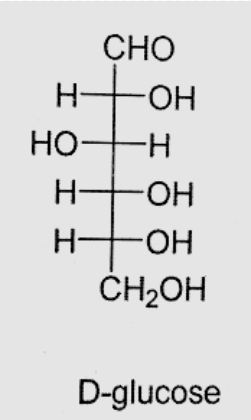
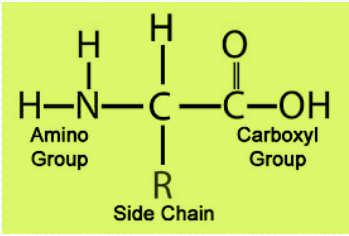
BIOLOGICAL NITROGEN FIXATION

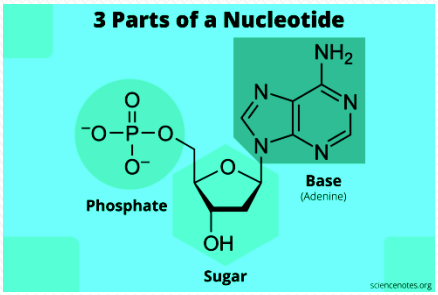
Dr. Mahesha H B

Professor and Head
Department of Sericulture
Yuvaraja's College,
University of Mysore, Mysuru, India

Nitrogen is a component of amino acids, proteins, enzymes, Hormones, nucleic acids, vitamins etc.,

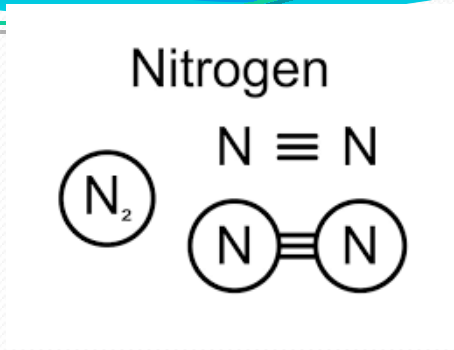




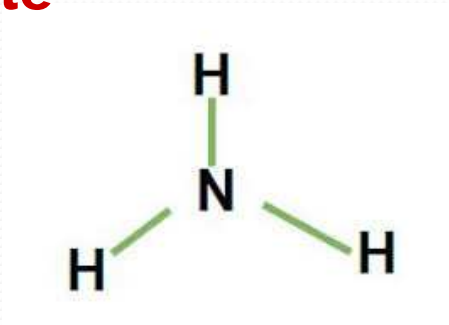


Nitrogen is available in atmosphere in high amount *i.e.*, 78% in the form of gas.

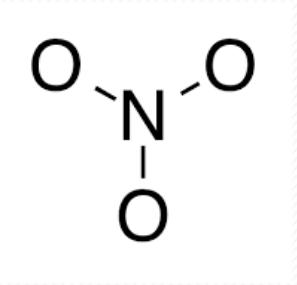
Diatomic nitrogen is inert because of the Presence of bonds between atoms.



It will be reactive or absorbed by the plants only when it is Combined with either hydrogen or oxygen as ammonia or nitrate



