BIOTECHNOLOGY OF SMALL SCALE INDOLE ACETIC ACID PRODUCTION BY *RHIZOBIUM* SP

Abstract

There are so many important physiological implications in plant during the indole acetic acid (IAA) production by the root nodule bacteria. Several researchers have been optimized the cultural requirements and measured IAA production in maximum amount by the pathogenic bacteria from root nodules produced by different legumes. Some of the mutant rhizobia were also able to synthesize IAA from its precursor molecules in culture. The knowledge of small scale production of IAA in best supplemented cultural condition at laboratory may enrich the biotechnological approach of large scale production in Industry. This chapter depicts a concise idea about the content of IAA in the nodule of leguminous roots, synthesis of IAA in small scale by the pathogenic bacteria from root nodules in culture and also the association of legume root nodule–*Rhizobium* considering the second line of host-pathogen symbiosis.

Keywords: Legume family, Indole acetic acid(IAA), *RHIZOBIUM* SP.

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I. INTRODUCTION

There are approximately 730 genera [1] of leguminous plants and over 20000 species [2] worldwide of which more than 20% were able to form nodules in the roots [3]. About a very small number of the leguminous plants had been only studied in relation to the nodule bacteria [4]. The persistent interest of researchers in the rhizobial root nodules of leguminous plants have documented in the elucidation of many facets of the physiology of *Rhizobium*-legume symbiotic association [5]. Development of a successful stable symbiotic association was due to the activity of a complex mechanism of host-pathogenicassociative interactions [6]. Root nodules were also formed on non-leguminous dicotyledonous plants [7] to form a suitable ecological niche with biotechnological potential in agriculture [8] and on a monocotyledonous plant [9]. Legumes showed their importance in agricultural economy. Seeds of which were the chief source of vegetable proteins for human consumption and a very good source of animal fodder. Some members of legumes were largely used as green manure because their root nodules served as trapping sites for atmospheric nitrogen and increase soil fertility. After nodule formation through the infection of root hairs of leguminous plants, *Rhizobium* spp. fixes atmospheric nitrogen to produce ammonia with the help of the nitrogenase complexenzyme. The host plants ultimately assimilate this ammonia. From the pathogen point of view, the development of a successful symbiotic relationship with leguminous host plants depends on the expression of Nod factor signals and secretion of the correct surface and/or extra cellular polysaccharides (EPS) [10].

Biological nitrogen fixation accounted for more than 90% of terrestrial nitrogen turn over [11] and a large part of this nitrogen was fixed by the *Rhizobium* spp. in association with legumes. The balance of the fixed nitrogen of about 60% or 150 to 190 million metric tons annually throughout the world were accounted for by living organisms due to the reduction of nitrogen to ammonia [3]. The ratio between chemically fixed nitrogen and biologically fixed nitrogen ranged approximately from 1:4 to 1:2.5 [12].

The mechanism of nitrogen fixation, its regulation and the enzyme nitrogenase had been well investigated [3] with many other parts of the plant physiology of *Rhizobium*-legume association [5]. The fixation of nitrogen and its supply to the host was thought to be the only function of nodules for many years among the scientists. Again the amount of hormone within the root nodules gained much attention during the last few years, the measurable amount of many phytohormones [13] present in the root nodules played important biochemical roles in genesis and development [14] and formation [15] of root nodules. Hunter (1989) [16] reported that symbionts were responsible for IAA production in the root nodules and ultimately in association along with other phytohormones; this IAA expected to be involved to develop symbiotic association in several steps [16]. Transport of IAA from the root nodules to other plant parts was established [17, 18], though some aspects of *Rhizobium*-legume symbiosis were still partly understood.

Rhizobia are Gram-negative bacteria capable to fix atmospheric nitrogen, under the family Rhizobiaceae. Leguminous plants produced nodules with the help of the *Rhizobium* and a symbiotic relationship was developed by which hosts got nitrogen through the bacteria's essential nitrogen-fixing processes. Fabaceae (formerly Leguminosae) holds the third position among most of the largestangiospermous family membersafter Orchidaceae, the first one and Asteraceae, the second [19]. Legumes are second afterPoaceae (the grass

family) in economic and agricultural importance. Pulses, among the most explored herbs, studied in connection with nitrogen fixation within the root nodules.

The International Year of Pulses in 2016declared by The Food and Agriculture Organization (FAO) of the United Nations after focusing on the contribution of pulses in food production and nutritional diversity to help eradicate hunger and malnutrition [20]. Seeds of legumes are important dietary source of carbohydrates and proteins both in underdeveloped and developed countries. The immature pod contains vitamins A and C, while dry seeds contain protein, carbohydrates and also some of the essential minerals. Protein and phosphoric acid are also found within thelegume seeds as important dietary sources. A large proportion of rural people of India consume legume seeds as their sole source of protein as these are cheaper than animal sources. Leguminous plants produced nodules with the help of the *Rhizobium* and a symbiotic relationship was developed. A successful symbiosis between the host and the symbiont was established due the effect of a series of interactions [21]. The symbionts contributedin many ways such as supply of hormones to the nodule [22]. Rhizobia could also produce IAA in culture supplemented with precursor [23].Fixation of nitrogen and production of phytohormones both has played an important role in genesis, and development of root nodules [24].

