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Symbiotic Nitrogen Fixation: An Overview

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Introduction

Symbiotic nitrogen-fixing prokaryotes dwell within nodules, the special organs of the plant host that enclose the nitrogen-fixing bacteria. In the case of *Gunnera*, these organs are existing stem glands that develop independently of the symbiont. In the case of legumes and actinorhizal plants, the nitrogen-fixing bacteria induce the plant to form root nodules. Grasses can also develop symbiotic relationships with nitrogen-fixing organisms, but in these associations root nodules are not produced. Instead, the nitrogen-fixing bacteria seem to colonize plant tissues or anchor to the root surfaces, mainly around the elongation zone and the root hairs. For example, the nitrogen-fixing bacterium *Acetobacter diazotrophicus* lives in the apoplast of stem tissues in sugarcane.

Legumes and actinorhizal plants regulate gas permeability in their nodules, maintaining a level of oxygen within the nodule that can support respiration but is sufficiently low to avoid inactivation of the nitrogenase. Nodules contain an oxygen-binding heme protein called leghemoglobin. Leghemoglobin is present in the cytoplasm of infected nodule cells at high and gives the nodules a pink color. The host plant produces the globin portion of leghemoglobin in response to infection by the bacteria; the bacterial symbiont produces the heme portion. Leghemoglobin has a high affinity for oxygen. Its function is to help transport oxygen to the respiring symbiotic bacterial cells in a manner analogous to hemoglobin transporting oxygen to respiring tissues in animals.

Rhizobia are diazotrophs (prokaryotic organisms that carry out dinitrogen fixation) that form a symbiotic association with legumes. About 2500 sp of legumes can form symbiotic association with rhizobia. This association is symbiotic in that both the plant and rhizobia benefit. The plant supplies the rhizobia with energy in the form of amino acids and the rhizobia fix nitrogen from the atmosphere for plant uptake. The reduction of atmospheric dinitrogen into ammonia is the second most important biological process on earth after photosynthesis.

Rhizobium bacteria are also categorized based on the species of legume that they nodulate. This type of grouping is known as cross-inoculation groups and are as follows:

1. Clover groups - *R. trifolii* infects and nodulates plants of genus Trifolium (clovers/trefoil)
2. Alfalfa groups - *R. meliloti* infects and nodulates the roots of medicago, melilotus
3. Bean group - *R. phaseoli* infects and nodulates plants of genus Phaseolus (e.g. beans)
4. Lupine group - *R. lupine* nodulates lupines
5. Pea group - *R. leguminosarum* infects and nodulates pea, sweet pea, lentil
6. Soybean group - *R. japonicum* nodulates soybean
7. Cowpea group - *Rhizobium sp*. nodulates cowpea, pegionpea, groundnut

Mechanism

The symbiosis between legumes and rhizobia is not obligatory. Legume seedlings germinate without any association with rhizobia, and they may remain unassociated throughout their life cycle. Rhizobia also occur as free-living organisms in the soil. Under nitrogen-limited conditions, however, the symbionts seek out one another through an elaborate exchange of signals. This signaling, the subsequent infection process, and the development of nitrogen fixing nodules involve specific genes in both the host and the symbionts. Plant genes specific to nodules are called nodulin (*Nod*) genes; rhizobial genes that participate in nodule formation are called nodulation (*nod*) genes. The *nod* genes are classified as common *nod* genes or host-specific *nod* genes. The common *nod* genes—*nodA*, *nodB*, and *nodC*—are found in all rhizobial strains; the host-specific *nod* genes—such as *nodP*, *nodQ*, and *nodH*; or *nodF*, *nodE*, and *nodL*—differ among rhizobial species and determine the host range. Only one of the *nod* genes, the regulatory *nodD*, is constitutively expressed and its protein product (NodD) regulates the transcription of the other *nod* genes. A particular legume host responds to a specific Nod factor. The legume receptors for Nod factors appear to be special lectins (sugar-binding proteins) produced in the root hairs. Nod factors activate these lectins, increasing their hydrolysis of phosphoanhydride bonds of nucleoside di- and triphosphates. This lectin activation directs particular rhizobia to appropriate hosts and facilitates attachment of the rhizobia to the cell walls of a root hair.

