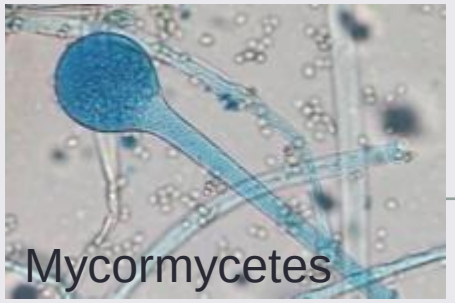
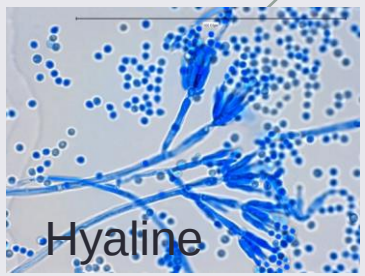
# Mycology Update 2020

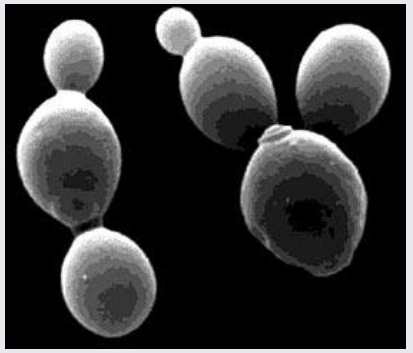
**Margie Morgan, PhD, D(ABMM)**



# Starting point

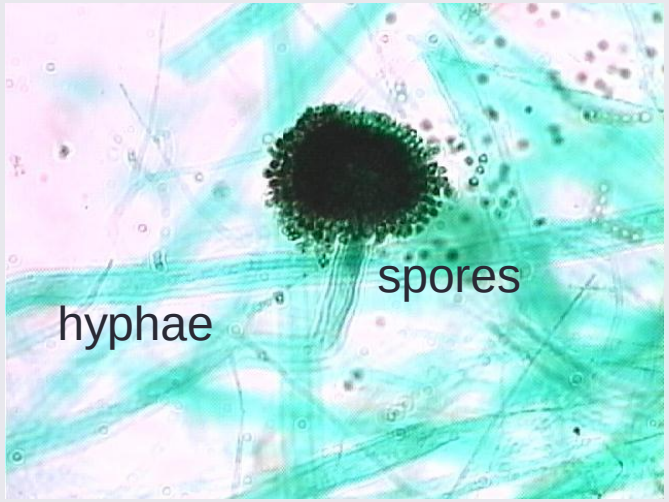


## Yeast are:

- Unicellular and bud to produce daughter cells
- Growth on solid media usually white to beige and appear much like bacterial colonies
- A few genera produce pigment ( ex. Rhodotorula = orange/red)
- Some genera produce mucoid colonies (ex. Cryptococcus)

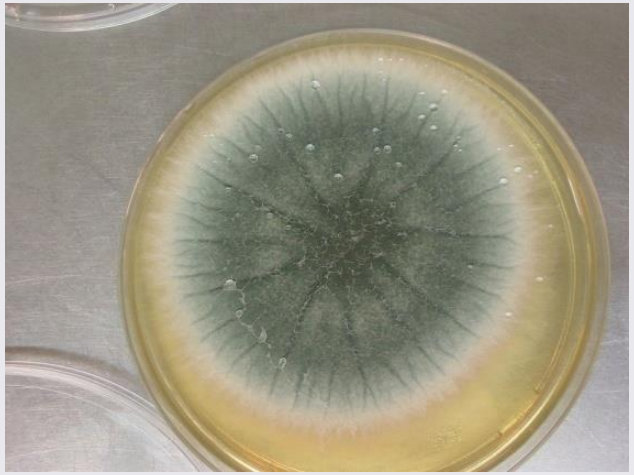
Yeast colonies on blood agar

# Starting point




## Molds:

- Produce hyphae and conidia [spores]
- Growth on solid media usually downy, fluffy, or cottony
- Most mold colonies produce pigment, which aid in identification



# Specimen Collection and Transport

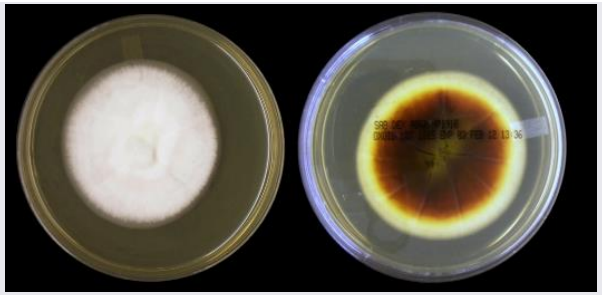
- Fungi are very hardy organisms
- No special requirements for transport media
- Sterile containers necessary to prevent bacterial contamination / Room temperature transport is adequate
- Numerous anatomic sites are appropriate for culture
  - Respiratory specimens – sputum, bronchial lavage, brushings, nasal sinuses
  - Tissue biopsies
  - Cutaneous - Skin scrapings, material from lesions
  - Ocular
  - Sterile body fluids,
  - Cerebrospinal fluid (CSF)
  - Blood
  - Bone Marrow

# Fungal Culture Media

- **Sabouraud glucose agar (SABS)**
  - All purpose Fungal media – antibiotic free
  - Contains 2% glucose, pH @7.0
  - Best use is for subculture of fungi
  - for identification and susceptibility workup

- **Inhibitory mold agar (IMA)**
  - Selective and enriched agar - contains chloramphenicol and gentamicin to inhibit bacterial growth
  - Good for the primary recovery of pathogenic fungi

# Fungal Culture Media



- **Mycosel / Mycobiotic agar**
  - Selective SABs like agar containing chloramphenicol and cycloheximide
  - Used for selective culture of dermatophytes – fungi that cause skin, hair and nail infections
  - Should not be used as a primary plating media for all fungi due to inhibitory agents.
    - Cycloheximide can suppress some species of pathogenic fungi from growing: these include *Trichosporon, Candida tropicalis and Cryptococcus neoformans/gattii*

- **Brain heart infusion agar** / with or without blood
  - Primary recovery of fungi
  - Inhibits bacterial growth by adding chloramphenicol and gentamicin
  - Addition of blood can be added to nurture for systemic fungi

# Processing of Fungal Cultures

- Inoculate specimen onto media and streak for isolation
- Seal plates with oxygen permeable tape to prevent environmental contamination and protect laboratory workers from plates opening and releasing pathogenic fungal spores
- Can use tubes or vials, but more difficult to visualize fungal growth
- Incubate media at 30˚C for 4 weeks (extended time for the detection of systemic fungi
- If growth occurs - perform appropriate identification test method

# Yeast Identification

- Biochemical reactions have been used for decades to identify yeast. Recent taxonomic updates and new emerging pathogens has made biochemical identification less definitive.
- Manual and automated systems have methods for biochemical identification

- Newer methods with improved accuracy of identification include:

(1) MALDI-TOF (Matrix Assisted Laser Desorption/Ionization Time of Flight)

Yeast is superheated and converted into charged particles (proteins) based on mass and charge form identification peaks characteristic for each yeast species

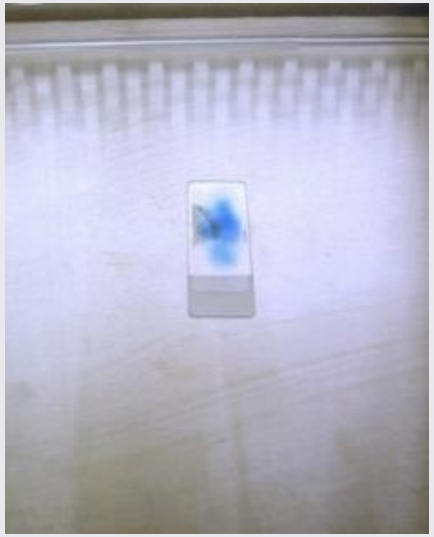
(2) 16S rRNA sequencing – particularly helpful for rare and difficult to identify yeast species/ based on the detection of sequence differences (polymorphisms) in the16S rRNA gene which is present in all yeast

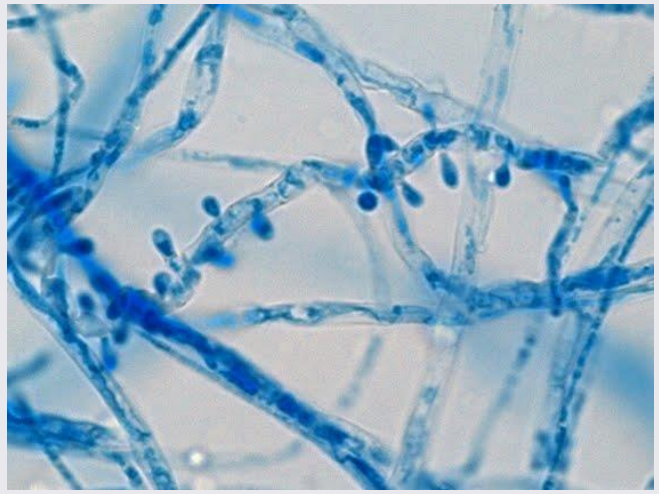
# Mold Identification methods

For years, Lactophenol Cotton Blue [LCB] adhesive tape preparation has been used to assist with mold identification.

LCB mounting medium consists of phenol, lactic acid, glycerol and aniline cotton blue dye.

Clear adhesive tape touches mold colony, picking up fungal hyphae/conidia. Prep then gently pressed into one drop of LCB on a microscope slide.





Newer/ more exact identification methods include:

(1) MALDI-TOF
(2) 16S rRNA sequencing

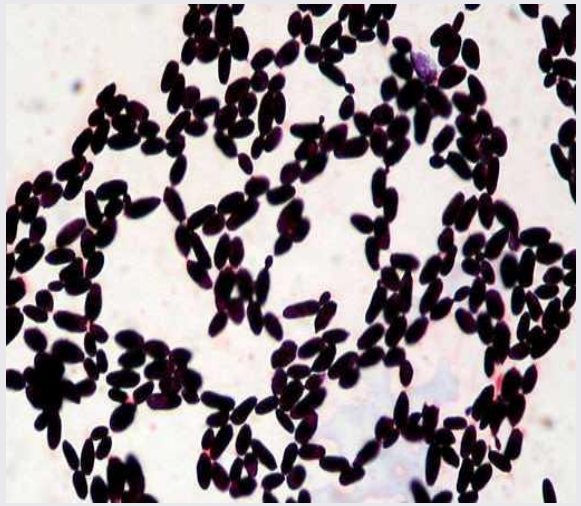
# Safety in the Mycology Laboratory

- All identification work on molds must be performed in a BSL-2 biosafety cabinet with HEPA filtration
- Yeast identification testing can be performed on the bench top
- All fungal media plates should be sealed to prevent contamination from mold producing spores being released into the laboratory.

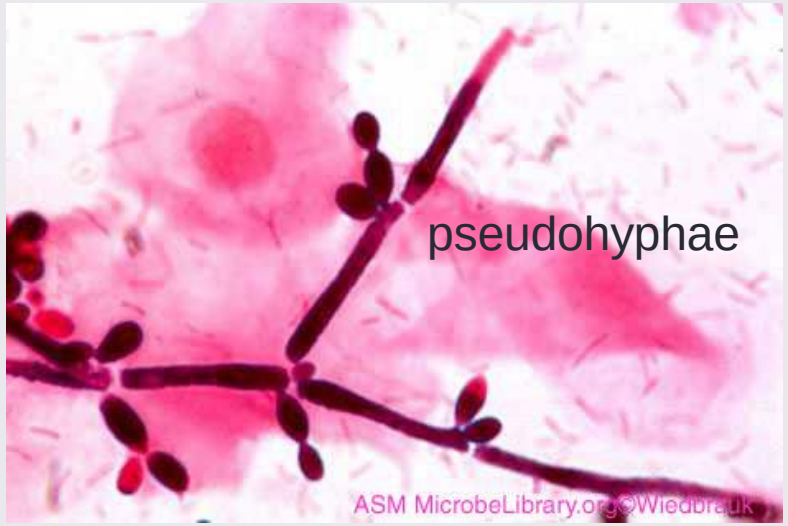
# Direct Exam of Specimens

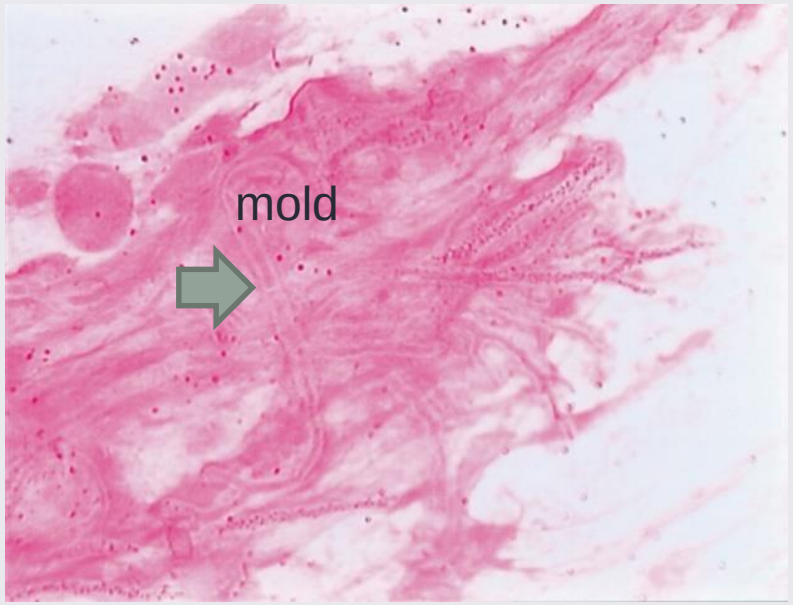
## Gram stain

- Yeast cells stain positive (blue) using Gram stain procedure
- Examine Gram stain for budding cells to confirm that it is a yeast and not a staining artifact.
- Yeast pseudo-hyphae can often be seen on Gram stain
- Mold (true hyphae) can be difficult to visualize on Gram stain



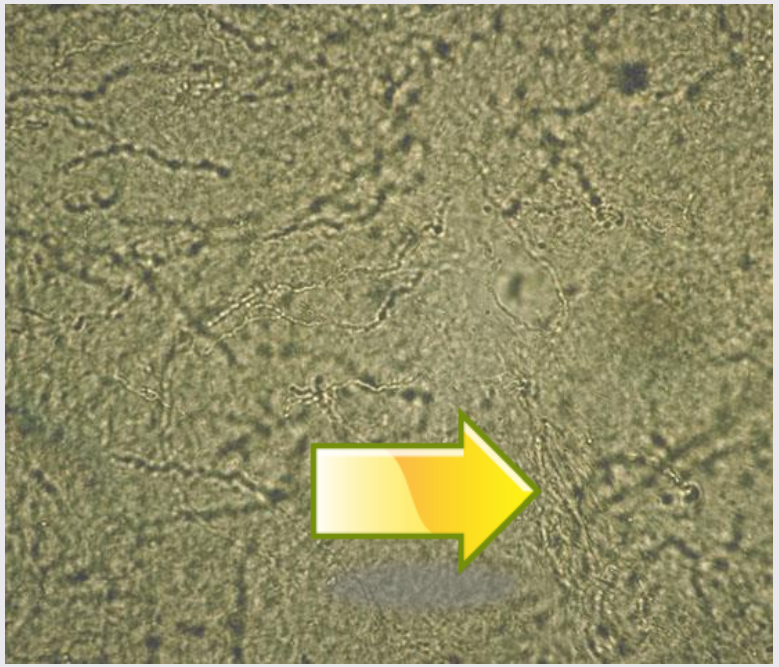
X100 oil immersion



# Potassium Hydroxide Prep / KOH

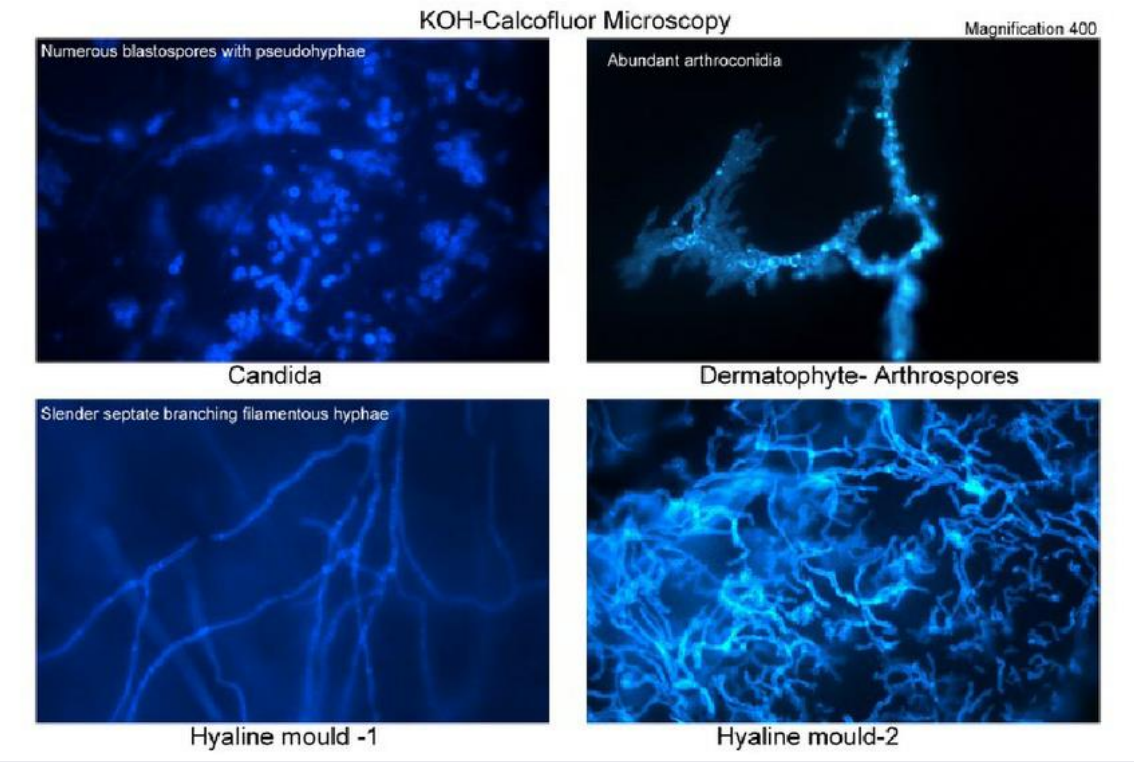
- KOH mount is used to detect yeast, pseudohyphae, and hyphae from skin, hair, and nail specimens
- KOH dissolves the keratin in cellular material and frees fungal hyphae and yeast cells so they can be visualized. –
- Careful! Hyphae can be confused with cell borders



X40
Light microscopy

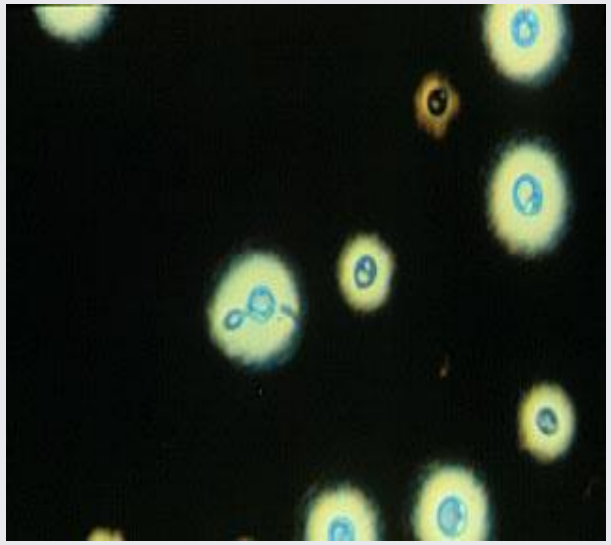
# Calcofluor White Stain

- Yeast, pseudo-hyphae, and mycelial fungi bind with the Calcofluor white stain reagent and fluoresce
- Read stain using a fluorescence microscope (40X)
- More sensitive and specific than a KOH preparation.



# India Ink – for Cryptococcus detection in CSF

One drop of black ink is placed into one drop of CSF on a microscope slide – examine using light microscope (40X)

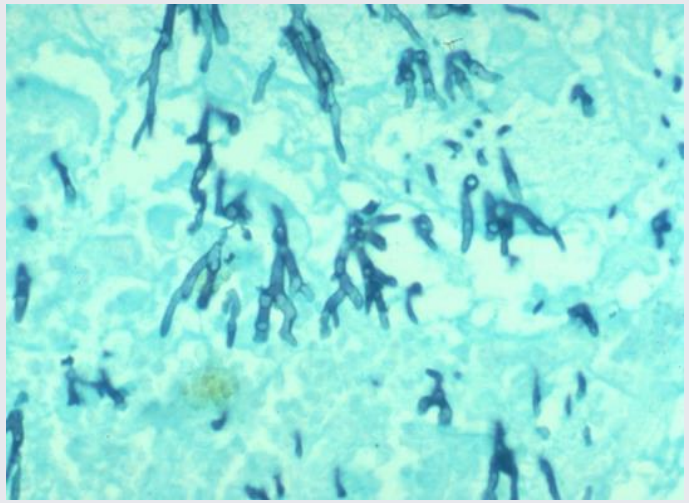


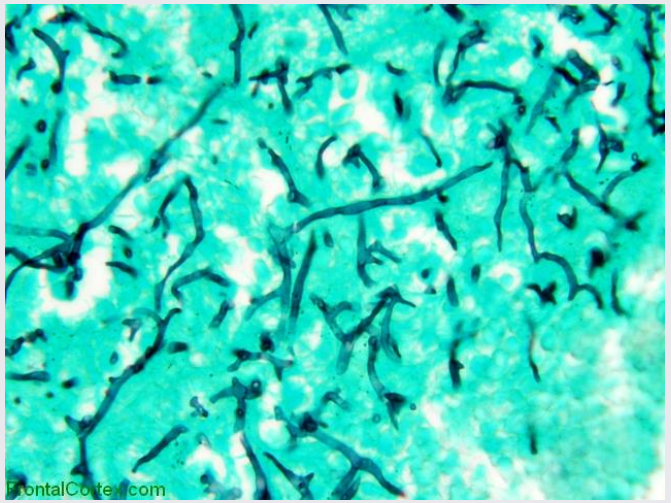
It is a “negative” stain technique – stains the background of prep not the yeast cell or capsule. The clearing around the yeast is due to the polysaccharide capsule produced by *Cryptococcus neoformans* and *C. gattii.*

India ink is less sensitive than the Cryptococcal antigen test for cryptococcal meningitis diagnosis.  Cryptococcal antigen is considered the gold standard test.

# Examination of fungi in fixed tissue

**Grocott's Methenamine Silver Stain [GMS]** – yeast and hyphae stain grey to black. Stains both living and dead yeast and hyphae

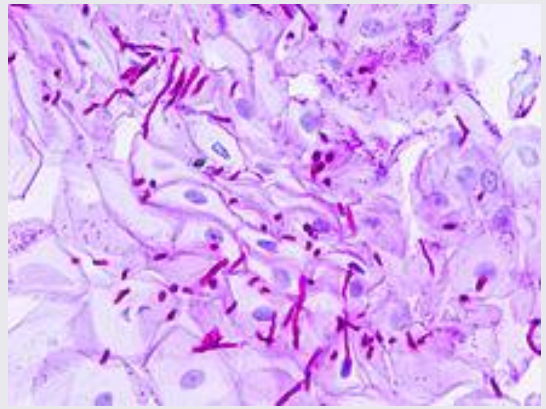


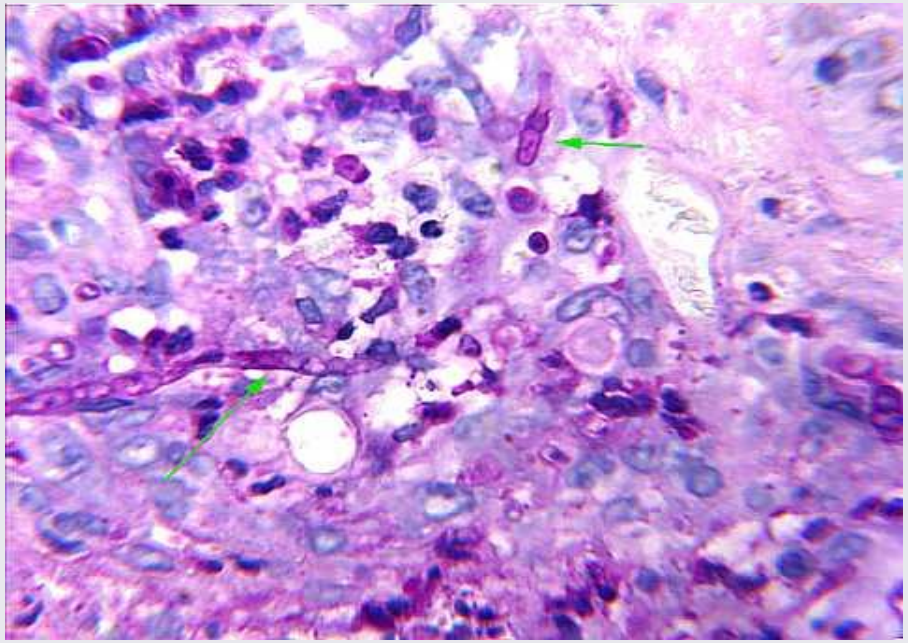


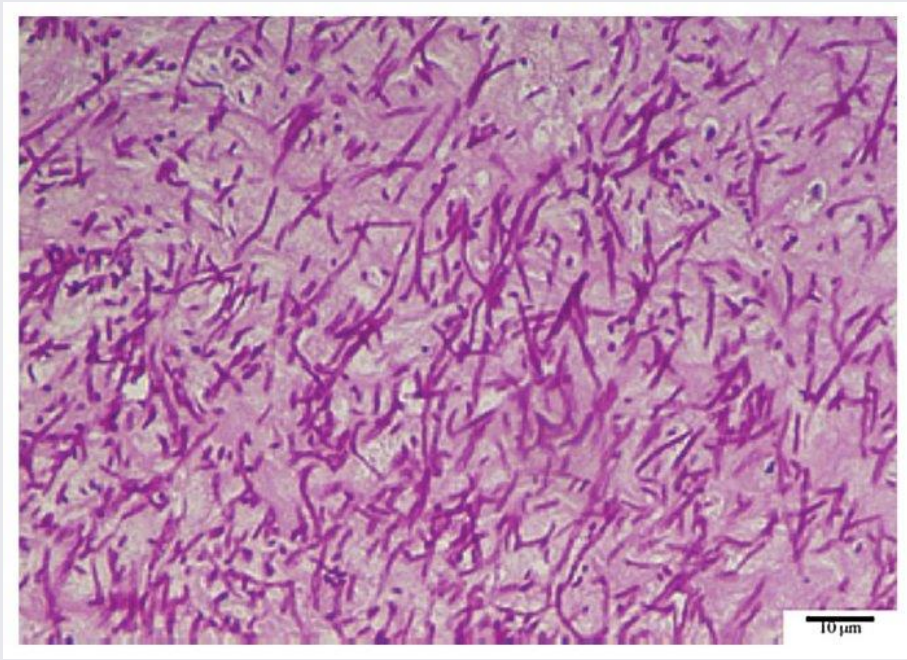
- Observe the width of the hyphae, presence of hyphae septations and angle of branching
- Observe the size and budding pattern of yeast, is pseudohyphae present ?
- Will explain on later slides how these observations can assist in identification

# Periodic Acid Schiff [PAS]

Positive staining hyphae are magenta – will also stain structures containing carbohydrate macromolecules (glycogen, glycoprotein, proteoglycans) Only stains living fungi.

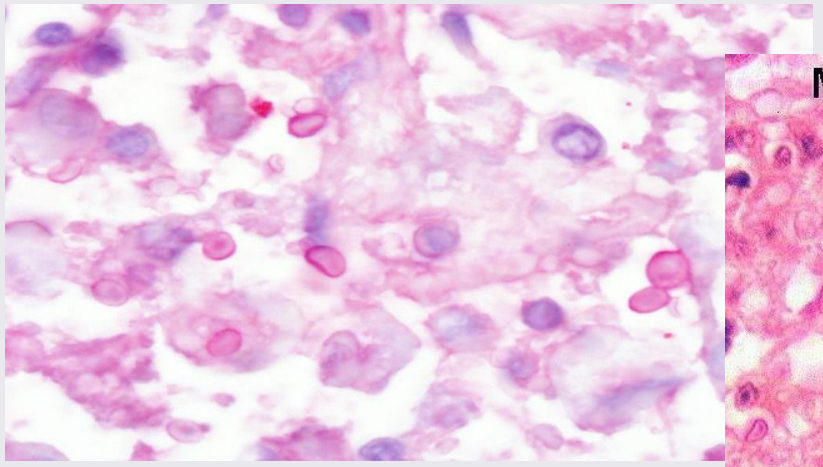


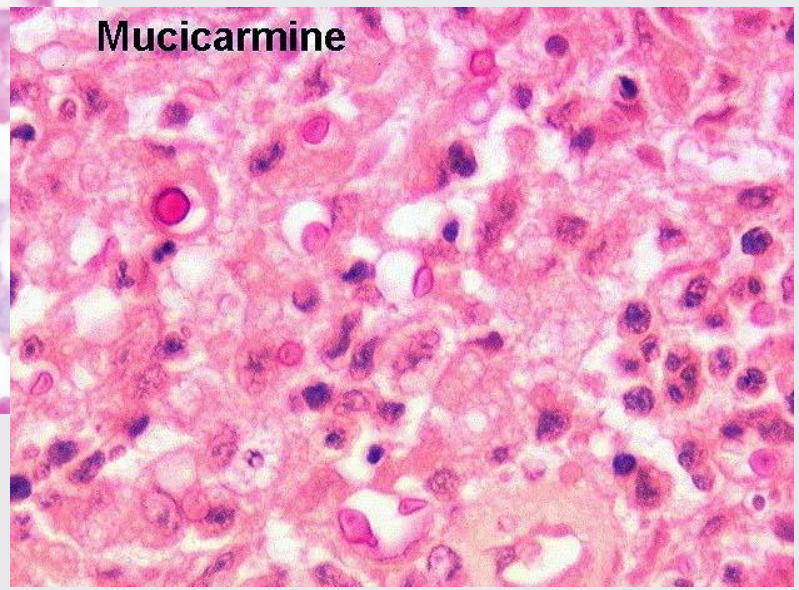


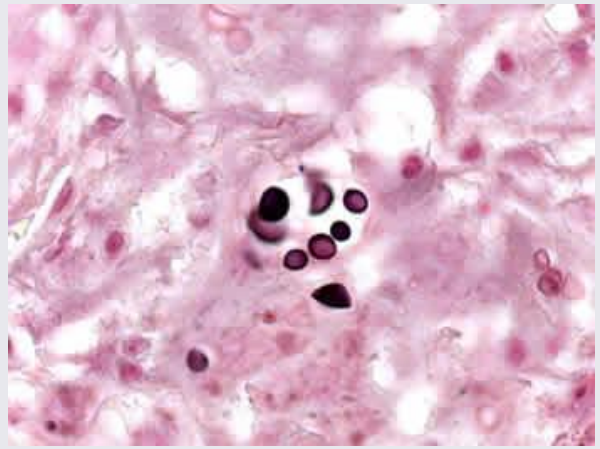


# Mucicarmine [Mucin] stain

Mucicarmine stains the polysaccharide capsule of *Cryptococcus neoformans* and *C. gatti* pink. Will also stain mucin in fixed tissue.

