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Review Article

An Effective and Beneficial Plant Growth Promoting Soil Bacterium "*Rhizobium*": A Review

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Abstract: *Rhizobium* is a soil habitat, gram negative bacterium which is associated symbiotically with the roots of leguminous plants. The symbiosis is based on specific recognisation of signal molecules produced by bacteria and its plant partners. Process of symbiosis results in Biological Nitrogen Fixation in which atmospheric nitrogen (N_2) is converted into ammonia (NH_3) and is subsequently available for plants, and in turn plants provide nutrients to the bacterium. This genus of bacteria is aerobic in nature and produces acidic reaction on Mineral Salt Medium or carbohydrates. Presently, *Rhizobium* is classified into groups *Rhizobium, Bradyrhizobium, Mesorhizobium, Azorhizobium and Sinorhizobium*. As a Plant Growth Promoting Rhizobacteria (PGPR), *Rhizobium* promotes overall growth and development of the plant through direct and indirect mechanisms. To reduce the utilization of chemical fertilizers which disturbs the ecosystem, biofertilizers are prepared commercially. Bioinoculant of *rhizobia* can effectively improve the agricultural yield and productivity which indicates that *Rhizobium* is an effective Plant growth promoting microbe.

Keywords: *Rhizobium*, Biological Nitrogen Fixation, Symbiosis, PGPR, Biofertilizer

Introduction

The soil contains many types of microorganisms – microscopic forms of animal life such as bacteria, actinomycetes, fungi and algae. Soil microorganisms are important because they affect the soils physical, chemical and biological properties. For example the process of the decay, breakdown, and disappearance of dead plant and animal materials occurs due to the action of many different types of microorganisms.

Beneficial free-living soil bacteria are usually referred to as Plant Growth Promoting Rhizobacteria (PGPR). Independent of the mechanisms of vegetal growth promotion, PGPRs colonalizes the rhizosphere, the rhizoplane or the roots itself [1]. 1-2% of bacteria promote plant growth in the rhizosphere. The rhizosphere is the volume of soil surrounding and under the influence of plant roots, and rhizoplane is the plant root surfaces and strongly adhering soil particles [2].

Among the soil bacteria there is a unique group called *Rhizobia* that have beneficial effect on the growth of legumes. *Rhizobium* is soil inhabiting bacteria that form the root nodules where symbiotic biological Nitrogen fixation occurs [3]. In the soil the bacteria are free living and motile, feeding on the remains of dead organisms. Free living *rhizobia* cannot fix nitrogen and they have a different shape from the bacteria found in root nodules. They are regular in structure, appearing as straight rods; in root nodules the nitrogen-fixing form exists as irregular cells called bacteriods which are often club and Y-shaped.

When *Rhizobia* live in the soil, they are called saprophytes. Many soils contain *rhizobia* that live on the organic matter without legume partner; (called native *rhizobia*) while those the farmers add as inoculant, are called introduced rhizobia. The population of native rhizobia can be very diverse, including species and many distinct strains within each species. Numbers can range from zero to more than one million *rhizobia* per gram (gm) of soil.

Rhizobium inoculant was first made in USA and commercialized by private enterprise in 1930s and strange situation at that time chronicled by Smith, [4]. The *Rhizobial* inoculants are commonly applied to seeds of legume crops to ensure effective Nitrogen

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fixation by *Rhizobium*, thereby making this essential nutrient available to crop. The inoculants are often used together with agrochemicals (pesticides), which besides containing essential nutrients also contain contaminants and toxic elements. [5]. Pesticides may influence nodulation and biological nitrogen fixation in legumes by affecting *rhizobia*, plant or both.

