

COURSE: MSc Part -II

PAPER – IX (2)

TOPIC- Biochemistry(Group B)

PREAPARED BY: Ajai Kishore Sharan

Topic-1

Vitamins

Vitamins are nutrients your body needs to function and fight off disease. Your body cannot produce vitamins itself, so you must get them through food you eat or in some cases supplements. There are 13 vitamins that are essential to your body working well. Knowledge of the different types and understanding the purpose of these vitamins are important for good health.⁶

Types and Examples of Foods

There are two types of vitamins: fat-soluble and water-soluble. Fat-soluble vitamins are stored in your fat cells, consequently requiring fat in order to be absorbed. Water-soluble vitamins are not stored in your body; therefore, they need to be replenished daily. Your body takes what it needs from the food you eat and then excretes what is not needed as waste. Here is a list of some vitamin types and common food sources:

Fat-Soluble Vitamins

Vitamin A - comes from orange colored fruits and vegetables; dark leafy greens, like kale

Vitamin D - can be found in fortified milk and dairy products; cereals; (and of course, sunshine!)

Vitamin E - is found in fortified cereals; leafy green vegetables; seeds; nuts

Vitamin K - can be found in dark green leafy vegetables; turnip/beet greens

Water-Soluble Vitamins

Vitamin B1, or Thiamin - come from whole grains; enriched grains; liver; nuts; seeds

Vitamin B2, or Riboflavin - comes from whole grains; enriched grains; dairy products

Vitamin B3, or Niacin - comes from meat; fish; poultry; whole grains

Vitamin B5, or Pantothenic Acid - comes from meat; poultry; whole grains

Vitamin B6, or Pyridoxine - comes from fortified cereals; soy products

Vitamin B7, or Biotin - is found in fruits; meats

Vitamin B9, or Folic Acid (Folate) - comes from leafy vegetables

Vitamin B12 - comes from fish; poultry; meat; dairy products

Vitamin C - comes from citrus fruits and juices, such as oranges and grapefruits; red, yellow, and green peppers

. For the most part, vitamins are obtained from the diet, but some are acquired by other means: for example, microorganisms in the gut flora produce vitamin K and biotin; and one form of vitamin D is synthesized in skin cells when they are exposed to a certain wavelength of ultraviolet light present in sunlight

Water soluble vitamins

Vitamin B1 (thiamine)

The structure of vitamin B1 contains substituted thiazole nuclei and pyrimidine. Biologically active form is called thiamine pyrophosphate (TPP, thiamine diphosphate) and its formation involves a special transferase located in brain and liver tissue.

Thiamine is found in large quantities in the outer layers of the coating covering cereal grains, in yeast (which generally contain vitamins of B group), legumes, pork meat or milk.

Vitamin B2 (riboflavin)

Chemical structure of riboflavin (from Latin flavus – yellow) contains alcohol called ribitol connected to a heterocyclic core.

Our bodies phosphorylate and transform riboflavin to one of its active forms – flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). They both form prosthetic groups of a set of oxidoreductases called flavoproteins. One of the most well-known enzymes are part of the respiratory chain – NADH- dehydrogenase or succinate dehydrogenase. Riboflavin is present in yeast, liver, kidneys, eggs or milk. The recommended daily intake is around 1.4mg.

Vitamin B3 (niacin)

Vitamin B3 is a collective term for two compounds: nicotinic acid and nicotinamide. Niacin used to be termed vitamin PP (pellagra- preventive).

Biologically active forms of niacin are nicotinamide adenine dinucleotide (NAD⁺) and its phosphorylated derivative – nicotinamide adenine dinucleotide phosphate (NADP⁺). Our body can synthesize it with the available amino acid source..

Vitamin B5 (pantothenic acid)

Vitamin B5 is made of six-carbon branched hydroxy acid called pantoic acid bound to β -alanine. The name pantothenic acid is derived from greek pantothen – from everywhere. Vitamin B5 is indeed present in many foods of plant or animal origin .As it already has been mentioned above, pantothenic acid is present in many kinds of foodstuff, including legumes, whole grain products, meat, offal or yeast.

