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MYCOLOGY -ALGOLOGY -VIROLOGY MODULE

INTENDED FOR STUDENTS OF THE MICROBIOLOGY DEGREE

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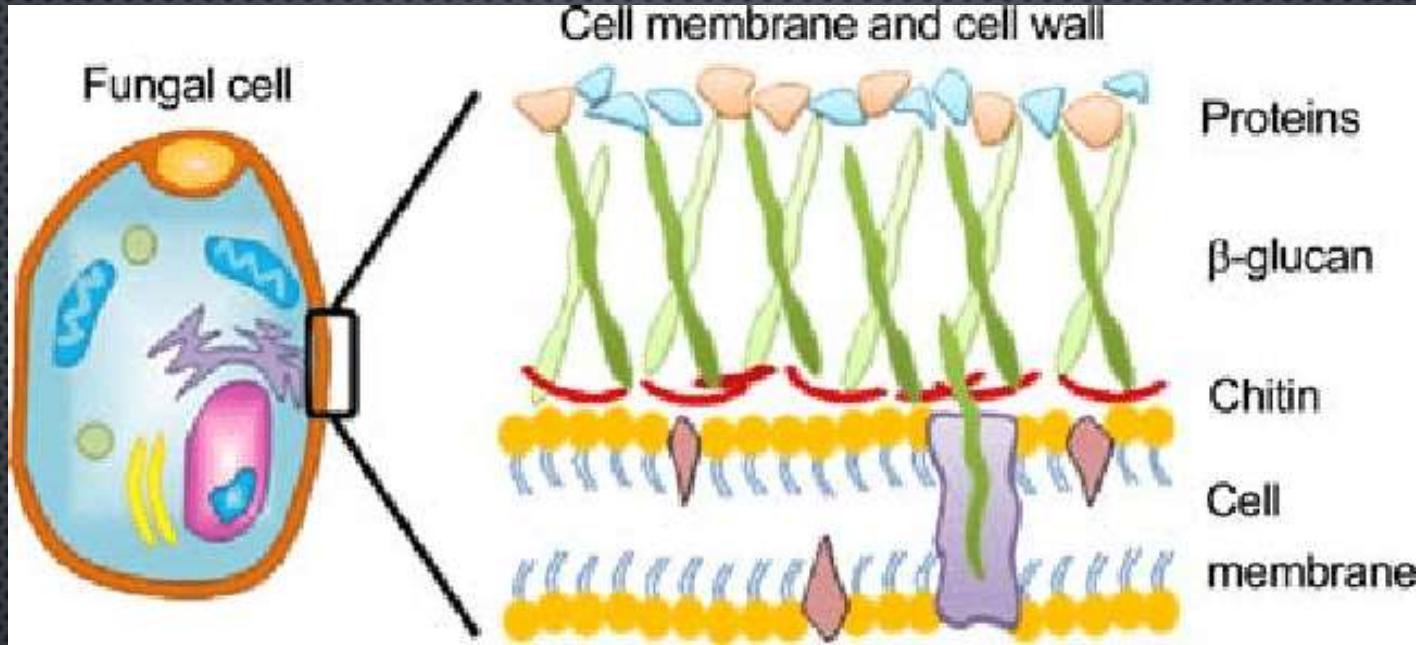
# MYCOLOGY

## I. General Characteristics of Fungi (Molds and Yeasts)

Fungi constitute a separate kingdom of eukaryotic organisms. They are heterotrophic, obtain their nutrients by absorbing organic compounds from their environment, thus acting as essential decomposers in ecosystems. Molds and yeasts are two common forms of fungi. Molds are multicellular fungi that form filaments called hyphae, while yeasts are unicellular fungi.

### -Chemical Composition and Cell Structure

Cell Wall is primarily composed of chitin, a fibrous polysaccharide. It also contains glucans and manno-proteins that strengthen the structure and enable interactions with the environment. This composition is a prime target for antifungal drugs.



Plasma membrane: It contains ergosterol, a sterol that performs the same functions as cholesterol in animals. Ergosterol synthesis is an important target for many antifungal drugs.

