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Legume-Rhizobium Symbiosis

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SUMMARY

It is a well-established fact that legume plants under symbiotic association with Rhizobia are able to convert atmospheric nitrogen (N2) to ammonia (NH4). This association however, is a complex process that requires a series of chemical and biological processes which results in formation of root nodules in the host plants, which houses Rhizobium and help in fixing atmospheric nitrogen. This article attempts to highlight in brief, the mechanism involved in legume-Rhizobium symbiosis.

INTRODUCTION

The term 'rhizobium' comes from two Greek words, '*rhizo*' indicating root and 'b*ium*' meaning home, combined conveying the meaning 'root dweller'; 'rhizobium' is a single bacterium, but 'rhizobia' refers to a group of bacteria. This cannot be spelled as Rhizobia, since Rhizobium is the official scientific name of a bacterial genus. Late 19th century onwards, all legume root-nodule bacteria have been classified within the Rhizobium genus (Frank 1889). Over time, it became apparent that they were rather different. Several slow-growing rhizobia have been separated into the new genus Bradyrhizobium. All rhizobia were classified in the family Rhizobiaceae in the 1984 edition of Bergey's Manual of Systematic Bacteriology (Krieg and Holt 1984). Since then, the number of bacterial genera representing rhizobia has expanded significantly rhizobia are now classified in genera that were formed to describe non-nodulating bacteria as well (Willems 2006). Consequently, the genus name is no longer a reliable indicator of whether a bacterium is a rhizobium.

Table- Rhizobium species and its host range

Species	**Host Range**
Rhizobium *leguminosarum*bv. *phaseoli*	Common bean
Rhizobium *leguminosarum*bv.*trifoli*	Clover
Rhizobium *leguminosarum*bv. *viceae*[a]	Pea, Vetch
Rhizobium *tropici*	Common bean
Rhizobium *etli*[a]	Common bean
Mesorhizobium*loti*[a]	Lotus japonicus
Azorhizobium*caulinodans*	Sesbania
Sinorhizobium*meliloti*[a]	Alfalfa, Medicago truncatula
Sinorhizobium*fredii*	Soybean
Bradyrhizobium*japonicum*[a]	Soybean,Cowpea, mungbean
Bradyrhizobium*elkanii*	Soybean,Cowpea, mungbean

[a] Species for which the genomic sequence is known or from whuch sequencing projects are underway

Source- Stacey, G. (2007). The Rhizobium-legume nitrogen-fixing symbiosis. In *Biology of the nitrogen cycle* (pp. 147-163). Elsevier.

Rhizobium symbiosis

Anton de Bary is credited for introducing the word "symbiosis" (from the Greek for "living together") into biology. Since the nineteenth century, nitrogen-fixing bacteria in the root nodules of legumes have been crucial to theories about symbiosis. Beginning with two independent living entities, the rhizobium-legume symbiosis culminates in close cellular coexistence. Since plants are incapable of metabolising the plentiful molecular nitrogen (N_2) in the atmosphere, the symbiotic relationship between legumes and rhizobia is of immense importance wherein the excess NH4+ formed in the nodule is exchanged for plant-exuded sugars. The whole global nitrogen cycle is underpinned by symbiotic nitrogen fixation. As we know that nitrogen is among the major nutrients limiting plant growth in the agroecosystem. The significance of Nitrogen acquisition and assimilation for the development and growth of the plant is second only to photosynthesis (Newbould, 1989).

Process of Nodulation

The nodulation and nitrogen fixation process in legume-rhizobium symbiosis can be divided into several stages:

1. Attachment: The rhizobia get attached to the surface of the root hairs of the leguminous plant using pili (hair-like structures) or other adhesins.
2. Penetration: The rhizobia enter the root hairs and are transported through the plant's cortex (the tissue surrounding the vascular cylinder) to the nodule primordium (the area where the nodule will form).
3. Nodule formation: The infection thread, a specialized cell that surrounds the rhizobia, differentiate into nodules. The nodules are spherical structures that contain the rhizobia and the host plant cells.
4. Nitrogen fixation: The rhizobia within the nodules transform into nitrogen-fixing bacteroids. Using the enzyme nitrogenase, the bacteroids convert atmospheric nitrogen (N_2) into ammonia (NH_3). The ammonia is subsequently transformed into a form that the plant can utilise, such as nitrate (NO_3^-).5. Transport: The fixed nitrogen is transported to the plant, where it is used for growth and development.