Characterstics of *Rhizobium*

A typical *Rhizobial* cell is small to medium in size (0.5-0.9 X 1.2 – 3.0 μm), Gram negative, motile rod and contains the granules of poly-β-hydroxybutyrate which are refractile as observed by phase contrast microscopy. These bacteria are aerobic, possessing respiratory type of metabolism with oxygen as terminal electron acceptor. The bacteria are able to grow well at oxygen tension less than 1.0kPa. This genus of the bacteria produces an acidic reaction in mineral salts medium or carbohydrates. Most strains produce sticky gum like structures of varying compositions. The bacteria are pleomorphic form (bacteriods) which are normally involved in the fixation of atmospheric nitrogen into utilizable form by plant. Molecular G + C content of DNA is 59.64 Tm. On the basis of dry matter, the bacteria contains 52-55% carbon and 4-5% nitrogen [6].Cells of alfalfa, clover, pea and soybean *Rhizobia* are characterized by low content of basic nitrogen [7]. Movement in this bacterium is due to thread like structure called flagella.

Growth Characteristics of *Rhizobium* in laboratory: In laboratory, *Rhizobia* are grown on a special medium called Yeast Extract Mannitol Agar (YEMA) medium. They are grouped into two main genera-the Fast growing *Rhizobium* species and slow growing *Bradyrhizobium* species. When cultured on YEMA, the *Rhizobium* species produce visible growth in 2-3 days. They produce an acid growth reaction, which can be detected by addition of Bromothymol blue (BTB) to the medium. *Rhizobia* isolated from pea, bean, clover, alfalfa, chickpea and Leucaena are all fast growers. The soybean and cowpea are slow growers. Temperature required for the growth of *Rhizobium* on YEM is 25-42^{o}C and pH range for this genus of bacteria is 4-9.The extensive literature with nutritive requirement was reviewed by Wilson [8]. Fast growing responds to the additions of biotin. Slow growing strains of cowpea and soybean are not stimulated. The growth factors of *Rhizobium sp*. is shown in Table 1.

Table 1: Growth factors of *Rhizobium* species

Vitamin	*R.melliloti*	*R.trifolli*	*R. leguminosarum*	*R.phaseoli*	*R.lupini*	*R.japonicum*	*Rhizobium sp.*
Biotin	S,+	+	+	+	+	_	_
Thiamine	_	_	_	_	_	_	_
Riboflavin	_	S, +	_	+	+	_	_
Nicotinic Acid	_	+	_	+	_	+	_
β-Alanine	_	+	_	_	+	_	_
Pantothenic acid	S	_	_	_	_	_	+

"S" means vitamin is synthesized, "+" vitamin is essential; "-" vitamin is not essential

Classification of Rhizobium

Rhizobium has been classified in *Bergey's Manual of Determinative Bacteriology* in such diverse families as *Azotobacteriaceae, Mycobacteriaceae, Myxobacteriaceae, and Pseudomonadaceae*. *Bergey's Manual of Determinative Bacteriology*, was first published in which cataloguing of information of identifying bacteria was included. Much Revision, by the American Society of Bacteriology (now American Society for Microbiology) provided such references which are followed by *Bergey's Manual of systematic Bacteriology* [9]. Speciation of *Rhizobium* is based on the cross-inoculation grouping given by the classical studies of Fred [10].

The basis for cross-inoculation grouping lies in the ability of an isolate of *Rhizobium* to form nodules on roots of a limited species of legumes which are related to one another. Based on this principle, *Rhizobia* that can form nodules on roots of certain legumes have been collectively taken as a species.

Cross Inoculation groups of *Rhizobium:* Scientists have studied the matching system for many important food, forage, and tree legumes. They have categorized *Rhizobia* and their legume partners into Cross Inoculation Groups. Each of these consists of the entire legume species that will develop nodules when inoculated with *Rhizobia* obtained from any other member of the same group (Table 2).

Table 2: Cross Inoculation Groups

Rhizobium Spp.	Cross Inoculation Grouping	Legume Types
R. leguminosarum	Pea group	Pisum, Vicia, Lens
R. phaseoli	Bean group	Phaseolus
R. trifolii	Clover group	Trifolium
R. meliloti	Alfalfa group	Melilotus, Medicago, Trigonella
R. lupini	Lupini group	Lupinus, Orinthopus
R. japonicum	Soybean group	Glycine
Rhizobium sp.	Cowpea group	Vigna, Arachis

The system of cross-inoculation grouping of *Rhizobia* is not free from limitations because *Rhizobia* have been often found to cross-infect or interchange between groups. However, there is no better alternative to this system. Therefore, many rhizobiologists have come to regard cross-inoculation grouping is a convenient and satisfactory way to classify *Rhizobia* into species.

