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INFLUENCE OF CARBON ON PRODUCTION OF CATECHOL-TYPE OF SIDEROPHORE

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ABSTRACT – Iron is present for metabolic activity but is not present in soluble form under aerobic and neutral pH. microorganisms have efficient high affinity iron uptake system, to fulfil their requirements. *Bradyrhizobium* strains were isolated from the nodule of pea nut plant (*Arachis hypogaea* L.) and characterized according to Bergy's Manual of Determinative Bacteriology. Strains were screened for siderophore production. Effect of different carbon such as mannitol, lactose, glucose, maltose, sucrose on siderophore was analyzed. Out of 15, only six *Bradyrhizobium* strains BRP-1, BRP-3, BRP-4, BRP-8, BRP-11, BRP-15 showed change in colour i.e. orange halo around colony in Chrome-azurol S (CAS) assay medium (CAS) i.e. siderophore positive. All strains were produced maximum peak at wavelength at 510nm which showed that strains produced catechol type siderophore. All strains produced the siderophore in medium containing different carbon source mannitol, lactose, sucrose, glucose, maltose ranging from 12-31 µg ml^{-1}. BRP-1 showed maximum siderophore production (31 µg ml^{-1}) in mannitol.

Kay words **:** *Bradyrhizobium*, siderophore, catechol, carbon.

INTRODUCTION

Although iron is the fourth most abundant element on the earth, it is mainly present in its oxidized state mentioned by Guerinot (1991). At pH 7.0, free available iron at a concentration of no more than 10^{-17} M, which is far below that required for microbial and plant growth, was reported by Shenker *et al* (1995). Neilands ((1981) mentioned that micro-organisms have high affinity iron uptake system, to fulfill their requirements and in this process siderophore that is low molecular weight iron (III) chelating agents, are synthesized.

Iron is very essential for the growth of plant. If iron is not soluble form or in very low concentration then plant cannot utilize it. Microorganism can chelate iron as siderophore. Rroco *et al* (2003) reported that Fe acquisition and growth of rape (*Brassica napus*) when the plant was grown in sterile soil but normal growth could be restored by adding Fe-EDHA to the sterile soil or spray EDTA-Fe to the leaves.

Rhizobia are known to produce a wide variety of siderophores (Deshwal *et al*, 2003a). Microbial siderophore may stimulate plant growth directly by increase the availability of iron rhizosphere (Marek-Kozarczuk *et al*, 1996). Siderophore producing *Rhizobium* and *Bradyrhizobium* strains considered as a potential way to improve production of leguminous plants (Deshwal *et al*, 2003a; Deshwal and Vig, 2010). These reports suggest that rhizobial strains produce siderophore. Reports suggested the importance of carbon in siderophore production (Mahmoud and Abd-Alla, 2001; Sridevi *et al*, 2008). So, aim of present study was to investigate screening of the siderophores producing *Rhizobium* strains and evaluate the effect of carbon on siderophore production.

MATERIALS AND METHODS

Isolation of *Rhizobium* strains, *Bradyhizobium* strains were isolated from nodules of peanut plant (*Arachis hypogeae*) by standard microbiological techniques (Deshwal *et al*, 2003b). Isolated strains were purified by streaking plate method and pure culture of *Rhizobium* strains were maintained on yeast extract mannitol agar (YEMA) at 4°C.

Characterization of *Bradyrhizobium* strains, Pure culture of *Bradyrhizobium* strains were characterized on the basis of gram staining and specific biochemicals tests done as per guidelines of Bergey's Manual of Determinative Bacteriology (Holt *et al*, 1994).

Glassware preparation, To remove iron from glassware, all the glassware was cleaned in 20% HCL to remove iron and rinsed in deionised water.

Siderophore production, *Bradyrhizobium* strains were screened on the basis of siderophore production by two different methods.

