MLAB 1331: MYCOLOGY LECTURE GUIDE

I. OVERVIEW OF MYCOLOGY

A. Importance of mycology

1. Introduction

Mycology - the study of fungi

Fungi - molds and yeasts
Molds - exhibit filamentous type of growth
Yeasts - pasty or mucoid form of fungal growth

50,000 + valid species; some have more than one name due to minor variations in size, color, host relationship, or geographic distribution

2. General considerations

Fungi stain gram positive, and require oxygen to survive
Fungi are eukaryotic, containing a nucleus bound by a membrane, endoplasmic reticulum, and mitochondria. (Bacteria are prokaryotes and do not contain these structures.)

Fungi are heterotrophic like animals and most bacteria; they require organic nutrients as a source of energy. (Plants are autotrophic.)

Fungi are dependent upon enzymes systems to derive energy from organic substrates

- saprophytes - live on dead organic matter
- parasites - live on living organisms

Fungi are essential in recycling of elements, especially carbon.

3. Role of fungi in the economy

a. Industrial uses of fungi

(1) Mushrooms (Class Basidiomycetes)
Truffles (Class Ascomycetes)
(2) Natural food supply for wild animals
(3) Yeast as food supplement, supplies vitamins
(4) Penicillium - ripens cheese, adds flavor - Roquefort, etc.
(5) Fungi used to alter texture, improve flavor of natural and processed foods

b. Fermentation

(1) Fruit juices (ethyl alcohol)
(2) *Saccharomyces cerevisiae* - brewer's and baker's yeast.
(3) Fermentation of industrial alcohol, fats, proteins, acids, etc.

c. Antibiotics

First observed by Fleming; noted suppression of bacteria by a contaminating fungus of a culture plate.

d. Plant pathology

Most plant diseases are caused by fungi

e. Medical importance

(1) 50-100 species recognized human pathogens
(2) Most prefer to be free-living saprophytes; and only accidentally become pathogens

(3) To be pathogenic, they must tolerate the temperature of the host site and possess enzymatic system that allows them to parasitize animal tissues.

(4) Increased incidence of fungal infections in recent times.

4. Importance of medical mycology

a. History

During the time period between 1941 - 1973, the number of reported deaths in the U.S. due to scarlet fever, typhoid, whooping cough, diphtheria, dysentery and malaria decreased from 10,165 cases to 107; but the reported deaths due to mycoses during the same time period, increased from 324 to 530.

b. Modern man

(1) Increased mobility - Travel to a geographical area where a fungus exists as part of the commensal flora of the local population, or is endemic to the area.

(2) The immunocompromised / immunosuppressed

(a) AIDS
(b) Drugs - tetracycline (acne); birth control pills; indiscriminate use of antibiotics; immunosuppressant drugs used in organ transplant patients, cancer and leukemia patients.
(c) Environmental factors - air & water pollution; over processed & "fast" foods; fad diets etc.

(3) Ageing population

c. Immunology of the Mycoses

(1) Antibody mediated immunity (B-cell humoral)

(a) Antibodies are often produced in response to a fungal infection

(b) Serological tests for identification of fungal diseases.

(2) Cellular mediated immunity (T-cell)

(a) T-cell immunity is effective in resistance to fungal infections.

II. Definitions - basic terms as they relate to mycology

A. Basic Structures

1. Hypha (hyphae plural) - fundamental tube-like structural units of fungi.

a. Septate - divided by cross walls

b. Aseptate - lacking cross walls

2. Mycelium - a mass / mat of hyphae forming the vegative portion of the fungus

a. Aerial - growing or existing in the air

b. Vegetative - absorbs nutrients

c. Fertile - bears conidia or spores for reproduction

B. Spores

Sporulation & Spores - preferred terms used when there is a merging of nuclear material / genes combine. Fusion of nuclear material.

Self-fertile = homothallic. Mating types = heterothallic.

Sexual spores - fusion of nuclei

1. **Ascospore** - spore formed in a sac-like cell known as an ascus, the shape of which aids in identification of the fungus. Often eight (8) spores formed. (sexual). (Ascomycetes)

2. **Basidiospore** - sexual spore (union of two nuclei) produced on a specialized club-shaped structure, called a basidium. (Basidiomycetes)

3. **Zygospore** - a thick-walled spore formed during sexual reproduction in the Phycomycetes

Asexual spores - most common type

4. **Conidia** - asexual fungal spores borne externally in various ways from a conidiophore; often referred to a macro- and microconidia. (Ascomycetes & Deuteromycetes). Macroconidia are multicellular
Microconidia are unicellular

5. **Arthroconidium** (Arthrospore) - special type of asexual spore formed by disarticulation of the mycelium.

6. **Blastoconidia / Blastospore** - asexual spore formed from a budding process along the mycelium or from another blastospore. (class Ascomycetes)

7. **Chlamydospore** - thick-walled resistant asexual spore formed by direct differentiation of the mycelium (concentration of protoplasm and nutrients)

8. **Sporangiospore** - an asexual spore contained in a sporangium at the end of a sporangoiphore of the taxonomic class Phycomycetes

9. **Thallospore** - asexual spore produced on a thallus (hypha). (Deuteromycetes)

C. Miscellanous terms

1. **Ascus** - sac-like structure containing (usually eight) ascospores developed during sexual reproduction in the Ascomycetes.

