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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC. MICROBIOLOGY

UNIT – I – Medical Mycology – SMB3103

General characteristics & classification of fungi

Characteristics of Fungi

1. Fungi is a separate kingdom

2. Fungi are Eukaryotic organism

3. Morphology:

- Fungi exists in two fundamental forms, filamentous or hyphal form (MOLD) and singe celled or budding form (YEAST).
- But for the classification of fungi, they are studied as mold, yeast, yeast like fungi and dimorphic fungi.
- Yeast is Unicellular while Mold is multicellular and filamentous

4. Fungi lacks Chloroplast.

5. Mode of nutrition:

- Fungi are organotropic heterotrophs.
- Mostly Fungi are saprophytic and some are Parasitic

6. Fungi grow best in acidic environment (tolerate acidic pH).

7. Fungi can tolerate high sugar concentration and dry condition

8. Most of the fungi are Obligate aerobes (molds) and few are facultative anaerobes (yeasts)

9. Optimum temperature of growth for most saprophytic fungi is 20-30 C while (30-37) C for parasitic fungi.

10. Growth rate of fungi is slower than that of bacteria.

11. Cell wall is composed of chitin

12. Cell membrane consists of ergosterol

13. Reproduction: both asexual (Axamorph) and sexual (Teliomorph) mode of reproduction

- Asexual methods: fragmentation, fsomatic budding, fission, asexual spore formation
- Sexual methods: gametic copulation, gamate-gametangium opulation, gametangium copulation, somatic copulation and Spermatization.

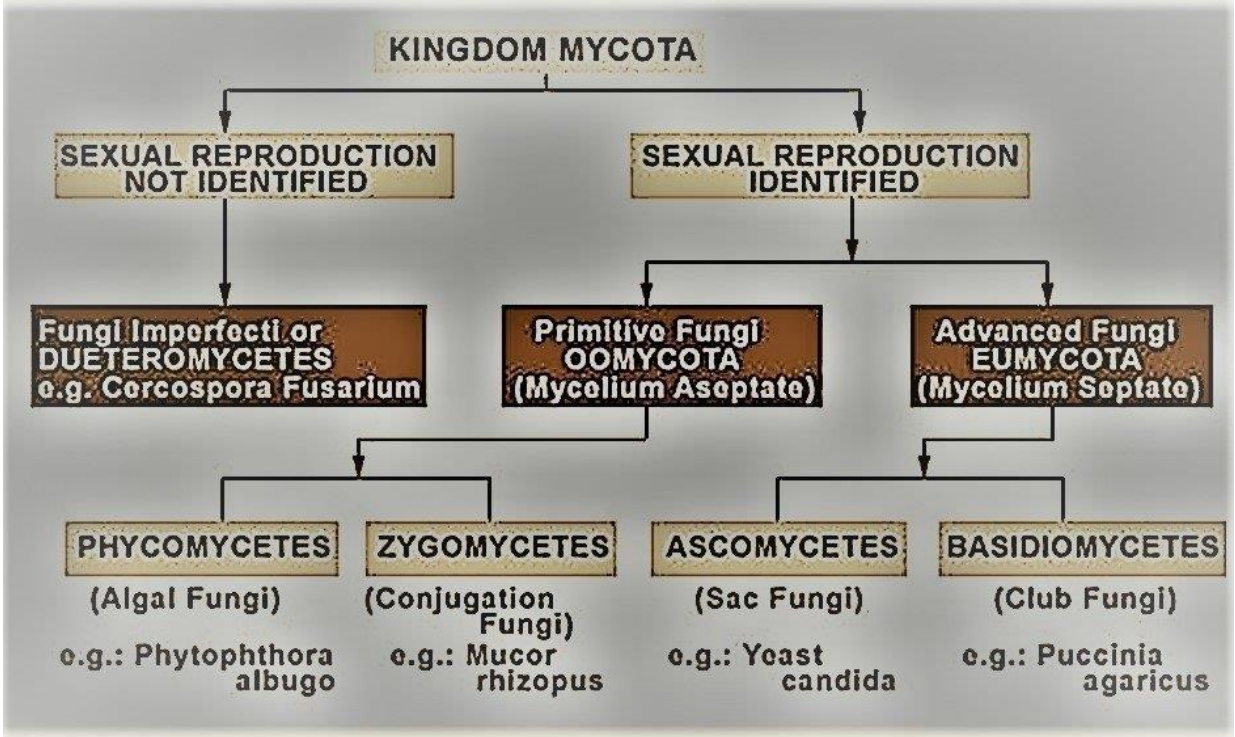
14. More than 2,00,000 fungi species are known.

15. More than 100 fungi are responsible for human infection.

16. More than 20 species are responsible to cause severe systemic human infection, 35 species causes less severe systemic disease or might causes cutaneous or sub cutaneous infection and 45 species causes superficial cutaneous infection.

17. Some fungi shows mutualistic relationship with higher plants, eg Mycorrhiza is symbiotic associated with root of gymnosperm

CLASSIFICATION OF FUNGI



Fungi are classified as follows:-

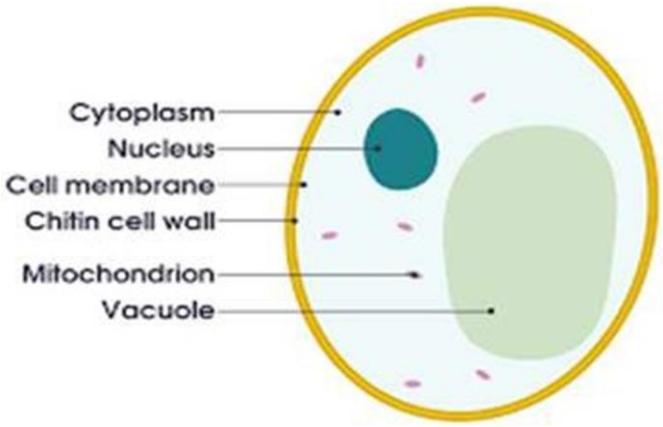
A.) MORPHOLOGICAL CLASSIFICATION OF FUNGI

⇒ From a diagnostic point of view, fungi may be classified depending on cell morphology into four groups:

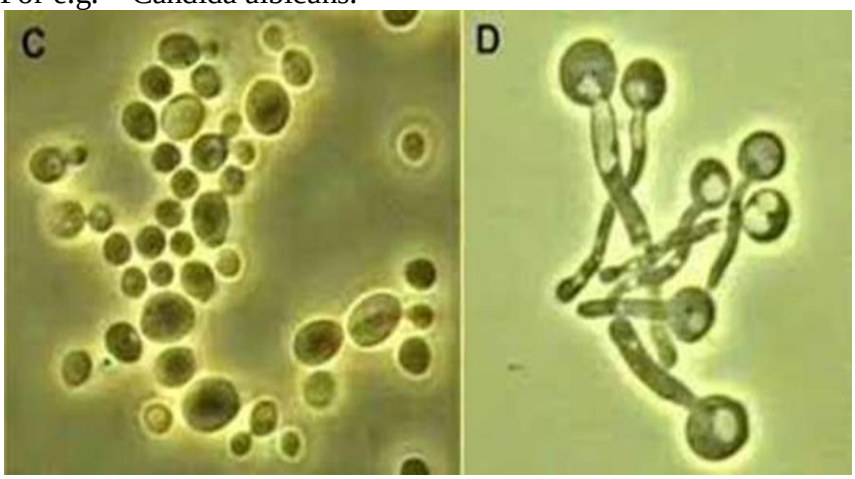
a. Yeast
b. Yeast-like fungi
c. Moulds
d. Dimorphic fungi

⇒ Yeasts – Characteristics:-

- Yeast is a Unicellular fungus that has a single nucleus and reproduces either asexually by budding [bud = blastospore (blastoconidium)] or sexually by true spore formation.
- Each bud that separates can grow into new yeast.
- Macroscopically appears as creamy mucoid colonies on the culture media
- Microscopically appears as oval to round (3-15 microns in diameter) in tissues and in culture.
- The most important pathogenic yeast is *Cryptococcus neoformans*.

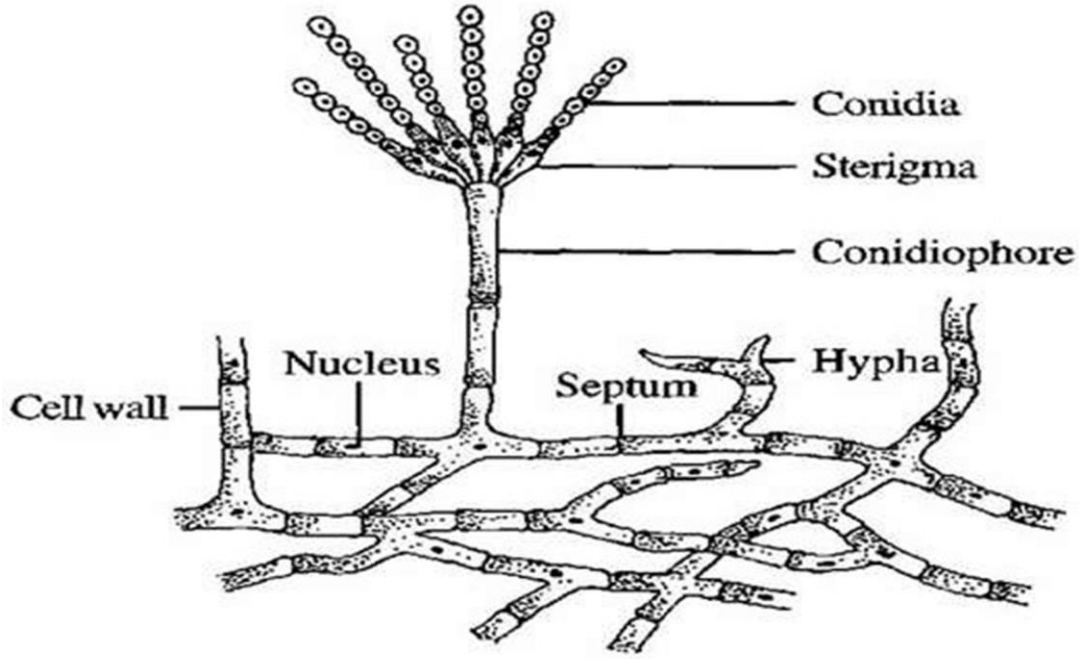


⇒ Yeast-like fungi – characteristics:-

- Unicellular fungi that reproduce by budding or by fission.
- Macroscopically appears as pasty colonies on the culture media.
- Microscopically appears as spherical or oval structure; filamentous structures may be seen due to the chains of elongated budding cells joined end to end (pseudohyphae) in tissues and in culture.
- For e.g. – Candida albicans.
- 

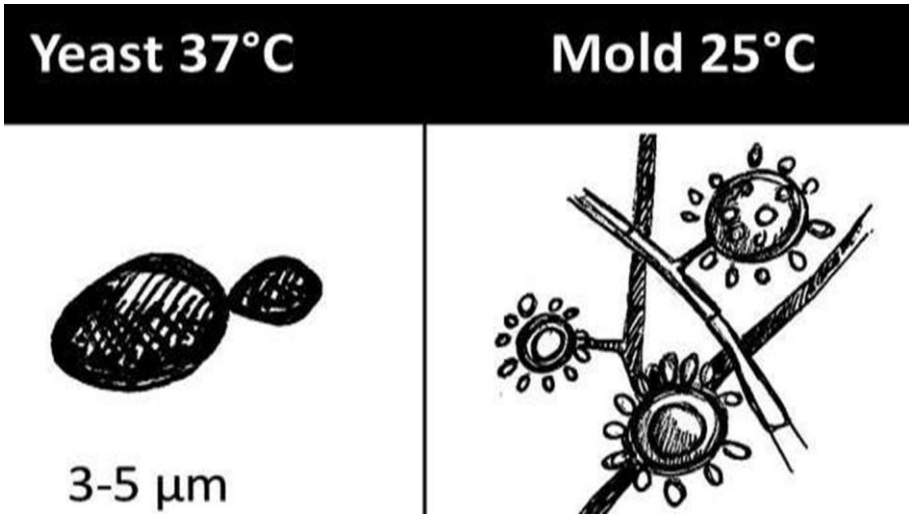
⇒ Moulds – Characteristics:-

- Multicellular fungi that reproduce by asexual means (spore formation); some exhibit sexual reproduction.
- Moulds are composed of Hyphae which may have cross-walls or septa or may lack septa (coenocytic).
- Macroscopically appears as cottony/ woolly/ velvety/ granular growth on the culture media.
- Microscopically appears as thread-like filamentous Hyphae (2-10 microns) seen in tissues and in culture.
- For e.g. – *Aspergillus fumigatus, Penicillium notatum*



⇒ Dimorphic fungi – characteristics:-

- Many fungi especially those that cause disease in human and animal are dimorphic i.e. they have two forms.
- Dimorphic fungi can change from the yeast form in the animals to the moulds or mycelium form in the external environment in response to change in various environmental factors.
- The shift is called as Y M shift.
- They exist as yeasts in the host tissue and in the cultures at 37°
- Grows as hyphal (mycelial) form in the soil and in the cultures at 22-25°.
- For e.g. – *Blastomyces dermatitidis*



B.) TAXONOMICAL / SYSTEMIC CLASSIFICATION OF FUNGI

Fungi are placed in phylum *Thallophyta*. This classification of fungi is based on the sexual spore formation. There are four classes of fungi as follows :

a. Phycomycetes/Zygomycetes
b. Ascomycetes
c. Basidiomycetes
d. Deuteromycetes/Hyphomycetes/Fungi imperfecti

DIVISIONS OF FUNGI

DIVISION	COMMON NAME	APPROXIMATE NO. OF SPECIES
Zygomycota	Zygomycetes	600
Ascomycota	Sac fungi	35,000

DIVISION	COMMON NAME	APPROXIMATE NO. OF SPECIES
Basidiomycota	Club fungi	30,000
Deuteromycota	Fungi imperfecti	30,000

⇒ Zygomycetes

- The division Zygomycota contains the fungi called Zygomycetes.
- These are lower fungi that have non-septate Hyphae and produce endogenous asexual spores, called Sporangiospores, contained within swollen sac-like structures called Sporangia.
- The Hyphae of Zygomycetes known as Coenocytic, with many haploid nucleoids.
- Zygomycetes also produce sexual spores known as oospores in some fungi and Zygospores in others.
- Zygospores are tough thick walled zygotes called Zygospores that can remain dormant when the environment is too harsh for the growth of the fungus.
- They usually reproduce asexually but if food becomes starve or environmental condition unfavorable it begins sexual reproduction.
- The Zygomycetes also contributes to human welfare for e.g. – Rhizopus is used in Indonesia to produce food; another Zygomycetes is used with soybean to make a curd called Sufu.

⇒ Ascomycetes

- The division Ascomycota contains the fungi called Ascomycetes are commonly known as sac fungi.
- They have septate Hyphae ad form exogenous asexual spores called conidia.
- It forms sexual spores (Ascospores) are present within a sac or Ascus.
- Asexual reproduction is common in the Ascomycetes and takes place by means of Conidiospores.
- It includes both yeasts and filamentous fungi. Many yeast genera are classified specifically within Ascomycetes because of their sexual reproduction.
- Many species are quite familiar and economically important for example – most of the Red, Brown and blue-green moulds that cause food spoilage are Ascomycetes.

⇒ Basidiomycota

- The division Basidiomycota contains the Basidiomycetes commonly known as the club fungi.
- It forms sexual spores called Basidiospores on a basidium or base. A basidium is produced at the tip of Hyphae and normally in club shape.

- The Basidiomycetes affects humans in many ways; many mushrooms are used as food throughout the world.
- For e.g. – Basidiomycetes includes Smuts, Jelly fungi, Mushrooms & Bird net fungi.

⇒ Deuteromycota

- When a fungus lacks the sexual phase (perfect stage) or if this phase has not been observed it is placed within the division Deuteromycota, commonly called as Deuteromycetes or Fungi imperfecti.
- Most Deuteromycetes reproduce by means of Conidia.
- Most fungi imperfecti are terrestrial; with only a few being reported from freshwater and marine habitats.
- Many Deuteromycetes directly affects human welfare and causing numerous diseases such as Ringworm.
- The chemical activities of many fungi are important to industries such as some species of Penicillium used in the synthesis of antibiotics.

C.) PATHOLOGICAL CLASSIFICATION OF FUNGI:

⇒ Infection caused by the fungus is known as Mycoses.

⇒ Based on the pathogenic potential, fungi may be considered as :

- **Primarily pathogenic –** Those fungi which are able to cause infections in healthy individuals; for e.g., thermally dimorphic fungi.
- **Opportunistic pathogens –** Those fungi that are able to cause infection in patients who are Immuno-compromised due to some other infections or diseases or who are receiving immunosuppressive drugs etc.

⇒ Pathogenic fungi may cause:

- **Actual infection of tissues (Mycoses) –** Fungal infections or Mycoses in humans can be classified according to the tissues involved into –
  i. Superficial Mycoses
  ii. Subcutaneous Mycoses
  iii. Systemic or Visceral Mycoses
  iv. Opportunistic Mycoses
- **Mycotoxicoses –** These are diseases due to toxic metabolic products released by fungi. For e.g. – aflatoxicosis due to consumption of grains containing aflatoxins secreted by *Aspergillus flavus* contaminating the groundnuts, corn, and peas. The fungus does not necessarily have to be present in the tissues to exert its pathogenic effect since its toxic metabolites are present. There is no invasion of tissues by the fungus.
- **Hypersensitivity (Allergic reactions) –** A type I and/or type III hypersensitivity reaction is provoked by inhalation of fungal spores. For e.g. – Allergic Bronchopulmonary Aspergillosis (due to spores of *Aspergillus fumigatus*) and allergic fungal rhinosinusitis. There is no invasion of tissues by the fungus.

Reproductive Processes Of Fungi

Following a period of intensive growth, fungi enter a reproductive phase by forming and releasing vast quantities of spores. Spores are usually single cells produced by fragmentation of the mycelium or within specialized structures (sporangia, gametangia, sporophores, etc.). Spores may be produced either directly by asexual methods or indirectly by sexual reproduction. Sexual reproduction in fungi, as in other living organisms, involves the fusion of two nuclei that are brought together when two sex cells (gametes) unite. Asexual reproduction, which is simpler and more direct, may be accomplished by various methods.

Asexual reproduction

Typically in asexual reproduction, a single individual gives rise to a genetic duplicate of the progenitor without a genetic contribution from another individual. Perhaps the simplest method of reproduction of fungi is by fragmentation of the thallus, the body of a fungus. Some yeasts, which are single-celled fungi, reproduce by simple cell division, or fission, in which one cell undergoes nuclear division and splits into two daughter cells; after some growth, these cells divide, and eventually a population of cells forms. In filamentous fungi the mycelium may fragment into a number of segments, each of which is capable of growing into a new individual. In the laboratory, fungi are commonly propagated on a layer of solid nutrient agar inoculated either with spores or with fragments of mycelium.

Budding, which is another method of asexual reproduction, occurs in most yeasts and in some filamentous fungi. In this process, a bud develops on the surface of either the yeast cell or the hypha, with the cytoplasm of the bud being continuous with that of the parent cell. The nucleus of the parent cell then divides; one of the daughter nuclei migrates into the bud, and the other remains in the parent cell. The parent cell is capable of producing many buds over its surface by continuous synthesis of cytoplasm and repeated nuclear divisions. After a bud develops to a certain point and even before it is severed from the parent cell, it is itself capable of budding by the same process. In this way, a chain of cells may be produced. Eventually, the individual buds pinch off the parent cell and become individual yeast cells. Buds that are pinched off a hypha of a filamentous fungus behave as spores; that is, they germinate, each giving rise to a structure called a germ tube, which develops into a new hypha.

Although fragmentation, fission, and budding are methods of asexual reproduction in a number of fungi, the majority reproduce asexually by the formation of spores. Spores that are produced asexually are often termed mitospores, and such spores are produced in a variety of ways.

Sexual reproduction

Sexual reproduction, an important source of genetic variability, allows the fungus to adapt to new environments. The process of sexual reproduction among the fungi is in many ways unique. Whereas nuclear division in other eukaryotes, such as animals, plants, and protists, involves the

dissolution and re-formation of the nuclear membrane, in fungi the nuclear membrane remains intact throughout the process, although gaps in its integrity are found in some species. The nucleus of the fungus becomes pinched at its midpoint, and the diploid chromosomes are pulled apart by spindle fibres formed within the intact nucleus. The nucleolus is usually also retained and divided between the daughter cells, although it may be expelled from the nucleus, or it may be dispersed within the nucleus but detectable.

Sexual reproduction in the fungi consists of three sequential stages: plasmogamy, karyogamy, and meiosis. The diploid chromosomes are pulled apart into two daughter cells, each containing a single set of chromosomes (a haploid state). Plasmogamy, the fusion of two protoplasts (the contents of the two cells), brings together two compatible haploid nuclei. At this point, two nuclear types are present in the same cell, but the nuclei have not yet fused. Karyogamy results in the fusion of these haploid nuclei and the formation of a diploid nucleus (i.e., a nucleus containing two sets of chromosomes, one from each parent). The cell formed by karyogamy is called the zygote. In most fungi the zygote is the only cell in the entire life cycle that is diploid. The dikaryotic state that results from plasmogamy is often a prominent condition in fungi and may be prolonged over several generations. In the lower fungi, karyogamy usually follows plasmogamy almost immediately. In the more evolved fungi, however, karyogamy is separated from plasmogamy. Once karyogamy has occurred, meiosis (cell division that reduces the chromosome number to one set per cell) generally follows and restores the haploid phase. The haploid nuclei that result from meiosis are generally incorporated in spores called meiospores.

Fungi employ a variety of methods to bring together two compatible haploid nuclei (plasmogamy). Some produce specialized sex cells (gametes) that are released from differentiated sex organs called gametangia. In other fungi two gametangia come in contact, and nuclei pass from the male gametangium into the female, thus assuming the function of gametes. In still other fungi the gametangia themselves may fuse in order to bring their nuclei together. Finally, some of the most advanced fungi produce no gametangia at all; the somatic (vegetative) hyphae take over the sexual function, come in contact, fuse, and exchange nuclei.

Fungi in which a single individual bears both male and female gametangia are hermaphroditic fungi. Rarely, gametangia of different sexes are produced by separate individuals, one a male, the other a female. Such species are termed dioecious. Dioecious species usually produce sex organs only in the presence of an individual of the opposite sex.

Sexual incompatibility

Many of the simpler fungi produce differentiated male and female organs on the same thallus but do not undergo self-fertilization because their sex organs are incompatible. Such fungi require the presence of thalli of different mating types in order for sexual fusion to take place. The simplest form of this mechanism occurs in fungi in which there are two mating types, often designated + and − (or *A* and *a*). Gametes produced by one type of thallus are compatible only with gametes produced by the other type. Such fungi are said to be heterothallic. Many fungi, however, are homothallic; i.e., sex organs produced by a single thallus are self-compatible, and a second thallus is unnecessary for sexual reproduction. Some of the most complex fungi

(e.g., mushrooms) do not develop differentiated sex organs; rather, the sexual function is carried out by their somatic hyphae, which unite and bring together compatible nuclei in preparation for fusion. Homothallism and heterothallism are encountered in fungi that have not developed differentiated sex organs, as well as in fungi in which sex organs are easily distinguishable. Compatibility therefore refers to a physiological differentiation, and sex refers to a morphological (structural) one; the two phenomena, although related, are not synonymous.

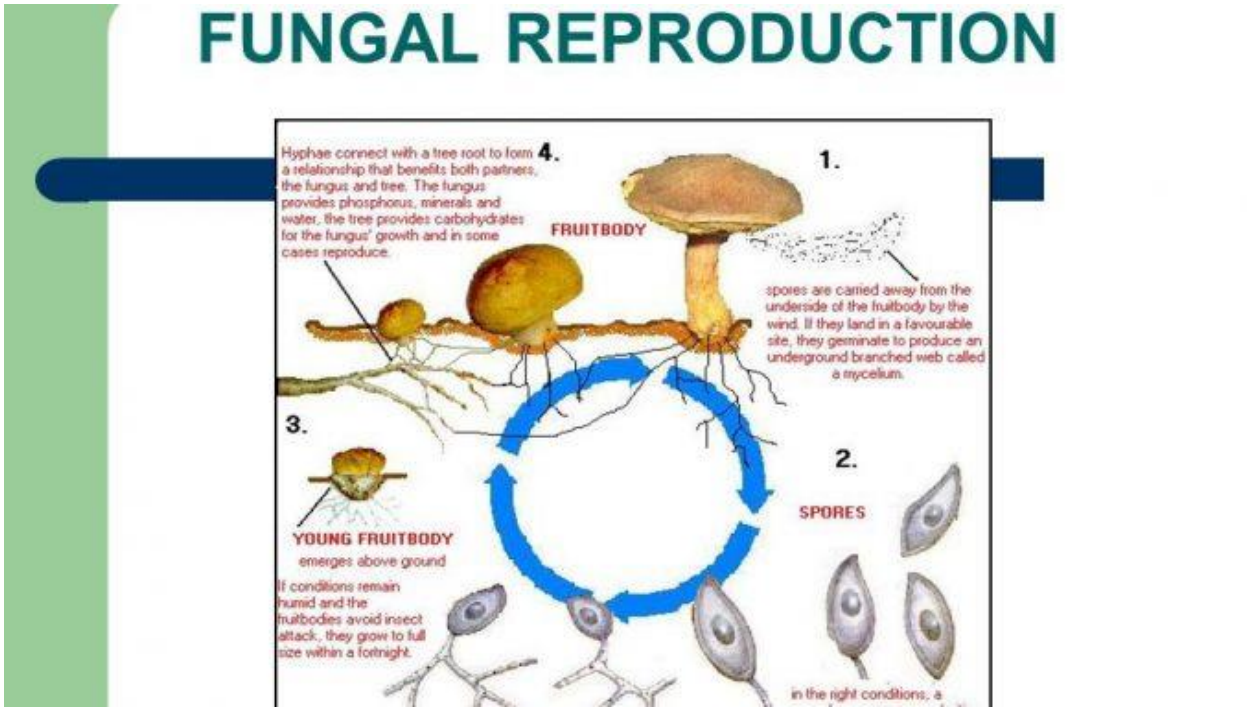
Sexual pheromones

The formation of sex organs in fungi is often induced by specific organic substances. Although called sex hormones when first discovered, these organic substances are actually sex pheromones, chemicals produced by one partner to elicit a sexual response in the other. In *Allomyces* (order Blastocladiales) a pheromone named sirenin, secreted by the female gametes, attracts the male gametes, which swim toward the former and fuse with them. In some simple fungi, which may have gametangia that are not differentiated structurally, a complex biochemical interplay between mating types produces trisporic acid, a pheromone that induces the formation of specialized aerial hyphae. Volatile intermediates in the trisporic acid synthetic pathway are interchanged between the tips of opposite mating aerial hyphae, causing the hyphae to grow toward each other and fuse together. In yeasts belonging to the phyla Ascomycota and Basidiomycota, the pheromones are small peptides. Several pheromone genes have been identified and characterized in filamentous ascomycetes and basidiomycetes.

Reproduction in fungi: asexual and sexual methods

Asexual reproduction in fungi:

1. fission of somatic cell
2. Budding of somatic cell
3. Fragmentation or disjoining of hyphae
4. Asexual spore formation



1. Fission:

- In binary fission a mature cell elongates and its nucleus divides into two daughter nuclei.
- The daughter nuclei separates, cleaves cytoplasm centripetally in the middle till it divides parent protoplasm into two daughter protoplasm.
- A double cross wall is deposited in the middle to form two daughter cell.
- Ultimately the middle layer of double cross wall degenerates and daughter cells are separated.
- Examples: Saccharomyces pobbe, Psygosaccharomyces

2. Budding:

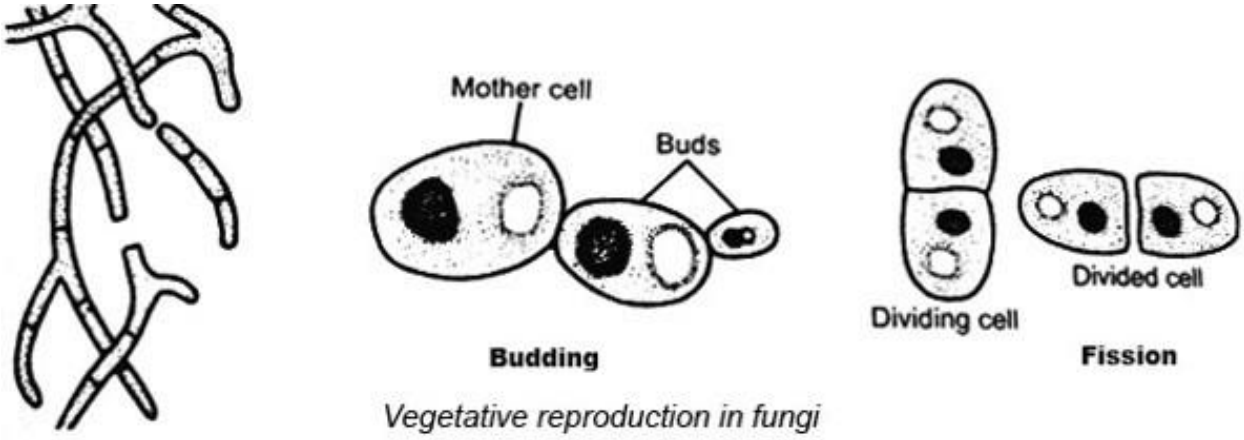
- The cell wall bulge out and softens in the area probably by certain enzymes brought by vesicles.
- The protoplasm also bulge out in this region as small protuberance.
- The parent nucleus also divides into two, one of the daughter nucleus migrates into bud, the cytoplasm of bud and mother remain continuous for some time
- As the bud enlarges, a septum is laid down at the joining of bud with mother cell. Then bud separates and leads independent life.
- Some time, bud starts reproducing while still attached with mother cell. This gives branching appearance.
- Budding is the typical reproductive characteristics of Ascomycetes.
- Examples: yeast

3. Fragmentation:

- In some fungi, fragmentation or disjoining of hyphae occurs and each hyphae become a new organism

4. Asexual spore of fungi:

- Spore formation is the characteristic feature of fungi.
- Different fungi forms different types of spore,



Vegetative reproduction in fungi

Types of asexual spore:

i. Sporangiospore:

- These asexual spore are produced in a sac like structure called sporangia (singular;saprangium).
- Sporangium are produced at the end of special aerial hyphae called sporangiophore
- Sporangium contains large numbers of haploid spores, which are released by rapture of sporangial wall
- Examples: *Rhizopus*

ii. Conidiospore:

- Conidiospore or conidia are single celled, bicelled or multicelled structure born on the tip or side of aerial hyphal structure called conidiophore
- Conidia are different from sporangiospore as these are not produced inside sporangium or any sac like structure.
- Conidia are born singly or in chain
- Examples: *Penicillium, Apergillus*

iii. Arthrospore:

- Arthrospore are very primitive type of spore formed by the breaking up of fungal mycelium
- A spore is formed by separation followed by fragmentation of hyphae
- Examples: *Trichosporium, Geotrichum, Coccididious imitis*

iv. Chlamydospore:

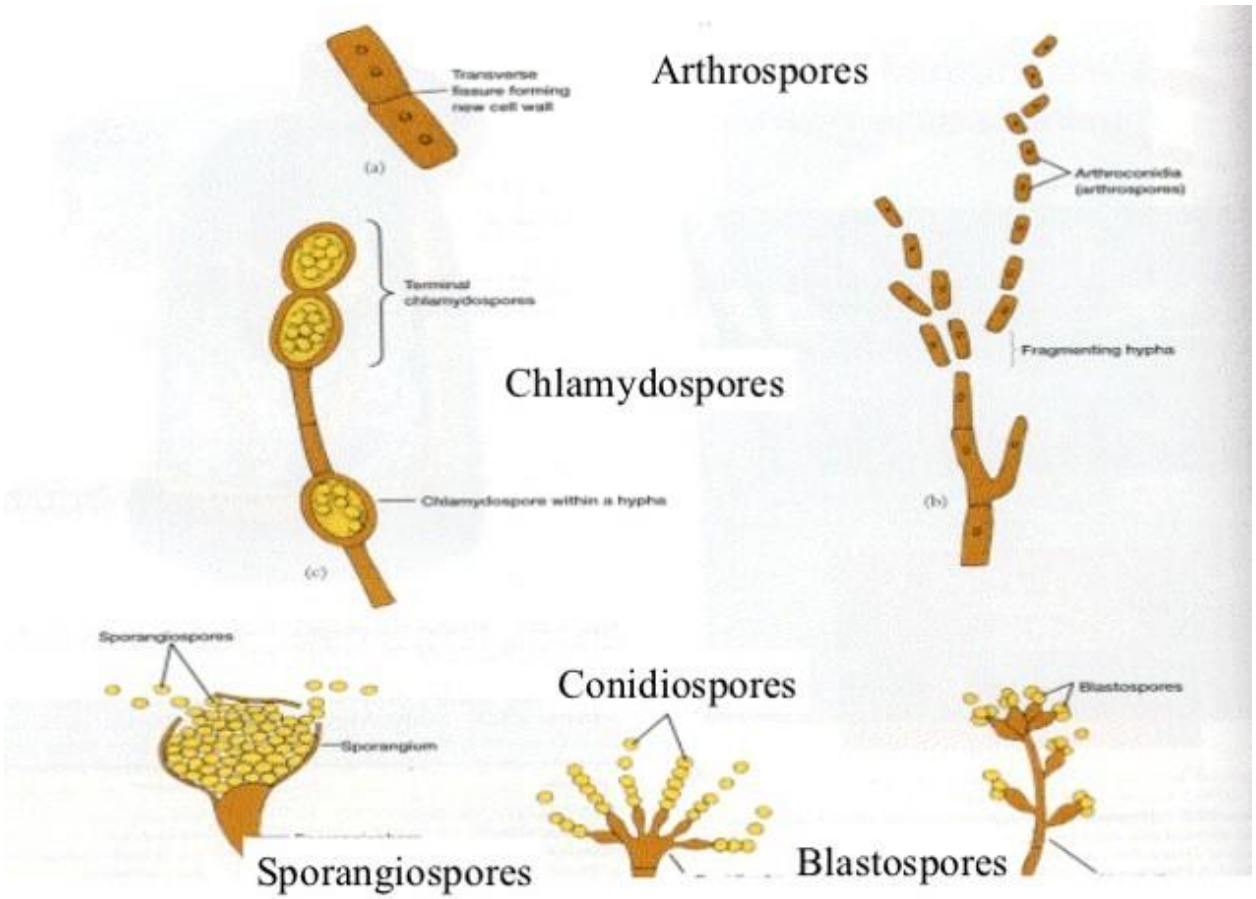
- These are usually formed during unfavorable condition and are thick walled single celled spore, which are highly resistant to adverse condition.
- Hyphal cell or portion of hyphae contracts, loose water, round up and develops into thick walled chalmydospore.
- When favorable condition returns, each chlamydospore give rise to a new individual fungi.
- Examples: ascomycetes, basidiomycetes, zygomycetes,
- *Histoplasma capsulatum, Candida albicans*

v. Blastospore:

- It is a budding spores usually formed at the terminal end of hyphae.
- These spore may remains attached to hyphae and bud further to gibe branching chain of blastospores
- Examples: ascomycetes, basidiomycetes, zygomycetes

Sexual reproduction in fungi:

- Sexual reproduction is carried out by diffusion of compatable nuclei from two parent at a definite state in the life cycle of fungi.
- The process of sexual reproduction involves three phases:
  - Plasmogamy: fusion of protoplasm
  - Karyogamy: fusion of nucleus
  - Meiosis: reductional nuclear division
- Various methods by which compatible nuclei are brought together in plasmogamy. Some are:
  - Gametic copulation
  - Gamete-gametangial copulation
  - Gametangial copulation
  - Somatic copulation
  - Spermatization



1. Gametic copulation:

- Fusion of two naked gametes, one or both of them are motile
  - Isogamous
  - Anisogamous
  - Oogamous

2. Gamete-gametangial copulation:

- Male and female gametangia comes into contact but do not fuse.
- A fertilization tube formed from where male gametangium enters the female gametangium and male gamate passes through this tube

3. Gametangial copulation;

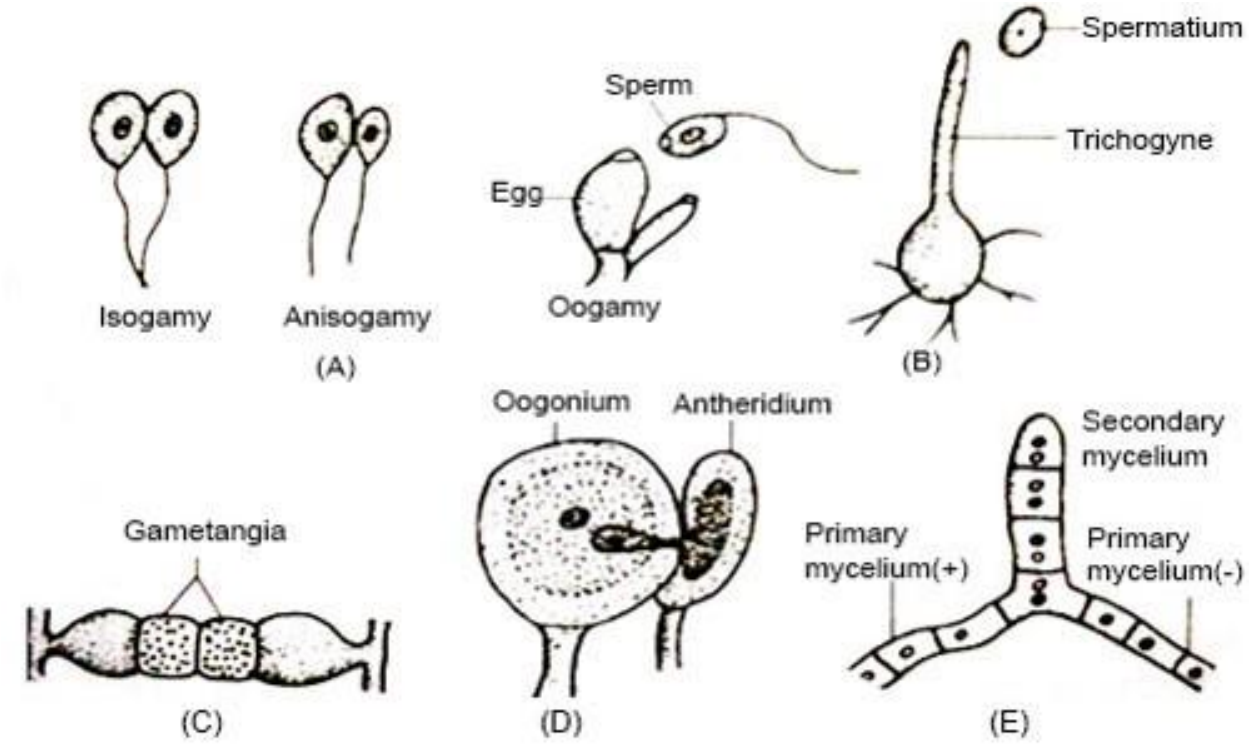
- Two gametangia or their protoplast fuse and give rise to zygospore

4. Somatic copulation:

- Also known as somatogamy.
- In this process fusion of somatic cell occurs
- This sexual fusion of undifferentiated vegetative cell results in dikaryotic hyphae, so the process is also called dikarotization

5. Spermetization**:**

- It is an union of special male structure called spertatium with a female receptive structure.
- Spermatium empties its content into receptive hyphae during plasmogamy



Sexual spores of fungi

- As a result of sexual reproduction sexual sores are produced.
- Sexual spores are fewer in number than asexual spores.

Types of sexual spores

i. Ascospore:

- It is usually single celled produced in a sac called ascus (plural;asci) and usually there are 4-8 ascospore in an ascus but the number may vary from species to species
- The ascospore are usually arranged in a linear order. In some case ascospores are long, narrow and are arranged in parallel order.

ii. Basidiospore:

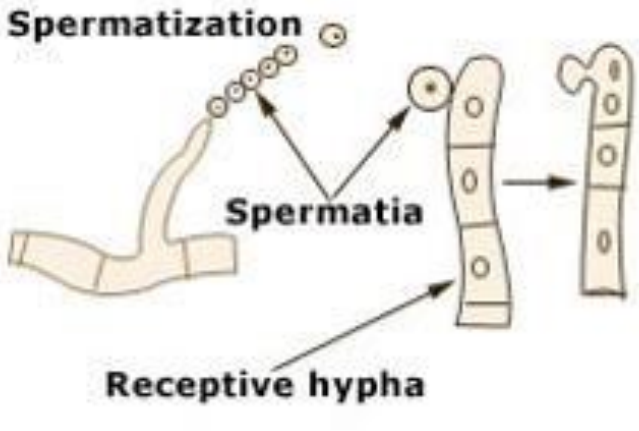
- It is a reproductive spore produced by basidiomycetes.
- This single celled spores are born in a club shaped structure called basidium
- These basidiospore aerves as main air dispersal unit for the fungi.

