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Siderophore Production by *Rhizobium nepotum* isolated from "Stem nodule of *Aeschynomene indica*"

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Abstract

Iron stress is one of the reasons for bacteria to produce iron chelating molecule known as siderophores. The *Rhizobium nepotum* (LT560376) isolated from stem nodules of *Aeschynomene indica* was studied for its ability to produce chelating molecule. On Chrome-Azyrol S agar medium *Rhizobium nepotum* is able to produce siderophore after 4 hours of incubation. Maximum siderophore production observed after 48 hours incubation.

Keywords: *Aeschynomene*, siderphore, Rhizobium nepotum, CAS agar.

Introduction

Symbiotic relationship undergoes in leguminous plant with respective root and stem module bacteria. The relationship is iron dependent, nodule formation require iron as well as nitrogenase system and leghaemoglobin for nitrogen fixation (Raychaudhuri etal, 2005).

Aerobic atmosphere caused surface iron to oxidiz to insoluble oxyhydroxide Polymer and reduced the level of free iron, hence bacteria choose the way for iron uptake by producing iron chelating molecule known as siderophore.

The compound is secreted by bacteria solublize and bind iron and transport back into microbial cell (Payne, 1994)

Aeschynomene indica is a weed legume growing in waterlogged soil in Ratnagiri Konkan region of Maharashtra. It possesses stem nodules in addition to root nodule.

The cultural, morphological and biochemical characteristics of *Rhizobium* were determined and identified based on Bergey's Manual of Systematic bacteriology. In addition to bio-chemical analysis, *Rhizobium* subjected to partial sequencing of 16S ribosomal RNA gene and found to *Rhizobium nepotum* (LT 560376).

The aim of this study is to investigate Siderphore production by *Rhizobium nepotum* isolated from stem nodules of *Aeschynomene indica*. Detection of Siderophores is done by using iron limited media (Neilands J.B.1995). Most of siderphore is either hydroxamates or catechols (Guan L.L.Kgnoh K and Kamino K, 2001) (Xie et. al 2006).

The Chrome Azurol Sulfonate (CAS) assay widely used to detect the siderphore producing capacity of bacteria (Louden B.C.Hoarmann D and Lynne A.M. 2011)

Materials and Methods

Isolation of *Rhizobia*

The *Rhizobia* is isolated from free healthy stem modules of *Aeschynomene indica* plants, which grow in waterlogged condition and collected from Shivaji University Campus. Plants were uprooted with intact root system and brought into laboratory for isolation of *Rhizobia*, using CRYEMA medium. Identification of *Rhizobia* was carried out on the basis of morphological cultural and biochemical characteristics on CRYEMA by using standard methods (Holt etal, 1994) and 16S ribosomal RNA sequencing for siderophore producing capacity is detected by using Chrome Azurol Sulfonate agar (CAS) medium.

Identification of *Rhizobium*

Identification of *Rhizobial* traits evaluated on the basis of colony characteristic Gram staining and motility and utilization of carbohydrate.

Confirmation of *Rhizobium* was done by using confirmatory test, Congo Red Dye Absorption Test (Skinner & Lovelock 1979) Nile Blue Reduction Test (Bergersen 1980), Ketolactose test (Subba Rao 2006) and Growth on Glucose Peptone Agar (skinner & Lovelock 1979) to confirm isolates is *Rhizobium*.

Table 1 : Results of confirmation test of *Rhizobium*

Sr. No.	Confirmatory test	Results
1	Congo Red dye Absorption	-
2	Nile Blue Reduction	-
3	Ketolactose	-
4	Growth on Glucose peptone	-

Table 2 : Results of Biochemical tests of *Rhizobium*

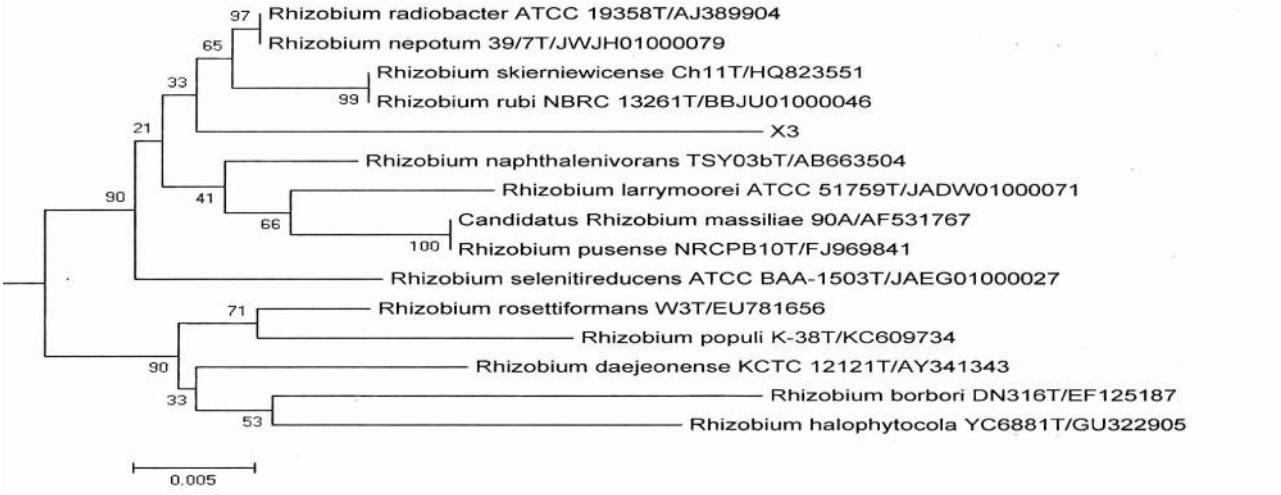
Sr. No	Test	Results
1	Ala-Phe-Pro Arylamidase	-
2	Adonitol	+
3	L-Arabitol	+
4	D-Cellobiose	-
5	Beta-Galactosidase	-
6	H_2S Production	-
7	Beta-N-Acetyle Glucosyminidase	+
8	D-Glucose	
9	Gamma-Gluamyl-Transferase	-
10	Fermentation of Glucose	-
11	D-Maltose	-
12	D-Mannitol	-
13	D-Mannose	-
14	Beta-Xylosidase	-
15	Lipase	-
16	Urease	+
17	D-Sorbitol	-
18	D-Trehalose	-
19	Citrate	-
20	Malonate	-
21	Alpha-Glucosidase	+
22	Succinate alkalization	-
23	Phosphatase	-
24	Ornithine Decarboxilase	-
25	Lysten Decarboxylase	-
26	L-Histidine assimahation	-
27	L-Malate assimilation	-
28	L-Lactose assimilation	-

