Multiple Choice Questions

1. The process in which NO_3^- serves as electron acceptor in place of O_2, is known as:
(a) Ammonification
(b) Nitrate reduction
(c) Nitrate respiration
(d) Denitrification
2. Nitrate uptake into roots of the plants from soil is facilitated by:
(a) Uniporters
(b) $2H+/NO_3^-$ symporters
(c) $2H+/NO_3^-$ antiporters
(d) Nitrate transporters belonging to chloride channel family
3. During reduction by nitrate reductase, electrons are passed to NO3_:
(a) From heme cofactor of the enzyme
(b) From FAD cofactor at C-terminal of the enzyme
(c) From reduced molybdenum of MoCo at the C-terminal of the enzyme
(d) From reduced molybdenum of MoCo at the N-terminal of the enzyme
4. Electrons for NO2_ reduction are obtained from:
(a) NADH
(b) Reduced ferredoxin
(c) NADPH
(d) Reduced glutathione
5. An example of symbiotic association of non-leguminous plants with rhizobium is:
(a) Gunnera
(b) Anthoceros
(c) Casuarina
(d) Parasponia
6. Chemical nature of Nod factors is:
(a) Lipoproteins
(b) Oligosaccharides
(c) Lipochitooligosaccharides
(d) Oligopeptides
7. The term symbiosome is used for depicting:
(a) Vesicular structure formed in the infected cells of nodules
(b) The symbiotic relationship in between the endosymbiont and host plant
(c) The infection thread formed at the time of nodulation
(d) Infected roots cells of nodules in legumes
8. Site for N2 reduction in nitrogen-fixing enzyme is present in:
(a) Dinitrogenase reductase
(b) P cluster of dinitrogenase
(c) M cluster of dinitrogenase
(d) O-side chain of serine from the peptide backbone of P cluster
9. N2 fixed by bacteroids is released in the cytosol of the infected cell as:
(a) NH3
(b) NH4+
(c) Glutamine
(d) Ureides
10. Which of the following statements is true?
(a) nif D and nif K encode for nodulins.
(b) Fe protein is encoded by nif F.
(c) The nif genes are required for nitrogen fixation only by the symbiotic bacteria and not by free-living nitrogen fixers.
(d) fix genes are essential for nitrogen fixation in symbiotic nitrogen fixers but do not have counterparts in free-living forms.
Answers
1. c 2. b 3. d 4. b 5. d 6. c 7. a 8. c 9. b 10. D
11. What is the first stable product of nitrogen fixation in the root nodules of leguminous plants?
(a) Glutamate
(b) NO_3^-
(c) Ammonia
(d) NO_2^-
Answer: (c)
12. Which of the following is required for the action of the nitrogenase enzyme?
(a) Light
(b) High input of energy
(c) Super oxygen radicals
(d) Mn^{2+}
Answer: (b)
13. Leghaemoglobin is present in the root nodules of legumes. What is the function of leghaemoglobin?
(a) Oxygen removal
(b) Inhibition of nitrogenase activity

(c) Expression of nif gene
(d) Nodule differentiation
Answer: (a)
14. An aquatic fern that performs nitrogen fixation is ________.
(a) *Nostoc*
(b) *Azolla*
(c) *Salvinia*
(d) *Salvia*
Answer: (b)
15. Which of the following is correct for nitrifying bacteria?
(a) They convert free nitrogen to nitrogen compounds
(b) They oxidize ammonia to nitrates
(c) They reduce nitrates to free nitrogen
(d) They convert proteins into ammonia
Answer: (b)
16. Which of the following prevents the inactivation of nitrogenase by oxygen?
(a) Cytochrome
(b) Carotene
(c) Xanthophyll
(d) Leghaemoglobin
Answer: (d)
17. Nitrogen is absorbed by plants as ______.
(a) Nitrites
(b) Ammonium
(c) Nitrites
(d) All of the above
Answer: (d)
18. Industrial nitrogen fixation is carried out by ______.
(a) Friedel Crafts reaction
(b) Helmonts process
(c) Haber process
(d) None of the above
Answer: (c)
19. This element plays a key role in the nitrogen fixation.
(a) Manganese
(b) Molybdenum
(c) Zinc
(d) Copper
Answer: (b)
20. Cells where nitrogen fixation takes place in *Nostoc* are known as ______.
(a) Hormogonia
(b) Heterocysts
(c) Akinetes
(d) Nodules
Answer: (b)
21. The mutually beneficial symbiotic relationship between legumes and rhizobia is mainly reflected in___________
A. Legumes obtain nitrogen-containing inorganic substances from rhizobia, and rhizobia obtain carbohydrates from legumes
B. Legumes obtain nitrogen-containing organic matter from rhizobia, and rhizobia obtain nitrogen-containing inorganic matter from legumes
C. Legumes obtain N_2 from rhizobia , and rhizobia obtain organic matter from legumes
D. Legumes obtain NO_3^- from rhizobia, and rhizobia obtain NH_3 from legumes .
Answer: A
22. Nitrogen is absorbed by plants as ______.
A. Nitrites
B. Ammonium
C. Nitrites
D. All of the above
Answer: D
23. What is the first stable product of nitrogen fixation in the root nodules of leguminous plants?
A. Glutamate
B. NO_3^-
C. Ammonia
D. NO_2^-
Answer: C
24. Which enzyme is involved in nitrogen fixation?
A. Nitrogenases
B. Isomerase
C. Denitrogenase
D. Methyl Transferase
Answer: A