It is also possible to differentiate *Rhizobia* on the basis of growth on a defined substrate, as fast growers and slow growers. Studies have been done on morphological and physiological character (colonial character, vitamin, carbohydrate and nitrogen nutrition, antibiotic sensitivities and infective attributes) of *Rhizobia* to find out if a better method of rhizobial classification can be proposed. The results have shown that the genus *Rhizobium* and *Agrobacterium* need not be split at the generic level and *Agrobacterium* need not necessarily be merged with *Rhizobium* [11].

Rhizobia are able to produce acid or alkali on YEMA medium. Based on this criterion, the fast-growing *R. phaseoli, R. trifolii, R. leguminosarum, and R. meliloti* could be grouped as acid producers while the slow-growing *R. japonicum, R. lupine, and Rhizobium sp.* (cowpea) could be grouped as non-acid producers. The slow-grouping, which are non-acid-producing rhizobia, are associated with primitive tropical legumes growing in alkaline environment [12].

The base composition of pure DNA (expressed as molar percent-age of gunanine and cytosine) of several *Rhizobia* has been analyzed and a suggestion has been made to regroup *Rhizobia* as fast-growing peritrichous strains having a low percentage (G + C) composition in the range of 58.6-63.1 percent which belong to *R. leguminosarum* and *R. meliloti*. On the contrary, it has been suggested that the sub-polarly flagellated, slow-growing strains having a somewhat higher percentage (G+C) mostly in the range of 62.8-65.5 percent come under *R. japonicum* [13]. However, more studies are needed in this direction before we can come to any definite conclusions on the use of DNA base composition data in the classification of nodule bacteria [14].

As per the ninth edition of *Bergey's Manual of Determinative Bacteriology* the genus *Rhizobium* is classified into following classification [15]

Genus I: *Rhizobium*

Rhizobium leguminosarum (biovars trifolii, phaseoli, viceae), R. meliloti, R. loti- fast-growing, sub-polar flagellated strains from Lotus and Lupinus with strong affinity for *L. corniculatus, L. densiflorus, and Anthyllis vulneraria* (but also nodulate *Orinthopus sativum*). Include the fast-growing strains nodulating *Cicer, Sesbania, Leucaena, Mimosa, and Lablab.*

Genus II: *Bradyrhizobium*

Slow-growing, polar, or sub-polar flagellated strains nodulating soybean, Lotus *uliginosus, L. pendulatus, and Vigna.* Includes those slow-growing strains nodulating *Cicer, Sesbania, Leucaena, Mimosa, Lablab and Acacis*. The possibility exists that other species will eventually be defined within the genus, but for the present it is suggested that, other than *B. japonicum* (the type species), the various cultures can be designated as *Bradyrhizobium sp. (Vigna), Bradyrhizobium sp. (Cicer)* etc.

Rhizobia currently consist of 61 species belonging to 13 different genera *Rhizobium, Mesorhizobium, Sinorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium, Methylorhizobium, Burkholdera, Cupriavidus, Devosia, Herbaspirillum, Orchobactrum and Phyllobacterium*. The taxonomy is in constant flux [16].

Plant Growth Promoting Rhizobacteria (PGPR)

The complexity of the soil ecosystem is established by numerous and diverse interactions among its physical, chemical and biological components [17]. Especially, the variable genetic and functional activities of the heterogeneous microbial populations have a vital effect on soil functions, as such microbes are considered powerful forces for specific enzyme mediated fundamental metabolic processes [18]. The unique physico-chemical and biological characteristics of the soils that are associated with the roots, compared to the soils away from the root and root surface are responsible for the enhanced microbial diversity together with the increased numbers and activity of the microorganisms in the rhizosphere [19]. During plant-microbe interaction, plant roots exude the organic materials which are used up by root associated microorganisms as nutrients as well as organic material is also supplied to the soil micro biota through the death of dead and decay plants as either growth substrates and structural components or signal molecules. Microbial activity in the rhizosphere affects the rooting pattern and supply of nutrients to the plants [20].

