The Study of the Cultural and Biochemical Characteristics of Endosymbionts of both Effective and Ineffective Nodule of Vignaradiata

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***Abstract*: The present study was conducted to assess the biochemical responses during both effective and ineffective nodules of Vigna Radiata. Proline content was calculated by using the acid ninhydrin method. Protein content at both stages was estimated by Bradford's method. Nodulation test with bacterial strains isolated from Acacia nodules in the field have shown that although nodules are formed on the roots. Effective nodule formed by effective strains are well developed pink in colour due to leghemoglobin and the bacteroid tissue is well as organized with plenty of bacteroids. Ineffective nodules which are generally small and contain poorly developed bacteroid tissue showing accumulation of starch in host cells. In order to compare the effective and ineffective root nodules, the root modulating tropical legumes, Vigna radiata, that produce both effective and ineffective nodules were selected. The seeds of *vigna radiata* along with *Bradyrhizobium* strains S24 (effective) and S24A06 (ineffective) were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore**

***Keywords*: VignaRadiata, Effective root nodules, Ineffective root nodules, Bradyrhizobium.**

Introduction:

Mung bean (Vigna radiata) is a warm season annual grain legume. The optimum temperature range for good production is 27-30°C (Imrie, 1998). Mung bean requires 60-75 days to mature. It is useful crop in drier areas and has a good potential for crop rotation and relay cropping with cereals using residual moisture. The mung bean was also known as green gram or golden gram and is mainly cultivated in the Indian subcontinent. Now days; it is being cultivated after harvesting of Rabi crops (wheat, mustard, lentil, etc.).

Mung bean is one of the major calories (347-k cal. food energy) and protein (19.5% to 28.5%) sources in Asia, especially for the vegetarian population. High lysine content, which makes mung bean a good complementary food for rice-based diets, is usually the first limiting amino acid (Chen et al.1987). It is a popular food among vegetarians since it contains a lot of proteins and fiber and the main advantage of mung bean is that it helps in digestion and controls the amount of cholesterol content in our body. Mung bean contains a lot of minerals like calcium and potassium which was essential for enhancing the strength of bones and teeth. Fat content in mung bean is very low so it is highly recommended for people who want to minimize fat from their body. It is a store house of nutrients and it is a nutrition giving food and they are rich in Vitamin B, vitamin C, Manganese and a lot of other essential nutrients required for effective functioning of the human health (AVRDC, 1988).. Root nodules are classified into effective and ineffective. Ineffective strains of rhizobia form ineffective nodules which are generally small and contain poorly developed bacteroid tissue showing accumulation of starch in host cells which do not contain *Rhizobium* and dextran in host cells infected by *Rhizobium*. On the other hand, effective nodules formed by effective strains are well developed, pink in colour due to leghemoglobin and the bacteroid tissue is well organized with plenty of bacteroids (Vincent, 1982; Venkataraman and Kannaiyan, 1993).

Biological Nitrogen Fixation:

Biological nitrogen fixation by leguminous green manure crops in symbiotic association with Rhizobium is a low cost input for rice crop. The cultivation of nitrogen fixing legume green manure crop would-be an efficient way of improving soil fertility. The formation of nodules in the roots is an external manifestation of the symbiotic association of a bacterium, Rhizobium, with the roots of the leguminous plants.

Rhizobium - Legume Symbiosis:

Symbiosis is a biological phenomenon involving dynamic changes in the genome, metabolism and signalling network. A multidirectional comprehension of these interactions is required when studying symbiotic organisms. In plant–microbe interactions, two symbiotic systems have been actively studied for many years (Kistner and Parniske, 2002; Bonfante and Genre, 2010).

