Unit 2:

Biological Interactions

With the development of microbial communities, the demand for nutrients and space also increases. As a result, there has been a development of different strategies to enable microorganisms to persist in an environment. Cell–cell interactions may produce cooperative effects where one or more individuals benefit, or competition between the cells may occur with an adverse effect on one or more species in the environment. The nature and magnitude of interaction will depend on the types of microorganism present as well as the abundance of the microorganisms and types of sensory systems of the individual organisms.

Classification of microbial interactions

In addressing microbe–microbe interactions, it is important to determine whether the interaction is between cells of different genera or within the same species.

Various types of interaction of a microorganism with another microorganism and specific examples of the processes associated with microbe–microbe interaction are presented in table 1 and 2.

Table 1: Types of Interaction between Microorganisms and Hosts

Example	Characteristic	Species A	Species B
Neutralism	No interaction	Not affected	Not affected
Mutualism and symbiosis	Interaction needed to survive in the habitat, and specific species are required	Benefits	Benefits
Protocooperation	Interaction needed to survive in the habitat, but specific species are not required	Benefits	Benefits
Synergism (syntrophism)	Growth of one is improved by another	Benefits	Benefits
Commensalism	One benefits and the other is not harmed or helped	Benefits	Not affect
Competition	Organism in the environment attempts to acquire limiting nutrient	Harmed	Harmed
Parasitism and predation	Host is usually compromised	Benefits	Harmed
Amensalism (antagonism)	Products of one impact another	No effect or benefits	Harmed

Microorganisms can be physically associated with other organisms in a number of ways

- Ectosymbiosis-microorganism remains outside the other organism
- Endosymbiosis-microorganism is found within the other organism
- Ecto/endosymbiosis-microorganism lives both on the inside and the outside of the other organism
- Physical associations can be intermittent and cyclic or permanent

Table 2: Examples of Microbial Interactions

Type	Example
Mutualism and symbiosis	Lichens, mycorhizae, root nodules;[a] Microorganisms associated with sponges, jellyfish, sea anemones, and corals;[b] Bacteria associated with insects and aphids[b]
Protocooperation	
Synergism (syntrophism)	Interspecies hydrogen transfer—specific species required; cross-feeding of acetate between bacterial species
Commensalism	Nitrification with *Nitrosomonas* oxidizing ammonia to nitrite and *Nitrobacter* oxidizing nitrite to nitrate
Competition	Soil bacteria compete with fungi for soluble nutrients
Parasitism and predation	*Bdellovibrio* sp. and BALO require Gram-negative host for growth; cells of myxobacteria move as wolfpacks and digest other bacteria; protozoa engulf bacteria for nutrients
Amensalism (antagonism)	End products of metabolism inhibit other bacteria; production of antibiotics and bacteriocins; production of viruses active against bacteria and other microorganisms

a – plant microbe interaction; b - animal microbe interaction

Neutralism

Neutralism occurs when microorganisms have no effect on each other despite their growth in fairly close contact. It is perhaps possible for neutralism to occur in natural communities if the culture density is low, the nutrient level is high, and each culture has distinct requirements for growth. It has been suggested that neutralism may occur in early colonization of an environment without either harmful or beneficial interactions by the microorganisms introduced.

Microbe-Microbe Interactions

1. Positive interaction: Beneficial Interactions

Some interactions provide benefits to the different partners.
(1) Mutualism (2) Protocooperation and (3) Commensalism

2. Negative interaction: Conflictual Interactions

(1) Predation (2) Parasitism and (3) Amensalism (4) Competition

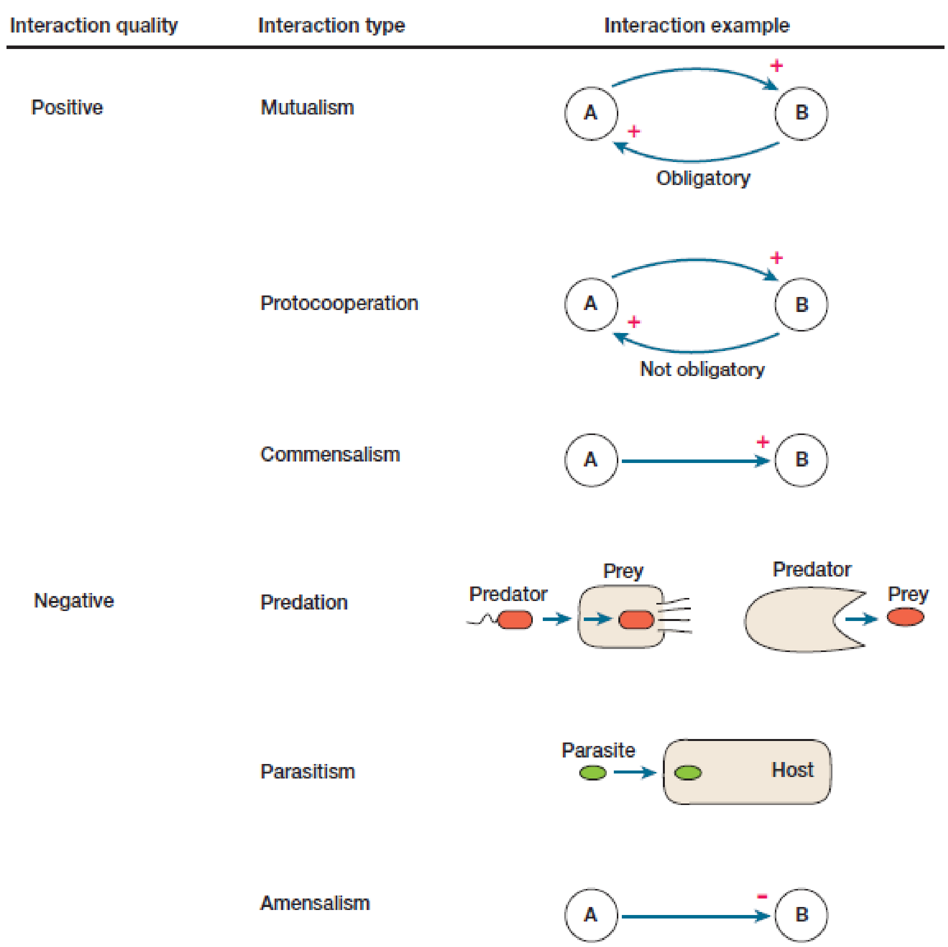


Figure: Microbial Interactions: Basic characteristics of positive (+) and negative (-) interactions that can occur between different organisms

Symbiotic associations

The term symbiosis has been used to characterize various situations where different species are found living together. In the broadest definition, symbiosis has been used to describe biological interactions known as mutualism, commensalisms, and parasitism.

Obligate versus facultative

Relationships can be obligate, meaning that one or both of the symbionts entirely depend on each other for survival. They cannot survive without each other.

Relationship may be facultative, meaning that one or both of the symbionts are not entirely depend on each other and they may live independently.

1. Positive interaction: Beneficial Interactions

Mutualism

Mutualism defines as an obligatory association that provides some reciprocal benefit to both partners. This is an obligatory relationship in which the mutualist and the host are metabolically dependent on each other.

Lichen is an excellent example of microbe-microbe mutualism interaction. Lichen is the association between specific ascomycetes (the fungus) and certain genera of either green algae or cyanobacteria. In a lichen, the fungal partner is termed the mycobiont. Algal photobionts are called phycobionts and cyanobacteria photobionts are called cyanobionts.

The fungi benefit for the carbohydrates produced by the algae or cyanobacteria via photosynthesis. The fungus obtains nutrients from its partner by haustoria (projections of fungal hyphae) that penetrate the phycobiont cell wall. It also uses the O_2 produced during phycobiont photophosphorylation in carrying out respiration.

The fungus provides space for algae or cyanobacteria by creating a firm substratum within which the phycobiont can grow. It also protects the phycobiont from excess light intensities and other environmental stress.

Protocooperation (Synergism)

Protocooperation is a mutually beneficial relationship, similar to that which occurs in mutualism, but in protocooperation, this relationship is not obligatory. The beneficial complementary resources are provided by each of the paired microorganisms. The organisms involved in this type of relationship can be separated, and if the resources provided by the complementary microorganism are supplied in the growth environment, each microorganism will function independently.

