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Production and Metabolism of Indole Acetic Acid (IAA) by Root Nodule Bacteria (*Rhizobium*): A Review

Sisir Ghosh, Pallab Ghosh and T.K. Maiti*

Microbiology Laboratory, Department of Botany, The University of Burdwan, Golapbag, Rajbati, Burdwan - 713 104 India.

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The production of indole acetic acid (IAA) by the root nodule bacteria is considered to have many important physiological implications. The biosynthesis, enzymatic oxidation and metabolism of IAA in *Rhizobium* have been reviewed. The existence of two different types of IAA biosynthesis pathways in plants and two different types of IAA biosynthesis pathways in *Rhizobium* and factors influencing the biosynthesis of its have described. The biological activity of the oxidation products of IAA and production of IAA in culture has also been discussed. Role of ACC deaminase on IAA metabolism, and VAM induced IAA production and role of IAA in legume-*Rhizobium* symbiosis are also critically reviewed. Isolation, identification and estimation of IAA by rhizobacteria have also been included with 140 references. 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* association.

Key words: Indole Acetic Acid (IAA), Root nodule, Rhizobacteria, Legume-*Rhizobium* symbiosis.

Indole acetic acid (IAA), earlier known as heteroauxin, was isolated from urine[1], cultures of yeast plasmolysate[2], *Rhizopus suinus*[3] and a large number of higher plants[4]. The term auxin (Greek 'auxein', "to increase") was first used by Went (1928)[5], who discovered that some unidentified compound probably caused curvature of Oat coleoptiles towards light[6]. Since the 1930's when K. V. Thimann (1935)[3] first observed the synthesis of IAA in the mould *Rhizopus suinus*, which had been fed the amino acid tryptophan[7].

Many physiological activities of plants are regulated by a variety of plant growth regulators and phytohormones. A large amount of information regarding phytohormones, nitrogen fixation, nodules (development, morphogenesis and physiology) and hormone production by microorganisms had enriched the literature from time to time. Among which IAA is play a critical role in nodule and nodule bacteria for the nodule development and symbiotic relationship. IAA has been implicated in virtually all aspects of plant growth and development[8, 9].

Recently advancement in understanding the IAA signaling pathway in plants has been established. The advancement in plant IAA signaling has also intensified research on the various aspects of bacterial IAA synthesis, including its role in bacteria-plant interactions. The role of IAA in plant-microorganisms interactions appears diverse in different bacterial species. The detail biochemical pathways of bacterial IAA synthesis and their regulation have provided some

* To whom all correspondence should be addressed.
E-mail: tkmmicrobiology@gmail.com
tkmbu@yahoo.co.in

clues on the possible interactions between plants and IAA producing bacteria. The present review mainly includes the IAA bioproduction and metabolism by Rhizobacteria and their role in physiology of root and nodule in legume plants.

Metabolism of IAA

Biosynthesis of IAA

German botanist Julius Van Sachs (1832-1897) proposed that chemical messenger is responsible for formation and growth of different plant organs. After that Charles Darwin performed phototrophism experiment in which demonstrated the presence of a transported signal originating from the plant apex, able to promote differential cell elongation in the seedling lower parts resulting in its bending toward the light source[10]. This signal was subsequently isolated and named auxin by Went (1935)[11]. Since the 1930's when K.V. Thimann first observed the synthesis of IAA in the mould *Rhizopus suinus*, which had been fed the amino acid tryptophan[7].

Several pathways are known regarding IAA biosynthesis. In higher plants, synthesis of IAA occurs in three steps, beginning with the conversion of tryptophan involving donation of the amino group to a á-keto acid by a transamination reaction to form indole-3-pyruvic acid and then decarboxylation of indole-3-pyruvate to form indole-3-acetaldehyde, oxidized to IAA in the last step[6, 7] (Fig- I)[12]. Indole-3-acetaldehyde may also be reversibly reduced to indole-3-ethanol. The second pathway involved an initial decarboxylation of tryptophan to tryptamine[7]. Tryptamine was converted to indole-3-acetaldehyde by amine oxidase, which was in turn oxidized to IAA (Fig -I)[12]. However, mutants of maize deficient in tryptophan synthesis formed large amounts of IAA, although tryptophan synthesis was blocked. Possibly there were alternative pathways for IAA synthesis in different plants[13]. Ostin *et al*. (1999)[14] reported the presence of tryptophan independent pathway of IAA synthesis in maize (Fig -II)[12]. Biosynthesis of IAA via indole-3-pyruvate (IPA) and indole-3-acetaldhyde pathways were found to occur in *Rhizobium meliloti*[15] and in *Bradyrhizobium* sp.[16]. *Rhizobium leguminosarum* bv. *phaseoli* was shown to convert tryptophan to indole-3-ethanol (Ieth), IAA and indole-3-methanol (IM); Ieth to IAA and IM; and IAA to IM[17]. Kobayashi *et al*. (1995)[18] proposed a pathway; common in plant associated bacteria, inwhich indole-3-acetonitrile (IAN) was converted to indole-3-acetamide (IAM) by nitrile hydratase and IAM was then converted to IAA by amidase in *Rhizobium* spp. Fast growing *Rhizobium* spp. and *Bradyrhizobium* sp. produced IAA as well as a number of IAA precursors in free living state[17, 19].

The biosynthesis of IAA is not restricted to only root nodule bacteria, it also exist in different bacteria including plant growth promoting Rhizobacteria. However there are different IAA biosynthesis pathway exist in different bacteria[20]. The overview of bacterial IAA biosynthesis pathways is given in Figure-III [20]. Tryptophan has been identified as a main precursor for IAA biosynthesis in bacteria. There are following two types of IAA biosynthetic pathway exist in different species of *Rhizobium*.

Indole-3-acetamide (IAM) pathway

Indole-3-acetamide (IAM) pathway is a two step pathway where tryptophan is first converted to IAM by the enzyme tryptophan – 2-monooxygenase encoded by the *iaaM* gene. Later IAM is converted to IAA by the enzyme IAM hydrolase encoded by the gene *iaaH* (Fig – III). The gene *iaaM* and *iaaH* have been cloned and characterized in *Rhizobium* sp. and *Bradyrhizobium* sp. The IAM pathway was described previously as bacterial specific pathway, but Pollmann (2002, 2003)[21, 22] first stated that this pathway also operated in plant *Arabidopsis thaliana.*

Indole-3-pyruvate (IPyA) pathway

Indole-3-pyruvate (IPyA) pathway is considered as major pathway for biosynthesis in plants. But the key genes or enzymes have not been identified yet in plants. In the first step tryptophan is converted into IPyA by the enzyme aminotransferase through transamination. The second step is rate limiting step where IPyA is decarboxylated to indole -3-acetaldehyde (IAAld) by indole-3-pyruvate decarboxylase. In the last step IAAld is oxidized in IAA (Fig- III) [20]. This pathway has been described in a broad range of bacteria like *Bradyrhizobium, Rhizobium, Azospirillum, Enterobacter cloacae* and cyanobacteria.

Factors influence IAA biosynthesis in bacteria

There are several factors alters the levels

of IAA biosynthesis and / or the IAA biosynthesis gene expression in bacteria. But it is not possible to integrate these factors into a comprehensive regulatory scheme of IAA biosynthesis in bacteria. This is may be due to the diversity of regulation of IAA biosynthesis across bacteria and also lack of integrated studies following IAA expression in different bacteria and different environments.

Environmental factors modulating IAA biosynthesis

IAA biosynthesis in different bacteria is related to environmental stress including pH[23], osmotic and matrix stress[24] and carbon limitation[23, 25]. Although the diversity of stress factors associated with modulating IAA expression in different bacteria, a common feature can be identified. The expression of IAA biosynthesis genes are in a fine tuned to encounter environmental stresses associated with the soil and plant environment. A typical rhizosphere environment is acidic pH due to proton extrusion through membranes of root cells. Similarly, matrix potential is an important condition in the environment of epiphytic bacteria[24].

Another class of factors regulating bacterial IAA production are contains of plant extracts. In the symbiotic bacteria *Rhizobium* sp., flavonoids produced by the host plant stimulate IAA production in a NodD1-, NodD2-, and SyrM2-dependent manner.NodD1, NodD2 and SyrM2 form a regulatory cascade that coordinates the expression of genes under influence of flavonoids[26]. In *Xanthomonas axonopodis* IAA production increased in the presence of plant leaf extracts from *Citrus cinensis*. The question as to which specific compound is responsible for this induction remains unclear[27].

Tryptophan is an important molecule that alter the level of IAA and identified as the main precursor for IAA .The addition of exogenous tryptophan was shown to increase the IAA production in various bacteria[28, 29] . There are two sources of tryptophan in rhizosphere viz. one from degrading root and microbial cells[30] and other from root exudates[31]. Although the amount of tryptophan in root exudates is rather low, exogenous tryptophan can be efficiently absorbed by bacteria. The K_m value indicate that the affinity of the transporter for tryptophan is low and the affinity of bacteria for tryptophan is high which makes them good scavengers for tryptophan even at low concentration[32]. Besides the role of the precursor tryptophan in the regulation of IAA production, IAA biosynthesis can also be modulated by the end-product IAA and by its intermediates[18].

Genetic factors modulating IAA biosynthesis

There are different genetic factors have been involved in level of IAA biosynthesis in bacteria firstly, the location of IAA biosynthesis gene in the genome, either plasmid[33] or chromosomal[34] has been shown to modulate the level of IAA production. Secondly, the mode of expression, constitutive or induced of IAA biosynthesis genes was observed to differ across biosynthesis pathways and across bacterial species. Further, RpoS – transcriptional regulators, regulates transcription of genes in response to stress conditions and starvation and the two component system GacS/GacA controls the expression of genes[35, 36].