Cytoplasm and organelles: Fungal cells have a well-defined nucleus, mitochondria that serve as "power plants," and other organelles common to eukaryotes. In molds, hyphae can be septate (divided by septa) or coenocytic (non-septate), a characteristic used for species classification.

## -Growth and reproduction

Fungi have complex life cycles, and their growth and reproduction strategies are adapted for the rapid colonization of new substrates.

**Molds:** Mold growth is apical, occurring through the elongation of hyphal tips. These filaments extend and branch to form mycelium. Reproduction can be:

**Asexual:** production of millions of asexual spores (conidiospores, sporangiospores, etc.) that are easily dispersed by wind or water, allowing rapid multiplication.

**Sexual:** Less common, this method involves the fusion of two hyphae, resulting in the production of sexual spores (zygospores, ascospores, basidiospores), which are often more resistant to harsh conditions.

**Yeasts:** Yeast reproduction occurs primarily by budding. A small outgrowth forms on the parent cell, grows, and then breaks off to become a new, independent cell, leaving a "birth scar" on the parent cell.



### **-Laboratory and large-scale cultivation**

Laboratory cultivation: Fungi are grown on solid (agar-based) or liquid nutrient media. Culture conditions (temperature, pH) are controlled to promote fungal growth while avoiding contamination. Observation of colonies allows species to be identified based on color, texture, and shape.

Large-scale cultivation: This practice is at the heart of biotechnology. Fungi are grown in tanks (fermenters or biogenerators), where conditions (oxygen, pH, temperature) are rigorously controlled. Species such as *Aspergillus niger* are used for the production of organic acids (citric acid) and enzymes, while *Saccharomyces cerevisiae* is essential for the production of biofuels and alcoholic beverages.

## II. Classification of fungi

### -Yeast

The majority of yeasts, including the best-known ones such as *Saccharomyces cerevisiae*, belong to the subphylum Saccharomycotina (phylum Ascomycota). Other yeasts, such as those of the genus *Cryptococcus*, are classified in the phylum Basidiomycota (class Tremellomycetes). This distribution clearly shows that the yeast life form appeared independently several times during the evolution of fungi.

-Zygomycetes includes most known species, such as bread molds (*Rhizopus*) and arbuscular mycorrhizae. They are characterized by the production of a thick-walled zygosporre during sexual reproduction. The Zoopagomycota includes parasites of invertebrates and other fungi. These fungi are often characterized by non-septate (coenocytic) hyphae.

-Chytridiomycetes are a basal group of the fungal kingdom, often considered the most primitive of the fungi. Their unique characteristic is the production of motile zoospores with a single posterior flagellum. This feature, absent in other "true" fungi, has long served as a classification criterion.

-Oomycetes (Oomycota), formerly called "fungi-algae," are no longer part of the Fungi kingdom. They belong to the Stramenopiles kingdom, a group of protists that also includes diatoms and brown algae. Oomycetes have a cell wall composed of cellulose, and their zoospores are biflagellate, which clearly distinguishes them from fungi.

They are very important plant pathogens, causing diseases such as grapevine blight (*Plasmopara viticola*) and potato rot (*Phytophthora infestans*).

