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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 the 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 osmiotrophic; 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 37oC and as moulds in their natural habitat and in vitro at room temperature. Eg: Histoplasma capsulatum, Blastomyces dermatidis, Paracoccidiodes 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. Chlamydospore (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 Candia 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 psueudohyphae at the point of budding, while the germ tube has no constriction.

Some yeast such as Cryptococcus and the yeast form of Blastomyces dermatatidis 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 cerviciae 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 gamentagia. 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. Sporangiophores 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

Genera of medical importance include:

1. Teleomorph of Cryptococcus neoformans, which is Filobasidiella neoformans

2. Agents of basidiomycosis such as Coprinus and Schizophyllium

3. Mushroom poisoning by Aminita, 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 Malasezzia 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 37oC.

• 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 be effector cells (polymorphonuclear leucocytes) and professional phagocytes (monocytes and macrophages) and specifically by T lymphocytes. The phagocytes are very important in defence against Candia, 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 n 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 hypersensivities. 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

Mycotoxin

A **mycotoxin** (from Greek μ   (mykes, mukos) "fungus" and
(toxikon) "poison") is a toxic secondary metabolite produced by
organisms of the fungi kingdom, commonly known asmolds. The term
'mycotoxin' is usually reserved for the toxic chemical products produced
by fungi that readily colonize crops. One mold species may produce
many different mycotoxins, and the same mycotoxin may be produced by
several species

Overview

Most fungi are aerobic (use oxygen) and are found almost everywhere in extremely small quantities due to the minute size of their spores. They consume organic matter wherever humidity andtemperature are sufficient. Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high. The reason for the production of mycotoxins is not yet known; they are necessary for neither growth nor the development of the fungi. Because mycotoxins weaken the receiving host, the fungus may use them as a strategy to better the environment for further fungal proliferation. The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms Some of the health effects found in animals and humans include death, identifiable diseases or health problems, weakened immune systems without specificity to a toxin, and as allergens or irritants. Some mycotoxins are harmful to other micro-organisms such as other fungi or even bacteria; penicillin is one example. It has been suggested that mycotoxins in stored animal feed are the cause of apparent sex change in hens.

 Mycotoxins can appear in the food chain as a result of fungal infection of crops, either by being eaten directly by humans or by being used as livestock feed. Mycotoxins greatly resist decomposition or being broken down in digestion, so they remain in the food chain in meat and dairy products. Even temperature treatments, such as cooking and freezing, do not destroy some mycotoxins.

Although various wild mushrooms contain an assortment of poisons that are definitely fungal metabolites causing noteworthy health problems for humans, they are rather arbitrarily excluded from discussions of mycotoxicology. In such cases the distinction is based on the size of the producing fungus and human intention. Mycotoxin exposure is almost always accidental whereas with mushrooms improper identification and ingestion causing mushroom poisoning is commonly the case. Ingestion of misidentified mushrooms containing mycotoxins may result in hallucinations. The cyclopeptide-producing *Amanita phalloides* is well known for its toxic potential and is responsible for approximately 90% of all mushroom fatalities. The other primary mycotoxin groups found in mushrooms include: orellanine, monomethylhydrazine, disulfiram-like, hallucinogenic indoles, muscarinic, isoxazole, and gastrointestinal (GI)-

specific irritants. The bulk of this article is about mycotoxins that are found in microfungi other than poisons from mushrooms or macroscopic fungi.

Many international agencies are trying to achieve universal standardization of regulatory limits for mycotoxins. Currently, over 100 countries have regulations regarding mycotoxins in the feed industry, in which 13 mycotoxins or groups of mycotoxins are of concern. The process of assessing a need for mycotoxin regulation includes a wide array of in-laboratory testing that includes extracting, clean-up and separation techniques. Most official regulations and control methods are based on high-performance liquid techniques (e.g., HPLC) through international bodies. It is implied that any regulations regarding these toxins will be in co-ordinance with any other countries with which a trade agreement exists. Many of the standards for the method performance analysis for mycotoxins is set by the European Committee for Standardization (CEN). However, one must take note that scientific risk assessment is commonly influenced by culture and politics, which, in turn, will affect trade regulations of mycotoxins

Food-based mycotoxins were studied extensively worldwide throughout the 20th century. In Europe, statutory levels of a range of mycotoxins permitted in food and animal feed are set by a range of European directives and Commission regulations. The U.S. Food and Drug Administration has regulated and enforced limits on concentrations of mycotoxins in foods and feed industries since 1985. It is through various compliance programs that the FDA monitors these industries to guarantee that mycotoxins are kept at a practical level. These compliance programs sample food products including peanuts and peanut products, tree nuts, corn and corn products, cottonseed, and milk. There is still a lack of sufficient surveillance data on some mycotoxins that occur in the U.S., which is due largely to the lack of reliable analytical methods.