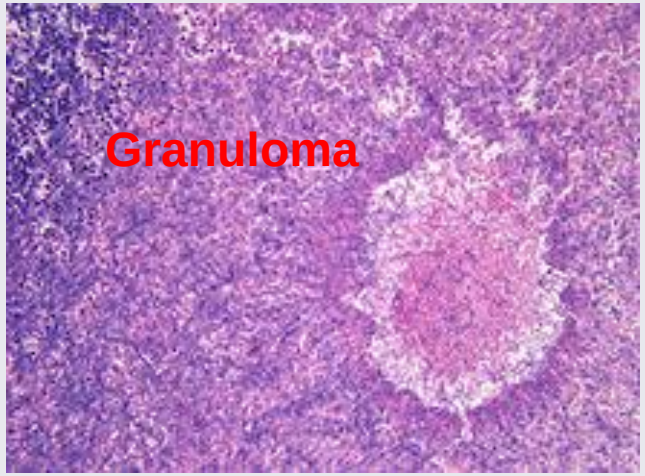




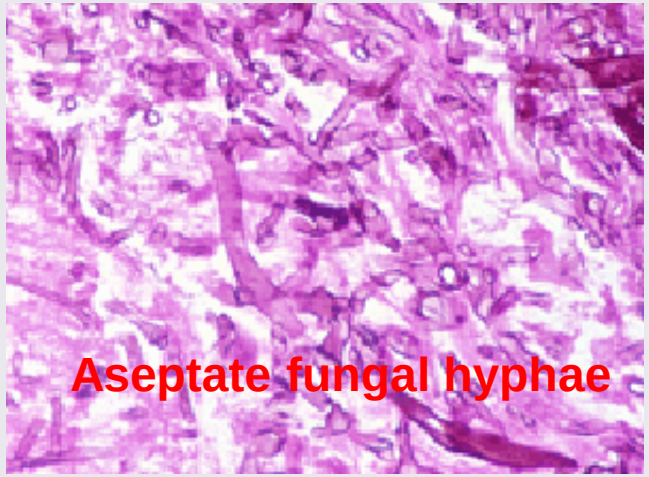



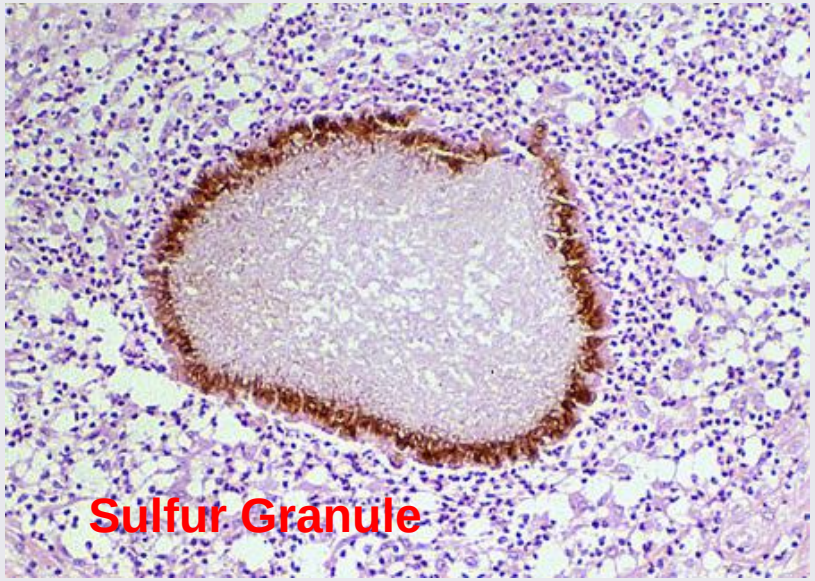
Fontana-Masson silver stain detects melanin, can be used in the rare situation when capsule deficient cryptococcus organisms are encountered.

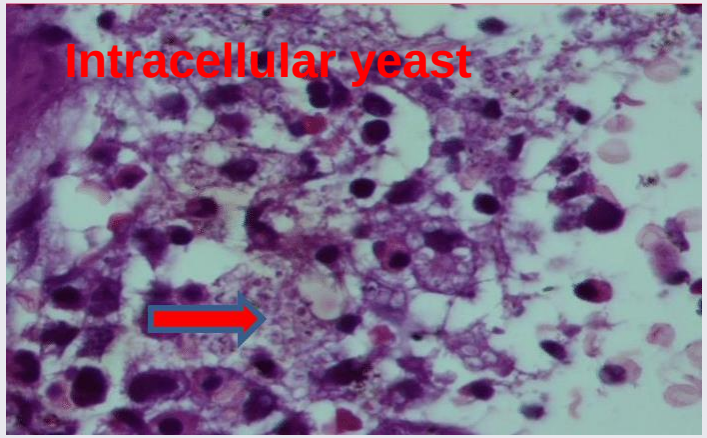
# Hematoxylin and Eosin Stain




Helpful for the description of cellularity in tissue and morphologic assessment of size, septations and branching pattern of molds and the size and budding pattern of yeast.  Can visualize structures such as sulfur granules.



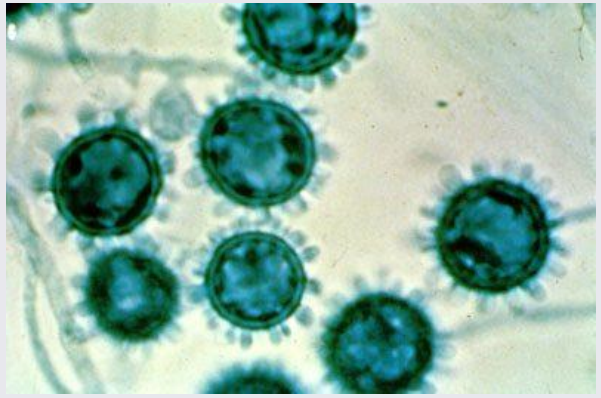
# DIMORPHIC FUNGI

Important systemic pathogens with unique characteristics

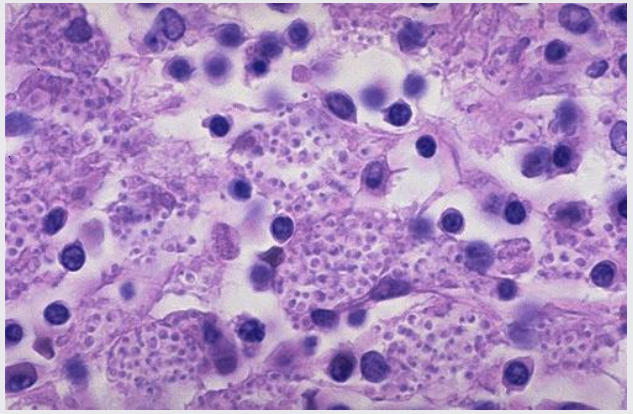
# What is a Dimorphic Fungus?

- Depending on temperature and conditions of environment, one fungi demonstrates two forms:
  - **(1) Mycelial form** - Hyphae and conidia (spores)
    - Occurs free living form nature and growth in laboratory at temperature <=30˚C
  - **(2) Yeast or yeast like form**
    - Parasitic phase found in human tissue or growth in the lab at temperatures >= 35˚C
    - Body temperature increase transforms the mycelial form to yeast

*Histoplasma capsulatum* –
**Mycelial** form in nature and grown at 30˚C



*Histoplasma capsulatum* –
**Yeast** in tissue exam and grown at 35˚C

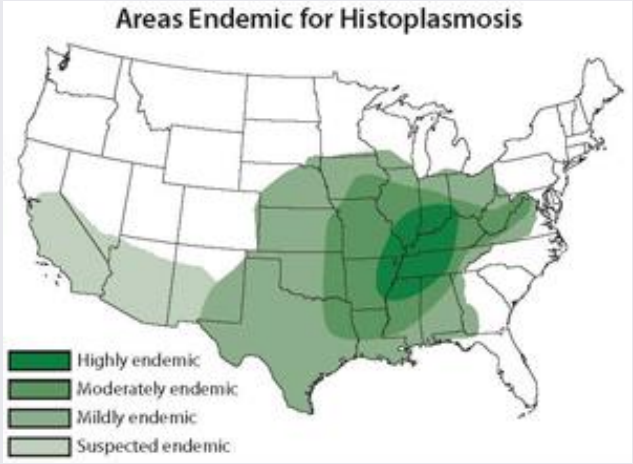


# Most Common Dimorphic Fungi

- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- *Coccidioides immitis and C. posadasii*
- *Paracoccidioides brasiliensis complex*
- *Sporothrix schenckii complex*
- *Penicillium marneffei*
  *(new name: Talaromyces marneffei)*

# *Histoplasma capsulatum*

- **Epidemiology**: World wide distribution, in the USA endemic in Ohio, Missouri, and Mississippi River valleys. Over time has spread over a larger geographic area.




- **Environmental sources**: Aerosolized bat guano so infections occur in Spelunkers (cave explorers), bird droppings, and farmers due to bird exposure

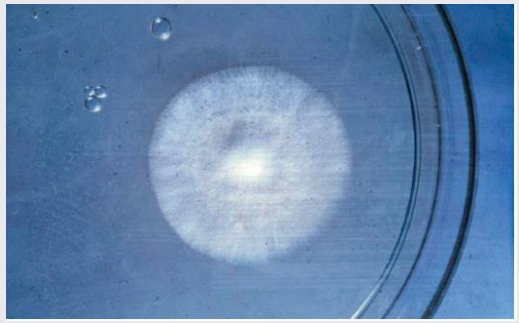
# Histoplasmosis

- 95% of infections are subclinical
- 5% infections are pulmonary with or without dissemination
- Who gets progressive Histoplasmosis?
  - HIV/AIDS / primary and reactivation
  - Organ transplant / primary and reactivation
  - Taking medications such as corticosteroids or TNF-inhibitors
  - Infants
  - Adults aged 55 and older / primary and reactivation disease
- Examination of bone marrow is useful in diagnosing disseminated infection
- Mucocutaneous lesions are a unique and common site of dissemination

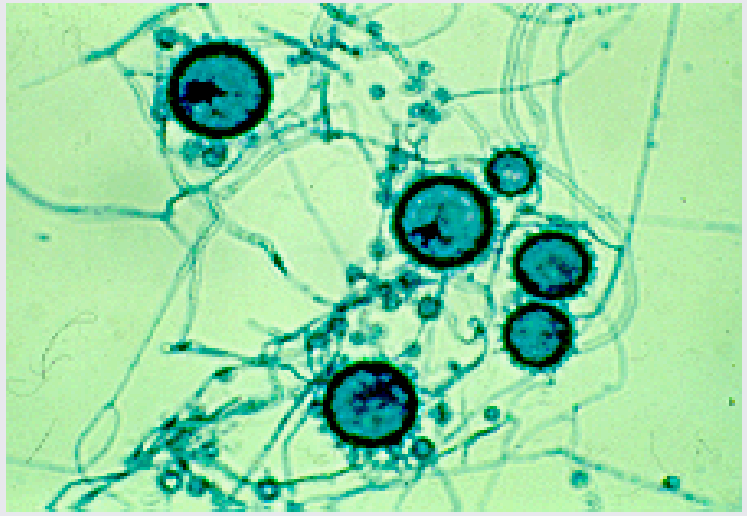
# Histoplasmosis Diagnosis


- **Antigen detection in urine**
  - Quantitative enzyme immunoassay
  - Performed on random collected urine specimen
  - Most sensitive (>=85%) for the diagnosis of disseminated and chronic pulmonary Histoplasmosis
  - Helpful in Histoplasma diagnosis in immune suppressed patients that do not produce a detectable antibody response
- *H. capsulatum* antibody tests are available, but have mostly been replaced by screening for antigen in urine

# *H. capsulatum* Culture

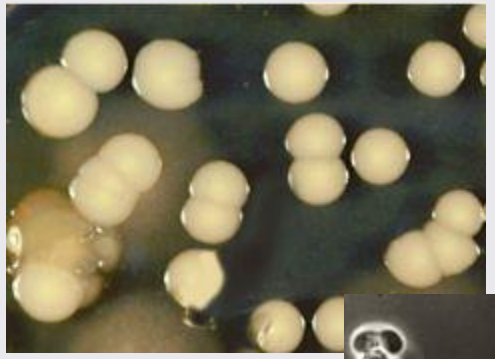


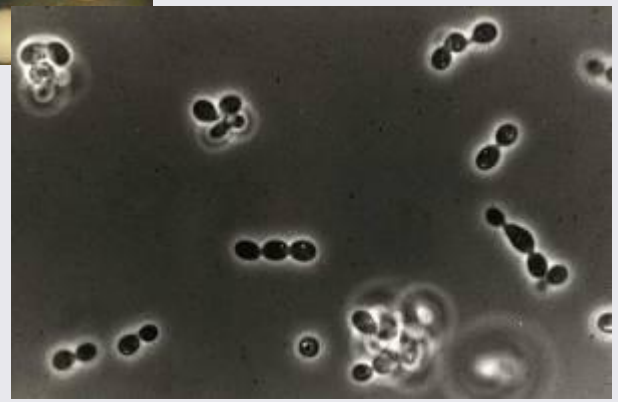
- **Incubated at 30˚C – Growth of Mold**
  - Requires 2-8 weeks to grow
  - Colony - white to brown and cottony
  - Microscopic appearance on scotch tape mount
    - Tuberculate macroconidia are large & round (8 – 16 µM)
    - Small microconidia (2 - 4µM)
    - Mold grows in nature, microconidia are inhaled / capable of penetrating deep into the lung and beginning infection



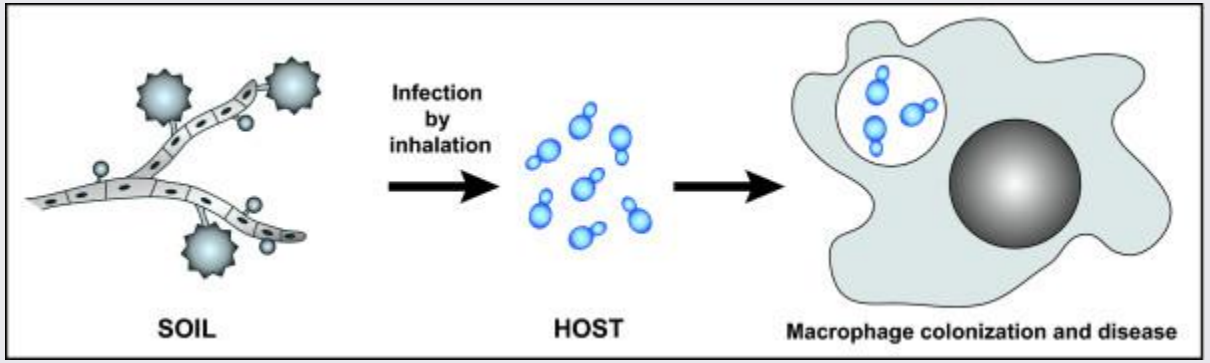
  - MALDI-TOF, DNA hybridization probe, or 16S rRNA sequencing necessary to confirm identification
  - Confirmation testing is required due to look alike fungi, for example *Sepedonium* species – which is usually considered not to be pathogenic

# *H.capsulatum* Culture

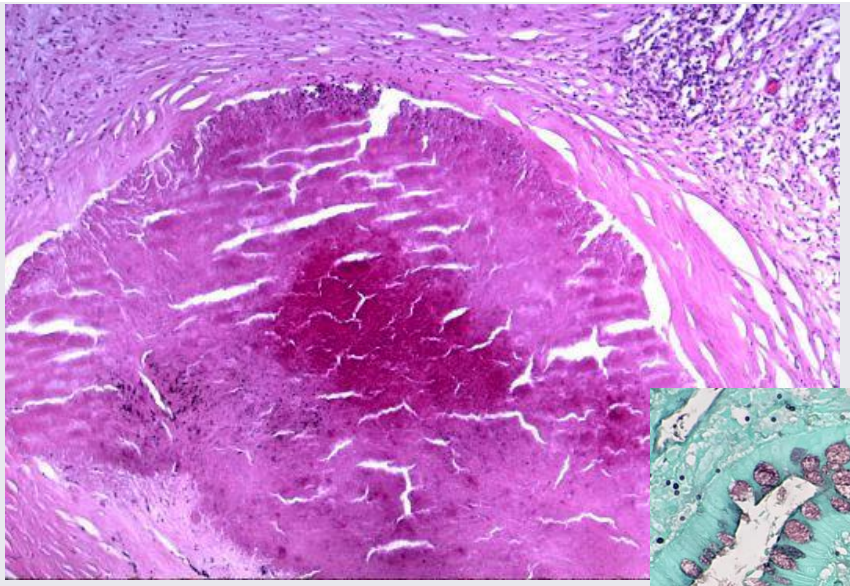


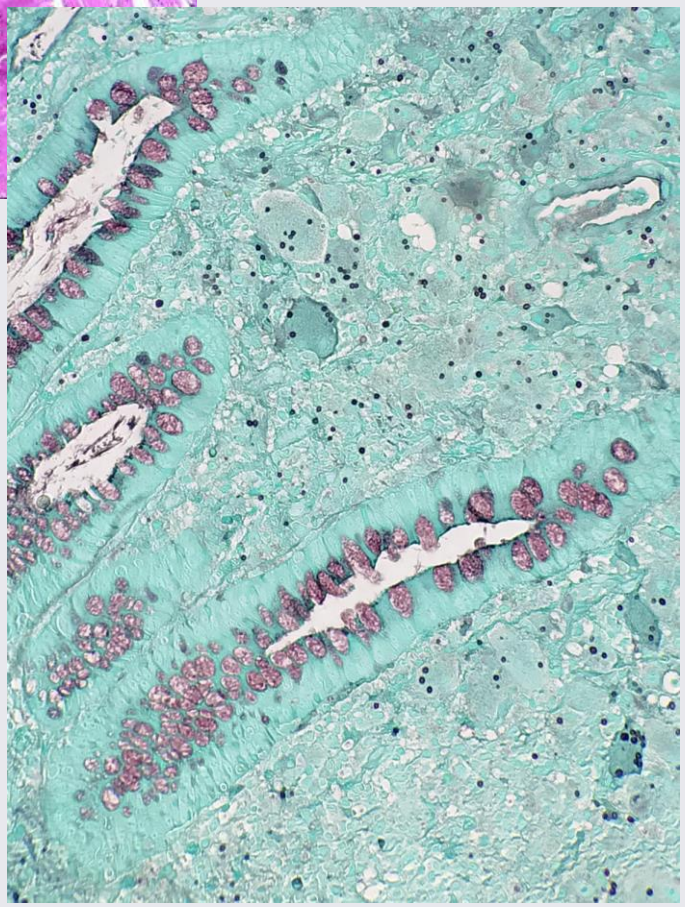


- **Incubated at 37*C – Growth of Yeast**
  - Requires 4-8 weeks to grow
  - Colony – white to beige, creamy
  - Microscopic appearance
    - 2-4 um in size, oval to round
  - Yeast is the form found in human tissue
    - Increase in temperature in human/laboratory induces the mycelial phase to undergo transformation to the yeast phase



# *H. capsulatum* in fixed tissue





- Infection begins by inhalation of the microconidia
- Granulomas produced – can be either caseating or non-caseating
- Can disseminate from lung to organs of the Reticuloendothelial System (RES) – with high % of dissemination to bone marrow

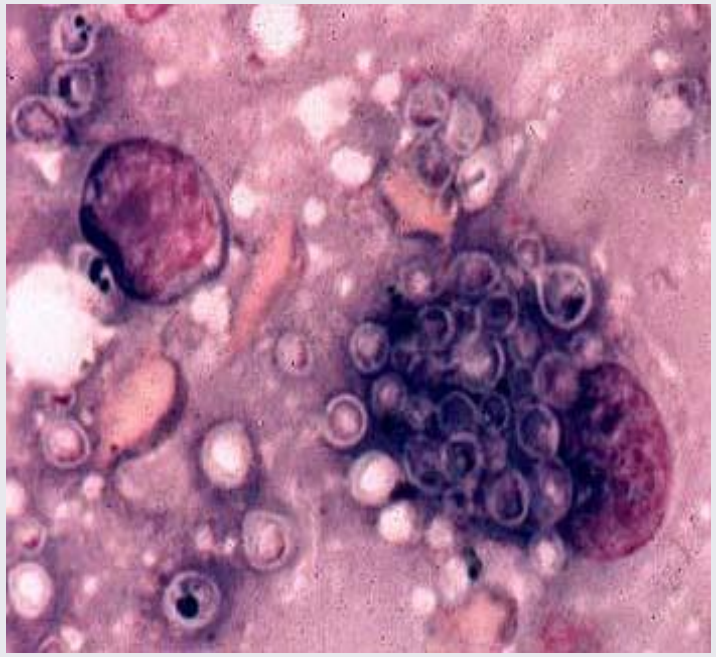
# *Histoplasma capsulatum* – Yeast in fixed tissue

Usually **i**ntracellular within macrophages

Small yeast 2-4 um, minimal size variation, narrow neck bud, oval to round.

Appear to be encapsulated due to staining artifact – which is caused by the shrinkage of the yeast away from the cell wall

Yeast stains well with a variety of stains.



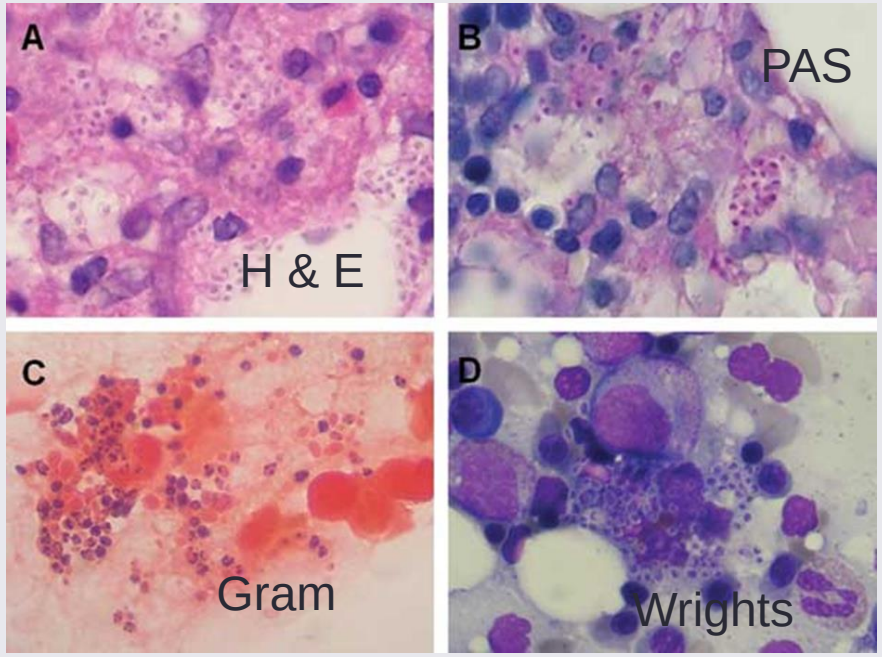
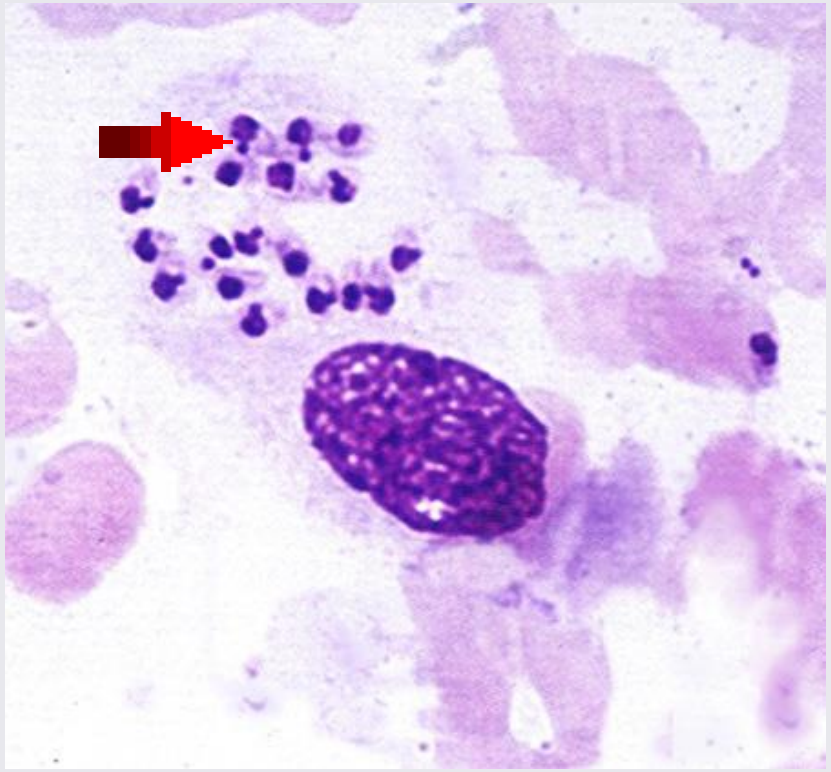


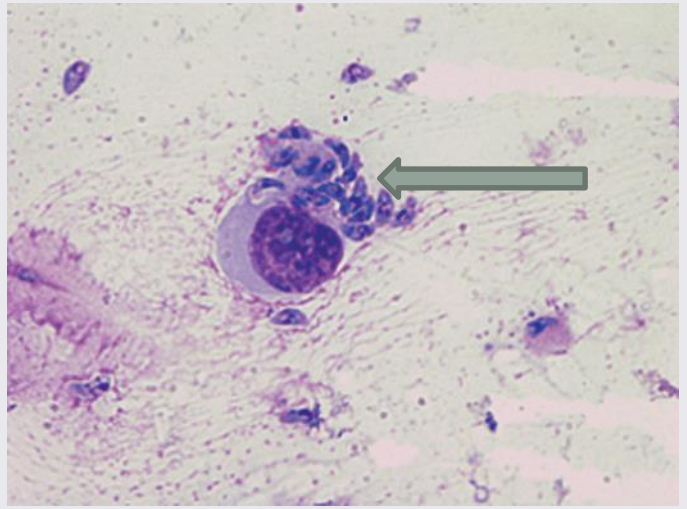
**Table 3. Morphologic Features of the Common Fungi Causing Granulomatous Lung Disease**

| | *Histoplasma* | *Cryptococcus* | *Coccidioides* | *Blastomyces* |
|---|---|---|---|---|
| Visualized on hematoxylin-eosin | No (in necrotizing granulomas)<br>Yes (in disseminated histoplasmosis) | Yes | Yes | Yes |
| Spherules and endospores | No | No | Yes | No |
| Usual size[a] | Small<br>3 μm | Small<br>4–7 μm | Large<br>30–60 μm (spherules)<br>2–5 μm (endospores) | Large<br>8–15 μm |
| Shape | Mostly oval, often tapered at one or both ends | Round | Round or fragmented (spherules)<br>Round (endospores) | Round |
| Size variation | Minimal | Marked | Marked | Considerable |
| Budding | Narrow based | Occasional | Absent | Broad based |
| Nuclei | Single (when intracellular) | None | None | Multiple |
| Staining with mucicarmine | Absent | Usual, strong | Absent | Occasional, weak |

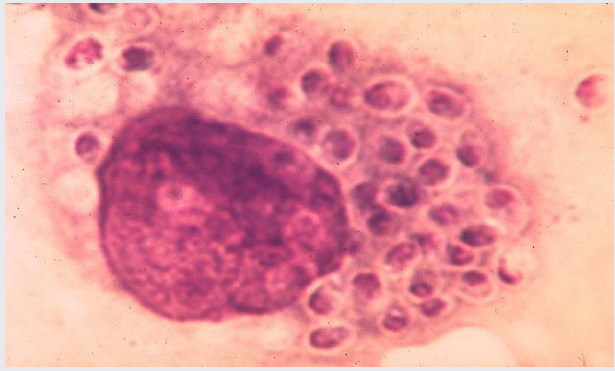
# Beware of look-a-likes



**Leishmania amastigote**
Note: kinetoplast (mitochondrial DNA) next to nucleus



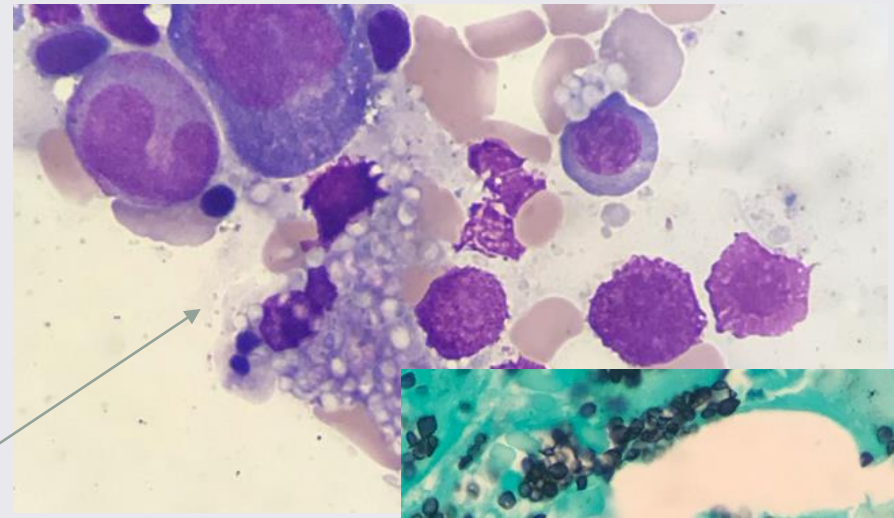
**Toxoplasma tachyzoites**
oval to crescent in shape, no capsule like clearing around cell