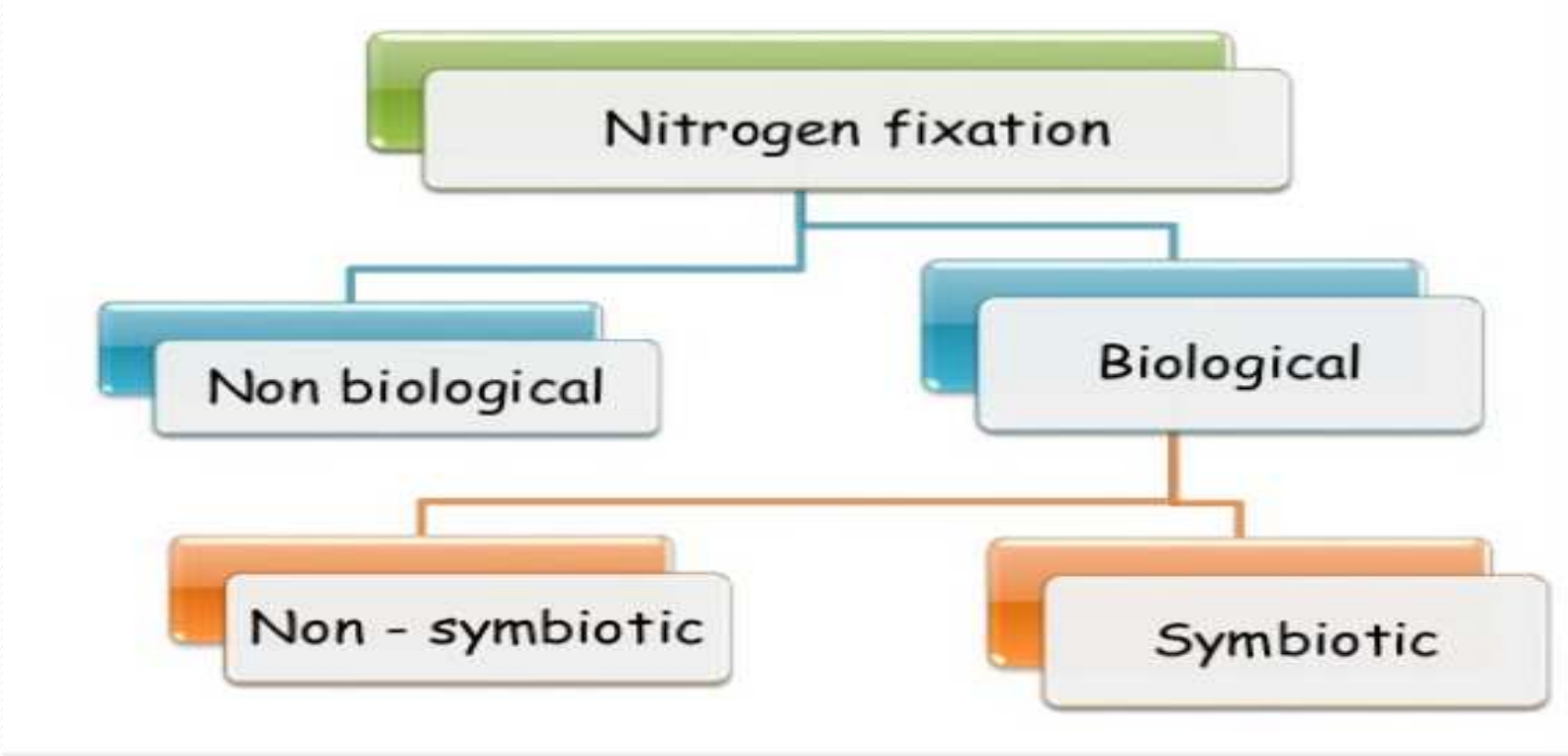
Ammonia



Nitrate

Nitrogen fixation is a process by which atmospheric nitrogen (N_2) is converted into ammonia (NH_3)

TYPES OF NITROGEN FIXATION



Non Biological/Natural Nitrogen Fixation

- During lightening and thunderstorms some amount of nitrogen is converted as nitrogen oxides, which then Dissolves in moisture to form nitrates

- During Volcanic emissions/Automobile emissions as Nitrogen oxides

- Industrial – Haber-Bosch Process is the method in Producing ammonia from nitrogen and hydrogen

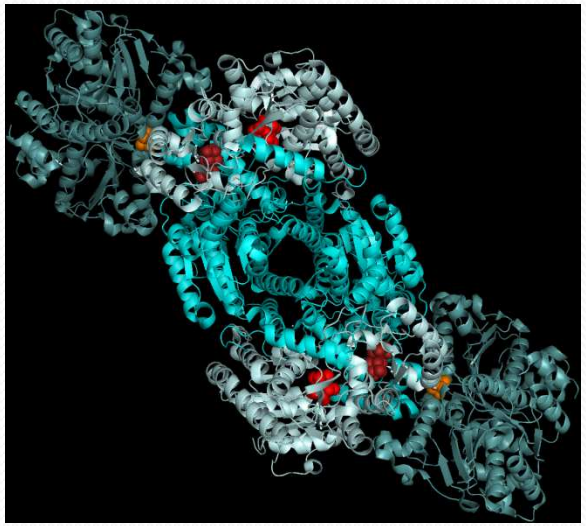
Biological Nitrogen Fixation

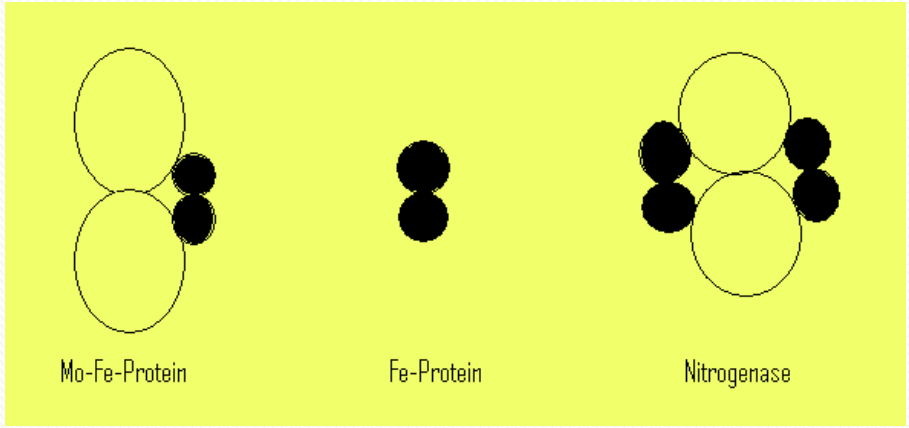
Examples of nitrogen-fixing bacteria (* denotes a photosynthetic bacterium)			
Free living		Symbiotic with plants	
Aerobic	Anaerobic	Legumes	Other plants
Azotobacter *Beijerinckia* *Klebsiella* (some) Cyanobacteria (some)*	*Clostridium* (some) *Desulfovibrio* Purple sulphur bacteria* Purple non-sulphur bacteria* Green sulphur bacteria*	*Rhizobium*	*Frankia* *Azospirillum*