Major groups

Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*. The umbrella term aflatoxin refers to four different types of mycotoxins produced, which are B_1, B_2, G_1, and G_2. Aflatoxin B_1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many animal species. Aflatoxins are largely associated with commodities

produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios and maize.

Ochratoxin is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species. The three forms differ in that Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form Ochratoxin A. *Aspergillus ochraceus* is found as a contaminant of a wide range of commodities including beverages such as beer and wine. *Aspergillus carbonarius* is the main species found on vine fruit, which releases its toxin during the juice making process OTA has been labeled as a carcinogen and a nephrotoxin, and has been linked to tumors in the human urinary tract, although research in humans is limited by confounding factors.

Citrinin is a toxin that was first isolated from *Penicillium citrinum*, but has been identified in over a dozen species of *Penicillium* and several species of *Aspergillus*. Some of these species are used to produce human foodstuffs such as cheese (*Penicillium camemberti*), sake, miso, and soy sauce (*Aspergillus oryzae*). Citrinin is associated with yellow rice disease in Japan and acts as a nephrotoxin in all animal species tested. Although it is associated with many human foods (wheat, rice, corn, barley , oats, rye, and food colored with Monascus pigment) its full significance for human health is unknown. Citrinin can also act synergistically with Ochratoxin A to depress RNA synthesis in murine kidneys.

Ergot Alkaloids are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of *Claviceps*, which are common pathogens of various grass species. The ingestion of ergot sclerotia from infected cereals, commonly in the form of bread produced from contaminated flour, cause ergotism the human disease historically known as St. Anthony's Fire. There are two forms of ergotism: gangrenous, affecting blood supply to extremities, and convulsive, affecting the central nervous system. Modern methods of grain cleaning have significantly reduced ergotism as a human disease, however it is still an important veterinary problem. Ergot alkaloids have been used pharmaceutically.

Patulin is a toxin produced by the *P. expansum*, *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal species. *P. expansum* is especially associated with a range of moldy fruits and vegetables, in particular rotting apples and figs. It is destroyed by the fermentation process and so

is not found in apple beverages, such as cider. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals. In 2004, the European Community set limits to the concentrations of patulin in food products. They currently stand at 50 µg/kg in all fruit juice concentrations, at 25 µg/kg in solid apple products used for direct consumption, and at 10 µg/kg for children's apple products, including apple juice.

Fusarium toxins are produced by over 50 species of *Fusarium* and have a history of infecting the grain of developing cereals such as wheat and maize. They include a range of mycotoxins, such as: the **fumonisins**, which affect the nervous systems of horses and may cause cancer in rodents; the **trichothecenes**, which are most strongly associated with chronic and fatal toxic effects in animals and humans; and **zearalenone**, which is not correlated to any fatal toxic effects in animals or humans. Some of the other major types of *Fusarium* toxins include: beauvercin and enniatins, butenolide, equisetin, and fusarins.

Binding agents and deactivators

In the feed and food industry it has become common practice to add mycotoxin binding agents such as Montmorillonite or bentonite clay in order to affectively adsorb the mycotoxins. To reverse the adverse effects of mycotoxins, the following criteria are used to evaluate the functionality of any binding additive:

- Efficacy of active component verified by scientific data
- A low effective inclusion rate
- Stability over a wide pH range
- High capacity to absorb high concentrations of mycotoxins
- High affinity to absorb low concentrations of mycotoxins
- Affirmation of chemical interaction between mycotoxin and adsorbent
- Proven *in vivo* data with all major mycotoxins
- Non-toxic, environmentally friendly component

Since not all mycotoxins can be bound to such agents, the latest approach to mycotoxin control is mycotoxin deactivation. By means of enzymes (esterase, epoxidase), yeast (*Trichosporon mycotoxinvorans*) or bacterial strains (Eubacterium BBSH 797), mycotoxins can be reduced during pre-harvesting contamination. Other removal methods include physical

separation, washing, milling, heat-treatment, radiation, extraction with solvents, and the use of chemical or biological agents. Irradiation methods have proven to be effective treatment against mold growth and toxin production.

