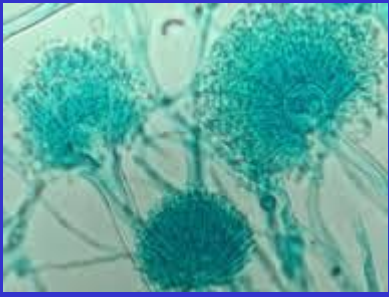




Medically important fungi





- Approximately 400 medically important fungi (yeast and moulds).
- Approximately 50 species cause more than 90% of the fungal infections in humans.
- **Mycoses** are fungal infections and their origin may be endogenous or exogenous.



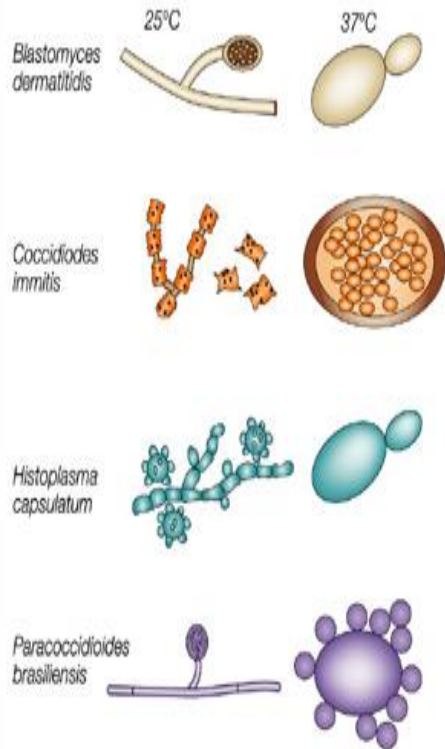
Mycoses

Clasification by their most common portal of entry and initial site of involvement:

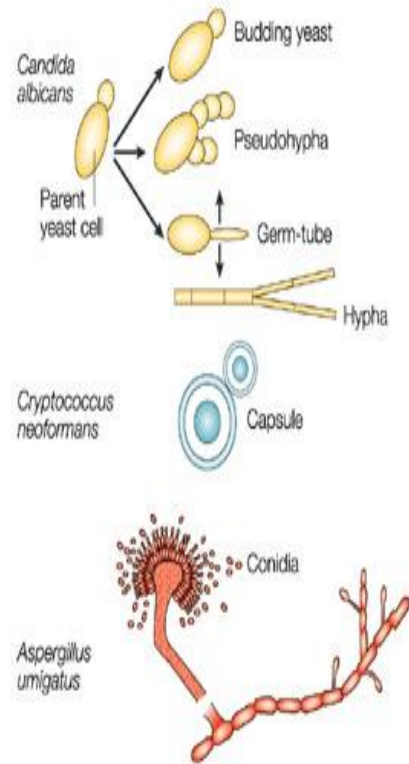
1. Superficial
2. Cutaneous
3. Subcutaneous
4. Systemic (endemic or opportunistic)

Systemic mycosis

Dimorphic fungi



Opportunistic fungi



Nature Reviews | Immunology

1. Endemic (primary) caused by dimorphic fungi.
2. Opportunistic caused by opportunistic fungi.

Taxonomy

Earlier classification was based on phenotypic data, while today is modified by molecular systematics.

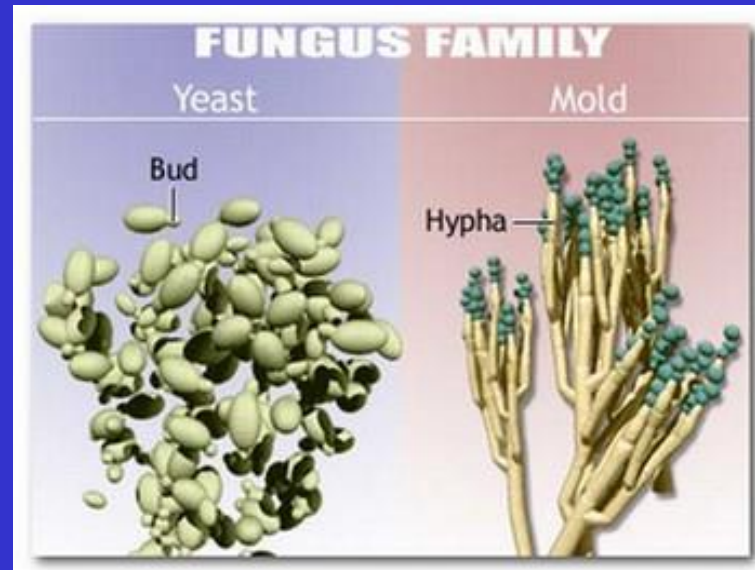
Most of the pathogenic fungi are members of the following phyla:

1. Phylum Ascomycota (*Candida*, *Trichophyton*)
2. Phylum Basidiomycota (*Cryptococcus*)
3. Phylum Glomerulomycota (*Mucor*)

1. Phylum *Ascomycota* (Ascomycetes)

- Sexual reproduction, producing ascospores
- Asexual reproduction, via conidia
- Two hundred sixty families
- Includes yeasts (*Candida*, *Saccharomyces*) and molds (*Coccidioides*, *Trichophyton*, *Blastomyces*)
- Some of them are opportunists and others are primary pathogens

Yeasts versus Molds

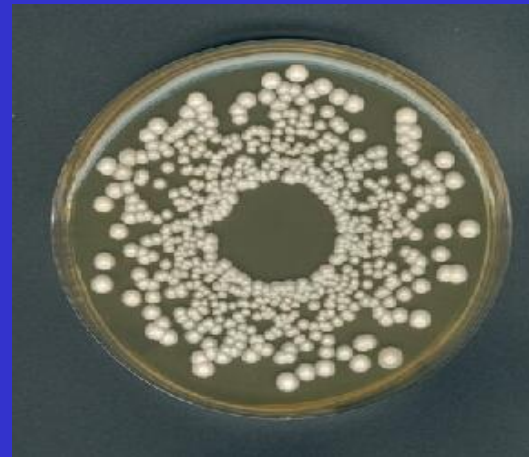
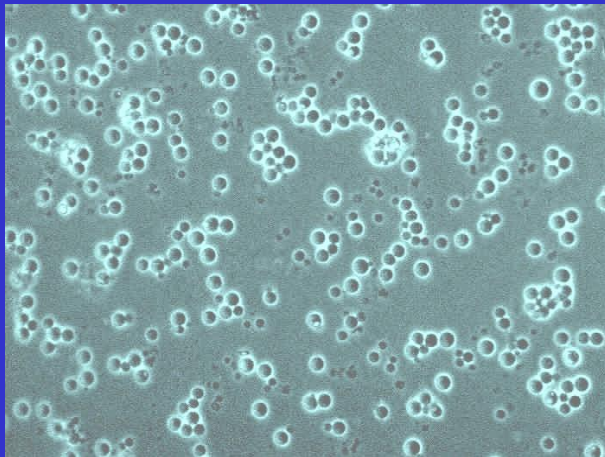


Yeasts are single cells.

Molds (Moulds) are multicellular fungi.

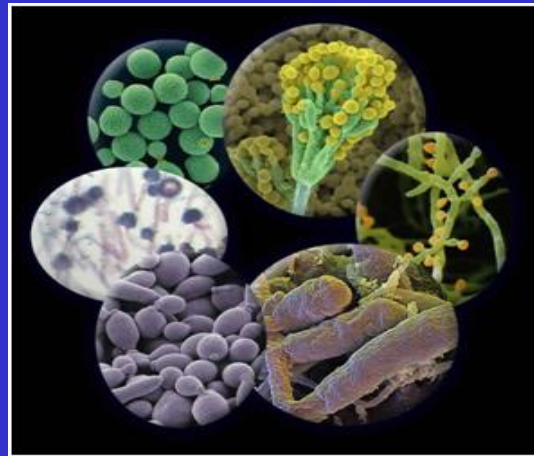
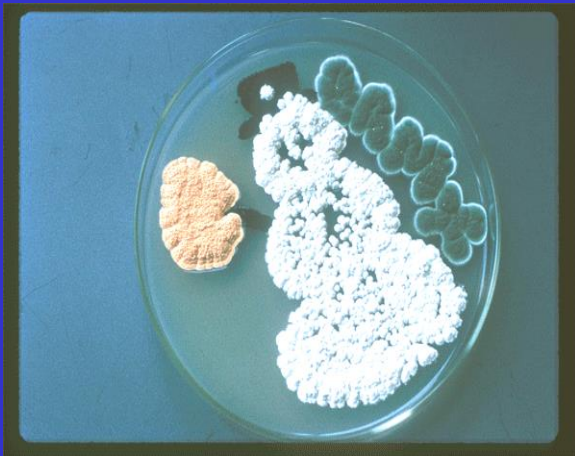
Yeasts

- Cells are spherical to ellipsoid, from 3 to 15 μm in diameter
- Reproduce by **budding**
- Elongated buds that fail to detach are called **pseudohyphae**.
- Some species (*Candida albicans*) can produce hyphae
- Colonies are soft, cream-colored and quite similar among different species on selective media (SDA).



Molds (Moulds)

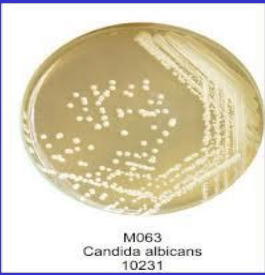
- Cells are branching cylindric tubules, from 2 to 10 μm in diameter, called **hyphae**
- Hyphae accumulate during growth and form **mycelium**. There are two forms of mycelium: vegetative and aerial.
- Vegetative hyphae absorb nutrients from supporting medium
- Aerial hyphae bear the reproductive structures.
- Genus and species identification is determined by macroscopic appearance on selective media and microscopic morphology





Genus *Candida*

- Part of a normal microbiota
- Endogenous opportunistic yeasts
- The most common are *C. albicans*,
C. parapsilosis, *C. tropicalis*, *C.*
glabrata, *C. guilliermondii*, *C.*
dubliniensis, *C. krusei* and *C.*
lusitaniae.

