CHAPTER 1

1.1. Introduction & Historical Background

The word **Mycology** is derived from the Greek word mykes = cap; mushrooms and logos = discourse. Alike, it is the scientific study of fungi. The English word **fungus** is directly adopted from the Latin fungus that meaning "mushroom", used in Horace and Pliny (*Simpson, 1979).* The latter is derived from the Greek word **sphongos**/σφογγος ("**sponge**"), referring to certain mushrooms and molds.

The name fungus has different words in each language ***Viz***; Lat. **Fungus**, originally sfungus, cognate with spongia from Greek sphongis, a sponge, **Champignon** (France), **Ciuperca** (Romanian), **Cogumelo** (Portgalese), **fungo** (Italian), **gljiva** and **guba** (Croat), **goba** (Slovene), **gomba** (Hungrian), **grzyb** (Poland), **hongo** (Spanule), **Kavak** (Hindi), **Këphurdhe** (Albanian), **Pilz** (Germane), **zwam** (Dutch), **siene** (Finland), **Jalam** (Tamil), **seen** (Estonia). According to Oxford dictionary = **the term fungus** (nown). (Plural = **fungi** and pronounced as **fungai**) = plant without green colouring-matter.

Fungi and animals probably share a common ancestor with choanoflagellates (collar-flagellates) based on genetic data. Cell wall components and other complex biosynthetic pathways are similar between fungi and animals.

Fungi are a very large and diverse group of organisms, with a unique life-style. They are cosmopolitans and utilize many different substrates. They can simply but not precisely define as an eukaryotic, heterotrophic organism devoid of chlorophyll that obtains its nutrients by absorption, and reproduces by spores.

Fungi are variable in form and structures, versatile in solving the environmental problems. Thus, it is difficult to give a precise definition of a fungus. Ainsworth (1973) and Webster (1980) have listed their main characteristics in replacement of a definition; it is **old but is still acceptable:**

1. Nutrition: heterotrophic (lack photosynthesis) and absorptive (ingestion rare).

2. Thallus:

a. on the substratum and **plasmodial** amoeboid or **pseudoplasmodial**;

b. in the substratum and **unicellular** or **filamentous** consists of hypahe that form mycelium.

c. The hyphae are septate or nonseptate;

d. Typically nonmotile (with protoplasmic flow through the mycelium) but motile states (e.g. zoospores) may presents.

3. Cell wall: well-defined, typically **chitinised** (cellulose **in Oomycota**).

4. Nuclear status: eukaryotic, multinucleate, the mycelium being homo-or heterokaryotic, haploid, dikaryotic, or diploid, the last being usually of limited duration.

5. Sexuality: asexual or sexual and homo- or heterothallic.

6. Sporocarps: microscopic or macroscopic and showing limited tissue differentiation.

7. Habitat: omniprésent as saprobes, symbionts, parasites, or hyperparasites.

8. Distribution: cosmopolitan.

Why study fungi?

Fungi are among the most diverse organisms on the globe, and follow insects in diversity (Nature, 2000; Fig.1.1). They are not able to photosynthesis and hence they are enforced for a heterotrophic existence as follow.

A. As saprophytes (Decomposers):

1. Fungi are essential in carbon cycle and are among the few organisms that can break down lignin**.**

2. They share with bacteria and animals the role of decay of complex plant and animal remains in the soil. Without this essential process of decay, the growth of plants, would eventually cease for lack of raw materials.

3. Destructive effects of saprophytic fungi on human economy are seen rotted food, timber, and textiles.

4. **Fermentation** as in brewing, production of antibiotics or citric acid they are also important. It is also important in food processing such as baking, cheese making, or wine fermentation.

5. **Fungi** also used in carrying out chemical transformations in the pharmaceutical industry.

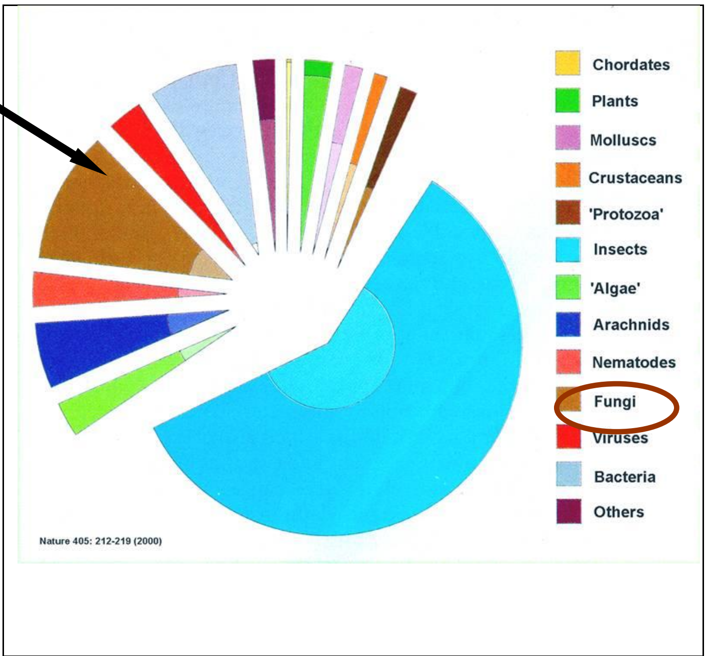

Fig.1. 1. Diversity of fungi on Earth.(Nature, 2000).

B. As symbioses:

1. Soil fertility thought to be correlated with fungal activity.

2. The roots of most green plants are infected with **mycorrhiza** and absorption of minerals may be enhanced following infection. In fertile natural soils, the success of the higher plant may depend on mycorrhizal infection (**Harley, 1969**).

3. Other a**ssociations, are Lichens Endophytes and Mutualists.**

C. As parasites:

1. Fungi cause diseases in plants and animals and Over 70% of plant diseases are caused by fungi.

2. Although fungal infections of plants have been known since human records began, it has been waited until the impact of potato blight on the population of Ireland in the mid-nineteenth century, **potato famine** that gave the impetus to the scientific study of plant pathology and their control **(Large, 1958).**

4. Comparable to bacteria and viruses, fungi are commonly less severe, although some are lethal.

D. Possible autotrophy:

1. Fungi are considered as heterotrophic organisms as mentioned above and so evolved a remarkable metabolic adaptability that allows most of them to utilize a large variety of substrates for growth (*Marzluf, 1998; Heynes, 1994).*

2. Recently, possible autotrophic nourishment has been existed. The research raises the possibility that some fungi utilize the **melanin** pigments to extract **energy** from ionizing radiation, such as **gamma radiation** for "**radiotrophic**" growth. It is recommended that this process might resemble **photosynthesis** in plants (*Dadachova et al., 2007). N*nevertheless, a scientific detailed biochemical evidences needed to support this hypothesis.

E. Fungi have a long history to be used as laboratory tools.

1. For instants, ***Neurospora*** was find to be ideal for investigation by physiologist, microbiologist, biochemist and geneticist, who also found yeasts ideal tool for understanding of respiration.

2. Investigations into the **bakanae** disease of rice caused by ***Gibberlla Fujikuroi*** led to the discovery of the group of plant growth regulators (hormones) called gibberellins (Webster, 1980).

History of Mycology

There is a big history made by fungi and mycologist. It is interesting to mention that before knowing the word fungus the sporophores of mushrooms were popular among Roman potters and Sculptors. A series of ancient stone sculptors (1000 Bc. – 200 A.D.) bearing designs of mushrooms have been collected from several countries e.g. Guatemala, and other central American countries.

In the late of 17th century, with the invention of the first microscope by **Leeuwenhoek**, vision to the hitherto unknown world of Microorganisms was become real. Consequently, with the invention of printing in Europe in 15th century, **Clusius (1601)** described many mushrooms with the help of water-colour drawing in his "**Rariorium Plantarum Historia**", which contain section on fungi. The first book specialized entirely on fungi was "**Theatrum Fungorum of het Toomeel der**

campernoellen" by **Van Sterbeeck (1675).** The book aimed to correct identification of edible fungi from poisonous ones. The first picture of micro fungi was made by **Robert Hooke (1635 – 1703*)*** in his book "**Micrographia**" (**1665**). **He** believed that the fungi initially arose spontaneously, which later produced seeds for further propagation. On the contrary, **Micheli (1729)** stated that fungi originated from their spores colonies of the same molds developed on the inoculated areas. He got the honor of being called the "***Founder and Father of Mycology***" and put an end to the theory of origin of fungi from decaying materials. **Linnaeus (Carl Von Linne, 1707 – 1778)** failed to appreciate the importance of fungi.

The fungi were then carefully studied by several able systematists like **Bulliard**, **Batch**, **Persso**, **Link**, **Schweinitz** and **Fries**. The two important works of this period are the **"*Synopsis Methodica Fungorum*" (1801)** by **Persson** and **"*Systema Mycologicum*" (1821 – 1832)** by ***Fries***. **The** first book gave the frame work on which ***Fries*** and all the later fungal systematists based their classifications. **Fries**, in his systema put approach dominated for over 100 years **"*Friesian approach*",** which had, and still has, the attraction of differentiating Basidiomycota based on field characters without the help of compound microscope. He said, these fungi depend on a diseased condition of the plant.

