belonging to different districts of Haryana. Mostly high siderophore producers belong to district Bhiwani whereas, low siderophore producers mainly belong to district Hisar and Mahendergarh. From this study, it is concluded that *Rhizobium* strains differ significantly in terms of siderophore production. There is variation in siderophore production with location also. The most efficient siderophore producing *Rhizobium* strains belonged to Bhiwani district of Haryana which may be exploited as bio-fertilizer for these areas.

We successfully isolated rhizobia from root nodules of cluster bean and grown on specific medium YEMA. It is tested for siderophore production capacity by using CAS agar assay and found to be forming orange to yellow color halo ground the well. A total of six rhizobial isolates were identified as siderophore producing rhizobia among which two efficient significant producing rhizobia belongs to Bhiwani district of Haryana. It is concluded that presence of such growth promoting rhizobia accountable for the beneficial effects on cluster bean growth and yield.

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