



INTRODUCTION TO MYCOLOGY

The term "mycology" is derived from Greek word "mykes" meaning mushroom. Therefore mycology is the study of fungi.

The ability of fungi to invade plant and animal tissue was observed in early 19th century but the first documented animal infection by any fungus was made by Bassi, who in 1835 studied the muscardine disease of silkworm and proved that the infection was caused by a fungus *Beauveria bassiana*.

In 1910 Raymond Sabouraud published his book *Les Teignes*, which was a comprehensive study of dermatophytic fungi. He is also regarded as father of medical mycology.

Importance of fungi: Fungi inhabit almost every niche in the environment and humans are exposed to these organisms in various fields of life.

Beneficial Effects of Fungi:

1. Decomposition - nutrient and carbon recycling.
2. Biosynthetic factories. The fermentation property is used for the industrial production of alcohols, fats, citric, oxalic and gluconic acids.
3. Important sources of antibiotics, such as Penicillin.
4. Model organisms for biochemical and genetic studies. Eg: *Neurospora crassa*
5. *Saccharomyces cerviciae* is extensively used in recombinant DNA technology, which includes the Hepatitis B Vaccine.
6. Some fungi are edible (mushrooms).
7. Yeasts provide nutritional supplements such as vitamins and cofactors.
8. Penicillium is used to flavour Roquefort and Camembert cheeses.
9. Ergot produced by *Claviceps purpurea* contains medically important alkaloids that help in inducing uterine contractions, controlling bleeding and treating migraine.
10. Fungi (*Leptolegnia caudate* and *Aphanomyces laevis*) are used to trap mosquito larvae in paddy fields and thus help in malaria control.

Harmful Effects of Fungi:

1. Destruction of food, lumber, paper, and cloth.
2. Animal and human diseases, including allergies.
3. Toxins produced by poisonous mushrooms and within food (Mycetism and Mycotoxicosis).
4. Plant diseases.
5. Spoilage of agriculture produce such as vegetables and cereals in the godown.
6. Damage the products such as magnetic tapes and disks, glass lenses, marble statues, bones and wax.

General properties of fungi:

1. They are eukaryotic; cells contain membrane bound cell organelles including nuclei, mitochondria, golgi apparatus, endoplasmic reticulum, lysosomes etc. They also exhibit mitosis.
2. Have ergosterols in their membranes and possesses 80S ribosomes.
3. Have a rigid cell wall and are therefore non-motile, a feature that separates them from animals. All fungi possess cell wall made of chitin.
4. Are chemoheterotrophs (require organic compounds for both carbon and energy sources) and fungi lack chlorophyll and are therefore not autotrophic.
5. Fungi are osmotrophic; they obtain their nutrients by absorption.
6. They obtain nutrients as saprophytes (live off of decaying matter) or as parasites (live off of living matter).
7. All fungi require water and oxygen and there are no obligate anaerobes.
8. Typically reproduce asexually and/or sexually by producing spores.
9. They grow either reproductively by budding or non-reproductively by hyphal tip elongation.
10. Food storage is generally in the form of lipids and glycogen.

Classification of fungi:

Fungi were initially classified with plants and were a subject of interest for botanists; hence the influence of botany can be seen on their classification. In 1969 R.H Whittaker classified all living organisms into five kingdoms namely Monera, Protista, Fungi, Plantae and Animalia.

Traditionally the classification proceeds in this fashion:

Kingdom - Subkingdom - Phyla/phylum - Subphyla - Class - Order - Family - Genus- Species

This classification is too complicated to be dealt here.

There are alternate and more practical approaches, one based on sexual reproduction and the other based on morphology of the thallus (vegetative structure).

Based on Sexual reproduction:

1. Zygomycetes: which produce through production of zygospores.
2. Ascomycetes: which produce endogenous spores called ascospores in cells called asci.
3. Basidiomycetes: which produce exogenous spores called basidiospores in cells called basidia.
4. Deuteromycetes (Fungi imperfecti): fungi that are not known to produce any sexual spores (ascospores or basidiospores). This is a heterogeneous group of fungi where no sexual reproduction has yet been demonstrated.