2. **Asexual reproduction** - spores (reproductive bodies of a fungus) are formed directly from the vegetative mycelium or from specialized hyphae.

3. **Chromoblastomycosis** - a subcutaneous mycosis often the result of traumatic inoculation of a dematiaceous fungus into the skin; etiologic agents include species of *Cladosporium, *Fonsecaea, Exophiala, & Phialophora*

4. **Coenocytic** - a cell or an aseptate hypha containing numerous nuclei.

5. **Conidiophore** - a specialized branch of hypha on which conidia are developed.

6. **Dematiaceous** - pigmented, dark in color, usually gray to black.

7. **Dermatophyte** - fungi that cause superficial mycoses.

8. **Diphasic** (dimorphic) - the ability of some fungi to grow as either yeast or filamentous stages, depending on conditions of growth.

9. **Ectothrixic** - ability of the fungus to grow on the outside of a hair shaft.

10. **Endothrixic** - ability of the fungus to grow and penetrate into the hair shaft.

11. **Endogenous** - derived from internal source.

12. **Exogenous** - derived from external source.

13. **Eukaryotes** - organisms possessing a true nucleus (such as fungi) as opposed to prokaryotes which do not contain a nuclear membrane (such as bacteria).

14. **Fungemia** - fungal blood infection

15. **Fungi Imperfecti** - a large class of fungi with septate hyphae in which the asexual state of reproduction is known, but not the sexual state. They are also called Deuteromycetes and include the majority of medically significant fungi.

16. **Germ Tube** - small projections which arise from cells of certain yeasts; indicates the onset of hyphal formation.

17. **Hyaline** - colorless, clear.

18. **Mold** - term generally referring to filamentous fungi

19. **Mycetoma** - a clinical syndrome of localized, tumorous lesions in cutaneous and subcutaneous tissues due to infections, often a foot, with actinomycetes or fungi.

20. **Mycosis** - a disease caused by a fungus

21. **Mycotoxins** - toxins of fungal origin

22. **Oospore** - also called zygospore, a sexual spore produced through the fusion of two unlike nuclei (class Phycomycetes)

23. **Perfect fungi** - fungi having sexual and asexual reproductive stages

24. **Phycomycetes** - a class of fungi forming a coenocytic mycelium with stiff sporangiophores that bear sporangiospores contained in a sporangium

25. **Pseudohyphae** - a chain of elongated budding cells that have failed to detach (not true hyphae)

26. **Ringworm** - term used to describe circular or ring-like skin lesions produced by dermatophytes

27. **Rhizoids** - root-like structures

28. **Saprobe** (Saprophyte) - any plant organism that obtains its nourishment from dead organic matter

29. **Sexual reproduction** - zygote / spore formation follows the fusion of two haploid nuclei

30. **Sporangiophore** - a special aerial hypha or stalk bearing a sporangium

31. **Sporangium** - a sac or cell containing spores produced asexually

32. **Spore** - generally the reproductive body of a fungus; occasionally, a resistant body for adverse environment

33. **Sterigmata** - a specialized structure that arises from a basidium and supports basidiospores

34. **Sporotrichosis** - mycosis the result of inoculation with *Sporothrix schenckii*, lymphocutaneous type is most common

35. **Telemorph** - the sexual form of a fungus

36. **Thallospore** - spore formed by a change in portions of the thallus

37. **Thallus** - the vegetative body of a fungus

38. **Tinea** (Ringworm) - Prefix used with the infected area of the body to indicate a cutaneous mycosis

39. **Yeast** - pasty or mucoid form of fungus growth, microscopically shows a predominance of budding cells

40. **Zoophilic** - dermatophytes which are parasitic on lower animals as well as man

III. Classifications of Fungi

A. *Geographic grouping*

Classification by geographic distribution. Certain fungal diseases are considered endemic to particular areas

Histoplasmosis - Central Mississippi Valley and Ohio Valley fever
Coccidioidomycosis - San Joaquin Valley fever

B. *Epidemiologic grouping*

Concerned with how fungal disease is transmitted.

Few are contagious - i.e. ringworm of the scalp. Some are inhaled, others must be directly introduced into deeper tissue such as by a puncture from a thorn.
Most mycoses are dependent on the susceptibility of the individual host.

C. *Taxonomy grouping*

Scientific grouping according to morphologic and cultural characteristics; varies somewhat, depending on author.

One example of a taxonomic grouping:

KINGDOM: Plantae
PHYLUM: Thallophyte - Entire plant is somatic; no roots, or leaves.
SUBPHYLUM: Schizomycotina
CLASS: Schizomycetes (bacteria)
ORDER: Actinomycetales
FAMILY: Actinomycetaceae
GENUS: Actinomyces
SPECIES: *A. isarelii*
A. bovis
A. naeslundii
GENUS: Nocardia
SPECIES: *N. asteroides*
N. brasiliensis
N. carviae

FAMILY: Streptomycetaceae
GENUS: Streptomyces
SPECIES: *S. madurae*
S. pelletierii
S. paraquavensis
S. somaliensis

SUBPHYLUM: Myxomycotina
CLASS: Myxomycetes (slime molds)

SUBPHYLUM: Eumycotina (true fungi)
CLASS: Phycomycetes (algae fungi)
GENUS: *Rhizopus sp.*
Mucor sp.
Others

CLASS: Ascomycetes
GENUS: *Penicillium sp.*
Aspergillus sp.
Many others

CLASS: Basidiomycetes
GENUS: *Amanita sp.* (many poisonous toadstools)
Agaricus sp. (edible mushrooms)

CLASS: Deuteromycetes (fungi imperfecti)
Yeast that reproduce by budding.