Beneficial, root colonizing, rhizosphere bacteria: Plant Growth Promoting bacteria are the group of bacteria that colonalizes the roots and survive the microhabitats associated with root surface to promote plant growth and protection. These are free living, soil-borne bacteria which when applied to seeds, soil or crops, enhances the growth of plants by providing nutrients and reducing the damage from the soil borne pathogens [21]. Interaction of plants with rhizosphere bacteria affects the overall crop yield and agricultural productivity commercially.

PGPR helps in the growth of the plants through direct and indirect mechanisms. Direct mechanisms include (i) Biological Nitrogen Fixation (ii) Phosphate Solubilization (iii) Phytoharmones (iv) Siderophore Production (v) Hydrogen Cyanide (HCN) Production. Indirect mechanism includes (i) Stress and Biocontrol Action (ii) Antibiotic Production (iii) Synthesis of antifungal metabolites.

Role of *Rhizobium* as a PGPR is as follows:

Direct Mechanisms

Biological Nitrogen Fixation: Biological Nitrogen Fixation is the process in which atmospheric nitrogen (N_2) is converted into ammonia (NH_3) and is subsequently available for plants. This is perhaps a symbiotic process in which plant supplies the *Rhizobia* with energy in the form of nutrients and *Rhizobia* fix dinitrogen from atmosphere for plant uptake. The reduction of atmospheric dinitrogen into ammonia is second most important biological process on earth after photosynthesis [22]. It has been estimated that 1gm of soil may contain a community of 10^9 micro-organisms, with *Rhizobia* representing 0.1% of soil microbes or 10^6 *Rhizobia* g^{-1} soil [23].

Process of Biological N_2 fixation involving reduction of N_2 into NH_3 (as depicted in Figure 1) is an energy intensive process. It requires 16 molecules of ATP and a complex set of enzymes to break N_2 bonds so that it can combine with hydrogen. Its reduction is:

$$N_2 + 8\ H_2 + 16\ ATP \rightarrow 2\ NH_3 + 2\ H_2 + 16\ ADP + 16\ Pi \quad \ldots (1)$$

The immediate electron donor is the potent reducing agent ferrodoxin and the reaction is driven by hydrolysis of ATP for each electron transferred.

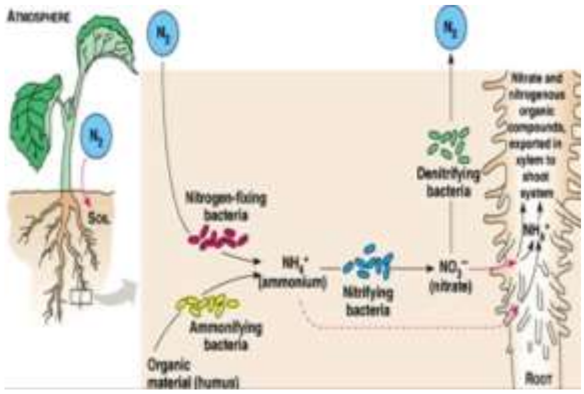

Figure 1: Process of Biological Nitrogen Fixation

Root Nodule Formation: Nodulation is a highly host specific interaction in which, specific *Rhizobia* strains infect a limited range of plant hosts. N_2 fixing nodules are formed by interactions of *Rhizobia* and legume host plants.

Sets of genes in the bacteria control different aspects of the nodulation process. One *Rhizobium* strain can infect certain species of legumes but not others e.g. the pea is the host plant to *Rhizobium leguminosarum* biovar *viciae,* whereas clover acts as host to *R. leguminosarum* biovar *trifolii*. Specificity genes determine which *Rhizobium* strain infects which legume. Even if a strain is able to infect a legume, the nodules formed may not be able to fix nitrogen. Such rhizobia are termed as ineffective. Effective strains induce nitrogen-fixing nodules. Effectiveness is governed by a different set of genes in the bacteria from specificity genes. Nod genes direct the various stages of nodulation.