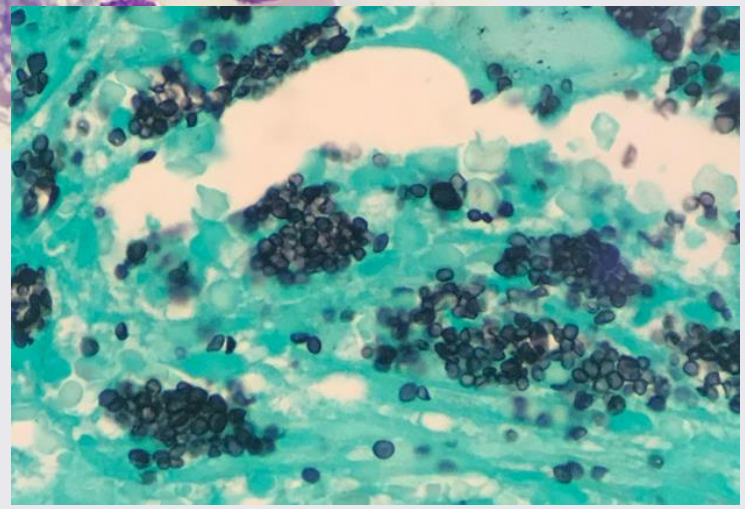


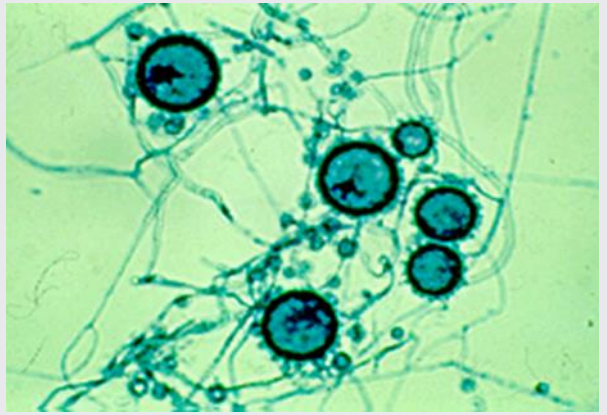
**Histoplasma yeast**
appear to have capsule

# **Case History**:

38 year old male
Newly diagnosed HIV disease/AIDS
CD4 count =3,
HIV Viral Load =220,000 copies
Anemia
Thrombocytopenia
Bilateral pulmonary infiltrate
Bone marrow aspiration procedure
Stain and
Culture performed



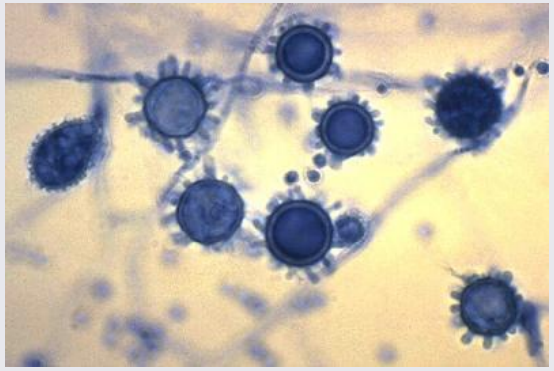




| Component |
| --- |
| Histoplasma Ag Urine (Abnormal) |
| >25.0 ! |
| Comment: |
| REFERENCE RANGE: <0.5 ng/mL |

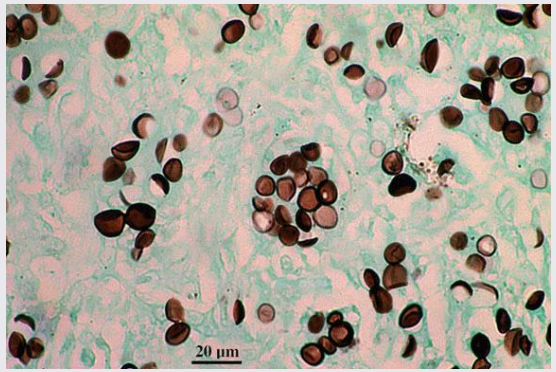
# Unusual variant of Histoplasma – *variate duboisii*

Endemic area is central, east and west Africa
Primary infection is in skin and bone

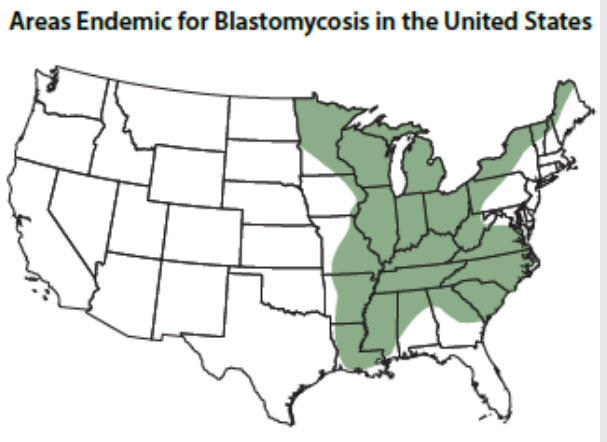


The mold phase is identical to *H. capsulatum*.

Difference is the size of the yeast cell:
Note yeast cell of *H. duboisii* is 8-10 um,
which is 2X the size of *H. capsulatum* yeast cell.

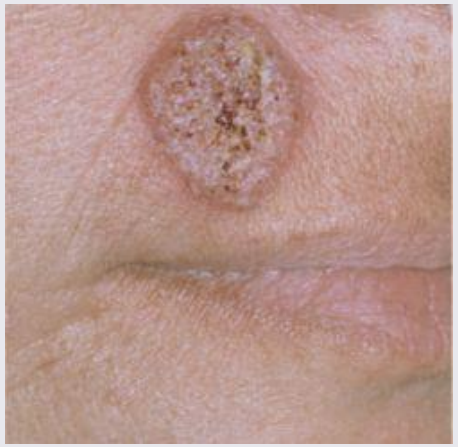


# *Blastomyces dermatitidis*




- Epidemiology
  - Ohio and Mississippi River valleys
  - No association with animal or specific activity
    - Found in forested areas and river banks
  - Primary pulmonary pathogen with small

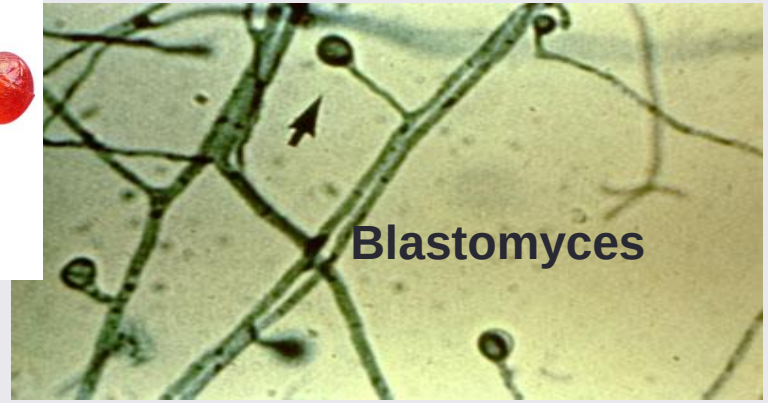
    % of patients that disseminate to skin and bone/ dissemination most often in immune suppressed patients



- Well demarcated skin lesions can be seen in disseminated cases of Blastomycosis.

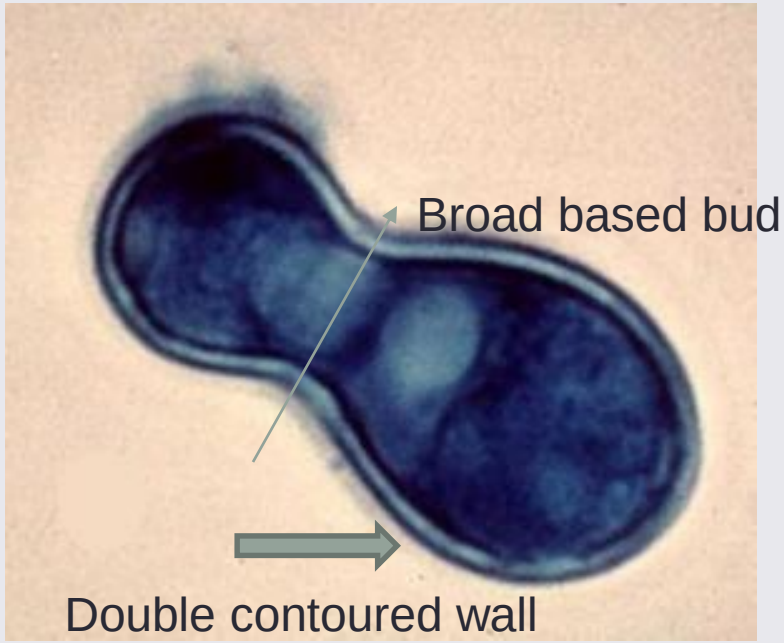
# *Blastomyces dermatitidis*

- Culture at 30˚C - mold
  - Grows in 2-3 weeks
  - Fluffy white – buff colored mold, prickly
  - Microscopic - pear shaped conidia at the end of supporting hyphae – looks like a lollipop
  - Look-alike fungus – *Chrysosporium* species and possibly others (?)
  - Require MALDI-TOF, DNA probe of sequencing to confirm identification



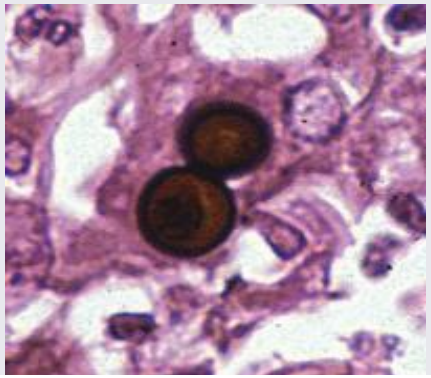

# *Blastomyces dermatitidis*

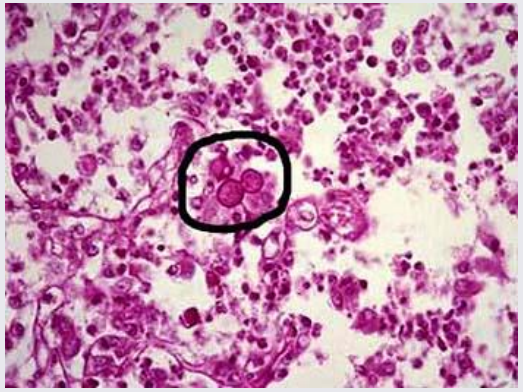
- Yeast phase grows at 35*C
- Slow growing taking up to 4 weeks
- Large yeast cell with considerable size variation, 8-20 um in size, round to slightly oval
- Unique “Broad Based Budding” pattern with thick/double contoured wall.

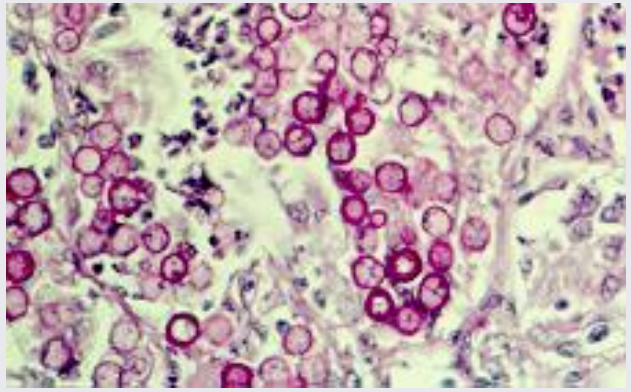


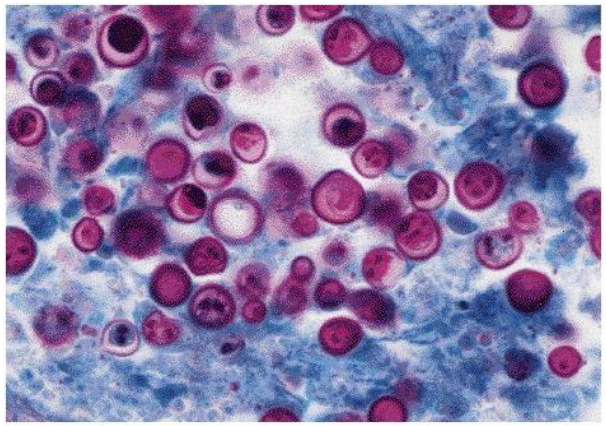
# *Blastomyces dermatitidis* Histopathology

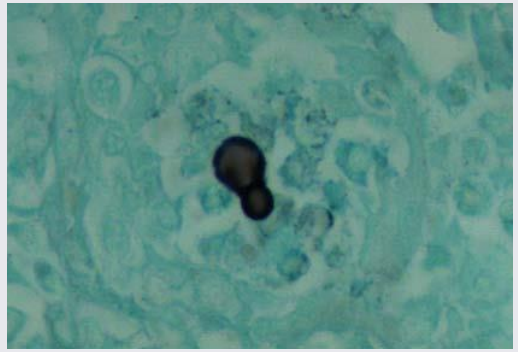
- Mixed pyogenic and granulomatous inflammation is observed in tissue
- Stains well with H and E, can stain weakly positive on mucicarmine
- Broad based budding yeast cell is the classic structure

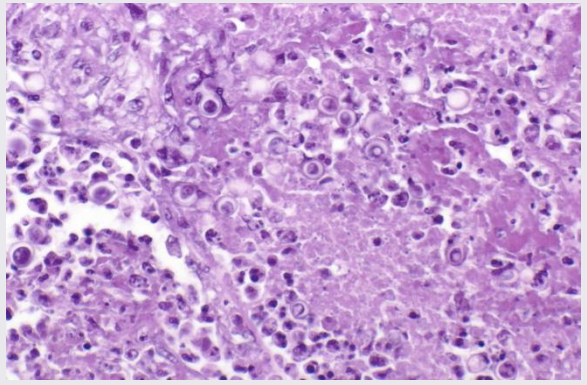












# *Coccidioides immitis and C. posadasii*

- Taxonomy update: 2 species, *Coccidioides posadasii* and *C. immitis, close* genetic relatedness
  - The two species are found in different geographic regions, *C.immitis* (California) and *C. posadasii* (outside CA)
- Identical disease process and react the same in serologic and morphology based diagnostic tests
- Endemic in SW USA (San Joaquin Valley), Mexico, and South America, in areas known as the Sonoran life zone / warm and desert sands
- Infection is from inhalation of fungal particles (arthroconidia) found in the sandy soil in the Sonoran life zone areas

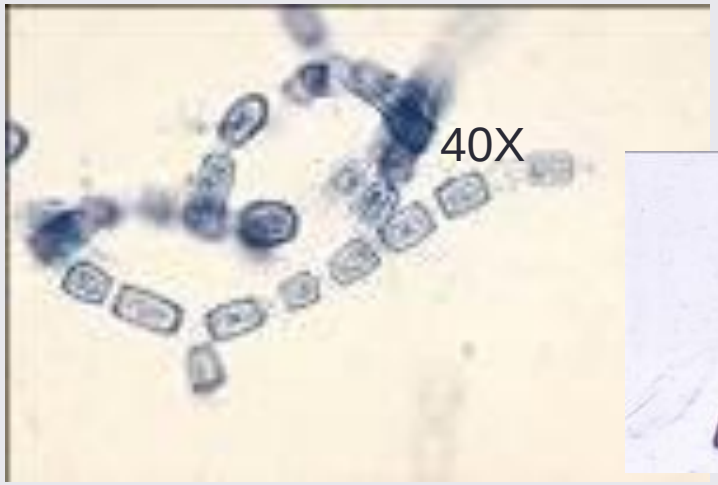

# Coccidioidomycosis

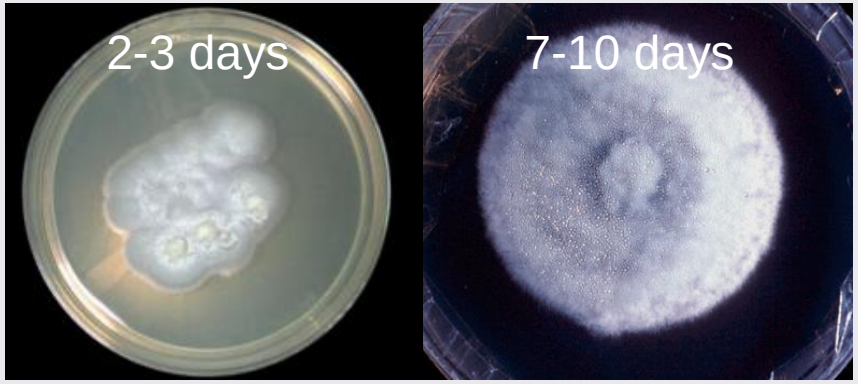
- 60% of patients have asymptomatic infections
- 30% of patients have limited pulmonary disease
- The remaining 5-10% experience chronic disease, progressive pulmonary or disseminated infections
  - Tropism to the Central Nervous System (CNS)
  - Dissemination to CNS carries a high fatality rate.
- Risk factors for severe or disseminated coccidioidomycosis include:
  - African-American race or Filipino ethnicity, HIV/AIDS, use of immunosuppressive medications, organ transplant, diabetes mellitus, or pregnancy

# Serologic diagnosis of Coccidioidomycosis

- **Enzyme immunoassay (EIA):** Sensitive and commonly used method for diagnosing coccidioidomycosis, detects IgG and IgM antibodies
- **Immunodiffusion (ID):** detects IgM antibodies; positive early in the course of infection, not as specific as EIA detection of antibodies and can have “false” positive results, must confirm infection with additional testing
- **Complement Fixation (CF):** detects IgG antibodies and allows for assessment of disease severity.

# Coccidioides Culture



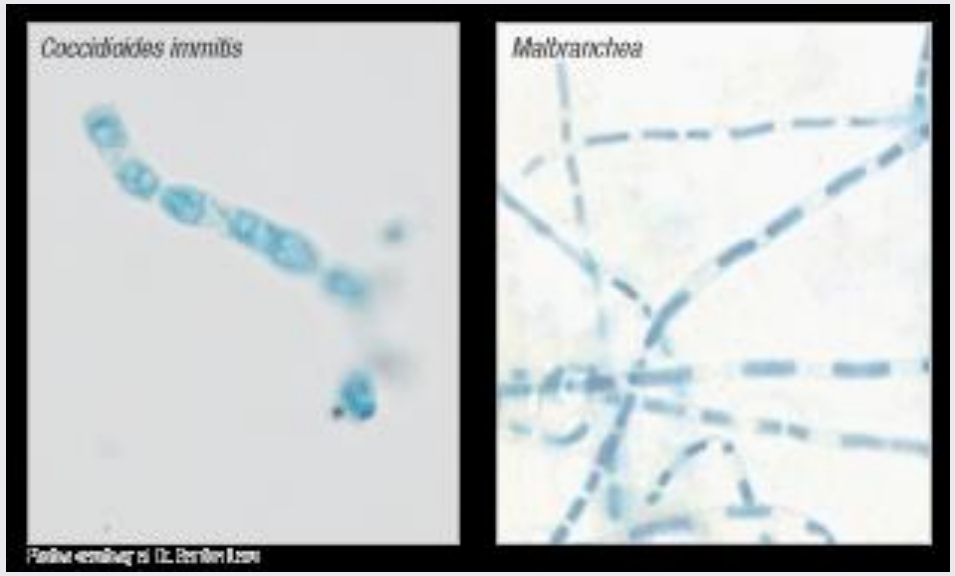




- **Culture at 30˚C grows the Mold phase**
  - Growth in 2-3 days, colony starts waxy and becomes wooly in around 7–10 days
  - Microscopically (40X) one observes areas with septate hyphae and thick walled alternating barrel shaped arthroconidia
  - Barreled arthroconidia breakoff and become the infectious particle in nature
  - Beware! Mature culture is hazardous to laboratory personnel

# Coccidioides Look-a-likes

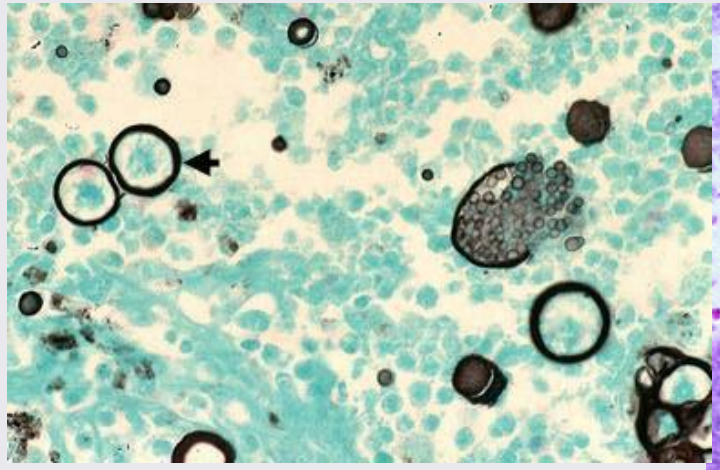
- *Malbranchea* species can look somewhat like *C. immitis and C. posadasii* under the microscope using an adhesive tape prep
- Must confirm any fungi suspected to be Coccidioides using MALDI-TOF, molecular DNA probe or 16S rRNA sequencing

**Coccidioides** **Malbranchea**

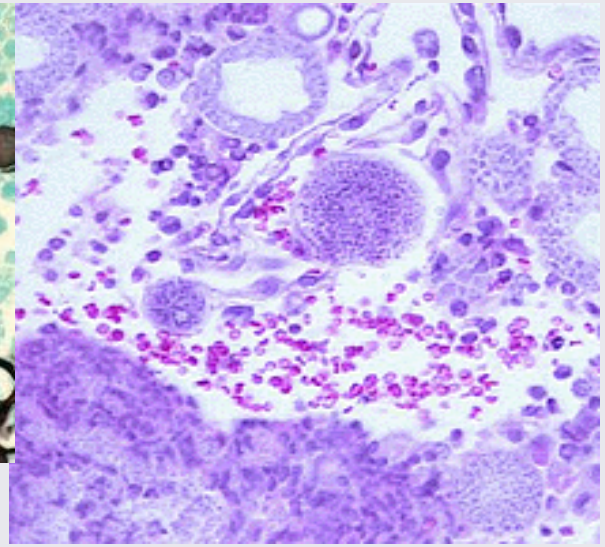


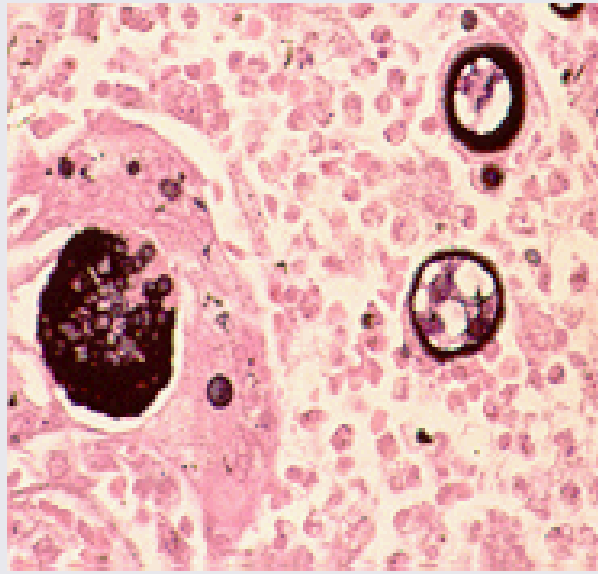
# Coccidioides Histopathology

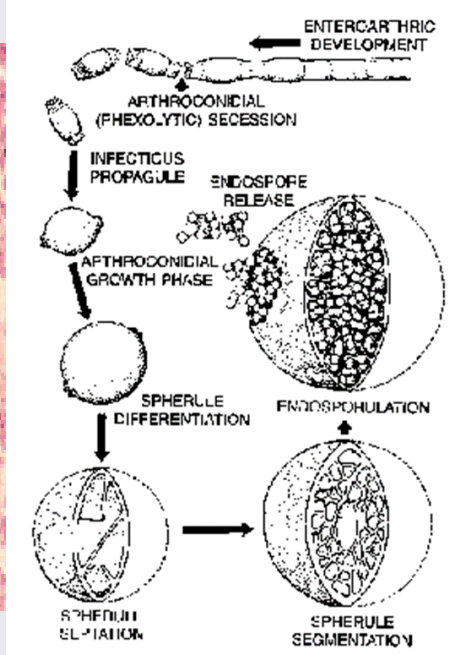
- No yeast cell produced in human tissue: Thick walled spherules variable in size (10 – 80 uM) with endospores are the structures observed in tissue.
- Spherules can be in all stages of development- fragmented spherules to well formed mature cysts with endospores (2 – 5 uM)
- Granulomatous inflammation with caseation is usually observed



Position of Coccidioides spherules can mimic the broad based bud of Blastomyces







# Case History:

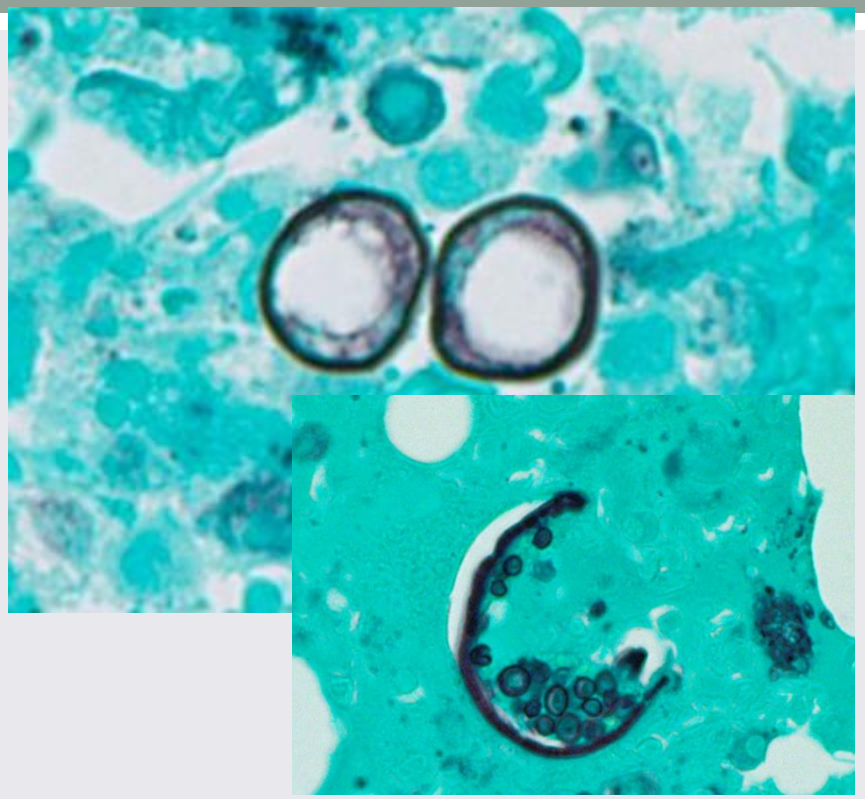
75 year old male
Right Pleural Effusion
Lung nodule

Past medical history: Gout, hypertension, anemia, obesity

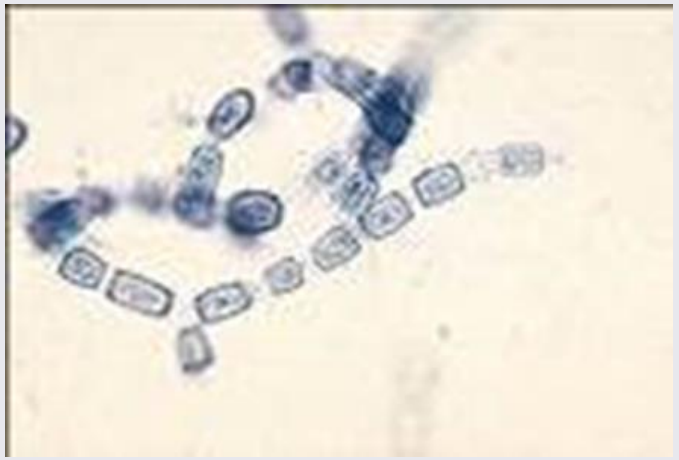
Fungal serology testing:
Histoplasma urine Ag: negative
Cryptococcal Ag: negative
Coccidioides IgM: Indeterminate
Coccidioides IgG: Weak Positive
Titer 1:4

Epidemiology: Lives on ranch in Simi Valley, California

Biopsy of lung nodule stain/ GMS

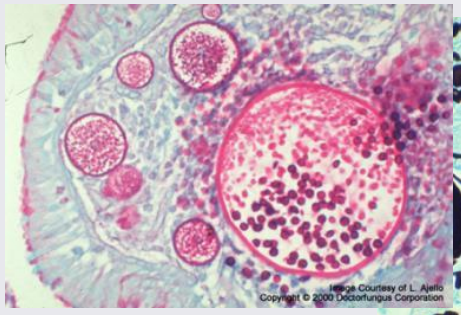


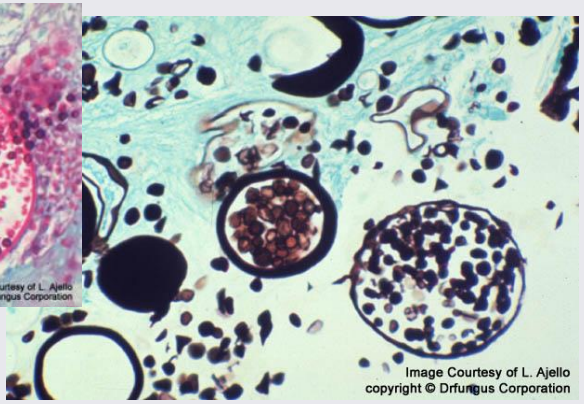
Culture of lung nodule incubated at 30° C



# Coccidioides is not the only spherule forming organism!

- ***Rhinosporidium seeberi*** (an aquatic parasite) forms spherules that are larger than those of Coccidioides
- *Rhinosporidium* spherules can be > 80 uM in size compared to those of Coccidioides <=30 uM
- *R. seeberi* cause oral or nasal mass lesions






**Oral /nasal mass lesions**

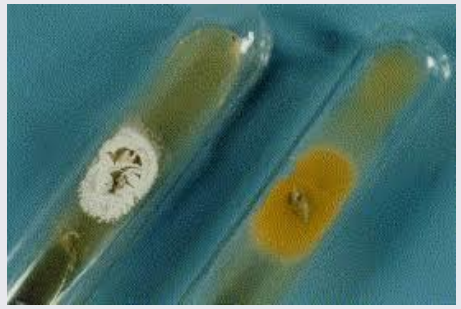
**Prototheca wickerhamii** produce structures that mimic spherules of Coccidioides/structures known as morulas. The are mostly seen in skin lesions

