SEMESTER- III
SEC – A : UNIT-2
Non-Symbiotic Nitrogen Fixers

- **Symbiotic N_2-Fixing Bacteria:**

The heterotrophic bacteria that fix di-nitrogen gas (N_2) from the atmosphere in plant root nodules (symbiotic bacteria) have a mutually beneficial relationship with their host plants. Legumes (pod-bearing plants such as peas, beans, alfalfa and clovers, etc.) had a beneficial effect upon both companion and whatever crop was planted next in the same soil. It is evident that the fixation of atmospheric nitrogen in the legume is due to the formation of root nodules. Symbiotic bacteria initially start by infecting root hairs, causing an invagination (enclosing-like sheaths) inward through several cells. Surrounding plant cells proliferate quickly, perhaps because of auxin, a phytohormone produced by the infecting bacteria. As the bacteria enter the nodule cells, they form enclosing membranes and produce meta-haemoglobin, an oxygen-carrying pigment (the nodule may be pink in cross-section). The haemoglobin like material may be an oxygen sink or trap to keep the bacteria in an anaerobic environment, which is necessary for N_2 fixation.

The di-nitrogen (N_2) fixation is performed by the enzymes nitrogenase. This enzyme lowers the activation energy (the energy required to perform the reaction). The fixation proceeds in reduction stages from di-nitrogen (N = N) through uncertain intermediates HN=NH and $H_2N\text{-}NH_2$ to produce 2 NH_3. Finally, the ammonium is transformed into some organic compounds such as amino acids. All of this will take place when the nitrogen is bonded to the enzyme(s).

The lifetime of a bacterium may be only a few hours and the bodies of a portion of the bacterial population are continuously dying, decomposing, and releasing NH_4^+ and NO_3^- ions for the utilization by the host plant. Most of the nitrogen fixed is excreted by the bacteria and made available to the host plant and to the other plants growing nearby. The well-known symbiotic bacteria belong to the genus *Rhizobium*. Symbiotic heterotrophic bacteria specific to the crop to be grown are frequently applied or inoculated, in a dried powder from the crop seed to ensure that nitrogen fixing organisms are present. The same bacterial species will not inoculate all legumes. *Sesbania rostrata* (dhaincha) was found to form nodules both in roots and stems and it is most important host plant for the symbiotic N_2-fixation.

- **Non-Symbiotic N_2-Fixing Bacteria:**

The non-symbiotic nitrogen fixing bacteria do not require a host plant. In 1891, Winogradsky observed that when soil was exposed to the atmosphere, the nitrogen content of the soil was recorded to be increased.
The anaerobic bacterium *Clostridium pasteurianum* was found responsible for such an increase of the nitrogen content in soil. In 1901, Beijerinck proved that there were also free-living aerobic bacteria, *Azotobacter chroococcum* that could fix atmospheric nitrogen.

Non-Symbiotic Nitrogen Fixation:

- **Diazotrophs:**

Microorganisms which pass independent life and fix atmospheric nitrogen are known as free living diazotrophs. There are two groups of such micro-organisms: bacteria and cyanobacteria (blue-green algae). Based on the mode of nutrition (carbon, nitrogen and oxygen and requirement of reducing groups) bacteria are divided into
(i) Aerobic bacteria *(Azonomas, Azotobacter, Beijerinckia, Mycobacterium, Methylomonas),*
(ii) Facultative anaerobic bacteria (*Bacillus, Enterobacter, Klebsiella,* etc.),
(iii) Anaerobic bacteria (*Clostridium, Desulfovibrio,* etc.) and
(iv) Photosynthetic bacteria *(Rhodomicrobium, Rhodopseudomonas, Rhodospirillum, Chromatium, Chlorobium,* etc.).

Among cyanobacteria, both heterocystous and non-heterocystous forms fix atmospheric nitrogen, for example, *Anabaena, Anabaenopsis, Aulosira, Calothrix, Cylindrospermum, Gloeocapsa, Lyngbya, Nostoc, Oscillatoria, Plectonema, Scytonema, Stegonema, Tolypothrix, Trichodesmium,* etc.