The initial interaction between the host plant and free-living *Rhizobia* is the release of a variety of chemicals by the root cells into the soil. Some of these encourage the growth of the bacterial population in the area around the roots (the rhizosphere). Reactions between certain compounds in the bacterial cell wall and the root surface are responsible for the *Rhizobia* in recognizing their correct host plant attaching to the root hairs. Flavonoids secreted by the root cells activate the nod genes in the bacteria which then induce nodule formation. The whole nodulation process is regulated by highly complex chemical communications between the plant and the bacteria.

Once bound to the root hair, *Rhizobia* excrete nod factors. *Rhizobium* in the soil secretes nodD which is a protein that recognizes Flavonoids released by the plants. Interaction with the plants activates nodD, which further induces the transcription of nodABC protein which modifies nodD factor in response to Flavonoids released by the plants.

Structure of Nod Factors: The chemical structures of Nod factors (Figure 2) produced by 730 *Rhizobial* strains have been studied. All the Nod factors produced by *Rhizobia* consists of oligosaccharide backbone β-1, 4 linked N-acetyl D- glucosamine. A fatty acyl group is always attached to the nitrogen of non-reducing saccharide. Because of resemblance of the oligosaccharide backbone to the fragment of chitin, Nod factors are also called Lipo-Chitin Oligosaccharide (LCO). All *Rhizobia* produce complex mixtures of LCO complexes.

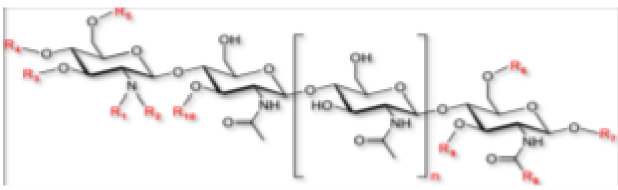

Figure 2: Generic structure of NOD factor

Process of Biological Nitrogen Fixation: These nod factors stimulate the hair to curl. *Rhizobia* then invade the root through the hair tip where they induce the formation of an infection thread as shown in Figure 3. This thread is constructed by the root cells and not the bacteria and is formed only in response to infection. The infection thread develops from the inner primary cell wall, which grows inwards in the form of invagination enclosing bacterial cells. The infection thread further grows inwards and invades the cortex and finally it finds its way into pericyclic region where the end of the infection thread bursts open releasing bacterial cells. As the infection grows inwards bacterial cells multiply by cell division and the process of multiplication continues even after they are released into the host cells. Bacterial cells assume various shapes and also they aggregate into groups. Such bacterial clusters surrounded by a thin membrane are called bacteroids.

With the entry of rhizobial cells into pericyclic cells, if the host cell is a tetraploid cell, the cell undergoes transformation into actively dividing cells otherwise they do not respond to bacterial infection. However, the infected tetraploid cells then divide and redivide to produce a mass of cells which assume nodular form. The growth and the development of a nodule require the secretion of indole acetic acid (IAA) by the bacterial cells. With entry of bacterial cells into host cells, bacterial cellular components stimulate host genome, where globin genes and other related genes get expressed. As a result globin proteins and other factors are synthesized in significant quantities. The globin protein produced in leguminous root nodules is called leghaemoglobin, whose amino acid sequence and structure is similar to that of animal globin proteins. On the contrary, host cellular factors in turn activate the expression of nitrogen fixing genes found in rhizobial cells. The nif genes remain unexpressed if the rhizobial cells are free from host cells

Role of Nitrogenase in Biological Nitrogen Fixation: An enzyme called nitrogenase catalyses the conversion of nitrogen gas into ammonia in nitrogen-fixing organisms. Nitrogenase exists in three forms that include molybdenum nitrogenase, vanadium nitrogenase and iron nitrogenase. Mo-containing nitrogenase is the enzyme utilized by *Rhizobium* [24]. In legumes it only occurs within the bacteriods. The reaction requires hydrogen as well as energy from ATP. *Rhizobium* controls oxygen levels in the nodule with leghaemoglobin. This red, iron-containing protein has a similar function to that of haemoglobin; binding to oxygen. This provides sufficient oxygen for the metabolic functions of the bacteriods but prevents the accumulation of free oxygen that would destroy the activity of nitrogenase. It is said that leghaemoglobin is formed through the interaction of the plant and the *Rhizobia* as neither of them can produce it independently.

