UNIT 2: MICROORGANISMS IN FOOD

Before the discovery of microorganisms, all known living things were believed to be either belonging to plant or animal kingdom. No transitional types were thought to exist. During the 19th Century, however, it became clear that the microorganisms combine plant and animal properties in all possible combinations.

As more and more was learned about microorganisms, many biologist lead by German Zoologist E.H. Haeckel in 1866 believed that they should be placed in a separate kingdom, a third kingdom called the *protista*. Members of protista kingdom are prokaryotes, which are distinguished from plant, and animals by their simple organisation i.e. they are unicellular, or if multicellular, their tissue shows little differentiation.

2.1 BACTERIA

2.1. Shape and Size

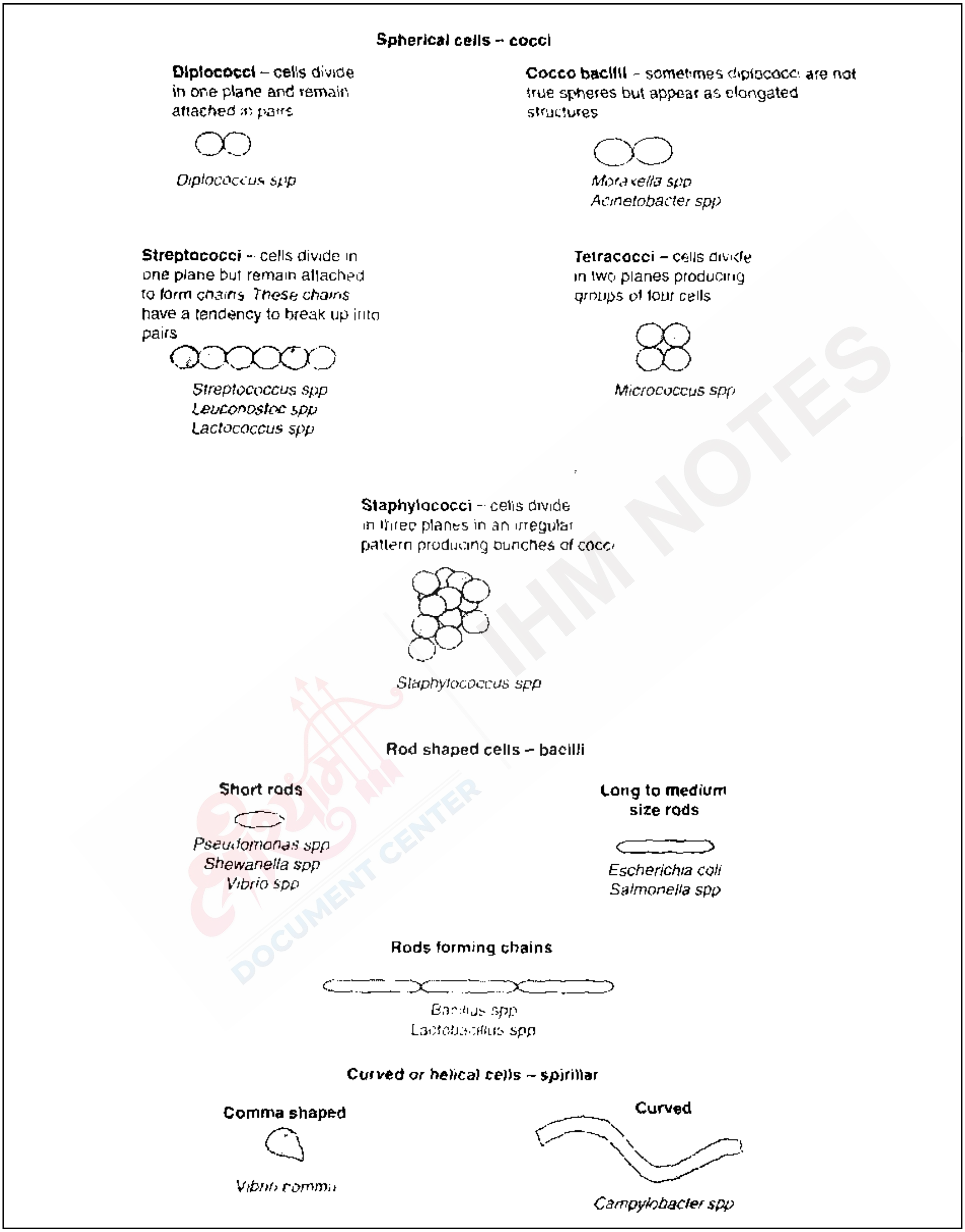
Bacteria are organisms characterized by small size approximately 0.5 to 2.5 μm in diameter and unicellular in nature. The major characteristics of bacterial cells are their size, shape, structure and arrangements. There are three distinct forms viz,. (1) bacilli (singular-bacillus, rodshaped) (2) cocci (singular-coccus spherical or ellipsoidal) (3) spiral forms (curved rods or spiral cells). The most common of all the three form is the bacillus i.e., the rod shape which are cylindrical and may vary considerably in length and breadth according to the species. The next comes the coccus and the least common form is the spiral in which the cell is curved spirally. Although these three forms are well recognized, there are variations of these shapes. The shapes of the each species of bacteria also do not always maintain a definite shape at all times. Some species even exhibit a variety of shapes.