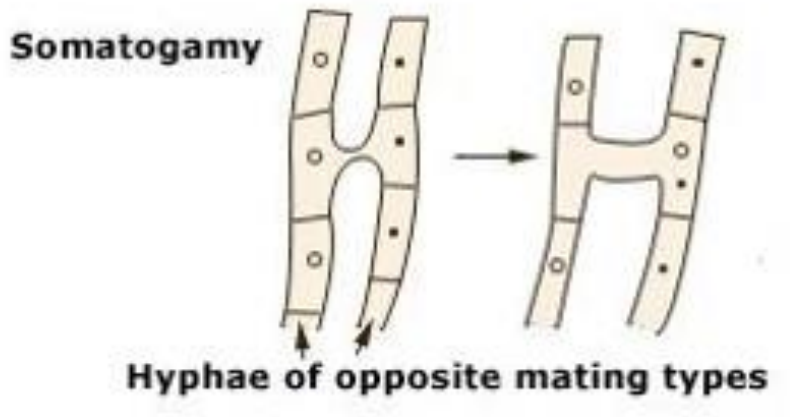
iii. Zygospore:

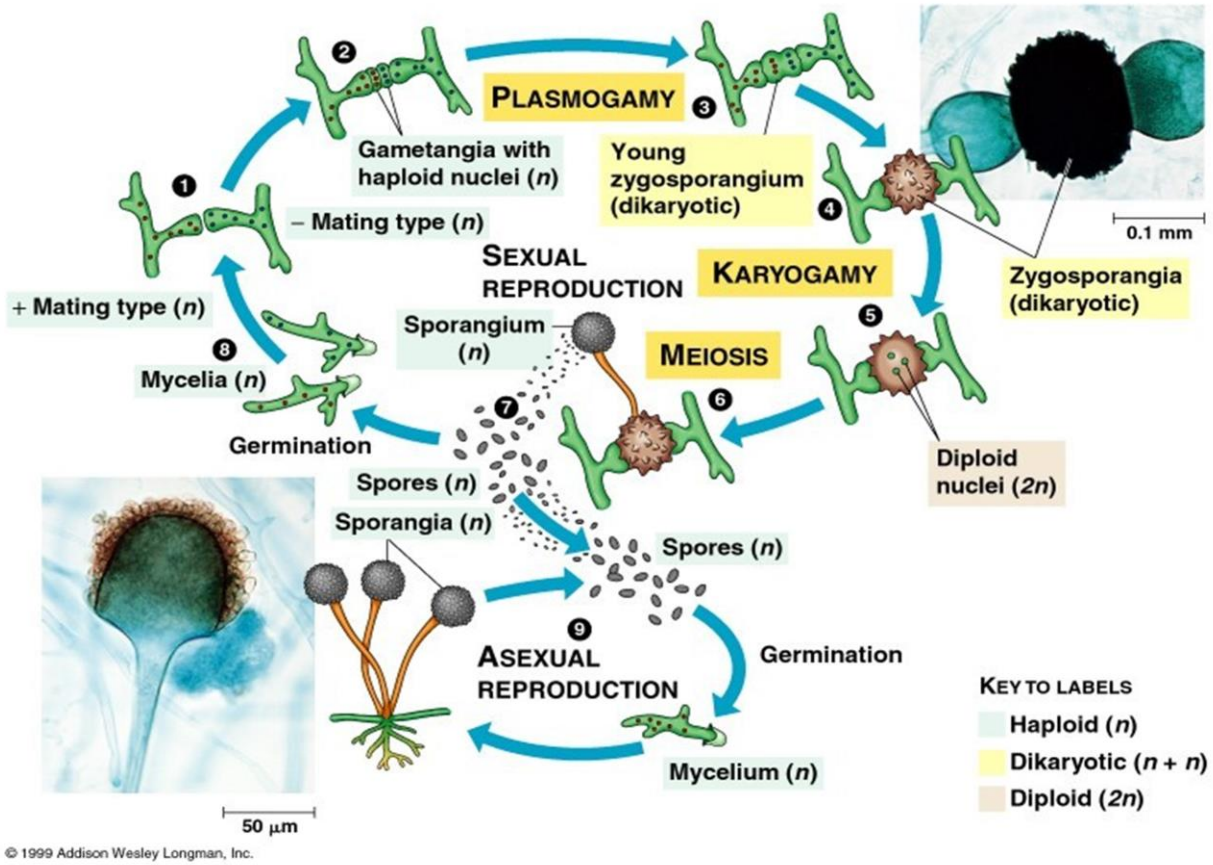
- Zygospores are thick walled spores formed when two sexually compatible hyphae or gametangia of certain fungi fuse together.
- In suitable condition, zygospore germinates to produce a single vertical hyphae which forms a aporangium and releases its spores

iv. Oospore:

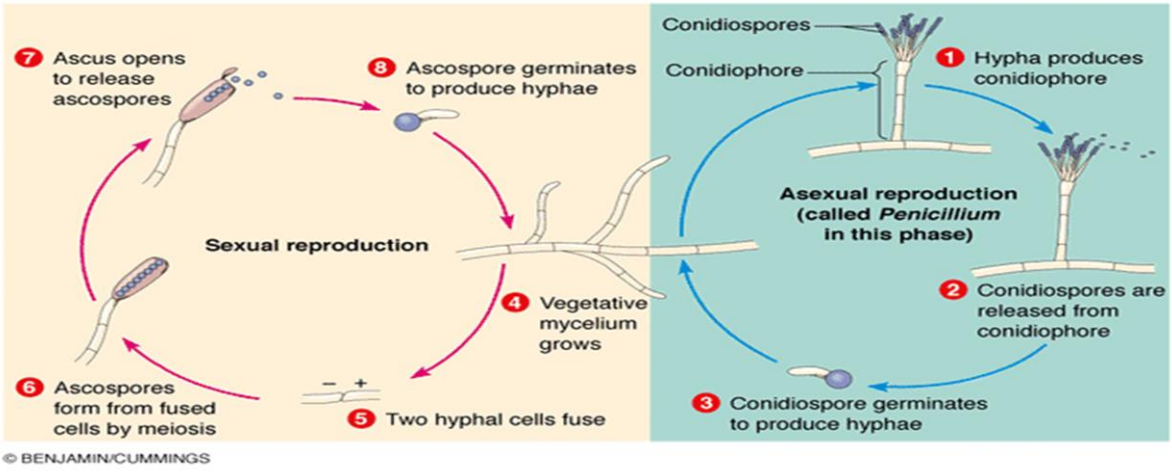
- These are formed within a special female structure called Oogonium.
- Fertilization of egg by male gamete in female sex organ give rise to oospoes.
- There are one or more oospores in each oogonium.



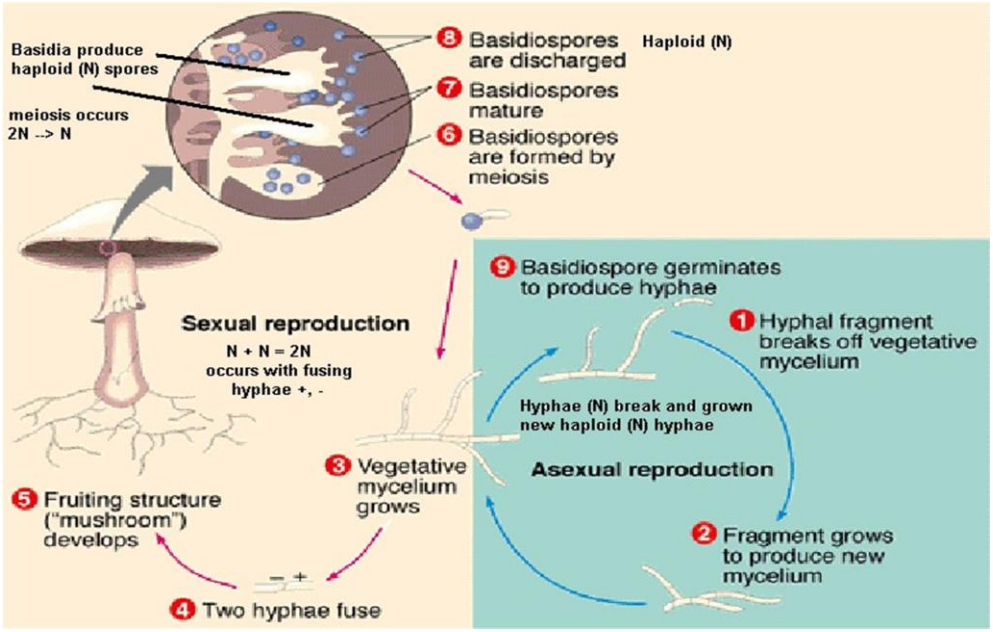




1. **Reproductive Structures of Zygomycete (*Rhizopus*) Sporangia (asexual) and Zygospore (sexual)**



2. **Life Cycle of *Eupenicillium* (Ascomycete) Reproduces Asexually and Sexually**



3. **Life Cycle of Basidiomycete Fungi.**

Pathogenesis of fungal infections

General Concepts

Entry

Fungi rarely cause disease in healthy immunocompetent hosts. Disease results when fungi accidentally penetrate host barriers or when immunologic defects or other debilitating conditions exist that favor fungal entry and growth.

Adaptation and Propagation

Fungi often develop both virulence mechanisms (e.g., capsule and ability to grow at 37°C) and morphologic forms (e.g., yeasts, hyphae, spherules, and sclerotic bodies) that facilitate their multiplication within the host.

Dissemination

Dissemination of fungi in the body indicates a breach or deficiency of host defenses (e.g., endocrinopathies and immune disorders).

Host Factors

Healthy, immunologically-competent individuals have a high degree of innate resistance to fungi. Resistance to fungi is based primarily upon cutaneous and mucosal physical barriers. Severity of disease depends on factors such as inoculum, magnitude of tissue destruction, ability of fungus to multiply in the tissue, and the immune status of the host.

Fungal Factors

Enzymes such as keratinase, the presence of capsule in *Cryptococcus neoformans*, the ability to grow at 37°C, dimorphism, and other as yet undefined factors contribute to fungal pathogenesis which involves a complex interplay of many fungal and host factors.

Introduction

Fungi are ubiquitous in nature and exist as free-living saprobes that derive no obvious benefits from parasitizing humans or animals. Since they are widespread in nature and are often cultured from diseased body surfaces, it may be difficult to assess whether a fungus found during disease is a pathogen or a transient environmental contaminant. Before a specific fungus can be confirmed as the cause of a disease, the same fungus must be isolated from serial specimens and fungal elements morphologically consistent with the isolate must be observed in tissues taken from the lesion. In general, fungal infections and the diseases they cause are accidental. A few fungi have developed a commensal relationship with humans and are part of the indigenous microbial flora (e.g., various species of *Candida*, especially *Candida albicans*, and *Malassezia furfur*). Although a great deal of information is available concerning the molecular basis of bacterial pathogenesis, little is known about mechanisms of fungal pathogenesis. Infection is defined as entry into body tissues followed by multiplication of the organism. The infection may be clinically inapparent or may result in disease due to a cellular injury from competitive metabolism, elaboration of toxic metabolites, replication of the fungus, or an immune response. Immune responses may be transient or prolonged and may be cell-mediated, humoral (with production of specific antibody to components of the infecting organism), or both. Successful infection may result in disease, defined as a deviation from or interruption of the normal structure or function of body parts, organs, or systems (or combinations thereof) that is marked by a characteristic set of symptoms and signs and whose etiology, pathology, and prognosis are known or unknown.

Entry

Fungi infect the body through several portals of entry (Table 74-1). The first exposure to fungi that most humans experience occurs during birth, when they encounter the yeast *C. albicans* while passing through the vaginal canal. During this process the fungus colonizes the buccal cavity and portions of the upper and lower gastrointestinal tract of the newborn, where it maintains a life-long residence as a commensal.

Table 74-1

Summary of Disease Mechanisms of Fungi.

Another fungus, *Malassezia furfur,* is common in areas of skin rich in sebaceous glands. How it colonizes the skin is not known, but both *M furfur* and *C albicans* are the only fungi that exist as commensals of humans and are considered part of the indigenous flora. Only under certain unusual circumstances have they caused disease. Other fungi that have been implicated in human diseases come from exogenous sources, where they exist as saprobes on decaying vegetation or as plant parasites. Fungi rarely cause disease in healthy, immuno-competent hosts, even though we are constantly exposed to infectious propagules. It is only when fungi accidentally penetrate barriers such as intact skin and mucous membrane linings, or when immunologic defects or other debilitating conditions exist in the host, that conditions favorable for fungal colonization and growth occur. When *C albicans,* for example, is implicated in disease processes, it may indicate that the patient has a coexisting immune, endocrine, or other debilitating disorder. In most cases, the underlying disorder must be corrected to effectively manage the fungal disease.

Adaptation and Propagation

Although most fungal diseases are the result of accidental encounters with the agent, many fungi have developed mechanisms that facilitate their multiplication within the host. For example, the dermatophytes that colonize skin, hairs, and nails elaborate enzymes that digest keratin. *Candida albicans* as a commensal organism exists in a unicellular yeastlike morphology, but when it invades tissues it becomes filamentous; conversely, the systemic fungi *Histoplasma capsulatum, Blastomyces dermatitidis,* and *Paracoccidoides brasiliensis* exist as molds in nature and change to a unicellular morphology when they cause disease. Other properties, such as capsule production by *C neoformans* and the adherence properties of *Candida* species to host tissues, also contribute to their pathogenicity. In general, the fungi that cause systemic disease must be able to grow and multiply at 37°C.

Dissemination

Disseminated fungal diseases usually indicate a breach in host defenses. Such a breach may be caused by endocrinopathies or immune disorders, or it may be induced iatrogenically. Effective management of the fungal infection requires a concerted effort to uncover and correct the underlying defects.

Host Factors

The high degree of innate resistance of humans to fungal invasion is based primarily on the various protective mechanisms that prevent fungi from entering host tissues. Fungal growth is discouraged by the intact skin and factors such as naturally occurring long-chain unsaturated fatty acids, pH competition with the normal bacterial flora, epithelial turnover rate, and the desiccated nature of the stratum corneum. Other body surfaces, such as the respiratory tree, gastrointestinal tract, and vaginal vault, are lined with mucous membranes (epithelium) bathed in fluids that contain antimicrobial substances, and some of these membranes are lined with ciliated cells that actively remove foreign materials. Only when these protective barriers are breached can fungi gain access to, colonize, and multiply in host tissues. Fungi gain access to host tissues

by traumatic implantation or inhalation. The severity of disease caused by these organisms depends upon the size of the inoculum, magnitude of tissue destruction, the ability of the fungi to multiply in tissues, and the immunologic status of the host.

Fungal Factors

Most of the fungi that infect humans and cause disease are classified by tissue or organ levels that are primary sites of colonization. These are discussed below.

Superficial Fungal Infections

Superficial fungal infections involve only the outermost layers of the stratum corneum of the skin (*Phaeoannellomyces werneckii* [syn. *Exophiala werneckii*] and *M furfur*) or the cuticle of the hair shaft (*Trichosporon beigelii* and *Piedraia hortae*). These infections usually constitute cosmetic problems and rarely elicit an immune response from the host (except occasionally *M furfur* infections). Recently *T beigelii* and *M furfur* were implicated as opportunistic agents of disease, particularly in immunosuppressed or otherwise debilitated patients. Patients are accidentally infected with these common organisms via indwelling catheters or intravenous lines. Virtually nothing is known concerning the pathogenic mechanisms of these fungi.

Dermatophyte Infections

The dermatophytes are fungi that colonize skin, hair, and nails on the living host. These fungi possess greater invasive properties than those causing superficial infections, but they are limited to the keratinized tissues. They cause a wide spectrum of diseases that range from a mild scaling disorder to one that is generalized and highly inflammatory. Studies have shown that the disease-producing potential of these agents depends on various parasite and host factors, such as the species of organism, immunologic status of the host, type of clothing worn, and type of footwear used. Trauma plays an important role in infection. These organisms gain entry and establish themselves in the cornified layers of traumatized or macerated skin and its integument and multiply by producing keratinase to metabolize the insoluble, tough fibrous protein. The reason why these agents spread no deeper is not known, but it has been speculated that factors such as cell-mediated immunity and the presence of transferrin in serum inhibit fungal propagation to the deeper tissue layers and systemic disease does not occur. Some dermatophytes have evolved a commensal relationship with the host and are isolated from skin in the absence of disease. Little is known about specific pathogenic mechanisms of the dermatophytes, but they do not cause systemic disease.

Subcutaneous Mycoses

The fungi that have been implicated in the subcutaneous mycoses are abundant in the environment and have a low degree of infectivity. These organisms gain access to the subcutaneous tissues through traumatic implantation. Again, little is known about mechanisms of pathogenesis. Histopathologic evidence indicates that these organisms survive in the subcutaneous tissue layers by producing proteolytic enzymes and maintaining a facultative microaerophilic existence because of the lowered redox potential of the damaged tissue. In eumycotic mycetoma there is extensive tissue damage and production of purulent fluid, which

exudes through numerous intercommunicating sinus tracts. Microabscesses are common in chromoblastomycosis, but the clinical manifestation of disease indicates a vigorous host response to the organism, as seen by the intense tissue reaction that characterizes the disease (pseudoepitheliomatous hyperplasia).

Although most of the fungi implicated in this category of disease exist in a hyphal morphology, the agents of chromoblastomycosis and sporotrichosis are exceptions. Chromoblastomycosis is caused by a group of fungi that have several features in common. They are all darkly pigmented (dematiaceous) and exhibit a pleomorphism consisting of two distinct morphologies: the organism may exist in a mycelial state or as a thick-walled spherical cell that divides by cleavage. The latter cell morphology, called a muriform cell, sclerotic cell or Medlar body, is the pathologic morphology seen in tissue sections. However, transition to the sclerotic morphology may not be a crucial requirement for pathogenesis. Several dematiaceous fungi cause a disease called phaeohyphomycosis, which clinically consists of a broad group of diseases characterized by the presence of various darkly pigmented yeastlike to hyphal elements, but not sclerotic cells, in pathologic specimens. Alternatively, the immune reaction of the host may dictate the morphology that the organism assumes. Again, there is no information about mechanisms or the role of morphogenesis in the pathogenesis of this group of fungi.

Sporotrichosis is caused by *Sporothrix schenckii*, which grows as a mold in nature or when cultured at 25°C, but as yeastlike cells when found in tissues. The clinical manifestations of disease caused by *S schenckii* vary, depending on the immune status of the patient. The classic condition, subcutaneous lymphanigitic sporotrichosis, is characterized by numerous nodules, abscesses, and ulcerative lesions that develop along the lymphatics that drain the primary site of inoculation. The disease does not extend beyond the regional lymph nodes that drain the site of the original infection. Alternatively, infection may result in solitary lesions or pulmonary disease. Clinical manifestations of pulmonary infections vary depending on the immune status of the patient. The immunocompetent individual has a high degree of innate resistance to disease, and when infection occurs the organism is often a secondary colonizer of old infarcted or healed cavities of the lungs. If the patient is immunocompromised, dissemination can occur. There is no information about mechanisms of pathogenesis of this dimorphic fungus.

Systemic Mycoses

Of all the fungi that have been implicated in human disease, only the six agents that cause the systemic mycoses have the innate ability to cause infection and disease in humans and other animals. The primary site of infection is the respiratory tract. Conidia and other infectious particles are inhaled and lodge on the mucous membrane of the respiratory tree or in the alveoli, where they encounter macrophages and are phagocytosed. To successfully colonize the host these organisms must be able to survive at the elevated temperature of the body and either elude phagocytosis, neutralize the hostility they encounter, or adapt in a manner that will allow them to multiply.

Several factors contribute to infection and pathogenesis of these organisms. Of the six systemic agents, five, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, and *Penicillium marneffei* are dimorphic, changing from a mycelial to a unicellular morphology when they invade tissues, except *C immitis* that forms spherules. The change from mycelial to yeast morphology in *H. capsulatum* appears critical for

pathogenicity. Several physiologic changes occur in the fungus during the transition, which is induced by the temperature shift to 37°C. The triggering event is a heat-related insult: the temperature rise causes a partial uncoupling of oxidative phosphorylation and a consequent decline in the cellular ATP level, respiration rate, and concentrations of electron transport components. The cells enter a period of dormancy, during which spontaneous respiration is maintained at a decreased level. Then there is a shift into a recovery phase, during which transformation to yeast morphology is completed. Mycelial cells of *H capsulatum* that are unable to undergo this morphologic transition are avirulent. Similar observations have been made when mycelia of *B dermatitidis* and *P brasiliensis* are shifted from 25°C to 37°C, and it has been implied that transformation to the yeast morphology is critical for infection.

Coccidioides immitis is also dimorphic, but its parasitic phase is a spherule. Little is known about the role of morphologic transformation in infection and disease of this organism. Dimorphism does not appear to play a role in *C neoformans* pathogenesis since the organism is an encapsulated yeast both at 25°C and in host tissues. The sexual phase of *C neoformans*, *Filobasidiella neoformans*, is known, and the organism assumes a filamentous morphology, producing small basidiospores. It has been suggested that these propagules are relevant in infection.

In addition to adjustment to the elevated temperature of the host, the infectious propagules must deal with the hostile cellular environment of the lungs. Studies with mutants of *C neoformans* have shown that the acidic mucopolysaccharide capsule is important in pathogenesis. Acapsular variants of the yeast are either avirulent or markedly deficient in pathogenicity. Since these mutants were obtained by mutagenesis, it is difficult to rule out the contribution of other genetic defects to their decreased pathogenicity. However, at the cellular level, the capsular polysaccharide inhibits phagocytosis of the yeast. Encapsulated *C neoformans* cells are highly resistant to phagocytosis by human neutrophils, whereas acapsular variants are effectively phagocytosed. The active component of the capsular polysaccharide has been identified as glucoronoxylomannan. In addition, the capsular polysaccharide is poorly immunogenic in humans and laboratory animals, and the glucoronoxylomannan component persists for extended periods in the host.

In addition to the capsular polysaccharide, elaboration of phenyl oxidase (an enzyme that catalyzes the oxidation of various phenols to dopachrome) by *C neoformans* appears to be a determinant of virulence, although the role of this enzyme in virulence is unknown. The infectious propagules of *H capsulatum*, *B dermatitidis*, *P brasiliensis*, and *C immitis* are readily phagocytosed by alveolar macrophages. To survive phagocytosis and to multiply, these fungi must neutralize the effects of the phagocytes. The production of reactive oxygen metabolites by phagocytic cells is an important host defense against microorganisms. Studies have shown that the yeast phase of *H capsulatum* fails to trigger release of reaction oxygen metabolites in unprimed murine macrophages despite extensive phagocytosis. How they avoid destruction by the fungicidal mechanisms within lysosomes is unclear. Arthroconidia of *C immitis* inhibit phagosome-lysosome fusion and survive within normal murine peritoneal macrophages. Phagosome-lysosome fusion takes place after *H capsulatum* infection, but the yeast cells survive in the phagolysosome. It has been speculated that the fungus neutralizes the fungicidal components of the lysosome by a mechanism not yet elucidated.

There is very little information about mechanisms of fungal pathogenicity, in contrast to what is known about molecular mechanisms of bacterial pathogenesis. Fungal pathogenesis is complex and involves the interplay of many factors. Studies to elucidate these mechanisms are needed because of the increasing incidence of opportunistic infections.

Isolation and identification of fungi

Method of Fungi isolation from soil

- **Procedure:**
  - Sterile slide—> add the molten agar and allow to solidify —> cut the material making two half —-> place cover slip ——> seal the coverslip with wax or petroleum jelly making small area free at the side if cut —-> buried in a soil gently in a tray à allowed to incubate for few days ——-> remove gently ——> remove coverslip and observe under microscope.
- 1. **Bait method:**
  - Many molds have quite specific nutrient requirements and are specialized to use materials that other fungi use with difficulty or not at all.
  - We can isolate fungi by presenting a particular substance to the environment for colonization then later recovering it for isolation of the fungi that occupied it.
  - Different types of baits might be pieces of wood, insects, carrot chunks, plastics, hair etc.
  - The bait can be submerged in a specific habitat in nature or in a moist chamber.
  - E.g. To isolate dermatophytes, we can place a hair on moist soil in a most chamber and examine it periodically for sporulating molds.
  - Many cellulolytic fungi can be isolated from cellulose containing material.
  - Then the mycelium is transferred into medium like PDA, SDA, Czapels agar for cultivation.
- **Soil dilution plating:**
  - If hyphae cannot be dissected from field material for identification, they must be induced to grow cut as visible colonies onto an artificial culture medium.
  - The dilution plate method, in which a dilution series is prepared from a soil suspension and the selected dilution incorporated in an agar medium (PDA-SDA).
  - After few days of incubation, colonies will appear in varying densities and the number of spores present in the original sample can be calculated.

II. Method of isolation of fungi from Organic components:

- Fungi can also be isolated from rotten fruits, from roots etc.
- Surface sterilized —-> crushed in distilled water ——> inoculate in suitable agar medium.

III. Method of Fungi isolation from clinical specimens: Isolation of pathogenic Fungi

- Fungal cultures are microbiology laboratory tests to detect or role out the presence of fungi in specimens taken from patients or animals.
- The laboratory employs optimal conditions to grow and identify any fungus present in the specimen.
- The specimen is cultured on various agar media and then incubated and examined.
- Specimen could be the skin scrapping, nail scrapping, infected hair etc.
- **Plate exposure method:**
  - Useful method for airborne fungi.
  - Certain molds are likely to get their spores into the air and these spores may be serve as an infective agent of plant diseases and same allergies.
  - These can be isolated by —> plate containing PDA is exposed to air for few minutes —-> covered à incubate at 25ºC or room temperature for few days —-> observe.
- **Imprint method:**
  - Fungi present on leaf surface or root of the plant can be isolated by pressing leaf or root on a suitable culture media and then incubate at 28ºC or at room temperature.
- **Other methods:**
  - Direct transfer, moist chamber, direct soil plate method etc.

Identification of fungi:

- **<u>Criteria for identification of Fungi</u>**

Methods of Identification of Fungi

1. Wet mount (tease mount) method for fungal hyphae identification:

- **Procedure of wet mount preparation:**
  - Take a grease free slide and plate with fungus culture.
  - With the help of sterile scalpel or 90º bent wire, remove fungal colonies from plate (which might contain a small amount of supporting agar.
  - Place the portion of culture into a slide to which has been added to a drop of lactophenol cotton blue or aniline blue.
  - Place the coverslip into position and apply the gentle pressure to dispose the general growth and agar.
  - Examine microscopically.
- **Drawbacks:**
  - Characteristic arrangement of spores might be disrupted when pressure is applied to the coverslip.

2. Adhesive (scotch) tape preparation for fungal spore identification:

- **Procedure of scotch tape preparation:**
  - Touch the adhesive side of a cellophane tape to the surface of the colony.

- Adhere the tape to the surface of a microscopic slide to which has been added a drop of lactophenol cotton blue or aniline blue.
- For the characteristic shape and arrangement of the spores, microscopical examination is required.
- This method allows to observer in a way it sporulates in culture and easy method to identify organism.
- In instances where spores are not observed, a wet mount should be made as a backup step.

3. Microslide culture technique for fungi identification :

- **Procedure of microslide culture technique**:
  - Take sterile petri dish—-> remove its lid —–> place sterile filter paper over it and add distilled water to moisten the filter paper —–> put glass slide over glass rod ——> add $5mm^2$ PDA agar on glass slide from agar plate ——> inoculate spores of fungi on that agar and cover with coverslip —–> incubate at room temperature for 7 days ——> remove coverslip and mounted in next slide and observe under microscope.

4. Coverslip culture technique for fungi identification :

- This technique is simple, less time-consuming technique which produces high quality permanent mounts and is suitable for clinical isolate identification, student teaching, examination of fungi at different stages of their development without disturbing the arrangement of spores and hyphal structure.
- It is advantageous over slide culture technique that if the first preparation fails to demonstrate adequate sporulation, there are still left to be examined of weekly intervals.
- The ability of aerial mycelia to adhere to a glass surface has been utilized as a basis of this technique.
- **Procedure of coverslip culture method:**
  - Insert 4-5 coverslips on petri-plate containing POA at an angle of 45^o —–> inoculate organism at the point of media and coverslip ——> incubate the plate at room temperature for 7 days ——> after incubation remove the coverslip gently and mounted with lactophenol cotton blue and observe under microscope.

Lactophenol Cotton Blue (LPCB) Staining

Lactophenol Cotton Blue (LPCB) Staining is a simple histological staining method used for the microscopic examination and identification of fungi.

Principle of Lactophenol Cotton Blue (LPCB) Staining

Lactophenol Cotton Blue (LPCB) Staining method works on the principle of aiding the identification of the fungal cell walls.

- Fungi are eukaryotic organisms with both macroscopic and microscopic characteristics.

- The fungal spore cell wall is made up of chitin of which the components of the Lactophenol Cotton Blue solution stains for identification.
- The lactophenol cotton blue solution acts as a mounting solution as well as a staining agent.
- The solution is clear and blue in color and it is made up of a combination of three main reagents:
  - **Phenol**: It acts as a disinfectant by killing any living organisms
  - **Lactic acid**: To preserve the fungal structures
  - **Cotton blue**: To stain or give color to the chitin on the fungal cell wall and other fungal structures
- The stain will give the fungi a blue-colored appearance of the fungal spores and structures, such as hyphae.

Reagents of Lactophenol Cotton Blue (LPCB) Staining

A preparation of 50ml Lactophenol cotton Blue staining solution is made up of:

- Distilled water 50ml
- Cotton Blue (Aniline Blue) 0.125g
- Phenol Crystals ($C_6H_5O_4$) 50g
- Glycerol 100ml
- Lactic acid ($CH_3CHOH\ COOH$) 50ml
- 70% ethanol

Note: Lactophenol Cotton Blue solution is prepared at least 2 days before use.

Preparation of Lactophenol Cotton Blue solution

Lactophenol Cotton Blue solution is prepared for over two days leaving the reagents undisturbed to allow dissolving and maturation.

1. Day 1: Dissolve the cotton blue in distilled water and leave to rest overnight. This eliminates insoluble dye.
2. Day2: Using protective gloves, add phenol crystals to lactic acid in a glass beaker and stir using a magnetic stirrer until the crystals dissolve.
3. Add glycerol
4. Filter the Cotton blue and the distilled water into the phenol + glycerol +lactic acid solution and mix.
5. Store at room temperature.

Procedure of Lactophenol Cotton Blue (LPCB) Staining

1. On a clean microscopic glass slide, add a drop of 70% ethanol
2. Add the fungal specimen to the drop of alcohol using a sterile mounter such as an inoculation loop (from solid medium), depending on the sample of use.
3. Tease the fungal sample of the alcohol using a needle mounter, to ensure the sample mixes well with the alcohol.
4. Using a dropper or pipette, add one or two drops of Lactophenol Cotton Blue Solution (prepared above) before the ethanol dries off.
5. Carefully cover the stain with a clean sterile coverslip without making air bubbles to the stain.

6. Examine the stain microscopically at 40X, to observe for fungal spores and other fungal structures.

Results and Interpretation

Fungal spores, hyphae, and fruiting structures stain blue while the background stains pale blue. For example,

- *Aspergillus niger* stains the hyphae and fruiting structures a delicate blue with a pale blue background.
- *Trichophyton mentagrophytes* also stains the hyphae and fruiting structures a delicate blue with a pale blue background.

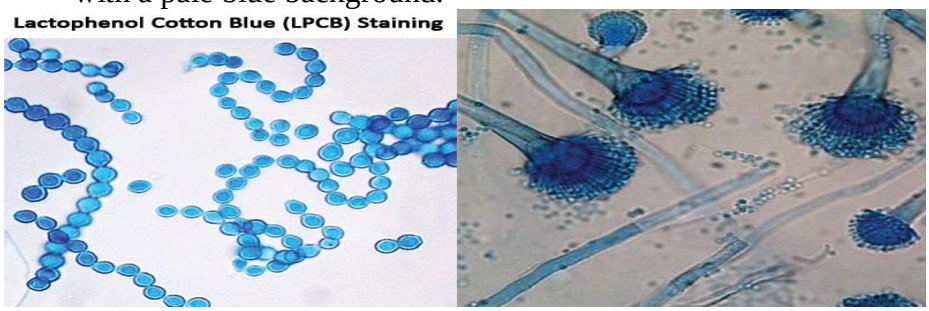


Figure: First: *Scopulariopsis* species on a lactophenol cotton blue stain. Image Source: stylish_streaking and Senthil Prabhu.

Limitations

- It can only be used as a presumptive identification method of fungi which should be followed up with other diagnostic tools such as biochemical and cultural examination.
- The components of the solution should be used before expiry, including the use of the solution before it expires.
- The solution may disrupt the original morphology of the fungi.
- The stain can only be used to identify mature fungi and its structures and not the young vegetative forms of fungi.
- The stain can not be stored for a long period of time.

Applications

- Used in the identification of suspected fungal samples.
- General identification of fungi and its structures.

KOH Preparation Test: Principle, Procedure, Results and Uses

Potassium hydroxide (KOH) preparation is used for the rapid detection of fungal elements in clinical specimen, as it clears the specimen making fungal elements more visible during direct microscopic examination.

KOH is a strong alkali. When specimen such as skin, hair, nails or sputum is mixed with 20% w/v KOH *(preparation of KOH is posted at the end of this post)*, it softens, digests and clears the tissues (e.g., keratin present in skins) surrounding the fungi so that the hyphae and conidia (spores) of fungi can be seen under microscope.

Uses:

In diagnostic laboratories, KOH mount is one of the main methods of investigating fungal infections. It is used as a primary screening tool, it detects fungal elements present but may not necessarily identify the species of the fungi. (**Note:** To identify the fungal isolate, specimen must be cultured in either general purpose fungal culture media such as **Sabouraud Dextrose Agar (SDA)** or specific media based on the type of anticipated isolate.)

KOH preparation is recommended in the following suspected conditions (this is not the exclusive lists);

Suspected conditions	**Specimen**	**Diagnostic characteristics**
Aspergillus infection	Sputum	Septate hyphae with V-shaped branching
Dermatophytes (ringworm fungi)	skin scrapings, nails or hair	Septate hyphae or spherical yeast cells depending on the nature of etiologic agents involved.
Blastomyces dermatitidis infection	Pus, sputum or skin specimens	Yeast cells (large budding yeast cells with distinct broad base) of *Blastomyces dermatitidis.* *B.dermatitidis* is a **dimorphic fungus** with yeast cells in tissue.
Mucormycosis	Exudates from infected lesions or tissue	Aseptate hyphae of fungi causing mucormycosis.

Procedure of KOH Preparation

1. Place a drop of KOH solution on a slide.
2. Transfer the specimen (small pieces) to the drop of KOH, and cover with glass. Place the slide in a petri dish, or other container with a lid, together with a damp piece of filter paper or cotton wool to prevent the preparation from drying out. **Note:** To assist clearing, hairs should not be more than 5 mm long, and skin scales, crusts and nail snips should not be more than 2 mm across.

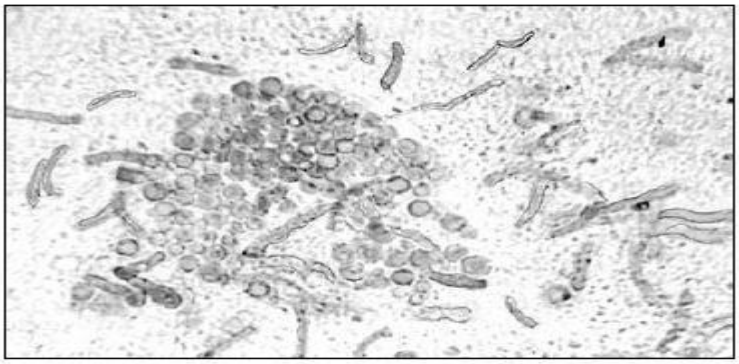


Image 2: *Malassezia furfur* yeast cells and hyphae in KOH blue–black ink preparation.

As soon as the specimen has cleared, examine it microscopically using the 10X and 40X objectives with the condenser iris diaphragm closed sufficiently to give a good contrast. If too intense a light source is used the contrast will not be adequate and the unstained fungi will not be seen.

Disadvantages of KOH preparation method

- Experience required since background artifacts are often confusing.
- Clearing of some specimens may require an extended time

Procedure to make 100 ml of KOH 20% w/v solution:

1. Weigh 20 g potassium hydroxide (KOH) pellets.
2. Transfer the chemical to a screw-cap bottle.
3. Add 50 ml distilled water, and mix until the chemical is completely dissolved, add remaining distilled water and make the volume 100 ml.
4. Label the bottle and **mark it corrosive**. Store it at room temperature. The reagent is stable for up to 2 years.
5.

Caution: Potassium hydroxide is a **highly corrosive** deliquescent chemical, therefore **handle it with great care** and make sure the stock bottle of chemical is **tightly stoppered** after use.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC. MICROBIOLOGY

UNIT – II – Medical Mycology – SMB3103

Candida

Candidiasis is also known as **moniliasis,** is a fungal disease that is classified under opportunistic fungal infection, because of its occurrence in persons with an immune-suppressed system, especially newborns, HIV/AIDS patients, patients on antibiotic therapies, cancer therapy patients.

- It usually does not cause disease in healthy individuals.
- It is an overgrowth in the gut by *Candida albicans* species of yeast-like fungi.
- The yeast-like fungi mutate into a fungal form, proliferating and invading the gastrointestinal tract and its walls, to cause candidiasis.

Causative agent of Candidiasis

- It is caused by a yeast-like fungus, known as Candida, and most commonly by a specific species known as *Candida albicans.*
- *Candida albican*s are normal gut inhabitants living in various sites of the body including the gastrointestinal tract, respiratory tract, vagina, and mouth.
- They are dimorphic fungi, existing in both mold and yeast forms.
- They are strictly aerobic, utilizing oxygen in its metabolisms and growth, therefore it is highly adaptive to the human mucous membranes.
- Their growth is naturally suppressed by other normal microbiota but when these microbiotas are disrupted, Candida multiplies rapidly, producing candidiasis disease.
- They have also been found to commonly live in hospitals, causing nosocomial blood infections in hospitalized patients.

Transmission of *Candida albicans*

- Besides being a normal flora, it is a transmissible fungal pathogen.
- Some modes of transmission include:
  - Mother-to-infant transmission through childbirth, remaining as normal microflora, unless there is an overgrowth which can result in symptomatic disease, hence candidiasis.
  - Child-to-mother transmission during breastfeeding, whereby the candida on the child's tongue/mouth binds to the breast nipples on the mother during breastfeeding.
  - Sexual transmission, this form is rare but it can occur and it has been listed by CDC as a sexually transmissible disease.
  - Nosocomial transmission whereby immunocompromised patients acquire the yeast form healthcare workers, from invasive devices such as catheters and respirators. They account for 10% of nosocomial bloodstream infections.

Pathogenesis and pathology of Candidiasis

Candida albicans colonization of the host is based on the virulence factors it elicits making it adherent to the host organ systems.
These virulence factors include:

- The dimorphic nature of *Candida albicans*. It exists as both mold and/or yeast.
- Presence of adhesins and invasins, Als-3 proteins that enable it to attach and invade the surfaces.
- They also form biofilms during adherence which enables it to survive and thrive on the host.

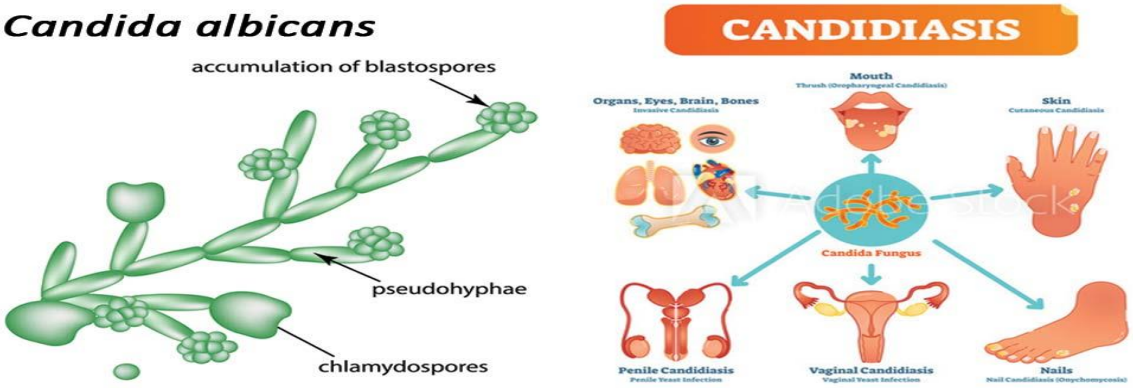
- They also secrete proteases, phospholipases, and lipases which allows it to penetrate into the host cells.

