# PRACTICAL 8 IDENTIFICATION OF FUNGI AND YEASTS

**Structure**



## 8.1 INTRODUCTION

In the last Practical we learnt about different staining techniques used to visualize bacterial cell morphology. What about identification techniques of fungi and yeast? Are the processes/techniques similar to techniques used for bacteria? We will look at these aspects in this practical. Practical 8 briefly describes the structure, nutrition and classification of fungi. Identifying features of some commonly isolated fungi have been enumerated. You may recall we have already studied about this earlier in our theory Course in Unit 2. We suggest you look up section 2.4 now. The purpose of this Practical is to acquaint you with the morphological characteristics of both the yeasts and moulds and help you visualize and identify the common unknown fungi.

**Objectives**

After going through this practical and undertaking the exercises given herewith, you will be able to:

- describe the structure and morphological characteristics of yeasts and moulds,
- identify the specific features of some commonly isolated fungi,
- classify the different fungi, and
- visualize and identify common unknown fungi.

# 8.2 INTRODUCTION TO FUNGI

The fungi are spore-bearing eukaryotic organisms without chlorophyll and having absorptive nutrition. These reproduce sexually as well asexually. Primarily, these are terrestrial microorganisms, though some are present in aquatic environment also, both in marine and fresh water. Many are pathogenic to plants, animals and humans. Some fungi are present in beneficial relationship with other organisms e.g. Mycorrhizae (an association between fungi and roots of vascular plants), lichens (an association between algae and fungi) etc. The branch of microbiology which deals with the study of fungi is called *mycology*. The fungi are placed in a separate kingdom myceteae, as you will learn later. Let us start our study about fungi with a discussion on the importance of fungi.

## 8.2.1 What is the Importance of Fungi?

Fungi are widely distributed in nature. It is estimated that there are about 1.5 million species of fungi on earth. Fungi are both beneficial and harmful to humans.

The beneficial and detrimental effects of fungi are highlighted herewith. You may recall studying about this already in the Principles of Food Science Course in Unit 12. We suggest you look up the Unit once again now.

So from our discussion above, it must be clear that fungi are important microorganisms with implications in food industry. Next, let us look at the structure of fungi.

| Beneficial Effects | Detrimental Effects |
|---|---|
| These act as decomposers and can degrade complex organic molecules in dead plants and animals to simple organic compounds and inorganic molecules, making the soil fertile. | These cause diseases of animals and humans. These either cause superficial mycoses (infection of skin, hair and nails) or systemic mycoses (infection of subcutaneous and deeper tissues such as lungs, urinary tract etc.) in human. |
| Fungi, especially the yeasts are important in brewing and baking industry. These yeasts, such as *Saccharomyces*, are used for making beer and bread. Many wild yeasts are involved in production of wine. | These are the major cause of plant diseases. |
| Fungi also play a role in production of some foods like, cheese, soy sauce, sufu etc., in manufacturing of organic acids like citric and galic acid and in making of certain drugs, like ergometrine, cortisone etc. | Many fungi produce toxins such as mycotoxins and hallucinogens about which you have already studied in Unit 5 in Food Microbiology and Safety Course. |
| Many antibiotics, e.g. Penicillin, Griseofulvine and immunosuppressive drugs, e.g. cyclosporine are manufactured by fungi. | These are involved in spoilage of foods like bread, citrus fruit etc. |
| In addition, fungi are used for making many industrial enzymes. | Many harmful yeasts result in fermentation of fruit sugar to alcohol and spoil fruit juices. |
| Fungi are also important as research tools in the study of fundamental biological processes and is used regularly by geneticists, cytologists, biochemists, microbiologists etc. for their research purpose. | |
| Many yeasts are valuable as a food supplement because of their high vitamin content. | |

## 8.2.2 Structure of Fungi

The vegetative structure of a fungus is called *thallus*. It varies in complexity and size, ranging from unicellular yeasts to multicellular moulds.

A yeast is a unicellular fungus, producing moist to waxy colony in culture. It is about 5-10 times larger than bacteria and reproduces asexually by budding or transverse division and sexually through spore formation, as we will learn later in sub-section 8.2.4. Look up Unit 2, section 2.4 to learn about the budding process. Morphologically, these are usually spherical or ellipsoidal in shape.

A mould produces leathery, cottony or profuse powdery growth on medium. It consists of a mass of intercoiling branched, threadlike structures called *hyphae* as illustrated in Figure 8.1. The mass is known as *mycelium.* Depending on the fungal type, the hyphae may be *septate*, i.e., have cross wall or septum to produce multicells [refer to Figure 8.1 (b)] or *aseptate*, i.e., septum is absent and the cytoplasm is coenocetic [refer to Figure 8.1 (a)]. The hyphae grows on or within the surface of nutrient media to get nutrients. This represents vegetative mycelium. Some of the specialized hyphae arise upward away from the medium surface. It is called the aerial mycelium on which the reproductive structure or spores are formed.

**Figure 8.1: Fungal hyphae**

Some fungi are dimorphic, that is, at 37$^{o}$C these grow as yeast (Y) but at 25$^{o}$C change to mould (M) structure. This shift is known as *YM shift.*

Next, let us see how the fungi are classified.