Based on Morphology:

1. Moulds (Molds): Filamentous fungi Eg: *Aspergillus sps*, *Trichophyton rubrum*
2. Yeasts: Single celled cells that buds Eg: *Cryptococcus neoformans*, *Saccharomyces cerviciae*
3. Yeast like: Similar to yeasts but produce pseudohyphae Eg: *Candida albicans*
4. Dimorphic: Fungi existing in two different morphological forms at two different environmental conditions. They exist as yeasts in tissue and in vitro at 37°C and as moulds in their natural habitat and in vitro at room temperature. Eg: *Histoplasma capsulatum*, *Blastomyces dermatidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*

Some 200 "human pathogens" have been recognized from among an estimated 1.5 million species of fungi.

Morphology of fungi:

Fungi exist in two fundamental forms; the filamentous (hyphal) and single celled budding forms (yeast). But, for the classification sake they are studied as moulds, yeasts, yeast like and dimorphic fungi.

All fungi have typical eukaryotic morphology. They have rigid cell wall composed of chitin, which may be layered with mannans, glucans and other polysaccharides in association with polypeptides. Some lower fungi possess cellulose in their cell wall. Some fungi such as *Cryptococcus* and yeast form of *Histoplasma capsulatum* possess polysaccharide capsules that help them to evade phagocytosis.

Inner to the cell wall is the plasma membrane that is a typical bi-layered membrane in addition to the presence of sterols. Fungal membranes possess ergosterol in contrast to cholesterol found in mammalian cells. The cytoplasm consists of various organelles such as mitochondria, golgi apparatus, ribosomes, endoplasmic reticulum, lysosomes, microtubules and a membrane enclosed nucleus. A unique property of nuclear membrane is that it persists throughout the metaphase of mitosis unlike in plant and animal cells where it dissolves and re-forms. The nucleus possesses paired chromosomes.

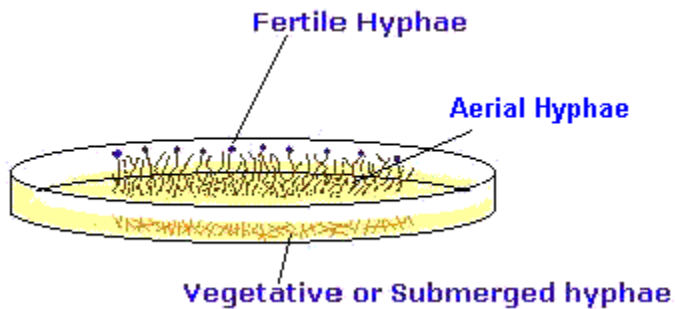
Moulds:

The thallus of mould is made of hyphae, which are cylindrical tube like structures that elongates by growth at tips. A mass of hyphae is known as mycelium. It is the hypha that is responsible for the filamentous nature of mould. The hyphae may be branched or unbranched. They may be septate or aseptate. Hyphae usually have cross walls that divide them into numerous cells. These cross walls, called septa have small pores through which cytoplasm is continuous throughout the hyphae. Therefore all hyphal fungi tend to be coenocytic (multinucleate). With exception of zygomycetes (*Rhizopus*, *Mucor*), all moulds are septate. Non-septate hyphae are considered to be more primitive because if a hyphal strand is damaged the entire strand dies. When a septate hyphal strand is damaged, the pores between adjacent compartments can be plugged, thus preventing death of the whole hyphal strand.

Mycelium are of three kinds:

1. **Vegetative mycelium** are those that penetrates the surface of the medium and absorbs nutrients.