2.1.2 The arrangement of bacterial cells

The bacterial cells are arranged in a characteristic manner according to the species. Th cocci exist either singly, or in pairs or in long chains depending upon the manner in which they divide and then adhere to each other after division, whereas, the bacilli can be seen as single cell, pairs or in short or long chains. The length of cocci chains is an identifiable characteristic but in case of bacilli it is not so (refer Fig. 2.1).

2.1.3 The cell structure

Like other living cells, bacterial cell essentially has an outer wall, cell membrane, cytoplasm and nuclear material. The outer part of the bacterial cell is made up of cell wall, cytoplasmic membrane and slime layer. The cell wall gives shape to the cell and it

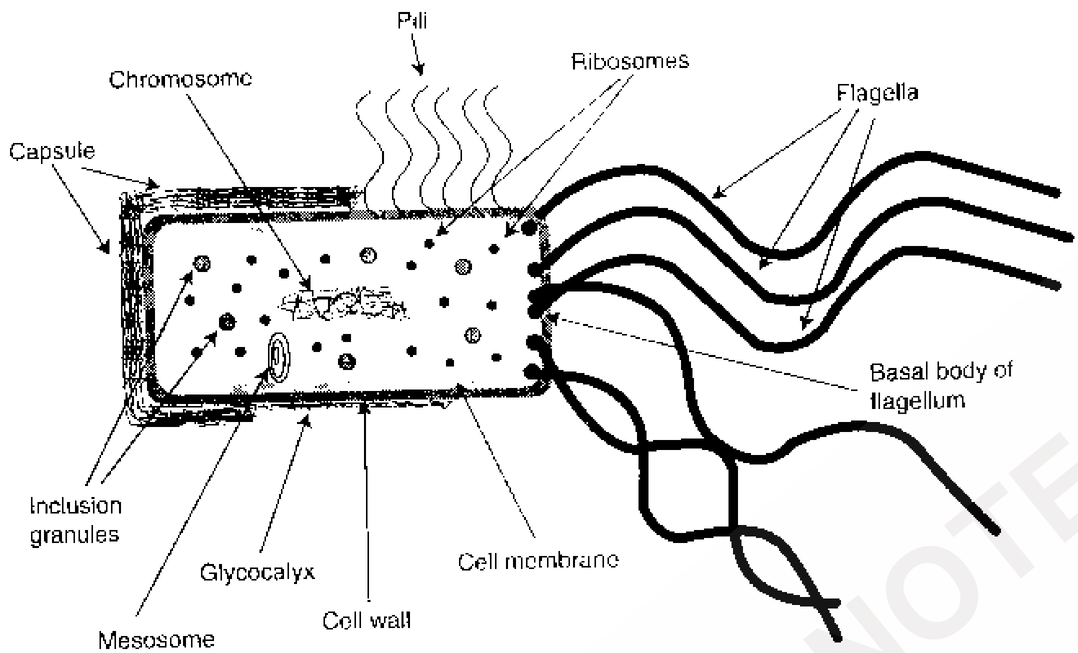

Spherical cells – cocci
Diplococci – cells divide in one plane and remain attached in pairs
Diplococcus spp
Cocco bacilli – sometimes diplococci are not true spheres but appear as elongated structures
Moraxella spp
Acinetobacter spp
Streptococci – cells divide in one plane but remain attached to form chains. These chains have a tendency to break up into pairs
Streptococcus spp
Leuconostoc spp
Lactococcus spp
Tetracocci – cells divide in two planes producing groups of four cells
Micrococcus spp
Staphylococci – cells divide in three planes in an irregular pattern producing bunches of cocci
Staphylococcus spp
Rod shaped cells – bacilli
Short rods
Pseudomonas spp
Shewanella spp
Vibrio spp
Long to medium size rods
Escherichia coli
Salmonella spp
Rods forming chains
Bacillus spp
Lactobacillus spp
Curved or helical cells – spirillar
Comma shaped
Vibrio comma
Curved
Campylobacter spp