## 8.2.3 Classification of Fungi

The fungi, as you may recall reading above, is now placed in a separate kingdom called *Myceteae*. In sub-section 2.4.4 in Unit 2 of the Food Microbiology and Safety theory Course, we have briefly described the classification of fungi. Traditionally, fungi were divided into four sub-divisions based on the mode of sexual reproduction as – Zygomycota, Ascomycota, Basidiomycota and Deuteromycota. Now, molecular microbiologists have put Deuteromycotina among their closed relatives in Eumycota (true fungi). The new sub-divisions are *Zygomycota, Ascomycota, Basidiomycota* and *Chytridiomycota* based on 18s rRNA studies.

Let us get to learn about these different sub-divisions of fungi.

*Zygomycota* – Fungi belonging to zygomycota are called *zygomycetes*. Hyphae in this sub-class are coenocytic. Asexual spores develop in sporangia at the tips of aerial hyphae. Sexual spores called zygospores, as illustrated in Figure 8.2 (a), are formed by fusion of different mating types designated positive and negative, because male and female are morphologically indistinguishable. Zygomycetes are involved in production of foods, like tempeh, sufu. It is also used in commercial preparation of some anaesthetics, birth control agents, industrial alcohol, meat tenderizers etc. Example: *Rhizopus, Mucor*.

**Figure 8.2: Fungi divisions**

*Ascomycota* – Ascomycetes or sac-like fungi have septate mycelium. These are called so because sexual reproduction involves the formation of an *ascus* - a sac-like structure containing ascospores, as shown in Figure 8.2 (b). Asexual reproduction is through development of conidiospores. Many yeast are also classified within the ascomycetes because of their sexual reproduction. Examples: *Claviceps, Neurospora.*

Ascomycetes are important as many of these are involved in food spoilage, cause diseases in plants and are used as research tool in genetics and biochemistry. Few ascomycetes produce toxins.

*Basidiomycota* – Fungi in this division are called *basidiomycetes* or *club fungi*. Sexual structure is known as *basidium*, as shown in Figure 8.2 (c), which is club-shaped structure producing basidiospores e.g. rusts, mushrooms, puffballs etc. Basidiomycetes are involved in decomposing plant debris. Many basidiomycetes causes plant, animal and human diseases. Toxins or hallucinogens are produced by certain mushrooms.

*Deuteromycota* – Deuteromycetes or fungi imperfecti reproduce by means of conidia. These have either lost the capacity for sexual reproduction or it has never been observed. Sexual spores if observed in the members of Deuteromycota, it is reclassified into a different genus and placed accordingly into appropriate division.

Molecular systematics places the Deuteromycota among their closest relatives in Eumycota.

Fungi imperfecti are important as many of them are human pathogens. Few are important industrially and are involved in antibiotics and food production. Some produce mycotoxins, which are highly toxic and carcinogens to animals and humans. Example: *Aspergillus, Penicillium.*

*Chytridiomycota* – Look at the Figure 8.3, which illustrates chytridiomycota. These are the simplest among true fungi and are commonly called *chytrides*. Reproduction is asexual by means of motile zoospores as shown in Figure 8.3. When sexual reproduction occurs, it results in formation of sporangium. The entire organism is microscopic in size and may consist of a single cell, a small multinucleate mass or a true mycelium.



**Figure 8.3: Chytridiomycota**

Having looked at the classification above, it must be clear to you that fungi can be divided into four sub-divisions based on the mode of sexual reproduction. In the next sub-section, let us learn a bit more about the reproduction of fungi.

### 8.2.4 Reproduction in Fungi

Fungi, we have seen above, reproduce both sexually and asexually. Asexual mode of reproduction include budding, binary fission and more commonly through spore production. Figure 8.4 illustrates the budding process and binary fission process. Asexual spores include anthrospores, chlamydospores, sporangiospores, conidiospores and blastospores. Sexual reproduction involves union of compatible nuclei to produce zygospores, ascospores and basidiospores, as you have already seen in the Figure 8.2. The spores are important means in fungal dissemination and are also useful in identification of fungal species.

**Figure 8.4: Budding and binary fission process**

Finally, let us find out what food do the fungi need?

### 8.2.5 Nutrition of Fungi

Fungi grow in moist environment and are chemoorganoheterotrophs. What do we mean by *chemoorganoheterotrophs*? We have already read about this earlier in Practical 3, section 3.3. The term chemotroph describes organism that generate energy by oxidation of organic or inorganic compounds. Chemoorganoheterotrophs use organic compound as a source of energy, electron and carbon.

Most of the fungi grow on dead organic matter by producing exoenzymes. These are saprophytes. Few fungi are parasitic. Fungi are usually aerobic though some are anaerobic e.g., as in rumen of cattle. Yeasts are mostly facultative anaerobes (i.e., they can survive either in the presence or absence of oxygen).

With this, we end our study on basic structure, characteristics and nutrition of fungi. In the next section, we will learn about how to identify fungi.

## 8.3 IDENTIFICATION OF FUNGI

A fungus can be identified by observing its growth on slide culture and plate culture, and also by observing its hyphae and sporulating structures microscopically under low and high power. Unknown fungus can be assigned to a particular group by consulting various monographs and books on fungal systematics.

Both macroscopic and microscopic characteristics can be considered for identification. These characteristics are highlighted herewith:

*Macroscopic*

Under the macroscopic characteristics are included the:

(1) Colonial morphology – waxy, leathery, powdery, fluffy, cottony, velvety, etc.

(2) Colony colour and size.

(3) Underside of plate.