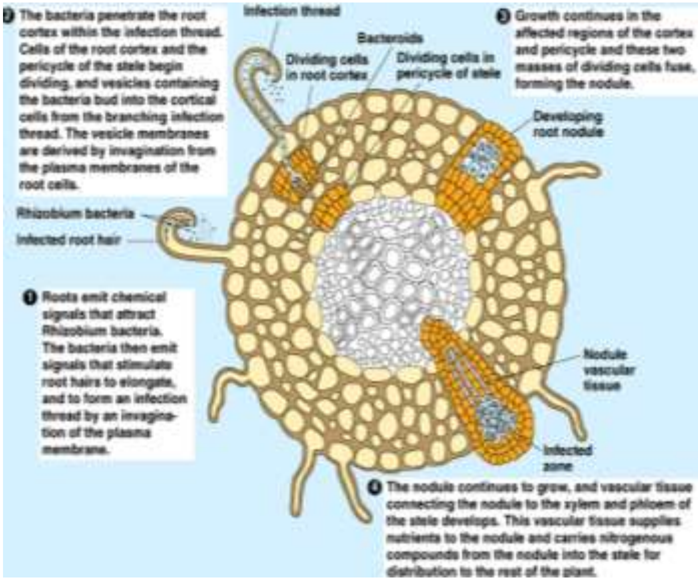

Figure 3: Nodule Formation during biological nitrogen fixation

Importance of Biological Nitrogen Fixation: Biological Nitrogen Fixation is regarded as a renewable resource for sustainable agriculture as it helps to reduce fertilizer Nitrogen requirements and thus increases economic returns to producer [25]. Furthermore, it plays a key role in assessment of *Rhizobial* diversity contributed to worldwide knowledge of biodiversity. Nitrogen fixation by *Rhizobia* is of great importance in agriculture in several ways.

Legumes such as peas, beans, lentils, soybeans, alfalfa and clover help to feed the meat-producing animals of the world as well as humans. Crop yields are greatly improved in nodulated plants; legumes can grow well in poor soils where there is not enough fixed nitrogen to support other types of plants. After harvest legume roots left in the soil decay, releasing organic nitrogen compounds for uptake by the net generation of plants. Farmers take advantage of this natural fertilization by rotating a leguminous one.

Nitrogen fixation by natural mean cuts down on the use of artificial fertilizers. This not only saves money but helps to prevent the many problems brought about by excessive use of commercial nitrogen and ammonia fertilizers such as eutrophication of rivers and lakes, generation of acid rain, and overgrowth of agricultural land by non-food crops. Symbiotic nitrogen fixation is of great importance not only in production of leguminous crops but also in global N_2 cycle [26].

Phosphate Solubilization: Phosphorous (P) is a major essential macronutrient for biological growth and development. The ability of some microorganisms to convert insoluble inorganic phosphorous (P) to accessible form, (i.e. orthophosphate) is an important characteristic feature of PGPR [27, 28]. The rhizospheric phosphate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture [29]. The use of phosphate solubilizing bacteria as inoculants increases the phosphorous uptake by plants. Phosphorous is present in both organic and inorganic forms in the soils. Few rhizobial strains have ability to solubilize phosphorous i.e. *Bradyrhizobium* strains were able to solubilize inorganic phosphate [30]. *Rhizobium* isolates from root nodules of *Cassia absus*, *Vigna trilobata* and three strains from *Sesbania sesban* showed zone of tricalcium phosphate (TCP) solubilization [31].

