Faculty of Science
Department of Botany,
Telangana University
M.Sc. Botany II Year, IV Semester,
Paper: **Physiology and Molecular Biology of Nitrogen Fixation**
Internal Assessment: II
Question Bank

---

1. The member of the Enterobacteriaceae with ability to fix nitrogen [a]
   a. *Klebsiella pneumonia*
   b. *E. coli*
   c. *Rhizobium*
   d. *Azotobacter*
   e. All of these
2. Genetic studies in *Klebsiella* have identified a total of *nif* genes [a]
   a. 20
   b. 15
   c. 23
   d. 24
   e. 26
3. *nifH*, *nifD, nifK* genes in *Klebsiella* are [a]
   a. Structural
   b. Regulatory
   c. Inducer
   d. Repressor
   e. All of these
4. The genes required for assembly and incorporation of the metal centres in nitrogenase in *Klebsiella* [e]
   a. *nifE, nifN*
   b. *nifU, nifS, nifV, nifW, nifX*
   c. *nifM*
   d. *nifB, nifQ*
   e. All of these
5. Genes that encode proteins required for electron transfer to nitrogenase in *Klebsiella* [a]
   a. *nifF* and *nifJ*
   b. *nifH*
   c. *nifD*
   d. *nifK*
   e. All of these
6. Genes that encode proteins that regulate *nif* gene expression in *Klebsiella* [a]
   a. *nifL, nifA*
   b. *nifH*
   c. *nifD*
   d. *nifK*
   e. All of these
7. *nif* genes in *Klebsiella* are clustered in ___ region of the chromosome [a]
   a. 24 kb
   b. 20 kb
   c. 15 kb
   d. 23 kb
   e. 21 kb
8. The genus, *Klebsiella*, named after the microbiologist [a]
   a. Edwin Klebs
   b. Hans Krebs
   c. Hans Christian gram
   d. Kurt
   e. Koch
   f. All of these
9. Key signal molecules that play a pivotal role during initiation of nodule development and bacterial invasion [a]
   a. Nod factors
   b. Co-factors
   c. Proteins
   d. Hormones
   e. All of these
10. In response to plant flavonoid compounds, the bacteria produce _____ factors, which in turn elicit nodule development on roots of the legumes [a]
    a. Nod factors
    b. Co-factors
    c. Proteins
    d. Hormones
    e. All of these
11. Nod factors are [a]
    a. lipo-chito-oligosaccharide molecules
    b. fatty acids
    c. nucleic acids
    d. nucleotides
    e. all of these
12. *Rhizobium* nodulation (*nod*, *nol*, and *noe*) genes involved in the production of [a]
    a. Nod factors
    b. Co-factors
    c. Proteins
    d. Hormones
    e. All of these
13. genes involved in the production of Nod factors are located primarily on [b]
    a. chromosomes
    b. plasmids
    c. transposons
    d. insertion elements
    e. all of these
14. The genes for production of Nod factors are located on large plasmids called [a]
    a. symbiotic plasmids or pSyms
    b. Ti plasmids
    c. NOL plasmids
    d. NIF plasmids
    e. All of these
15. An initial stage of root nodule formation on legumes is triggered by production of [a]
    a. Flavonoids
    b. Lipids
    c. Alkaloids
    d. Terpenoids
    e. All of these
16. Plant flavonoids that induce transcription of the common nodulation genes [a]
    a. nodA, nodB, nodC
    b. *nifH*
    c. *nifD*
    d. *nifK*
    e. All of these
17. Natural *nod* gene inducers from various legumes are [a]
    a. Flavonoids
    b. Lipids
    c. Alkaloids
    d. Terpenoids
    e. All of these

