Biological Nitrogen fixation

- Microorganisms function as-
- **populations** or assemblages of similar organisms, and as
- **communities,** or mixtures of different microbial populations

- Microbes - evolved while interacting with inorg. world and higher organisms, play beneficial and vital roles; disease-causing organisms are only a minor component of the microbial world..

- **ecosystem,** or self-regulating biological communities and their physical environment, is the sum total of all organisms and abiotic factors in a particular environment, such as soil, water, animal body etc.
- Includes many **habitats**, portions of ecosystems suited to one or small no. of microbial populations,

- Knowledge of these interactions is important in understanding both microbial contributions to the natural world and microbial roles in disease processes.

Rolee Sharma, CSJM University, Kanpur

Nitrogen and sulphur must be assimilated in plant

- Plants – autotrophs that can synthesize organic molecular components out of inorganic nutrients from the environment.
- This process- essential for plant growth and development, and is necessary for production of a wide range of organic substances including nucleic acids, amino acids, enzymes, cofactors, pigments and lipids.
- Many mineral nutrients are absorbed from soil into roots and then incorporated directly into organic compounds.
- In contrast, N and S must both be assimilated into organic metabolites via a complex series of high energy requiring biochemical reactions.
- N- mainly used for production of macromolecules, while S – crucial role in catalytic or electrochemical functions of biomolecules.
- Plants need source of inorganic N2. 99.95% of N2 on biosphere is present in atmosphere or dissolved N2, but this is available to only v. few plants.
- Most plants get their cellular Nitrogen from nitrate in cultivated or aerated neutral soils, or ammonium in acid or waterlogged soils.
- In some plants, N is fixed via a symbiotic relationship with N-fixing bacteria, such as Rhizobium or via a mycorrhizal relationship between plants and actinomycete fungi, converting molecular N2 into ammonia.
- Nitrate assimilation initially converts nitrate via nitrite and ammonium into the amide nitrogen of glutamine for its subsequent incorporation into organic material.
- Sulfate assimilation involves conversion of sulfate via adenosine 5’-phosphosulphate to sulphite and sulphide before being converted into amino acid cysteine.

- Nitrogen is the most abundant element in plants.
- N2 – clearly important for plant growth, and the most abundant source of global inorganic N2 is in atmosphere (~80% by vol).
- However, much of this N2 –not directly accessible to living organisms. In contrast to other plant nutrients, as PO4 or SO4-, NO3- cannot be delivered by weathering of rocks. Ammonia, the primary product for synthesis of Nitrate fertilizer, is produced by **Haber-Bosch process**, that requires production of high pressure and temperature (400-500ºC). 3H2 + N2 ---□ 2NH3 (ΔH -92 kJ/mol)
- To obtain N from atm. it is necessary to break the extremely stable triple covalent bond b/w 2 N atoms to form ammonium. Once fixed into ammonium, N enters the biogeochemical cycle, passing through a number of organic or inorganic forms before returning to molecular N2. This interconversion b/w different oxidation states contributes to global N2 cycle.
- Reductive conversion or N-fixation, occurs through a no. of biological or non-biological routes.
- Small amounts of NO3 are generated by lightening and carried into soil by rain water.
- N-fixation - process by which nitrogen in the earth's atmosphere is converted into ammonia, a substance required for the biosynthesis of amino acids.
- Microorganisms have a key role in N_2 fixation (availability) and thus support life on earth. N-fixation can be carried out by aerobic or anaerobic procaryotes and does not occur in eucaryotes.
- Both bacteria and plants benefit by this association. The bacteria provide NH_3 (fixed N_2) to plants and in turn bacteria derive nutrients from tissues of the plants.