GENUS & SPECIES:
Cryptococcus neoformans
Candida albicans

Diphasic yeast (yeast-like when grown at 37 degrees)
GENUS & SPECIES:
Blastomyces dermatitidis
Blastomyces brasiliensis
Histoplasma capsulatum
Coccidioides immitis
Sporotrichum schenkii

Filamentous fungi - no yeast stage
GENUS & SPECIES:
Geotrichum sp.
Microsporum sp.
Trichophyton sp.
Epidermophyton sp.

D. *Topographic Grouping* - Classification as to type of mycoses produced.

1. Superficial
Confined to the outermost layers of the skin and hair.

No host cellular / inflammatory response due to organisms being remote from living tissue. Essentially no pathology; the disease is recognized purely on cosmetic basis.

2. Cutaneous
Have particular affinity for the keratin of the skin, nails, and hair.

Most cutaneous infections are caused by a closely related group of fungi, called the dermatophytes.

These keratinophilic prefer the non-living cornified layers. The disease caused by these organisms is called Adermatophytosis@ or "dermatomycosis". Host response is patchy scaling or eczema forming eruptions; inflammation may occur. They are classified according to the area of the body that is involved.

3. Subcutaneous
Involve the deeper layers of skin and often muscle tissue.

Man is an accidental host following inoculation of fungal spores via some form of trauma. This type of fungal infection is often tentatively identified by the presence of a characteristic tissue reaction or granule.

4. Systemic
Attack the deep tissues and organ systems; often create symptoms that resemble other diseases.

Two categories of systemic disease.

a. Those caused by truly pathogenic fungi with the ability to cause disease in the normal human host when the inoculum is of sufficient size.

Histoplasma capsulatum
Blastomyces dermatitidis
Coccidioides immitis
Paracoccidioides brasiliensis

b. Opportunistic fungi, low virulence organisms, require the patient's defenses to be lowered before the infection is established.

Aspergillus sp.
Candida albicans
Cryptococcus neoformans

IV. Laboratory Methods in Medical Mycology

A. Collection, handling and processing of clinical mycology specimens

1. Importance

2. Collection - usually by physician or nursing staff

a. Skin - cleaned with 70% alcohol to remove dirt, oil and surface saprophytes

b. Nails - cleaned same as for skin. Usually clipped; need to be finely minced before innoculating to media

c. Hair - obtained from edge of infected area of scalp,. Use a Wood's lamp (fluorescence) to help locate infected hair. Hair can be obtained by plucking, brushing, or with a sticky tape.

d. Body fluids - normal sterile collection procedures

3. Preparation of specimens for immediate transport to laboratory

a. Hair & nails sent in a dry envelope, inside proper container.

b. Other specimens are usually sent frozen or on dry ice.

c. Packaging - biohazard regulations. Any growing cultures must be on tube media (not plates). Aluminum screw-capped mailing tube with outer cardboard mailing tube.

d. Inside labeling information: patient ID, specimen source, suspected organism.

e. Outside labeling information: must state
WARNING: POTENTIAL PATHOGEN

4. Appropriate processing of specimen to recover fungus

a. Skin, nails, & hair - direct exam following KOH preparation

b. Body fluids

(1) CSF - centrifuged; examine sediment microscopically, inoculate media

(2) Pleural fluid, sputum, and bronchial aspiration - specimen must be fresh as saprophytes would overgrow pathogens such as *H. capsulatum*. Specimens may be refrigerated up to 2 hours.

(3) Gastric washings - same as for pleural fluids

(4) Genito-urinary specimens - first morning specimen preferred; centrifuge

(5) Blood/bone marrow - generally inoculated directly to BHI broth and BHI slant. Extra specimen should be inoculated to other fungal media.

(6) Wound abscess or drainage - should be cultured anaerobically, especially if actinomycosis is suspected.

(7) Tissue specimens - examine for pus, caseous material or granules; mince aseptically , can use small amount of sterile saline and the supernatant also inoculated.

B. Direct examination of specimens

1. Direct exam required on any biological material sent to lab for fungus culture. Look for spores, hyphae, mycelial elements, budding yeast, mycotic granules.

2. Wet mount prep - good for yeast; examination is done in natural environment, so loss of fragile structure is minimal.

3. KOH prep - Potassium hydroxide; done on skin scrapings, hail, nails, sputum, vaginal specimens, etc. The KOH digests and clears the specimen's tissue cells, mucous, etc., so fungal elements can be seen.

C. Stains

1. Lactophenol Cotton Blue (LPCB) - very popular for quick evaluation of fungal structures; will stain the chitin in cell walls of fungi.

2. Periodic Acid - Schiff Stain (PAS) - stains certain polysaccharide in the cell walls of fungi. Fungi stain pink-red with blue nuclei.

3. Gomori Methenamine Silver Stain - silver nitrate outlines fungi in black due to the silver precipitating on the fungi cell wall. The internal parts of hyphae are deep rose to black, and the background is light green.