Rhizobia were able to convert tryptophan to indole compound [25]. Production of IAA by *Rhizobium* spp. in culture supplemented with tryptophan was reported by many other workers [23, 26]. The purpose of this study was to throw some light on the production of IAA by the mutant species of *Rhizobium* without supplementation of any isomer of its precursor, tryptophan, of indole production in culture medium. It is expected to create a better knowledge on the legume-*Rhizobium* symbiotic association in the root nodules of legumes.

Fixation of nitrogen in leguminous plants and its supply within plant body were the main focused area of legume-*Rhizobium* symbiosis. Modern scientific research put emphasis on the hormone content of the nodule and its supply because of their involvement in the formation and development of root nodule. But till now the research regarding the content and metabolism of plant hormone in the nodules is restricted only in few legumes most of which are tree. Present investigation is meant to focus on pulse producing legumes, which have been unfolded to some extent. Therefore, the proposed work will reveal *Phaseolus mungo - Rhizobium* symbiosis. An effort was directed to correlate all these findings to explain the beneficial aspects of the symbiosis in this chapter.

1. **Brief Idea about Legume Family:** The members of the legume family ranges from pulse crops to many other species harvested for oils, fibre, fuel, timber, medicines, chemicals and horticultural varieties. It includesfrom large trees and extends up to annual herbs and is well established throughout the world from temperate to tropics[27].Forests members in tropics exhibit dry habitsin a particular season and temperate shrublands members tailored by extreme climates. They are not found to less represent in mesic temperate habitats, including many arctic and alpine regions. The legumes of semi-arid to arid are very much related to its environment of nitrogen-demanding metabolism [28]. Nitrogen fixation of symbiotic bacteria is one of several ways (like mycorrhizal association) in which legumes fix high levels of nitrogen to meet the demands of their metabolism [29]. By the formation of root nodules legumes play a vital role interrestrial

nitrogen cycle [29]. Though the family regarded as a tropical family of late Cretaceous origin (about 65–70 Mya), it has continuous and an abundant fossil record from the Tertiary [30].

2. **Root Nodule: General Characters And Importance**: The root nodules of leguminous and non-leguminous plants have created a great interest among scientists for a long period of time. Successful interactions between host plants and the soil microorganisms resulted in the formation of such specialized organs on leguminous and non-leguminous plants. There are over 20000 species of legumes [2]; but only 20% have been determined for nodulation, 90% of those do form nodules [3] and only about 0.5% of the leguminous plants studied in relation to the nodule bacteria [4]. The persistent interest of researches in the rhizobial root nodules of leguminous plants resulted in the elucidation of many aspects of plant physiology of *Rhizobium-* legume symbiotic association[5, 31]. In leguminous plants three taxonomically closely related genera *Rhizobium, Bradyrhizobium,* and *Azorhizobium* possessed the capacity to induce nitrogen-fixing nodules [3]. Formation of nodule and fixation of nitrogen in symbiotic conditionare restricted to an uniquesingle clade of plants of leguminous species [32]. Only 3% of Caesalpinioideae, 90% of Mimosoideae and 97% of Papilionoideaewere nodulated among the all legumes[32]. Formation of nodules in the root could have arisen independently during the course of evolution in legumes, including in the genus *Chamaecrista* (see Scheme- I) producing root and stem nodules on legumes, which are divided into six genera- *Rhizobium, Azorhizobium, Mesorhizobium, Sinorhizobium*, *Allorhizobium*, and *Bradyrhizobium*[33].