Based on these virulence factors and the site of colonization of the fungal agent, candida infections (candidiasis) can be classified as follows:

- **Mucosal Candidiasis** affects the mucosal lining of the host such as oral candidiasis, candidal vulvovaginitis, gastrointestinal candidiasis, and respiratory candidiasis.
- **Cutaneous candidiasis** affects the skin, skin pores and nails, for example, candidal folliculitis, candidid, chronic mucocutaneous candidiasis, congenital cutaneous candidiasis, diaper candidiasis, candidal onychomycosis
- **Systemic candidiasis** affects the deep-seated organs and the bloodstream, for example, candidemia, a form of fungemia that causes sepsis, invasive candidiasis, chronic systemic candidiasis (hepatosplenic candidiasis).
- **Antibiotic candidiasis** is also known as iatrogenic candidiasis.

Disease cycle

- On the fungal colonization on the epithelial lining of the host, the fungi acquire nutrients from the lining and its cells, providing a suitable platform for growth.
- They cause superficial infection by degrading the surface proteins and if there is no prompt treatment, the infection persists into the tissues.
- Invasion of the tissues can affect the vascular system, through the spread and escaping immune interventions.
- The disease then disseminates into the endothelial tissues of various organs causing disseminated disease.



Clinical Manifestations of Candidiasis

- Candidiasis infection affects the cutaneous layer and the mucous membrane, which provides suitable conditions for growth. These manifestations affect the mouth, skin, vagina, and intestines.
- The most common types of candidiasis include:

Cutaneous and Mucosal candidiasis

- This infection is most common among persons who are immune-compromised including AIDS patients, pregnant mothers, diabetes, infants and children, women on birth control pills, and trauma patients (burns, maceration of the skin).

Oral candidiasis is also known as **thrush or oropharyngeal candidiasis** is the most common cutaneous and mucous candidiasis affecting the mouth cavity.

- It majorly affects newborns, who acquire it from the mother during childbirth, whose vagina is infected with Candida albicans.
- The fungi colonize the upper respiratory tract of the newborn during passage through the birth canal.
- Normally, when newborns are born, they do not harbor any microbiota, which allows *Candida albicans* growth.
- This enables the fungi to thrive and grow without competition or inhibition.
- Thrush occurs on the tongue, lips, gums, or the palates causing patchy to confluent, whitish pseudomembranous lesions made up of epithelial cells, yeast, and pseudohyphae.
- Other risks of developing Oral thrush include treatment with corticosteroids and antibiotics, high glucose levels, and cellular immunodeficiency.
- Another common cutaneous and mucosal is **candidal vulvovaginitis.**
  - This is a yeast invasion of the vaginal mucosa characterized by irritation, pruritus, and vaginal discharge.
  - Those at-risk are pregnant women, diabetic patients, and persons taking antibacterial drugs.
  - These factors alter the normal microflora, local acidity, and secretions
    -
    - Other forms of cutaneous and mucosal candidiasis include skin invasions that occur due to trauma such as burns or surgical wounds.
    - **Intertriginous infection** occurs in moist, warm parts of the body such as the axillae, groin, and intergluteal or inframammary folds; it is most common in obese and diabetic individuals forming red and moist vesicles.
    - **Onychomycosis** is a candidal invasion of the nails and the nail plates causing painful erythematous swelling on the nail folds which resemble pyogenic paronychia, which destroys the nails,

Systemic Candidiasis

- This is also known as candidemia.
- Those most at risk are patients which invasive devices such as catheters, surgery patients, intravenous drug invasives, invasive drug abusers, aspirator patients, skin abrasions such as wounds, and gastrointestinal tract patients.
- Patients with an immune-compromised system especially the phagocytic defenses, develop occult lesions in the kidney, skin (macronodular lesions), eyes, heart, and meninges.
- Systemic candidemia is associated with chronic administration of corticosteroids or immune-suppressive agents, especially in persons with leukemia, lymphoma, and aplastic anemia.
- Candidal endocarditis can occur when there are deposition and growth of yeast and pseudohyphae on the heart valves.
- Systemic kidney infection can also occur associated with urinary tract infection in persons with foley catheters, diabetes, pregnancy, and antibacterial antibiotics

Chronic Mucocutaneous Candidiasis

- It is a rare form of candida infection that normally starts in early childhood. It is associated with cellular immunodeficiencies and endocrinopathies causing chronic superficial disfiguring infection affecting any areas of skin or the mucosa.
- Chronic mucocutaneous candidiasis affects effective Th17 immune responses.

Laboratory Diagnosis of Candidiasis

Specimen and specimen preparation

- Oral swabs and scrapings from lesions, vaginal swabs, blood, spinal fluid, tissue biopsies, urine, exudate, intravenous catheter materials.
- Spinal fluids are prepared by centrifugation.

Microscopic Examination

- Using tissue biopsies, centrifuged spinal fluid, tongue and mouth swabs, and scrapings, examine the specimens by Gram staining for identification of pseudohyphae and budding cells
- Skin or nail scrapings can be analyzed using KOH wet mount and calcofluor white dye for observation of the pseudohyphae and formation of a germ tube.

Cultural examination

- The specimens can be examined by culture methods using fungal media and/or bacterial media at room temperature or at 37°C.
- The colonies are examined for the formation of pseudohyphae or chlamydospores.
- A 10% KOH wet mount after culturing is used for the identification of pseudohyphae and the germ tube formed by *Candida albicans.*
- Candidemia is primarily diagnosed through blood cultures.

Treatment of Candidiasis

- Candidiasis is treated with antifungal drugs such as clotrimazole, nystatin, fluconazole, voriconazole, amphotericin B, and echinocandins. Fluconazole and echinocandins are administered intravenously. Caspofungin is used for the treatment of immunocompromised individuals.
- Localized infections such as mouth and throat candidiasis can be treated with topical medications and oral antifungals.
- Candidal skin infections are treated with topical antifungal treatment such as nystatin and miconazole.
- Gentian violet is used to treat oral thrush in breastfeeding babies and topical miconazole for breastfeeding mothers with breast candidal infection.
- Esophagitis candida infection can be treated with oral or intravenous amphotericin B.
- Vaginal candidiasis is treated with topical antifungal agents majorly fluconazole, imidazole or triazole some using vaginal suppositories or medical douches. This can also be done during pregnancy.
- Candidemia can be treated orally or intravenously using fluconazole, echinocandin like caspofungin, and also amphotericin B can be used.

Prevention and control

- Maintain a healthy lifestyle to reduce the overgrowth of *Candida albicans.*
- Good hygienic practices such as brushing teeth
- Proper nutrition
- Careful use of antibiotics

Cryptococcus spp

- *Cryptococcus* spp is a fungal group that belongs to the Phylum Basidiomycota (Basidiomycetes).
- They undergo sexual reproduction forming dikaryotic hyphae and basidiospores supported by a club-shaped basidium with hyphae that has a complex septate.
- There are four known species of Cryptococcus:
  - *Cryptococcus neorformans*
  - *Cryptococcus gattii*
  - *Cryptococcus albidus*
  - *Cryptococcus laurentii*
  - *Cryptococcus inuguttulatus*
- *C. albidus and C.laurentii* are the most common species and *C.neorformans* is the most pathogenic of these species, for both humans and animals, while *C. laurentii and C.gittii* are mildly pathogenic.
- *Cryptococcus neorformans* distinguishes from other fungal yeasts by the presence of a polysaccharide capsule, the formation of melanin, and urease activity, which all function as virulence determinants.
- Its human pathogenicity is via inhalation of desiccated yeast cells or small basidiospores. The yeast then migrates to the central nervous system causing meningoencephalitis.
- This infection is otherwise known as cryptococcosis, which is a systemic infection primarily involving the lungs and central nervous system.
- *Cryptococcus neoformans* affect immunocompetent persons but more often in patients with HIV/AIDS, tuberculosis, hematogenous malignancies, and hospitalized patients majorly those with invasive devices.

Habitat of *Cryptococcus neoformans*

- *Cryptococcus neoformans* are commonly found in the environment growing as unicellular yeast and they reproduce by budding.
- In the natural environment, it exists as a saprophytic forming a large budding yeast morphology.
- It is an encapsulated yeast-like fungus that is isolated from dried avian (particularly pigeon) and bat excreta, and in dust contaminated with such droppings.

Morphology of *Cryptococcus neoformans*

- *Cryptococcus neoformans* is a yeast fungus, producing yeast cells during reproduction.
- The yeast cells are dry, mildly encapsulated, and light, making them easy to aerosolize.
- In culture, they produce whitish mucoid colonies which are spherically budding cells 5–10 μm in diameter and are surrounded by a thick nonstaining capsule.

Cultural characteristics of C*ryptococcus neoformans*

- In culture, they produce whitish mucoid colonies within 2-3 days.
- They have spherically budded yeast cells of 5–10 μm in diameter with a thick nonstaining capsule surrounding it.
- They lack hyphae.
- It has the ability to grow at 37°C, producing laccase, which is a phenol oxidase that catalyzes the formation of melanin from phenolic substrates such as catecholamine, which can be detected by biochemical characterization.

Life Cycle of *Cryptococcus neoformans*

- *Cryptococcus neoformans* is a facultative intracellular pathogen, which uses human phagocytes to spread within the body.
- The fungus has the ability to colonize phagocytic cells and undergoes vomocytosis, hence it escapes phagocytosis without processing.
- The yeast-like fungus gains entry into the human host via inhalation of aerosolized basidiospores.
- They then disseminate into the Central nervous system where it causes meningoencephalitis.
- When it gains entry into the lungs, they get phagocytosed by the alveolar macrophages. the macrophages produce oxidative and nitrosative agents which normally kill microorganisms but *Cryptococcus neoformans* have the ability to escape killing by Oxidative reaction, by upregulation of expressing genes of oxidative stress.
- They then remain latent in the macrophages which have been associated with disease dissemination and resistance to antifungal drugs.

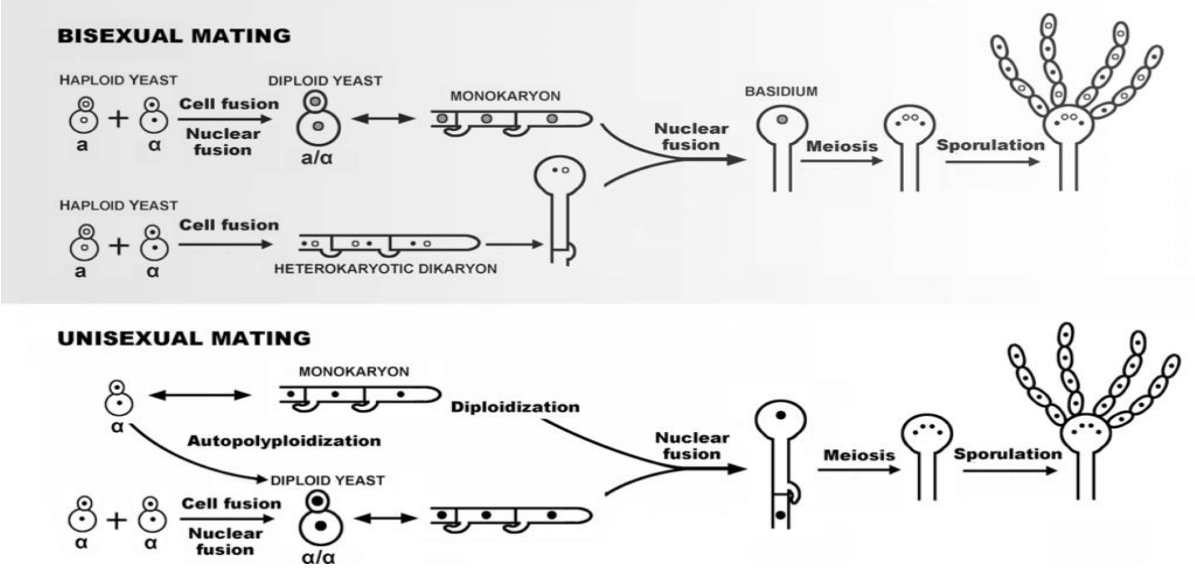


Figure: The life cycle of Cryptococcus neoformans. The upper panel depicts the traditional bisexual mating involving partners of opposite mating types a and α, and the lower panel depicts unisexual mating that occurs between partners of the same mating-type or with itself. Image Source: Elsevier Inc.

They can also undergo sexual reproduction of meiosis by the alpha types of the fungi.

- The filaments of the mating types alpha have a haploid nucleus, which undergoes diploidization forming diploid cells called blastospores. Blastospores have the ability to undergo meiosis to form haploid basidiospores that are easily dispersed. This mechanism is known as monokaryotic fruiting.
- During this process of meiotic reproduction, they can also promote DNA repair from damages of oxidative and nitrosative stress. This produces the monokaryotic fruit which contributes to the fungal virulence.

Pathogenesis and Clinical Features of *Cryptococcus neoformans* Infection

Virulence Factors

- Their virulence is enhanced by the production of laccase and capsule, and the presence of the capsular polysaccharide.
- The capsular polysaccharides are long, unbranched polymers consisting of an α-1,3-linked polymannose backbone with β-linked monomeric branches of xylose and glucuronic acid.
- On infection, the capsular polysaccharide solubilizes in spinal fluid, serum, or urine, increasing the yeast's virulence of infection and disease spread.

Infection

- The infection of *Cryptococcus neoformans* is initiated by the inhalation of yeast cells, which are dry and lightweight making them easy to aerosolize.
- The fungus gains entry into the body through the respiratory tract system, causing a minimal pulmonary infection that is transitory.
- The primary pulmonary infection can be asymptomatic, mimicking influenza infection and it resolves spontaneously.
- For immunocompromised patients, the yeast replicates and spreads to other parts of the body and majorly to the Central Nervous system.
- Affecting about 15% of HIV/AIDS patients, it starts by causing mild infections of the pulmonary system, which spreads to the skin, bones, viscera, and the central nervous system.
- When the CNS is affected, it eventually causes cryptococcal meningitis.
- Other common sites of dissemination include the skin, adrenals, bone, eye, and prostate gland.
- There is a minimal inflammation-causing granulomatous reaction.
- Patients who get cryptococcal meningitis and are under Highly Active Antiretroviral Therapy (HAART) are likely to develop immune reconstitution inflammatory syndrome (IRIS), which greatly exacerbates the illness. This is also common in AIDS patients with Tuberculosis.

Clinical features of *Cryptococcus neoformans* infection

- *C. neoformans* causes a fungal infection commonly known as cryptococcosis.
- This is an infection that majorly affects persons with an immune-deficient system and majorly AIDS patients are associated with symptoms such as:
  -
  - Flu-like symptoms
  - Chronic meningitis resembling brain tumor, brain abscess, degenerative central nervous system
  - Mycobacterial mimicked disease
  - Fungal meningitis
  - Patients with meningitis symptoms have complications of severe headaches, neck stiffness, and disorientation
  - They may also have lesions in skin, lungs, bone weakness with muscle dystrophy and inability to move.
  - Untreated cases may end in fatality.

Laboratory Diagnosis of *Cryptococcus neoformans*

Specimen and specimen preparation

- Cerebrospinal fluid, tissue, exudates, sputum, blood, cutaneous scrapings, and urine.
- Spinal fluid is centrifuged before microscopic examination and culture.

Staining and Microscopic examination:

Gram staining

- The cells of *Cryptococcus neoformans* may appear round with Gram-positive granular inclusion on a pale lavender cytoplasmic background or as Gram-negative lipoid bodies.

Negative staining

- Using India Ink for visualizing the cerebral spinal fluid, indicating that particles of ink pigments can not enter the cell capsule surrounding the yeast cells. This forms a zone of clearance known as a halo, around the cells. This is a quick method for the identification of *Cryptococcus neoformans.*

Mucicarmine stain

- This stain is specific for the identification of polysaccharide cell walls of *Cryptococcus neoformans* in tissue samples.

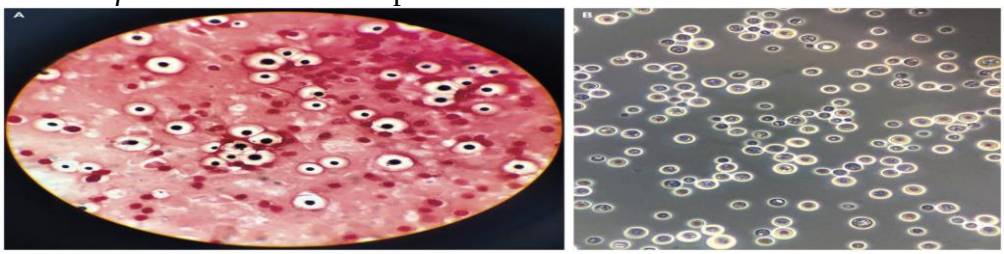


Figure: Gram's stain (Panel A) and India ink stain (Panel B) revealed abundant encapsulated, round yeasts, with some budding forms. Image Source: Massachusetts Medical Society (NEJM).

Cultural examination

- Culture examination uses Sabouraud Dextrose Agar (SDA).
- The yeast develops colonies within a few days on most cultural media at room temperature or 37°C.
- Media with cycloheximide inhibit Cryptococcus and should be avoided.
- Detection of urease production in the culture media.
- Alternatively, on an appropriate diphenolic substrate, the phenoloxidase (or laccase) of *C. neoformans* produces melanin in the cell walls, and colonies develop a brown pigmentation.

Biochemical characterization

- Demonstrating the production of urease and laccase or a specific pattern of carbohydrate assimilations immediately after the cultural examination

Serological Examination

Antigen detection

- Detection of the capsular polysaccharide antigen of *Cryptococcus* is the idlest diagnostic test by using spinal fluid or serum or urine by the application of enzyme immunoassays or agglutination of latex particles coated with antibodies to the polysaccharide antigen.
- This test is sensitive and specific for antigen detection in cases of cryptococcal meningitis.

Antibody Quantification

- Antibodies produced against the polysaccharide capsule antigen can be quantified

Treatment of Cryptococcosis

- Infection that does not spread to the CNS can be treated with fluconazole.
- Cryptococcal meningitis is treated by combination therapy which is the standard treatment. Administration of intravenous administration of amphotericin B and oral fluconazole for

two weeks. This is then followed with oral fluconazole for 10 weeks and a lower dose a year to improve the patients' CD4 count.

- For patients who develop side effects to amphoterin B, intravenous ambisome can be used.

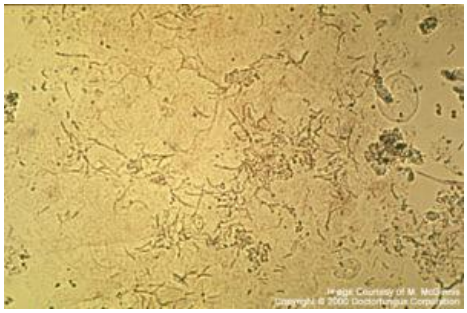
Prevention and Control

- Immunosuppressed people should avoid contact with birds and avoid digging and dusty activities in areas heavily contaminated with bird droppings.
- Avoiding areas with dried pigeon feces.
- Wearing masks may help to prevent the inhalation of *Cryptococcus neoformans*.

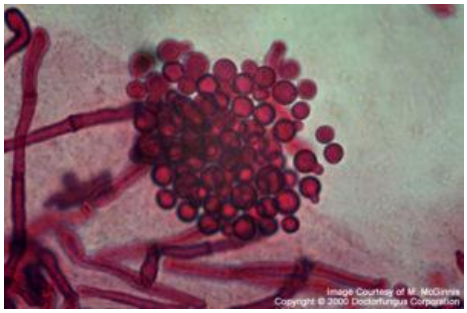
Malassezia

Taxonomic Classification

Kingdom: **Fungi**
Phylum: **Basidiomycota**
Class: **Hymenomycetes**
Order: **Tremellales**
Family: **Filobasidiaceae**
Genus: ***Malassezia***



Malassezia furfur Yeast in skin



Malassezia furfur Cluster of yeast cells

Description and Natural Habitats

Malassezia is a lipophilic yeast found on skin and body surfaces of humans and animals. It has been shown that colonization with *Malassezia* may occur as early as neonatal period [790]. It is a member of the normal skin flora in as much as 90{64e6c1a1710838655cc965f0e1ea13052e867597ac43370498029d1bc5831201} of adults and may occasionally cause superficial and deep mycoses. *Malassezia* has no known teleomorphic phase.

Species

There are seven proposed species in the genus *Malassezia* based on molecular, morphological, and biochemical profiles [276, 924, 948, 1361, 2077, 2395]. The most common and well-known species are *Malassezia furfur* and *Malassezia pachydermatis* [1216]. See the complete list of active species and summary of synonyms for the Malassezia spp.

Pathogenicity and Clinical Significance

Malassezia infections are mostly endogenous and originate from the colonized skin [240]. They may occur in otherwise healthy individuals as well as immunocompromised hosts, such as bone marrow transplant recipients, patients with cancer or AIDS [55, 755, 908, 1251, 1464, 1582].

The most common clinical picture caused by *Malassezia furfur* is pityriasis versicolor [947]. It may also cause seborrheic dermatitis [55], folliculitis [1019], neonatal pustulosis [1874], blepharitis [2250], and white piedra [1364]. Given the lipophilic nature of the fungus, fungemia, catheter-related infections and sepsis due to *Malassezia furfur* may occur particularly in patients who are on parenteral nutrition with lipids [129, 146, 176, 510, 1603, 2050]. Noteworthy, colonization of the catheters with *Malassezia* may occur in absence of lipid administration as well [298]. *Malassezia globosa* and *Malassezia sympodialis* are also common causes of pityriasis versicolor in humans [947, 1684].

Malassezia pachydermatis is a distinctive species due to its well-known zoophilic nature [417, 1859]. It causes canine otitis externa and is prevalent in carnivores. However, according to current knowledge, *Malassezia pachydermatis* is not the only *Malassezia* species associated with infections or colonization in animals [1859]. Some lipid-dependent species of *Malassezia* may also be isolated as occasional causes of canine otitis externa [481]. *Malassezia pachydermatis* may cause disseminated infections in humans as well [1294, 1533].

Macroscopic Features

Malassezia colonies grow rapidly and mature in 5 days at 30-37°C. Growth is weak when it is incubated at 25°C. The colonies are raised and smooth initially and get dry and wrinkled in time. The color of the *Malassezia furfur* colonies is creamy yellow to brown while those of *Malassezia pachydermatis* are cream-colored initially and become buff to orange beige in time [531, 1295, 2202].

One of the most important differences between *Malassezia furfur* and *Malassezia pachydermatis* is their relative lipid dependence. *Malassezia furfur* requires long chain fatty acids for growth. The most practical and commonly applied method to supplement the medium with these fatty acids is to overlay the medium with a thin layer of olive oil. Unlike *Malassezia furfur*, *Malassezia pachydermatis* does not require fatty acids.

Microscopic Features

Yeast-like conidia are the predominant structures. These cells are globose to ellipsoidal in shape and round at one end and blunt at the other. The blunt site is where bottle-shaped, unipolar budding occurs from the phialides with small collarettes. The width of the bud with respect to

that of the mother cell may vary from one species to other. Occasionally, few rudimentary hyphal elements are also observed. Sexual spores do not exist [531, 1295, 2202].

Direct microscopic examination of the skin scrapings reveals yeast cells and hyphal fragments. This appearence resembles to and is called spaghetti and meatballs [2202].

Laboratory Precautions

No special precautions other than general laboratory precautions are required.

Susceptibility

Very limited data are available. The results of the in vitro susceptibility studies done so far showed that amorolfine, bifonazole, itraconazole, and terbinafine MICs varied considerably among isolates of *Malassezia furfur*. The MICs obtained for other less commonly encountered species were also variable [1047, 1615, 1767, 1887]. In another comparative study, ketoconazole appeared to be more active than econazole and miconazole against *Malassezia* isolates. Of note, *Malassezia furfur* was the least susceptible *Malassezia* species to ketoconazole, econazole, miconazole, and tea tree oil [977]. For MICs of various antifungal drugs for *Malassezia*, see our N/A(L):susceptibility database.

Oral ketoconazole or itraconazole are commonly used for treatment of pityriasis versicolor [2199]. Antifungal therapy and removal of the catheter are the guidelines for treatment of catheter-related infections. Amphotericin B therapy may be used in these patients with catheter infections [2050].

Trichosporon

Trichosporon species are urease-positive, non-encapsulated basidiomycetous yeasts characterised by the development of hyaline, septate hyphae that fragment into oval or rectangular arthroconidia. Some blastoconidia are also seen. The colonies are usually raised and have a waxy appearance, which develop radial furrows and irregular folds. They are widely distributed in the environment and many have different habitats, usually occupying narrow ecological niches. Some are soil borne and others are associated with humans and animals (Colombo *et al.* 2011, Sugita 2011, Arendrup *et al.* 2014).

The genus has undergone major taxonomic revision (Gueho *et al.* 1992, de Hoog *et al.* 2000, Rodriguez-Tudela *et al.* 2005). In particular, the name *Trichosporon beigelii* is now obsolete, and previously described infections reported in the literature under this name could in fact be due to any one of the species listed below.

Six species are of clinical significance: *T. asahii*, *T. asteroides*, *T. cutaneum*, *T. inkin*, *T. mucoides* and *T. ovoides*. Other species reported from human and animal infections include *T. dermatis*, *T. domesticum*, *T. faecale*, *T. jirovecii*, *T. loubieri* and *T. mycotoxinovorans* (Rodriguez-Tudela *et al.* 2005, Chagas-Neto *et al.* 2008, Colombo *et al.* 2011).

Trichosporon species are a minor component of normal skin flora, and are widely distributed in nature. They are regularly associated with the soft nodules of white piedra, and have been involved in a variety of opportunistic infections in the immunosuppressed patient. Disseminated infections are most frequently (75%) caused by *T. asahii* (Arendrup *et al.* 2014) and have been associated with leukaemia, organ transplantation, multiple myeloma, aplastic anaemia, lymphoma, solid tumours and AIDS. Disseminated infections are often fulminate and widespread, with lesions occurring in the liver, spleen, lungs and gastrointestinal tract. Infections in non-immunosuppressed patients include endophthalmitis after surgical extraction of cataracts, endocarditis usually following insertion of prosthetic cardiac valves, peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD), and intravenous drug abuse.

Geotrichum

Geotrichum candidum is a fungus which is a member of the human microbiome, notably associated with skin, sputum and feces where it occurs in 25–30% of specimens. It is common in soil and has been isolated from soil collected around the world, in all continents.

G. candidum is the causative agent of the human disease geotrichosis, the plant disease **sour rot** which infects citrus fruits, tomatoes, carrots, and other vegetables. It can affect harvested fruit of durians such as *Durio graveolens*. *G. candidum* is used widely in the production of certain dairy products including rind cheeses such as Camembert, Saint-Nectaire, Reblochon and others. The fungus can also be found in a Nordic yogurt-like product known as viili where it is responsible for the product's velvety texture.

Morphology

Anamorph

G. candidum colonies are thin, spreading, soft, creamy and white in the anamorph state. The fungus *G. candidum* is characterized by hyphae that appear creeping, mostly submerged and septatee. The hyphae colour appears to be hyaline or lightly pigmented. When the hyphae becomes airborne it changes shape from arthroconidia to cylindrical or barrel-shaped or ellipsoidal. Chlamydospores are subglobose, solitary, borne on undifferentiated hyphae. Blastoconidia sometimes develop on hyphae laterally. Conidia appear arthrosporous, terminal or intercalary, aerial on an agar surface. The conidia size ranges from 4.8–12.5 μm x 2.4–2.5 μm.

Teleomorph

G. candidum is thought to be homothallic but most isolates are self-sterile. Sexual reproduction was first observed in strains isolated from soils in Puerto Rico. The fungus produces globose asci that contain a single, thick walled, uninucleated, globose to oval ascospore measuring 6–7 μm by 7–10 μm.[1] The ascospores have a smooth inner wall and a furrowed outer wall.[15] The septa are perforated by microspores, arranged in a ring structure. The colonies appear to be growing faster in the sexual stage than the asexual stage. Colonies grow at a rate of 5–7 mm daily at 24 °C (75 °F).

Growth

Geotrichum candidum forms a fast growing colony that can grow to 5–6 cm diameter at 5 days on Sabouraud-glucose agar, wort agar and synthetic media. Microscopically, the growth is characterized by the production of dichotomously branched hyphae that resemble tuning forks along the colony margin. The condial chains become aerial, erect or decumbent and measure 6–12(−20) x 3–6(−9) µm. The fungus can grow on a variety of citrus fruits and cause Sour Rot. It tends to cause rotting in fruits that are stored at 0–5 °C (32–41 °F). The conidia are colourless and have a slimy coating.[16] *G. candidum* is also found occasionally in the human gut, feces, sputum and on skin. The fungus grows in soil, water, sewage, various plant substrates, baker's dough, husks of fermentation, bread, milk and milk products[1] The optimal temperature for growth is 25 °C (77 °F) with a pH range of 5.0–5.5.[8] The temperature range changes depending on the surface that the fungus grows on. For example, in plants the optimum temperature ranges from 25–27 °C (77–81 °F). In animals the optimum temperature ranges from 30–31 °C (86–88 °F).[17] The maximum temperature for growth is 35–38 °C (95–100 °F).[1][12] Fungal growth can be supported by D-glucose, D-mannose, D-xylose, L-sorbose, D-fructose, D-galactose, sucrose, D-mannitol, SorbitolD-sorbital, ethanol and glycerol. Sporulation often requires a balance of carbon and nitrogen.[1]

Distribution

G. candidum is extremely common in soil and has been isolated from substrates in Canada, United States, Britain, Germany, Austria, India, South Africa, Japan, Brazil and Peru.[1] It is also found as a causal agent in sour rot in citrus fruits— a soft rot associated with the emission of a fruity odour.[18] The fungus is also known as a post-harvest spoilage agent of muskmelon, squash and cucumber. It plays a role in tomato fruit rot when it is stored at 0–5 °C (32–41 °F).[1]

It is a naturally occurring colonist of certain dairy products particularly cheeses and is sometime used to inoculate wash-rind and bloomy rind cheeses.[8][9][19]

Commercial uses

G. candidum can be used commercially to inoculate wash-rinds and bloomy rind cheeses.[8][9][19] Cultures can be added to milk, brine or sprayed onto cheese surface. The optimum pH range for growth on cheese ranges from 4.4 to 6.7. The fungus colonizes nearly the entire surface of the cheese during the early stages of ripening. It is found on soft cheeses like Camembert cheese and semi-hard cheese Saint-Nectaire and Reblochon.[8] For the Camembert cheese the fungi grows on the outside of the cheese forming a rind.[19] The fungus is responsible for the uniform, white, velvety coat on Saint-Marcellin cheese.[8] Lipases and proteases from *G. candidum* release fatty acids and peptides that provide the cheese with distinctive flavors. *G. candidum* reduces the bitterness in Camembert cheese through the activity of the aminopeptidases that hydrolyze low molecular weight hydrophobic peptides. Aminopeptidases also contributes an aroma in traditional Norman Camembert. The fungus also neutralizes the curd by catabolizing lactic acid produced by bacteria. *G. candidum* prepares the cheese for colonization of other acid sensitive bacteria such as *Brevibacterium*. The fungus inhibits growth of the bacteria *Listeria monocytogenes*.[20] Commercial strains of *G. candidum* are available for cheese ripening.[8]

Human colonization and disease

Geotrichum candidum is also a frequent member of the human microbiome, notably associated with skin, sputum and feces where it occurs in 25-30% of specimens.[2][3] The fungus can cause an infection known as geotrichosis, affecting the oral, bronchial, skin and bronchopulmonary epithelia.[2] The inoculum may arise from endogenous or exogenous sources.

In 1847 Bennett described *Geotrichum candidum* causing a superinfection in the tuberculous cavity.[4][5] Bennett was able to differentiate infection by *Geotrichum candidum* from *candidiasis*, and diagnose the first case of geotrichosis. Other early medical case reports in 1916 and 1928 also described lung infections.[4] Most cases affect the bronchopulmonary tree, although other sites can be involved, such as oral mucosa and vagina.[4] Skin and gut infections are also known.[4] Reported cases of geotrichosis have been characterized with symptoms of chronic or acute bronchitis. Exogenous geotrichosis may arise from contact with contaminated soil, fruits or dairy products.[6]

- **Pulmonary geotrichosis** is the most frequent form of geotrichosis. The symptoms appear to be secondary symptoms of tuberculosis. This includes symptoms such as light, thick, grey sputum,[4] which in some cases may be blood-tinged.[4][7] Patients often have a cough that produces clear or yellow sputum.[8] Another symptom of pulmonary geotrichosis includes fine to medium rales.[4] Patients may develop fever, rapid pulse and leukocytosis.[7] The condition appears chronic with the presence of a little debilitation and fever.[4] There is no chest pain and occasional wheezing can occur.[7]
- **Bronchial geotrichosis** does not involve the lung instead the disease persists within the bronchial. *Geotrichum candidum* grows in the lumen of the bronchi. The disease is characterized as an endobronchial infection. Bronchial geotrichosis is similar to the allergic reaction of aspergillosis. Symptoms include prominent chronic cough,[7] gelatinous sputum, lack of fever and medium to coarse rales.[4] Patients with the bronchial condition their pulse and respiration are rarely elevated.[7] Fine mottling may be present in the middle or basilar pulmonary region. Colonization of the bronchi can be associated with *Candida albicans* and usually occur with patients with chronic obstructive lung disease.[4]
- **Oral and vaginal geotrichosis** is similar to thrush in its appearances and was often confused with this infection. The difference between oral and vaginal geotrichosis can be determined using microscope analysis.[4] The infected area forms a white plaque and patients usually report burning sensation in the affected areas.[9] The vaginal geotrichosis is more common in pregnant women and is often associated with vaginitis.[4]
- **Gastrointestinal geotrichosis** is enterocolitis associated with glutamic therapy. The symptoms usually stop once the glutamic therapy is discontinued. Establishment of the etiology of the fungi is difficult since *G. candidum* is found within the gut normal flora. The difference between normal gut flora form and the disease causing form is the production of toxins.[4]
- **Cutaneous geotrichosis** has two different types of variants which include superficial and deep infection. The superficial form the infection occurs on skin folds including submammary, inguinal, perianal and interdigital folds. The deep form

develops nodules, tumours and ulcers on legs, face and hands.[9] Geotrichosis can cause a cystic lesion appears as soft tissue on the skin.[4]

Diagnosis

Laboratory culture

The diagnoses of geotrichosis cannot be determined without using culture or microscopic measurements.[5] The laboratory diagnosis of geotrichosis involves collected fungi samples areas of infections without contamination.[6] Scraping of the mouth lesions and the ulcers can provide a sample of *G. candidum.* Samples can also be collected from pus and mucus can be obtained from the feces.[5] Sputum can be searched for the mucoid-like white flakes for further examination.[6][5] Culturing the cylindrical barrel-shaped or elliptical fungi in considerable numbers in oral lesions is an indicator that a patient may have geotrichosis.[6] Under the microscope the fungi appears yeast-like and septate branching hyphae that can be broken down into chains or individual arthrospores. Arthrospores appear rectangular with flat or rounded ends.[3][5] Under the microscope the arthroconidia size range from 6-12µm x 3-6µm. Arthroconidia and coarse true hyphae can be observed can be observed under the microscope.[3] Another identification method for *G. candidum* is selective isolation method. A selection isolation method based on the fungi tolerance to novobiocin and carbon dioxide can determine if *G. candidum* is the cause of illness.[2]

Diagnostic imaging

X-rays can be used to examine the lung tissue, however it can not be used to positively diagnose geotrichosis. X-rays may show cavitation that is located the walls of the lungs tissues. The lung tissue resemble the early signs of tuberculosis.[5] The results of an x-ray examination of pulmonary geotrichosis presents smooth, dense patchy infiltrations and some cavities. Bronchial geotrichosis shows peribronchial thickening with fine mottling may be present on middle or basilar pulmonary fields.[4] Bronchial geotrichosis usually present itself as non-specific diffuse peribronchical infiltration.[7]

Treatment

Geotrichosis generally has a good prognosis and patients generally have successful recovery.[10] However, there is not a standard treatment for geotrichosis.[3] There are several types of antimicrobial or antifungal compounds that can be used for geotrichosis treatment.[11] Another method of treatment involves symptomatic care, bed rest, iodine therapy,[5] aerosol nystatin and amphotericin B.[4] Azole drugs including isoconazole and clotrimazole are used for geotrichosis treatment.[11] Associated treatment for pulmonary geotrichosis includes the use of potassium iodide, sulfonamides or colistin.[10] The associated asthma can be treated with desensitization and prednisolone.[10][4] Amphotericin B, clotrimazole and S-fluorocytosine have become more susceptible to *G. candidum*. Antimycotic resistance can appear due to repeated treatment.

Saccharomyces

Kingdom – Mycota – Division – Eumycota

Sub- division – Ascomycotina – Class – Hemiascomycetes

Order – Endomycetales – Family – Saccharomycetaceae

Genus – Saccharomyces – Species – cerevisiae

Saccharomyces or Yeast is saprophytic unicellular eukaryotic fungus which grows on sugary solution, grapes etc.

Structure of Saccharomyces:
In a culture of Saccharomyces 2 types of somatic cells can be seen

(a) 'Dwarf strain' yeast cells:
They are small (3 x 2µm) haploid spherical yeast cells which exist in 2 opposite mating types (+ and – strains)

(b) 'Large strain' yeast cells:
They are comparatively larger (15 x I0 µm) diploid, ellipsoidal yeast cells. Structurally both strains of Saccharomyces are unicellular, uninucleate, hyaline, holocarpic, non-mycelial thallus. In a favourable sugary medium as many as 64 cells found temporarily connected to form a pseudomycelium. The cell surface has one concave birth scar and on e or many convex bud scars. Like a plant cell, it consists of protoplast surrounded by cell wall. Cell wall composed of glucan (30-40%), mannan (30%), proteins, lipids and chitin.

The granular cytoplasm contains various organelles and reserve foods (glycogen and oil globules). The centril cytoplasm (endoplasm) contains a large vacuole with a nucleus at one end. From nucleus fine dark staining strands extend around the vacuole. The vacuole enclosed by tonoplast and fills with solution of volutin composed of RNA, lipoprotein and poly-phosphate granules.

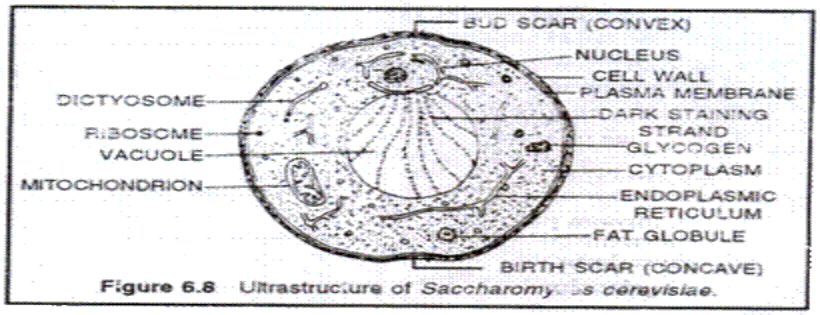


Figure 6.8 Ultrastructure of *Saccharomyces cerevisiae*.

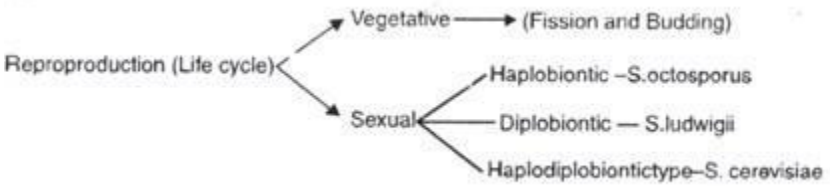
Nutrition:

Yeast cells are facultative aerobe. They contain Zymase (an enzyme complex) which brings about alcoholic fermentation when placed in sugar solution. Alcohol fermentation is ectothermic process which goes on rapidly in absence of oxygen. Fermentation process occurs inside the cell, as zymase confined within the cells, except invertase which can diffuse out of cell.

$$C_6H_{12}O_6 \xrightarrow{\text{Zymase}} \underset{\text{Ethyl alcohol}}{2C_2H_5OH} + 2CO_2 + \underset{\text{available to yeasts}}{22\ \text{cal}}$$

Life Cycle of Saccharomyces:

In the life cycle of Saccharomyces cereviceae alternation of generations seen where 2 types of vegetative individuals alternate with each other's i.e. the 'dwarf strain' haploid yeasts ceils alternates with 'large strain' diploid yeast cells. The 'dwarf stain' cells represent haplophase or gametophyte phase while large strain' cells are diplophase or sporophyte phase. Saccharomyces undergo asexual cycle in favourable period and sexual cycle under unfavourable period.