In the first 75 years of the 19th century, with development of the microscope and staining processes, all sorts of fungi, e.g. rusts, smuts, various moniliales and pycnidial fungi were studied and roughly identified. **De Bary** initiated the study of life history and soon it was discovered for several fungi as mycetozoa. He is really the founder of modern mycology (1831 – 188). **De Bary** elucidate the delicate mechanisms of parasitism and saprophytism, the nature of lichens, heteroecism in rusts and development and sex of several fungi. He also **the father of plant pathology**, as it was he the first to proved that the fungus Phytophthora infestans was the cause of the potato blight and hence commenced the study of plant pathology. Another important approach is studying fungi in pure culture under varying condition which evolved by Oscar Bcefeld (1839 – 1925)**. Saccardo P.A. (1845 – 1920)** collected and brought together the scattered knowledge on systematic mycology. He classified ***Fungi Imperfecti*** (Mitosporic fungi) on spore group, which has been in use ever since it was published in 1880.

In the last quarter of the 19th century, with the diminish of theory of spontaneous generation, the germ theory of disease was established by **Robert Koch, Louis Pasteur** and **De Bary.** The area of physiology, ecology, and genetics of fungi became a subject of great controversy. **The** discovery of enzymes (in fermentation ***zymase***) gave birth to a new science, **Biochemistry**.

Before **the First World War** mycology was mostly devoted to taxonomy and cytology with the elucidation of the phylogenetic relationships as the main objective. **In the second quarter of 20th century,** greater attention was paid to other aspects like ecology, artificial cultivation, nutrition, physiology, and biochemistry of fungi. **Pasteur** was "convinced that a day would come when molds will be utilized in certain industrial operations on account of their power of destroying organic matter".

These led to list some of the major contribution of fungi to science and humanity.

Major contribution of fungi to science

1- **Commercial production of enzymes** e.g. **Proteolytic, Pectolytic** and **diastase** enzymes**.**

2- **Production of chemicals** especially **Citric Acid**. **Pasteur** was the manufacture of citric acid from ***Aspergillus niger***.

3- **Development** of **Fungicidal** Research. **Millardet (1882)** was early discovered the inorganic fungicides like **Bordeaux** mixture, which was further enriched by the discovery of **dithiocarbamats** by Du Pont company of USA (**1930**).

4- **Agriculture**-especially fungus transformation in soils. Soil fungi are the primary, rather most important, degraders of cellulose, which forms the bulk of decomposing plant remains.

5- **Food Manufacture** notably cheese e.g. **Roquefort**, **camembert**, **Gorgonzola** and **silton**, which have a different tastes depend upon the quality of curd used and the used mold strain (e.g. ***Penicilliu*****)**.

6- **Food Spoilage**. Fungi growing on food stuffs render them useless and sometimes even toxic.

7- **Rotting of Textiles Fibers**. fungi are able attach and degrade cotton by enzymic degradation mechanisms. Thus fungi are able to spoil clothes in humid warm weather.

8- **Antibiotics**. The first antibiotic reported was penicillin produces by a fungus, ***Penicillium notatum*** (Alexander fleming, 1928). Since then many antibiotics have been discovered.

9- ***Neurospora crassa*****:** initiated the field of haploid genetics led to development of many modern genetic theories, Beadle & Tatum: Nobel prize for one gene-one enzyme.

10- Many fungi produce **mycotoxins**, referring to their fungal origin and toxic activity. Mycotoxins such as **aflatoxins** (produced by *Aspergillus flavus* and *A. parasiticus*) which are insidious liver toxins and highly carcinogenic metabolites, **amatoxins** (produced by ***Amanita* spp.), ochratoxins**, **patulin**, **ergot** alkaloids, **trichothecenes** and **fumonisins**. All have significant impact on human food supplies or animal livestock (*van Egmond et al. 2007).*

11- Biological control of pests

I- In agricultural settings, some fungi may be used to suppress growth or eliminate harmful plant pathogens, such as insects, mites, weeds, nematodes and other fungi that cause diseases of important crop plants (Becker, 1998).

II- **Endophytic** fungi of grasses of the genus ***Neotyphodium***, such as ***N. coenophialum*** produce alkaloids that are toxic to a range of invertebrate and vertebrate herbivores. These alkaloids protect the infected grass plants from herbivory, but some endophyte alkaloids can cause poisoning of grazing animals, such as cattle and sheep (*Bush et al., 1997).*

1.2. General Characteristic

Objectives

- To know the general morphological characters of fungi.
- To determine the basic mode of life
- To discuss the various methods of reproduction.

1-Vegetative Growth and development

Growth of fungi is adapted to efficient absorption of nutrients from the environment, by secretion of extracellular enzymes that breakdown the complex substrates, such as polysaccharides, proteins, lipids into simple molecules (Farrar, 1985; Pereira, et al., 2007; Schaller et al., 2007).

The **vegetative** fungal **thalli** are extremely varied, ranging from the simple, single-celled yeasts to more complex, or extensive filamentous structures or both (**dimorphic**). The **vegetative** phase of most fungi is composed of cylindrical filaments, known as **hyphae**. Many hyphae together, known as **mycelium**, make up the **thallus** or colony of a fungus **(Fig. 1.2)**.

2- Thallus

The thallus is normally, **eucarpic**, is differentiated into a **vegetative** part that absorbs nutrients, and a **reproductive** part. However, in some fungi, the entire thallus becomes converted into reproductive organ and so called “**holocarpic**”. The unicellular type of thallus is typical of yeasts and yeast-like fungi (e.g. ***Saccharomyces***). Some fungi, especially animal pathogens, can exist either in the **filamentous** or **yeast-like** phase, and this is known as “**dimorphism**”.

The hyphae may be **septated** or **non-septated**. **Oomycetes, Chytrids** and Z**ygomycota** are generally **non-septated**, whilst ***Ascomycota***, ***Basidiomycota*** and **their anamorphs** are **septated**. In non-septate forms, the mycelium contain numerous nuclei which are of course, not separated from each other by cross walls, but lie in a common mass of cytoplasm (i.e. **Coencytic**).

In septate forms, the hyphal segments may contain one, two or more nuclei (Fig.1. 3). The mycelium may be **homokaryotic** nuclei of same genotype) or **heterokaryotic (**nuclei of different genotype). As results of **mutation** or **anastomosis** of hyphae. The cell in ***Bosidiomycota***, may contain two genetically distinct nuclei (**dikaryotic**) or single, genetically identical nucleus (**monokaryotic**) (Fig 1.3).

3- Wall structure

The cell wall structure in fungi is essential, because it serves several important functions. The fungal cell wall mainly consists of chitin (brown) located close to the cell membrane, β-1,3- and β-1,6-glucan (green) adjacent to the chitin fibers and mannoproteins (red) as the outermost part of the cell wall.

Chitin is synthesized by transferring N-acetylglucosamine residues from uridine diphosphate-N-acetylglucosamine (UDPGlcNAc; brown hexagon) to a growing fiber that is shuttled through the cell membrane by the transmembrane chitin synthase (light blue). β-1,3-glucan is synthesized by a β-1,3-glucan synthase (yellow) that uses uridine diphosphate-N-glucose (UDPGlc; green hexagon) as a donor to transfer glucose to the extruded β-1,3-glucan fiber (Fig. 1.4.).

The gross chemical composition of the cell wall differs greatly between taxonomic groups of fungi.

The main components of fungal walls are **polysaccharides** (80-90% wall dry mass). The bulk of the carbohydrate appears as **glucans** (group of **D-glucose** polymers, linked by different **glycosidic bonds)**. β-(1, 3)-linked glucan occurs, with side branches that often have β-(1, 6)-linkages (Fig. 1.5). These **non-cellulosic glucans** occur as both **microfibrills** and **matrix** materials in walls and often are composite together. These found nearly in all fungi except **Zygomycota**.

In ***Oomycetes***, **cellulose** forms up to 45% of the wall polysaccharide (Fig. 1.6). Their walls also contain the unusual aminoacid **hydroxyproline**. Cellulose, $(C6H10O5)n$, is a polysaccharide that consists of a long unbranched chain of glucose units linked by (1→4)-β-glycoside bonds.

Chitin is the important and major wall component of the members of kingdom **fungi**. It may up to 60% of the dry material of the wall. Chitin is a nitrogen-containing linear polysaccharide of ß(1->4) linked units of N-acetyl-ß-d-glucosamine. The structure of chitin is similar to cellulose except for the replacement hydroxyl group (-OH) at the carbon 2 with an acetyl amine group (–NH–CO–CH3). After cellulose, chitin is the second most abundant biopolymer in nature. It is insoluble in water, organic solvents, weak acids and lyes. (**Fig. 1.7**).

Mannans often replace chitin or glucans in the walls of yeasts, when the chitin is absent the rigidity of the wall is probablyestablished by a **mixture** of **glucan**. Additional wall materials are found in the oldest parts of hyphae and many spores; the most notable are **melanin** and **lipids**.

4- Ultrastructure of Hyphal compartment.