Role of ACC deaminase on IAA metabolism in *Rhizobium*

The enzyme ACC deaminase cleaves ACC, the immediate precursor of ethylene in plants, to form ammonia and a- ketobutyrate. This multimeric enzyme is a common component of many soil microorganisms, both bacteria and fungi. The pioneering work in elaborating the biochemical properties of ACC deaminase is largely the results of studies carried out by Honma and his co-worker[37, 38]. ACC deaminase is a multimeric enzyme (homodimeric or homotrimeric) with a subunit molecular mass of approximately 35-42 kDa. It is a sulfhydryl enzyme in which one molecule of the essential co-factor pyridoxal phosphate (PLP) is tightly bound to each subunit. Interestingly, the enzyme ACC deaminase is cytoplasmically localized[39] so that the substrate ACC must be exuded by plant tissues[40] and subsequently taken up by an ACC deaminase-containing microorganism before it is cleaved[41]. The PLP-dependent enzymes catalyze a wide variety of biochemical reactions, many of which are involved in the metabolism of amino acids. In most of these reactions two basic chemical properties of the PLP have been conserved: (i) PLP forms an external aldimine between its aldehyde group and the or-amino group of the substrates and (ii) PLP acts as an electron sink, withdrawing electrons from the substrate[42]. In higher plants, the

enzyme S-adenosyl-L-methionine (SAM) synthetase catalyzes the conversion of methionine to SAM[43]; ACC synthase catalyzes the hydrolysis of SAM to ACC and 5'methylthioadenosine[44]; and ACC oxidase catalyzes the conversion of ACC to ethylene, carbon dioxide, and hydrogen cyanide[42].

The plant hormone ethylene plays an important role in root initiation and elongation, nodulation, senescence, abscission and ripening as well as in stress signaling[45]. During periods of environmental stress, plants produce high levels of "stress ethylene". Moreover, much of the growth inhibition that occurs as a consequence of an environmental stress is the result of the response of the plant to the increased levels of stress ethylene which exacerbates the response to the stressor. In addition, inhibitors of ethylene synthesis can significantly decrease the severity of some environmental stresses. Thus, if ACC deaminase-containing bacteria can lower plant ethylene levels, treatment of plants with these organisms should provide some protection against the inhibitory effects of these stresses.

The ACC deaminase- containing plant growth-promoting bacteria bind to the surface of either the seed or root of a developing plant-some endophytic bacteria are also located inside of the plant root (Fig.-IV)[41]. In response to root exudates, including the amino acid tryptophan, the bacteria synthesize indoleacetic acid (IAA). Plant cells take up some of the IAA that is secreted by the bacteria and, together with the endogenous plant IAA, can stimulate plant cell proliferation and/or elongation as well as induce the synthesis of the enzyme ACC synthase. Some of the ACC, already present or newly synthesized by the plant, is exuded and taken up by the ACC deaminase-containing bacteria. This ACC is cleaved by ACC deaminase to form ammonia and alpha-ketobutyrate, both of which are readily metabolized by the bacteria. As a

Table 1. IAA content in root nodules of some legumes and IAA production by their symbionts

Name of the Plant	Name of the symbiont	Max IAA present in root nodules (µg/g)	Max IAA produced by the symbiont (µg/ml)	Reported by
Pongamia pinnata	*Rhizobium sp.*	206	-	Sinha *et al*. (1981)[118]
Phaseolus aureus	*Rhizobium sp.* (P5)	7.0	169.29	Dangar (1987)[119]
Pterocarpus mursupium	*Rhizobium sp.* (M9)	11.5	160.18	
Lens esculenta	*Rhizobium sp.* (L1)	14.5	187.47	
Butea monosperma	*Rhizobium sp.* (B3)	21.0	188.99	
Erythrina indica	*Rhizobium sp.*	65.0	152	Bhowmick (1987)[120]
Sesbania grandiflora	*Rhizobium sp.*	50.0	152.0	
Pterocarpus santalinus	*Rhizobium sp.*	46.86	131	
Samanea saman	*Rhizobium sp.*	5.4	83.0	Chattopadhyay (1989)[121]
Dalbergia sisso	*Rhizobium sp.*	4.2	89.5	
Crotalaria juncea	*Rhizobium sp.*	3.8	53.5	
Arachis hypogaea	*Rhizobium sp.*	2.5	102.2	Roy (1990)[122]
Clitoria ternatea	*Rhizobium sp.*	3.77	47.1	
Erythrina indica	*Rhizobium sp.*	42.0	82.0	
Mimosa pudica	*Rhizobium sp.*	2.27	60.0	
Dalbergia lanceolaria	*Rhizobium sp.*	7.42±0.09	82.7±2.0	Ghosh (1999)[123]
Aschynomene aspera	*Rhizobium sp.*	25.4±0.04	50.9±1.0	
Roystonia regia	*Rhizobium sp.*	16.90±0.95	122.0±2.0	
Melilotus alba	*Rhizobium sp.*	13.1±0.35	298.3±3.0	Datta (2000)[124]
Dolichos biflorus	*Rhizobium sp.*	5.3±0.11	309.7±2.75	
Cajanus cajan	*Rhizobium sp.*	6.7±0.15	168.0±1.55	
Phaseolus mungo	*Rhizobium sp.*	22.2	162	Ghosh *et al*. (2008)[29]
Crotalaria saltiana	*Rhizobium sp.*		212.5	Mazumdar et al. (2008)[138]
Vigna mungo	*Rhizobium sp.*	-	-	Mondal et al.(2009)[139]

consequence of lowering the level of ACC within a plant, the amount of ethylene that can form is reduced. Thus, the net result of the interaction of ACC deaminase- containing plant growth-promoting bacteria with plant cells is that the bacteria act as a sink for ACC. Direct consequences of this interaction are significantly increased plant root and shoot length, an increase in biomass, and protection of plants from inhibitory effects of ethylene synthesized as a direct consequence of a variety of biotic and abiotic stresses. In practice, ACC deaminase-containing plant growth promoting bacteria have been used to protect plants against growth inhibition caused by: flooding, both in the laboratory[46] and in the field[47]; the presence of organic toxicants such as polyaromatic hydrocarbons (PAHs), polycyclic biphenyls (PCBs) and total petroleum hydrocarbons (TPHs) both in the laboratory[48, 49]; the presence of a variety of different metals including nickel, lead, zinc, copper, cadmium, cobalt and arsenic, both in the laboratory[50] and high salt[51]; phytopathogens[52]; drought[53]; and infection of plant roots by various strains of Rhizobia[54].

While it is well known that IAA can activate the transcription of ACC synthase[55], it is less well known that ethylene may inhibit IAA transport and signal transduction[56]. This feedback loop of ethylene inhibition of IAA synthesis and/or functioning limits the amount of ACC synthase, ACC and, ultimately, ethylene following every stressful event of the plant. When an ACC deaminase- containing plant growth-promoting bacterium lowers the ethylene concentration in plant roots, this relieves the ethylene repression of auxin response factor synthesis, and indirectly increases plant growth[57]. Thus, ACC deaminase-containing plant growth-promoting bacteria facilitate plant growth by (i) decreasing ethylene inhibition of various plant processes and (ii) permitting IAA stimulation of cell proliferation and elongation without the negative effects of increasing ACC synthase and plant ethylene levels.

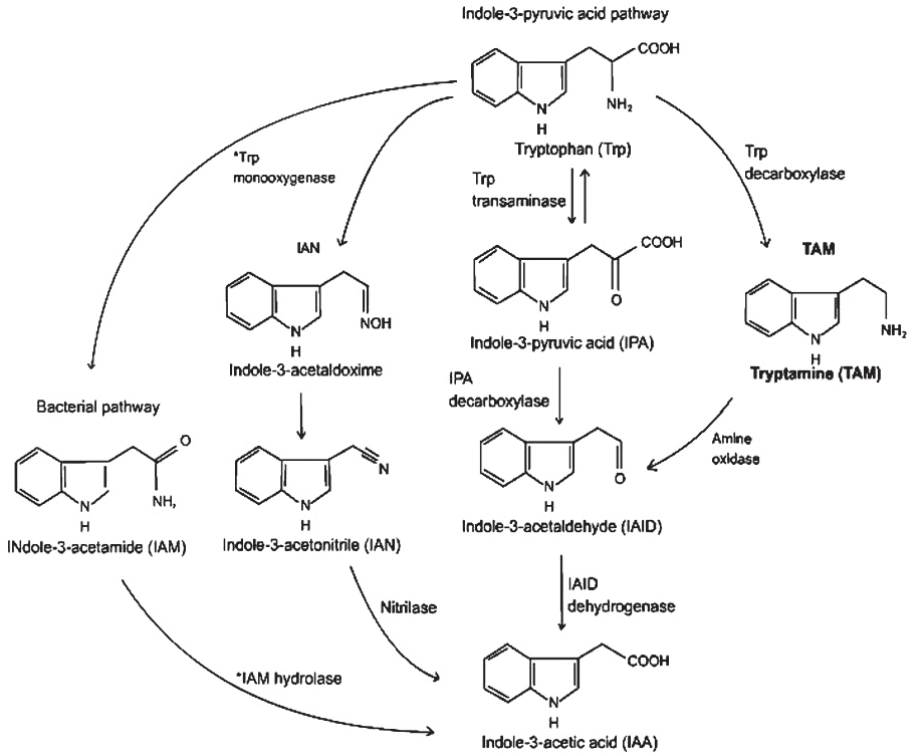


Fig. 1. Tryptohan-dependent pathways of IAA biosysthesis in plants and bacteria.
The enzymes that are present only in bacteria are marked with an asterisk (After Bartel 1997).

