Nitrogen Metabolism:

BIOLOGICAL NITROGEN FIXATION

Nitrogen Cycle

- **Nitrogen (N_2)** is the most abundant gas and an important constituent of biomolecules in living organisms.
- **Nitrogen cycle** is a vital biogeochemical cycle by which inert nitrogen is converted into various chemical compounds and circulated within the atmosphere and earth ecosystems.
- In a series of Chemical reactions, nitrogen from the atmosphere is fixed in compounds in soil or water and assimilated into proteins by plants and animals,
- Eventually N_2 is released to the soil and water through decomposition, and returned to the atmosphere through denitrification.

Nitrogen Fixation

- Most of the plants obtain nitrogen from soil in the form of nitrate or ammonium ion, but it is limited
- As dinitrogen (N_2) gas is very stable hence fixation of nitrogen is required
- The conversion of N_2 to ammonia (NH_3) is called Nitrogen Fixation
- First discovered by the Hermann Hellrigel and Martinus Beijerinck

Nitrogen Fixation is carried out by two ways

1. Abiotic Nitrogen fixation

- Fixation by Lightening: During lightning N_2 can be oxidized to Nitric Oxide NO_2 during lightening process.
- These oxides are carried to the ground by rain and deposited as HNO_2 or HNO_3

Nitrogen Fixation is carried out by two ways

2. Biological Nitrogen Fixation

The conversion of N_2 to NH_3 is called Biological Nitrogen Fixation.

Two mechanisms of fixation:

- **Non symbiotic nitrogen fixation:** carried out by free living bacteria (*Azotobacter*, *Azospirillum)* and Cyanobacteria (blue green algae)

- **Symbiotic Nitrogen fixation:** Symbiotic relationship between plants and N_2-fixing bacteria

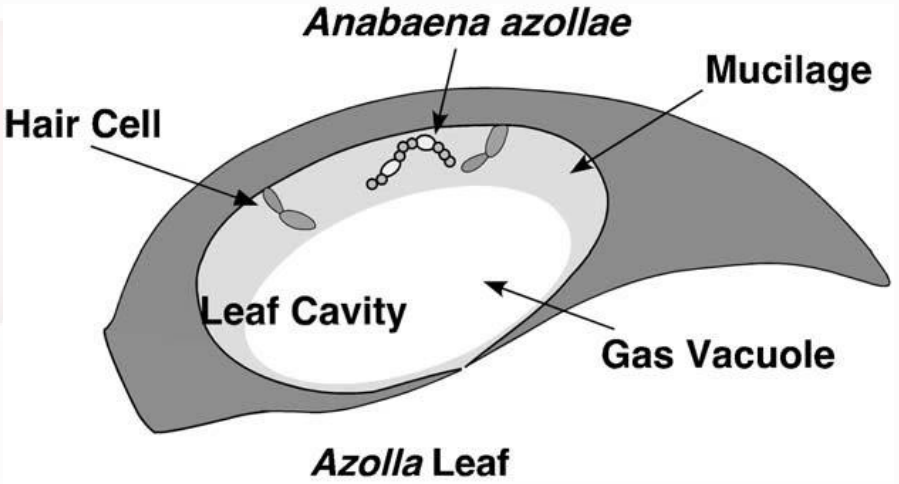
Symbiotic Nitrogen fixation

Symbiotic relationship between plants and N_2-fixing bacteria

- Microbe receives carbohydrates. Plant also provides suitable environment (anaerobic site) for N_2 fixation .
- In return plant receives fixed nitrogen N_2

1. Symbioses involving Cyanobacteria

- **Cyanobacteria associate with Nonvascular plants:** Liverworts, hornworts, mosses



Azolla (aquatic Fern): with *Anabaena* symbiont

Other examples: *Gunnera* with *Nostoc* symbiont

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2. Nodule-forming symbioses

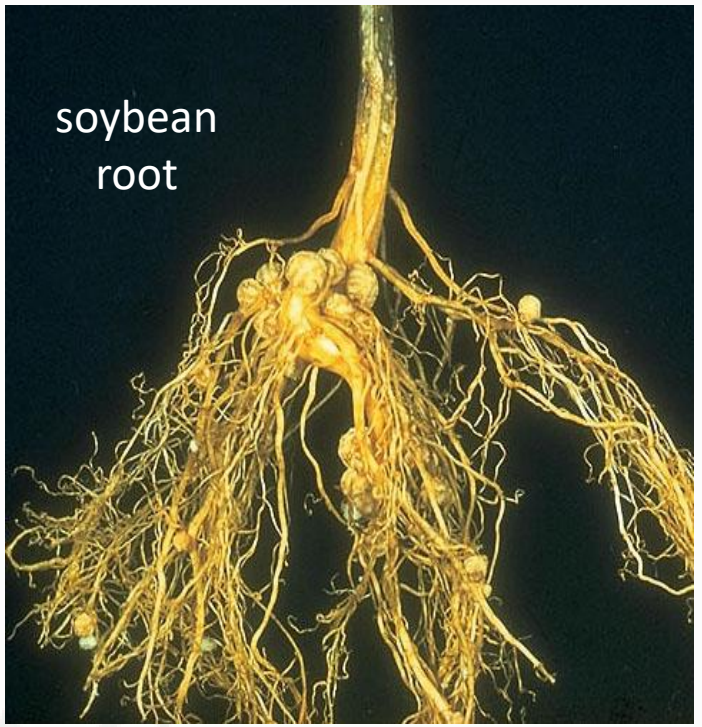
- Nitrogen-fixing soil bacteria belonging to genus *Rhizobium* form nodule forming symbiotic association with leguminous plants.