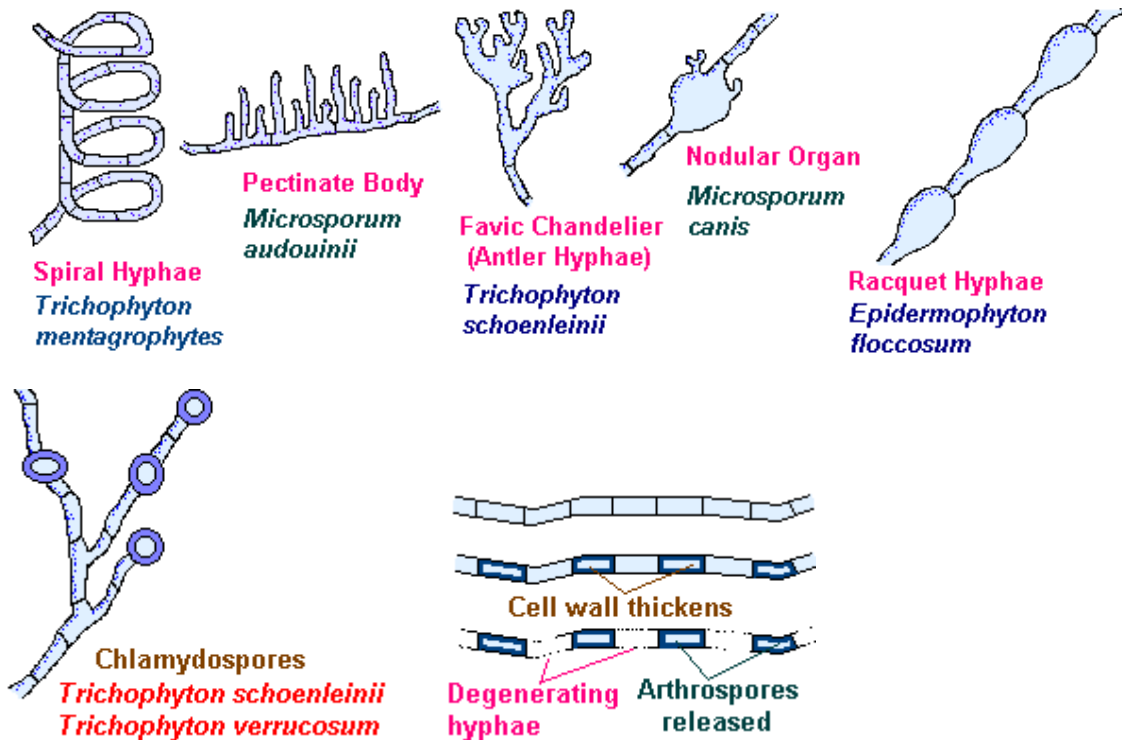
2. **Aerial mycelium** are those that grow above the agar surface
3. **Fertile mycelium** are aerial hyphae that bear reproductive structures such as conidia or sporangia.



Since hypha is the structural unit of mould, the mycelium imparts colour, texture and topography to the colony. Those fungi that possess melanin pigments in their cell wall are called phaeoid or dematiaceous and their colonies are coloured grey, black or olive. Examples are species of *Bipolaris*, *Cladosporium*, *Exophiala*, *Fonsecaea*, *Phialophora* and *Wangiella*. Those hyphae that don't possess any pigment in their cell wall are called hyaline. Hyphae may have some specialised structure or appearance that aid in identification.

Some of these are:

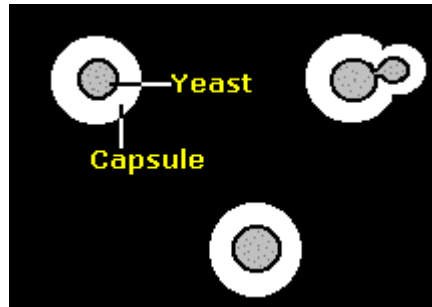
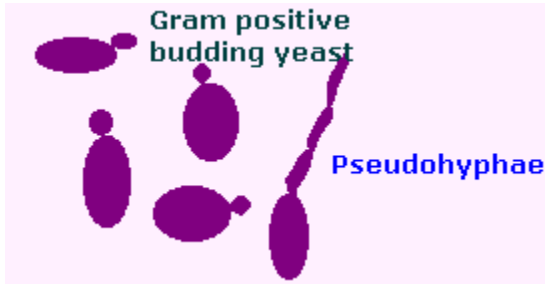
- a) **Spiral hyphae**: These are spirally coiled hyphae commonly seen in *Trichophyton mentagrophytes*.
- b) **Pectinate body**: These are short, unilateral projections from the hyphae that resemble a broken comb. Commonly seen in *Microsporum audouinii*.
- c) **Favic chandelier**: These are the group of hyphal tips that collectively resemble a chandelier or the antlers of the deer (antler hyphae). They occur in *Trichophyton schoenleinii* and *Trichophyton violaceum*.
- d) **Nodular organ**: This is an enlargement in the mycelium that consists of closely twisted hyphae. Often seen in *Trichophyton mentagrophytes* and *Microsporum canis*.
- e) **Racquet hyphae**: There is regular enlargement of one end of each segment with the opposing end remaining thin. Seen in *Epidermophyton floccosum*, *Trichophyton mentagrophytes*.
- f) **Rhizoides**: These are the root like structures seen in portions of vegetative hyphae in some members of zygomycetes.
- g) There are structures in the hyphae, which arise out of modification of a single cell and transform into thick walled resting cells. Chlamyospore (or chlamydoconidia), which are produced by *Trichophyton schoenleinii* and *Trichophyton verrucosum* are thick walled cells that are larger than other cells and arranged singly or in groups. In some fungi such as *Trichosporon beigeilli* and *Coccidioides immitis* some alternating cells become thick walled and subsequently the intervening cells disintegrate leaving behind arthrospores (or arthroconidia).