NITROGENASE

- **Nitrogenase is a complex enzyme containing two oxygen sensitive components.**
- **Component-I has 2 α-proteins subunits and 2β-protein subunits,24 molecules of iron,2molecules of molybdenum and an iron-molybdenum cofactor(FeMoCo).**
- **Component –II possess 2 α-proteins subunits and a large number of iron molecule.**

Structure of Nitrogenase





Component 1 Component 2 Nitrogenase Complex

Component - I

Dinitrogenase, $2.2X10^5$, Mo -2 atoms/mol, Fe-32 atoms/mol, S-30 atoms/mol

Component-II

Dinitrogenase reductase, $5X10^4$, Fe-4 atoms mol, S-4 atoms/ mol

HYDROGENASE

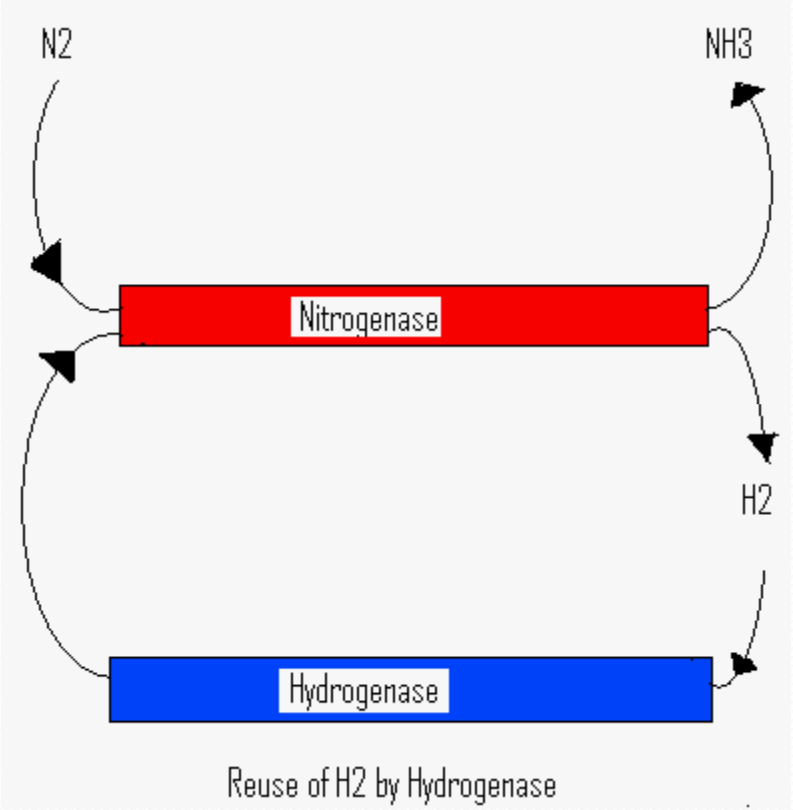
- **It is a enzyme which uses hydrogen as the energy source.**
- **During the coarse of nitrogen fixation by nitrogenase, an undesirable reaction also occurs *i,e*., reduction of H^+ (hydrogen ion) --->H_2 (molecular hydrogen).**
- **For the production of hydrogen ATP is utilized, it is rather wasted.**

Role of Hydrogenase

Many iazotropes evolve Hydrogen (H_2) during Nitrogen fixation, which inturn Inhibits nitrogen fixation.

Hydrogenase recycles H_2.

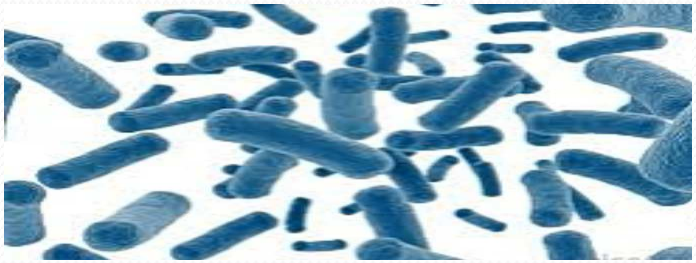
Reutilization of H2 produces More ATP and improves the efficiency of N_2 fixation