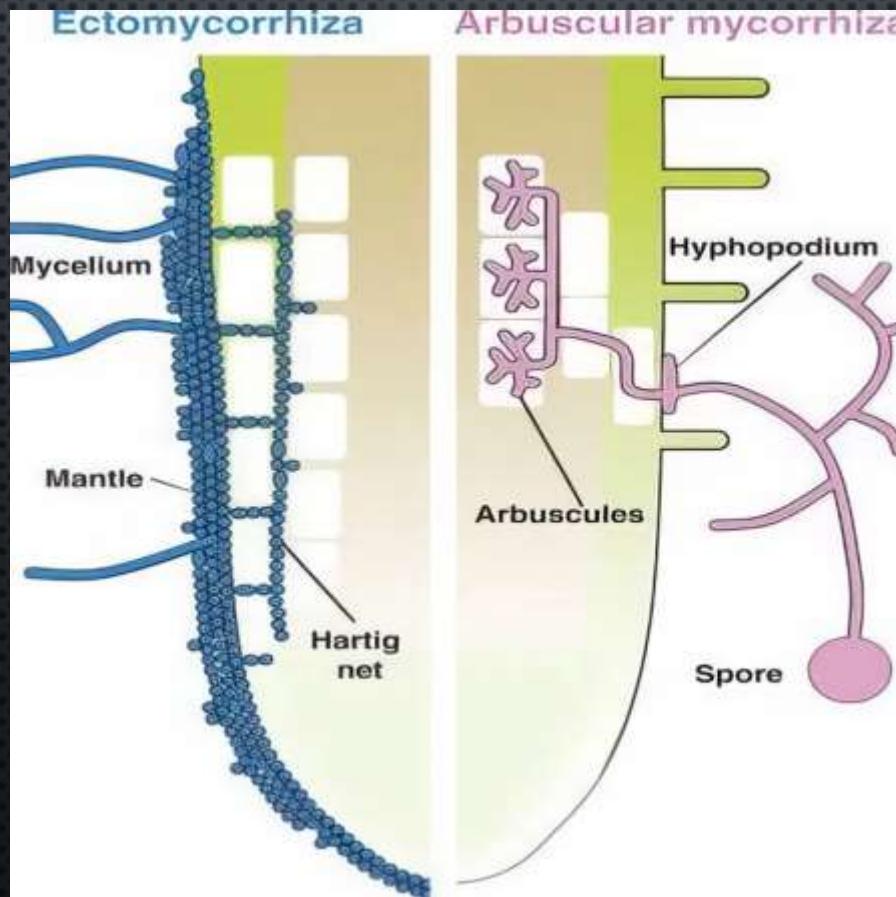
-Ascomycetes (Ascomycota) represents the largest phylum in the fungal kingdom, encompassing a great diversity of life forms. They are defined by the production of ascospores in a structure called an ascus. The subphylum Saccharomycotina includes the majority of yeasts, while the subphylum Pezizomycotina includes species that form complex fruiting bodies (ascocarps), such as truffles and morels.

- Imperfect Fungi: They classified into the Ascomycota or Basidiomycota. Example, the genus *Penicillium*, known for its antibiotic production, is now classified in the phylum Ascomycota.

-Basidiomycetes (Basidiomycota) includes the most familiar fungi. Their main characteristic is the basidium, a club-shaped structure that produces basidiospores on its surface.

-Ectotrophic and Endotrophic Mycorrhizae: Mycorrhizae are mutualistic symbioses between plant roots and fungi. Ectomycorrhizae: This symbiosis are formed by Basidiomycota and Ascomycota. The fungus forms a fungal mantle that surrounds the roots. It does not penetrate the cells.

Endomycorrhizae: The fungus penetrates the cells of the root cortex to form internal structures. The most common type is arbuscular mycorrhizae (AM), formed by fungi of the phylum Glomeromycota. These fungi create arbuscules (branched structures) and vesicles that facilitate the exchange of nutrients between the plant and the fungus. This symbiosis is extremely widespread, occurring in over 80% of plant species.



### III. Interest in using fungi in: food, agriculture, and public health

#### A. Agri-Food

##### 1. Uses of molds:

###### - The phases of Mold growth

- ❖ Lag Phase: During the introductory stage, fungal elements such as spores or hyphal fragments undergo a period of adaptation to their environment. Crucial conditions like pH balance, temperature, and water activity play a major role in determining the duration of this adjustment phase.
- ❖ Exponential (Log) Phase: This stage marks a rapid and continuous expansion of the mycelium, leading to a significant increase in biomass. It is also defined by the maximum generation of primary metabolites.

- ❖ **Stationary Phase:** During this phase, microbial growth stabilizes as the rate of cell division equals the rate of cell death. This balance typically occurs when the nutrients, such as carbon or nitrogen, are depleted and toxic metabolic byproducts accumulate. To endure these unfavorable conditions, microbes often adapt by differentiating and shifting their metabolic priorities to secondary processes, reflecting a change in their physiological behavior.
- ❖ **Death Phase:** This stage is marked by a gradual decline in fungal biomass as the mycelium undergoes autolysis and structural degradation. Cells actively participate in self-digestive mechanisms during this period, eventually breaking down entirely until complete disintegration is achieved.