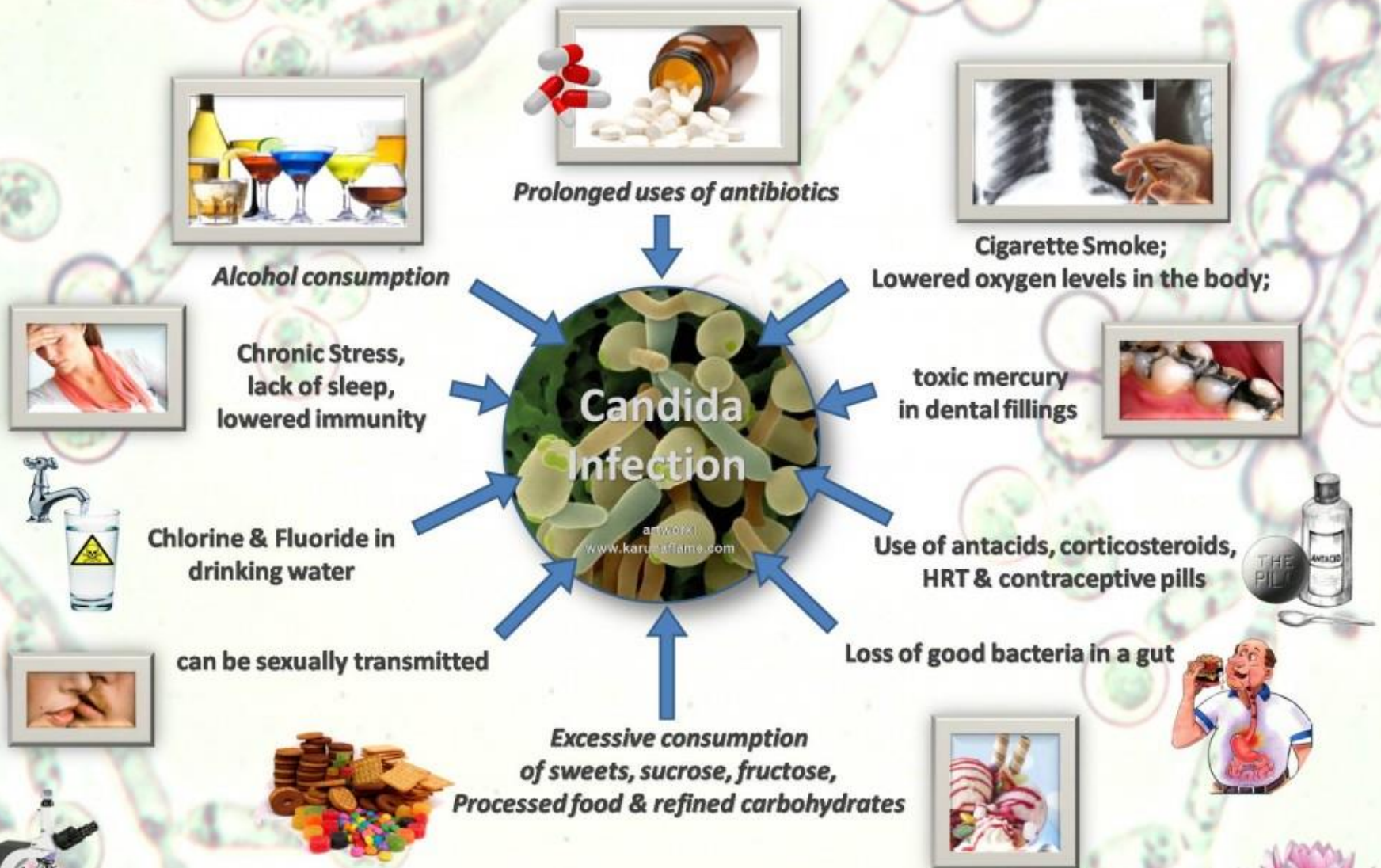


Candida albicans

Member of the normal flora
(gastrointestinal tract, skin and
mucous membranes)

Risk faktors for colonization and
infection: (patients with compromised
host defenses, previous antibiotic
treatment or immunosuppressive
therapy, pregnancy, diabetes,
leukemia, AIDS)

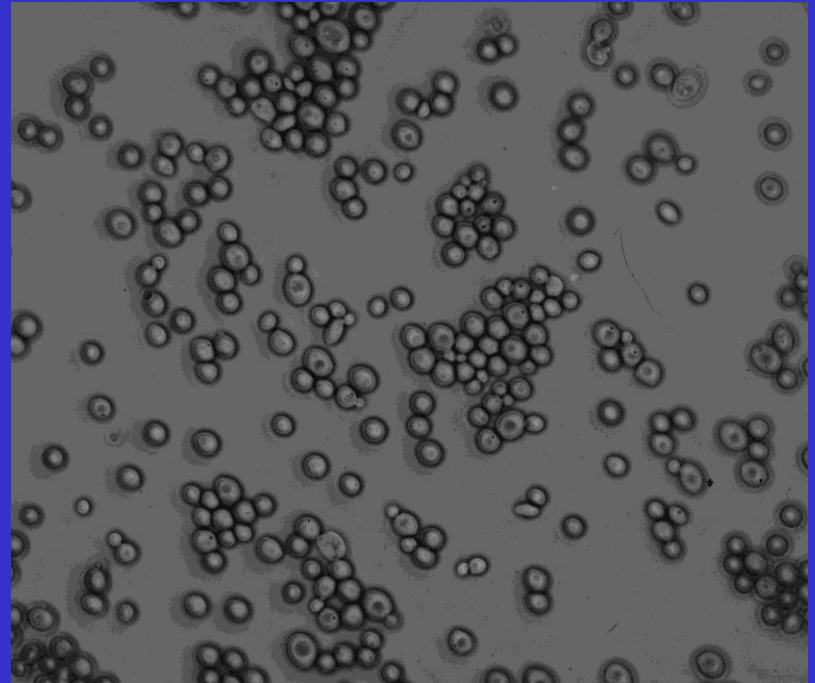
What Causes Candida / Yeast Infection ?



Morphology

- *Candida* species grows as oval, budding yeast cells, in tissue or culture.

Figure. Blastoconidia, 400x



Morphology



Buds continue to grow ,
producing chains of
elongated cells that are
constricted at the
septations between
cells, but fail to detach.
This forms are called
pseudohyphae.

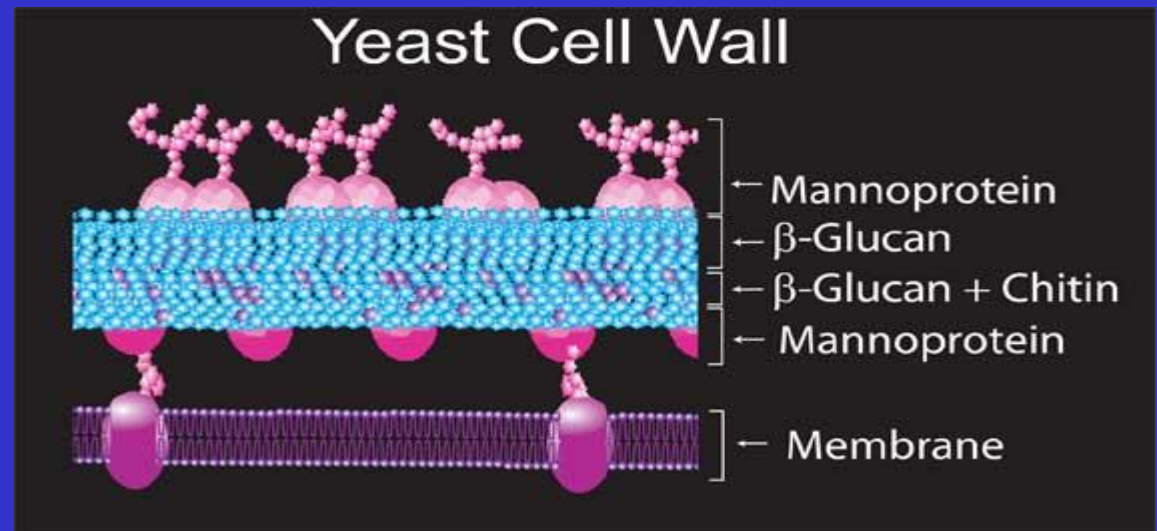
Figure. Budding yeast cells, pseudohyphae and hyphae.
400x



Antigenic Structure

There are two serotypes of *C. albicans*, A and B.

Cell wall components (mannans, glucans) and enzymes often elicit production of antibodies.

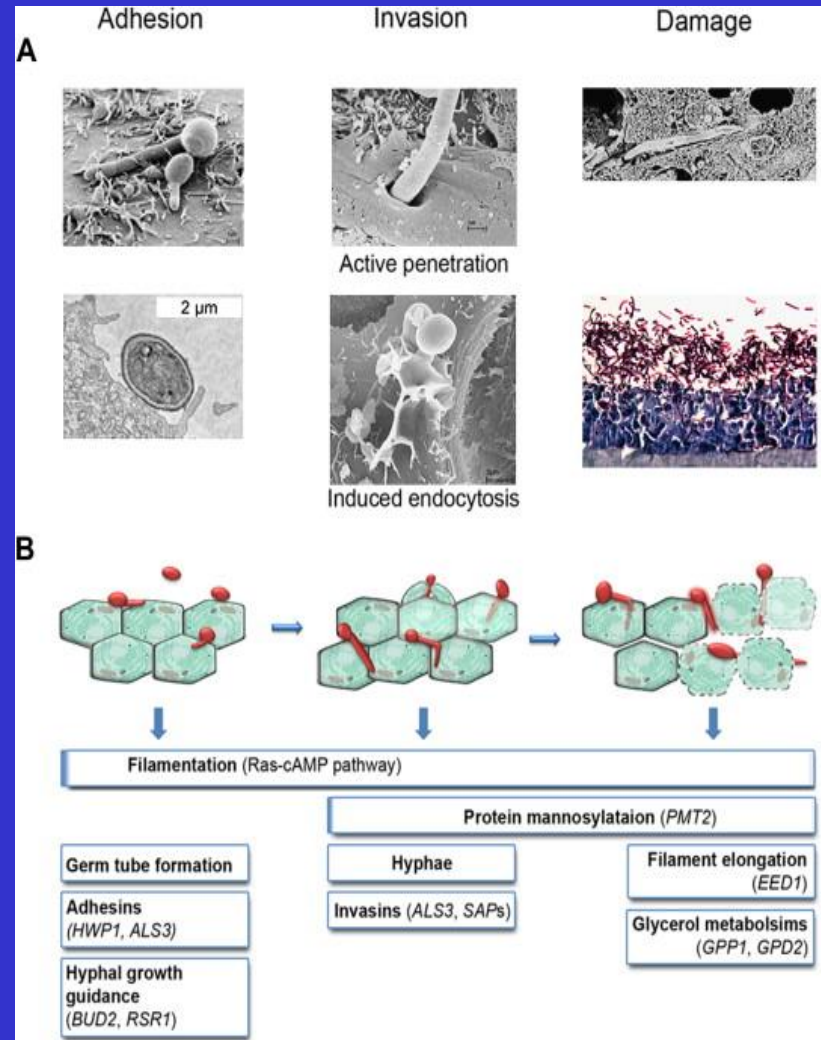
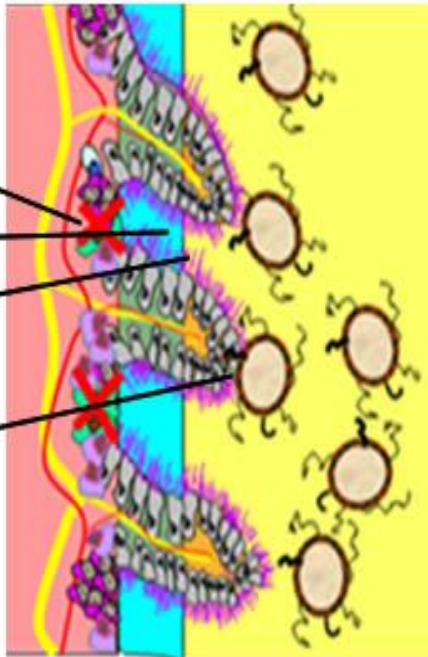


Pathogenesis

Three distinct stages: ADHESION - INVASION - TISSUE DAMAGE

Illustration of early Candida Invasion

1. Mucus producing cells are downregulated by NSAIDS.
2. Mucus layer is reduced.
3. Antibiotics have stripped the intestine of beneficial bacteria.
4. Intestinal absorption cells become vulnerable to yeast attachment and subsequent invasion.