18. *nod* gene inducers such as anthocyanidins, chalcones, flavanones, flavones, flavonols, and isoflavones are [a]
    a. Flavonoids
    b. Lipids
    c. Alkaloids
    d. Terpenoids
    e. All of these
19. Nodules characterized by a round-shaped appearance, initiation of nodule primordia in the outer cortex, and meristematic activity that disappears early after nodule initiation are called [a]
    a. Determinate
    b. Indeterminate
    c. Disseminate
    d. Uniform
    e. All of these
20. Nodules formed on tropical and subtropical legumes (e.g., soybean, bean) are [a]
    a. Determinate
    b. Indeterminate
    c. Disseminate
    d. Uniform
    e. All of these
21. Nodules characterized by oval-shape, nodule primordia initiate in the inner cortex, the meristematic activity is persistent, and the central tissue consists of a number of distinct zones [b]
    a. Determinate
    b. Indeterminate
    c. Disseminate
    d. Uniform
    e. All of these
22. Nodules usually form on roots of temperate legumes (e.g., pea, alfalfa, vetch) [b]
    a. Determinate
    b. Indeterminate
    c. Disseminate
    d. Uniform
    e. All of these
23. One of the key *nod* genes encoding an acyl transferase have been discovered in symbiotic *Methylobacterium* sp. and *Burkholderia* sp. [a]
    a. *nod A*
    b. *nifH*
    c. *nifD*
    d. *nifK*
    e. All of these
24. *Medicago truncatula* and *Lotus japonicus* are considered the best model legumes, their genomes are being sequenced to efficiently determine plant responses occurring during all stages of _____ development [a]
    a. Nodules
    b. Galls
    c. Tumors
    d. Rust
    e. All of these
25. Nod factor synthesis depends on the expression of nodulation (*nod*) genes, comprising the [a]
    a. *nod*, *nol*, and *noe*
    b. *nifH*
    c. *nifD*
    d. *nifK*
    e. All of these
26. Nod factors act in concentrations as low as [a]
    a. 10^{-9} to 10^{-12} M
    b. 10^{-9} to 10^{-11} M
    c. 10^{-9} to 10^{-15} M
    d. 10^{-9} to 10^{-22} M
    e. All of these
27. Nod factors preferentially migrate into [a]
    a. Root hair cell walls
    b. Root hair cortex
    c. Root hair medulla
    d. Root hair cytoplasm
    e. All of these
28. Shepherd's crooks is [a]
    a. Curling of root hair
    b. Increase in length
    c. Decrease in length
    d. Swelling
    e. All of these
29. The first responses to Nod factor treatment is an increase in intracellular, cytosolic pH with more than 0.2 U that lasts for 5 min is called [a]
    a. Alkalinization
    b. Neutral effect
    c. Acidic effect
    d. Mild acidic effect
    e. All of these
30. The Nod factor–induced depolarization of the membrane potential and extracellular alkalinization of the alfalfa root surface are inhibited on deactivation of the plasma membrane [a]
    a. H^+ ATPases
    b. Na^+ ATPases
    c. K^+ ATPases
    d. Mg^+ ATPases
    e. All of these
31. Nod factors induce several plant genes that are expressed during early stages of nodulation, called [a]
    a. Early nodulin
    b. Late nodulin
    c. Nitrogenase
    d. Mid-nodulin
    e. All of these
32. Genes that are expressed for early nodulin are [a]
    a. *ENOD* genes
    b. *nod A*
    c. *nifH*
    d. *nifD*
    e. *nifK*
33. First expressed *ENOD* genes in root hairs [a]
    a. *PsENOD12*
    b. *PsENOD1*
    c. *PsENOD2*
    d. *PsENOD10*
    e. *PsENOD22*
34. *PsENOD12*, encode [a]
    a. hydroxyproline-rich protein
    b. hydroxylysine-rich protein
    c. hydroxycysteine-rich protein
    d. hydroxyalanine-rich protein
    e. All of these
35. *PsENOD12* expression is induced after _____ amount of Nod factors in *R. leguminosarum* bv. *viciae* [a]
    a. 10^{-8} M
    b. 10^{-7} M
    c. 10^{-6} M