Phytoharmones: Phytoharmones function to coordinate plant growth and development. Phytoharmones are chemical messengers produced and translocated in plants to produce plant responses and substantial increase in growth and yield of plants. Role of Phytoharmones like auxins, cytokinins, gibberellins, ethylene and Abscisic Acid (ABA) when applied to the plants have shown a substantial increase in growth and yield of plants [32]

Indole-3-acetic acid a main auxin in the plants is known to control many important physiological processes of plants such as cell enlargement, cell division, root initiation, growth rate, phototropism and apical dominance [33]. An important molecule that alters the level of IAA synthesis is the amino acid tryptophan which is the major precursor of IAA and helps in modulating the biosynthesis of IAA. Starting with tryptophan five different pathways have been described for synthesis of IAA [34]: (i) IAA formation via indole-3-pyurvic acid and indole-3-acetic aldehyde found in bacteria like *Rhizobium, Azospirillum, Bradyrhizobium, Agrobacterium*, (ii) Conversion of tryptophan into indole-3-acetic aldehyde may involve an alternative pathway in which tryptamine is formed as in *pseudomonad* and *azospirilla*,(iii) IAA biosynthesis via indole-3-acetamide formation for phytopathogenic bacteria (iv) IAA biosynthesis that involved tryptophan conversion into indole-3-acetonitrile is found in cyanobacterium and (v) the tryptophan-independent pathway found in cyanobacteria.

Most rhizobial species produces IAA. Inoculation with *Rhizobium leguminosarum bv. viciae* wherein IAA biosynthetic pathway had been introduced produced nitrogen fixing root nodules containing 60-fold more IAA than nodules formed by wild type counterpart in *Vicia hirsute* [35]. A *Rhizobium* sp. isolated from the root nodules of a leguminous pulse-yielding shrub, *Cajanus cajan,* was found to produce high amounts (99.7 microgram/ml) of indole acetic acid (IAA) during growth in basal medium supplemented with L-tryptophan [36].

Siderophore Production: For effective growth and development, iron is an essential element required by all living organisms. To fulfill the nutritional requirement, microbes have evolved specific pathways that produce low molecular weight iron chelating compounds called "Siderophores". Siderophores are small, chelating compounds which are released by microbes to scavenge iron from mineral phases by formation of soluble Fe^{3+} complexes that are taken up by active transport mechanism Siderophore production and utilization by Rhizobacteria is important because of iron in the nitrogen fixation and assimilation process. *Rhizobium* strains isolated from the root nodules of S*esbania seban* (L) Merr, showed the ability to produce hydroxamate type siderophore [37]. *Rhizobial* isolate belonging to *Rhizobium* and *Mesorhizobium sp*. produced catecholate type siderophore [38].

Hydrogen Cyanide (HCN) Production: In order to control the weeds, some PGPR's are able to produce HCN, a gas which can negatively affect root metabolism and root growth. Some rhizospheric microorganisms have known to protect their host plants through inhibition of growth of pathogens by HCN production. *Rhizobia* are relatively less efficient in HCN production. It was observed by Alvarez *et al*, 1995 [39] that less than 1% rhizobia isolated from tomato rhizosphere showed positive results for HCN production. Among rhizobia 12.5% and 3% were found to be HCN producers. Among 256 bacterial strains isolated, *Rhizobium NBRI 19513* completely inhibited the growth of *Fusarium oxysporum,* and *Pythium sp*. in vitro [40].

Indirect Mechanism

Stress and Biocontrol Action of *Rhizobium* against certain pathogens: Rhizobacteria are a group of microbes which effectively play a major role in controlling plant pathogen and suppressing the diseases induced by them. They can suppress a broad spectrum of bacterial, fungal and nematode disease. Greenhouse and field tests indicated that *Bradyrhizobium* have variation in the fungicide tolerance, peanut *Bradyrhizobium* strains from different peanut cultivators have been previously reported [41]. Rhizobacteria have been shown to suppress disease by inducing a resistance mechanism in the plant called "Induced Systemic Resistance". Induced Resistance is the state of enhanced defensive ability developed by plants when appropriately stimulated [42]. Greater application of PGPR is possible in agriculture for Biocontrol of plant pathogens and biofertilization [43].