The fungal cells (Fig. 1.6) are **eukaryotic** but lack plastids. It contains several other organelles. The nuclear membrane does not always break down as in most other organisms after mitotisis, but may constrict in the middle to separate two sister nuclei (**Karyochosis**).

Mitosis in fungi occurs in several ways: (Fig. 1.9)

1. Intranuclear: The nuclear membrane doesn't breakdown during mitosis.

2. Centric: in flagellated forms where typical centrioles of eukaryotes are present.

3. Noncentric: in non-flagellated forms, where **spindle pole bodies** (SPBs). In yeast, ***S. cerevisiae*** microtubules are organized by the spindle pole body (SPB).

The SPB is the fungal equivalent of the **centrosome**, and both are microtubule organizing centers (MTOC). Microtubules provide the physical means by which chromosomes are segregated during mitosis, and by which nuclei fuse during mating. SPBs organize the nuclear microtubules of the mitotic spindle and the cytoplasmic microtubules.

The centrosome consists of two centrioles surrounded by **pericentriolar** material, whereas the **SPB** is a multilayered cylindrical structure.

The *S. cerevisiae* SPB is an electron-dense, short, cylindrical structure composed of several layers (Fig. 1.10). It is a permanent structure: it is always embedded in the nuclear envelope, and no nuclear envelope breakdown occurs during mitosis in ***S. cerevisiae***. This is in contrast with most other eukaryotes, where nuclear breakdown is concomitant with mitosis. Although permanence of the nuclear envelope is a common feature of yeast mitosis, a permanent association of the SPB with the nuclear envelope is not. In ***Schizosaccharomyces pombe***, for example, the SPB is not permanently associated with the nuclear envelope.

The central plaque of the SPB is co-planar with the nuclear envelope and is interposed between the inner and outer plaques, from which the nuclear and cytoplasmic microtubules, respectively, emanate throughout the cell cycle (Fig. 1.10). Separated by the nuclear envelope, the cytoplasmic and nuclear arrays are always separated compartmentally. Early in the cell cycle, a single SPB exists within the nuclear envelope. Some of the proteins that comprise the layers of the SPB have been identified (for example, Spc98p, Spc42p, and Spc110p/Nuf1p) (1-3). Some proteins are thought to play a structural role, whereas others are more actively involved in association with microtubules. The essential phosphoprotein Spc42p, for example, is present at the central plaque.

Eukaryotic organells: Mitochondria, endo-plasmic reticulum ER, Cytoplasmic microtubules**,** Golgi apparatus, Cytoplasmic inclusions (lipids, and glycogen) and Vacuole. Plasmalemma is a unit membrane. **Lomasomes**, vesicles appeared in pockets between the cell wall and the plasmamlemma.

<u>Septa in fungal cells are of three main kinds.</u>

a.complete, i.e. delimits reproductive structures**.**

b.simple and perforated lying at right angles to the axis of the hypha e.g. Ascomycota

c.complex e.g. Basidiomycota (excluding the rust and smut fungi). The septum central pore is thickened to form a barrel-shaped structure surrounding the pore termed as toroidal swelling. It is termed as dolipore septum. It is often overlaid by perforated ER called parenthesomes.

5- Hyphal growth

The hyphae are growing by extension at the extreme apex (Fig. 1.12). This accompanied with rapid synthesis of new wall and membrane components to provide the increase in area of the walls and plasmalemma. In **non-septated** fungi as ***Pythium ultimum*** Grove et al. (1970), stated that dictyosomes receiving membrane materials from blebs of ER from proximal side giving rise to vesicles on the distal face, that migrate toward hypal apex and fuse with the membrane and release their contents into the wall (Fig. 1.12). In **Zygomycota** there is a specialized organell called **chitosome** if it is incubated with **chitin precursor**, uridin diphospho N-acetyl-D-glucose amine, chitin microfibrils well produced. Thus, it is considered that **chitosome** microvesicles **are** transporting **chitin synthases** to growing cell wall. Chitosomes are either proctoid (anus like) or cycloid.

In septated fungi, specific organelles are involved in cell wall growth ***vize***; **Spitzenkörper** and **chitosome** (Fig. 1.13)**.** Spitzenkörper **is** associated with growing hyphal tips. It is only appeared as dark body in the growing hyphal apex and disappeared when growth cease (Grove et al., 1970).

6- Fungal tissues

Fungal tissue is called **plectenchyma (**Fig. 1.14). **Plectenchyma** occurs in **two** types:

(1) **Prosenchyma:** where hyphae are loosely woven and obvious as it such.

(2) **Pseudoparenchyma:** composed of closely packed hyphae which in sections resemble the parenchymatous tissues of higher plants.

7. Hyphal aggregation:

Hyphae may be **aggregated** and give rise to: mycelial strands**,** rhizomorphs, sclerotia (thick-walled resting bodies containing food reserves), harting network and reproductive structure

a) Mycelial strands (Fig. 1.15)**:**

It is an aggregation of parallel, relatively undifferentiated hyphae. It is quite common in ***Basidiomycota*** and in some ***Ascomycota*** and **their anamorphs**.

b) Rhizomorphs (Fig. 1.16).

It is a highly differentiated aggregations of hyphae with a well developed **apical** meristem, a central core of larger, thin-walled, elongated cells, and coat of smaller, thicker-walled cell which are often darkly pigmented e.g. ***Armillaria mellea***, (serious parasite of trees and shrubs). It helps the fungus to extended underground from one root to root

c) Sclerotia

Sclerotia are **pseudoparaenchymatous** aggregations of hyphae, which serve in survival that may be for long periods, sometimes for several years. They are common amongst plant pathogens. Sclerotia germinates by the development of mycelium, conidia, or by the formation of ascocarps or basidiocarps. They are present in several types: **loose,** e.g. ***Rhizoctonia solani***, **terminal *e.g. Botrytis allii*** and **strand,** e.g. ***Sclerotinia gladioli***.

(d) Harting network

A continuous sheath of mychorizal mycelium that covered the root-tips of many coniferous and deciduous trees, are extends outwards into the litter area of the soil, and inwards, intercellular between the cortical cells of the root, to form a **Harting network** (Fig. 1.17).

(e) Reproductive organs

In ***Ascomycota***, ***Basidiiomycota*** and ***Deuteromycota***, hyphae may become aggregated together to form fruiting structures (e.g. Ascocarps, basidiocarps and pycnidia).

8-Reproduction:

Reproduction in fungi normally occurs either asexually or sexually.

Sexual reproduction takes place by spores from **meiotically** derived nuclei. The fungus my be **Homothallic** (self) or **Heterothallic** (out-crossing).

Asexual reproduction occurs by spores with **mitotically** derived nuclei.

Spores are varied in structures shapes and size. They are unicellular or multicellular, colourless or pigmented, thin- or thick-walled. They may be produced asexually or sexually, thick-walled zygospores (or oospores), ascospores and basidiospores are spores formed as a result of sexual reproduction. These are called “**meiospores**”. Asexual spores are called “**mitospores**”.

I-Asexual Reproduction

Asexual reproduction in fungi either occurs by mitotically derived spores and conidia or by hyphal fragmentation, budding as in some unicellular fungi.

i. Asexual spores:

They are of two main types: sporangiospores and conidia.

1. Sporangiospores (Fig. 1.18):

In ***zygomycota*** and especially in ***Mucorales***, the sexual spores are contained in globose sporangia or cylindrical sacs termed as **aplanospores**. The motile sporingiospore is termed as "**Zoospores**". They are self-propelled by means of flagella and occurs in three kinds:

a. Posterioly uniflagellated zoospores with flagella of **whiplash** type, e.g. **Chytridiomycota**.

b. **Anterioly uniflagellated** zoospores with flagella of **tinsel** type e.g. **hyphochytridiomycetes**.

c. **Biflagellate zoospores,** with anterioly or laterally attached flagella one of which is of the whiplash type and the other of the tinsel type e.g. **Oomycetes**.

2. Conidia:

They are formed exogenously on hyphae or morphologically differentiated conidiophores. They are found in ***Ascomycota*** and ***Basidiomycota*** **anamorphs**.

There is a great variation in conidial ontogeny. The conidial development may be **thallic** or **blastic** (Fig. 1.19).

1- Thallic: used to describe development where there is no enlargement of the conidial initial,

2- Blastic:, conidial initial is enlarged before it is delimited by a septum: it is either "**Holoblastic** or **Enteroblastic**. The later either **tretic** or **phialidic**.

3.Chlamydospore:

It occurs in almost all **fungi** as terminal or intercalary segments of the mycelium, which become packed with food reserves and develop thick walls. The wall may be colourless or **melaninized**. (Fig. 1.120). They form important organs of asexual survival, e.g. ***Absidia glauca.*** In ***Saprolegnia*** spp, they break free from the mycelium and be dispersed in water current, this termed as **gemmae**.