An example of this type of relationship is the association of Cellulomonas and Azotobacter. Azotobacter uses glucose provided by a cellulose-degrading microorganism such as Cellulomonas, which uses the nitrogen fixed by Azotobacter.

Another example of this type of relationship is the association of Desulfovibrio and Chromatium, in which the carbon and sulfur cycles are linked. The organic matter (OM) and sulfate required by Desulfovibrio are produced by the Chromatium while reduction of CO_2 to organic matter and oxidation of sulfide to sulfate required to Chromatium carried out by Desulfovibrio.

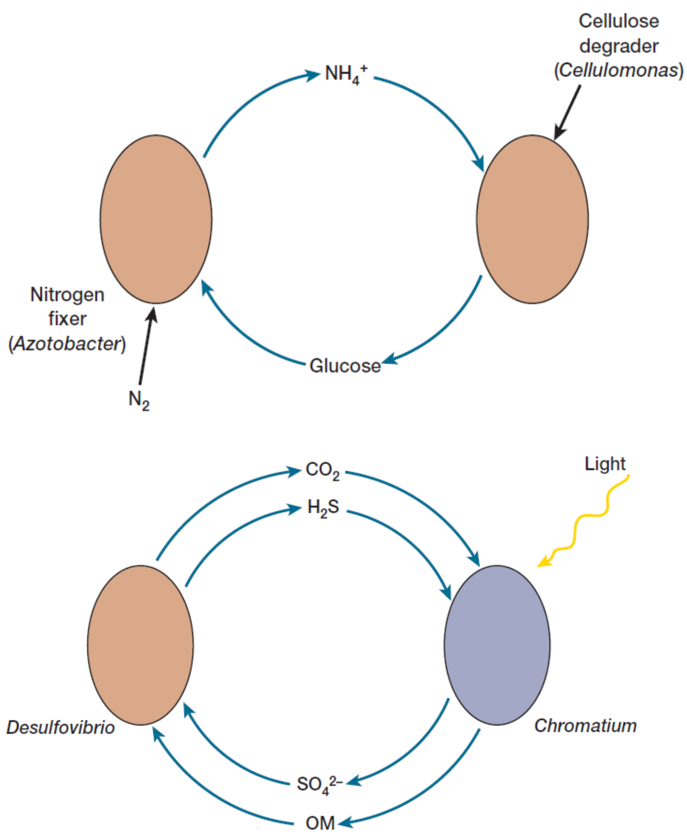


Fig. 2: Examples of Protocooperative Symbiotic Processes

Syntrophism is a protocooperative symbiotic process in which phototrophic and chemotrophic bacteria not only exchange metabolites but also interact at the level of growth coordination.

Commensalism

The commensalistic relationship involves two microorganisms where one partner (the commensal) benefits while the other species (the host) is not harmed or helped.

There are several situations under which commensalisms may occur between microorganisms

(1) Commensalistic relationships between microorganisms include situations in which the waste product of one microorganism is the substrate for another species.

An example is nitrification, the oxidation of ammonium ion to nitrite by microorganisms such as *Nitrosomonas*, and the subsequent oxidation of the nitrite to nitrate by *Nitrobacter* and similar bacteria. *Nitrobacter* benefits from its association with *Nitrosomonas* because it uses nitrite to obtain energy for growth.
(2) Commensalistic associations also occur when one microbial group modifies the environment to make it more suited for another organism.
For example, in the intestine the common, nonpathogenic strain of *Escherichia coli* lives in the human colon, but also grows quite well outside the host, and thus is a typical commensal. When oxygen is used up by the facultatively anaerobic *E. coli*, obligate anaerobes such as *Bacteroides* are able to grow in the colon.
(3) One species releasing vitamins, amino acids and other growth factors that are needed by a second species.

2. Negative interaction: Conflictual Interactions

Predation

Predation is a widespread phenomenon where the predator engulfs or attacks the prey. In the world of eukaryotes, it is common that the larger animal eats the smaller one; however, with microorganisms the predator may be larger or smaller than the predator, and this normally results in the death of the prey.
Several of the best examples are *Bdellovibrio*, *Vampirococcus*, and *Daptobacter*.

Each of these has a unique mode of attack against a susceptible bacterium.
(1) epibiotic predator with growth on the surface of the prey. Ex. – *Vampirococcus*
(2) periplasmic predator, with growth in between the inner and outer membranes of bacteria. Ex. *Bdellovibrio*
(3) cytoplasmic predator, with growth in the cytoplasm of the prey. Ex. *Daptobacter*

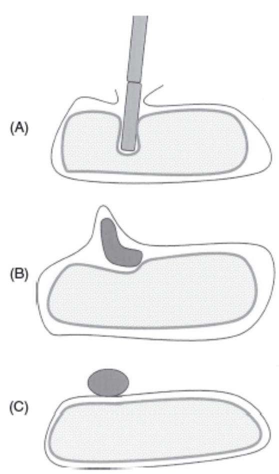


Figure: Cell associations: bacteria as predators on other bacteria. Bacterial parasites may be found growing (A) in the cytoplasm, (B) in the periplasm, or (C) on the surface of a bacterial host.

Parasitism

Parasitism occurs when one species obtains nutrients from another for the purpose of cell growth. Parasites display two types: (1) direct lifecycle that does not require an intermediate host and (2) indirect lifecycle that requires an intermediate host.

In parasitism, one organism (parasite) benefits from another (host); there is a degree of coexistence between the host and parasite that can shift to a pathogenic relationship (a type of predation).

The host may be microbes, plants or animals.

Microbe-microbe parasitism

(1) Mycoparasitism (Fungus-Fungus Interaction)

When one fungus is parasitized by the other fungus, this phenomenon is called mycoparasitism. The parasitizing fungus is called hyper parasite and the parasitized fungus as hypoparasite.

Barnett and Binder (1973) divided mycoparasitism into (i) necrotrophic parasitism, in which the relationships result in death of the host thallus, and (ii) biotrophic parasitism, in which the development of the parasite is favored by a living rather than a dead host structure. The antagonistic activity of necrotrophic mycoparasites is attributed to the production of antibiotics, toxins, and hydrolytic enzymes. It is used as biocontrol agent. Ex. - The fungal genus, *Trichoderma* produces enzymes such as chitinases which degrade the cell walls of other fungi

(2) Mycophagy

Mycophagy or Fungivory is the process of organisms consuming fungi.
Bacterial mycophagy - mechanisms by which bacteria feed on fungi. Ex.- Bacteria *Aeromonas caviae* feed on fungus *Rhizoctonia solani* and *Fusarium oxysporum*.
Many amoebae are also known to feed on pathogenic fungi. The antagonistic soil amoebae are *Arachnula*, *Gephyramoeba*, *Geococcus*, *Saccamoeba*, *Vampyrella* etc.

(3) Bacterivores

Bacterivores are free-living, generally heterotrophic organisms, exclusively microscopic, which obtain energy and nutrients primarily or entirely from the consumption of bacteria. Many species of amoeba are bacterivores, as well as other types of protozoans. i.e. *Vorticella*

(4) Bacteriophage

A bacteriophage, also known as a phage, is a virus that infects and replicates within Bacteria and Archaea. i.e. ds DNA phases (T4 – phase, lambda phase), ssDNA phase (M13 phase, ΦX174).

Amensalism

Amensalism describes the negative effect that one organism has on another organism. This is a unidirectional process based on the release of a specific compound by one organism which has a negative effect on another organism.
A classic example of amensalism is the production of antibiotics that can inhibit or kill a susceptible microorganism. Ex. - the destructive effect of the bread mold *Penicillium* on certain bacteria by the secretion of penicillin.

Competition

When two or more species use the same nutrients for growth, some of the populations will be compromised. Competition between microbial species may be attributed to availability of nitrogen source, carbon source, electron donors and acceptors, vitamins, light, and water. Microbes also compete with their neighbors for space and resources. Competition for a limiting nutrient among microorganisms leads to exclusion of slower growing population.
For ex. - during decomposition of organic matter the increase in number and activity of microorganisms put heavy demand on limited supply of oxygen, nutrients, space, etc. The microbes with weak saprophytic survival ability are unable to compete with other soil saprophytes for these requirements.