VAM induced IAA production in root nodule

The nitrogen-fixing interaction between bacterial members of Rhizobiaceae and legumes are the two most commonly studied symbioses[58].The association between *Rhizobium* sp., legumes and that between vesicular arbuscular mycorrhizal fungi (VAM)[59] and most of the land plants display a remarkable degree of similarity. Both the events of symbiosis involve the recognition of the host for entry and coexistence within the plant roots, with the development of a specialized interface that always separates the two partners and at which nutrient exchange occurs. Development of both the fungal and bacterial symbioses can be explained as an analogous progression of events like attraction, recognition, contact, entry, growth, development, and differentiation. The wide spread occurrence of VAM fungi in nodulated legumes and the mycorrhizal role in improving nodulation and *Rhizobium* activity within the nodules are universally recognized[60]. The beneficial effect of VAM in symbiotic activity of *Rhizobium* and in nodulation and N_2 fixation is due to the relatively high phosphorus demand during N_2 fixation process by *Rhizobium* which is mitigated with

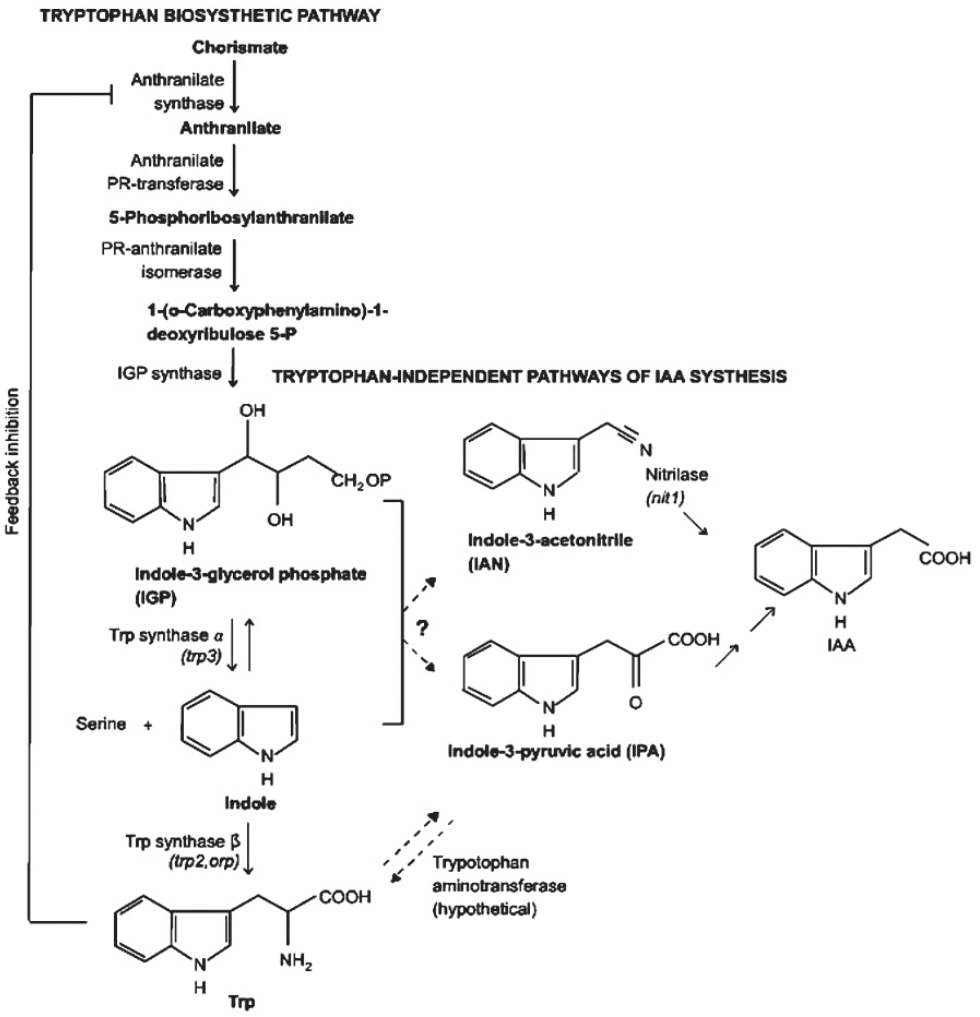


Fig. 2. Tryptopahan-dependent pathways of IAA biosysthesis in plants. The tryptophan (Trp) biosysthetic pathway is shown on the left. Mutants are indicated in parentheses is uncertain (indole-3-glycerol phosphate or indole), and IAN and IPA are two possible intermediates. PR, phosphoribosly (After Bartel 1997)

Phosphorous supplied by the mycorrhizal fungus. However, nutrients other than P, such as Zn, Cu, Mo, and Ca can affect the infectivity and symbiotic efficacy of *Rhizobium*. Enhanced uptake of different nutrients like P, K, N, Ca [61], Zn and S [62] by the VAM fungi has been demonstrated using radioactive isotopes. Therefore, enhanced uptake of these elements could also be involved in the synergistic interaction of VAM fungus and *Rhizobium* conversely. Supply of Nitrogen by N_2 fixation, as carried out by rhizobial activivity, could be critical to maintain a balanced physiological status in the host plant, which is important for mycorrhiza formation and functioning[63]. Dual effect of VAM fungus, *Glomus fasciculatum* and the nitrogen-fixing bacteria, *Rhizobium* sp. on the nodulation of roots of *V. mungo* was studied. It was recorded that the roots which were inoculated simultaneously with both the symbionts i.e., *G. fasciculatum* and *Rhizobium* exhibited greater amount of IAA production than the non-inoculated roots[64].

Enzymatic oxidation IAA, inhibitors and co-factors of the enzyme

IAA in aqueous solution is readily degraded by a variety of agents, including acids, ultraviolet rays and ionizing radiation, visible light, and in the presence of sensitizing pigments such as riboflavin. The most prevalent form of IAA degradation, however, appears due to oxygen and peroxides either separately or in combination, in the presence of a suitable redox system[7]. It became clear that plants synthesize, inactivate, and catabolize IAA by multiple pathways and multiple genes could encode a particular enzyme within a pathway[65]. IAA is destructed by two basic oxidative processes: enzymatic reactions and photooxidation[66]. There are two pathways of enzymatic IAA catabolism (Fig V). The first involved oxidation by oxygen and loss of the carboxyl group as carbon dioxide. The products were variable, but 3-methyleneoxindole (Meox) was usually a principal one[6] and other reported products of oxidation of IAA were 3-

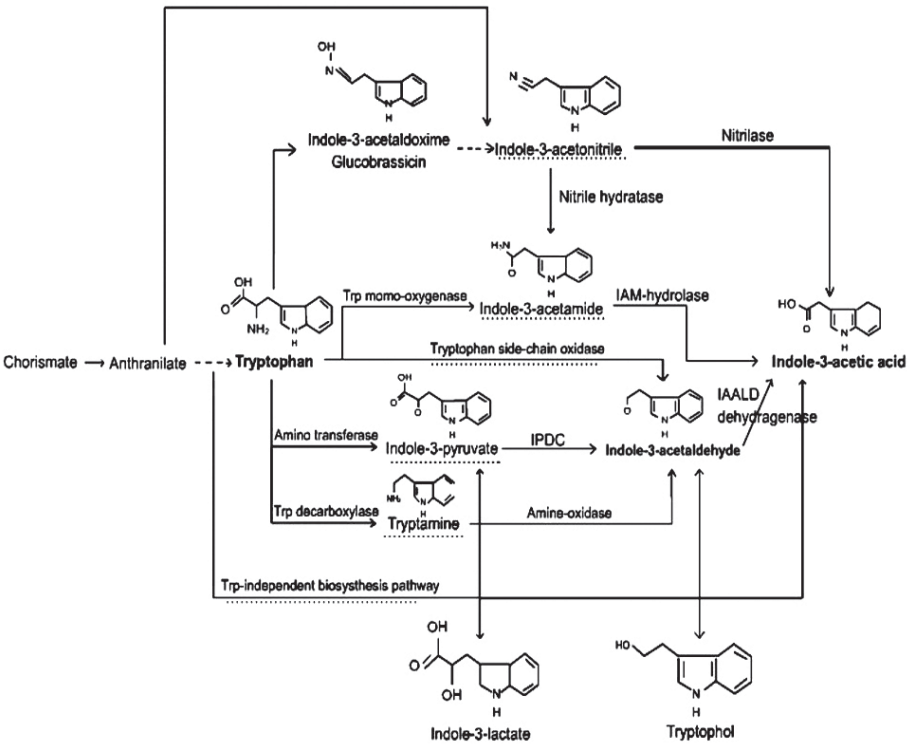


Fig. 3. Overview of the different pathways to synthesize IAA in bacteria. The intermediate referring to the name of pathway of the pathway itself is underlined with a dashed line. IAAId, indole-3-acetaldehyde; IAM, indole-3-acetamide, IPDC, indole-3-pyruvate decraboxylase, Trp, tryptophan (after Spaepen et al 2007)

hydroxymethyl oxindole (HMO), indole-3-carboxylic acid[66], oxindole-3-acetic acid (OxIAA), indole-3-acetyl aspartic acid (IAAsp) and indole-3-acetyl glutamate (IAGlu)[67]. Formation of indole-3-methanol was also reported by Gazaryan *et al*. (1999)[68]. The enzyme that catalyzed this reaction was IAA oxidase. In plants Meox was formed by enzymatic conversion of HMO[69]. Meox was enzymatically converted into 3-methyl oxindole[70]. Level of IAA is regulated in tissues by synthesis, formation of conjugated auxin, or by degradation.