is made up of proteins and complex carbohydrates or polysaccharides; large amounts of fat or lipid may also be present. Presence of outer membrane made up of lipopolysaccharides (LPS) differentiates gram negative bacteria from gram positive. Apart from giving protection to the cytoplasm, the cell wall also plays a role in cell division. It regulates the passage of various materials between the external and internal environment. The slime layer, is like a jelly, which surrounds the bacteria. In some bacteria, the slime becomes very thick and covers the entire bacteria. It is known as a capsule. The slime layer gives some protection to the bacteria against invasion.

The cell membrane which is inside the cell wall initiates, the cell division and also controls the entrance of food into cells and removal of the waste products. The cytoplasm is a complex substance containing the nuclear body known as nucleoid. It lacks nuclear membrane and is rich in dexoyribonucleic acid (DNA). It also contains ribosomes and fluid portion which contains various dissolved nutrients.

Many bacteria can swim by means of small appendages called flagella (flagellum, singular). In bacteria, this is the only means of locomotion. They are usually several times the length of the cell body but are extremely thin. The flagella of the motile bacteria are distributed over the surface depending on the bacteria. In some cases they may be found, all over the surface of the cell or they may be restricted to one or both ends. The common form is the former type A typical bacterial cell is shown in Fig.2.2.

Fig. 2.2 : A typical bacterial cell

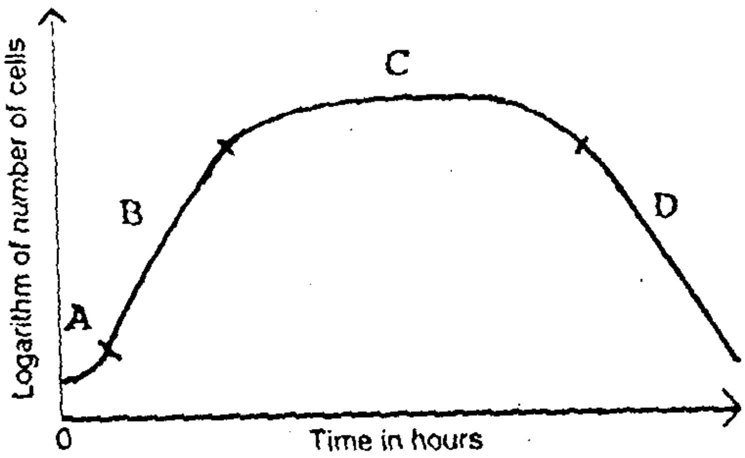


2.1.4 The endospore

Some bacteria like *Bacillus* or *Clostridium* produce resting structures known as endospores, which are produced within cell (one spore is formed within single bacterial cell). These are highly resistant bodies. The endospore is physiologically dormant and it can resist extreme unfavourable conditions both physical and chemical (like heat, UV light and chemicals). When conditions are favourable, these spores will germinate and produce fresh vegetative cells. This process of sporulation is a mode of reproduction.