Plant –microbe Interactions

Earth is richly populated with plants, and many different types of microorganisms grow in close association with them. Although, many microbial activities are detrimental to plant growth, however, microorganisms are also beneficial to plants.

Root-microbe interaction

Plant roots have a lot of extensions from the central root, and bacteria may become localized on the root surface. In general, plants benefit from two types of microbe–plant associations: (1) highly specialized interaction, where there is considerable specificity found in mutalistic activities; and (2) commensalism resulting from nutrient secretion from plants when bacteria and fungi grow in close proximity to the roots.
The three best characterized symbiotic systems are the fungus–root system (Mycorrhizae), bacterium–root nodule system and cyanobacteria-root system.

Mycorrhizae

Mycorrhizae are fungus-root associations, first discovered by Albert Bernhard Frank in 1885. The term “mycorrhizae” comes from the Greek words meaning fungus and roots. These microorganisms contribute to plant functioning in natural environments, agriculture, and reclamation. The roots of about 95% of all kinds of vascular plants are normally involved in symbiotic associations with mycorrhizae.

Symbiosis begins when fungal spores germinate and emerging threadlike structures, called hyphae, enter the epidermis of plant roots. After colonization of the root, the fungus sends out a vast network of hyphae throughout the soil to form a greatly enhanced absorptive surface area. This results in improved nutrient acquisition and uptake by plant roots, particularly elemental phosphorus (P), zinc (Zn), manganese (Mn) and copper (Cu) and water. In return, the plant provides carbohydrates for the fungi.

Rhizospheric interactions of terrestrial fungi are influenced by the number of microorganisms, type of microorganisms, and specific plant root exudates. Mycorrhiza is mainly of two types, endomycorrhizae and ectotrophic mycorrhizae. Growth on the exterior of the root is characteristic of endomycorrhizae or ectotrophic mycorrhizae, while growth inside the root is attributed to the endomycorrhizae or endotrophic mycorrhizae.

(1) Ectomycorrhizae

Ectomycorrhizae are commonly found on tree roots of gymnosperms or woody angiosperms, and fungal partners include members of the nonseptate fungi. Majority of the fungi belong to Agaricales of Hymenomycetes under Basidiomycotina. Ectomycorrhiza is commonly called "sheathing mycorrhiza". The fungus enters the cortex, grows between the plant cells and forming 'Hartig net', but never goes inside the endodermis or stele. They form a mantle of varying thickness. Such ectomycorrhizae, including *Cennococcum*, *Pisolithus* and *Amanita*, form irregular structures that are easy to recognize.

The fungal hyphae extend into the soil, where they sequester phosphorus, nitrogen, sulfur, calcium, zinc, iron, and numerous minerals for fungal growth. When the plant becomes mineral-deficient or when the fungal cells die, the appropriate ion will be passed on to the roots.

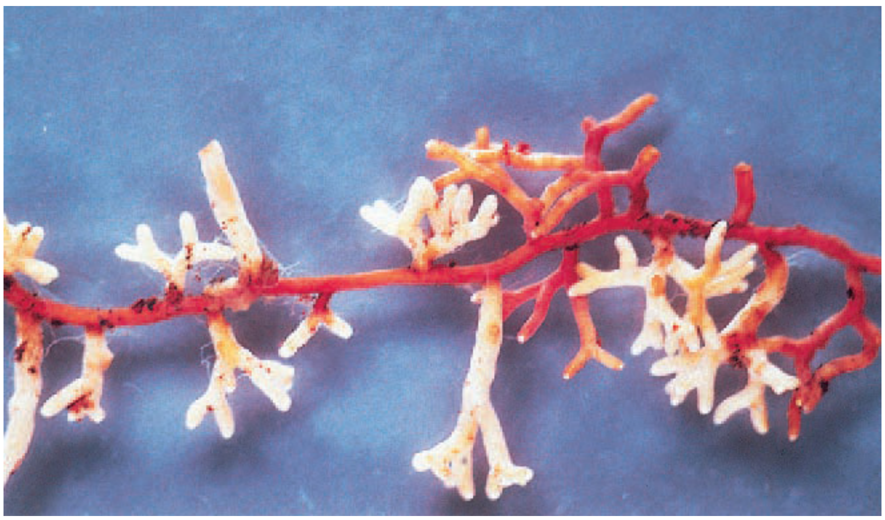
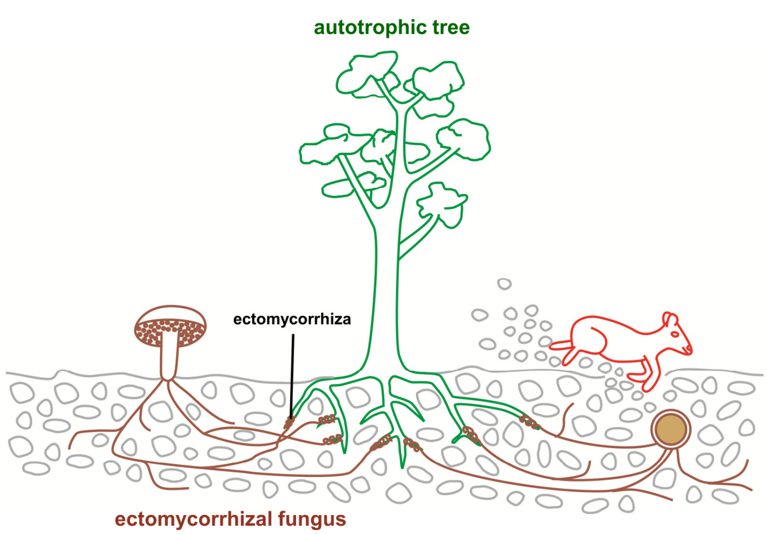


Fig. Ectomycorrhizae as Found on Roots of a Pine Tree



(2) Endomycorrhizae

In this association the fungal hyphae penetrate the outer cortical cells of the plant root, where they grow intracellularly and form coils, swellings, or minute branches. The fungi penetrate the root with growth between epidermal cells or through root hairs and invade cortex cells. Endomycorrhizae are found on most herbaceous plants, including lower vascular plants such as ferns and bryophytes.

Vesicular-arbuscular mycorrhizae (VAM) is a type of endomycorrhizal association, where both vesicles and arbuscles are developed together. VAM is by far the commonest of all mycorrhizae and has been reported in more than 90% of land plants.