Vitamin B6 (pyridoxine)

Vitamin B6 includes three related pyridine derivatives with the same biological function – pyridoxine (pyridoxol), pyridoxal and pyridoxamine. All three of them have to be transformed and phosphorylated (with the help of an enzyme pyridoxal kinase, present in most of body tissues) into pyridoxal-5-phosphate (PLP), the biologically active form of vitamin B6.

Vitamin B7 (biotin, formerly vitamin H)

Vitamin B7 belongs to a group of imidazole derivatives. It acts as a cofactor of enzymes catalyzing carboxylation reactions, where it serves as a carrier for CO₂ molecule. In order to perform its function it forms an active intermediate product called carboxybiotin. Examples of reactions, where biotin plays active role, include fatty-acid biosynthesis (cofactor of CoA carboxylase) or anaplerotic reactions of oxaloacetate synthesis from pyruvate (cofactor of pyruvate carboxylase). Majority of biotin is supplied to our body through a biosynthesis

performed by intestinal bacteria and biotin present in food (e.g. in liver, meat, yeast or nuts) is not so important.

Vitamin B9 (folic acid)

Folic acid (lat. folium – leaf) and its derivatives, folates, are composed of pteroyl-glutamic acids containing pteridin attached to p-aminobenzoic acid (PABA) and glutamic acid. These compounds (folate and folic acid) are collectively termed as folacin. Dietary folates are mostly present in the form of polyglutamates (containing more glutamate residues within the molecules), which are enzymatically broken into monoglutamates (with much higher absorbency) in small intestine. Intestinal cells convert the absorbed folate first into dihydrofolate and subsequently reduce it into its active form tetrahydrofolate (THF).

Vitamin B12 (cobalamin)

The structure of vitamin B12 resembles that of the porphyrin ring. The core of the molecule is the corine ring, with a cobalt ion attached in its centre. Cell metabolism utilizes active forms of cobalamin – methylcobalamin (synthesized in cytosol) and deoxyadenosylcobalamin (formed in mitochondria), which both act as enzyme cofactors. Cobalamin can only be synthesized by microorganisms, but animals have the ability to store it within their bodies. Nutritionally important sources of vitamin B12 are liver, offal, meat, fish, eggs, milk and dairy products.

Vitamin C (L-ascorbic acid)

The structure of vitamin C resembles glucose, which is at the same time a substrate for its synthesis (via D-glucuronic acid). Most animal species are able to synthesize vitamin C, the inability is rather exceptional – it includes primates and a few other species (like guinea pig). The widespread notion that citrus fruits contain large quantities of vitamin C is not entirely correct. The highest content of vitamin C has been measured in blackcurrant, cabbage, cauliflower or tomatoes. Citrus fruits, on the other hand, stand somewhere in the middle of the ranking.

Fat-soluble vitamins

Vitamin A

Vitamin A is a term for a group of substances with the biological activity of vitamin A. Natural and synthetic forms are collectively referred to as retinoids. Within the body they are represented mainly by an alcohol – retinol. Less numerous are retinal and retinoic acid. Sources of vitamin A are animal products, particularly fish oil, butter, eggs and liver. Effective absorption requires bile. Human body is able to produce vitamin A from its provitamin called β -carotene, a tetraterpene present in certain vegetables (mainly in carrot). β -carotene undergoes oxidation (using molecule of O_2) and breaks into two molecules of retinol or, at lesser extent, oxidizes to retinoic acid. Retinol is stored in Ito cells in liver.

Vitamin E (tocopherol)

Vitamin E collectively refers to four tocopherol (α , β , γ a δ) and four tocotrienol isomers (α , β , γ a δ), of which the higher biological activity is found in D- α -tocopherol. All of them feature chromanol ring and a hydrophobic phytol side chain being responsible for its poor water solubility and lipophilicity. Tocopherols easily penetrate the cell membranes and

become their parts. Chromanol ring binds one hydroxyl group that can donate a hydrogen atom, which is responsible for its antioxidative effects and methyl groups as well, which determine the particular type of tocopherol. The most abundant D- α -tokoferol also has the highest antioxidative activity.