The enzyme peroxidase, in concert with a flavoprotein, was shown to catalyze the oxidation of IAA releasing CO_2. Oxidative decarboxylation of IAA is now known to be catalyzed by peroxidases from a variety of plant sources. There are dissimilar requirements for enzymes from different sources and even multiple isozymes within a single species[7]. The ratio of the various products depended on several factors including enzyme substrate ratio, co-factors and pH of the reaction[25]. IAA oxidase from higher plants required Mn^{+2}.

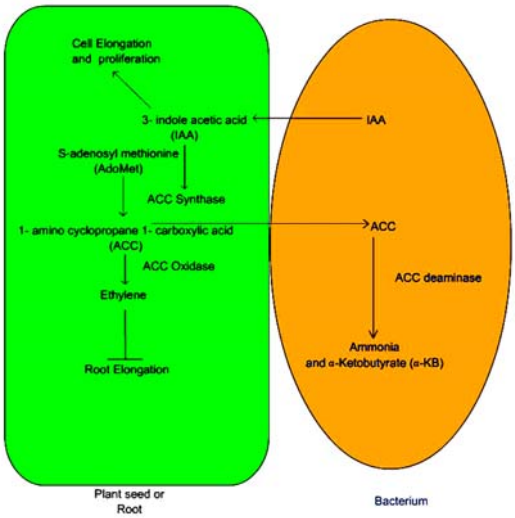


Fig. 4. Schematic representation of how a plant growth promoting bacterium bound to either a seed or a plant root lowers the ethylen concentration and there by prevents ethylene inhibition of root elongation. The arrows indicate chemical orphysical steps in the mechanisms and the symbol-indicates inhibition of root elongation by ethylene (Adopted from Glicet al. and J. Thear. Biol 1998)

The reaction catalysed by IAA oxidase was accelerated by monophenol (e.g. 2, 4-dichlorophenol) and inhibited by orthodiphenols such as catechol and pyrogallol. Flavonols acted in the same way, as kaempferol promoted and quercetin inhibited IAA oxidase. Rigaud and Puppo (1975)[71] reported an IAA oxidase activity in preparations of soybean bacteroids and in cytosol from root nodules. In a study with beech and horseradish, for example, 20 peroxidase isozymes were found, and all had IAA-oxidase activity[72]. Synthetic auxins and conjugated auxins were also resistant to IAA oxidases. The horseradish peroxidase system catalyzed oxidative decarboxylation without added cofactors, although manganese ion, simple monophenols, and m-diphenols increased the reaction rate. On the other hand, p-diphenols, o-diphenols and polyphenols generally inhibited IAA oxidation[7]. Hare (1964)[73] reported that IAA oxidase and peroxidase activities were found by the same enzyme. Presence of both peroxidase and non peroxdative forms of IAA-oxidase were also reported[74]. Several IAA oxidase isozymes were present, and all or nearly all were identical to the peroxidases[6].

IAA could be oxidized via two mechanisms: a conventional hydrogen-peroxide-dependent pathway, and other one that was hydrogen-peroxide independent and required oxygen. Only plant peroxidases were able to catalyze the reaction of IAA oxidation with molecular oxygen[75]. Presence of hydrogen peroxide-independent pathway was also demonstrated in peanut by Gazaryan *et al.* (1999)[68].

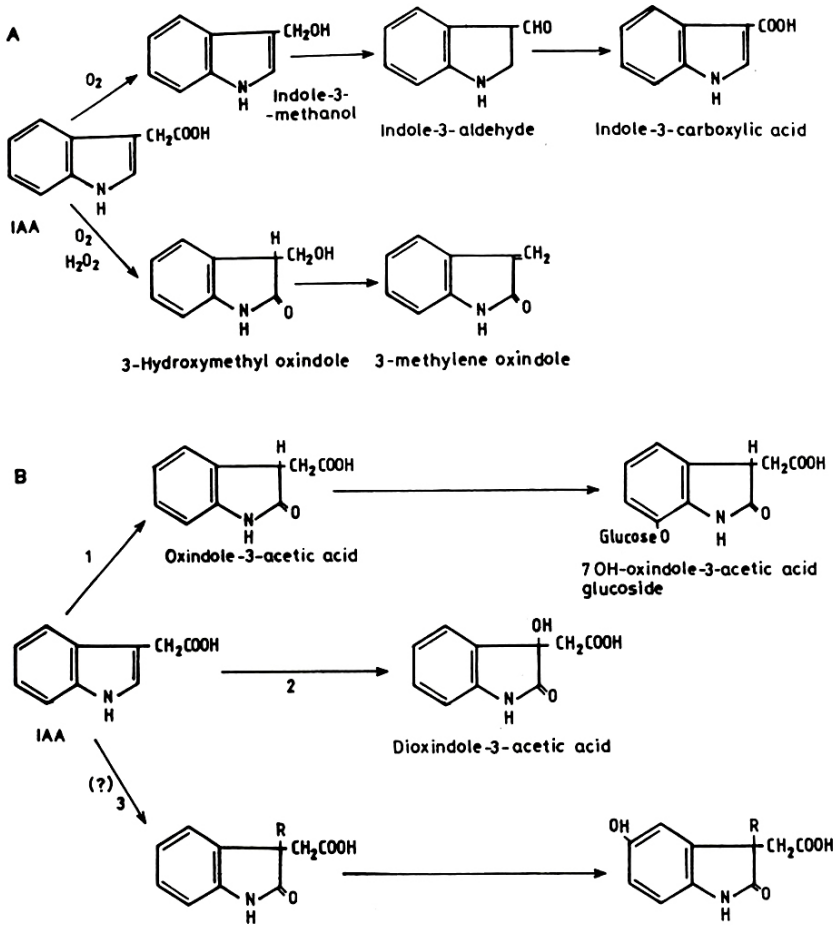
A second pathway for degradation of IAA had been found to occur in dicots and monocots[76]. In this pathway the carboxyl group of IAA was not removed, but carbon 2 of the heterocyclic ring was oxidized to form oxindole-3-acetic acid. Also, dioxiindole-3-acetic acid exists in various species, and it had been oxidized both at carbons 2 and 3 of the heterocyclic ring. Details of this degradative pathway are still unknown[6]. Besides enzymatic oxidation, IAA could be oxidized by photooxidation. Riboflavin, eosin and other fluorescent dyes were effective catalyst for photooxidation of IAA. Products of photooxidation of IAA were like that of enzymatic reaction[66]. IAA oxidase activity is generally higher in older, non growing tissues than then it is in

younger, actively growing tisssues, which have a high auxin requirement[7].

Although much is known about bacterial IAA producers (BIPs), little is known about bacteria that have the converse property i.e. the ability to destroy IAA. This is surprising given the early realization that, like BIPs, bacterial IAA degraders (BIDs) are abundantly associated with plants[77]. Actually, BIDs were also considered a source of contamination, since their ability to destroy IAA obscured true levels of IAA in plant tissues[78]. Ironically, there have been more studies dedicated to successfully eliminate BID activity in measurements of plant IAA, e.g., by the use of antibiotics[77] than there have been serious attempts for trying to understand the abundance, origin, and significance of these bacteria and their unique ability to destroy IAA[79].

Effect of phenol and phenol metabolizing enzyme on IAA production

The degradation of IAA was catalysed by IAA oxidase. The enzyme IAA oxidase was accelerated by monophenol (e.g. 2, 4-dichlorophenol) and inhibited by orthodiphenols such as catechol and pyrogallol. More phenol in root nodules provided more di- and polyphenols



A) Oxidative decarboxylation pathway for IAA
B) Nondecarboxylation oxidative pathway for IAA in *Zea mays* (1), *Vicia faba* (2) and *Oryza sativa* (3) (after Moore, 1989).

Fig. 5. Pathways of IAA catabolism

as inhibitors of IAA oxidase[66] which superseded the activity of monophenols having some promotive effect on the enzyme. The levels of peroxidase and polyphenol oxidase generally changed inversely with the phenol level both in nodules and roots and these enzymes were less active in nodules, a condition which favoured the higher phenol level in root nodules[80]. Flavonols acted in the same way, as kaempferol promoted and quercetin inhibited IAA oxidase. Rigaud and Puppo (1975)[71] reported an IAA oxidase activity in preparations of soybean bacteroids and in cytosol from root nodules. In a study with beech and horseradish, for example, 20 peroxidase isozymes were found, and all had IAA-oxidase activity[72]. Synthetic auxins and conjugated auxins were also resistant to IAA oxidases. The horseradish peroxidase system catalyzed oxidative decarboxylation without added cofactors, although manganese ion, simple monophenols, and m-diphenols increased the reaction rate. On the other hand, p-diphenols, o-diphenols and polyphenols generally inhibited IAA oxidation[7]. The cause of difference of phenol levels in different parts would also be due to the variation in the metabolism and synthesis of phenols in different tissues by phenylalanine-ammonia lyase and tyrosine-ammonia lyase[81, 82].

Biological activity of the oxidation products of IAA

Many workers have shown the biological activities of the oxidation products of IAA. The elongation of wheat coleoptiles was stimulated by hydroxymethyl oxindole (HMO) has been reported earlier[83]. HMO also stimulated ethylene evolution and increased protein and RNA content of wheat coleoptiles[84]. Methylene oxindole (Meox), an oxidation product of IAA, stimulated growth by desensitization of regulatory proteins at low concentration, but it inhibited such process at high concentration[83]. Meox was also reported to be 10 fold more effective than IAA as a plant auxin in promoting growth of pea and mung bean segment[83] and wheat coleoptiles[85]. Frenkel *et al*. (1975)[86] reported that Meox promoted softening of the pears as well as increased evolution of ethylene in the same during ripening. HMO was reported to be more effective than IAA in inducing female flower buds[87, 88] and ethylene evolution in cucurbits[89]. HMO and Meox increased the elongation of *Avena* coleoptiles[90].

