SYMBIOTIC N_2 FIXATION

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Introduction

- **The capacity for fixing atmospheric nitrogen is widespread among free-living bacteria.**
- **However, while N_2-fixing bacteria are common in soils, it has been estimated that the bulk of terrestrial biological nitrogen fixation is due to symbiotic associations between plants and specific types of bacteria.**
- **Nitrogen fixation requires a high energy expenditure as well as anaerobic or microaerobic conditions, both of which can be provided by plants (Table 1), and this explains the prevalence of symbiotic N_2 fixation.**
- **Symbiotic nitrogen fixation can be defined as the part of a mutualistic relationship in which plants provide a niche and fixed carbon to bacteria in exchange for fixed nitrogen.**
- **It has been estimated that about half of Earth's biological nitrogen fixation is due to legume cultivation.**

TABLE 1. Established Examples of Symbiotic Nitrogen Fixation

Hosts	N_2-Fixing Symbionts	Symbiont-Containing Organs
Legumes	Rhizobia	Root nodules
Various woody plants	*Frankia*	Root nodules
Cycads	*Nostoc*	Coralloid roots
Azolla (fern)	*Anabaena*	Leaf cavities

Symbiotic N_2 Fixation in Legumes

- Rhizobia (legumes root nodulating bacteria belongs to several genus viz. *Rhizobium, Bradyrhizobium, Sinorhizobium, Allorhizobium* – all of them commonly known as rhizobia) occur as free-living bacteria in soils.
- They are relatively rare in soils in which legumes have not been grown over a period of many years, but are especially numerous in the rhizosphere (the soil surrounding roots) of legumes.
- Rhizobia multiply around germinating legumes.

Root Nodule Development

- The symbiosis is triggered by nitrogen starvation of the host plant which has to select its rhizobia partner from billions of bacteria in the rhizosphere.
- The complex infection process-appears to involve a series of molecules produced by the host pant that lead to the exchange of recognition signals.
- **The Recognition Process:** occurs when a potential root nodule forming microbe approaches the plant root and is assumed to be an alien invader.
- Plants responds with an oxidative burst (producing mixture of superoxide radicals, H_2O_2, N_2O) determining the fate of infection process.
- Rhizobia that uses antioxidant defenses survive and continue the infection process.
- Plant root releases flavonoids that activate NodD1 and start the sequence of Nod (nodulation factor) synthesis by bacteria.
- The bacterium contains a large plasmid (also called sym-plasmid) that encodes information that is not used when it grows as a free-living organism in the soil, but is vital for infection and nodulation of the susceptible host plant. Plasmid-encoded genes also influence the range of host plants that rhizobia can nodulate.

Infection process-Early events

Attachment of Rhizobia: Process begins after chemotaxis of rhizobia towards root hairs followed by adhesion to root hairs. Adhesion depends on specific lectins produced by the host plant, and on specific polysaccharide (rhicadhesins) cell coatings produced by the bacteria, that affect the pattern of attachment and *nod* genes expression.

Early events in root nodule formation:

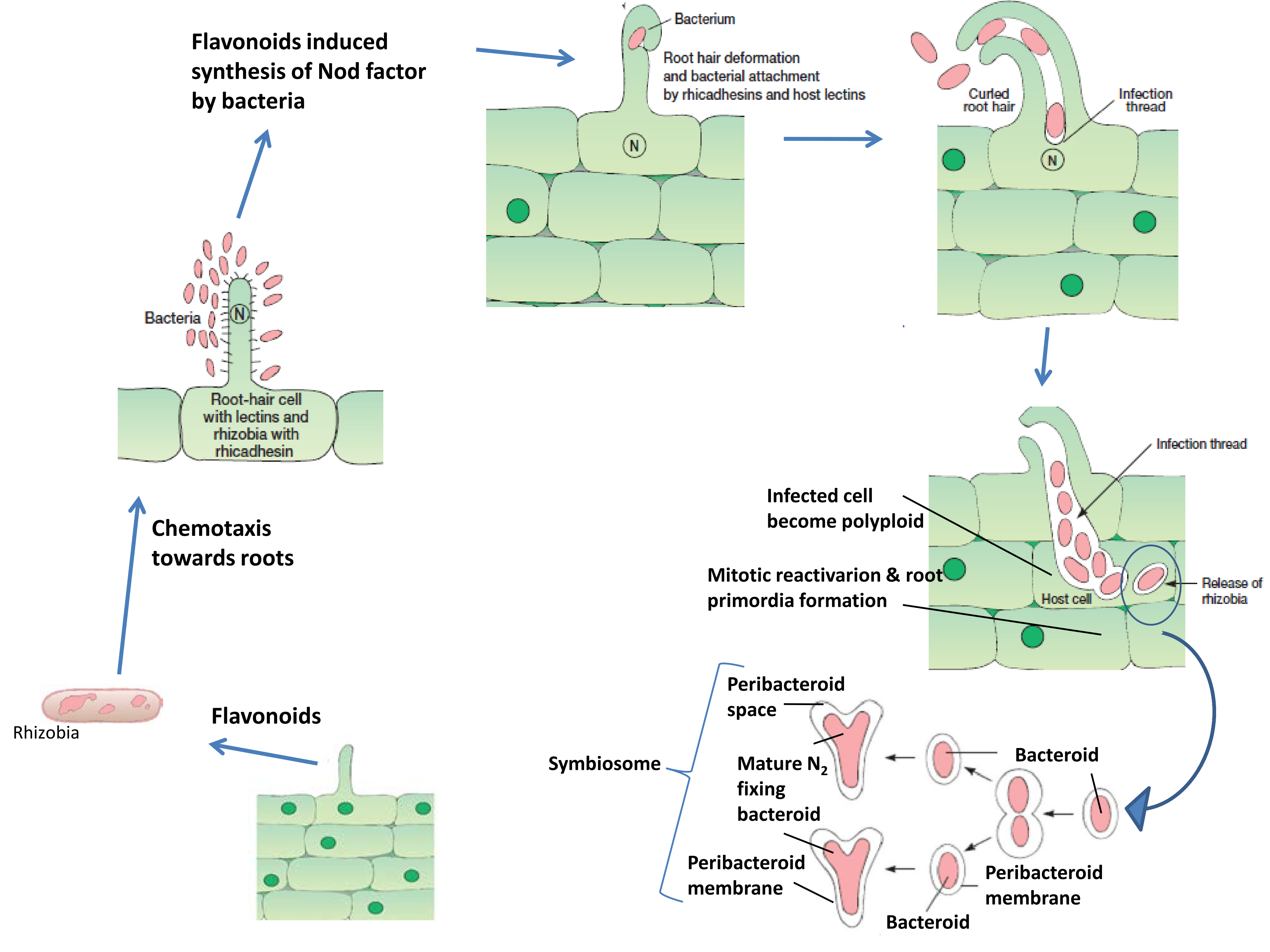
- *nod* genes are required for the production of bacterial signal molecules; the Nod factors (NFs- are lipochitooligosaccharide molecules) which trigger the nodule developmental program in the host plant.
- Plant infection and nodule formation are intricate processes; Nod factors play distinct roles in nodule organogenesis and root hair infection. Moreover, beside Nod factors, various bacterial surface polysaccharides are crucial for efficient infection.
- Nod D protein serves as a positive transcription activator for *nod* genes (*nod ABC*). *nod ABC* genes common to all rhizobia are required for the synthesis of a lipopoysaccharide (Nod factor) that triggers root nodule formation.
- Addition of various substituents to this core compound under the influence of *nodH* and *nodQ* and the *nodFEL* operon, imparts host specificity to the lipoligosaccharide.
- After bacterial attachment, Nod factor promotes root hair curling and Nod factor enter root hairs and migrate to their nuclei. Nod I and Nod J proteins play a role in the normal development of infection thread.
- Initiation of bacterial penetration into the root hair cell and infection thread growth coordinated by the plant nucleus.