Tocopherols are plentiful in oleaginous seeds (soya, sunflower etc.) and oils. The recommended daily dose is 12 mg. The transport of vitamin E in blood plasma is carried out by lipoproteins and it is stored in adipose tissue.

Vitamin D

Vitamin D also called calciferol or calciol occurs in several forms: in plants as ergocalciferol (vitamin D₂), in animals as cholecalciferol (vitamin D₃, calciol). In the strict sense, these substances represent prohormones (both being equally effective), which are converted in human body to their active form calcitriol (D₂- or D₃ - calcitriol).

We obtain vitamin D (mainly in the form of vitamin D₃) in diet (liver, fish, eggs, milk), but our bodies are able to synthesize it by themselves from its provitamin 7-dehydrocholesterol. Vitamin D₃ forms through photolysis by UV light in the epidermal cells.

Vitamin K

From the chemical point of view, group of vitamin K belongs to the naphthoquinone derivatives. Plants possess vitamin K₁ (phylloquinone), animals have vitamin K₂ (menaquinones), which are also synthesized by bacteria present in large intestine of our bodies. Vitamin K₃ (menadione) does not occur naturally, but it also has biological activity.

Vitamins K are obtained through diet (they are most abundant in leafy vegetables, oatmeal or live), but in the case of their absence in diet, they can fully be substituted by the vitamin K synthesized by the intestinal bacteria. That is why hypovitaminosis K resulting from an insufficient intake normally does not occur.

Topic-2

Secondary metabolites

Secondary metabolites (SM) are compounds that are not necessary for a cell (organism) to live, but play a role in the interaction of the cell (organism) with its environment. These compounds are often involved in plants protection against biotic or abiotic stresses. Secondary metabolites are from different metabolites families that can be highly inducible in response to stresses. Primary metabolites perform essential metabolic roles by participating in nutrition and reproduction. A few SMs are used as especially chemical such as drugs, flavours, fragrances, insecticides, and dyes and thus have a great economic value. These new technologies will serve to extend and enhance the continued usefulness of the higher plants as renewal sources of chemicals, especially medicinal compounds. A continuation and intensification efforts in this field is expected to lead to successful biotechnological production of specific, valuable and as yet unknown plant chemicals.

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(1). Alkaloids are additional class of SMs, which are nitrogenous organic molecules biosynthesized mainly from amino-acids, e.g., tryptophan, tyrosine, phenylalanine, lysine and arginine using many unique enzymes

(2). Many of the most important therapeutic agents are alkaloids. The sites of biosynthesis are compartmentalised at cellular or sub-cellular level. However SMs can be transported long distances and accumulate from their location of synthesis. Metabolites of Plants and their Role

(2). Sometimes it is hard to discriminate primary and secondary metabolites. For example, both primary and secondary metabolites are found among the terpenoids and the same

compound may have both primary and secondary roles. Secondary metabolites are a broad range of compounds from different metabolite families that can be highly inducible in stress conditions. Carotenoids and flavonoids are also involved in cell pigmentation in flower and seed, which attract pollinators and seed dispersers. Therefore, they are also involved in plant reproduction

(3). Plant primary products refer to the compounds of nucleic acids, proteins, carbohydrates, fats and lipids and are related to structure, physiology and genetics, which imply their crucial role in plant development. In contrast, secondary metabolites usually take place as minor compounds in low concentrations. Primary metabolism refers to the processes producing the carboxylic acids of the Krebs cycle. Secondary metabolites, on the other hand, are non-essential to life but contribute to the species' fitness for survival. In fact, these specific constituents in a certain species have been used to help with systematic determination, groups of secondary metabolites being used as markers for botanical classification (chemotaxonomy).

