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Morphological, Cultural and Biochemical Characteristics of Rhizobium Japonicum of Soyabean (Glycine max L.)

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ABSTRACT: Rhizobium Japonicum was isolated from root nodules of Soybean (Glycine max L.) on yeast extract mannitol agar (YEMA) medium and its morphological, cultural and biochemical characteristics were studied. Two to three days old culture grown on YEM agar plate examined for colony characters, colonies of Glycine max L, were circular, convex, whitish pink and glistering with entire margin. Slow growing strains of Glycine max L produce white, opaque, circular, granular colonies, which do not exceed 1mm in diameter after prolonged incubation. The bacterium was gram negative, rod shaped, aerobic, non-spore forming and motile. It showed negative chemical reaction for indole, methyl red, Voges-Proskaur. While it showed positive reaction for citrate utilization and catalase test.

KEYWORDS: Soybean, Rhizobium Japonicum, root nodules.

I. INTRODUCTION

Rhizobium is able to enter into symbiotic relationship with legumes. They fix atmosphere nitrogen and thus not only increase the production of the inoculated crops, but also leave a fair amount of nitrogen in the soil, which benefits the leguminous crop plants. Following groups of rhizobium have been recognized for inoculating legumes in India. R. leguminosarun, R. melilotti, R.trifoli, R.phaseoli, R.lupinli, R.japonicious etc. Rhizobium as a bacteria are a tiny and lower most components of any food chain, but these tiny members have their own importance, without these rhizobium bacteria we can't imagine legumes and nitrogen cycle in atmosphere. So it is very important especially in area where large amount of soil and air polluted, by various pollutants such as effluent, which is directly dumped in soil. Direct and indirect dumping of effluent may alter the characters of leguminous plants and bacteria present in soil and nodule of those plants.

Pribac and Ardelean (2008) reported that the most convenient method of obtaining rhizobia from nature is by isolation from root nodules. Contrary to popular belief, many of the bacteroids in nodules are viable, is impractical to isolate rhizobia directly from the soil because of their fastidious growth requirements and the presence of numerous less fastidious fast growing soil microorganisms.

II. MATERIAL AND METHODS

The mature root nodules from Soybean plant were collected from experimental pots washed thoroughly under tap water and surface sterilized with 0.1% mercuric chloride. Surface sterilized rood 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.

Biochemical characteristics of the Rhizobium isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Catalase test (Aneja 1996, 2008). The biochemical tests were carried out in medium at 28˚C for 48 hours incubation.

Indole Test - The tube containing SIM agar medium at pH 7.3 were inoculated by isolated Rhizobium japonicum bacterial culture, after 24-48 hr of inoculation on addition of Kovac's reagent, no change in color of media indicate indole negative. (Table-2).

Methyl Red - The tubes containing (MR-VP Broth) at pH 7.3 were inoculated by isolated Rhizobium japonicum bacterial culture. After 24-48 hours of incubation on addition of Methyl red reagent, change in color of the medium was observed that red color was not appeared showed MR negative (Table-2).

Voges Proskauer- The tubes containing (MR-VP Broth) at pH 7.3 was inoculated by isolated Rhizobium japonicum bacterial culture. After 24-48 hours of incubation on addition of Barritt's reagent A and B, change in color of the medium was observed and find that there was no color change it indicate VP negative (Table-2).

Citrate Utilization- The tube containing (Simmons Citrate Agar) at pH-6.9 were inoculated by Rhizobium japonicum bacterial culture. After 24-48 hours of incubation change in the color of the media was observed and result indicate citrate positive (Table-2).

Catalase Test- Tubes containing 2-3 drops of (Trypticase soya Agar) at pH- 7.3 were inoculated by 24-48 hours isolated Rhizobium meliloti bacterial culture. After few seconds on addition of 3% hydrogen peroxide observe the change on slide. Appearance of bubbles showed catalase positive (Table-2).

III. OBSERVATION AND RESULTS

Morphological Characters:
The Rhizobium japonicum was Gram negative, aerobic, non-spore forming and motile rods. In general, the colonies were circular, convex, whitish pink and glistering with entire margin.

Table 1: Cultural and Morphological Characters of Rhizobium japonicum (Glycine max L.)

Character	Result
Shape	Circular
Color/Pigmentation	Whitish pink and glistering
Elevation	Convex/Raised
Opacity	Opaque/Semitransparent
Motility	Motile
Bacterium shape	Rod
Spore formation	Non-sporeforming
Oxygendemand	Aerobic
Gram's nature	-ve

Biochemical Characters:
The bacterium showed well-marked growth on YEMA medium at pH 7.0. The bacterium was gram negative, rod shaped, aerobic, non-spore forming and motile. It showed negative chemical reaction for indole, methyl red, Voges-Proskaur. While it showed positive reaction for citrate utilization and catalase test.

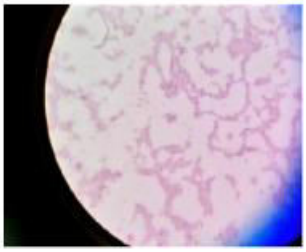
IV. DISCUSSION

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. It was clearly observed that Indole was not produced after incubation of isolated rhizobial inoculants in tryptophan broth. Similarly Methyl red and Voges-Proskauer reaction were examined in glucose phosphate broth by adding methyl red and α-napthol solution with KOH respectively report negative results. Citrate was utilized as a carbon source in Simon's citrate medium and represent as color change. Catalase activity was observed by stirring the culture in a drop of hydrogen peroxide (10% by W/V). The bacterium showed positive test for Citrate and Catalase activity. Mahana, et. al., (2000) and Maheshwari et. al., (2012) reported Catalase activity in some isolates from Vigna mungo and E.coli. The bacterium is negative for MR-VP and Indole reaction reported by Gachande and Khansole, (2011). Deka and Azad, (2006) studied the rhizobial isolates from 6 common pulses justify our results. 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. Similar morphological, physiological and biochemical characters of rhizobial isolates have been reported by Lalitha and Immanuel (2013) and Rasool et. al., (2015).

Table 2: Biochemical Characters of Rhizobium japonicum (Glycine max L.)

S.No.	Test	Remark
1.	Productionofindolefrom tryptophan	-Ve
2.	Methylred test	-Ve
3.	Voges-Proskaur test	-Ve
4.	Citrateutilizationassourceofcarbon	+Ve
5.	Catalase test	+Ve
6.	Effect of pH on growth of Rhizobium	pH7.0
7.	Effect of temperature on growth of Rhizobium	Room temp

(A)Root nodules from *Glycine max* L.



(C)Microscopic view of Bacteria

(B)Culture Plate of *Glycine max* L.

(D)Pure Culture Plate of Bacteria

Various Stages of Rhizobium Culture in Glycine max L.

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