4. Gridley Stain - Hyphae and yeast stain dark blue or rose. Tissues stain deep blue and background is yellow.

5. Mayer Mucicarmine Stain - will stain capsules of *Cryptococcus neoformans* deep rose.

6. Fluorescent Antibody Stain - simple, sensitive, and extremely specific method of detecting fungi in tissues or fluids. Applications for many different fungal organisms.

7. Papanicolaou Stain - good for initial differentiation of dimorphic fungi. Works well on sputum smears.

8. Gram Stain - generally fungi are gram positive; Actinomyces and Nocardia are gram variable.

9. Modified Acid-Fast Stain - used to differentiate the acid-fast Nocardia from other aerobic Actinomyces.

10. Giemsa Stain - used for blood and bone marrow specimens. *Histoplasma capsulatum* is an intra cellular organism, which appears as small oval to

pear-shaped yeast-like cells with crescent shaped red-stained protoplasm surrounded by clear halo in segmented neutrophils.

11. India Ink - demonstrates the capsule of *Cryptococcus neoformans* in CSF specimens.

D. Fungal Culturing

1. Media introduction

a. Generally tube media is used rather than plated media because:
(1) there is less chance for spore release into the environment.
(2) less chance for dehydration
(3) ease of storage.

b. The agar in a tube is inoculated in a straight line. Preliminary identification is based on differential growth patterns on various media

2. Media (Media should be carefully selected, and more than one media should be used)

a. Sabouraud's dextrose agar (Sab-Dex) - classic medium, recommended for most studies.

b. Sabouraud's dextrose agar with chloramphenicol - chloramphenicol inhibits bacterial growth.

c. Mycosel agar - commercially produced agar containing chloramphenicol to inhibit bacterial growth, and cycloheximide to inhibit saprophytic fungi and some yeasts (including *C. neoformans).*

Notes:
(1) Aspergillus and Scopulariopsis (saprophytes) are <u>opportunistic</u> pathogens. Cycloheximide will prevent their growth.

(2) *Cryptococcus neoformans* is also inhibited.

(3) Bacteria-like fungi (such as Actinomycetes) are inhibited by chloramphenicol.

d. Brain heart infusion slant (BHI) - more enriched than Sab-Dex. Used in recovery of *H. capsulatum*.

e. Potato-dextrose agar (PDA) and Corn-meal agar - are used in slide cultures; as they induce spore formation, which greatly aids in identification.

3. Special applications agar

a. Caffeic Acid Agar - *Cryptococcus neoformans* will produce melanin resulting in black colonies. (protect media from light)

b. Birdseed Agar - used to isolate *Cryptococcus neoformans* from contaminated cultures.

c. KT Medium & Kelley Agar - used to convert dimorphic fungus *Blastomycetes dermatitidis* from mycelial to yeast form.

d. Modified Converse Liquid Medium (Levine's) - used to promote spherule production by *Coccidioides immitis*.

4. Fungal growth requirements

a. Temperature - Room temperature (25-30 C) for most fungi.

Notes: (1) *Nocardia sp.* and some dimorphic organisms grow best at 37 degrees C.

(2) Any fungus capable of growing at 37 C, should be considered potentially pathogenic.

b. Atmosphere - True fungi are aerobic; there are a few anaerobes among the bacteria-like fungi.

c. Time - Some yeasts grow overnight. Saprophytes are fast growers (several days). Generally cultures are held at least 4 weeks. *

Exceptions*: Paracoccidioides brasiliensis* may require 4-5 weeks, & 10 weeks are recommended if *Histoplasma capsulatum* is suspected.

V. Techniques for Identification of Fungi & Laboratory ID

A. Inoculation

1. Plates - Inoculated like a large "S", so that rapid growing fungi can removed.

2. Slants - Inoculated with a straight line.

B. Incubation

1. Aerobic (and anaerobic if Actinomycetes are suspected)
2. Room temperature & also sometimes at 37E if dimorphic fungus is expected.

C. General considerations

1. Type of media used. Does it contain antibiotics?
2. Growth rate & age of the culture.

a. Routine cultures are kept for 4 weeks & should be examined every other day.

b. Most systemic pathogens require 10 days to 2 weeks, while saprophytic fungi grow usually grow within 1 week.

D. Colony Morphology (macroscopic features)

1. Surface topography - Some fungal colonies may be free growing, covering the entire surface of agar in a particular manner; others grow in a restricted manner.

2. Surface texture -examples: cottony or wooly (floccose), granular, chalky, velvety, powdery, silky, glabrous (smooth, creamy), waxy, etc.

3. Pigmentation - Fungi may be colorless or brightly colored. Color may be on fungus itself, on its sporulating apparatus, on the agar, or on the bottom of the colony (reverse pigmentation). Pigment color is due to the color of the sporulating apparatus. The pigment can be diffused into the agar. It is important to note the top pigment (obverse) and the discoloration of the agar medium (reverse). See *Dematiaceous* in definitions.

4. Mycelium
   a. Vegetative mycelium - provides nutrition
   b. Aerial mycelium - reproductive

E. Microscopic evaluation

1. Methods

   a. Teased Preparation
   b. Slide Culture Techniques - best as it gives undisturbed microscopic morphology.
   c. Transparent Tape Preparation

2. A review of terms associated with the microscopic features is advisable.

   a. Hyphae structure. Hyphae (plural); hypha (singular)

      (1) Septate vs. non-septate (aseptate)
      (2) Dematiaceous vs. hyaline

   b. Spore bearing structures

   c. Spores - Many terms addressing reproduction are provided in the terms section and in the text, please review (perhaps categorize them).