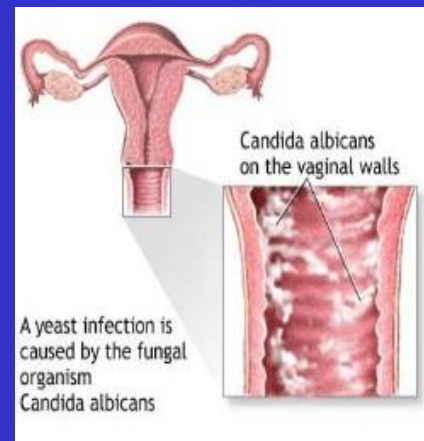
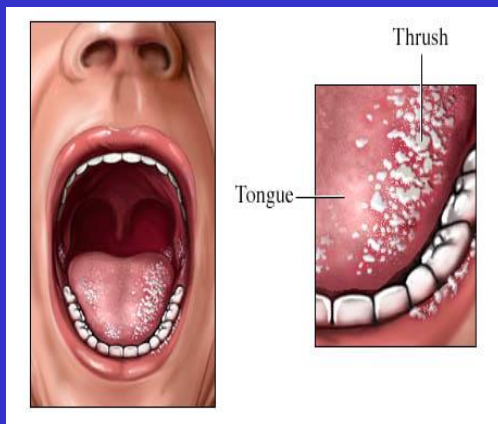
Clinical findings

I. Cutaneous and Mucosal Candidiasis

- thrush (whitish pseudomembranous lesions)
- vulvovaginitis (pruritus, discharge)
- cutaneous candidiasis (red moist skin, vesicles)
- onychomycosis (painful, erythematous swelling)

II. Chronic Mucocutaneous Candidiasis

- chronic superficial disfiguring in immunocompromised



Clinical findings

III. Systemic Candidiasis (Candidemia)

- transient in in patients with normal host defenses (after damage of the skin or gastrointestinal tract, aspiration, surgery)
- patients with compromised innate phagocytic defenses: development of occult lesion anywhere (skin, eye, heart, meninges...)





Diagnostic Laboratory Tests

Specimens:

- swabs (mucosa), scrapings (skin)
- tissue biopsies and exudates
- blood
- removed intravenous catheters
- spinal fluid
- urine



I Direct examinations of specimens

1. Microscopic Examinations

- Gram-stained smears
- histopathologic slides
- skin or nail scrapings in a drop of 10% KOH or calcoflourwhite

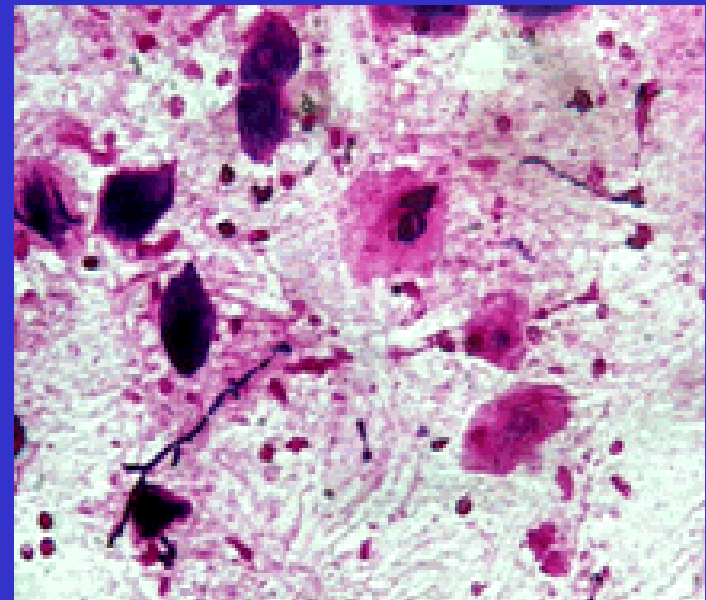


Figure. Blastoconidia, hyphae and pseudohyphae in tissue with Gram stain, 1000x.

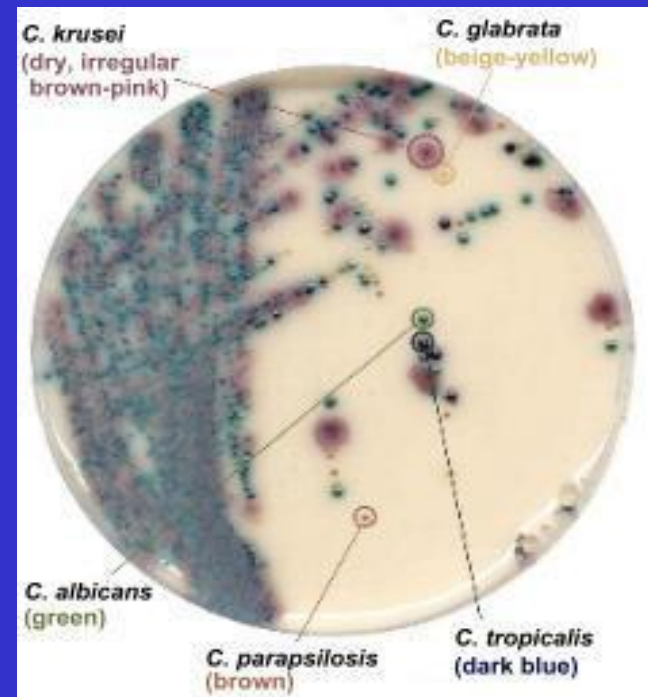
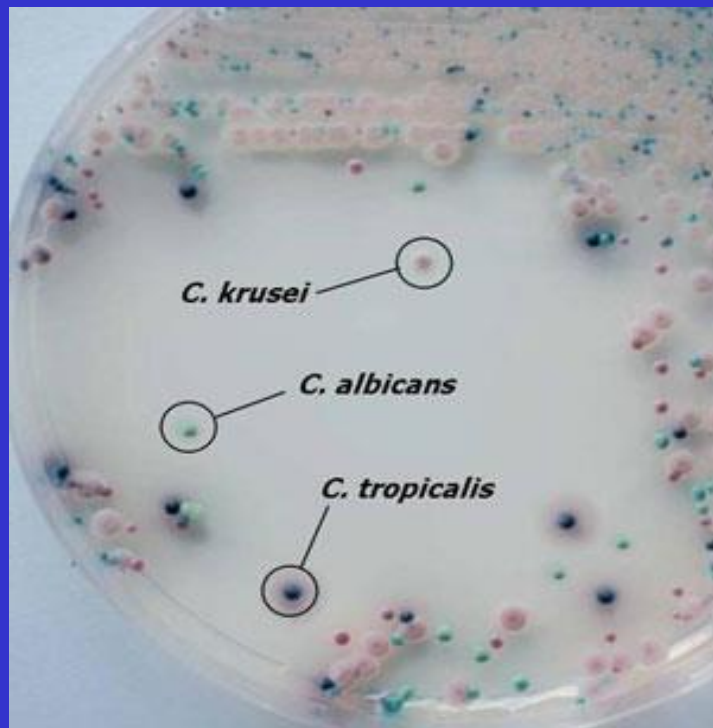
I Direct examinations of specimens

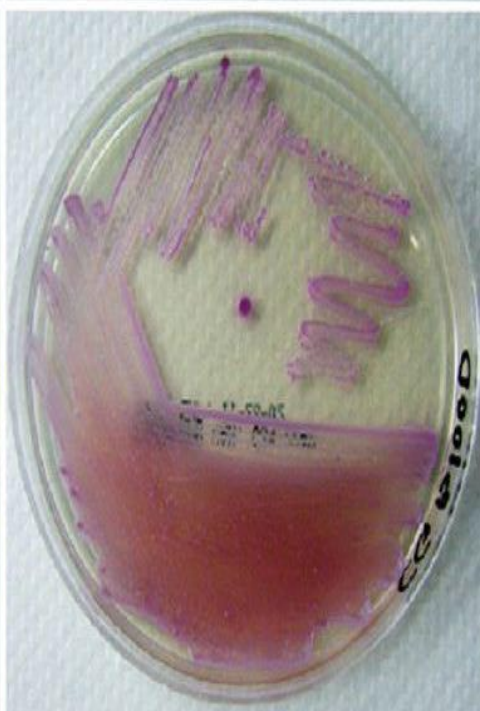
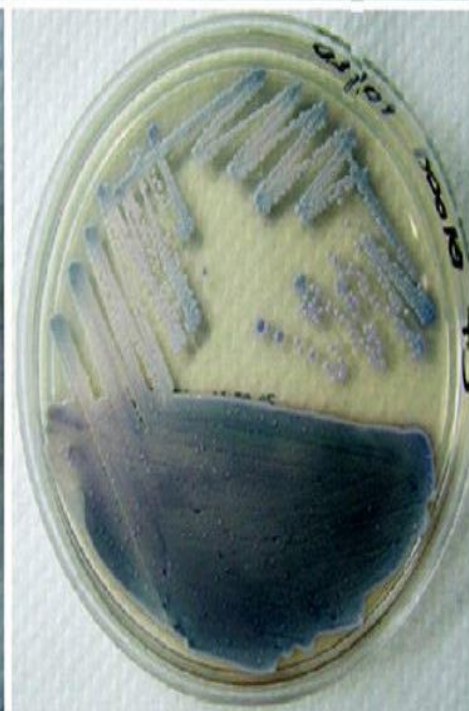
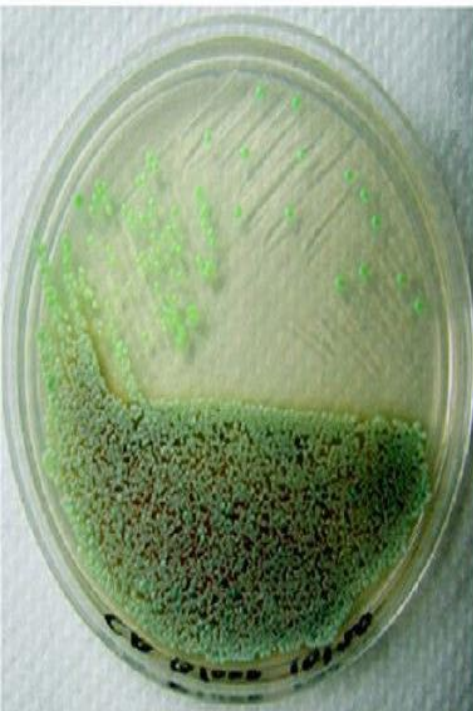
2. Culture and Identification

- grow well on bacteriologic and selective fungal media at room temperature and 37°C
- on Sabouraud's agar (fungal media) within 24 hours, all species grow as cream-colored, soft colonies with a yeasty odor



- on chromogenic media (chromagar), different species of *Candida* grow in different colours.



