BOT 316 MYCOLOGY AND FUNGAL PHYSIOLOGY

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INTRODUCTION

HISTORICAL BACKGROUND

Mycology is a classical translation of the Greek word *Mykes logos* which means mushroom discussion, thus mycology is the study of fungi. In the past this area of science was limited to the study of mushrooms but as science developed, the scope of the subject widened far beyond the objects seen with the naked eyes with the discovery of microscopes. The development of mycology cannot be isolated from that of science.

The ancestry of fungi is ancient, dating back to the Devonian and Precambrian eras. The history is also influenced by calamities and man has always kept record from time and as such the first record of fungi was not that of observing fungi directly but that of their harmful effects.

The Romans and Greeks have a lot in their records. Even in the Holy Bible there are many references of the fungi and their effects; Leviticus 14: 4-48, 1Kings 8:37, Deuteronomy 28:22. The first indication that man saw fungi as food was a report of death at Icarius.

The first book devoted to fungi is the Van Sterbeek's "Theatrum Fungerium" in 1675 and this work distinguished the edible from the poisonous mushrooms. The discovery of the microscope led to the systematic study of the fungi. Robert Hooke was credited with the first illustration of micro fungi in 1667 in his work titled *Micrographa*.

The Greeks and Romans regarded fungi as mysterious things. They were regarded as the "evil formats of the earth originating from the mouth of vipers". This was in the era of spontaneous generation and the classical view that fungi originated from the leaf tissues and other organic matter.

What is a fungus?

First, just what is a fungus? Over the last 40 years the way that we have defined "fungi" has changed several times. In the early 20th. Century, until around the 1950's, botanist used the term fungi to include all members of the "plant kingdom" that did not have stems, roots, leaves and chlorophyll (Alexopoulos, 1952). By this definition, bacteria would even be included with the fungi. However, by the 1950's botanists, slowly, began classifying bacteria as a distinct group, separate from the fungi, but still classified as plants. In 1952, Constantine Alexopoulos, the author of the popular mycology text book, Introductory

Mycology, defined fungi, in the first edition of his textbook as being "nucleated, achlorophyllous organisms which typically reproduce sexually and asexually, and whose usually filamentous branched somatic structures are surrounded by cell walls".

The following attributes can be used to characterize those organisms that we classify as fungi:

- a. They are **heterotrophs**. That is, they cannot manufacture their own food from simple compounds as plants are able to do. So they are dependent on other organisms to produce their foods, e.g., sugars, starches, proteins, fats, etc. Fungi can be further divided into **saprobes**, **parasites**, **symbionts**, **facultative parasites** and **facultative saprobes**.
- b. The food gathering part of a fungus is made up of either filamentous, hollow, branched tubes called **mycelium** or are single cells called **yeasts**.
- c. Structures called **spores** reproduce the fungus in the form of mycelium or yeast cells.
- d. They have **cell walls**. This is a characteristic generally attributed to plants, but unlike plants, most fungal cell walls are composed of **chitin**, a carbohydrate, and is the same material which makes up the exoskeletons of insects. Plant cell walls are composed of **cellulose**.
- e. Fungi are **eukaryotes** as are most other organisms with which you are familiar. However, bacteria differ from fungi in that they are **prokaryotes**.
- f. **Absorption**: The process by which fungi "eat". This differs from the way in which we eat in that a fungus will digest its food *before* eating it. Bacteria are the only other group of organisms that eat in this fashion.

Achlorophyllous

This term indicates means that fungi cannot produce their own food. Such organisms are called **heterotrophs**. This is important because it is commonly believed that fungi are plants, and once upon a time fungi were classified in the plant kingdom.

The Fungus Body: Mycelium and Yeasts

At one time or another, we have all come across food that has become contaminated in some forgotten corner of our refrigerator and observed the filamentous growth of fungi. The filamentous growth is called **mycelium** (pl.=mycelia) and represents the "body" of the fungus. A fragment of mycelium is referred to

as a **hypha** (pl.=hyphae). A less familiar fungus body, is the **yeast**. The yeast fungus is unicellular. Whether the fungus body is composed of mycelium or yeast, both will function in feeding and reproduction. Let us first discuss the more familiar filamentous appearance of the mycelium.

Mycelium

If we place a single fungal **spore** in the center of a petri plate, hyphae will germinate from the spore and grow radially, to form a circular growth of mycelium (Figure 1a). The radial growth of the mycelium can be small as shown in the petri plate below or may be extensive. Recall from the video "The Moldy World About Us" where a circle of mushrooms, called a "**fairy ring**", was formed.



Figure 1a. Mycelium with approximately circular colony (left) and Figure 1b. Mycelium as seen through the microscope (right)

In this case the mycelium that produced the mushrooms is hidden from our eyes in the soil beneath the grass (Figures 2a and b). In either case, if this growth is observed under a microscope, you will be able to see that it is composed of branched, filaments (Figure 1b). Each branch will form more branches at a given interval which will in turn branch further. Growth will occur only at the ends of the cells, not from the extension of all of the mycelial cells.



Fig. 2a. *Chlorophyllum molybdites* mushrooms (left) andFig. 2b. Fairy ring of same species (right): Note that the grass is greener inside the ring than out.

When mycelium form a *continuous* tubular growth it is said to be **coenocytic** (Figure 3a) or it can be divided into cells that are attached end to end and is said to be **septate** (Figure3b). The cell walls that divide the mycelium into cells are referred to as **septa** (sing.=septum):



Fig. 3a. Coenocytic hypha fragment (left) and Fig. 3b. Septate hypha fragment (right).

