**Biotechnology of small scale Indole Acetic Acid production by *Rhizobium* sp.**

**Sisir Ghosh**

Assistant Professor, Department of Botany, Sreegopal Banerjee College, Bagati, Mogra, Hooghly

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 production of IAA in maximum amount by the bacteria isolated from root nodules of different legumes. Some of the mutant rhizobia were also able to produce 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, production of IAA in small scale by the bacteria isolated from root nodules in culture and also the association of legume root nodule–*Rhizobium* considering the second line of host-pathogen symbiosis.

**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 0.5% 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 the result of a complex series of host-pathogen 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 complex enzyme. 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 in several steps of the symbiotic relationship [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) is the third largest family after Orchidaceae and Asteraceae among angiosperms [19]. Legumes are second after Poaceae (the grass family) in economic and agricultural importance. Most of the explored herbs are pulses studied in relation to nitrogen fixation by the root nodules and play an vital role in Indian diet. The International Year of Pulses in 2016 declared 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 the legume 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 contributed in 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 have 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.

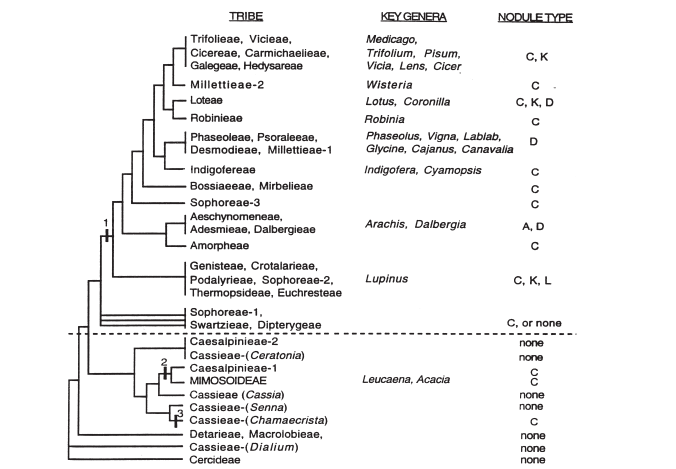
**Brief idea about Legume family**

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 includes large trees to annual herbs and is well represented throughout the world from temperate to tropical regions [27]. Members in tropical forests exhibit seasonally dry habits and temperate shrublands members tailored by xeric climates. They are absent to poorly represent in mesic temperate habitats, including many arctic and alpine regions and the understory of cool temperate forests. The legumes of semi-arid to arid habitats is 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 in terrestrial nitrogen cycle [29]. Though the family regarded as a tropical family of late Cretaceous origin (about 65–70 Mya), it has an abundant and continuous fossil record since the Tertiary [30].

**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 condition are restricted to a single clade of plants of legume and actinorhizal species [32]. Only 3% of Caesalpinioideae, 90% of Mimosoideae and 97% of Papilionoideae were nodulated among the all legumes [32]. Formation of nodules in the root could have arisen independently on several occasions 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].

**SCHEME- I**



**Scheme-I:** 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. Tribes appearing more than once on the tree are polyphyletic. The numbered rectangles indicate stages in evolution beyond which most species are nodulated. C and K, A and D, and L refer to indeterminate, determinate, and lupinoid (collar) nodule shapes, respectively [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 GA3, 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 plants benefited 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].

**Formation of root nodules**

A mature root nodule is an unique organized structure formed as a result of symbiotic relationship which normally occurs 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 offer a suitable environment for the survival of soil bacteria 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 released from the host for their growth may help to secrete metabolites into the signalling compounds by the pathogen and the compounds are perceived by neighbouring cells within the same habitat [42, 43]. In *Rhizobium*-legume symbiosis the plant releases flavonoid compounds that act as signals for the bacterium to secrete Nod factors. Nod factors secreted are perceived by root hairs of legumes and act to induce root nodules by which the bacterium can fix nitrogen molecule from atmosphere. 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 plant and establishes the concept of ‘plant growth-promoting rhizobacteria’ (PGPR): in nitrogen-poor environments and limiting nutrients [44].

So many mechanisms of rhizobacterial growth promotion 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 for growth also made available through the secretion of many organic acids [46].

Other mechanisms of growth promotion involve modulation of plant regulatory mechanisms through the production of hormones or other compounds that influence plant development [47]. It has also been documented that there are many bacterial species are capable of producing auxin and/or ethylene, and synthesis of 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 curling of 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].