Plants secondary metabolites can be divided into three chemically distinct groups viz: Terpenes, Phenolics, N (Nitrogen) and S (sulphur) containing compounds

I) Terpenes : Terpenes comprise the biggest group of secondary metabolites and are free by their common biosynthetic origin from acetyl-coA or glycolytic intermediates. An immense bulk of the diverse terpenes structures produced by plants as secondary metabolites that are supposed to be concerned in defense as toxins and feeding deterrents to a large number of plant feeding insects and mammals. In Gymnosperms (conifers) α -pinene, β -pinene, limonene and myrcene are found.

(II) Phenolic compounds : Plants produce a large variety of secondary products that contain a phenol group, a hydroxyl functional group on an aromatic ring called Phenol, a chemically heterogeneous group also. They could be an important part of the plants defence system against pests and disease including root parasitic nematodes

(III) Sulphur containing secondary metabolites: They include GSH, GSL, Phytoalexins, Thionins, defensins and alliin which have been linked directly or indirectly with the defence of plants against microbial pathogens. GSH is the one of the major form of organic sulphur in the soluble fraction of plants and has an important role as a mobile pool of reduced sulphur in the regulation of plant growth and development and as a cellular antioxidant in stress responses, reported as a signal of plant sulphur sufficiency that down regulates sulphur assimilation and sulphur uptake by roots.

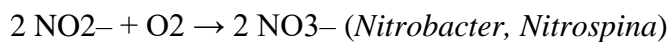
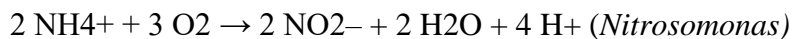
Topic-3

Nitrification

Nitrification is the process by which ammonia (NH₃) or ammonium (NH₄⁺) is converted to nitrate (NO₃⁻). Nitrification is the net result of two distinct processes: oxidation of ammonium to nitrite (NO₂⁻) by nitrosifying or ammonia-oxidizing bacteria and oxidation of nitrite (NO₂⁻) to nitrate (NO₃⁻) by the nitrite-oxidizing bacteria. Nitrification is an important step in the nitrogen cycle in soil. Nitrification is an aerobic process performed by small groups of autotrophic bacteria and archaea.

Chemistry of Nitrogen Compound Oxidation

Nitrification is a process of nitrogen compound oxidation (effectively, loss of electrons from the nitrogen atom to the oxygen atoms):



Both of these processes are extremely energetically poor, which leads to very slow growth rates for both types of organisms.

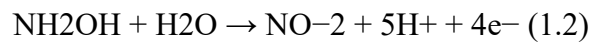
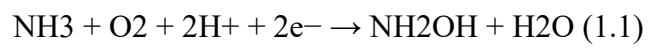
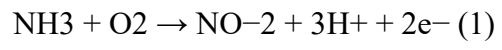
Molecular mechanism of ammonium oxidation by AOB

Ammonia oxidation in autotrophic nitrification is a complex process that requires several enzymes, proteins and presence of oxygen. The key enzymes, necessary to obtaining energy during oxidation of ammonia to nitrite are ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO). First is a transmembrane copper protein which catalyzes the oxidation of ammonia to hydroxylamine (1.1) taking two electrons directly from the quinone pool. This reaction requires O₂.

The second step of this process has recently fallen into question.

For the past few decades, the common view was that a trimeric multiheme c-type HAO converts hydroxylamine into nitrite in the periplasm with production of four electrons (1.2). The stream of four electron are channelled through cytochrome c554 to a membrane-bound cytochrome c552. Two of the electrons are routed back to AMO, where they are used for the oxidation of ammonia (quinol pool). The remaining two electrons are used to generate a proton motive force and reduce NAD(P) through reverse electron transport.

Recent results, however, show that HAO does not produce nitrite as a direct product of catalysis. This enzyme instead produces nitric oxide and three electrons. Nitric oxide can then be oxidized by other enzymes (or oxygen) to nitrite.