Yeast

A less familiar fungus body is the yeast. Under the microscope, you can observe that yeast is composed of single cells that continually divides, by **budding** or **fission** to form lots and lots of cells. To the naked eye, this growth will appear to be a thick, syrupy growth (Figure 4a). Fission is merely division of a yeast cell into two cells and will not be described here. The process of budding is illustrated in Figure 4b. A yeast cell that is about to bud has a predetermined area of the cell that becomes blown out forming a new cell, the so-called bud. The nucleus will divide by mitosis, with one nucleus migrating into the new cell. When the new cell is approximately the size of the original cell, the cells will seal off the opening and separate, giving rise to two yeast cells. Some species have both a yeast and mycelial stage (Figure 4c). Such species are said to be **dimorphic**.



Fig. 4a. Yeast colony of *Rhodotorula* (right), Fig. 4b. Yeast cell undergoing budding (middle) and Fig. 4c. Dimorphic species having both mycelium and yeast.

Spores

Fungi reproduce by **spores**. Spores are usually composed of one to a few cells. They may be **sexual** or **asexual** and are variable in shape, size and color (Figure 5):



Fig. 5. Spores of various shapes and sizes

Spores are often borne directly on modified mycelial structures. In the colony of *Penicllium notatum* pictured below (Figure 6a), the green, central portion of the mycelium is where the spores are borne on the modified mycelial structures called **conidiophores** (Figure 6b). The specific types of spores produced are asexual spores called **conidia** (sing.=conidium) (Figure 6b):



Fig. 6a. Colony of *P. notatum* (right), and Fig. 6b. Conidia and conidiophores from green portion of colony, as observed under the microscope.

An example of a mushroom that forms fairy rings that occurs in Hawai'i is *Chlorophyllum molybdities* (Fig. 6a and 6b). The grass was greener inside the fairy ring because the mushroom mycelium having grown through the area, inside the circle, had decomposed the organic material, within. Provided with more minerals, the grass growth was greener and lusher, inside the circle:

Instead of producing spores directly on the mycelium, the mushroom mycelium becomes very tightly interwoven, to produce the large mushrooms that you saw in Figs.3a and b, where the spores are borne.

Cell Wall

The mycelium or yeast cell is surrounded by a cell wall that is typically composed of **chitin**, the same material that makes up an insect's exoskeleton. However, one group of fungi that we will be studying has cell wall composed of **cellulose**, which is is the same material that is found in plant cells. The presence of a cell wall, regardless of its composition, was once used as evidence for fungi being closely related to plants, but presently the two groups are not thought to be closely related. During the early 1970's Solomon-Barnicki Garcia proposed that only those organisms that produce chitin in their cell wall should be defined as fungi. The characteristic of a chitinous cell wall is still one of the accepted criterion for defining a fungus. However, those "fungi" with cellulose cell walls are no longer believed to be closely related to the fungi and have even been classified in a kingdom of their own.

Fungal Tissues

During certain stages in the life cycle of some fungi, the mycelium becomes transformed into loosely or compact mass of woven tissue called **Plectenchyma**. This is of two types;

- (1) **Prosenchyma**: This is a compact mass of hyphae growing parallel to one another and distinguished as such.
- (2) **Pseudoparenchyma**: This occurs as numerous isodiametric, oval shaped mass of hyphae, resembling the parenchyma of vascular plants.

Prosenchyma and pseudo parenchyma tissues form various types of somatic and reproductive organs found in fungi, examples stroma and sclerotia.

Mycelial Growth

Until the 1990's fairy rings were thought to be the largest examples of mycelial growth (actually rhizomorphs in this case) in fungi. In April 2, 1992 the war of the *humongous fungus* began. Smith, *et al.* (1992) published an article of what at that time was considered to be the world's largest organisms. This article reported that what genetically was determined to be an individual of *Armillaria bulbosa*, a species of mushroom, produced rhizomorphs that covered approximately 37 acres. It was estimated to be at least 1500 years old and weighing in at about 100 tons. On May 18, 1992, a still bigger mass of rhizomorph was discovered. On Mt. Adams, in Washington State, rhizomorphs of *Armillaria ostoyae* covered 1500 acres. However, even this example does not represent the largest example of radial, mycelial growth. The most incredible growth of mycelium is one that was found in eastern Oregon, on August 1, 2000. The mycelium was found to belong to a mushroom identified as *Armillaria ostoyae*. Researchers determined that the mycelium of this mushroom covered 2,200 acres and estimated it to be over 2400 years old. The full text of this story was written by Volks (2002).

Mode of Nutrition: Absorption

The mode of nutrition or the matter in which fungi "eat" is called **absorption**. Among eukaryotes, absorption is unique to the fungi. Fungi obtain their food by transporting it through their cell walls, but first, how does a fungus "find" its food since like plants, they are not mobile organisms and cannot seek out their food. The answer is fungi *do not* have to find their food. In order to eat, the spores that give rise to fungi must be dispersed to a location where there is food and after the spore germinates, the mycelium

of the fungus must grow into its food. In other words, usually fungi must live in their food if they are to eat. If the food is composed of simple molecules such as glucose or sucrose, soluble food can be immediately transported through their cell walls. However, most food that a fungus might consume is composed of complex, organic compounds, e.g., cellulose, lignin, pectin, starch, etc., which is insoluble. In order for this food to be utilized by the fungus, it must be broken down into simpler molecules that can be transported through their cell walls. Another way in which you can think of this is that the cell wall is like a sieve that will allow only particles of a certain size to enter. The fungus breaks down the complex material by secreting digestive enzymes through their cell wall that will digest the complex organic compounds and convert them into simple molecules that can readily be transported through their cell walls. For example, if a fungus is growing in wood, digestive enzymes would be secreted from the fungus, into the wood, and break down the complex compounds of wood, e.g. cellulose and lignin into simpler materials, such as simple sugars, which then can be transported into the mycelium.