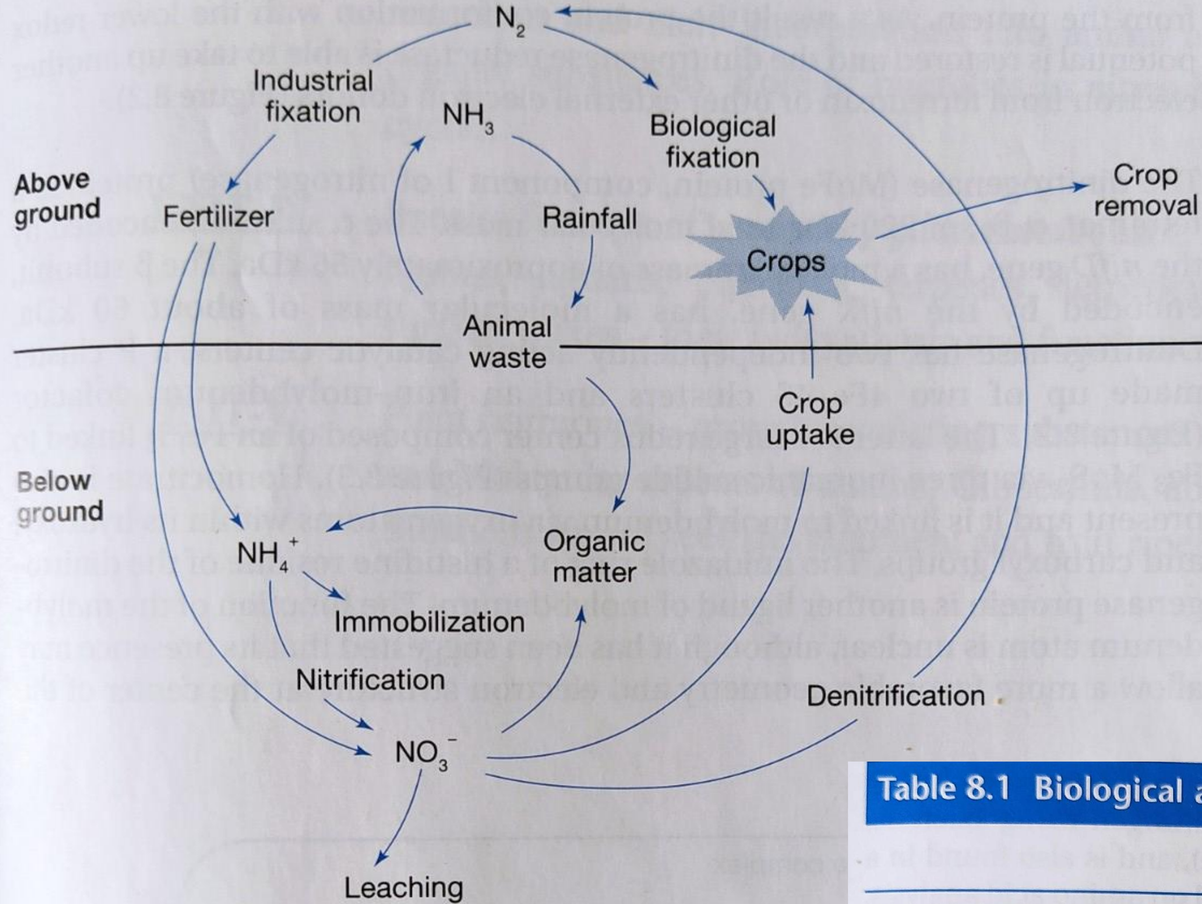


Table 8.1 Biological and nonbiological nitrogen fixation processes

	Total nitrogen fixed per year
Non-biological nitrogen fixation	
Atmospheric fixation via lightning and photochemical conversion of molecular nitrogen to ammonium	10 Tg
Industrial fixation of nitrogen via Haber-Bosch reaction used in nitrogenous fertilizer production	50 Tg
Fossil fuel combustion	20 Tg
Biological nitrogen fixation	
Agricultural land	90 Tg
Forest and nonagricultural land	50 Tg
Marine	35 Tg

The standard unit of measurement is the teragram, Tg, which is equivalent to 10^6 metric tons.

Nitrogen fixation: some plants obtain N_2 from atmosphere via a symbiotic association with bacteria, process by which nitrogen in the earth's atmosphere is converted into ammonia, a substance required for the biosynthesis of amino acids.