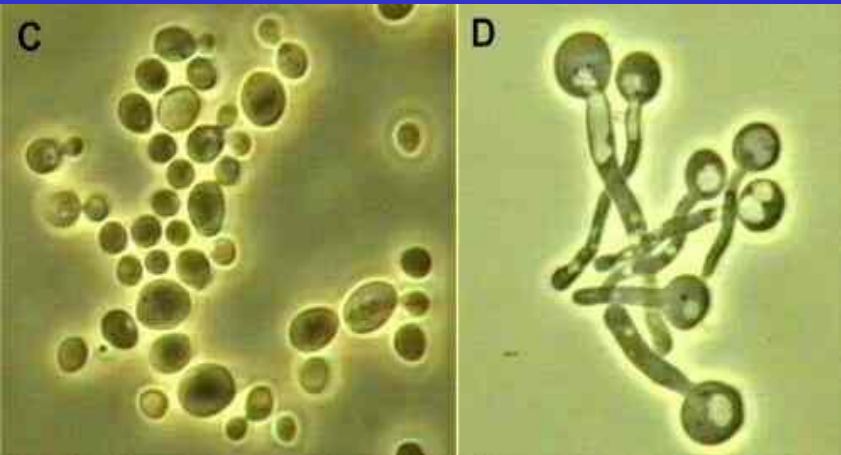
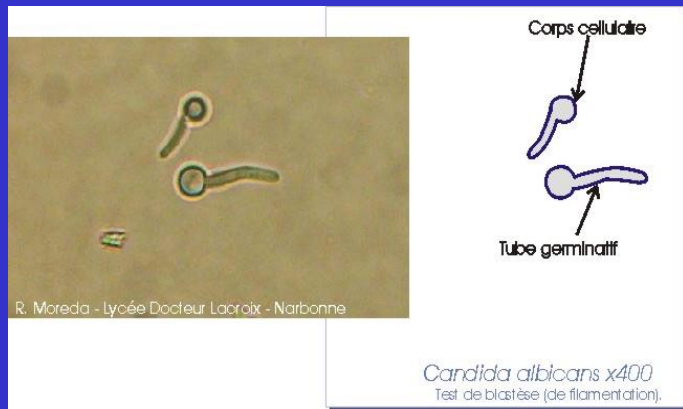


Identification

1. Germ tube test

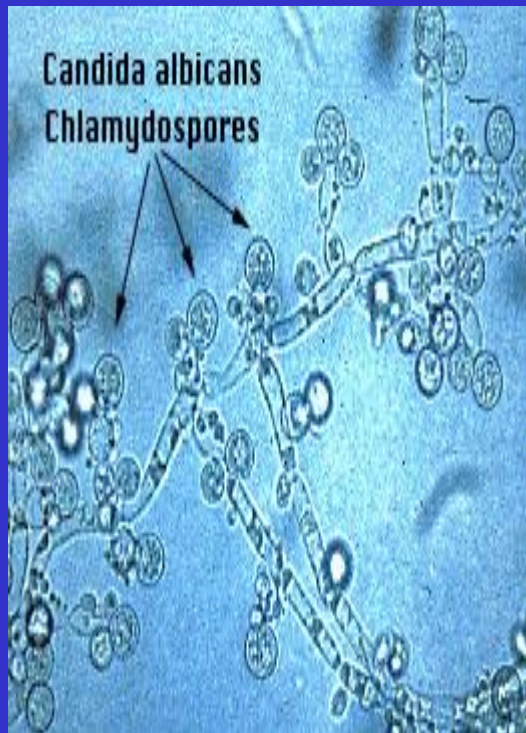
- distinguish *C. albicans* from other *Candida* species
- preparation: mix well yeast cells in serum, incubate at 37°C for about 90 minutes
- results:

C. albicans will form true hyphae or germ tubes, unlike all other species of *Candida*

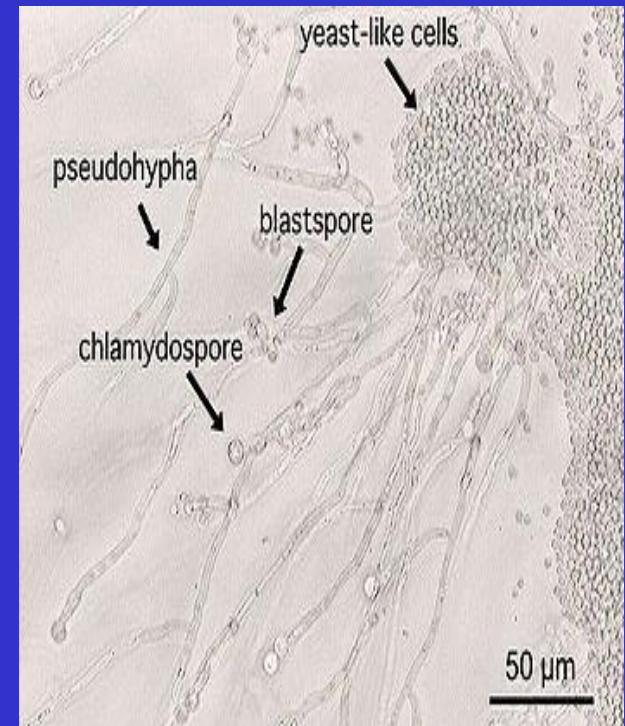


2. Grow on nutritionally deficient media

- *C. albicans* produces large, spherical chlamydospores

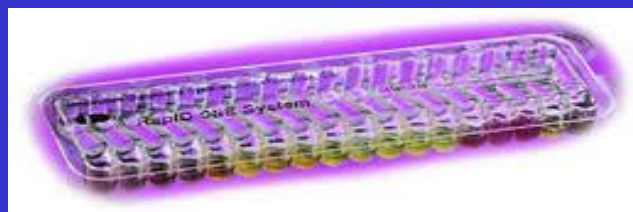


Terminal chlamydospores



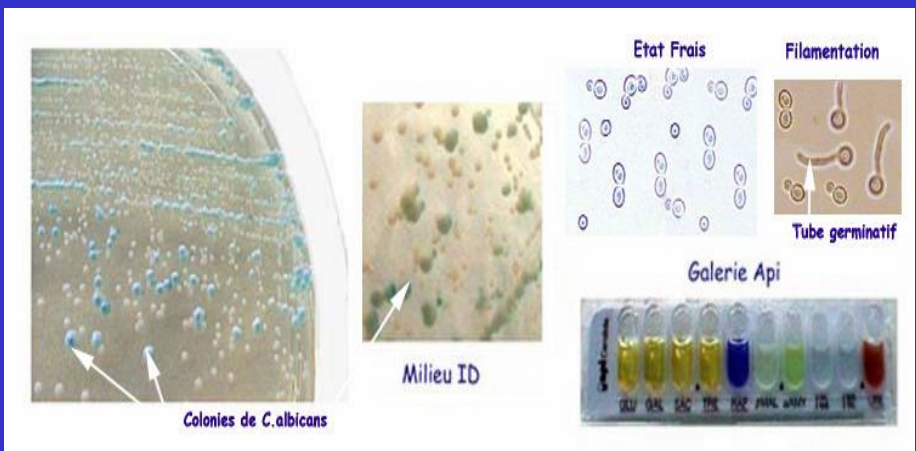
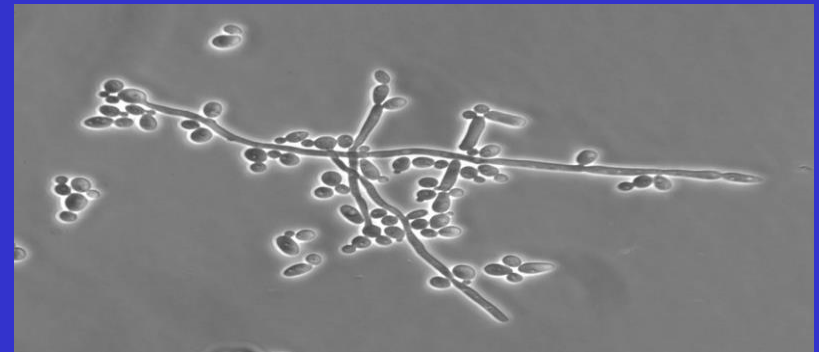
3. Series of biochemical tests

Sugar fermentation and assimilation tests for identification of different common species of *Candida* (each small compartment contains a dehydrated powder that can be inoculated from a culture, after incubation, the colorimetric changes can be scored and match to specific species).



Key concepts for identification of *Candida albicans*

1. Budding yeast cells, hyphae (!) and pseudohyphae
2. Germ tube test positive.
3. Colored colonies on chromagar (usually green), cream colonies on Sabouraud' agar, chlamydospores on nutritionaly deficient media.
4. Specific pattern of sugar fermentation.



II. Serology

1. Detection of circulating cell wall mannan

- LATEX AGLUTINATION

- ENZYME IMMUNOASSAY

Specific test but with low sensitivity.

2. Tests for circulating β -glucan

Not specific for *Candida*, because it is found in the cell walls of many fungal species.