II. SCHEME- I
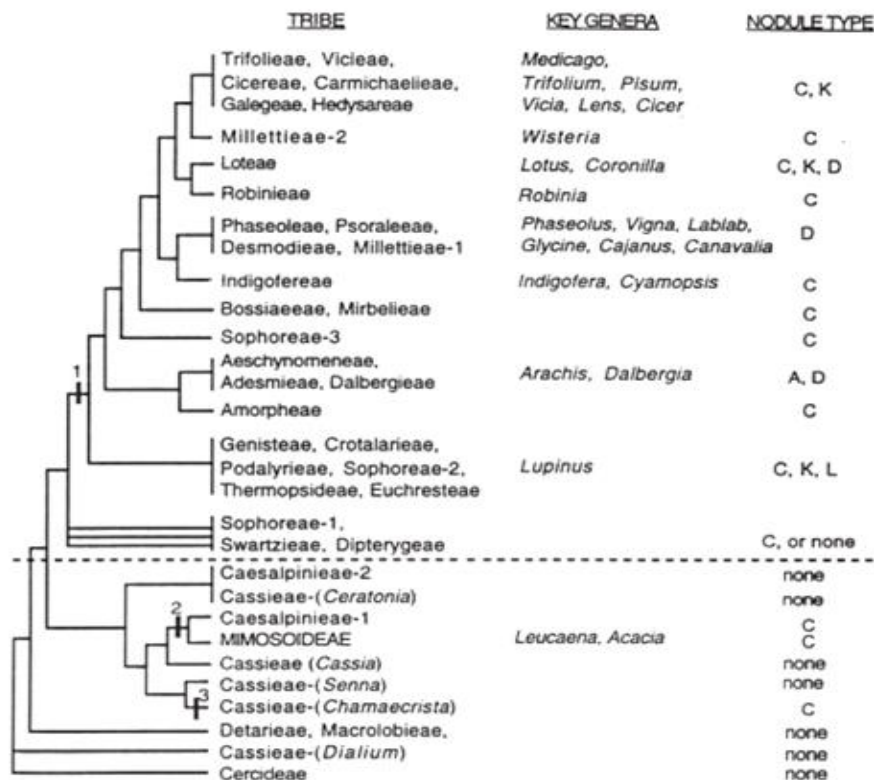
Scheme-I is about 40 species (http://www.dsmz.de) are now recognized [34]. Root nodules were also formed on non-leguminous plants such as *Parasponia aspera* (Ulmaceae). Phylogeny of the Leguminosae based on rbc L DNA-sequence data [32].

Mature root nodules are made up of largely of tetraploid cells containing bacteroids and some diploid cells without bacteroids. Several thousands of bacteroids usually occur in the cytoplasm, each group surrounded by a membrane called the peribacteroid membrane. Outside the peribacteroid space in the plant cytosol is a protein called leghaemoglobin [35]. Leghaemoglobin, which is much more dilute in nodules of non-leguminous plants, give nodules a pink colour [35].\

On the basis of shape and meristematic activity of the nodules Vance (1983) [36] divided the *Rhizobium* induced root nodules into three categories. These were (a) elongate- as found in pea, clover, alfalfa etc., (b) spherical- as in common bean, soybean etc. and (c) collar type- as in lupine. Determinate nodules which are usually spherical in shape, with determinate or nonpersistent internal meristematic activity, transport fixed nitrogen as ureides and the vascular system is closed over the apex of the nodule [7]. Indeterminate nodules which are elongate, cylindrical, with indeterminate or persistent apical meristematic activity, transport fixed nitrogen as amides and the vascular system does not close over the apex [7]. Small newly formed nodules are white and contain numerous actively dividing cells [37]. As nodule development proceeds, the nodule appears pink because of leghaemoglobin.

Microorganisms in the nodules carried out nitrogen fixation and thereby maintained symbiotic association with higher plants. In addition to nitrogen fixation in nodules by microorganisms, root nodules also contained large amounts of different plant hormones like indole acetic acid (IAA) [38, 39]. Transports of IAA from the root nodules to other plant parts were established [17, 18]. Root nodules were also found to contain GA_3, cytokinin and ABA like substances [18]. Verma *et al.* (1992) [14] reported that plant hormones played an important role in the genesis and development of the nodules.The roots of the surrounding plantsbenefited due to the activities of the roots of nitrogen fixing plants, either through supply of nitrogen from nodules or through microbial decomposition of nodules [40]. Mixture of legumes and grasses often used as pastures as an important contribution in agriculture contributed in [35].

1. **Formation of Root Nodules:** A mature root nodule is an unique developed structure formed as a final result ofrelationship of symbiotic association which normally happens between legumes and the bacterial genus *Rhizobium*.

    Several plant growth-promoting rhizobacteria (PGPR) have shown potential to enhance nodulation of legumes when co-inoculated with *Rhizobium* sp. [41]. Plant roots gave a suitable environment for the survival of soil rhizosphericbacteria that takes their nutrients from root exudates and lysates. The rhizosphere may contained up to hundred times higher population than the normal density of soil bacteria and form microcolonies of different strains of rhizobacteria. Utilization of nutrients synthesized by the host plant for growth may help to secrete compound metabolites into the signalling compounds by the pathogen and the compounds are taken by associated cells [42, 43].Flavonoid compounds released by host plants act as signals and these signalling molecules help symbionts to secrete Nod factors, perceived by roots of host plants and induced for

nodule formation, in legume-*Rhizobium* symbiosis. The excess amount of carbohydrates released by the host may help the bacteria to grow and provide fixed nitrogen to the host for amino acid biosynthesis [42]. It is an intimate relationship between a soil bacterium and the host plantand establishes the concept of 'plant growth-promoting rhizobacteria' (PGPR): in nitrogen-poor environments and limiting nutrients [44].