2.1.5 Growth of bacteria

Growth of bacteria can be defined as an increase in mass of bacteria per unit volume of medium. The bacteria divide by binary fission i.e., a division of a cell produces two new cells which are assumed to be identical in all relevant properties.



Under favourable conditions of moisture, pH, nutrition and temperature, the growth of bacteria takes place. There are several factors like exhaustion of food supply, accumulation of waste products etc. which can limit the growth of bacteria. The growth of bacteria is generally expressed in four principal stages viz. (1) lag phase (2) lograthmic or exponential phase (3) the stationary phase and (4) the death phase. A few viable cells from a culture incubated in a suitable medium at the optimum temperature generally go through the four phases as shown in the figure 2.3.

Fig. 2.3: A typical growth curve illustrating the phases of growth. A–Lag Phase;

B–Logarithmic growth phase; C–Stationary phase and D–Death phase

During the initial lag phase i.e. in a period of one to few hours, there may be little or no increase in the cells. But when the growth begins due to cell division, the cells proliferate rapidly and lograthmically i.e. one cell divides to 2 cells and then 2 cells to 4 cells and so on. The average generation time is mostly constant for a given species under similar conditions. In the stationary phase, after rapid growth, the cell multiplication gets stagnant due to the exhaustion of nutrients or accumulation of toxic waste products or any other factors. The stationary phase can be maintained for hours or days by the balance between the death of some cells and the continued division of others. The decline or death phase where the cells die, occurs, when the medium is not changed as it has become incapable of supporting further growth.

2.1.6 Factors affecting bacterial growth

There are several important factors, which have a bearing on the growth of bacteria. The principal influencing factors are nutrition, oxygen, temperature, pH, moisture, osmotic pressure, light, and the presence of inhibitory substances like chemicals. Although each of the factors mentioned limits the growth of bacteria, the growth depends more on the combined effects of these factors.

(a) Nutrition

The nutritional requirements of bacteria differ from species to species. The nutrients are required by bacteria not only as a source of energy but also for manufacturing cellular components. The majority of the bacterial species use naturally occuring organic material viz. carbohydrates, proteins and fats as source of energy and the essential elements like carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. Other elements like iron, magnesium, potassium are required in minute quantities.

Generally based on the nutritional requirement, the bacteria could be divided into two groups namely *autotrophic* and *heterotrophic* bacteria. The autotrophic bacteria's requirements of carbon are derived from carbondioxide or from carbonates and nitrogen from gaseous nitrogen or nitrites and nitrates. They have the ability to synthesize its other essential requirements from inorganic substances like sodium chloride, dipotassium hydrogen phosphate (K_2HPO_4) etc. for growth and survival. From these simple substances, they synthesize complex structures like carbohydrates, proteins, vitamins, enzymes etc. The heterotrophic bacteria require one or several preformed organic compounds, which are readily available for their growth. These requirements range from a single vitamin to several complex organic compounds. By far *the heterotrophic bacteria are the most commonly found type of bacteria and are widely distributed.*

Even the autotrophic and heterotrophic bacteria are subdivided further depending on the mode of deriving the energy source. They are chemosynthetic and photosynthetic. The chemosynthetic bacteria get their energy from the oxidation of inorganic chemical reactions, whereas the photosynthetic bacteria have the ability to get their energy requirement from the sunlight.

(b) Oxygen

The presence of oxygen in the atmosphere is essential for the survival of microorganisms also but many bacteria have the ability to thrive in the absence of oxygen or free air. These bacteria are known as *strict* or *obligate anaerobes*. These organisms die when exposed to air or oxygen. But there are only few obligate anaerobes. There is another category of bacteria, which can survive either in the presence, or absence of oxygen. They are known as *facultative anaerobes*.

The other kinds of bacteria, which cannot survive in the absence of oxygen, are called *obligate aerobes*. There is yet another category of bacteria known as *micro-aerophiles* which require oxygen for survival albeit at low concentrations than present in air.