Yeasts:

Yeasts are unicellular spherical to ellipsoid cells. They reproduce by budding, which result in blastospore (blastoconidia) formation. In some cases, as the cells buds the buds fail to detach and elongate thus forming a

chain of elongated hyphae like filament called pseudohyphae. This property is seen in *Candida albicans*. The same species also have the ability to produce true hypha, which is seen as germ tube. The difference between the two is that there is a constriction in pseudohyphae at the point of budding, while the germ tube has no constriction.



Some yeast such as *Cryptococcus* and the yeast form of *Blastomyces dermatitidis* produce polysaccharide capsule. Capsules can be demonstrated by negative staining methods using India ink or Nigrosin. The capsule itself can be stained by Meyer Mucicarmine stain.

Some yeasts are pigmented. *Rhodotorula sps* produces pink colonies due to carotenoid pigments while some yeasts such as *Phaeoannellomyces werneckii* and *Piedraia hortae* are dematiaceous, producing brown to olivaceous colonies.

True yeasts such as *Saccharomyces cerevisiae* don't produce pseudohyphae. Yeast-like fungi may be basidiomycetes, such as *Cryptococcus neoformans* or ascomycetes such as *Candida albicans*.

Reproduction in fungi:

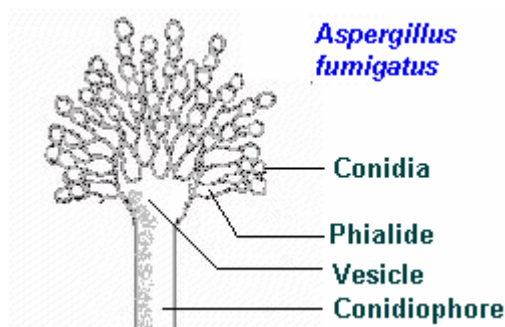
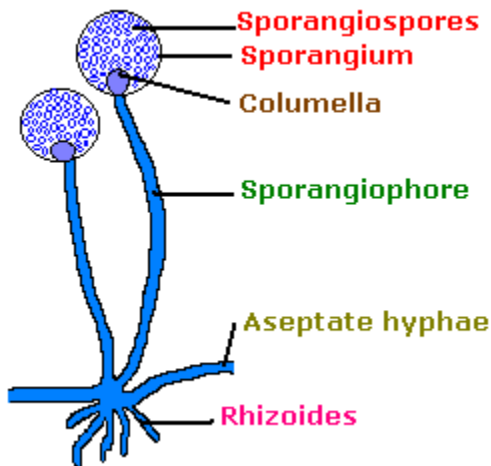
Fungi reproduce by asexual, sexual and parasexual means.

Asexual reproduction is the commonest mode in most fungi with fungi participating in sexual mode only under certain circumstances. The form of fungus undergoing asexual reproduction is known as anamorph (or imperfect stage) and when the same fungus is undergoing sexual reproduction, the form is said to be teleomorph (or perfect stage). The whole fungus, including both the forms is referred as holomorph. (*Taxonomically, the teleomorph or the holomorph is used, but practically it is more convenient to use the anamorph.*)

Asexual reproduction:

Asexual propagules are termed either spores or conidia depending on their mode of production. Asexual spores are produced following mitosis where as sexual spores are produced following meiosis.

The asexual spores of zygomycetes, which are known as sporangiospores form within sac like structure known as sporangia. The sporangiospores result from the mitotic cleavage of cytoplasm in the sporangium. The sporangia are borne on special hyphae called sporangiophore. This endogenous process of spore formation within a sac is known as sporogenesis.



Conidia arise either by budding off conidiogenous hyphae or by differentiation of preformed hyphae. These develop following mitosis of a parent nucleus and are formed in any manner except involving cytoplasmic cleavage. This exogenous process is known as conidiogenesis, a process that occurs both in yeasts and moulds. Conidia are borne on specialised structures called conidiophore.

