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Topic- Role of microbes in nitrogen fixation.

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ROLE OF MICROBES IN NITROGEN FIXATION

Nitrogen forms the main bulk of the atmosphere (78%) as well as the biological systems. Various nitrogenous compounds, e.g., proteins, enzymes, chlorophylls, nucleic acids, etc. play vital roles in the life processes of organisms.

The atmospheric nitrogen is chemically inert and is not directly taken by most of the living organisms. The latter, therefore, depend on a source of combined nitrogen or organic nitrogen compounds for their growth.

They obtain nitrogenous compounds from soil and convert them into essential biomolecules needed for their healthy development. In addition, a part of the great reservoir of the atmospheric nitrogen is converted into an organic form by certain free living microorganisms and by plant-microorganism associations which make it available to the plants.

Animals obtain it from plants. The percentage of nitrogen in the atmosphere remains constant by the operation of a nitrogen-cycle in nature.

Nitrogen is continually entering in the air by the action of denitrifying bacteria and continually returning to the cycle through the action of nitrogen-fixing microorganisms, lightning, and industrial production of artificial fertilizer.

This sequence of changes from free atmospheric nitrogen to fixed inorganic nitrogen, to simple organic compounds, to complex organic compounds in the tissues of microorganisms, plants and animals, and the eventual release of this nitrogen back to atmospheric nitrogen is dealt under the ‘nitrogen cycle’.

Microorganisms which play a significant role in the operation of nitrogen cycle may be divided into following four major groups:

Group 1:

Those microorganisms which are capable of fixing atmospheric nitrogen (biological nitrogen fixation), i.e., of combining free nitrogen with other elements or compounds.

Group 2:

Those microorganisms which bring about the production of ammonia, i.e., ammonification.

Group 3:

Those microorganisms which oxidise ammonia to nitrite and nitrite to nitrate, i.e., nitrification.

Group 4:

Those microorganisms which are capable of transforming nitrates to nitrogen or nitrous oxide, i.e., denitrification.

The following points highlight the top three processes of nitrogen fixation in plants. The processes are: 1. Atmospheric Nitrogen Fixation 2. Biological Nitrogen Fixation 3. Industrial Nitrogen Fixation.

Process # 1. Atmospheric Nitrogen Fixation:

The enormous energy of lightning breaks nitrogen molecules and enables them to combine with the oxygen in the air forming nitrogen oxides. These dissolve in water forming nitrates, that are carried to the earth. Atmospheric nitrogen fixation probably contributes about 5-8% of the total nitrogen fixed.

Process # 2. Biological Nitrogen Fixation:

Biological nitrogen fixation occurs when atmospheric nitrogen (N=N) is reduced to ammonia by complex set of enzymes called nitrogenase and huge expenditure of ATP. Although the first stable product is ammonia, it is quickly incorporated into protein and other organic nitrogen compounds.

It can be represented by the following equation:

$N_2 + 8H^+ + 8e^- + 16ATP = 2NH_3 + H_2 + 16ADP + 16Pi$

Nitrogenase enzyme

Two molecules of ammonia are produced from one molecule of nitrogen gas, at the expense of 16 molecules of ATP and a supply of electrons and protons (H ions), in the presence of enzyme complex nitrogenase. The enzyme is a biological catalyst found naturally only in certain microorganisms called diazotrophs.

Micro-organisms that fix atmospheric nitrogen are:

(a) Aerobic:

Bacteria Azotobacter, Beijerinckia, Klesbiella, Cyanobacteria e.g., Nostoc. Anabaena

(b) Anaerobic:

Bacteria Clostridium, Desulfovibria, Purple sulphur bacteria. Green sulphur bacteria.

(c) Symbiotic with plants:

(i) Legumes:

Leguminous plants of family Fabaceae (Taxa such as Clovers. Soya-beans, Alfalfa, pea-nuts etc.)contain symbiotic bacteria called Rhizobium (Bacillus radicola) within the nodules in their root system. These crops are also referred as green manure.

(ii) Other Plants:

Frankia (soil bacteria) forms nitrogen fixing root nodules in actinorrhizal plants e.g., Alder, Bayberry etc. (sometimes called actinorrhizae). Parasponia also able to interact with Rhizobium and forms root fixing nodules.Azospirillum, Azotobacter (free living nitrogen fixing bacteria) grow in the rhizosphere of grasses and several other plants and fix atmospheric nitrogen.Spirillum lipoferm is associated with cereal grasses.