Reuse of H2 by Hydrogenase

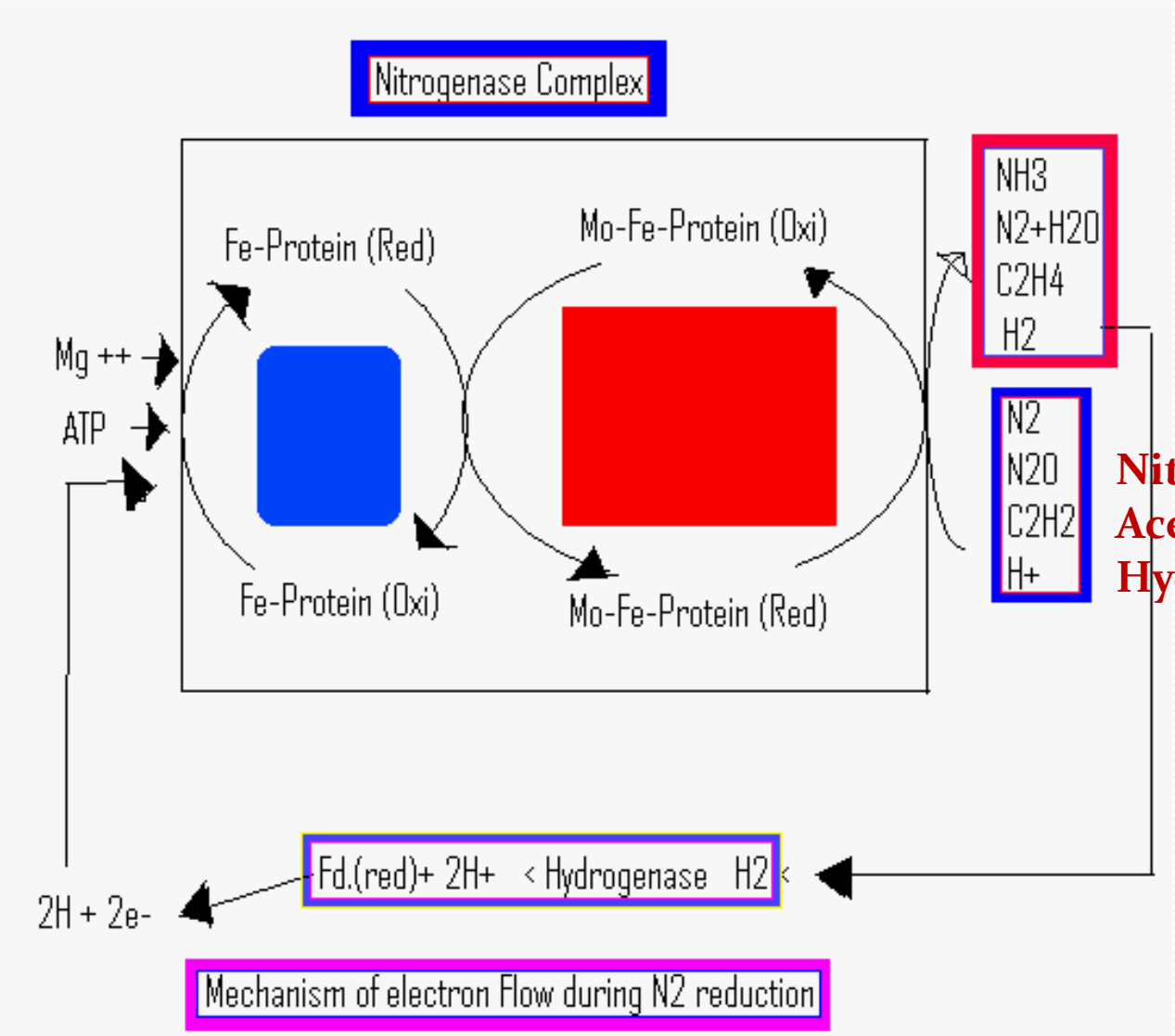
ASYMBIOTIC OR NONSYMBIOTIC

- **The gaseous nitrogen of the atmosphere is directly and independently utilized to produce nitrogen rich compounds.**
- **When non symbiotic organisms die, they enrich the soil with nitrogenase compounds.**



- **Ex:-Clostridium, Pasteuranium, Azatobacter chrooccum.**

Mechanism of Nitrogen fixation in free living organisms



Overall Reaction in free living organism

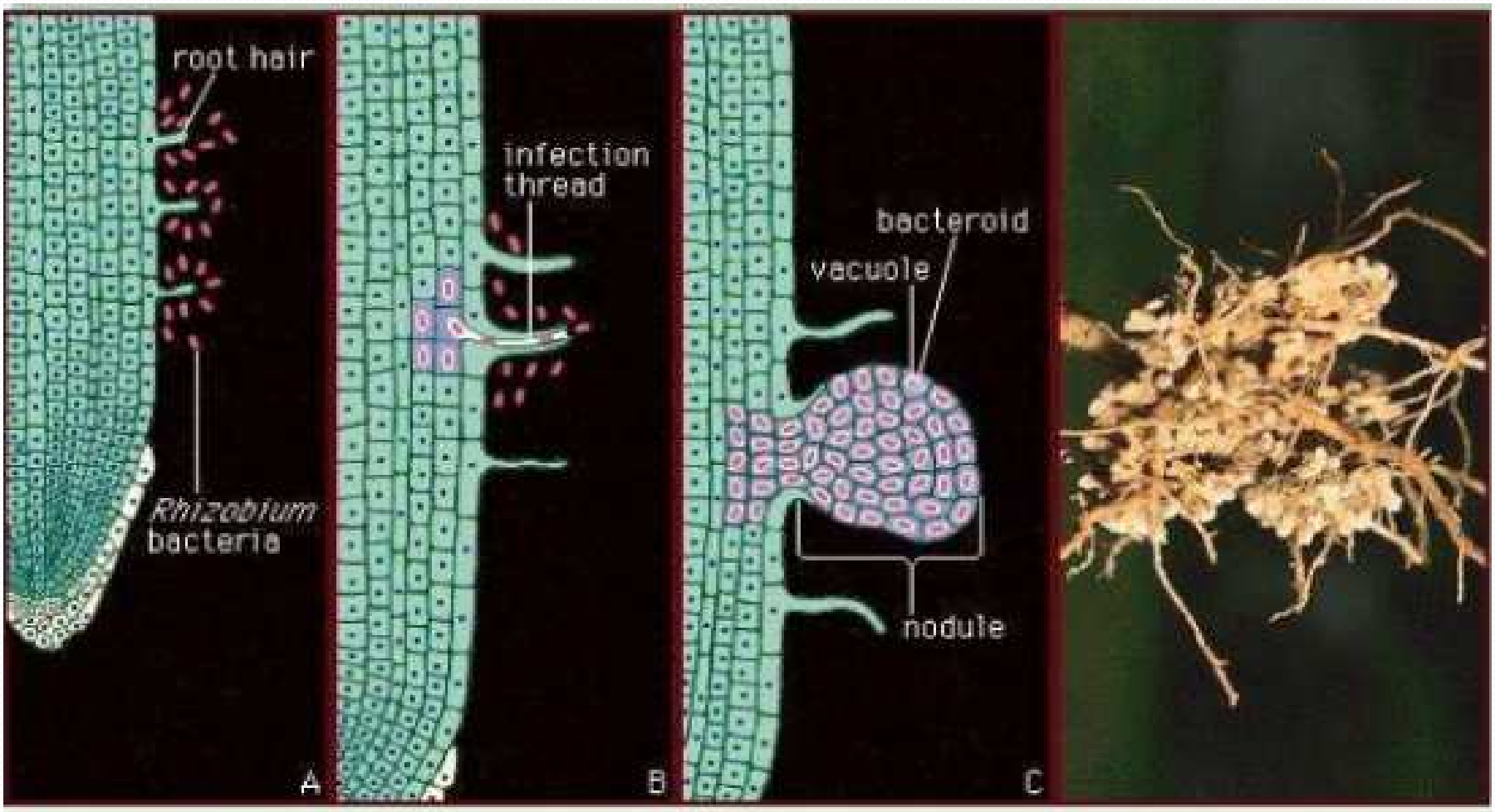
$$N_2 + 12\ Mg^{++}\ ATP + 6\ (H^+ + e^-) \longrightarrow 2\ NH_3 + 12\ (Mg^{++}\ ADP + pi)$$

