

**B.Sc. Botany**  
**Part-I (2019-21)**  
**Paper-II: Microbiology Fungi and Plant Diseases**  
**GROUP-B**

**ROLE OF MICROBES IN NITROGEN FIXATION**

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## ROLE OF MICROBES IN NITROGEN FIXATION

Nitrogen is one of the major limiting nutrients in plant growth. Nitrogen fixation refers to the conversion of atmospheric nitrogen to ammonia and then to nitrogen containing organic compounds that can be available to all forms of life. Nitrogen can be fixed by non-biological processes, such as lightening or the Haber-Bosch process used to produce fertilizer products such as urea. Biological nitrogen fixation is carried out only by prokaryotes, which are belonging to diverse groups.

Biological nitrogen fixation requires three components:

- I. a strong reducing agent,
- II. ATP to transfer hydrogen atoms to dinitrogen ( $N\equiv N$ ), and
- III. the enzyme systems.

The reducing agent (FAD) and ATP are provided by photosynthesis and respiration.

### Nitrogen fixing microbes (Diazotrophs)

Diazotrophs are generally active in rhizosphere. They are classified according to their mode of fixation.

- I. Free living nitrogen fixers
- II. Associative nitrogen fixers
- III. Endophytic nitrogen fixers
- IV. Symbiotic nitrogen fixers

**I. Free living nitrogen fixers:-** They are capable of fixing atmospheric nitrogen independently of other living organism.

- *Azotobacter*- obligate aerobic
- *Clostridium*- anaerobic
- Cyanobacteria- *Anabaena\_cylindrica*, *Gloeocapsa*, *Nostoc\_muscorum*
- *Bacillus polymyxa*- facultative anaerobic

**II. Associative nitrogen fixers :-** Johannah Dobreiner observed a loose association of *Azospirillum\_lipoferum*, a nitrogen fixer, with roots of certain Brazilian grasses and maize in 1975. These are widespread in the soils of tropical, subtropical and temperate regions. *Azospirillum* bacteria are aerobic non-fermentative chemoorganotrophic vibroid to S-shaped containing polyhydroxyalkanoate granules (PHA).

IV. **Endophytic nitrogen fixers**:-Endophytes multiply and spread within plant tissues without causing damage . e.g. *Gluconacetobacter\_\_diazotrophicus*: an acetic acid bacterium first isolated from sugarcane plants.

V. **Symbiotic nitrogen fixers**:-This is mutually beneficial relationship between microbes and plant. e.g.

- **Rhizobium** (Rhizobium-legume association)
- **Bradyrhizobium** (Bradyrhizobium-soybean association)
- **Azorhizobium** (Azorhizobium –*Sesbania\_\_rostrata* association)- Form stem nodule along with root nodule
- **Actinomycetes**  
e.g. Frankia (Frankia-Casuarina association)
- **Cyanobacteria**: Cyanobacteria are one of very few groups of organisms that can convert inert atmospheric nitrogen into an organic form, such as nitrate or ammonia. Nitrogen fixation cannot occur in the presence of oxygen, so nitrogen is fixed in specialized cells called **heterocysts**. These cells have an especially thickened wall that contains an anaerobic environment.
  - \* Lichens (Cyanobacteria-fungus association)
  - \* Bryophytes (Anabaena-Anthoceros)
  - \* Pteridophytes (*Anabaena\_azollae*-*Azolla*)-*Azolla* is a small fast growing aquatic fern. *Anabaena\_azollae* , a cyanobacterium lives in cavities of *Azolla* leaves. It fixes nitrogen from the air and excretes the nitrogenous compounds into the leaf cavity of fern. *A.pinnata* is an excellent biofertiliser for rice.
  - \* Gymnosperms (Nostoc-Cycads), Coralloids root
  - \* Angiosperms (Nostoc-Gunnera)

**Legume Rhizobium symbiosis**:- Rhizobium is predominant symbiotic nitrogen fixing bacterium. It is rod shaped, motile, gram-negative, non-spore forming bacterium and utilize organic acid salts as carbon sources. The bacteria are mostly rhizospheric microorganisms, despite its ability to live in soil for long period of time. Each bacterial sps.infects only certain sps. of plants.

**Nitrogenase:** The biological nitrogen fixation is catalyzed by a multimeric enzyme complex nitrogenase. The enzyme nitrogenase is synthesized by the bacteria.

- It consists of two conserved proteins: an iron (Fe) containing dinitrogenase reductase and a molybdenum iron (Mo-Fe) dinitrogenase, neither of which has catalytic activity by itself.
- It is O<sub>2</sub> labile ( Fe protein ,irreversibly inhibited by molecular oxygen and reactive oxygen species).
- Other substrate for nitrogenase are N<sub>2</sub>O,N<sub>3</sub>, C<sub>2</sub>H<sub>2</sub> etc.

**Leghemoglobin:-** A red iron-containing protein pigment similar to blood hemoglobin is found in the root nodules (between bacterioids and the envelopes surrounding them) of leguminous plant.

- i. The prosthetic group protohaem is synthesized by the bacterioids, whilst the synthesis of the protein part (globin) involves the plant cells.
- ii. It acts as a oxygen scavenger for nitrogenase enzyme and facilitates the diffusion of oxygen to symbiotic bacterioids in order to promote nitrogen fixation.

Recently, it has been known that an excessive use of chemical fertilizer (nitrogenous fertilizer) induces environmental pollution, Therefore, concerns on biological nitrogen fixation, a natural nitrogen supplying source are increasing. Further they are eco-friendly and are compatible with long term sustainability.