So many mechanisms for promotion of growth in rhizobacteria have been discovered [44]. The atmospheric nitrogen fixing ability is also present in various free-living and associated bacterial species [45]. Inorganic nutrients, which are poorly soluble, are rate-limiting growth factors also made available after the secretion of many organic acids [46].

Other mechanisms for promotion of growth in rhizobacteria involve modulation through hormone production in plant regulatory mechanisms and many other compounds help in plant development [47]. It has also been documented that there are many bacterial species are able to produce auxin, ethylene,gibberellins and cytokinins [48].

The development and stability of a functional root nodule clearly demands a high degree of regulation. Plant hormones are involved in triggering the initiation of the formation root nodules, and that hormonal balance is an important factor in the control of nodule development, maintenance, and senescence [17]. Once rhizobia gain intracellular access to their host, legumes also strongly influence the process of bacterial differentiation that is required for nitrogen fixation. Even so, symbiotic rhizobia play an active role in promoting their goal of host invasion and chronic persistence by producing a variety of signal molecules that elicit changes in host gene expression [49].

Root nodule formation has been described to occur primarily via well described pathway of curlingof root hair, infection thread formation, initiation of a nodule meristematic cell as described in many legumes [50]. During the differentiation of root nodules the development of specialized tissues from primordial and meristem is closely coupled to the subcellular process of tissue and cell invasion by *Rhizobium* spp. [51].

Reciprocal exchange in signalling mechanisms were also noticed, which allow the symbionts to enter into the roots of the host plants during the early step of infection by *Medicagotruncatula* and *Sinorhizobiummmeliloti* [21].

After the initiation of the signal in the form of molecular dialogue in association with the specific rhizobia within the soil and replying by the secretion of factors lipochitooligosaccharidic associated with nodulation after permitting the entry of the pathogen within the host [52]. A number of nodule specific proteins contributed by the host cells for the development of an active nodule. The secretion of proteinis most important among all others in determining the outcome of the interaction as well [53]. These proteins, called nodulins [3] encoded by *nod* genes situatedwithin the host cell genome. The essential genes of rhizobia for establishment of symbiosis are compartmentalized eitherin symbiotic islandsor in plasmids within the cell. Five symbiotic genome compartmentshave been entirely sequenced. The symbiotic parts of rhizobia genomes are mosaic structures, frequently associatedwith recombination, horizontal transfer and transposition [54]. A range of host-specificity occurs due to

presence of host specific lectin- surface polysaccharide interactions results in the attachment of the *Rhizobium* to the host root hair [3].

Most of the researches focused on the first phase of this interaction like morphogenesis of nodule and the starting point of nitrogen fixation concerning the interaction between legumes and rhizobia [55].

Nodule senescence is also characterized by an increase in proteolytic activities especially with respect to the degradation of leghemoglobin[56] and an increase of reactive oxygen species [57]. It was comes from the re-isolation studies that a more direct indication of a non-negligible part of nodule bacteroids may escape lysis and redifferentiation into growing symbiont [58].

In soybean root nodules during senescence upon the treatment of nitrate or herbicide, the number of viable bacteria estimated from the nodules did not decrease [59].

During the very early stage of plant science research with auxin, the existence of bacterial IAA producers (BIPs) was recognized. On the way to measurement of IAA, the BIPs associated with plants haveconsidered as a source of contamination in plant tissues [60]. Later researches depict that BIPs cause the symptoms in many plants along with thebacterial diseases like gypsophila gall [61], knot disease in olive and oleander plants [62], russet of fruits inpear [63], and also in plant-growth-promoting rhizobacteria (PGPR) associated plants [64]. The discovery of BIPs on the biology, ecology, and pathology, genetics and biochemistry also enriched the scientific information of bacterial IAA production [65].

Another intriguing possibility of legume-*microbes* association is that the bacteriahave the capacity to resist the fungal infections to the plants by many ways [66].

The compound IAA might represent as a effective measureto select the specific microbial symbiontover the surfaces [67]. Further researchmay reveal to detect whether and how BIDs shows the beneficial effect to the host plants. It may be noted that plant growth stimulating substances were produced by BIDs and thereby production of siderophores are the effect of pathogen infection [68].