...Early events

- In most legumes, the rhizobia enter the host via the root hairs where by invagination of the plasma membrane an infection thread (IT) is formed that contains the multiplying bacteria and grows towards the root cortex. (A less frequent and ancient mode of infection occurs via cracks on the root surface of certain legumes like groundnut).
- The rhizobia spreads within the infection thread into the underlying root cells. The bacteria then invade root cells and transform into bacteroids; they swell and become deformed in various ways.
- The bacteria released from the IT are present in the host cytoplasm as organelle-like structures, called symbiosomes. The bacteria have no direct contact with cytoplasm as they are surrounded by a peribacteroid membrane, known also as symbiosome membrane (SM). The bacteroid, the SM and the space between them comprise the symbiosome.
- The bacteroids multiply in the growing host nodule cells to a certain cell density, adapt to the endosymbiotic life-style and microaerobic conditions and mature to nitrogen-fixing bacteroids.
- The N_2 fixing bacteroids are irreversibly transformed to polyploid, enlarged, non-cultivable endosymbionts (under host control). These terminally differentiated bacteroids can be elongated and even branched and 5- to 10-fold longer than the free-living cells or can be spherical from 8 to at least 20-fold amplified genome depending on the host.
- These events also induce the root to form nodules that host the infected cells.

Late events-Root nodule formation