The early steps in the invasion of barrel medic (*Medicago truncatula*) and alfalfa (*Medicago sativa*) roots by *Sinorhizobium meliloti* are characterized by the reciprocal exchange of signals that allow the bacteria to use the plant root hair cells as a means of entry [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 protein is most important among all others in determining the outcome of the interaction as well [53]. These proteins, called nodulins [3] encoded by *nod* genes situated within the host cell genome. The essential genes of rhizobia for establishment of symbiosis are compartmentalized either in plasmids or in symbiotic islands within the cell. Five symbiotic genome compartments have been entirely sequenced. The symbiotic compartments of rhizobia genomes are mosaic structures, frequently tailored by 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 initial phase of this interaction like morphogenesis of nodule and the onset 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 bacteria [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 as compared to non-senescencing control nodules [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 have considered as a source of contamination in plant tissues [60]. Later, BIPs were identified as the cause of symptoms in many plants associated with severe bacterial plant diseases such as gypsophila gall [61], knot disease of olive and oleander [62], and russet of pear fruit [63], or, in other cases, as benefactors of plants, e.g., nitrogen-fixing *Bradyrhizobium japonicum* in root nodules and plant-growth-promoting rhizobacteria (PGPR) such as *Pseudomonas putida* [64]. An impressive body of scientific information has been established after the discovery of BIPs on the biology, ecology, and pathology, and much of the genetics and biochemistry of bacterial production of IAA has been elucidated [65].

Another intriguing possibility of legume-*microbes* association is that the bacteria can protect plants from fungal infections by keeping plant surfaces free of IAA: fungal pathogens use IAA as a chemical cue that signals plant presence and induces mechanisms for invasion [66]. It is not clear whether and in what amount plants supply or secrete IAA and how much of it could be used by bacterial IAA degraders (BIDs).

The compound IAA might represent as a means for the plant point of view to select for a highly specific microbial population over the surfaces [67]. Further investigation may reveal to see whether and how such a selection for BIDs shows the beneficial effect to the host plants. It may be noted that BIDs produce plant growth stimulating substances that are antagonistic to pathogens due to the production of siderophores [68] or secondary metabolites which have the antimicrobial activity.

**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 variety of legume genera and is to some extent determined 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]. Plasmid transfer 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.

**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 the IAA 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 inactive but released free, active indole compounds upon extraction with the solvent, hydrolysis with the alkaline solution or *in vivo* enzymatic hydrolysis [3]. There are common there broad categories of assay methos 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 (including HPLC and GC) followed by mass spectrometry (MS) to obtain the proof of structure and iii) immuno assay [35].

**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 another intermediate product during IAA synthesis. Another pathway involved the decarboxylation of tryptophan to tryptamine [3] and its conversion into 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 pathway for the biosynthesis of IAA via indole-3-pyruvate (IPA) and indole-3-acetaldhyde were found in *Rhizobium meliloti* [78] and in *Bradyrhizobium* sp. [79]. Indole-3-methanol (IM) in the place indole-3-ethanol (Ieth) 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, converted to indole-3-acetamide (IAM) by the enzyme nitrile hydratase 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*.*

**Cultural requirements of *Rhizobium* spp. for growth and IAA production**

There are so many carbohydrates which has 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 NH4+, glutamine and glutamate were most effective but nitrate was less preferred as nitrogen source [84]. A *Rhizobium* sp. from *Tephrosia purpurea* preferred KNO3­ and NaNO3 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 of *Rhizobium* sp. Chakraborti *et al.* (1981) [84] reported many strains of *R. japonicum*, which showed little or no response to any vitamin in culture. In some cases pantothenate and p-amino benzoic acid were the most common vitamins or as precursor molecules of vitamins for growth of *Rhizobium* sp.