3. Biochemical studies - generally used to ID yeast and yeast-like organisms.

   a. Carbohydrate fermentation

(1) Growth and utilization of a carbohydrate under anaerobic conditions as determined by acid and gas production.

(2) Specimen is inoculated beneath broth so that it is completely covered. *Bromcresol purple* is the indicator. Acid production turns purple to yellow. Gas is detected by appearance of bubbles trapped in the fermentation tube. Observe every 48 hours for 14 days.

b. Carbohydrate assimilation

(1) Ability to utilize a carbohydrate as sole source of carbon.

(2) *Bromcresol purple* indicator turns from purple to yellow.

(3) Tubes unchanged (as determined by comparing to a blank tube) by 10 days are negative.

c. Nitrogen assimilation

(1) Utilizes 3 tubes with differing sources of nitrogen. *Bromthymol blue* is the indicator (blue to yellow is positive).

d. Growth on specific agars

(1) Christensen's urea agar

(a) Urea is hydrolyzed by some yeast to form ammonia (pH increases) which turns media from yellow to dark pink.

(2) Caffeic acid medium (protect media from light)

(a) Production of melanin by *Cryptococcus neoformans* resulting in black colonies.

4. Other tests

a. Germ tube - *Candidia albicans & Candidia stellatoidea* produce germ tubes when incubated in a protein medium.

Candida germ tube

b. Demonstration of chlamydospores - Yeast is inoculated by jabbing appropriate agar (Cornmeal with tween 80) and observed every 24 hours for 3 days for chlamydospore production.

5. Stains (covered previously)

6. Hypersensitivity (seromycology; skin tests) and serological tests

a. Skin tests - demonstrates T-cell immunity (cellular) to a fungus

b. Serological tests - demonstrates B-cell (humoral) immunity to a fungus; sera should be drawn in pairs (acute and convalescent).

(1) Complement fixation
(2) Agglutination tests
(3) Precipitin tests
(4) Immunofluorescence
(5) Immunodiffusion techniques
(3) Counterimmunoelectrophoresis

7. Antifungal susceptibility testing - some progress has be made in the attempt to standardize susceptibility testing as a means in evaluating unusual fungal isolates, determining the cause(s) of patient relapse and treatment failure.

8. Determining serum concentration of antifungal agents - can be very important for patients with renal or liver problems or in cases where poor absorption is a concern. HPLC has proven to be an excellent method due to its accuracy and ability to analyze more than one drug in a specimen.

VI. Saprophytes

A. Contaminates? or opportunistic pathogens?

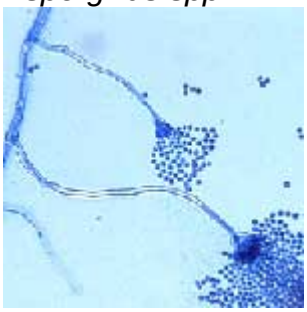
B. Most are inhibited by cycloheximide

C. Grouped by type of mycelia produced

1. Septate vs. aseptate
2. Hyaline vs. dematiaceous

D. Members - hyaline

1. *Aspergillus spp*.:

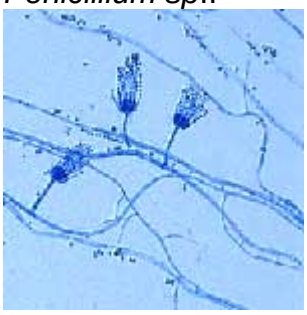


Growth rate varies, colors vary, surface velvety to cottony. Mycelium - septate and hyaline with unbranched condiophores (compare to *Syncephalastrum*, which appears similar, but is aseptate). Of the species commonly listed, *A. fumigatus* is considered potential pathogen, especially if from a pulmonary source (grows well at 37C.).

2. *Paecilomyces sp*.:

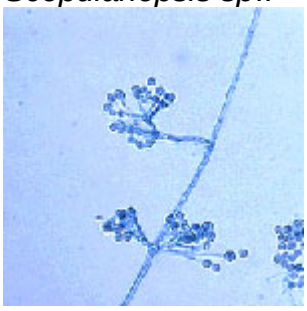
Rapid grower, colors vary. Brush-like conidiophores. Long, tapered sterigmata.

3. *Penicillium sp*.:



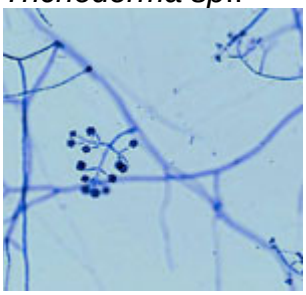
Commonly rapid growing; white to bluish-green. Conidiophores characteristically form a brush-shaped structure. Sterigmata are flask shaped.

4. *Scopulariopsis sp*.:



Moderately slow growing. White turning brown with age. Branched or unbranched conidiophores; sterigmata are coarsely roughened.

5. *Trichoderma sp*.:



Moderately rapid growth. Flask-shaped conidiophores, conidia are clustered.

6. *Fusarium sp*.:

Rapid growth, white colonies may be come brightly colored.
Short conidiophores often branched, have macro- and micro-conidia, which are oval to sickle-shaped. Has been reported in eye infections.