In the indoor environment

Buildings are another source of mycotoxins and people living or working in areas with mold increase their chances of adverse health effects. Molds growing in buildings can be divided into three groups — primary, secondary, and tertiary colonizers. Each group is categorized by the ability to grow at a certain water activity requirement. It has become difficult to identify mycotoxin production by indoor molds for many variables, such as

(i)   they may be masked as derivatives
(ii) they are poorly documented and
(iii) the fact that they are likely to produce different metabolites on building materials. Some of the mycotoxins in the indoor environment are produced by *Alternaria*, *Aspergillus* (multiple forms), *Penicillium*, and *Stachybotrys Stachybotrys chartarum* contains a higher number of mycotoxins than other molds grown in the indoor environment and has been associated with allergies and respiratory inflammation. The infestation of *S. chartarum* in buildings containing gypsum board, as well as on ceiling tiles, is very common and has recently become a more recognized problem. When gypsum board has been repeatedly introduced to moisture *S. chartarum* grows readily on its cellulose face. This stresses the importance of moisture controls and ventilation within residential homes and other buildings. The negative health effects of mycotoxins are a function of the concentration, the duration of exposure and the subject's sensitivities. The concentrations experienced in a normal home, office or school are often too low to trigger a health response in occupants.

In the 1990s, public concern over mycotoxins increased following multi-million dollar toxic mold settlements. The lawsuits took place after the Center for Disease Control (CDC) did a study inCleveland, Ohio and claimed that there was an association between mycotoxins from *Stachybotrys* spores and pulmonary hemorrhage in infants. However, in

2000, based on internal and external reviews of their data, the CDC concluded that because of flaws in their methods, the association was not proven. *Stachybotrys* spores in animal studies have been shown to cause lung hemorrhaging, but only at very high concentrations. One study by the Center of Integrative Toxicology at Michigan State University investigated the causes of Damp Building Related Illness (DBRI). They found that *Stachybotrys* is possibly an important contributing factor to DBRI. So far animal models indicate that airway exposure to *S. chartarum* can evoke allergic sensitization, inflammation, and cytotoxicity in the upper and lower respiratory tracts. Trichothecene toxicity appears to be an underlying cause of many of these adverse effects. Recent findings indicate that lower doses (studies usually involve high doses) can cause these symptoms.

Some toxicologists have used the Concentration of No Toxicological Concern (CoNTC) measure to represent the airborne concentration of mycotoxins that are expected to cause no hazard to humans (exposed continuously throughout a 70–yr lifetime). The resulting data of several studies have thus far demonstrated that common exposures to airborne mycotoxins in the built indoor environment are below the CoNTC, however agricultural environments have potential to produce levels greater than the CoNTC.

Human health effects

Mycotoxicosis is the term used for poisoning associated with exposures to mycotoxins. The symptoms of mycotoxicosis depend on the type of mycotoxin; the concentration and length of exposure; as well as age, health, and sex of the exposed individual. The synergistic effects associated with several other factors such as genetics, diet, and interactions with other toxins have been poorly studied. Therefore it is possible that vitamin deficiency, caloric deprivation, alcohol abuse, and infectious disease status can all have compounded effects with mycotoxins. In turn, mycotoxins have the potential for both acute and chronic health effects via ingestion, skin contact, and inhalation. These toxins can enter the blood stream and lymphatic system; they inhibit protein synthesis, damage macrophage systems, inhibit particle clearance of the lung, and increase sensitivity to bacterial endotoxin.

 In 2004 in Kenya, 125 people died and nearly 200 others were treated after eating aflatoxin-contaminated maize. The deaths were mainly

associated with homegrown maize that had not been treated with fungicides or properly dried before storage. Due to food shortages at the time, farmers may have been harvesting maize earlier than normal to prevent thefts from their fields, so that the grain had not fully matured and was more susceptible to infection.

Antifungal medication

An **antifungal medication** is a pharmaceutical fungicide used to treat mycoses such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcalmeningitis, and others. Such drugs are usually obtained by a doctor's prescription or purchased over-the-counter.