Topic-4

Nucleic acid

Chemical Nature of DNA and RNA

The concept of Nucleic acid started during 1869 when Friedrich Miescher isolated a new molecule from the pus cells and called it as Nuclein. Miescher determined that chemically is made up of hydrogen, oxygen, nitrogen and phosphorus. Miescher could also report an unique ratio of phosphorus of nitrogen in the Nuclein. In his further attempt Nuclein was also found in Salmon sperm. In 1889 Richard Altman discovered that Nuclein have acidic properties and hence the name was changed to Nucleic acid. In 1891 Albert Kossel discovered that Nuclein is made up of four bases and sugar molecule for which he got noble prize in medicine. In 1897 Eduard Zachans reported that Nuclein in an integral part of chromosome and his (1897) proposed the concept of chromatin with the chemical substance as Nuclein. Until 1940-1950, Nuclein as considered to be protein, until Avery et al (1944) and Hershey and chase (1952) proved that DNA is the genetic material in *Escherichia coli* (Bacteria) and T2 virus (A Bacteriophage of *E.coli*).

Basic Structure of DNA and RNA

The basic structure of DNA and the RNA is the same and has the following structural configuration.

- Thread likes in structure
- Made up of long chain of Polynucleotide.
- Each nucleotide consists of a nitrogen containing aromatic base attached to a pentose (five carbon) sugar, which in turn attached to a phosphate group.
- Each Nucleic acid contains four of five nitrogen base such as:
 1. Adenine (A), Guanine (G), Cytosine (C) and Thymine (T) – in DNA.
 2. Adenine (A), Guanine (G), Cytosine (C) and Uracil (U) – in RNA.
- A and G are categorized as purines and C, T and U are collectively categorized as Pyrimidines.
- A, T, C, G are common for DNA A, U, C, G makes the RNA.
- The Pentose sugar of DNA differs from pentose sugar (Ribose) by the absence of a hydroxyl group (–OH) from carbon position two and hence the name deoxyribose.
- The Phosphate group connects to the sugar group by 5` – hydroxyl group (known as 5` prime end) and the 3` – hydroxyl group (known as 3` prime end), by two ester bonds called as phospho-di-ester bond. This phospho-di-ester bond is common for both DNA and RNA.

Chemical Nature and Types of Ribonucleic Acid (RNA)

The nucleic acid in the cell has two important functions to perform. DNA is responsible for inheritance and its transfer to next generation; RNA is concerned with carrying out metabolic function. DNA can remain present in the cell, since birth. RNA has to be synthesized by DNA to carry out metabolic function. A sustainable life, appears to be interaction of DNA and RNA.

It is often postulated that life began as RNA molecule, concept becomes evident as some of the viruses (Influenza virus, foot and mouth virus, Rous sarcoma virus, Reovirus and Bacteriophage, Tobacco mosaic virus) contain RNA as genetic material.

Thus the RNA can be

1. Genetic
 2. Non-Genetic
1. The Genetic RNA

The basic structure of RNA

Every RNA has two aspect of structure and function. The structural aspect is concerned with its unique assemblage of molecules to construct it, while the functional aspect deals with the modification of structure to perform an assigned job.

The RNA is a single stranded nucleic acid made up of four nucleotides; A, C, G and U joined together with a back alternating sequence of Phosphate and ribose sugar. It shows resemblance to DNA molecule so far as union of the three molecules is concerned. The Pentose sugar of RNA is Ribose sugar and the nitrogenous base Thymine of DNA is replaced by Uracil. It appears that functional DNA acquires the structure of RNA when functional aspect has to deal in the cell. By doing so the DNA (heritable material of cell) conserves itself. RNA is concerned with performing various metabolic functions of the cells, and hence acquires various forms and shapes to encounter various types of enzymes (acting as catalytic agent).