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Rhizobia are free living in the soil until they are able to sense flavonoids, derivatives of 2-phenyl-1.4-benzopyrone. Once the bacteria detect these chemicals, they actively swim towards and attach to the legume root. In addition to attracting the bacteria, these chemicals (flavanoids in particular) also play an important role of activating genes involved in producing Nod factors. Here, then, attraction to the legume roots is followed by transcription of *nod* genes in preparation of the symbiotic relationship. Flavonoids promote the DNA binding activity of NodD which belongs to the LysR family of transcriptional regulators and triggers the secretion of nod factors after the bacteria have entered the root hair. A second mechanism, used especially by rhizobia which infect aquatic hosts, is called crack entry. In this case, no root hair deformation is observed. Instead the bacteria penetrate between cells, through cracks produced by lateral root emergence.

Recently, a *Bradyrhizobium* strain was discovered to form nodules in *Aeschynomene* without producing nod factors, suggesting the existence of alternative communication signals other than nod factors, possibly involving the secretion of the plant hormone cytokinin. It has been observed that root nodules can be formed spontaneously in *Medicago* without the presence of rhizobia. This implies that the development of the nodule is controlled entirely by the plant and simply triggered by the secretion of nod factors.

Nodule forming process:

Flavonoids are released by the host root. The flavonoid is at the highest concentration at the root and interacts with the product of bacterial *nodD* gene. The *nodD* gene produces the protein, *nodD*, which is the sensor that recognizes chemicals excreted by host plant roots. Rhizobia colonize the soil in the vicinity of the root hair in response to the flavonoids. This process is autoregulated where favonoids stimulate Nod factor production, which stimulates flavonoid secretion. Response to Nod factors is extremely rapid and the disruption of cell wall happens very quickly. Disruption of crystallization of cell walls take place, thereby allowing entrance by the rhizobia. At the same time Rhizobia multiply in the rhizosphere. The root hair is then stimulated and curls to the side where the bacteria are attached which stimulates cell division in the root cortex. A "shepherd's crook" is formed and entraps the rhizobia which then erode the host cell wall and enter near the root hair tip. An infection thread is formed as rhizobia digest the root hair cell wall. Free-living *Rhizobium* bacteria are converted to bacteroids as the infection elongates by tip growth down root hair and toward

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epidermal cells. Infection thread branches and heads toward the cortex and a visibly evident nodule develops on the root as the plant produces cytokinin and cells divide. Nodules can contain one or more rhizobial strains and can be either determinant (lack a persistent meristem and are spherical) or indeterminate (located at the distal end of cylindrically shaped lobes). Many infections are aborted due to a breakdown in communication between rhizobia and the host plant leaving nodule number strictly regulated by the plant. Once inside the nodule, rhizobia are released from the infection thread in a droplet of polysaccharide. A plant-derived peribacteroid membrane, which regulates the flow of compounds between the plant and bacteroid, quickly develops around this droplet via endocytosis. This process keeps the microbes "outside" the plant where the rhizobia are intracellular but extracytoplasmic. The loss of the ammonium assimilatory capacity by bacteroids is important for maintaining the symbiotic relationship with legumes.

Nitrogen fixation

Nitrogen fixation in the nodules begins when the nodules fully mature. Here, it is worth noting that nitrogen fixation involves the conversion of atmospheric nitrogen into organic compounds (particularly ammonia) that can be used for plant development. This process requires two important genes (*nif* and *fix*). These genes play an important role of producing several crucial enzymes that are involved in the nitrogen fixation.The nitrogenase enzyme complex catalyzes this reaction. The nitrogenase enzyme complex can be separated into two components—the Fe protein and the MoFe protein. The Fe protein is the smaller of the two components and has two identical subunits of 30 to 72 kDa each, depending on the organism. Each subunit contains an iron–sulfur cluster (4 Fe and 4 S_2^-) that participates in the redox reactions involved in the conversion of N_2 to NH_3. The MoFe protein has four subunits, with a total molecular mass of 180 to 235 kDa, depending on the species. Each subunit has two Mo–Fe–S clusters.