- Nitrogen is fixed, or combined, in nature as nitric oxide by lightning and UV rays, but more significant amounts of nitrogen are fixed as ammonia, nitrites, and nitrates by soil microorganisms.
- >90 percent of all nitrogen fixation is effected by them. Two kinds of nitrogen-fixing microorganisms are recognized:

1. **Non-symbiotic (free-living) bacteria** in soil, including aerobic cyanobacteria, *Anabaena, Nostoc, Azotobacter spps., Beijerinckia, Clostridium pasteurianum (a spore forming anaerobe).*
   -N_2 fixed by non-symbiotic bacteria range from 22-55 kg/hectare.*
   -Process important in flooded rice fields, barren/rock surfaces & cultivated arid soils.

2. **Symbiotic bacteria** as *Rhizobium,* (nodule inducing bacteria) associated with leguminous plants (soyabean, lentils, pea, clover) and various *Azospirillum* spps, associated with cereal grasses.

Symbiotic N2 fixation- is of particular importance to agriculture. Thus legumes are important as green manure and in crop rotation, are inexpensive alternative to artificial fertilizers. *e.g. Rhizobium* – legumes,

- Water fern *Azolla* with cyanobacterium ***Nostoc*** supplies rice fields with N_2.
- N_2 fixing actinomycetes of the genus ***Frankia*** forms symbiosis with woody plants as *Alder* or Australian casurina trees.
- *Anabaena* - Azolla and others *hectare (ha)= 100 sq m)

TABLE 12.2
Examples of organisms that can carry out nitrogen fixation

Symbiotic nitrogen fixation	
Host plant	**N-fixing symbionts**
Leguminous: legumes, *Parasponia*	*Azorhizobium*, *Bradyrhizobium*, *Photorhizobium*, *Rhizobium*, *Sinorhizobium*
Actinorhizal: alder (tree), *Ceanothus* (shrub), *Casuarina* (tree), *Datisca* (shrub)	*Frankia*
Gunnera	*Nostoc*
Azolla (water fern)	*Anabaena*
Sugarcane	*Acetobacter*

Free-living nitrogen fixation	
Type	**N-fixing genera**
Cyanobacteria (blue-green algae)	*Anabaena*, *Calothrix*, *Nostoc*
Other bacteria	
Aerobic	*Azospirillum*, *Azotobacter*, *Beijerinckia*, *Derxia*
Facultative	*Bacillus*, *Klebsiella*
Anaerobic	
Nonphotosynthetic	*Clostridium*, *Methanococcus* (archaebacterium)
Photosynthetic	*Chromatium*, *Rhodospirillum*

- Under **aerobic** conditions a wide range of
  - free-living microbial genera

    Aerobic *Azotobacter, Azospirillum* contribute to this process.

    Anaerobic *Clostridium*, purple and green bacteria
  - Symbiotic - (bacteria that develop symbiotic associations with plants.)