Treatment

1. Mucocutaneous candidiasis

- Nystatin, topical
- Ketoconazole, oral
- Fluconazole, oral

2. Systemic candidiasis

- Amphotericin B
- Caspofungin
- Amphotericin B + flucytosine
- fluconazole





Aspergillus

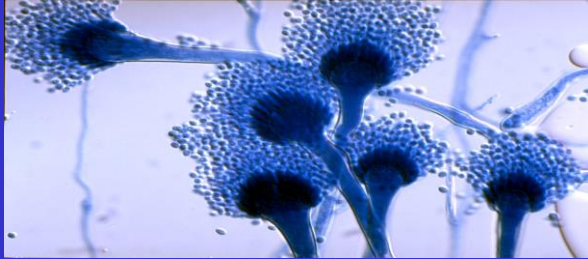


- Approximately 200 species
- Ubiquitous saprobes in nature
- Isn't part of a normal microbiota



Aspergillus

1. *A. fumigatus*, the most common as human pathogen



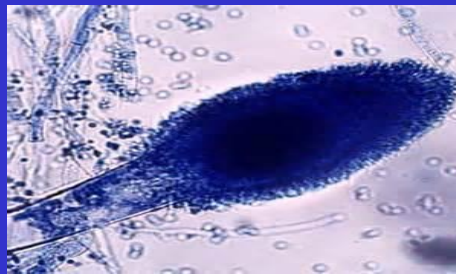
2. *A. flavus*



3. *A. niger*



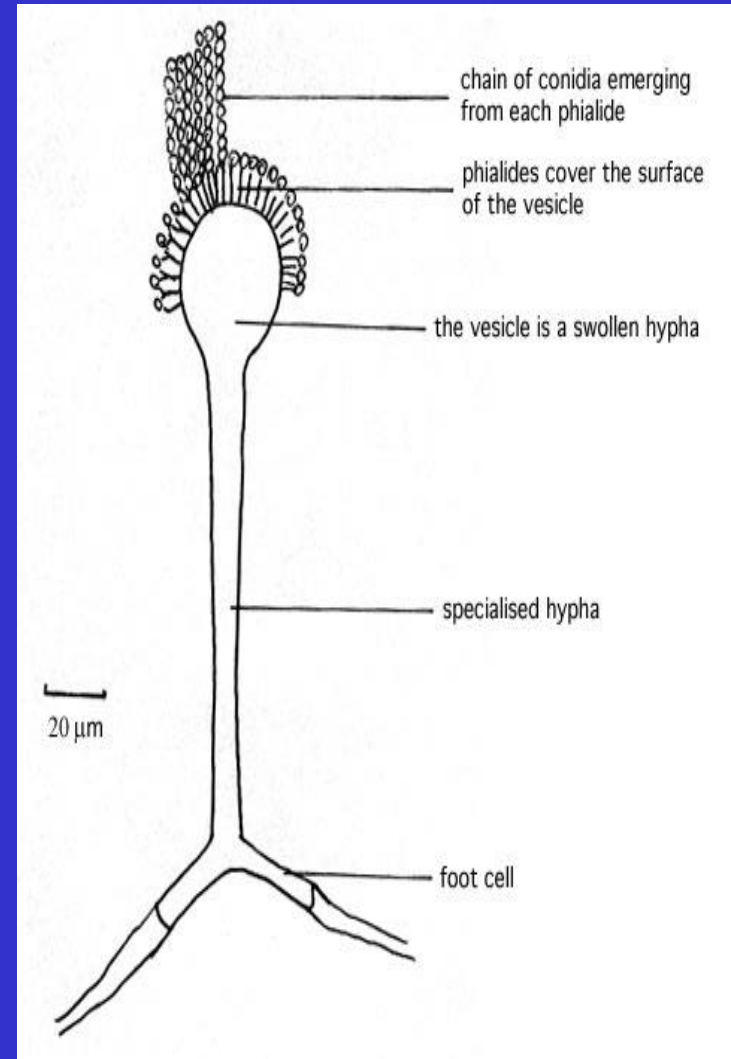
4. *A. clavatus*



Other: *A. terreus*, *A. nidulans*, *A. Lentulus*...

Morphology

- Long conidiophora with terminal vesicle
- Vesicle has phialides
- Phialides produce chains of conidia
- There are differences in these structures among species (very useful for identification of different species)



Aspergillosis

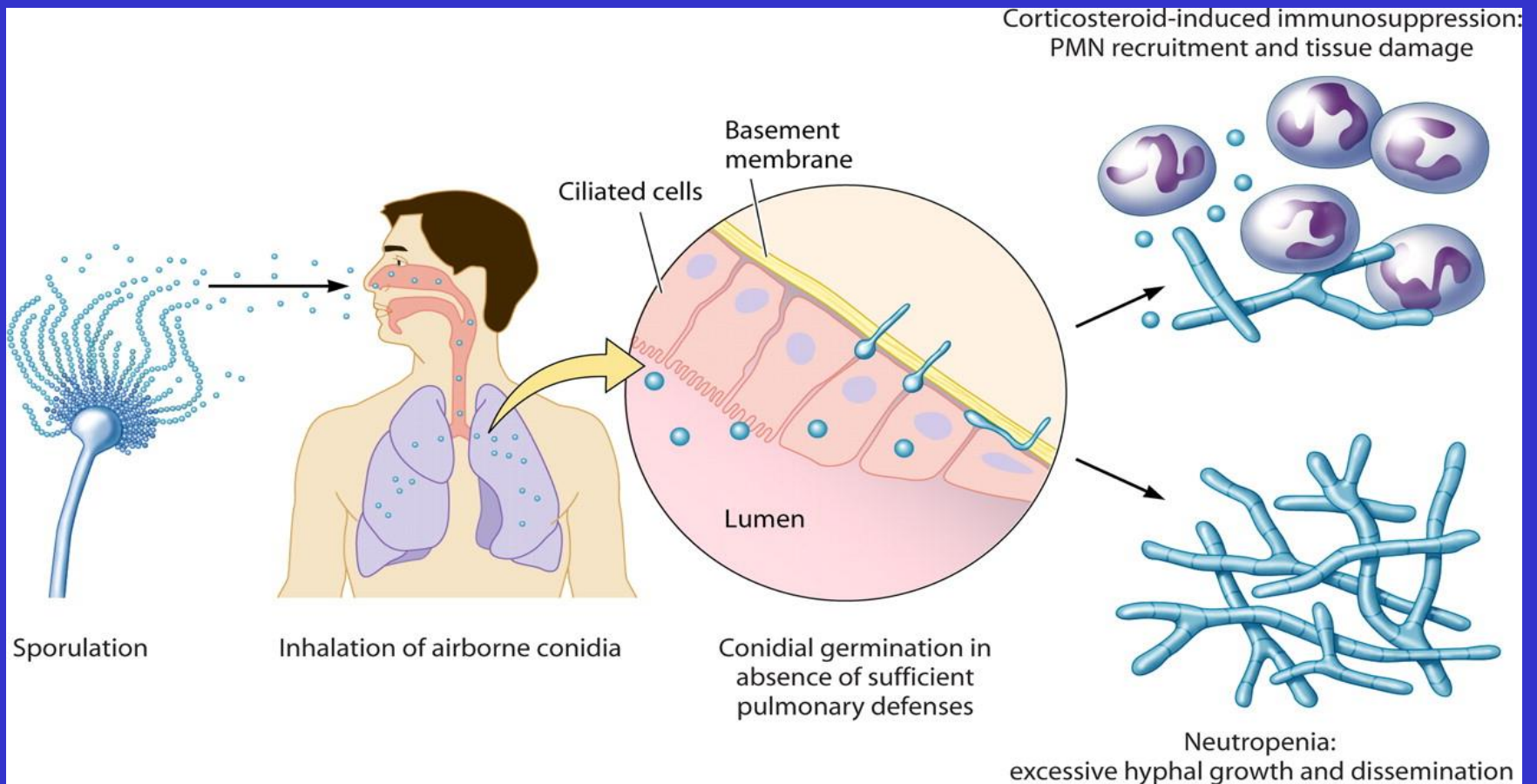
Spectrum of diseases caused by *Aspergillus* species.

Exogenous infection following inhalation of conidia.

Risk factors:

- atopic individuals
- immunocompromised patients
(corticosteroids, leukemia, stem cell transplant patients)
- preexisting lung cavities

Pathogenesis



Clinical Findings:

1. DISEASE IN NORMAL HOST

- toxicosis (mycotoxins)
- allergic manifestations
- superficial infections
- invasive infection

2. INFECTIONS ASSOCIATED WITH TISSUE DAMAGE

- Keratitis
- Burn wound
- Aspergilloma
- Prosthetic valve endocarditis

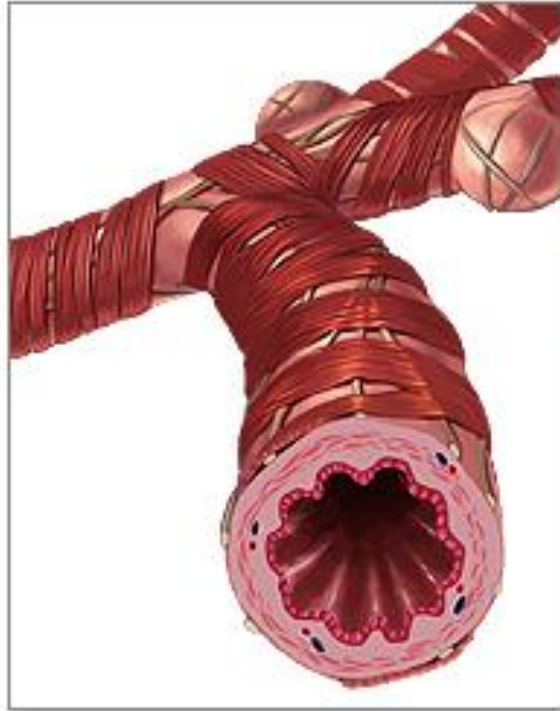
3. INFECTIONS IN THE COMPROMISED HOST

- pulmonary aspergillosis
- central nervous system aspergillosis
- invasive (disseminated) aspergillosis

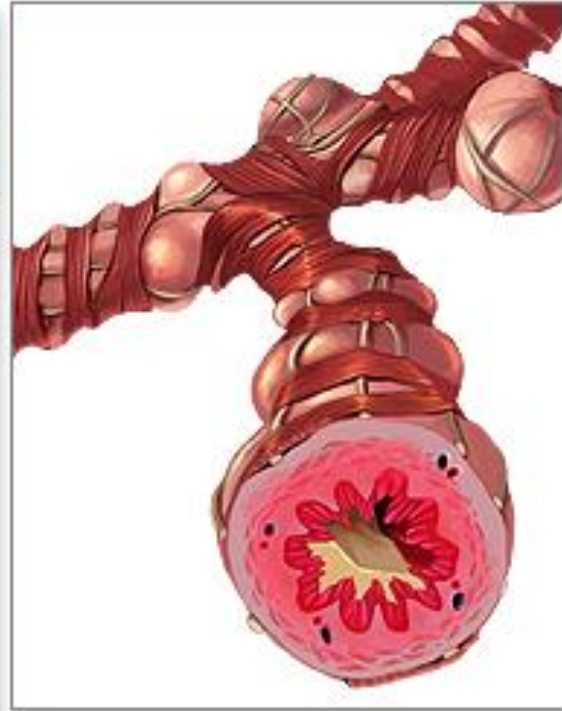
Clinical Findings:

1. Allergic Forms

Normal bronchiole

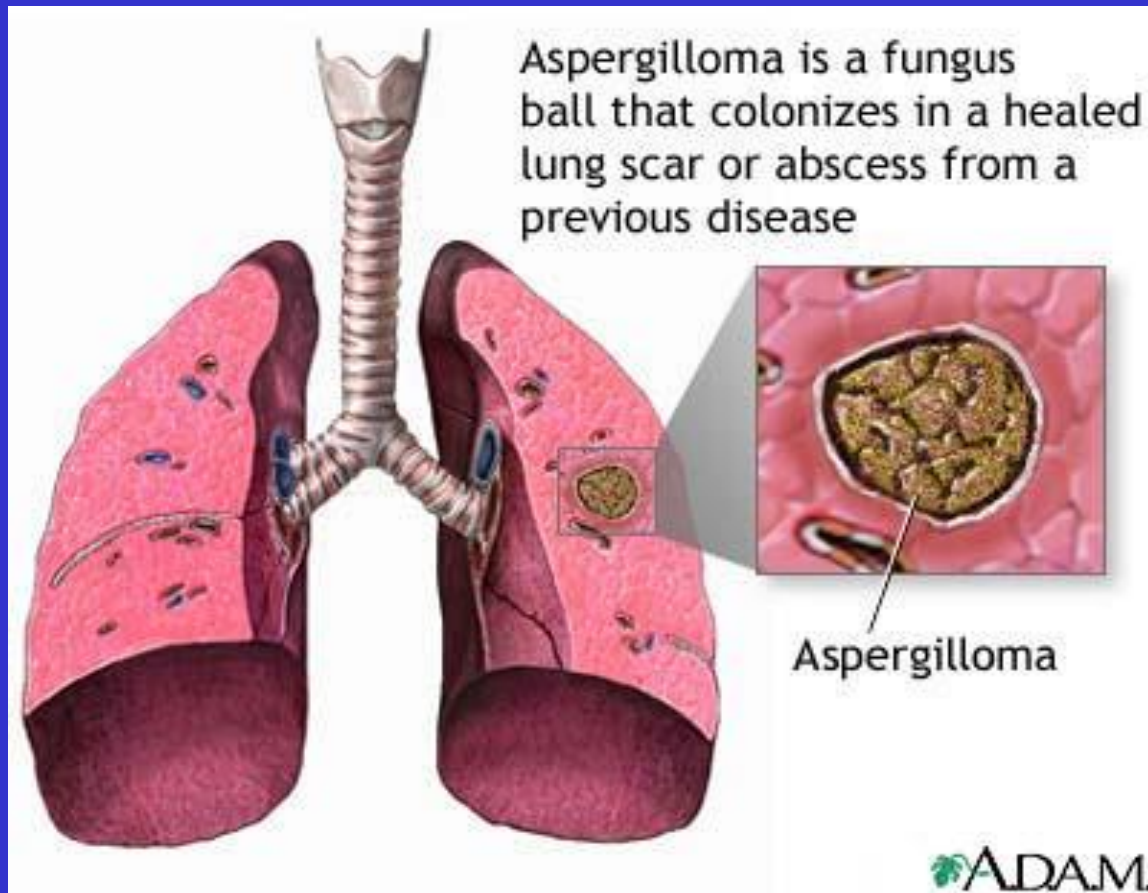


Asthmatic bronchiole



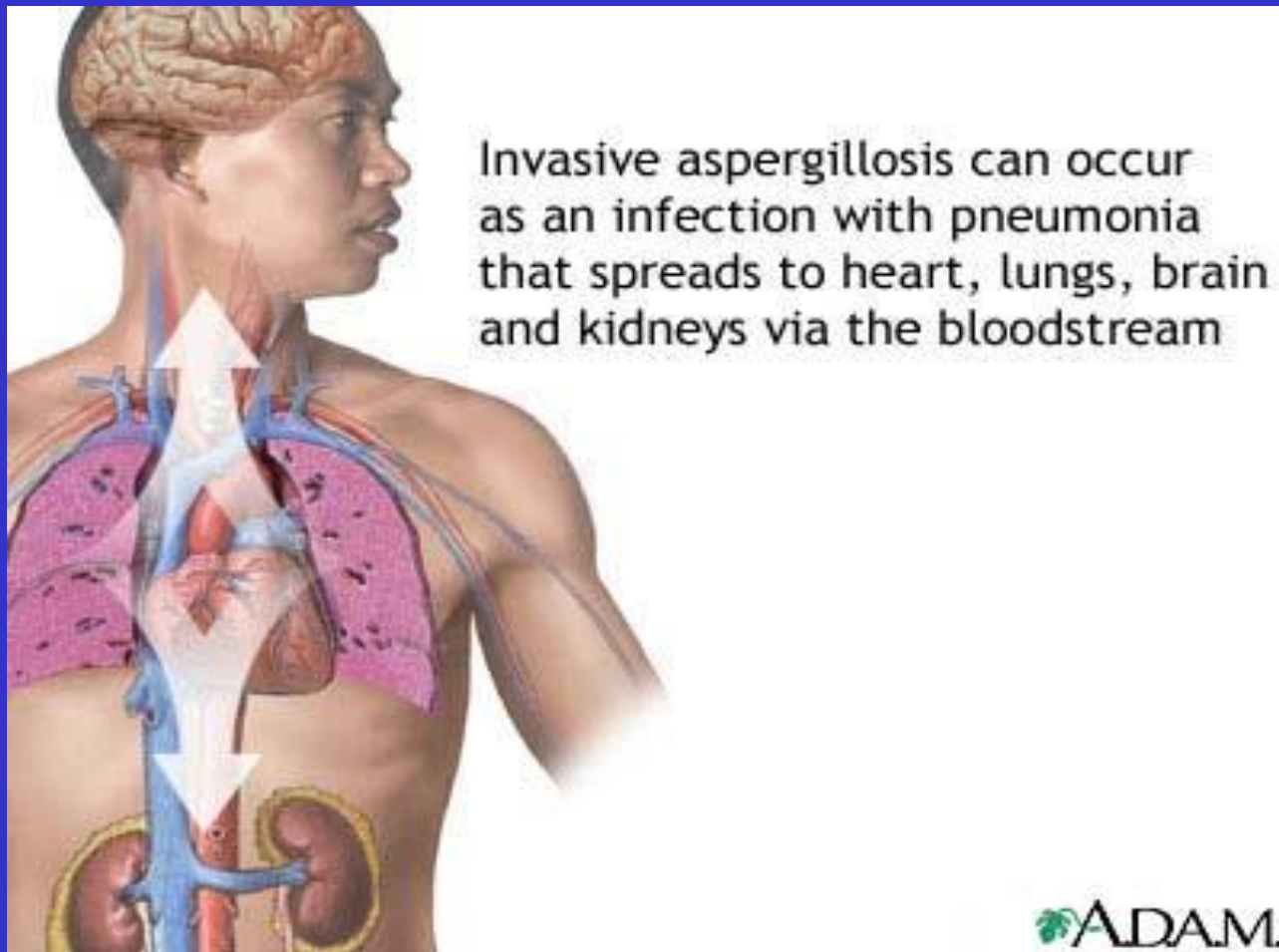
Clinical Findings:

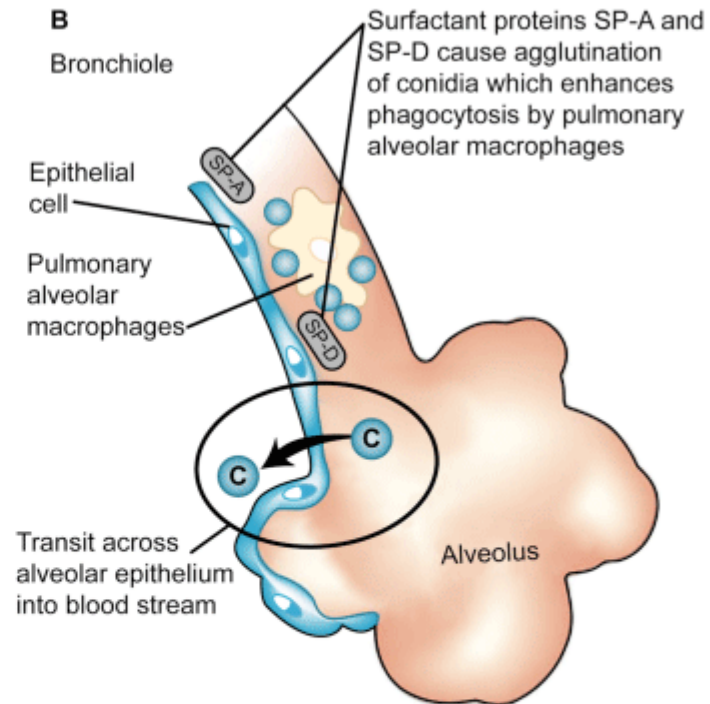
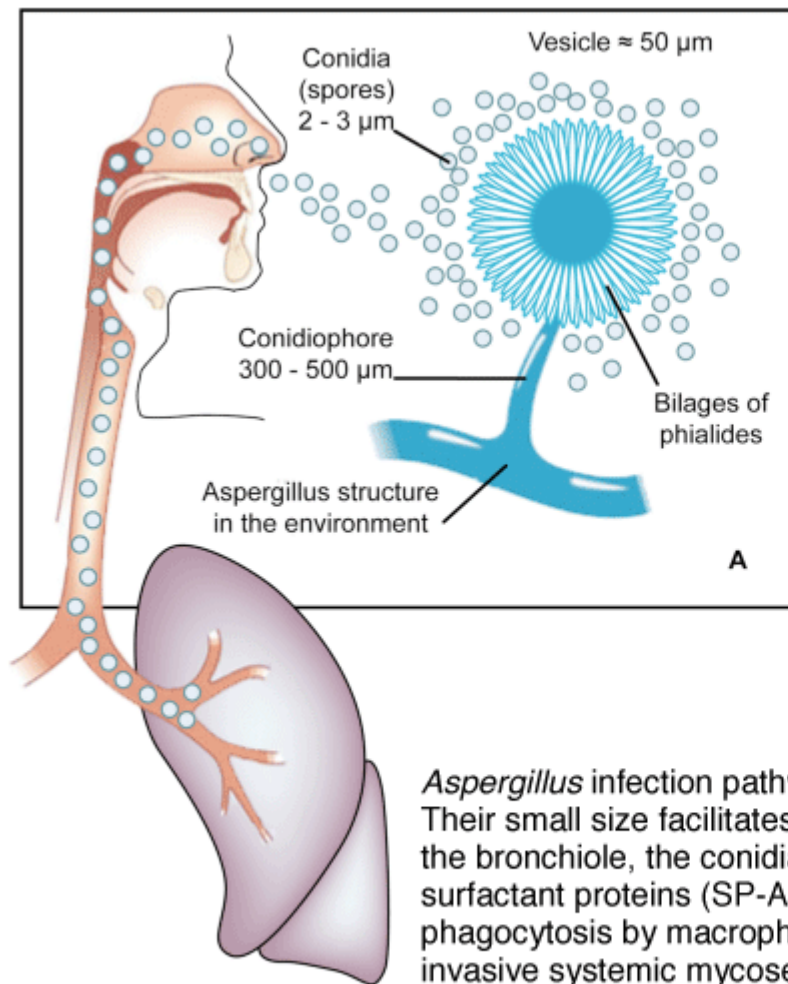
2. Aspergilloma



Clinical Findings:

3. Invasive Aspergillosis





Aspergillus infection pathway. (A) *Aspergillus* conidia are inhaled into the lung. Their small size facilitates their entry into the alveoli. (B) Once they have entered the bronchiole, the conidia must evade the first-line immunity presented by surfactant proteins (SP-A and SP-D), which trap conidia and promote their phagocytosis by macrophages. (C) *Aspergillus* lung infection can progress to invasive systemic mycoses by crossing the alveolar epithelium (adapted from Williams (2000)).