On the contrary, auxin activity of Meox was not found in root initiation in pea stem cuttings, in extension of growth of etiolated pea segments and *Avena* first internode[91], in coleoptiles and pea stem segments[92], in xylem differentiation in explants of lettuce pith parenchyma cultures[93]. Meox was found to be a potent bacteriostatic and a potent inhibitor of SH-containing enzymes[66]. Lau *et al*. (1978)[94] reported that Meox and other oxidation products of IAA such as indole-3-carboxylic acid and dioxindole-3-acetic acid (DioxIAA) were unable to stimulate ethylene production. The oxindole-3-acetic acid (OxIAA), another oxidation product of IAA, was reported to be generally inactive in growth promotion[94, 95], although a contrary report has appeared[96].

Role of IAA in legume Rhizobium Symbiosis

Rhizobium spp. have shown to produce IAA and changes in IAA level in the host plant are a prerequisite for nodule organogenesis[97]. IAA is involved in cell division, differentiation and vascular bundle formation in root and nodule also. But exact role of IAA in the different stages of *Rhizobium*-plant symbiosis remains unclear.

It was established that the specific nod inducers, flavonoids, stimulate the IAA production by *Rhizobium*[98]. Moreover, in *Rhizobium* sp. NGR234 this flavonoid induction is dependent on the transcriptional regulators NodD1 and NodD2 also presence of the nod-box NB15 upstream of the y4wEFG operon required for IAA biosynthesis[26]. In addition root nodules contain more IAA than nonnodulated roots[80, 99, 100] and IAA might be important for maintaining root nodules[101].

Nod factors initiate multiple responses that are essential for bacterial invasion of the legume plant host. One of the earliest plant responses to the correct Nod factor structure is an increase in the intracellular levels of calcium in root hairs, followed by strong calcium oscillations (spiking) and alterations to the root hair cytoskeleton. These responses are followed by curling of the root hairs, which traps rhizobial bacteria within what is known as a tight colonized curled root hair (CCRH). Simultaneously, Nod factor stimulates root cortex cells to reinitiate mitosis, a process that is dependent on the inhibition of auxin transport by rhizobia and plant

flavonoids. These cells will form the nodule primordium, and give rise to the cells that will receive the invading bacteria. In developing indeterminate nodules, infection threads grow through the developing meristematic zone to the underlying cell layers through which the wave of mitotic activity has already passed. These layers of invasion-competent cells have exited mitosis and become polyploid owing to cycles of genomic endoreduplication without cytokinesis. Plant cell endoreduplication is required for the formation of functional nodules, and is dependent on the degradation of mitotic cyclins by anaphase-promoting complex (APC), an E3 ubiquitin ligase. The advantages of cellular polyploidy are a higher transcription rate and a higher metabolic rate than cells with the normal 2n DNA content-characteristics that might be important for the invaded plant cells to support bacterial nitrogen fixation[102].

Recently, it has been demonstrated that a cytokinin receptor (*HITI* gene) was involved in the Nod factor signaling cascade in *Lotus japonicus* and *Medicago*[103, 104]. Since the effect of cytokinins is dependent on the IAA/ cytokinin balance, these findings may give a new dimension to the role of IAA in nodule formation.

Bacterial IAA producers (BIPs) have the potentiality to interfere with any of these processes by input of IAA into the plant's auxin pool. The consequence for the plant is usually a function of (i) the amount of IAA that is produced and (ii) the sensitivity of the plant tissue to changes in IAA concentration. A root, for instance, is one of the plant's organs that are most sensitive to fluctuations in IAA, and its response to increasing amounts of exogenous IAA extends from elongation of the primary root, formation of lateral and adventitious roots, to growth cessation[105]. Infection of the roots of legumes by *Rhizobium* spp. initiated a coordinated series of morphological and physiological events, which resulted in the formation of the organized structures of the root nodules. Presence of relatively large quantities of auxin in leguminous root nodules was reported by many workers [101, 106, 107]. Root nodules contained higher amount of IAA than their corresponding roots[108, 109]. It was also reported that the production of plant hormones such as auxins, cytokinins and gibberellins, which allow certain bacteria and fungi to direct a plant's physiology toward their own advantage, is also a striking feature[110].

IAA production by *Rhizobium* spp. in culture

Rhizobium sp. can produced a huge amount of IAA in culture rather than IAA produced in symbiotic condition (Table 1). *Rhizobium* spp. could utilize a wide range of carbohydrates as carbon source[111]. Carbon of the incubation medium was a determining factor for the amount of IAA produced by *R. trifolii* in culture[112]. Different carbon sources supplemented at 1% level promoted the bacterial growth and IAA production by a *Rhizobium* sp. to different extent and mannitol was the most effective promoter[113]. Nitrate was less preferred nitrogen source for some strains of *Rhizobium*, whereas glutamate, glutamine or NH_4^+ (except *R. trifolii*) was generally preferred as nitrogen source[114]. De and Basu (1996)[115] reported that KNO_{3-} and $NaNO_3$ increased both growth and IAA production by a *Rhizobium* sp. isolated from *Tephrosia purpurea*.

Jordan (1984)[111] reported that biotin and other waste soluble vitamins were required for growth of *Rhizobium* spp. But some *R. japonicum* strains were inhibited by biotin[116]. Growth of *Rhizobium* sp. might be promoted by biotin. Riboflavin could enhance the growth of a *Rhizobium* sp.[117]. Chakraborti *et al.* (1981)[114] reported that out of the 31 strains of the cowpea rhizobia and 14 strains of *R. japonicum*, approx 50% of the population showed little or no response to any vitamin added individually or in mixture. For the slow growing strains, pantothenate and p-amino benzoic acid are the most common vitamins or precursors of vitamins showing promoted growth, although some strains required the amine, biotin and choline.

Effects of IAA on Rhizobia

Very little information accumulated about the effect of IAA on microorganisms. IAA could enhance the incorporation of glycine in proteins[125] and also increase the formation of bacteroids[126] of *Rhizobium.* IAA was reported to increase the cell surface of *Rhizobium*[127]. Nodules of plants inoculated with mutant *Bradyrhizobium japonicum* strains overproducing IAA contain higher amounts of IAA in comparison to nodules derived from wild-type bacteria[128], suggested a major contribution of bacteria to IAA accumulation in nodules. Rescently it has been shown that IAA

induces cell defense systems[129, 130] and TCA pathway[131, 132]

Isolation, identification and estimation of IAA

IAA might exist either in free form or bound with sugars, nucleic acids, proteins, amino acids etc. in plants[4]. Bound auxin was now recognized as IAA that formed chemical conjugates. IAA-conjugates were themselves inactive but released free, active IAA upon solvent extraction, alkaline hydrolysis or *in vivo* enzymatic hydrolysis[7]. Bioassay was frequently used to measure partially purified hormones. Different bioassay techniques were used for measurement of auxin. The most common assay of IAA fall within three broad categories: a) biological assay or bioassay of straight growth measurement of *Avena*[133] or wheat coleoptile[134] and the curvature test of *Avena* coleoptile[6] or *Cephalaria* sp. and *Raphanus sativus* hypocotyls[135]; split pea curvature and root growth inhibition of lentil and wheat were also sometimes used for auxin bioassay[134], b) instrumental analysis - by the use of modern instruments of separation and quantization, including high performance liquid chromatography (HPLC), gas chromatography (GC) and nuclear magnetic resonance (NMR) along with thin layer chromatography (TLC), column chromatography, UV spectrophotometry and fluorometry which are followed by the use of mass spectrometry (MS) to obtain proof of structure and c) immuno assay[6] - which are more rapid and extremely sensitive (about 10000 times more than any bioassay) detection method. Apart from these plant hormones can also be detected by immuno-affinity chromatography[136]. The identification of IAA was done by checking the colour reactions with Ehrlich or Salkowski's reagent. Its property of being oxidized by IAA oxidase and peroxidase and the characteristics of its other biological activity were also used for identification[137].

CONCLUSION

The production of IAA by the nodule bacteria is considered to have important physiological implications as it seemed reasonable to suspect that IAA, alone or in conjunction with other plant hormones, might be involved in several stages of the symbiotic relationship and might be transported to other parts of the plant as Wheeler *et al.* (1979)[140] showed that phytohormones from the nodules were transported to other parts of the plant. N_2 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 nitrogen fixation in the nodules and supply to the host can be considered as the first-line 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* association.

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REFERENCES

1. Kogl, F., Haagen, S. A. J., Erxleben H. Uber einneus Auxin ('Heteroauxin') aus Horn, XI. *Z. Physiol. Chem.*, 1934; **228**: 90-103.
2. Kogl, F., Kostermans, D. G. F. R. Heteroauxin als stoffwechselprodukt niederer pflanzlicher organismen isolierung aus Hefe, XIII. *Z. Physiol. Chem.*, 1934; **228**: 113-121.
3. Thimann, K. V. On the plant growth hormone produced by *Rhizopus suinus*. *J. Biol. Chem.*, 1935; **109**: 279-291.
4. Leopold, A. C., Kriedemann, P. E. Plant Growth and Development, 2nd ed, Tata Mc Graw-Hill Publ. Co. Ltd., New Delhi, 1975: 109-194.
5. Went, F. W. Wichstoff and Wachstum. *Recl. Trav. Bot. Neerl.*, 1928; **25**: 1-116.
6. Salisbury, F. B., Ross, C. W. Plant Physiology, 4th ed, Wadsworth Publishing Company, Belmont, California, 1992: 357-406.
7. Hopkins, W. G. Introduction to Plant Physiology, 2nd edition, John Wiley and Sons Inc., New York 1999.
8. Woodward, A. W. Bartel, B. Auxin: regulation, action, and interaction. *Annal. Bot.* (London), 2005; **95**: 707–735.
9. Teale, W. D., Paponov, I. A., Palme, K. Auxin in action: signalling, transport and the control of plant growth and development. *Nat. Rev. Mol. Cell Biol.*, 2006; **7**: 847–859.
10. Darwin, C., Darwin, F. The power of movement in plants. John Murray, London 1880.
11. Went, F. W. Auxin, the plant growth hormone. *Bot. Rev.*, 1935; **1**:162–182.