# *Paracoccidioides brasiliensis complex*

- South American Blastomycosis – 80% of cases reported from Brazil
- Most prevalent systemic fungal infection in Latin America
- Infection acquired from inhaling infectious particle from soil
- >95% of infections in males, possibly due to estrogen inhibition of the fungus mycelial to yeast transformation
- Disease presentation:
  1. Primarily a pathogen of Pneumonia
  2. Disseminated infection can occur
  3. Extrapulmonary lesions on the face and oral mucosa are common site of dissemination

# Paracoccidioides

- Cultures at 30*C – mold, cultures usually not performed due to slow growth and nonspecific sporulation



- Culture at 35-37˚C - yeast
  - Slow growing yeast – 3 weeks
  - Large (10 – 30um), thick walled, with

    2 or more tear drop daughter buds (2–10 um)
  - Unique multiply budding yeast cell known as the Mariner's wheel or Pilot's wheel yeast

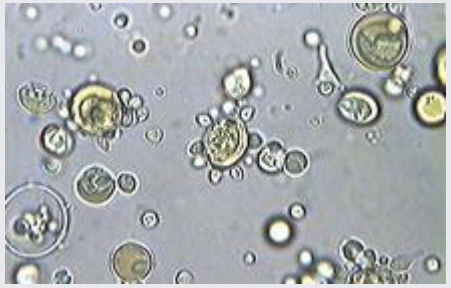
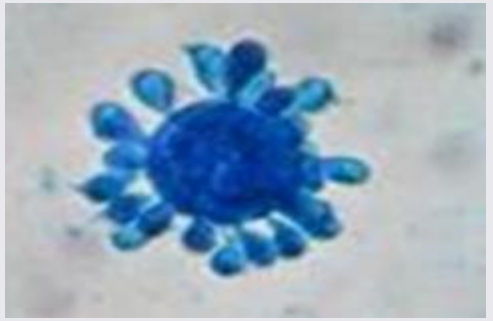
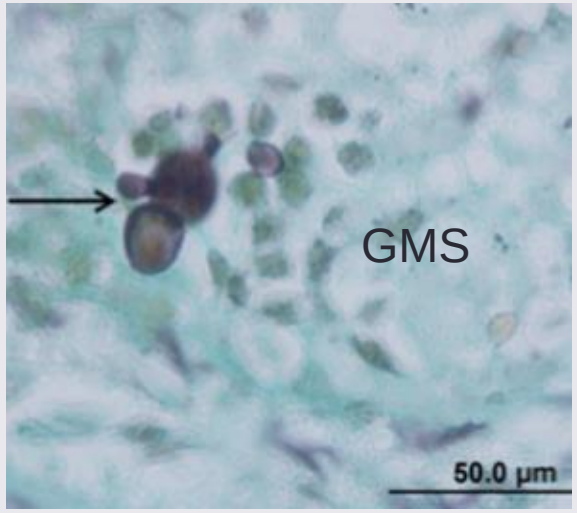
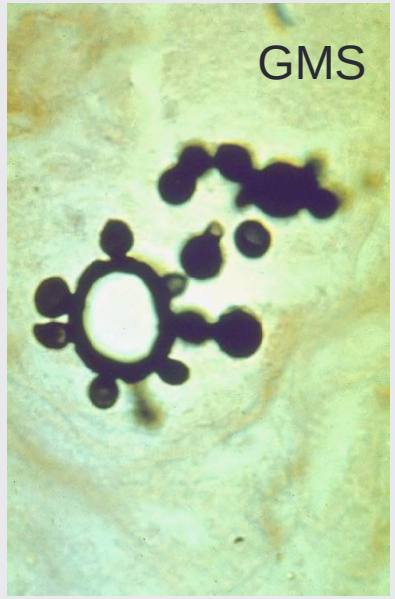


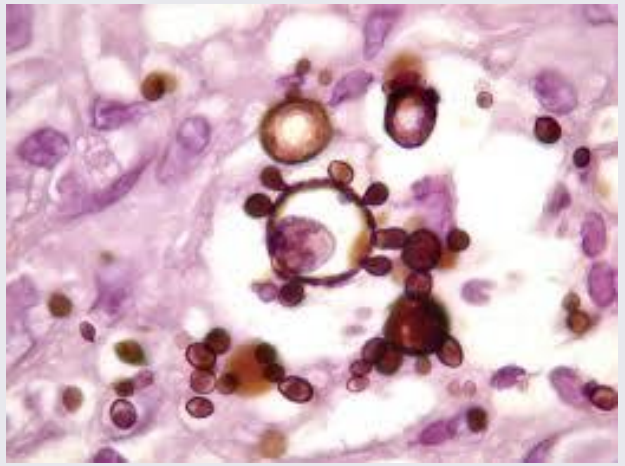
Fig. 1 - Polygemulating yeast and wooden ship's wheel.

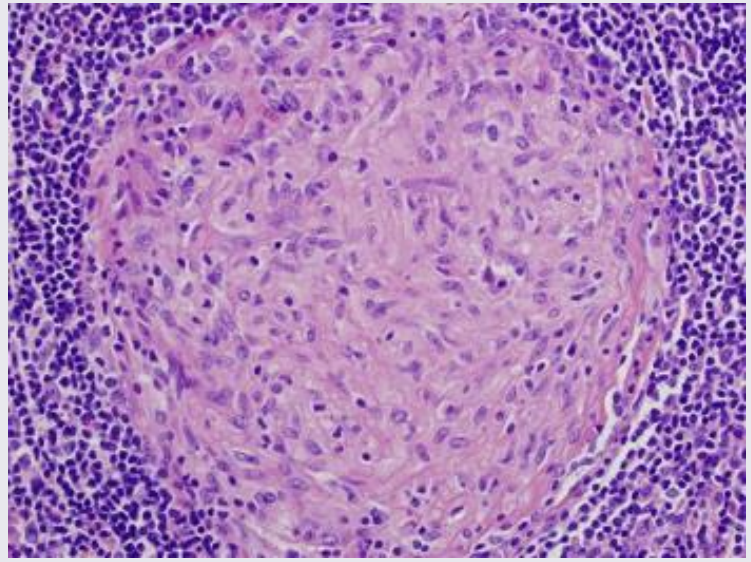


# Tissue Exam of *Paracoccidioides brasiliensis complex*



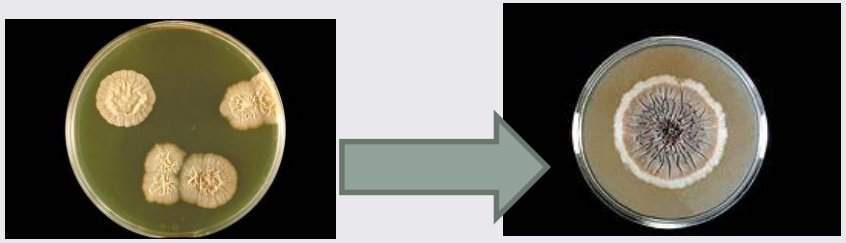


- Granulomatous inflammation with giant cells formed in tissue
- Mariner’s wheel yeast forms

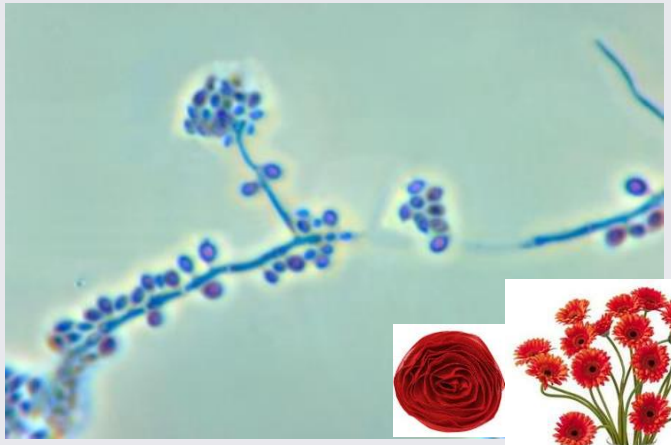
# *Sporothrix schenckii complex*

- Sporotrichosis
  - Cutaneous inoculation from penetrating injury, such as cut or scrape with contaminated plant thorns
  - Known as “Rose gardener’s disease” due to acquisition from bushes
  - Usually begins as skin lesion with or without ulceration and leads to a subcutaneous disease
  - Lesion can progress with lymphocutaneous spread and possible dissemination to bone and other organs
  - Pulmonary and CNS infections occur, but rarely

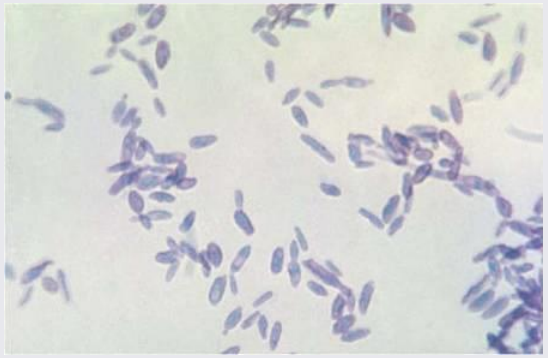
# *Sporothrix schenckii complex*



- 30˚C culture grows mold in 3 -5 days, starts beige eventually turns brown/black
- Microscopic: septate hyphae with conidia in daisy wheel pattern or rosette

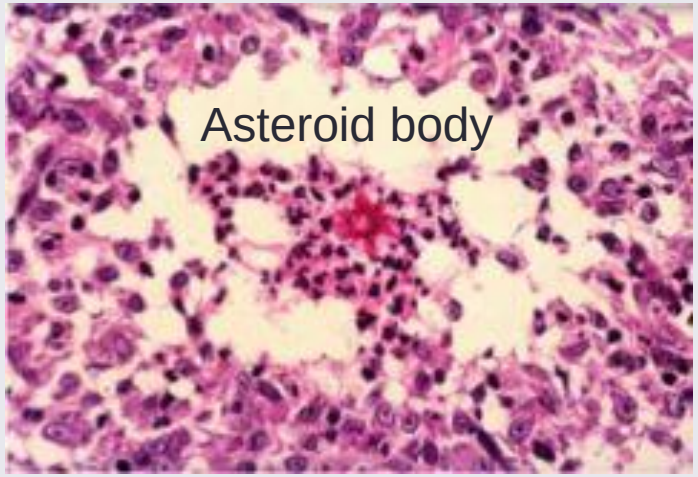


- 37˚C culture grows yeast in 7 days as small oval and elongated yeast cells,2 – 5 µm, described as cigar bodies



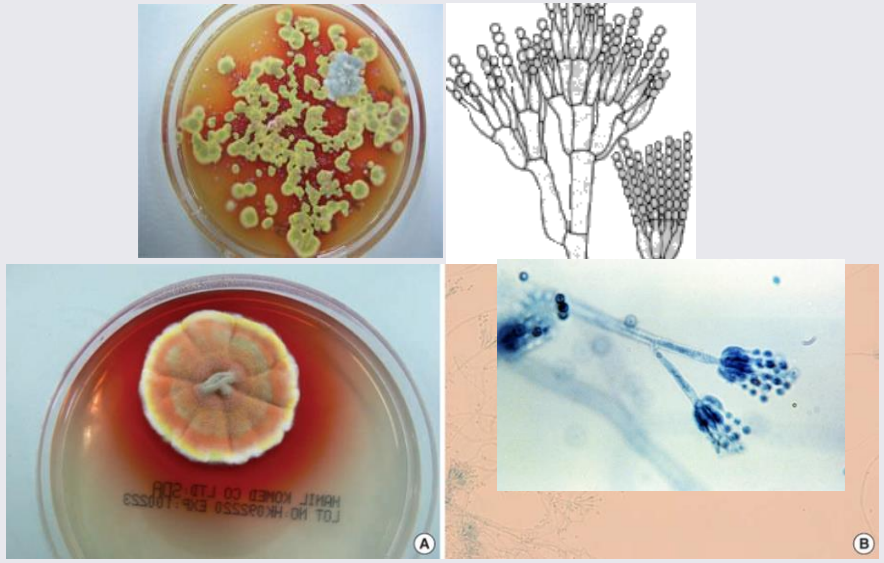
# *Sporothrix schenckii* Histology

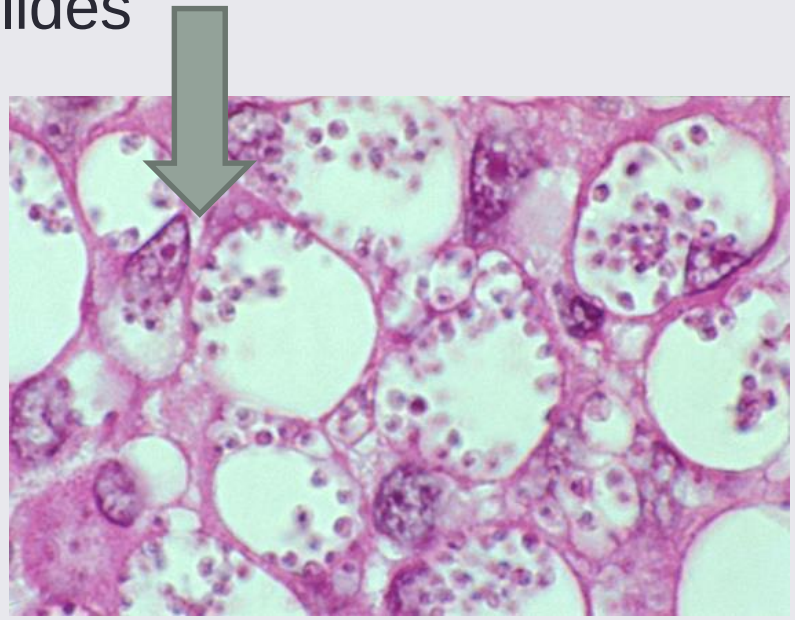
- Pyogenic to granulomatous inflammation
- Yeast cells seldom observed in human tissue
- If seen, elongated yeast described as cigar shaped
- More common structure seen is an Asteroid body known as Splendore-Hoeppli phenomenon (SHP)
- SHP are not unique to Sporotrichosis,  also seen in infections with:
  - Mucormycetes (Mucor, Rhizopus)
  - Aspergillus
  - Blastomyces
  - Candida spp




# *Penicillium (Talaromyces) marneffei*

- Disease: Cutaneous lesions most often, but Pneumonia or systemic infection in HIV/AIDS
- Endemic in the tropics and SE Asia
- Mold grows in 2-3 days, green colony with red diffusible pigment
- Microscopically looks like a typical Penicillium with branched conidiophore and sporulation in culture at <=30* C.
- Yeast like cells seen in fixed tissue slides

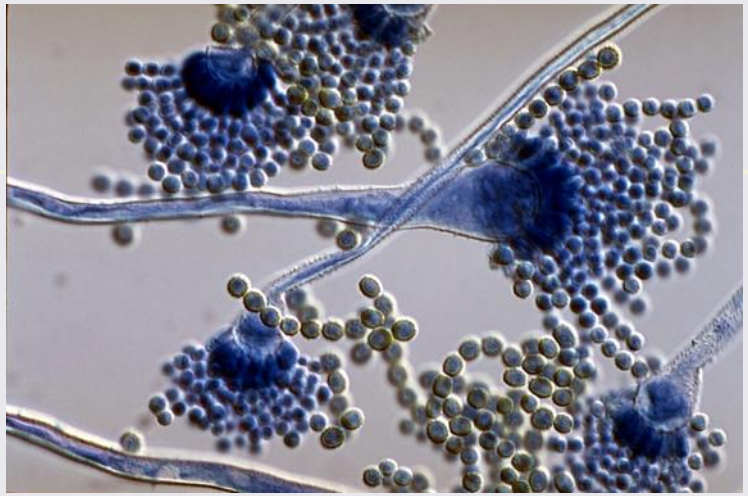




# MYCOLOGY II

## YEAST, FUNGI THAT CAUSE CUTANEOUS AND SUBCUTANEOUS INFECTIONS, AND THE OPPORTUNISTIC FUNGI

Margie Morgan, PhD, D(ABMM)
Medical Director Microbiology
Cedars Sinai Medical Center
Los Angeles, CA



# SUBCUTANEOUS FUNGAL INFECTIONS

Mycetoma

Chromoblastomycoses

Phaeohyphomycosis

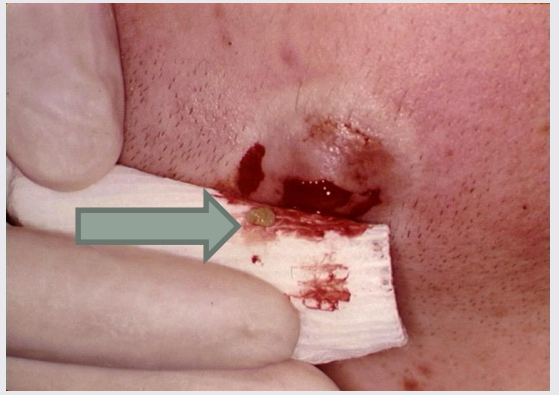
# Mycetoma

- This subcutaneous infection most commonly occurs in hot temperate parts of the world
- Causative organisms grow on organic soil debris
- Infection begins with trauma implanting organism into the subcutaneous tissue

- Three criteria define mycetoma:
  - -Swollen extremity from lesion progression
  - -Develop draining sinus tracts
  - -Sulfur granules drain from sinus tracts and can be observed in tissue histology slides

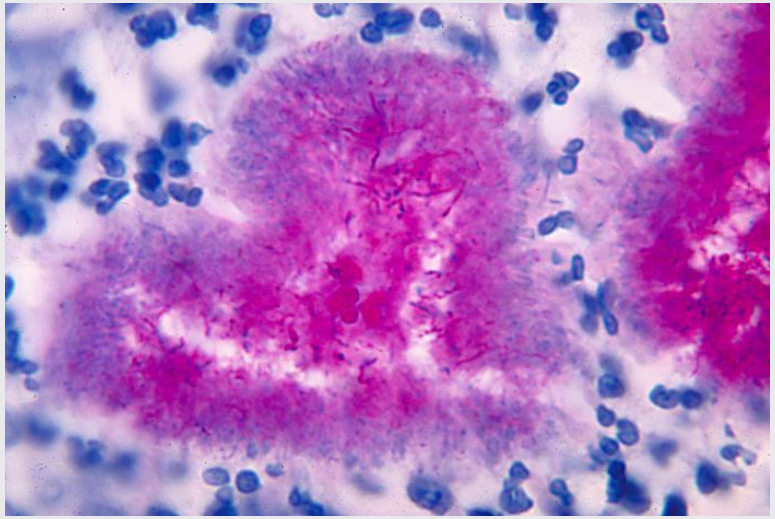
Two types of mycetoma:

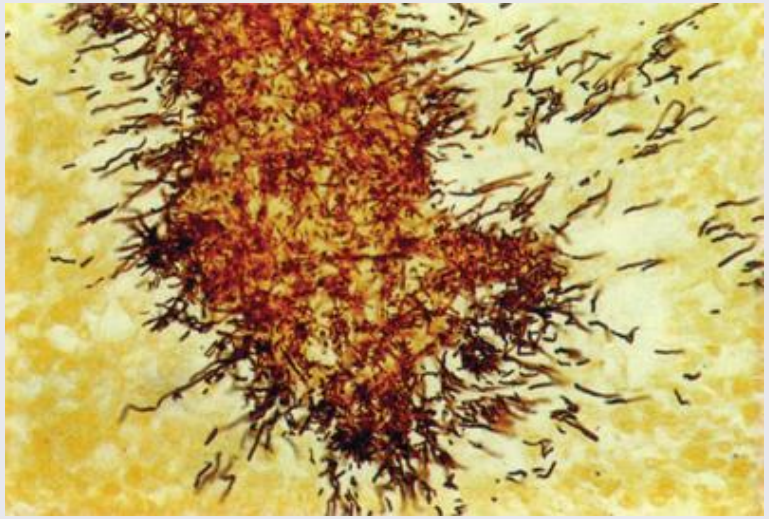
1. **Actinomycotic** – caused by higher bacteria
2. **Eumycotic** – caused predominately by black molds

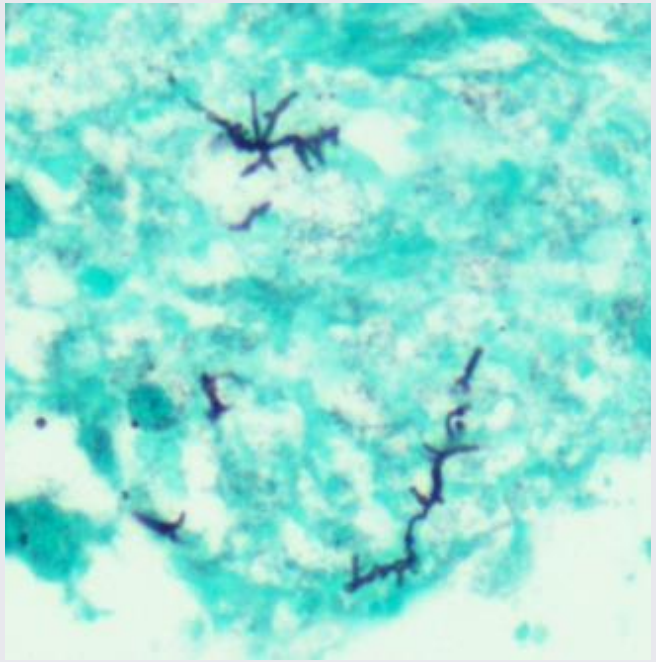



# Actinomycotic Mycetoma

- Nocardia species causative in 98% of cases
- Sulfur granules are formed in tissue.  The granules vary in color depending on the Nocardia species causing infection
- The granules contain a matrix of filamentous bacteria that can be visualized at the edge of the stained granule
- Nocardia stain by GMS in tissue samples as thin filamentous branching organisms

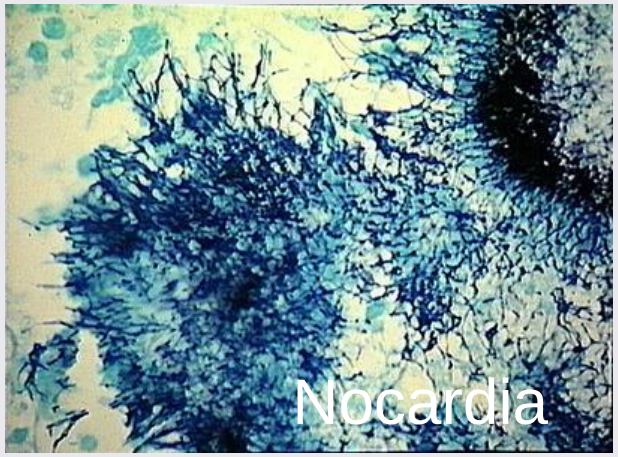


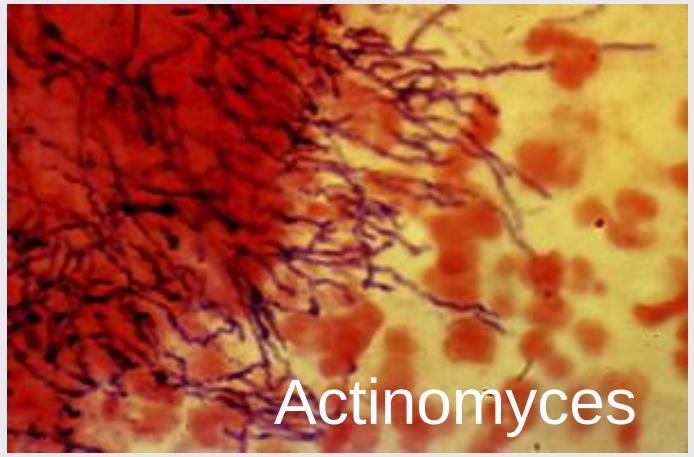


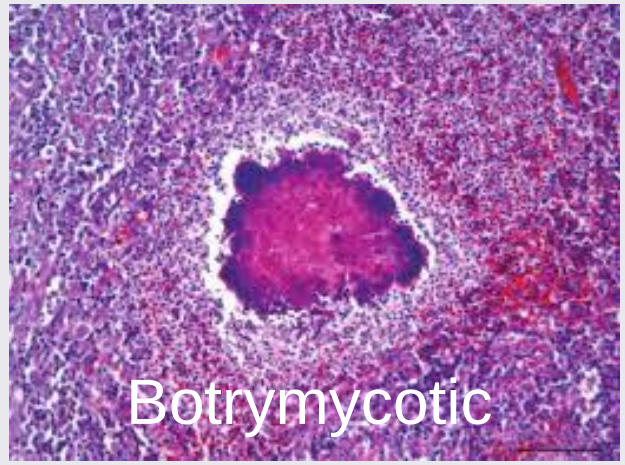


# Actinomycotic sulfur granule vs Not

Look-a-like granules: (1) Sulfur granules due to infection with *Actinomyces* species (an anaerobic Gram positive bacilli) and (2) Botryomycotic "pseudo-sulfur" granules (chronic bacterial abscesses) caused by aerobic bacteria spp.



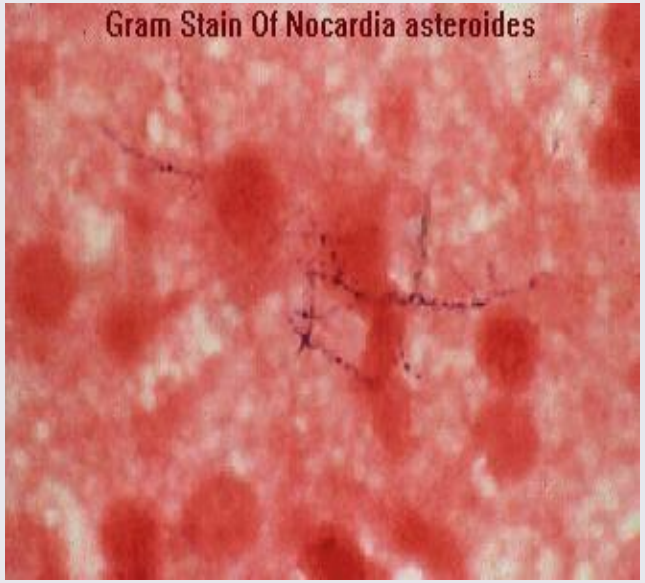



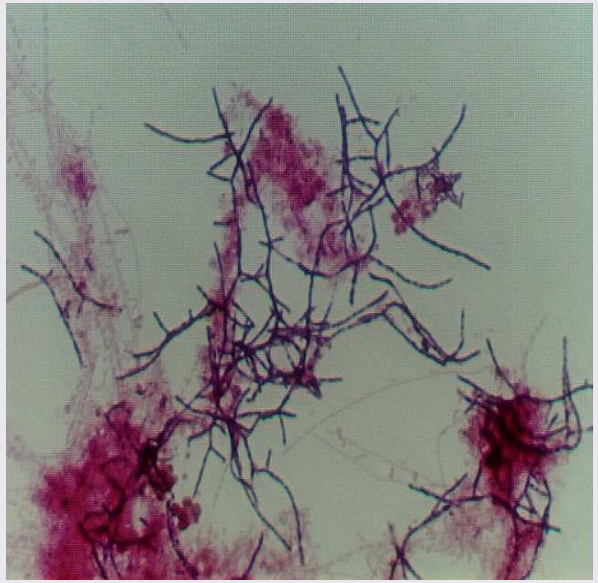




How can you determine the causative agent?
Best solution is to identify the organism in culture

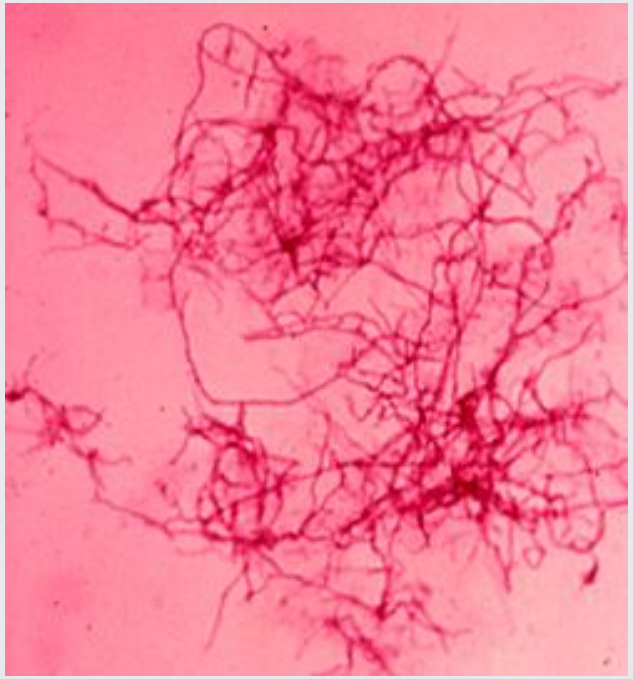
# Nocardia

Gram stain filamentous Gram positive bacilli which can stain poorly and appear speckled.
Kinyoun modified partial Acid-Fast Stain (PAF) is positive and stain red



**Gram stain**



**Kinyoun modified partial acid-fast stain**

# Nocardia species

Besides mycetoma, Nocardia spp can also cause primary pulmonary with dissemination to brain. These infections usually occur in severely immune suppressed patients.