E. Members - dematiaceous

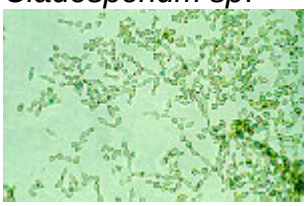
1. *Alternaria sp.:*

Rapid growth; colonies become very dark with age, but may become overgrown with looser white to gray aerial mycelium. Conidiophores bear single or branched chains of large, brown conidia.

2. *Curvalaria sp*.:

Rapid growth. Velvety colonies vary in color from grayish-brown to black. Spirally arranged brown conidia are borne at the tips or brown, septate, unbranched conidiophores.

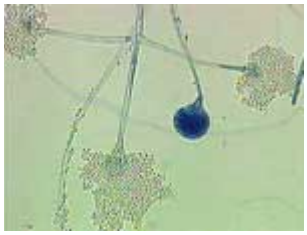
3. *Cladosporium sp*.



Rapid growth. Green colonies, reverse black. Septate, dematiaceous mycelium; conidia are borne in chains and resemble trees.

F. Members - Phycomycetes (aseptate). All are susceptible to cycloheximide. Rapid growers. Some have root-like structures - rhizoids. Spore (sporangia) bearing structures called sporangiophores.

1. *Mucor* –

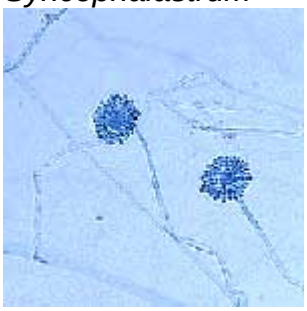


Can fill a culture tube in one day. Unbranched sporangiophores. No rhizoids.

2. *Rhizopus* –

Rhizoids are present; sporangiophores nodal in origin.

3. *Syncephalastrum* –

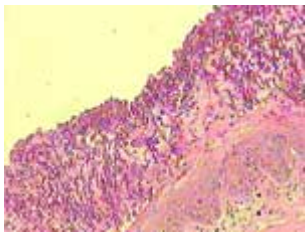


Very rapid growth. White to dark gray colonies with dense, cottony, aerial mycelium. Aseptate, hyaline mycelium, with short, branched sporangiophores, terminating into tips with many tubular sporangia containing chains of spores.

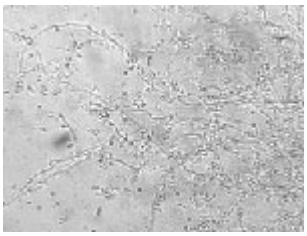
VII. Yeasts

A. *Candidia albicans* –

C. albicans culture



Oral Thrush



Candida vaginitis

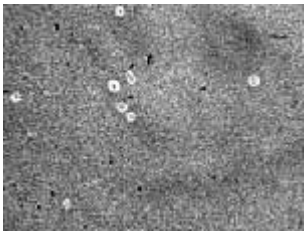
Oral thrush, cutaneous infections, buy can go systemic. ID usually by positive germ tube test, any or production of chlamydospore on cornmeal agar.

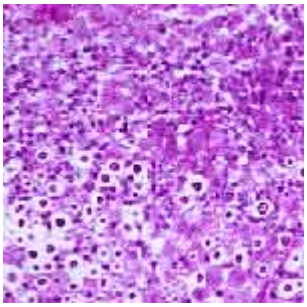
B. *Torulopsis glabrata* – (now included in the Genus Candida)

ID by carbohydrate fermentation tests. Frequently found in urine of debilitated persons, is. elderly diabetics

C. *Cryptococcus neoformans* –


C. neoformans culture


Capsule with India ink


C. neoformans brain infection

Pigeon feces. Has capsule, grows at 37 degrees & produces melanin-like pigment on certain agars. ID in CSF by India ink stain for capsule.

D. *Geotrichum* –

A saprophyte; commonly present in GI tract; implicated in respiratory infections; frequently a secondary invader (lungs, mouth, intestine, vagina skin). Causes disease in immunosuppressed.

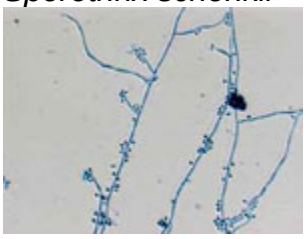
VIII. Bacteria - Like Fungi

A. Some produce Asulfur granules@ , esp. *Actinomycetes israeli*

B. Often require special media, stains, & conditions for growth: anaerobic *Actinomycetes*, and partially acid-fast *Nocardia*

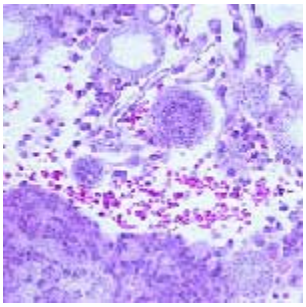
IX. Biphasic

A. *Sporothrix schenkii* –



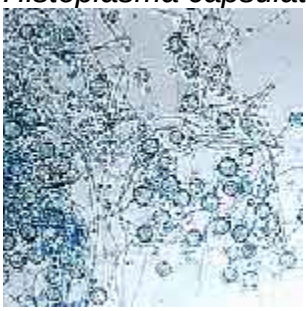
Rose fever, gardeners often affected.