Classes

Polyene antifungals

A polyene is a molecule with multiple conjugated double bonds. A polyene antifungal is a macrocyclic polyene with a heavily hydroxylated region on the ring opposite the conjugated system. This makes polyene antifungals amphiphilic. The polyene antimycotics bind with sterols in the fungal cell membrane, principally ergosterol. This changes the transition temperature (Tg) of the cell membrane, thereby placing the membrane in a less fluid, more crystalline state. (In ordinary circumstances membrane sterols increase the packing of the phospholipid bilayer making the plasma membrane more dense.) As a result, the cell's contents including monovalent ions (K^+, Na^+, H^+, and Cl^-), small organic molecules leak and this is regarded one of the primary ways cell dies. Animal cells contain cholesterol instead of ergosterol and so they are much less susceptible. However, at therapeutic doses, some amphotericin B may bind to animal membrane cholesterol, increasing the risk of human toxicity. Amphotericin B is nephrotoxic when given intravenously. As a polyene's hydrophobic chain is shortened, its sterol binding activity is increased. Therefore, further reduction of the hydrophobic chain may result in it binding to cholesterol, making it toxic to animals.

- Amphotericin B
- Candicidin
- Filipin – 35 carbons, binds to cholesterol (toxic)

- Hamycin
- Natamycin – 33 carbons, binds well to ergosterol
- Nystatin
- Rimocidin

Imidazole, triazole, and thiazole antifungals

Azole antifungal drugs inhibit the enzyme lanosterol 14 -demethylase; the enzyme necessary to convert lanosterol to ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth.

Imidazoles

- ***Bifonazole***

- Butoconazole
- Clotrimazole
- Econazole
- Fenticonazole
- Isoconazole
- Ketoconazole
- Miconazole
- Omoconazole
- Oxiconazole
- Sertaconazole
- Sulconazole
- Tioconazole

Triazoles

Albaconazole

- Fluconazole
- Isavuconazole
- Itraconazole
- Posaconazole
- Ravuconazole
- Terconazole
- Voriconazole

Thiazoles

Abafungin

Allylamines

Allylamines inhibit squalene epoxidase, another enzyme required for ergosterol synthesis:

- Amorolfin
- Butenafine
- Naftifine
- Terbinafine

Echinocandins

Echinocandins may be used for systemic fungal infections in immunocompromised patients, they inhibit the synthesis of glucan in the cell wall via the enzyme 1,3- glucan synthase:

- Anidulafungin
- Caspofungin
- Micafungin

Echinocandins are poorly absorbed when administered orally. When administered by injection they will reach most tissues and organs with concentrations sufficient to treat localized and systemic fungal infections.

Others

Benzoic acid – has antifugal properties, but must be combined with a keratolytic agent such as in Whitfield's ointment

Ciclopirox – (ciclopirox olamine) – is a hydroxypyridone antifungal which interferes with active membrane transport, cell membrane integrity, and fungal respiratory processes. It is most useful against tinea versicolour.

- Flucytosine or 5-fluorocytosine – an antimetabolite pyrimidine analog
- Griseofulvin – binds to polymerized microtubules and inhibits fungal mitosis
- Haloprogin – discontinued due to the emergence of more modern antifungals with fewer side effects

- Polygodial – strong and fast-acting *in-vitro* antifungal activity against *Candida albicans*.
- Tolnaftate – a thiocarbamate antifungal, which inhibits fungal squalene epoxidase (similar mechanism to allylamines like terbinafine)
- Undecylenic acid – an unsaturated fatty acid derived from natural castor oil; fungistatic, antibacterial, antiviral, and inhibits *Candida morphogenesis*
- Crystal violet – a triarylmethane dye, it has antibacterial, antifungal, and anthelmintic properties and was formerly important as a topical antiseptic.
- Alternatives

Research conducted in 1996 indicated the following substances or essential oils had antifungal properties:

 Oregano – the most powerful anti-fungal of the essential oils, and possess significant activity against *Candida albicans*. The minimum inhibitory concentration against *C. albicans* has been found to be <0.1μg per ml. In contrast, caprylic acid (a mixture of calcium and magnesium salts, a natural anti-fungal fatty acid), is 0.5μg.