SYMBIOTIC NITROGEN FIXATION

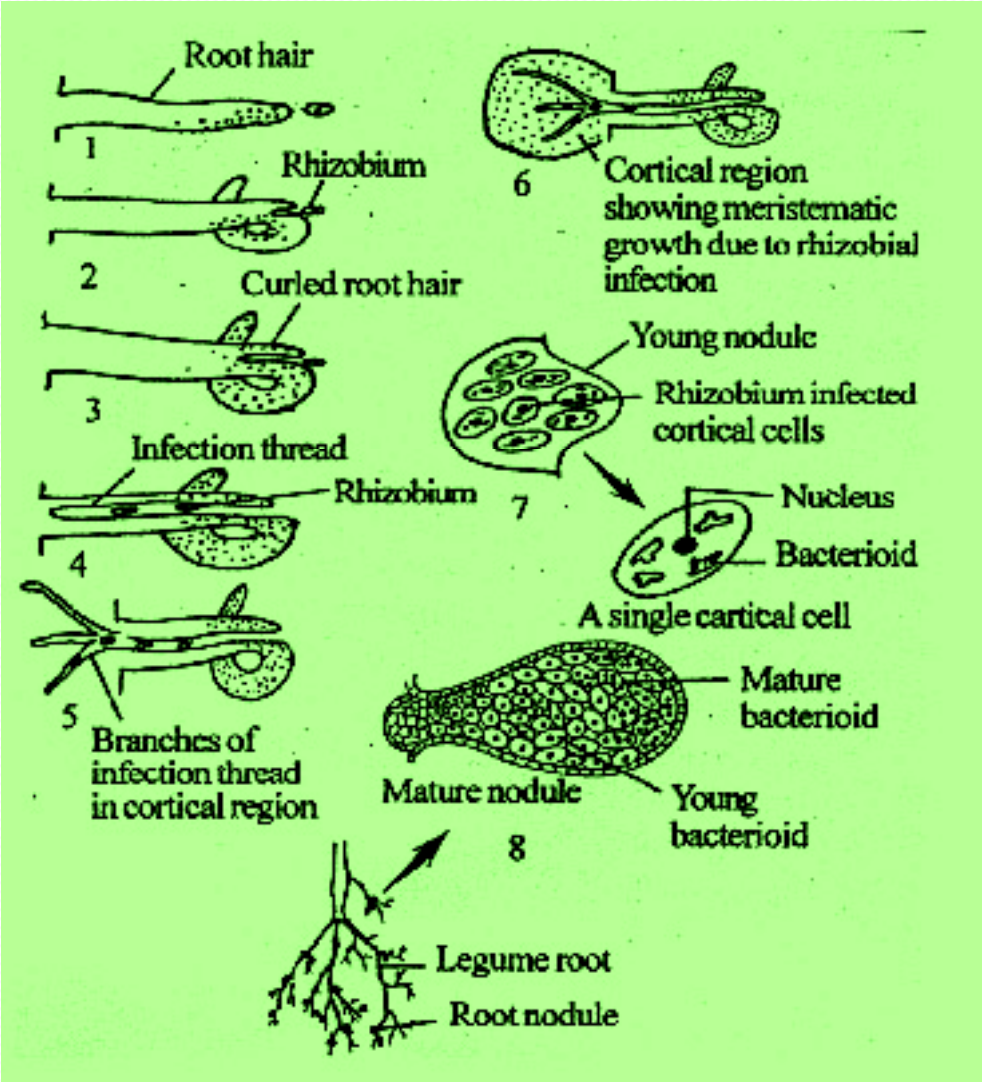
- **Microorganisms live together with the plants in a mutually beneficial relationship. This phenomenon is referred to as 'Symbiosis'.**
- **Most important microorganisms involved in symbiosis belong to the genera Rhizobium and Bradyrhizobium.**
- **The host plant harbouring these bacteria are known as legumes.**