d. 10^{-5} M
e. All of these

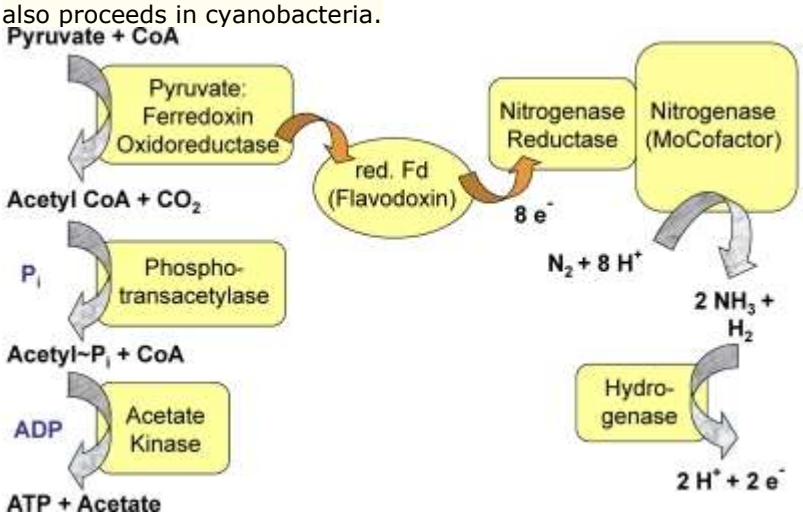
36. The chitin backbone of Nod factors can be hydrolyzed by [a]
a. Chitinases
b. Cellulases
c. Amylases
d. Pectinases
e. All of these
37. Antifungal activity, carrot somatic embryo development, and plant development are activities of [a]
a. Plant chitinases
b. Cellulases
c. Amylases
d. Pectinases
e. All of these
38. Chitinases are present in the cortex of soybean nodules, induced by *Bradyrhizobium japonicum* protect central tissues against [a]
a. Pathogen
b. Parasites
c. Bacteria
d. Viruses
e. All of these
39. Carbohydrate-binding lectin proteins function as [a]
a. Nod factor receptors
b. Nod factor signals
c. Nod factor inhibitors
d. Nod factor suppressors
e. All of these
40. Strains of the soil bacteria *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Azorhizobium* collectively called [a]
a. Rhizobia
b. Azotobacter
c. Azospirillum
d. Derxia
e. All of these
41. Nitrogen-fixing root nodules on plants of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) are formed by [a]
a. *Rhizobium leguminosarum*
b. *Bradyrhizobium japonicum*
c. *Sinorhizobium rostrata*
d. *Rhizobium galegae*
e. *Rhizobium etli*
42. The stages of symbiotic establishment [e]
a. root hair curling
b. infection thread formation
c. Penetration
d. nodule formation and function
e. all of these
43. Plant nodulation and nitrogen fixation processes in nature are affected by [e]
a. Soil temperature,
b. pH, texture
c. moisture, salinity
d. deficiencies in essential elements
e. all of these
44. Specific flavonoid molecules such as naringenin and hesperetin are normally present in the rhizosphere of pea and lentil, and induce *nod* gene expression of [a]
a. *Rhizobium leguminosarum*
b. *Bradyrhizobium japonicum*
c. *Sinorhizobium rostrata*
d. *Rhizobium galegae*
e. *Rhizobium etli*
45. As a result of *nod* gene induction, a lipochitin oligosaccharide (Nod factor) is produced by the bacterial symbiont which, in turn, elicits root hair deformation and cortical cell division in the plant [a]
a. Root
b. Leaf
c. Shoot
d. Flower
e. Fruit
46. In addition to flavonoids, plant signals to rhizobia include [e]
a. Betaines
b. aldonic acid
c. xanthones
d. simple phenolics and jasmonates
e. All of these
47. Compounds synthesized by rhizobia encompass not only Nod factors and surface polysaccharides but also [e]
a. types I, III and IV secreted proteins
b. *N*-acyl homoserine lactones (AHL)
c. bradyoxetin, hopanoids, lumichrome
d. indole-3-acetic acid (IAA)
e. all of these
48. The first flavonoid *nod* gene inducers, luteolin from *Medicago sativa* and 7,4′ dihydroxyflavone from *Trifolium repens*, had been discovered in [a]
a. 1986
b. 1985
c. 1956
d. 1966
e. 1962
49. A major byproduct of nitrogenase activity during nodule function [a]
a. Hydrogen
b. Methane
c. Nitrogen
d. Helium
e. Argon
50. The host proteins involved in the regulation of infection-thread growth are [a]
a. lectins
b. pectins
c. lignin
d. amylopectin
e. all of these