Next let us consider the microscopic characteristics.

*Microscopic*

(i) Hyphae structure and colour – septate/coenocytic; coloured/colourless

(ii) Reproductive structures – Ascocarp/basidiocarp, simple conidiophores/synnamata/ sporocladia/pycnidia.

(iii) Type of spores (sexual, asexual or both).

Sexual Spore – Ascospore/basidiospore/oospores or zygospore.

Asexual Spores – Zoospores/bporangiospore/conidium/blastospore.

(iv) Colour, size and shape of the spore.

(v) Arrangement of conidia – solitary/masses/chains

Based on this understanding, let us get to learn about the characteristics of some common fungi in the next section. We begin with the study of the characteristics of *Rhizopus*.

## 8.4 CHARACTERISTICS OF SOME COMMON FUNGI

Can you name a few common fungi? Yes, *Rhizopus* (found in bread, fruits, vegetables), *Aspergillus* (used in food industry for fermentation), *Mucor, Penicillium* etc. Let us get to know about their characteristics one by one. We begin with *Rhizopus*.

### 8.4.1 *Rhizopus*

*Classification*

| | |
|---|---|
| Kingdom | Mycetae |
| Division | Amastigomycota |
| Class | Zygomycetes |
| Order | Mucorales |
| Family | Mucoraceae |
| Genus | *Rhizopus* |

You will find the classification and identifying features of *Rhizopus* highlighted in this sub-section.

*Rhizopus* is a common laboratory contaminant. It is a spoiling mould and found frequently on the surface of bread, fruits and vegetables. It can grow as weak facultative parasite under certain conditions, causing soft rot of sweet potato and leak of peach, raspberry, strawberry and some other fungal diseases in animals and human. Various species of *Rhizopus* are exploited industrially for production of cortisone (*R. stolonifer)*, alcohol (*R. oryzae*), lactic acid and fumaric acid (*R. oryzae, R. stolonifer*).

Next let us look at the identifying features.

*Identifying features of Rhizopus:*

1. Macroscopically *Rhizopus* appears as a white cottony mass (look at rhizopus growth on media above) growing rapidly and spread over entire plate during vegetative phase. Later black pinhead like structures formed in reproductive phase giving blackish appearance as shown in Figure 8.5. Therefore, *Rhizopus* is also called *black mould.*

**Figure 8.5. The *Rhizopus***

2. Microscopically, hyphae are aseptate and coenocytic. There are 3 kinds of hyphae:

   (a) *Stolon* – These grow horizontally on substratum surface. Look at the Figure 8.5 and identify the stolon. Stolon runs horizontally for some distance and then arches over so that its tip touches the substratum and forms node-like structure from which fresh stolon arise. These hyphae help in the spread of the mycelium.

   (b) *Rhizoids* – These are brown slender root like structures, as you may have seen in Figure 8.5, which arise in cluster from each node of the stolon. These penetrate the substratum and helps in anchorage and the absorption of nutrients from the substratum. Rhizoids and stolons are present during vegetative phase.

   (c) *Sporangiophore* – Tufts of special, erect unbranched, hyphae growing in air arise from stolon just opposite to rhizoids, as illustrated in the Figure 8.6. These are sporangiophores which appear during reproductive phase

3. Sporangiophores swell at the tip into a spherical knob like structure called *sporangium.* It has two zones.

   - Central dome shaped zone called *columella*, and
   - Peripheral sporiferous zone in which black spores called *sporangiospores* are formed. Because of sporangiospores, sporangium appears black at maturity.

4. Sporangiospores are non-motile, colourless or brown, multi-nucleated, globose to oval structure.

5. *Rhizopus* usually reproduce asexually, but under unfavourable conditions, sexual reproduction (isogamous) occur resulting in formation of zygospores – thick, black, rough structures as you have already seen in Figure 8.2. Zygospores on germination produces a hyphae that bears an asexual sporangium and the cycle begins anew.

Next we shall get to know about the *Mucor*.

### 8.4.2 *Mucor*

*Classification*

| | |
|---|---|
| Kingdom | Mycetae |
| Division | Amastigomycota |
| Class | Zygomycetes |
| Order | Mucorales |
| Family | Mucoraceae |
| Genus | *Mucor* |

*Mucor* is present as a food contaminant. It resembles *Rhizopus* in life history and colonies appear just like *Rhizopus* as can be seen in Figure 8.6. However, important differences between the two exist. Can you identify these differences by comparing Figures 8.5 and 8.6? Tally your response with the different characteristics highlighted herewith:

**Figure 8.6: Mucor species**

*Characteristic features of Mucor*

1. Stolons characteristics of *Rhizopus* are absent in *Mucor*. The aerial mycelium in *Mucor* consists of branched hyphae, which grow over the surface.
2. Rhizoids are absent in *Mucor*.
3. In *Mucor*, sporangiophores arise singly instead of a cluster of two or more sporangiophores as in case of *Rhizopus*.
4. Dehiscence of mature sporangia and dispersal of spores is different. In *Mucor*, sporangial wall dissolves instead of cracking into fragments. Only a small collar-like narrow rim of the wall called collarete persists round the columella at the base. The spore mass is embedded in the liquid as a sporangial drop, which may be dispersed by insects.

So *Mucor* has:

(1) Nonseptate mycelium
(2) Single sporangiophore arises at a point.
(3) Globular sporangium containing a columella.
(4) Spores are oval
(5) No rhizoids.