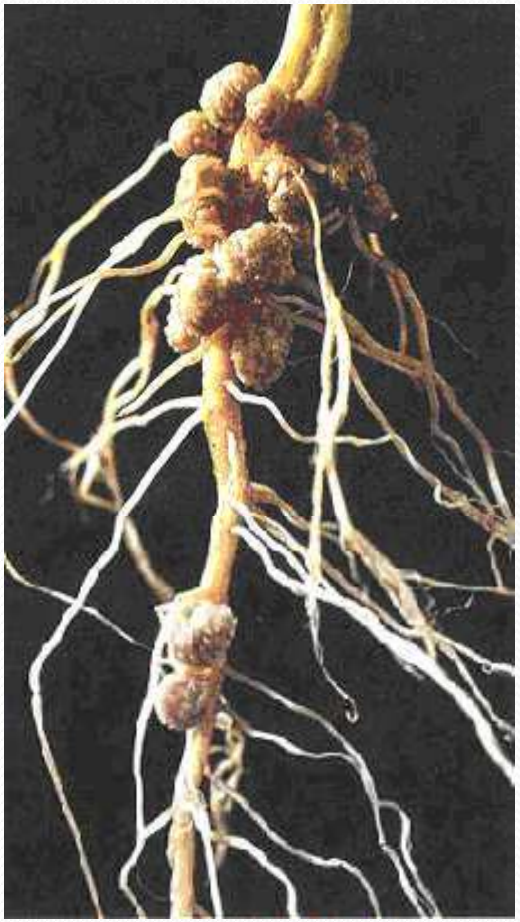
  Ex:- Soyabean, Peas, Beans, Alphapha, Peanuts and cloves.

MECHANISM OF NITROGEN FIXATION IN SYMBIOTIC ORGANISMS



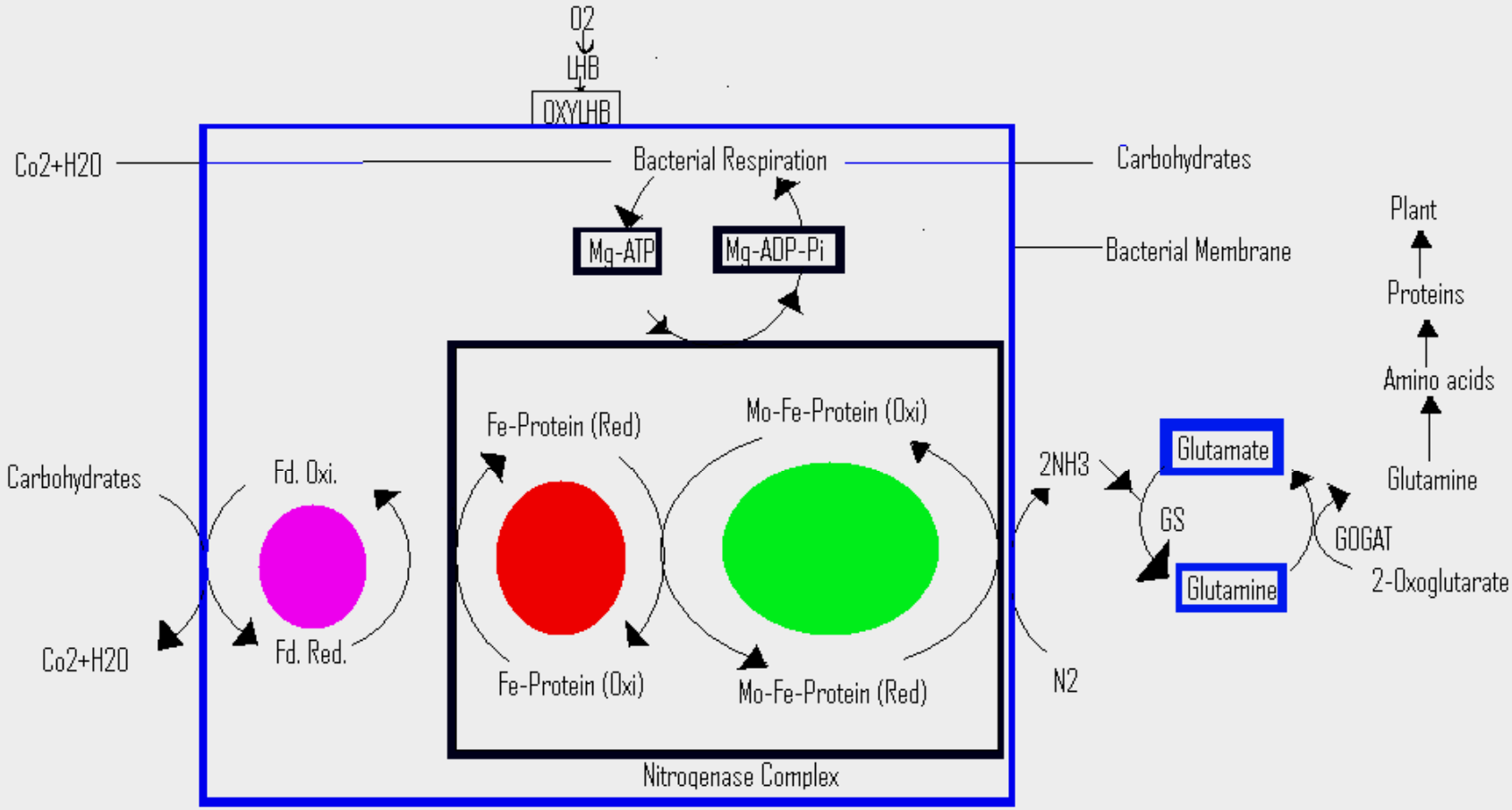
Process of Symbiotic Nitrogen Fixation.