Grows in 3-5 days at 30-35*C on many agars including SABs and 5% Sheep's blood agar
Colony is dry/crumbly with a musty odor

Total of 85 species of Nocardia
*Nocardia asteroids complex* most common

Identification: MALDI -TOF Mass spec
16s rRNA gene sequencing

# Eumycotic Mycetoma

Infection most often with numerous species of pigmented/black fungi (dematiaceous molds) found in soil and debris

-Cause @ 2% of mycetoma cases

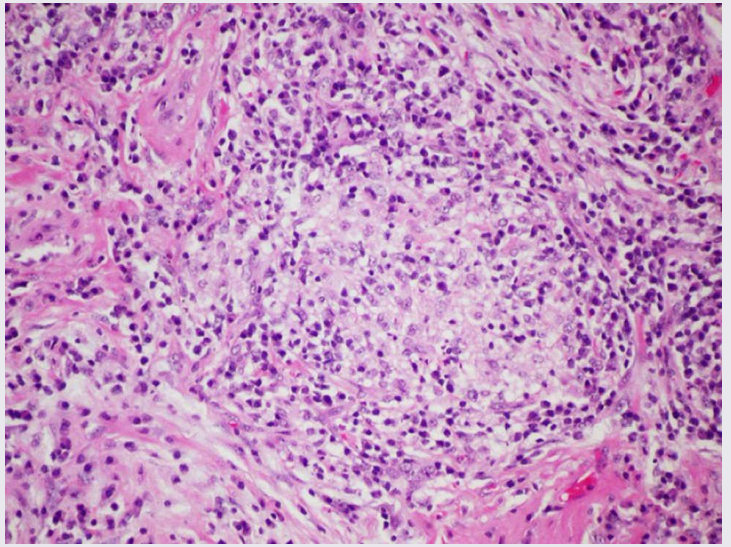
-Infection begins with traumatic implantation of the fungus into the subcutaneous tissue



Notice the thick hyphae on the edge of the sulfur granule

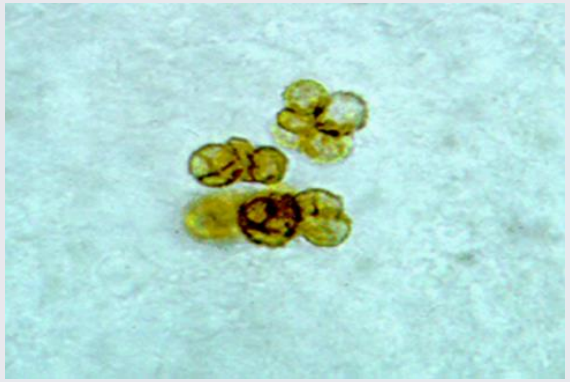
# Thermoactinomycetes

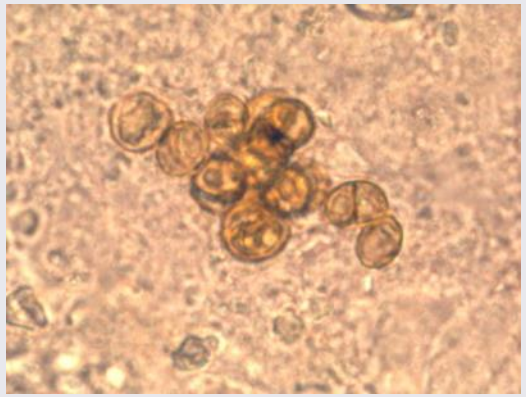
- Gram positive filamentous rods related to Nocardia species
  - *Thermoactinomyces* and *Saccaropolyspora*
  - Flourish in areas of high humidity and high temperature (40*-60*C)
  - Preavalent in moist and hot agricultural environments – stacks of hay
- Agents of Farmer's lung – hypersensitivity pneumonitis or extrinsic allergic alveolitis, occupational disease
  - Immunologically mediated inflammatory disease of the lung
  - Inhalation exposure to the thermophilic actinomycetes
  - Pathology:  Loose, non-necrotizing granulomas
- Diagnosis
  - Detailed environmental history
  - Serology testing



# Chromoblastomycosis (Chromomycosis)

- Wart like lesions (scarred and nodular) in subcutaneous and cutaneous tissues/ tropical and subtropical areas
- Skin abrasion and implantation of fungi into tissue
- Infection caused by black pigmented fungi (dematiaceous)



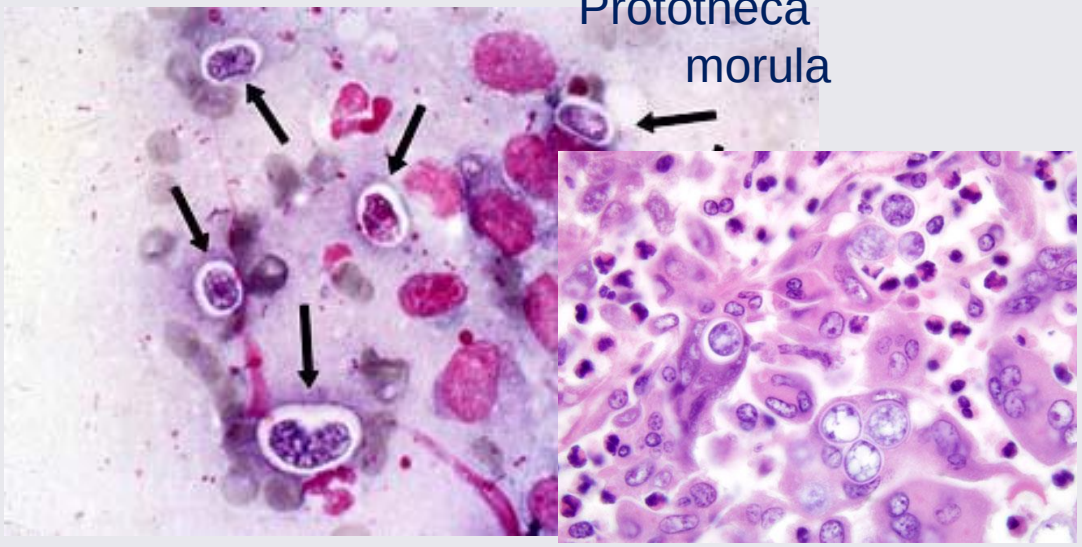



Naturally brown in color, septate structures known as muriform bodies, medlar bodies or copper pennies is the unique structure found in tissue – they break up and pieces extend into the subcutaneous tissue

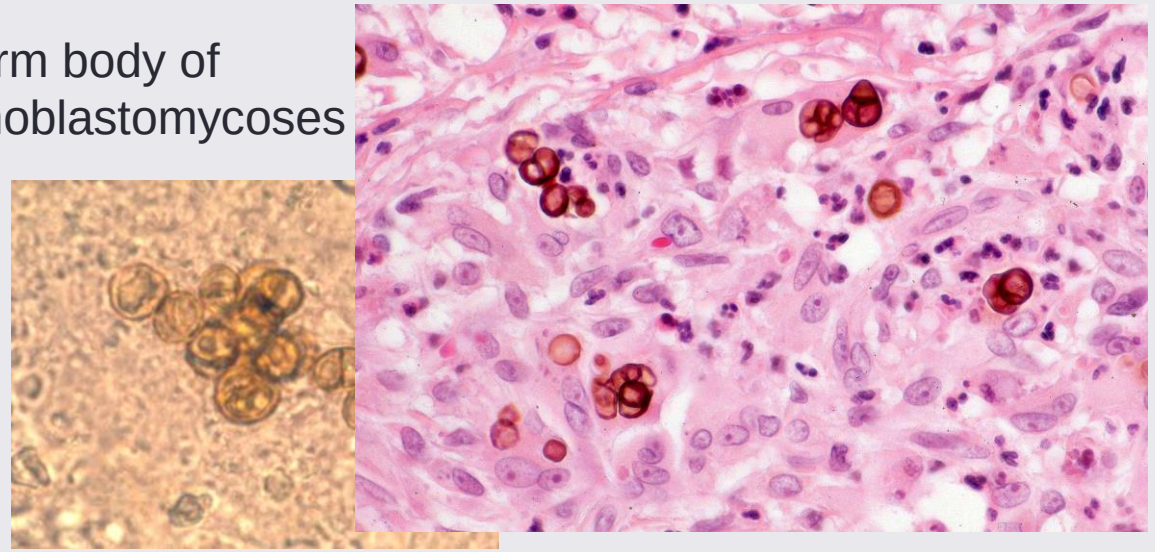
# *Prototheca wickerhamii* (Protothecosis)

- Algae without chlorophyll
- Causes nodular skin lesions
- Most common in patients with suppressed immune system
- Compare morula of Protothecosis to muriform body of Chromoblastomycoses
  - Morula of Protothecosis is Not naturally brown in color

Prototheca morula



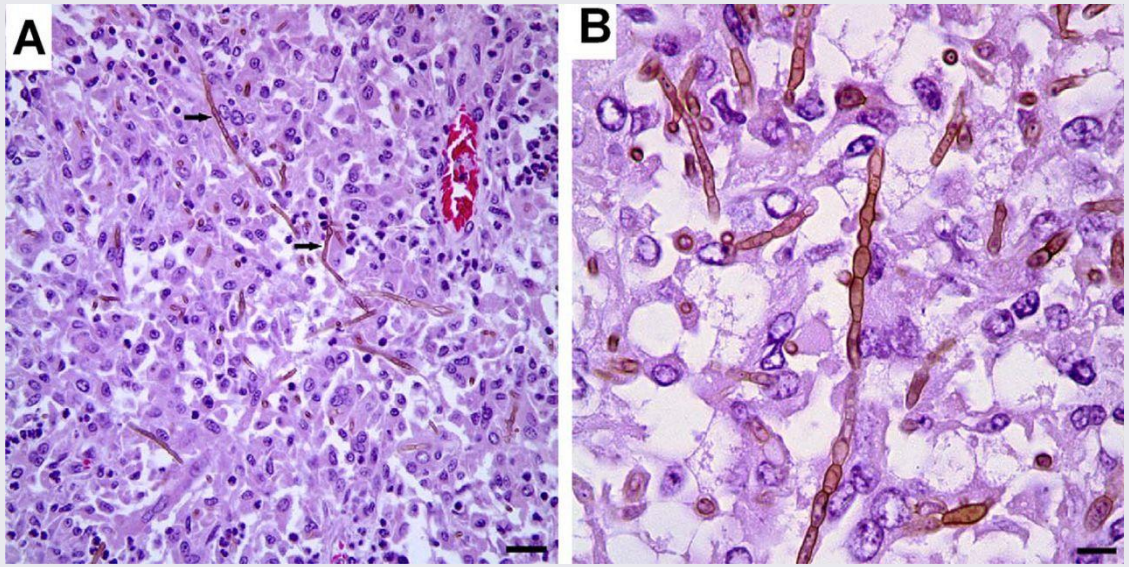
Muriform body of Chromoblastomycoses

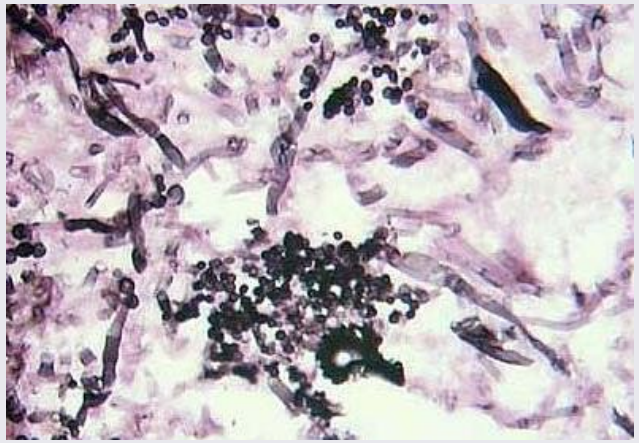


# Phaeohyphomycosis

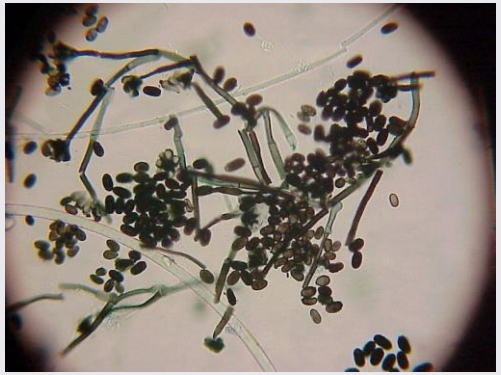
Traumatic implantation of dark fungi into subcutaneous tissue

- Infection usually nodular skin lesions or cysts
- Usually confined to skin but can disseminate, particularly to brain
- In fixed tissue, dark brown colored swollen hyphae and yeast like cells
- *Alternaria, Curvularia, Exophiala and Phialophora* spp most often



Fontana-Masson stain can intensify the melanin pigment



# Black molds / Dematiaceous molds

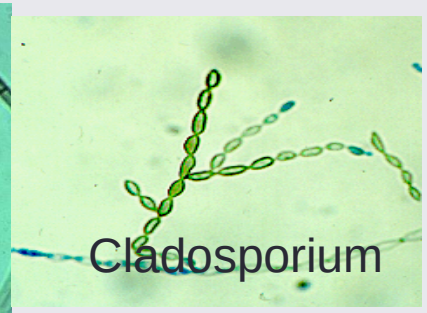
- Black colored colonies; both topside and the reverse [underside of colony]
- Naturally brown colored hyphae and spores due to melanin production
- Commonly found in soil and areas damaged by flooding

Most common black molds include:

*Cladophialophora carrionii*
*Cladophialophora bantiana*
*Phialophora verrucosa*
*Fonsecaea pedrosoi*
*Exophiala species*
*Wangiella species*
*Rhinocladiella species*

# Black (Dematiacious) Molds

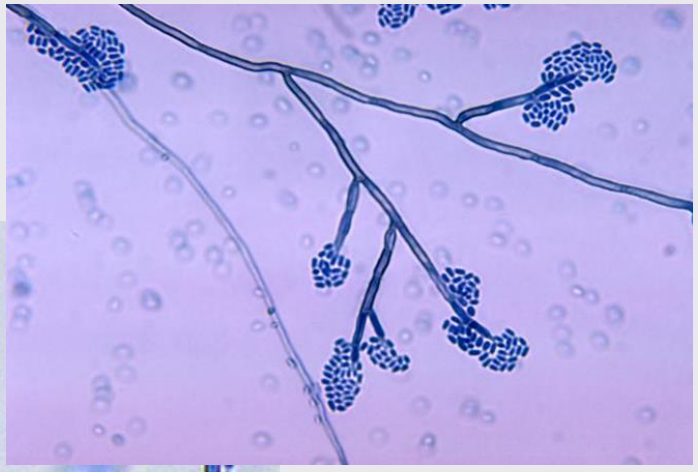
- Numerous species
- Difficult to identify using just morphology, requires MALDI-TOF or 16S rRNA sequencing
  - All have at least one of these four types of sporulation
    - Rhinocladiella-like
    - Cladosporium-like
    - Phialophora-like
    - Acrotheca-like
  - Growth in 7 days at 30*C

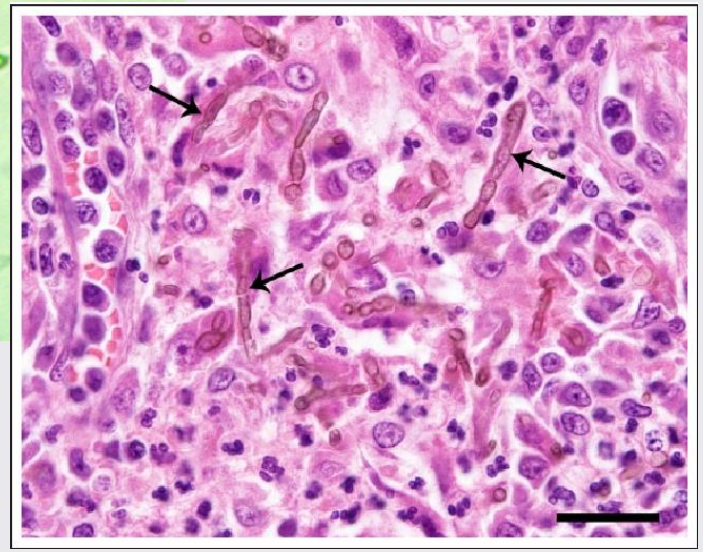


# Most Common Black Molds

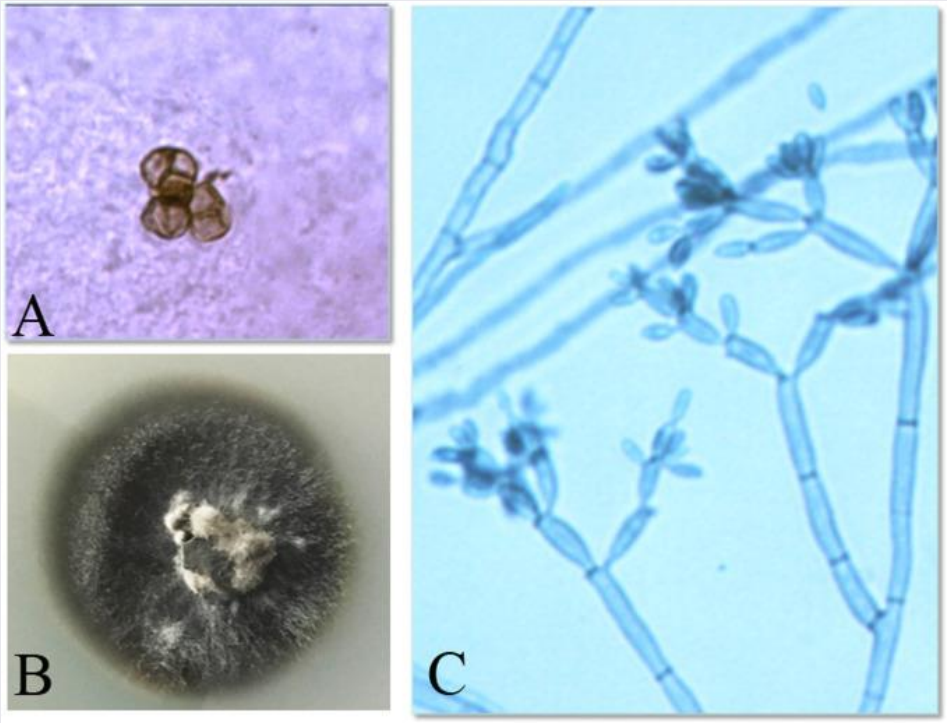
***Exophiala*** **species –**
**Cutaneous and subcutaneous Infections/ may disseminate to the brain**



***Cladophialophora bantiana*** **-**
**Associated with infections that disseminate to brain**



*Fonsecaea species* – common cause of Chromoblastomycosis



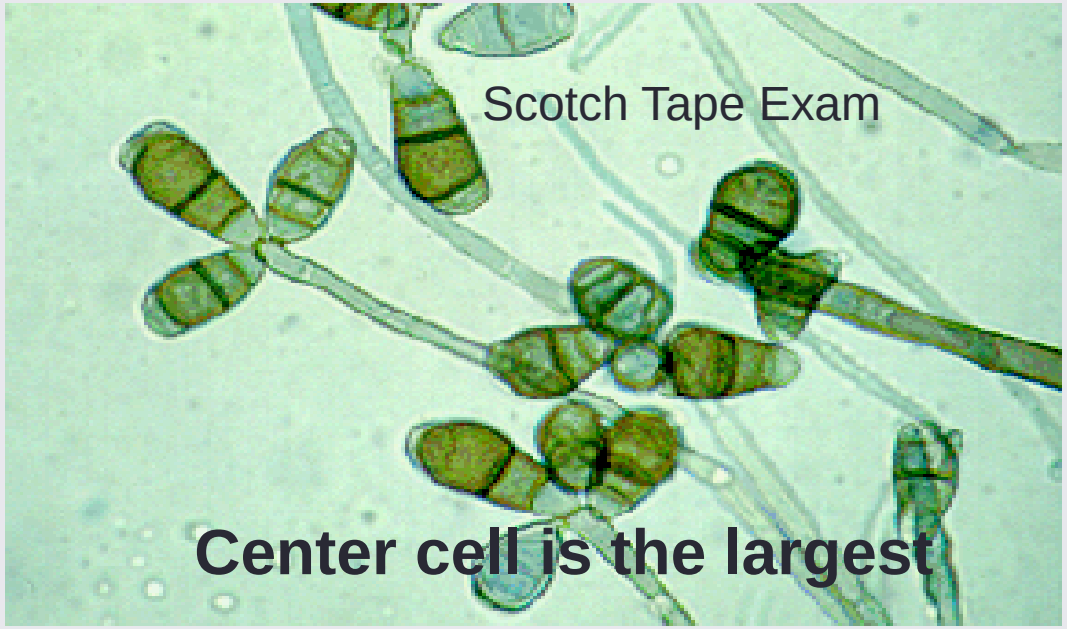

*Phialophora species*

chromoblastomycosis, phaeohyphomycosis with potential dissemination to brain

# *Alternaria species*–

- Opportunistic fungal pathogen commonly found in nature
- Sinusitis and phaeohyphomycosis most often
- Rare infection in nails or eyes

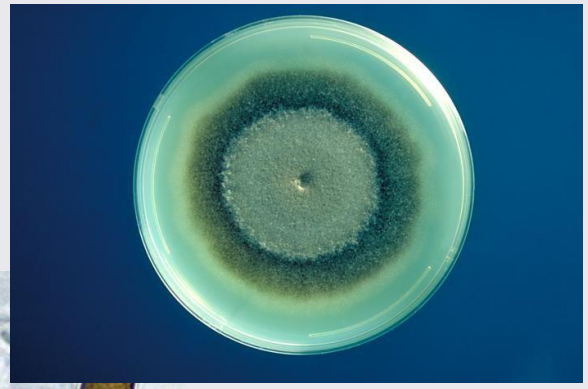

# *Curvularia lunata*



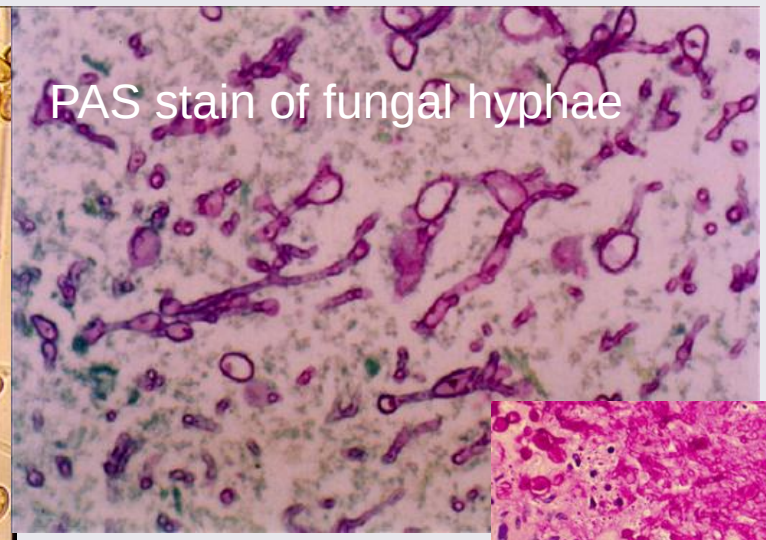

- Opportunistic species/ disseminated infections are rare, Reports of infection in patient with aplastic anemia receiving a bone marrow transplant
- More often reported to cause phaeohyphomycosis, keratitis, mycetoma and sinusitis

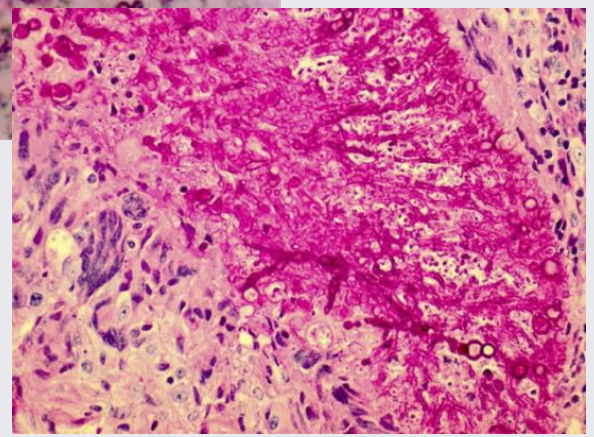
# Exserohilum rostrum

- Causative fungus in an outbreak due to a compound product of methylprednisolone in 2012 contaminated with dust during manufacturing
- Contaminated steroid injected into lumbar spine and knee joints for pain management and led to serious and fatal infections:
  - Meningitis
  - Spinal abscess
  - Synovial infections
- Usually a plant pathogen, rare cases of phaeohyphomycosis







# *Scedosporium apiospermum complex/*

## *(previously Pseudallescheria boydii complex)*

- Cat fur-like gray colony
- Lollipop like spore production with brown colored spores
- Opportunistic pulmonary pathogen from inhalation exposure
- Can invade vessels, particularly in the lung, and lead to infarcts
- Difficult to distinguish from Aspergillus in fixed tissue stains – can branch at 45*
- Intrinsically resistant to Amphotericin B

# IMPORTANT YEAST CAUSING HUMAN INFECTION

*Candida species*

*Cryptococcus neoformans*

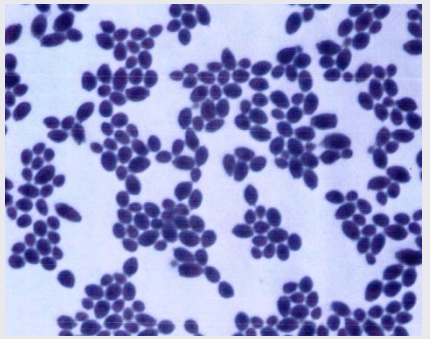
*Cryptococcus gattii*

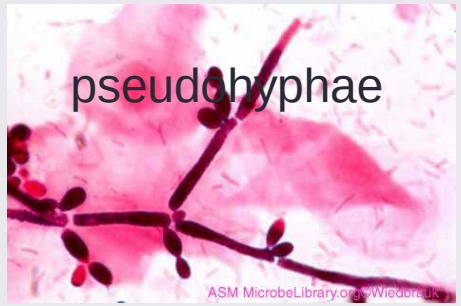
*Trichosporon species*

# Candida species

- Candida species (@ 10 species infect humans)
  - Yeast are usually found as normal flora in GI, GU and skin
  - Candida can be opportunistic pathogens involving skin or mucous membranes from excessive exposure to moisture, escessive antibiotics, or immune suppression
    - Thrush, vaginitis, skin and nail lesions, and diaper rash
  - Can also lead to more serious infections such as fungemia, endocarditis, and systemic tissue infection.

# Candida species






- Grow in 24-48 hours at 30-35*C
  - Growth on many agars SABS, IMA, Blood agar… others
- Bacteria-like colony – pasty white
- Most are oval shaped @ 7-8 um in size**
- Form pseudohyphae in human tissue.  Not true hyphae, the yeast do not detach from pseudohyphae and the yeast cells elongate
- Identify using biochemicals, MALDI-TOF, or 16 sRNA sequencing

- Exception:  ***Candida glabrata and C. auris are* small  @ 4 µM in size and do NOT form pseudohyphae

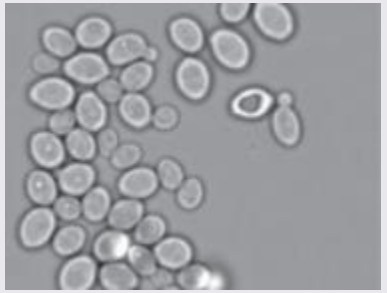
# Most Common *Candida* species

- *Candida albicans* cause @ 60% of Candida infections,
  - Usually susceptible to fluconazole and other antifungals
- *C. parapsilosis is a* pathogen of children and common in IV line infections (forms biofilms effectively on plastics)
  - Usually susceptible to fluconazole and other antifungals

- *C. glabrata*
- *C. krusei* (Pichia kudriavzevi)*
- *C. tropicalis*
- Species causing higher % of infections over last decade due to prophylactic use of fluconazole that select for these more resistant yeast
  - C. glabrata and C. tropicalis likely to be resistant to fluconazole
  - C. krusei (P. kudriavzevi) intrinsically resistant to fluconazole

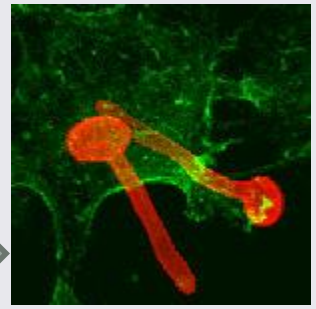
# Candida auris

- Emerging nosocomial pathogen
- Reported from bloodstream, urine, respiratory tract, biliary fluid, wounds, and ear canals
- Axilla and groin best sites for surveillance cultures
- Identification
  - Growth on media can be slower than most yeast (@ 36-48hr)
  - Small yeast (@4 um) and does not produce pseudohyphae
  - Biochemical testing is not definitive
  - MALDI-TOF mass spectrometry and 16 sRNA gene sequencing for identification

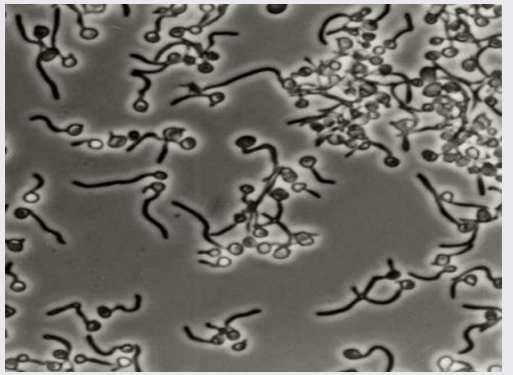


# *Candida albicans* Identification

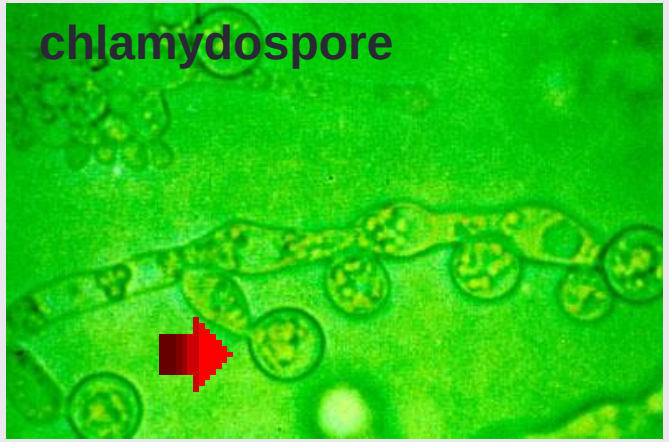
Starry appearing Colony on BAP

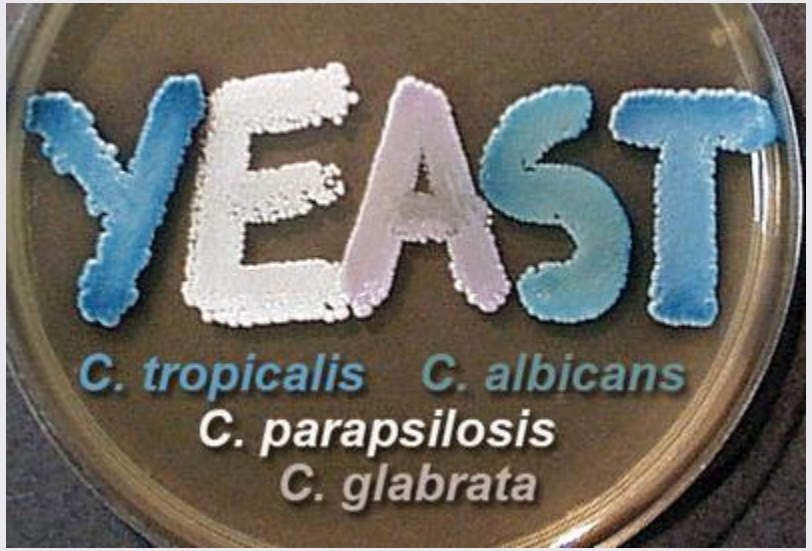


- Germ tube formation
  - Incubate yeast in serum for 3-4 hrs at 35 ˚C
  - Growth extension from yeast cell = germ tube positive
  - If incubate >4 hrs – *C. tropicalis can produce a* false positive germ tube reaction
  - *Note: Test is not specific for C. albicans, C. dubliniensis* can also form germ tubes



- Chlamydospore formation
  - Growth on cornmeal agar for 48 hrs at 30*C
  - Form rudimentary structures known as chlamydospores



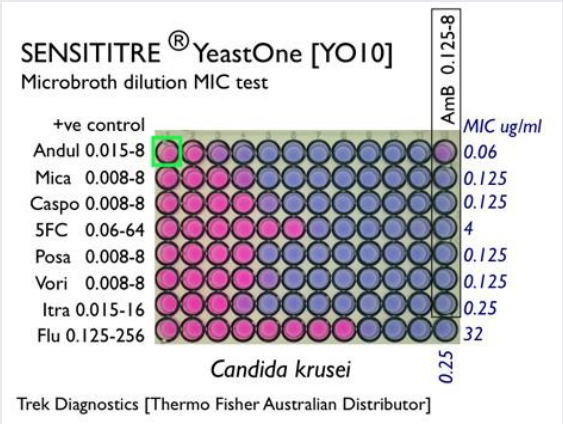



**ChromAgar**

- Can be used for the preliminary identification of  4 Candida species
- Each Candida spp turn a unique color due to action on select chromogenic substrates

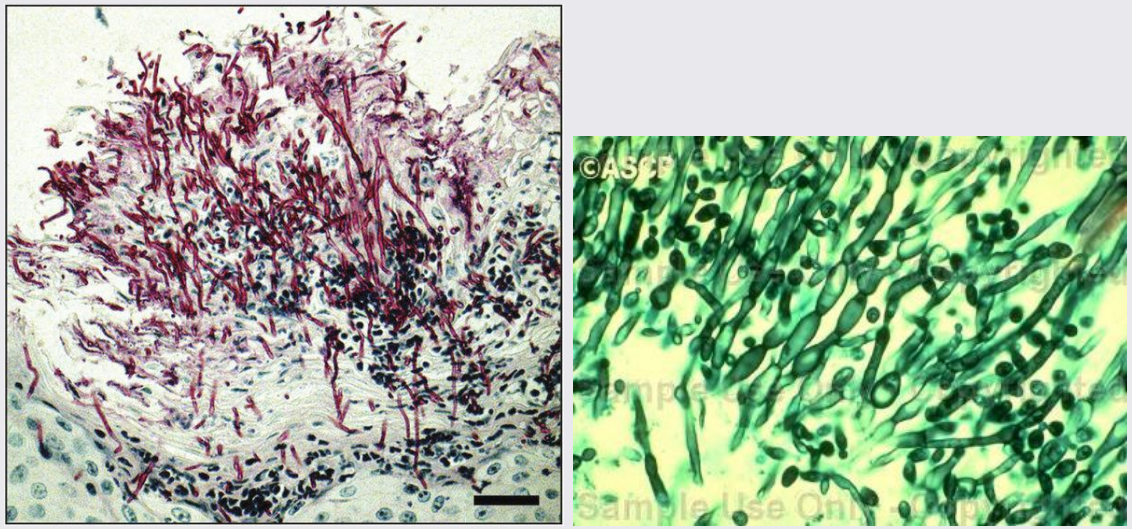
**Susceptibility testing**

Much like bacteria, using broth dilution and Etest methods, regulatory guidelines established by CLSI.