Eighty percent (80%) of the atmosphere is nitrogen gas (N_2). Unfortunately, N_2 is unusable by most living organisms. Plants, animals, and microorganisms can die of nitrogen deficiency, if surrounded by N_2 they cannot use. All organisms use the ammonia (NH_3) form of nitrogen to manufacture amino acids, proteins, nucleic acids, and other nitrogen-containing components necessary for life. Biological Nitrogen Fixation (BNF) process changes inert N_2 to useful NH_3. This process is mediated in nature only by bacteria and certain species of *Actinomycetes*. In the free-living system, plants gain benefit when bacteria die and release nitrogen to the environment, or when the bacteria are loosely associated with the roots of plants. In legumes and a few other plants, bacteria live in small club-like growths on the roots called nodules. Within these nodules, N_2 fixation occurs, and the NH_3 produced is directly absorbed by the plant. Nitrogen fixation by legumes is a close/symbiotic relationship between a *Rhizobium* bacterium and a legume host plant. Biological nitrogen fixation takes many forms in nature from the:

1) symbiotic forms including blue-green algae (*Nostoc*),
2) lichens, *Actinomycetes*, non-legumes
3) associative symbiosis and
4) free-living soil bacteria.

These types of N_2 fixation contribute significant quantities of NH_3 to natural ecosystems. Nitrogen fixation by legumes can be in the range of 25-75 pounds of N_2 per acre per year in a natural ecosystem and several hundred pounds in a cropping system. Symbiotic N_2 fixation occurs through associations of plant roots with nitrogen-fixing bacteria. The symbioses are:

1) between many leguminous species and *Rhizobium* or *Bradyrhizobium*, forming nodules on roots;
2) between a small number of non-leguminous genera and *Frankia*.

The utilization often associative BNF technology in grass and cereal crops was found to be useful in the development of profitable agriculture technologies. The findings of several authors revealed existing associations of tropical grasses with nitrogen-fixing bacteria, that which under favourable conditions, may be contributing significantly to the N_2 economy of these plants. *Azospirillum* as a "biofertilizer" is particularly important in agricultural systems where fertilizer inputs are either impractical (rangelands), undesirable (organic farming), or not possible (subsistence agriculture). Experiments on inoculation of crops with

Azospirillum or other diazotrophs often resulted in enhanced plant growth or nitrogen content under environmental conditions, improve nutrient assimilation, alter root size and function. Numerous studies have shown greater N2 fixation activities in inoculated plants than in uninoculated controls. In addition to N_2 fixation, inoculation with *Azospirillum* results in the following benefits:

1. Promotion of root hair development and branching;
2. Increased uptake of N, P, K and microelements;
3. Improved water status of plants and,
4. Increased dry matter accumulation and grain yield.

Inoculated plants when examined under the electron microscopes revealed invasion of the cortical layer. From the roots of “talahib” a native grass (*Saccharum spontaneum L.*) several nitrogen-fixing bacteria were isolated which when used as inoculants were found capable of enhancing shoot growth, root density and yield of rice, corn and sugar cane. The bacteria were found to possess at least 57 % of the characteristics of the genus *Azospirillum*. Several reports from field tests in different regions of the country confirmed the significant contribution of these nitrogen-fixing bacteria to yield improvement of corn, rice and a few vegetable species. These associative N_2 fixing bacteria were found to be capable of producing growth regulators like gibberellins and cytokinins, which were thought to contribute to the stimulated plant growth. The bacteria belong to the genus *Azospirillum* and are the most promising microorganisms that colonize roots of economically important grasses and cereals. *Azospirillum* species are described as Gram negative, rod-shaped, 1mm in diameter, very motile. Cells are about 1.0 um x 3.5 mm in size single flagellum when grown in MPSS broth while lateral flagella when grown on MPSS agar at 30 °C. They also form wrinkled, dark pink colonies when grown on MPSS agar. A formation of a white veil or bacteria band, is visible when inoculated into an Nfb and Dobereiner’s liquid medium. *Azospirillum* utilizes glucose, lactate, succinate, fructose, malate, pyruvate, fumarate, as carbon source, reduced nitrate and does not require biotin. The N_2 source used by *Azospirillum* for their growth are: Ammonium, Nitrate, Amino acids, elemental N_2.

Why Nitrogen Fixation is important?