Types of RNA

The Non-genetic role of RNA are many, and the role with RNA has to play originates from the DNA. So, RNA is made by DNA by a process called as Transcription. The three RNA formed are

- a. Messenger RNA (m RNA)
- b. Ribosomal RNA (r RNA)
- c. Transfer RNA (t RNA)

In order to remain functional, the cell, with the interaction of DNA and RNA has to perform many metabolic function for growth and development. Various kinds of proteins has to be made to perform this function. The DNA transfers knowledge for synthesis of proteins

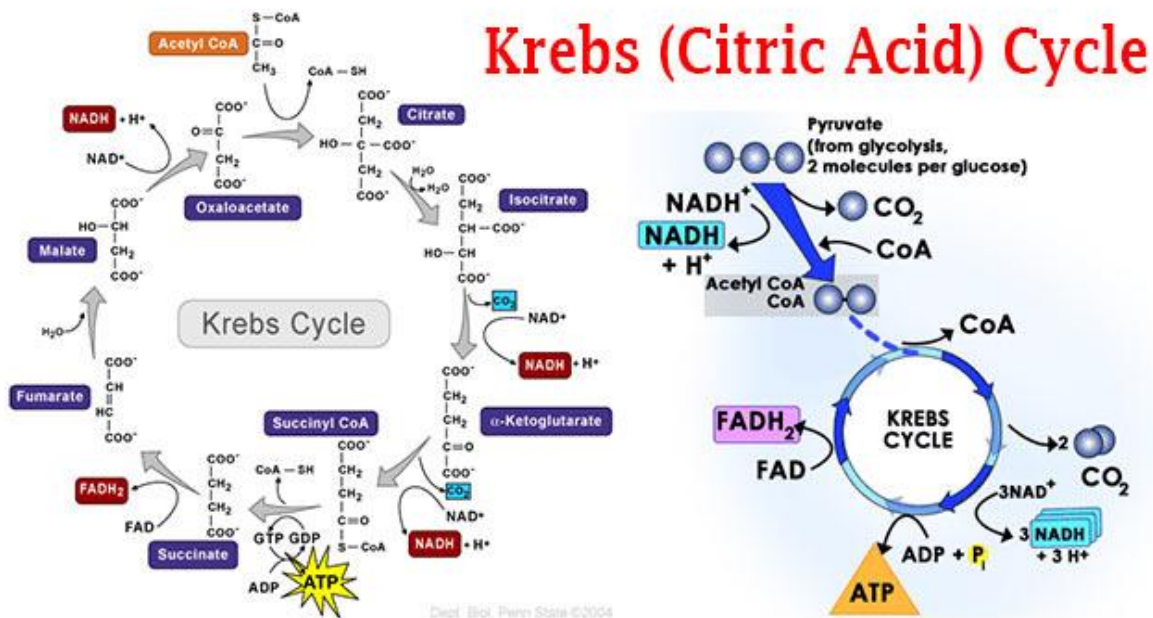
and the three RNA molecules helps in effective performance of this activity. The three RNA molecules are assigned with three separate jobs, such as:

- The mRNA carries the information from the DNA.
- The tRNA is concerned with supply of amino acids and
- The rRNA serves as a factory for alignment of different amino acids for the synthesis of a specific protein.

Topic-5

Krebs cycle

Most organisms use glucose as a major fuel source, but must break down this glucose and store the energy in ATP and other molecules. The Krebs cycle is contained within mitochondria. Within the mitochondrial matrix, the reactions of the Krebs cycle add electrons and protons to a number of electron carriers, which are then used by the electron transport chain to produce ATP. The Krebs cycle starts with the products of glycolysis, which are two three-carbon molecules known as pyruvate. This molecule is acidic, which is why the Krebs cycle is also called the tricarboxylic acid cycle (TCA). Throughout a number of reactions, these molecules are further broken down into carbon dioxide. Energy from the molecules is moved to other molecules, called electron carriers. These molecules carry the stored energy to the electron transport chain, which in turn creates ATP. Then, the cell uses this ATP to power various cellular reactions, such as the activation of enzymes or transport proteins. The Krebs cycle is the second of 4 different processes which must happen to extract the energy from glucose. Altogether, the Krebs cycle consists of 9 sequential reactions.