Although this process may seem very different than our own means of obtaining food. It is not that different. The essential difference between fungi and animal digestive systems is that fungi digest their food first and then "eat" it, while animals eat their food before digesting it. The basic process of digestion is otherwise more or less the same. Our digestive system requires that our food is chewed by teeth, go through the esophagus, stomach, intestine and many associate organs. So there are a lot of things that can go wrong when we eat our food. The fungal digestive system is much more simplified and one which has been very successful for them.

It is important to understand here that different kinds of fungi will secrete only a specific number of different enzymes. This means that they can only "eat" certain materials. For example, a fungus that is usually found in your stored food, probably will not be able to "eat" wood because it does not have the enzymes that is needed to break down or "rot" the wood. Some fungi have a very broad range of enzymes. Species of *Penicillium*, for example, can be found on a number of different "food"; leather, cloth, paper, wood, manure, animal carcasses, ink, syrup, paint, glue, hair, literally thousands of products. A summary of absorption is illustrated in the figure below:



Illustration of the process of absorption, the mechanism by which fungi consume their food.

Although yeasts are quite different in their appearance than mycelial fungi, their means of obtaining food is identical.

Heterotrophs can be divided into several categories:

- 1. Saprobe: Heterotroph that derives its food from *non-living* organic carbon sources. Many old text books use the term saprophyte literally meaning "rotting plant" (showing the botanical origin of this word). These types of fungi are very important as nature's recyclers. They consume dead organic material and break down into their most basic components, i.e. minerals, which can then be utilized by plants to produce more food. So even though we say that these fungi cause "rot" and "decay", that isn't necessarily a bad thing. This is a very important process in nature's effort to recycle. The down side of decomposition is that fungi do not distinguish between fallen branches and logs, and your furniture. To a fungus, wood is wood, regardless of the source. We will talk more on this topic later in the semester.
- 2. **Parasite**: Heterotroph that derives its food from the *living* cells of another organism referred to as the **host**. Many fungi fit into this category, but not all, and not even most. This is just one of the many biases that we have of fungi, i.e., the common belief that most fungi are parasites.

- 3. **Facultative Parasite**: Heterotroph that is primarily a saprobe, but when opportunity presents itself, can be a parasite.
- 4. **Facultative Saprobe**: Heterotroph that is primarily a parasite, but when opportunity presents itself, can become a saprobe.
- 5. **Symbiont** (used here in the mutualistic sense): Heterotroph that derives its food from another living organism, but the relationship is mutually beneficial to both organisms involved, e.g. lichens = fungus and alga. As you'll see later in the semester, fungi that are in this category are very important, i.e. if they did not exist, the world as we know it also would not exist

The latter two categories of fungi are of more concern to plant pathologists than those that are obligate parasites. An obligate parasite will normally weaken its host, but not kill it since killing the host would almost certainly guarantee the death of the parasite. However, a facultative parasite or facultative saprobe, when they are in their parasitic mode are more likely to be aggressive parasites that will kill their host.

Fungi found in the terrestrial habit can be categorized as follows;

- (i) Lignicolous (wood fungi)
- (ii) Cellulolytic (cellulose loving fungi)
- (iii) Coprophilous (dung loving fungi)
- (iv) Pyrophilous (burnt ground loving fungi)

Mycological Terms

Absorption: The means by which fungi obtain their food. Process begins with the release of digestive enzymes, from the fungus, through their cell walls to digest the food that is around them. The digested food is then "absorbed" through their cell wall.

Budding: A form of asexual reproduction in which an outgrowth (="bud") developing on a parent yeast cell detaches to produce a new individual.

Cell Wall: The rigid outermost cell layer found in plants and certain algae, bacteria, and fungi but characteristically absent from animal cells.

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Cellulose: A complex carbohydrate, $(C_6H_{10}O_5)n$, that is composed of glucose units, forms the main constituent of the cell wall in most plants and some fungi.

Chitin: A complex, primarily nitrogen-containing carbohydrate, which forms the principal component of arthropod exoskeletons, ex. insects, and the cell walls of most fungi.

Clone: An organism descended asexually from a single ancestor, such as a plant produced by bulbs or fungi by asexual spores.

Conidium (plural: Conidia): Asexually produced spores produced on conidiophores.

Conidiophore: A specialized hypha on which conidia are borne.

Dimorphic: Fungi that have both a yeast and mycelial phase.

Eukaryote: Organisms whose cells contain a distinct membrane-bound nucleus.

Fruiting body: A specialized spore producing structure found especially in fungi.

Hyphae (sing: Hypha): Threadlike filamentous fragment of the mycelium of fungi.

Mitosis: The division of the nucleus, which results in the formation of two new nuclei. The two nuclei are identical and contain a complete copy of the parental chromosomes.

Mycelium: The vegetative part of the fungus that consisting of a mass of branching hyphae.

Rhizomorph: An aggregation of mycelial strands that forms a root-like structure formed in some fungi.

