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BENEFITS OF NITROGENFIXATION TO THE NITROGENFIXING BACTERIA

Biological Science

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ABSTRACT

The state of some plants being deprived from the availability of nitrogen causing nitrogen starvation leads to the phenomenon of Biological Nitrogen Fixation . Microorganisms are employed to enhance the availability of nitrogen to these plants. The major N2 - fixing systems involve the symbiotic association between rhizobia soil bacteria and legumes. The enzymatic conversion of free nitrogen to ammonia occurs as a part of this symbiotic relationship. The significant role of this phenomenon is enhancing the fertility of the soil and in the growth of the host plant that would otherwise be nitrogen limiting. This process has fascinated researchers in the agricultural sector for the yield of legume crops. This review article focuses on the benefits that Rhizobium earns on being in mutualistic symbiosis with the leguminous plants.

KEYWORDS

INTRODUCTION :

● ***What Is Nitrogen Fixation ?***

Nitrogen is the element that has been a crucial part be it food, fertilizers or poison. Nitrogen gas contributes to about 80% of the total gases in the air. Chlorophyll constitutes of Nitrogen that is very crucial in photosynthesis. Nitrogen fixation is the biological process in which the dinitrogen having 2 molecules of nitrogen present in the air is converted to the naturally available form of nitrogen that is ammonia (Nh3). For any living organism Nitrogen Fixation becomes essential as inorganic nitrogen compounds that are fixed are required for the biosynthesis of organic nitrogen compounds, such as amino acids and proteins, nucleoside triphosphates and nucleic acids. Being a part of the nitrogen cycle, this organic nitrogen compounds play an important role in areas like agriculture and the manufacture of fertilizer. Bacteria and Archaea are the two domains that have been fixing atmospheric nitrogen for millions of years now. This biological nitrogen fixation contributes to a major amount of the nitrogen input of natural systems. Biological fixation in prokaryotes is performed by the nitrogenase complex, which is a metalloenzyme complex that contains catalytic protein dinitrogenase, along with an ATP-dependent electron-donating iron protein, the dinitrogenase reductase. This catalytic domain contains a molybdenum-iron cofactor while some species use rest two classes of dinitrogenases that is distinguished by the presence of vanadium-iron or only iron cofactors. All the biological reactions hat involve nitrogen fixation are catalyzed by enzymes nitrogenases. The nitrogen molecule is made up of two nitrogen atoms linked by a triple covalent bond, that makes the molecule inert and nonreactive. The enzyme nitrogenases catalyzes the breaking of the linkage and there is an addition of three hydrogen atoms to each nitrogen atom.

Microorganisms that carry out the fixation of nitrogen require 16 moles of adenosine triphosphate (ATP) that carries out the function of reduction of each mole of nitrogen. Microorganisms obtain the energy by carrying out oxidation of organic molecules. Non-photosynthetic microorganisms obtain the required energy from other organisms, while photosynthetic microorganisms, such as cyanobacteria, make use of sugars produced during photosynthesis. Symbiotic nitrogen fixing microorganisms obtain energy from their host plants.

●***Nitrogen- Fixing Organisms :***

Numerous bacteria show the ability to form nitrogen fixing nodules with legumes. The classic examples of symbiotic association developed by Rhizobium sp. are found in about 12,900 or more leguminous plants, herbs , shrub and trees. Organisms include alpha proteobacterium, *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium , Sinorhizobium and Rhizobium*. They are all collectively called the **rhizobia.**

Rhizobium is a soil species with a nature of Gram negative and is also capable of causing infection, nodule formation, carry out symbiosis and nitrogen fixation. Rhizobium shows a prominent growth on media containing organic nutrients. They are also more likely to be seen in the rhizosphere of leguminous plants. Rhizobium cells show presence of genes for nitrogen fixation (*nif* genes) that have been evolved by lateral gene transfer.

The plant with which rhizobia us associated belong to the Leguminosae/Fabaceae family. Nitrogen fixing symbiosis has evolved in several lineages, but not all legumes form symbiosis.

The symbiosis is seen to be initiated by initial starvation of nitrogen by the host plant which then selects its Rhizobium partner from enormous bacteria present in the rhizosphere. The interaction between Rhizobium and host plant range from surface colonization through facultative symbiotic relationships to obligate intracellular pathogen or endosymbiont lifestyles. Legume roots colonization by rhizobia results in the curling of root hairs. Plants that contribute to nitrogen fixation contain symbiotically associated rhizobia bacteria in nodules in their root systems, that produce nitrogen compounds which help the plant in growth and also help compete with other plants. When the plant perish, the nitrogen that was fixed is released by making it available to other plants also allowing it to fertilize the soil.