### **-Examples of cultures on solid and liquid media**

#### *Liquid Media (Submerged Fermentation):*

Widely used for large-scale metabolite production, this method is known for its outstanding efficiency. It has become the foundation for deep-tank fermentation processes, which are integral to producing antibiotics like penicillin (generated by *Penicillium chrysogenum*) and

various industrial enzymes.

Primary Benefits:

It delivers greater control over vital environmental factors, including pH and dissolved oxygen levels, facilitating optimal growth conditions. Furthermore, this approach enhances post-fermentation workflows by simplifying the purification of targeted metabolites, making it an ideal choice for industrial applications.

*Solid Medium (Solid-State Fermentation - SSF):*

Application: Mimics the natural growth patterns of fungi on porous substrates like rice or wheat bran, commonly utilized for cultivating Koji mold (*Aspergillus oryzae*) in the production of soy sauce, sake, and for enhancing enzyme output, particularly proteases and amylases.

Benefits: Promotes the development of higher concentrations of valuable secondary metabolites and enzymes, often accompanied by lower energy consumption during the process.

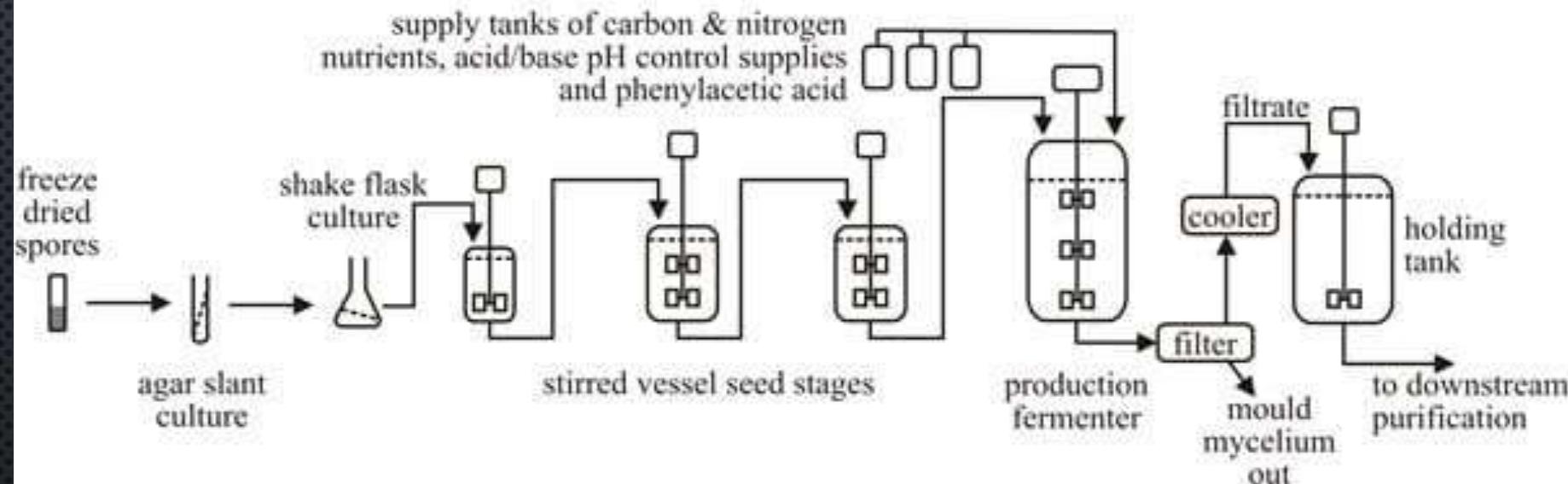
*Selective Media:* Specialized media such as Dichloran Glycerol Agar (DG18) are crucial in food microbiology, aiding in the isolation of xerophilic molds that significantly contribute to the spoilage of dried and semi-dried food items.

preserved culture → 1 l → 10 l → 100 l → 1000 l → 10,000 l → 100,000 litre

Laboratory stages

Stirred vessel seed stages

Production stage



## -Development and differentiation

Changes in mold structure are closely linked to the production of secondary metabolites.