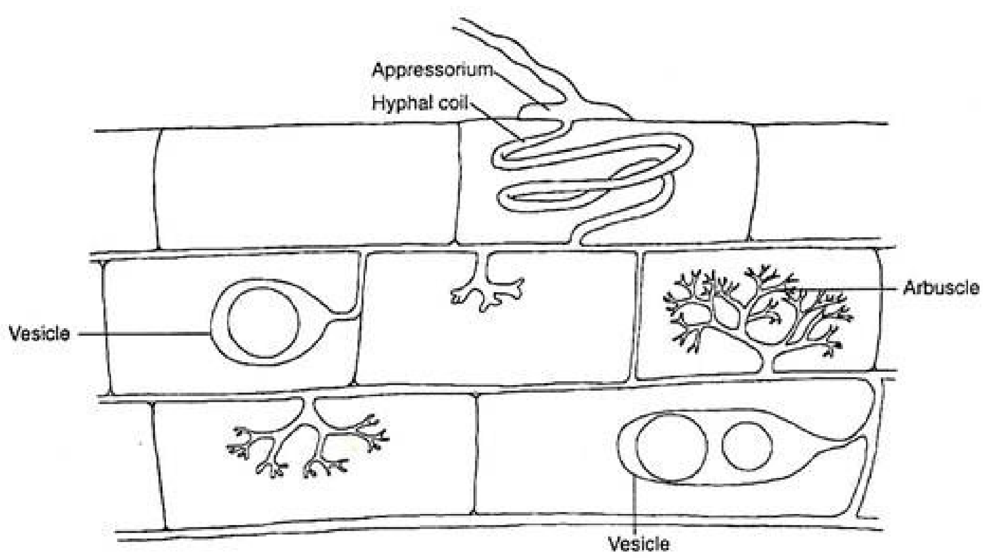


Fig. Endomycorrhizae of the vesicular–arbuscular type

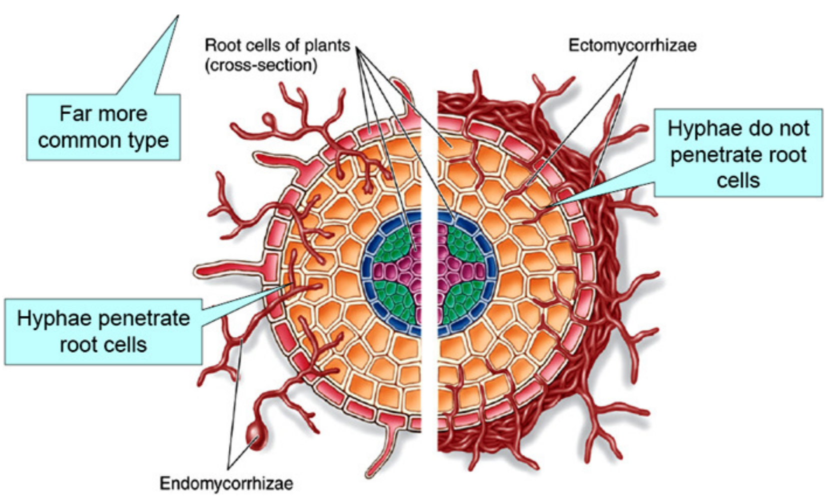


Fig. Comparison of endo and ectomycorrhiza

Role of Mycorrhizae in Agriculture and Foresty:

- The mycorrhizal association helps in the formation of dichotomous bran-ching and profuse root growth, thus enhances plant growth.

- Ectotrophic mycorrhiza helps in uptake of mineral ions and also acts as reservoir. They also help in absorption of nutrients. In nutrient deficient soil, the mycelial association helps in the absorption of N, Ca, P, Zn, Fe, Na and others.

- Mycorrhizal association is obligatory for the germination of orchid seeds. Mycorrhizal growth in orchids (Rhizoctonia repens with Orchis militaris tuber tissues) causes the synthesis of phytoalexins — orchinol and hirsinol. Both the compounds act as a barrier to protect infection by other pathogens.

- Inoculation of VAM as biofertiliser provides a distinct possibility for the uptake of P in phosphorus-deficient soil.

- Mycorrhiza plays an important role to establish forest in unfavourable location, barren land, waste lands etc.

- The application of mycorrhizal fungi in forest bed enhances the formation of mycorrhizal association that prevents the entry of fungal root pathogens. This method is very much effective in the root of *Pinus clausa* against *Phytophthora cinnamoni* infection.

- Mycorrhiza mixed nitrogenous compounds such as nitrate; ammonia etc. is available to the plants. Thus it helps in plant growth, especially in acid soil.

Bacterium–root nodule system

Cyanobacteria-root system (Coralloid Root)

Details in biological nitrogen fixation

Mutualism relationship of aerial plant surfaces with microorganism

Phyllosphere Microorganisms

A wide variety of microorganisms are found on and in the aerial surfaces of plants, called the phyllosphere. These include microorganisms that have interactions with leaf and sometimes stem at various stages of development. The plant leaves and stems release organic compounds, and this can lead to massive development of microbes. Some important phyllosphere bacteria are *Sphingomonas*, *Pseudomonas*, and *Methylobacterium*. The genera present on plant leaves and stems include *Sphingomonas*, which is especially equipped to survive with the high levels of UV irradiation occurring on these plant surfaces. Phyllosphere microorganisms play important roles in protection and possibly harm to the plant.

Biological Nitrogen Fixation

The conversion of atmospheric nitrogen into the nitrogenous compounds through the agency of living organisms is called biological nitrogen fixation. The process is carried out by two main types of microorganism: those which live in close symbiotic association with other plants and those which are "free living" or non-symbiotic.

Biological nitrogen fixation (BNF) is the process whereby atmospheric nitrogen is reduced to ammonia in the presence of 'Nitrogenase'. Nitrogenase is a biological catalyst found naturally only in certain microorganisms such as the symbiotic *Rhizobium* and *Frankia*, or the free-living *Azospirillum* and *Azotobacter* and blue green algae (BGA).

Nitrogen Fixers

Among the earth's organisms, only some prokaryotes like bacteria and cyanobacteria can fix atmosphere nitrogen. They are called nitrogen fixers or diazotrophs. Diazotrophs may be symbiotic or asymbiotic (free living) such as given below:

(1) Symbiotic Nitrogen Fixing Bacteria

Rhizobium is aerobic, gram negative nitrogen fixing bacterial symbionts of leguminous roots. *Sesbania rostrata* has *Rhizobium* in root nodules and *Aerorhizobium* in stem nodules. *Frankia* is symbiont in root nodules of many non-leguminous plants like *Casuarina* and *Alnus*. *Parasponia*, a tropical genus in the Cannabaceae (Non legume) also able to interact with rhizobia and form nitrogen-fixing nodules. Several species of *Rhizobium* live in the soil but are unable to fix nitrogen by themselves. They do so only as symbionts in the association of roots of legumes.

Several other genera are also able to form nitrogen-fixing nodules with legumes. These include *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Sinorhizobium*.
Nodule formation

It involves multiple interactions between free-living soil *Rizobium* and roots of the host plant. The roots of young leguminous plants secrete a group of chemical attractants like flavonoids and betaines. In response to these chemical attractants specific rhizobial Tells migrate towards the root hairs and produce nod (nodulation) factors. The nod factors found on bacterial surface bind to the lectin proteins present on the surface of root hairs. This lectinnod factor interaction induces growth and curling of root hairs around *Rhizobia*. At these regions wall degrades in response to node-factors and Rhizobia enter the root hair invagination of plasma membrane called infection thread. The infection thread filled with dividing Rhizobia elongate through the root hair and later branched to reach different cortical cells. The infected cortical cells divide to form nodule