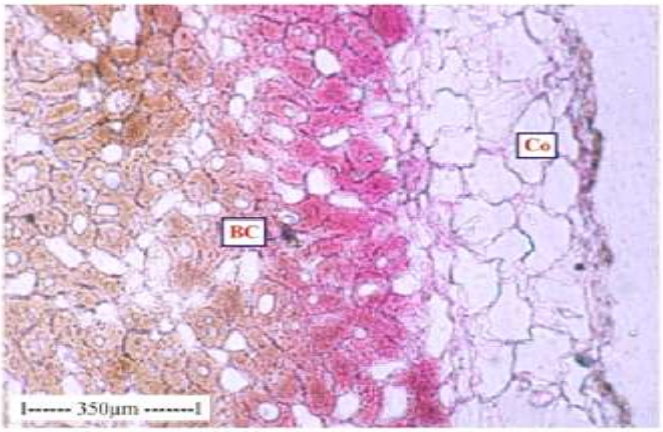
Rhizobia have two different life-styles, either as free-living soil bacteria or as nitrogen-fixing endosymbionts within root nodules of legume host plants. In a well-balanced physiological interaction, the microsymbiont fixes atmospheric nitrogen and provides ammonia as a nitrogen source to the plant in exchange for a carbon and energy source generated by photosynthesis (Udvardi and Day, 1997; Djordjevic et al., 2000; Long, 2001; Prell and Poole, 2006; Pessi et al., 2007).

MATERIALS AND METHODS

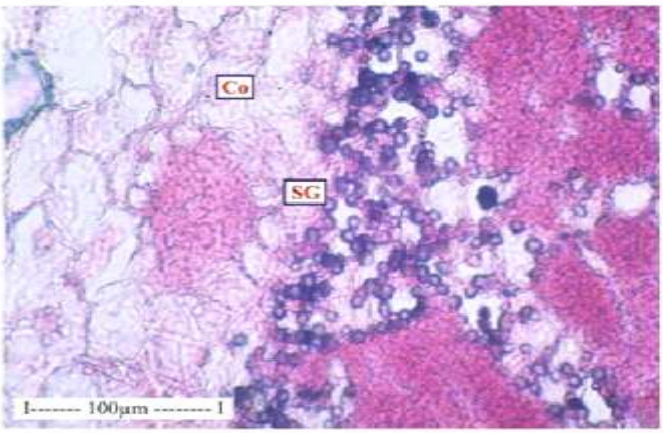
SAMPLE COLLECTION

Root nodules were obtained from one species mung bean (Vigna radiata). These species are collected as root masses taken from agricultural fields(Tamil Nadu) and Collection sites and soil. samples Soil samples were collected from 5 regions and root nodules were picked up from 3 areas in which Vigna had previously been cultivated These areas are located in diverse agro ecological regions with contrasting climates, topographies, and soils (Table 1). Soil samples from each area are a composite of 2 sub-samples prepared by mixing soils obtained from depths of 0–20 cm. No bacterial inoculations had previously been performed in these areas. Therefore, these strains Vigna-nodulating rhizobia are genetically diverse (Risalet al., 2012). They were previously characterized as slow growing bradyrhizobia (Allen and Allen, 1981). Vigna has been characterized for establishing symbioses in most countries, mainly with bradyrhizobia. These bradyrhizobialegume interactions have been examined in Asia, Africa, and the Americas (Lambrides and Godwin, 2007; Risal et al., 2012). Recent studies reported that some Vigna species also successfully establish N2-fixing symbiosis with Ensifer and Rhizobium species.