B. *Coccidioides immitis* – San Joaquin valley fever

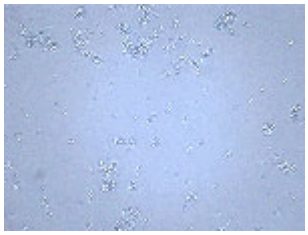


1. mycelial phase
2. parasitic phase (tissue)

C. *Histoplasma capsulatum*



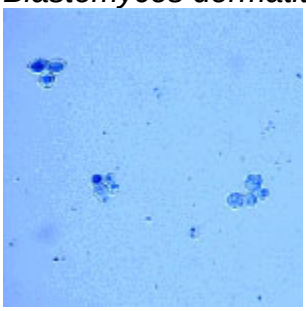
H. capsulatum mold phase

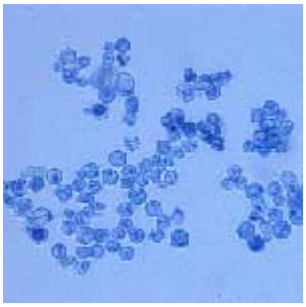


H. capsulatum yeast phase

Mississippi Valley fever \ Darling=s disease - bird droppings, man inhales spores, disease mimics TB. ID - found frequently in reticuloendothelial cells as extracellular inclusions. Skin testing - A negative skin test indicates only that there is not an active or recent infection.

D. *Blastomyces dermatitis* –





North American Blastomycosis / Gilcrest disease. Large yeast cells with single bud.

E. *Paracoccidioides braziliensis* –

South American Blastomycosis / Large yeast cells with multiple buds.

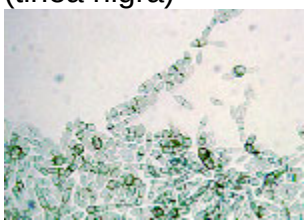
X. Types of mycoses based on body site (review earlier pages of lecture guide)

A. Superficial mycoses

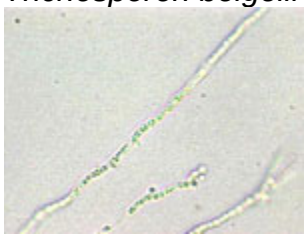
1. Involve outer keratinized tissues of the skin and hair
2. Little inflammatory response or tissue destruction results
3. Causative agents

a. *Malassezia furfur* - cause of pityriasis versicolor (tinea versicolor)

b. *Phaeoannellomyces werneckii* (formerly *Cladosporium werneckii* or *Exophiala werneckii* - cause of superficial phaeohyphomycosis (tinea nigra)

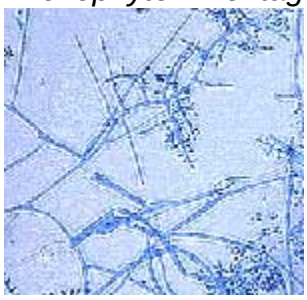


c. *Trichosporon beigelii* - cause of white piedra



d. *Piedraia hortae* - cause of black piedra

e. *Trichophyton mentagrophytes* - cause of Arural ringworm@



B. Cutaneous mycoses (dermatomycoses - fungal infections involving the dermis, includes *C. albicans* & other yeasts as well as dermatophytes)

1. Three genera: *Microsporum, Epidermophyton, & Trichophyton*

2. Dermatophytoses: - infection of skin, hair & nails caused by :

   a. Microsporum - hair, skin, rarely nails (frequently in children, rarely in adults) often spontaneous remission occurs.
   b. Trichophyton - hair, skin & nails. In both children & adults - chronic.
   c. Epidermophyton - skin, nails, rarely hair. In adults, rarely children.

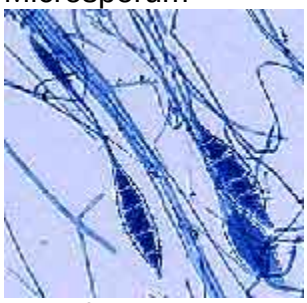
3. Dermatophytes produce septate, hyaline mycelium.

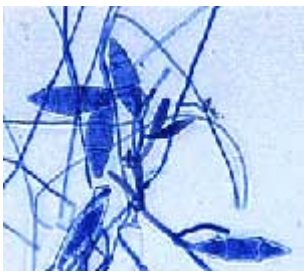
4. Host preference

   Microsporum -children, rarely adults
   Trichophyton - both children and adults
   Epidermophyton - adults, rarely children

5. Macroconidia of each genus

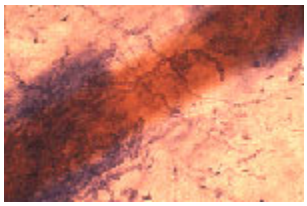
   a. Microsporum –

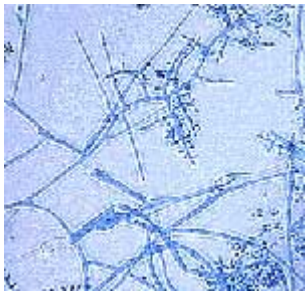

M. canis


M. gypseum

attached singly, has thick walls & mature forms are echinulate (spiny)

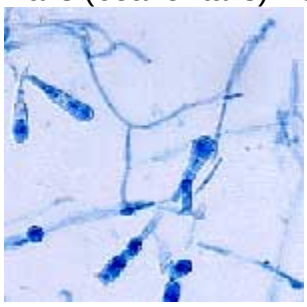
   b. Trichophyton - attached singly, have smooth walls


Ectothrix infection – hair shaft


T. mentagrophytes


T. rubrum

c. Epidermophyton - attached in multiples, moderately thick, smooth walls (beaver tails). Grows slowly.