(i) Screen method, Siderophore production by *Bradyrhizobium strains* was tested by using chrome-

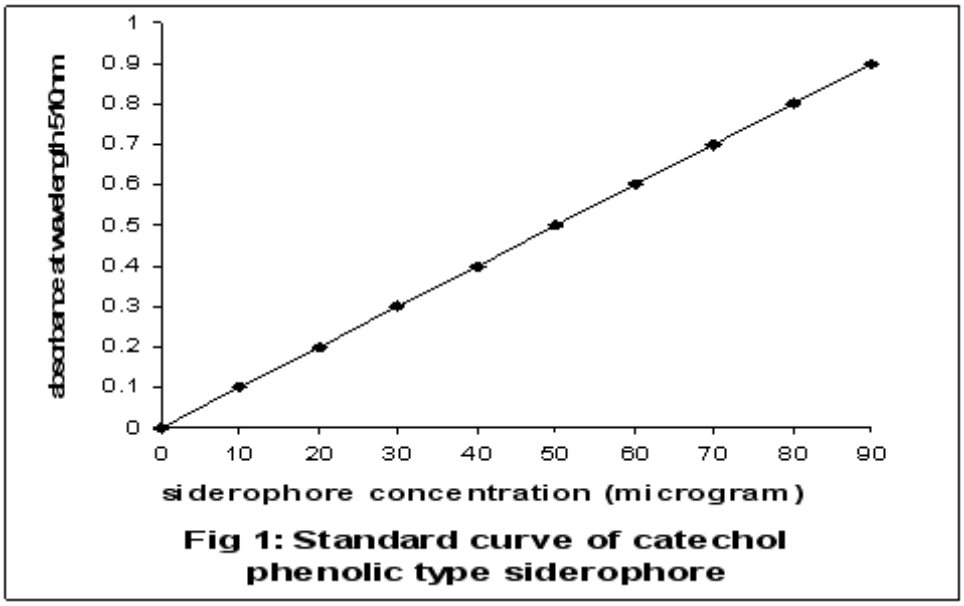

Fig 1: Standard curve of catechol phenolic type siderophore

azurol S (CAS) assay medium (Schwyn and Neliands, 1987). *Bradyrhizobium* strains were spread over YEM agar and incubated at 28 ± 1 ^{0}C for 48-72 h. Thereafter, a thin layer of CAS reagent in 0.7% agar was spread over the colonies of *Bradyrhizobium* and plates were re-incubated at 30^{0}C for 24 h. Observe change in colour blue to orange confirm siderophore.

(ii) Qualitative and quantitative analysis of siderophore, The bacterial culture filtrate (48 h) was used to determined the presence of catechol and hydroxamate type siderophores respectively by Arnow (1937); Gibson and Magrath (1969).

Qualitative analysis of catechol type siderophores

Catechol type siderophore in culture supernatant was detected at 510 nm by phenolic acid assay. The standard was prepared with 2,3 dihydroxy benzoic acid (2,3 DHBA). One unit of activity was g of 2,3 DHBA equivalent per ml (Arnow *et al*, 1937).

Effect of different carbon source on siderophore production

YEM broth was prepared and pH of medium adjusted at pH 7 with buffer solution. Different type of carbon sources viz. lactose, sucrose, glucose and maltose were added separately in place of equal quantity of mannitol (1g) in YEM broth. Similar, yeast extract was served as nitrogen source. One loop culture of individual *Bradyrhizobium* strain was transferred separately in the medium amended with different carbon sources. YEM broth containing mannitol served as control medium. The growth and siderophore production was calculated by spectrophotometric method.

RESULTS AND DISCUSSION

Bradyrhizobium strains were isolated from the nodules of peanut plant (*Arachis hypogaea* L.). Fifteen pure culture of *Bradyrhizobium* strains were characterized according to the Bergey's mannual of determinative bacteriology (Holt *et al*, 1994). On the basis of the biochemical tests, it confirmed that isolated strains were *Bradyrhizobium*.