These are also some other symbiotic nitrogen fixing associations for e.g., Cyanobacteria (Nostoc, Anabaena) in the coralloid roots of Cycas and thallus of Anthoceros, mosquito – fern Azolla and some species of lichens such as Loberia and Peltigera etc. Biological nitrogen fixation was discovered by the Gereman agronomist Hermann Hellriegel and Dutch microbiologists M. Beijerinck.

Process # 3. Industrial Nitrogen Fixation:
Under great pressure, at temperature of 600°C and with the use of catalyst, nitrogen and hydrogen (usually derived from natural gas or petroleum) can be combined to form Ammonia (NH_3). It can be used directly as fertilizer but most of it is further processed to form urea and ammonium nitrate, (NH_4NO_3).
Artificial fertilizer production is now the largest source of human-produced fixed nitrogen in the Earth's ecosystem. The most common method is Haber process. This process requires a high pressure (200 atms) and high temperature (at-least 400°C). Nitrogen fixation is essential for agriculture and manufacturing of fertilizers. It is also an important process in the manufacturing of explosives e.g., gunpowder, dynamite, TNT etc.

These nitrates are absorbed by the plants for the manufacture of complex nitrogenous compounds. Plants in turn are eaten by animals. When the plants and animals die, certain bacteria, fungi and other microbes start acting on them and decompose the protein of dead organisms into ammonia.

Such bacteria are called ammonifying bacteria and the process is known as ammonification. Ammonia is converted into soluble ammonium compounds which are released in the soil or water. Enzymes involved in ammonification are GS (Gin synthetase, GOGAT (Glu 2-oxoglutarate aminotransferase), GDH (Glu dehydrogenase) etc. Ammonia is converted into soluble ammonium compounds which are released in the soil or water. Ammonia or the ammonium compounds are converted into nitric acid and then into nitrate by nitrifying bacteria.

This process is known as nitrification and occurs in two steps:
Step I: Ammonia is converted into nitrite.

$$\underset{\text{Ammonia}}{2NH_3} + 3O_2 \xrightarrow{\text{Bacteria } \textit{Nitrosomonas}} \underset{\text{Nitrite}}{2HNO_2} + 2H_2O + 79\ \text{kcal.}$$

Step II: Nitrite are converted into nitrates

$$\underset{\text{Nitrite}}{2HNO_2} + O_2 \xrightarrow{\text{Bacteria } \textit{Nitrobacter}} \underset{\text{Nitrate}}{2HNO_3} + 21.6 \text{ kcal}$$

In reverse reaction, some bacteria break of nitrates, nitrites and ammonium compounds to molecular nitrogen which is returned to atmosphere to complete the nitrogen cycle. Such bacteria are called denitrifying bacteria e.g., Pseudomonas denitrificans, Thiobacillus denitrificans, Micrococcus, Bacillus licheniformis etc. and this process is known as de-nitrification. This inorganic nitrogen is again recycled into the organic system upon absorption by the plants (fig. 1).

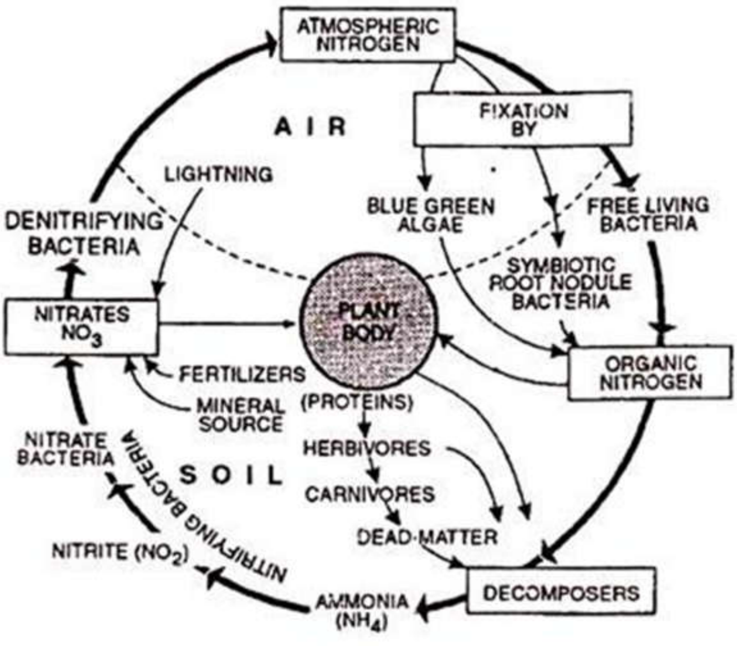

Fig. 1. Nitrogen cycle