Saprobes: An organism that derives its nutrition from nonliving or decaying organic material.

Yeast: Unicellular fungi that reproduce asexually by budding (a small outgrowth, or "bud", on the cell's surface increases in size until a wall forms to separate the new individual) and fission.

Questions to Think About

- 1. Why are facultative saprobes or facultative parasites more of a problem, as pathogens, than those that are obligate parasites?
- 2. Even though fungi are all around us and are very common, people are generally unaware of their existence. What are some reasons that you can give for this lack of awareness?
- 3. Certain organisms are no longer considered to be fungi that were previously classified as such. Name these types of organisms and give the reasons that they have been excluded.
- 4. How does sexual and asexual reproduction differ? What are the advantages and disadvantages of both?

Reproduction in Fungi

Reproduction is the formation of new individuals having all the characteristics typical of the species (Alexopoulos and Mims (1982). Reproduction in fungi can be broadly categorized into types; sexual and asexual.

Sexual vs. Asexual Reproduction

Sexual reproduction is a subject that can probably be best understood if we discuss it in human terms rather than using plants or fungi. You are all aware that this type of reproduction must involve two parents, and that the children from two parents will inherit characteristics from each parent. For example, all of you have features that can be recognized as being maternally or paternally inherited. This will also be true for any siblings that you may have. However, you and your siblings are genetically unique in appearance and personality because the process of sexual reproduction is such that no two individuals will be exactly alike unless they are identical twins.

Asexual reproduction requires only a single parent and the "children" produced would be genetically identical to the parent. Genetically, identical individuals are said to be **clones**. Although some animals, naturally, have this type of reproduction, there are far more examples of asexual reproduction in plants. Asexual reproduction occurs when a part of an *individual* regenerates itself into another individual. Since this new individual was originally part of the parent, the two are genetically identical. Many agricultural plants are reproduced asexually because if you have a plant with all the qualities that you want, growing clones of this individual will ensure that everything you are growing will also have these qualities.

In fungi this process occurs by physical break up of parts of the filament or modification of vegetative parts to give rise to new individuals. Asexual reproduction takes place under favorable conditions and most fungi use this method to propagate themselves several times during the season. Spores vary in shape and size, colored or hyaline, moistly unicellular.

Modes of asexual reproduction

- Fragmentation: the fungal hypha breaks into smaller pieces and each may later grow into new mycelium. This is a common laboratory technique where a piece of fungal segment is transferred into fresh medium which afterwards develops into a fungal colony
- 2) Fission: this is a common method in unicellular fungi and some lower fungi. A single cell divides on a lateral plane to give rise to two daughter cells.
- 3) Budding: this is common in yeasts. A small outgrowth emerges on the parent cell. The nucleus divides into two. One of the daughter nuclei passes into the bud. The bud increases in size while it is still attached to the parent cell but soon breaks off. Sometimes the daughter bud is not separated and produces another bud in the same manner. This process is repeated forming a chain of buds appearing like a mycelium called pseudomycelium.
- 4) Oidia: sometimes hyphae break up into small components called oidia or arthospores. They are one celled and on germination form a new plant. These are usually formed in *Eryshiphe* and Basidiomycetes.
- 5) Chlamydospores: in some fungi like *Fusarium* and *Mucor*, cells of hypha become thick walled either singly or in chains after accumulation of food material in them. They may be terminal or intercalary in position and are capable of forming a new plant on return of favorable condition.
- 6) Gemmae: are chlamydospores like in structure but not so durable and thick walled. They are formed in S*aprolegnia* and others.
- 7) Zoospores: these are naked, motile and flagellated spores.
- 8) Sporangiospores: spores produced in special structures called sporangia (singular; sporangium)
- 9) Conidia: asexual spores borne in chains on the tip of specialized structures called conidiophores.
- 10) Urediospores and pycniospore

Reproduction by spores provides the fungus advantages as follows;

a) A crop of spores provide the individual in a large number and repetition of the same many times under favorable conditions increases chances of survival.

- b) The methods of dissemination through air, water, animals etc. affords the fungus of fresh supply of food.
- c) Comparatively spores are more resistant than vegetative hyphae and can tolerate/withstand unfavorable conditions.

Sexual Reproduction

Sexual reproduction in fungi takes place by the fusion of compatible parent nuclei. It begins with the coming together of two sex cells or reproductive structures or mating types. Enzymes are produced to digest the walls of the cells and fusion of cytoplasm of the two cells takes place. This is known as plasmogamy. The two haploid (n) nuclei of the two mating types fuse together. This is called karyogamy. Karyogamy results in the formation of a diploid (2n) nucleus (zygote). Immediately after karyogamy, meiosis takes place reducing the chromosome number to haploid state. These sexually produced spores divide mitotically to produce multicellular individuals.

The fungal structure that produces gametes is known as gametangium (plural gametangia) and two compatible gametangia are morphologically identical. In some species, the male and female gametangia are morphologically different. In such cases the male gametangium is termed antheridium (pl. antheridia) and female is known as archogonium or ascogonuim (pl. archegonia, ascogonia).

Types of Sexual Reproduction in Fungi

A. Planogametic Copulation

This involves fusion of two naked gametes of opposite (+ and -) mating strains. One or both gametes could be motile. This is characteristic of lower fungi.

B. Gametangial Contact

The two gametangia grow parallel and close to each other, form a bridge known as fertilization tube. The male gamete migrates through the tube to the female gametangium and fertilizes the female gamete.