(a) Asexual cycle (budding):

Under favourable conditions both 'dwarf strain' cells and 'large strain' cells multiply by asexual process called budding. During the process, near one pole cell wall softens by enzymatic action as a result an outgrowth appears. The nucleus divides mitotically and passes one daughter nucleus along with a part of vacuole into the outgrowth. The outgrowth now known as a bud that enlarges, and then constricts as a young yeast cell from the parent. Rapid budding may form pseudomycellium (Fig. 6.9).

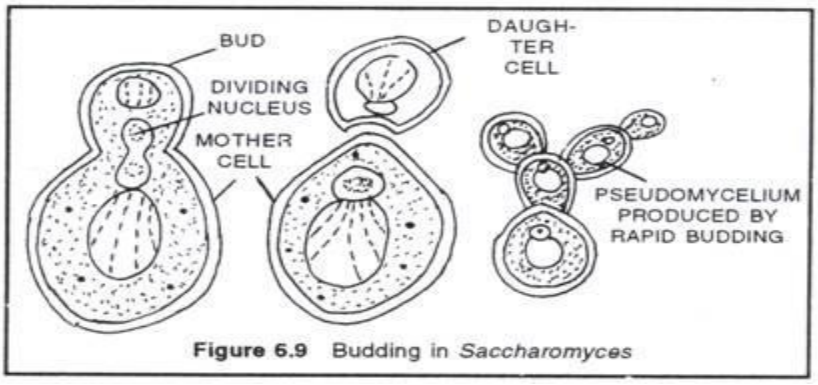


Figure 6.9 Budding in *Saccharomyces*

(b) Sexual cycle (Haplo- diplobiontic cycle):

Under unfavourable condition Saccharomyces cerevisiae undergo sexual cycle which is haplo-diplobiontic type. Here both haploid and diploid phases are equally represented. The haploid dwarf cells have two genetically distinct mating types or strains (+ and -) (Fig. 6.10).

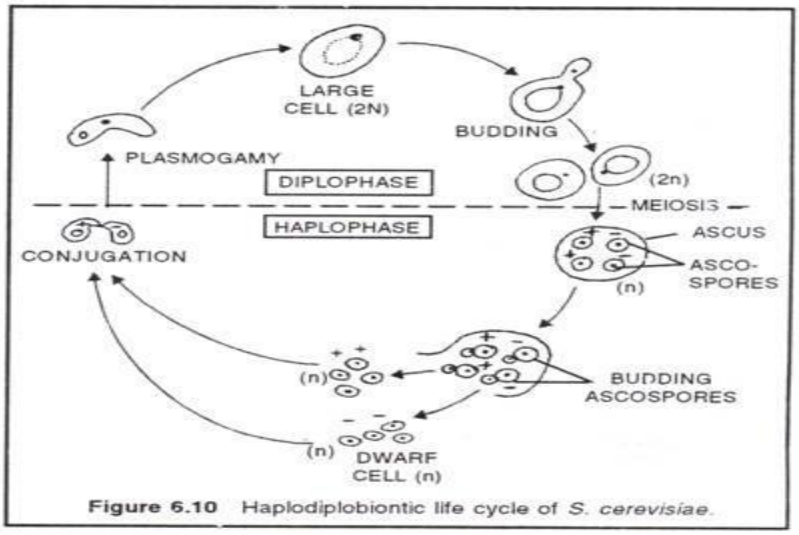


Figure 6.10 Haplodiplobiontic life cycle of *S. cerevisiae*.

The haploid dwarf cells of opposite strains function as gametangia and perform gametangial conjugation. It involves plasmogamy (cytoplasmic fusion) and karyogamy (fusion of + nucleus and – nucleus). As a result of sexual fusion zygote or large yeast cell develop. In favourable period large diploid yeast cells directly act as asci and divides by meiosis to form four haploid ascospores. The ascospores upon liberation form a number of haploid dwarf cells of opposite strains by budding.

Herman (1971) and Hartwell (1974) have observed that when dwarf cells of opposite mating types (i.e. – and +) are not in contact with each other, a sex hormone is secreted. This hormone induces enlargement and elongation in the yeast cells, and consequently they grow towards the opposite mating types, and conjugation follows.

Such a fusion of cells or nuclei of opposite mating types of the same species is called legitimate copulation and the phenomenon is called heterothallism (Blakeslee, 1904). Rarely, cells of the same mating types may also fuse and depolarization takes place. Such a conjugation between the same mating types is called illegitimate copulation.

Pathology

Saccharomyces cause food spoilage of sugar-rich foods, such as maple sap, syrup, concentrated juices and condiments. Case report suggest extended exposure to *S. cerevisiae* can result in hypersensitivity.

Aspergillus

Aspergillus niger is the most common species of aspergillus.

- Its name is adapted from the Latin name aspergillum, which means holy water sprinkler because it has a sprinkler like an appearance when viewed under a microscope.
- It is known commonly to cause black mold in fruits and vegetables like grapes, apricot, onions, and peanuts.
- It is also known to cause food contaminations or food spoilages.
- It also has mild opportunistic characteristics of causing opportunistic respiratory infections associated with pneumonia in immunocompromised individuals than the other species of Aspergillus such as *Aspergillus flavus, Aspergillus fumigatus,* and *Aspergillus clavatus.*
- It is found ubiquitously in soil and sometimes indoors, appearing black, hence the black mold.
- Beneficially, Aspergillus niger has been used for centuries in the production of citric acid that is a common food preservative in canned fruits, shampoos, and blood preservative.
- Some strains of *Aspergillus niger* produce mycotoxins, including ochratoxin A, isoflavone orobol inhibitor.

Aspergillus fumigatus

History of *Aspergillus niger*

- *Aspergillus niger* is the most common and the most studies species in Aspergillus in relation to its morphology, physiology, benefits, and effects.
- Due to this, it is well known to be less pathogenic to both humans and animals.
- In 1917, a food chemist named James Currie discovered that Aspergillus niger produces citric acid in high concentrations when cultivated in sugar-containing medium. Of which he extracted the acid and vastly studies its benefits, as a food preservative.
- Other studies also revealed that it can be used in the production of glucoamylase, α-galactosidase, and many other industrially significant enzymes.
- Based on these findings and other studies involving morphological research, scientists concluded that Aspergillus niger has different strains with different properties.
- In 2004, several species similar to Aspergillus niger were discovered by a group of researchers studying the characteristic production of Ochratoxins by *Aspergillus niger*. These species include subgenus *Circumdati*, section *Nigri* which has 15-related black-spores which are very similar to those *of Aspergillus niger*. They are *A. tubingensis, A. foetidus, A. carbonarius,* and *A. awamori*.

Aspergillus flavus

Habitat of *Aspergillus niger*

- *Aspergillus niger* is highly thermotolerant therefore they can thrive in extreme temperatures including extremely low and extremely high conditions.
- Coupled with its asexual form of reproduction which makes it grown in any kind of environment when the conditions are favorable, and therefore it is also opportunistic.
- It ideally lives in decaying vegetation like compost piles and dead leaves, in soil and it can also be found in a lot of places including on grain stored with stored grains, dried fruits, dry nuts, and polyester.
- A recent study on International space stations showed that Apsergillusniger is highly adaptive to space radiations, an environment that is extremely affected with UV radiations, X-rays, and solar flares. This also is an indicator of how highly adaptive Aspergillus niger is to extremities.

Morphology of *Aspergillus niger*

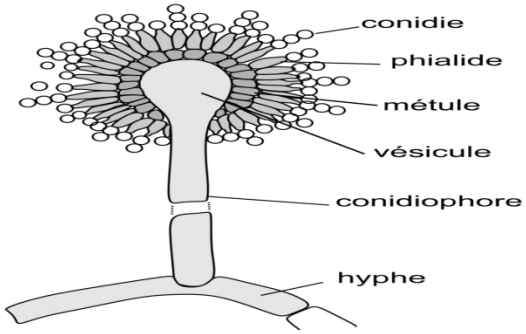


Figure: Aspergillus niger, conidiophore. Image Source: Wikipedia.

- *Aspergillus niger* is a filamentous fungus, forming filamented hyphae that make them appear like small plants.
- Macroscopic observation of *Aspergillus niger* reveals that their growth is initially white but they change to black after a few days producing conidial spore. The edges of the colonies appear pale yellow producing radial fissures.
- A microscopic view of Aspergillus niger reveals that Aspergillus niger has smooth colored conidiophores and conidia. The conidiophores are protrusions from a septate and hyaline hyphae. The conidial heads appear radial and they split into columns (biseriate). the conidiophore vesicle produces sterile cells known as metulae which support the phialides on the conidiophores,
- The Conidiophores are 400-3000um long, they are smooth and hyaline.
- The conidiophore becomes dark at the apex and terminating in a globose vesicle which is 30-75um in diameter.
- The metulae and phialides cover the vesicle.
- The phialides produce conidia that have a rough texture, are dark brown colored, and have a diameter of 4-5um.

Cultural Characteristics of *Aspergillus niger*

- Generally, they have a cottony appearance; initially white to yellow and then turning black. Made up of felt-like conidiophores. The reverse is white to yellow. In microscopy, the conidial heads are radiate with conidiogenous cells biseriate. Conidia brown.
- Macroscopic observation of colonies on potato dextrose agar at 25°C is initially white, which quickly becomes black with conidial production. The reverse is pale yellow and growth may produce radial fissures in the agar.
- **Malt Extract Agar –** an incubation for 7 days at 25ºC and 37ºC producing slightly brown colonies smooth-walled colonies of conidia.
- **Czapek Yeast Agar –** after 5 days of incubation at 25ºC and 37ºC, they produce black colonies with wooly smooth-walled colonies of conidia.

Figure: *Aspergillus niger* colony on Malt Extract Agar (MEA). Image Source: Paul Cannon

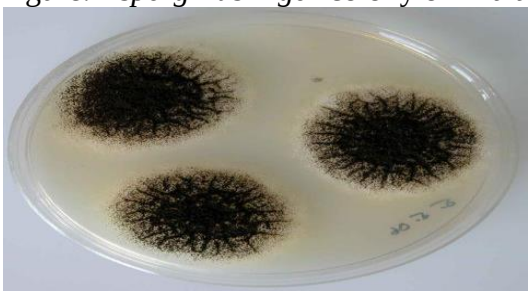


Figure: *Aspergillus niger* colony on Czapek yeast agar. Image Source: Paul Cannon

Life Cycle of *Aspergillus niger*

- *Aspergillus niger* reproduces asexually by forming conidial spores.
- The life cycle starts with the dispersion of the conidia onto a platform with favorable conditions of at least 25-40°C.
- The conidia then germinate forming a vegetative cell
- The cells develop into hyphal mycelium which branches dichotomously forming aerial hyphae.
- The aerial hyphae then grow to form conidiophores which swell at the apex forming the vesicle part of the conidiophore.
- From the vesicles, form the primary sterigmata known as the phialides.
- The sterigmata form the secondary sterigmata that start to produce the conidial spores.
- The spores are arranged in columns (several rows) on to of the phialides.

Pathogenesis of *Aspergillus niger*

Plant manifestations

- *Aspergillus niger* causes a black mold of onions and ornamental plants by infecting onion seedlings. It can disseminate and become systemic and manifest when conditions are favorable.
- *A. niger* causes a common postharvest disease of onions, in which the black conidia can be observed between the scales of the bulb.
- It can also cause disease in peanuts and in grapes.

Human and animal disease

- It is less likely to cause disease in humans and animals unlike the other common species of *Aspergillus*.
- In rare cases, however, it can cause opportunistic invasive pulmonary aspergillosis in individuals with immune-compromised systems on inhalation of a damaged epithelial lining and respiratory tract system, causing severe lung disease.
- It can also cause aspergillosis in horticultural workers who are frequently exposed to inhalation of peat dust which is rich in *Aspergillus* spores.
- It is also a common cause of otomycosis, a type of fungal ear infection associated with temporary impaired hearing, pain, and severe cases that can damage the ear canal and the tympanic membrane.

Laboratory Diagnosis of *Aspergillus niger*

- Microscopic examination to reveal the dark brown rough-edged conidia spores, brown conidiophore.
- Cultural examination using Potato dextrose Agar, Czapek yeast Agar and Malt Yeast Agar.
- Genomic sequencing of the fungi can be done for identification and differentiation from other fungi.
- Thin-layer chromatography can be used for the identification and quantification of ochratoxin mycotoxin.

Treatment of *Aspergillus niger* Infections

- Use of antifungal drugs for the treatment of opportunistic invasive aspergillosis such as itraconazole, Amphotericin B.
- For otomycosis use of itraconazole and antiinflammatory none steroids to relieve the pain, such as acetaminophen.

Prevention and control of *Aspergillus niger*

Removal of Aspergillus niger spores and growth using chemical and antifungal treatment by using:

- 70% ethanol or isopropyl alcohol for about 10 minutes, which is effective in penetrating the spore's cell wall and its hyphae, and it effectively kills them.
- Phenols kill *Aspergillus niger* spores within 20 minutes. It can be added to scrub soaps, mouthwashes, and surface disinfectants.
- Bleach containing hypochlorite inhibits the growth of spores.

For prevention of otomycosis infection:

1. Avoid getting water in your ears while swimming or surfing.
2. Dry your ears after showering.
3. Avoid putting cotton swabs inside your ears.
4. Avoid scratching the skin outside and inside your ears.

5. Use acetic acid ear drops after getting water in your ears

Industrial Uses of *Aspergillus niger*

- *Aspergillus niger* is known for its production of citric acid that is majorly used as a food preservative for canned fruits, dry nuts, and dry fruits.
- It also produces glycoside hydrolase, an enzyme used to convert biomass into biofuels by breaking down the cellulose and hemicellulose from plant cell walls into a substance that can be converted into ethanol.
- This species can also be used to produce bioactive metabolites, as well as other pharmaceutical products.
- *Aspergillus niger* can be adapted to produce large amounts of fructooligosaccharides, due to the observably high transfructosylating activity of the enzymes within its surface.
- *Aspergillus niger* has strong bioabsorption abilities and there its colonies are used to increase the absorption abilities of certain dyes such as Congo red and Blue 9 dye and removal of their impurities.

Zygomycetes

Salient Features of Zygomycetes:

The class Zygomycetes includes those members in which the resting spore (zogospore) develops by the fusion of two gametangia. They do not have motile cells (zoospores) in any stage of their life-cycle.

The salient features of the class are as follows:

(i) The members are saprobes or weak parasites on plants to specialized parasites on animals. A few occur on dung thus coprophilous in nature.

(ii) The thallus usually consists of well developed, branched, filamentous, and coenocytic mycelium; some members possess very much reduced septate mycelium. In some cases, the coenocytic mycelium produces rhizoids and adheres to hard surfaces with their help.

(iii) The cell wall is mainly composed of chitosan-chitin.

(iv) The asexual reproduction takes place usually by means of non-motile sporangiospores, called aplanospores, but some also reproduce by chlamydospores or by oidia formation.

(v) The sexual reproduction takes place by means of gametangial copulation, resulting in the formation of thick-walled zygospores.

(vi) The zygospore germinates by producing a germ sporangiophore that terminally bears a germ sporangium.

Significance of Zygomycetes:

(i) Many members of Zygomycetes (especially those of order Mucorales) grow rapidly and are often the first species that participate in the decay of vegetable matter by utilizing the simplest

carbohydrates (sugars) efficiently leaving complex poly-saccharides (cellulose, hemicellulose, pectin, etc.) for other microorganisms to attack. Because of this, these fungi have often aptly been referred to as the "sugar fungi".

(ii) Various species of Rhizopus, the bread-mould fungus, are used for commercial production of lactic acid; R. stolonifer for fumaric acid and R. oryzae for alcohol. Species of Mucor and Actinomucor elegans are utilized for making 'Sufu' or Chinese cheese from soybeans. 'Tempeh', a solid food prepared with soybeans, is processed with R. oligosporus.

(iii) The phenomenon of heterothellism was discovered by A.F. Blakeslee in 1904 in Mucor mucedo and M. hiemalis.
(iv) Many Zygomycetes are involved in spoilage of food, textiles, and leather.

The Zygomycota, or conjugation fungi, include molds, such as those that invade breads and other food products. The identifying characteristics of the Zygomycota are the formation of a zygospore during sexual reproduction and the lack of hyphal cell walls except in reproductive structures. Many (~100 species) are known plant root symbionts.

Structure
The mycelia of Zygomycota are divided into three types of hyphae. The rhizoids reach below the surface and function in food absorbtion. Above the surface, sporangiophores bear the spore-producing sporangia. Groups of rhizoids and sporangiophores are connected above the surface by stolons. Cell walls separating individual cells are absent in all but reproductive structures, allowing cytoplasm and even nuclei to move between cells.

Whenever you crack open a dystopian novel (you know, to distract yourself from the real-life dystopian nightmare that we wake up to daily), there are a couple things you can expect. For instance, you can expect everyone to be wearing drab clothing. It's like literary…

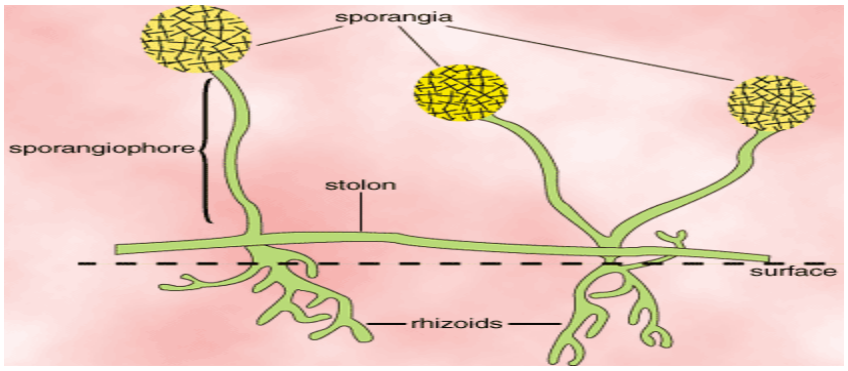


Figure %: Structure of the Zygomycota

Reproduction

Like all fungi, Basidiomycota can undergo both asexual and sexual reproduction. Asexual reproduction in Zygomycota is similar to that in other types of fungi, while sexual reproduction bears some similarity to that in Ascomycota.

Asexual Reproduction

Asexual reproduction in Zygomycota varies greatly among orders and species. Spores may be formed by the separation and thickening of hyphal cells. They may also be produced in specialized organs, whose structure is also widely varied.

Sexual Reproduction

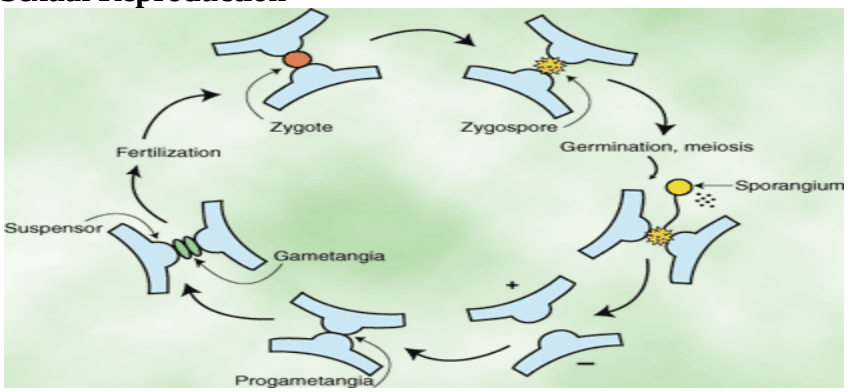


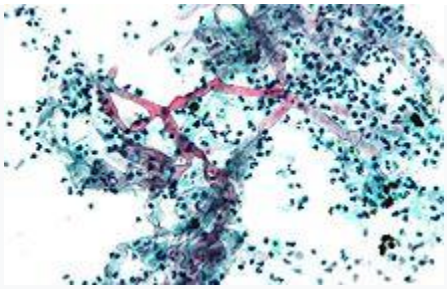
Figure %: Sexual Reproduction in the Zygomycota

Like Ascomycota, some Zygomycota have two mating types, though individual species may only have one mating type. When hyphae from opposite mating types meet, they produce structures called progametangia that are dense and multinucleate. Cell walls form to separate the tips of the progametangia into gametangia, which continue to be attached to the mating hyphae by the remaining suspensors. Plasmogamy then occurs between the two gametangia to form a zygote. Next, karyogamy takes place within the zygote. The cell walls of the zygote are thin at first, but later thicken into a zygospore. Germination begins when the diploid nucleus undergoes meiosis and a sporangium develops at the end of a germ tube. Spores are produced within the sporangium.

Zygomycosis is the broadest term to refer to infections caused by *bread mold fungi* of the zygomycota phylum. However, because zygomycota has been identified as polyphyletic, and is not included in modern fungal classification systems, the diseases that zygomycosis can refer to are better called by their specific names: mucormycosis (after Mucorales), phycomycosis (after Phycomycetes) and basidiobolomycosis (after Basidiobolus). These rare yet serious and potentially life-threatening fungal infections usually affect the face or oropharyngeal (nose and mouth) cavity.[4] Zygomycosis type infections are most often caused by common fungi found in soil and decaying vegetation. While most individuals are exposed to the fungi on a regular basis, those with immune disorders (immunocompromised) are more prone to fungal infection.[2][5][6] These types of infections are also common after natural disasters, such as tornadoes or earthquakes, where people have open wounds that have become filled with soil or vegetative matter.

The condition may affect the gastrointestinal tract or the skin. In non-trauma cases, it usually begins in the nose and paranasal sinuses and is one of the most rapidly spreading fungal infections in humans.[2] Common symptoms include thrombosis and tissue necrosis.[8] Treatment consists of prompt and intensive antifungal drug therapy and surgery to remove the infected tissue.[9][10] The prognosis varies vastly depending upon an individual patient's circumstances.

Causes



Micrograph showing a zygomycetes infection.

Pathogenic zygomycosis is caused by species in two orders: Mucorales or Entomophthorales, with the former causing far more disease than the latter.[11] These diseases are known as "mucormycosis" and "entomophthoramycosis", respectively.[12]

- Order Mucorales (mucormycosis)
  - Family Mucoraceae
    - *Absidia* (*Absidia corymbifera*)
    - *Apophysomyces* (*Apophysomyces elegans* and *Apophysomyces trapeziformis*)
    - *Mucor* (*Mucor indicus*)
    - *Rhizomucor* (*Rhizomucor pusillus*)
    - *Rhizopus* (*Rhizopus oryzae*)
  - Family Cunninghamellaceae
    - *Cunninghamella* (*Cunninghamella bertholletiae*)
  - Family Thamnidiaceae
    - *Cokeromyces* (*Cokeromyces recurvatus*)
  - Family Saksenaeaceae
    - *Saksenaea* (*Saksenaea vasiformis*)
  - Family Syncephalastraceae
    - *Syncephalastrum* (*Syncephalastrum racemosum*)
- Order Entomophthorales (entomophthoramycosis)
  - Family Basidiobolaceae
    - *Basidiobolus* (*Basidiobolus ranarum*)
  - Family Ancylistaceae
    - *Conidiobolus* (*Conidiobolus coronatus/Conidiobolus incongruus*)

Epidemiology

Zygomycosis has been found in survivors of the 2004 Indian Ocean earthquake and tsunami and in survivors of the 2011 Joplin, Missouri tornado.[13]

Other animals

The term oomycosis is used to describe oomycete infections.[14] These are more common in animals, notably dogs and horses. These are heterokonts, not true fungi. Types include pythiosis (caused by *Pythium insidiosum*) and lagenidiosis.

Zygomycosis has been described in a cat, where fungal infection of the tracheobronchus led to respiratory disease requiring euthanasia

Pseudallescheria boydii

Pseudallescheria boydii is a species of fungus classified in the Ascomycota.[2] It is associated with some forms of eumycetoma, maduromycosis[3] and pseudallescheriasis. Typically found in stagnant and polluted water, it has been implicated in the infection of immunocompromised and near-drowned pneumonia patients. Its asexual (anamorphic) form is ***Scedosporium apiospermum***.[4] Treatment of infections with *P. boydii* is complicated by its resistance to many of the standard antifungal agents normally used to treat infections by filamentous fungi.[5]

Pseudallescheria boydii fungal infection was the cause of death in three athletes submerged in the Yarkon River after a bridge collapsed, during 1997 Maccabiah Games.

Taxonomy

The fungus was originally described by American mycologist Cornelius Lott Shear in 1922 as a species of *Allescheria*. Shear obtained cultures from a patient of the Medical Department of the University of Texas. The microbe was apparently associated with a penetrating thorn the patient had incurred in his ankle while running barefoot 12 years before. The diseased area was found to contain hyphae-containing granules that, when cultured, led to the growth of the organism. Shear considered the fungus most closely related to *Eurotiopsis gayoni* (now called *Allescheria gayoni*). The specific epithet *boydii* refers to Dr. Mark F. Boyd, who sent Lott the specimen.[7] David Malloch moved the species to the newly created genus *Petriellidium* in 1970.[8] The species was transferred to the genus *Pseudallescheria* in 1982 when examination of the type specimens of *Petriellidium* and *Pseudallescheria* revealed that they were the same genus.[9]

Ecology

An ability to tolerate minimal aeration and high osmotic pressure[10] enables *P. boydii* to grow on soil, polluted and stagnant water and manure.[11] Although this fungus is commonly found in temperate climates, it is thermotolerant and can survive in tropical climates and in environments with low oxygen pressure.[10] Growth of *P. boydii* can be seen in environments where nitrogen-containing compounds are common, usually due to human pollution. Its ability to use natural gas and other volatile organic compounds suggests a capacity for bioremediation.[10]

Growth and morphology

Pseudallescheria boydii is a saprotrophic fungus with broad hyphae growing up to 2–5 μm in width.[12] Colonies change in colour from white to pale brown and develop a cottony texture with maturity.[11][13] After a 2–3 week incubation period, cleistothecia may form[13] containing asci filled with eight fusiform, one-celled ascospores[14] measuring 12–18 × 9–13 μm in diameter.[15] This fungus grows on most standard media, maturing in 7 days.[15] Its primary nutrients are the sugars xylose,[11] arabinose,[11] glucose,[11] sucrose,[16] ribitol,[16] xylitol[16] and L-arabinitol.[16] It cannot assimilate maltose or lactose; however, it is able to assimilate urea, asparagine, potassium nitrate and ammonium nitrate.[10] The optimal temperature for growth is 25 °C (77 °F) and the fungus is generally considered to be mesophilic,[13] although it can grow at higher temperatures (up to 37 °C (99 °F)) as well.[10] Asexual reproduction manifests in one of two forms: the *Scedosporium* type (the most common type) and the *Graphium* type. *Scedosporium apiospermum* forms greyish-white colonies with a grey-black reverse. The conidia are single-celled, pale brown and oval in form. Their size ranges from 4–9 x 6–10 μm and their development is annellidic.[15]

Pathogenicity

Pseudallescheria boydii is an emerging opportunist.[11] Immune response is characterized by TLR2 recognition of *P. boydii* derived α-glucans, while TLR4 mediates the recognition of *P. boydii* derived rhamnomannans.[17] Human infection takes one of two forms: mycetoma (99% of infections), a chronic, subcutaneous disease,[11] and pseudallescheriasis, which includes all other forms of the disease commonly presented in the central nervous system, lungs, joints and bone.[18] The former can also be distinguished by the presence of sclerotia, or granules, which are typically absent in pseudallescheriasis-type infections.[15] Infection is initiated via inhalation or traumatic implantation in the skin.[18] Infection can lead to arthritis,[11] otitis,[11] endocarditis,[11] sinusitis, and other manifestations.[11] Masses of hyphae can form "fungus balls" in the lungs.[11] While "fungus balls" can also form in other organs, they are commonly derived from host necrotic tissue resulting from nodular infarction and thrombosis of lung vessels following infection.[10]

This species is second in prevalence after *Aspergillus fumigatus* as a fungal pathogen in cystic fibrosis lung. It causes allergic bronchopulmonary disease and chronic lung lesions that resemble aspergillosis.[15] Infections can also occur in immunocompetent individuals, usually in the lungs and upper respiratory tract.[10] Infections in the CNS, which are rare, present as neutrophilic meningitis or multiple brain abscesses[19] and have a mortality rate of up to 75%.[15] Infections have also been observed in animals, notably corneal infection, abdominal mycetoma and disseminated infections in dogs and horses.[13] Transient colonization is more likely than disease. However, invasive pseudoallescheriasis can be found in patients with prolonged neutropenia, high-dose corticosteroid therapy and allotransplantation of bone marrow.[18] *Pseudallescheria boydii* has also been implicated in pneumonia subsequent to near-drowning events with infection developing anywhere between a few weeks to several months after exposure yielding high mortality. Dissemination of the organism to the central nervous system has been observed in some cases.[20] This species is also known as a non-invasive colonist of the external ear and airways of patients with poor lung or sinus clearance, and the first documented case of human pseudallescheriasis involved the ear canal.[21] It has also been implicated in infection of joints

following traumatic injury, and these infections can progress to osteomyelitis. Infections of the skin and cornea have also been reported. Typical host-related risk factors for infection include lymphopenia, steroid treatment, serum albumin levels of < 3 mg/dL and neutropenia.[22]

Diagnosis

Detection and diagnosis of *S. apiospermum* is possible through isolation of the fungus in culture or through cytology and histopathology in the tissues of diseased individuals.[10] In mycetoma-type infections, a confluence of symptoms is necessary for diagnosis, including tumefaction, draining sinuses and extrusion of grains. Furthermore, *P. boydii* grains and hyphae should be cultured and observed microscopically after staining with H&E, periodic acid–Schiff stain, Tissue Gram or Grocott's methenamine silver stain.[10] A radiological diagnosis may be helpful in elucidating the extent of the disease in terms of bone and soft tissue involvement. Scedosporium-caused eumycetomas have been found to have thick-walled cavities and grains appearing as hyperreflective echoes on scans, while actinomycetomas show fine echoes at the bottom of cavities.[10]

Direct detection is possible in samples histochemically stained in 20% KOH followed by fluorescence microscopy with antibody. The characteristic shape, texture and colour of tissues can help identify *S. apiospermum* grains, which are often surrounded by an eosinophilic zone.[10] Histopathologically, hyalohyphomycotic fungi like *Scedosporium* spp., *Aspergillus* spp., *Fusarium* spp. and *Petriella* spp. are similar in that they show septation of hyphae at regular intervals, have dichotomous branching and invade blood vessels. However, *Scedosporium* presents more irregular branching, sometimes with terminal or intercalary chlamydospores.[10] In serum, Scedosporium infections can be detected by counterimmunoelectrophoresis.[23] Molecular diagnostics appear to be promising in complementing current conventional diagnostic methods.[10]

Culture detection is accomplished by rinsing "grains" in 70% ethanol and sterile saline solution to avoid bacterial contamination prior to inoculation on growth medium. Selection of *Scedosporium* growth can be achieved on Leonian's agar supplemented with 10 g/mL benomyl, or on media containing cycloheximide or amphotericin B.[10] Optimal incubation is at a temperature of 25–35 °C (77–95 °F).[10]

Treatment

Pseudallescheria boydii is resistant to amphotericin B [18] and nearly all other antifungal drugs. Consequently, there is currently no consistently effective antifungal therapy for this agent.[18] Miconazole has shown the best *in vivo* activity; however, itraconazole, fluconazole, ketoconazole and voriconazole have also been used in treatment, albeit with less success.[15][20] In an *in vitro* environment, terbinafine has been found to work in synergy with azoles against *P. boydii*. Echinocandins, such as caspofungin and sordarins, have shown promise in *in vitro* assays. CMT-3, a chemically modified tetracycline, has also shown to be active *in vitro* against *P. boydii*.[13]

Epidemiology

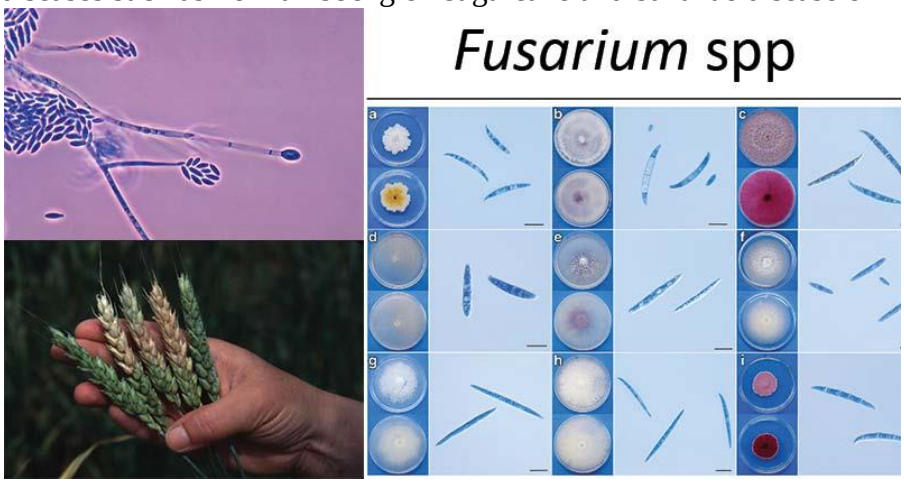
In the United States, *P. boydii* is the most common causal agent of eumycetoma, and tends to be more common in men than in women, particularly in the 20- to 45-year-old age group.[10] In the

United States, the incidence of infection by *S. apiospermum* between 1993 and 1998 was 0.82; this figure increased to 1.33 by 2005.[10] *Pseudallescheria boydii* infection was implicated in the deaths of three athletes injured during the opening ceremony of the 1997 Maccabiah Games when the Maccabiah bridge collapsed in the Yarkon River.[6]

Fusarium

Fusarium is a large group of filamentous fungi belonging to the hyphomycetes. Commonly distributed widely in the soil they are saprophytic fungi known to associate with plants, causing a wide range of plant diseases. This is because of their ability to produce mycotoxins especially in cereal crops, which can cause disease in human and animal hosts if ingested. *Fusarium spp* majorly produces fumonisins and trichothecenes mycotoxins.

Fusarium spp does not commonly cause diseases in humans because some exist as commensals in the skin, but it has been found to cause opportunistic infections in immune compromised individuals. It is vastly known for its pathological effects on plants and animals. Some of the common plant diseases caused by *Fusarium spp* include crown rot, head blight, and scab on cereal grains; vascular wilts on a wide range of horticultural crops; root rots; cankers; and other diseases such as Pokkah boeng on sugarcane and bakanae disease of rice.



Classification of *Fusarium spp*

- Research on the classification and taxonomy of *Fusarium spp* has indicated that there are many species, several populations within the species, and several unidentified groups in the genera. This explains the wide range of variations exhibited by these groups with respect to morphology, cultural characterization, and physiology of the fungal group.
- And these variations might be an explanation as to the ability of the fungi to colonize a wide range of environments globally.
- *Fusarium* classification and taxonomy are as follows:

Kingdom: Fungi

Division: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Nectriaceae

Genus: *Fusarium*

- Presently, the genus *Fusarium* has at least 300 phylogenetically distinct species, 20 species complexes, and nine monotypic lineages of which some have been identified to be opportunistic *Fusarium* pathogens
- Some of these species include:
  - *Fusarium solani* represents a complex of over 45 phylogenetically distinct species of which at least 20 are associated with human infections.
  - *Fusarium oxysporum* complex is phylogenetically diverse
  - *Fusarium incarnatum-equiseti* complex is diverse
  - *Fusarium chlamydosporum* complex is equally diverse.
  - *F. fujikuroi* complex
  - *F. dimerum* complex is less diverse
  - *F. sporotrichioides* complex is less diverse

The opportunistic pathogenic Fusarium groups for plants, animals, and humans majorly belong to the *F. solani* complex, *F. oxysporum* complex, and *F. fujikuroi* complex.

Habitat of *Fusarium* spp

- *Fusarium spp* is commonly found in soil and environmental habitats, with many growing and thriving in tropical and temperate regions and even in desert regions, the alpine, the arctic regions with harch cold conditions, they seem to prevail.
- *Fusarium species* are widely distributed in soil and on subterranean and aerial plant parts, plant debris, and other organic substrates.
- Commonly and abundantly found in soil, they seem to thrive in fertile cultivated soil and therefore they are classified as soilborne fungi where they closely associate with plant roots saprophytically or parasitically.
- They disperse in the atmosphere and become airborne, thus colonizing aerial plants and causing diseases.
- Therefore, *Fusarium* species are widely distributed and have efficient dispersal mechanisms thus growing in a wide range of substrates as well.

Morphology of *Fusarium spp*

Figure: *Fusarium verticillioides*. Image Source: Wikipedia.

- *Fusarium spp* reproduces asexually and produces three kinds of fungal spores known as macroconidia, microconidia, and chlamydospores.

- Some species of Fusarium produce all three types of spore while others produce singularly.
- These spores especially the microconidia are held by microconidiophores.
- These conidiophores may be either mono-phialides only or both mono-phialides and poly-phialides in a given species producing microconidia.
- Macroconidia are produced in a sporodochium, which is an erumpent crowded cluster of conidiophores arising from stroma to form a cushion-like mass that supports the macroconidia.
- Macroconidia are also produced on mono-phialides (a conidiophore with a single opening through which an endoconidia is released) and poly-phialides (two or more openings or pores from which the endoconidia are forced out) on aerial mycelium.
- The macroconidia vary in size and shape.
- The Major producers of macroconidia are *Fusarium semitectum, Fasarium avenaceum and Fusarium suglutinans*.
- Microconidia are produced in the aerial mycelium.
- The microconidia can be produced on false heads or false chains on mono-phialides or poly-phialides. False Heads are a result of moisture drops on the conidiophore and they contain the endoconidia as they are produced.
- Microconidia have different shapes and sizes. the microconidia produced in chains have a truncate base.
- Chlamydospores are thick-walled spores filled with lipid-like material that carries the spores overwinter in the soil.
- Chlamydopsores are sometimes airborne occurring in pairs, in clumps, or in chains.
- They have an outer wall which can be smooth or rough.

Cultural characteristics of *Fusarium* spp

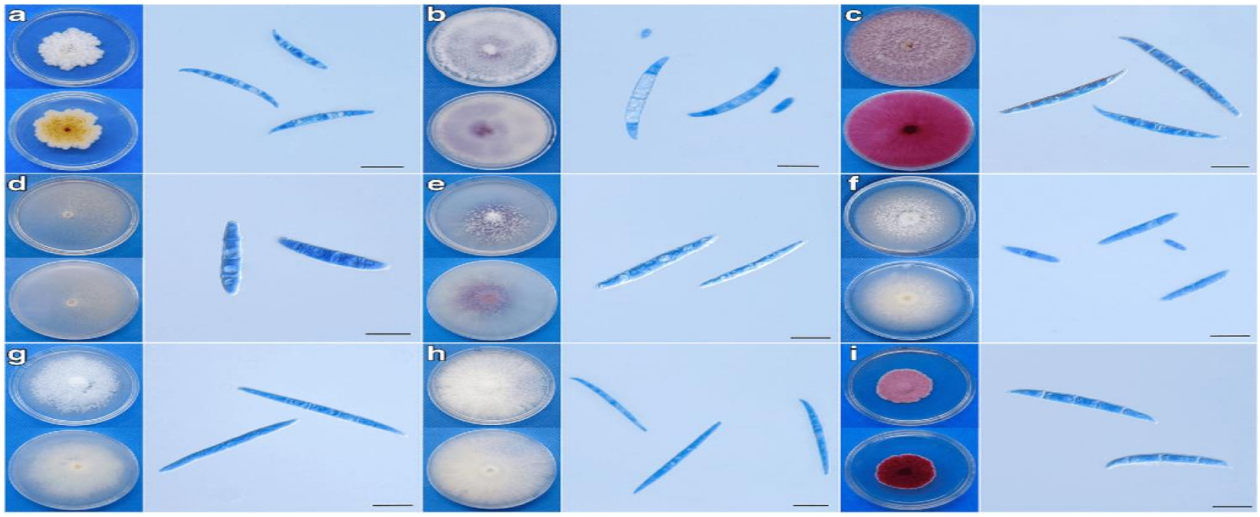


Figure: Typical colonies and macroconidia of *Fusarium* isolates from soybean root rot in Sichuan, China. Typical colonies of the representative Fusarium isolates were observed on PDA after 5 days and macroconidia in Carboxymethyl cellulose Medium after 5 days grown. a F. equiseti; b F. oxysporum; c F. graminearum; d F. solani; e F. commune; f F. verticillioides; g F. proliferatum; h F. fujikuroi; i F. avenaceum. Scale bar = 20 μm. Image Source: https://doi.org/10.1007/s10658-017-1410-7

Carnation leaf agar

- It promotes sporulation and suppresses mycelial growth. It produces conidia and conidiophores in large numbers and specialized morphologies of the spores are distinct. Carnation leaf agar has low carbohydrates with complex substances that provide a natural environment that promotes *Fusarium* growth.