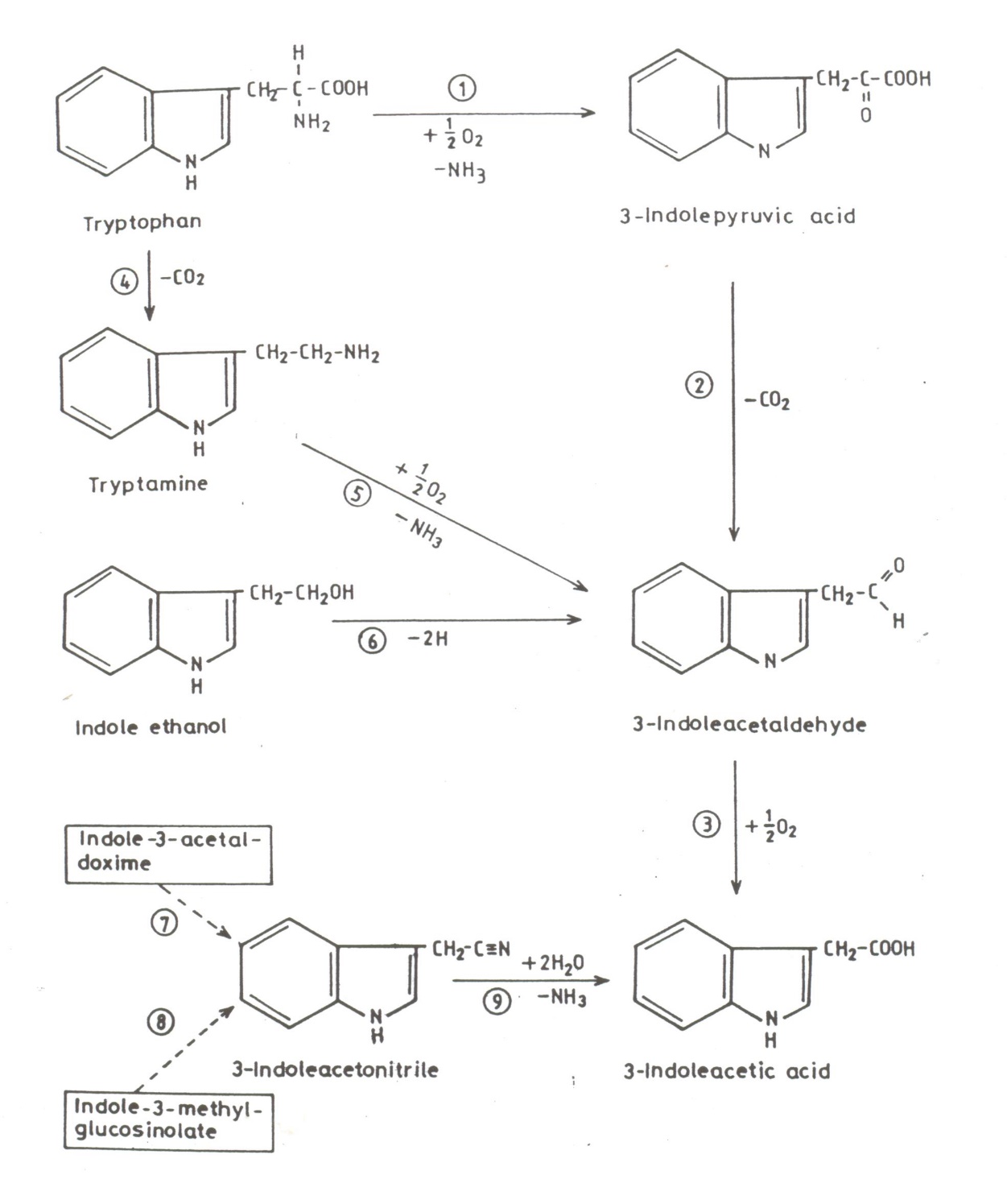
The production of indole compound by the root nodule bacteria (Figure-1 and 2) is considered to have important physiological function within the plants as it seemed reasonable to suspect that the plant hormones might be involved in several stages of the symbiotic relationship and transported to other plant parts. Wheeler *et al*. (1979) [18] showed that phytohormones from the root nodules were transported to other parts of the plant though nitrogen fixation and supply to the host was thought to be the only function of the root nodule–*Rhizobium* symbiosis.

The above data and the works of other authors show that, if the fixation of nitrogen in the root nodules and supply to the host can be considered as the first-line of symbiosis, the IAA content in the nodule and supply to the host may be taken as the second-line of symbiosis in the root 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]

**SCHEME- II**



**Scheme-II: 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].

**Indole Acetic Acid (IAA) production by 5-fluro-tryptophan****resistant mutant strains of *Rhizobium* sp.**

A number of mutant strains of *Rhizobium* sp. were reported as analogue resistant mutant strains viz. 5FTR 5A, 5FTR 5B, 5FTR 5C and 5FTR 5D which were isolated from 5.0 mM 5-fluro-tryptophan supplemented medium and the mutant strains were designated as analogue resistant mutant strains viz. 5FTR 10A, 5FTR 10B, 5FTR 10C and 5FTR 10D isolated from 10.0 mM 5-fluro-tryptophan supplemented medium (Table 2) [89].

Earlier it was found that the *Rhizobium* spp. could generally produce IAA in culture when 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 made to check the ability of IAA production by the tryptophan producing 5FT resistant rhizobial strain. It was observed that among the eight tryptophan producing 5-fluro-tryptophan resistant mutants all the strains were capable to produce IAA in culture (Table 2). The production of IAA was greatly varied with the amount of tryptophan produced by the mutants. This result strongly support that the tryptophan was the precursor of IAA production as already established earlier [79, 91].

