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ISOLATION AND CHARACTERIZATION OF RHIZOBIA BACTERIA FROM ROOT NODULE: A REVIEW

PATKAR R¹, UPADHAYAY D², BHATTACHARYA I², ANDHARE P² AND MARCHAWALA F^{2*}

1: Student, MSc Biotechnology, Parul Institute of Applied Sciences, Parul University, Post Limda,
Waghodia, Gujarat, 391760

2: Assistant Professor, Parul Institute of Applied Sciences, Parul University, Post Limda, Waghodia,
Gujarat, 391760

*Corresponding Author: Farhat Marchawala: E Mail: farhat.marchawala82133@paruluniversity.ac.in; Tel:
+918758737462

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ABSTRACT

Nitrogen is critical element for plant growth and development which is supplied by mutual symbiosis of rhizobia in cultivated legume plants. Biological process could help to strengthen agricultural productivity and ensure food security. Rhizobia are documented as a plant growth promoter and nitrogen fixers. Many indigenous rhizobial strains with the plant growth promoting ability have already been identified and characterized from different legume plants. This study could suggest potential application of Rhizobia for development of the sustainable agriculture and a suitable candidate of biofertilizer which help in soil fertilization without applying chemical fertilizers. Rhizobium-legume symbioses are of great ecological and agronomic importance, thanks to their ability to repair large amounts of atmospheric nitrogen. These symbioses end in the formation on legume roots of differentiated organs called nodules, during which the bacteria reduce nitrogen into ammonia employed by the host plant. Rhizobia have the capacity to repair nitrogen (N₂) from the atmosphere. These bacteria live either freely within the soil or in beneficial association with leguminous plants, including important crops like peas, beans and soybeans.

Keywords: Plant Growth Promotion, Sustainable agriculture, Biofertilizers, Nitrogen Fixing Bacteria, Legumes

INTRODUCTION

Soil microbiology is the study of microorganism in soil, their function, and how they affect soil properties. Soil microorganism can be differentiated into bacteria, protozoa, fungi, algae, actinomycetes. Each microorganism has definite character and function in soil fertility. Microorganism colonizes minerals in the soil which overall increases the overall health of the soil [1]. Soil nutrients are very vital for the continual and efficient production of the crops and healthy food to satisfy the stress of accelerating population. Their sufficiency is extremely vital component of sustainable agriculture. Agriculture is totally hooked in to fertilizers for increasing the yield of the crops. The fertilizers used can either be chemical, organic or biofertilizers and therefore the characteristics of every of the fertilizer are different and possesses its own ability to the enhance growth of the crop and soil fertility [2]. Soil microorganisms represent the world's largest reservoir of biological diversity and a crucial to the functioning of terrestrial ecosystems. This microbial diversity significantly enhances the rates of Carbon and N₂ Cycle within the ecosystem. They need a profound impact in every face of human life. The beneficial microbes are fascinating, versatile and

perform extremely useful processes that can't be achieved by other physical and chemical means. Biological nitrogen fixation is carried out by either symbiotic or free-living prokaryotic, it has well documented that biological N₂ fixation mediated by nitrogenase enzymes may be a process important to the biological activity of soil. Soil micro-organism that has capability of fixing N have often been reported as plant growth promoters [3].

Nitrogen (N₂)

N₂ is an important element for all living organisms. it is required for synthesizing amino acids, proteins, nucleic acids and other nitrogen containing compounds necessary for all times. It is a significant component of amino acids, the building blocks of proteins. Nitrogen is additionally a component of energy transfer compounds, ATP (Adenosine triphosphate), nucleic acids like DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid). It's also a component of chlorophyll, the compound by which plants perform photosynthesis. Nitrogen in the form of chemical fertilizer is a convenient way to supply nitrogen in the soil. Urea, which is the most ordinarily used nitrogenous fertilizer, has now become a costly input for many of the farmers. In the last few decades, there is a

huge increase in the utilization of chemical fertilizers. This increased utilization of fertilizers results in drastic effects on environment such as water eutrophication, increased emissions of nitrogen oxides [4].

Nitrogen Fixation

Nitrogen (N_2) \longrightarrow Nitrogenous Ammonia (NH_3)

Nitrogen fixation is a process in which nitrogen in the atmosphere is converted into usable form such as ammonia. The fixation process frees up the nitrogen atoms from their triply bonded diatomic form. Generally, nitrogen fixation can take place in 3 different ways natural nitrogen fixation, industrial nitrogen fixation and biological nitrogen fixation. Nitrogen fixation is very important as a result of it formed inorganic nitrogen compounds are essential for biogenesis of nitrogen containing compounds like amino acids, proteins, nucleic acid and nucleoside triphosphate at intervals the method of ammonification, the microorganism facilitate in decomposing the animal and plant matter, that indirectly helps to wash up the atmosphere. Nitrates and nitrites square measure discharged into the soil, that helps in enriching the soil with necessary nutrients needed for cultivation. The nitrogen fixing ability of Rhizobium makes legumes less dependent on chemical and fertilizers than most of the other non-legume crops. Rhizobia

are classified into slow (*Bradyrhizobium*) and fast-growing rhizobia [5].

Nitrogen fixation by free living microbes

Free living nitrogen fixers live in the soil and fix significant levels of nitrogen without the direct interaction with other organisms. These bacteria live close to the root such that the atmospheric nitrogen fixed by the bacteria is taken up by the plant. However, free living nitrogen fixing bacteria provide only a small amount of the fixed nitrogen that the host plant requires. Examples of this type of free-living nitrogen fixing bacteria include species of *Azotobacter*, *Azospirillum*, *Bacillus*, *Paenibacillus* and *Klebsiella*. These free-living nitrogen fixers find their own source of energy by oxidizing organic molecules released by other organisms or from decomposition.