Potato dextrose agar

- This is the most valuable medium for *Fusarium* growth-producing gross morphological appearance and colony colorations. The medium which contains a high carbohydrate content which promotes sporulation, however, takes longer to grow in this medium. The conidia produced are misshapen and atypical.

KCl medium

- KCI medium is used to observe the formation of microconidia in chains by species. The species that do form chains of microconidia form more abundant, longer chains on this medium. The chains are easier to observe because there is less moisture on the surface of the agar and fewer droplets of moisture in the aerial mycelium.

Soil agar

- Soil agar promotes rapid chlamydospore formation in a number of *Fusarium* species. Large inoculum with actively growing fusarium inoculates produces chlamydospores within 3-4 days but secondary inoculates produce chlamydospores in 30 days.

Pathogenesis and Clinical Features of *Fusarium* spp

Fusarium spp causes disease in plants, animals, and human hosts, but most commonly in plants. These pathogenesis have been linked to the toxigenicity of the species associated with the production of mycotoxins such as trichothecenes (types A and B), fusaric acid and fumonisins

Plant pathologies caused by *Fusarium* species

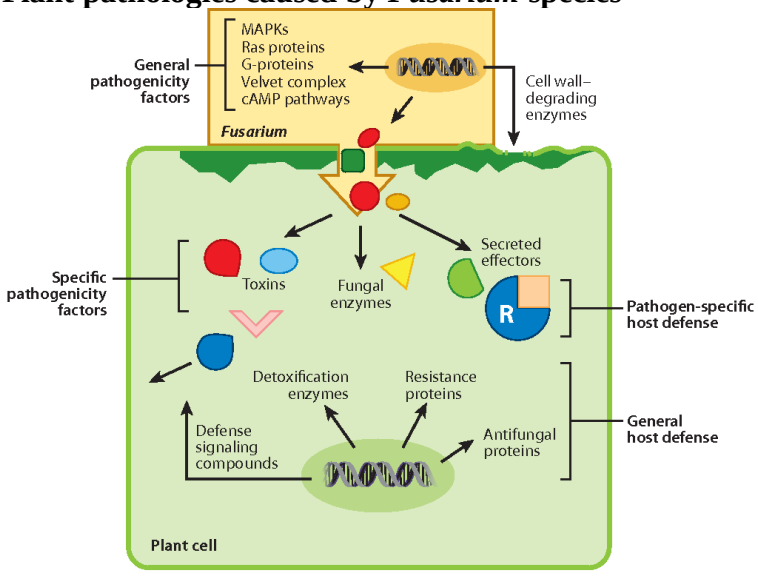


Figure: *Fusarium* pathogenicity and host defense mechanisms. Image Source: https://doi.org/10.1146/annurev-micro-092412-155650

- The effect of *Fusarium* spp on plants affects and infects specific parts such as grains, seedlings, heads, roots, or stems.
- The plant infections by *Fusarium* spp are caused by the secretion of mycotoxins by various group complexes of *Fusarium*.

- They cause various plant diseases which contribute to decreased quality and production yield of these crops.
- *Fusarium solani* species complex (FSSC) causes diseases in many agriculturally important crops, such as ***Fusarium*** **Rot and/or Root Rot** and necrosis of the infected host plant.
- The infection by FSSC causes symptoms, such as wilting, stunting, and chlorosis. Necrosis depends on the severity of fungal development.
- Two of the most serious diseases of wheat known globally are *Fusarium* Crown Rot (FCR) and *Fusarium* Fusarium Head Blight (FHB).
- Plant pathologies include:
  - *Fusarium* head blight (FHB) caused by *F. graminearum*, which contributed to the loss of starch and proteins in cereals.
  - Footrot (FR) and root rot (RR)
  - Crown rot (CR)
  - *Fusarium* wilts a destructive disease in bananas caused by *F. oxysporum.*

Fusarium Head Blight

Figure: Fusarium head blight in wheat. Image Source: The American Phytopathological Society (APS)

- It is caused by *Fusarium graminearum.*
- This is a disease that commonly affects cereals such as wheat, barley, oats, rye, and triticale.
- The disease infects the heads of the crop, reducing grain yield.
- The disease cycle has three stages of infection:
  - *Fusarium graminearum* multiplies rapidly on inoculation on the plant site making large biomass within the first 2 days. This allows the fungal spores to germinate forming superficial hyphae on the leaf sheath.
  - Stage two involves a decrease in the fungal biomass due to penetration of the hyphae into the leaf sheath base from the outer leaf sheath.
  - The third stage included the increase in biomass of the fungus allowing colonization on the cereal crops especially the crown head of wheat parenchyma.

Footrot and root rot

- This is commonly caused by *Fusarium solani*, saprophytic fungus.
- The fungus colonizes the dead or decaying plant tissues allowing the fungal invasion of the stem of the nodes or the soil line and opportunistically infecting the plant wound.
- The fungus spores then germinate in the affected area (wound), favored by high humidity and temperatures.

Fusarium wilt

Figure: A tobacco plant suffering from Fusarium wilt. Image Source: Wikipedia (R.J. Reynolds).

- It is caused by *Fusarium oxysporum* as the only pathogenic group of Fusarium known to grow inside the plant vessels and spreads upwards inside the plant.
- It is a saprophytic fungus that can survive in soil between crop cycles in infected plant debris.
- Survival morphologies can be in the mycelial form or in spore forms.
- The fungus infects the root tips directly or through damaged tissues allowing mycelial growth into the root cortex into the xylem and later affecting the whole vascular tissue.
- This causes a reduction of water and nutrient intake leading to leaf wilting and plant death.

Human pathologies of *Fusarium* species

- *Fusarium species* cause superficial, locally invasive, and diffuse infections in humans.
- Localized infection includes septic arthritis, endophthalmitis, osteomyelitis, cystitis, and brain abscess.
- Invasive infections are as a result of surgery and oral antifungal therapy.
- Disseminated infection occurs when two or more noncontiguous sites are involved.
- The infections are opportunistic and they are majorly caused by *F. solani* complex including *F. solani*, *F. oxysporum*, *F. verticillioides*, and *F. proliferatum, F. moniliforme and F. fujikuroi species* complex.
- They cause opportunistic infections in immunocompromised patients.
- The elderly and diabetics with prevalent meningospondylodiscitis are opportunistically infected by *F. oxysporum*. *F. sacchari*, *F. anthophilum*, *F. chlamydosporum*, and *F. dimerum.*
- A perinephric abscess caused by *F. chlamydosporum* is common in children who have been reported before.
- Corneal infections (endophthalmitis) caused by *Fusarium oxysporum* and *Fusarium solani* also occur because of the adherence of the Fungi to the corneal membrane causing eye damage.
- Some *Fusarium* species, such as *F. dimerum*, are associated with keratomycosis, particularly in the bad hygiene conditions.
- Mycotoxicosis caused by Fusarium species is common in the ingestion of the mycotoxins produced by the fungi.

***Fusarium* infection in animals**

- *Fusarium* spp can cause *Fusarium* infections in animals including mycotoxicosis which affects the growth, reproduction, and hormonal condition of the animal.

- The effect of these mycotoxins on animals depends on the quantity of mycotoxin intake. After intake, these mycotoxins arrive at the gastrointestinal epithelial cell layer. In high doses, the mycotoxins cause abdominal distress, diarrhea, cardiac insufficiency, emesis, and even death in pigs and equine leukoencephalomalacia (ELEM) in horses.

Identification and diagnosis of *Fusarium* species

- Use of mycological and blood culture methods to identify hyaline, banana-shaped, and multicellular macroconidia.
- Use of molecular methods such as genus-specific PCR, 28 s rRNA gene sequencing, sequence-based PCR, multiplex tandem PCR, and automated repetitive sequence-based PCR for differentiation of the various *Fusarium species* groups.
- The diagnosis of Fusarium infection in humans and animals can be may be done using histopathology, gram stain, mycology, blood culture, or serological identification of the fungal antigens.

Treatment, Prevention, Control, and Management of *Fusarium*

- For human and animal infections can be treated with intravenous administration of itraconazole, oral amphotericin B.
- Plant infections can only be controlled by used on control agents and measures.
- Biocultural control by the use of mycoparasitic fungi such as *T. harzianum* may be used as a biocontrol agent against *Fusarium*. The parasite has shown to have an antagonistic effect against Fusarium that causes footrot, root rot, and crown rot.
- Fungicides have very minimal effects with likely resistance development on most of the Fusarium species groups.
- Resistant cultivars can be used to control Fusarium Head Blight by controlling the plant lines that allow the release of mycotoxins. This reduces fungal growth and lowers mycotoxin contamination.

Piedra

Piedra is a hair disease caused by a fungus, which causes formation of nodules on the hair shaft.[2][3]

Types include:

- White piedra
- Black piedra

White piedra (or **tinea blanca**) is a mycosis of the hair[1] caused by several species of fungi in the genus *Trichosporon*. It is characterized by soft nodules composed of yeast cells and arthroconidia that encompass hair shafts.

White piedra can occur on the hair of the scalp; *Trichosporon ovoides* is likely the cause in this case.[2] White piedra on scalp hair is rarely caused by *Trichosporon inkin*; pubic hair with white piedra is what *T. inkin* is mainly associated with.[3] White piedra can occur on pubic hair; *T. inkin* likely causes this.[2]

Trichosporon beigelii

Treatment

There are several approaches to treat this infectious disease. One approach involves shaving the affected areas. Another approach involves the use of antifungal medication.

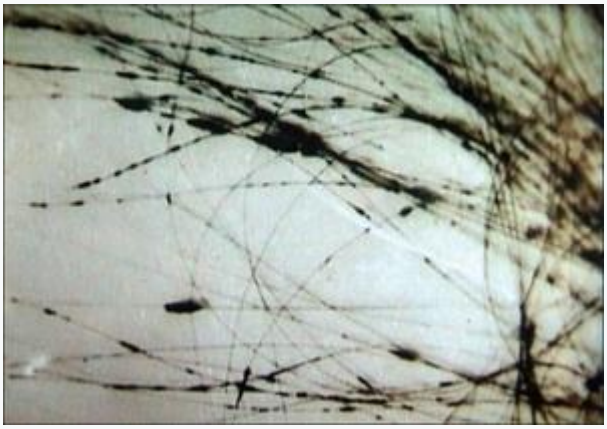
Piedraia hortae is a superficial fungus that exists in the soils of tropical and subtropical environments and affects both sexes of all ages.[2] The fungus grows very slowly, forming dark hyphae, which contain chlamydoconidia cells and black colonies when grown on agar. *Piedraia hortae* is a dermatophyte and causes a superficial fungal infection known as black piedra, which causes the formation of black nodules on the hair shaft and leads to progressive weakening of the hair.[3] The infection usually infects hairs on the scalp and beard, but other varieties tend to grow on pubic hairs. The infection is usually treated with cutting or shaving of the hair and followed by the application of anti-fungal and topical agents. The fungus is used for cosmetic purposes to darken hair in some societies as a symbol of attractiveness.

Morphology

When grown on agar at 25 °C (77 °F) *Piedraia hortae* grows very slowly[4] to form black-greenish, limited and pointed colonies.[4] *Piedraia hortae* taken from infected hairs have dark brown nodules,[5] which are made up of ascostroma. The nodules have a gritty feel, organized in a stromatic fashion[6] and have a high concentration of chitin and melanoid pigments.[3] The colonies produce a red pigment and remain smooth and covered with short aerial hyphae. Microscopically, *P. hortae* produces short, dark hyphae containing thick-walled resting cells. The ascomata consist of irregularly shaped pseudothecia that are black in colour. Each ascoma contains a single ascus containing eight ascospores.[3] The ascospores are dark, curved and become very narrow at the ends forming whip-like appendages.[7] Affected hairs develop stone-like black nodules affixed to the hair shaft that cause weakness of the hair. Infected hairs treated with potassium hydroxide fluoresce under ultraviolet light despite that the fungus itself does not normally fluoresce. Fluorescence of the piedra indicates secondary contamination by bacteria.[8] Identification is easily achieved by microscopic examination of the hair nodules, and can be confirmed by sequence analysis of the nuclear ribosomal internal transcribed spacer region.[9]

Pathology

Black piedra



Black piedra nodules on hair

Piedraia hortae causes the formation of nodules on the hair shaft, a clinical superficial disease commonly known as **black piedra**.[10] Black piedra is usually seen in tropical regions and it usually targets humans of all ages and targets the scalp, moustache and occasionally pubic hair. The source of the infection is usually in soils, poor hygiene, long hair, cultural use of veils and the application of plant oils to wet hair favours the growth of the infection.[8] Black piedra is a superficial fungal infection, which means that it is restricted to the stratum corneum and causes no inflammation.[11] The infection of the hair shaft results in the formation of nodules on the scalp, moustache and pubic hair. The nodules are hard and gritty,[2] which produce a metallic sound when the hair is combed. The nodules colonize the hair shaft, which causes progressive weakness of the hair and leads to breakage of the hair in severe cases, which can lead to hair loss and baldness. The fungus also has the potential to destroy the cuticular layers of the hair and move into the cortex. *Piedraia hortae* survives in the scalp is due to the slow rate of the keratin degradation near the cortex and the compact formation of the nodules[6] and the hyphae are tightly packed in black piedra cases.[12] The initial invasion of human hair by *P. hortae* is achieved by using an eroding hyphae, which force their way beneath or between the cuticular layer.[8] The force applied between or beneath the hair cuticle arises from the growth of the fungus itself. The breakdown of keratin is mainly due to enzymatic processes and corresponds to the abundance of localized mitochondria. The breakdown of keratin begins with the cementing material and progresses to the cortex of the hair shaft.[8] In the cortex two types of degradative patterns are produced which are either parallel or vertical to the axis hair shaft. The parallel pattern arises from hyphal separation of the external cortical layers. The vertical pattern is produced by direct hyphal penetration which creates channels that increase in size as the cortex degrades.[8]

Treatment

The infection cannot easily be removed mechanically,[5] although further proliferation of infection can be achieved by avoidance of moisture. Removal generally involves cutting or shaving of the hair,[2] but chemical treatments may be similarly useful. For women some individuals use a fine comb to remove as much of the infection as possible[13] and then they cut or shave their hair. This is then followed by the application of a sublimate solution in 60% alcohol solution to the scalp. Historical treatments have used alcoholic tinctures of heavy metals,

such as mercury bichloride.[14] The application of antifungal shampoos such as pyrithione zinc, formaldehyde and salicylic acid is effective against black piedra. Oral therapy with itraconazole or terbinafine also causes nodules to break down over time.[7][15] Removal of affected hair and treatment with topical agents is also effective and results in very low recurrences rates.[*citation needed*] However, even in the absence of treatment, spontaneous remission may occur.[12]

Terbinafine has been used in the treatment.[16]

Cosmetic uses

Black piedra is sometimes cultivated for cosmetic purposes due to social factors that favour a specific hair colour, which makes them more attractive in their society. Several Indian tribes located from Panama have been known to use several methods in order to darken the hair of albino individuals within their community. One of these methods is the cultivation of black piedra for an extensive period of time in the individuals hair.[17] In Malaysia the nodules of black piedra are very attractive and women are encouraged to sleep with their hair buried in the soil to encourage growth of the black nodules.[18]

Similar taxa

The genus *Piedraia* contains another species known as *Piedraia quintanilhae*, which is more common in chimpanzees than humans. It differs from *P. hortae* in terms of the ascospores do not have any attachments.[19] Another species known as *Trichosporon biegelii* is commonly known to cause white piedra . White piedra is more common in temperate and semitropical climates,[20] such as South America, Asia, Europe, Japan, and parts of the southern United States. Black piedra usually affects scalp hair, whereas white piedra is more commonly found in pubic hair, axillary hair, beards, moustaches, and eyelashes.[21][22] White piedra affects horses and monkeys, in addition to humans and the nodules are white and brown in colour and can be easily detached from the hair shaft. White piedra is treated by using topical and antifungal agents, but a more effective approach is to use itraconazole therapy.[20] Recent studies have shown that the black, lichen-colonizing fungus, *Xanthoriicola physciae*, is closely related to *P. hortae*.[23]

Hyphomycetes

Hyphomycetes are a form classification of Fungi, part of what has often been referred to as Fungi imperfecti, Deuteromycota, or anamorphic fungi. Hyphomycetes lack closed fruit bodies, and are often referred to as moulds (or molds). Most hyphomycetes are now assigned to the Ascomycota, on the basis of genetic connections made by life-cycle studies or by phylogenetic analysis of DNA sequences; many remain unassigned phylogenetically. Identification of hyphomycetes is primarily based on microscopic morphology including: conidial morphology, especially septation, shape, size, colour and cell wall texture, the arrangement of conidia as they are borne on the conidiogenous cells (e.g. if they are solitary, arthrocatenate, blastocatenate, basocatenate, or gloiosporae), the type conidiogenous cell (e.g. non-specialized or hypha-like, phialide, annellide, or sympodial), and other additional features such as the presence of sporodochia or synnemata.

Taxonomic and nomenclatural history

Because asexual forms of fungi usually occur separately from their sexual forms, when microscopic fungi began to be studied in the early 19th century, it was often unknown when two morphologically different forms were actually part of one species. The tendency for some organisms to apparently only have asexual forms, or for their sexual forms to be discovered long after the asexual forms, meant that an independent taxonomy was developed for asexual fungi. Near the beginning of the 20th century, when it became clearer that many asexual and sexual forms were related, the concept of 'form taxa' was developed. The independent taxonomy of asexual forms was regarded as artificial, not representative of evolutionary relationships, and intended to be practical for identification purposes. The taxonomy of the sexual states was considered the true classification. The result was that many fungal species ended up with two accepted Latin binomials, one for the asexual form (or anamorph) and the other for the sexual form (teleomorph). This dual nomenclature was only abandoned in January 2012,[4] and the transition to a single name system, with one name representing all morphs of a fungus, is still incomplete.[5]

Ecological importance

Aquatic or **Ingoldian** hyphomycetes are common on submerged decaying leaves and other organic matter, especially in clean running water with good aeration. Colonised leaves fall from the tree into the river. Their branched, septate mycelium penetrates through the leaf surface and spreads through leaf tissue. Conidiophores project into the water and bear conidia, which are often sigmoid, branched or tetraradiate structures. Aquatic hyphomycetes play an important role in the breakdown of organic matter in rivers, because their extracellular enzymes break down leaf tissue, which in turn is made more palatable to invertebrates. Leaves with fungi (conditioned) are a more nutritious source of food than unconditioned leaves.[6]

Coprophilous or dung-loving hyphomycetes are part of the succession of fungi occurring on many kinds of herbivore faeces, playing an important role in breaking down cellulose.[7] Several species are found only on dung, such as *Angulimaya sundara*, *Onychophora coprophila*, *Pulchromyces fimicola*, *Sphondylocephalum verticillatum* and *Stilbella fimetaria*.

Entomogenous, entomopathogenic or insect-pathogenic hyphomycetes infect and kill insects (and spiders) and are especially diverse in tropical and subtropical regions, especially in Asia.[8] Most are asexual states of or phylogenetically related to the Ascomycete families, Cordycipitaceae and Ophiocordycipitaceae. Insect hosts are infected by asexual spores, which germinate and grow to fill the host body with mycelium or hyphal bodies, then produce sporulating structures on the insect carcass. They are often found on dead insects under bark or in soil, but some affect insect behaviour ("zombie fungus"), causing infected hosts to climb towards the light, ensuring that air-borne infective spores will be released higher up in the canopy of the forest or meadow.[9] Well-known entomogenous hyphomycetes are classified in *Beauveria*, *Metarhizium* and *Tolypocladium*; famous anamorphic generic names such as *Akanthomyces*, *Gibellula*, *Hirsutella*, *Hymenostilbe* and *Isaria* are now subsumed in genera formerly considered sexual, such as *Cordyceps*, *Ophiocordyceps* and *Torubiella* under fungal single-name nomenclature.[10] Species of *Beauveria* and *Metarhizium* show some promise as biological control agents against pest insects.[11] *Tolypocladium inflatum* was the original source of cyclosporine A, used as a drug to prevent rejection of organ transplants.[12]

Many **food-borne fungi** are hyphomycetes. Species of *Penicillium* and *Aspergillus* are particularly common agents of food spoilage and also produce important mycotoxins that affect human health.[13] Some species, such as *Penicillium digitatum* on citrus fruits, and *Penicillium expansum* on apples, are common on specific foods, while others are less specialized and grow on many different kinds of food.

Nematophagous or nematode-trapping hyphomycetes either live their life-cycles in the bodies of dead nematodes or trap and kill nematodes in order to supplement their nitrogen requirements.[14] Species of the hyphomycete genus *Arthrobotrys*, phylogenetically related to or being the asexual forms of *Orbilia*, produce constricting loops that quickly shut to grab nematodes, or non-constricting loops or hyphal networks that entangle nematodes, or sticky knobs that adhere to nematodes as they swim by. Attempts to exploit these fungi as biological control agents against agriculturally harmful nematodes have generally been unsuccessful.[15]

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.SC. MICROBIOLOGY

UNIT – III – Medical Mycology – SMB3103

Histoplasmosis

Histoplasmosis is an infection caused by breathing in spores of a fungus often found in bird and bat droppings. The infection is most commonly spread when these spores are inhaled after taking to the air, such as during demolition or cleanup projects.

Soil contaminated by bird or bat droppings also can spread histoplasmosis, putting farmers and landscapers at a higher risk of the disease. In the United States, histoplasmosis commonly occurs in the Mississippi and Ohio River valleys, though it can occur in other areas, too. It also occurs in Africa, Asia, Australia, and in parts of Central and South America.

Most people with histoplasmosis never develop symptoms and aren't aware they're infected. But for some people — primarily infants and those with compromised immune systems — histoplasmosis can be serious. Treatments are available for even the most severe forms of histoplasmosis.

Symptoms

The mildest forms of histoplasmosis cause no signs or symptoms, but severe infections can be life-threatening. When signs and symptoms occur, they usually appear three to 17 days after exposure and can include:

- Fever
- Chills
- Headache
- Muscle aches
- Dry cough
- Chest discomfort
- Fatigue

Some people with histoplasmosis also get joint pain and a rash. People who have a lung disease, such as emphysema, can develop a chronic form of histoplasmosis.

Signs of chronic histoplasmosis can include weight loss and a bloody cough. The symptoms of chronic histoplasmosis sometimes mimic those of tuberculosis.

Severe histoplasmosis

The most severe variety of histoplasmosis occurs primarily in infants and in people with compromised immune systems. Called disseminated histoplasmosis, it can affect nearly any part of your body, including your mouth, liver, central nervous system, skin and adrenal glands. If untreated, disseminated histoplasmosis is usually fatal.

Causes

Histoplasmosis is caused by the reproductive cells (spores) of the fungus Histoplasma capsulatum. They float into the air when dirt or other material is disturbed.

The fungus thrives in damp soil that's rich in organic material, especially the droppings from birds and bats. It's particularly common in chicken and pigeon coops, old barns, caves, and parks.

Histoplasmosis isn't contagious, so it can't be spread from person to person. If you've had histoplasmosis, you can get it again. However, if you do get it again, the illness will likely be milder the second time.

Risk factors

The chances of developing histoplasmosis symptoms increase with the number of spores you inhale. People more likely to be exposed include:

- Farmers
- Pest control workers
- Poultry keepers
- Construction workers
- Roofers
- Landscapers and gardeners
- Cave explorers
- Demolition workers

Most at risk of severe infection

Children younger than age 2 and adults age 55 and older have weaker immune systems, so they're more likely to develop disseminated histoplasmosis — the most serious form of the disease. Other factors that can weaken your immune system include:

- HIV or AIDS
- Cancer chemotherapy
- Corticosteroid drugs, such as prednisone
- Tumor necrosis factor inhibitors, often used to control rheumatoid arthritis
- Medications that prevent rejection of organ transplants

Complications

Histoplasmosis can cause a number of serious complications, even in otherwise healthy people. For infants, older adults and people with compromised immune systems, the potential problems are often life-threatening.

Complications can include:

- **Acute respiratory distress syndrome.** Histoplasmosis can damage lungs to the point that the air sacs begin filling with fluid. This prevents good air exchange and can deplete the oxygen in your blood.
- **Heart problems.** Inflammation of the sac that surrounds your heart (pericardium) is called pericarditis. When the fluid in this sac increases, it can interfere with the heart's ability to pump blood.
- **Adrenal insufficiency.** Histoplasmosis can harm your adrenal glands, which produce hormones that give instructions to virtually every organ and tissue in your body.
- **Meningitis.** In some cases, histoplasmosis can cause this inflammation of the membranes surrounding your brain and spinal cord.

Prevention

It's difficult to prevent exposure to the fungus that causes histoplasmosis, especially in areas where the disease is widespread. But taking the following steps might help reduce the risk of infection:

- **Avoid exposure.** Avoid projects and activities that might expose you to the fungus, such as cave exploring and raising birds, such as pigeons or chickens.
- **Spray contaminated surfaces.** Before you dig soil or work in an area that could harbor the fungus that causes histoplasmosis, soak it with water. This can help prevent spores from being released into the air. Spraying chicken coops and barns before cleaning them also can reduce your risk.
- **Wear a respirator mask.** Consult the National Institute for Occupational Safety and Health to determine which type of mask will provide protection for your level of exposure.

Blastomycosis

Blastomycosis is a pulmonary disease caused by inhaling spores of the dimorphic fungus *Blastomyces dermatitidis*; occasionally, the fungi spread hematogenously, causing extrapulmonary disease. Symptoms result from pneumonia or from dissemination to multiple organs, most commonly the skin. Diagnosis is clinical, by chest x-ray, or both and is confirmed by laboratory identification of the fungi. Treatment is with itraconazole, fluconazole, or amphotericin B.

In North America, the endemic area for blastomycosis includes

- Ohio–Mississippi River valleys (extending into the middle Atlantic and southeastern states)
- Northern Midwest
- Upstate New York
- Southern Canada

Rarely, the infection occurs in the Middle East and Africa.

Immunocompetent people can contract this infection. Although blastomycosis may be more common and more severe in immunocompromised patients, it is a less common opportunistic infection than histoplasmosis or coccidioidomycosis.
B. dermatitidis grows as a mold at ambient temperature in soil enriched with animal excreta and in moist, decaying, acidic organic material, often near rivers.
In the lungs, inhaled spores convert into large (15 to 20 micrometers) invasive yeasts, which form characteristic broad-based buds.

Once in the lungs, infection may

- Remain localized in the lungs
- Disseminate hematogenously

Hematogenous dissemination can cause focal infection in numerous organs, including the skin, prostate, epididymides, testes, kidneys, vertebrae, ends of long bones, subcutaneous tissues, brain, oral or nasal mucosa, thyroid, lymph nodes, and bone marrow.

Symptoms and Signs

Pulmonary

Pulmonary blastomycosis may be asymptomatic or cause an acute, self-limited disease that often goes unrecognized. It can also begin insidiously and develop into a chronic, progressive infection. Symptoms include a productive or dry hacking cough, chest pain, dyspnea, fever, chills, and drenching sweats.

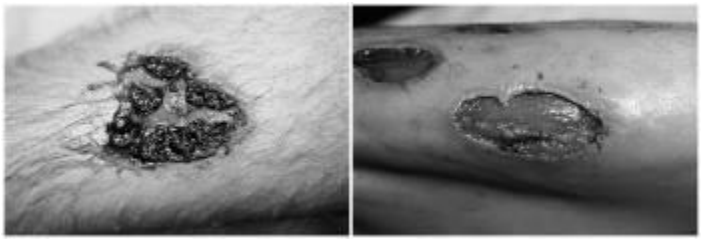
Pleural effusion occurs occasionally. Some patients have rapidly progressive infections, and acute respiratory distress syndrome may develop.

Extrapulmonary

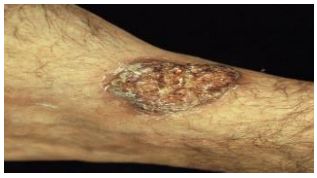
In extrapulmonary disseminated blastomycosis, symptoms depend on the organ involved.

Skin lesions are by far the most common site of dissemination; they may be single or multiple and may occur with or without clinically apparent pulmonary involvement. Papules or papulopustules usually appear on exposed surfaces and spread slowly. Painless abscesses, varying from pinpoint to 1 mm in diameter, develop on the advancing borders. Irregular, wartlike papillae may form on surfaces. Sometimes bullae develop. As lesions enlarge, the centers heal, forming atrophic scars. When fully developed, an individual lesion appears as an elevated verrucous patch, usually ≥ 2 cm wide with an abruptly sloping, purplish red, abscess-studded border. Ulceration may occur if bacterial superinfection is present.

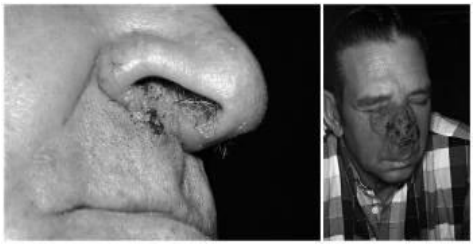
Images of Extrapulmonary Blastomycosis



Ulcers may have marked exudate at the base, as shown in the photo on the left, or a relative lack of exudate, as shown in the photo on the right.



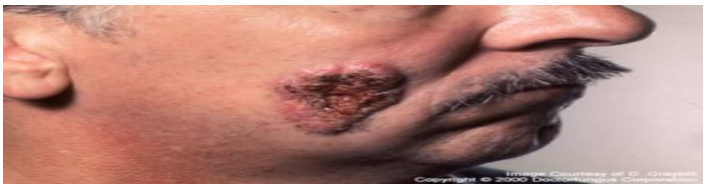
This image shows a raised, crusted, irregularly bordered ulcerative skin lesion with small microabscesses at the periphery of the lesion.



The photo on the left shows a mildly noticeable papule beneath the nare. The photo on the right shows destructive, infected, verrucous, ulcerative lesions on the nose.

Extrapulmonary blastomycosis may manifest in the skin or genitals. Skin lesions manifest as papules, pustules, or bullae. This photo shows a bullous skin lesion.

Image courtesy of the Public Health Image Library of the Centers for Disease Control and Prevention.



Extrapulmonary blastomycosis may occur in the skin or genitals. Skin lesions may develop wartlike papillae or a verrucous appearance. This photo shows a verrucous skin lesion.

If **bone lesions** develop, overlying areas are sometimes swollen, warm, and tender.
Genital lesions cause painful epididymal swelling, deep perineal discomfort, or prostatic tenderness detected during rectal examination.
Central nervous system involvement can manifest as brain abscess, epidural abscess, or meningitis.

Diagnosis

- Fungal cultures and smear
- *Blastomyces* urine antigen

If blastomycosis is suspected, a chest x-ray should be taken. Focal or diffuse infiltrates may be present, sometimes as patchy bronchopneumonia fanning out from the hilum. These findings must be distinguished from other causes of pneumonia (eg, other mycoses, tuberculosis [TB], tumors).

Skin lesions can be mistaken for sporotrichosis, TB, iodism, or basal cell carcinoma. Genital involvement may mimic TB.

Blastomycosis—Pulmonary

IMAGE COURTESY OF DR. HARDIN VIA THE PUBLIC HEALTH IMAGE LIBRARY OF THE CENTERS FOR DISEASE CONTROL AND PREVENTION.

Cultures of infected material are done; they are definitive when positive. Because culturing *Blastomyces* can pose a severe biohazard to laboratory personnel, the laboratory should be notified of the suspected diagnosis. The organism's characteristic appearance, seen during microscopic examination of tissues or sputum, is also frequently diagnostic.
Serologic testing is not sensitive but is useful if positive.

A urine antigen test is useful, but cross-reactivity with *Histoplasma* is high.
Molecular diagnostic tests (eg, polymerase chain reaction [PCR]) are becoming available.

Treatment

- For mild to moderate disease, itraconazole
- For severe, life-threatening infection, amphotericin B

(See also Antifungal Drugs and the Infectious Diseases Society of America's Practice Guidelines for the Management of Blastomycosis.)
Untreated blastomycosis is usually slowly progressive and is rarely ultimately fatal.

Treatment of blastomycosis depends on severity of the infection.

For **mild to moderate disease,** itraconazole 200 mg orally 3 times a day for 3 days, followed by 200 mg orally once a day or 2 times a day for 6 to 12 months is used. Fluconazole appears less effective, but 400 to 800 mg orally once a day may be tried in itraconazole-intolerant patients with mild disease.
For **severe, life-threatening infections,** IV amphotericin B is usually effective. The Infectious Diseases Society of America's guidelines recommend a lipid formulation of amphotericin B at a dosage of 3 to 5 mg/kg once a day or amphotericin B deoxycholate 0.7 to 1.0 mg/kg once a day for 1 to 2 weeks or until improvement is noted.
Therapy is changed to oral itraconazole once patients improve; dosage is 200 mg 3 times a day for 3 days, then 200 mg 2 times a day for ≥ 12 months.
Patients with central nervous system blastomycosis, pregnant patients, and immunocompromised patients should be treated with IV amphotericin B (preferably liposomal amphotericin B), using the same dose schedule as for life-threatening infection.

Voriconazole, isavuconazole, and posaconazole are active against *B. dermatitidis*, but clinical data are limited, and the role of these drugs has not yet been defined.

Key Points

- Inhaling spores of the dimorphic fungus *Blastomyces* can cause pulmonary disease and, less commonly, disseminated infection (particularly to the skin).
- In North America, blastomycosis is endemic in the regions around the Great Lakes and the Ohio–Mississippi River valleys (extending into the middle Atlantic and southeastern states).
- Diagnose using cultures of infected material; serologic testing is specific but not very sensitive.
- For mild to moderate disease, use itraconazole.
- For severe disease, use amphotericin B.

Coccidioides

Coccidioides is a genus of dimorphic ascomycetes in the family Onygenaceae. Member species are the cause of coccidioidomycosis, also known as San Joaquin Valley fever, an infectious fungal disease largely confined to the Western Hemisphere and endemic in the Southwestern United States.[2] The host acquires the disease by respiratory inhalation of spores disseminated in their natural habitat. The causative agents of coccidioidomycosis are *Coccidioides immitis* and *Coccidioides posadasii*. Both *C. immitis* and *C. posadasii* are indistinguishable during laboratory testing and commonly referred in literature as *Coccidioides*.[3]

Coccidioides immitis is a pathogenic fungus that resides in the soil in certain parts of the southwestern United States, northern Mexico, and a few other areas in the Western Hemisphere.

Epidemiology

C. immitis, along with its relative *C. posadasii*,[3] is most commonly seen in the desert regions of the southwestern United States, including certain areas of Arizona, California, New Mexico, Nevada, Texas, and Utah; and in Central and South America in Argentina, Brazil, Colombia, Guatemala, Honduras, Mexico, Nicaragua, Paraguay, and Venezuela.[4]

Precise location

C. immitis is largely found in California, but also Baja California and Arizona, while *C. posadasii* is regularly found in Texas, northern Mexico and in Central and South America. Both *C. immitis* and *C. posadasii* are present in Arizona.[5] C. immitis is more common west of the Tehachapi mountains, while *posadasii* east of it.[6] Coccidioides spp. are found in alkaline, sandy soils from semi-desert regions with hot summers, gentle winters, and annual rainfall between 10 and 50 cm. These fungi are usually found 10 to 30 cm beneath the surface.[7]

Clinical manifestation

C. immitis can cause a disease called coccidioidomycosis (valley fever).[8][9][10] Its incubation period varies from 7 to 21 days.[11] Coccidioidomycosis is not easily diagnosed on the basis of vital signs and symptoms, which are usually vague and nonspecific. Even a chest X-ray or CT scan cannot reliably distinguish it from other lung diseases, including lung cancer. Blood or urine

tests are administered, which aim to discover *Coccidioides* antigens. However, because the *Coccidioides* creates a mass that can mimic a lung tumor, the correct diagnosis may require a tissue sample (biopsy). A Gomori methenamine silver stain can then confirm the presence of the *Coccidioides* organism's characteristic spherules within the tissue. The *C. immitis* fungus can be cultured from a patient sample, but the culture can take weeks to grow and requires special precautions on a part of the laboratory staff while handling it (screw cap vials and sterile transfer hoods are recommended).[12] It is reported as the tenth-most often acquired infection in the laboratory conditions with two documented deaths.[2] Until October 2012, *C. immitis* had been listed as a select agent by both the U.S. Department of Health and Human Services and the U.S. Department of Agriculture, and was considered a biosafety level 3 pathogen.

Treatment

- Most *Coccidioides* infections have an incubation period from one to four weeks[2] and resolve without specific therapy; few clinical trials have assessed outcomes in less-severe disease.
- Commonly used indicators to judge the severity of illness include:[13]
  - Continuous fever for longer than 1 month
  - Body-weight loss of more than 10%
  - Intense night sweats that persist for more than 3 weeks
  - Infiltrates that involve more than half of one lung or portions of both lungs
  - Prominent or persistent hilar adenopathy
  - Anticoccidioidal complement fixation IgG titers of 1:16 or higher
  - Absence of dermal hypersensitivity to coccidioidal antigens
  - Inability to work
  - Symptoms that persist for more than 2 months
- Risk factors for dissemination (for which treatment should be initiated):
  - Primary infection during infancy
  - Primary infection during pregnancy, especially in the third trimester or immediately *post partum*
  - Immunosuppression (e.g., patients with HIV/AIDS, transplant recipients, patients receiving high-dose corticosteroids, those receiving antitumor necrosis factor medications)
  - Chronic debilitation or underlying disease, including diabetes mellitus or preexisting cardiopulmonary disease
  - High inoculum exposures
  - Certain ethnicities, such as Filipino, Black, or Hispanic
  - Age greater than 55 years

Azoles

The introduction of azoles revolutionized treatment for coccidioidomycosis,[14] and these agents are usually the first line of therapy. However, none of these azoles are safe to use in pregnancy and lactation because they have shown teratogenicity in animal studies.

Of the azoles, ketoconazole is the only one approved by the U.S. Food and Drug Administration (FDA) for treatment of coccidioidomycosis. Nevertheless, although it was

initially used in the long-term treatment of nonmeningeal extrapulmonary disease, more-potent, less-toxic triazoles (fluconazole and itraconazole) have replaced it. Itraconazole (400 mg/day) appears to have efficacy equal to that of fluconazole in the treatment of nonmeningeal infection and have the same relapse rate after therapy is discontinued. However, itraconazole seems to perform better in skeletal lesions, whereas fluconazole performs better in pulmonary and soft tissue infection. Serum levels of itraconazole are commonly obtained at the onset of long-term therapy because its absorption is sometimes erratic and unpredictable. Complications can include hepatic dysfunction.

For patients who are unresponsive to fluconazole, options are limited. Several case reports have studied the efficacy of three newer antifungal agents in the treatment of disease that is refractory to first-line therapy: posaconazole and voriconazole (triazole compounds similar in structure to fluconazole) and caspofungin (glucan synthesis inhibitor of the echinocandin structural class). However, these drugs have not been FDA approved, and clinical trials are lacking. Susceptibility testing of *Coccidioides* species in one report revealed uniform susceptibility to most antifungal agents, including these newer drugs.

In very severe cases, combination therapy with amphotericin B and an azole have been postulated, although no trials have been conducted. Caspofungin in combination with fluconazole has been cited as beneficial in a case report of a 31-year-old Asian patient with coccidioidal pneumonia. In a case report of a 23-year-old Black male with HIV and coccidioidal meningitis, combination therapy of amphotericin B and posaconazole led to clinical improvement.

Posaconazole has been approved by the European Commission as a salvage therapy for refractory coccidioidomycosis. Clinical trials are now ongoing for further evaluation. Voriconazole is also being studied in salvage therapy for refractory cases. A case report indicated that voriconazole in combination with amphotericin B as salvage therapy for disseminated coccidioidomycosis was successful.

Several case reports have studied caspofungin, with differing results. Caspofungin 50 mg/day following administration of amphotericin B in a patient with acute pulmonary coccidioidomycosis who had undergone transplantation showed promising results. In a patient with disseminated coccidioidomycosis, first-line therapy with amphotericin B and caspofungin alone failed to elicit a response, but the patient was then given caspofungin combined with fluconazole, with good results. A published report described a patient with disseminated and meningeal coccidioidomycosis in whom conventional therapy with fluconazole, voriconazole, and amphotericin B failed; caspofungin 50 mg/day after a loading dose of 70 mg intravenously was also unsuccessful.

Amphotericin

Amphotericin B, introduced in 1957, remains the treatment of choice for severe infections. It is usually reserved for worsening disease or lesions located in vital organs such as the spine. It can be administered either in the classic amphotericin B deoxycholate formulation or as a lipid formulation. No studies have directly compared amphotericin B with azole therapy. Complications include renal toxicity, bone marrow toxicity, and local systemic effects (fever, rigors).