1. Hydrogenases are enzymes capable of producing or uptaking molecular hydrogen.
2. Algal hydrogenases are among to the most efficient hydrogen (H_2) generating biocatalysts and use low-potential electrons from the photosynthetic light reactions
3. Photobiological H_2 evolution by eukaryotic microalgae represents a sustainable alternative to the energy intensive industrial production of H_2 based on fossil fuels.
4. Most of the known hydrogenases are iron-sulfur proteins with two metal atoms at their active site, either a Ni and an Fe atom (in [NiFe]-hydrogenases) or two Fe atoms (in [FeFe]-hydrogenases).
5. The key enzyme involved in the metabolism of H_2 is hydrogenase.
6. The enzyme hydrogenase. catalyzes the simplest chemical reaction: 2H+ + 2e- → H2.
7. The reaction by hydrogenase is reversible, and its direction depends on the redox potential of the components able to interact with the enzyme.
8. In the presence of H2 and an electron acceptor, it will act as a H2 uptake enzyme hydrogenase
9. In the presence of an electron donor of low potential, hydrogenase may use the protons from water as electron acceptors and release H2
10. The first classification of hydrogenase enzymes was based on the identity of specific electron donors and acceptors, namely, NAD (hydrogenases of EC class 1.12.1.12), cytochromes (class 1.12.2.1), coenzyme F420 (class 1.12.99.1), or ferredoxins (class 1.18.99.1)
11. Nitrogenase activity results in the evolution of hydrogen as a result of a side reaction intrinsic to the activity of this enzyme.
12. Some rhizobia, and also other nitrogen fixers, induce a NiFe uptake hydrogenase (Hup) to recycle hydrogen produced by nitrogenase, thus improving the efficiency of the nitrogen fixation process.
13. The two cyanobacterial Ni hydrogenases are differentiated as either uptake or bidirectional hydrogenases.
14. The [FeFe] hydrogenases have a unique active center (the H cluster) which produces about 100-fold higher activity than the other hydrogenases
15. The simplest [FeFe] hydrogenase occurs in green algae with only the H cluster as the prosthetic group
16. The H cluster contains two Fe atoms and the two ligands CO and CN^{-}, which are attached to both of the Fe atoms.
17. In green algae, the H cluster is directly reduced by ferredoxin
18. [FeFe] hydrogenase of *Desulfovibrio vulgaris* is involved in the utilization of H_2 in sulfate reduction
19. The majority of hydrogenases in prokaryotes are Ni-containing enzymes.
20. Bidirectional hydrogenase is widespread in cyanobacteria
21. Bidirectional hydrogenase is present in unicellular, filamentous, and heterocystous cyanobacteria species, where it occurs in both heterocysts and vegetative cells
22. The *hyp* genes required for the synthesis of the hydrogenase
23. In photosynthetic eukaryotic algae, hydrogenase is located in plastids
24. The *nod* genes are the key bacterial determinants of the signal exchange between the two symbiotic partners.
25. Purified Nod factors induce at very low concentrations (down to 10^{-12} mol l^{-1}) on the roots of host plants, a number of developmental responses which are similar to those induced by rhizobial cells: root hair deformation, division of cortical cells, and formation of nodule primordia.
26. *NodPQ* encode ATP sulfurylase and adenosine 5′-phosphosulphate (APS) kinase
27. ATP sulfurylase and adenosine 5′-phosphosulphate (APS) kinase catalyze the formation of 5′-phosphoadenosine 5′-phosphosulphate (PAPS)
28. In *Sinorizobium meliloti*, the *nodH* and *nodPQ* are required for the sulfation of Nod factors.
29. In rhizobia, the terminal non reducing GlcNAc residue was specifically N-deacetylated by the NodB protein to generate a free amine group to which the fatty acid chain was attached by acyl tranferase encoded by *nodA*
30. The co-expression of *nodB* and *nodC* in *E. coli* resulted in the production of chitooligosaccharides
31. The sulfation of Nod Factors by the sulfotransferase encoded by *nodH*
32. The impact of Ni on the regulation of nod factors expression mainly arises due to its control over phenylalanine ammonia lyase (PAL) activity and nod gene expression.
33. The phenylalanine ammonia lyase is one of the preliminary enzymes in the phenylpropanoid pathway whose upregulation in response to metal stress enhances phenol, flavanones, and isoflavanones production
34. Plants secretes flavonoids in the form of signals that are sensed by appropriate bacteria in the rhizosphere, thereby producing Nod factors (NF) that triggers the early events in the nodulation process
35. In legume nitrogen-fixing symbioses, rhizobial *nod* genes are obligatory for initiating infection thread formation and root nodule development.
36. The two main classes of nitrogen-fixing genes, the nif genes and fix genes, are present in rhizobia.
37. The nif genes encode nitrogenase and show structural and functional resemblance with the nitrogen-fixing genes present in *Klebsiella pneumoniae* and other microbial groups
38. Most of the *nif* genes are found on plasmids of rhizobia, it was also reported to be found on chromosomes of *Bradyrhizobium*
39. The process of nitrogen fixation, either in symbiotic or non symbiotic microorganisms, is catalyzed by the enzyme nitrogenase and this enzyme complex is encoded by genes *nifDK* and *nifH*
40. The nonsymbiotic *K. pneumoniae* carries at least 20 *nif* genes which are organized in about eight operons.
41. *NifA* (a positive activator of transcription) and *NifL* (a negative regulator) control the regulation of all *nif* genes.
42. The regulation of *nif* genes are affected by the concentration of both oxygen and nitrogen