Rhizobia have a good potential to be used as biological control agents against some plant pathogens. Strains of *S. meliloti* are antagonistic to *Fusarium oxysporum* [44] and *Rhizobia* antagonistic to *F. solani f*. sp. *phaseoli* isolated from commercial snap bean, appeared to have a good potential for controlling *fusarium* rot [45]. Ehteshamul-Haque and Ghaffar [46] observed under field conditions that *S. meliloti*, *R. leguminosarum* bv. *viciae*, and *B. japonicum* used either as seed dressing or as soil drench reduced infection of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp., in both leguminous (soybean; *Glycine max* and mungbean; *Vigna radiata*) and non-leguminous (sunflower; *Helianthus annuus)*

Biofertilizers: A Rising Boom to Agriculture

Biofertilizers are specific formulations of beneficial microorganisms that promote the growth of crops by converting the unavailable form of nutrients into available form. Classification of different types of biofertilizers and their groups is shown in Table 3. These biofertilizers induce resistance in plants against pests. Biofertilizers are also commonly called Microbial Inoculants, which are the artificially multiplied cultures of certain soil organisms that improve the soil fertility and crop productivity. Biofertilizers helps in nutrient management, as they are cost effective and renewable source of plants against chemical fertilizers. Farmers can stimulate Biological Nitrogen fixation by applying the correct *Rhizobia* to legume crops, by a process called Inoculation

Table 3: Classification of Different types of Biofertilizers and their groups with examples

S. No.	Group	Examples
	N_2 fixing biofertilizer	
1.	Free living	*Azotobacter, anaebena*
2.	Symbiotic	*Rhizobium, Frankia*
3.	Associative Symbiotic	*Azospirillum*
	P solubilising Biofertilizer	
1.	Bacteria	*Bacillus cirulans*
2.	Fungi	*Penicillin sp.*
	P mobilizing biofertilizer	
1.	*Arbuscular mycorrhiza*	*Colomus sp.*
2.	*Ectomycorhiza*	*Laccaria sp.*
3.	*Conicoid mycorrhiza*	*Perizella ericae*
4.	*Orchid mycorrhiza*	*Rhizoctonia soloni*
	Biofertilizers for mononutrients	
1.	Silicate and zinc solubilizers	*Bacillus sp.*
	Plant growth promoting Rhizobacteria	
1.	*Pseudomonas*	*Pseudomonas fluorescens*

***Rhizobium* Biofertilizer:** This belongs to bacterial group and the classical example is symbiotic nitrogen fixation.It has been estimated that 40-250 kg N/ha/year is fixed by different crops by microbial activities of *Rhizobium.* Liquid formulations of *Rhizobium* biofertilizer are dull white in color; do not have bad smell and no foam formation with pH 6.8-7.5 whereas carrier biofertilizer are applied in seed inoculants form.

Conclusion

Rhizobium is the most well known species of group of bacteria that are engaged in symbiotic relationship with leguminous plants. They obtain their nutrients from the plants and produce nitrogen fixing root nodules through a process called Biological Nitrogen Fixation. The bacterial enzyme system supplies a constant source of reduced nitrogen to host plant and plant furnishes energy in the form of nutrients for the metabolic activities of bacterium. Extensive literature survey suggests that *Rhizobium* is gram negative, motile rod which contains the granules of β-hydroxybutyrate. These bacteria are aerobic and can grow well on a synthetic medium i.e. Yeast Extract Mannitol Agar (YEMA) Medium. *Rhizobia* may be classified into slow and fast growing. Presently, *Rhizobium* is classified into *Rhizobium, Bradyrhizobium, Mesorhizobium, Azorhizobium* and *Sinorhizobium*. Plant Growth Promoting Rhizobacteria (PGPR) are a group of naturally occurring soil bacteria inhabiting on the root surface which are directly or indirectly involved in the plant growth and development. Direct mechanism of action of PGPR includes Biological Nitrogen Fixation, Phosphate Solubilization, Phytoharmones, Siderophores, HCN Production whereas indirect mechanism involves Stress, Biocontrol Action, and Antibiotic Production etc. Extensive study of Literature suggests that *Rhizobium* has been an effective PGPR and helped in plant growth promotion and crop production. It has been seen that Microbial biofertilizers are an effective supplement in agriculture against utilization of chemical fertilizer. Farmers can stimulate Biological Nitrogen fixation by applying the correct rhizobia to legume crops, a process called Inoculation. *Rhizobium* biofertilizers fixes atmospheric nitrogen to a level of 20-40 kgs hectare and overall helps in crop yield.

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