Duration of therapy and costs

The objectives of treatment are resolution of infection, decrease of antibody titers, return of function of involved organs, and prevention of relapse. The duration of therapy is dictated by the clinical course of the illness, but it should be at least 6 months in all patients and often a year or longer in others. Therapy is tailored based on a combination of resolution of symptoms, regression of radiographic abnormalities, and changes in CF IgG titers. Immunocompromised patients and patients with a history of meningeal involvement require lifelong treatment.

Paracoccidioides brasiliensis

Paracoccidioides brasiliensis is a dimorphic fungus and one of the two species that cause paracoccidioidomycosis (the other being *Paracoccidioides lutzii).*[1][2][3][4] The fungus has been affiliated with the family Ajellomycetaceae (division Ascomycota) although a sexual state or teleomorph has not yet been found.

History

Paracoccidioides brasiliensis was first discovered by Adolfo Lutz in 1908 in Brazil.[6] Although Lutz did not suggest a name for the disease caused by this fungus, he made note of structures he called "pseudococcidica" together with mycelium in cultures grown at 25 °C.[6] In 1912, Alfonse Splendore[7] proposed the name *Zymonema brasiliense* and described the features of the fungus in culture.[6] Finally in 1930, Floriano de Almeida created the genus *Paracoccidioides* to accommodate the species, noting its distinction from *Coccidioides immitis*.[6]

Physiology

Paracoccidioides brasiliensis is a nonphotosynthetic eukaryote with a rigid cell wall and organelles very similar to those of higher eukaryotes.[3][8] Being a dimorphic fungus, it has the ability to grow an oval yeast-like form at 37 ℃ and an elongated mycelial form produced at room temperature.[9] The mycelial and yeast phases differ in their morphology, biochemistry, and ultrastructure.[8] The yeast form contains large amounts of α-(1,3)-linked glucan.[10][11] The chitin content of the mycelial form is greater than that of the yeast form, but the lipid content of both phases is comparable.[10] The yeast reproduces by asexual budding, where daughter cells are borne asynchronously at multiple, random positions across the cell surface. Buds begin by layers of cell wall increasing in optical density at a point that eventually gives rise to the daughter cell.[3] Once the bud has expanded, a cleavage plane develops between the nascent cell and the mother cell. Following dehiscence, the bud scar disappears.[8] In tissue, budding occurs inside the granulomatous center of the disease lesion, as visualized by hematoxylin and eosin (H&E) staining of histologic sections.[10] Nonbudding cells measure 5–15 μm in diameter, whereas those with multiple spherical buds measure from 10–20 μm in diameter.[10] In electron microscopy, cells with multiple buds have been found to have peripherally located nuclei and cytoplasm surrounding a large central vacuole.[12] In the tissue form of *P. brasiliensis,* yeast cells are larger with thinner walls and a narrower bud base than those of the related dimorphic fungus, *Blastomycosis dermatitidis*.[10] The yeast-like form of *P. brasiliensis* contains multiple nuclei, a porous two-layered nuclear membrane, and a thick cell wall rich in fibers, whereas the mycelial phase has thinner cell walls with a thin, electron-dense outer layer.[8]

Dimorphism

The mycelial form of *P. brasiliensis* can be converted to the yeast form *in vitro* by growth on brain heart infusion agar or blood-glucose-cysteine agar when incubated for 10–20 days at 37 °C.[10] Under these conditions, hyphal cells either die or convert to transitional forms measuring 6–30 µm in diameter, which ultimately detach or remain on the hyphal cells, yielding buds.[10] New buds develop mesosomes and become multinucleated.[10] In contrast, yeast-like cultures can be converted to the mycelial form by reducing the incubation temperature from 37 to 25 °C.[13] Initially, nutritional requirements of both the yeast and mycelial phases of *P. brasiliensis* were thought to be identical;[14] however, later studies demonstrated the yeast form to be auxotrophic, requiring exogenous sulfur-containing amino acids including cysteine and methionine for growth.[15]

Ecology

Although the habitat of *P. brasiliensis* remains unknown, it is commonly associated with soils in which coffee is cultivated.[5][16][17] It has also been associated with the nine-banded armadillo, *Dasypus novemcinctus*.[18] The disease caused by *P. brasiliensis* is mostly geographically restricted to Latin American countries such as Brazil, Colombia, and Venezuela, with the greatest number of cases seen in Brazil.[10] The endemic areas are characterized by hot, humid summers, dry temperate winters, average annual temperatures between 17 and 23 °C, and annual rainfall between 500 and 800 mm.[19] However, the precise ecology regularities of the fungus remain elusive, and *P. brasiliensis* has rarely been encountered in nature outside the human host.[3] One such rare example of environmental isolation was reported in 1971 by Maria B.de Albornoz and colleagues who isolated *P. brasiliensis* from samples of rural soil collected in Paracotos in the state of Miranda, Venezuela.[20] In *in vitro* studies, the fungus has been shown to grow when inoculated into soil and sterile horse or cow excrement.[21] The mycelial phase has also been shown to survive longer than the yeast phase in acidic soil.[22] Despite a sexual state not having been documented, molecular investigations suggest the existence of recombining populations of *P. brasiliensis*, potentially by means of an undiscovered sexual state.[23]

Epidemiology

P. brasiliensis causes a disease known as paracoccidioidomycosis characterized by slow, progressive granulomatous changes in the head mucosa, notably the nose and sinuses or the skin. Uncommonly, the disease affects the lymphatic system, the central nervous system, the gastrointestinal tract, or the skeletal system.[10] Due to the high proportion of cases affecting the oral mucosa, these tissues were originally thought to be the primary route of entry of fungus.[3] However, strong evidence now indicates the respiratory tract is the chief point of entry[10] and *P. brasiliensis* lung lesions occur in nearly a third of progressive cases.[24] The disease is not contagious.[10] Paracoccidioidomycosis is more frequently seen in adult males than females.[10][25] The hormone estrogen is thought to inhibit the transformation of the mycelial to the yeast form, as supported by *in vitro* experimental data, and this factor may account for the relative resistance of women to infection.[26]

Detection and surveillance

A number of serologic tests have been employed for the diagnosis of paracoccidioidomycosis.[10] Double diffusion in agar gel and complement fixation test, are

amongst the most commonly used tests in serodiagnosis.[10] Culture extracts of the yeast or mycelia are exploited to produce effective, quick, and reproducible antigens.[10][27] A study reported detection of 43 kD antigen in pooled sera of affected individuals, which might provide a basis for the development of a diagnostic test.[28] Tests targeting the presence of serum antibodies to *P. brasiliensis* simultaneously detect both active and historical infections and cannot discriminate active infection. The evaluation of populations in endemic zones has shown roughly equal rates of seroconversion between men and women, suggesting equal rates of exposure, despite the strong male predominance shown by the clinical disease.[10]

Clinical manifestations

P. brasiliensis causes mucous membrane ulceration of the mouth and nose with spreading through the lymphatic system. A hypothesis for entry of the fungus to the body is through periodontal membrane.[29][30] The route of infection is assumed to be inhalation following which the infective propagule gives rise to the distinctive multipolar budding yeast forms in the lung resembling a "ship's wheel" seen in histological sections.[9][31] Both immunologically normal and compromised people are at risk for infection.[9] The lungs, lymph nodes, and mucous membrane of the mouth are the most frequently infected tissues.[10] The pathological features of paracoccidioidomycosis are similar to those seen in coccidioidomycosis and blastomycosis.[32] However, in the former, the lesions first appear in the lymphoid tissue and then extend to mucous membranes,[32] producing localized to diffusive tissue necrosis of the lymph nodes.[32] The typically extensive involvement of lymphoid tissue and the limited occurrence of the gastrointestinal tract, bone and prostate set the clinical picture of paracoccidioidomycosis apart from that of blastomycosis.[10][32]

Diagnosis and Testing

Healthcare providers use a patient’s symptoms as well as laboratory tests and imaging tests, such as a chest X-ray, to diagnose paracoccidioidomycosis. [7] Often, a healthcare provider will perform a biopsy, which is a small sample from the body part that is affected. The sample is sent to a laboratory for a fungal culture or to be examined under the microscope. A blood test can also help diagnose paracoccidioidomycosis.

Treatment

Paracoccidioidomycosis can be treated with antifungal medicines such as itraconazole and amphotericin B. [7] Another medicine often used to treat paracoccidioidomycosis is trimethoprim/sulfamethoxazole (TMP/SMX), which is also known as co-trimoxazole and by several different brand names, including Bactrim, Septra, and Cotrim. Patients usually need treatment for about one year.

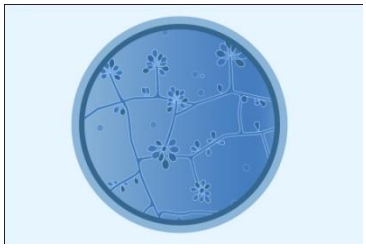
Sporotrichosis

What is sporotrichosis?

Sporotrichosis (also known as "rose gardener's disease") is an infection caused by a fungus called *Sporothrix*. This fungus lives throughout the world in soil and on plant matter such as sphagnum moss, rose bushes, and hay.[1,2] People get sporotrichosis by coming in contact with the fungal spores in the environment. Cutaneous (skin) infection is the most common form of the infection. It occurs when the fungus enters the skin through a small cut or scrape, usually after someone touches contaminated plant matter. Skin on the hands or arms is most commonly affected.

Types of sporotrichosis

- **Cutaneous (skin) sporotrichosis** is the most common form of the infection. It usually occurs on a person's hand or the arm after touching contaminated plant matter.
- **Pulmonary (lung) sporotrichosis** is rare but can happen after someone breathes in fungal spores from the environment.
- **Disseminated sporotrichosis** occurs when the infection spreads to another part of the body, such as bones, joints, or central nervous system. This form of sporotrichosis usually affects people with health problems or who take medicines that lower the body's ability to fight germs and sickness, such as people living with HIV (see Risk & Prevention).



Medical illustration of *Sporothrix schenckii*.

Sporotrichosis has been caused by scratches or bites from animals, particularly cats. Learn more about *Sporothrix brasiliensis*, a fungus that cats are spreading in Brazil and other areas of South America.

Symptoms

The symptoms of sporotrichosis depend on where the fungus is growing in the body. Contact your healthcare provider if you have symptoms that you think are related to sporotrichosis.

Sporotrichosis usually affects the skin or tissues underneath the skin. The first symptom of **cutaneous (skin) sporotrichosis** is usually a small, painless bump that can develop any time from 1 to 12 weeks after exposure to the fungus. The bump can be red, pink, or purple, and

usually appears on the finger, hand, or arm where the fungus has entered through a break in the skin. The bump will eventually grow larger and may look like an open sore or ulcer that is very slow to heal. Additional bumps or sores may appear later near the original one.

Pulmonary (lung) sporotrichosis is rare. Symptoms include cough, shortness of breath, chest pain, and fever.

Symptoms of **disseminated sporotrichosis** depend on the body part affected. For example, infection of the joints can cause joint pain that may be confused with rheumatoid arthritis. Infections of the central nervous system can involve difficulty thinking, headache, and seizures.

Risk & Prevention

Who gets sporotrichosis?

People who touch plant matter such as sphagnum moss, rose bushes, or hay are more likely to become infected. For example, sporotrichosis outbreaks have occurred among forestry workers, people who work in tree nurseries and garden centers, and people who handle hay bales.

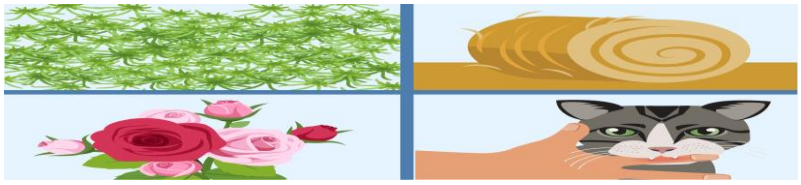
The severe forms of sporotrichosis (those that affect the lungs, bones or joints, or central nervous system) usually affect people with weakened immune systems or other diseases including diabetes, chronic obstructive pulmonary disease (COPD), alcoholism, or HIV.[1,3,4]

How can I lower the chance of developing sporotrichosis?

You can lower the chance of getting sporotrichosis by wearing protective clothing such as gloves and long sleeves when touching plant matter that can cause minor cuts or scrapes.

In Brazil, people have gotten sporotrichosis from contact with cats. This form of sporotrichosis (*Sporothrix brasiliensis*) has not been found in the United States. Be careful with unfamiliar animals, particularly cats. Cat bites and scratches can spread the fungus that causes sporotrichosis, and other diseases. This fungus is most often spread by stray cats and pet cats that are allowed outdoors. Learn more about sporotrichosis from cats.

Sources



Sporotrichosis is often linked to sphagnum moss, rose bushes, hay, or animal scratches or bites.
The fungus that causes sporotrichosis lives in the environment.

Sporothrix, the fungus that causes sporotrichosis, lives in the environment in soil and on plant matter such as sphagnum moss, rose bushes, hay, or wood. The microscopic fungus can enter the skin through small cuts or scrapes. In rare cases, breathing in the fungus can cause a pulmonary (lung) infection. The type of sporotrichosis found in North America is not contagious and can't spread from person to person. However, in South America, a type of sporotrichosis caused by *Sporothrix brasiliensis* spreads through scratches or bites from animals, particularly cats. (This fungal illness is not cat-scratch disease, a bacterial illness spread by cats – which occurs worldwide, wherever cats live.)

Diagnosis & Testing

Your healthcare provider will take a small tissue sample (biopsy) of the infected area of the body for laboratory tests. The laboratory will usually perform a fungal culture to find out what is causing the infection. Blood tests can help diagnose severe sporotrichosis, but usually can't diagnose skin infections.

Treatment

Most cases of sporotrichosis only involve the skin or the tissues underneath the skin. These infections are not life-threatening, but must be treated with prescription antifungal medicine for several months. The most common treatment for this type of sporotrichosis is itraconazole, taken by mouth for 3 to 6 months. Supersaturated potassium iodide (SSKI) is another treatment option for skin sporotrichosis. SSKI and azole drugs like itraconazole should not be used if you are pregnant.

If you have severe sporotrichosis that affects your lungs, bones, joints, or central nervous system, you'll probably receive intravenous amphotericin B medicine, which is given through a vein. After the first treatment with amphotericin B, you may receive itraconazole by mouth, for a total of at least 1 year of antifungal treatment. People with sporotrichosis in the lungs may also need surgery to cut away the infected tissue.

Healthcare providers: For detailed treatment guidelines, please refer to the Infectious Diseases Society of America's Clinical Practice Guidelines for the Management of Sporotrichosis pdf icon[PDF – 11 pages]external icon.

Statistics

Diagnosed sporotrichosis is rare, but the number of cases is difficult to determine because there is no national surveillance for it in the United States. Population-based incidence estimates for sporotrichosis were obtained from laboratory surveillance in the San Francisco Bay Area during 1992–1993 and suggested a yearly rate of less than one case per 1 million population.[5] However, more mild infections may not be diagnosed. Sporotrichosis may be more common in other parts of the world, such as Latin America.[2] For example, in the state of Rio de Janeiro, Brazil, more

than 2,200 cases were reported during 1998–2009.[6] Another study suggested a rate of 48 to 60 sporotrichosis cases per 100,000 population in the south central highlands of Peru.[7]

Sporotrichosis outbreaks

In the United States, sporotrichosis outbreaks have occurred among people who touched sphagnum moss or hay, such as forestry workers,[8,9] tree nursery and garden center workers,[10-12] and people who worked with or played on hay bales.[13-16] Sporotrichosis outbreaks also have been reported in several other countries, including Australia, Brazil, China, Guatemala, and South Africa.[2] Healthcare providers who are concerned about an unusual number of new cases should contact their state or local public health agency.

An ongoing outbreak of sporotrichosis is occurring in some cities in Brazil, where the infection has become common in outdoor cats and can spread to humans through bites or scratches. Learn more about this problem.

Penicillium marneffei

Talaromyces marneffei, formerly called *Penicillium marneffei*,[1] discovered in 1956, is now regarded as one of the world's ten most feared fungi.[2] It is a particularly important cause of disease due to weakened immunity (i.e. an opportunistic infection) in people living in southeast Asia whose immune systems have been weakened by HIV infection.[3][4]

When it was classified as a *Penicillium*, it was the only known thermally dimorphic species of that genus that caused a lethal systemic infection (talaromycosis), with fever and anaemia similar to disseminated cryptococcosis. This contrasted with related *Penicillium* species that are usually regarded as unimportant in terms of causing human disease.

Epidemiology

There is a high incidence of talaromycosis in AIDS patients in SE Asia; 10% of patients in Hong Kong get talaromycosis as an AIDS-related illness. Cases of *T. marneffei* human infections (talaromycosis) have also been reported in HIV-positive patients in Australia, Europe, Japan, the UK and the U.S.. All the patients, except one,[5] had visited Southeast Asia previously. The disease is considered an AIDS-defining illness.

Discovered in bamboo rats (*Rhizomys*) in Vietnam,[6] it is associated with these rats and the tropical Southeast Asia area. *Talaromyces marneffei* is endemic in Myanmar (Burma), Cambodia, Southern China, Indonesia, Laos, Malaysia, Thailand and Vietnam.

Although both the immunocompetent and the immunocompromised can be infected, it is extremely rare to find systemic infections in HIV-negative patients. The incidence of *T. marneffei* is increasing as HIV spreads throughout Asia. An increase in global travel and migration means it will be of increased importance as an infection in AIDS sufferers.

Talaromyces marneffei has been found in bamboo rat faeces, liver, lungs and spleen. It has been suggested that these animals serve as a reservoir for the fungus. It is not clear whether the rats are affected by *T. marneffei* or are merely asymptomatic carriers of the disease.

One study of 550 AIDS patients showed that the incidence was higher during the rainy season, which is when the rats breed. But this season also has conditions that are more favorable for production of fungal spores (conidia), which can become airborne and be inhaled by susceptible individuals.

Another study could not establish contact with bamboo rats as a risk factor, but exposure to the soil was the critical risk factor. However, soil samples failed to yield much of the fungus.

It is not known whether people get the disease by eating infected rats, or by inhaling fungi from their faeces.

One HIV-positive physician is known to have been infected while attending a course on tropical microbiology. He did not handle the organism, though students in the same laboratory did. It is presumed he contracted the infection by inhaling aerosol containing *T. marneffei* conidia. This shows that airborne infections are possible.

Clinical presentation

Patients commonly present with symptoms and signs of infection of the reticuloendothelial system, including generalized lymphadenopathy, hepatomegaly, and splenomegaly. The respiratory system is commonly involved as well; cough, fever, dyspnea, and chest pain may be present, reflecting the probable inhalational route of acquisition. Approximately one-third of patients may also exhibit gastrointestinal symptoms, such as diarrhea.[7][8][9]

Laboratory diagnosis

The fact that *Talaromyces marneffei* is thermally dimorphic is a relevant clue when trying to identify it. However, it should be kept in mind that other human-pathogenic fungi are thermally dimorphic as well. Cultures should be done from bone marrow, skin, blood and sputum samples.

Plating samples out onto two Sabouraud agar plates, then incubating one at 30°C and the other at 37°C, should result in two different morphologies. A mold-form will grow at 30°C, and a yeast-form at 37°C.

Mycelial colonies will be visible on the 30°C plate after two days. Growth is initially fluffy and white and eventually turns green and granular after sporulation has occurred. A soluble red pigment is produced, which diffuses into the agar, causing the reverse side of the plate to appear red or pink. The periphery of the mold may appear orange-coloured, and radial sulcate folds will develop.

Under the microscope, the mold phase will look like a typical *Penicillium*, with hyaline, septate and branched hyphae; the conidiophores are located both laterally and terminally. Each conidiophore gives rise to three to five phialides, where chains of lemon-shaped conidia are formed.

On the 37°C plate, the colonies grow as yeasts. These colonies can be cerebriform, convoluted, or smooth. There is a decreased production in pigment, the colonies appearing cream/light-tan/light-pink in colour. Microscopically, sausage-shaped cells are mixed with hyphae-like structures. As the culture ages, segments begin to form. The cells divide by binary fission, rather than budding. The cells are not yeast cells, but rather arthroconidia. Culturing isn't the only method of diagnosis. A skin scraping can be prepared, and stained with Wright's stain. Many intracellular and extracellular yeast cells with crosswalls are suggestive of *T. marneffei* infection.

Smears from bone marrow aspirates may also be taken; this is regarded as the most sensitive method. These samples can be stained with the Giemsa stain. Histological examination can also be done on skin, bone marrow or lymph nodes.

The patient's history also is a diagnostic help. If they have traveled to Southeast Asia and are HIV-positive, then there is an increased risk of them having talaromycosis.

Antigen testing of urine and serum, and PCR amplification of specific nucleotide sequences have been tried, with high sensitivity and specificity. Rapid identification of talaromycosis is sought, as prompt treatment is critical. Treatment should be provided as soon as talaromycosis is suspected.

Treatment

Treatment of talaromycosis depends on the degree of immunosuppression and organ involvement, but most isolates of *Talaromyces marneffei* display low MIC's to amphotericin B as well as itraconazole, posaconazole and voriconazole.

Dermatophytes

Dermatophytes (from Greek δέρμα *derma* "skin" (GEN δέρματος *dermatos*) and φυτόν *phyton* "plant")[1] are a common label for a group of three types of fungus that commonly causes skin disease in animals and humans.[2] These anamorphic (asexual or imperfect fungi) mold genera are: *Microsporum*, *Epidermophyton* and *Trichophyton*.[3] There are about 40 species in these three genera. Species capable of reproducing sexually belong in the teleomorphic genus Arthroderma, of the Ascomycota (see Teleomorph, anamorph and holomorph for more information on this type of fungal life cycle).

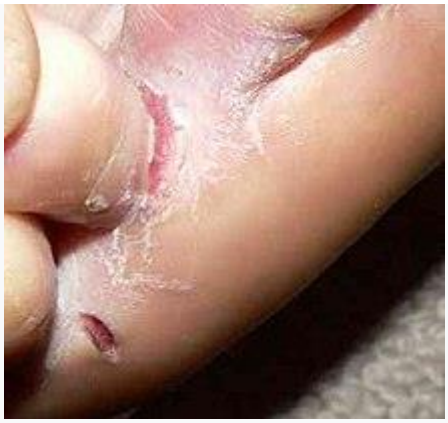
Dermatophytes cause infections of the skin, hair, and nails, obtaining nutrients from keratinized material.[4] The organisms colonize the keratin tissues causing inflammation as the host responds to metabolic byproducts. Colonies of dematophytes are usually restricted to the nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immunocompetent host. Invasion does elicit a host response ranging from mild to severe. Acid proteinases (proteases),[5] elastase, keratinases, and other proteinases reportedly act as virulence factors. Additionally, the products of these degradative enzymes serve as nutrients for the fungi.[5] The development of cell-mediated immunity correlated with delayed hypersensitivity and an inflammatory response is associated with clinical cure, whereas the lack of or a defective cell-mediated immunity predisposes the host to chronic or recurrent dermatophyte infection.

Some of these skin infections are known as ringworm or tinea (which is the Latin word for "worm"), though infections are not caused by worms.[3][6] It is thought that the word tinea (worm) is used to describe the snake-like appearance of the dermatophyte on skin.[6] Toenail and fingernail infections are referred to as onychomycosis. Dermatophytes usually do not invade living tissues, but colonize the outer layer of the skin. Occasionally the organisms do invade subcutaneous tissues, resulting in kerion development.

Types of infections

Infections by dermatophytes affect the superficial skin, hair, and nails are named using "tinea" followed by the Latin term for the area that is affected.[3] Manifestation of infection tends to involve erythema, induration, itching, and scaling. Dermatophytoses tend to occur in moist areas and skin folds.[7] The degree of infection depends on the specific site of infection, the fungal species, and the host inflammatory response.[7]

Although symptoms can be barely noticeable in some cases, dermatophytoses can produce "chronic progressive eruptions that last months or years, causing considerable discomfort and disfiguration." [7] Dermatophytoses are generally painless and are not life-threatening.[7]



Tinea pedis also known as athletes foot.

Tinea pedis or athlete's foot

Contrary to the name, tinea pedis does not solely affect athletes. Tinea pedis affects men more than women, and is uncommon in children.[8][3] Even in developed countries, tinea pedis is one of the most common superficial skin infections by fungi.[8]

The infection can be seen between the toes (interdigital pattern)[9] and may spread to the sole of the foot in a "moccasin" pattern. In some cases, the infection may progress into a "vesiculobullous pattern" in which small, fluid-filled blisters are present.[9] The lesions may be accompanied by peeling, maceration (peeling due to moisture), and itching.[3]

Later stages of tinea pedis might include hyperkeratosis (thickened skin) of the soles, as well as bacterial infection (by streptococcus and staphylococcus) or cellulitis due to fissures developing between the toes.[3][10]

Another implication of tinea pedis, especially for older adults or those with vascular disease, diabetes mellitus, or nail trauma, is onychomycosis of the toenails.[3] Nails become thick, discolored, and brittle, and often onycholysis (painless separation of nail from nail bed) occurs.[3]

Tinea cruris or jock itch

More commonly occurs in men than women. Tinea cruris may be exacerbated by sweat and tight clothing (hence the term "jock itch").[3][9] Frequently, the feet are also involved. The theory is that the feet get infected first from contact with the ground. The fungus spores are carried to the groin from scratching from putting on underclothing or pants. The infection frequently extends from the groin to the perianal skin and gluteal cleft.

The rashes appear red, scaly, and pustular, and is often accompanied by itch. Tinea cruris should be differentiated from other similar dermal conditions such as intertriginous candidiasis, erythrasma, and psoriasis.[3]

Tinea corpora or ringworm of the body

Tinea Corporis of the arm with an active border and central clearing.

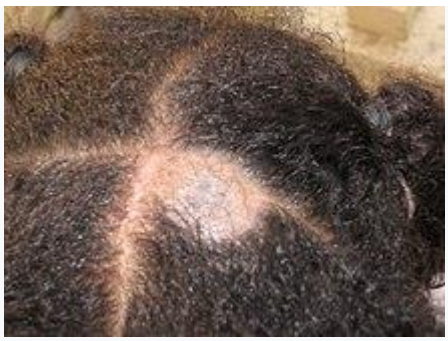
Lesions appear as round, red, scaly, patches with well-defined, raised edges, often with a central clearing and very itchy (usually on trunk, limbs, and also in other body parts). The lesions can be confused with contact dermatitis, eczema, and psoriasis.[3]

Tinea faciei or facial ringworm

Round or ring shaped red patches may occur on non-bearded areas of the face.[10] This type of dermatophytosis can have a subtle appearance, sometimes known as "tine incognito". [10] It can be misdiagnosed for other conditions like psoriasis, discoid lupus, etc. and might be aggravated by treatment with immunosuppressive topical steroid creams.[11]

Tinea capitis or scalp ("blackdot") ringworm

Children from ages 3 to 7 are most commonly infected with tinea capitis.[3] Trichophyton tonsurans is the most common cause of out breaks of tinea capitis in children, and is the main cause of endothrix (inside hair) infections. Trichophyton rubrum is also a very common cause of favus, a form of tinea capitis in which crusts are seen on the scalp.



Tinea capitis is characterized by irregular or well-demarcated alopecia (balding) and scaling.

Infected hair shafts are broken off just at the base, leaving a black dot just under the surface of the skin, and alopecia can result.[3] Scraping these residual black dot will yield the best diagnostic scrapings for microscopic exam. Numerous green arthrospores will be seen under the microscope inside the stubbles of broken hair shafts at 400x. Tinea capitis cannot be treated topically, and must be treated systemically with antifungals.[12]

Tinea manuum or ringworm of the hands

In most cases of tinea manuum, only one hand is involved. Frequently both feet are involved concurrently, thus the saying "one hand, two feet".

Onychomycosis, tinea unguium, or ringworm of the nail

See Onychomycosis

Tinea incognito

Ringworm infections modified by corticosteroids, systemic or topical, prescribed for some pre-existing pathology or given mistakenly for the treatment of misdiagnosed tinea.

Pathogenesis

In order for dermatophytoses to occur, the fungus must directly contact the skin.[7] Likelihood of infection is increased if the skin integrity is compromised, as in minor breaks.[7]

The fungi use various proteinases to establish infection in the keratinized stratum corneum.[7] Some studies also suggest that a class of proteins called LysM coat the fungal cell walls to help the fungi evade host cell immune response.[7]

The course of infection varies between each case, and may be determined by several factors including: "the anatomic location, the degree of skin moisture, the dynamics of skin growth and desquamation, the speed and extent of the inflammatory response, and the infecting species."[7]

The ring shape of dermatophyte lesions result from outward growth of the fungi.[3] The fungi spread in a centrifugal pattern in the stratum corneum, which is the outermost keratinized layer of the skin.[3]

For nail infections, the growth initiates through the lateral or superficial nail plates, then continues throughout the nail.[3] For hair infections, fungal invasion begins at the hair shaft.[3]

Symptoms manifest from inflammatory reactions due to the fungal antigens.[3] The rapid turnover of desquamation, or skin peeling, due to inflammation limits dermatophytoses, as the fungi are pushed out of the skin.[7]

Dermatophytoses rarely cause serious illness, as the fungi infection tends to be limited to the superficial skin.[8] The infection tends to self-resolve so long as the fungal growth does not exceed inflammatory response and desquamation rate is sufficient.[7] If immune response is insufficient, however, infection may progress to chronic inflammation.[7]

Immune response

Fortunately, dermatophytoses soon progress from the inflammatory stage to spontaneous healing, which is largely cell-mediated.[7] Fungi are destroyed via oxidative pathways by phagocytes both intracellularly and extracellularly.[7] T-cell-mediated response using TH1 cells are likely responsible for controlling infection.[7] It is unclear whether the antifungal antibodies formed in response to the infection play a role in immunity.[7]

Infection may become chronic and widespread if the host has a compromised immune system and is receiving treatment that reduces T-lymphocyte function.[7] Also, the responsible species for chronic infections in both normal and immunocompromised patients tends to be *Trichophyton rubrum;* immune response tends to be hyporeactive.[7] However, "the clinical manifestations of these infections are largely due to delayed-type hypersensitivity responses to these agents rather than from direct effects of the fungus on the host."[7]

Diagnosis and identification

Usually, dermatophyte infections can be diagnosed by their appearance.[3] However, a confirmatory rapid in-office test can also be conducted, which entails using a scalpel to scrape

off a lesion sample from the nail, skin, or scalp and transferring it to a slide. Potassium hydroxide (KOH) is added to the slide and the sample is examined with a microscope to determine presence of hyphae.[3] Care should be taken in procurement of a sample, as false-negative results may occur if the patient is already using an antifungal, if too small a sample is obtained, or if sample from a wrong site is collected.[8]

Additionally, a Wood's lamp examination (ultraviolet light) may be used to diagnose specific dermatophytes that fluoresce.[10] Should there be an outbreak or if a patient is not responding well to therapy, sometimes a fungal culture is indicated.[3] A fungal culture is also used when long-term oral therapy is being considered. [10]

Fungal culture medium can be used for positive identification of the species. The fungi tend to grow well at 25 degrees Celsius on Sabouraud's agar within a few days to a few weeks.[7] In the culture, characteristic septate hyphae can be seen interspersed among the epithelial cells, and the conidia may form either on the hyphae or on conidiophores.[7] Trichophyton tonsurans, the causative agent of tinea capitis (scalp infection) can be seen as solidly packed arthrospores within the broken hairshafts scraped from the plugged black dots of the scalp. Microscopic morphology of the micro- and macroconidia is the most reliable identification character, but both good slide preparation and stimulation of sporulation in some strains are needed. While small microconidia may not always form, the larger macroconidia aids in identification of the fungal species.[7]

Culture characteristics such as surface texture, topography and pigmentation are variable, so they are the least reliable criteria for identification. Clinical information such as the appearance of the lesion, site, geographic location, travel history, animal contacts and race is also important, especially in identifying rare non-sporulating species like Trichophyton concentricum, Microsporum audouinii and Trichophyton schoenleinii.

A special agar called Dermatophyte Test Medium (DTM) has been formulated to grow and identify dermatophytes.[14] Without having to look at the colony, the hyphae, or macroconidia, one can identify the dermatophyte by a simple color test. The specimen (scraping from skin, nail, or hair) is embedded in the DTM culture medium. It is incubated at room temperature for 10 to 14 days. If the fungus is a dermatophyte, the medium will turn bright red. If the fungus is not a dermatophyte, no color change will be noted. If kept beyond 14 days, false positive can result even with non-dermatophytes. Specimen from the DTM can be sent for species identification if desired.

Often dermatophyte infection may resemble other inflammatory skin disorders or dermatitis, thus leading to misdiagnosis of fungal infections.[8]

Transmission

Dermatophytes are transmitted by direct contact with an infected host (human or animal)[3] or by direct or indirect contact with infected shed skin or hair in fomites such as clothing, combs, hair brushes, theatre seats, caps, furniture, bed linens, shoes,[15] socks,[15] towels, hotel rugs, sauna, bathhouse, and locker room floors. Also, transmission may occur from soil-to-skin contact.[3] Depending on the species the organism may be viable in the environment for up to 15 months.

While even healthy individuals may become infected,[8] there is an increased susceptibility to infection when there is a preexisting injury to the skin such as scars, burns, excessive

temperature and humidity. Adaptation to growth on humans by most geophilic species resulted in diminished loss of sporulation, sexuality, and other soil-associated characteristics.

Classification

Dermatophytes are classified as anthropophilic (humans), zoophilic (animals) or geophilic (soil) according to their normal habitat.

- Anthropophilic dermatophytes are restricted to human hosts and produce a mild, chronic inflammation.
- Zoophilic organisms are found primarily in animals and cause marked inflammatory reactions in humans who have contact with infected cats, dogs, cattle, horses, birds, or other animals. Infection may also be transmitted via indirect contact with infected animals, such as by their hair.[5] This is followed by a rapid termination of the infection.
- Geophilic species are usually recovered from the soil but occasionally infect humans and animals. They cause a marked inflammatory reaction, which limits the spread of the infection and may lead to a spontaneous cure but may also leave scars.

Sexual reproduction

Dermatophytes reproduce sexually by either of two modes, heterothallism or homothallism.[16] In heterothallic species, interaction of two individuals with compatible mating types are required in order for sexual reproduction to occur. In contrast, homothallic fungi are self-fertile and can complete a sexual cycle without a partner of opposite mating type. Both types of sexual reproduction involve meiosis.

Frequency of species

In North America and Europe, the nine most common dermatophyte species are:

- *Trichophyton: rubrum, tonsurans, mentagrophytes, verrucosum, and schoenlenii*[8]
- *Microsporum: canis, audouinii, and gypseum*[8]
- *Epidermophyton: floccosum*[8]

- About 76% of the dermatophyte species isolated from humans are *Trichophyton rubrum*.
- 27% are *Trichophyton mentagrophytes*
- 7% are *Trichophyton verrucosum*
- 3% are *Trichophyton tonsurans*
- Infrequently isolated (less than 1%) are *Epidermophyton floccosum*, *Microsporum audouinii*, *Microsporum canis*, *Microsporum equinum*, *Microsporum nanum*, *Microsporum versicolor*, *Trichophyton equinum*, *Trichophyton kanei*, *Trichophyton raubitschekii*, and *Trichophyton violaceum*.[*citation needed*]

The mixture of species is quite different in domesticated animals and pets (see ringworm for details).

Epidemiology

Since dermatophytes are found worldwide, infections by these fungi are extremely common.[3]

Infections occur more in males than in females, as the predominantly female hormone, progesterone, inhibits the growth of dermatophyte fungi.[3]

Medications

General medications for dermatophyte infections include topical ointments.[3]

- Topical medications like clotrimazole, butenafine, miconazole, and terbinafine.
- Systemic medications (oral) like fluconazole, griseofulvin, terbinafine, and itraconazole.

For extensive skin lesions, itraconazole and terbinafine can speed up healing. Terbinafine is preferred over itraconazole due to fewer drug interactions.[3]

Treatment

Tinea corpora (body), tinea manus (hands), tinea cruris (groin), tinea pedis (foot) and tinea facie (face) can be treated topically.

Tinea unguum (nails) usually will require oral treatment with terbinafine, itraconizole, or griseofulvin. Griseofulvin is usually not as effective as terbinafine or itraconizole. A lacquer (Penlac) can be used daily, but is ineffective unless combined with aggressive debridement of the affected nail.

Tinea capitis (scalp) must be treated orally, as the medication must be present deep in the hair follicles to eradicate the fungus. Usually griseofulvin is given orally for 2 to 3 months.[17] Clinically dosage up to twice the recommended dose might be used due to relative resistance of some strains of dermatophytes.

Tinea pedis is usually treated with topical medicines, like ketoconazole or terbinafine, and pills, or with medicines that contains miconazole, clotrimazole, or tolnaftate.[17] Antibiotics may be necessary to treat secondary bacterial infections that occur in addition to the fungus (for example, from scratching).

Tinea cruris (groin) should be kept dry as much as possible.

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DEPARTMENT OF BIOTECHNOLOGY

B.SC. MICROBIOLOGY

UNIT – IV – Medical Mycology – SMB3103

Mycetoma

Mycetoma is a disease caused by certain types of bacteria and fungi found in soil and water. These bacteria and fungi may enter the body through a break in the skin, often on a person's foot. The resulting infection causes firm, usually painless but debilitating masses under the skin that can eventually affect the underlying bone. Mycetoma can be caused by bacteria (actinomycetoma) or fungi (eumycetoma). The number of people with mycetoma worldwide is not known, but there were 17,607 cases reported in a 2017 review of scientific articles between 1950 and 2017. The actual number of cases is likely substantially higher.

Mycetoma affects people of all ages and is more common in men. This disease primarily affects poorer people in rural regions of Africa, Latin America, and Asia that are located near the equator and have dry climates. People affected by mycetoma often live in remote areas where they have limited access to healthcare and medications. Mycetoma can cause severe physical disabilities that can force people to stop working and cause stigma.

Mycetoma has rarely been reported in the United States in recent decades. A review of the literature from 1890 to 2020 showed fewer than 80 cases occurring in the United States. People who travel from the United States to areas where mycetoma has been reported are unlikely to get mycetoma. Developing mycetoma requires repeatedly exposing broken skin to soil and water that contain the microbes that cause mycetoma over long periods of time. Travelers are unlikely to have enough exposure to be at risk.

Diagnosis requires laboratory evaluation of a biopsy, or small tissue sample, of the infected area. The treatment for mycetoma includes antibiotics or antifungal medicine, depending on whether the disease is caused by bacteria or fungi. Surgery is sometimes needed to cut away the infected tissue. Wearing shoes might help prevent mycetoma.

Symptoms

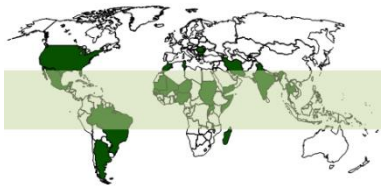
Man with a swollen foot caused by mycetoma.

Symptoms are similar for bacterial and fungal mycetoma. Both appear as firm, painless masses under the skin. These masses usually appear on a person's foot but can form anywhere on the body. The mycetoma masses start small, but over time they can grow larger, develop oozing sores, and cause the affected limb to become deformed or unusable. If mycetoma is not treated or if treatment fails, it can spread to other areas of the body. Long-term mycetoma can eventually destroy the underlying muscle and bone.

Risk & Prevention

Mycetoma is a health problem in equatorial regions of Africa, Latin America, and Asia known as the "mycetoma belt." Fungal mycetoma (eumycetoma) is the most common type in Africa, while

bacterial mycetoma (actinomycetoma) causes most cases in South and Central America and some Asian countries.



Countries shown in dark green indicate those in which cases of mycetoma have been reported in the medical literature. The mycetoma belt region is shaded in light green.
Mycetoma affects people of all ages and is more common in men. Many people with mycetoma work in agricultural jobs, such as farmers and livestock herders.

Health care providers and researchers believe that wearing shoes might prevent injuries that can lead to mycetoma. Shoes protect the feet while someone is walking or working outside in areas where the germs that cause mycetoma are common in water and soil. Early detection and treatment, before symptoms cause serious effects, can reduce disabilities from mycetoma and may cure the condition.

Transmission

The bacteria and fungi that cause mycetoma live in soil and water. These germs can enter the body through wounds or other small skin injuries, like a thorn prick. It is not known why some people develop mycetoma and others do not, but aspects of the environment and living conditions are likely involved. Mycetoma does not spread between people.

Diagnosis

A doctor can diagnose mycetoma by taking a small sample (biopsy) of the infected area of the body and sending it to a laboratory. The laboratory may examine the sample under a microscope, but this test may not always determine if the infection is caused by bacteria or fungi and cannot determine what type of bacteria or fungi is the cause of the mycetoma. A culture (growing the bacteria or fungi in the laboratory) can determine the specific type of bacteria or fungus causing the infection. A doctor may also do an imaging test such as an X-ray or ultrasound to diagnose mycetoma and see how much damage has taken place to muscle and bone. Patients can avoid long-term infection and amputation by seeking care and detecting and treating mycetoma early.