25. Which of the following statements about nitrogen-fixing bacteria is incorrect__________
A. A rhizobia can invade all species of legumes
B. Mutual symbiosis between legumes and the rhizobia in their nodules
C. The rhizobia in the soil cannot fix nitrogen
D. Legumes with root nodules can use nitrogen as a nitrogen source
Answer: A
26. Which crop is helpful in nitrogen fixation?
A. beans,
B. peanuts
C. soy
D. All of these
Answer: D
27. Leghaemoglobin is present in the root nodules of legumes. What is the function of leghaemoglobin?
A. Oxygen removal
B. Inhibition of nitrogenase activity
C. Expression of nif gene
D. Nodule differentiation
Answer: A
28. Which of the following is symbiotic nitrogen fixing bacteria?
A. Rhizobium trifolii
B. Clostridium pasteurianum
C. Azotobacter sp.
D. Escherichia coli
Answer: A
29. What is true about biological nitrogen fixation?
A. Nitrogen-fixing microorganisms reduce nitrogen in the air to ammonia
B. Legumes are capable of biological nitrogen fixation
C. Autogenic nitrogen-fixing microorganisms are autotrophs
D. The energy source of symbiotic nitrogen-fixing microorganisms is light energy
Answer: A
30. Which of the following is correct for nitrifying bacteria?
A. They convert free nitrogen to nitrogen compounds
B. They oxidize ammonia to nitrates
C. They reduce nitrates to free nitrogen
D. They convert proteins into ammonia
Answer: B
31. Rhizobium is a nitrogen-fixing microorganism, one of its biological characteristics is_________
A. Able to fix nitrogen when living independently in the soil
B. Aerobic heterotrophic bacteria
C. The energy required is provided by its own mitochondria
D. unicellular eukaryotes
Answer: B
32. Which of the following statements about rhizobia is correct?
A. Rhizobium can also fix nitrogen outside plant roots
B. Rhizobium cannot survive without the root of the plant
C. When the soil is flooded, nitrogen fixation by rhizobia decreases
D. The nitrogen required for the growth of soybean plants comes from the rhizobia
Answer: C
33. How many ATP are required for nitrogen fixation?
A. Microbes fixing nitrogen need 16 moles of ATP for the reduction of each mole of nitrogen.
B. Microbes fixing nitrogen need 32 moles of ATP for the reduction of each mole of nitrogen.
C. Microbes fixing nitrogen need 8 moles of ATP for the reduction of each mole of nitrogen.
D. Microbes fixing nitrogen need 24 moles of ATP for the reduction of each mole of nitrogen.
Answer: A
34. Which of the following statements about root nodules and rhizobia is correct__________
A. The genes that control nitrogen fixation traits are located on nucleoid DNA molecules
B. Rhizobium can survive in soil but cannot fix nitrogen
C. A rhizobia can only invade one type of legume
D. The shape of root nodules is determined by the genes of legumes
Answer: B
35. Which of the following statements about biological nitrogen fixation is incorrect__________
A. Some bacteria, actinomycetes, cyanobacteria, etc. can carry out biological nitrogen fixation, and their cells have no nuclear membrane
B. Azotobacter rotundus can not only fix nitrogen, but also promote plant growth and fruit ripening
C. Rhizobium can provide the fixed N element to legumes in the form of NH_3
D. The metabolic type of Rhizobium is heterotrophic and aerobic, and its nitrogen-fixing gene has no exons and introns
Answer: B

