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Isolation and characterization of endosymbiont of *Pithecellobium dulce (*Benth)

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Abstract
Legumes are the macro symbionts of bacteria, the microsymbiont that form root nodules and fix atmospheric nitrogen indirectly increasing the crop yield *Pithecellobium dulce* in a N_2 fixing, root nodulating, multipurpose tree legume. In this present investigation, the nodules were isolated from *P.dulce* at different localities. These isolates were characterized by carrying out a number of standard morphological, ecological and biological tests and compared with Bergey's manual of determination bacteriology. The results revealed that the nodulating bacteria of *P. dulce* was confirmed as the geneus Rhizobium and belongs to the *Rhizobium spp*., cowpea group.

Keywords: *Pithecellobium dulce,* rhizobial isolates, Agar *(YEMA)* medium

1. Introduction
Legumes are the macrosymbionts of bacteria, the microsymbionts that from root nodules and fix atmospheric nitrogen indirectly increasing the crop yield. The oldest agricultural records available indicate that leguminous trees have been cultivated for centuries and they were valued for fuel, fodder and soil enrichment. Long before, their ability to work symbiotically with bacteria was understood [6]. The endosymbiotic association reduces the dependency of agricultural crops on fossil fuel-derived nitrogenous fertilizers.
The high cost of fertilizers, rapid depletion of non-renewable energy sources, release of pollutants during fertilizer production, disruption of nutrient balance in the soil, leaching of nutrient into ground water and pollution in surface water has emphasized the need of characterization to increase productivity in legumes. Growing concerns about the environment, energy nutrition and agriculture and forestry sustainability make the need for biological nitrogen fixation research even more compelling.
Among the leguminous trees, *Pithecellobium dulce* Benth, are nitrogen fixing, root nodulating, multipurpose tree legumes, widely recognized throughout the world for its potential in a forestation and rehabilitation of wastelands especially in tropical and subtropical regions of Tamil Nadu.
The perusal of the literature proved that there had been no extensive works on the legumes, especially in relation to the nodulation and taxonomy of rhizobia [16]. Keeping these points in view the present investigation is aimed in studying the characterization and identification of endosymbiont of *Pithecellobium dulce*.

2. Materials and Methods
2.1 Study Material
Pithecellobium dulce Benth. Belongs to the sub-family Mimosoideae of Leguminosae. It produces club shaped, elongated nodules on the roots of the seedlings and trees.

2.2 Morphology of Nodules
Nodules were collected from *P. dulce* trees at three different localities, by using standard techniques [15]. Their morphometric characteristics such as size, shape, colour, nodule number per plant and nodule weight were studied. In addition nitrogenic activity [13]. Leghaemoglobin content of the nodules were also estimated.

2.3 Isolation of Rhizobium
Nodules were collected from the roots of *P. dulce* and surface sterilized by using standard Techniques [15]. These sterilized root nodules were crushed simply with pestle and mortar. The extract was serially diluted with distilled water and inoculated into a Yeast Extract mannitol

Agar (YEMA) medium. The *Rhizobium* was confirmed by Congo red test, Hofer alkaline test and staining of poly ß-hydroxy butyrate test [10] and mass cultured.

2.4 Characterization studies on *Rhizobium*

The size and shape of the cells of rhizobia were studied by using micrometric and gram's staining techniques.

Effect of temperature, pH and salinity tolerance on the growth of rhizobial isolates of

P. dulce also carried out by using standard techniques.

The biochemical tests were conducted by the methods of [9] as described by [1] to identify the bacteria.

3. Results and Discussion

The endosymbionts of *P. dulce* was studied in detail to characterize, classify and establish their ability as biofertilizer.

It has been well established that the nodules developed on the roots of the legume host showed nodule diversity in the shape, size, number and distribution which is often influenced by the host and the associated *rhizobium* [2, 3]. The seedlings of *P. dulce* showed profusely branched roots with yellowish to brown colour nodules and were elongated and palmately branched [14] reported that the red colour of the nodules is due to the development of the red pigment called Leghaemoglobin. [4, 8] surveyed many strains of rhizobia and used many cultural tests, pointed out that the rhizobia typically show slow growth on peptone glucose agar, from little or no H2S from bismuth sulphite and give no precipitate in glycerol phosphate agar. In the present study, the bacteria isolated from the nodules of *P. dulce* grew poorly on peptone glucose agar, but grew well on YEMA in the form of white or watery colonies within two days, which clearly indicated that the isolated bacterium could possibly be the fast growing *Rhizobium*.

They produced no H2S from bismuth sulphite and gave no precipitate in glycerophosphate agar indicating the presence of *Rhizobium*. However, *Rhizobium* can easily be distinguished from *Agrobacterium* by not absorbing congo red from YEMA containing cong red, unable to grow in Hofer's alkaline broth with high pH (11) in which *Agrobacterium* can grow and not able to utilize [7, 5]. In the present investigation, the isolates of *P.dulce* failed to absorb Congo red from YEMA medium, able to grow in Hofer's alkaline broth with high pH (11) and did not utilize lactose from the medium. Moreover the isolates grow well in temperature ranging from 26^0 to $30^0 \pm 2^0$ which is conducive for *Rhizobium*.

Based on the above discussion, it can be concluded that isolates of *P.dulce*, there by lending support to the earlier investigations [12].

Table 1: Morphological Characteristics of rhizobial isolates of *P.dulce*

S. No	Morphological Characters	Rhizobial isolates of *P.dulce*
1	Colony form	Filliform
2	Colony size	Moderate
3	Colony diameter	2-3 mm
4	Colony shape	Circular
5	Colony colour	Milky white colony
6	Colony elevation	Convex
7	Colony margin	Entire
8	Optical characteristics of colony	Translucent
9	Cell shape	Rod
10	Cell length (µm)	2 to 3
11	Cell diameter (µm)	1 to 5
12	Motility	+
13	Gram's reaction	Gram negative
14	Poly –ß hydroxy butyrate	+

Table 2: Biochemical characteristics of rhizobial isolates of *P.dulce*

S. No	Biochemical Test	Rhizobial isolates of *P.dulce*
1	Mac conkey test	+
2	Indole	+
3	Voger proskaur	-
4	Citrate utilization	+
5	Methyl red	-
6	Triple sugar iron	-
7	Carbohydrate fermentation	+
8	Gelatin Hydrolysis	+
9	Starch	+
10	Urea hydrolysis	+
11	H2S production	+
12	Cytochrome oxidation	+
13	Catalse	+
14	Oxidase	-
15	Nitrate reduction	+

+ Positive result - Negative result

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