Treatment

Therapies that work against mycetoma are limited, may have to be taken for a long time, and can be expensive. The treatment for mycetoma depends on whether it is caused by bacteria (actinomycetoma) or fungi (eumycetoma).

- Actinomycetoma is usually treatable with antibiotics, and surgery is usually not needed.

- Eumycetoma is usually treated with long-term antifungal medicine, but treatment may not be completely effective. In this case, surgery or amputation are sometimes needed to cut away the infected tissue.

keratomycosis

Fungal keratitis or keratomycosis refers to an infective process of the cornea caused by any of the multiple pathologic fungi capable of invading the ocular surface. It is most typically a slow, relentless disease that must be differentiated from other types of corneal conditions with similar presentation; especially its bacterial counterpart, which accounts for the majority of the microbial corneal infections.

Disease Entity

Fungal Keratitis

Disease

Fungal keratitis is a serious ocular infection with potentially catastrophic visual results. Caused by any of the many species of fungi capable of colonizing human tissue, its occurs worldwide and its incidence is increasing in frequency.

Etiology

The list covers many fungi including but not limited to yeasts of Candida spp., filamentous with septae such as Aspergillus spp., Fusarium spp., Cladosporium,spp., Curvularia, and non septated such as Rhizopus. Bare in mind that any agent capable of infecting humans is a potential infectious agent, especially if the host has a debilitating disease.

Risk Factors

Risk factors include trauma, ocular surface disease, and topical steroid use. Risks and type of fungi also might vary by geographic location and climate.[1] [2] [3] [4] [5] [6] [7] In warmer climates the rule is that the most common organisms are filamentous fungi, like Fusarium spp and Aspergillus spp. with a strong relationship to trauma. Reports from Brazil show the most common isolates in descending order were Fusarium spp in 67%, Aspergillus spp in 10.5%, and Candida spp in 10%. About 40% of the infections were related to trauma.[1]

In the northern USA, corneal infection by fungus was, until recently, more common in debilitated or immunocompromised patients and the causative organism being a yeast, such as Candida albicans. Filamentous fungi in these latitudes were then rarely reported. A few years ago a breakout of Fusarium keratitis associated with a type of contact lens solution[8] displaced yeasts as the most common fungal corneal infection in some areas. This trend persists in the most recent epidemiological reports. Still they are, in most cases, related to contact lens use.[9] It should be noted that the incidence of contact lens-related fungal keratitis was increasing before the Fusarium outbreak.

This new distribution means that we no longer can rely on the geographical distribution only to initiate empirical treatment. Broad-spectrum treatment should be administerd once there is a strong probability of a mycotic infection.

General Pathology

Even though fungi can be classified as a kingdom due to their complexity and unique characteristics, a simple practical classification for ocular infection is used. Under this method, morphology and type of reproductive method define the type of fungi. They are classified for our purpose as yeast, filamentous septated pigmented and non-pigmented and filamentous without septae. Fungi are present worldwide and can be part of the ocular flora. They are eukaryotic with a defined nucleus surrounded by a membrane. They can be either saprophytic, free organisms that subsist on decaying organic matter or pathologic and require a living host for perpetuation.

The American Academy of Ophthalmology's Pathology Atlas contains virtual microscopy images of tissue samples with the following:

- Aspergillus Keratitis
- Fungal Keratitis

Pathophysiology

The infection probably starts when the epithelial integrity is broken either due to trauma or ocular surface disease and the organism gains access into the tissue and proliferates. Proteolytic enzymes, fungal antigens and toxins are liberated into the cornea with the resulting necrosis and damage to its architecture thus compromising the eye integrity and function.

Primary prevention

Wearing safety glasses while gardening will diminish the risk of ocular trauma. Also, general hygiene, proper contact lens care, and avoidance of nonessential steroid use should diminish the probability of mycotic infection.

Diagnosis

A high degree of suspicion from the physician accounts for early diagnosis and treatment, which are paramount for a successful resolution of the fungal keratitis. Corneal ulcers unresponsive to broad-spectrum antibiotics, the presence of satellite lesions, and scanty secretions in a large ulcer are some signs that should raise flags to the attending professional about the possibility of a mycotic agent.

History

Blurred vision, redness, tearing, photophobia, pain, foreign body sensations, secretions related to trauma, ocular surface disease and topical steroid use are all important characteristics to ascertain in the history.

Physical examination

After establishing the patient's general condition, the examiner should look for evidence of ocular surface disease and determine the amount and type of secretions and lid swelling. The upper eyelid should be everted to exclude a retained foreign body. The examiner should measure

the size and depth of the lesion as well as the presence of satellite lesions. The intraocular pressure should also be ascertained. Anterior chamber reaction and evidence of hypopyon should also be recorded. Vitreous reaction if present may suggest intraocular spread of the disease.

Signs

With filamentary fungi, the corneal lesions have a white/gray infiltrate with feathery borders. There might be satellite lesions with a hypopyon and conjunctival injection as well as purulent secretions. Ulcers caused by yeast are plaque-like and slightly more defined, similar to bacterial keratitis.

Symptoms

Symptoms are similar to any corneal infection including blurred vision, redness, tearing, photophobia, pain, foreign body sensation and secretions. In some cases the lesion are rather indolent which help to delay the diagnosis and hence delay the treatment. Suspicion should be high in cases of trauma with vegetable matter.

Clinical diagnosis

Under the slit lamp, early on the lesion might look like an unhealed corneal abrasion with scanty infiltrates and no secretions. With time however, the ulcer develops thicker infiltrates and fuzzy margins. The presence of satellite lesions strongly suggests a fungal infection. Redness and periocular edema are also common. This combined with a history of trauma, especially with vegetable matter, ocular surface disease or chronic use of topical steroids should alert the practitioner to the possibility of a mycotic etiology.

Diagnostic procedures

Corneal scrapings are taken from deep within the lesion with a surgical blade or sterile spatula. To perform a corneal biopsy, a dermatological 2mm punch can be used.

Laboratory test

For a definitive diagnosis, scrapings taken from deep within the lesion should be made and inoculated in Sabouraud agar. The shortcoming is that it can take up to 3 weeks to grow and identify the organism. For a faster result, smears with special stains such as Gomori, PAS, acridine orange, calcofluor white or KOH should be performed. The drawback is that not all laboratories have these stains available, so, again we might need to rely on the patient's evolution and the physician's clinical acumen. If all labs and cultures are negative, corneal biopsy should be considered to obtain a specimen.

Differential diagnosis

Fungal infections can mimic any microbial keratitis secondary to other causes: Bacteria, which is the most common cause of corneal infections; Acanthamoeba, related to swimming with contact lenses and or the use of tap water in their cleaning; or herpes simplex or herpes zoster for which recurrences are frequent. Other conditions such as a retained foreign body, sterile infiltrates, marginal ulcers due to Staphylococcal hypersensitivity or chronic epithelial defect should also be ruled out. Again, a high index of suspicion is important in the diagnosis of fungal keratitis.

Management

In general, management consists of medical therapy with the use of topical and/or systemic anti-fungal medications alone or in combination with surgical treatment.

General treatment

Topical antifungals, either commercially available or compounded from systemic preparation into eye-drops are the backbone for the management of fungal keratitis. In resistant cases, the addition of systemic antifungal have shown effectiveness. If those treatments fail, then conjunctival flaps , lamellar or penetrating keratoplasty might be needed.

Medical therapy

Fungal ulcers are inherently difficult to treat. The diagnosis is often delayed and medications available for ocular therapy are limited and are deficient in their ability to penetrate deep into the cornea. The mainstay of treatment is the use of antifungal drops but only a polyene, natamycin 5%, is FDA approved and commercially available for topical ocular use. Also, due to the rarity of the diagnosis it is sometimes available only as a special order. Furthermore, the complexity of the disease and possible visually devastating outcomes require a rather aggressive approach to its treatment. Hence, many other antimycotics are used to fight this infection. These compounded eye drops are made by diluting the intravenous medication into concentrations that provide enough medication to eradicate the organism while being tolerated by the eye.

Prior to the development of natamycin the most commonly used antifungal was amphotericin b, a polyene, in a 0.15% dilution in sterile water (one 50mg vial of amphotericin b diluted in 30cc sterile water gives a 0.166% dilution). It is still used today alone and in combination with natamycin with relatively good results. Since they both are readily available, they are both a good choice as initial therapy.

Voriconazole, a triazole antifungal agent derived from fluconazole, can be used either topically at 1% dilution, orally at 400 mg twice a day and even has being injected in the corneal stroma around the fungal lesion (50 micrograms/0.1 ml)[10] [11] [12]

Oral posaconazole, a new generation triazole, has been successful eradicating deep infections of resistant Fusarium[13]Subconjuctival antifungals are not generally used because the produce severe pain and some might even induce tissue necrosis.

Other antifungals available include the azoles like miconazole, clotrimazole ketoconazole, posaconazole, and fluconazole and the echinocandin antifungal agents caspofungin, and micafungin. The echinocandins are not active against Fusarium.

Medical follow up

All corneal infections should be followed daily until there is a marked improvement. Since fungal infections run a protracted course, their follow up is longer and after a few days the interval between evaluations increases according to its progress. Complete healing might take weeks and even months. The intraocular pressure should be closed monitored during the episode. It should be noted that epithelialization does not necessary means that the ulcer is healing. In fact it might hinder the penetration of the fungicide. Confocal microscopy might be an effective additional method to follow the success or failure of the therapy. It does so by direct examination of the organism, inflammation, and corneal stromal cells.[14]

Surgery

Periodic debridement is commonly used in the management fungal keratitis. The procedure removes necrotic tissue and diminishes the organism load but mostly it enhances the penetration of the drugs. It can be performed every 24 to 48 hours.

If everything fails, a conjunctival flap might deter the infection. If there is no response, then a lamellar or penetrating keratoplasty could be needed. If there is a perforation, a patch graft or a therapeutic transplant should be performed. The infected cornea should be sent for cultures and pathological evaluation. It is performed in the usual manner but it should extend about 1 to 1.5mm beyond the margins of the lesion.

Surgical follow up

Close follow up for at least 2 weeks with topical antimycotics is recommended. Systemic medication may be added as well. If the edges of the specimen are found by pathology to have organisms the use of topical and systemic antifungals should be extended.

Complications

Adverse results range from mild to severe corneal scarring, corneal perforation, anterior segment disruption and glaucoma to endophthalmitis resulting in evisceration.

Prognosis

The aftermath of fungal keratitis can be dismal . There is severe visual loss in 26% to 63% of patients. Fifteen to twenty percent may need evisceration. Penetrating keratoplasty is required in 31 to 38% of cases.[1][15]

Otomycosis

Otomycosis is an infection caused by a fungus. There are several different types of fungus that can cause this infection, but most otomycosis infections are related to *Aspergillus* species or, less commonly, *Candida*.

People come into contact with fungi every day in the environment, but fungi do not typically pose a problem.

However, those with weakened immune systems can catch an infection more easily than others when they come into contact with a fungus.

Also, people who live in hot or tropical climates are more likely to experience otomycosis, as fungi thrive in warm, damp places.

Other risk factors include:

- trauma to the ear from hearing aids or cotton swabs
- chronic skin conditions, such as eczema
- having diabetes mellitus

- participating in water sports, including swimming or surfing
- swimming in contaminated water
- lack of cerumen, or earwax, which suppresses bacterial or fungal growth and stops the ear canal drying out

Symptoms

Typical symptoms of otomycosis include:

- hearing loss, which can be mistaken for deafness
- a feeling of fullness in the ear
- redness of the outer ear
- itching, a more common symptom of fungal infections than bacterial ones
- pain
- inflammation or swelling
- flaky skin
- ringing in the ears
- discharge from the ear, which can be white, yellow, gray, black, or green

These symptoms typically occur in one ear, but it is possible that both ears can be affected at the same time.

Diagnosis

Symptoms of otomycosis should always be evaluated by a doctor in order to get the correct diagnosis and treatment.

The doctor will take a thorough medical history to determine if any risk factors are present. They will perform a physical exam with an instrument called an otoscope to look inside the ear canal and eardrum.

The doctor may also take a sample of cells or fluid from the ear and look at them under a microscope. This will help them to differentiate between a fungal or bacterial infection.

Treatment

 Share on PinterestEardrops may help to cure the infection and prevent it from reoccurring.

A doctor will prescribe the correct treatment once a diagnosis of otomycosis is made. Treatment can be eardrops, topical cream, or oral medication.

Cleaning

Firstly, a doctor usually needs to clean the ear. They may use a rinse or a suction tool to do this. Cleaning will get rid of debris or a buildup of material and allow the medication to work better.

Next, the ear is cleaned and dried, as much as possible, to inhibit further growth of fungus.

Note that a person should not attempt to clean their own ears with cotton swabs or other tools, as this could worsen the situation.

Eardrops or topical agents

A doctor may prescribe eardrops that contain an antifungal agent.

Research has shown that 1 percent clotrimazole eardrops show high rates of cure and prevention of recurrence.

Eardrops may also contain econazole, miconazole, or amphotericin B, among other chemicals.

Antifungals may also be in the form of a topical cream that is applied to the outer ear.

Other topical medications might include:

- aluminum acetate
- salicylic acid
- hydrogen peroxide

These agents can help to treat the fungus or soften the crust that forms to help other medications penetrate better.

Oral medications

Oral medications, such as itraconazole or voriconazole, are usually reserved for more severe infections, or infections that are difficult to get rid of with topical agents. Some fungus species are resistant to antifungal eardrops.

Oral antifungals can be a problem for people who have liver disease.

Over-the-counter pain relievers, such as acetaminophen or ibuprofen, can be used to ease any minor pain.

Complications

Although uncommon, complications can arise from otomycosis.

Otomycosis can become a chronic condition if not adequately treated, or if it does not respond to treatment. This can also happen if a person has continued exposure to contaminated water that contains a fungus.

Otomycosis can invade further than the outer ear and perforate the eardrum or travel to places that may include the inner ear or base of the skull.

These types of infections typically require oral antifungal treatment and surgical management. A complication such as this is more likely to occur in those who have a weakened immune system or diabetes mellitus.

Prevention

Share on PinterestDrying the ears thoroughly after swimming and bathing can help to prevent otomycosis.

There are a few factors that can help prevent otomycosis, including:

- leaving a small amount of earwax in the ears for its natural anti-fungal properties
- drying the ears well after swimming and bathing
- using earplugs when swimming to keep water out
- using a hairdryer on low speed to dry ears, being careful not to burn the skin
- avoiding scratching the ears as this may damage the skin and make it easier for a fungus to invade
- avoiding putting cotton swabs in the ears

Outlook

In general, otomycosis is not dangerous, and it is easily treated with antifungal treatments.

Otomycosis can become chronic if someone does not respond to treatment or has a weakened immune system, diabetes mellitus, or a chronic skin condition, such as eczema.

Otomycosis can usually be prevented by keeping the ears dry and avoiding contaminated water sources.

Pneumocystis

Pneumocystis pneumonia (PCP) is a serious infection caused by the fungus *Pneumocystis jirovecii*.

Most people who get PCP have a medical condition that weakens their immune system, like HIV/AIDS, or take medicines (such as corticosteroids) that lower the body's ability to fight germs and sickness. In the United States, people with HIV/AIDS are less likely to get PCP today than before the availability of antiretroviral therapy (ART). However, PCP is still a substantial public health problem.[1-3] Much of the information we have about PCP and its treatment comes from caring for patients with HIV/AIDS.
Scientists have changed both the classification and the name of this organism since it first appeared in patients with HIV in the 1980s. *Pneumocystis jirovecii* used to be classified as a protozoan but is now considered a fungus.[4] *Pneumocystis jirovecii* used to be called *Pneumocystis carinii*. When scientists renamed *P. carinii* to *P. jirovecii*, some people considered using the abbreviation "PJP," but to avoid confusion, *Pneumocystis jirovecii* pneumonia is still abbreviated "PCP."

Symptoms
The symptoms of PCP can develop over several days or weeks and include [1,6 – 8]

- Fever
- Cough
- Difficulty breathing
- Chest pain
- Chills
- Fatigue (tiredness)

Contact your healthcare provider if you have symptoms that you think are related to PCP.
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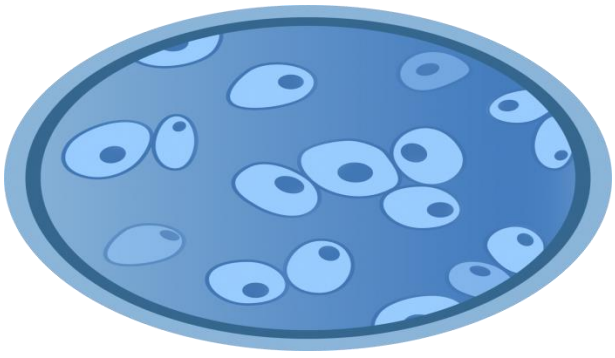
Risk & Prevention
Who gets PCP?



Pneumocystis jirovecii, the fungus that causes *Pneumocystis pneumonia*.

PCP is extremely rare in healthy people, but the fungus that causes this disease can live in their lungs without causing symptoms. In fact, up to 20% of adults might carry this fungus at any given time, and the immune system removes the fungus after several months. [8]

Most people who get PCP have weakened immune systems, meaning that their bodies don't fight infections well. About 30-40% of people who get PCP have HIV/AIDS. [7,9] The other people who get PCP are usually taking medicine (such as corticosteroids) that lowers the body's ability to fight germs or sickness or have other medical conditions, such as:[7,9]

- Chronic lung diseases
- Cancer
- Inflammatory diseases or autoimmune diseases (for example, lupus or rheumatoid arthritis)
- Solid organ or stem cell transplant

How can I prevent PCP?

There is no vaccine to prevent PCP. A healthcare provider might prescribe medicine to prevent PCP for people who are more likely to develop the disease. The medicine most commonly used to prevent PCP is called trimethoprim/sulfamethoxazole (TMP/SMX), which is also known as co-trimoxazole and by several different brand names, including Bactrim, Septra, and Cotrim. Other medicines are available for people who cannot take TMP/SMX.

Medicine to prevent PCP is recommended for some people infected with HIV, stem cell transplant patients, and some solid organ transplant patients. [9-13] Healthcare providers might also prescribe medicine to prevent PCP in other patients, such as people who are taking long-term, high-dose corticosteroids.

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How *Pneumocystis* pneumonia Spreads

PCP spreads from person to person through the air. [15-17] Some healthy adults can carry the *Pneumocystis* fungus in their lungs without having symptoms, and it can spread to other people, including those with weakened immune systems. [8]

Many people are exposed to *Pneumocystis* as children, but they likely do not get sick because their immune systems prevent the fungus from causing an infection. [18] In the past, scientists believed that people who had been exposed to *Pneumocystis* as children could later develop PCP from that childhood infection if their immune systems became weakened. [8,19] However, it is more likely that people get PCP after being exposed to someone else who has PCP or who is carrying the fungus in their lungs without having symptoms.

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Diagnosis and Testing

PCP is diagnosed using a sample from a patient's lungs. The sample is usually mucus that is either coughed up by the patient (called sputum) or collected by a procedure called bronchoalveolar lavage. Sometimes, a small sample of lung tissue (a biopsy) is used to diagnose PCP. The patient's sample is sent to a laboratory, usually to be examined under a microscope.

Polymerase chain reaction (PCR) can also be used to detect *Pneumocystis* DNA in different types of samples. A blood test to detect β-D-glucan (a part of the cell wall of many different types of fungi) can also help diagnose PCP. [21]
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Treatment and Outcomes

PCP must be treated with prescription medicine. Without treatment, PCP can cause death. The most common form of treatment is trimethoprim/sulfamethoxazole (TMP/SMX), which is also known as co-trimoxazole and by several different brand names, including Bactrim, Septra, and Cotrim. This medicine is given by mouth or through a vein for 3 weeks.

TMP/SMX can cause side effects such as rash and fever. Other medicines are available for patients who cannot take TMP/SMX.

Rhinosporidium seeberi

Rhinosporidium seeberi is a eukaryotic pathogen responsible for rhinosporidiosis, a disease which affects humans, horses, dogs, and to a lesser extent cattle, cats, foxes, and birds.[1] It is most commonly found in tropical areas, especially India and Sri Lanka.[1][2]

The pathogen was first identified in 1892, and was comprehensively described in 1900 by Seeber.

Many aspects of the disease and of the pathogen *Rhinosporidium seeberi* remain problematic and enigmatic. These include the pathogen's natural habitat, some aspects of its 'lifecycle', its immunology, some aspects of the epidemiology of the disease in humans and in animals, the reasons for the delay at *in vitro* culture, and establishment of disease in experimental animals, hence paucity of information on its sensitivity to drugs, and the immunology of the pathogen. Thankamani isolated an organism believed to be *R. seeberi* and gave the name "UMH.48." It was originally isolated from the biopsies and nasal swabs of rhinosporidiosis patients. The various developmental stages of UMH.48 showed a strong resemblance with the structures seen in hisopathological sections of rhinosporidiosis in tissue samples. The spores of UMH.48 were found to be viable even after a decade of preservation in the refrigerator without any subculture, resembling the features of *Synchytrium endobioticum*, a lower aquatic fungus that causes black wart disease in potatoes. However, carefully performed molecular studies showed the definitive identity of the organism.[3][4][5]

Arseculeratne, Sarath N; Atapattu, Dhammika N. (2011). Rhinosporidiosis in Humans and Animals & Rhinosporidium seeberi. Faculty of Medicine, University of Peradeniya. ISBN 978-9555891578. discusses recent research developments and clinical associations of this enigmatic disease.

Phylogeny

For most of the 20th century, the classification of *R. seeberi* was unclear (being considered either a fungus or a protist), but it was shown to be part of a group called the Mesomycetozoea[6] (or "DRIP clade"),[7] which includes a number of well-known fish pathogens such as *Dermocystidium* and *Sphaerothecum destruens*. The Mesomycetozoea are neither part of the

fungi nor of animals, but diverged from them close to the time when they diverged from each other.[5][8]

Rhinosporidium is generally classified as having a single species, although some evidence indicates that different host species may be infected by different strains.[9]

Epidemiology

Infection in humans with this organism has been reported from about 70 countries, with the majority of cases (95%) reported from India and Sri Lanka; per capita, Sri Lanka has the highest incidence in the world. The disease is also found in other parts of the world.[2][10][11]

An all-India survey conducted in 1957 found that this disease was absent from the states of Jummu and Kashmir, Himachal Pradesh, Punjab, Haryana and the north eastern states of India. In Tamil Nadu, four endemic areas were identified in the survey—(Madurai, Ramnad, Rajapalayam, and Sivaganga). The common factor found in these areas was the practice of bathing in common ponds.

Transmission and dissemination

1. Demellow's theory of infection
2. Karunarathnae's autoinoculation theory
3. Haematogenous spread – to distant sites
4. Lymphatic spread – causing lymphadenitis (rare)

Demellow postulated that while bathing in common ponds, the nasal mucosa came into contact with infectious material. Karunarathnae proposed that the satellite lesions in skin and conjunctival mucosa arose as a result of autoinoculation.

Because of its relationship to fish pathogens, *Rhinosporidium* is presumed to have evolved from aquatic pathogens similar to the other Mesomycetozoea and evolved to infect mammal and bird hosts. If this happened once or more than once is unknown.[9]

Natural habitat

Karunarathnae also proposed that *Rhinosporidium* existed in a dimorphic state—a saprotroph in soil and water and a yeast form inside living tissues. Recent studies done using fluorescent *in situ* hybridization techniques provide evidence that its natural habitat is reservoir water, and perhaps, soil contaminated with this water.[12]

Pathology

One report indicates that patients with rhinosporidiosis possess anti-*R. seeberi* IgG to an inner wall antigen expressed only during the mature sporangial stage. This finding suggests that the mapping of antigenic proteins may lead to important antigens with the potential as vaccine candidates.

Humoral and cell-mediated immune responses in human patients and in experimental mice have been defined; several mechanisms of immune evasion by *R. seeberi* have been identified.

A novel method for the determination of the viability of rhinosporidial endospores by MTT-reduction led to the study of the sensitivity of endospores to biocides and antimicrobial drugs (paper in preparation for submission).

Clinical features

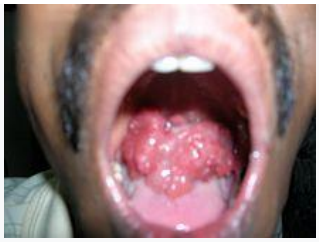


Image showing a large rhinosporidial mass in the oropharynx of a patient

This organism infects the mucosa of the nasal cavity, producing a mass-like lesion. This mass appears to be polypoidal in nature with a granular surface speckled with whitish spores. The rhinosporidial mass has been classically described as a strawberry-like mulberry mass. This mass may extend from the nasal cavity into the nasopharynx and present itself in the oral cavity. These lesions commonly cause bleeding from the nasal cavity.

R. seeberi can also affect the lacrimal gland and also rarely the skin and genitalia.

Common sites affected:

1. Nose – 78%
2. Nasopharynx – 68%
3. Tonsil – 3%
4. Eye – 1%
5. Skin – very rare

Treatment

Treatment is generally by surgical removal of the infected tissues.[2]

Povidone-iodine and antifungal drugs such as amphotericin B, dapsone, and silver nitrate have been suggested as possible antiseptics

Lobomycosis

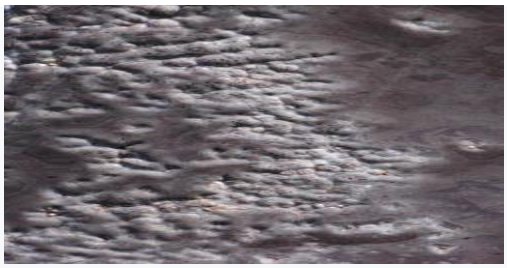
Lobomycosis[2] is a blastomycosis, a fungal infection of the skin caused by *Lacazia loboi* (formerly named *Loboa loboi*),[3] and discovered by Brazilian dermatologist Jorge Lobo. Other names which were given to the disease are: **keloidal blastomycosis**, **Amazonian blastomycosis**, **blastomycoid granuloma**, **miraip** and **piraip**. These last two names were given by natives of the Amazon and mean *that which burns*.

Infected dorsal fin of wild bottle-nosed dolphin, Gulfo Dulce, Costa Rica

This disease is usually found in humans[4] and bottlenose dolphins, with the possible risk of transmission from one species to the other.

Presentation

The disease is endemic in rural regions in South America and Central America.Infection most commonly develops after minor scratches or insect bites, but many patients cannot recall any skin trauma. Human-to-human transmission does not occur, and the disease is only acquired from the environment.[6] The disease manifests as chronic keloidal nodular lesions on the face, ears, or extremities.



Lobomycosis lesions on the skin of a bottlenosed dolphin

Diagnosis of Lobo's disease is made by taking a sample of the infected skin (a skin biopsy) and examining it under the microscope. *Lacazia loboi* is characterized by long chains of spherical cells interconnected by tubules. The cells appear to be yeast-like with a diameter of 5 to 12 μm. Attempts to culture *L. loboi* have so far been unsuccessful.

Diagnosis

Differential diagnosis

The disease is often misdiagnosed as *Blastomyces dermatitidis* or *Paracoccidiodes brasiliensis* due to its similar morphology.

Treatment

Surgical excision or cryosurgery is the treatment of choice.[7] Treatment with antifungals has been considered ineffective, but the use of clofazimine and dapsone in patients with leprosy and lobomycosis has been found to improve the latter. This treatment regimen, with concomitant itraconazole, has been used to prevent recurrence after surgery.[8]

Animals

Lesions in dolphins occur on the dorsal fin, head, flukes, and peduncle. In January 2006, a potential epidemic of lobomycosis was reported in dolphins of the Indian River Lagoon in Florida.

Actinomyces

Actinomyces is a genus of the Actinobacteria class of bacteria. They all are gram-positive. *Actinomyces* species are facultatively anaerobic (except *A. meyeri* and *A. israelii* both obligate anaerobe), and they grow best under anaerobic conditions.[1] *Actinomyces* species may form endospores, and, while individual bacteria are rod-shaped, *Actinomyces* colonies form fungus-like branched networks of hyphae.[2] The aspect of these colonies initially led to the incorrect assumption that the organism was a fungus and to the name *Actinomyces*, "ray fungus" (from Greek *actis*, ray, beam and *mykes*, fungus).

Actinomyces species are ubiquitous, occurring in soil and in the microbiota of animals, including the human microbiota. They are known for the important role they play in soil ecology; they produce a number of enzymes that help degrade organic plant material, lignin, and chitin. Thus their presence is important in the formation of compost. Certain species are commensal in the skin flora, oral flora, gut flora, and vaginal flora[3] of humans and livestock. They are also known for causing diseases in humans and livestock, usually when they get an opportunity to gain access to the body's interior through wounds. As with other opportunistic infections, people with immunodeficiency are at higher risk. In all of the preceding traits and in their branching filament formation, they bear similarities to *Nocardia*.[4]

Like various other anaerobes, *Actinomyces* species are fastidious and thus not easy to culture and isolate. Clinical laboratories do culture and isolate them, but a negative result does not rule out infection, because it may be due simply to reluctance to grow in vitro.

Pathology

Actinobacteria are normally present in the gums and are the most common cause of infection in dental procedures and oral abscesses. Many *Actinomyces* species are opportunistic pathogens of humans and other mammals, particularly in the oral cavity.[6] In rare cases, these bacteria can cause actinomycosis, a disease characterized by the formation of abscesses in the mouth, lungs, or the gastrointestinal tract.[7] Actinomycosis is most frequently caused by *A. israelii*, which may also cause endocarditis, though the resulting symptoms may be similar to those resulting from infections by other bacterial species.[8][9] *Aggregatibacter actinomycetemcomitans* has been identified as being of note in periodontal disease.

The genus is typically the cause of oral-cervicofacial disease. It is characterized by a painless "lumpy jaw". Lymphadenopathy is uncommon in this form of the disease. Another form of actinomycosis is thoracic disease, which is often misdiagnosed as a neoplasm, as it forms a mass that extends to the chest wall. It arises from aspiration of organisms from the oropharynx. Symptoms include chest pain, fever, and weight loss. Abdominal disease is another

manifestation of actinomycosis. This can lead to a sinus tract that drains to the abdominal wall or the perianal area. Symptoms include fever, abdominal pain, and weight loss.[10] *Actinomyces* species have also been shown to infect the central nervous system in a dog "without history or evidence of previous trauma or other organ involvement."[11]

Pelvic actinomycosis is a rare but proven complication of use of intrauterine devices. In extreme cases, pelvic abscesses might develop. Treatment of pelvic actinomycosis associated with intrauterine devices involves removal of the device and antibiotic treatment.[12]

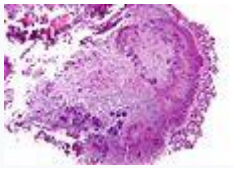
Diagnosis

Actinomycosis may be considered when a patient has chronic progression of disease across tissue planes that is mass-like at times, sinus tract development that may heal and recur, and refractory infection after a typical course of antibiotics.[10]

Treatment

Treatment for actinomycosis consists of antibiotics such as penicillin or amoxicillin for 5 to 12 months,[13] as well as surgery if the disease is extensive.[10]

Additional images

- 

Micrograph of actinomycosis, H&E stain

Nocardiosis

Nocardiosis is a disease caused by bacteria found in soil or standing water. It starts in your lungs or skin, and can cause serious problems if it gets into your bloodstream and infects other parts of your body.

Nocardiosis comes in two forms. You get the pulmonary (lung) version from breathing in the bacteria. The second type is primary cutaneous (skin). That’s when the bacteria gets into an open wound like a scratch.

Between 500 and 1,000 people get it each year in the United States. Men are three times more likely to be infected by it than women -- middle-aged men working outdoors are at the highest risk. You also may have a greater chance of getting it if you have a weak immune system because of a condition like diabetes, HIV, or cancer, or if you’ve had a bone-marrow or organ transplant. If you’ve taken high doses of powerful steroids (drugs that help with inflammation), it also increases your chances.

Symptoms

The signs can be different, depending on which type you have. Pulmonary nocardiosis is the most common, and its symptoms are a lot like ones you might have with pneumonia or tuberculosis:

- Chest pain
- Coughing
- Sweating
- Chills
- Feeling weak
- Lack of appetite
- Unexplained weight loss
- Shortness of breath or a hard time breathing

The most common signs of primary cutaneous nocardiosis are skin abscesses on your hands, chest, or rear end. These are bumps on or below the skin's surface that are usually filled with a fluid (pus). You also might have a fever.

If not treated, the infection can spread through your bloodstream to your brain or, more rarely, to your kidneys, intestines, or other organs. It can be very serious. Signs that the infection has spread to your brain include:

- Bad headaches
- Motor skills problem, like balance or hand-eye coordination
- Extreme sensitivity to loud sounds or bright lights

Diagnosis

It can be hard to tell the difference between pulmonary nocardiosis and pneumonia or tuberculosis. And primary cutaneous nocardiosis looks a lot like several other, more common skin infections.

To find out for sure, your doctor probably will take a small sample of tissue or fluid from the area where you're infected. This might include tissue or mucus from your lungs or tissue from your skin.

If the infection is in your lungs, you might get a chest X-ray -- or a CT scan, which takes X-rays from several angles and puts them together to make a more detailed image.

Treatment

Nocardiosis usually can be cured with antibiotics, but not all of them will work against the bacteria. Your doctor might need to run some lab tests to see which ones will work best for you. Then you might need to take them for 6 weeks up to a year, depending on how serious your infection is.

In some cases, you might need surgery to remove or drain abscesses in infected areas.

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B.SC. MICROBIOLOGY

UNIT – V – Medical Mycology – SMB3103

Fungal contamination

Fungi are more evolutionarily advanced forms of microorganisms, as compared to the prokaryotes (such as bacteria). Fungi are commonly divided into two distinct morphological forms: yeasts and hyphae (or filamentous). Yeasts are unicellular fungi which reproduce asexually by blastoconidia formation (budding) or fission[4]. Fungal contamination in pharmaceutical products represents a potential hazard for two reasons. First, it may cause product spoilage; the metabolic versatility of fungi is such that any formulation ingredient from simple sugars to complex aromatic molecules may undergo chemical modification in the presence of a suitable organism. Spoilage will not only affect therapeutic properties of the product but may also discourage the patient from taking the medication. Second, product contamination represents a health hazard to the patient, although the extent of the hazard will vary from product to product and patient to patient, depending on the types and numbers of organisms present, the route of administration, and the resistance of the patient to infection.

The ability of fungi to produce degradative spoilage in products depends on their ability to synthesise appropriate enzymes. Pharmaceuticals, cosmetics, foods and other products are at risk because fungi are extremely versatile and adaptive in their ability to synthesise degradative enzymes. A consequence of degradation is that low-molecular-weight substrates such as sugars, amino acids, organic acids and glycerol are broken down by primary catabolic pathways. The enzymes for these pathways are constitutive in a wide range of fungi. Fungal contaminants like *Aspergillus* and *Penicillium* spp. are the most common source of proteinase and peptidase enzymes causing breakdown of compounds such as gelatine.
It has been estimated that around half of the fungi found in the environment could cause infections in people (mycosis)[5]. With pharmaceutical products, the two major hazards are air, through the inhalation of spores (such as through inhalers); and skin, through the rubbing in of creams and ointments (many fungi live off keratin, a protein that makes up the skin, hair and nails).

Product recalls

Fungal incidents have increasingly accounted for product recalls for medicinal products associated with microbiological incidents[6]. This is evident by an examination of data provided by the US Food and Drug Administration (FDA). A recall is a firm's removal or correction of a marketed product that a regulator like the FDA considers to be in violation of the laws it administers, and against which the regulator would initiate legal action (such as seizure of the medicinal product).

Dividing recent recall notification into two periods (1990 – 1999 and 2000 – 2012) shows that for 1990 – 1999, five per cent of microbiological related recalls were due to fungal contamination; whereas for 2000 – 2012, fungal innocents accounted for 21 per cent of recalls; making fungal contamination the second highest microbial related reason (after water-associated Gram-negative bacteria). With these fungal incidents, some of the reasons given for the recalls

were package integrity deficiencies; media fill failures, improper sterilisation validation and numerous deficiencies during aseptic processing.

Further analysis indicates that a total of 38 non-sterile products were recalled due to fungal contamination by FDA during 1997 – 2012. Among these incidents, with 29 recalls, the fungi causing the contaminated were not speciated (probably due to inadequacies within quality control departments). The main types of fungi that were speciated were filamentous (moulds), with contamination from yeast being less common.

Some of the main types of fungi and the range of products contaminated have been summarised in **Table 1**. Aside from these, one of the most significant fungal contamination incidents relating to sterile products was the case relating to the New England Compounding Center (NECC) in 2012. A series of sterility assurance failures triggered a multistate outbreak of fungal infections among patients who received contaminated preservative-free methylprednisolone acetate steroid injections manufactured by the NECC. The infections identified as part of this investigation include fungal meningitis, a form of meningitis that is not contagious, localised spinal or paraspinal infections, and infections associated with injections in a peripheral joint space, such as a knee, shoulder, or ankle[7]. By October 2013, some 751 patients had become infected and of these 64 had died.
The primary fungal contaminant was *Exserohilum rostratum* (a dematiaceous, or black, mould containing melanin in its cell wall. It is widely found in the environment, on plant debris, in soil, and in water)[8]. The contamination was traced to several sterility assurance failures which were identified by the FDA. These included concerns with cleanroom management (including disinfection), aseptic filling practices, and the improper use of autoclaves for equipment sterilisation.

Contamination control

One of the most important areas in pharmaceutical process control is the systems to control the number, survival and proliferation of microorganisms during manufacturing of non-sterile and sterile pharmaceutical products. To prevent microbial contamination, the pharmaceutical production facilities (environment), materials and personnel should provide an environment that will minimise the survival, growth and distribution of microorganisms.

Moulds are ubiquitous in nature and, therefore they pose a risk to pharmaceutical manufacturing operations. *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Trychophyton* spp., and other filamentous fungi have, in some cases, caused significant microbial contamination issues in production environments and manufactured products. Such moulds are detected by effective environmental monitoring regimes using a range of air and surface monitoring techniques and appropriate microbiological culture media.

Within cleanrooms and controlled environments, risk areas where fungi can occur include:

- Door kick back plates
- Bags
- Incubators
- Boxes
- Cart wheels
- Ceiling tiles
- Poor flooring
- Vibrations from construction
- Light fixtures
- Faulty HVAC systems (a fungus like *Cladosporium* can reside within Blower wheel fan blades, ductwork and cooling coil fins)[9]
- Items transferred into cleanrooms.

The most commonly isolated species to cleanrooms are *Cladosporium* spp., *Aspergillus* spp., *Pencillium* spp. and *Aurebasidium* spp.
In addition to the cleanroom sources, the extent that personnel carry fungi appears to be greater than previously though. US National Institutes of Health researchers sequenced the DNA of fungi at skin sites of healthy adults and found the heel and toes to carry high levels of fungi, with populous areas also relating to the head, neck and eyebrows. The main species found on the human body were *Malassezia* spp., *Pencillium* spp. and *Aspergillus* spp[10]. Such high numbers mean that cleanroom gowning procedures and cleanroom behaviour disciplines need to be of a high standard.

In terms of the proliferation of fungi within cleanrooms, the main risk factors are poorly ventilated areas or cleanrooms with insufficient air changes; where areas are damp; and where there are ridges or cracks in finishes. In addition, plaster and paint can provide a nutritional source for moulds, although the addition of a fungistat to paint (such as percentage pentachlorophenol) can be effective at inhibiting fungal growth.

Materials transferred into cleanrooms also present a risk, particularly raw materials where fungal flora are associated with materials of plant origin such as gum acacia, tragacanth, agar, and starches. Packaging materials can also be a source. Here the primary risk factors are dust, cardboard and transport.

To these hazards can be added ‘influencing factors’, which can affect the likelihood of fungi surviving. These factors include changes to environmental conditions (such as a rise in ambient temperature; air humidity); personnel behaviour and hygiene; biocide efficacy; ineffective cleaning; the time of year; and the geographical environment.

As indicated earlier, one of the problems with tracking contamination through a pharmaceutical facility is the lack of species identification performed. To develop the effective corrective action when out of specification results are obtained, accurate fungal identification is needed if the contamination source is to be determined and tracked. A corrective action is not effective if wrong information is used to develop a proper solution to a given problem. Moreover, it is of interest that many of the main species recovered from cleanrooms and materials are the same as those species of fungi associated with pharmaceutical product recalls.

Risk assessment

With non-sterile products, where a fungus is detected as the result of end-product testing, an assessment is required as to whether the fungus is objectionable. According to Sutton[1], this risk analysis should incorporate a minimum of four separate considerations. These are absolute numbers of organisms seen; the microorganism's characteristics; product characteristics; and the potential impact on patients.