(c) Temperature

Temperature is one of the important factors affecting the process of growth in bacteria as it has a direct influence on chemical reactions. The temperature at which the maximum growth occurs is known as the *optimum temperature*. Based on the temperature at which the maximum growth occurs, the bacteria are divided into three main categories.

1. The species of bacteria, which grow rapidly between 45-65°C, although they may grow anywhere between 45-75° C, are called *thermophiles*.

2. The species of bacteria, which grow rapidly between 20-45°C where the optimum range is between 30-40°C, are known as *mesophiles*.

3. The bacterial species which grow rapidly at 0°C (and even below) have optimum temperature between 10-20°C are known as *psychrophiles*.

Although the bacteria have been grouped into three categories based on temperature, there is no certainty that there is no overlapping between the bacterial groups. This division is based on broad range of species.

(d) Water activity

Moisture is one of the important factors for the survival of living species even microorganisms. Water usually accounts for 80-90% of the total weight of the microbial cells. The water requirement of bacteria varies from species to species. Although bacteria require water, the growth largely depends upon the available water, which is known as *water activity* (a_w). This is expressed as

Water activity (a_w) = *vapour pressure of the solution* / *vapour pressure of pure water*

where the water activity of pure water is taken as 1.00

The nutrition and temperature have been found to have an effect on moisture. With the increase in temperature, the available water will be reduced and the availability of nutrients determines the growth. Most bacteria have been shown to grow well in media with water activity (a_w) between 0.990-0.998. Water activity is an important factor in the control of growth of microorganisms.

(e) Osmotic pressure

Bacterial cell is contained by a cell membrane. This membrane allows water to pass in an out of the cell. When bacteria are placed in heavy sugar syrup or salt brine, water in the cell move out through the membrane and into the concentrated syrup or brine, which may, contained 30-40% water. This is known as *osmosis*. The tendency to equalise water concentration inside and outside the cell in this case causes partial dehydration, where the cell shrinks and is called *plasmolysis*. Instead, if the bacterial cell is placed in distilled water, the water enters the cell and causes it to burst. This is known as *plasmoptysis*.

The tendency of the cell membrane to allow water to pass from inside to outside or vice versa is to maintain the equilibrium between the cell contents and its fluid surroundings. As the hydrostatic pressure causes osmosis it is known as osmotic pressure.

The osmotic pressure is related to the water activity of solutions and foods, solutions high in solute concentrations have a high osmotic pressure and a lower water activity. Dilute solutions are lower in osmotic pressure and have a high water activity. Accordingly, the rate of bacterial growth is affected.

(f) pH

The hydrogen ion concentration has significant role to play in the growth of bacteria as every microorganism has minimal, maximal and an optimum pH at which it can exist and thrive.

The pH is defined as the logarithm of the reciprocal of the hydrogen ion concentration.

i.e., pH = log 1/ (H+)

The pure water when ionized contains 10^{-7} moles each of H^+ and OH^-. As there is a balance between H^+ and OH^- ions, the solution (i.e., water) has neutral (pH 7). A pH scale has been devised to indicate the pH of various foods. The pH of water being neutral (pH7), it is mid point of the scale. The pH scale extends form 0-14. Any substance whose pH is below 7 is known to be acidic whereas a substance is known to be alkaline if the pH is above 7. Most of the bacteria prefer a pH near 7 (neutrally) whereas, there are some bacteria which prefer alkaline or acidic medium.

(g) Light

Although visible light is beneficial to the photosynthetic bacteria, the ultraviolet light (UV) is , however, harmful to the bacteria. The UV light is absorbed by the nucliec acid present in the cells, which get denatured and may result in the death of cells. Due to this property , UV light is used in surface sterilization.

2.2 FUNGI

Fungi exhibit a wide range of different forms, which include moulds, yeasts and mushrooms. The important forms are yeasts, which are unicellular, and the moulds, which are filamentous and multicellular. Mushrooms in addition to being filamentous and multicellular have definite fruiting body, which is prominent.