Conidia production may be blastic or thallic. In blastic development the conidium begins to enlarge and a septum is formed. Here the conidium originates from part of parent. In thallic mode of development the conidium is differentiated by a septum before its differentiation. Thus the conidium results from the conversion of entire parent cell into the conidium.

The cell that gives rise to a conidium is called a conidiogenous cell. Conidiophores are specialised hyphae that bear conidia or conidiogenous cells. In many cases conidiogenous cells are referred as phialides.

Sexual Reproduction:

Sexual propagules are produced by the fusion of two nuclei that then generally undergo meiosis.

The first step in sexual methods of reproduction involves plasmogamy (cytoplasmic fusion of two cells). The second step is karyogamy (fusion of two compatible nuclei), resulting in production of diploid or zygote nucleus. This is followed by genetic recombination and meiosis. The resulting four haploid spores are said to be sexual spores, e.g. zygospores, ascospores and basidiospores.

If a sexual spore is produced only by fusion of a nucleus of one mating type with a nucleus of another mating type (+ and - strains), the fungus is said to be heterothallic. In contrast, homothallic moulds produce sexual spores following the fusion of two nuclei from the same strain. For sexual reproduction to occur, two compatible isolates are required.

Zygospores, which are the sexual spores of zygomycetes are round, thick walled reproductive structures that result from the union of two gametangia. Ascomycetes produce sexual spores called ascospores in a special sac like cell known as ascus. In basidiomycetes the basidiospores are released from basidium, which is the terminal cell of a hyphae.

Parasexual reproduction:

Parasexual reproduction, first seen in *Aspergillus* is known to occur in basidiomycetes, ascomycetes and deuteromycetes. The process involves genetic recombination without the requirement of specific sexual structures.

Importance of Spores:

A. Biological

- 1) Allows for dissemination
- 2) Allows for reproduction
- 3) Allows the fungus to move to new food source.
- 4) Allows fungus to survive periods of adversity.
- 5) Means of introducing new genetic combinations into a population

B. Practical

- 1) Rapid identification (also helps with classification)
- 2) Source of inocula for human infection
- 3) Source of inocula for contamination

ZYGOMYCETES

Commonly known as bread moulds, these are fast growing, terrestrial, largely saprophytic fungi. Hyphae are coenocytic and mostly aseptate. Asexual spores include chlamydoconidia, conidia and sporangiospores. Sporangioophores may be simple or branched. Sexual reproduction involves producing a thick-walled sexual resting spore called a zygospore.

Medically important orders and genera include:

1. Entomophthorales: *Conidiobolus* and *Basidiobolus* are involved in subcutaneous zygomycosis
2. Mucorales: *Rhizopus*, *Mucor*, *Rhizomucor*, *Absidia* and *Cunninghamella* are involved in subcutaneous and systemic zygomycosis (formerly called Mucormycosis)

BASIDIOMYCETES

They exist as saprobes and parasites of plants. Hyphae are dikaryotic and can often be distinguished by the presence of clamp connections over the septa. Sexual reproduction is by the formation of exogenous basidiospores, typically four, on a basidium. Occasional species produce conidia but most are sterile.

Genera of medical importance include:

1. Teleomorph of *Cryptococcus neoformans*, which is *Filobasidiella neoformans*
2. Agents of basidiomycosis such as *Coprinus* and *Schizophyllum*
3. Mushroom poisoning by *Amanita*, *Lepiota*, *Coprinus* and *Psilocybe* etc.

ASCOMYCETES

They exist as saprophytes and parasites of plants. Hyphae are septate with simple septal pores. Asexual reproduction is by conidia. Sexual reproduction is by the formation of endogenous ascospores, typically eight, in an ascus.

Medically important genera include the:

1. Teleomorphs of known pathogenic fungi e.g. *Arthroderma* (of *Trichophyton* and *Microsporum*), *Ajellomyces dermatitidis* (of *Blastomyces dermatitidis*), *Pseudallescheria boydii* (of *Scedosporium apiospermum*)
2. Agents of mycetoma, like *Leptosphaeria*
3. Agents of black piedra, like *Piedraia hortae*.

DEUTEROMYCETES

Deuteromycetes are also known as Fungi Imperfecti because of absence of sexually reproducing forms (teleomorph or perfect stage). As their teleomorph continue to be discovered, they would be classified among the previous categories, until then this remains an artificial and heterogeneous group.