    Rhizobium, Bradyrhizobium with legumes,

    Frankia in association with many woody shrubs, and

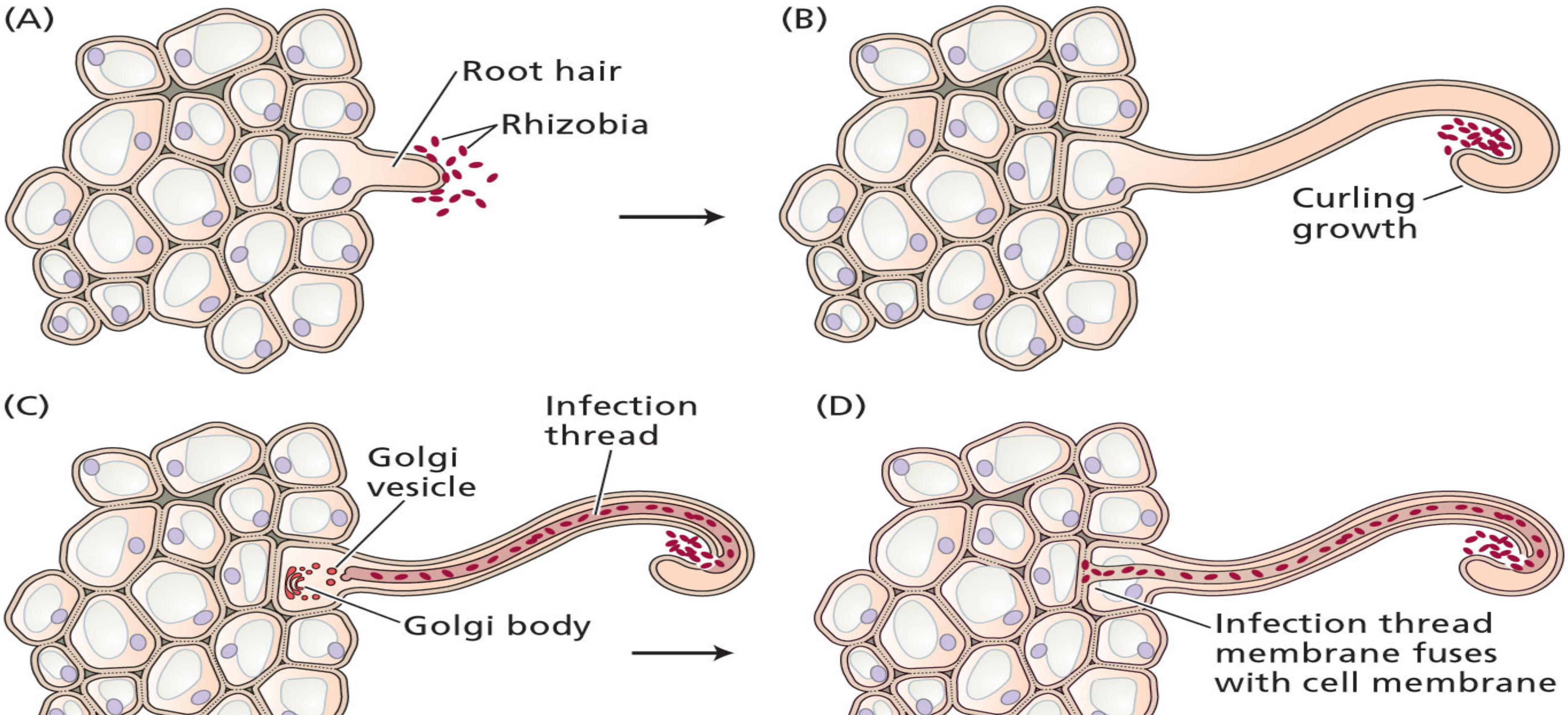
    Anabaena, with *Azolla,* a water fern important in rice cultivation.

- The most efficient & best studied system of symbiotic N_2 fixation is that of **Rhizobium bacteria**, growing attached to roots of legumes (plants that bear seeds in pods) such as peas, beans, gram etc.
- Nodule inducing bacteria include *Rhizobium, Azorhizobium* and *Bradyrhizobium,* collectively called **rhizobia.**

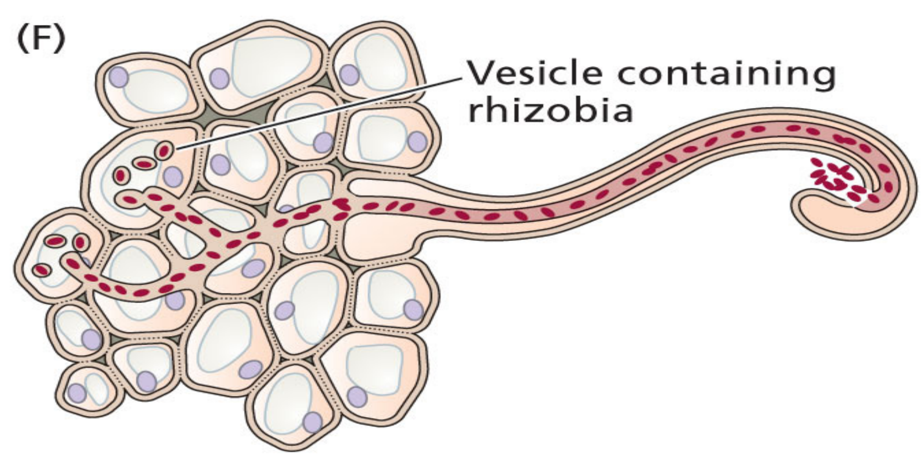
- Estimated- amount of N_2 fixed by a legume crop ranges b/w **55-550 kg/ha**. In order to achieve effective N_2 fixation in Rhizobium legume system, it is necessary to select the proper strain of *Rhizobium* bacteria. To ensure sufficient nodule formation and optimum growth of legumes (e.g., alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium.