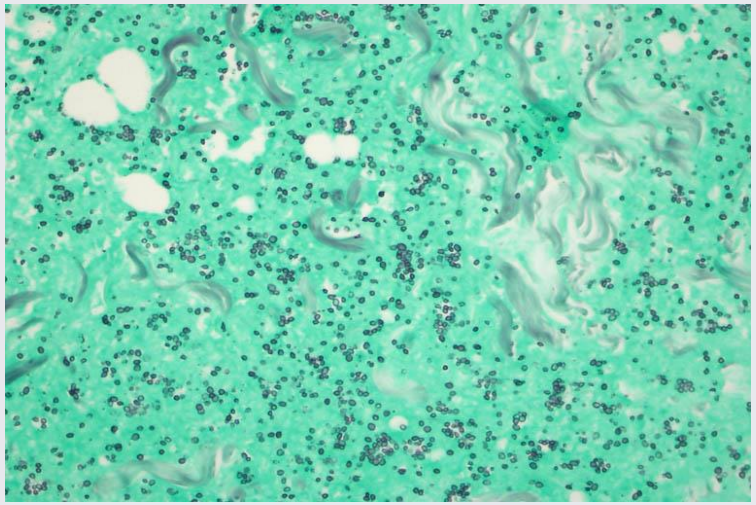



# Candida Histopathology

- Pyogenic to granulomatous inflammation
- Most Candida species produce oval yeast cells (8 um) and pseudohyphae in tissue stains
- *C. glabrata and C. auris* forms smaller yeast cells (4 um) and there is an absence of pseudohyphae



**Candida species not glabrata**



***Candida glabrata* GMS stain**

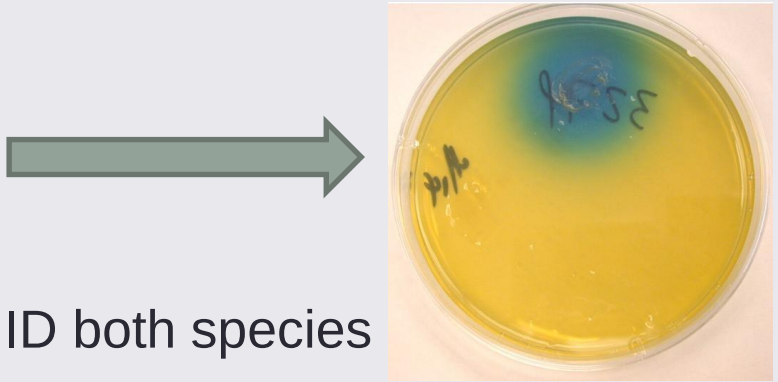
# *Cryptococcus neoformans*

- In nature, a 2um non-encapsulated yeast cell
- Grows well in bird droppings (esp. pigeon)
  - Growth enriched by nitrogen, humidity and warmth in the heaped droppings
- The small, non-encapsulated yeast cells are inhaled – travel through the pulmonary system with hematogenous spread to brain and meninges
- Infection of the compromised host most often
- One of the major AIDS defining infections

# *Cryptococcus gattii* – closely related to *C. neoformans*

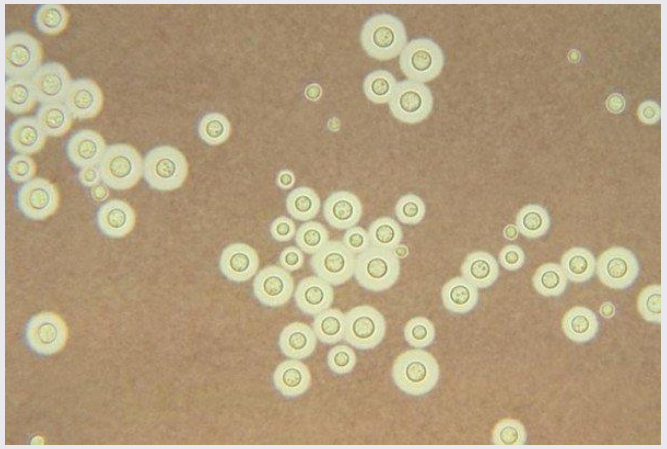
- Isolated from forested areas of the Pacific Northwest Columbia, Washington, Oregon, and California
  - Associated with soil debris and tree bark
- Infection of both normal and immune suppressed hosts
  - Primarily a pulmonary disease [Cryptococcoma] but can disseminate to the central nervous system like C. neoformans
- Culture, biochemical & staining characteristics identical to *C. neoformans*
  - One *C. gattii* defining biochemical reaction
    - L Canavanine glycine bromthymol blue medium –

    *C. gatti* = blue  *C. neoformans* = colorless

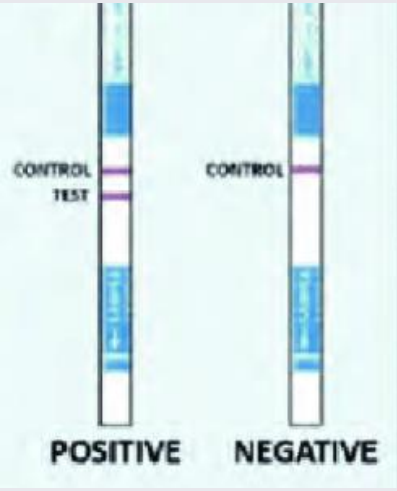


MALDI-TOF and 16sRNA sequencing will correctly ID both species

# *C. neoformans and C. gattii*

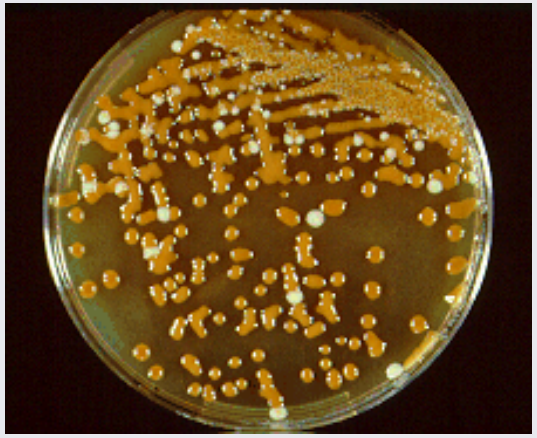


- Variable sized yeast cells (2 – 20 um)
- Virulence factor:  Polysaccharide capsule
- Polysaccharide capsule is basis of diagnostic testing:
  - **India ink exam-**  CSF placed in one drop of black ink. It is a negative staining method – background is stained but not the polysaccharide capsule or yeast cell.  Sensitivity @ >=50%
  - **Cryptococcal antigen test** – Detection of capsular polysaccharide that leached off the yeast cell and detected in CSF or serum using lateral flow enzyme immunoassay
    - Best test for diagnosis with sensitivity >=99%, can also follow patient recovery with decreasing titer of polysaccharide present in the sample



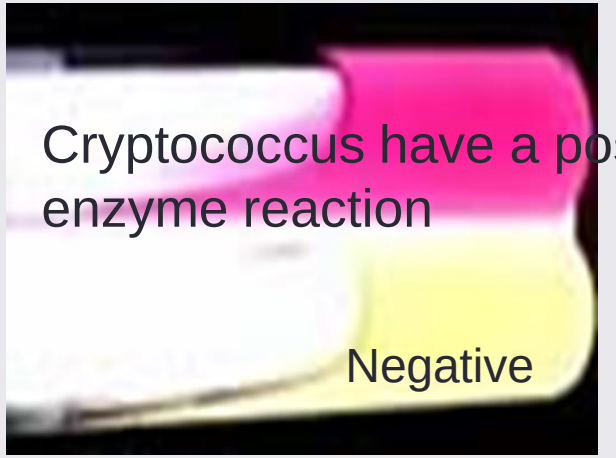
# C. neoformans and C. gattii


Grows on most fungal media in 1-3 days at 30-35*C
Mucoid colonies due to capsular polysaccharide



Both C. neoformans & C. gattii form brown colonies on Birdseed agar

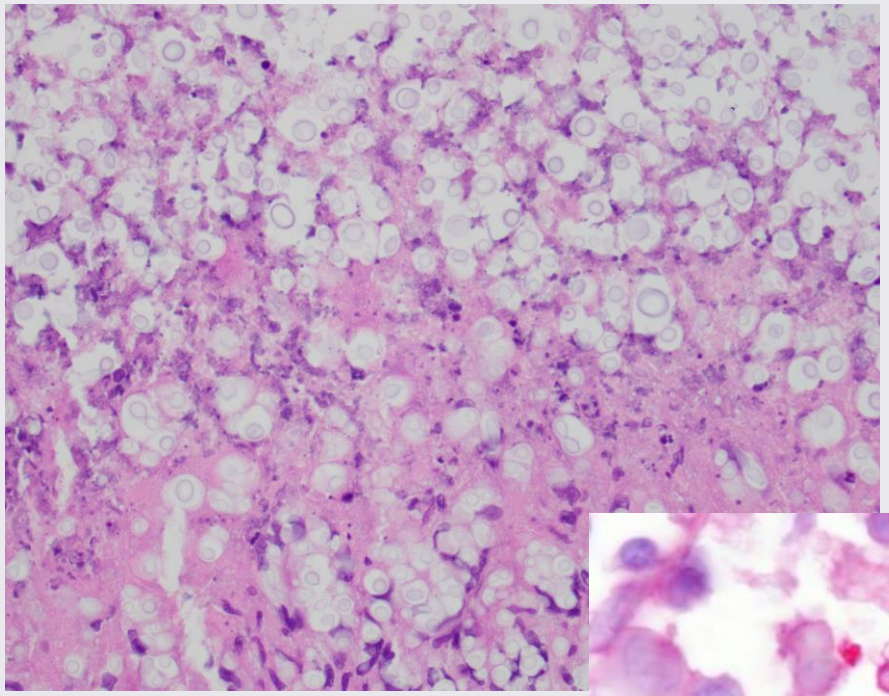
Gram stain /
stains as blue blobs due to polysaccharide capsule



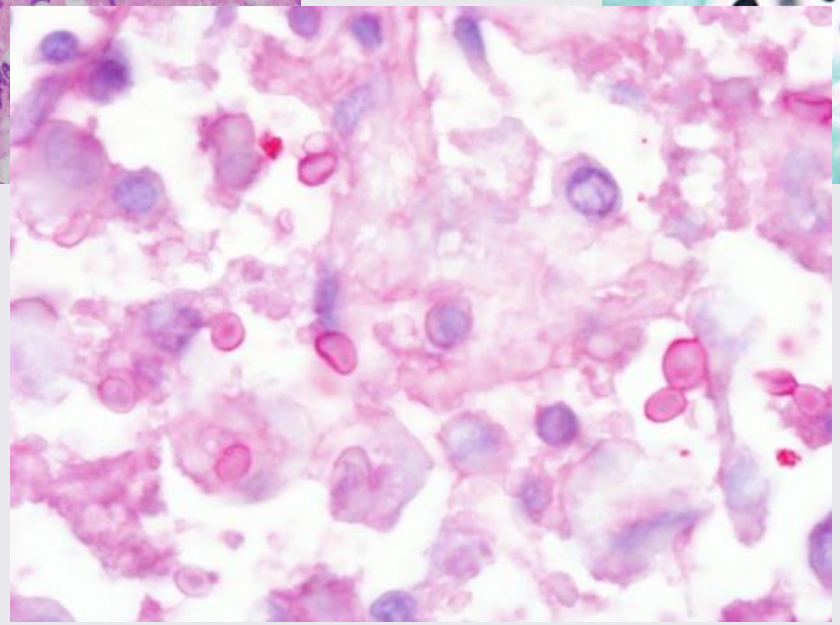
Cryptococcus have a positive urease enzyme reaction

Negative

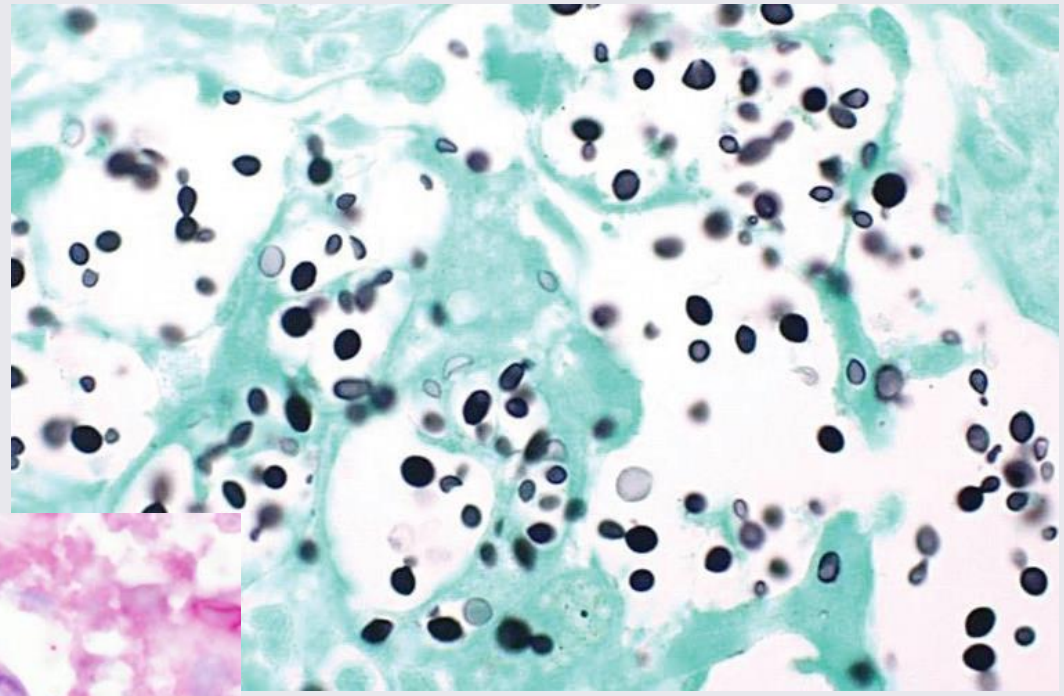
# Cryptococcus in tissue



Cryptococcus has visible clearing due to capsule/ yeast stains poorly with H&E stain



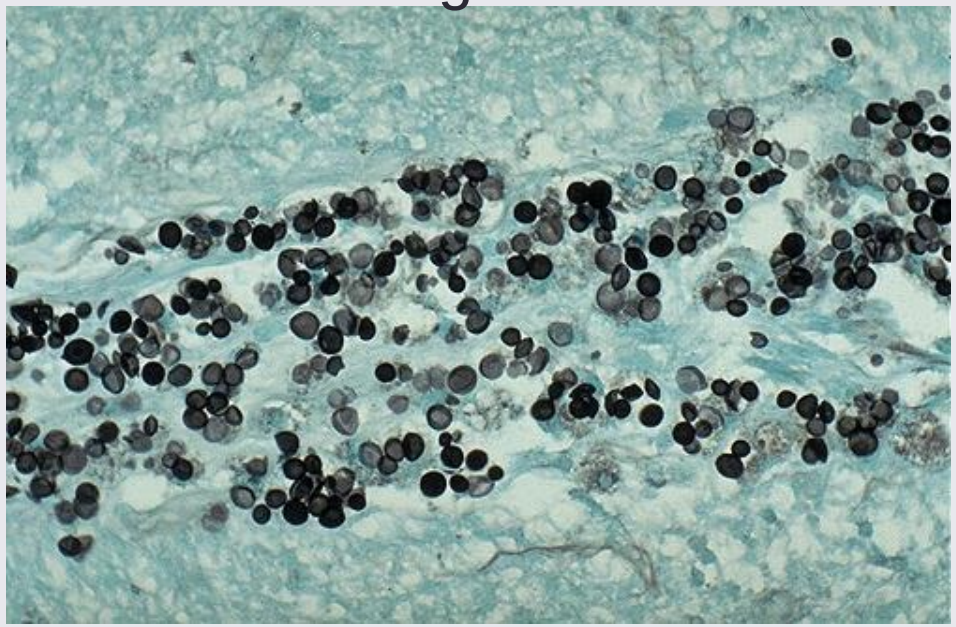
GMS stain –the yeast are variable in size.



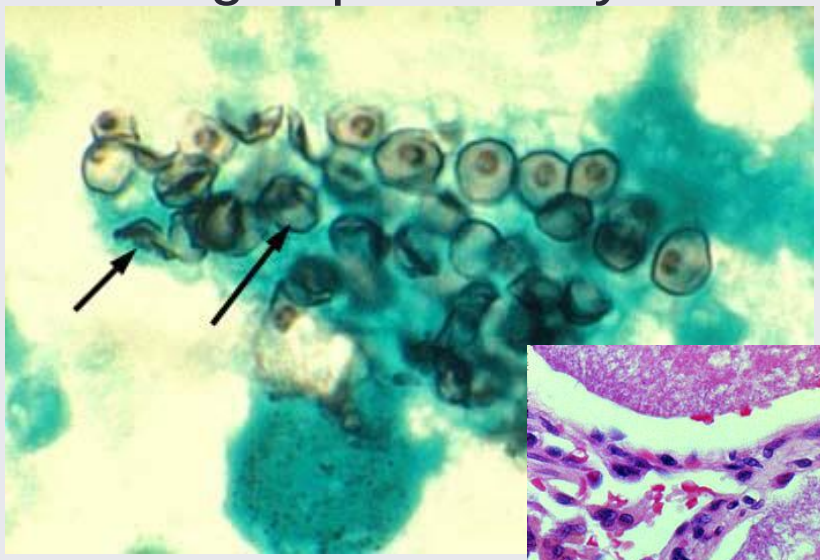
Mucicarmine stains the capsular polysaccharide of C. neoformans and C. gattii.

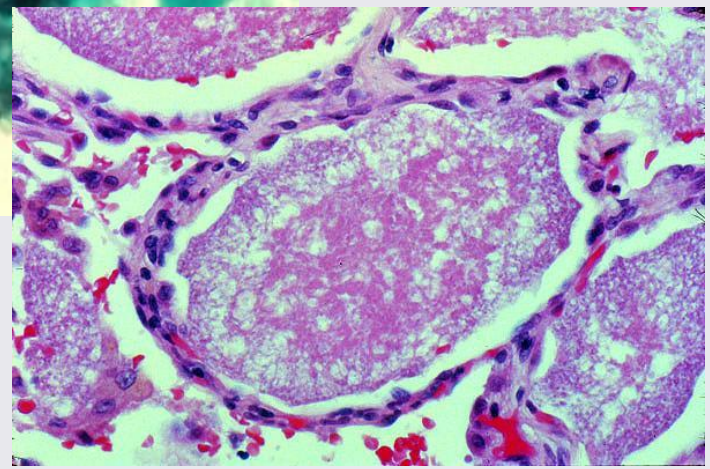
# Cryptococcus vs. Pneumocystis

*C. neoformans/ C. gattii* – no nuclear staining



*Pneumocystis jiroveci* (yeast like fungus) could be confused with C. neoformans – Careful! Central nuclear staining in pneumocystis

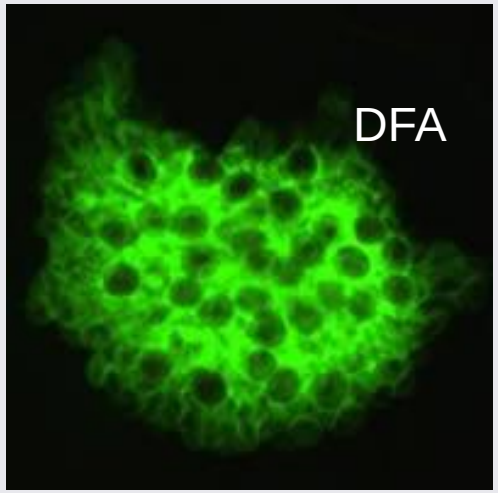


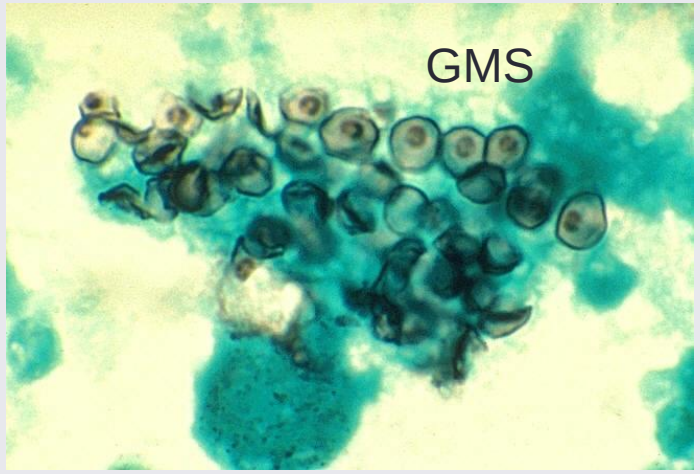


Pneumocystis froth on H & E

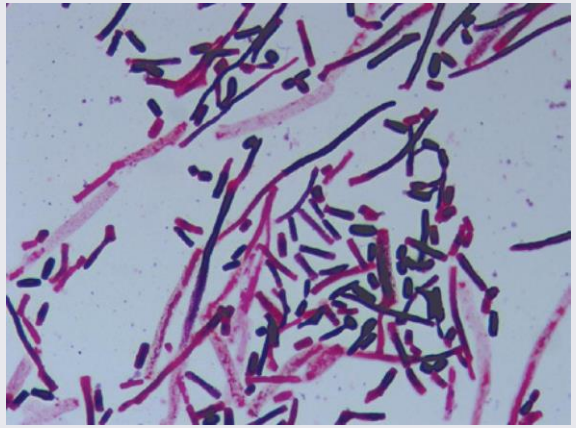
# *Pneumocystis jiroveci*

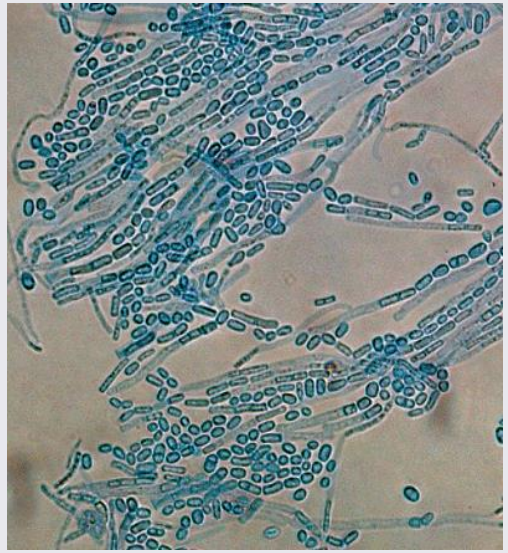
- Yeast like fungus
- Used to be named *Pneumocystis carinii* and considered a protozoan parasite
- Causes pneumonia in the immunocompromised host (PCP) particularly HIV/AIDS
- Diagnosis:  Bronchial lavage, lung biopsy tissue, induced sputum using direct fluorescent antibody (DFA) and GMS.



# Trichosporon





- *T. asahii* most common species
- Commonly inhabit the soil
- Colonize the skin/ gastrointestinal tract of humans
- Rectangular shaped yeast cells (3 x 8 uM)
- Cause of superficial infection, White piedra, a distal infection of the hair shaft, also nail infections (onychomycosis), cutaneous, and fungemia
- Uncommon cause of disseminated yeast infections in humans
  - Most usually recovered from patients with corticosteroid use, solid tumors, HIV/AIDS, and intravascular devices, including catheters and prosthetic heart valves
  - Difficult to treat, most antifungal drugs have elevated MICs against species of Trichosporon
  - Mortality 50 – 80%

# CUTANEOUS AND SUPERFICIAL MYCOSES

*Malassezia furfur*

Dermatophytes that cause Dermatophytoses

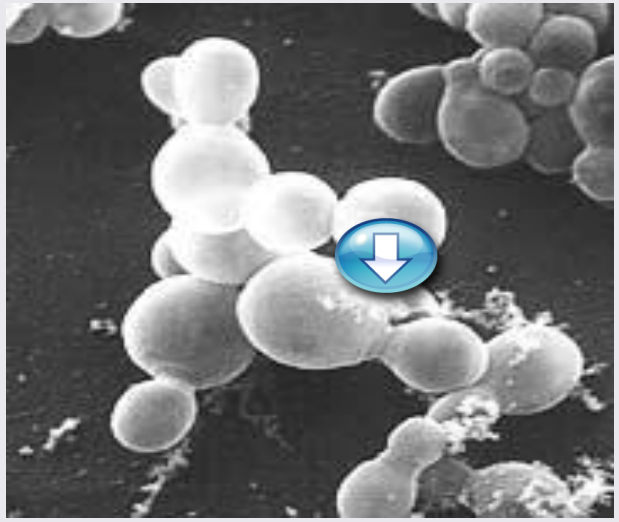
- *Microsporum species*
- *Trichophyton species*
- *Epidermophyton floccosum*

# *Malassezia furfur*

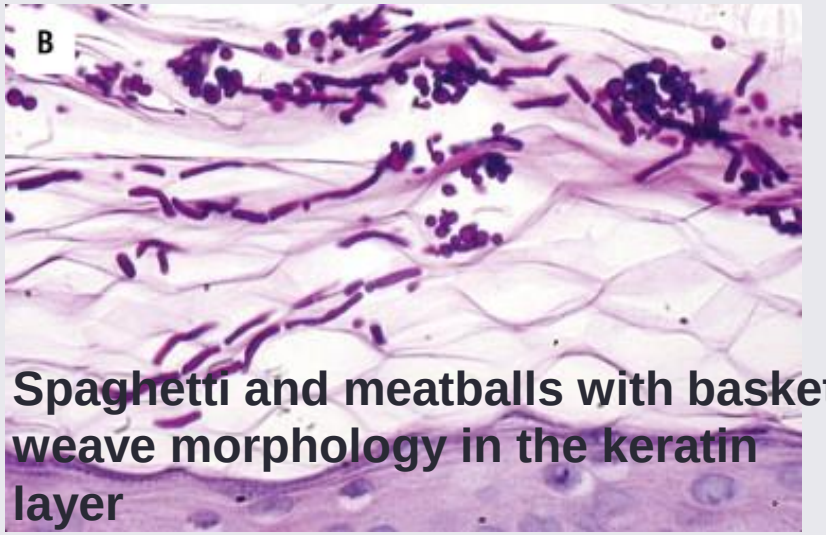
- Most superficial of the dermatomycoses
- M. furfur can be normal flora on human skin
  - More common on oily skin or patients with high use of skin oils
  - Warm and humid climates
- Diseases:
  - Skin:  macules, papules, patches, plaques on chest back and shoulders with either hypo or hyper pigmentation – does not invade into deeper tissues – known as Pityriasis versicolor
  - Fungemia: caused by tunneling in with IV lipid feeding lines (parenteral nutrition) – in neonates or rarely adults

# Malassezia furfur

- Lipophilic yeast – oil required for growth
  - Media for isolation must contain oil or use an oil overlay
- Small budding yeast 2 – 4 µm with collarette (appears like necklace at junction of mother and daughter yeast cell)
- In tissue described as “Spaghetti and Meatballs” due to budding yeast and short hyphal fragments.