Mechanism of host specificity in rhizobium

Rhizobia are a group of bacteria that form symbiotic relationships with plants, specifically legumes. The host specificity of rhizobia refers to their ability to only form this symbiotic relationship with certain plants. This specificity is achieved through a combination of several mechanisms, including:

1. Surface polysaccharides: Rhizobia produce specific surface polysaccharides that enable them to bind to the root hairs of certain plants.
2. Nod gene specificity: Rhizobia also produce nodulation (Nod) genes that are responsible for the formation of nodules on the roots of plants. Different rhizobia strains have different Nod gene variants, which determines the plant species they can infect.
3. Quorum sensing: Rhizobia use chemical signals called Nod factors to communicate with plant roots. Different rhizobia strains produce different Nod factors, which allows them to differentiate between different plant species.
4. Plant receptor: Plant species also have specific receptors on their root hair cells that can recognize the specific rhizobia strains that infect them.

Together, these mechanisms allow rhizobia to form a specific symbiotic relationship with certain plant species, while remaining incompatible with others.

Role of flavonoids in nodulation

Flavonoids are a class of secondary metabolites present in several plants, including legumes, and play a role in a variety of physiological processes, including the legume-rhizobium symbiosis. Flavonoids have a function in the detection and signalling between the host plant and rhizobia during nodulation. They are chemical signals generated by the host plant that aid rhizobia in recognising and infecting the host. Flavonoids also contribute to the creation and growth of nodules, since they influence the differentiation of the infection thread and the nodule primodium. Flavonoids serve a role in the control of nitrogen fixation in addition to their role in nodulation. It has been demonstrated that they influence the expression of genes involved in nitrogen fixing and modify the activity of the nitrogenase enzyme.

Nodulation genes

Rhizobia are a group of bacteria that can form a symbiotic relationship with certain plants by colonizing their roots and fixing atmospheric nitrogen. The process by which the bacteria infect the plant and form nodules on the roots is called nodulation. Genes involved in nodulation include: nif (nitrogen fixation) genes, which encode enzymes necessary for the conversion of atmospheric nitrogen to a form that plants can use nod (nodulation) genes, which encode proteins that are involved in the infection and colonization of the plant roots by the bacteria fix (fixation) genes, which encode proteins involved in the transfer of fixed nitrogen from the bacteria to the plant. Some specific examples of Rhizobium nodulation genes include: nifA, nifB, nifH, nifD, nifK, nifEN nodC, nodD, nodE, nodF, nodO, nodA, nodB, nodG, nodI, nodJ, nodL, nodM, nodN, nodP, nodQ, nodR, nodS, nodT, nodU, nodV, nodW, nodX, nodY. These genes are usually regulated at transcriptional level, by the presence of specific signal molecules coming from the plant, that are perceived by the bacteria, and by the presence of specific transcriptional regulators in the bacteria.

Nitrogen fixation process and it's importance

The process of nitrogen fixation by legumes occurs inside a specialized organ known as nodules. The bacteroid zones are the core of the mature nodules, surrounded by several layers of cortical cells. The bacteroid are the primary site of nitrogen fixation. A functional, healthy, and effective nodule is characterized by large and pink (due to leghaemoglobin), with well-developed and organized bacteroid tissue. Leghaemoglobin are oxygen-carrying phytoglobin and the prefix 'leg' indicates they were first identified from the root nodules of leguminous plants. The role of leghaemoglobin in the nitrogen fixation of legumes was given by Wittenberg et al. (1975). The primary role of leghaemoglobin is to regulate the diffusion of free oxygen in the root nodules to a level that does not affect the activity of oxygen-sensitive nitrogenase but at the same time, supply enough oxygen for aerobic respiration of bacteroid to carry out the process of nitrogen fixation. Nitrogenase are enzyme produced by the bacteroid for the conversion of N_2 to ammonia. The nitrogenase complex are sensitive to oxygen and are made of two metallo components. The first is the Fe-Mo protein complex known as dinitrogenase and the second is the Fe protein known as dinitrogenase reductase. The bacterial *nif* genes encode for various components of nitrogenase enzyme complex. *nif*H, *nif*D and *nif*K. *Nif* genes encode the structural subunit of dinitrogenase reductase and 2 subunits of dinitrogenase.