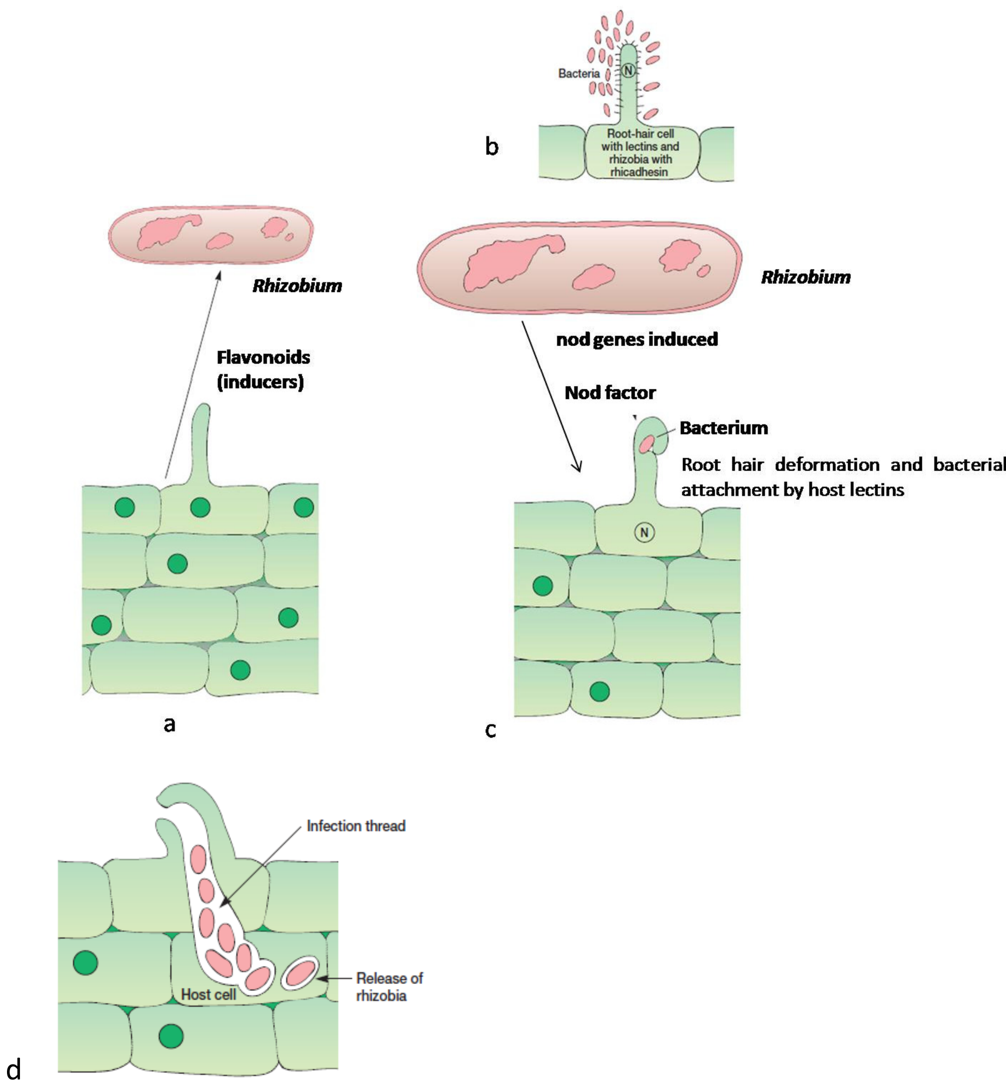


Fig. Root Nodule Formation by Rhizobium. (a) The plant root releases flavonoids that stimulate the production of various Nod metabolites by *Rhizobium*. There are many different Nod factors that control infection specificity. (b) Attachment of *Rhizobium* to root hairs involves specific bacterial proteins called host plant lectins that affect the pattern of attachment and nod gene expression. (c & d) Nod factor that promotes root hair curling and plant cortical cell division.

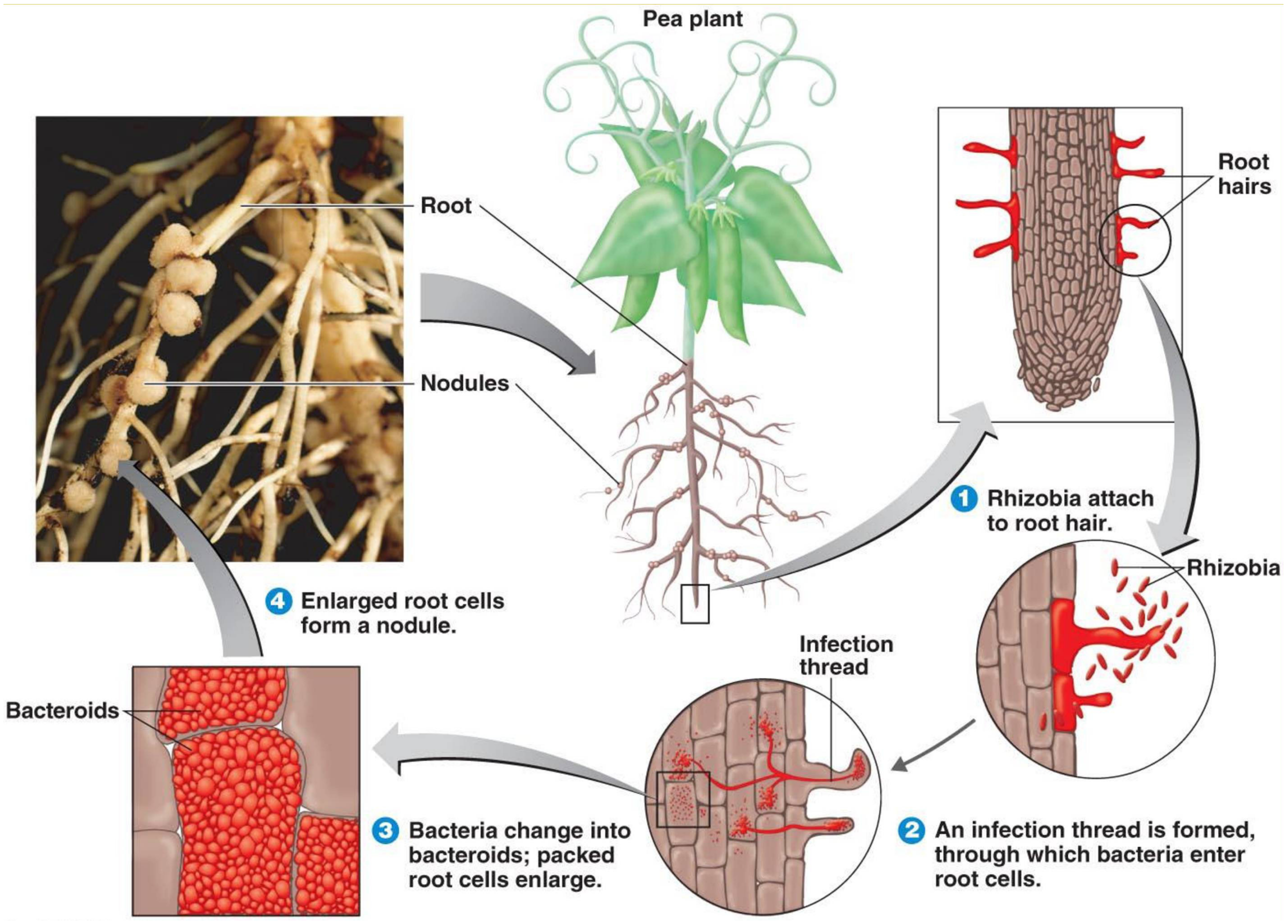


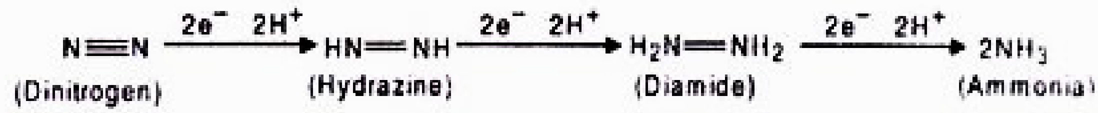
Fig. Root Nodule Formation by *Rhizobium*

Mechanism of nitrogen fixation

The nodule serves as site for N_2 fixation. It contains all the necessary bio-chemicals such as the enzyme complex called nitrogenase and leghaemoglobin (leguminous haemoglobin). The nitrogenase has 2 components i.e. Mo-Fe protein (molybdoferredoxin) and Fe-protein (azoferredoxin). The nitrogenase catalyzes the conversion of atmosphere di-nitrogen (N_2) to $2NH_3$. The ammonia is the first stable product of nitrogen fixation.

$$N_2 + 8H^+ + 8e^- + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

During nitrogen fixation, the free di-nitrogen first bound to MoFe protein and is not released until completely reduced to ammonia. The reduction of di-nitrogen is a stepwise reaction in which many intermediates are formed to form ammonia (NH_3) which is protonated at physiological pH to form NH4+. In this process ferredoxin serves as an electron donor to Fe-protein (nitrogenase reductase) which in turn hydrolyzes ATP and reduce MoFe protein, the MoFe protein in Turn reduce the substrate N_2. The electrons and ATP are provided by photosynthesis and respiration of the host cells.



The intermediates of N_2 fixation.

The ammonia produced by nitrogenase is further used in synthesis of amino acid by assimilation of ammonia and again in nitrate assimilation (Nitrogen cycle).

(2) Free Living Nitrogen Fixing Bacteria

Azotobacter, *Beijerinckia* (both aerobic) and *Clostridium* (anaerobic) are saprophytic bacteria that perform nitrogen fixation. *Desulphovibrio* is chemotrophic nitrogen fixing bacterium. *Rhodopseudomonas*, *Rhodospirillum* and *Chromatium* are nitrogen fixing photoautotrophic free living bacteria.

(3) Free living Nitrogen Fixing Cyanobacteria

Many free living blue-green algae (also called cyanobacteria) perform nitrogen fixation, e.g., *Anabaena*, *Nastoc*, *Aulosira*, *Cylmdrospermum* etc. These are also important ecologically as they live in water-logged sods where denitrifing bacteria can be active. *Aulosira fertilissima* is the most active nitrogen fixer in Rice fields, while *Cylindrospermum* is active in sugarcane and maize fields.

(4) Symbiotic Nitrogen Fixing Cyanobacteria

Anabaena and *Nostoc* species are common symbionts in lichens, *Anthoceros*, *Azolla* and cycad roots. *Azolla pinnata* (a water fern) has *Anabaena azollae* in its fronds. It is often inoculated to Rice fields for nitrogen fixation.