Nodule endophytic bacteria

Nodule endophytic bacteria are those bacteria which are incapable of inducing nodules by themselves, but they coexist with nodulating rhizobia inside nodules without causing any harm to the host plant. Many previous reports have suggested the presence of endophytic bacteria in the root nodules of legume plants. These endophytic bacteria can colonize different parts of the plant such as root, stem, nodule, etc. Till date it is not very clear how endophytic bacteria enter nodules. The

endophytic bacteria find safe space inside the nodules to protect themselves from environmental stress and microbial competition. In return, these bacteria benefit the growth of the host plant by suppressing plant disease causing bacteria via competition for nutrients, production of phytohormones and by solubilizing mineral phosphates [6].

Rhizobia

Rhizobia a group of gram negative, rod shaped bacteria are one of the best examples of symbiotic nitrogen fixer. It forms nodules in the roots of leguminous plants and fixes atmospheric nitrogen [7]. Beijerinck was the

first to isolate bacteria from plant root nodules, which he named *Bacillus radicolica*. In 1889, Frank renamed this bacterium as *Rhizobium leguminosarum*. The term “rhizobia” was originally used to name bacteria belonging to the genus *Rhizobium*. However, further research on rhizobial diversity revealed that only a small fraction of rhizobia belongs to this *Rhizobium* genus and many other genera were identified, such as *Azorhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Sinorhizobium* and *Mesorhizobium* [8] (Table 2).

Table 1: Isolation of Rhizobia from different plants

Name of Bacteria	From which plant rhizobia is isolated	Reference
<i>Rhizobium leguminosarum</i>	Cow pea, Soybean	11
	Spinach	11
	Chickpeas	11
<i>Rhizobium phaseoli</i>	Beans	12
<i>Rhizobium japonicum</i>	Soybeans	12

Table: 2 General classification of Rhizobium

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rhizobiales
Family	Rhizobiaceae
Genus	Rhizobium

Characteristics of rhizobia

Rhizobia are gram negative, rod-shaped (0.5 - 0.9 µm in width and 1.2 - 3.0 µm in length) and heterotrophic in nature. They do not form endospores and consist of 2 - 6 peritrichous flagella. Rhizobia generally grow at 25 - 30°C of temperature and in the pH range of 6 - 7. Rhizobia growth normally occurs under

aerobic conditions. However, when fixing nitrogen, low levels of oxygen are required to protect the enzyme nitrogenase [9-11]. Rhizobia utilize simple carbohydrates and amino compounds as a source of energy. The number of native rhizobia in any soil can be very diverse, including many species and several distinct strains within one species.

Several factors affect the number of rhizobia. These include vegetation, cropping history and soil conditions.

Plant growth promoting properties of rhizobia

Rhizobia are an important member of PGPR and many previous studies reported the plant growth promoting abilities of rhizobia. Plant growth promoting rhizobacteria (PGPR), are the bacteria which is able to enhance plant growth by a decent type of mechanisms. They are the natural resource which colonizes roots of plants and stimulates growth and yield directly and indirectly. Direct promotion of growth by PGPR includes fixation of nitrogen, production of plant hormones such as auxins, cytokinins, gibberellins and through the solubilization of minerals like phosphorus. Indirect plant growth promotion includes production of HCN, antibiotics, siderophores that decrease or prevent the deleterious effects of plant pathogenic microorganisms [12-13].

DISCUSSION

Rhizobium is a genus of Gram-negative soil bacteria that fix nitrogen. Rhizobium species form an endosymbiotic nitrogen-fixing association with roots of legumes. The bacteria colonize plant cells within root nodules, where they convert atmospheric nitrogen into ammonia using the enzyme

nitrogenase then provide organic nitrogenous compounds like glutamine to the plant. The plant, in turn, provides the bacteria with organic compounds made by photosynthesis. This mutualist relationship is true of all of the rhizobia. Rhizobium is additionally capable to solubilize phosphorus. Nitrogenase is an enzyme produced by certain microbes like *Rhizobium* and *Cyanobacteria* and is responsible for reducing Nitrogen to ammonia. *Rhizobium* can also be used as biofertilizer. Rhizobium as a biofertilizer is applied to plant surfaces, seeds or soil. Here, the *Rhizobium* bacteria colonizes the rhizosphere or within of the plant to plug growth by enhancing the supply or nutrients availability to the host plant. Rhizobium uses the host plant to repair atmospheric nitrogen and convert it in to useful atmospheric compound benefiting both the bacteria and thus the plant.

CONCLUSION

Rhizobium is a soil bacteria that fixes atmospheric nitrogen once it finds a base inside the roots of the leguminous plants. It attaches to the roots of the legume and produces nodules. These nodules fix atmospheric nitrogen and convert it into ammonia, which is employed by the plant for its growth and development. Rhizobium is in symbiotic association with the nodules of the

leguminous plants. Fixation of nitrogen cannot be done independently that is why rhizobium requires a plant host. Rhizobium can be a vital source of nitrogen to agricultural soils including those in arid regions. They convert dinitrogen into ammonia. Ammonia, being toxic in nature is rapidly absorbed into organic compounds. The presence of rhizobia bacteria would not only fix the present situations, but would also provide higher crop yield. Rhizobia are often utilized in multiple situations in agriculture including restoration, plant growth and pathogen defense.

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