**Table 2: Production of tryptophan and IAA by the analogue resistant mutant**

The bacteria were grown in Bergersen’s medium pH 7.0 at 30±2oC. 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 an 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].

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Bacterial strains** | | **Growth OD at 540nm** | **Tryptophan production (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 | 5FTR 5A | 0.23 | 28.0 | 26.0 |
| 5FTR 5B | 0.23 | 25.0 | 22.0 |
| 5FTR 5C | 0.23 | 17.0 | 22.0 |
| 5FTR 5D | 0.21 | 17.0 | 18.0 |
| Analogue resistant mutant isolated from 10.0 mM 5FT supplemented medium | 5FTR 10A | 0.28 | 43.0 | 37.0 |
| 5FTR 10B | 0.21 | 25.0 | 18.0 |
| 5FTR 10C | 0.29 | 25.0 | 18.0 |
| 5FTR 10D | 0.27 | 13.0 | 15.0 |
|  | Critical difference at P=0.05 | 0.02 | 0.3 | 0.4 |

**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 introduce a new idea of second-line of association in the root nodule– *Rhizobium* symbiosis.

**References**

[1] G. P. Lewis , B. D. Schrire , B. A. Mackinder and J. M. Lock, “Legumes of the World”, Royal Botanic Gardens, Kew, Richmond, 2005).



[2] F. Stagnari, A. Maggio, A. Galieni, M. Pisante, “Multiple benefits of legumes for agriculture sustainability: an overview”, [Chemical and Biological Technologies in Agriculture](https://www.biomedcentral.com/openurl?doi=10.1186/s40538-016-0085-1), 4(1):2, 2017.

[3] W. G. Hopkins, “Introduction to Plant Physiology”, 2nd edition. John Wiley and Sons Inc., New York 1999.

[4] D. C. Jordan, “Rhizobiaceae. In: Bergey’s Manual of Systematic Bacteriology”, Vol. 1. (Eds. Krieg N. R. and Holt J. G.). Williams and Wilkins Co., Baltimore, USA, pp. 234-256, 1984.

[5] H. P. Cheng and G. C. Walker, “Succinoglycan is required for initiation and elongation of infection threads during nodulation of alfalfa by *Rhizobium meliloti*”,J. Bacteriol. **180 No. 19:** 5183-5191, 1998.

[6] B. J. Pellock, H. P. Cheng and G. C. Walker, “Alfalfa root nodule invasion efficiency is dependent on *Sinorhizobium meliloti* polysaccharides”, J. Bacteriol. **182:** 310-313, 2000.

[7] D. C. Smith and A. E. Douglas, “The biology of symbiosis”, Edward Arnold, Great Britain, 1987.

[8] E. Hassan, “Root nodules of legumes: A suitable ecological niche for isolating non-rhizobial bacteria with biotechnological potential in agriculture**”, Current Research in Biotechnology. Vol: 4, 78-86, 2022,** <https://doi.org/10.1016/j.crbiot.2022.01.003>.

[9] P. S. Basu, A. C. Ghosh and T. K. Dangar, “*Roystonea regia*, a monocotyledonous tree, bears rhizobial root nodules”, Folia Microbiol. **42**: 601-606, 1997.

[10] K. P. J. Price, “Carbohydrate determinate of *Rhizobium*-legume symbiosis”, Carbohydr. Res., **317:** 1-9, 1999.

[11] D. J. Fisher, “Effects of some fungicides on *Rhizobium trifolii* and its symbiotic relationship with white clover”, Pestic. Sci.**, 7:** 10, 1976.

[12] N. S. Subba Rao, “Biofertilizers in Agriculture”, Oxford and IBH Publishing Co. New Delhi, India, 1982.

[13] P. S. De and P. S. Basu, “Content of different phytohormones and indole acetic acid metabolism in root nodules of *Derris scandens* BENTH”, J. Basic Microbiol.,. **36:** 299-304, 1996.

[14] D. P. S. Verma, C. A. Hu and M. Zhang, “Root nodule development: origin, function and regulation of nodulin genes”, Physiol. Plant., **85:** 253-265, 1992.

[15] N. P. Kefford, J. Brockwell and J. A. Zwar, “The symbiotic synthesis of auxin by legumes and nodule bacteria and its role in nodule development”, Aust. J. Biol. Sci., **13:** 456-457, 1960.