  Examples: Rhizobium sp.with Legumes and *Rhizobium* sp. with *Parasponia* (Cannabaceae)

- Other examples actinomycetes *Frankia* association with actinorhizal plants (woody angiosperms)

Rhizobium-legume symbioses

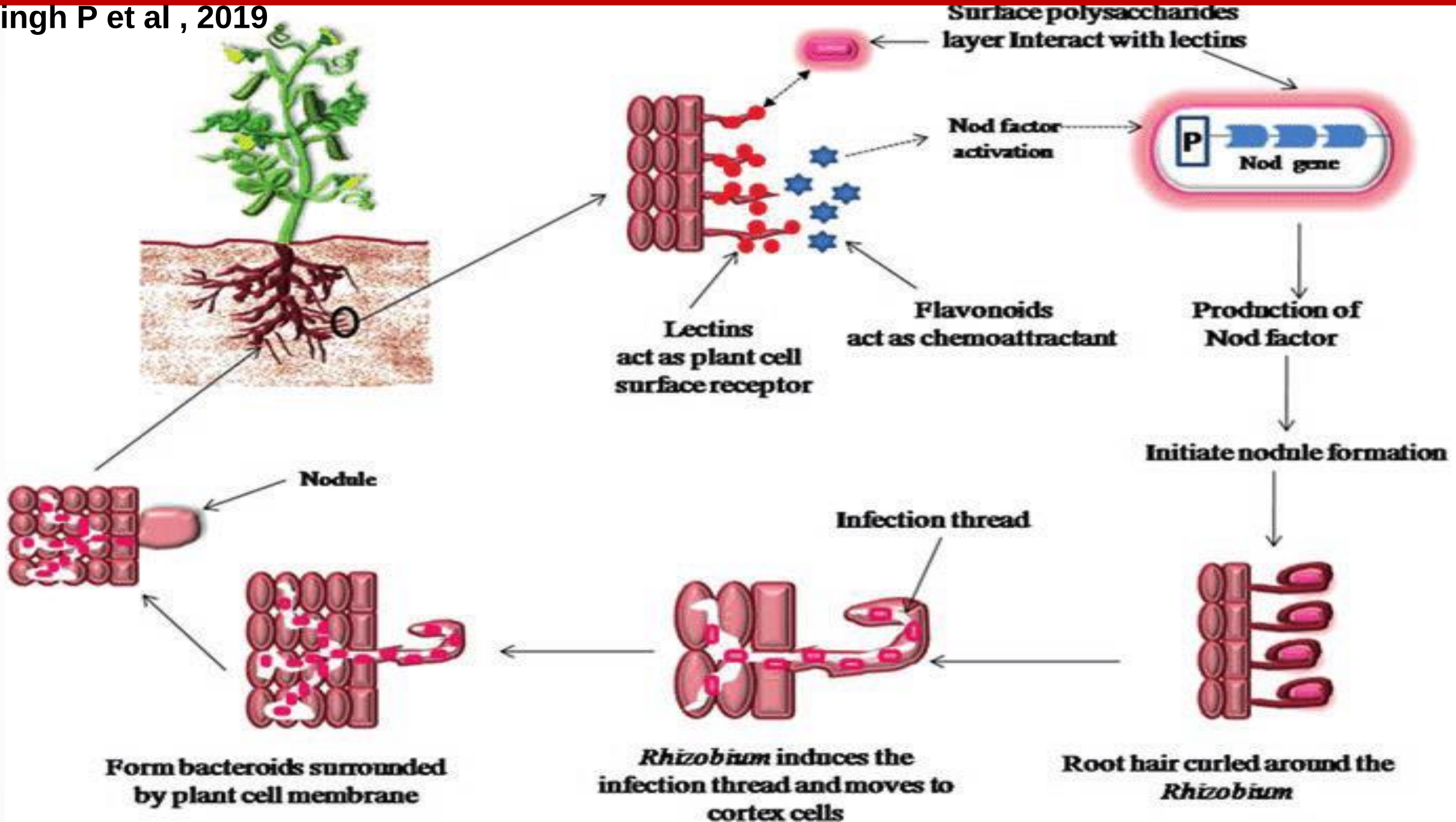
Host plant legumes	Bacterial symbiont
Alfalfa	*Rhizobium meliloti*
Clover	*Rhizobium trifolii*
Soybean	*Bradyrhizobium japonicum*
Beans	*Rhizobium phaseoli*
Pea	*Rhizobium leguminosarum*