2. **Taxonomy of Rhizobiaceae:** In Bergey's Manual of Systematic Bacteriology (second edition), Kuykendall (2005) [33] divided the family Rhizobiaceae into 7 genera – *Rhizobium, Agrobacterium, Allorhizobium, Carbophilus, Chelatobacter, Ensifer*and*Sinorhizobium*. The family Rhizobiaceae is a phenotypically heterogeneous assemblage of aerobic, Gram-negative rod-shaped bacteria and is based solely on 16S rRNA gene sequence analysis [33]. Nodules were formed on the roots of leguminous plants by different strains of *Rhizobium, Sinorhizobium* and *Allorhizobium* of the family Rhizobiaceae, *Mesorhizobium* of family Phylobacteriaceae and*Bradyrhizobium* of family Bradyrhizobiaceae and also on leaves of some plants of Myrsinaceae and Rubiaceae by strains of *Phyllobacterium* belong to the family Phyllobacteriaceae [33]. The genus *Azorhizobium* under the family Hyphomicrobiaceae[33] formed stem nodules on some hydrophytic lgumes[69]. The bacteria were present in root nodules as pleomorphic forms (bacteroids), normally involved in fixing atmospheric nitrogen. Monotrichous flagella were present in*Bradyrhizobium* (polar or subpolar) andalso in some strains of *Rhizobium*

(polar or subpolar), *Phyllobacterium* (polar, subpolar, or lateral), *Mesorhizobium* (polar or subpolar) and *Agrobacterium*whereas peritrichous flagella present in most species of *Rhizobium* (2-6 peritrichous flagella), *Agrobacterium* (2-4 peritrichous flagella) and *Mesorhizobium*. Fimbriae have also been described on some strains of *Rhizobium*[33].

Nitrogenase activity was present in *Rhizobium, Sinorhizobium, Allorhizobium*, *Mesorhizobium,Bradyrhizobium*[33] and *Azorhizobium* but absent in naturally occurring strains of *Agrobacterium*. They also considered the characters such as optimal temperature, pH range, NaCl tolerance, fast growing nature and diameter of the colony, production of EPS in carbohydrate media, mol % G+C of DNA, and amplified 16S rDNA restriction analysis, comparative 16S rDNA sequence analysis with GenBank accession number, DNA-DNA reassociation and nutritional data for differentiating the genera of Rhizobiaceae. Most rhizobial strains showed poor or no growth on glucose-peptone medium [70].

Development of 'cross inoculation group' concept for classifying rhizobia was considered by many workers [71]. Most rhizobia were restricted to the nodule formation with a limited number of specific host plants while others were highly specific, infecting only one legume host species [3]. Plant host specificity usually may include a wide range of legume genera and is to some extent by the chemical structure of the lipochito-oligosaccharide *Nod* factors produced [33]. Cross inoculation grouping system gradually lost its credibility and was replaced by information regarding bacterial genome [4]. The fusion of the former species *R. phaseoli* and *R. trifolii* with *R. leguminosarum* as biovars and retention of *R. meliloti* as a separate species were based on extensive evidences employing numerical taxonomy including DNA-DNA homology, characterization of cellular proteins, serology, composition of extra-cellular gum and findings involving the transfer of infectivity via plasmids [4]. Transfer of plasmid between species resulted in the expression and stable inheritance of the particular plant-interactive properties of the plasmid-donor species [33]. The biovars of *R. leguminosarum* were based largely but not entirely on host plant specificity there were reasons to suspect that biovars of *R. phaseoli* was more distinctly related to *R. leguminosarum* than the other two biovars such as *R. viceae* and *R. trifolii*[72]. From the literature it is appeared that the taxonomy of Rhizobiaceae has yet remained controversial and needed more study.

3. **Indole Acetic Acid (IAA): Isolation, Identification and Estimation:** The Greek term 'Auxin', meaning 'to increase' used by Went for the first time in the year 1928 [73].Went (1928) discovered that there were some unidentified compound which were responsible for the curvature of Oat coleoptiles towards the source of light [35]. It was K.V. Thimann(during 1930's), who first observed that theIAA synthesis within the mould *Rhizopus suinus* from the amino acid tryptophan [3]. The indole compound was present in plants either in the free form or in the bound form with many other macromolecules [74]. The chemical conjugates were formed from the bounded form of those indole compounds. The conjugate forms were not active still released active indole compoundsafter extraction with the solvent, hydrolysis with the alkaline solution or *in vivo* enzymatic hydrolysis [3]. There are common there broad categories of assay methods for IAA: i) bioassay or biological assay [75], ii) analysis with the help of the instruments- use of modern instruments for separation and quantification, and also for

chromatography-mass spectroscopy (including HPLCand GC-MS)to get structure and iii) immunometric assay [35].