[16] W. J. Hunter, “Indole-3-acetic acid production by bacteroid from soyabean root nodules”, Physiol. Plant,**76:** 31-36, 1989.

[17] J. Badenoch-Jones, B. G. Rolfe and D. S. Letham, “Phytohormones, *Rhizobium* mutants, and nodulation in legumes. III. Auxin metabolism in effective and ineffective pea root nodules”, Plant Physiol., **73**: 347-352, 1983.

[18] C. T. Wheeler, I. E. Henson and M. E. Mc Laughlin, “Hormones in plants bearing actinomycete nodules”, Bot. Gaz., **140(suppl.):** S 52-S 57, 1979.

[19] D. J. Mabberley, “The plant book”, 2nd ed. Cambridge University Press, Cambridge, UK, 1997.

[20] M. J. Considine, K. H. Siddique, C. H. Foyer, “Nature’s pulse power: legumes, food security and climate change”, [Journal of Experimental Botany](https://academic.oup.com/jxb/article-abstract/68/8/1815/3813857), 1;68(8):1815-8, 2017.

[21] K. M. Jones, H. Kobayashi, B. W. Davies, M. E. Taga, G. C. Walker, “How rhizobial symbionts invade plants: the *Sinorhizobium*–*Medicago* model”, Nature Revs Microbiol.,5:619-633, 2007.

[22] G. P. Lewis , B. D. Schrire , B. A. Mackinder, J. M. Lock, “Legumes of the World”, Royal Botanic Gardens, Kew, Richmond, 2005.



[23] S. Ghosh, C. Sengupta, T. K. Maiti, P. S. Basu, “Production of 3-Indolylacetic acid in root nodules and culture by a Rhizobium species isolated from root nodules of the leguminous pulse *Phaseolus* *mungo*”, Folia Microbiol., 53 (4): 351-355, 2008.

[24] D.P.S. Verma, C.A. Hu, M. Zhang, “Root nodule development: origin, function and regulation of nodulin genes”, Physiol Plant, 85: 253-265, 1992.

[25] T. Kaneshiro, M.E. Slodki and R.D. Plattner, “Tryptophan catabolism and IAA by *Rhizobium japonicum* L. 259 mutants”, Curr. Microbiol. 8:301-306, 1983.

[26] M. Sridevi, K.V. Mallaiah, “Bioproduction of indole acetic acid by *Rhizobium* strains isolated from root nodules of green manure crop, *Sesbania sesban* (L.)”, Merr. Iranian J Biotech, 5 (3): 178-182, 2007.

[27] R. W. Rundel, “Ecological success in relation to plant form and function in the woody legumes: In [eds. Stirton C. H. and Zarucchi J. L.], Advances in legume biology”, Monographs in Systematic Botany from the Missouri Botanical Garden, **29:** 377–398,1989.

[28] D. McKey, “Legumes and nitrogen: the evolutionary ecology of a nitrogen-demanding lifestyle. In: Advances in legume systematics 5, the nitrogen factor (Eds. Sprent J. I. and McKey D.)”, Royal Botanic Gardens, Kew, pp.211–228, 1994.

[29] J. I. Sprent, “Nodulation in legumes”, Royal Botanic Gardens, Kew, UK, 2001.

[30] P. S. Herendeen, W. L. Crepet and D. L. Dilcher, “The fossil history of the Leguminosae: phylogenetic and biogeographic implications. In Advances in legume systematics (Eds. Herendeen P. S. and Dilcher D. L.), part 4, The fossil record”, Royal Botanic Gardens, Kew, UK, pp. 303–316, 1992.

[31] M. A. Matamoros, D. A. Dalton, J. Ramos, M. R. Clemente, M. C. Rubio and M. Becana, “Biochemistry and Molecular Biology of Antioxidants in the Rhizobia-Legume Symbiosis”,Plant Physiol.**, 133:** 499–509, 2003.

[32] P. H. Graham, “Nodule formation in legumesMicroorganisms, In: The Encyclopedia of Microbiology (Ed. Schaechter M. *Consulting Editor* LederbergJ.)” pp. 715-724, 2004.

[33] L. D. Kuykendall, “Rhizobiales. In: Bergey’s Manual of Systematic Bacteriology, Second Edition, Vol. II, Part C. (Editor-in-Chief Garrity G. M.)”. Springer, East Lansing, USA, pp. 324-574, 2005.

[34] L. Moulin, A. Munive, B. Dreyfus and C. Boivin-Masson, “Nodulation of legumes by members of the β-subclass of Proteobacteria”, Nature**411:** 948–950, 2001.