C. Gametangial Copulation

Here the entire contents of the two contacting gametangia fuse. This takes place by the dissolution of the contacting walls of the two gametangia, resulting in a common cell in which the two protoplasts mix. This method is also called conjugation or gametangiogamy. Example is zygospore formation.

D. Spermatization

Some fungi bear numerous, minute multinucleate, spore-like male structures termed spermatia (singular; spermatium). The spermatia are usually carried by wind and deposited on female gametangial hyphae, to which they become attached. A pore develops at the point of contact, and the contents of the spermatium pass into the particular receptive structure that serves as the female organ.

E. Somatogamy (Somatic Copulation)

The vegetative cells, not gametes or gametangia are involved in this type of sexual reproduction. Two hyphae meet and fuse, growing dikaryotically (n + n). The two nuclei fuse to form a diploid nucleus and further meiosis gives rise to spores.

CLASSIFICATION OF FUNGI

Taxonomic Hierarchies

The following are the suffices for the various ranks/hierarchies in fungal classification as recommended by the International Code of Fungal Nomenclature (ICFN)

•	The name of Division of fungi ends in	-	mycota
•	The Sub-divisions	-	mycotina
•	Classes	-	mycetes
•	Sub-classes	-	mycetidae
•	Orders	-	ales
•	Families	-	aceae
•	Genera and species	-	no specific suffices

There are five phyla that are commonly recognized in the Kingdom: Fungi:

- 1. Chytridiomycota: Flagellated zoospores and gametes
- 2. Zygomycota: Zygospore supported by suspensors on either side.
- 3. Ascomycota: Ascospores borne with asci.
- 4. Basidiomycota: Basidiospores borne on sterigmata of basidia
- 5. Deuteromycota: Sexual stage absent, reproduction by conidia borne on conidiophores.

Dichotomous keys are a means of identifying organisms without the aid of a teacher. This was one of the contribution for which Linnaeus was recognized. When constructing such a key, two choices are offered and the student selects one of the choices based on which choice seems to best fit the organism being identified. Let construct such a key for the five phyla of Fungi above:

A. Flagellated stages present, mostly aquatic, thallus unicellular to mycelial. If mycelial, coenocytic......Chytridiomycota

AA. Flagellated stages absent, usually not aquatic, Thallus mycelial or yeast or both......B

B. Mycelium usually coenocytic, Sexual spore, zygospore, asexual spores borne in sporangia......Zygomycota

BB. Thallus mycelial, if present, septate, or yeast, or dimorphic. Sexual spore if present, not zygospore, asexual spore, conidia.....C

CC. As asci	above,	but	sexual	spores	not D	ascospores	borne	in
D. Sexua basidia	1	spores		basidiospo	res, B	born Basidiomycota	l I	on
DD. conidia	Sexual	stage	mostly	absent, Deutero	repr	oduction	asexual	by

Usually, after you arrive at a name, the description of the organisms is described after the key. Just as there are different groups of plants, example, ferns, mosses, conifers, flowering plants, etc., there are different groups of fungi.

<u>Chytridiomycota description and images</u> (Figs. 8-10), the terrestrial Fungi (Figs. 11-16) are classified according to the types of *sexual* spores that are produced. The Chytridiomycota can readily be distinguished from the terrestrial fungi being the only Fungi that have swimming zoospores and gametes. These stages are propelled by a single hair-like appendages referred to as a flagellum.



Figure 8: Zoospore of Chytridiomycota (left) and Allomyces gametangia

Figure 9: Male and female gametes Figure 10: Zoospores of released from *Allomyces* gametangia

Allomyces released from Zoosporangium

Zygomycota and Ascomycota descriptions and images: The remaining fungi are mostly terrestrial without flagellated stages. The Zygomycota can be defined by its thick walled zygospore (Fig. 11), produced ruing sexual reproduction and its asexual spores borne in sporangia. The Ascomycota is defined by asci and ascospores (Fig. 12) produced during sexual reproduction that may be formed on a complex fruiting body Fig. 13)



Figure 11: Zygospores are sexual spores characteristic of the division Zygomycota. Spores have thick, black cell walls and are supported by two cells called suspensors.

Figure 12: Ascospores are sexual spores Figure 13: An example of a diskborne in cylindrical cells called asci shaped Fruiting body in the (sing.=ascus) belong in the division Ascomycota. Asci and Ascomycota. Asci and ascospores are ascospores form a continuous usually produced in fruiting bodies. There layer on the red hymenial are typically eight ascospores/ascus. surface.

Basidiomycota description and images: This phylum is defined by the basidia and basidiospores (Fig. 14-15) during sexual reproduction. These structures may be borne on fruiting bodies such as the gills of mushrooms (Fig. 16).



Figure 14: A low magnification of a piece of lamella from a mushroom fruiting body. Elliptical objects are basidiospores.

Figure 15: A close-up of two basidiospores borne on a basidium. A characteristic feature of the division Basidiomycota, which includes mushrooms.

Figure 16: *Cortinarius clelandii* mushroom. basidiospores and basidia are borne on the lamella of mushrooms.

Deuteromycota description and images: This phylum is defined by its lack of sexual reproduction, but this is not strictly true. Some species have been induced to reproduce sexually and can be reclassified as usual a member of the Ascomycota and less commonly the Basidiomycota. Reproduction is mostly by conidia. Some examples can be seen in Figs. 17-19This phylum is said to be a "form taxon" because the fungi classified in this phylum are not known to reproduce sexually. There are species in which the sexual stage has been induced in the laboratory and found in nature. When this occurs, they sexual stage is usually referable to the Ascomycota or less frequently to the Basidiomycota.