4. **Biosynthesis of IAA:** Many pathways are established regarding the biosynthesis of IAA. IAA synthesis occurs in higher plants (Figure-1) in three steps from the tryptophan as a precursor via indole-3-pyruvate and indole-3-acetaldehyde (see Scheme-II) [3, 35]. Indole-3-ethanol may also produce from Indole-3-acetaldehyde after reduction (see Scheme-II). Tryptamine is the intermediate product during IAA synthesis. Another pathway involved the decarboxylation of tryptophan to tryptamine [3] and its conversioninto indole-3-acetaldehyde and then finally oxidized to IAA (see Scheme-II). Alternative pathways in different plants were also reported for IAA synthesis [76]. A tryptophan independent pathway for IAA synthesis present in maize plant was also reported by Ostin *et al.* (1999) [77]. Alternative pathwayof IAA biosynthesis via indole-3-pyruvate (IPA) and indole-3-acetaldhyde (IAld) were measured in members of *Rhizobium meliloti*[78] and*Bradyrhizobium*sp.[79]. Indole-3-methanol (IM) in the place indole-3-ethanol (Ie^th) was also reported in *Rhizobium leguminosarum*bv. *Phaseoli* which convert tryptophan to IM and IAA in final step[80]. Indole-3-acetonitrile (IAN), another intermediate product of IAA biosynthesis, conversion of indole-3-acetamide (IAM) by the nitrile hydratase enzyme in a different pathway for indole synthesis and form IAM to IAA in the final step by amidase enzyme in *Rhizobium* spp was also proposed by Kobayashi *et al.* (1995) [81]. However, within legume-*Rhizobium* symbiotic association the pathway leading to IAA from tryptophan by*Rhizobium* sp.has not yet been fully understood.

5. **Cultural Requirements of *Rhizobium* Spp. For Growth and IAA Production:** There are so many carbohydrates which have been utilized by *Rhizobium* spp. as their carbon sources during growth and metabolic processes [4]. The carbohydrates supplemented within the medium were the determining factors for the growth and metabolite production in culture [82]. Mannitol among the most supplemented carbon sources at 1% level was showed most effective performance for the promotion of growth and the metabolite production by *Rhizobium* sp. Isolated from different legumes [83]. Among the many other nitrogen sources NH_4^+, glutamine and glutamate were most effective but nitrate was less preferred as nitrogen source [84]. A*Rhizobium* sp. from *Tephrosia purpurea* preferred KNO_3 and $NaNO_3$ as nitrogen source for maximum growth and production of indole compounds was also reported by De and Basu (1996) [85].

Regarding the most preferred vitamin sources *Rhizobium* spp. utilized biotin and other water soluble vitamins for their growth was reported by Jordan (1984) [4]. But exceptions were also there; in some strains of *R. japonicum*inhibition of growth by biotin was also reported [86]. Riboflavin has also the growth enhancing property of several strains of*Rhizobium* sp. [87]. Vitamins were also showed little or no effect in growth in culture of *Rhizobium* sp. Research conducted by Chakraborti *et al.* (1981) [84] showedmany strains of *R. japonicum*, which exhibitedalmost no response with vitaminsin culture condition. In some cases pantothenic acid and p-amino benzoate were usedas precursor molecules of vitamins for growth of *Rhizobium* sp. in culture

The indole compound production of by the symbiotic of root nodule (Figure-1 and 2) is considered to have important physiological function within the plants as it seemed