- ✓ Metabolic transition: The shift from a nutrient-rich environment (growth phase) to a nutrient-poor environment (stationary phase) signals the triggering of regulatory mechanisms (such as the velvet complex in *Aspergillus*), which mediate the switch from primary to secondary metabolism.
- ✓ Function of secondary metabolites: Most secondary metabolites are produced during the stationary phase, which coincides with sporulation. This phase suggests they play a role in survival, serving as pigments (such as melanin) or competitive tools (such as toxins).
- ✓ Mycotoxin production: The production of harmful toxins (such as aflatoxins) is often associated with genetic pathways regulating asexual development. This demonstrates the important biological connection between growth and secondary metabolic processes.

- Production of metabolites (primary and secondary)

\*Major Metabolites:

*Organic Acids*: In addition to citric acid, *Aspergillus niger* also produces itaconic acid (used in polymers) and fumaric acid (used as a food preservative).

*Industrial Enzymes*: Glucoamylase (from *Aspergillus*) is used to break down starch into sugars, pectinase is used to clarify fruit juice, and lipase is used to modify fats.

\*Secondary Metabolites (SM):

*Polyketides (PKs)*: Produced from acetyl-CoA. These compounds include medically important statins (cholesterol-lowering drugs) and harmful aflatoxins (potent mycotoxins).

*Terpenoids*: These compounds include plant growth hormones such as gibberellins, which are mimicked by some plant pathogens.

*Nonribosomal Peptides (NRPs)*: Produced by a wide range of enzyme complexes called nonribosomal peptide synthetases (NRPSs). These include important compounds such as siderophores, which help fungi absorb iron and are essential for survival.

- Uses in the preparation of dairy products

- *Penicillium roqueforti* provides vital enzymes for blue cheeses, including Roquefort: The breakdown of milk fat into free fatty acids is known as lipolysis. Blue cheese gets its unique, sharp, and peppery flavor from the conversion of these acids into methyl ketones.
- Proteolysis: This process increases the complexity of flavors by dissolving proteins into smaller peptides and amino acids.
- Soft-Ripened Cheeses: *Penicillium camemberti* works with other molds and yeasts, like *Geotrichum candidum*, to produce soft-ripened cheeses.
- Deacidification: The pH rises as a result of the lactic acid on the surface being consumed. This alteration sets off the action of milk proteases, which gradually degrade the protein structure to produce the desired creamy texture from the rind's surface inward.

- Edible Fungi

They are prized for their flavor as well as for being wholesome foods and sources of health-promoting substances. Fungi are a unique non-animal source of vitamin (Ergocalciferol) when exposed to UV light.

They also provide high-quality protein and dietary fiber, particularly beta-glucans, which may lower cholesterol and improve immune response. Lentinan, a type of polysaccharide found in shiitake (*Lentinula edodes*), has been researched for possible immune-boosting properties. In addition to its mild texture, *Hericium erinaceus* has been studied for compounds that could promote nerve growth and enhance brain function. Because of its texture and high fiber content, mycoprotein (*Fusarium venenatum*) is a sustainable protein source derived from microorganisms that can be used in place of meat.

## 2. Uses of yeasts

### - Beer production

The production of beer is fundamentally based on the fermentation of sugars derived from malted cereals, with the yeast *Saccharomyces cerevisiae* playing a pivotal role in the process. This production is carried out through several critical stages:

- The process begins with the mashing phase, during which the malted cereals are combined with hot water to convert starches into fermentable sugars.
- Following mashing is the boiling phase, wherein the resultant liquid, known as wort, is heated, and hops are added. The addition of hops imparts bitterness while enhancing the aromatic profile of the beer.
- Subsequently, the wort is cooled and undergoes fermentation, during which the yeast metabolizes the sugars, producing alcohol and carbon dioxide as primary by-products.
- After fermentation, the beer enters a maturation phase referred to as conditioning. During this step, the flavors are further refined and developed to achieve greater complexity.
- The final stage is packaging, which prepares the finished product for distribution and consumption.



## -Bread fermentation:

The fermentation of bread represents a critical stage during which the starches present in the dough undergo a transformation, resulting in the production of carbon dioxide and ethanol through the action of *Saccharomyces cerevisiae*. This biochemical process is primarily responsible for the dough's rise, imparting the light, airy, and tender texture characteristic of well-prepared bread. Enzymatic activity facilitates the breakdown of complex compounds within the dough, creating a favorable structural matrix while enhancing the flavor and aroma of the final product.