Figure 17: Trichocladium conidia

Figure 18: Periconia conidia

Figure 19: *Pencillium* conidia

There are also two additional phyla that were once thought to be "Fungi", but are no longer classified as such. The phylum Oomycota (Figs. 20-22) has all of the appearances of a fungus and most of combination of characteristics, but does not have a chitinous cell wall. It is characterized by the large oogonia that contain eggs (Fig. 22), which is named for the phylum, literally "egg fungus". Note that the male tube containing the sperm nuclei is also attached. There are other characteristics, but this would be beyond the scope of this course. Asexual reproduction also commonly occurs by zoospores (Fig. 20) borne in zoosporangium (Fig. 21)The Oomycota causes some of the more serious plant diseases and has had a tremendous, historically, on several occasions that we will cover.



Figure 20: Two types of Figure 21: Zoosporangium of Oomycota. Figure 22: Oogonium and antheridium zoospores released during Note dark color at tip. The tip is full of of Oomycota. zoospores.

The phylum Myxomycota is commonly called the slime molds because of its plasmodial stage (Fig. 22). It is no longer classified as belonging to the fungi because they lack mycelium, cell walls in its vegetative stage and do not absorb their food. Slime molds are called the "mold that walks" because they have amoeba and plasmodial stages that are mobile as they search for food. Probably the only reason that they were classified as fungi is because they reproduce with spores.



Figure 22: The plasmodium is the stage that eats as it creeps along.

Figure 23: *Stemonitis*, The plasmodium becomes reproductive and gives rise to sporangia

Figure 24: *Lycogala* is another genus with much larger sporangia.

PHYLUM CHYTRIDOMYCOTA

Class Chytridiomycetes

The unique characteristic of members of this class is the presence of motile zoospores (planogametes). They are referred to as uniflagellates and are usually posteriorly flagellated. They have chitin and β -glucan as the chief components of their cell walls. They have coenocytic mycelia or thalli. Basically they have no nuclei and also exhibit pseudo septa structures. Members are generally found in soil and aquatic habitats. Some are parasitic on various fresh water plants and animals, terrestrial plants and as saprobes on a wide variety of plants and animals. A few are found in marine water and they may be easily recovered from soil and water by baiting techniques.

They bear resting spores or sporangia and are holocarpic (the whole thallus of the fungi takes part in sexual reproduction). They also bear rhizopodia and rhizomycelia. Some may be monocentric (a condition whereby the rhizoidal system bear a simple reproductive system), while others could be polycentric (condition where the rhizoidals bear more than one reproductive system. The sporangia could be operculate or inoperculate (that is presence or absence of a defined circular cap at the tip of the discharge operculum). They reproduce both sexually and asexually by spores. They form resting spores or sporangia from the zygote. The zygote in a few species germinates into a diploid coenocytic thallus. Sexual reproduction is accomplished by one of the following;

a) (i) Planogametic copulation (conjugation of isogamous planogametes). Example, *Olpidium, Synchytrium*.

(ii) Conjugation of anisogamous planogametes. Example found only in members of Order Blastocladiales.

- (iii) Fertilization of non-motile gamete (egg) by a motile gamete (antherizoid). Examples are members of Order Monoblepharidales.
- b) Gametangial copulation; this involves the transfer of the entire protoplast of one gametangium to the other.
- c) Somatogamy; fusion of the rhizomycelial filaments. This process precedes resting spore formation in some chytridiomycetes.

Life cycle

Asexual reproduction is by means of zoospores which are produced by the cleavage of the zoosporangial cytoplasm. The zoospore settles on the surface of suitable host and empties its protoplasmic content into the host tissue leaving an empty membrane on the outside. The *in vivo* thallus develops into a pro sorus which moves towards the base of the host cell and enlarges as well as undergoes cytological changes such as vacuole formation. Development is usually completed in six days at 30°C but twice as much at 20°C. The prosorus then gives rise to a sorus, a cluster of sporangia (the cytoplasm of the pro sorus flows out slowly through the papilla into a vesicle within 6 hours and the vesicle eventually becomes the sorus). The number of zoospores produced by any species depends on the size of the zoosporangia and the size of the zoosporangia can be modified by pH, temperature, vitamins and nutrients available. The release of zoospores is through one or more discharge papillae which appear like nipple shaped prostrations on the apex of each zoosporangium.

In the sexual cycle, the zoospores become gametes. Plasmogamy occurs about 1 to 3 hours after pairing and karyogamy occurs about 30 minutes later. The resulting zygote penetrates the plant and forms an intercellular thallus. Thallus enlarges and develops into a resting spore. The content of the host cell disintegrates and may appear as residue on the resting spore. Following a period of dormancy, the resting spore germinates to produce pro sori. Each pro sorus forms an exit tube that penetrates through the host wall and forms a sorus. The diploid nucleus from the resting spore migrates into the sorus and divides meiotically and mitotically respectively. Zoospores are produced and released to produce young thalli.

Sexual reproduction is probably absent in some species. When present, it involves the fusion of gametes eg. *Synchytrium brownii*; gametangia e.g. *Polyphagus euglenae*; or rhizoids of thalli e.g. *Chytriomyces*.