reasonable to suspect that the plant hormoneswere involved in symbiotic relationship and moved to other plant parts. Wheeler *et al*. (1979) [18] proved that nitrogen fixation was not the only function in legume- *Rhizobium* association but production of phytohormonesand its utilization in different parts of the host plants after transportation was also the another function. Research from the many workers show that, if nitrogen fixation by symbiont in legume nodule and its supply to other parts can be treated as the primary function in symbiotic relationship, then the hormone production and metabolism may be considered as the secondary function of symbiosis in nodule–*Rhizobium* symbiotic association.
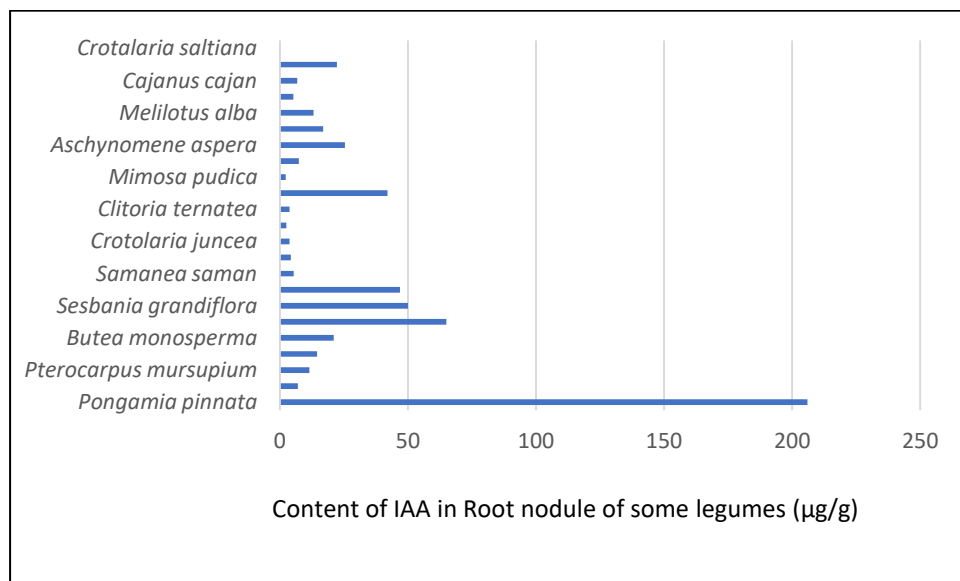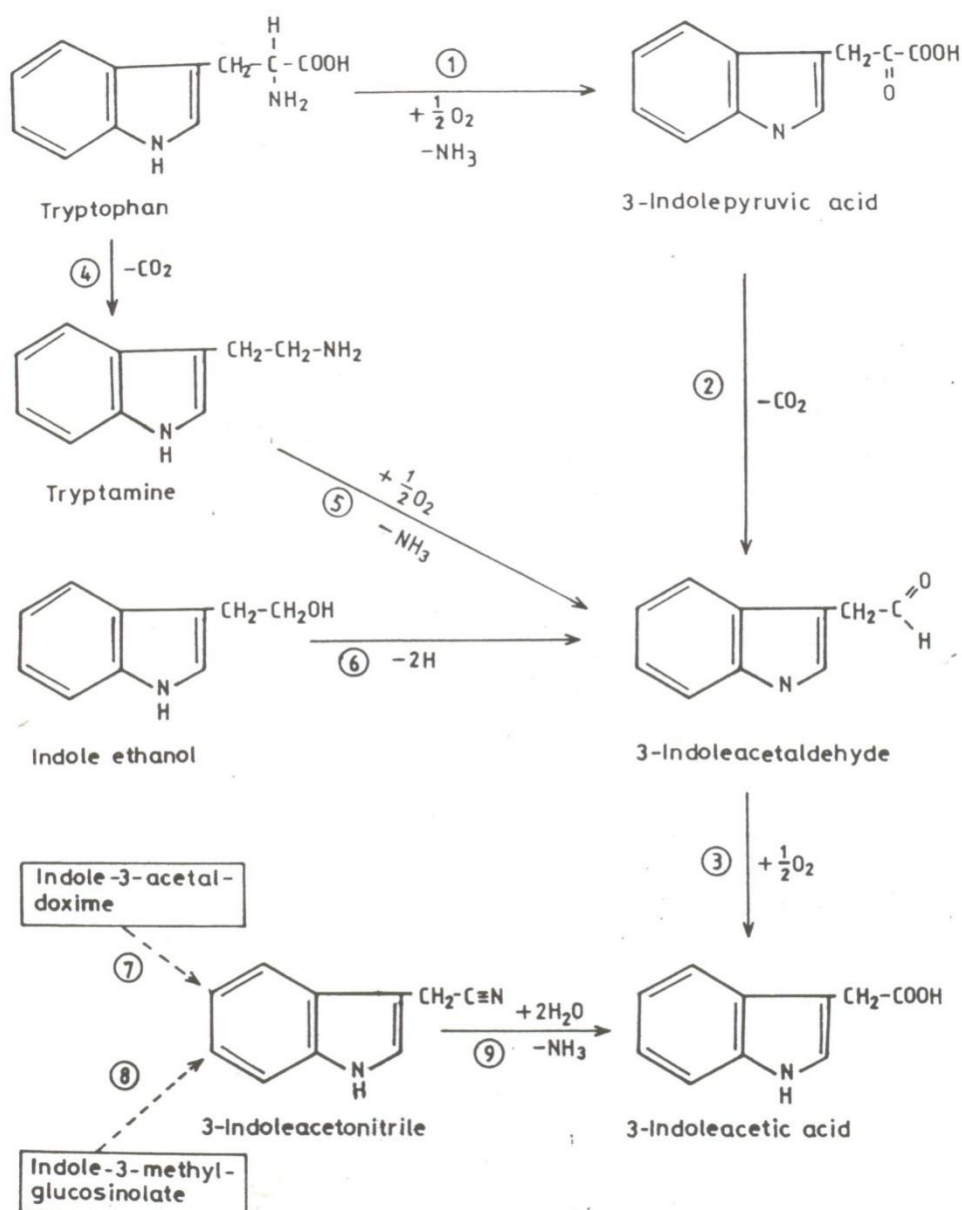