Animal-Microbe Interactions

There are many kinds of microorganisms that interact with different groups of animals and develop a variety of mutualism relationships.

Ruminant Symbiosis: Role of Microbes in Ruminants

The herbivorous mammals (e.g. catties, sheep, goats, camels, etc) are known as ruminants because they have a special region of gut which is called rumen. These animals use plant cellulose as the source of carbohydrate which is not digested in normal gut. The cellulosic material is digested in rumen which acts as incubation chamber teeming with protozoa and bacteria. In some animals like cow, the size of rumen is very large.

Some of anaerobic cellulose-digesting bacteria (e.g. *Bacteroides succinogens*, *Ruminococcus flavofaciens*, *R. albus* and *Botryovibrio fibrisolvens*) develop mutualistic symbiosis, and hydrolyse cellulose and other complex polysaccharides to simpler forms which in turn are fermented to fatty acids (.g. acetic acid, propionic acid, butyric acid) and gases (methane and carbon dioxide).

Some of the bacteria are capable of digesting proteins, lipids and starch as well. Lignin fraction of plant remains undigested.

The rumen bacteria ferment proteins and lipids and produce hydroggen and carbon dioxides gase, which in turn is converted into methane by *Methanobacterium ruminantium*. The bacteria of rumen multiply into a large population. However, most of them are passed into stomach along with undigested material where they are killed by proteases and other enzymes. The fatty acids in rumen are absorbed and gases are passed out.

Nematophagus fungi

Nematophagous fungi are carnivorous fungi which depend on nematodes. The nematophagous fungi are of three main types on the basis of ecological habit: 1. Nematode-Trapping Fungi 2. Endoparasitic Fungi and 3. Egg Parasites.

1. Nematode-Trapping Fungi

Fungi capturing nematodes are called nematode-trapping fungi. Such fungi have evolved structural adaptations to trap or penetrate their prey. They may be predatory or endoparasites.

2. Endoparasitic Fungi

Unlike nematode-trapping fungi, the endoparasitic fungi do not extensively produce mycelium external to nematode body. But they attack nematodes through many modifications brought about in conidia. The endoparasitic fungi are species of Cephalosporium, Meria, Verticillium, Catenaria, Meristacrum, etc.

3. Egg Parasites

There are a few saprophytic fungi which attack on nematode eggs. When a fungal hypha comes in contact of an egg, a swollen structure at terminal portion develops at the point of contact. It gets attached to the egg where from a narrow infectious tube develops that penetrates the shell of the egg. Examples of egg parasites are Dactyllela oviparasitica and Paecilomyces lilacinus that penetrate root-knot or cyst nematode.

Nematophagus fungi in Biological control

Some species of nematophagous fungi are being investigated for use in biological pest control. *Purpureocillium lilacinum*, for example, infests the plant-parasitic *Meloidogyne incognita*, which attacks the roots of many cultivated plants. *Arthrobotrys dactyloides* shows promise at controlling the cosmopolitan plant-parasitic root-knot nematode *Meloidogyne javanica*.

Luminescent bacteria

Luminous bacteria are the most widely distributed light-emitting organisms with the majority existing in seawater and the remainder living in the terrestrial or freshwater environment. While most species of luminescent bacteria are capable of living free, the majority are found in nature associated in symbiosis with host organisms i.e., fishes, squids, crabs, nematodes, etc

In symbiosis, the bacteria are nourished with readily available food sources for growth, and at the same time the host utilizes the adopted illumination to communicate, to attract prey, and to masquerade itself from predators.

There are three major genera, into which most luminous bacteria are classified; Photobacterium, Vibrio, and Photorhabdus. Species existing in the marine environment are mainly categorized into the Photobacterium and Vibrio genera, and the terrestrial species are classified into the Photorhabdus (previously designated as Xenorhabdus) genus. Species within the Photobacterium genus are generally light organ symbionts of marine animals, whereas the Vibrio species exist as free-living forms as well as symbionts in the sea.

Bacterial luciferase is the enzyme that catalyzes light emission. However, the catalytic machinery involved in continuous light production in luminous bacteria includes not only bacterial luciferase, but also the enzymes that supply and regenerate the substrates of bacterial luciferase. The DNA sequences coding the proteins in the luminescent system are termed the lux genes.

This lux gene is used as reporter gene in many genetic engineering experiments.

Unit 3: Microbial biogeochemical processes of nutrient cycling and biodegradation

Carbon cycle:

Carbon is present in reduced forms, such as methane (CH_4) and organic matter, and in more oxidized forms, such as carbon monoxide (CO) and carbon dioxide (CO_2). Reductants (e.g., hydrogen, which is a strong reductant) and oxidants (e.g., O_2) influence the course of biological and chemical reactions involving carbon.

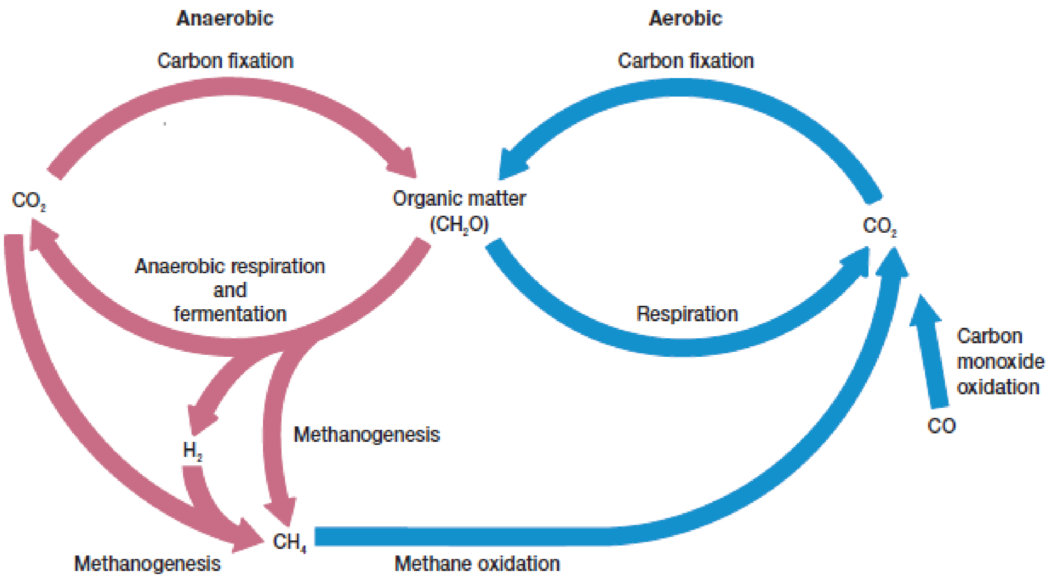


Figure 28.19 The Basic Carbon Cycle in the Environment. Carbon fixation can occur through the activities of photoautotrophic and chemoautotrophic microorganisms. Methane can be produced from inorganic substrates ($CO_2 + H_2$) or from organic matter. Carbon monoxide (CO)—produced by sources such as automobiles and industry—is returned to the carbon cycle by CO-oxidizing bacteria. Aerobic processes are noted with blue arrows, and anaerobic processes are shown with red arrows. Reverse methanogenesis will be discussed in chapter 29.

Microbial degradation of polysaccharide (cellulose, hemicellulose, lignin, chitin)

Plant cell wall polysaccharides

In nature, cellulose, hemicellulose and lignin (lignocellulosic complex) are major sources of plant biomass; therefore, their recycling is indispensable for the carbon cycle. Each polymer is degraded by a variety of microorganisms. Microorganisms, especially fungi, are able to degrade the plant cell wall through a set of acting synergistically enzymes. This phenomenon leads to glucose being released in a free form, which can enter the metabolism of the microorganism, providing its energy.

Plant cell wall polysaccharides (cellulose, hemicelluloses, pectin and lignin) are the most abundant organic compounds found in nature. These compounds consist mainly of polysaccharides such as cellulose, hemicelluloses and pectin, as well as the phenolic polymer lignin. Cellulose, the major constituent of plant cell wall consists of ß-1,4 linked D-glucose units that form linear polymeric chains of about 8000-12000 glucose units. Hemicelluloses are heterogeneous polysaccharides consisted by different units of sugars, being the second most abundant polysaccharides in plant cell wall. For ex.- galacto(gluco)mannans, which consist of a backbone of ß-1,4-linked D-mannose (mannans) and D-glucose (glucomannans) residues with D-galactose side chains. Lignin is a phenolic polymer that confers strength to plant cell wall. Lignin is a highly insoluble complex branched polymer of substituted phenylpropane units, which are joined together by ether and carbon-carbon linkages, forming an extensive cross-linked network within the cell wall.