Classification of Chytridiomycetes

The Chytridiomycetes are further classified into the following Orders below;

Order Chytriales eg. *Chytriomyces, Synchytrium* Order Blastocladiales eg. *Coelomomyces, Allomyces.* Order Harpochytriales eg. *Oedogoniomyces.* Order Monoblepharidales eg. *Gonapodya, Monoblepharis,*

PHYLUM OOMYCOTA

This class is a large and heterogeneous group of fungi. Morphologically, the thalli range from simple unicellular forms to holocarpic endobiotic freely branched forms. Majority of them are eucarpic (develop reproductive structures independent of somatic body). Habitats are equally diverse as they may live as parasites of simple animals (example rotifers), simple plants (eg algae) or higher plants or animals. Many are also saprophytic in nature. They occur predominantly in aquatic environment or moist soil, but some also occur in the aerial parts of plants. Their cell walls show the presence of cellulose, which is very rare in most of the other fungi.

They are characterized by the formation of biflagellate zoospores. One flagellum is tinsel and directed forward while swimming, the second flagellum is whiplash and directed backward. The flagella are attached in the lateral or anterior position. The zoospores are pyriform and are devoid of cell wall. They are also produced inside zoosporangia. In some higher forms the sporangia are borne on specialized reproductive hyphae called sporangiophores. Many oomycetes produce only one kind of zoospore which, germinates directly into a new plant. Such species are said to be monoplanetic. Some members are diplanetic and are thus referred to as exhibiting dimorphism; a phenomenon in which primary (pear-shaped) and secondary (bean-shaped) zoospores are produced in a life cycle. Sexual reproduction is oogamous, where sexual phase has a clear differentiation into large female and small male structures, termed oogonia and antheridia respectively.

Most oomycetes cause serious diseases of fish (trouts and salmon) and their eggs. Some also cause diseases of vascular plants example, late blight disease of potato caused by *Phytophthora infestans*, downy mildew caused by *Perenospora parasitica*, *Plasmopora veticola* causes diseases of the cabbage family and of grapes, *Pythium* causes damping off disease of seedlings etc. There are three orders in the class Oomycetes; Saprolegniales, Lagenidiales and Perenosporales.

Order Saprolegniales

Many members of this order are commonly referred to as water molds. This term though applicable to a number of other fungal groups as well, is customarily used to designate the Saprolegniales because most of them occur abundantly in clear waters and are easily isolated. Many species are also soil-inhabiting, majority is saprobes, and some are parasitic and cause diseases of fish and fish eggs. Members have both apical and lateral biflagellate zoospores and equivalent stages. Their hyphae lack cross walls (coenocytic). The oogonia lack periplasm. Conspicuous members of this order are found in the Families Leptomitaceae and Saprolegniaceae.

Asexual reproduction is usually initiated by starvation or under adverse conditions. Elongated or spherical zoosporangia often produced at the terminal part of the thallus. The zoosporangium may also proliferate internally. They could be monomorphic or dimorphic. In dimorphic situation, based on the order of appearance of the zoospores, one can classify them as primary and secondary zoospores respectively. Old culture may develop resting spores called gemmae which germinate under suitable conditions.

In sexual reproduction, the gametangial copulation type is present. The antheridium grows towards the oosphere containing oogonium. Fusion results in oospore formation. Some species such as *Dictuchus* and *Achyla* are heterothallic (self sterile) or dioecious. Most species of Saprolengiales are homothallic (self fertile) monoecious or hermaphroditic. It should be noted that there is much variation in the Saprolegniales, both within and between species and genera; the pattern of zoospore formation, activity and encystment.

Life cycle of Genus Saprolegnia

Members are parasitic example, *Saprolegnia parasitica*. They produce elongated zoosporangia which may be more than one in a sporangiophore. The zoosporangia rupture on maturity releasing primary zoospores. They are usually biflagellate. One essential feature of the life cycle of *Saprolegnia* species is diplanetism. The secondary zoospores germinate to produce somatic hyphae which may produce

gametangia. The antheridium copulates with the oogonium fulfilling plasmogamy, dikaryogamy and karyogamy; the three processes necessary for sexual reproduction. After fusion, the fertilized oosphere (oospore) germinates to produce a new somatic hypha.



Order Lagenidiales

This order comprises of a group of simple holocarpic fungi which may or may not be closely related. They are facultative parasites and occur chiefly within algae and other aquatic fungi, although some are parasitic within various forms of animal life (endobiotic), such as nematodes, daphne, mosquito larvae and other insects. They occur principally in freshwater habitats, although there are marine forms. The thallus may be multinucleate, single, more or less amoeboid mass as in *Olpidiopsis*, or it may be tubular

or filamentous structure as in *Lagendium* and *Haliphthoros*. In unicellular thalli, such as those formed by species of *Olpidiopsis*, each thallus is converted into a zoosporangium or gametangium on maturity.

Order Perenosporales

This order represents a higher development of oomycetes. Some members are highly specialized obligate parasites, example, *Pythium debaryanum*, which causes damping off disease of seedlings; *Phytophthora infestans*, causes late blight of potato, etc. They inhabit water or damp soils and live as saprophytes or weak parasites.

They are monomorphic and their oogonia are differentiated into ooplasm and periplasm. Sexual reproduction is through the formation of oospore within the oogonium. Their coenocytic hyphae are more slender than those of *Saprolegnia* and are freely branched. Based on the species the zoosporangia may germinate by;

- a) Releasing mature zoospores into a vesicle
- b) Releasing mature zoospore into the environment, or
- c) Releasing apparently undifferentiated cytoplasm into the vesicle where it is later cleaved to form numerous zoospores.