There are three classes of Fungi Imperfecti.

1. **Blastomycetes:** These include asexual budding forms of *Cryptococcus*, *Candida*, *Torulopsis* and *Rhodotorula*. Depending on the presence of melanin in their cell walls, they may be non-dematiaceous or dematiaceous.
2. **Hyphomycetes:** A class of mycelial moulds which reproduce asexually by conidia on hyphae. Hyphae are septate. This class contains the majority of medically important fungi. Dematiaceous hyphomycetes are those conidial fungi that produce dark brown, green-black, or black colonies and are the causative agents of phaeohyphomycosis. Hyaline hyphomycetes include those conidial fungi, which are not darkly pigmented; colonies may be colourless or brightly coloured. These include the agents of hyalohyphomycosis, aspergillosis, dermatophytosis and the dimorphic pathogens, like *Histoplasma capsulatum*.
3. **Coelomycetes:** These produce acervuli, which are tightly bound mats of hyphae on which conidia are produced.

Pathogenesis of fungal diseases (Mycoses):

Most fungi are saprophytic or parasitic to plants and are adapted to their natural environment. Infection in humans is a chance event, occurring only when conditions are favourable. Except for few fungi such as the dimorphic fungi that cause systemic mycoses and dermatophytes, which are primary pathogens, the rest are only opportunistic pathogens.

Human body is a hostile environment and offers great resistance to fungal invasion. Most fungi are saprophytic and their enzymatic pathways function more efficiently at the redox potential of non-living substrates than at the relatively more reduced state of living metabolizing tissue. Some fungi such as *Candida* and *Malassezia* have adapted to human environment and exist as commensals.

The complex interplay between fungal virulence factors and host defence factors will determine if a fungal infection will cause a disease. Infection depends on inoculum size and the general immunity of the host.

Fungal Pathogenicity (virulence factors):

- Ability to adhere to host cells by way of cell wall glycoproteins
- Production capsules allowing them to resist phagocytosis
- Production of a cytokine called GM-CSF by *Candida albicans* that suppress the production of complement.

- Ability to acquire iron from red blood cells as in *Candida albicans*
- Ability to damage host by secreting enzymes such as keratinase, elastase, collagenase
- Ability to resist killing by phagocytes as in dimorphic fungi
- Ability to secrete mycotoxins
- Having a unique enzymatic capacity
- Exhibiting thermal dimorphism
- Ability to block the cell-mediated immune defences of the host.
- Surface hydrophobicity

Host defence factors:

- Physical barriers, such as skin and mucus membranes
- The fatty acid content of the skin
- The pH of the skin, mucosal surfaces and body fluids
- Epithelial cell turnover
- Normal flora

- Chemical barriers, such as secretions, serum factors
- Most fungi are mesophilic and cannot grow at 37°C.
- Natural Effector Cells (polymorphonuclear leucocytes) and the Professional Phagocytes (monocytes and macrophages)

Factors predisposing to fungal infections:

- Prolonged antibiotic therapy
- Underlying disease (HIV infection, cancer, diabetes, etc.)
- Age
- Surgical procedures
- Immunosuppressive drugs
- Irradiation therapy
- Indwelling catheters
- Obesity
- Drug addiction
- Transplants
- Occupation

Immunity to fungal infections:

Mechanism of immunity to fungal infections can be innate or acquired. The non-specific immunity includes the physical barriers offered by skin and mucus membranes along with their secretions and normal flora. The pH, body temperature and serum factors along with phagocytic cells play an important part in providing non-specific immunity. Even though body mounts both humoral and cell mediated immunity, it is the latter that is the mainstay of host defence.

Cell mediated immunity:

Immunity is provided non-specifically by effector cells (polymorphonuclear leucocytes) and professional phagocytes (monocytes and macrophages) and specifically by T lymphocytes. The phagocytes are very important in defence against *Candida*, *Aspergillus* and *Zygomycetes* as is evidenced by their severity in granulomatous diseases, myeloperoxidase deficiency and cytotoxic chemotherapy.

Expression of T-cell-mediated immunity to fungi includes:

- delayed-type hypersensitivity
- contact allergy
- chronic granulomatous reactions

Humoral immunity:

Even though antibodies are produced against many fungi, their role in protection is not very clear. However, antibodies help in clearing fungal pathogens through opsonisation, which is important against *Candida* and *Cryptococcus*. Another component of humoral immunity is the complement, which can act as opsonins and may even cause damage to their cells through complement activation. Antibodies are important to fungal serodiagnosis.