43. The soil concentrations of ammonia (NH_3 or NH_4) is high, nitrogen fixation is slowed down by *NifL* to act as a negative controller or gene expression by preventing *NifA* to act as an activator
44. NifA recognizes and binds to enhancer elements located about 100–200 base pairs upstream of the transcriptional start sites of its target genes
45. The NifA protein is the central regulator of N_2-fixation, which (under [–N] conditions) activates transcription of all the other *nif* genes in proteobacteria
46. Common to all NifA proteins is a modular structure consisting of an N-terminal domain, which is involved in control of NifA activity, a highly conserved central domain, which interacts with RNA polymerase, and a C-terminal DNA-binding domain containing a helix turn–helix motif.
47. The consensus sequence for the NifA-binding site (upstream activator-binding site, UAS) is TGT–N_{10}–ACA.
48. NifA-dependent target genes are the structural genes of nitrogenase, *nifHDK,* and all the other *nif* genes (except *nifA* itself).
49. NifA proteins activate transcription in concert with RNA polymerase containing the alternative sigma factor RpoN (σ^{54}, NtrA).
50. RpoN binds to a characteristic sequence motif, CTGG–N_8–TTGC, that is typically located at position –24/–12 upstream of the transcription start site.
51. RpoN-dependent activators are unique among transcriptional activators in that they must hydrolyze ATP (or other nucleoside triphosphates) to activate transcription
52. Biological N2 fixation (BNF) accounts for 65% of the N currently utilized in agriculture and will be increasingly important in future crop productivity
53. The nitrogen fixation provides Earth's ecosystems with about 200 million tons N per year
54.*Azoarcus*, *Arthrobacter*, *Azospirillum*, *Enterobacter*, *Azotobacter*, *Bacillus*, *Klebsiella*, *Gluconobacter*, *Herbaspirillum*, *Serratia*, and *Pseudomonas*, have been screened from rhizosphere of several crops, that play a useful role in supplying fixed N to the host plants

Hydrogenases
Hydrogenases catalyze the reversible oxidation of dihydrogen. They can be divided into three phylogenetically distinct classes, i.e., [Ni-Fe], [Fe-Fe], and [Fe] hydrogenases, according to the type of catalytically active metal site. Whereas most [Ni-Fe] hydrogenases are of the hydrogen uptake type, [Fe-Fe] hydrogenases mainly produce molecular hydrogen, and [Fe] hydrogenases catalyze a specific reaction utilizing H_2. All hydrogenases contain at least one iron atom in the active site, which carries non-protein ligands, i.e., carbon monoxide and cyanide. A common mechanistic step of all three types of hydrogenases is the heterolytic splitting of dihydrogen that takes place at a metal center.

Hydrogen uptake by hydrogenase.
A simple scheme showing the relationship between pyruvate degradation, ammonium and hydrogen formation by nitrogenase, and hydrogen uptake by hydrogenase. This pathway is typical in strict or facultative anaerobes but also proceeds in cyanobacteria.



Nod factors
Nod factors consist of a tetra- or pentameric chitooligosaccharide backbone to which a long unsaturated fatty acid (C16 or C18) is attached at its non reducing end. Nod factors are lipochitooligosaccharides that are secreted by rhizobia to trigger the developmental process that leads to the formation of nitrogen-fixing root nodules in leguminous plants. Host specificity is determined by the structure of the fatty acid chain, the length of the chitooligosaccharide and by various substituents that can be attached to the two terminal residues of the chitin backbone. Nod factors induce the formation of root nodules on the host plant roots at concentrations of 10^{-9}–10^{-12} M, and genuine nodule structures can be induced by the application of Nod factors alone without bacteria in alfalfa and soybeans, indicating that these glycofactors can be the sole chemical inducer for nodule formation.