Rhizobium – *Parasponia* association: Only non-legume plant known to form nodules with *Rhizobium*

Process of root nodulation in plant

Singh P et al , 2019



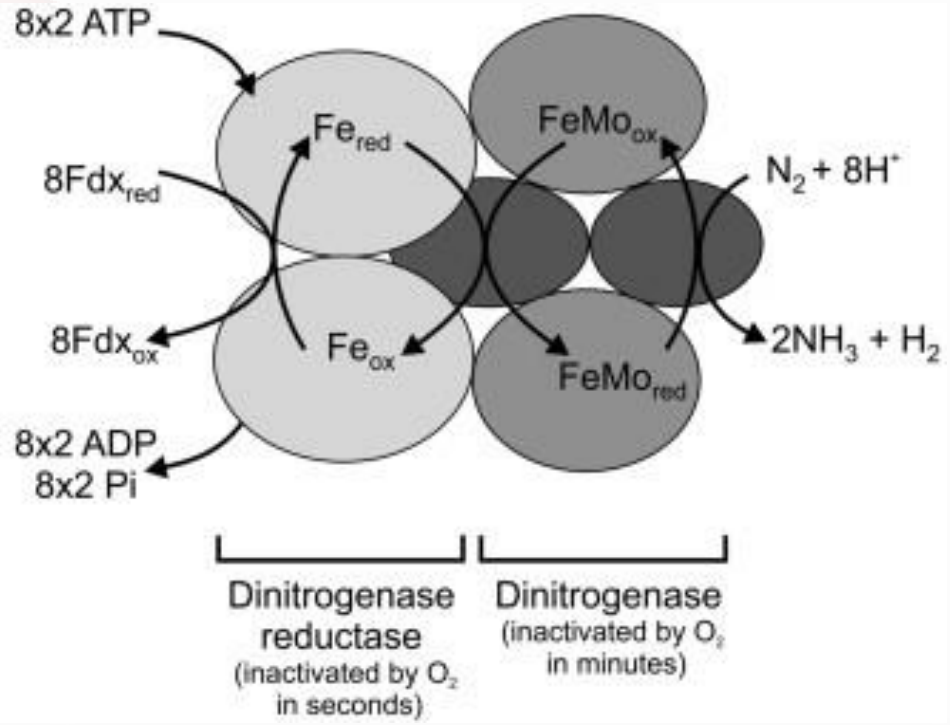
Process of *Rhizobium* attachment , infection and root nodulation in plant

1. Free-living *Rhizobium* are attracted to root exudates; attach to root and multiply
2. Symbiotic genes are activated in both plant and bacteria
3. Flavenoids activate *nodD1* and start synthesis of Nod protein (lipooligosaccharide) which act as nodulation factor.
4. Nod factors induce root hair curling and initiate nodule development
5. Bacteria enter root, root cortex cells divide to form nodule.
6. Bacteria fix nitrogen which is transferred to plant cells in exchange for fixed carbon

In root nodules, bacteria perform nitrogen fixation by converting atmospheric nitrogen to ammonia by an enzyme called **nitrogenase.**

This enzyme is encoded by *nif* genes and is composed of two protein components:

- **Component I (Fe protein)** : dinitrogenase reductase
- **Component II (MoFe protein)** : dinitrogenase



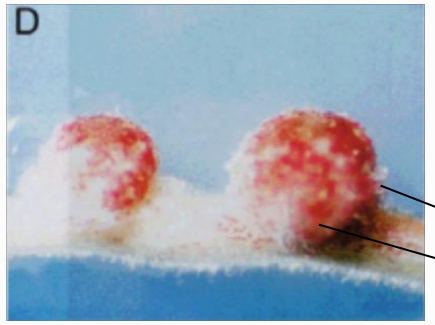
$$N_2 + 8H^+ + 8\ e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$$

Nitrogenase enzyme is very sensitive to O_2

Protective mechanisms:

- reduced oxygen tension (*Azospirillum*)
- anaerobic heterotrophs (*Clostridium*, *Desulfovibrio*, others)
- anoyxygenic phototrophs (*Chromatium*, *Rhodospirillum*, others)
- protective structures – root nodule for *Rhizobium*, heterocysts in cyanobacteria

Root nodules contain leghaemoglobin, a oxygen-scavenging molecules



R. leguminosarum **nodules**

Pea Plant

- Leghaemoglobin regulate the supply of oxygen to the nodule tissues. It delivers O_2 to the otherwise anaerobic nodule.
- Leghaemoglobin is found only in the nodules (responsible pink color of nodules)
- Not produced by either the bacterium or the plant when grown alone.

References

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Thank you