II-Sexual Reproduction

The reproductive organs in fungi are known as gametangia, which is the structure that contains sex cells called gametes. gametangia are Isogametangia or heterogametangia that produce isogametes or heterogametes. The male gametophyte is called Antheridium and the female gametophyte is called Oogonium. Sexual reproduction occurs by fusion of two compatible nuclei and occurs in the following three sequential steps:

1- **Plasmogamy** (both protoplasts fused together)

2- **Karyogamy** (nuclear fusion). This may be delayed and the result is a **dikaryotic** stage, thus the mycelium have both **monokaryotic** and **dikaryotic** stage, the first called primary mycelium and the second called secondary mycelium as in **Basidiomycota.** Nuclei fused later.

3- **Meiosis**. Followed it immediately.

These steps either followed by Development of fruiting bodies or other structures.

Sexual reproduction in fungi is varied, it is three main types, **isogamous**, **anisogamous** and **heterogamous** (i.e. morphologically distinguishable gametes, gametes are different in size and morphologically similar but differ in size). Fungi are either **homothallic** and called self-fertile, or **heterothallic** and termed as self-sterile.

Methods of sexual reproduction:

1-Planogametic Copulation (Mating of motile gametes) Fig. 9a:

It involves the fusion of two naked gametes, one or both of which are motile. The motile gametes are known as planogametes. The most primitive fungi produce motile isogametes while Anisogametes of similar morphology and of varying size are produced by only one group of primitive fungi. The female gamete is non-motile (Oosphere) and the male gamete is mobile (Antherozoid) and the latter penetrates into the oogonium for fertilization.

2- Gametangial Contact

Where two heterogamous gametes touch, but both the male and female gamete are immobile and the gametes are never released from the gametophyte to the outside, but they move directly from one gametophyte to another, and one or more gametophytic nuclei move from the male gametophyte to the female, and There is no actual fusion between the gametangia or they lose their autonomy by any means during the sexual process. In some species, the male nuclei penetrate through a hole formed as a result of the dissolution of the gametophyte walls at the point of contact, and in other types, a fertilization tube emerges from the antheridium (male) that is used as a passage for the male nuclei. After completing the passage of the nuclei, the oogonium continues to reveal it in various ways, and the antheridus soon dissolves.

3. Gametangial Copulation

This pathway is characterized by the fusion of all the contents of two touching gametangia, and such fusion occurs in one of two ways:

1-Passage of the contents of one gametangium over another through a hole formed in the walls of the gametangia at the point of contact, and this method is characterized by some fully fruiting fungi.

2-Direct fusion between two gametangia to form one cell, and this fusion takes place by the dissolution of the contact walls between the two gametangia, resulting in a common cell in which the protoplasts of the two cells mix.

4. Spermatization:

Some fungi carry tiny, numerous, single-nuclear male structures, similar to spores and known as spermatia. They are produced in various ways and are transmitted to the female gametangia by insects, wind or water, where they attach to specialized receptor fungal filaments or even to the somatic fungal hyphae themselves. A hole is formed at the point of contact through which the contents of the spermatia are transmitted to the special receptor structure, which is used as a female organ.

5-Somatogamy:

In higher fungi such as Ascomycetes and Basidiomycetes, sometimes sexual organs do not form at all in many higher fungi, and the somatic cells perform the sexual function (Fig. 1.25). The sexual process is carried out simply by the fusion of two fungal hyphae belonging to opposite strains. Post-fertilization changes lead to the production of a fruitful body

7- Parasexuality

We mentioned earlier that the three stages of sexual reproduction are plasma fusion, nuclear fusion, meiosis, and Karyogamy Plasmogamy, and they occur in a specific place and time and in succession regularly, but in special cases the process of producing sexual individuals occurs and passes in three stages as mentioned previously, but its stages do not fall in a way sequential and not in one specific place as in the normal sexual process.

This process occurs in the imperfect fungi that have not discovered a sexual phase to form generations that do not resemble the parents. This process was first discovered in 1956 in Pontecorvo at the University of Glasgow in the United Kingdom.

The benefit of the paragenital process in the imperfect fungi is that they form individuals that are not similar to the parents, and thus evolution occurs in successive generations.

The successive processes of parasexuality are:

(1). Formation of **heterokaryotic** hyphae, which are the hyphae that contain more than one nucleus that carry genetic codes that are not the same, i.e. sexually compatible. This process occurs in fungi in several ways, including genetic mutations, synapsis of hyphae, or fission of binary nuclei.

(2). **Karyogamy** is the union of compatible and incompatible nuclei where in some cases the cytoplasm contains at least five types of haploid or diploid nuclei.

(3). **Miotic** crossing over, which is the transfer of some parts of a chromosome to another chromosome, which leads to a change in the positions of the nitrogenous bases in the DNA and a change in the genetic traits that it carries and the emergence of generations that are not similar to the parents in the deficient fungi.

(4). Sorting out of diploid: haploid nuclei unite to form diploid nuclei, which grow into diploid nuclei.

(5). **Haploidization** is the process of reducing the number of chromosomes in diploid nuclei to haploid nuclei.

i. Sexual spores

1. Ascospores:

Ascospores are the characteristic spores of large group of fungi known as ***Ascomycota*** **(Fig 1.26)**. They are mostly found in a cylindrical sac called **Ascus**. Ascospores may be uninucleate or multinucleate, unicellular or multicellular, divided up by transverse or by transverse or longitudinal septa. The wall of the spore is multilayered, as in ***Neurospora***; the **endosporium**, **episporium**, and **perisporium**. Spore **germination** occurs by the production of germ tubes from germ pore, or thin area in the episporium, at either end of the spore.

2.Basidiospores:

Basidiospores are the characteristic spores of Basidiomycota. They are attached to the tip of **sterigma** that projecting from the apex of the **basidium (**Fig. 1.28**)**. The point of attachment is called **hilum**, that is usually found at the tip of a short conical projections, the **hilar** appendix. They are unicellular, and **rarely** septates, varied in shape, colour and size The spores are normally **binucleate**. Comparatively, they are more identical than ascospores. The wall is multilayered, composed of five layers that develop gradually from the outside in wards; ended with **endosporium** (electron – leucent) and **episporium** (electron – opaque, lamellar organisation), followed by **exosporium** and **ectosporium**. **Germination** occurs through a germ pore, usually at the apex of the spore where, the wall layers except endosporium are thin.

3. Zygospore: (Fig. 1.29)

It is the perfect stage of ***Zygomycota***, e.g. ***Mucorales.*** Zygospores are sexually produced following plasmogmy between gametangia that are usually equal in size.

4. Oospore:

is the sexual spore of **Oomycetes**. It is a result of gametangial copulation between male and female gametes

<u>7. Life cycles</u>

1- Sexual cycle

The two essential stages that usually governed the type of life cycles are karyogamy and meiosis.

(a) Haplobiontic A Life Cycle.

Haplobiontic means single generation, where meiosis occurs immediately after karyogamy and then mitosis as follow: Nuclear fusion - meiosis - mitosis. It is common in ***Mucorales*** and ***Ascomycota***.

(b) Haplobiontic B life cycle.

It occurs as follow: Nuclear fusion - mitosis - meiosis. Thus, the vegetative stage thallu is diploid e.g. many ***Oomycota*** and ***Saccharomyces cervisiae***. In ***Basidiomycota*** the cycle is modified: the primary mycelium with each is **monokaryotic**. After plasmogamy a **dikaryotic** mycelium has produced, with clamp connection and basidia are produced on this mycelium.

(c) Diplobiontic life cycle.

Alteration of generations between a haploid and diploid stages. It occurs as follow: Nuclear fusion - mitosis - meiosis - mitosis It is unusual**,** but has been described in certain members of the ***Blastocladiomycota***, e.g. ***Allomyces***:

2- Non-sexual cycle:

It occurs in asexual fungi which called **Mitosoric fungi** (***Deutermycota, Fungi Imperfecti***).

3- Parasexual cycles: Why anamorphic fungi are remarkably abundant and successful in all habitats, in spite of lacking sexual cycle. Such fungi could have some mechanism for recombination enable them to adapt to environmental changes in any ecosystem. This is a kind of recombination without **meiotic** segregation and termed as **parasexual** recombination (Roper, 1966). Example: try of plant breeders to breed resistant plant varieties to species of ***Fusarium*** and ***Verticillium*** have frequently been frustrated by the appearance of “new” races of pathogens capable of causing severe disease symptoms in resistant varieties. This is a result of **genetic recombination**. The important events:

1- A**nastomosis** of genetically distinct hyphae to form **heterokaryon**,
2- Nuclear fusion to form diploid nuclei.
3- Multiplication of diploid heterozygous nucleus at the main time with the parent haploid nuclei.
4- Eventually, strain of homokarytic diploid mycelium was produced
5- Mitotic crossing over during multiplication of diploid nuclei.
6- Vegetative haplodisation of the diploid nuclei.

True meiosis does not occur. The outcome of these **events** are:

It is possible to follow inheritance in anamorphic fungi, it is possible to breed anamorphic fungi.

It explains how variation can occur in anamorphic fungi.

1.3. Evolution and Phylogeny

Phylogeny

Phylogeny is the evolution of a group of organisms through time. It is based on comparative morphology, cytology, hyphal wall chemistry, ultcastracture, and occasionally fossils.