Table: Characteristics of plant cell wall polysaccharides

			Elements Present in Large Quantity					*Degradation*	
Substrate	**Basic Subunit**	**Linkages (if Critical)**	**C**	**H**	**O**	**N**	**P**	**With O_2**	**Without O_2**
Starch	Glucose	$\alpha(1\rightarrow 4)$ $\alpha(1\rightarrow 6)$	+	+	+	–	–	+	+
Cellulose	Glucose	$\beta(1\rightarrow 4)$	+	+	+	–	–	+	+
Hemicellulose	C6 and C5 monsaccharides	$\beta(1\rightarrow 4)$, $\beta(1\rightarrow 3)$, $\beta(1\rightarrow 6)$	+	+	+	–	–	+	+
Lignin	Phenylpropane	C–C, C–O bonds	+	+	+	–	–	+	–
Chitin	*N*-acetylglucosamine	$\beta(1\rightarrow 4)$	+	+	+	+	–	+	+

Microbial degradation of plant cell wall polysaccharides

Saprophytism, one of the most common lifestyle of microorganisms, involves living in dead or decaying organic matter, mainly composed by plant biomass. In this context, microorganisms developed cellular mechanisms in order to take energy from plant biomass, and one of this mechanisms involves the production and secretion of carbohydrate-active enzymes. These enzymes degrade the plant cell wall, releasing sugars monomers that can be used as substrates for the metabolism of the microorganism. The microbial use of plant biomass is pivotal for life on Earth, because it is responsible for large portions of carbon flux in the biosphere. In addition, plant cell wall-degrading enzymes (CWDEs) have a broad range of industrial applications, such as within the food and feed industry and for sustainable production of many chemicals and fuels.

Degradation of cellulose is performed by cellulases, a high specific class of enzymes able to degrade the cellulose glycosidic bonds. The fungi *Trichoderma reesei* is the most important organism used in cellulase production. The filamentous fungi *Aspergillus niger* is known to produce a wide range of hemicellulose-degrading enzyme (hemicellulases) and it has been used for many industrial applications.

Bacterial degradation of cellulolytic material is more restrict to biomass containing low amounts of lignin, once bacteria are poor producers of lignanases. Some important cellulolytic bacteria are *Clostridium*, *Ruminococcus*, *Caldicellulosiruptor*, *Butyrivibrio*, *Acetivibrio*, *Cellulomonas, Erwinia, Thermobifida, Fibrobacter, Cytophaga,* and *Sporocytophaga*.

Concerning to lignin degradation, many white-rot basidiomycetes and some actinomycetes are able to produce lignin-degrading enzymes, especially peroxidases. For instance, *Phanerochaete chrysosporium* and *Phlebia radiata* are well known producers of extracellular peroxidases, as well as *Coriolus tersicolor*, which was shown to produce the intracellular haem peroxidase upon the induction by phenolic compounds. A white-rot basidiomycete, *Rigidoporous lignosus*, is known to secrete two oxidative enzymes, laccase and Mn peroxidase, responsible for solubilizing the lignin in a synergistic way.
Glucose presents approximately 60% of the total sugars available in cellulosic biomass. The yeast *Saccharomices cerevisiae* is the most important microorganism able to ferment glucose (hexose), generating ethanol.

Chitin degradation

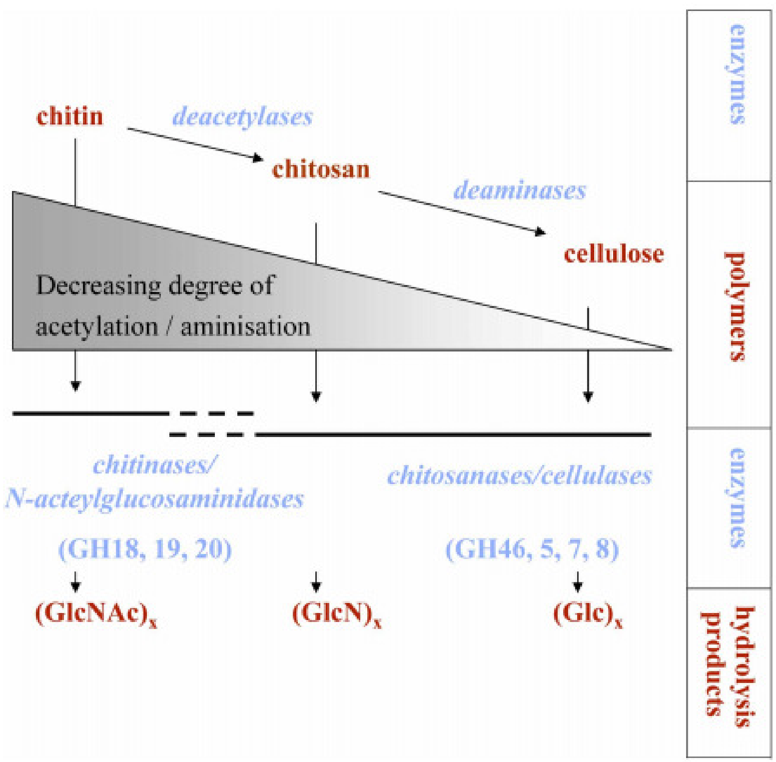


Fig. - Mechanism of chitin degradation

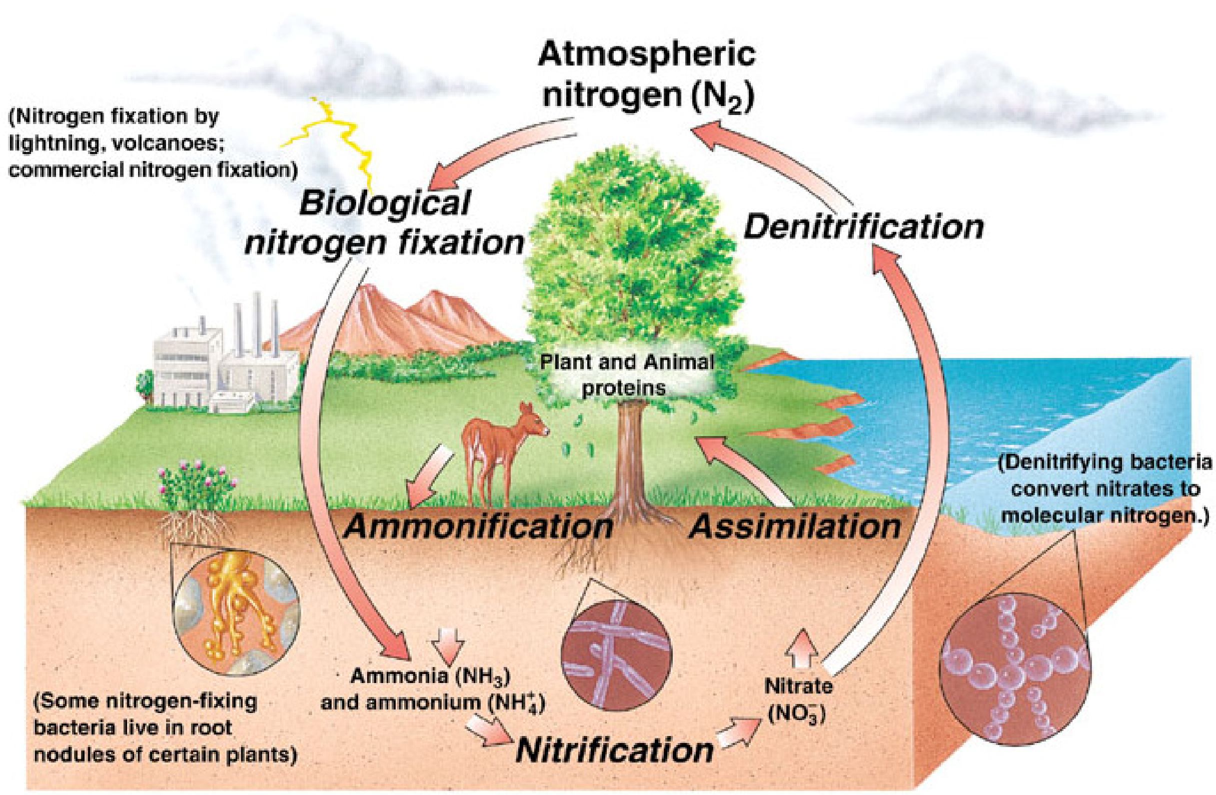
Chitin is one the most abundant polymers in nature and interacts with both carbon and nitrogen cycles. The occurrence of chitin is widespread in nature and chitin serves as a structural element in many organisms, e.g., fungi, crustaceans and insects. Chitin is the polymer of (1→4)-β-linked