Figure 1: Content of IAA in Root Nodules of Some Important Legumes (After Ghosh *Et Al*. 2011) [88]
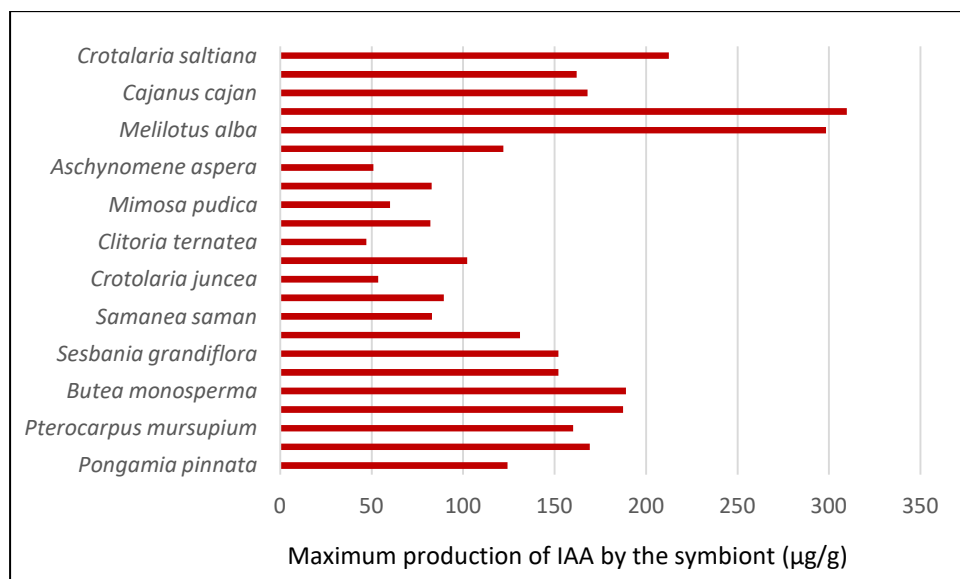
Figure 2: Production of IAA by Symbionts (*Rhizobium* spp.) of Some Important Legumes (After Ghosh *et al*. 2011) [88]

III. SCHEME- II

Scheme-II is the pathways of IAA biosynthesis. Enzymes are 1) tryptophan transaminase; 2) indole pyruvate decarboxylase; 3) indole acetaldehyde oxidase/ dehydrogenase; 4) tryptophan decarboxylase; 5) amine oxidase; 6) indole ethanol oxidase; 7) indole acetaldoxime dehydratase; 8) myrasinase; 9) nitrilase.(After Ghosh *et al.* 2011) [88].

1. **IAA Production by Mutant Strains of *Rhizobium* Sp. Having 5-Fluro Tryptopha resistant Capacity:** A number of mutant strains of *Rhizobium* sp. were reported as analogue resistant mutant strains viz. 5FT[R] 5A, 5FT[R] 5B, 5FT[R] 5C and 5FT[R] 5D which were taken from 5.0 mM 5-fluro-tryptophan supplemented medium and theywere marked as analogue resistant mutant strains viz. 5FT[R] 10A, 5FT[R] 10B, 5FT[R] 10C and 5FT[R] 10D taken from 10.0 mM 5-fluro-tryptophan supplemented medium (Table 2) [89].

Earlier it was found that the *Rhizobium* spp. normallysynthesize IAA in culture if the culture media were supplemented with tryptophan exogenously [79, 90]. To see the production of IAA in culture media without supplementation of any tryptophan as precursor molecule exogenously, experimental design were develop to detect the capacity of IAA production by the tryptophan synthesizing 5FT resistant rhizobialsymbiont. Many of the tryptophan synthesizing 5-fluro-tryptophan resistant mutantstrains were capable of IAA production in culture by using tryptophan as precursor (Table 2) as proposed earlier [79, 91].

Table 2: Production of Tryptophan and Indole Compound by Analogue Resistant Mutant Strains

Bacterial strains		Growth OD A_{540nm}	Tryptophan (µg/ml)	IAA production (µg/ml)
Parent *Rhizobium* strain	PM 25 (*Rhizobium* sp.)	0.36	ND	ND
Analogue resistant mutant isolated from 5.0 mM 5FT supplemented medium	5FT^R 5A	0.23	28.0	26.0
	5FT^R 5B	0.23	25.0	22.0
	5FT^R 5C	0.23	17.0	22.0
	5FT^R 5D	0.21	17.0	18.0
Analogue resistant mutant isolated from 10.0 mM 5FT supplemented medium	5FT^R 10A	0.28	43.0	37.0
	5FT^R 10B	0.21	25.0	18.0
	5FT^R 10C	0.29	25.0	18.0
	5FT^R 10D	0.27	13.0	15.0
	Critical difference at P=0.05	0.02	0.3	0.4

The bacteria were grown in Bergersen's medium pH 7.0 at 30 ± 2°C. The incubation period for maximum IAA production was 24 h. Growth of the bacteria was measured by checking turbidity using a spectrophotometer at 540 nm against a uninoculated control broth. Tryptophan and IAA were estimated spectrophotometrically comparing the OD values with a standard curve from the tryptophan and IAA. Data presented here are the mean of three replicates (after Ghosh *et al*. 2014) [89].

IV. CONCLUSION

The above data and the research works of other authors show that, the knowledge of small scale production of IAA by root nodule bacteria can be taken as doorstep of the technology of large scale production of IAA. The comparative account of IAA production may also help enrich the idea of another line of symbiosis apart from nitrogen fixation in the nodules by nodule bacteria. The production of IAA by mutant strains of *Rhizobium* sp. also introduces a new idea of secondary function in the root nodule– *Rhizobium* symbiotic association.

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