Going back early of the last century, **two kingdoms (Plantae, Animalia)** are proposed as simplified in Fig. 1.31. The fungi were accepted as a single kingdom along with bacteria (Monera), plants (Plantae), animals (Animalia) and protists (Protista) according to the five kingdoms of Whittaker (1969) (Fig. 1.32). Advances in ultrastructural biochemical, and molecular biology, fungi were distributed into **3** kingdoms. **Thus** at least **7 kingdoms** are recognized: **Eubacteria**, **Archaebacteria**, **Animalia**, **Plantae, Fungi, Chromista, Protoctista (Protozoa, Protista)** (Fig. 1. 33).

The **three** kingdoms including fungi (Chromists, Protozoa & Fungi) are shown by molecular studies of 16S–like rRNA sequences to be phylogenatically remote. According to the molecular evidence, Fungi have originated from Protozoan ancestors before the kingdom **Animalia** and **Plantae** split.

The more advanced Classification

s-RNA gene sequence analysis as a tool for microbial systematics and ecology

Ribosomes are organized into a large subunit and a small subunit, as shown in Figure 1.34 prokaryotic ribosomes contain over 50 different types of protein and those of eukaryotic cells contain more than 80 different types of protein). The large subunit contains two different ribosomal RNAs in prokaryotes and three in eukaryotes, while the small subunits contain one ribosomal RNA'. Both prokaryotic and eukaryotic ribosomes can be broken down into two subunits (the S in 16S represents **Svedberg units**), nt= length in nucleotides of the respective rRNAs, for exemplary species *Escherichia coli* (prokaryote) and human (eukaryote).

In **prokaryotes** a **small 30S** ribosomal subunit contains the **16S ribosomal** RNA. The **large 50S** ribosomal subunit contains two rRNA species **(the 5S and 23S ribosomal RNAs).** In contrast, **eukaryotes** generally have many copies of the rRNA genes organized in tandem repeats. The **18S rRNA** in most eukaryotes is in the small ribosomal subunit, and the large subunit contains three rRNA species **(the 5S, 5.8S and 28S in mammals, 25S in plants, rRNAs).**

Type	Size	Large subunit (LSU rRNA)	Small subunit (SSU rRNA)
prokaryotic	70S	50S (5S : 120 nt, 23S : 2906 nt)	30S (16S : 1542 nt)
eukaryotic	80S	60S (5S : 121 nt,[1] 5.8S : 156 nt,[2] 28S : 5070 nt[3])	40S (18S : 1869 nt[4])

Phylogenetic analysis of rRNA gene sequences (particularly ss-rRNA, i.e., the small subunit rRNA) has led to important advances in microbiology, such as the discovery of a third branch on the tree of life (the archaea) and the realization that the microbes that can be grown in pure culture represent but a small fraction, in terms of both phylogenetic types and total numbers of cells of the microbes, found in nature.

The power of ss-rRNA for phylogenetic analysis can be attributed to many factors, including its presence in all cellular organisms, its favorable patterns of sequence conservation that enable study of both recent and ancient evolutionary events, and the ease with which this gene can be cloned and sequenced from new organisms.

life
Domain I: Eukarya

Eukaryote name, comes from the Greek **ευ**, meaning "**good/true**", and **κάρυον**, that means "**nut**". The last few years with advances in molecular phylogenetics, particularly in analytical methods, and in the diversity of organisms for which data are available, as well as a maturing of knowledge about some key features of eukaryotic cells.

The figure on the left is the eukaryotic phylogenetic tree from 1993 (based on small subunit ribosomal (r)RNA sequences provided an intuitively appealing evolutionary tree of eukaryotes. Complex eukaryotes, including animals, fungi, plants and most algae, emerged as a broad radiation usually called the 'eukaryotic crown'. Below this 'crown', more bizarre, and generally simpler, organisms diverged in a ladder-like succession. The small subunit rRNA tree was 'rooted' with mitochondrion-lacking unicellular eukaryotes such as diplomonads, parabasalids and microsporidia forming the basal branches (**Figure 1.35**). Simpson and Roger explain (2002) explain that animals, fungi and their relatives may be at the 'basal' position in the eukaroytic tree fits more comfortably with front-loading than the old 'crown' view. It's almost as if these complex, ancestral eukaryotic cells were poised to become animals very early on, while the algae, diatoms, or paramecia missed the cue and shot off on tangential pathways.)

Eukaryotes at least can now be divided into just a **6 major groups** that are probably all monophyletic. The following sections introduce each of these major groups:

1-**Opisthokonts** (Animals, fungi, choanoflagellates, etc.
2- **Amoebozoa** (Most lobose amoebae and slime moulds)
3-**Rhizaria** (Foraminifera, Radiolaria, and various other amoeboid protozoa)
4-**Excavates** (Various flagellate protozoa)
5- **Archaeplastida** (or Primoplantae) Land plants, green algae, red algae, and glaucophytes
6-**Chromalveolates** Heterokonts, Haptophytes, Cryptomonads, and Alveolates.

Two larger **clades**, the **unikonts (**uniflagellar) and the **bikonts (**biflagellate), were recognized (Fig. 1.36). The **opisthokonts** and **amoebozoans** are considered **unikonts**, and the rest are **bikonts**.

The protein phylogenies and a complex fusion of pyrimidine synthesis genes suggest that **Opisthokonta** is most closely related to **Amoebozoa**. This grouping has been called '**unikonts**'. Interestingly, a different fusion character, involving the genes for **dihydrofolate reductase** and **thymidylate synthase**, suggests that all other major groups of eukaryotes might be specifically related to each other. Pending confirmation from other evidence, these data imply that the root of eukaryotes falls between **unikonts** and everything else, along the branch indicated by an arrow in Figure . 1.35.

Clade : Opisthokonta

Opisthokonts (Greek: **οπίσθω-** (opisthō-) = "rear, posterior" + **κοντός** (kontos) = flagellum) Large monophyletic group of eukaryotes, Opisthokonta contains **animals** and **true fungi**, as well as several unicellular groups, including the free-living choanoflagellates, a diverse range of parasitic forms called Ichthyosporea or Mesomycetozoea, and a group of free-living amoebae called the nucleariids. Two significant groups of spore-forming parasites, myxozoa and microsporidia, often considered as protists, turn out to be animals and fungi, respectively (Fig. 1.36). Motile cells are posteriory flagellated (so the group's name). In contrast, flagellate cells in other eukaryote groups propel themselves with one or more **anterior** flagella. **Cavalier-Smith** (1987) suggested a close relationship between **animals** and **fungi** that confirmed later by genetic studies. He and **Stechmann** dispute that **unikonts** split off from **bikonts**, shortly after they evolved.

Kingdom 1: Fungi

Fungi" are pragmatically defined as **eukacyotic**, **heterotrophic**, develop branching filaments (or rare more rarely single-called) and reproduce by spores.

Scientific classification

Domain: Eukarya
(**unranked**) Opisthokonta
Kingdom: Fungi (Moore, 1980)
Phyla: Chytridiomycota,Blastocladiomycota, Neocallimastigomycota, Glomeromycota, Zygomycota
Subkingdoms: Dikarya; Ascomycota, Basidiomycota and their anamorphs.

General characters

Fungi are generally have thallus made of hyphae (microscopic filaments of between 2-10 microns in diameter and up to several centimetres in length), which combined to form mycelium. Some fungi, such as yeasts, grow as single ovoid cells.

I. Characteristics shared with animals:

1- Fungi are heterotrophic organisms, lack chlorophyll.

2- **Glycogen** (Lomako et al., 2004) is stored form of carbohydrates.

3- Cell wall is mainly composed of chitin (N-containing carbohydrate) (Bowman and Free 2006). Chitin also present in some animals, such as the insects and crustaceans

4. Fungi have flattened mitochondrial cristae like most animals,

5. Fungi, synthesize lysine by the **α**-amino adipic acid (AAA) pathway as do animals .

II- Characteristics shared with plants:

1. The presence of vacuoles in the cell, (Shoji et al., 2006).
2. Similar pathway in the biosynthesis of **terpenes** using **mevalonic** acid and **pyrophosphate** as biochemical precursors; **plants** however use an additional terpene biosynthesis pathway in the chloroplasts that is apparently absent in fungi (Wu et al., 2007).
3. **Production** of a **plethora of secondary metabolites** functioning as defensive compounds or for niche adaptation; however, biochemical pathways differ markedly between fungi and plants (Siewers et al., 2004; Tudzynski, 2005).
4. They absorb nutrients from the surrounding environment.
5. Fungi are normally lack an efficient vascular system, but some fungi, such as ***Armillaria***, form **rhizomorphs** or **mycelial cords** (Mihail and Bruhn, 2005) resembling and functionally related to, but morphologically distinct from, plant roots.
6. Chromoalveolates synthesize lysine by the diaminopimelic acid (DAP) pathway like green algae, vascular plants and some bacteria (Xu et al., 2006).
7. Chromoalveolates fungi have tubular mitochondrial cristae.
8. Chromoalveolates and plant cell have cellulose in their walls.

Evolution

For a long time as mentioned in this chapter, taxonomists considered fungi to be members of the Plant Kingdom. However, fungi are now considered a separate kingdom, distinct from both plants and animals, from which they appear to have diverged approximately one billion years ago (Bruns, 2006).