Members produce lemon-shaped or oval sporangia on distinct sporangiophores. These sporangia can sometimes be deciduous (sporangia fall off at certain time) or wind disseminated. They produce single oosphere with very few exceptions. There are three families in this order; Pythiaceae, Albuginaceae and Peronosporaceae.

PHYLUM ZYGOMYCOTA (zygote fungi)

- 1. Hyphae have no cross walls between nuclei
- 2. Reproduce asexually by spores in sporangia or sexually by production of zygospores
- 3. Asexual: sporangia on tips of hyphae, haploid spores formed by mitosis
- 4. Sexual: hyphae specialized to become "+" and "-," zygote becomes a zygospore, zygospore sits around until it is time to undergo meiosis, meiosis produces a new sporangium (on hypha)
- 5. Most are saprophytes and attack stored foods

Class Zygomycetes

The Class Zygomycetes includes the fungi that do not have motile cells or zoospores during any stage in their life cycle, and the sexual spores are present in the form of thick walled zygospores. They are mainly saprophytic and in the parasitic forms the mycelia are immersed in the host cell/tissue. Some occur on dung, showing coprophilous nature. Most Zygomycetes produce well developed and branched mycelium, consisting of coarse, grey or white, coenocytic hyphae. Cell wall is mainly composed of chitosan-chitin. Asexual reproduction takes place by non-motile sporangiospores, called aplanospores produced in very large number within the sporangia. Some reproduce by chlamydospores and many produce modified sporangial units functioning as conidia or by true conidia, which may be borne singly or in chains.

Sexual reproduction takes place by gametangial fusion. Two fusing gametangia may arise from the same mycelium or from different mycelia. Gametangial fusion results in the production of thick walled resting spore called zygospore. The zygospore develops within a zygosporangium. The zygospore remains surrounded by a thick wall, which is highly resistant to desiccation and other unfavorable factors. The zygospore wall is pigmented and sculptured in many species. At germination, a hypha emerges and bears a terminal sporangium. Meiosis occurs during germination.

The Class Zygomycetes has two orders Mucorales and Entomophthorales.

Order Mucorales

Members of this order are mostly terrestrial saprophytes, living on a wide variety of organic substrates including bread, cooked food, and dung and decaying animal and plant matter. A few are obligate parasites or weak parasites causing diseases of higher plants and animals. The mycelia consist of stout, well branched coenocytic hyphae. Septa are formed at the bases of reproductive organs. Motile zoospores are absent.

Asexual reproduction takes place by unicellular, non-motile sporangiospores (aplanospores) produced in large number in sporangia borne on sporangiophores. A sporangiophore may be simple or branched, uninucleate or multinucleate. A flat or bulged septum cuts the sporangium from the sporangiophore. In the presence of bulged septum the sporangiophore contains an inflated head called columella.

Sexual reproduction takes place by the fusion of two multinucleate gametangia usually of equal size; rarely unequal. Members are either homothallic or heterothallic. The resulting diploid zygote develops a thick wall and changes into a resistant resting spore called zygospore

Genus Rhizopus stolonifer

This fungus occurs very frequently on bread, and is commonly known as bread mold. It is a frequent contaminant of laboratory cultures of bacteria and fungi. It is considered a weed of laboratory cultures. *Rhizopus* occurs worldwide in soil, on decaying fruits, dung and vegetation. It is also a parasite, causing rot of sweet potato or fruit rot of apple, strawberry and tomato. Some species cause mucomycosis in domestic animals.

Laboratory culture of Rhizopus

The fungus can be grown on dead organic matter such as bread and butter by keeping them in dark and damp atmosphere. By first, exposing a moistened piece of bread in a Petri dish for about 24hrs at room temperature, and then covering it for a few days. *Rhizopus* appears in the form of white tuft of mycelium. The young mycelium consists of many well branched, white, tubular or filamentous hyphae, which are multinucleate and without cross walls. Later, when the mycelium enters the reproductive stage, the hyphae are differentiated into rhizoids, stolons and sporangiophores.

Rhizoids are the repeatedly branched hyphae that penetrate the substratum. They absorb water and nutrients from the substratum. They are commonly known as holdfast.



Stolons are the hyphae that grow horizontally above the substratum for some distance and then bend down into the substratum. They are aerial and unbranched.

Sporangiophores are the erect, aerial, unbranched and negatively geotropic hyphae, which grow upwards in tufts at the point where stolons from rhizoids. They are reproductive in function. Each sporangiophore bears a terminal sporangium.

Asexual reproduction takes place by the formation of sporangiospores and rarely by chlamydospores. Sexual reproduction is by conjugation and majority of species are heterothallic (self sterile). Thus zygospores are formed only when two compatible strains of different mating types come together. These special hyphae, which are capable of developing into progametangia are called zygophores.

The progametangia of opposite strains adhere together, enlarge, pushing the zygophores apart. The tip of each progametangium is cut off by a septum. The small terminal cell so formed is called gametangia whereas the long tubular part is called a suspensor. A pore develops in the adjoining cells of the gametangia allowing their protoplast to fuse and form a zygospore. The zygospore soon becomes surrounded by thick, black, warty wall. The zygospore undergoes a resting period before germination. Meiosis takes place only at the time of zygospore germination. Segregation of strains also takes place during meiosis to ensure that different sets of spores are released. Half of the resultant nuclei belong to strain (+) and the other half strain (-). Zygospores germinate by formation of promycelium.

BOT 316 MYCOLOGY AND FUNGAL PHYSIOLOGY