Fill in the Blanks

1. Plants are able to absorb nitrogen from the soil as nitrates or ammonium.
2. Absorption of nitrate and ammonium ions is facilitated by the receptors present on plasma membrane of the root cells.
3. Excess nitrates can be stored in the vacuoles, while excess ammonium, if not metabolized, becomes toxic for the plants.
4. Nitrate can also be translocated to leaves and is metabolized there.
5. There are two enzymes, i.e., nitrate reductase and nitrite reductase, which catalyze reduction of nitrate to ammonium in cytosol and plastids of the plant cell, respectively.
6. Nitrate reductase is an inducible enzyme, while nitrite reductase is a constitutive enzyme.
7. Molecular nitrogen present in the air can be fixed by some of the prokaryotes, which are called diazotrophs.
8. Diazotrophs can either be free living or grow in symbiotic associations with plants.
9. Two most important nitrogen-fixing symbiotic associations are Rhizobium, legume, and Frankia, actinorhizal.
10. A great degree of specificity exists in between the specific strain of rhizobia and the leguminous plant, which is due to the interaction of biochemical signals produced by the plants in the form of flavonoids, and the Nod factors of rhizobia.
11. Specific Nod factors produced by the rhizobia species are identified by the Nod receptors present on the plasma membrane of the root hair. This is followed by the formation of an infection thread and subsequently nodules.
12. Effective symbiosis is established in the form of symbiosomes due to the involvement of both rhizobial and host genes.
13. Bacteroids are the nitrogen-fixing forms of rhizobia because of nitrogenase being synthesized by them, which is responsible for the reduction of molecular nitrogen to ammonium ions.
14. Nitrogen-fixing genes of the bacteria, i.e., nif genes, are involved in the synthesis of nitrogenase.
15. Ammonium ions, either absorbed by the plants or produced as a result of nitrate reduction or nitrogen fixation, need to be assimilated which is carried out by glutamine synthetase and 2-oxoglutarate aminotransferase
16. 80–90% of the nitrogen available to the plants originates through biological nitrogen fixation
17. Frankia are the gram-positive actinomycetes, which grow in symbiotic associations with as many as 20 genera of dicots belonging to 8 or more families.
18. Alnus, Casuarina, Ceanothus, and many other species are known as actinorhizal plants
19. One non-leguminous plant - Parasponia spp. family Ulmaceae also forms symbiotic associations with rhizobia
20. In both actinorhizal and rhizobial symbiotic associations, root nodules are formed due to division of cortical and hypodermal cells.
21. Nitrogen-fixing bacteria colonize in the apoplastic regions in the stem tissues of sugarcane.
22. Gunnera in which Anabaena is present in the glands found at the base of leaf petioles
23. Symbiotic association between cyanobacteria e.g., Anabaena and diverse group of plants includes liverworts such as Anthoceros, coralloid roots of cycads, Azolla a water fern Anabaena grow in the leaf cavities
24. The rhizobia includes species of Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium, and Sinorhizobium
25. The core structure of Nod factor consists of a backbone of three to four N-acetyl-D-glucosamine which are linked by β-1,4 linkages. It is linked to a fatty acid on the terminal sugar.

1. Name the microorganisms that show symbiotic relationship with the following:

(i) A bacterium that produces aerial nodules on the stem of *Sesbania rostrata* and *Aeschynomene aspera*.	*Azorhizobium*
(ii) With non-legume genera like *Alnus*, *Casuarina* and *Myrica*	*Frankia* (an Actinomycetes)
(iii) Wetland rice ecosystem or agriculture	*Anabaena azollae*, cyanobacteria (free-living)

2. List the 'genes' controlling nitrogen fixation.
Ans. There are three classes of genes controlling N2 fixation *in situ*. They are *nod genes*, *nif genes* and
fix genes
1. *nif genes*: involved in the formation of functional nitrogenase enzyme.
2. *nod genes*: involved in the formation of root nodules.
3. *'fix' genes*: required for the maintenance of N2 fixation by the nodule.

3. Give **one** example each of free-living (asymbiotic) and symbiotic nitrogen-fixing organisms, together with their host.
Ans.

1. Asymbiotic	
Aerobic	*Azotobacter*
Anaerobic	*Clostridium*
Blue-green algae	*Nostoc*
2. Photosynthetic bacteria (anaerobic)	*Chromatium*
3. Symbiotic	
Legumes	*Rhizobium*
Non-legume (*Casuarina*)	*Frankia* (an Actinomycetes)
Liverwort (*Anthoceros*)	*Nostoc*
Fern (*Azolla*)	*Anabaena azollae**
Cycads	Blue-green algae *(Anabaena)*
Lichen	Blue-green algae *(Nostoc, Scytonema)*
4. A flowering plant (with a cyanophyceaen symbiont)	*Gunnera*
5. A flowering plant with aerial nodules visible on the stem.	*Sesbania rostrata (with Rhizobium)*

* The nitrogen-fixing cyanobacterium *Anabaena azollae* are present not in root nodules but in cavities within the leaves of an aquatic fern (*Azolla*). The *Azolla*-rice-duck agricultural system is practiced in rice paddies. The *Azolla-Anabaena* symbiosis provides fixed nitrogen to the rice plants while ducks graze on the *Azolla* and their faecal matter is rich in other nutrients required by the rice.