At the most, fungi appear to be more closely related to animals than to plants, and are placed with the animals in a monophyletic group called **opisthokonts** (Sitte et al., 1991) (Fig. 1.37).

In addition to the above treats, RNA analyses declared that fungi are closer to **Animalia** than **Plantae**. It is also indicated that animal lineage is monophylytic and includes choanoflagellates. Thus it was inferred that animals and fungi share a unique evolution history and that their last common ancestor was a flagellated protest similar to existing choanoflagellates (Wainright et al., 1993). Thus all the above data support **atkinson's** (1909) opinion that fungi were derived from colourless organisms "lower" than the chytridiomycota. Finally, analyses using molecular phylogenetics support a monophyletic origin of the Fungi. (Hibbett, *et al.* 2007a).

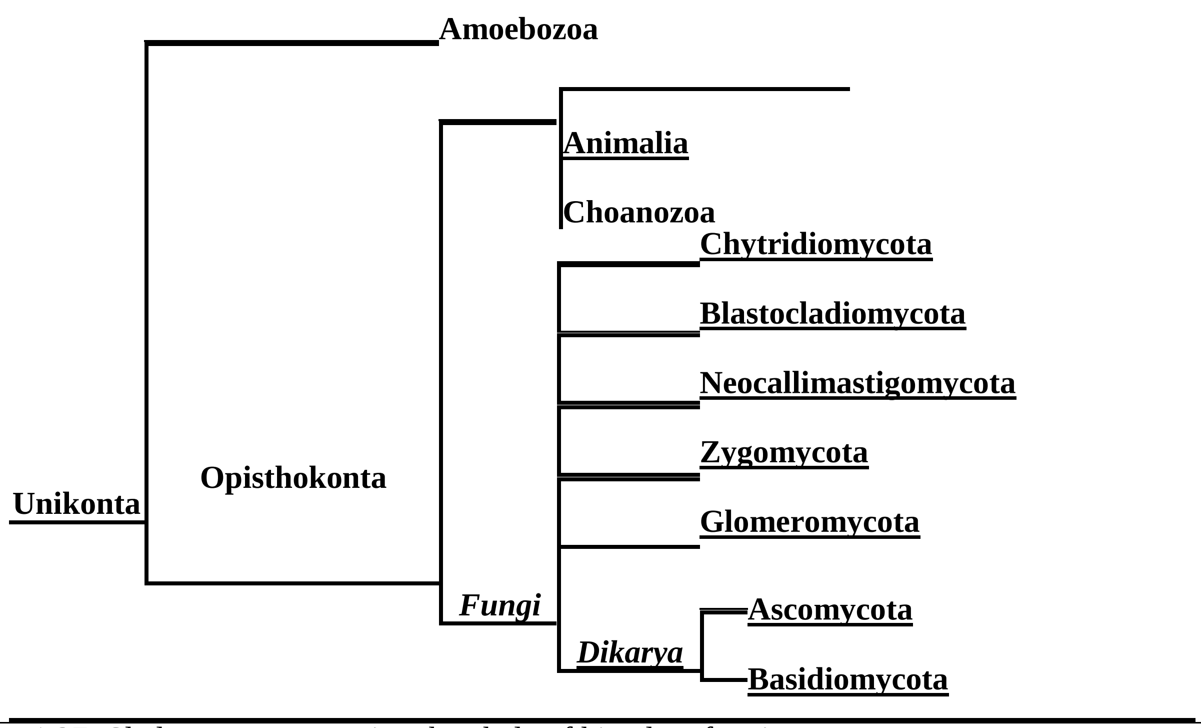

Fig. 1.37. Cladogram representing the phyla of kingdom fungi.

Fungal era

Fungi appear in **Paleozoic** Era as aquatic organisms similar to **Chytrids** (James et al., 2006). The Terrestrial fungi appeared in the **Silurian**, right after the first land plants appeared, and shortly after the **Permian-Triassic** extinction event, a fungal spike, detected as an unique abundance of fungal spores in sediments. This indicates that fungi were dominant during this period—nearly 100% of the fossil record available from this period (Eshet et al. 1995).

Clade: Amoebozoa

Most of the cells that move and feed using broad or finger-like pseudopodia are grouped together as the Amoebozoa. They are a major group of amoeboid protozoa, including the majority that moves by means of internal **cytoplasmic** flow.

General characters

1. **Pseudopodia** are characteristically blunt and finger-like, called lobopodia.
2. Most are unicellular. It is also include the slime moulds, multinucleate or multicellular forms that produce spores and are usually visible to the unaided eye.
3. Common in soils and aquatic habitats.
4. **Most Amoebozoa** are free-living heterotrophs that engulf other cells using their pseudopodia (some large amoebae eat small animals!).
5. There are several facultative or obligate parasites, for example the amoebic dysentery agent ***Entamoeba histolytica***, responsible for 40,000–100,000 deaths per year worldwide.
6. The slime moulds are amoebae that periodically form a stalked spore-producing phase called a fruiting body. For this reason alone slime moulds used to be treated as fungi, when other Amoebozoa were considered to be animals..
7. **Size: they are greatly variable in size, many small (10-20 µm in size), and many are of large size** e.g. ***Amoeba proteus*** (800 µm in length), **multinucleate amoebae** e.g. ***Chaos*** and ***Pelomyxa*** (several **millimetres** in length), and some **slime moulds** cover several square feet.

Classification

This kingdom passed with several modifications in their classification (Cavalier-Smith and Chao,1996; Cavalier-Smith, 1998; Patterson, 1999; Baldauf, *et al*., 2000;). In fact, amoebozoa (based in proteomes) form a **stem** group to animals and fungi, diverging from this lineage after it had split from the plants, (Eichinger, *et al.* 2005).

Kingdom: Amoebozoa (Lühe, 1913 emend)

Phyla: Mycetozoa (slime molds), **Archamoebae**, **Tubulinea**, **Flabellinea**

Uncertain placement: Acanthopodida, Stereomyxida

<u>Phylum: Mycetozoa</u>

Slime Molds is a popular term of this phylum, often referring to roughly six groups of Eukaryotes. This group fit into the **unikont** supergroup **Amoebozoa**, whereas the others fit into various **bikont** groups. Originally, they were considered primitive fungi, but now they are **amoebozoa** and split into the following groups:

1. **Myxogastria**, Liceida, Echinosteliida, Trichiida, Stemonitida, Physarida. (plasmodial slime molds).
2. **Protostelia,** Protosteliida: smaller plasmodial slime molds.
3. **Dictyostelia,** Dictyosteliida: unicellular slime molds.
4. **Acrasidae**: similar life style to Dictyosteliids, but of uncertain taxonomy.
5. **Plasmodiophorids**: cabbage club root disease.
6. **Labyrinthulomycetes**: slime nets.

Clade: Plantae

Primary endosymbiosis' describes the origin of a eukaryotic organelle by the engulfment, enslavement and genomic reduction of a prokaryotic cell. Three photosynthetic groups have plastids (chloroplasts) that originated by primary endosymbiosis: land plants (embryophytes) and green algae such as Chlamydomonas; red algae (rhodophytes); and an obscure group called the glaucophytes. Phylogenies of several plastid genes and the organisation of plastid genomes suggest that the plastids of these groups form a single lineage specifically related to cyanobacteria. So primary plastid endosymbiosis seems to have happened just once in eukaryotic evolution, with the host being a common ancestor of these three groups.

Clade: Chromalveolata

Chromalveolata was first proposed by Cavalier-Smith as a modification of kingdom **Chromista** that was proposed in 1981 by the same author. It is now currently regarded as one of six major clades of eukaryotes, and considered as kingdom in this course. It is descended from a **bikont** which carry out secondary **endosymbiosis** with a red alga, and includes the following **four groups**:

Heterokontophyta , Haptophyta , Cryptophyta and **Alveolata.**

In '**secondary endosymbiosis'** a eukaryote already containing a primary plastid is engulfed by another host eukaryote, and over time is reduced to an organelle. The new plastid-containing host is termed a 'secondary alga'. **Secondary endosymbiosis** has happened more than once in eukaryotes, but mounting evidence from plastid gene trees and a distinctive gene replacement event suggests that most groups of secondary algae descend from one particular endosymbiosis involving a red algal symbiont. These organisms, plus their many non-photosynthetic relatives, comprise the group **Chromalveolata**.

The chromalveolates unites four major groups of eukaryotic algae: **dinoflagellates**, **cryptophytes**, **haptophytes** and **stramenopiles** (**~heterokonts**), and many non-photosynthetic forms (see below). The first three groups are unicellular, with a few colonial forms. **Stramenopiles**, however, range from tiny unicells, through to elaborate unicells and colonies, for example diatoms, and truly multicellular and massive life forms, such as kelps. **Dinoflagellates** and **diatoms** are the dominant 'large' phytoplankton in the ocean.

