

Morphological, Cultural and Biochemical Characteristics of *Rhizobium Meliloti* of Alfalfa (*Medicago sativa* L.)

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Abstract:

Rhizobium meliloti was isolated from root nodules of Alfalfa (*Medicago sativa* L.) on yeast extract mannitol agar (YEMA) medium and its morphological, cultural and biochemical characteristics were studied. It was observed that the colonies were circular, convex, whitish pink and glistening with entire margin. The bacterium was gram negative, rod shaped, aerobic, non-spore forming and motile. It showed negative chemical reaction for indole, methyl red, Voges-Proskauer. While it showed positive reaction for citrate utilization and catalase test.

Key words: Alfalfa, *Rhizobium meliloti*, root nodules.

1. Introduction:

Nitrogen is the most limiting nutrient for growth of leguminous plants like Common beans, Soya beans, Cow peas and Garden peas because that present in the soil cannot support growth (Howieson et. a., 2007). Nitrogen is essential in plant cells for synthesis of enzymes, proteins, chlorophyll, DNA and RNA, thus essential for plant growth and production of food and feed (Matiru et. al., 2004). Rhizobia can live on plant residues (saprophytes) or entirely within plants (endophytes) or (rhizobacteria) or in close association with the plant roots (Mohammadi, K. and Sohrabi, Y., 2012, Anwer, M.A., 2013). Legumes have been suggested as appropriate crops for the enhancement of bio productivity and improvement of marginal lands, because these plants not only yield good fodder, protein rich seeds and fruits, but they also enrich soil nitrogen in symbiotic association with *Rhizobium*. Sprent, (2001) studied that among plant-microbe interactions, legumerhizobium interactions are unique because they supply 80-90% of total nitrogen requirements of legumes. It involves a complex interaction among host, microbialsymbiont and environment.

2. Material and Methods:

The mature root nodules from Alfalfa plant were collected from experimental pots washed thoroughly under tap water and surface sterilized with 0.1% mercuric chloride. Surface sterilized root nodules were crushed in small quantity of sterile distilled water. Rhizobial suspension was inoculated on pre sterilized YEMA medium and incubated at 26 ±30 C temperature for 24-48 hrs. The isolates were maintained on slopes of YEMA medium as described by Graham and Parker (1964). These isolates were used to study the morphological, cultural and biochemical characteristics. Five days old culture was used for Gram's reaction, morphology and colony characters.

Production of Indole was noticed in inoculated tryptophan broth after 7 days of incubation by adding Kovac's reagent. The reduction of methyl red and Voges-Proskauer reaction was examined in glucose

phosphate broth by adding methyl red and α -naphthol solution with KOH respectively. Citrate utilization was observed by using Simon’s citrate medium with Bromothymol blue in basal medium. The Gram’s staining technique was followed as suggested by Graham and Parker 1964. Catalase activity was observed by stirring the culture in a drop of hydrogen peroxide (10% by W/V).

Table 1: Cultural/Morphological and Staining Characters of *Rhizobium meliloti*

Characters	Result
Shape	Circular
Color/Pigmentation	Whitish pink and glistening
Elevation	Convex/ Raised
Opacity	Opaque/Semitransparent
Motility	Motile
Bacterium shape	Rod
Spore formation	Non-spore forming
Oxygen demand	Aerobic
Gram’s nature	-ve

3. Results and Discussion:

Morphological Characters:

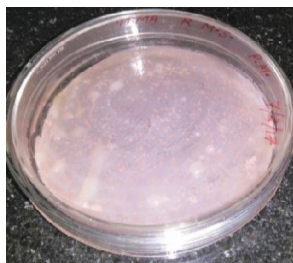
The *Rhizobium meliloti* was Gram negative, aerobic, non-spore forming and motile rods. In general, the colonies were circular, convex, whitish pink and glistening with entire margin.

Biochemical Characters

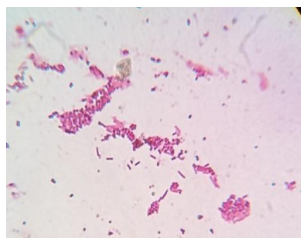
The bacterium showed well-marked growth on YEMA medium at pH 7.0. Mahana et. al.(2000) reported that the *Rhizobium* isolated from *Vigna mungo* L. showed marked Variations in growth with respect to time period on YEMA. The bacterium showed positive test for citrate and catalase activity. Our these findings are in close agreement with Elsheikh and wood (1989); Javed and Asghari(2008) who also reported characterized the rhizobium from soil and sunflower root nodules with the same positive biochemical tests. Mahana et. al. (2000) reported catalase activity in some isolates from *Vigna mungo* L. The bacterium is negative for MR-VP and indole reaction. Similarly, Shahzadet al. (2012) isolated *Rhizobium* from root nodules of Alfalfa (*Medicago sativa*) plant and characterized on the basis of various biochemical tests. Similarly, Singh et.al. (2008) also characterized *Rhizobium* strains on the basis of biochemical tests. *Rhizobium* is symbiotic bacteria which form nodule in leguminous plant.



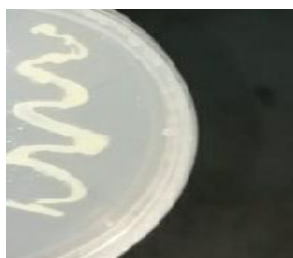
(A) Root nodules from *Medicago sativa* L.



(B) Culture Plate of *Medicago sativa* L.



C) Microscopic view of Bacteria



D) Pure Culture Plate of Bacteria

Table 2: Biochemical Characters of *Rhizobium meliloti*

S. No.	Test	Remark
1.	Production of indole from tryptophan	-Ve
2.	Methyl red test	-Ve
3.	Voges-Proskaur test	-Ve
4.	Citrate utilization as source of carbon	+Ve
5.	Catalase test	+Ve
6.	Effect of pH on growth of Rhizobium	pH 7.0
7.	Effect of temperature on growth of Rhizobium	Room temp

4. References:

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