Achieving optimal results depends on the meticulous management of several key factors, including fermentation temperature, process duration, and the choice of leavening agent, with yeast being the most commonly utilized option. A careful balancing of these elements is essential to ensure that the bread attains the desired balance of flavor and texture, hallmarks of excellence in baking.

**SPIKED WHITE FLOUR**  
(100 µg/kg of each TeA, AOH,  
AME in flour)

**INGREDIENTS**  
(2.5% baker's yeast, 2.0% salt,  
58% water)

Kneading  
(25 °C, 15 min)

DOUGH

Fermentation  
(37 °C, 60 min)

FERMENTED DOUGH

Dividing (15 g pieces)/ Final  
proof (37 °C, 50 min)

DOUGH PIECES

Baking (250 °C, 8 min)

BREAD

## B. Pharmaceutical Industry

Fungi producing metabolites: vitamins, antibiotics, and enzymes

### *Origin*

Pharmaceutical fungal strains are derived from a wide range of sources, including:

- Cultivated and wild species, such as baker's yeast (*Saccharomyces cerevisiae*) and macrofungi.
- Soil microorganisms and those associated with organic matter, particularly molds such as *Aspergillus* and *Penicillium*.
- Endophytic fungi, which are strains isolated from within plant tissues, often functioning as a defensive mechanism for the host plant.

### *Isolation*

Isolation involves obtaining a pure strain of the desired fungus. This process is carried out in two main steps:

1. Sample Collection: This step entails gathering samples from the source material, which could include plants, soil, or decomposing organic matter.
2. Selective Culturing: In this phase, specific growth media, such as Czapek yeast agar or malt extract agar, are used. These media are designed to promote the growth of the target species while simultaneously inhibiting the proliferation of contaminants.

*Purification and Extraction:* To ensure the genetic uniformity of the production strain, the culture is derived from a single spore or cell through successive transfers to fresh Petri dishes. Preparing a pure strain for large-scale fermentation in a bioreactor is referred to as inoculum preparation.

The target metabolite is extracted and refined from the culture medium through the following steps:

1. Recovery: If the product is extracellular (enzymes and antibiotics), it can be easily isolated by simply filtering the fungal biomass. For intracellular products, which are contained within the cells, the fungal cells must be physically lyse).

Using specific solvents, the chemical product is separated from other components. The physicochemical properties of the metabolite (such as methanol, ethyl acetate, etc.) dictate the choice of solvent and procedure, which may include maceration, solvent extraction, or supercritical fluid extraction, etc.

Chromatographic methods are used to refine the crude extract into a pure form depending on variations in molecule size, charge, or polarity:

TLC stands for thin-layer chromatography.

One of these is HPLC, or high-performance liquid chromatography.

-Therapeutic applications and uses

Many pharmaceuticals are derived from fungal metabolites, encompassing various therapeutic applications:

- Anti-infective agents or antimicrobial (antibiotics): The most renowned example is penicillin, which effectively combats a wide range of bacterial infections.
- Immunosuppressants: Cyclosporin A, obtained from *Tolypocladium inflatum*, plays a crucial role in minimizing organ transplant rejection.
- Cardiovascular medications: Lovastatin, classified as a statin, is employed to lower cholesterol levels. This compound is derived from *Aspergillus terreus* and *Monascus purpureus*.
- Anticancer agents: The antitumor and immunomodulatory properties of certain fungal extracts, particularly those derived from medicinal mushrooms such as *Ganoderma lucidum*, have been extensively investigated.

## IV. Pathological Aspects

### A. In Humans and Animals

#### -Candidiasis

Candidiasis refers to infections caused by yeasts of the *Candida* genus, predominantly *Candida albicans*. These yeasts are part of the normal commensal flora (mouth, gastrointestinal tract, vagina, skin). Infection arises primarily when the microbial balance is disrupted or when the host's immune system is compromised, making the host more susceptible.

#### *Clinical Manifestations*

- Superficial and mucosal Candidiasis: These are the most frequently encountered forms and include oral candidiasis (thrush), vulvovaginal candidiasis (vaginal thrush), and intertrigo (infections affecting skin folds).
- Invasive (systemic) Candidiasis: These forms are considered the most severe and are typically observed in immunocompromised patients, such as those with cancer, HIV/AIDS, or organ transplants, as well as hospitalized individuals undergoing invasive procedures (e.g., usage of catheters or post-surgical care).