12. Bartel, B. (1997) Auxin biosynthesis, *Annu. Rev. Plant Physiol.Plant Mol. Biol*. 48:51-66
13. Mohr, H., Schopfer, P. Plant physiology. Springer Verlag, Berlin 1995.
14. Ostin, A., Ilic, N., Cohen, J. D. An *in vitro* system from maize seedlings for tryptophan-independent IAA biosynthesis. *Plant Physiol.,* 1999; **119(1):** 173-178.
15. Williams, M. N. V., Signer, E. R. Metabolism of tryptophan and tryptophan analogs by *Rhizobium meliloti. Plant Physiol.,* 1990; 92**:** 1009-1013.
16. Costacurta, A., Vanderleyden, J. Synthesis of phytohormones by plant associated bacteria. *Critt. Rev. Microbiol.,* 1995; **21(1):** 1-18.
17. Ernstsen, A., Sandberg, G., Crozier, A., Wheeler, C. T. Endoginous indoles and the biosynthesis and metabolism of IAA in cultures of *Rhizobium phaseoli. Planta,* 1987; **171:** 422-428.
18. Kobayashi, M., Suzuki, T., Fujita, T., Masuda, M., Shimizu S. Occurrence of enzymes involved in biosynthesis of IAA from indole-3-acetonitrile in plant-associated bacteria, *Agrobacterium* and *Rhizobium*. *Proc. Natl. Acad. Sci.,* 1995; **92:** 714-718.
19. Kaneshiro, T., Slodki, M. E., Plattner R. D. Tryptophan catabolism and IAA by *Rhizobium japonicum* L. 259 mutants. *Curr. Microbiol.,* 1983; **8:** 301-306.
20. Spaepen, S., Vanderleyden, J., Remans, R. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol. Rev.,* (2007). doi:10.1111/j.1574-6976.2007.00072.
21. Pollmann, S., Muller, A., Piotrowski, M., Weiler, E. W. Occurrence and formation of indole-3-acetamide in Arabidopsis thaliana. *Planta,* 2002; **216**: 155–161.
22. Pollmann, S., Neu, D., Weiler, E. W. Molecular cloning and characterization of an amidase from Arabidopsis thaliana capable of converting indole-3-acetamide into the plant growth hormone, indole-3-acetic acid. *Phytochemistry,* 2003; **62**: 293–300.
23. Vande Broek, A., Gysegom, P., Ona, O., Hendrickx, N., Prinsen, E., Van Impe, J., Vanderleyden, J. Transcriptional analysis of the *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene and identification of a cis-acting sequence involved in auxin responsive expression. *Mol. Plant–Microbe Interact.,* 2005; **18**: 311–323.
24. Brandl, M. T., Lindow, S. E. Environmental signals modulate the expression of an indole-3-acetic acid biosynthetic gene in *Erwinia herbicola*. *Mol. Plant–Microbe Interact.,* 1997; **10**: 499–505.
25. Ona, O., Van Impe, J., Prinsen, E., Vanderleyden, J. Growth and indole-3-acetic acid biosynthesis of *Azospirillum brasilense* Sp 245 is environmentally controlled. *FEMS Microbiol. Lett.,* 2005; **246**:125–132.
26. Theunis, M., Kobayashi, H., Broughton, W. J. Prinsen, E. Flavonoids, NodD1, NodD2, and nod-box NB15 modulate expression of the y4wEFG locus that is required for indole-3-acetic acid synthesis in *Rhizobium* sp. strain NGR234. *Mol. Plant–Microbe Interact.,* 2004; **17**: 1153–1161.
27. Costacurta, A., Mazzafera, P. Rosato, Y. B. Indole-3-acetic acid biosynthesis by Xanthomonas axonopodis pv. citri is increased in the presence of plant leaf extracts. *FEMS Microbiol. Lett.,* 1998; **159**: 215–220.
28. Datta, C., Basu, P. S. Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. *Microbiol. Res.,* 2000; **155:** 1-5.
29. Ghosh, S., Sengupta, C., Maiti, T. K., Basu, P. S. 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.,* 2008; **53 (4):** 351-355.
30. Omay, S. H., Schmidt, W. A. Martin, P. Indoleacetic acid production by the rhizosphere bacterium *Azospirillum brasilense* Cd under in vitro conditions. *Can. J. Microbiol.,* 1993; **39**: 187–192.
31. Kravchenko, L. V., Azarova, T. S., Makarova, N. M. Tikhonovich, I. A. The effect of tryptophan present in plant root exudates on the phytostimulating activity of rhizobacteria. *Microbiology,* 2004; **73**: 156–158.
32. Marlow, J. L., Kosuge, T. Tryptophan and indoleacetic acid transport in olive and oleander knot organism *Pseudomonas savastanoi* (e F Smith) *Stevens. J. Gen. Microbiol.,* 1972; **72**: 211.
33. Mazzola, M., White, F. F. A mutation in the indole-3-aceticacid biosynthesis pathway of *Pseudomonas syringae* pv syringae affects growth in *Phaseolus vulgaris* and syringomycin production. *J. Bacteriol.,* 1994; **176**: 1374–1382.
34. Patten, C. L., Glick, B. R. Bacterial biosynthesis on indole-3-acetic acid. *Can. J. Microbiol.,* 1996; **42:** 207–220.

35. Patten, C. L., Glick, B. R. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Appl. Env. Microbiol.,* 2002; **68:** 3795–3801.
36. Gysegom, P. Study of the transcriptional regulation of a key gene in indole-3-acetic acid biosynthesis in *Azospirillum brasilense*. PhD thesis, K.U. Leuven. 2005.
37. Honma, M., Shimomura, T. Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agric. Biol. Chem*., 1978; **42**: 1825–1831.
38. Ose, T., Fujino, A., Yao, M., Watanabe, N., Honma, M., Tanaka, I. Reaction intermediate structures of 1-aminocyclopropane-1-carboxylate deaminase. *J. Biol. Chem.,* 2003; **278**: 41069–41076.
39. Jacobson, C. B., Pasternak, J. J., and Glick, B. R. Partial purification and characterization of ACC deaminase from the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Can. J. Microbiol*., 1994; **40**:1019–1025.
40. Penrose, D. M., Glick, B. R. Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) in exudates and extracts of canola seeds treated with plant growth-promoting bacteria. *Can. J. Microbiol*., 2001; **47**: 368–372.
41. Glick, B. R., Penrose, D. M., Li, J. P. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *J. Theor. Biol.,* 1998; **190**: 63–68.
42. John, R. A. Pyridoxal phosphate-dependent enzymes. *Biochim. Biophys. Acta.,* 1995; **1248**: 81–96.
43. Giovanelli, J., Mudd, S. H., Datko, A. H. Sulfur amino acids in plants. **In**: *Amino Acids and Derivatives, The Biochemistry of Plants: A Comprehensive Treatise*, Vol. 5, pp. 453–505. Miflin, B. J., Ed., Academic Press, New York 1980.
44. Kende, H. Enzymes of ethylene biosynthesis. *Plant Physiol*., 1989; **91**: 1–4.
45. Arshad, M., Frankenberger, W. T. Microbial production of plant hormones. *Plant Soil,* 1991; **133**: 1–8.
46. Grichko, V. P., Glick, B. R. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. *Plant Physiol. Biochem*., 2001; **39**: 11–17.
47. Farwell, A. J., Vesely, S., Nero, V., McCormack, K., Rodriguez, H., Shah, S., Dixon, D. G., Glick, B. R. Tolerance of transgenic canola (*Brassica napus*) amended with ACC deaminase-containing plant growth-promoting bacteria to flooding stress at a metal-contaminated field site. *Environ. Poll*., 2007; **147**: 540–545.
48. Huang, X.-D., El-Alawai, Y., Gurska, J., Glick, B. R., Greenberg, B. M. A multi-process phytoremediation system for decontamination of Persistent Total Petroleum Hydrocarbons (TPHs) from soils. *Microchem. J*., 2005; **81**: 139–147.
49. Greenberg, B. M., Huang, X. D., Gurska, Y., Gerhardt, K. E., Wang, W., Lampi, M. A., Zhang, C., Khalid, A., Isherwood, D., Chang, P., Wang, H., Dixon, D. G., Glick, B. R. Successful field tests of a multi-process phytoremediation system for decontamination of persistent petroleum and organic contaminants. **In**: *Proceedings of the Arctic and Marine Oilspill Program*, Vol. 1, pp. 389–400. Environment Canada, Ottawa (2006).
50. Safranova, V. I., Stepanok, V. V., Engqvist, G. L., Alekseyev, Y. V., Belimov, A. A. Root-associated bacteria containing 1-aminocyclopropane-1- carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. *Biol. Fertil. Soils*, 2006; **42**: 267–272.
51. Cheng, Z., Park, E., Glick, B. R. 1-Aminocyclopropane-1-carboxylate (ACC) deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Can. J. Microbiol*., 2007; in press.
52. Wang, Y. Q., Ohara, Y., Nakayashiki, H., Tosa, Y. Mayama, S. Microarray analysis of the gene expression profile induced by the endophytic plant growth-promoting rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. *Mol. Plant–Microbe Interact*., 2005; **18**: 385–396.
53. Mayak, S., Tirosh, T., Glick, B. R. Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper. *Plant Sci*., 2004; **166**: 525–530.
54. Shaharoona, B., Arshad, M., Zahir, Z. A. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). *Lett. Appl. Microbiol*., 2006; **42**: 155–159.
55. Kende, H., Zeevaart, J. A. D. The five "classical" plant hormones. *Plant Cell,* 1997; **9**: 1197–1210.
56. Prayitno, J., Rolfe, B. G., Mathesius, U. The Ethylene-insensitive sickle mutant of *Medicago truncatula* shows altered auxin transport regulation during nodulation. *Plant Physiol*., 2006; **142**: 168–180.