- Concomitantly with the progression of infection processes from the epidermis into the cortex, a fraction of cortical cells beneath the site of the infection undergo mitotic reactivation, begin to divide and form a root nodule primordium.
- The infection thread releases the bacteria into the submeristematic cells.
- The cytoplasm of a nitrogen-fixing symbiotic cell hosts about 50,000 bacteroids. To accommodate such a high number of endosymbionts, the host cells grow.
- The growth of infected cells occurs due to repeated rounds of genome duplication leading to the formation of gradually growing polyploid cells .
- Nodule premordia then differentiates into nitrogen-fixing root nodules providing microaerobic condition in the central zone for functioning of the oxygen sensitive nitrogenase enzyme in the bacteroids.
- At this point, specific nodule components such as leghemoglobin, which protect the nitrogen fixation enzymes from oxygen, are produced to complete the nodulation process.



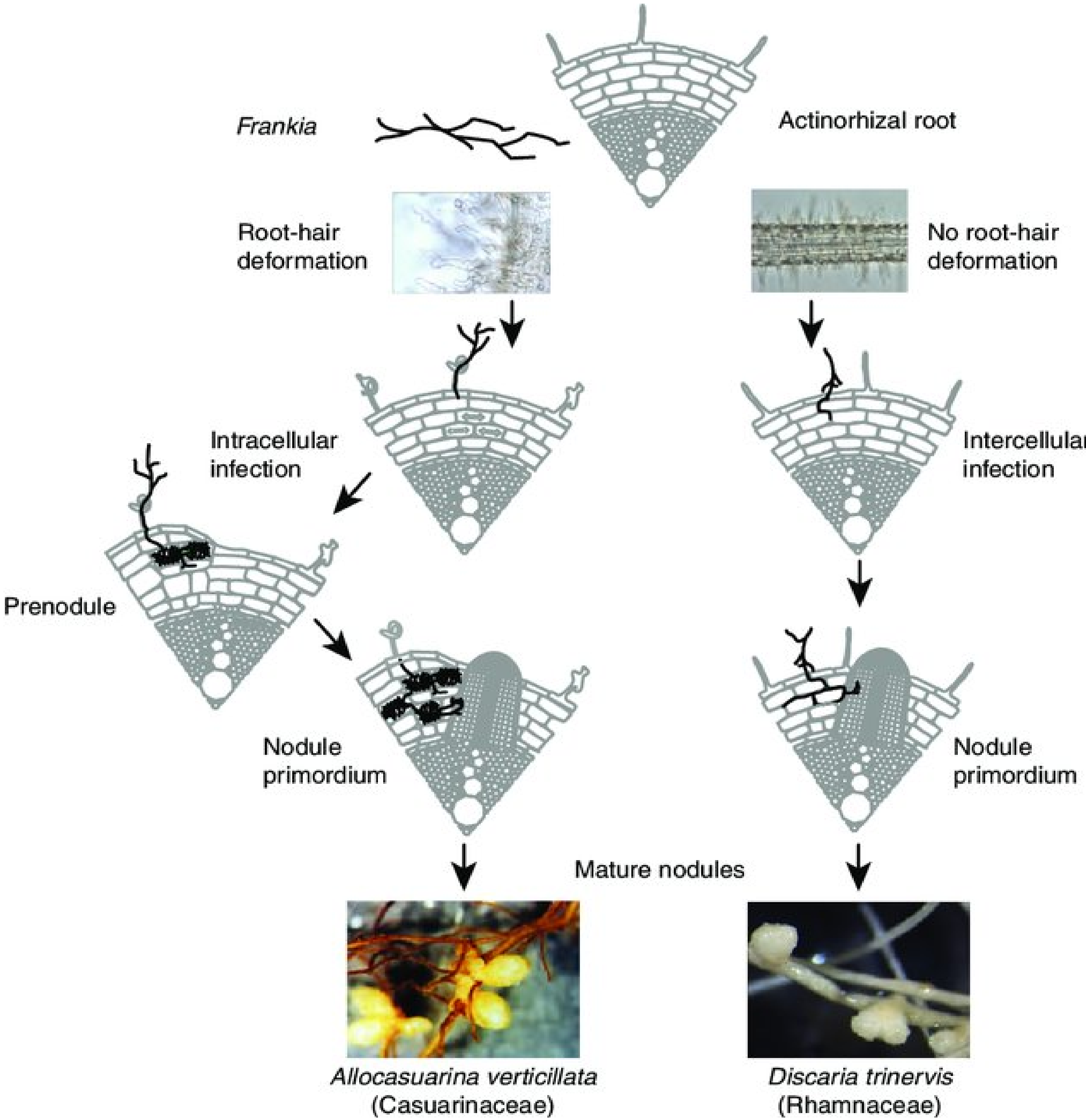
Flavonoids induced synthesis of Nod factor by bacteria
Bacterium
Root hair deformation and bacterial attachment by rhicadhesins and host lectins
N
Curled root hair
Infection thread
N
Bacteria
N
Root-hair cell with lectins and rhizobia with rhicadhesin
Chemotaxis towards roots
Infection thread
Infected cell become polyploid
Mitotic reactivarion & root primordia formation
Host cell
Release of rhizobia
Rhizobia
Flavonoids
Peribacteroid space
Symbiosome
Mature N_2 fixing bacteroid
Bacteroid
Peribacteroid membrane
Peribacteroid membrane
Bacteroid