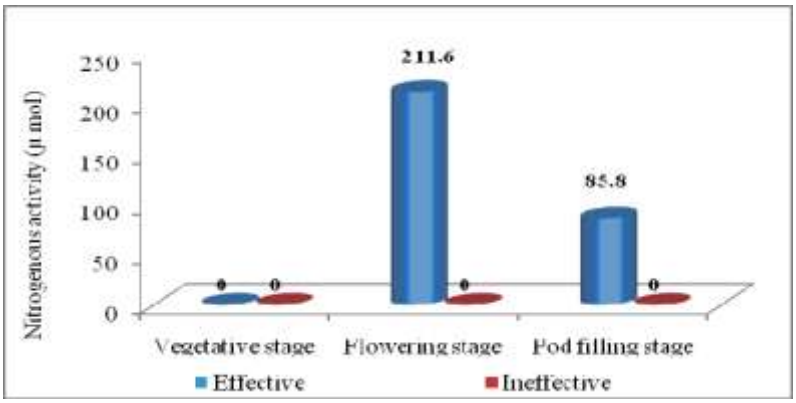
In order to compare the effective and ineffective root nodules, the root nodulating tropical legumes, Vigna radiata, that produce both effective and ineffective nodules were selected. The seeds of *vigna radiata* along with *Bradyrhizobium* strains S24 (effective) and S24A06 (ineffective) were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore. The obtained rhizobial strains were sub cultured and maintained in yeast extract agar nitol (YEMA) slants as well as in broth.



A - Effective nodule without starch grains



B - Ineffective nodule showing starch grains and calcium oxalate crytals



Nitrogenase activity of the root nodules of Vigna radiata

Cultural and Biochemical characteristics of endosymbionts

This was done to confirm the identity of the isolates from effective and ineffective root nodules of *Vigna radiata*

Isolation of endosymbionts from effective and ineffective root nodules ((Vincent, 1970)

Healthy and well developed root nodules (effective and ineffective) from were collected and washed 4 to 5 times with sterile distilled water. They were surface sterilized using 0.1 per cent acidified mercuric chloride solution for 4-5 min and then washed with sterile distilled water. Then they were dipped in 70 per cent ethyl alcohol and finally washed in sterile water to remove all the traces of sterility. The surface sterilized root nodules were crushed with the help of pestle and mortar by adding small aliquots of sterile water. From this, 1 ml was taken and mixed with 9 ml of sterile water to make 10^{-1} dilution. Thus the suspension was serially diluted up to 10-7. The diluted suspensions 10-5 -10-7 were selected and 0.1 ml of suspension from each dilution was inoculated into Petriplates containing sterile yeast extract mannitol agar (YEMA) medium with congo red. The inoculated plates were incubated at 30 ± 2˚C for 6 days. At the end of the incubation period, the colonies of endosymbiont were removed and purified by repeated streaking. The pure cultures were maintained on YEMA slants at 4˚C.

Cultural characterization

The test isolates were subjected to the following cultural and microscopic tests in order to confirm their identity as *Bradyrhizobium* and differentiate it from an allied genus *Agrobacterium***.**

Colony shape and size

The rhizobial isolates were grown on YEMA medium with congo red. After 6 days, the colony shape and size (diameter) were measured and recorded.

Motility test

Petroleum jelly was applied around the cavity slide. A loopful of rhizobial culture was placed in the center of a clean coverslip. The petroleum jelly applied slide was placed with the concave surface facing down over the coverslip. Then the slide was quickly turned upside down and it was observed under the microscope. The motility of the rhizobial isolates was recorded.

Gram staining

A thin smear of rhizobial isolates was separately made on a clean glass slide and heat fixed. Then the smear was stained using crystal violet for 1 min and then washed with water followed by flooding with Gram's iodine. After 1 min, the slide was washed again in tap water and decolourized. Then the smear was counterstained with safranin for 1 min. Finally the slide was washed, air dried and observed under microscope.

Capsular staining (Mc Kinney, 1953).

Bacterial smear was prepared, heat fixed and stained with 1 per cent alcian blue in 95 per cent ethanol for 1 min. The excess stain was washed with sterile water and allowed to air dry. Zihel-Neelsen carbol fuchsin was used as counter stain for 1 min. The slide was washed, air dried and finally observed under microscope.