Next let us study about the *Aspergillus*.

### 8.4.3 *Aspergillus*

| *Classification* | |
|---|---|
| Kingdom | Mycetae |
| Division | Amastigomycota |
| Class | Ascomycetes |
| Subclass | Plectomycetidae |
| Order | Eurotiales |
| Family | Eurotiaceae |
| Genus | *Eurotium* (*Aspergillus*) |
| Form Class | Deuteromycetes |
| Form Order | Moniliales |
| Form family | Moniliaceae |
| Form Genus | *Aspergillus* |

*Aspergillus* is a common laboratory contaminant. Its conidia are usually present in air. *Aspergillus* species are used industrially for manufacturing citric, gluconic and gallic acid and for fermenting soy sauce. Wide range of enzymes is produced by *A. niger* and *A. oryzae*. *Aspergillus* sp. also causes various diseases of plants.

Different species of *Aspergillus* like, *Aspergillus fumigatus* is responsible for aspergillosis in human. Certain *Aspergillus* species e.g. *A. flavus* produces aflatoxins, which are highly toxic, and carcinogens in human, causing liver cancer. We have already studied about this in Unit 5 in the Food Microbiology and Safety theory Course. *Aspergillus* species are also responsible for spoilage of food, leather, cotton fabric etc., thus reducing their commercial value. It can grow on decaying vegetables, butter, ghee, bread, rice, jam, jellies etc. easily by producing large number of enzymes.

The identifying features of *Aspergillus* are illustrated next.

*Identifying features of Aspergillus:*

Look at the Figure 8.7. It illustrates the *Aspergillus*.

**Figure 8.7: Aspergillus**

The identifying features include:

1. Macroscopically *Aspergillus* colonies are powdery and are of different colours like green, blue, black, yellow, brown etc.
2. Microscopically mycelium consists of branched, bright or pale coloured hyphae some of which grow within the substrate while others grow on the substrate.
3. From these vegetative hyphae, long, unbranched, nonseptate erect hyphae arise called *conidiophores*. The cell from which conidiophore arise is called *foot cell*. It is thick walled and T-shaped and one conidiophore arises from each foot cell.
4. Conidiophores terminate into a globular structure called *vesicle*, as you can see in the Figure 8.7.
5. Around the vesicle, there are 1-2 layers of flask shaped structures called *phialides* or *sterigmata*.
6. At the tip of the sterigmata, a chain of small unicellular spores called *conidia* arises. These conidia are formed in basipetal manner (oldest is at the top). These are arranged compactly side by side. The whole structure consisting of the foot cell, the upright hypha, the vesicle, the metullae and the phialides constitutes the conidiophore.
7. Sexual Reproduction occurs by formation of ascus and ascospores.

The next fungi covered in this section is *Penicillium.*

## 8.4.4 *Penicillium*

| *Classification* | |
|---|---|
| Kingdom | Mycetae |
| Division | Amastigomycota |
| Class | Ascomycetes |
| Subclass | Plectomycetidae |
| Order | Eurotiales |
| Family | Eurotiaceae |
| Genus | Talromyces (*Penicillium*) |
| Form Class | Deuteromycetes |
| Form Order | Moniliales |
| Form Family | Moniliacea |
| Genus | *Penicillium* |

*Penicillim* is cosmopolitan in distribution. It is called green or blue mould (look of growth or media above) though exists in different colours. *Penicillium* is used in industries for production of organic acids like oxalic, fumaric and citric acid. It is also a source of antibiotics like *Penicillin* and *Griseofulvin* which are produced by *P. chrysogenum* and *P.griseofulvin*. In cheese industries *Penicillium* (example: *P. camemberti* and *P.roqueforti*) is employed to impart distinctive flavour and odour to the product. On the other hand, *Penicillium* damage leather goods, fabrics and wood furniture. It also spoils bread, cheese, butter, jam, jelly and other food stuffs. Some are plant pathogens, e.g., soft rot disease of citrus fruit is caused by *P.italicum* and *P.digitatum.* Mycotoxins and ochratoxins are produced on cereal grains by *P. viridicatum.*

Let us next study the identifying features of *Penicillium.*

*Identifying features of Penicillium:*

Figure 8.8 illustrates the *Penicillium* as observed through the microscope. The features include:

**Figure 8.8: *Penicillium***

The identifying features of *Penicillium* are:

1. Mycelium consists of colourless, septate and branched hyphae, some of which grow inside the substratum to get nutrients and the rest spread on the surface. Former are called *haustaria hyphae*.
2. Erect, tubular septate hyphae called conidiophores grow outward in the air from any cell of the mycelium. No foot cells are present in *Penicillium.* Only one conidiophore arises from one cell.
3. Unlike *Aspergillus*, conidiophores branches once, twice or even more times to produce primary, secondary or tertiary branches. The ultimate branches bears tufts of flask shaped structures called *sterigmata* (phialides). These branches are called the *metulae* while lower branches which support the metulae are called *rami*. At the tip

of the sterigmata, a long chain of conidia arise in a basigenous arrangement. The conidia are shed continuously. The conidiophore along with remi, metulae, sterigmata and conidia gives artist's brush or broom like appearance and the structure is called penicillus, as illustrated in the Figure 8.8.

4. The conidia are tiny, uninucleate and unicellular, globose, solid, elliptical or pyciform structures. These may be smooth or rough.