Root nodulation in legume.

The overall process of nitrogen fixation in a bacterioid of Rhizobium



Biological N2 Fixation in Root Nodules. GS - Gultamine Synthetase; GOGAT-Glutamine oxoglutarate aminotransferase

Overall Reaction in Symbiotic Organisms

$$N_2 + 16ATP + 8e^- + 10H^+ \xrightarrow[Mg^{++}]{} 2NH_4^+ + H_2 + 16\,ADP + 16\,pi$$

LEGHAEMOGLOBIN

- **It is a oxygen binding protein. It contains iron and is red in colour.**
- **Lhb facilitates the appropriate transfer of oxygen to bacteria for respiration to produce ATP.**
- **The energy required for the nitrogen fixation is utilized in the form of ATP.**
- **The haem part of Lhb is synthesized by bacteria while the protein part (globin) portion is produced by host plant.**

NITROGEN FIXING GENE OR Nif GENE

- **The nif genes are located as a single cluster, occupying approximately 24kb of the bacterial genome.**
- **There are 7 distinct operon that encodes 20 different proteins.**
- **The nif genes are under the regulatory control of 2genes namely nif-A and nif-L.**

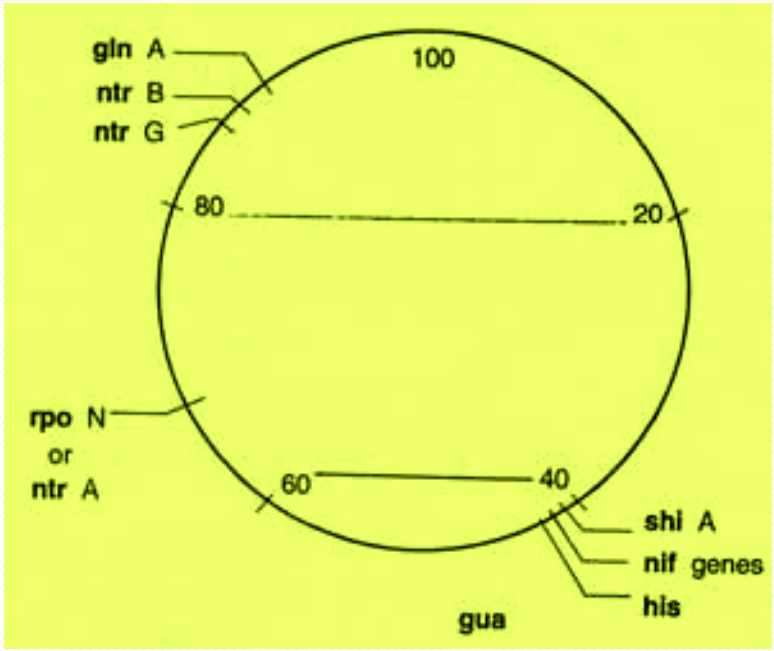
Table 1. The *nif-gene* Products and Their Role (Known or Proposed) in Nitrogen Fixation

nif-GENE	IDENTITY/ROLE
nifH	Dinitrogenase reductase. Obligate electron donor to dinitrogenase during nitrogenase turnover. Also is required for FeMo-co biosynthesis and apodinitrogenase maturation
nifD	α subunit of dinitrogenase. Forms an $a_2ß_2$ tetramer with ß subunit. FeMo-co, the site of substrate reduction, is present buried within the α subunit of dinitrogenase
nifK	ß subunit of dinitrogenase. P-clusters are present at the ß subunit-interface
nifT	Unknown
nifY	In *K. pneumoniae*, aids in the insertion of FeMo-co into apodinitrogenase
nifE	Forms $a_2ß_2$ tetramer with NifN. Required for FeMo-co synthesis. Proposed to function as a scaffold on which FeMo-co is synthesized
nifN	Required for FeMo-co synthesis
nifX	Involved in FeMo-co synthesis. Specific role is not known
nifU	Involved in mobilization of Fe for Fe-S cluster synthesis and repair
nifS	Involved in mobilization of S for Fe-S cluster synthesis and repair
nifV	Homocitrate synthase, involved in FeMo-co synthesis
nifW	Involved in stability of dinitrogenase. Proposed to protect dinitrogenase from 0_2 inactivation
nifZ	Unknown
nifM	Required for the maturation of NifH
nifF	Flavodoxin. Physiologic electron donor to NifH
nifL	Negative regulatory element
nifA	Positive regulatory element
nifB	Required for FeMo-co synthesis. Metabolic product, NifB-co is the specific Fe and S donor to FeMo-co
fdxN	Ferredoxin. In *R. capsulatus*, serves as electron donor to nitrogenase
nifQ	Involved in FeMo-co synthesis. Proposed to function in early MoO_4^{2-} processing
nifJ	Pyruvate:flavodoxin (ferredoxin) oxidoreductase. Involved in electron transport to nitrogenase