Diagnostic Laboratory Tests

Specimens:

- respiratory tract specimens (sputum, transtracheal aspirates, lung biopsy tissue)
- blood (very rare positive samples!)
- tissue biopsy (any organ, abscesses, necrotic lesions)

I Direct examinations of specimens

1. Microscopic Examinations

- sputum (direct examination with 10% KOH or calcofluor white)
- histopathologic slides

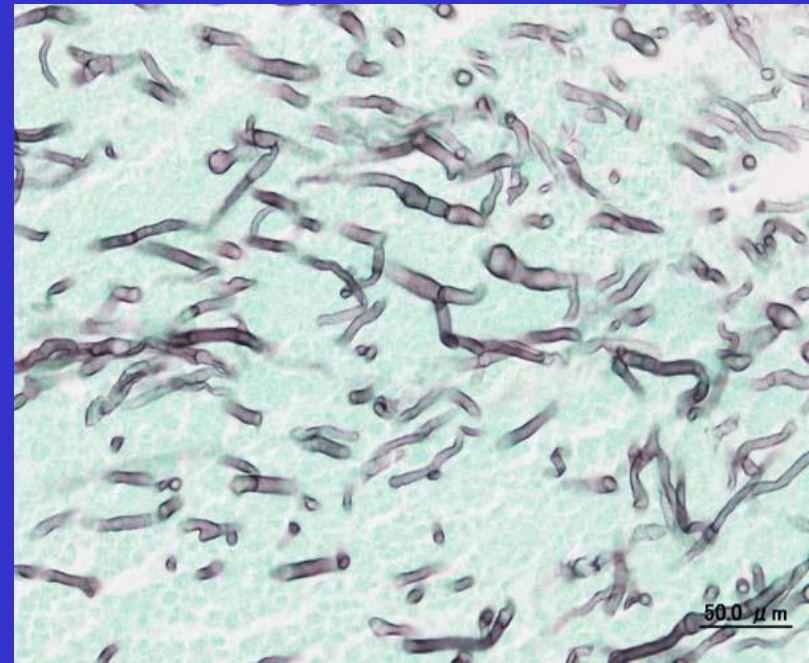
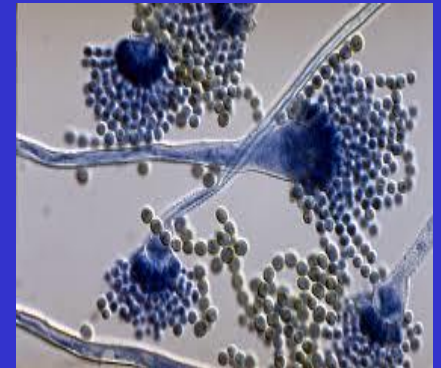


Figure. Branching septate hyphae in lung tissue stained with methenamine silver, 1000x.

I Direct examinations of specimens

2. Culture and Identification

- grow within a few days on most media (selective and nonselective) at room temperature
- species identification according to macro (grow on selective media) and micro morphology (microscopic examination of their conidial structures)



Contaminant or pathogen?

- Isolation of the same fungus from a repeat specimen
- Detection in the specimen of hyphal elements which are compatible with the morphology of the isolated mould

II. Serology

1. Detection of Antibody
 - Precipitins to a *A. fumigatus* (for aspergilloma and allergic forms, not for invasive aspergillosis)
2. Detection of Antigen (for early, accurate diagnosis of invasive infection, aspergillosis or candidiasis)
 - Circulating galactomannan
 - Detection of β -glucan

Treatment

Aspergilloma

1. itraconazole
2. amphotericin B

Invasive aspergillosis

1. amphotericin B
2. voriconazole
3. posaconazole

(for amphotericin B-resistant strains)

Pneumocystis species

Taxonomic problems have plagued the organisms since their original description in 1909 by Chagas, who thought them to be developmental stages of protozoan parasite *Trypanosoma cruzi*.

Pneumocystis species

In 1912, these organisms were provided an identity of their own;

- "pneumo" - reflected their predilection for the lung
- "cystis"- the characteristic morphological form

Pneumocystis jirovecii

Potential fungal nature was raised in the 1950s, and the controversy of the protozoan or fungal nature continued to the late 20th century.

In the late 1980s, phylogenetic analyses based on the nuclear small-subunit rRNA sequence alignments showed conclusively that *P. jirovecii* is a member of the fungal kingdom, phylum *Ascomycota*.

Natural habitats

P. jirovecii inhabit the human lungs (*P. carinii* is found in rats).

They can exist without consequence in mammalian hosts with intact immune systems (part of the normal flora?).

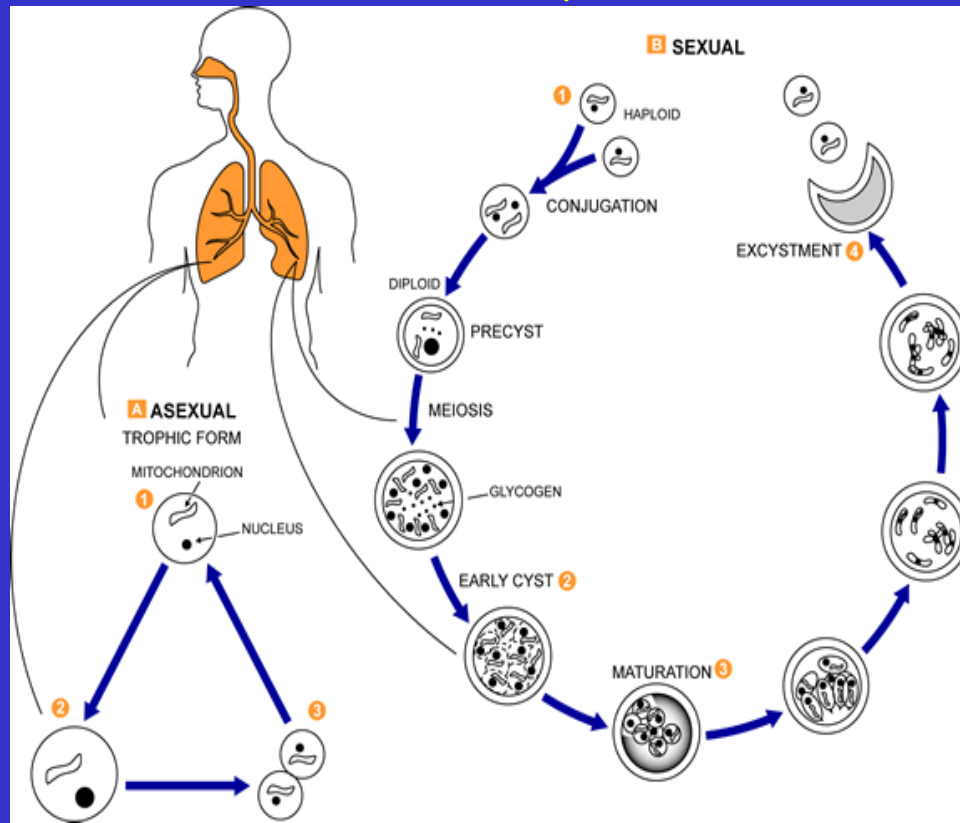
Once the immune system becomes compromised, the organisms proliferate within the lung alveoli and cause lethal pneumonia if the infection is left untreated.

Life cycle

Reproduce **extracellularly** within mammalian lung alveoli, on the surfactant layer above alveolar epithelium).

There are three developmental forms:

1. **TROPHIC STAGE** (the trophozoite)
2. **SPOROCTE** (the precyst)
3. **SPORE CASE** (the cyst)



Trophic forms reproduce asexually by **binary fission**.

They also participate in the **sexual** mode of reproduction.

Subsequent to conjugation, **sporogenesis** is initiated, resulting in formation of the **precyst**.

Following meiosis, an additional mitotic replication occurs.

The end product of sporogenesis is the **spherical cyst** with **eight spores**.

Clinical Findings and Diagnostic Laboratory Tests

1. Interstitial plasma cell pneumonitis

- in malnourished infants and immunosuppressed Patients

2. Interstitial pneumonitis without plasma cells

- in AIDS patients

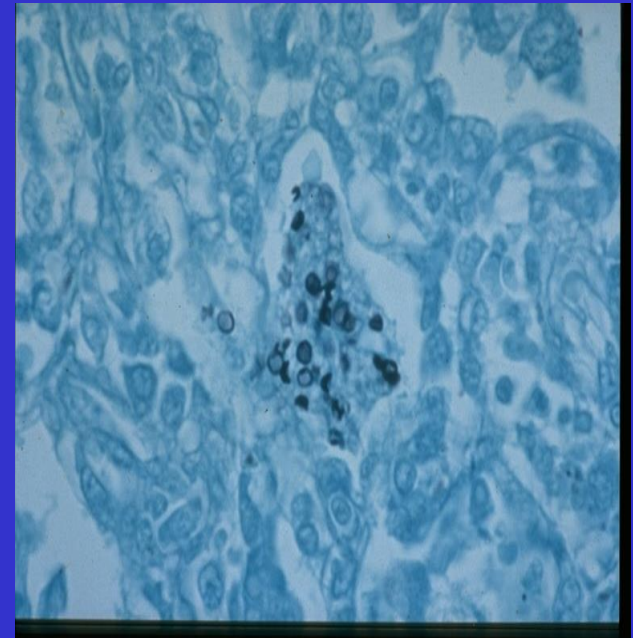
SPECIMENS:

- bronchoalveolar lavage (BAL), lung biopsy tissue, induced sputum

Diagnostic Laboratory Tests and Treatment

1. Microscopic Examination

- specimens are stained (Giemsa, methenamine silver, calcofluor white) and examined for the presence of cysts or trophozoites
- direct fluorescent examination with a specific monoclonal antibody



2. Serology

Only to establish the prevalence of infection.