57. Dharmasiri, N., Dharmasiri, S., Estelle, M. The F-box protein TIR1 is an auxin receptor. Nature, 2005; **435:** 441–445.
58. Newman, E. I., Reddell, P. The distribution of mycorrhizas among families of vascular plants. *New Phytol.,* 1987; **106:** 745–751.
59. Barea, J. M., Azcon-Aguilar, C. Mycorrhizae and their significance in nodulating nitrogen-fixing plants. *Adv. Agron.,* 1983; **36:** 1–54.
60. Crush, J. R. Plant growth responses to vesicular-arbuscularmycorrhizae. VII. Growth and nodulation of some herbage legumes. *New Phytol.,* 1974; **73:** 743–752.
61. Liu, A., Hamel, C., Hamilton, R. I., Bl Ma, Smith, D. L. Acquisition of Cu, Zn, Mn and fe by mycorrhizal maize (Zea mays L.) grown in soil at different different P and micronutrient levels. *Mycorrhiza,* 2000; 9**:** 331–336.
62. Cooper, K. M., Tinker, P. B. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. II. Uptake and transfer of Phosphorus, zinc and Sulphur. *New Phytol.,* 1978; **88:** 43–52.
63. Hayman, D. S. The physiology of vesicular-arbuscularmycorrhizal symbiosis. *Can. J. Botany,* 1983; **61:** 944–963.
64. Chakrabarti, J., Chatterjee, S., Ghosh, S., Chaterjee, N. C., Dutta, S. Synergism of VAM and *Rhizobium* on Production and Metabolism of IAA in Roots and Root Nodules of *Vigna mungo*. *Curr. Microbiol.,* 2010; **61**: 203-209.
65. Normanly, J., Bartel, B. Redundancy as a way of life-IAA metabolism. *Curr. Opinion Plant Biol*., 1999; **2(3)**: 207-213.
66. Moore, T. C. Biochemistry and Physiology of Plant Hormones, 2nd ed., Springer Verlag New York Inc., 1989: 28-93.
67. Ostin, A., Kowalyczk, M., Bhalerao, R. P., Sandberg, G. Metabolism of indole-3-acetic acid in *Arabidopsis*. *Plant Physiol*., 1998; **118 (1)**: 285-296.
68. Gazaryan, I. G., Chubar, T. A., Mareeva, E. A., Lagrimini, L. M., Van Huystee, R. B., Throneley, R. N. F. Aerobic oxidation of indole-3-acetic acid catalysed by anionic and cationic peanut peroxidase. *Phytochem*., 1999; **(2)**:175-186.
69. Basu, P. S., Tuli, V. Enzymatic dehydration of 3-hydroxymethyl oxindole. *Plant Physiol*., 1972; **50**: 503-506.
70. Basu, P. S. Oxindole pathway of IAA metabolism. In Pal, P. K., (Eds.) Contemporary Thoughts in Plant Sciences. Academic Staff College, The University of Burdwan, Burdwan, 1996: 163-175.
71. Rigaud, J., Puppo, A. Indole-3-acetic acid catabolism by soybean bacteroids. *J. Gen. Microbiol*., 1975; **88**: 223-228.
72. Gove, J. P., Hoyle, M. C. The isoenzymic similarity of indole acetic acid oxidase to peroxidase in birch and horseradish. *Plant Physiol*., 1975; **56**: 684-687.
73. Hare, R. C. Indole acetic acid oxidase. *Bot. Rev.,* 1964; **30**: 129-165.
74. Bryant, S. D., Lane, F. E. Indole-3-acetic acid oxidase from peas. I. Occurrence and distribution of peroxidative and non-peroxidative forms. *Plant Physiol*., 1979; **63**: 696-699.
75. Savitsky, P. A., Gazaryan, I. G., Tishkov, V. I., Lagrimini, L. M., Ruzgas, T., Gorton, L. Oxidation of indole-3-acetic acid by dioxygen catalysed by plant peroxidases: specificity for the enzyme structure. *Biochemical J*., 1999; **340**: 579-583.
76. Reinecke, D. M., Bandurski, R. S. Auxin biosynthesis and metabolism. Davies, P. J. Eds., In Plant Hormones and Their Role in Plant Growth and Development. Martinus Nijhoff Publishers, Boston, 1987: 24-42.
77. Wichner, S. Beziehungen zwischen Pflanzen und epiphytischen Bakterien hinsichtlich ihres Auxinstoffwechsels. IV. IES-Zerstoerung und Produktion von Inhibitoren der IES-Oxydase durch epiphytische Bakterien. *Flora Abt. A*., 1968; **159**: 141–166.
78. Nissl, D., Zenk, M. H. Evidence against induction of protein synthesis during auxin-induced initial elongation of *Avena* coleoptiles. *Planta,* 1969; **89**: 323–341.
79. Leveau, J. H. J., Lindow, S. E. Utilization of the Plant Hormone Indole-3-Acetic Acid for Growth by *Pseudomonas putida* Strain 1290. *Appl. Env. Microbiol*., 2005; **71(5)**: 2365–2371.
80. Ghosh, S., Basu, P. S. Production and metabolism of indole acetic acid in roots and root nodules of *Phaseolus mungo*. *Microbiol. Res.,* 2006: **161**: 362-366.
81. Stafford, H. A. The metabolism of aromatic compounds. *Ann. Rev. Plant Physiol.,* 1974: **25**: 459-486.
82. Vance, C. P. Comparative aspects of root and nodule secondary metabolism. *Phytochemistry,* 1978; **17**: 1889-1891.
83. Tuli, V., Moyed, H. S. The role of 3-methylene oxindole in auxin action. *J. Biol. Chem*., 1969; **244**: 4916-4920.
84. Sinha, B. K., Ghosh, S., Basu, P. S. 3-hydroxymethyl oxindole: its auxin action and

metabolism in wheat. *Biochem. Physiol. Pflanzen*., 1982; **177**: 447-459.
85. Basu, P. S., Tuli, V. Auxin activity of 3-methyleneoxindole in wheat. *Plant Physiol*., 1972; **50**: 499-502.
86. Frenkel, C., Haddon, V. R., Smallheer, J. M. Promotion of softening and ethylene synthesis in Bartlett Pears by 3-methyleneoxindole. *Plant Physiol*., 1975; **56**: 647-649.
87. Ghosh, S., Basu, P. S. Hormonal regulation of sex expression in *Momordica charantia*. *Physiol. Plant*, 1983; **57**: 301- 305.
88. Ghosh, S., Basu, P. S. Hormonal regulation of sex expression and some physiological and biochemical changes in *Luffa acutangula* Roxb. *Biochem. Physiol. Pflanzen*, 1984; **179**: 277-287.
89. Ghosh, S., Basu, P. S. Comparative effects of 3-hydroxymethyloxindole and indole-3-acetic acid on sexual tendency and ethylene evolution in *Cucumis flexuosus* L. and *Cucumis sativus* L. *Biochem. Physiol. Pflanzen*, 1983; **178:** 279-285.
90. Bhattacharyya, R. N., Chattopadhyay, K. K., Basu, P. S. Auxin activity of 3-hydroxymethyl oxindole and 3-methylene oxindole in oat. *Biol. Plant*, 1986; **28(3)**: 219-226.
91. Anderson, A. S., Moller, I., Hansen, J. 3-methyleneoxindole and plant growth regulation. *Physiol. Plant*, 1972; **27**: 105-108.
92. Evans, M. L., Ray, P. M. Inactivity of 3-methyleneoxindole as mediator of auxin action on cell elongation. *Plant Physiol*., 1973; **52**: 186-189.
93. Roberts, L. W. Does 3-methyleneoxindole possess auxin activity. *J. Exp. Bot*., 1974; **25**: 761-763.
94. Lau, O., John, W., Yang, S. F. Inactivity of oxidation products of indole-3-acetic acid on ethylene production in mung bean hypocotyls. *Plant Physiol*., 1978; **61**: 68-71.
95. Reinecke, D. M., Bandurski, R. S. Oxindole-3-acetic acid, an indole-3-acetic acid catabolism in *Zea mays*. *Plant Physiol*., 1983; **71**: 211-213.
96. Galston, A. W., Chen, H. R. Auxin activity of isatin and oxindole-3-acetic acid. *Plant Physiol*., 1965; **40**: 699-705.
97. Mathesius, U., Schlaman, H. R. M., Spaink, H. P., Sautter, C., Rolfe, B. G. Djordjevic, M. A. Auxin transport inhibition precedes root nodule formation in white clover roots and is regulated by flavonoids and derivatives of chitin oligosaccharides. *Plant J.*, 1998; **14**: 23–34.
98. Prinsen, E., Chauvaux, N., Schmidt, J., John, M., Wieneke, U., Degreef, J., Schell, J., Vanonckelen, H. Stimulation of indole-3-acetic acid production in *Rhizobium* by flavonoids. *FEBS Lett.*, 1991; **282**: 53–55.
99. Dullaart, J., Duba, L., Presence of gibberellin-like substances and their possible role in auxin bioproduction in root nodules and roots of *Lupinus luteus* L. *Acta Bot. Neerl.*, 1970; **19**: 290–297.
100. Theunis, M. IAA biosynthesis in rhizobia and its potential role in symbiosis. PhD thesis, Universiteit Antwerpen 2005.
101. Badenoch-Jones, J., Rolfe, B. G., Letham, D. S. Phytohormones, *Rhizobium* mutants, and nodulation in legumes. III. Auxin metabolism in effective and ineffective pea root nodules. *Plant Physiol*., 1983; **73**: 347-352.
102. Jones, K. M., Kobayashi, H., Davies, B. W., Taga, M. E., Walker, G. C. How rhizobial symbionts invade plants: the *Sinorhizobium–Medicago* model. *Nature Rev. Microbiol*., 2007; **5**: 619-633.
103. Murray, J. D., Karas, B. J., Sato, S., Tabata, S., Amyot, L. Szczyglowski, K. A cytokinin perception mutant colonized by Rhizobium in the absence of nodule organogenesis. *Science*, 2007; **315**: 101–104.
104. Tirichine, L., Sandal, N., Madsen, L. H., Radutoiu, S., Albrektsen, A. S., Sato, S., Asamizu, E., Tabata, S., Stougaard, J. A gain-offunction mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. *Science*, 2007; **315:** 104–107.
105. Davies, P. J. The plant hormone concept: concentration, sensitivity, and transport. Davies, P. J., Eds., In Plant hormones: physiology, biochemistry, and molecular biology. Kluwer Academic Publishers, Dordrecht, The Netherlands, 1995; 13–18.
106. Dullaart, J. The auxin content of root nodules and roots of *Alnus glutinosa* (L.). *Vill. J. Exp. Bot*., 1970; **21**: 975-984.
107. Hunter, W. J. Indole-3-acetic acid production by bacteroid from soyabean root nodules. *Physiol. Plant*, 1989; **76**: 31-36.
108. Dullaart, J. Quantitative estimation of IAA and indole carboxylic acid in root nodules and roots of *Lupinus luteus* L. *Acta Bot. Neerl*., 1967; **16**: 222-230.
109. Ghosh, A. C., Basu, P. S. IAA and its metabolism in root nodules of a leguminous tree *Dalbergia lanceolaria*. *Ind. J. Exp. Biol*., 1998; **36**: 1058-1060.
110. Lambrechet, M., Okon, Y., VandeBrock, A.,