Staining of Polyhydroxybutyrate (PHB) (Vincent, 1970)

A loopful of culture was spread over the microscopic slide and air dried. **1** ml of carbol fuchsin solution was added and allowed to stand for 30 min. The slide was washed in running water, air dried and observed under microscope. The red colour of the cells is indicative of positive result.

Congo red test (Allen and Allen, 1936)

The YEMA medium containing 1 per cent congo red solution can be used to differentiate *Rhizobium* from *Agrobacterium.* The bacterial isolates were inoculated into the petriplate containing the medium and incubated at 30 □ 2˚C for 48 h. *Rhizobium* stands out as white, translucent, glistening, elevated and Comparatively small colonies with entire margin in contrast to stained colonies of *Agrobacterium*.

Hofer's alkaline broth test (Hofer, 1941)

The isolated rhizobial cultures were spread on YEMA medium with high pH of 11 and observed for growth. The test is based on the fact that agrobacteria grow at higher pH levels, while rhizobia are unable to do so.

Growth in Lactose agar (Bernnerts and Delay, 1967)

The YEMA medium was prepared (by ading 1 per cent lactose instead of mannitol) and poured into sterile Petriplates. After solidification, 1 ml of rhizobial culture was spread over the medium. The plates were incubated at 30 □ 2˚C for 3 days, then flooded with Benedict's reagent and the results were recorded. Absence of yellow coloration indicates the presence of rhizobia.

Growth in Litmus milk agar (Vincent, 1970)

The Litmus milk medium (Appendix) was prepared and poured into sterile Petriplates. After solidification, 1 ml of rhizobial culture was spread over the medium and the plates were incubated at 30 ± 2˚C for 48 h. The growth rate was observed and recorded.

Growth in Glucose peptone agar (Subba Rao and Balasundaram, 1971)

1 ml of each isolate was transferred to sterile Petriplates. Liquefied and cooled glucose peptone agar (Appendix) (20 ml) was added to the Petriplates and mixed by rotating several times in clockwise and anticlockwise direction.

Discussion

The world population is expected to cross the ten billion mark by 2050. This is likely to create unprecedented pressures on the limited natural resource base of the planet, earth, making it pretty difficult to produce additional requirement of food, fibre and raw materials for the huge population. Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environmental pollution associated with indiscriminate use of agrochemicals (Suman et al., 2015). The chemicals used in agriculture are supposed to be taking a very heavy toll of this source of energy. The agricultural importance of nitrogen fixation is not only to provide ammonium to the crops but also to minimize pollution.. Chemical fertilizers and their exploitation cause air and ground water pollution by eutrophication of water bodies (Youssef et al., 2014). Though biofertilizers are ecofriendly and cost effective, its production, use and quality are to be strengthened for better exploitation under sustainable agriculture systems.

Conclusion

Scientists are looking for more viable and sustainable alternatives. They have identifiedmicroorganisms that convert atmospheric nitrogen and fix it into soils as a source. It has been observed that the total world biological nitrogen fixation by the microorganisms is about three times that of the industrially produced nitrogen. This proves that if the natural biological nitrogen cycles are harnessed through enhanced microbial population and activity, the need for nitrogen can be met more sustainably and economically (Sahoo and Bhardwaj Tuteja, 2013). Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environmental pollution associated with indiscriminate use of agrochemicals (Suman et al., 2015).In this study, the main aim is effective and in-effective root nodules with deal strain of Brady hizobiumSpp,and also observe the morphological characters and histo-chemical characters.Vigna-nodulating rhizobia are genetically diverse (Risalet al., 2012). They were previously characterized as slowgrowing bradyrhizobia (Allen and Allen, 1981). Vignahas been characterized for establishing symbioses in most countries, mainly with bradyrhizobia. These bradyrhizobialegume interactions have been examined in Asia, Africa, and the Americas (Lambrides and Godwin, 2007; Risal etal., 2012). Recent studies reported that some Vigna species also successfully establish N2-fixing symbiosis with Ensifer and Rhizobium species.

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