Hypersensitivity:

As a result of dermatophyte infection some fungus-free skin lesions of variable morphology occur elsewhere on the body, which are thought to result from hypersensitivity to the fungus. These reactions are called "id reaction". These reactions are also seen in *Candida* infections. An inflamed boggy lesion of the scalp called the kerion may result from a strong immune reaction to the dermatophyte. Granulomas due to intracellular fungi represent delayed hypersensitivities. Many fungi are significant allergens to humans, the allergens being spores, conidia, hyphae and other fungal products. On inhalation they may produce allergic pulmonary diseases such as allergic bronchopulmonary aspergillosis, farmer's lung, maple bark stripper's lung, bronchial asthma etc, which may be Type I or III hypersensitivity.

Fungal Diseases (Mycoses):

Mycoses can be conveniently studied as:

1. Superficial mycoses
 - I. Superficial phaeohyphomycosis
 - II. Tinea versicolor
 - III. Black piedra
 - IV. White piedra

2. Cutaneous mycoses
 - I. Dermatophytosis
 - II. Dermatomycosis
3. Subcutaneous mycoses
 - I. Chromoblastomycosis
 - II. Rhinosporidiasis
 - III. Mycetoma
 - IV. Sporotrichosis
 - V. Subcutaneous phaeohyphomycosis
 - VI. Lobomycosis
4. Systemic (deep) mycoses
 - I. Blastomycosis
 - II. Histoplasmosis
 - III. Coccidioidomycosis
 - IV. Paracoccidioidomycosis
5. Opportunistic mycoses
 - I. Candidiasis
 - II. Cryptococcosis
 - III. Aspergillosis
6. Other mycoses
 - I. Otomycosis
 - II. Occulomycosis
7. Fungal allergies
8. Mycetism and mycotoxicosis

Laboratory diagnosis of mycoses:

Specimen collection: specimen collection depends on the site affected. Different specimens include hair, skin scrapings, nail clippings, sputum, blood, CSF, urine, corneal scraping, discharge or pus from lesions and biopsy.

- All specimens must be transported to the laboratory without any delay to prevent bacterial overgrowth. In case of delay specimens except skin specimen, blood and CSF may be refrigerated for a short period.
- Infected hairs may be plucked using forceps. Those hairs that fluoresce under Wood's lamp may be selectively plucked. Hairs may be collected in sterilized paper envelopes.
- Surface of the skin must be disinfected with spirit before specimen collection. The advancing edge of the lesion is scraped with the help of a blunt forceps and collected in sterilized paper envelopes.
- Discoloured or hyperkeratotic areas of nail may be scraped or diseased nail clipping may be collected in sterilized paper envelopes.
- Specimens from mucus membranes (oral) must be collected by gentle scraping and transported to laboratory in sterile tube containing saline. Swabs may be collected from vagina.
- Corneal scrapings may be collected using a fine needle and inoculated at bedside.
- Pus may be collected by aspiration; use of cotton swabs may give false positive microscopic results.
- Clean catch urine may be collected in a sterile wide-mouthed container.
- Biopsy specimens must be transported in saline.

In certain cases, pus or exudates must be looked for presence of granules.

Microscopy: Microscopy is used to observe clinical specimens for the presence of fungal elements or to identify the fungus following culture. In the latter case, lactophenol cotton blue is stain of choice, which stains the fungal elements blue. Direct examination of clinical specimens could be stained or unstained.

- Wet mount: Candida may be observed in urine wet mounts
- 10-20% KOH mount: Several specimens are subjected to KOH mount for direct examination. The material is mixed with 20% KOH on a slide and a cover slip is placed. The slide is then gently heated by passing through the flame 2-3 times. The slide is observed on cooling. KOH serves to digest the protein debris and clears keratinised tissue and increases the visibility. Addition of Dimethyl sulphoxide (DMSO) permits rapid clearing in the absence of heat.
- Calcofluor white: This is a fluorescent dye, which binds selectively to chitin of the fungal cell wall. The specimen then can be observed under fluorescent microscope.
- India Ink: Capsules of *Cryptococcus neoformans* can be demonstrated by this negative staining technique.
- Periodic Acid-Schiff (PAS) stain: On staining by this stain, fungal elements appear bright magenta coloured while the background stains green. It is useful in staining tissue specimens.