Spaghetti and meatballs

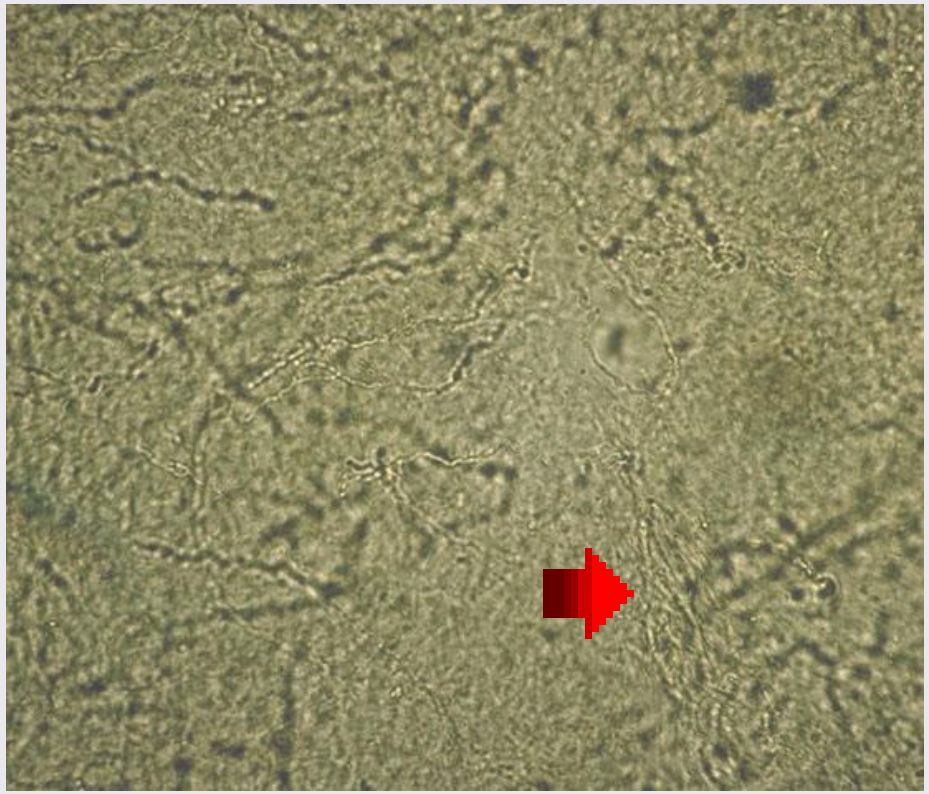



Spaghetti and meatballs with basket weave morphology in the keratin layer

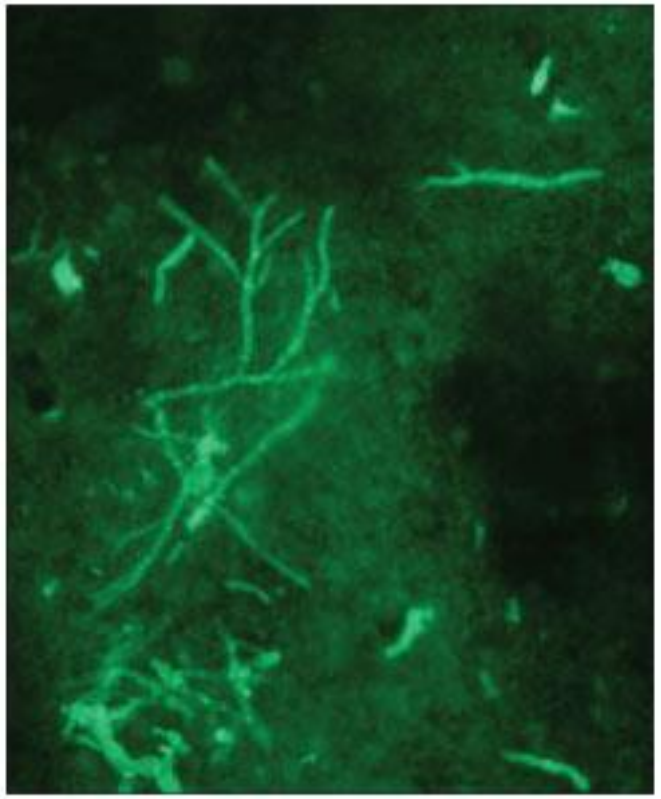
# Dermatophytes – Ringworm infections

- Hair, skin and nail infections
- 3 genera of fungi
  - Microsporum species (many)
  - Epidermophyton floccosum
  - Trichophyton species (many)
- Disease described by area of the body infected:  For example: tinea capitis (head), t. pedis (foot)
- Usually a clinical diagnosis not requiring culture
- KOH prep or Calcofluor white prep can be used to visualize fungal hyphae from skin scrapings

## Positive KOH prep Showing thin septate fungal hyphae



## Calcofluor white stain with fluorescence – thin fungal hyphae



***Microsporum canis***

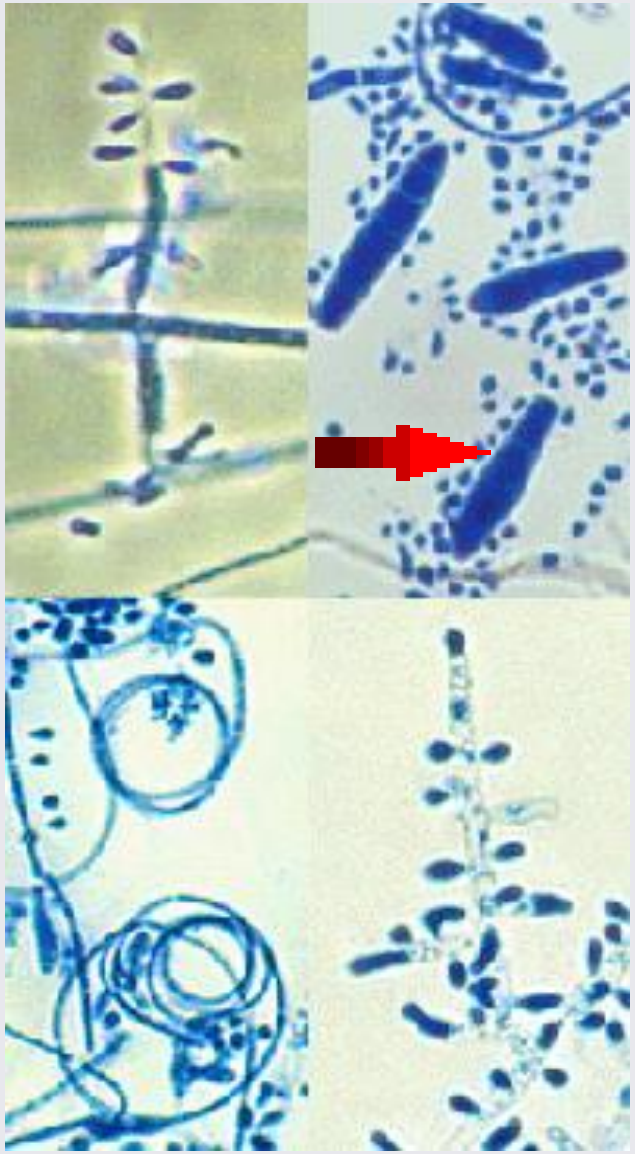
- Ringworm infection acquired from infected dog or cat
- White colony grows in 5-7 days at 30* C/ yellow on reverse (back) of colony
- Tuberculate thick walled macroconidia [tiny spiny projections]
- Few if any microconidia (small spores)

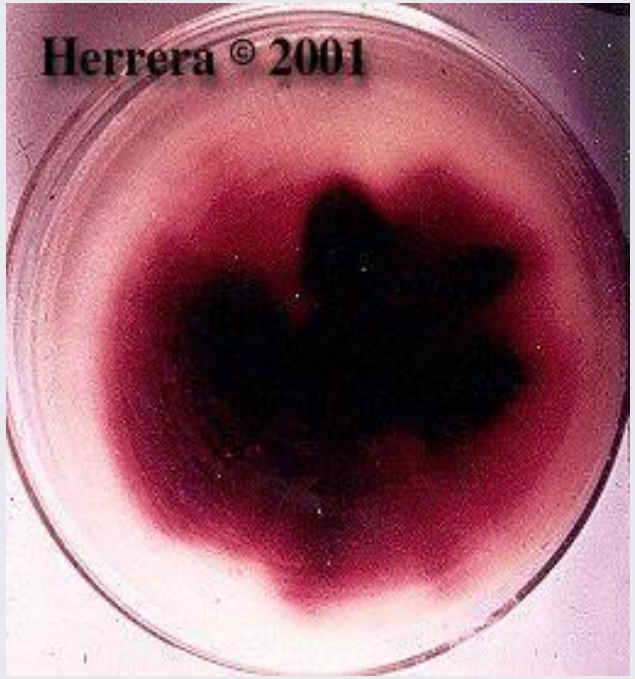
***Microsporum gypseum* –**

- Mostly skin infection from exposure to contaminated soil
- Sandy colored colony that grows in 5-7 days, 30*C
- Large macroconidia are produced, no microconidia produced

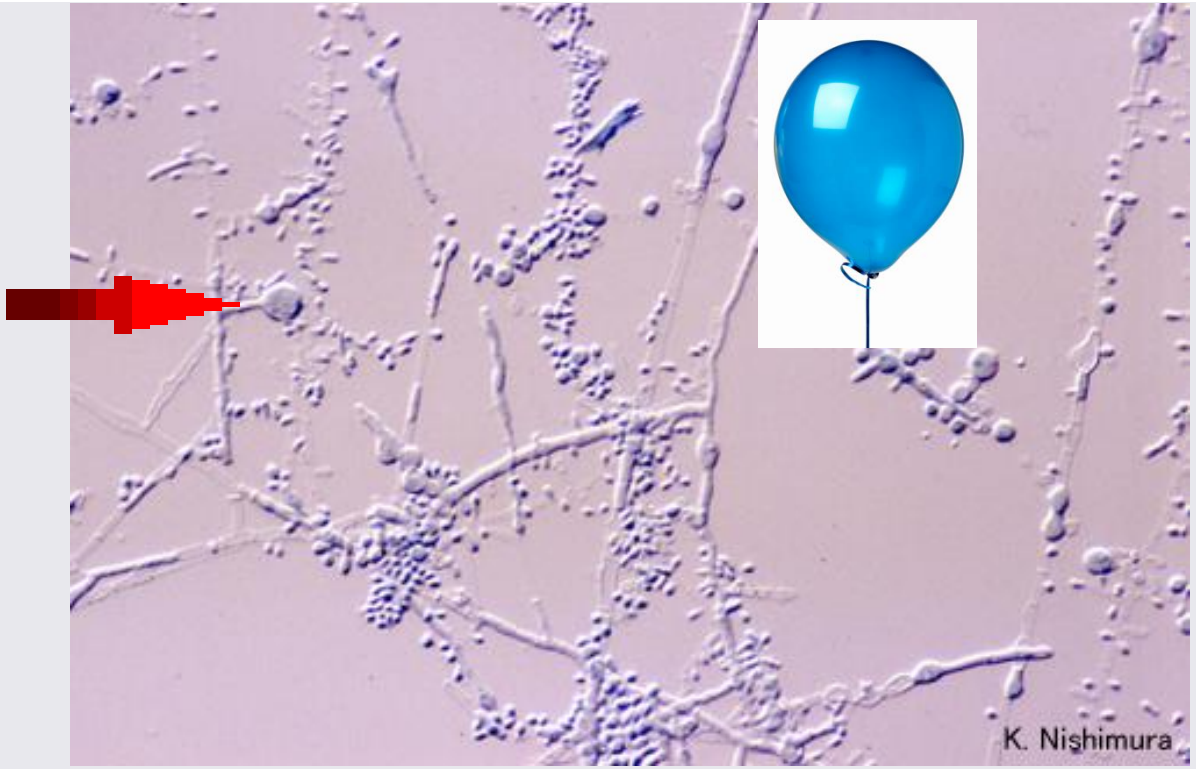
# *Trichophyton rubrum*

- Mostly infects skin and hair after exposure to fomites, such as wet towels or hairbrushes
- Grows in 5 – 7 days at 30*C. The colony is white and the back side of the colony has an intense red pigment that diffuses into the media
- Forms pencil shaped macroconidia/many micro-conidia (different than Microsporidium)






**Red diffusible pigment**




# *Trichophyton tonsurans*

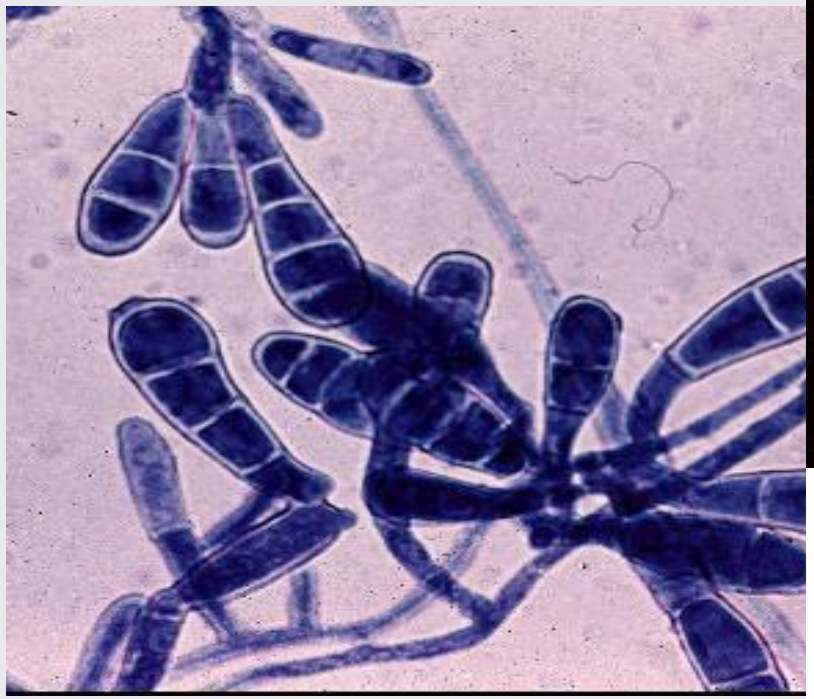
- White colony, grows in 5-7 days at 30* C
- Light yellow reverse

- No macroconidia
- Microconidia produced with <u>rare ballooning microconidia</u>

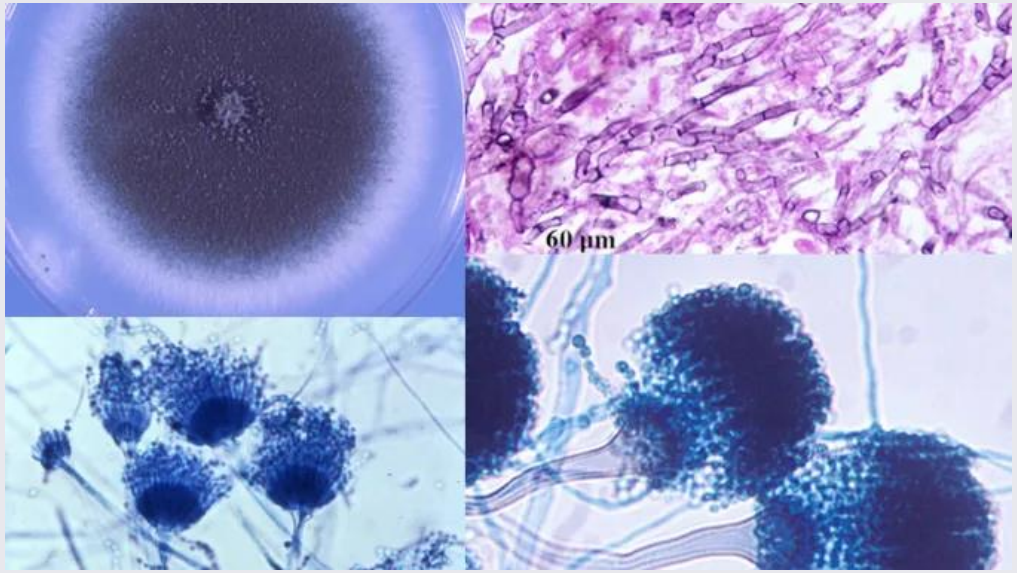
- Primary cause of epidemic scalp ringworm in children

# *Epidermophyton floccosum*

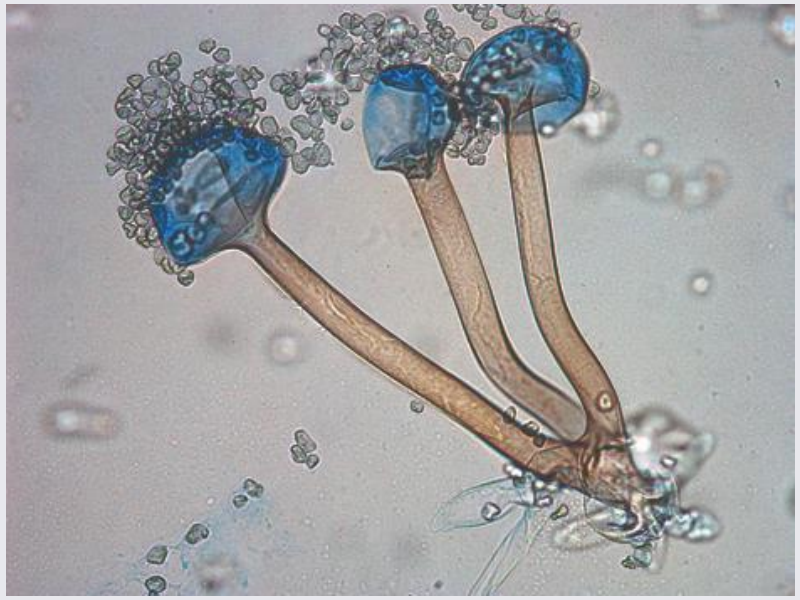
- Infections in skin and nails
- Khaki green colored colony that grows in 5-7 days at 30*C
- *Beaver tail shaped large macroconidia* – no microconidia



# Opportunistic Hyaline Molds
# Two groups:
# (1) Narrow septate hyphae
# (2) Broad non-septate hyphae




Infections in the immune suppressed host or special circumstances



# Hyaline/ narrow septate hyphae

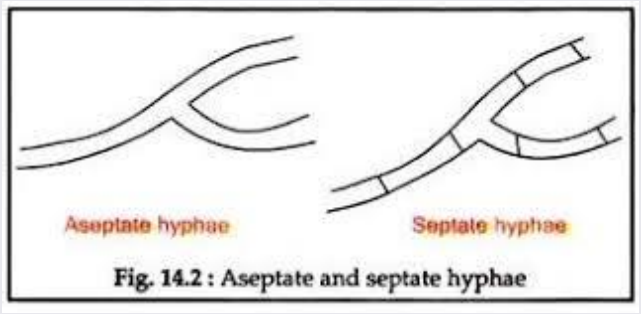
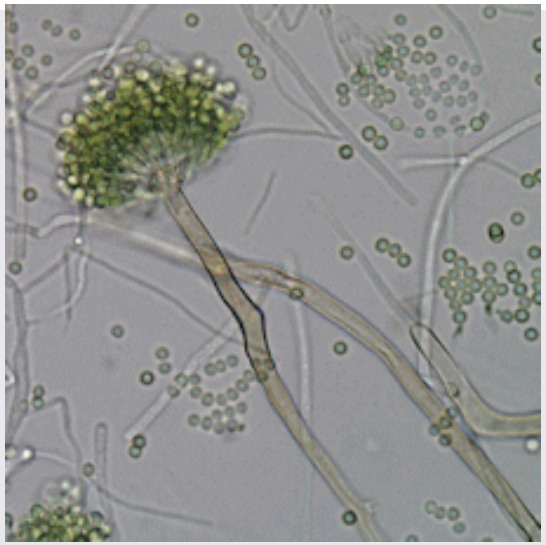
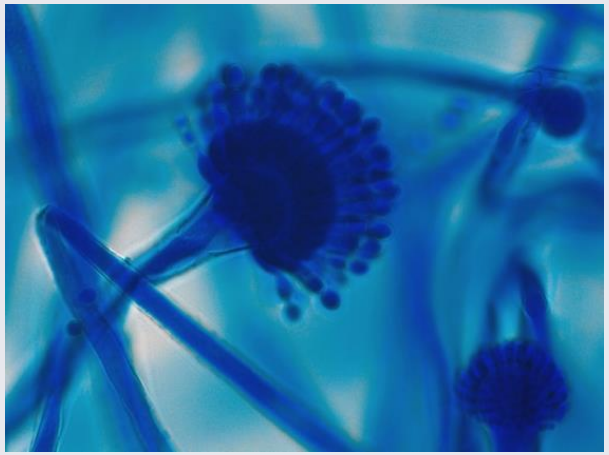


Fig. 14.2 : Aseptate and septate hyphae

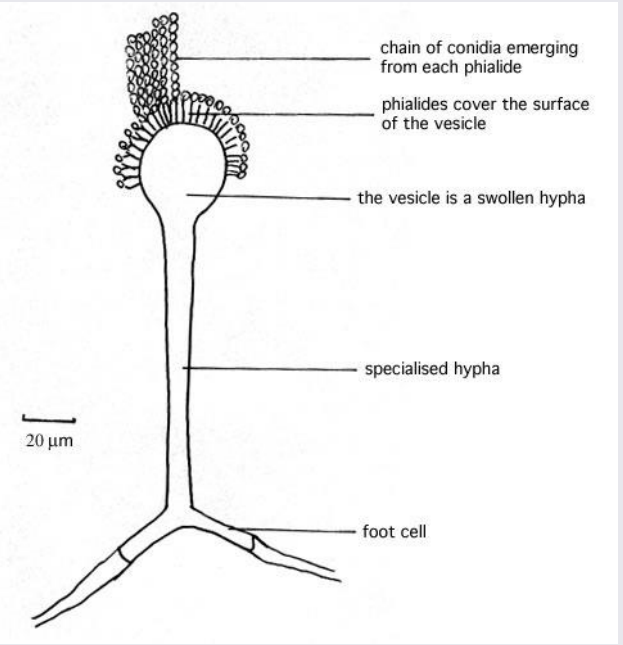
- Hyaline – no color to the hyphae
- Regular septations in hyphae
- Grow on a variety of agar media in 3-5 days at 35 or 30˚C
- Identification based on growth rate, color/texture of colony and microscopic structures
  - Definitive speciation: MALDI-TOF or 16sRNA sequencing

# Aspergillus species



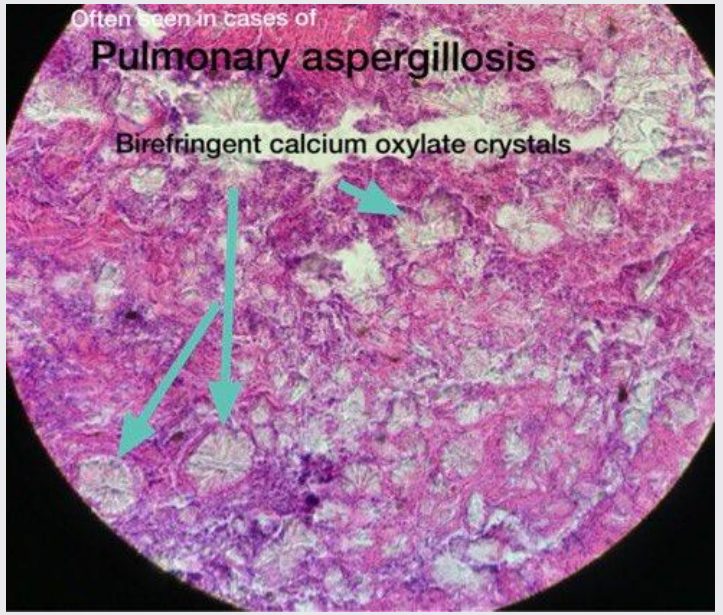


- Ubiquitous in nature @ 200 species
- Hyaline hyphae with regular septation
- Swollen vesicle support phialides
- Numerous round conidia emerge from the phialides
- Predominately a respiratory pathogen
  - Nasal sinus and lung
- Dissemination to other organs in immune suppressed
- Neutropenia can predispose to infection

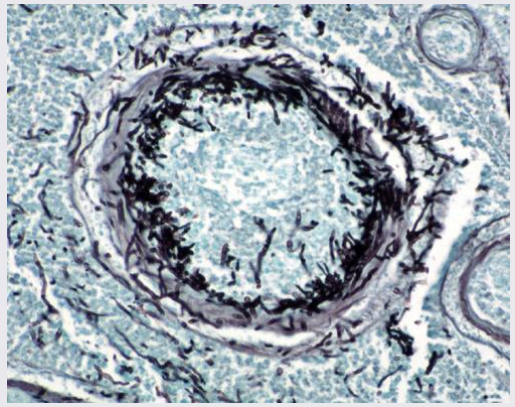


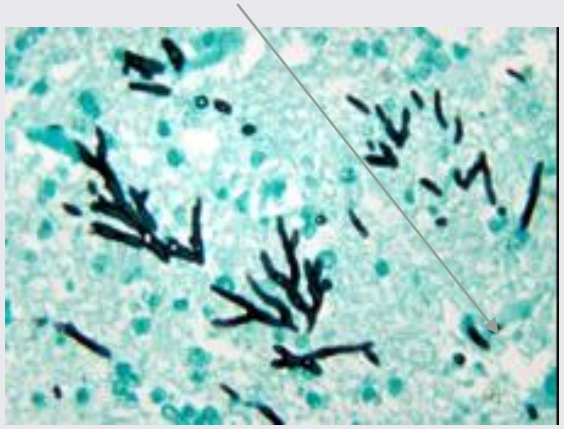
# Aspergillus species

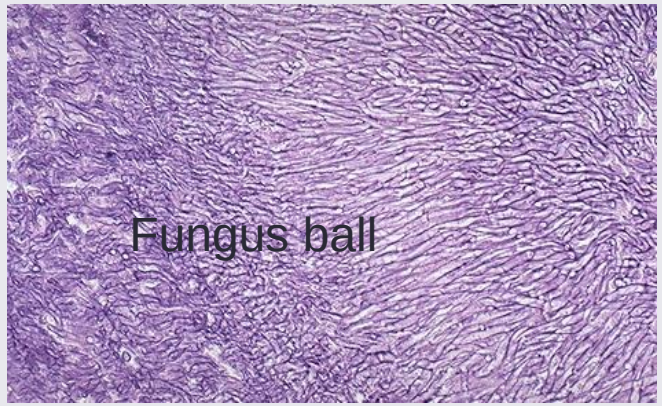
- Stains with many stains
- Thin septate hyphae
- 45* degree angle branching is helpful to ID
  - Branches can branch (Dichotomous)
- Invade vessels, cause thrombosis & infarctions
- Birefringent Calcium oxylate crystals can be present











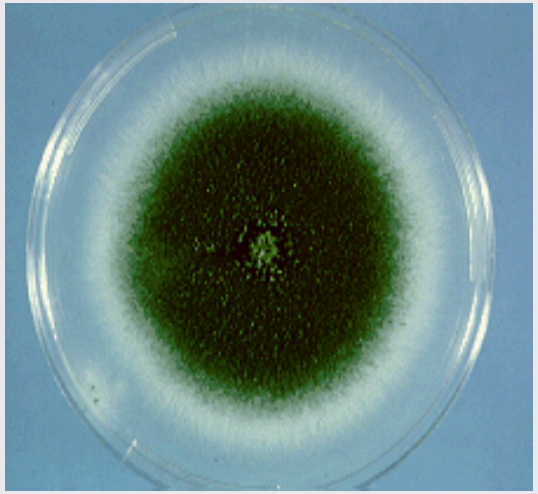
# Serology Tests Supportive not Conclusive for Diagnosis

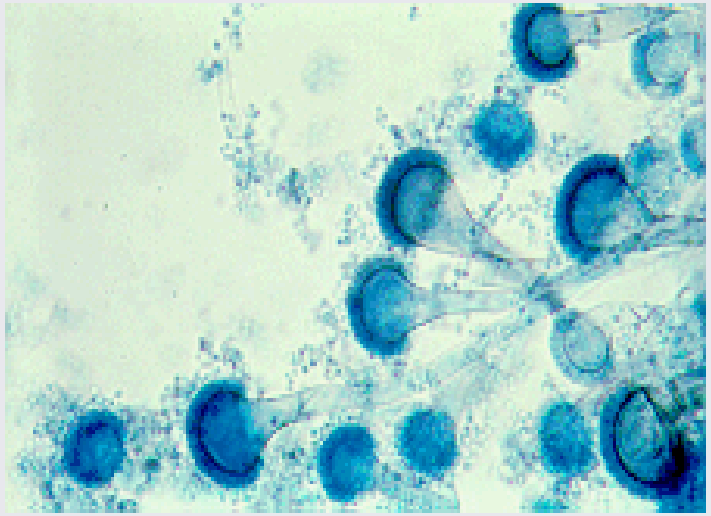
## Aspergillus Galactomannan Enzyme Immunoassay

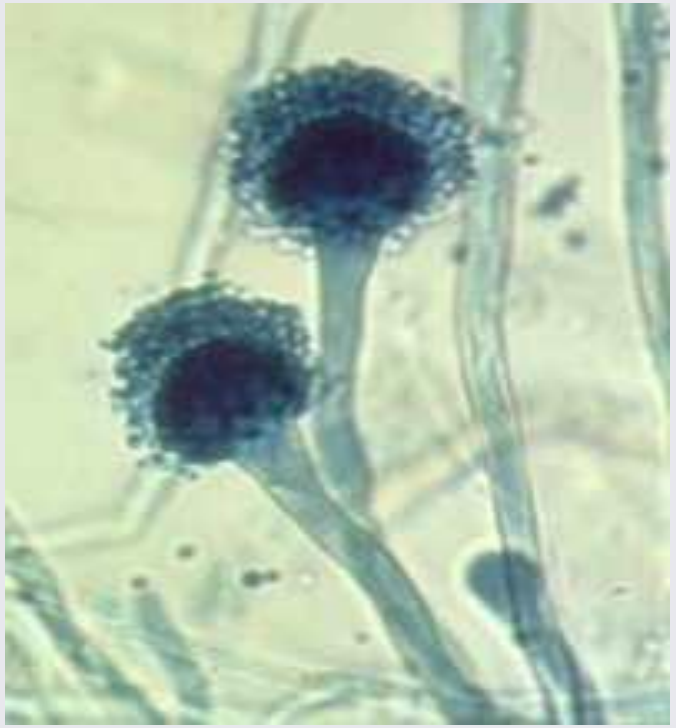
- Detects circulating Aspergillus antigen in the blood and/or bronchial lavage, most helpful in disseminated infections
- +/- sensitivity and specificity (PPV 68%/ NPV 96%)
- False positive reactions (10%) with Piperacillin/Tazobactam therapy, infection with *H. capsulatum,* and rice and pasta ingestion