Legumes form symbiosis with nodule-inducing bacteria

- Initially thought nodules-caused by plant disease, their function in N2 fixation was recognized by H. Hellriegel and H.Wilfarth in 1888. They found that beans containing these nodules were able to grow without N2 fertilizer.
- Rhizobia are strictly aerobic gram-negative rods, which live in the soil and grow heterotrophically in presence of organic compounds.
- Uptake of rhizobia into host plant is a **controlled infection.** The Rhizobia are capable of invading the root hair cells of host plants and establish a mutualistic partnership.
- Rhizobia form species specific nodulation factors (**Nod factors**). These are lipochito-oligosaccharides that acquire a high structural specificity (as by acetylation, sulfation etc)They are like security key with many notches and open house of specific host with which rhizobia associate. Nod factors bind to specific **receptor kinases** of host, that initiate signal transduction cascade. In this way, the ‘key’ induces the root hair of the host to curl and the root cortex cells to divide, forming the **nodule primordium.** Initially bacteria form an **infection thread** in root hair cells, the infection thread allows bacteria to penetrate deeper in cortex of roots, forms branches and infects cells of nodule primordium, infecting tissues and further cells.
- The infected root cells enlarge and divide at an increased rate, leading to development of large masses of cells called as **root nodules** (= enlargements of plant cells and bacteria in intimate association). Nodules are connected with root via vascular tissues, supplying them with products of photosynthesis.
- Bacteria in plant cell, are enclosed by **a peribacteroid membrane**, formed by plant, and is thus separated from cytoplasm of host cell in a so called **symbiosome**, where it differentiates to **bacteroids**. Several bacteroids are surrounded by a peribacteroid membrane.



Roots emit chemical signals that attract Rhizobium bacteria. The bacteria emit signals that stimulate root hairs to elongate, and to form an infection thread by an invagination of the plasma membrane



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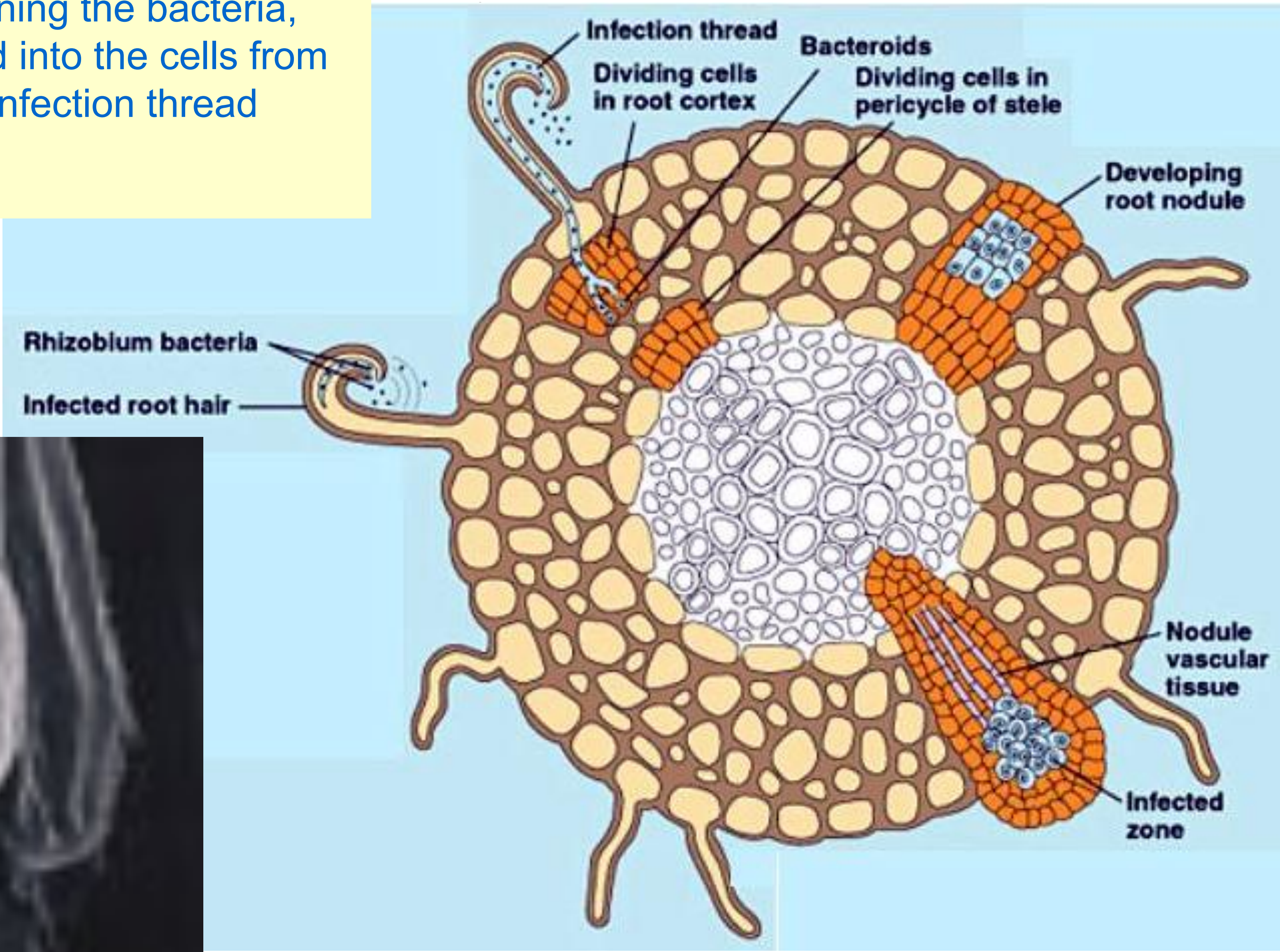
The bacteria penetrate the root cortex within the infection thread. Plant cells start dividing and vesicles containing the bacteria, bacteriods, bud into the cells from the branching infection thread