2.2.1 Yeasts

The yeast cells, in general, are either ellipsoidal, cylindrical or filamentous. The yeast cells are generally much are larger than bacterial cells and range from 3-5 □m wide by 5-10 □m long and they exists as single cells. The reproductive process in the case of yeast cells is by the process known as *budding*. Although in certain yeast, the process is by way of fission as in bacteria. The budding process involves the bulging of protoplasm outwardly and as the bulge grows in size, it separates from the parent cell after attaining maturity. Some type of yeasts reproduce sexually also and are known as true yeasts. In this process, the cells serve as ascus (sac). Here the nucleus undergoes division without the participation of the cell wall forming spores within the ascus. The spores of yeast are also resistant to some adverse conditions but get destroyed at temperature above 60°C whereas the bacterial spores are quite resistant to higher temperatures.

2.2.2 Moulds

The term mould is used to describe certain multicellular fungi consisting of a filamentous branching growth known as a mycelium which is composed of individual filaments called hyphae (singular, hypha). The mould can grow within the food or on the surface. Depending on the location, the aerial mycelium carries fertile hyphae, which acts as reproductive organ while the reminder of the mycelium absorbs food and moisture necessary for growth (submerged hyphae).

In moulds, reproduction is mainly by asexual spores whereas there are mould which reproduce by sexual spores. Based on the manner and type in which the spores are formed (refer Fig. 2.4 and 2.5), moulds are classified as :

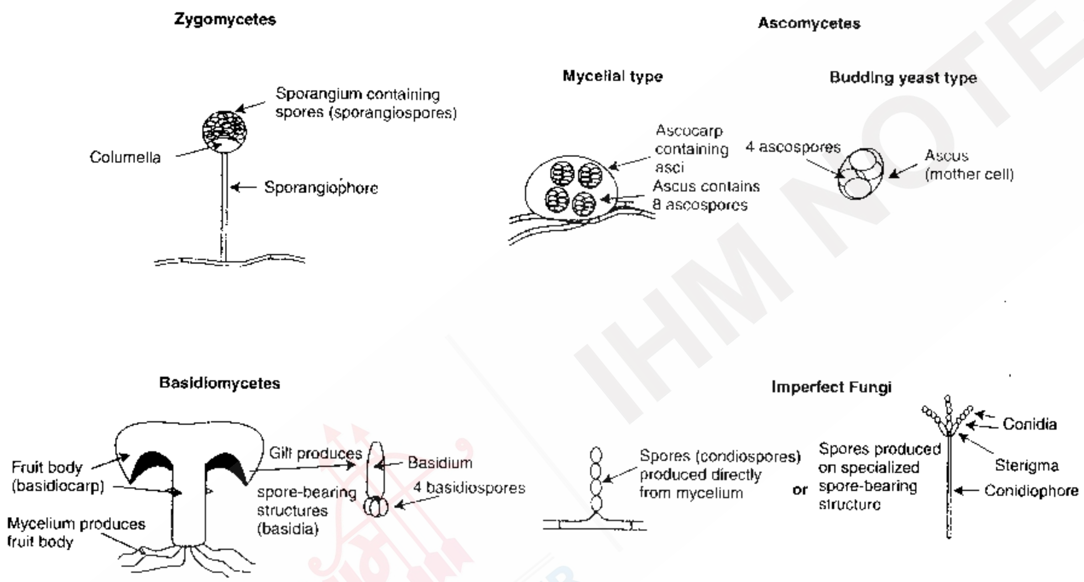


Fig. 2.4: Reproductive structures in the main groups of fungii

(a) Phycomyecetes

The moulds belonging to the group of phycomycetes contain several genera. These moulds produce hypae which are not divided into typical uninucleate cells and thus have no cross walls (septa). These non-septate hyphae have nuclei scattered throughout their length. Phycomycetes which are commonly found on food are members of the sub-class zygomycetes. The two most commonly found genera of this group are genus *Mucor* and genus *Rhizopus*. These moulds possess hypae that although apparently alike are able to conjugate and form a zygospore (sexual spores).