5. Sexual reproduction is observed in a few species by formation of asci containing ascospores. Ascospores are uninucleate, lens shaped structures.

From our discussion above you may have noticed that *Penicillium* like *Aspergillus* also grow conidiophore structure. But *Aspergillus* and *Penicillium* differ in their conidiophore structure. Former has a conidiophore which is nonseptate, unbranched and arising from a foot cell. It ends in a vesicle at its tip bearing sterigmata and conidia. Instead in *Penicillium*, a conidiophore is septate, branched and forms a broom-like structure – *Senicillus*. There is no foot cell and vesicles.

Next let us study about *Alternaria*.

### 8.4.5 *Alternaria*

*Classification*

| | |
|---|---|
| Kingdom | Mycetae |
| Division | Amastigomycota |
| Form Class | Deuteromycetes |
| Form Subclass | Hyphomycetidae |
| Form Order | Moniliales |
| Form Family | Dematiaceae |
| Form Genus | *Alternaria* |

*Alternaria* is widely distributed and is one of the common laboratory contaminants. Usually it grows as saprophyte on dead organisms but some are pathogenic to plants, animals and human e.g. early blight of potato (*A. solani*), leaf spot disease in crucifers (*A. brassicae*), leaf blight of wheat seedlings (*A. tenuis*), infection of mustard seeds (*A. brassiciae*), allergies and skin diseases in humans etc. *Alternaria* also causes decomposition of fruits and vegetables.

Let us next look at the identifying features of *Alternaria*.

Identifying features of *Alternaria*:

*Alternaria*, along with its identifying features is illustrated in the Figure 8.9.

**Figure 8.9: Alternaria**

1. *Alternaria* colony is wooly and compact. Underside is very dark coloured. Colony colour is grayish green or black with gray edges rapidly spreading over entire plate.
2. Mycelium consists of short septate, branched and light brown coloured hypae.
3. Multiply asexually by spore production or conidia formation, as shown in Figure 8.9.
4. Conidia reproduced at tips of short hyphae. Special structures such as conidiophores are not recognized.
5. Conidia are large, dark coloured, multicellular and beaked, as you may have noticed in Figure 8.9. Transverse, as well as, longitudinal septa are present. 8-14 or even more cells are present per spore.
6. Spores of *Alternaria* belongs to the category of dictyospore.

The next fungi we shall study is *Fusarium.*

### 8.4.6 *Fusarium*

| *Classification* | |
|---|---|
| Kingdom | Mycetae |
| Division | Amastigomycotina |
| Subdivision | Deuteromycotina |
| Class | Deuteromycetes |
| Order | Moniliales |
| Family | Tuberculariaceae |
| Genus | *Fusarium* |

*Fusarium* is the largest form genus of this family. It is found in soil. Many are saprophytic while others are mild facultative parasites and parasites. It causes stem canker, foot rot and wilt diseases in plants. Also, it causes rot of stored fruits, vegetables etc.

The identifying features of *Fusarium* are enumerated herewith:

Identifying features of *Fusarium:*

1. Wooly, white fuzzy colonies changing colour to pink, purple or yellow.
2. Mycelium consists of septate and branched hyphae. Hyphae are colourless or have a tinge of pink, purple or yellow when young, but at maturity become dark coloured.
3. Asexual reproduction takes place by formation of three kinds of spores – Microconidia, Macroconidia and Chlamydospores. Look at the Figure 8.10 which illustrates these three kinds of spores.
4. Microconidia are very small, rounded or oval and arise at the tips of simple or branched conidiophores in small masses. Look at the Figure 8.10 and identify the microconidia. The conidiophores are distinguishable from the vegetative hyphae.

**Figure 8.10: Fusarium**

5. Macroconidia, as you may have noticed in Figure 8.10, are large, elongated, sickle or crescent shaped, multi-cellular conidia having two to four cells. These are produced at the tips of simple or sparingly branched conidiophores, which are assembled to form a *sporodochium* type of fructification.

6. Chlamydospores are round, oval and thick walled cells formed singly or in chains of two or more. These are resting spores.

7. Compact resting bodies called sclerotia are also produced which serve as a storage organ and a means of perennation.

Finally let us get to know the *Cladosporium.*

### 8.4.7 *Cladosporium*

*Classification*

| | |
|---|---|
| Kingdom | Mycetae |
| Division | Amastigomycota |
| Form - Class | Deauteromycetes |
| Form - Order | Moniliales |
| Form - Family | Dematiaceae |
| Form - Genus | *Cladosporium* |

*Cladosporium* are found on dead and decaying plants. Figure 8.11 illustrates the *Cladosporium.*

**Figure 8.11: *Cladosporium***

The identifying features as highlighted in Figure 8.11 include:

Identifying Features of *Cladosporium:*

1. Colonies are small, heaped, powdery and greenish black in colour.

2. Mycelium is septate and usually brownish.

3. Spores (conidia) develop at the end of the complex conidiophores.

Before we end, let us quickly also study the identifying features of yeasts (e.g., *Saccharomyces*).