The understanding of pathogenic and objectionable microorganisms has become more complicated through the findings of the Human Microbiome Project. It is not yet fully known what the relationships are between the bacteria that normally reside within the human body, those introduced into it, and the various genetic interactions that may occur[11].

A greater emphasis upon risk assessment and a fuller understanding of the tools and methods, coupled with an understanding of the nature of the fungal contamination, will help to minimise the numbers of non-sterile products that are most at risk from fungal contaminants.

Summary

This article has examined the risks posed by fungi to pharmaceutical products and has emphasised how this is an issue of growing importance (as seen by the extent of product recalls relating to fungal contamination). The article has further considered where fungi pose a risk within the manufacturing process. The article has gone on to argue that recalls relating to fungal contamination can be reduced through improved cleanroom design; risk assessment; and developing greater specialisms within quality control departments in order to be able to characterise, identify and to trace fungi. This way, the risks posed by fungi to pharmaceutical processes should receive the level of attention necessary, especially in light of the potential for certain products to become contaminated.

Mycotoxicoses

Mycotoxicosis is the consequence of ingestion of grains or forage containing toxic metabolites produced by certain fungi. Fungi that produce toxins often do so only under specific conditions of warmth, moisture and humidity. Factors that adversely affect plants or their seeds (grains) often influence mycotoxin production. Mycotoxins can develop in field grains, damaged grains or improperly stored feeds.

Of the over 200 mycotoxins identified to date, at least seven have been reported to cause disease in swine. Some fungi produce more than one mycotoxin. Several different fungi can produce different mycotoxins in a single mixed feed. The toxins may be additive or may potentiate one another. When metabolized, they may be converted into other toxic substances. While toxicologic effects are numerous and often confusing, one should be careful not to implicate mycotoxins in disease processes without credible evidence.

Mycotoxins produce their toxic effects in several ways, including impairment of metabolic, nutritional or endocrine functions. Many mycotoxins damage the liver, reduce average daily feed intake, growth and feed efficiency. Some are teratogenic or carcinogenic. Some are immunosuppressive and predispose pigs to secondary diseases. Several mycotoxins decrease the reproductive performance of sows. Metabolites sometimes are passed in the milk of sows to their litters. The effect of mycotoxins may vary with the amount ingested, the time over which it is consumed, and the age of exposed swine. Young pigs usually are much more susceptible than adults. Within a herd there can be great variability in response to a mycotoxin.

Mycotoxicoses can present with either chronic or acute onsets. Most exposures are probably chronic or subacute as a result of consuming small amounts of toxin over a long period of time. In these instances, there may be few signs of toxicosis other than decreased appetite, slow growth, and increased susceptibility to secondary diseases. Acute outbreaks may have more obvious signs and will vary for each of the different mycotoxins. Diagnosis of chronic mycotoxicosis is often difficult because clinical signs are seldom overt and lesions are not specific. By the time a mycotoxicosis is considered, the suspected feed has often already been consumed with none having been collected and stored properly for analysis.

Prevention of mycotoxicosis is largely through careful selection and proper storage of high quality grains and other feed ingredients and the careful maintenance and cleanliness of feed preparation equipment. It often is worthwhile to properly dry and store samples of representative batches of grain that are used on a farm in the event they are needed for analysis later. Producers anticipating problems should locate a competent laboratory and get information on appropriate sample collection and storage techniques.

Aflatoxicosis

This mycotoxicosis is caused by mycotoxins produced by Aspergillus flavus, Aspergillus parasiticus or Penicillium puberulum. Four major toxins (B1, B2, G1, G2) are produced. B1 is of greatest significance and is a potent hepatotoxin. Fungi growing on peanuts, corn, wheat and several other cereal grains commonly produce the toxins. Maximum aflatoxin formation occurs

under conditions related to the specific grain, its moisture content, storage temperature and humidity.

There is a marked age-related difference in susceptibility to aflatoxicosis. Young nursing or weaned growing pigs are much more susceptible than adults. When aflatoxin is ingested by a lactating dam, toxic metabolites are passed in her milk and serve as a source of exposure to the nursing pigs. These toxins reduce feed intake, average daily gain and feed efficiency. Since aflatoxins are immunosuppressive, signs of toxicosis often include an increase in previously controlled secondary diseases.

Acute aflatoxicosis is uncommon in swine. It is usually a subacute to chronic disease caused by daily ingestion of smaller amounts of aflatoxin over several weeks. Lesions often vary noticeably among pigs in the same affected group but are predominantly those of a hepatopathy. In the more acute cases there are sudden deaths, hemorrhages in multiple tissues, and icterus. The liver may be swollen, fatty, and have areas of necrosis. There may be a prolonged clotting time. With subacute to chronic hepatotoxicosis, the liver may be reduced in size, fibrotic, and ascites may be present.

Diagnosis is usually based on some combination of a history of slow growth (often accompanied by secondary diseases that seem unresponsive to treatment), an elevation of serum enzymes associated with hepatocellular damage, and gross lesions related to liver pathology. Microscopic hepatic lesions include bile duct hyperplasia and enlargement of hepatocytes. In swine, chronic aflatoxin toxicity can occur with at levels as low as 300 ppb in the feed; acute toxicity usually doesn't occur until concentrations beyond 1000 ppb. Aflatoxin is considered to be carcinogenic in humans.

Ergotism

Claviceps purpurea is a fungus of many grasses and several cereal grains, especially rye, oats and wheat. The sclerotium of the fungus is a dark, elongated body and often can be seen on cereal grain heads and in processed grains. The fungus produces three major alkaloids that cause ergotism. The primary lesions caused by the alkaloids include arteriolar vasoconstriction and endothelial cell injury that often leads to thrombosis. When present in low levels, the alkaloids can result in reduced growth rates. Larger amounts lead to ischemic necrosis followed by a dry, gangrenous sloughing of parts of extremities, especially tails, ears and hooves. Symptoms of ergotism are exacerbated by cold weather. In pregnant sows, ergotism can inhibit mammary development, reduce litter size, reduce birth weights, and cause a profound post-farrowing agalactia. The agalactia is believed to be related to inhibition of prolactin secretion.

Diagnosis of ergotism is based on lesions coupled with the gross or microscopic identification of significant numbers of ergot sclerotia in grains or the ground feed. Doubtful results may be verified by laboratory confirmation of significant amounts of alkaloids in the feed.

Fumonisin Toxicosis

The fumonisins include two principal toxins produced by *Fusarium moniliforme*. Signs of acute toxicity in growing and adult pigs are primarily related to the respiratory system and include dyspnea, cyanosis, weakness and death within four to ten days. Pulmonary lesions include marked pulmonary edema and hydrothorax. Pregnant sows that survive acute toxicity frequently abort in the days following their recovery. Growing pigs that survive the acute syndrome suffer from clinical signs related to a hepatotoxicosis. Their lesions may include icterus, hepatic necrosis, and megalocytosis. Differences in lesions and clinical presentation seem to be dose related.

Recent research has demonstrated that fumonisins decrease the ability of intravascular macrophages to clear blood-borne bacteria in swine, thereby potentially increasing susceptibility to respiratory disease. Fumonisin is a well known cause of leukoencephalomalacia in horses and is carcinogenic in humans at high concentrations.

Trichothecene Toxicoses

There are numerous structurally related toxic compounds produced by certain Fusarium species that are classified as trichothecene mycotoxins. At least three of these are of importance in pig production. Trichothecenes are cytotoxic to many cell types and are strongly immunosuppressive. Signs of trichothecene toxicity usually include feed refusal, salivation and, sometimes, vomiting. With chronic exposure there may be paresis, paralysis, or seizures. Lesions often include gastroenteritis, hemorrhagic diathesis, skin irritation, and necrosis.

Diagnosis of trichothecene-related toxicosis can be difficult. The presence of moldy or caked feed, along with a reluctance to consume it, may suggest the presence of a trichothecene toxicosis. Improvement following a change in feed suggests the original feed was contaminated.

T-2 toxin

In swine, the experimental administration of T-2 toxin, alone and with aflatoxin, has resulted in crusting and ulceration of the skin of the snout, lips, buccal commissures, and prepuce.

Deoxynivalenol (DON, vomitoxin)

Deoxynivalenol is a commonly occurring mycotoxin in corn and wheat. Despite its common name of “vomitoxin,” swine only rarely consume a large enough dose to produce vomiting; reduced feed intake is often the only sign present.

Zearalenone (F-2)

This mycotoxin is produced by Fusarium graminearum and may be present in moldy corn, standing corn, other grains, and in pelleted cereal feeds. It has an estrogenic effect that results in vulvovaginitis and precocious mammary development in prepuberal gilts. Swelling and enlargement of the vulva sometimes lead to tenesmus with prolapse of the rectum. Similar

estrogenic effects in gilts have occurred as a result of consuming estrogens from other sources, including alfalfa.

There are few, if any, highly effective treatments for most mycotoxicoses. A ration suspected of being toxic should be replaced with good quality feed. If a large quantity of the feed remains, it sometimes can be fed to less susceptible species or diluted with good quality feed so that the mycotoxin no longer is being fed at a toxic concentration. There are several mycotoxin "binders" on the market that can be used to prevent the absorption of some mycotoxin from the pig's gut.

Mycotoxins have been suggested as a cause of abortion in swine but none have shown the effect in an experimental setting. Astute practitioners should be reluctant to implicate mycotoxins as a cause of ill-defined maladies without evidence.

A **mycotoxin** (from the Greek μύκης *mykes*, "fungus" and τοξίνη *toxini*, "toxin")[1][2] is a toxic secondary metabolite produced by organisms of the fungus kingdom[3] and is capable of causing disease and death in both humans and other animals.[4] The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops.[5]

Examples of mycotoxins causing human and animal illness include aflatoxin, citrinin, fumonisins, ochratoxin A, patulin, trichothecenes, zearalenone, and ergot alkaloids such as ergotamine.[6]

One mold species may produce many different mycotoxins, and several species may produce the same mycotoxin.

Production

Most fungi are aerobic (use oxygen) and are found almost everywhere in extremely small quantities due to the diminute size of their spores. They consume organic matter wherever humidity and temperature 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 not necessary for the growth or the development of the fungi.[8] Because mycotoxins weaken the receiving host, they may improve the environment for further fungal proliferation. The production of toxins depends on the surrounding intrinsic and extrinsic environments and these substances vary greatly in their toxicity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms.[9]

Major groups

Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*.[10] The umbrella term aflatoxin refers to four different types of mycotoxins produced, which are B_1, B_2, G_1, and G_2.[11] 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.[10] Aflatoxins are largely associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios, and maize.[10][11]

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.[12] *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.[13] 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.[12][13]

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 yellowed rice disease in Japan and acts as a nephrotoxin in all animal species tested.[14] 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.[15]

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, causes 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.[15]

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.[16][17] 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.[16] 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.[16][17]

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.[18][19] 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.[20]

Occurrence

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.[15] 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.[21] The other primary mycotoxin groups found in mushrooms include: orellanine, monomethylhydrazine, disulfiram-like, hallucinogenic indoles, muscarinic, isoxazole, and gastrointestinal (GI)-specific irritants.[22] The bulk of this article is about mycotoxins that are found in microfungi other than poisons from mushrooms or macroscopic fungi.[15]

In indoor environments

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*.[23] *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.[24] 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.[25] 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 multimillion-dollar toxic mold settlements. The lawsuits took place after a study by the Center for Disease Control (CDC) in Cleveland, Ohio, reported 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.[26]

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.[24]

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.[27]

In food

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.

In 2004 in Kenya, 125 people died and nearly 200 others were treated after eating aflatoxin-contaminated maize.[28] 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.

Spices are susceptible substrate for growth of mycotoxigenic fungi and mycotoxin production.[29] Red chilli, black pepper, and dry ginger were found to be the most contaminated spices.[29]

Physical methods to prevent growth of mycotoxin-producing fungi or remove toxins from contaminated food include temperature and humidity control, irradiation and photodynamic treatment[30]. Mycotoxins can also be removed chemically and biologically using antifungal/anti-mycotoxins agents and antifungal plant metabolites [30].

In animal food

Dimorphic fungi, which include Blastomyces dermatitidis and Paracoccidioides brasiliensis, are known causative agents of endemic systemic mycoses.[31]

There were outbreaks of dog food containing aflatoxin in North America in late 2005 and early 2006,[32] and again in late 2011.[33]

Mycotoxins in animal fodder, particularly silage, can decrease the performance of farm animals and potentially kill them.[34] Several mycotoxins reduce milk yield when ingested by dairy cattle.[34]

Mycobacterium is derived from its "fungus-like" nature [35]

In dietary supplements

Contamination of medicinal plants with mycotoxins can contribute to adverse human health problems and therefore represents a special hazard.[36][37] Numerous natural occurrences of mycotoxins in medicinal plants and herbal medicines have been reported from various countries including Spain, China, Germany, India, Turkey and from the Middle East.[36] In a 2015 analysis of plant-based dietary supplements, the highest mycotoxin concentrations were found in milk thistle-based supplements, at up to 37 mg/kg.[38]

Health effects

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.[39] It has been suggested that mycotoxins in stored animal

feed are the cause of rare phenotypical sex changes in hens that causes them to look and act male.[40][41]

In humans

Mycotoxicosis is the term used for poisoning associated with exposures to mycotoxins. Mycotoxins have the potential for both acute and chronic health effects via ingestion, skin contact,[42], inhalation, and entering 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.[25]

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.[15] 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.[15]

Mitigation

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.[43]

Removal

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 effectively adsorb the mycotoxins.[44] 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, de-epoxidase), yeast (*Trichosporon mycotoxinvorans*), or bacterial strains (Eubacterium BBSH 797 developed by Biomin), mycotoxins can be reduced during pre-harvesting contamination. Other removal methods include physical separation, washing, milling, nixtamalization, 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.[44]

Regulations

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.[45] 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.[46] Most official regulations and control methods are based on high-performance liquid techniques (e.g., HPLC) through international bodies.[46] 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).[46] 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.[47]

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 EC 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.[48]

Anti fungal agents

An **antifungal medication**, also known as an **antimycotic medication**, is a pharmaceutical fungicide or fungistatic used to treat and prevent mycosis such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Such drugs are usually obtained by a doctor's prescription, but a few are available OTC (over-the-counter).

Types

There are two types of antifungals: local and systemic. Local antifungals are usually administered topically or vaginally, depending on the condition being treated. Systemic antifungals are administered orally or intravenously.

Of the clinically employed azole antifungals, only a handful are used systemically.[1] These include ketoconazole, itraconazole, fluconazole, fosfluconazole, voriconazole, posaconazole, and isavuconazole.[1][2] Examples of non-azole systemic antifungals include griseofulvin and terbinafine.

Classes

Polyenes

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 a cell dies.[3] 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

Azoles

Azoles inhibit conversion of lanosterol to ergosterol by inhibition of lanosterol 14α-demethylase.

Imidazoles

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

Triazoles

- Albaconazole
- Efinaconazole
- Epoxiconazole
- Fluconazole

- Isavuconazole
- Itraconazole
- Posaconazole
- Propiconazole
- Ravuconazole
- Terconazole
- Voriconazole

Thiazoles

- Abafungin

Allylamines

Allylamines[5] inhibit squalene epoxidase, another enzyme required for ergosterol synthesis. Examples include amorolfin, butenafine, naftifine, and terbinafine.[6][7][8]

Echinocandins

Echinocandins inhibit the creation of glucan in the fungal cell wall by inhibiting 1,3-Beta-glucan synthase:

- Anidulafungin
- Caspofungin
- Micafungin

Echinocandins are administered intravenously, particularly for the treatment of resistant *Candida* species.

Others

- Aurones - have been shown to possess antifungal properties[11]
- Benzoic acid – has antifungal properties, such as in Whitfield's ointment, Friar's Balsam, and Balsam of Peru.[12]
- Ciclopirox – (ciclopirox olamine) – is a hydroxypyridone antifungal that interferes with active membrane transport, cell membrane integrity, and fungal respiratory processes. It is most useful against tinea versicolour.[13]
- Flucytosine or 5-fluorocytosine – an antimetabolite pyrimidine analog [14]
- Griseofulvin – binds to polymerized microtubules and inhibits fungal mitosis[*medical citation needed*]
- Haloprogin – discontinued due to the emergence of more modern antifungals with fewer side effects [15]
- Tolnaftate – a thiocarbamate antifungal, which inhibits fungal squalene epoxidase (similar mechanism to allylamines like terbinafine)[*medical citation needed*]
- Undecylenic acid – an unsaturated fatty acid derived from natural castor oil; fungistatic, antibacterial, antiviral, and inhibits *Candida morphogenesis*[*citation needed*]
- Triacetin - hydrolysed to acetic acid by fungal esterases.[16]
- Crystal violet – a triarylmethane dye, it has antibacterial, antifungal, and anthelmintic properties and was formerly important as a topical antiseptic.[17]

- Castellani's paint
- Orotomide (F901318) - pyrimidine synthesis inhibitor.[18][19]
- Miltefosine disrupts fungal cell membrane dynamics by interacting with ergosterol [20]
- Potassium iodide is the preferred treatment for lymphocutaneous sporotrichosis and subcutaneous zygomycosis (basidiobolomycosis). The mode of action is obscure.[21]
- Nikkomycin blocks formation of chitin present in the cell wall of fungus.
- Coal tar
- Copper(II) sulfate[22]
- Selenium disulfide
- Sodium thiosulfate
- Piroctone olamine
- Iodoquinol(Diiodohydroxyquin), clioquinol
- Acrisorcin
- Zinc pyrithione
- Sulfur

Side effects

Apart from side effects like altered estrogen levels and liver damage, many antifungal medicines can cause allergic reactions in people.[23] 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 any 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.[24] Azole antifungals also are both substrates and inhibitors of the cytochrome P450 family CYP3A4,[24] causing increased concentration when administering, for example, calcium channel blockers, immunosuppressants, chemotherapeutic drugs, benzodiazepines, tricyclic antidepressants, macrolides and SSRIs.

Before oral antifungal therapies are used to treat nail disease, a confirmation of the fungal infection should be made.[25] Approximately half of suspected cases of fungal infection in nails have a non-fungal cause.[25] The side effects of oral treatment are significant and people without an infection should not take these drugs.[25]

Azoles are the group of antifungals which act on the cell membrane of fungi. They inhibit the enzyme 14-alpha-sterol demethylase, a microsomal CYP, which is required for biosynthesis of ergosterol for the cytoplasmic membrane. This leads to the accumulation of 14-alpha-methylsterols resulting in impairment of function of certain membrane-bound enzymes and disruption of close packing of acyl chains of phospholipids, thus inhibiting growth of the fungi. Some azoles directly increase permeability of the fungal cell membrane.

in vitro antifungal susceptibility tests

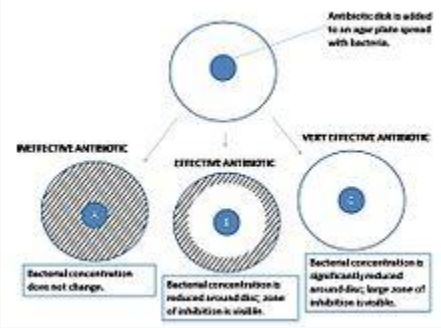
Disk diffusion test

The **disk diffusion test**, or **agar diffusion test**, or **Kirby–Bauer test** (disc-diffusion antibiotic susceptibility test, disc-diffusion antibiotic sensitivity test, KB test), is an antibiotic susceptibility test. It uses antibiotic discs to test the extent to which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible.[1][2] This is called a zone of inhibition.

The size of this zone depends on many factors, one being how effective the antibiotic is at stopping the growth of the bacterium. Another factor that will influence the size of a zone is the diffusion of the antibiotic within the agar medium and varies based on the molecular configuration of the antibiotic. Once the zone diameter is measured it must be compared to a database of zone standards to determine if the bacterium being studied is susceptible, moderately susceptible, or resistant to the antibiotic in question.

A pure bacterial culture is suspended in a buffer, standardized to turbidity, and swabbed uniformly across a culture plate. A filter-paper disk impregnated with the compound to be tested is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk and will decrease as the distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. This along with the rate of antibiotic diffusion are used to estimate the bacteria's susceptibility to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotic for that bacteria. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection.

Standardization



A close-up look at the results of an agar diffusion test.

Inoculation is made with a broth culture diluted to match a 0.5 McFarland turbidity standard, which is roughly equivalent to 150 million cells per mL.

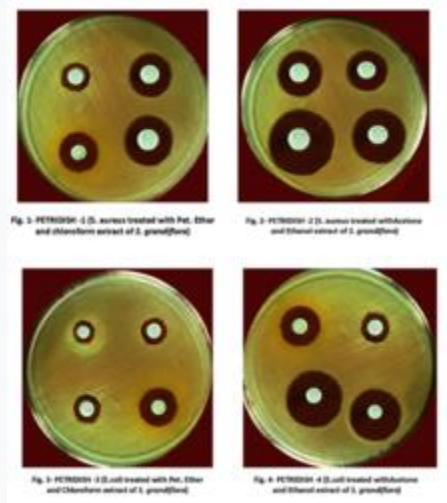
Kirby–Bauer testing: White wafers containing antibiotics shown on plate of bacteria. Circles of poor bacterial growth surround some wafers, indicating susceptibility to the antibiotic

Preparation

All aspects of the Kirby– Bauer procedure are standardized to ensure consistent and accurate results. Because of this, a laboratory must adhere to these standards. The media used in Kirby–Bauer testing must be Mueller-Hinton agar at only 4 mm deep, poured into either 100 mm or 150 mm Petri dishes. The pH level of the agar must be between 7.2 and 7.4.

Incubation procedure

1. Using an aseptic technique, place a sterile swab into the broth culture of a specific organism and then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube.
2. Using the swab, streak the Mueller-Hinton agar plate to form a bacterial lawn.
    - To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction.
    - Repeat this rotation 3 times.
3. Allow the plate to dry for approximately 5 minutes.
4. Use an antibiotic disc dispenser to dispense discs containing specific antibiotics onto the plate.
5. Using a flame-sterilized forceps, gently press each disc to the agar to ensure that the disc is attached to the agar.
6. Plates should be incubated overnight at an incubation temperature of 37 °C (98.6 °F).[4]



Petri dishes showing zone of inhibition by various extracts like petroleum ether, chloroform, ethanol and acetone

Oxford penicillin cup method

Disks containing increasing antibiotic concentrations are placed on seeded bacterial lawn on the agar surface and plates are incubated. Zone sizes are measured from the edge of the disk to the end of the clear zone. Interpretation is more complicated in mixed susceptibility populations (*cf.* BSAC). These are plotted as linear dimensions or squares of distances as a function of the natural logarithm of antibiotic concentration in the disks. The MIC is determined from the zero intercept of a linear regression fit through the data (*cf.* agardiffusion.com). The intercept itself is the logarithm of the MIC. The slope of the regression line is related to the diffusion coefficient of that particular antibiotic in the agar.[6]

2. Broth dilution method for MIC Determination

Broth dilution method for measuring minimum inhibitory concentration of antibiotics.

Minimum inhibitory concentration (MIC) is determined when a patient does not respond to treatment thought to be adequate, relapses while being treated or when there is immunosuppression.

The lowest concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the minimal inhibitory concentration (MIC).

Dilution methods can be carried out in 2 ways

A. Broth dilution

Broth dilution testing allows the option of providing both quantitative (MIC) and qualitative (category interpretation) results. MIC can be helpful in establishing the level of resistance of a

particular bacterial strain and can substantially affect the decision to use certain antimicrobial agents.

Broth dilution can again be performed by 2 ways

1. Macro dilution: Uses broth volume of **1 ml** in standard test tubes .
2. Microdilution: Uses about **0.05 to 0.1 ml** total broth volume and can be performed in a microtiter plate or tray .

The procedure for both macro and microdilution are same except the volume of the broth.

B. Agar dilution

MIC of an antibiotic using broth dilution method is determined by using the following procedure

1. Preparation of antibiotic stock solution
2. Preparation of antibiotic dilution range
3. Preparation of agar dilution plates
4. Preparation of inoculum
5. Inoculation
6. Incubation
7. Reading and interpreting results

Preparation of antibiotic Stock solution.

Antibiotic stock solution can be prepared by commercially available antimicrobial powders (with given potency). The amount needed and the diluents in which it can be dissolved can be calculated by using either of the following formulas to determine the amount of antimicrobial powder (1) or diluent (2) needed for a standard solution:

Prepare antimicrobial agent stock solutions at concentrations of at least 1000 µg/mL (example: 1280 µg/mL) or 10 times the highest concentration to be tested, whichever is greater.

Because microbial contamination is extremely rare, solutions that have been prepared aseptically but not filter sterilized are generally acceptable. If desired, however, solutions may be sterilized by **membrane filtration**. Dispense small volumes of the sterile stock solutions into sterile glass, polypropylene, polystyrene, or polyethylene vials; carefully seal; and store (*preferably at −60 °C or below, but never at a temperature warmer than −20 °C and never in a self-defrosting freezer)*. Vials may be thawed as needed and used the same day.

Preparation of antibiotic dilution range

- Use sterile 13- x 100-mm test tubes to conduct the test. If the tubes are to be saved for later use, be sure they can be frozen.
- Close the tubes with loose screw-caps, plastic or metal closure caps, or cotton plugs.
- Prepare the final two fold (or other) dilutions of antimicrobial agent volumetrically in the broth. A minimum final volume of 1 mL of each dilution is needed for the test.

Note: For, microdilution,only 0.1 ml is dispensed into each of the 96 wells of a standard tray.

Preparation of inoculum

1. Prepare the inoculum by making a direct broth suspension of isolated colonies selected from an 18- to 24-hour agar plate *(use a non-selective medium, such as **blood agar**).*
2. Adjust the suspension to achieve a turbidity equivalent to a **0.5 McFarland turbidity standard**. This results in a suspension containing approximately 1 to 2 x 10^8 colony forming units (CFU)/mL for *Escherichia coli* ATCC®a 25922.
3. Compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.
4. Optimally within 15 minutes of preparation, dilute the adjusted inoculum suspension in broth so, after inoculation, each tube contains approximately 5 x 10^5 CFU/mL.**Note:** *This can be accomplished by diluting the 0.5 McFarland suspension 1:150, resulting in a tube containing approximately 1 x 10^6 CFU/mL. The subsequent 1:2 dilution in step 3 brings the **final inoculum to 5 x 10^5 CFU/mL.***

Broth dilution method for measuring minimum inhibitory concentration of antibiotics. *(image source:labome.com)*

Inoculation

Within 15 minutes after the inoculum has been standardized as described above, add 1 mL of the adjusted inoculum to each tube containing 1 mL of antimicrobial agent in the dilution series (and a positive control tube containing only broth), and mix.

This results in a 1:2 dilution of each antimicrobial concentration and a 1:2 dilution of the inoculums.

Incubation:

Incubate the inoculated tubes at 35 ± 2 ºC for 16 to 20 hours in an ambient air incubator. To maintain the same incubation temperature for all cultures, do not stack microdilution trays more than four high.

Interpretation

Compare the amount of growth in the wells or tubes containing the antimicrobial agent with the amount of growth in the growth-control wells or tubes (no antimicrobial agent) used in each set

of tests when determining the growth end points. For a test to be considered valid, acceptable growth (≥ 2 mm button or definite turbidity) must occur in the growth-control well.

Quality Control

Use reference bacterial strains that are genetically stable and have well-defined MICs that are in the

middle range of the expected MICs of each antimicrobial agent to be tested. A dilution series should include at least two concentration increments above and below the previously established MIC for the reference organisms.

CLSI has established QC limits for dilution susceptibility tests ; an unacceptable QC result is one that falls outside these published limits. Reference strains recommended by the CLSI for

QC of dilution tests for aerobic bacteria are

E. coli ATCC 25922,

E. faecalisATCC 29212,

P. aeruginosa ATCC 27853, and

S. aureus ATCC 29213.

Troubleshooting Microdilution Assays

1. Inappropriate MICs report:
   - When **MICs are lower than expected**—the **inoculum may be too light.**
   - When **MICs are higher than expected**—the **inoculum may be too heavy.** In such conditions, repeat testing using McFarland 0.5 turbidity standard or standardizing device. Check steps in inoculum preparation and inoculation procedure.
2. **When MICs are either higher or lower than expected**—the composition of the cation-adjusted Müeller–Hinton broth may not be optimal. Check the **pH** and **calcium concentration** of in-house prepared media. Use an alternative commercial lot of media, or an alternative lot of commercial panels.
3. When there are skipped wells—may be caused by several problems:
   -
   - Check for contamination.
   - Panel may have been inadequately inoculated or the inoculum may have been inadequately mixed.
   - Drug concentration in the wells may be inaccurate.
   - Volume of broth in the wells may be inaccurate.
5. When several MICs too high or too low—may indicate a possible reading/transcription error. Recheck all of the readings and repeat testing using an alternative lot.

Hypersensitivity Reactions

Certain human disorders are attributed to activity of the immune system. These disorders are commonly known as hypersensitivities, states of increased immune sensitivity that are mediated by antibody or cellular factors. The disorders may also involve immunodeficiencies in which failures of antibody-mediated or cell-mediated immunity take place.

Normally the immune system plays an important role in protecting the body from microorganisms and other foreign substances. If the activity of the immune system is excessive or overreactive, a **hypersensitivity reaction** develops. The consequences of a hypersensitivity reaction may be injury to the body or death.

Most injury resulting from hypersensitivities develops after an interaction has taken place between antigens and antibodies or between antigens and sensitized T-lymphocytes. The general nature of and symptoms accompanying the reaction depend upon whether antibodies or sensitized T-lymphocytes are involved. When antibodies are involved, the reactions fall under the heading of **immediate hypersensitivity.** When T-lymphocytes are involved, the reactions are characterized as **delayed hypersensitivity.** Immediate hypersensitivity reactions include anaphylaxis, allergic reactions, cytotoxic reactions, and immune complex reactions. Delayed hypersensitivity reactions are generally characterized as contact dermatitis or infection allergies.

Immediate hypersensitivity. The reactions accompanying immediate hypersensitivity depend upon the nature of the antigen, the frequency and route of antigen contact, and the type of antibody reacting with the antigen. The initial dose of antigen is referred to as the **sensitizing dose.** This exposure is followed by a **latent period** and then a later dose of the same antigen, called the **eliciting dose** or **shocking dose.** The shocking dose sets off the hypersensitivity reaction, resulting in tissue damage.

Immediate reactions begin within minutes of contact with the eliciting dose of antigen. If antigens are introduced directly into the tissues, such as by insect sting or injection, the result is a systemic reaction such as **anaphylactic shock.** When the contact is a superficial one involving the epithelial tissues, the reaction is more localized, as occurs in asthma or allergic rhinitis (hay fever). These local reactions are commonly referred to as **allergy.** Another term used is **atopy.**

The antigens eliciting an immediate hypersensitivity are called **allergens**, particularly when they are involved in local allergic reactions. Hapten molecules such as penicillin molecules may be involved when they are bound to larger protein molecules. Foods, feathers, pollen grains, animal dander, and dust may be allergens. Animal sera, bee venoms, and wasp venoms are also allergens.

The antibodies involved in anaphylaxis reactions are of the type IgE. In cytotoxic and immune complex reactions, IgG and IgM are involved.

Anaphylaxis. Anaphylaxis, or **type I hypersensitivity**, is a whole-body, immediate hypersensitivity also known as **anaphylactic shock.** The allergens are introduced to the body directly to the tissues in a concentrated form (intramuscular or intravenous injection, for example).

After the sensitizing dose has been administered, IgE is produced by the plasma cells. The antibodies circulate in the blood and attach at the Fc end to **mast cells** of the tissues and **basophils** in the bloodstream (Figure 1). This activity occurs during the latent period. When

the eliciting dose of allergen is later administered, the antigens combine with antibodies on the surface of the mast cells and basophils.

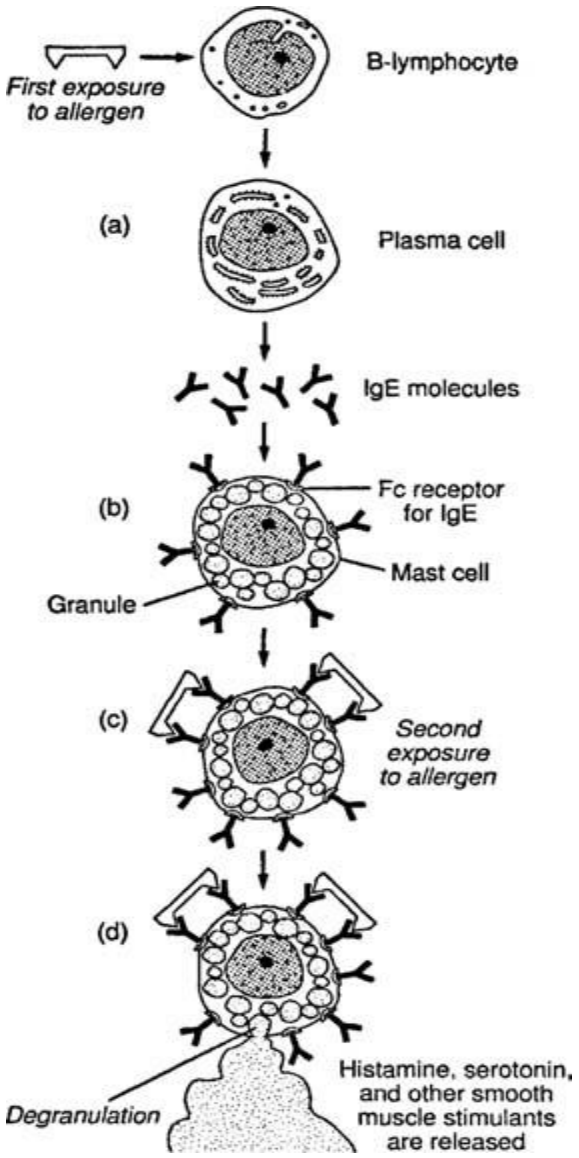


Figure 1

The process of anaphylaxis. (a) Allergens stimulate the production IgE antibodies, which (b) fix themselves to the surfaces of mast cells. (c) On second exposure to the allergens, a reaction occurs on the mast cell surface, and (d) the cellular granules release histamine and other stimulators of smooth muscle contraction.

After the antigen-antibody combination has taken place, the cells release a number of physiologically active substances including **histamine** and **serotonin.** These substances are

derived from granules within the cell. The histamine, serotonin, and other mediators induce spasms of the smooth muscle, such as in the bronchioles, small arteries, and gastrointestinal tract lining. A sudden drop in blood pressure occurs, followed by circulatory collapse and shock. Bronchospasms and edema cause constriction of the respiratory passageways, and breathing is very difficult. Facial edema occurs, and the heart rate increases due to constriction of the arteries. Swellings called "hives" develop at the site of injection and other areas of the skin. In severe cases, anaphylactic shock may result in death within several minutes to an hour. To relieve the symptoms, epinephrine is administered together with a smooth muscle relaxer, a drug such as cortisone to reduce swelling, and other drugs as appropriate.

Allergic reactions. Allergic reactions (allergy) are a milder, localized form of anaphylaxis. As noted, such things as foods, pollen grains, and animal dander can induce these localized reactions. IgE, basophils, and mast cells are involved, but much less than in anaphylaxis. There appears to be a genetic basis for allergic reactions, as evidenced by their distribution in families.
Cytotoxic reactions. Cytotoxic reactions are a form of immediate hypersensitivity, sometimes referred to as **type II hypersensitivity.** In these reactions, IgE and IgM are produced in response to stimulation by antigens. The antibodies unite with the antigens in the bloodstream, but they also unite with analogous antigens on the surface of the human body's cells. This union sets off the complement system, and destruction of the local tissue cells ensues.

An example of a cytotoxic reaction is **thrombocytopenia.** In this disease, antibody molecules are elicited by certain drug molecules. The antibodies unite with antigens on the surface of thrombocytes (platelets), and with complement activation, the thrombocytes are destroyed. The result is an impaired blood-clotting mechanism.

Another example of the cytotoxic reaction is **agranulocytosis.** In this immune disorder, antibodies unite with antigens on the surface of neutrophils. As these cells are destroyed with complement activation, the capacity for phagocytosis is reduced.

Cytotoxic reactions are also manifested by the **transfusion reaction** occurring when improper blood transfusions are performed. Another consequence is **erythroblastosis fetalis**, also known as **hemolytic disease of the newborn**, or Rh disease. In this condition, a pregnant woman produces Rh antibodies against the developing fetus, and when the Rh antibodies unite with Rh antigens on the surface of fetal red blood cells in a succeeding pregnancy, the red blood cells are destroyed (Figure 2).

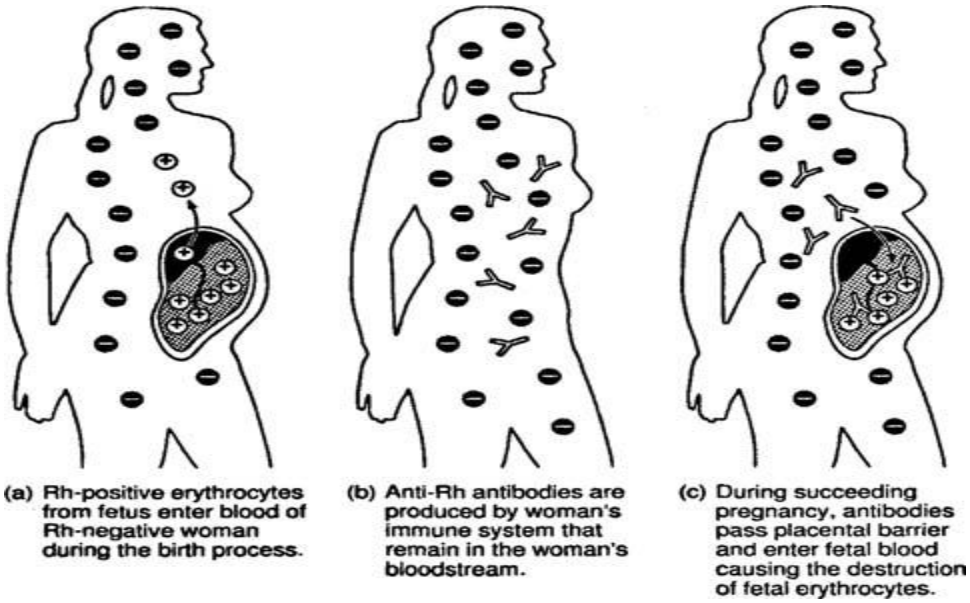


Figure 2

The cytotoxic reaction in erythroblastosis fetalis.

Immune complex disease. Immune complexes are combinations of antigen and antibody that have the ability to fix complement. The antibodies involved are IgM or IgG, and the antigens exist in fluid as soluble antigens. Proteins or nucleic acids may be involved.

An example of immune complex hypersensitivity is **serum sickness.** In this condition, animal serum is administered to humans, and its proteins elicit antibody production. When the antibodies and antigens unite, they form immune complexes, which activate the complement system and cause local tissue damage. The patient may display edema of the hands, face, and feet, as well as swelling of the upper respiratory tissues and impairment of normal respiration. An inflammatory response results.

Formation of immune complexes is also involved with numerous diseases including**systemic lupus erythematosus, rheumatoid arthritis**, and **glomerulonephritis.**Immune complex hypresensitivity is often called **type III hypersensitivity.**

Delayed hypersensitivity. T-lymphocytes rather than antibodies function in cases of**delayed hypersensitivity**, also called **type IV hypersensitivity.** Normally these are the T-lymphocytes involved in cell-mediated immunity. The T-lymphocytes produce lymphokines, which stimulate an influx of macrophages to perform phagocytosis. In delayed hypersensitivity, the result is an exaggeration of the immune response, and the phagocytes bring about the destruction of the local tissue.

Delayed hypersensitivity (also called cellular hypersensitivity) is so named because the reaction requires a day or more to develop. One manifestation of the reaction is**infection allergy**, as in the tuberculin skin test. A purified protein derivative (PPD) of*Mycobacterium tuberculosis* is applied to the skin superficially, and a skin reaction (swelling and redness) occurs 24 to 48 hours later if the person has had a previous exposure to the antigens of *Mycobacterium tuberculosis*, possibly during an episode of tuberculosis.

A second manifestation of delayed hypersensitivity is **contact dermatitis.** In many cases, the reaction is accompanied by large, blisterlike lesions in which vesicles are surrounded by a zone of erythema (redness). Usually, the vesicles itch intensely.

Antigens involved in contact dermatitis include metals such as nickel and mercury, cosmetics, disinfectants, and plant substances such as the resins of poison ivy, poison oak, and poison sumac. The individual can be tested to determine which antigen is the cause of allergy by performing a **patch test.** In this procedure, a patch containing a specific antigen is attached to the skin and left in place for 48 hours to determine if a reaction will take place.