Other Nitrogen Fixing Bacteria Are:

Non nodule forming diazotrophs example, *Azotobacter , Beijerinckia* . These are known to be associated with roots of certain plants like tropical grasses, sugarcane respectively. Other symbiotic nitrogen fixers include *Actinomycetes* , *Frankia sp.* that invade non-leguminous angiosperms like *Alnus, Casuarina, Myrica, Discaria forming root nodules. Azolla- Anabaena* complex is in symbiotic association with the rice plants.

Rhizobium Structure And Habitat :

Rhizobium bacteria are medium sized, rod - shaped cells, 0.5 to 0.9 micrometer in width and 1.2 to 3.0 micrometer in length. Rhizobium is a bacteria that is devoid of forming endospores, and uses a single polar flagellum is a source for motility. They are predominantly chemoorganotrophs .

When not in the symbiotic association in the root nodules of the host legume, they are seen to be found as normal soil microbiota. As a part of soil microbiota they are seen on the root surface (rhizoplane) , soil around and close to the surface (rhizosphere) and to some extent in non rhizosphere soil.

TABLE I.1 Species of Rhizobia in Genera I and II, and the Cross-Inoculation Groups of Legumes Nodulated by These Rhizobia

Rhizobia	Cross-Inoculation Group	Legumes in Cross-Inoculation Group
Genus I: *Rhizobium*		
Rhizobium leguminosarum bv. *viceae*	Pea	Peas (*Pisum* spp.), vetches; (*Vicia* and *Lathyrus* spp.); lentils (*Lens esculenta*)
R. leguminosarum bv. *trifolii*	Clover	Clovers (*Trifolium* spp.)
R. leguminosarum bv. *phaseoli*	Bean	Common beans (*Phaseolus vulgaris*); scarlet runner bean (*Phaseolus coccineus*)
R. meliloti	Alfalfa	Alfalfa/medics (*Medicago* spp.); sweet clovers (*Melilotus* spp.); fenugreek (*Trigonella foenumgraecum*)
R. loti	Lotus	Trefoils (*Lotus corniculatus* and *L. tenuis*); lupine (*Lupinus densiflorus*); serradella (*Ornithopus sativus*); kidney vetch (*Anthyllis vulneraria*)
R. galegae		Goat's rue (*Galega orientalis*)
R. fredii	Soybean	Soybean (*Glycine max*)
Rhizobium spp.		Leucaena (*Leucaena* spp); Gliricidia

		sepium, Sesbania grandiflora, Calliandra callothyrsus, Pithocellobium dulce, Prosopis pallida, P. juliflora, Acacia senegal, A. farnesiana, Robinia pseudoacacia
Rhizobium sp.	Chickpea	Chickpea (Cicer arietinum)
Genus II: *Bradyrhizobium*		
Bradyrhizobium japonicum	Soybean	Soybean (Glycine max)
Bradyrhizobium spp.	Cowpea	Pigeon pea (Cajanus cajan); peanut/ groundnut (Arachis hypogaea); Acacia mearnsii, A. mangium, A. auriculiformis; limabean (Phaseolus lunatus); winged bean (Phosphocarpus tetragonoloba); siratro (Macroptilium atropupureum); guar bean (Cyamopsis tetragonolobus); cowpea, mungbean, black/green gram, rice bean (Vigna spp.), Desmodium spp., Stylosanthes spp.; hyacinth bean (Lablab purpureus)

Type	Examples
1) Free-living nitrogen-fixing organisms	
i) Anaerobes	*Clostridium*
ii) Facultative anaerobes	*Klebsiella, Enterobacter*
iii) Microaerobes	*Azospirillum, Aquaspirillum Arthrobacter*
iv) Aerobes	*Azotobacter, Derxia*
v) Photosynthetic bacteria	*Rhodopseudomonas, Rhodospirillum,* Chromatium
vi) Cyanobacteria	*Anabaena, Nostoc*
2) Symbiotic systems	
i) *Rhizobium*-legume association	
a) Fast growers	*Pisum, Trifolium, Vicia*
b) Slow growers	*Arachis, Glycine, Vigna*
ii) *Rhizobium*-non-legume associations	
Slow growers	*Parasponia*
iii) Associated symbionts	*Azospirillum-brasiliense-sorghum*
iv) *Frankia*-(actinorhizal) associations	*Alnus, Casuarina, Myrica*
v) Cyanobacterial associations	
a) angiosperms	*Gunnera*
b) gymnosperms	*Agathis, Cycas, Macrozamia*
c) pteridophytes	*Azolla*
d) bryophytes	*Anthoceros, Blasia, Caricula*
e) lichens	*Collema, Lichina, Peltigera*