[35] F. B. Salisbury and C. W. Ross, “Plant Physiology”, 4th ed. Wadsworth Publishing Company, Belmont, California, pp. 357-406, 1992.

[36] C. P. Vance, “*Rhizobium* infection and nodulation: A benefitial plant disease”, Annu. Rev. Microbiol., **37:** 399-424, 1983.

[37] P. J. Dart, “Infection and development of leguminous nodules. In: A treatise on dinitrogen fixation”, sec. III, Biology. John Wiley and sons, Inc., New York, pp. 367-472, 1977.

[38] J. Dullaart, “The auxin content of root nodules and roots of *Alnus glutinosa* (L.)”, Vill. J. Exp. Bot.**, 21:** 975-984, 1970.

[39] P. S. De and P. S. Basu, “Content of different phytohormones and indole acetic acid metabolism in root nodules of *Derris scandens* BENTH”, J. Basic Microbiol.,**36:** 299-304, 1996.

[40] T. C. Ta and M. A. Faris, “Species variation in the fixation and transfer of nitrogen from legume associated grasses”,Plant Soil.**98:** 265-274, 1987.

[41] R. Remans, A. Croonenborghs, T. R. Gutierrez, J. Michiels and J. Vanderleyden, “Effects of plant growth-promoting rhizobacteria on nodulation of *Phaseolus vulgaris* L. are dependent on plant P nutrition”, Eur. J. Plant Pathol. **119:** 341-351, 2007.

[42] E. J. Gray and D. L. Smith, “Intracellular and extracellular PGPR: Commonalities and distinctions in the plant-bacterium signaling process”, Soil Biol. Biochem. **37:** 395-412, 2005.

[43] P. D. Kiely, J. M. Haynes, C. H. Higgins, A. Franks, G. L. Mark, J. P. Morrissey and F. O’Gara, “Exploiting new system-based strategies to elucidate plant-bacterial interactions in the rhizosphere”, Microbial Ecology, **51:** 257-266, 2006.

[44] L. C. van Loon, “Plant responses to plant growth-promoting rhizobacteria”, Eur. J. Plant Pathol., **119:** 243-254, 2007.

[45] S. Dobbelaere, J. Vanderleyden and Y. Okon, “Plant growth-promoting effects of diazotrophs in the rhizosphere”,Critical Rev. Plant Sciences, 22**:** 107-149, 2003.

[46] J. K. Vessey, “Plant growth promoting rhizobacteria as biofertilizers”, Plant and Soil**, 255:** 571-586, 2003.

[47] W. T. Frankenberger and M. Arshad, “Phytohormones in soils-microbial production and function”, Marcel Dekker, New York, 1995.

[48] C. M. J. Pieterse and L. C. van Loon, “Salicylic acid-independent plant defence pathways”, Trends in Plant Science, **4:** 52-58, 1999.

[49] K. E. Gibson, H. Kabayashi and G. C. Walker, “Molecular Determinants of a Symbiotic Chronic Infection”, Annu. Rev. Genet.**, 42:** 413-441, 2008.

[50] J. E. Olson and B. G. Rolfe, “Stem & root nodulation of the tropical legume *Sesbania rostrata* by *Rhizobium* strain ORS 571 & WE 7”, J. Plant Physiol.**, 121:** 199-210, 1985.

[51] S. Gucciardo, E. A. Rathbun, M. Shanks, M. Jenkyns, L. Mak, M. C. Durrant and N. J. Brewin, “Epitope tagging of legume root nodules extension modifies protein structure and cross linking in cell wall of transformed Tobacco leaves”, Amer. Phytopath. Soc.,**18***:* 24-32, 2004.

[52] W. J. [Deakin](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Deakin%20WJ%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus) and W. J. [Broughton](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Broughton%20WJ%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus) , “Symbiotic use of pathogenic strategies: rhizobial protein secretion system”, Nat. Rev. Microbiol*.* **7(4)**: 312-20, 2009.

[53] M. [Fauvart](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Fauvart%20M%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus) and J. [Michiels](http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Search&Term=%22Michiels%20J%22%5BAuthor%5D&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DiscoveryPanel.Pubmed_RVAbstractPlus),Rhizobial secreted proteins as determinants of host specificity in the rhizobium-legume symbiosis. [FEMS Microbiol Lett.](javascript:AL_get(this,%20'jour',%20'FEMS%20Microbiol%20Lett.');) **285(1):**1-9, 2008.