## (1,3)-Beta-D-glucan assay

- Detected in serum or BAL from the following pathogens: Candida spp., *Acremonium, Aspergillus spp., Coccidioides spp, Fusarium spp., Histoplasma capsulatum, Trichosporon spp, Sporothrix schenckii, Saccharomyces cerevisiae, and Pneumocystis jiroveci.*
- No reaction for *Cryptococcus or Zygomycetes*
- High values more meaningful, false positive reaction from the environment contaminated with many of these fungi

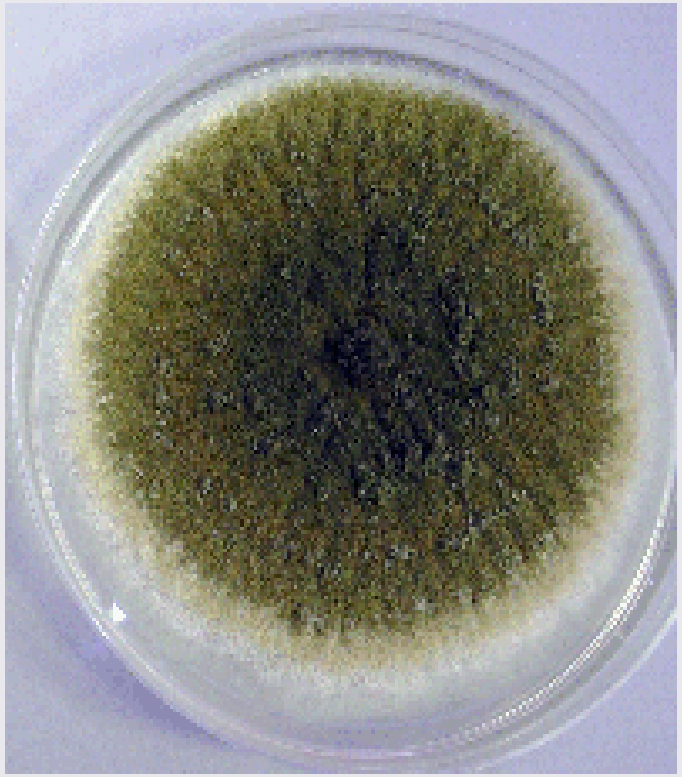






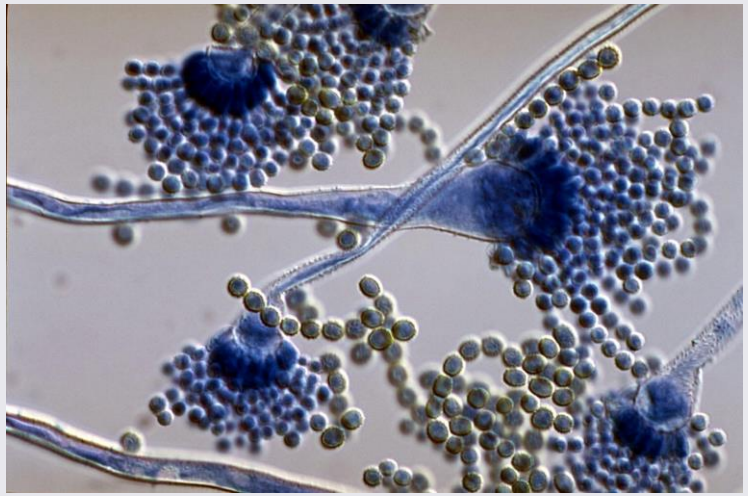
***Aspergillus fumigatus***

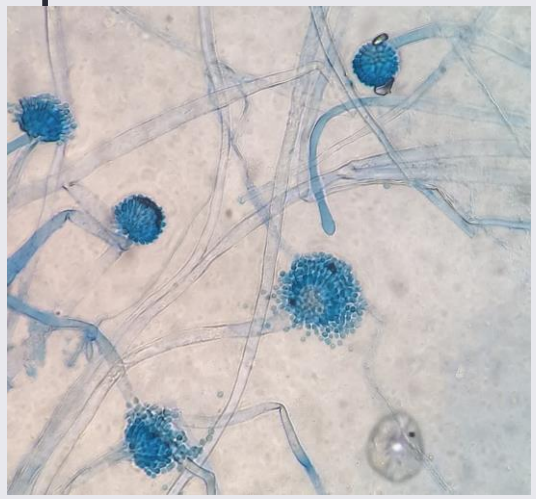
- Ubiquitous airborne fungus, inhale conidia in nature
- One of most common species causing pulmonary infection in immune suppressed, can disseminate from lung to other organs
- Blue/Green colony grows in 3 – 5 days at 30*C
- Phialides produce colorless spores, directed upward



## *Aspergillus flavus*

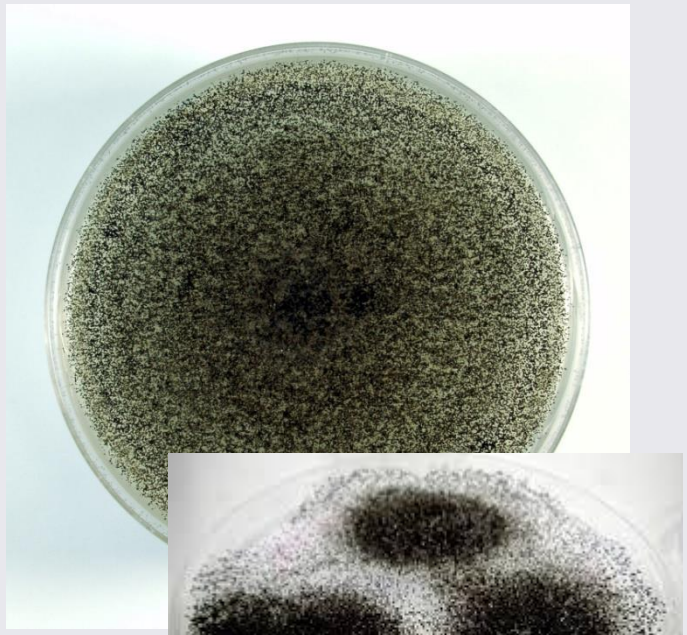
- Normally found in cereals, grains, legumes
- Same disease potential as A. fumigatus/ less common/ pulmonary infection with possible dissemination
- Green/yellow colony growing in 3-5 days 30*C
- Green/yellow hue to conidia produced on phialides that surround the swollen vesicle

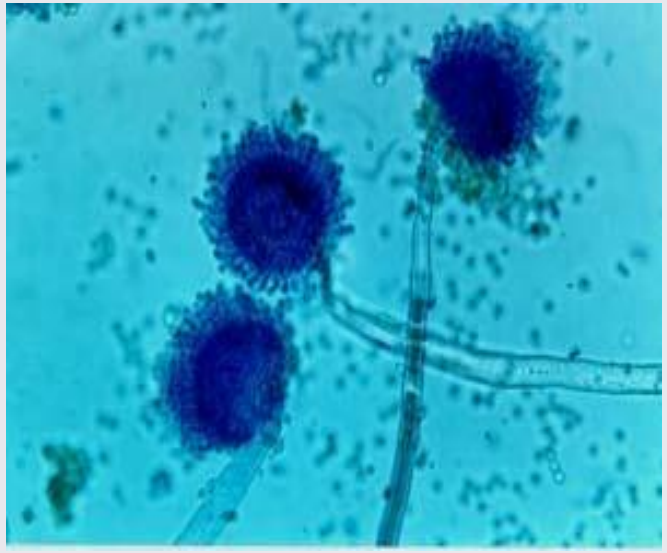


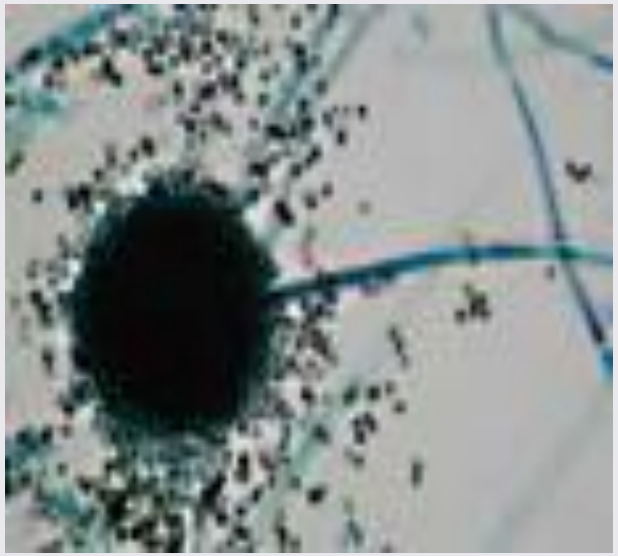


## *Aspergillus niger*

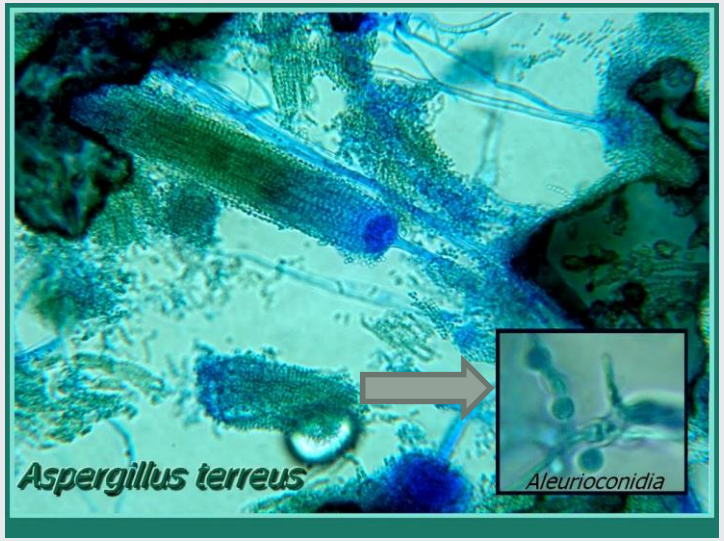
- Black colony – visible black fruiting heads grows in 2-5 days at 30*C
- Contaminate fruits and vegetables and found in soil
- Invasive disease uncommon, commonly isolated from ear infections
- Black conidia supported by phialides that surround the vesicle



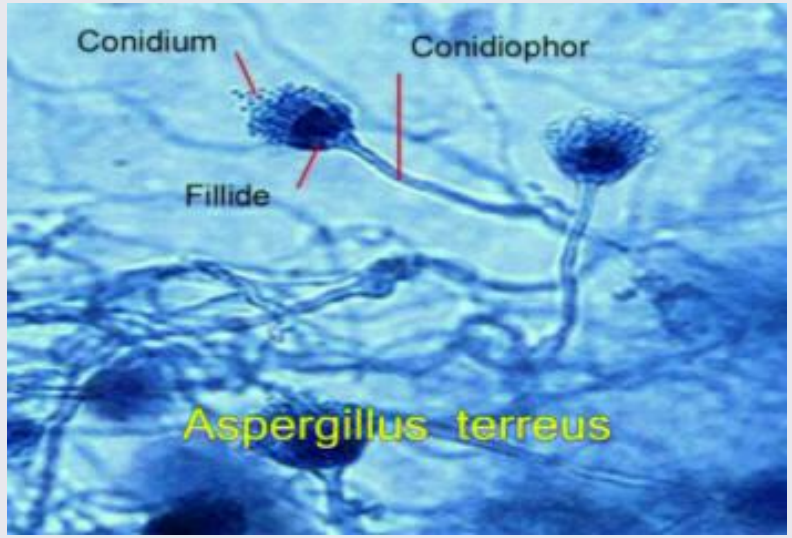




# Aspergillus terreus

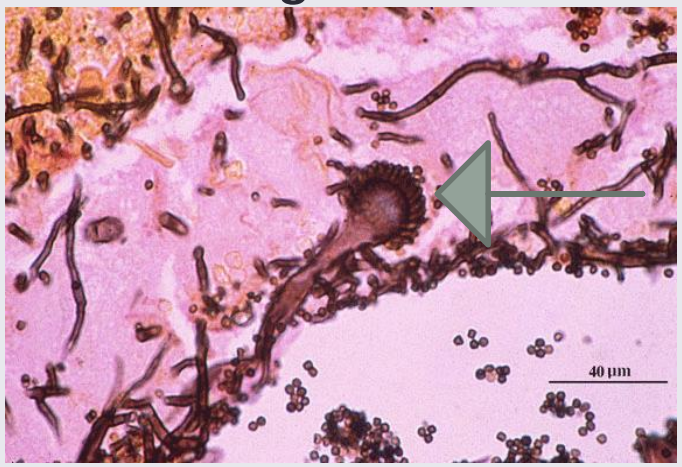


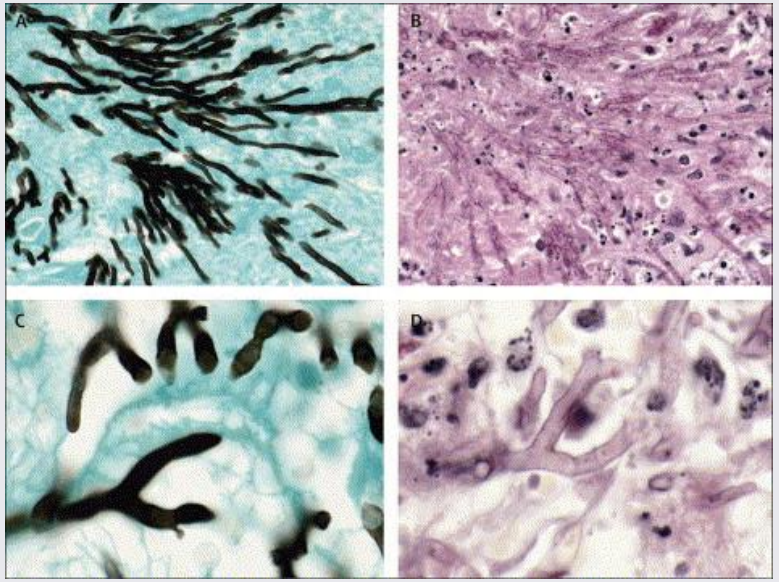

- Infection primarily in the immune compromised host
- Sandy colored colony grows in 3-5 days at 30*C
- Isolated from soil
- Colorless conidia supported by phialides, headed upward
- Aleurioconidia structure produced
- Intrinsic resistance to Amphotericin B

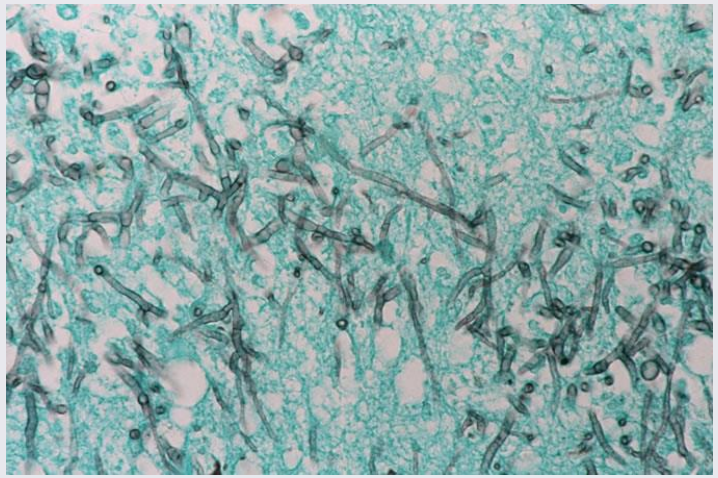


# Aspergillus – fruiting head seldom seen in fixed tissue

usually dichotomous (continuous) branching septate hyphae which branch at a 45* angle

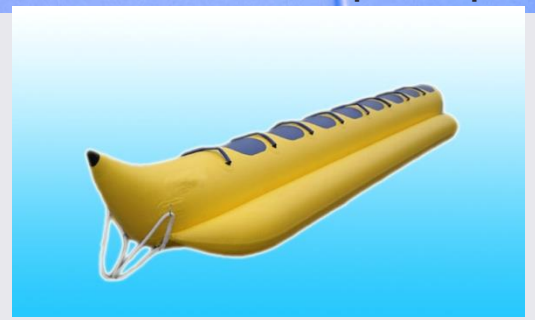


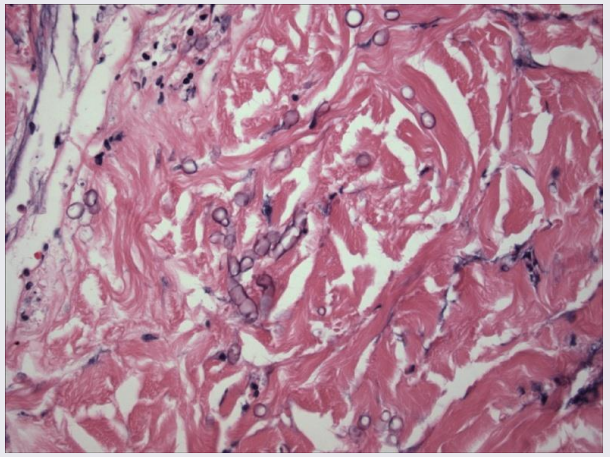
Can appear much like that of Scedosporium species, Growth in culture can differentiate these two fungi.

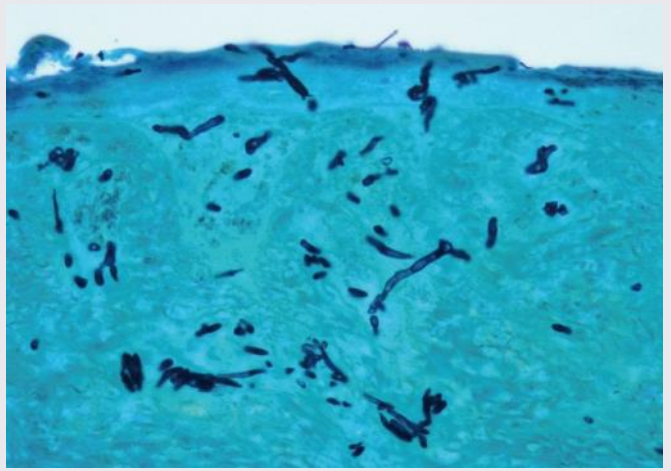
Banana boat shaped spore



# *Fusarium* species

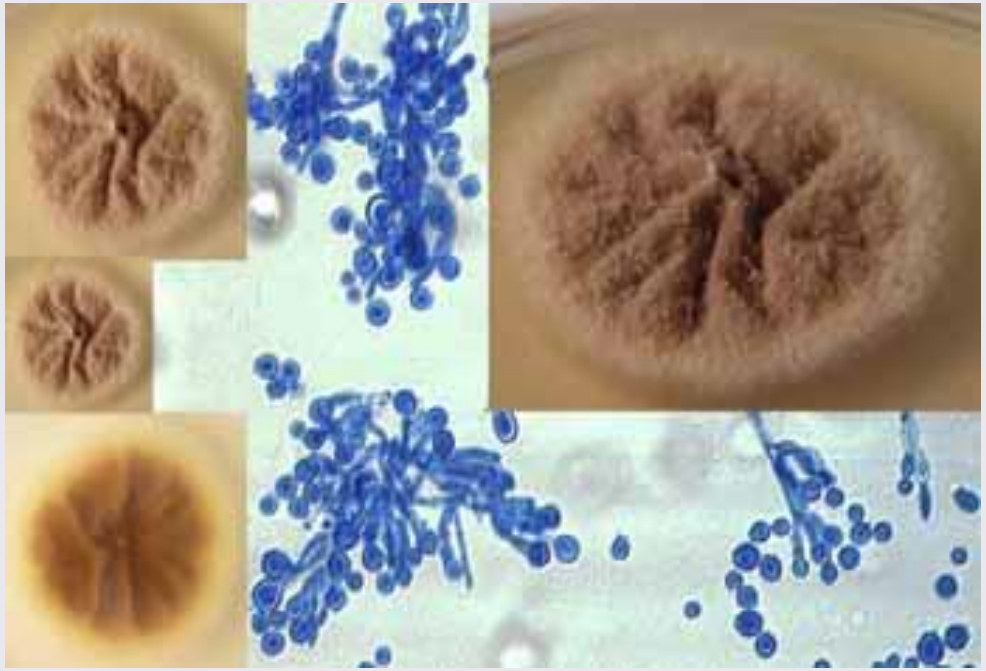
- Common in nature/plants
- Fuchsia colored colony grows in 3-5 days 30*C
- Disease related to immune status of host/ neutropenia/ severe immune suppression
- Infections reported:
- Disseminated disease in bone marrow transplants and corneal infections in contact lens wearers
- Random hyphae in fixed tissue

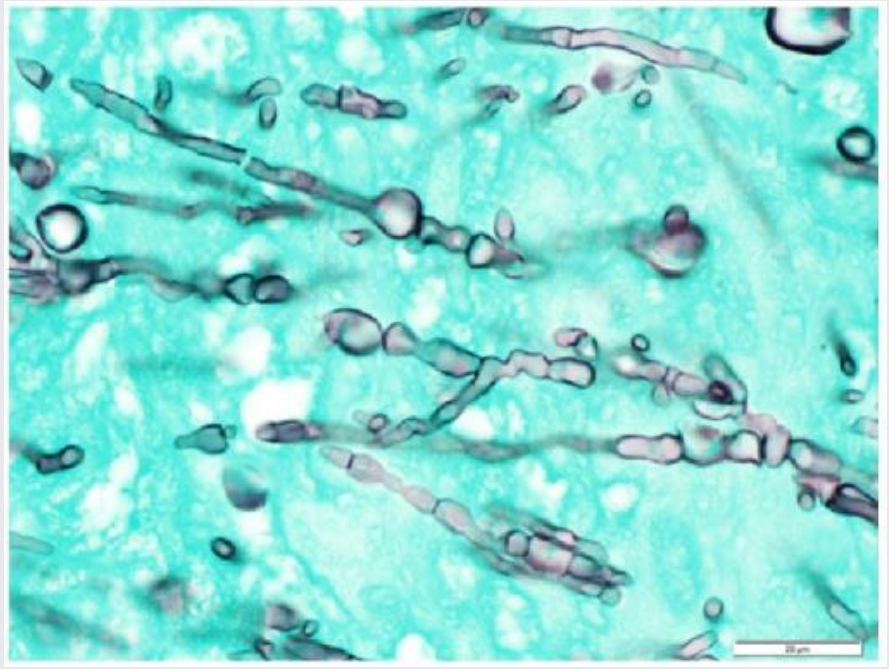




# *Scopulariopsis* species

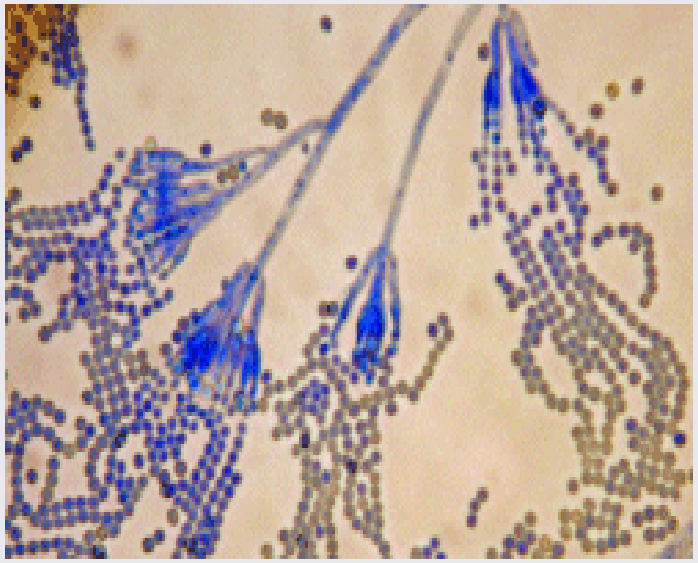
- Found in soil and grows on plant
- Infections:  Nail, skin, sinusitis, pulmonary and may disseminate in an immune suppressed host
- Sandy colored colony growing in 3-5 days at 30*C
- Very resistant to antifungal agents





# *Penicillium* species –

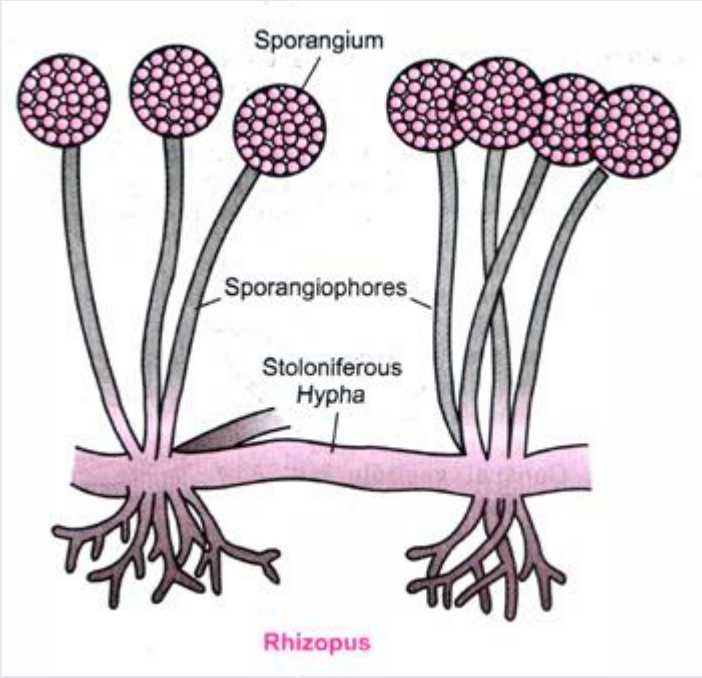
- One of the most common molds in the environment
- Common cause of bread mold
- Uncommon cause of human disease
- Can appear as a culture contaminate
- Blue/green colony grows in 3-5 days 30*C
- Branching hyphae with conidia production
- Appears like a bony hand



# MUCORMYCOSIS/ ZYGOMYCOSIS

Fungi in the Order Mucorales

- Hyaline
- Broad hyphae without septation
- Sporangium (sack) with spores



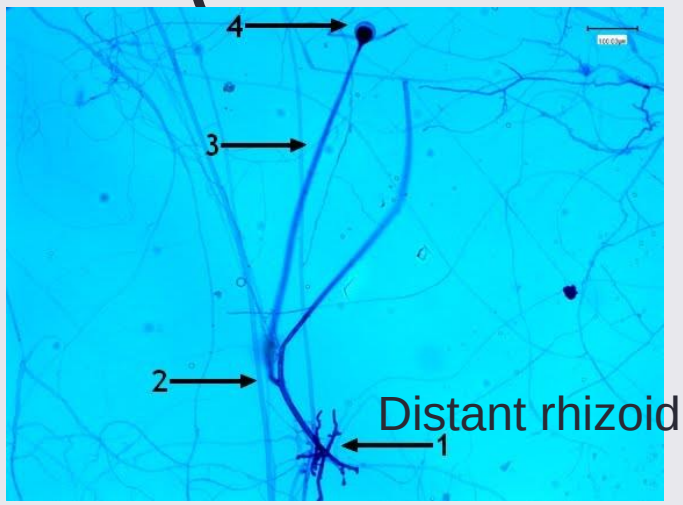
# Mucormycosis/Zygomycosis

- Found in soil, rotten fruit and vegetables
- Rhinocerebral mucormycosis - classic infection
  - Diabetics, the elevated glucose enriches fungal growth
  - Infection begins in nasal sinus - spreads to orbit of eye – then brain, high fatality rate
- Broad, hyaline, hyphae without septation produced
- Culture grows within 24-48 hrs, producing coarse aerial hyphae
- Mince do not grind infected tissue for culture, the grinding will kill the aseptate hyphae and they will not grow when cultured

# Most common Mucorales

## *Rhizopus species*

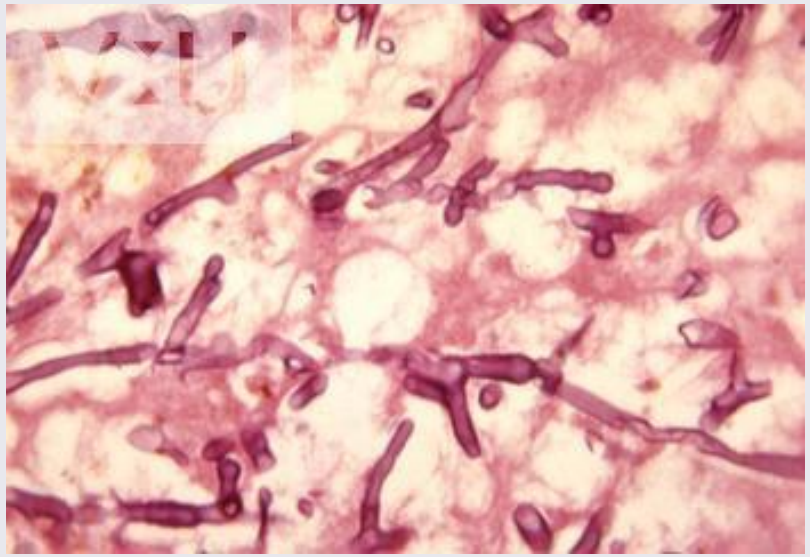

## *Absidia (Lichtheimia* complex)

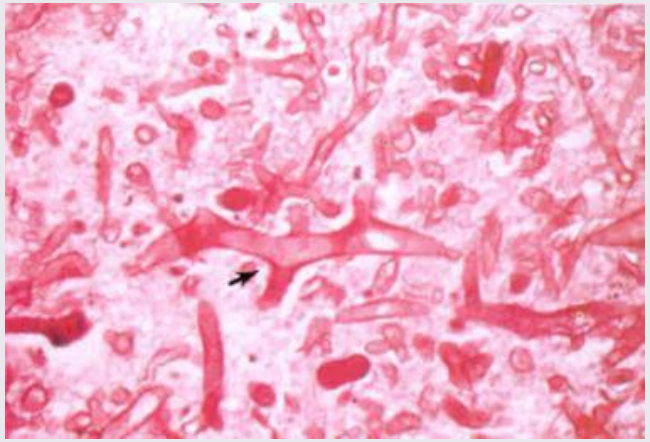



## *Mucor species*


# Zygomycete Fungi in Tissue

Hyaline, rare to no septations, ribbon like hyphae, 90* angle branching





**Invades vessels and can cause infarcts and thrombi**