Table 1 : Siderophore production by *Rhizobium* strains.

***Bradyrhizobium* strain**	**Siderophore production**	
	CAS agar medium	**Arnow method**
Bradyrhizobium BRP-1	+	+
Bradyrhizobium BRP-2	-	-
Bradyrhizobium BRP -3	+	+
Bradyrhizobium BRP -4	+	+
Bradyrhizobium BRP -5	-	-
Bradyrhizobium BRP -6	-	-
Bradyrhizobium BRP -7	-	-
Bradyrhizobium BRP -8	+	+
Bradyrhizobium BRP -9	-	-
Bradyrhizobium BRP -10	-	-
Bradyrhizobium BRP -11	+	+
Bradyrhizobium BRP -12	-	-
Bradyrhizobium BRP -13	-	-
Bradyrhizobium BRP -14	-	-
Bradyrhizobium BRP -15	+	+

- = negative; + = positive

Table 2 : Effect of carbon on siderophore production (µg ml^{-1}) by *Bradyrhizobium* strains

Bradyrhizobium Strains	Carbon source (Siderophore conc, µg ml^{-1})				
	Mannitol	Lactose	Sucrose	Glucose	Maltose
BRP-1	31	28	17	29	28
BRP-3	27	24	12	28	29
BRP-4	29	26	18	27	28
BRP-8	25	25	12	28	27
BRP-11	27	23	15	28	26
BRP-15	29	22	16	29	27

Values are mean of five replicates.

Siderophore producing strains showed orange halo around the colony and only 6 *Bradyrhizobium* strains were siderophore producer i.e. *Bradyrhizobium* BRP-1, BRP-3, BRP-4, BRP-8, BRP-11, BRP-15 (Table 1). Maximum peak observed at 510 nm when 300-700 nm range spectra analyzed as given in the guidelines (Arnow, 1937). It showed that *Bradyrhizobium* BRP-1, BRP-3, BRP-4, BRP-8, BRP-11, BRP-15 produced catechol type siderophore. Arora *et al* (2001) reported that *Rhizobium* strains produced catechol or hydroxamate type siderophore. Deshwal *et al* (2003b) was also mentioned the same information. Sridevi *et al* (2008) reported that *Rhizobium* sp. isolated from stem nodules of *Sesbania procumbens* (Roxb) produced catechol-type of siderophores. Joshi *et al* (2009) reported that rhizobial isolates belonging to genera (*Rhizobium* sp., *Mesorhizobium* sp.) produced catecholate type of siderophores.

Bradyrhizobium strains were produced different concentration of siderophore when different carbon was added in medium. All strains produced the siderophore in medium containing different carbon source mannitol, lactose, sucrose, glucose, maltose. All strains produced siderophore in range 25-31 µg ml^{-1} (mannitol), 22-28 µg ml^{-1} (lactose), 12-17 µg ml^{-1}(sucrose), 27-29 (glucose) and 26-29 µg ml^{-1} (maltose). BRP-1 showed maximum siderophore production (31 µg ml^{-1}) (Table 2). Similarly, Mahmoud and Abd-Alla (2001) observed that *Bradyrhizobium* strains produced siderophore production in mannitol, and glucose containing medium. Sayyed *et al* (2005) mentioned the importance of nutrient on siderophore production. Sridevi *et al* (2008) isolated the *Rhizobium* sp. isolated from stem nodules of *Sesbania procumbens* (Roxb.) and suggested that siderophore production was optimum in presence of mannitol.

Our results suggested that isolated *Bradyrhizobium* BRP-1, BRP-3, BRP-4, BRP-8, BRP-11, BRP-15 produced catechol type siderophore and various carbons such as mannitol, lactose, glucose, maltose, sucrose (least siderophore) enhanced siderophore production. It is also indicate that presence of carbon source in soil may affect the siderophore production.

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