Dinoflagellates and **stramenopiles** also include a wide diversity of heterotrophic forms (and **mixotrophs**, organisms that subsist by both photosynthesis and heterotrophy). <u>**Heterotrophic stramenopiles**</u> are very important consumers of bacteria in aquatic environments, but also include some animal parasites/commensals, and a diversity of fungal-like forms. For example, the Irish potato famine pathogen, ***Phytophthora infestans***, is an oomycete stramenopile. **Heterotrophic and mixotrophic** dinoflagellates are important micro-predators in the plankton, and there are numerous parasitic forms.

Despite its apparent algal origins, Chromalveolata includes two of the best known groups of non-photosynthetic microbial eukaryotes. **Ciliates** are dominant micro-predators in many habitats, and include several important laboratory models, such as ***Paramecium*** and ***Tetrahymena***. **Apicomplexa** are arguably the most successful group of specialist parasites on earth, and include the agents of toxoplasmosis, cryptosporidiosis, coccidiosis and, of course, malaria (Plasmodium). Although **Apicomplexa** live inside their hosts, usually in total darkness, most have retained a non-photosynthetic plastid, betraying their algal ancestry and providing a tempting target for novel drug therapies.

Evolutionary relationship:

Chromalveolata are descend from a heterotrophic bikonts and are more closer to **Archaeplastida** than with the other groups, in a clade **Corticata**. They are characterized with, 1. **cellulosic** cell walls. 2. **photosynthetic** ability, and rarely morphologically resemblance to the **Embryophyta. Thus** they considered as plants. After much research, **Chromalveolata** has been proposed as a **monophyletic** group, but the **monophyly** of this group is not yet established (Burki et al., 2007; Parfrey et al., 2006).

Scientific classification

Domain: Eukaryota
(**unranked**) Corticata
Kingdom: Chromalveolata (Cavalier-Smith, 1998)
Phyla : Heterokontophyta, Haptophyta, Cryptophyta & Alveolata

Ciliophora , Apicomplexa, Dinofilagellata

Phylum: Heterokontophyta (stramenopiles)

The **heterokonts** or stramenopiles are a major line of eukaryotes presently containing about 10,500 known species (Patterson, 1999). He named this extended group the **stramenopiles**, due to the presence of **tripartite mastigonemes**, mitochondria with **tubular cristae**, and **open mitosis**. Most are **algae**, ranging from the giant **multicellular kelp** to the **unicellular diatoms**, which are a primary component of plankton. **Others** (generally parasitic) are belong to **Oomycetes**, including ***Phytophthora*** of Irish potato famine infamy and ***Pythium*** which causes seed rot and damping off.

Classification

Classification are varies considerably, where the **heterokont algae** were treated as two divisions, first within the kingdom **Plantae** and later the **Protista** as follow:

Division Chrysophyta: Class Chrysophyceae (golden algae); Class Bacillariophyceae (diatoms)

Division Phaeophyta (brown algae)

The discovery that **Oomycetes** and **hyphochytrids** are related to these algae, rather than fungi as previously thought, has led many authors to include them among the **heterokonts** and in turn they evolved from colored ancestors, the group would be paraphyletic in their absence. Cavalier-Smith treats the **heterokonts** as identical in composition with the **stramenopiles**. He proposed placing **heterokonts** in a separate kingdom called **Chromalveolata**, besides **haptophytes**, **cryptomonads** and **alveolates**. This revision has not been generally adopted.

Typical classes

Colored groups		**Colorless groups**
Chrysophyceae (golden algae)	Bolidophyceae	**Oomycota** (water moulds)
Synurophyceae	Raphidophyceae	**Hyphochytridiomycota**
Actinochrysophyceae (axodines	Eustigmatophyceae	Bicosoecea
Pelagophyceae	Xanthophyceae (yellow-green algae)	**Labyrinthulomycota** (slime nets)
Phaeothamniophyceae	Phaeophyceae (brown algae)	Opalinea
Bacillariophyceae (diatoms)		Proteromonadea

Kingdom: Rhizaria

Rhizaria unites a wide diversity of free-living unicellular organisms, many of which feed using fine ‘filose’ pseudopodia, together with some fungi-like plant parasites, such as **plasmodiophorids**, and some animal parasites, for example ***Haplosporidia***. The best-known free-living Rhizaria are Foraminifera and Radiolaria. The exact relationships amongst all of these organisms is still uncertain – most likely Radiolaria are the sister group to all the others, most or all of which form a large subgroup called Cercozoa.

Kingdom: Excavata

Excavata are unicellular eukaryotes, most of which are heterotrophic flagellates. They include several groups that cause significant disease, such as trypanosomatids, diplomonads and parabasalids, which include the agents that cause sleeping sickness, giardiasis and trichomoniasis, respectively. But each parasitic group has free-living relatives that consume other microbes, often capturing them out of suspension using a distinctive feeding groove. Many excavates have greatly modified mitochondria that are not used for oxidative phosphorylation, and these cells are common in low-oxygen habitats, including animal guts. Members of one group, Heterolobosea, have evolved as broad-pseudopod-forming amoebae independently of Amoebozoa, and even include their own group of slime moulds, the acrasids. Another group, the euglenids, includes another independent lineage of secondary algae; the laboratory standard Euglena is an example.

CLASSIFICATION

Classification is the systematic arrangement of organisms into groups based on established criteria. **The science** of classification is called **taxonomy. Systematics** is the study of the relationships and classification of the living world in a system or conceptual hierarchy. Taxonomy, nomenclature are subcategories of systematics. **Taxonomy** is the study of the theory, practice and rules of classification of living and extinct organisms. **Nomenclature** is the "allocation of scientific names to the units a systematist considers to merit formal recognition." (Hawksworth et al., 1995. The Dictionary of the Fungi). **The nomenclature** of fungi is governed by the **International Code for Botanical Nomenclature**, latest edition 1994, as adopted by the International Botanical Congress in order to prevent the use of confusing or misleading terminology**.**

Depending on the criteria used for classification, taxonomy is of several kinds:

1- Classical taxonomy (classification of plants and animals is based on morphologY).
2- Biochemical taxonomy (based on distribution of chemical compounds).
3- Numerical taxonomy (applies mathematical procedures).
4- Cytotaxonomy (based on characters of somatic chromosomes).
5- Experimental taxonomy (based on analysis of various patterns of variations).

Thus, we have, Organisms are classified in a hierarchial series of groups.

Hierarchical Classification

Kingdom: Fungi
Phylum: Basidio<u>mycota</u>
Sub-phylum: Agaricomycotina
Class: Agaricomycetes
Order: Agaric<u>ales</u>
Family: Agaricac<u>eae</u>
Genus: Agaricus
Species: *Agaricus campestris* L.

Agaricus is the genus and *campestris* is the specific epithet. The genus plus species is the Latin binomial; note that the genus and species are in italics (or <u>underlined</u>), the genus is capitalized and the species epithet is in lower case. This is the type species of ***Agaricus***. L. stands for Linnaeus, the authority.

Typological Species Concept:

Species are as many as were created in the beginning by the Infinite (Linnaeus, 1758). Each species represented by a type specimen, designated in the original description and deposited in a recognized collection (eg., herbarium). The name is tied to the type specimen. The type specimen is not necessarily typical of the entire species!

Agaricus bisporus (Lange) Imbach. Lange and Imbach are both considered authorities for this species. Lange was the first to describe this fungus, but as *Coprinus bisporus* and Imbach later transferred the species *bisporus* to the genus *Agaricus*

Authority –the author of a scientific name of a taxon; the person/persons who formally described and published the name. What if there are several mycologists named Lange, how do we know which one did the work? Standard abbreviations of authority names can be found in the *Dictionary of the Fungi* (Hawksworth, *et al.* 1996).

What is a species?

Most fungi are **morphospecies**, and represented by a type specimen. A species may be

1. B**iological species:** is a population, or a group of populations, among which there is interbreeding. Two individuals might not be able to interbreed, but they are still members of the same species (**conspecific**) if they are part of the same gene pool.(**Futuyma, D.J. 1998. *Evolutionary Biology***).

2. P**hylogenetic species:** is "irreducible (basal) cluster of organisms diagnosably different from other such clusters, and within which there is a parental pattern of ancestry and descent" (**Cracraft, J. 1989, *Speciation and Its Consequences***).

CHAPTER 2

Kingdom: Amoebozoa

Classification

Fungi **which posses a** plasmodium **or** pseudoplasmodium**, rather than the "hypha" as the thallus or somatic phase, are placed in kingdom protozoa, in phylum** Mycetozoa**, within classes** Protostelia**,** Myxogastria**,** Dictyostelia and Plasmodiophorids**.** Ainsworth's **classification treated them parallel to fungi whereas in** Hawksworth *et al.* (1996), **they moved to kingdom protozoa. Ultimately they are included within kingdom:** Amoebozoa **(*Cavalier-Smith, 1998) and divided into six classes namely;***

1. **Myxogastria**/**Myxomycetes**: plasmodial or coenocytic slime molds.
2. **Protostelia**: smaller plasmodial slime molds.
3. **Dictyosteliida**: unicellular slime molds.
4. **Acrasidae**: similar life style to **Dictyostelids**, but of uncertain taxonomy.
5. **Plasmodiophorids**: cabbage club root disease.