Nitrogen fixation is an energy-expensive process that is compensated by legume photosynthates that act as an energy source and provide substrate for the generation of ATP by bacteroid. Ferrodoxins are 4Fe-4S electron carrier that transfers the electrons that are generated in vivo to the Fe protein part of the nitrogenase. This starts a series of oxidation-reduction cycle. The MgATP hydrolyses the reduced Fe protein resulting in the further transfer of electron into the Fe-Mo protein complex of the nitrogenase. The reduction of N_2 to NH_3 occurs in the Mo-Fe protein complex. The binding of N_2 by nitrogenase is a crucial step in nitrogen fixation. It is presumed that Fe is responsible for the binding of nitrogenase to N_2 while, Mo is responsible for weakening the N_2 bond. Another crucial factor in nitrogen fixation is the maintenance of free oxygen levels which is carried out by leghaemoglobin. The first stable product obtained is ammonia. The ammonia fixed in the cell of the nodule is then converted into glutamine by glutamine synthetase and then into glutamate by glutamine-oxoglutarate amidotransferase (GOGAT). In the process of nitrogen fixation, H^+ is also reduced to H_2 due to the activity of the hydrogenase enzyme. The proportion of H_2 to NH_3 fixed is dependent on the electron flux. Under low electron flux, H_2 is formed in a higher percentage compared to NH_3 and vice versa. The overall equation indicating the nitrogen fixation process is given as:

$$N_2 + 8e^- + 16MgATP + 8H+ \rightarrow 2NH_3 + H_2 + 16MgADP + P_i$$

In the process of nitrogen fixation, electron must be transferred six times to fix one molecule of N_2 and each electron transfer requires two MgATP. Thus, a total of 12 ATP is required for fixing one molecule of N_2. In addition to this, the conversion of H^+ to H_2 also requires two electron transfer. Therefore, a total of 16ATP is required for the reduction of N_2 (Cheng 2008).

Legumes are able to grow in nitrogen-poor soils and are able to fix nitrogen from the air and make it available to the plant, this means that farmers don't need to add synthetic nitrogen fertilizers to the soil, which is both costly and environmentally damaging. It is also important to note that not all legumes are able to form symbiotic relationships with rhizobia, and not all rhizobia are able to form symbiotic relationships with legumes. In order for the symbiosis to be successful, the rhizobia must be able to recognize and infect the appropriate host plant, and the host plant must be able to recognize and support the rhizobia. Additionally, there are also non-symbiotic nitrogen-fixing bacteria that can fix nitrogen in the absence of a host plant. These bacteria can be found in the soil and can fix nitrogen in the same way as rhizobia, however, they do not form nodules. In order to improve the efficiency of legume-rhizobium symbiosis, it is important to use rhizobia strains that are well-adapted to the specific legume crop and the environmental conditions in which it is grown. This can be achieved through the use of inoculants, which are commercial preparations of rhizobia that can be applied to the seed or the soil before planting.

Future line of work

Symbiosis between root nodule enables legumes to flourish in nitrogen-deficient soils, but crops without this symbiosis depend on fertilizers to attain high yields. Therefore, a fundamental knowledge of legume–rhizobium symbiosis might potentially allow the transfer of this capacity to nonsymbiotic plants. Engineering BNF in non-legume plants would have significant agronomic benefits and would allow for the reduction or elimination of N-fertilizers.

CONCLUSION

Legume-rhizobium symbiosis is a mutually beneficial relationship between leguminous plants and rhizobia, which allows the plants to thrive in nitrogen-poor soils and the bacteria to survive and reproduce. The relationship is complex and regulated by plant hormones and signaling molecules. Flavonoids play a crucial role in the legume-rhizobium symbiosis by regulating the recognition, signaling, development and protection of the host plant and the rhizobia. They are important compounds that can be used to improve the efficiency of the symbiosis, by modulating the expression of genes involved in nitrogen fixation, improving the resistance to biotic and abiotic stress and also by improving the plant's tolerance to environmental conditions. Understanding the structure, perception, and signaling pathway of nod factors is important for the development of sustainable agricultural practices and the improvement of crop yields. Legume-rhizobium symbiosis considered as one of the most important mutualistic relationships in agriculture and agroforestry, as it improves soil fertility and crop productivity

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