Species of Mucor genus are present in soil, organic matter fruits, vegetables, stored grains and other foods. Mucor species play a role in fermentation foods and have commercial value also. They sometime cause spoilage of foods. Species of Rhizopus are commonly associated with spoilage of stored foods (e.g. spoilage of bread by *R. stolonifer*)

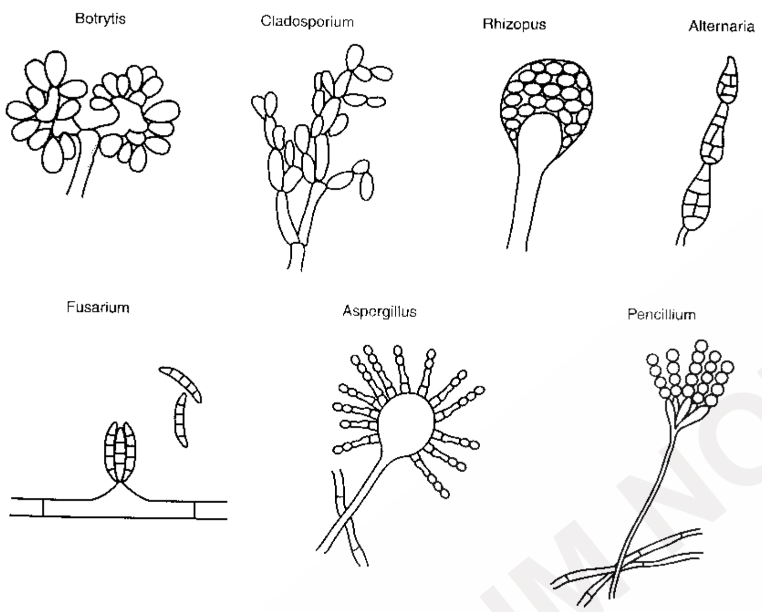
(b) Ascomycetes

These moulds have septate hypae and multiply asexually by separation at the tips of fertile hypae (conidiophores) to produce spores known as *conidia*, formed either singly, in chains or in irregular clusters on the conidiophores. In the ascomycetes, the sexual spores are termed ascospores. They are formed following the union of two cells from the same mucelium or from two different mycelia. A number of ascospores, usually eight, are subsequently formed formed within a sac known as ascus. The organisms of importance belong to this group of genus are *Claviceps, Neurosopra, Sclerotinia, Byssochlamys*.

(c) Basidiomycetes

This group also containing sepatate hypae and forms spores, usually, four in number. Most of the edible mushrooms belong to this class and the spores are produced in a club-shaped structure known as *basidium*. The spores are called basidiospores.

Fig. 2.5 : Common mould species found in foods



Most of the edible species of mushrooms are members of the genus *Agaricus*. In America the commercial production is dominated by the species, *Agaricus bisporus*. In East Asia, especially in Japan, *Lentinus edodes* which is grown on treetops is popular. In India, the oyster mushrooms, *Pleurotus destreatus* is grown and exported. Mushrooms are increasingly accepted as a source of food and considered a delicacy

(d) Fungi imperfecti

This group of fungi is known as the *fungi imperfecti* as the sexual stage of reproduction of many strains are yet to be discovered. Fungi imperfecti produce characteristic conidiophores and conidia. The organisms of importance of this class are *Alternaria, Aspergillus, Fusarium, Penicillium, Botrytis, Cladosporium* etc. The *Aspergillus* and *Penicilium* species are most commonly found and are significant storage fungi responsible for spoilage of foods

2.3 VIRUSES

Till the presence of viruses were demonstrated by Iwanowski in 1892, bacteria were considered the lowest forms of life. Viruses are called as obligate intracellular parasites since they are unable to carry out any of the typical life functions until they are inside a host cell. Once inside host-cell, they thrive and direct the host cell to produce more viruses.

The viruses are minute when compared to bacteria. Except for few viruses like the cow pox virus, used in vaccination against small pox, is 0.3 μm, whereas the smallest type, like the foot and mouth disease virus, is about 0.01 μm in size. They cannot be seen under ordinary microscope but can been seen only under electron microscope.