Topic-6

Nitrogen fixing Bacteria

Nitrogen is an important macronutrient because it is part of nucleic acids and proteins. Atmospheric nitrogen, which is the diatomic molecule N_2 , or dinitrogen, is the largest pool of nitrogen in terrestrial ecosystems. However, plants cannot take advantage of this nitrogen because they do not have the necessary enzymes to convert it into biologically useful forms. However, nitrogen can be “fixed.” It can be converted to ammonia (NH_3) through biological, physical, or chemical processes. Biological nitrogen fixation (BNF), the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3), is exclusively carried out by prokaryotes, such as soil bacteria or cyanobacteria. Biological processes contribute 65 percent of the nitrogen used in agriculture.

Nitrogen-fixing bacteria, microorganisms capable of transforming atmospheric nitrogen into fixed nitrogen (inorganic compounds usable by plants). More than 90 percent of all nitrogen fixation is affected by these organisms, which thus play an important role in the nitrogen cycle.

Two kinds of nitrogen-fixing bacteria are recognized. The first kind, the free-living (nonsymbiotic) bacteria, includes the cyanobacteria (or blue-green algae) *Anabaena* and *Nostoc* and genera such as *Azotobacter*, *Beijerinckia*, and *Clostridium*. The second kind comprises the mutualistic (symbiotic) bacteria; examples include *Rhizobium*, associated with leguminous plants (e.g., various members of the pea family); *Frankia*, associated with certain dicotyledonous species (actinorhizal plants); and certain *Azospirillum* species, associated with cereal grasses.

The symbiotic nitrogen-fixing bacteria invade the root hairs of host plants, where they multiply and stimulate formation of root nodules, enlargements of plant cells and bacteria in intimate association. Within the nodules the bacteria convert free nitrogen to ammonia, which the host plant utilizes for its development. To ensure sufficient nodule formation and optimum growth of legumes (e.g., alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium.

Nitrogen Fixing Bacteria that Live on Plant Roots

These bacteria form a symbiotic relationship with only legume plants, like soybeans, green beans, clover, and alfalfa. They form nodules on the root system.

Inside the nodule is an oxygen free zone where the nitrogenase enzyme reduces atmospheric nitrogen to nitrate and ammonia. The bacteria and the plant have a mutually beneficial relationship. The bacteria take certain food sources from the plant and in return they provide nitrogen to the plant.

Nitrogen Fixing Bacteria that Live in the Soil

Another important type of bacteria that has the ability to provide nitrogen to plants is Cyanobacteria. Cyanobacteria are beneficial bacteria in the soil that are free living. Cyanobacteria do not form nodules on plant roots. Instead, they work within the soil.

Nostoc commune is one type of cyanobacteria that can take nitrogen from the atmosphere and soil and transform it into nitrogen that plants can use. These cyanobacteria grow as chains of cells. On the chain, some of the microscopic cells will form what are called heterocysts. Inside the heterocyst is an oxygen free zone. The heterocyst is the place where the nitrogenase enzyme reduces atmospheric nitrogen to plant available ammonia. This ammonia is released into the soil chemistry and is then absorbed by plants.

Nitrogen Fixation In Nature

Nitrogen is fixed, or combined, in nature as nitric oxide by lightning and ultraviolet rays, but more significant amounts of nitrogen are fixed as ammonia, nitrites, and nitrates by soil microorganisms. More than 90 percent of all nitrogen fixation is effected by them. Two kinds of nitrogen-fixing microorganisms are recognized: free-living (nonsymbiotic) bacteria, including the cyanobacteria (or blue-green algae) Anabaena and Nostoc and genera such as Azotobacter, Beijerinckia, and Clostridium; and mutualistic (symbiotic) bacteria such as Rhizobium, associated with leguminous plants, and various Azospirillum species, associated with cereal grasses.

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