Recent classification

Domain**: Eukaryota**

(Unranked) Unikonta

Kingdom**: Amoebozoa Lühe, 1913 emend.**

Phylum**: Mycetozoa (slime molds)k**

Class1**: Protostelia (**Order **Protosteliida);** Class2**: Myxogastria (**Orders: **Liceida, Echinosteliida, Trichiida, Stemonitida, Physarida);** Class3**: Dictyostelia-**Order **Dictyosteliida;** Class4: Acrasidae; Class5: **Plasmodiophorids;**

Phylum: Mycetozoa (slime molds)

Mycetozoans fungi include cellular and plasmodial slime molds plus protostelids. Actin, elongation factor, and β-tubulin phylogenies place the plasmodial and cellular slime molds as a monophyletic group close to animals and fungi **(Baldauf and Doolittle, 1997). The names** Myxomycota **and** Mycetozoa **imply affinity toward fungi.**

Mycetozoa share fungi in the following characters:

1.The **fruiting phase** show a close relationship with fungi.

2.The **plasmodium** and **hypha** are similar; both **coenocytic** and show cytoplamic streaming.

3.The cytoplasm moves uninhibited in different directions within plasmodium (absence of **rigid wall**) while in a hypha it is confined within a **tubular track**.

4.The **wall-less somatic** phase is found also in some true fungi, like ***Coelomomyces***.

Salient feature

1- Common on moist soil, decaying wood and other organic substrata.

2- **Vegetative phase** (Fig. 2.1): free-living plasmodium.

3- **Nutrition**: plasmodium feed by engulfing bacteria, fungal cells, etc.,

4- **Sporophores:** arise from the plasmodium under suitable conditions; of diverse Shapes.

5- **Hypothallus:** Beneath the developing sporophores, deposited by the plasmodium **(**Fig. 2.2**)**.

6- **Spores** are dispersed by wind and germinate to form myxamoebae or zoospores.

7- **Myxamoeba**: reproduce asexually by fission, and the plasmodium is capable of fragmentation.

Sexual reproduction: by fusion of **myxamoebae** or **zoospores** to form **zygotes** from which plasmodia develop.

9- A plasmodium is multinucleate motile mass of protoplasm bounded by a plasma membrane but lacking a wall and generally **reticulate** and **coenocytic.**

10- Pseudoplasmodium: formed by the massing of separate myxamoebae (Fig. 2.3).

a-**When food supply becomes** exhausted or population reaches certain size, amoebae enter a starvation period, undergo developmental changes, Metabolic changes – shift from facultative aerobes to obligate aerobes, Use endogenous reserves, Cell surface antigens change, cells become more cohesive.

b. **Aggregation:** Certain amoebae secrete a **chemotactic substance – acrasin.** Acrasin in ***Dictyostelium*** is cyclic **AMP**, other species produce other substances. **Acrasin** causes other amoebae to migrate toward the center of production

c. **Amoebae aggregate** to form **pseudoplasmodium** (slug, grex). Transition from population of independent cells to a multicellular structure. **Pseudoplasmodium** in *D. discoideum* is 1-2 mm long and moves along gradients of temperature, light, humidity, is surrounded by a sheath of polysaccharide and protein.

in pulsating streams.

Class: Myxogastria

General characteristics

These organisms are true slime moulds, or plasmodial slime moulds, to distinguish them from the cellular slime moulds. They produce spores (endospores) internally, within fructification **that is first enclosed in a thin membrane called** peridium**.**

1-Spores

1. **Spores** are unicellular, usually globose, with smooth, spiny, or reticulately thickened walls, variously pigmented from colourless or yellow to brown or purple (Fig.2.6).
2. **The wall** is two-Layered (***Didymium nigripes)*** or three-Layers (***Physarum gyrosum***).
3. **Typically** are **uninucleate**: in some May be 2 to 8 per spore.

Table 2. 1. General characters of the main orders of **Myxogastria**

Comparative phase	Stemonitida	Physarida	Liceida	Trichiida
Example	Stemonitaceae *Stemonitis*	Physaraceae *Physarum*	Liceaceae *Licea*	Arcyriaceae *Arcyria*
Sporophores	epihypothalic	sub-hypothallic	sub-hypothallic	sub-hypothallic
Spore masses	-	black, violet-brown, dark purple-brown, ferruginous	pallid, white to bright coloured sometimes brown, rarely black	pallid, white to bright coloured sometimes brown, rarely black
Capillitium	-	-	True is lacking	abundant

4. Dispersion:
a. mostly by air currents.

b. by the **hygroscopic** twisting of spirally thickened "elaters", one end of which is fixed and the other rotates in response to changes in humidity (Fig.2.4) *Trichia* sp..

c.spores are interspersed within the fructification by a network of tubular, branched, anastomosing threads which hold them in place and allow them to be sifted out slowly by air currents. The network of threads is called **capillitium**.

5- **Viability:** spores may remain **viable** for many years. **Elliott (1949)** recorded the germination of spores of ***P. flavicomum*** 60 years

5.**Germination**: Germination of the spores occurs after cracking of the spore wall: Formation of one or more naked protoplasts, which may acquire one or two unequal **whiplash** flagella (**anisokont**) or not and behave as **myxamoebae**. Flagellate cells may revert to an amoeboid after collection stage. The two stages are readily inconvertible (Fig. 2.6) and feeding **phagocytically** on bacteria.

a. **myxamoebae** multiply following mitotic nuclear division. The flagella are withdrawn before or during prophase (before division).

b. It is encysted in drastic conditions and secrete a **galactosamine** wall to form **microcysts**.

c. At favorable conditions, microcysts germinate to produce either a myxamoeba or swarm cell.

2. <u>Syngamy</u>

1. Flagellate stage and myxaoebae can function as gametes.
2. Plasmogamy occurs followed by karyogamy, often within minutes to form zygote (**syngamy**).
4. Diploid nucleus in the zygote divides repeatedly and plasmodium produced.

3. The Plasmodium

1. Feeds **phagocytically** on bacteria, fungal cells, ect., and may also engulf **myxamoeba**.
2. It is capable of movement, and creeps over the substratum, often forming large sheets several centimeters in extent and under adverse conditions converted into **sclerotium (macrocyst)** within which the cytoplasm is divided up into a large number of multinucleate cells, termed as **spherules**. On the return of suitable conditions, the **sclerotia** may converted to **plasmodia**.

<u>**There are three types of plasmodia (Alexopoules, 1973 and Ewbster, 1980)**</u> (Fig. 2.6)**:**

i. Protoplasmodium: primitive microscopic plasmodium. e.g. ***Echinostelium minutum***.

ii. Aphanoplasmodium usually invisible type of plasmodium characteristic of members of the Stemonitales.. e.g. ***Stemonitis***

iii. Phaneroplasmodium: large conspicuous plasmodium, fan-shaped reticulate masses, yellow in color, showed reversible **cytoplasmic** flow (shuttle streaming) to order **Physarales**. e.g. <u>***Fuligo***</u>.

After a period of feeding, at suitable conditions, the plasmodium will converted into fruit bodies.

4. Sporulation

I. Sporulation occurred ***in vitro*** as the following conditions achieved e.g. ***Physarum polycephalum***:

1. Plasmodium at optimal age and at critical point of exposure to light of short wave length.
2. Decrease of α. amylase activity.
3. Decrease in ascorbic acid oxidase with concurrent increase in cytochrome oxidase.
4. Activation of enzyme system involved in production of melanin pigments.

5. Fructifications

Sporophores consist of mass of spores formed inside peridium, spores intermingled with one of the following (Fig. 2.7):

1- **Capillitium** ((pl. capillitia) sterile thread-like structures found within the spore mass of some slime moulds, (often ornamented).
2- **Elaters** (Threadlike, ornamented, not connected at ends).
3- **Pseudocapillitium** (pl. pseudocapillitia) irregular plates, tubes or thread-like elements within spore mass of aethalia.

Lime may be present on peridium, stalk, columella or capillitium, or nodes of pseudocapillitum.

The characteristic of mature fructifications (fruit-body) are varied, but generally they conform the following types:

1. Sporangia:

One to several thousand sporangia may develop form a single plasmodium.

1. Sporangia may be stalked or sessile, globose, cylindrical or cup-shaped (Fig. 2.8).
2. In stalked sporangium, the stalk may be extended into the body of the sporangium, as **columella**.
3. Branched **capillitiale** threads may found, but in many species capillitia are absent eg. ***Physarum***.

2. Plasmodiocarps:

It is a print of the original plasmodium where sporophore developing along veins of a phaneroplasmodium; and retains its original structure e.g. ***Hemitrichia.*** (Fig. 2.8).

3. Aethalia (sing. Aethalium):

a sessile flat encrusted fruiting body in several genera of the slime molds (class Myxomycetes) formed by the fusion of many plasmodia**,** a relatively large, stalkless, rounded fruiting body, e.g. *Fuligo septica*. **(Fig. 2.9 A).**

4. Pseudoaethalium (pl. pseudoaethalia) densely clustered group of distinct sporangia in various myxomycetes resemble aethalia, but are actually composed of individual, closely-packed sporangia**.** **(Fig. 2.9B)**