[54] V. González, P. Bustos, M. A. Ramírez-Romero, A. Medrano-Soto, H. Salgado, I. Hernández-González, J. C. Hernández-Celis, V. Quintero, G. Moreno-Hagelsieb, L. Girard, O. Rodríguez, M. Flores, M. A. Cevallos, J. Collado-Vides, D. Romero and G. Dávila, “The mosaic structure of the symbiotic plasmid of *Rhizobium* *etli* CFN42 and its relation to other symbiotic genome compartments”, Genome Biol. **4:** R 36, 2003.

[55] F. Sanchez, J. Padilla, H. Perez and M. Lane, “Control of nodulin genes in root nodule development and metabolism”,Annu. Rev. Plant Physiol. Plant Mol. Biol.**, 42:** 507-528, 1991.

[56] P-A. Vikman and J. Vessey, “Ontogenic changes in root nodule subpopulations of common bean (*Phaseolus vulgaris* L.) II. Protein content and carbohydrate pools”. J. Exp. Bot.**, 44:** 571-577, 1993.

[57] M. Matamoros, L. Baird, P. Escuredo, D. Dalton, F. Minchin, I. Iturbe-Ormaetxe, M. Rubio, J. Moran, A.Gordon and M. Becana, Stress-induced legume root nodule senescence. Physiological, Biochemical and Structural alterations”, Plant Physiol., **121:** 97-111, 1999.

[58] J. Muller, A. Wiemken and T. Boller, “Redifferentiation of bacteria isolated from *Lotus japonicus* root nodules colonized by *Rhizobium* sp. NGR 234”, J. Exp. Bot*.*, Vol. 52, no. 364, pp. 2181-2186, 2001.

[59] J. Muller, T. Boller and A. Wiemken, “Trehalose becomes the most abundant non-structural carbohydrate during senescence of soybean nodules”, J. Exp. Bot., **52:** 943-947, 2001.

[60] E. Libbert, S. Wichner, U. Schiewer, H. Risch and W.Kaiser, „The influence of epiphytic bacteria on auxin metabolism”, Planta **68:** 327–344, 1966.

[61] S. Manulis and I.Barash, “*Pantoea agglomerans* pvs. gypsophilae and betae, recently evolved pathogens”, Mol. Plant Pathol.**, 4:**307–314, 2003.

[62] S. E. Silverstone, D. G. Gilchrist, R. M. Bostock and T. Kosuge, “The 73-kb pIAA plasmid increases competitive fitness of *Pseudomonas syringae* subspecies *savastanoi* in oleander”, Can. J. Microbiol., **39:** 659–664, 1993.

[63] S. E. Lindow, C. Desurmont, R. Elkins, G. McGourty, E. Clark and M. T.Brandl, “Occurrence of indole-3-acetic acid-producing bacteria on pear trees and their association with fruit russet”, Phytopath., **88:** 1149–1157, 1998.

[64] C. L. Patten and B. R.Glick, “Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system”, Appl. Env. Microbiol., **68:** 3795–3801, 2002.

[65] M. Lambrecht, Y. Okon, A. VandeBrock and J. Vanderleyden, “Indole-3-acetic acid; a reciprocal signaling molecule in bacteria-plant interactions”,Trends Microbiol., **8:** 298-300, 2000.

[66] R. Prusty, P. Grisafi and G. R. Fink, **“**The plant hormone indoleacetic acid induces invasive growth in *Saccharomyces cerevisiae*”, Proc. Natl. Acad. Sci.**, 101:** 4153–4157, 2004.

[67] J. H. J. Leveau and S. E. Lindow, “Utilization of the Plant Hormone Indole-3-Acetic Acid for Growth by *Pseudomonas putida* Strain 1290”, Appl. Env. Microbiol.**, 71 (5):** 2365–2371, 2005.

[68] F. Persello-Cartieaux, L. Nussaume and C.Robaglia, “Tales from the underground: molecular plant-rhizobacteria interactions”, Plant Cell Env., **26:**189–199, 2003.

[69] F. J. de Bruijn, “The unusual symbiosis between the diazotrophic stem nodulating bacterium *Azorhizobium caulinodans* ORS 571 and its host, the tropical legume *Sesbania rostrata*. In: Plant-Microbe Interaction. (Eds. Kosuge T. and Nester E. W.)”, McGraw-Hill Publishing Company, New York, pp. 457-504, 1989.

[70] J. Klaczkowska, P. S. Nutman, F. A. Skinner and J. M. Vincent, “The identification and characterization of *Rhizobium*. In: Identification Methods for Microbiologists. Part B. (Eds. Gibb B. K. and Shepton D. A.)”, Academic Press, London and New York, pp. 51-65, 1968.