## 8.4.8 Yeast – *Saccharomyces*

| *Classification* | |
|---|---|
| Kingdom | Mycetae |
| Division | Amastigomycota |
| Class | Ascomycetes |
| Order | Endomycetales |
| Family | Saccharomycetaceae |
| Genus | *Saccharomyces* |

Yeasts are unicellular organisms, which are usually spherical or oval in shape as can be seen in Figure above. Some yeasts may be cylindrical. Few common examples of yeast are *Torula, Saccharomyces* etc. It is present on cheese and other foods. Colonies are white, pink, moist with unbroken even edges. Cells are oval, colourless.

Yeasts are facultative anaerobes and can survive well in various environments. Yeasts reproduce asexually by budding (budding yeast, e.g. *Saccharomyces*) or by binary fission (fission yeasts – *Schizosaccharomyces*). Look at the Figure 8.12, which illustrates the budding and binary fission process in yeasts

**Figure 8.12: Reproduction of Saccharomyces**

In budding, a small outgrowth called a bud arises. Parent nucleus divides and one nucleus migrates into a bud. Cell wall material is then laid down and the bud breaks away and grows to form a daughter cell. Fission yeast divides into two new cells by elongations followed by division into two.

Sexual reproduction is also observed in some yeasts. Ascospores are produced within the ascus through sexual reproduction, which are released and begin the cycle again.

As discussed earlier yeasts are both beneficial and harmful. Some yeasts causes disease in human e.g. *Candida albican* causes urinary and vaginal infections (moniliasis) and mouth infection (thrush).

Well then, that was an exhaustive review of the different fungi, which are important for sus and in the food industry. To understand them better, let us identify these in the laboratory. Let us get started with the exercises given in this practical. But first answer the review questions given in the section 8.5 so as to consolidate your understanding about fungi.

## 8.5 REVIEW QUESTIONS

1. Define mycelium and hyphae?

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2. What is coenocytic hyphae?

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3. What is fungal dimorphism? Give examples.

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4. How do the fungi differ from bacterium?

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5. On what basis fungi are classified?

.....

.....

6. Mention two harmful and two beneficial impacts of fungi.

.....

.....

7. Which stain is used for staining yeasts and moulds? Write its components and their role.

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.....

8. What is the industrial and medical significance of yeast?

.....

.....

9. Name the common methods used by yeast for multiplication.

.....

.....

10. Write about reproductive structures in *Penicillium*, *Aspergillus* and *Rhizopus*.

.....

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Now carry out Exercises 1-3 and identify the fungi for yourself. Please do not forget to get the answers to the review questions you have written above, evaluated by your counsellor.

# EXERCISE 1

# PREPARATION AND IDENTIFICATION OF TEMPORARY MOUNTS OF FUNGAL CULTURE

Date : .....

**Aim** : To make the temporary mounts of the given fungal cultures and identify them.

**Requirements** :

*Culture* : Asper*gillus, Alternaria, Fusarium, Mucor, Penicillium, Rhizopus*

*Reagent* : Lactophenol Cotton blue stain.

*Equipments & Glassware* : Clean glass slides, covers slips, ocular micrometer, bunsen burner, transfer needles.

**Theory/Principle:**

As discussed earlier, fungi are eukaryotic microorganisms having beneficial as well as harmful effects. These are important in the food chains because these decompose dead organic matter. Multicellular fungi identified on the basis of their physical appearance, including colony characteristics and reproductive structure. For yeasts identification biochemical tests are used. Lactophenol cotton blue solution is used for fungal staining. It contains lactic acid, (20.0 ml), phenol (20 gm), glycerol (40 ml), distilled water (20 ml) and aniline blue (0.05 gm). Glycerol gives viscosity while phenol kills the fungal cells. Lactic acid is used to colour the view. Cotton blue or aniline blue stains both the cell wall and cytoplasm. Few drops of cotton blue stain are used for staining purpose. After staining the sample is observed at low, as well as, at high power. Identifying characteristics are noted and used for identifying the fungal specimens.

**Procedure:**

Now carry out the exercise following the steps enumerated herewith:

(1) Observe mould culture for the colonial appearance and colour. Also observe the underside.

(2) Label the clean, non-greasy glass slide with the name of the organism using the permanent marker.

(3) Put a drop of lactophenol cotton blue stain on a slide. Gently take a fungal culture from the culture plate with the help of the dissecting needle and put it on the slide without damaging the fungal structure.

(4) Put a cover slip on the sample on the slide.

(5) Examine the preparations first under low power (10x) and then under high power (40x) objective.

(6) Take dimensions of various structures, i.e., hyphae, spores, reproductive bodies etc. if possible using calibrated ocular micrometer. Identify the culture, on the basis of colony morphology and microscopic observations.

(7) Record your observations and draw a representative microscopic field of your culture in the Observation and Result section herewith.

**Precautions:**

1. Aseptic conditions should be maintained.
2. No air bubbles should be present in the sample preparation.

**Observations and Result:**

Record your observations for all the given fungi in the format given herewith:

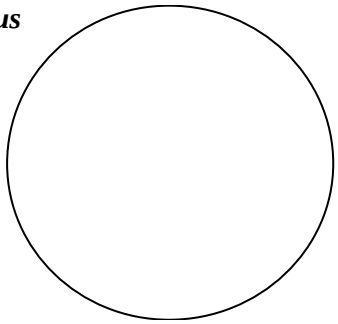
| | ***Aspergillus*** | ***Alternaria*** | ***Fusarium*** | ***Mucor*** | ***Penicillium*** | ***Rhizopus*** |
|---|---|---|---|---|---|---|
| Observations (as you observed) Colony morphology | | | | | | |
| Colour | | | | | | |
| Microscopic Structure* | | | | | | |

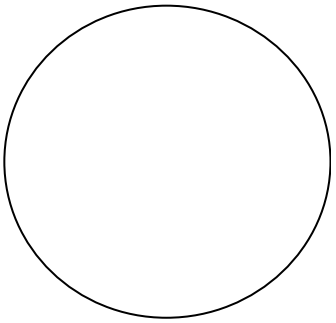
*(Comment about hyphae structure, reproductive structure, type of spore size, shape of spore, arrangement of conidia etc.).