C. Subcutaneous mycoses - chronic, supperative or granulomatous infections of the subcutaneous tissues, usually on an extremity (hands, feet); can extend through the lymphatics or form sinus tracts. Caused by a variety of fungi and bacteria-like fungi that live in the soil.

D. Chromoblastomycosis

1. Chromoblastomycoses are chronic, non-contagious skin diseases characterized by the development of a papule at the site of infection that spreads to form a warty or tumor-like lesion that has a Acauliflower@ appearance. Occurrence is usually on the legs or feet but can involve deeper tissues. Extensive fibrosis in deeper tissue may block the lymphatics and cause an Aelephantiasis@ syndrome. Complete cure is rare, but the condition usually remains localized. In the lab, crusts and exudates from lesions mounted in 10% KOH will reveal brown-pigmented, branching, septate hyphae. Biopsy material and pus may reveal thick-walled, round, brown cells called Asclerotic bodies.@ Causative agents: *Fonsecaea pedrosoi, Cladosporium spp.,* and *Phialophora* spp.

2. *Fonsecaea pedrosoi* is the most common etiologic agent of chromoblastomycosis worldwide.

3. Prevention: wear shoes, avoid trauma that would inoculate organisms, and use good hygiene.

4. Can be found throughout the world, but more in tropical areas.

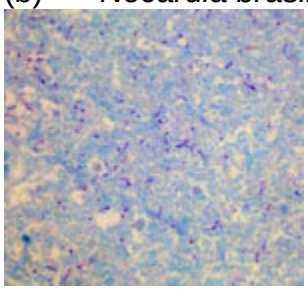
5. Mycetoma - clinical syndrome of localized, tumorous lesions in cutaneous and subcutaneous tissues usually the foot. Nodules are formed, and a collection of pus and formation of sinuses results. Actinomycotic mycetomas must be differentiated from Eumycotic (true fungi) mycetomas and as they have greatly differing treatments.

a. Actinomycotic mycetoma (some authors classify this as systemic)

(1) Lumpy-jaw named by Bollinger in 1976 because of its appearance in tissue. The initial nodules formed are firm and described as "woody" or "lumpy".

(2) Sulfur granules - Bread crumb-like aggregates of microorganisms and cellular debris which take on the appearance of a sulfur particle. True granules will appear microscopically as lobulated tangled masses of delicate gram-positive branching filaments or short bacillary elements.

(3) Etiological agents
(a) *Nocardia asteroides*
(b) *Nocardia brasiliensis*



(c) *Streptomyces somaliensis* (Africa, S. America, Arabia)
(d) *Streptomyces paraguayensis* (S. America)

(4) Prognosis - if diagnosed early, can be treated successfully. Advanced cases often require amputation due to physical impairment

b. Eumycotic Mycetoma

(1) Sometime termed Amaduromycotic mycetomas.@ Clinical picture similar to actinomycotic mycetomas. Disease is slowly progressive, with patients frequently dying of secondary infections. Treatment consists of excising early, localized lesions (amputation may be required for advanced infections). Anti-fungal drugs have little effect.

(2) Body tries to wall off the offending fungus. Invasion of deeper tissue may follow, with bone involvement, draining sinus tracts, or progression through the lymphatics. Can become systemic.

E. Systemic mycoses

1. Dissemination of any fungal agent, yeast, or bacteria-like fungus to involve any tissue or organ

2. Agents - all are dimorphic/diphasic

a. *Blastomyces dermatitidis* - blastomycosis

b. *Paracoccidioides brasiliensis* -paracoccidioidomycosis
c. *Coccidioides immitis* - coccidioidomycosis
d. *Histoplasma capsulatum* - histoplasmosis

3. *Histoplasma capsulatum*

a. Histoplasmosis - an infection of the reticuloendothelial system resulting in patchy bronchopneumonia containing yeast-laden phagocytic cells within alveolar spaces. The yeast cells multiply within the giant cells produced and can disseminate to other tissues.

b. Although worldwide in distribution, it is endemic in the Mississippi, Missouri, St Lawrence, and Ohio river valleys.
c. Strong association with bird and bat droppings.

4. *Coccidioides immitis*

a. Coccidioidomycosis - usually an asymptomatic or mildly symptomatic, self-limiting upper respiratory tract infection, but may become disseminated and fatal.

b. Called San Joaquin Fever, or Valley Fever, it is endemic in southwestern US, esp. Arizona and California.

c. Caution: cultures produce arthroconidia which are very easily inhaled. See related information

5. *Paracoccidioides brasiliensis*

a. Paracoccidioidomycosis - also called South American blastomycosis

6. *Blastomyces dermatitidis*

a. Blastomycosis - also called North American blastomycosis or Gilcrest disease.

XI. Treatment of Mycoses - treatments are variably effective.

A. Nystatin - seems to work well against *Candida sp.*

B. Pimaricin

C. Amphotericin B - useful on dermatophyte infections, may result in eye damage, not good for *Fusarium sp.*

D. 5-fluorocytosine - effective against *Cryptococcus* and *Candida.*

E. Miconazole