• *Biological Interactions :*

Mutualism is that form of symbiosis that involves association between two individuals of two different species in which both the partners benefit . The coordinated activities of legume and Rhizobium bacteria depends on the chemical interactions between the symbiotic partners. Roots of leguminous plants secrete chemical that tend to attract Rhizobium bacteria present in the nearby soil. Biological N2 fixation uptakes energy that comes at the expense of photosynthate (sucrose). The plant root is occupied by rhizobia and there is induction of a nodule thus then the bacteria reduces atmospheric nitrogen to ammonia and supply the plant with nitrogenous compounds . The plant along with this also attains the ability to grow in nitrogen deprived soils, and the bacteria is provided with a protected niche where they multiply and eventually escape back into the surrounding soil when the nodule senesces. These bacteria are capable of dealing with free oxygen. In plants infected with Rhizobium, the oxygen content in the root nodules results into the reduction of the activity of the nitrogenase. In such situations, the leguminous roots produce a protein leghemoglobin (also leghaemoglobin or legoglobin). Leghemoglobin results into the buffering of the concentration of oxygen present in the cytoplasm of infected plant cells to ensure the proper functioning of root nodules. Leghemoglobin is a nitrogen or oxygen carrier. It shows close chemical and structural similarities to hemoglobin. Leghemoglobin has a high affinity for oxygen, about 10-15 times higher than human hemoglobin. This affinity permits low oxygen concentration to allow nitrogenase to function but not so high so that all oxygen in the bacteria binds , providing the bacteria with oxygen for respiration.

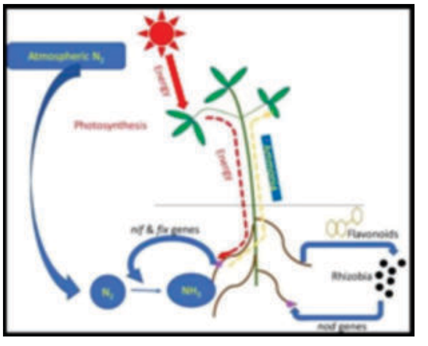
Interaction Of Other Organisms:

The symbiotic observed between Azolla- Anabaena is a mutualistic association where in Azolla is a water fern and Anabaena is a nitrogen fixing cyanobacterium . They are found flourishing over the surface of streams and ponds all through the tropical and temperate regions. Azolla provides a niche, water from surroundings and organic substrates and nutrients for the growth and replication of Anabaena . Azolla forms a dense layer on the surface of water and and absorbs nitrates from water. Anabaena carries out absorption of ammonia secreted . This partnership not only helps in the growth of rice fields but Azolla are also beneficial in biological control of water insects from invading the rice crop. In return this association is benefited with a proper niche , attachment to the host, growth of Azolla and replication of Anabaena.

• *Establishment Of Symbiosis :*

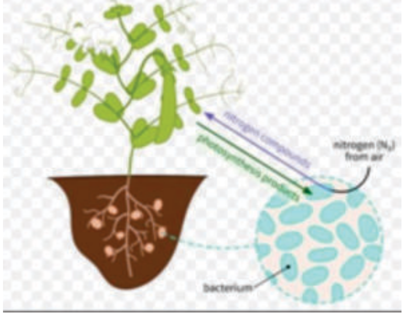
Rhizobium invasion and establishing inside the host root and further formation of root nodules is an intricate process.

1. **Host Specificity And Curling Of Roots:** Host recognition is brought about that initially depends on surrounding environmental conditions. Host plant secretes exudes in rhizosphere. Bacteria grows near the root in the rhizosphere. The primary host response results into root curling due to secretion of chemical substances like cytokinin , polymixin B , etc.
2. **Infection Of Root Hair:** Rhizobial aggregates have been observed at different sites on curled root hair leading to a infection thread that penetrates each cell. Plasmolysis is a crucial mechanism of root exudation.
3. **Nodule Formation**: The inner cortical cells are stimulated by growth hormones as the infection thread shows a continuous growth their the root tissue. Rhizobia are set free from the infected threads, multiply and acquire the center portion of root nodule. Once nodule formation occurs, multiple nodules are seen to be established in the root hair.



Concluding Remarks :

The potential of nitrogen fixing bacteria in the growth of plants has been well studied over decades. However much of the research has been confined to the boundaries of benefit to the plant ,moreover the research over benefit to bacteria is limited. The bacteria promotes plant growth by making nitrogen available for it. While in return the plant provides the bacteria with protected niche, energy, O2 for respiration, nutrients in the form of organic acids by the mechanism of photosynthesis, growth and replication factors. Microbes play an important role in ecosystems. If bacteria don't take an initiative in the nitrogen fixation process, then most of the photosynthesis would languish within a year.



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