Diagram of Microscopic appearance: Based on what you observe in the microscope, draw the structure here in the circle(s) given herewith.

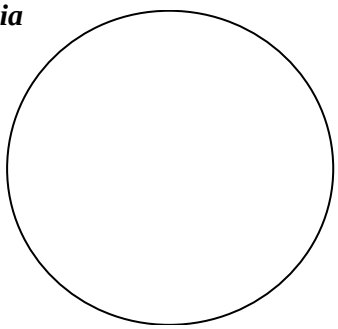
Low Power Magnification | High Power Magnification

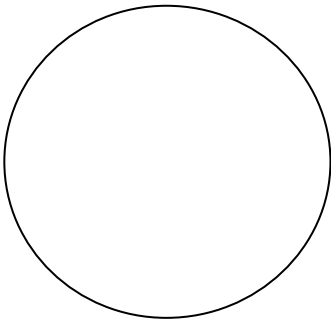
***Aspergillus***



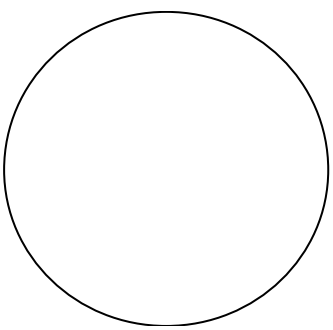


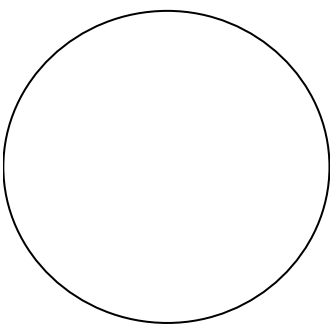
***Alternaria***



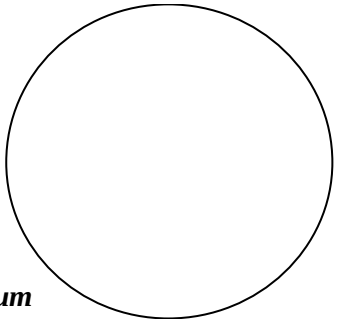


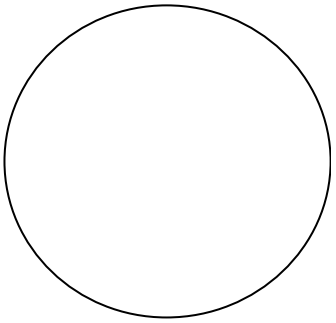
***Fusarium***



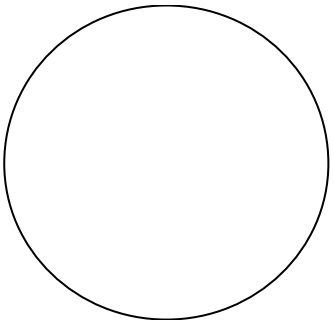


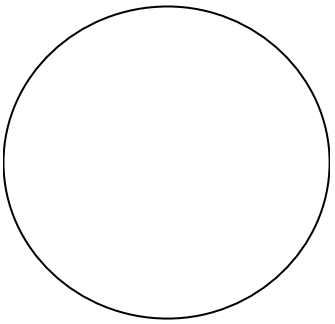
***Mucor***



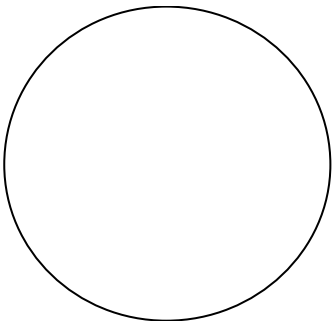


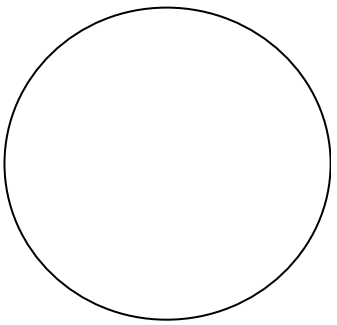
***Penicillium***





***Rhizopus***





**Inference/Conclusion:**

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**Submit the exercise along with the review questions for evaluation.**

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**Counsellor Signature**

# EXERCISE 2

## MORPHOLOGICAL CHARACTERISTICS OF YEAST CELLS

**Aim** : To study the morphological characteristics of Yeast Cells. Date : .....

**Requirements** :

*Culture* : Yeast S*accharomyces cerevisiae*

*Reagents* : Lactophenol Cotton blue stain, water-iodine solution

*Equipments & Glassware* : Bunsen burner, cover slip, inoculating loop and needle, glass slide, microscope.