Role of nod genes
The nod genes identified in *R. meliloti* and *R. leguminosarum bv. viciae* and *trifolii* have been broadly categorized into four classes: *nodD, nodA, nodB, nodC* (common nod genes), *hsn* (host-specific nod genes), and other nod genes. Bacterial *nod* genes (NodD) perceive plant signals of the flavonoid family. The *nod* genes trigger

the biosynthesis of lipochitooligosaccharides (LCOs). The LCOs induce the nuclear calcium spiking in the root cells of the host plant. The calmodulin-dependent kinase phosphorylates the transcription factor, which promotes gene expression and nodulation. At the initial stages of infection, calcium oscillations induce the Nodule Inception (NIN) factor that initiates the bacterial infection in the root epidermis. The development of root hairs is accomplished by tip growth in plants. During the growth of root hairs, vesicles containing plant cell membrane and cell wall move to the root hair tip, where they merge into cell membrane, whereby the cell membrane is added to the tip and the cell wall material is deposited outside the membrane. Nod factor production leads to reorientation of cell wall development, which causes root curling, hair deformation, and trapping of the infecting bacteria. Reorganization of the actin cytoskeleton is nod factor dependant, which diverts vesicle traffic from the root hair tip to a new site away from the center of the apical dome of the root hair. This deformation results in root curling and root hair deformation

***nif* Genes Products and Their Role in Nitrogen Fixation**

***Nif* Genes**	**Role in Nitrogen Fixation**
nifH	Dinitrogenase reductase
nifD	α-Subunit of dinitrogenase
nifK	B-subunits of dinitrogenase. B clusters are present at B subunit-interface
nifY	In *Klebsiella pneumoniae*, aids in the insertion of FeMo-co into apodinitrogenase
nifE	Forms a2 B2 tetramer with *nifN*. Required for FeMo-co synthesis
nifN	Required for FeMo-co synthesis
nifx	Involved in FeMo-co synthesis
nifU	Involved in mobilization of Fe–S cluster synthesis and repair
nifS	Involved in mobilization of S for Fe–S cluster synthesis and repair
nifV	Homocitrate synthesis involved in FeMo-co synthesis
nifW	Involved in stability of dinitrogenase. Proposed to protect dinitrogenase from O_2 inactivation
nifM	Required for the maturation of *nifH*
nifF	Flavodoxin, physiologic electron donor to *nifH*
nifL	Negative regulatory element
nifA	Positive regulatory element
nifB	Required FeMo-co synthesis. Metabolic product. NifB-co is the specific Fe and S donor to FeMo-co
fdxN	Ferredoxin serves as electron donor to nitrogenase
nifQ	Involved in FeMo-co synthesis. Proposed to function in early MoO42 processing
nifJ	Pyruvate flavodoxin (ferredoxin) oxidoreductase involved in electron transport to nitrogenase

***nif* genes**

The *nif* genes are genes involved in nitrogen fixation. They are found in nitrogen-fixing bacteria. They occur as an operon in free-living anaerobic nitrogen-fixing bacteria such as *Klebsiella pneumoniae*, *Rhodospirillum rubrum*, and *Rhodobacter capsulatus*. These genes may also be found on plasmids (together with the other genes, e.g. *nod* genes) in symbiotic bacteria, such as in *Rhizobium* inhabiting the roots of leguminous plants.
The *nif* genes code for proteins essential in nitrogen fixation, such as nitrogenase and certain regulatory proteins. NifA protein regulated the *nif* genes transcription. NifA protein is in turn regulated by NtrC. The expression of NifA protein is triggered when fixed nitrogen and oxygen levels are low. In contrast, a sufficient concentration of nitrogen or oxygen would stimulate the protein NifL. The latter inhibits the activity of NifA and this inhibits the formation of nitrogenase.

Regulation of *nif* genes

- In most bacteria, regulation of nif genes transcription is done by the nitrogen sensitive NifA protein.
- When there isn't enough fixed nitrogen available for the organism's use, NtrC triggers NifA expression, and NifA activates the rest of the nif genes.
- If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated.
- NifL inhibits NifA activity resulting in the inhibition of nitrogenase formation.

Regulation of *nif* genes in ***Klebsiella pneumoniae***

• The N2 fixation (nif) genes are organized into a regulon of 17 genes consisting of seven or eight operons each of which is transcribed into a single, usually polycistronic mRNA.

• Regulation of nif gene expression has Two elements: • an external system designated ntr(nitrogen regulatory) • an internal system mediated by nif A and nif L.

• The ntr system responds to conditions of nitrogen starvation by activating genes that enable the organism to utilize 'unusual' nitrogen sources such as arginine, proline, and histidine as well as N2 itself.