The viruses consist of protein, capsid, surrounding nucleic acid comprising either RNA or DNA. The important characteristic of viruses is that they are host specific. Most viruses infect only one species either animal or plant or else only very closely related species. The mammalian viruses does not affect any plant e.g., the polio virus infects humans and monkey and does not affect other animals whereas the tobacco mosaic virus which attacks plant does not affect humans.

Viruses are killed in a few minutes under pasteurization temperature i.e. 62°C for 30 minutes. They are affected by general disinfectants like phenols, formaldehyde, halogens and cresols. To a certain extent soaps and detergents inactivate them and UV light destroys all viruses. They are not affected by antibiotics unlike bacteria.

Viruses are known to cause illness although they do not grow on foods or produce toxins in foods. Food items merely act as vehicle for their transfer. They are the intestinal or enteric type and are food-borne. They spread from the hands of human carriers and from water to foods. The presence of viruses in foods, specially in the shell fish grown in sewage polluted water could be the significant route of illness in man. The hepatitis A virus which causes jaundice, spreads through foods but the etiology is difficult to be established as it has a long incubation period ranging from 15-50 days.

2.4 PARASITES

Several foods act as carriers of parasites which may cause illness when ingested. The parasitic infections of foods may occur due to contamination of food by a food handler directly or by polluted waters. In many parts of the World, the consumption of under cooked meat and fish are popular. The problem of contamination with parasites can occur when the food preparations are made with polluted water and under cooking prevents the parasites from destruction. Amoebic infection is the most important parasitic infection through contaminated food.

2.4.1 Amoeba

Entamoeba hystolytica, a protozoan, is the cause of amoebiasis, which is very common in a typical country like India. About 15% of the population suffer from this disease. The disease is transmitted through infected cyst. It remains viable only in moist form and gets destroyed at 60°C and at freezing conditions beyond 24 hours.

The foods like vegetables from fields irrigated with sewage polluted water are the vehicles of transmission. They get contaminated with amoebic cysts through polluted water and infected handlers. Viable cysts have been found on the hands and under the finger nails of the carriers. The common pests like flies, cockroaches and rodents may also harbour the cysts and contaminate food and drink. The symptoms of diarrhoea occur from several days to 4 weeks after ingestion of the contaminated food. The prevention of amoebiasis is by using food procured from reliable sources, preparing food in potable water and adequate cooking and proper storage after preparation. Use of filtered/boiled water, disinfection of uncooked vegetables with aqueous solution of iodine around 20 ppm or acetic acid (5-10%) or full strength vinegar are also recommended measures to minimise the problem.

Fig. 2.6 showing (A) *Entamoeba hystolytica* (B) *Giardia intestinalis*

2.4.2 Giardia

The disease giardiasis is caused by the flagellated protozoan *Giardia lamblia*. This disease occurs in areas where poor sanitation conditions prevail and it affects mostly children. The cyst of this organism is absorbed through the intestinal walls and the affected person excretes the Giardia cysts in faeces. The cysts get transferred to food when contaminated water is used for washing the vegetables. Consumption of such food containing the cysts leads to infection. The incubation period ranges between 2-25 days and the symptoms include discomfort, nausea and diarrhoea. Infected persons may simply be the carriers and may not always exhibit symptoms. The disease can be prevented by adopting good personal habits and proper faecal disposal methods and protecting the water supplies from faecal contamination.

2.4.3 Trichinella

The disease trichinosis is caused by the nematode, *Trichinella spiralis*. It is one of the commonly found parasitic foodborne infections in the populations consuming principally the undercooked pork. The parasite enters the human host in the form of larvae, which is found in the pork eaten by man. After consumption, the encysted larvae are released and they enter into the blood stream and they enter the duodenum-producing larvae, which gain entry into the blood stream and encyst in the muscle. The symptoms appear normally 2 days to one week after ingestion of the contaminated food with the larvae. The symptoms include fever, abdominal pain, nausea, vomiting and diarrhoea. In some cases edema of face and hands is also observed. The prevention of trichinosis is ensured when the food is thoroughly cooked to a temperature of 60°C, which destroys the larvae. The meat has to be cooked till it imparts grey colour. The parasite is also destroyed when held at temperatures below 25°C or lower for 10 days.