Leghemoglobin

- Leghemoglobin is one of the more notable features of the rhizobia-legume symbiosis.
- It is a true hemoglobin, the synthesis of which depends on symbiont genes for the heme moiety and plant host genes for the protein.
- Leghemoglobin is responsible for the pink colour seen when mature nodules are sectioned.
- Leghemoglobin, has a high affinity for O_2, maintains low oxygen tensions within nodules, thus protecting the highly oxygen-sensitive nitrogenase, while at the same time supplying the symbionts with enough oxygen to maintain a high rate of aerobic metabolism.
- This is essential for producing the large supply of ATP needed for nitrogen fixation.
- Dicarboxylic acids (succinate, malate and fumarate) provided by plant host are present in high concentration in the nodule and are the most effective substrates forrespiration and subsequent ATP utilizing N_2 fixation in the bacteroid.
- Mutant defective in dicarboxylic acid transport (gene *dct*) from ineffective nodules.
- Recent studies reveal that alanine, rather than ammonia, is the form of nitrogen transport to the plant.
- It has been shown that rhizobia can assimilate ammonia into pyruvate, forming alanine via alanine dehydrogenase.

Symbiotic N_2 Fixation in Non-leguminous plants

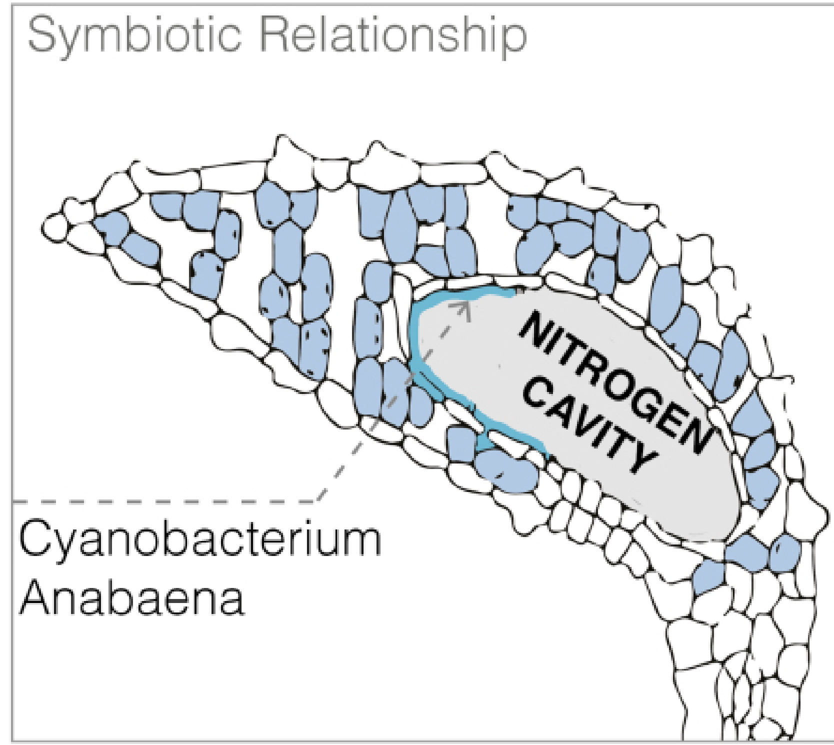
- Actinomycete associations with plant roots, called actinorhizae or actinorhizal relationships.
- Actinorhizal plants infected by *Frankia* (actinomycete) have been shown to fix nitrogen at comparable rates as legume symbioses.
- The actinorhizal plants that become infected by *Frankia* sp. are members of three phylogenetically related groups: Fagales (Betulaceae, Casuarinaceae, Myricaceae), Cucurbitales (Datiscaceae, Coriariaceae) and Rosales (Rosaceae, Elaeagnaceae, Rhamnaceae).
- *Frankia* cells enter symbiosis by root hair infection (for *Alnus*, *Casuanin*a, *Comptonia*, and *Myrica* species) or by a process of intercellular penetration of root epidermis and cortex.
- Root hair infection is characterized by root hair branching and curling.
- Cells of the hypodermis and cortex divide in response to the invasion to form a prenodule, and the *Frankia* hyphae penetrate through the prenodule tissue into the inner cortex of the root.
- The *Frankia* hyphae penetrate cells of the developing nodule lobe primordia to form the infected nodule.
- The infective hyphae of *Frankia* strains, as they enter the root hair, become encapsulated with a layer of plant cell wall-like material surrounded by host plasmalemma.
- The mature effective form of *Frankia* strains in infected cells is often but not exclusively associated with symbiotic vesicle formation, which is the site of nitrogen fixation.
- It was found that this bacterium would fix nitrogen only under low oxygen concentrations.



Frankia
Actinorhizal root
Root-hair deformation
No root-hair deformation
Intracellular infection
Intercellular infection
Prenodule
Nodule primordium
Nodule primordium
Mature nodules
Allocasuarina verticillata
(Casuarinaceae)
Discaria trinervis
(Rhamnaceae)

Azolla-Anabaena symbiosis and N_2 fixation

- ***Azolla*** leaf (water fern) consists of a thick, greenish (or reddish) dorsal (upper) lobe and a thinner, translucent ventral (lower) lobe emersed in the water.
- It is the upper lobe that has an ovoid central cavity, the "living quarters" for filaments of ***Anabaena azollae* (cyanobacteria)**. The cavity open to atmosphere through pore on upper side.
- The *Anabaena azollae* colony associated with Azolla's shoot apex comprises generative filaments without heterocysts and it is therefore unable to fix nitrogen from the atmosphere.
- In the cavity of mature leaves, the cyanobacteria filaments cease to grow and differentiate heterocysts, which reach a maximum of 25–45% of the cell population in leaves, coinciding with increased nitrogenase activity.
- Nitrogenase is oxygen labile and is protected in part by the thickened cell walls of the heterocyst, which results in them being immune to the oxygen concentrations in the *Azolla* leaf cavity.



Symbiotic Relationship
NITROGEN CAVITY
Cyanobacterium Anabaena

Questions

- What is symbiotic nitrogen fixation? Discuss common symbiotic system involve in nitrogen fixation.
- Write an essay on legume – rhizobia symbiotic nitrogen fixation.
- Write short note on:
  - Leghaemoglobin
  - Azolla-*Anabaena*symbotic nitrogen fixation
  - Frankia as symbiotic nitrogen fixer