Taxonomical characterization of *Rhizobium* using 16S ribosomal RNA Sequence

>X3

GCCAATGCCGCGTGAGTGATGAATGCCTTAGGGTTGTAAAGCTCTTTCACCGGAGAAGATAATGACGGTATCCGG
AGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGAATTACTG
GGCGTAAAGCGCACGTAGGCGGATATTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGAT
ACTGGGTATCTTGAGTATGGAAGAGGTAAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTACATATTCGGAGGA
ACACCAGTGGCGAAGGCGGCTTACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTAGCCGTCGGGCAGTATACTGTTCGGTGGCGCAGCTAA
CGCATTAAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGC
GGTGGAGCATGTGGTTTAATTCGAAGCACCGCGGCAGAACCTTACCAGCTCTTGACATTCGGGGTATGGGCATTN
GAGACGATGTCCTTCAGTTAGGCTGGCCCCAGAACAGGTGCCTGCATGGCTGTCCTCAGCTCGTGTCCTGGAAAT
GTTGGGATTAAGTCCCGCAACGAGCGCAACCCCTCCCCCCTTAATTTGCCAGCATTNATTTGGGCCCTCTAAGGGG
ACTGCCGGT

Strain Designation	Closest phylogenetic affiliation	Max ident
X3	*Rhizobium nepotum* 39/7 (T) 16S ribosomal RNA gene partial sequence (JWJH01000079)	97.59%

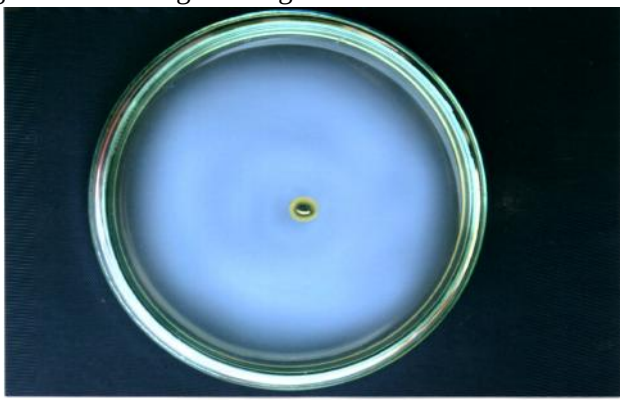


From above morphological & biochemical and phylogenic studies the isolate identified as *Rhizobium nepotum* (LT560376)

Detection of Siderophores

For Siderophore detection Chrome Azurol Sulfonate (CAS) agar medium (Schwyn and Neilands, 1987) was used.

The *Rhizobium neoptum* is grown on CRYEMA medium plate. Growth of organism centrifuged along with saline at 5000 rpm for 15 minutes. The supernatant was collected and used for CAS assay. For CAS assay cell free supernatant was applied to CAS plates containing wells. The plate was incubated at 28 $\pm$ 2 ^{0}C for 48 hrs and examined for growth and production of yellow halos surrounding the colonies.



Results and Discussion

Based on morphological, cultural and biochemical and phylogenic characteristic (16S ribosomal RNA) the isolate was identified as *Rhizobium nepotum*. When the supernatant of the culture *Rhizobium nepotum* was applied to the wells of CAS plates, orange to yellow color halo was produced around the well, indicating the *Rhizobium nepotum* produces siderophores. For confirmation, a halo was observed and the supernatant of *Rhizobium nepotum* is grown in iron restricted media and under high iron conditions creating no color change. CAS assay does not indicate the type of siderphore being produced.

Conclusion

We successfully isolated *Rhizobium Nepotum* from stem nodule of *Aeschynomene indica* plant and grown on specific medium CRYEMA. It is tested for siderphore production capacity by using CAS agar assay and found to be forming orange to yellow color halo ground the well.

For further detection of type of siderophore Arnows and Atkin's assay is to be carried out.

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