4. List only the essential requirements for biological (molecular) nitrogen fixation.
Ans. The following are the essential requirements for biological nitrogen (N2) fixation:
1. Nitrogenase is the enzyme that catalyzes the conversion of molecular N2 to NH3.
2. Cellular mechanism to protect nitrogenase action from oxygen inhibition.
3. Presence of natural physiological electron donors, reduced ferredoxin (Fdred)
4. Provision for abundant supply of ATP (provided by the host).
5. Availability of Mo and Fe, integral constituents of nitrogenase; Mg2+ divalent cations.
6. Absence of fixed nitrogen sources like nitrate or ammonia.
7. Presence of an 'uptake hydrogenase' enzyme system.

5. Explain the O2 protection mechanism for nitrogenase enzyme in cyanobacteria.
Ans. In cyanobacteria, such as *Nostoc* and *Anabaena*, the O2 protection mechanism for nitrogenase is provided by **heterocyst** with a specialized structure, that has lost its capability of PS II activity during development because of which there is reduced oxygen tension.
Heterocysts are the sites of both synthesis and activity of nitrogenase enzyme. During their differentiation from ordinary vegetative cells, PSII activity is lost, resulting in the loss of O2 production during photosynthesis. Secondly, during development, a glycolipid layer is newly laid in the wall of the heterocyst that constitutes a barrier to oxygen entry from the external environment.
The loss of photosystem II activity and formation of a new glycolipid layer in the wall are the two apparent mechanisms for oxygen protection for nitrogenase.

6. *Neither* the host plant *nor* the microorganism by itself can fix nitrogen. Comment.
Ans. The biological fixation of nitrogen in the root nodules of legume plants is controlled by the enzyme **nitrogenase** (now called **dinitrogenase**) that functions under anaerobic conditions. This anaerobic environment within the host cells is kept or maintained by leghaemoglobin (pinkish or reddish pigment).
The globin portion of the 'leghaemoglobin' is formed by the host plant in response to infection by the bacteria (*Rhizobium*) and the 'haeme' portion is formed by the bacterial symbiont. Through the symbiotic relationship, the leghaemoglobin would be synthesized and neither the bacterium nor the host is capable of synthesizing leghaemoglobin which is essential for the enzymatic activity of nitrogenase.

7. All nitrogen fixation systems are poisoned by even slight traces of oxygen. How have legume root nodules and cyanobacteria been able to overcome this problem?
Ans. The anaerobic environment at the site of nitrogen fixation is achieved through means such as:
1. Legume root nodule: It is accomplished by leghaemoglobin (LHb), a reddish, iron-containing pigment.

2. Nitrogen-fixing blue-green algae: The nitrogenase enzyme is localized in 'heterocysts', with specially thickened, non-photosynthetic, anaerobic cells with multilayered cell walls.

8. In which form is the fixed nitrogen transported from the root cells to different parts of the plant?
Ans. It is mainly in the form of four compounds – glutamic acid, glutamine, aspartic acid, and asparagine that fixed nitrogen is translocated from the root cells to remaining parts of the plant. In nodulated legumes, substituted urea derivatives (ureides such as allantoin, $C_4N_4H_6O_3$; allantoic acid, $C_4N_4H_8O_4$), as well as amides like asparagine ($C_4N_2H_7O_4$) have relatively high C : N ratios and are being transported upward *via* the xylem elements throughout the host plant.

9. Ammonia is toxic to plants cells. How have the plants overcome this problem of toxicity?
Ans. In plant cells, ammonia is not allowed to accumulate in substantial quantities as it reacts with α-ketoglutaric acid and oxaloacetic acid (by-products of Krebs cycle) to form glutamic acid and aspartic acid, and then with the addition of another NH_3 molecule, these are converted to glutamine and asparagine, respectively.

10. However, at high concentrations of ammonia (**The ammonia effect**), glutamic synthetase is converted to a 'form' that acts on 'nif genes', preventing transcription and ultimate synthesis of nitrogenase. Nitrogenase is synthesized when glutamine synthetase (GS) levels are high and that do not allow ammonia to accumulate. Thus, ammonia accumulation represses the synthesis of nitrogenase or dinitrogenase enzymes.

11. The enzyme nitrogenase needs the coordinated expression of several genes of the microsymbiont. Explain.
Ans. Nitrogenase is a large complex enzyme, composed of two major protein components:
1. Component I consists of both Mo and Fe and is called **Mo-Fe protein** (molecular weight of about 220 kD).
2. Component II contains just iron and is called **Fe-protein**. It is smaller with a molecular weight of about 24–36 kD and contains 4 iron atoms.
Components I and II are controlled by distinct genes of microsymbiont. *Neither* component I *nor* component II exhibit nitrogenase activity by themselves. The two together produce nitrogenase that is fully active.