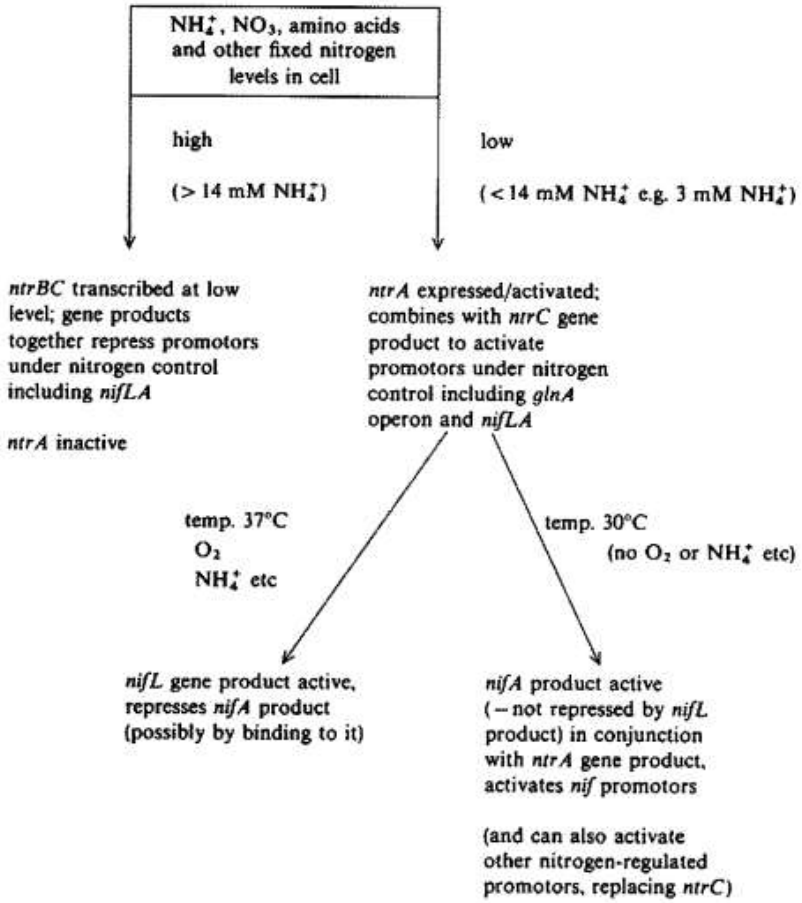
• The ntr A gene product (NtrA) is a-factor of RNA polymerase which recognize the nif and, other ntr - regulated genes. • These promoters have a structure different from that of typical bacterial promoters.

• NtrA allows RNA polymerase to bind at the nif promoters and to initiate transcription.

• The ntrB gene product (NtrB) is an enzyme that functions both as a protein kinase and as a phosphatase, the substrate of which is NtrC (the ntrC gene product).

• Whether kinase or phosphatase activity predominates depends upon the nitrogen status of bacterium, and the consequence of this is that, under condition of starvation, NtrC-P acts as an activator of, nifL and nif A.

• The nif A product is an activator of transcription of other nif genes, whilst the nif L product, in the presence of either intermediate concentrations of fixed nitrogen or inactivate the nif A product, thereby preventing transcription of other nif genes.



Organization of Nif Genes:

Nitrogen fixation is carried out by three groups of genes. These are; Nod gene (responsible for nodule formation), Nif gene (responsible for nitrogen fixation) and Hup gene (responsible for nitrogen uptake). All these three types of genes are present in a group on a single chromosome.

Nod gene:

Most of the biological nitrogen fixing bacteria contains a large plasmid called mega-plasmid. In several functions it is similar to Ti plasmid and contains genes responsible for auxin and cytokinin production. Excess production of these plant growth regulators helps in nodule formation. According to Rosenberg (1981) several special genes are present along with nod genes. Such plasmids are absent in non-symbiotic bacteria.

A nod gene is a group of genes containing Nod A, B, C, D genes having 8.5 kb length. These genes form polypeptides of different lengths (196, 197, 402, 211 amino acid). Nod genes of different rhizobium species have almost 70% homologies which are called common Nod genes.

Nif genes:

This gene is responsible for nitrogen fixation and present in the genome of symbiotic and non symbiotic nitrogen fixing bacteria. In symbiotic bacteria Rhizobium, it is present near nod genes on the megaplasmid, while in non-symbiotic cyanobacteria it is present on the main DNA. Initially Nif gene has been transferred in E. coli.

