BIOLOGICAL NITROGEN FIXATION

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BIOLOGICAL NITROGEN FIXATION

- ✓ The utilization of atmospheric di-nitrogen gas (N_2) as a source of cell nitrogen by way of its reduction to ammonia by a biological agent is called **biological nitrogen fixation**.

- ✓ The reduction of N_2 to ammonia is catalyzed by **nitrogenase**, a key enzyme-system of biological nitrogen fixation.

- ✓ The genes that code for the enzyme nitrogenase are collectively called ***nif genes***.

MODES OF NITROGEN FIXATION:

I) Non-symbiotic Nitrogen Fixation: This type of biological nitrogen fixation is effected by those microorganisms, which live freely and independently in the soil. A large number of bacteria and some cyanobacteria are capable of non-symbiotic N_2 fixation.

Examples: *Azotobacter, Azomonas*, *Mycobacterium, Thiobacillus, Clostridium, Klebsiella*, etc.

- *Azotobacter* spp. (aerobic) and *Clostridium* spp. (anaerobic) are the main nitrogen fixing free living bacteria.
- Cyanobacteria may fix ten times as much nitrogen as the other free-living bacteria. They are mainly responsible for maintaining the fertility and productivity of rice fields.
- *Anabaena* and *Nostoc* are good examples of nitrogen fixing cyanobacteria.

MODES OF NITROGEN FIXATION

II) Associative Symbiotic Nitrogen Fixation:

- They are usually found in association with the roots of grasses and cereal plants.
- These bacteria grow in the rhizosphere in close contact with the roots, sometimes invade the outer cortical regions of the roots, and fix nitrogen.
- Examples: *Azospirillum*, *Pseudomonas*, *Enterobacter*, *Bacillus*, etc.

MODES OF NITROGEN FIXATION:

III) Symbiotic Nitrogen Fixation:

1. Through nodule formation in legumes:

- This is an important plant-bacteria interactions; the plants being the legumes (soyabeans, clover, alfalfa, beans, peas) and the bacteria being *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium* and *Azorhizobium*.
- These bacteria are gram-negative motile rods.
- Infection of the roots of a leguminous plants by these genera leads to the formation of root nodules that are able to convert atmospheric nitrogen to ammonia by nitrogen-fixing mechanism.
- *Azorhizobium* develops nodules on roots as well as on stems.
- Legume-Rhizobium nitrogen fixation is of considerable agricultural significance as it leads to a greater quantitative enhancement of combined nitrogen in the soil.
- About 90% of all legume species become nodulated.

MODES OF NITROGEN FIXATION:

III) Symbiotic Nitrogen Fixation:

1. Through nodule formation in legumes (continued…):

- *Rhizobium* strains are highly specific to legume species.
- A group of *Rhizobium* strains capable of infecting a group of related legumes is referred to as cross-inoculating group.
- Before these bacteria can fix nitrogen, they must establish themselves in the root cortical cells of the host plant ultimately forming ‘root nodules’.

MODES OF NITROGEN FIXATION:

III) Symbiotic Nitrogen Fixation:

2. Through nodule formation in non-leguminous plants:

- There are certain non-leguminous plants that form nodules to fix nitrogen.
- The best known plant, in temperate regions, is **alder (*Alnus* spp.).**
- The bacterium involved in nodule formation in this case is an actinomycete, ***Frankia*** sp.
- This association has been shown to fix nitrogen quite efficiently and to have a significant effect on the nitrogen balance of some forest ecosystems and in some poor soils, such as glacial morains.
- Other non-leguminous plants that have root nodules are ***Casuarina*** in the tropics and ***Hippophae*** (buchthorn), which colonizes sand dunes and gravel soils.

MODES OF NITROGEN FIXATION:

III) Symbiotic Nitrogen Fixation:

3. Without nodulation:

- Non-nodulating nitrogen fixing associations are formed between **cyanobacteria and various plants**; and **bacteria (*Azospirillum*) and plants**.
- The cyanobacterium ***Anabaena azollae*** forms symbiotic association with ***Azolla***.
- Nostoc is found in the stem of ***Gunnera macrophylla***.
- ***Azotobacter paspali*** develops colonies below mucilaginous root sheath of ***Paspalum notatum.***
- ***Spirillum notatum*** lives associated in or around the roots of Digitaria, Sorghum, Maize, etc.

Root nodule formation in Rhizobium-Legume association:

An association of Rhizobium with leguminous roots and formation of nodules seems to be obligatory for fixing nitrogen.

The stages involved in root nodule formation includes the following:

1. Recognition and Attachment:

- In response to a variety of organic metabolites secreted by the roots of legume plants, the *Rhizobia* migrate towards and grow in the rhizosphere and built up to high population density.
- it is considered that a series of flavonoid signals that are present in the plant organic metabolites lead to the exchange of recognition signals thus attracting specific rhizobial species to specific legume root-hairs.
- ***Rhizobium*** sp. and ***Bradyrhizobium*** possess a specific adhesion protein, called **rhicadhesin** on their surface.
- Rhicadhesin is a calcium-binding protein and binds calcium complexes on the surface of root hairs.
- **Lectins**, carbohydrate containing proteins, also contribute in Rhizobium-legume attachment.

Root nodule formation in Rhizobium-Legume association (contnd..):

2. Penetration and travel:

- After attachment, the root hair curls as a result of the action of substances excreted by the Rhizobium sp. these substances are called nod-factors.
- After curling of the root hair, the bacteria penetrate and enter the root hair and induce the plant to develop a cellulosic tube, called infection thread, which extends inward to the root-hair.
- The Rhizobium cells then spread within the infection thread, move into the underlying root cells, and are released into cytoplasm of the host cell through the action of an organizer produced by the interaction between the rhizobial polysaccharides and components of root cells.
- Nod factors now stimulate root cell division eventually leading to the development of the root nodule.

Root nodule formation in Rhizobium-Legume association (contnd..):

3. Bacteroid formation and development of mature nodule:

- When the bacteria are released from the infection thread into the host cell cytoplasm, they get transformed into swollen, irregular shaped, branched structures called **bacteroids**.
- The bacteroids becomes surrounded singly or in small groups by a plant derived membrane, called **peribacteroiod** membrane, to form structures called **symbiosome**.
- Symbiosomes are the sites of nitrogen fixation.
- Symbiosomes secretes a hormone which enables the production of **leghaemoglobin**, which protects the nitrogen fixation enzymes from oxygen.
- Because of the presence of leghaemoglobin, nodules appear pink in color and those nodules which do not contain it generally fix less nitrogen.
- The ‘haeme’ component of the protein is synthesised by Rhizobium while the ‘globin’ comes from the legume.