Systemic infections can affect critical systems and organs, including the bloodstream (candidemia), heart, kidneys, brain, and other vital structures, often resulting in high mortality rates.

### *Virulence Factors*

The pathogenicity of *C. albicans* is heavily dependent on several virulence factors. These include its ability to undergo morphogenic shifts (transitioning from yeast to hyphal form), the secretion of hydrolytic enzymes (such as proteases and lipases), and the production of toxins, notably candidalysin. These mechanisms play a pivotal role in promoting infection and tissue invasion.

### -Dermatophytes

They are filamentous keratinophilic fungi classified within the genera *Microsporum*, *Trichophyton*, and *Epidermophyton*. These fungal species exhibit a distinct specialization for infecting keratin-containing tissues, including skin, hair, nails, and fur. The infections they cause are collectively referred to as dermatophytoses or ringworm.

## *Clinical manifestations*

Tinea pedis (Athlete's foot): Characterized by fungal infections of the feet, particularly occurring between the toes.

Tinea corporis (Ringworm or Skin Dartre): Involves circular lesions on the skin of the body.

Tinea capitis: Predominantly observed in children, it affects the scalp and can lead to hair loss (alopecia).

Onychomycoses: Fungal infections of the nails, known for their prolonged treatment duration.

## *Modes of transmission*

The dermatophytes are categorized based on their preferential host and associated transmission pathways.

Anthropophilic dermatophytes: These are transmitted from human to human (e.g., *Trichophyton rubrum*).

Zoophilic dermatophytes: Transmission occurs from animals to humans (e.g., *Microsporum canis*).

Geophilic dermatophytes: Acquired through contact with contaminated soil.

## B. At the plant

### -Storage fungi

Storage fungi, also referred to as post-harvest molds, are fungal species capable of thriving on agricultural commodities during storage, transport, or processing stages. Their development is primarily driven by suboptimal storage conditions, such as high humidity levels, elevated temperatures, inadequate ventilation, or physical damage to grains.

The presence of storage fungi results in considerable degradation in both the nutritional content and overall quality of agricultural produce and animal feeds. These fungi metabolize vital reserves such as carbohydrates, lipids, and proteins, diminishing the nutritional value of affected products.

**Unpleasant odors and flavors:** Contaminated produce may develop undesirable sensory attributes rendering it unfit for consumption.

**Reduced germination rate:** This is particularly problematic for seeds intended for sowing.

**Mycotoxin Production:** The release of mycotoxins by storage fungi represents a critical concern due to their adverse effects on human and animal health.

Examples: Prominent genera associated with storage fungi include *Aspergillus* and *Penicillium*, both notorious for their ability to proliferate under unfavorable storage environments and produce harmful mycotoxins

### -Mycotoxins

They are toxic secondary metabolites produced by certain strains of molds (fungi) that contaminate crops both in the field (pre-harvest) and during storage (post-harvest). The consumption of foods contaminated with mycotoxins is referred to as mycotoxicosis, representing a significant public health concern and a major economic challenge on a global scale.

### *Main Mycotoxins and their Producers*

Aflatoxins: These are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, commonly contaminating crops like maize, peanuts, and nuts. Aflatoxins are the most potent naturally occurring hepatocarcinogens.

Deoxynivalenol (DON) and Zearalenone: Produced by *Fusarium* species, they typically affect cereals such as wheat and barley. DON is associated with gastrointestinal disorders, while Zearalenone acts as an estrogenic compound.

Ochratoxin A (OTA): Generated by *Aspergillus* and *Penicillium* species, OTA is found in coffee, spices, cereals, and grapes. It is nephrotoxic and suspected to be carcinogenic. Health risks: Mycotoxins can cause a wide range of effects, from acute gastrointestinal disturbances to chronic outcomes such as immunotoxicity, nephrotoxicity, and carcinogenicity. Their regulation is tightly controlled by authorities such as the Food and Drug Administration (FDA) and the Codex Alimentarius to minimize risk to consumers