In higher plants, chloroplast is a cell organelle which might have been originated from prokaryotes, therefore attempt are made to transfer Nif gene into chloroplast. Easy availability of ATP and $NADPH_2$ in chloroplast also makes them ideal recipient for this gene transfer.

Most of the cereal plants are monocots and any such effort to transfer such Nif gene will revolutionize the yield, economics and environmental pollution. However, there are many difficulties in transferring, integration and expression of a prokaryotic gene into a monocot.

Hup gene:
Gene responsible for nitrogen uptake is Hup gene. In symbiotic bacteria this gene recycles the hydrogen produced during nitrogen fixation. Hydrogen produced at different steps is assimilated in the reduction of nitrogen. In most of the legumes 30-50% energy (in the form of ATP) is spent on hydrogen liberation. This results in loss in capacity of nitrogen fixation. If this hydrogen can be recycled by nitrogenase enzyme we can save a lot of energy, and this can be carried out by improved Hup gene.
Klebsiella pneumoniae strain M5 a1 (Enterobacteriaceae) is a free living bacteria which has been studied extensively for genetics of nitrogen fixation. This bacterial genome is quite similar to that of E. coli and Salmonella typhimurium. Therefore most of the techniques of genetic engineering can be applied to Klebsiella.

Nif Gene Organization in Klebsiella:
Several mutants of Klebsiella were developed by growing the bacteria on medium containing a mutagen, methyl-nitro nitro-so-guanidine. Different mutants obtained were used in transformation, transduction to map the Nif gene (Table 11.4). This provided the information that Nif gene is downstream to histidine operator (Fig. 11.4). On the basis of this Nif gene of Klebsiella was transferred in E. coli on the basis of homology in the plasmid and not in genomic DNA. Nif gene organization of Klebsiella is similar to that of Azotobacter, Asospirillum, Clostridium and prokaryotic blue-green algae.

Regulation of Nitrogen Fixation:
All the nif genes in Klebsiella are clustered and coordinately regulated. E. coli to which the nif genes of Klebsiella have been transferred can fix N_2. In both the original Klebsiella and the E. coli nitrogenase is expressed only in the absence of both O_2 and NH_3 in the growth medium. The nif genes are regulated by the nifLA operon. The nitrogen regulators NtrC (= GlnG), and NtrB determine whether or not the nifLA operon is expressed (depending on the presence of ammonia or organic nitrogen).
In the absence of ammonia or organic nitrogen the NtrC protein is phosphorylated by the NtrB protein. NtrC-P then binds to the upstream region of the nifLA operon and activates transcription. NtrA (= GlnF = RpoN = σ54) is the nitrogen sigma factor, which is needed for expression of the nifLA operon and the nif structural genes. NtrA is an alternative sigma factor used by RNA polymerase to recognize many genes involved in nitrogen metabolism which are not recognized by the standard sigma factor.
The nif A gene encodes a protein required for switching on all of the nif genes except the regulatory genes nifLA themselves. If nif A protein is made, its function is to activate the other nif genes. The nifL gene is required for O_2 repression. In the absence of NifL protein, nitrogenase is made in the presence of O_2 (but is inactivated by O_2). When oxygen is present, the nifL protein binds to nif A and prevents it from activating the other nif genes.

Transgenes with Nif Genes:
It is important to develop transgenic plants containing Nif genes to solve the problem of nitrogen feriliser supplement to crop plants. This will have beneficial effects of economics and environment also. For this purpose, Ti based plasmid and Cauliflower mosaic virus based promoter (CAM promoter) was used to transfer Nif genes in to non-legume plants. To test the efficacy of this system, phaseolin gene from legume (pulses) has been transferred to sunflower where it was expressed and produced phaseolin.
Protoplasts isolation from root nodules and preparations of rhizobia were used to develop hybrids by protoplasts fusion and organelles uptake.

Range of Rates of BNF Measured under Field Conditions by Different Diazotrophic Organisms and Their Associations

Diazotrophic bacteria and their associations	**N_2 fixed (kg ha^{-1} $year^{-1}$)**
Free-living bacteria (associated with wood decay, straw decomposition, cyanobacterial mats)	<1–10
Examples of plant–cyanobacterial associations	
Cryolithic crusts	10–80
Azolla	≤300
Examples of legume–rhizobial associations	
Soybean	60–115
Beans	50–100
Alfalfa	130–250
White clover	200
Examples of nonlegume–*Frankia* associations	
Alder	50–300
Ceanothus	50–60
Hippophae	10–60