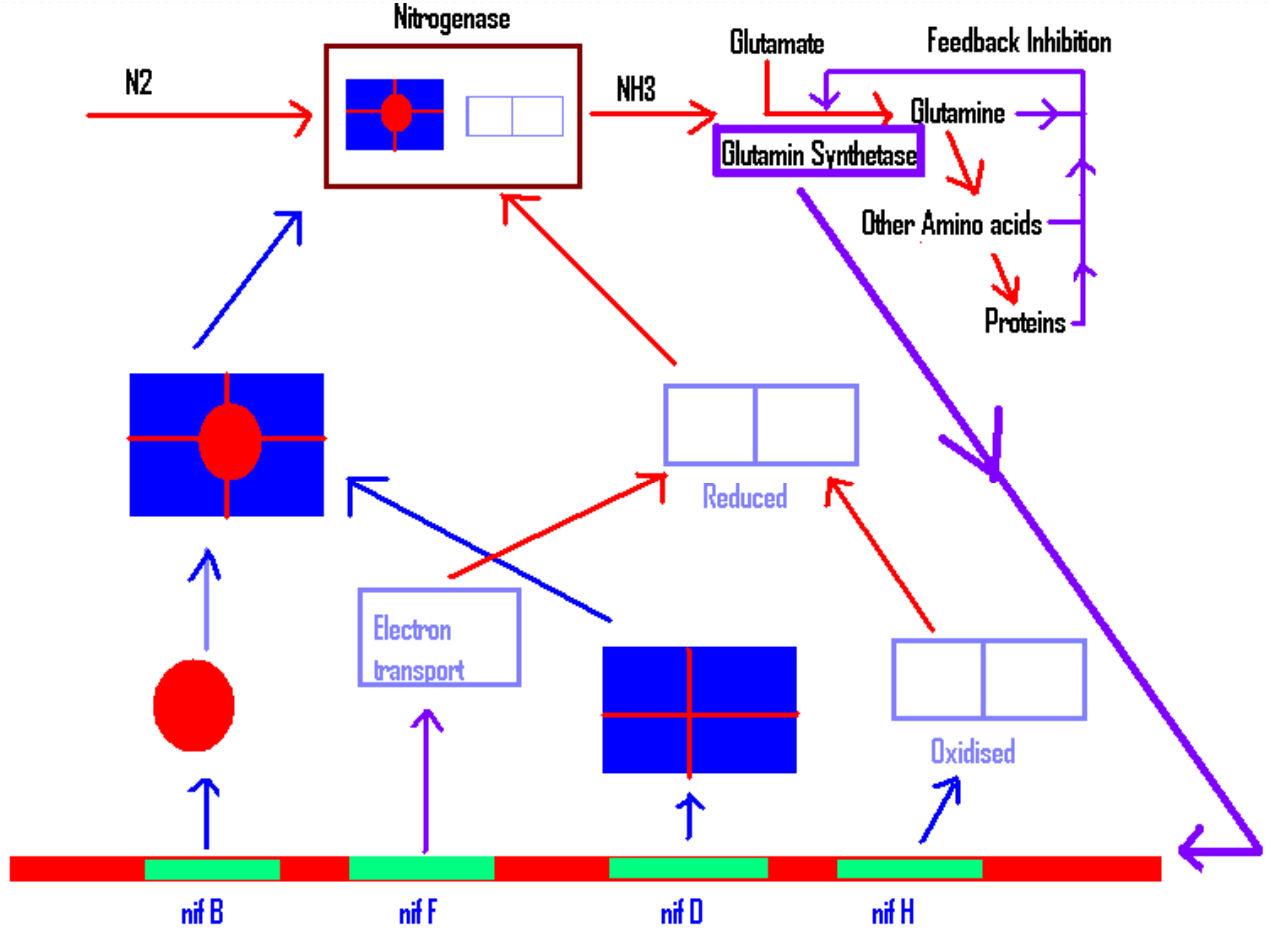
GENETICS OF NITROGEN FIXATION

- **It is absolutely necessary to identify, isolate and characterize the nitrogen fixing gene.**
- **Transfer of genes for nitrogen fixation to bacteria helps to infect several important crops.**
- **It can form a symbiotic relationship with non leguminous plants such as wheat ,rice and corn.**

A Map of *Klebsiella*-chromosome showing relative position of nif genes



REGULATION OF NITROGEN FIXATION



THANK YOU