Vanderleyden, J. Indole-3-acetic acid; a reciprocal signaling molecule in bacteria-plant interactions. *Trends Microbiol*., 2000; **8**: 298-300.

111. Jordan, D. C. Rhizobiaceae. In: Bergey's Manual of Systematic Bacteriology, Vol. 1. Krieg, N. R., Holt, J. G. Eds., Williams and Wilkins Co., Baltimore, USA, 1984: 234-256.
112. Yoshida, S., Yatazawa, M. Species characteristics and cultural conditions as affecting to rhizobial production of IAA. *Nippon Dojo Hiryogaku Zasshi*, 1973; **44**: 63-66.
113. Bhowmick, P. K., Basu, P. S. Indole acetic acid production by *Rhizobium* sp. from a leguminous tree, *Erythrinna indica*. *Folia Microbiol*., 1987; **32**: 140-148.
114. Chakraborti, S., Lee, M. S., Gibson, A. H. Diversity in nutritional requirements of strains of various *Rhizobium* species. *Soil Biol. Biochem*., 1981; **13(5)**: 349-354.
115. De, P. S., Basu, P. S. 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*., 1996; **151**: 71-76.
116. Elkan, G. H., Ewik, I. Nitrogen, energy and vitamin nutrition of *Rhizobium japonicum*. *J. Appl. Bacteriol*., 1968; **31**: 399-404.
117. Roy, M., Basu, P. S. Production of 3-indole acetic acid by a *Rhizobium* sp. from *Mimosa pudica*. *Folia Microbiol*., 1989; **34**: 120-126.
118. Sinha, B. K., Ghosh, S., Basu, P. S. Auxin activity of 3-hydroxymethyl oxindole in wheat. *Biochem. Physiol. Pflanzen.*, 1981; **176:** 228-235.
119. Dangar, T. K. Studies on plant hormons in relation to nitrogen fixation in root nodules of some Leguminous plants and in culture of the microsymbionts. Ph. D. Thesis. Department of Botany, The University of Burdwan, Burdwan, West Bengal, India ,1987.
120. Bhowmick, P. K. Studies on the plant growth hormones in root nodules and nodules bacteria of some Leguminous trees. Ph. D. Thesis. Department of Botany, The University of Burdwan, Burdwan, West Bengal, India, 1987.
121. Chattopadhyay, K. Metabolism of Auxin and its relation with other phytohormons in plant and root nodule – Rhizobium symbiosis Ph. D. Thesis. Department of Botany, The University of Burdwan, Burdwan, West Bengal, India, 1989.
122. Roy, M. Studies on nodules and nodule bacteria of some tropical Leguminous plants. Ph. D. Thesis. Department of Botany, The University of Burdwan, Burdwan, West Bengal, India, 1990.
123. Ghosh, A. C. Studies on root and stem nodules and their symbionts of two Legumes and a nonlegume with special reference to indole acetic acid production.Ph.D thesis, Department of Botany, Burdwan University Ph. D. Thesis. Department of Botany, The University of Burdwan, Burdwan, West Bengal, India, 1999.
124. Datta, C. Studies in root nodules and their symbionts of three leguminous plants with reference to indole acetic acid metabolism and extracellular polysaccharide production. Ph. D. Thesis. Department of Botany, The University of Burdwan, Burdwan, West Bengal, India ,2000.
125. Wiss, Z. Effect of IAA on the incorporation of glycine 1-^{14}C into the proteins of *Rhizobium phaseoli* and their composition. *Ernst-Moritz-Arndt-Univ. Greifsw. Math. Natorwiss. Reihe*., 1980; **29:** 81-83.
126. Labanok, E. B., Bakanchikova, T. I. Effect of IAA on the formation of bacteroid-like forms of *Rhizobium leguminosarum*. *Mikrobiologia*, 1979; *48***:** 901-905.
127. Gruodine, J. Effect of phytohormones on the cell size of kidney bean nodule bacteria. *Microbiol. Proyzvod* (Dokl. Kanf. Microbiol. Lit. SSSR 6 th. 1979), 1981: 144-157.
128. Hunter, W. J. Influence of 5-methyl tryptophan resistant *Bradyrhizobium japonicum* on soyabean root nodule indole-3-acetic acid content. *Appl. Environ. Microbiol.*, 1987; **53(5)**: 1051-1055.
129. Remans, R., Spaepen, S., Vanderleyden, J. Auxin signaling in plant defense. *Science,* 2006; **313:** 171.
130. Bianco, C., Imperlini, E., Calogero, R., Senatore, B., Amoresano, A., Carpentieri, A., Pucci, P., Defez, R. Indole-3-acetic acid improves Escherichia coli's defences to stress. *Arch. Microbiol.,* 2006; **185:** 373–382.
131. De Melo, M. P., Pithon-Curi, T. C., Curi, R. Indole-3-acetic acid increases glutamine utilization by high peroxidase activitypresenting leucocytes. *Life Sci*., 2004; **75:** 1713–1725. doi:10.1016/j.lfs.2004.03.021.
132. Bianco, C., Imperlini, E., Calogero, R., Senatore, B., Pucci, P., Defez, R. Indole-3-acetic acid regulates the central metabolic pathways in *Escherichia coli*. *Microbiol-Sgm.*, 2006; **152:** 2421–2431.
133. Nitsch, J. P., Nitsch, C. Studies on the growth

of the coleptile and first internode sections. A new sensitive straight growth test for auxins. *Plant physiol*., 1956; **31**: 94-111.

134. Audus, L. J. Plant growth substances, Vol. 1. Leonard Hill, London, 1972: 24-65.
135. Soding, H. Uber wuchsstoffteste. *Jb*. *Wiss*. *Bot*., 1937; **85**: 770-787.
136. Taiz, L., Zeiger, E. Plant Physiology. The Benjamin/ Cummings Pub. Co., Inc., Redwood city, California 94065, 1991: 398-489.
137. Thimann, K. V. The auxins. Wilkins, M. B. Eds., In Physiology of Plant Growth and development. Tata McGraw-Hill Publ. Co. Ltd., New Delhi, 1969:1-45.
138. Mazumder, P., Ghosh, S., Sadhu, S, Maiti, T.K, Production of Indole Acetic acid by *Rhizobium* sp. from Root Nodules of a Leguminous herb *Crotalaria saltiana* Andr. in Culture, *J. Pure & Appl. Microbiol., 200*8; **4**(1): 109-116.
139. Mandal, S., Mandal, M., Das, A., Pati, B., Ghosh, A. Stimulation of indoleacetic acid production in a *Rhizobium* isolate of *Vigna mungo* by root nodule phenolic acids. *Arch. Microbiol* 2009 ; **191**(4): 389-393.
140. Wheeler, C. T., Henson, I. E., Mc Laughlin, M. E. Hormones in plants bearing Actinomycete nodules. *Bot*. *Gaz*., 1979; **140** (suppl.): S 52-S 57.