- Giemsa's stain: It is particularly useful in the detection of *Histoplasma capsulatum* in the bone marrow smears.
- Haematoxylin and Eosin (H&E) stain: Useful for staining tissue sections.
- Gomori's methenamine silver nitrate (GMS) stain: Outlines of the fungi are black, internal parts stain pink-black while the background stains light green. *Candida* and *Aspergillus* may be missed in H&E stained sections, therefore GMS stained sections are essential for tissue pathology.
- Gridley's stain: It stains hyphae and yeasts dark blue-pink, tissues deep blue and background yellow.
- Meyer mucicarmine stain: Capsules of *C. neoformans* and inner walls of *Rhinosporidium seeberi*'s sporangium are stained pink.
- Gram stain: *Candida* is best demonstrated in clinical specimen by Gram stain.
- Masson-Fontana stain is helpful in staining phaeoid (dematiaceous) fungi in tissue.
- Immunofluorescence: Monoclonal antibody labelled with fluorescent dyes can be used to detect several fungi in the clinical specimens.

Culture: One of the most common media used to culture fungi in laboratory is Sabouraud's Dextrose Agar (SDA). It consists of peptone, dextrose and agar. High concentration of sugar and a low pH (4.5-5.5) prevents growth of most bacteria and makes it selective for fungi. Emmon's modification of SDA contains 2% dextrose and has pH of 6.8.

Other basal media to grow fungi include Potato Dextrose Agar, Malt Extract Agar etc. Most fungi are able to grow at room temperature while few pathogenic fungi (e.g, *Cryptococcus*, dimorphic fungi) can grow at 37°C. Saprophytic fungi grow much quickly than pathogenic fungi (e.g, dermatophytes). In such situations the saprophytic fungi can be inhibited by the addition of cycloheximide (actidione) to the SDA. Addition of antibiotics such as Chloramphenicol, Gentamicin or Streptomycin to SDA serves to inhibit bacterial multiplication. An example of SDA with cycloheximide and Chloramphenicol is Mycosel agar.

Other specialized media used for different fungi include:

- Brain Heart Infusion Agar general isolation of fungi and conversion of dimorphic fungi.
- Inhibitory Mould Agar, an isolation medium with Chloramphenicol to suppress most bacteria.
- Caffeic Acid Agar and Birdseed Agar for isolation of *Cryptococcus neoformans*.
- Corn Meal Agar: Enhances production of chlamydospores in *Candida albicans* and formation of conidia in fungi.
- Trichophyton Agars: Used for selective identification of Trichophyton species.
- Dermatophyte Test Medium: Used for recovery of dermatophytes from clinical specimens.
- Sabhi Medium: Isolation of *Histoplasma capsulatum*.
- 'CHROM agar *Candida*' is useful in identification of *Candida* species.

Conversion of mould to yeast phase must be demonstrated in vitro for identification of dimorphic fungi. Since some fungi grow slowly cultures should not be discarded for 4-6 weeks. Fungi are identified on the basis of colony morphology (including pigmentation) and microscopic observation by tease-mount preparation or slide culture technique.

Serology: Detection of anti-fungal antibody is helpful in diagnosis of sub-cutaneous and systemic mycoses, prognosis and response to anti-fungal drugs. Different serologic techniques that are used include agglutination, immunodiffusion, counter-immunoelectrophoresis, complement fixation test, immunofluorescence, RIA and ELISA.

Antigen detection: It is particularly useful in the diagnosis of cryptococcal meningitis from CSF specimens. The test is performed by Latex Agglutination or immunodiffusion tests. It is also helpful in the detection of *Aspergillus* and *Candida* antigens in systemic infections.

Skin tests: Delayed hypersensitivity reactions to fungal antigens can be demonstrated by skin tests. A positive skin does not necessarily indicate an active infection; it only indicates sensitization of the individual. Hence, its value is in epidemiological studies than diagnosis. These tests may be performed in Histoplasmosis, Candidiasis, Sporotrichosis, Coccidioidomycosis, Blastomycosis, Paracoccidioidomycosis and dermatophytosis.

Molecular techniques: Newer techniques such as DNA hybridization, PCR are useful in diagnosis of mycoses in a shorter period as well as detect those fungi that are difficult or dangerous to cultivate in vitro.