[71] M. Alexander, “Introduction to Soil Microbiology”, 2nd ed. John Wiley and Sons, Inc. New York, pp. 305-330, 1977.

[72] B. D. W. Jarvis, A. G. Dick and R. M. Greenwood, “DNA homology among strains of *Rhizobium trifolii* and related species”, Int. J. Syst. Bacteriol.**30:** 42-52, 1980.

[73] F. W. Went, Wichstoff and Wachstum, Recl. Trav. Bot. Neerl. **25:** 1- 116, 1928.

[74] A. C. Leopold and P. E. Kriedemann, “Plant Growth and Development”, 2nd ed. Tata Mc Graw-Hill Publ. Co. Ltd., New Delhi, pp. 109-194, 1975.

[75] J. P. Nitsch and C. Nitsch, “Studies on the growth of the coleptile and first internode sections. A new sensitive straight growth test for auxins”, Plant physiol.**31:** 94-111, 1956.

[76] H. Mohr and P. Schopfer, Plant physiology. Springer Verlag, Berlin, 1995.

[77] A. Ostin, N. Ilic and J. D. Cohen, “An *in vitro* system from maize seedlings for tryptophan-independent IAA biosynthesis”, Plant Physiol.**119(1):** 173-178, 1999.

[78] M. N. V. Williams and E. R. Signer, “Metabolism of tryptophan and tryptophan analogs by *Rhizobium meliloti*”, Plant Physiol. **92:** 1009-1013, 1990.

[79] A. Costacurta and J. Vanderleyden, “Synthesis of phytohormones by plant associated bacteria”, Critt. Rev. Microbiol. **21(1):** 1-18, 1995.

[80] A. Ernstsen, G. Sandberg, A. Crozier and C. T. Wheeler, “Endogenous indoles and the biosynthesis and metabolism of IAA in cultures of *Rhizobium phaseoli*”,Planta**171:** 422-428, 1987.

[81] M. Kobayashi, T. Suzuki, T. Fujita, M. Masuda and S. Shimizu, “Occurrence of enzymes involved in biosynthesis of IAA from indole-3-acetonitrile in plant-associated bacteria, *Agrobacterium* and *Rhizobium*”,Proc. Natl. Acad. Sci.,**92:** 714-718, 1995.

[82] S. Yoshida and M. Yatazawa, “Species characteristics and cultural conditions as affecting to rhizobial production of IAA”, Nippon Dojo Hiryogaku Zasshi **44:** 63-66, 1973.

[83] P. K. Bhowmick and P. S Basu, “Indole acetic acid production by *Rhizobium* sp. from a leguminous tree, *Erythrinna indica*”,Folia Microbiol. **32:** 140-148, 1987.

[84] S. Chakraborti, M. S. Lee and A. H. Gibson, “Diversity in nutritional requirements of strains of various *Rhizobium* species”, Soil Biol. Biochem.**, 13(5):** 349-354, 1981.

[85] P. S. De and P. S. Basu, “Growth behaviour and IAA production by a *Rhizobium* sp. isolated from root nodules of a leguminous medicinal herb *Tephrosia purpurea* Pers., in culture”, Microbiol. Res., **151**: 71-76, 1996.

[86] G. H. Elkan and I. Ewik, “Nitrogen, energy and vitamin nutrition of *Rhizobium japonicum*”,J. Appl. Bacteriol., **31:** 399-404, 1968.

[87] M. Roy and P. S. Basu, “Production of 3-indole acetic acid by a *Rhizobium* sp. from *Mimosa pudica*”,Folia Microbiol., **34:** 120-126, 1989.

[88] S. Ghosh, P. K. Ghosh and T. K. Maiti, “Production and Metabolism of Indole Acetic Acid (IAA) by root nodule bacteria (Rhizobium): A review”, J. Pure & Appl. Microbiol., 5(2), 2011.

[89] S. Ghosh, P. K. Ghosh and T. K. Maiti, “Production of Indole Acetic Acid by 5-Fluro-tryptophan resistant mutant of *Rhizobium* sp. isolated from root nodule of pulse legume  *Phaseolus mungo* L.”, Int. J. of Pharm. and Bio Sci., 5(2): (B) 206-213, 2014.

[90] E. A. Tsavkelova, T. A. Cherdyntseva, A. I. Netrusov, “Auxin production by bacteria associated with orchid roots”, Mikrobiologiia,74(1):55-62, 2005.

[91] M. N. V. Williams, E. R. Signer, “Metabolism of tryptophan and tryptophan analogs by *Rhizobium meliloti*”,Plant Physiol., 92: 1009-1013, 1990.