- Allicin – created from crushing garlic
- Citronella oil – obtained from the leaves and stems of different species of *Cymbopogon* (lemon grass)
- Coconut oil – medium-chain triglycerides in the oil have antifungal activities
- Iodine – Lugol's iodine
- Lemon myrtle
- Neem seed oil
- Olive leaf
- Orange oil
- Palmarosa oil
- Patchouli
- Selenium – in dietary supplements or natural food sources, particularly Brazil nuts
- Tea tree oil – ISO 4730 ("oil of melaleuca, terpinen-4-ol type")
- Zinc – in dietary supplements or natural food sources, including pumpkin seeds and chickpeas

- Horopito (*Pseudowintera colorata*) leaf contains the antifungal compound polygodial.
- Turnip
- Chives
- Radish
- Garlic

Researchers at Tel Aviv University's Department of Plant Sciences published a study in 2009 indicating carnivorous plants, such as the Venus flytrap, contain compounds that may be useful in providing a new class of antifungal drugs for use in humans, for fungal infections that are resistant to current drugs.

Adverse effects

Apart from side effects like liver damage or affecting estrogen levels, many antifungal medicines can cause allergic reactions in people. For example, the azole group of drugs is known to have caused anaphylaxis.

There are also many drug interactions. Patients must read in detail the enclosed data sheet(s) of the medicine. For example, the azole antifungals such as ketoconazole or itraconazole can be both substrates and inhibitors of the P-glycoprotein, which (among other functions) excretes toxins and drugs into the intestines. Azole antifungals also are both substrates and inhibitors of thecytochrome P450 family CYP3A4 causing increased concentration when administering, for example, calcium channel blockers, immunosuppressants, chemotherapeutic drugs, benzodiazepines, tricyclic antidepressants, macrolides and SSRIs.

Mechanism of action

Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism with fewer adverse effects to the host. Unlike bacteria, both fungi and humans areeukaryotes. Thus, fungal and human cells are similar at the biological level. This makes it more difficult to discover drugs that target fungi without affecting human cells. As a consequence, many antifungal drugs cause side effects. Some of these side effects can be life-threatening if the drugs are not used properly.

Antidandruff shampoos

ntifungal agents (such as ketoconazole) are often found in antidandruff shampoos. The antifungal drugs inhibit the yeast *Malassezia globosa* which encourages seborrhoeic dermatitis and tinea versicolor.

Agent	Trade names	Medical applications
Ketoconazole	Nizoral, Fungoral and Sebizole	Preliminary findings, research and studies including the completion of a small controlled clinical trial have produced data suggesting ketoconazole shampoo is effective as a hair loss treatment in men withandrogenic alopecia. Larger controlled clinical studies are still needed to evaluate the ideal dosage, formulation, and to determine the routine of treatment for this condition, thus ketoconazole shampoo is not FDA-approved for this indication.
Ciclopirox olamine	Loprox	The cream and lotion form of this agent is used to treat fungal infections of the skin. The lacquer form is used as part of a treatment plan to treat fungal infections of the nails. The shampoo form is used to treat and prevent dandruff or to treat seborrhoeic dermatitis.
Piroctone olamine	Octopirox and Nivea Complete Control	Piroctone olamine is sometimes used as an antifungal agent, and it often used in dandruff shampoos in lieu of zinc. Piroctone olamine is said to be less toxic than other antidandruff agents, often bypassing some of the normal FDA warnings, but still must be used with care, and only externally.

Zinc pyrithione	Head & Shoulders, Johnson and Johnson ZP-11, Clinic All Clear,Pantene Pro V and Sikkai Powder	An antifungal and antibacterial agent first reported in the 1930s, zinc pyrithione is best known for its use in the treatment of dandruff and seborrhoeic dermatitis. It also has antibacterial properties and is effective against many pathogens from the *Streptococcus* and *Staphylococcus* genera. Its other medical applications include treatments of psoriasis, eczema, ringworm, fungus, athletes foot, dry skin, atopic dermatitis, tinea, and vitiligo.
Selenium sulfide	Selsun Blue, Head & Shoulders and Vichy Dercos Anti-Dandruff Shampoo	Selenium sulfide is available as a 1% and 2.5% lotion and shampoo. In some countries, the higher-strength preparations require a doctor's prescription. The shampoo is used to treat dandruff and seborrhea of the scalp, and the lotion is used to treat tinea versicolor, a fungal infection of the skin.
Tar	Neutrogena T-Gel	Is effective as a therapeutic treatment to control scalp itching and flaking symptomatic of scalp psoriasis, eczema, seborrhoeic dermatitis and dandruff.
Tea tree oil	Dr. Bronner's Castile Soap	It is used topically as an ingredient in creams, ointments, lotions, soaps, and shampoos.