Growth continues in the affected regions of the cortex and pericycle and these fuse to form the nodule



The formation of nodules is due to a regulated interplay of expression of specific bacteria and plant genes

- Within the root nodules, the Rhizobium bacteria **nitrogenase system** convert free nitrogen gas to ammonia, which the host plant utilizes to make amino acids.
- Mol. biologists have characterized genes responsible for N2 fixation (*nif* and *fix* genes) encoding and regulating nitrogenase and *nod* genes inducing nodule formation.
- The host plant signals its readiness to form nodules by excreting several **flavonoids** as signal compounds.
- These flavonoids bind to a bacterial protein, encoded by a constitutive ***nod*** **gene.**
  - The protein to which flavonoid is bound, activates transcription of other *nod* genes. The proteins encoded by these *nod* genes are involved in the synthesis of Nod factors .
  - 4 ‘general’ *nod* genes – present in all Rhizobia, and 20 other *nod* genes – responsible for host’s specificity.
- Proteins required for nodule formation and synthesized by **host plant** during nodule formation = **nodulins**. These include leghemoglobin, enzymesof carbohydrate degradation, citric acid cycle, synthesis of glutamine and asparagine and also aquaporin of peribacteroid membrane.
- Plant genes encoding these proteins are early and late **nodulin genes.**
- Early nodulins are involved in process of infection & nodule formation, induced by signal substances released from rhizobia.
- Late nodulins are synthesized only after formation of nodules.

- **Metabolic products are exchanged between bacteroids and host cells**.
- Malate – provided by host cells to bacteroids.

In symbiotic nitrogen-fixing organisms such as *Rhizobium*, root nodules can contain *oxygen-scavenging molecules* such as ***leghaemoglobin***, which shows as a pink colour when the active nitrogen-fixing nodules of legume roots are cut open. Leghaemoglobin may regulate the supply of oxygen to the nodule tissues in the same way as haemoglobin regulates the supply of oxygen to mammalian tissues

- These plants thus protects the nitrogenase of bacteria from O_2. It does this by surrounding the bacteria with a red *O_2-binding pigment* k/a **Leghemoglobin** that resembles Hb of animal blood in many aspects..

Clover root nodules.

Leghaemoglobin is found only in the nodules and is not produced by either the bacterium or the plant when grown alone.