N-acetyl-D glucosamine (GlcNAc). Complete lysis of the insoluble chitin polymer is usually catalyzed by chitinases. Chitinase-catalyzed chitin degradation involves the hydrolysis of the (1→4)-β-glycoside bond and the process is called chitinolytic. Alternatively, chitin can be deacetylated to chitosan or possibly even cellulose-like forms, if it is further subjected to deamination.

Chitinases are produced by many genera of Gram negative and Gram positive bacteria, but not by Archaebacteria. Important bacterial genera which have chitinases activity are *Vibro*, *Photobacterium*, *Aeromonas*, *Cytophaga*, *Streptomyces*, *Photobacterium*, *Bacillus, Clostridium* and *Chromobacterium*. Most common Chitinolytic fungi are *Aspergillus*, *Trichoderma*, *Verticillium*, *Thielavia*, *Penicillium* and *Humicola*.

Nitrogen cycle: role of microorganisms

There are several aspect of the nitrogen cycle, the processes of nitrogen fixation, nitrification, denitrification, and Ammonification and nitrogen assimilation.



Nitrogen fixation – role of microorganism in nitrogen fixation is discussed in unit 2.

Nitrification

Nitrification is the aerobic process of ammonium ion (NH_4) oxidation to nitrite (NO_2^-) and subsequent nitrite oxidation to nitrate (NO_3^-). The conversion of ammonium to nitrate is

performed primarily by soil-living bacteria and other nitrifying bacteria. In the primary stage of nitrification, the oxidation of ammonium (NH_4^+) is performed by bacteria such as the *Nitrosomonas* species, which converts ammonia to nitrites (NO_2^-). Other bacterial species such as *Nitrobacter*, are responsible for the oxidation of the nitrites (NO_2^-) into nitrates (NO_3^-).
It is important for the ammonia (NH_3) to be converted to nitrates or nitrites because ammonia gas is toxic to plants.

$$NH_3 + 2\ O_2 \rightarrow NO_2{}^- + 2H^+ + H_2O \quad \text{by } \textit{Nitrosomonas}$$
$$NO_2{}^- + 0.5\ O_2 \rightarrow NO_3{}^- \quad \text{by } \textit{Nitrobacter}$$

Assimilation

Plants can absorb nitrate or ammonium from the soil via their root hairs. If nitrate is absorbed, it is first reduced to nitrite ions and then ammonium ions for incorporation into amino acids, nucleic acids, and chlorophyll.

Ammonification

When a plant or animal dies or an animal expels waste, the initial form of nitrogen is organic. Bacteria or fungi convert the organic nitrogen within the remains back into ammonium (NH_4^+), a process called ammonification or mineralization.

Denitrification

Denitrification is the reduction of nitrates back into nitrogen gas (N_2), completing the nitrogen cycle. This process is performed by bacterial species such as *Pseudomonas* and *Clostridium* in anaerobic conditions. They use the nitrate as an electron acceptor in the place of oxygen during respiration.

$$\underset{\text{Nitrate}}{NO_3^-} \rightarrow \underset{\text{Nitrite}}{NO_2^-} \rightarrow \underset{\text{Nitric oxide}}{NO} \rightarrow \underset{\text{Nitrous oxide}}{N_2O} \rightarrow \underset{\text{Dinitrogen}}{2\ N_2}$$

Phosphorous cycle: Phosphate immobilization and phosphate solubilization

Phosphorus is one of the most important constituent of several important compounds always present in organisms. It occurs both in organic (nucleic acids, nucleoproteins, phospholipids, etc.) and inorganic (phosphate) forms in the living organisms. Animals possessing bones have large amount of phosphorus in its inorganic form.

However, phosphorus is added to soil through chemical fertilizers, excrete and organism-residues. Though there is plenty of phosphorus present in the soil in unavailable inorganic forms, most of the plants obtain it only as orthrophosphate ions (soluble inorganic forms). However, mycorrhizae, when present, help the plants in obtaining phosphorus.

1. Mineralization: Conversion of Organic Phosphorus into Insoluble Inorganic Phosphates:

Many soil microorganisms produce enzymes that attack many of the organic phosphorus compounds in the soil and release inorganic phosphate. This process is comparable to the mineralization of organic nitrogen compounds. The enzymes involved in these reactions are collectively called ‘phosphatases’ which have a broad range of substrate specificity.

2. Solubilization: Conversion of Insoluble Inorganic Phosphates into Soluble Inorganic Phosphates:

The availability of phosphorus depends on the degree of solubilization by various organic and inorganic acids produced by microorganisms in soil. These are the solubilized form of insoluble inorganic phosphates which are taken in by the plants.

Fungi, e.g., *Aspergillus*, *Penicillium*, *Fusarium* are the most important of the soil microorganisms which produce substantial amounts of these acids; others are the bacteria, namely, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Flavobacterium*, etc.

Sulphur Cycle: Microbes involved in sulphur cycle

Sulphur is an essential part of all living matter because sulphur containing amino acids are always present in almost all kinds of proteins. Plants can absorb directly the sulphur containing amino acids, e.g., cystine, cysteine, and methionine but these amino acids fulfill only a small proportion or requirements for sulphur.

In plants, sulphur passes through a cycle of transformation mediated by microorganisms. It accumulates in the soil mainly as a constituent of organic compounds and has to be converted to sulphates to become readily available to the plants.

A) Degradation of Organic Compounds to Release H_2S:

Degradation of proteins (proteolysis) liberates amino acids which generally contain sulphur.

$$\text{Protein} \xrightarrow{\text{degradation}} \text{Amino acid}$$

Enzymatic activity of many heterotrophic bacteria results in the release of H_2S from further degradation of sulphur containing amino acids.

(B) Oxidation of Hydrogen Sulphide (H_2S) to Elemental Sulphur:

Hydrogen sulphide undergoes decomposition to produce elemental sulphur by the action of certain photosynthetic sulphur bacteria, e.g., members belonging to the families Chlorobiaceae (Chlorobium) and Chromatiaceae (Chromatium).

Example:

$$CO_2 + 2H_2S \xrightarrow[\text{bacteria}]{\text{Photosynthetic sulphur}} \underset{\text{carbohydrate}}{(CH_2O)_x} + H_2O + 2S$$

Some non-sulphur purple bacteria, e.g., Rhodospirillum, Rhodopseudomonas, and Rhodomicrobium, which are facultative phototrophs and grow aerobically in the dark and anaerobically in the light, can also degrade H2S to elemental sulphur.

(C) Oxidation of Elemental Sulphur to Sulphates:

Elemental form of sulphur accumulated in soil by earlier described processes cannot be utilized as such by the plants. It is oxidized to sulphates by the action of chemolithotrophic bacteria of the family Thiobacteriaceae (*Thiobcicillus thiooxidans*).

Example:

$$2S + H_2O + 3O_2 \xrightarrow[\textit{thiooxidans}]{\textit{Thiobacillus}} 2H_2SO_4$$

(d) Reduction of Sulphates:

Sulphate is first reduced to H_2S by sulphate reducing microorganisms under anaerobic conditions. Many bacteria including species of *Bacillus*, *Pseudomonas*, *Desulfovibrio* do this work. The mechanism of sulphate reduction to hydrogen sulphide involves, firstly, the reduction of sulphate to sulphite utilizing ATP and, secondly, reduction of sulphite to hydrogen sulphide.