**Theory/Principle:**

Look up section 8.6. Go to sub-section 8.4.8, which presents a brief write up on yeast. Based on your understanding, present a brief review on yeast in cell size, shape, the process of reproduction, beneficial and harmful effects etc. in the space provided herewith:

**Procedure:**

Now carry out the exercise following the steps enumerated herewith:

(1) Label the clean, non-greasy slide. Put few drops of water-iodine solution (Gram's Iodine-10ml, Distilled water - 30ml) on it.

(2) Suspend a loop full of yeast culture in the water-iodine solution and cover with the cover slip. Instead of water-iodine solution lactophenol – cotton blue stain can also be used.

(3) Observe the slide preparation under low and high power for the shape, size and budding or fission in the yeast. Draw a representative microscopic field.

**Precautions:**

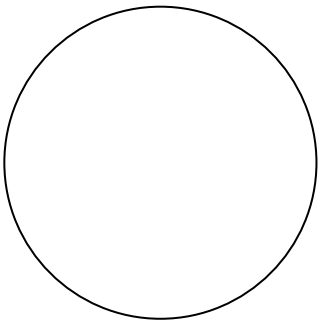
1. Observe aseptic conditions.
2. Use only a small sample for staining.
3. No air bubbles should be in the slide preparation.

## Observations and Results:

Record your observations in the format given herewith:

| Observations | Result |
|---|---|
| ***In water-iodine solution*** | |
| Under 10x | |
| Shape | |
| Size | |
| Budding | |
| Under 40x | |
| Shape | |
| Size | |
| Budding | |
| ***In lactophenol cotton blue solution*** | |
| Under 10x | |
| Shape | |
| Size | |
| Budding | |
| Under 40x | |
| Shape | |
| Size | |
| Budding | |

Draw the representative microscopic field here.



**Inference/Conclusion:**

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**Submit the exercise for evaluation.**

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**Counsellor Signature**

# EXERCISE 3

## MORPHOLOGICAL STUDY OF FUNGI BY SLIDE CULTURE TECHNIQUE

Date : .....

**Aim** : To study morphology of fungi by slide culture technique.

**Requirements** :

*Culture* : Culture of any fungus (e.g., *Aspergillus*).

*Media* : Czapek dox agar medium

*Equipments & Glassware* : Glass microscopic slide, sterile Petri-plate, cover slip, whatman filter paper, forceps, dissecting needles, transfer needles.

**Theory/Principle:**

The usual method of observing the moulds i.e., lifting a portion of the fungus and staining it after keeping it on a slide may disrupt the arrangement of spores and cells developing conidia. The best way to study morphological characteristics of the fungi without disturbing their structure is to use slide culture technique.

Write the theory related to slide culture technique in the space provided herewith:

You may refer to Practical 5, Section for details on slide culture media. Read through the theory given in this section and briefly describe the process in the space provided herewith:

**Procedure:**

Now carry out the exercise, following the steps enumerated herewith:

1. Prepare a humid chamber by placing two-three pieces of filter paper in a Petri plate and moisten with distilled water.
2. Sterilize the plate by autoclaving.
3. Put a Sterilized U shaped glass tube in a Petri plate over the moistened filter paper with a help of sterile forcep.
4. Put a clean, sterilized slide over U tube as shown in the Figure in the margin.
5. Cut a block of Czapek dox agar (10 × 10 mm) from medium plate with a flamed blade and inoculate its top and bottom surfaces with the given fungal culture.

6. Gently keep the agar block on the slide in plate with one of inoculated surface in contact with the slide surface.
7. Place gently a flamed cover slip on the upper inoculated surface of the block.
8. Incubate the whole plate at $28 \pm 2^{o}C$.
9. Observe the slide for the growth of the fungus and examine intermittently under microscope for conidial formation and arrangement.
10. Prepare semi-permanent mounts from cover slip and slide for observing conidiation/ sporulation.
11. For cover slip semi-permanent mount, remove the cover slip carefully from the upper surface of the agar block with the help of flame sterilized forceps. Put a drop of 95% ethanol on fungus side of the cover-slip. After draining excess of alcohol, put the fungus side of cover-slip on a drop of lactophenol cotton blue on a slide. Examine under the microscope.
12. In a similar manner slide semi-permanent mount is made. For the same, remove the agar block from the slide gently and put the block in the disinfectant solution. Put 95% alcohol on the fungus growth on the slide. Drain off the excess alcohol and stain with lactophenol cotton blue. After putting a coverslip observe under the microscope.

**Precautions:**

1. Use sterile forcep and needles for slide culture technique.
2. Aseptic conditions should be maintained.
3. Carefully remove the coverslip and agar block for semi-permanent mount so that morphologic characteristics of the fungus won't disturb.
4. Entry of air bubbles should be avoided when placing the cover-slip over a drop of stain.
5. Use sufficient amount of stain so that it completely covers the 18 mm cover-slip.
6. Remoisten the filter paper with sterile water when it becomes dry.

**Observations and Results:**

Record your observations of the slide in the format provided herewith:

| Observations | Result |
|---|---|
| **Fungal Structure** | |

**Inference/Conclusion:**

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Before you end this exercise, do answer the review questions given herewith which will help you to consolidate your understanding regarding morphological study of fungi by slide culture technique.

**Review Questions**

1. What is the importance of slide culture techniques?

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2. What precautions have to be taken while performing slide culture?

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3. What is the purpose of using a humid chamber and U shaped glass rod in the Petri dish?

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**Now submit the exercise for evaluation.**

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**Counsellor Signature**