Treatment: TMP-SMX or pentamidine

Dermatophytes

- Usual habitat is soil, animals or human (geophilic, zoophilic, anthropophilic).
- Cause cutaneous mycoses or dermatophytosis
- Traditionally terminology=Tinea (mistakenly described as ring-worm)
- Infect only the keratinized tissue; skin, hair and nails. Most are unable to grow at 37°C.
- Belong to three genera:
 - *Trichophyton*
 - *Microsporum*
 - *Epidermophyton*

Clinical Findings

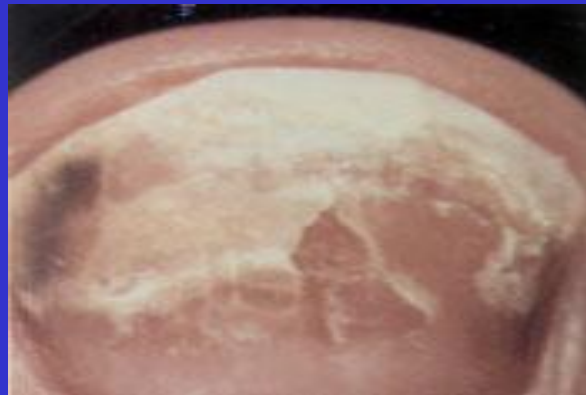
Tinea barbae



Tinea pedis



Tinea unguium



Morphology and Identification

1. *Trichophyton* species

- cylindric, smooth-walled macroconidia and characteristic microconidia
- infect skin, hair or nails

2. *Microsporum* species

- produce multicellular macroconidia with echinulate walls
- infect hair or skin

3. *Epidermophyton floccosum* - only pathogen in the genus!

- produces only macroconidia
- infects skin and nails

Diagnostic Laboratory Tests

Specimens:

- scrapings (skin, nail)
- hairs

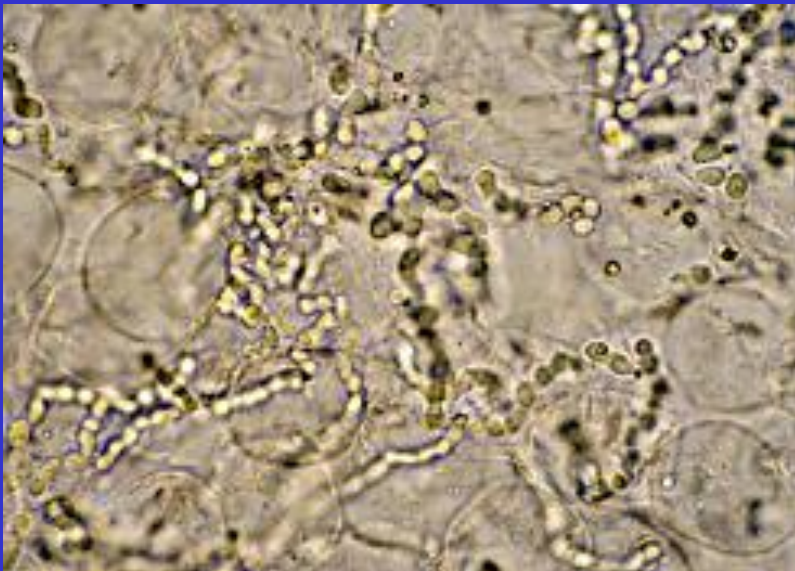
1. Microscopic Examination

2. Culture

Trichophyton



1. Ringworm lesion

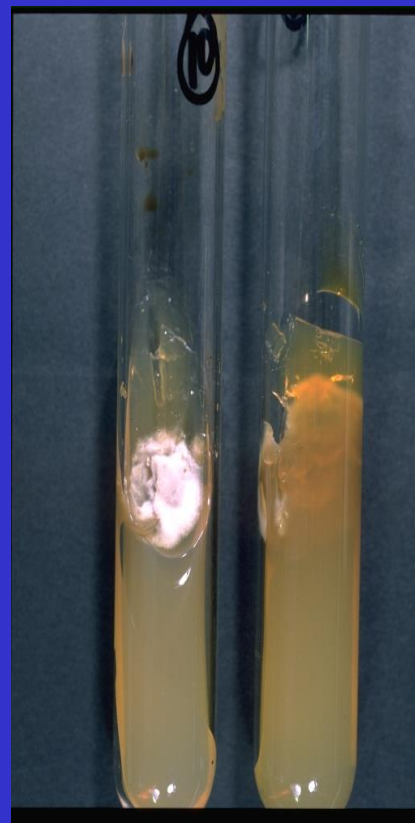


2. Microscopic
KOH preparation of
scrapings: hyaline
branching hyphae, 100x

T. mentagrophytes



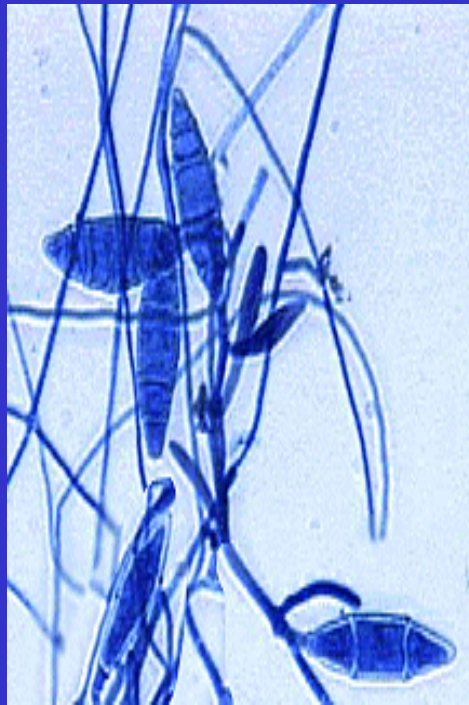
T. rubrum



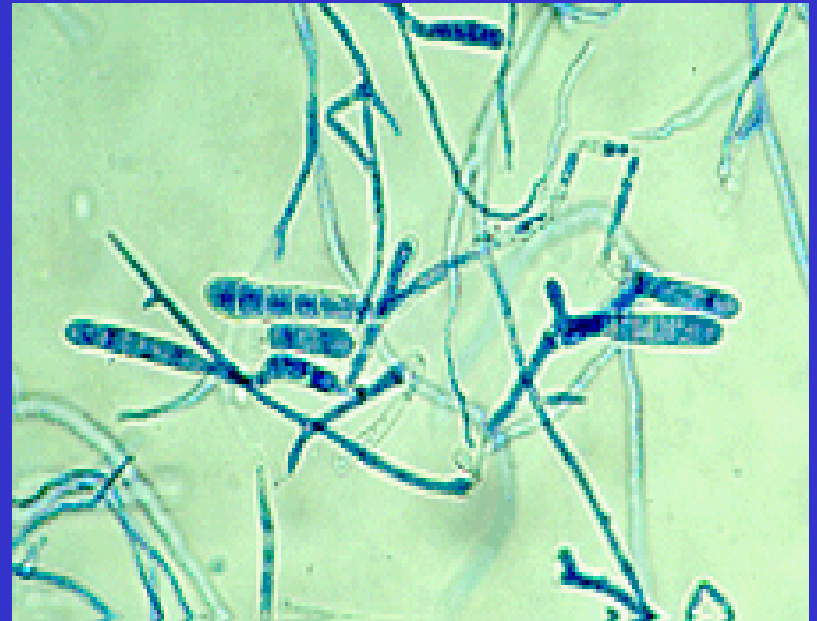
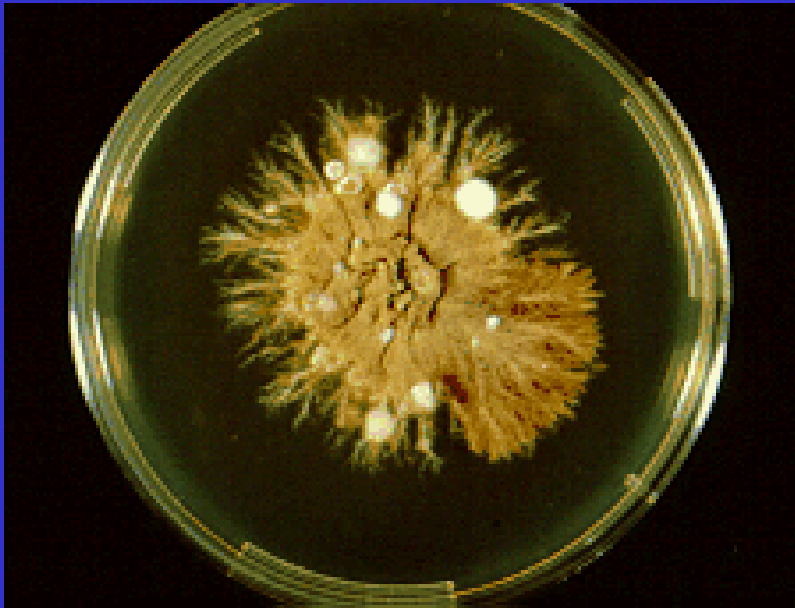


M. gypseum

M. canis



E. floccosum



Thermally dimorphic fungi

Geographically restricted to specific areas of **endemicity**.


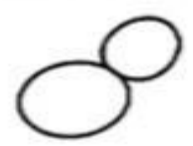

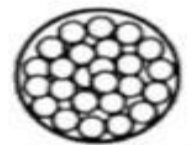

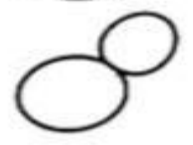
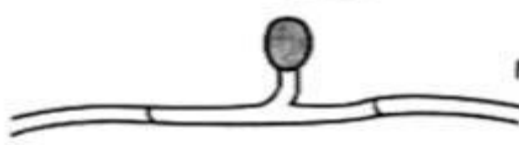


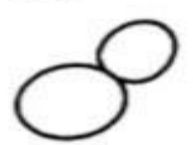
Infections are initiated in the lungs following inhalation of the **conidia**.

Most infections are asymptomatic; immunocompromised individuals (AIDS) have increased risk of serious infections.

Dimorphic fungi

- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- *Coccidioides immitis*
- *Paracoccidioides brasiliensis*
- *Sporotrichum schenkii*

Dimorphic fungi

Fungus	In vitro (25° C)	In vivo (37° C)
<i>Blastomyces</i>	 Mold	 Yeast
<i>Coccidioides</i>	 Mold	 Spherule
<i>Histoplasma</i>	 Mold	 Yeast
<i>Paracoccidioides</i>	 Mold	 Yeast
<i>Sporothrix</i>	 Mold	 Yeast

Histoplasma capsulatum

- In nature - soil saprophyte - soil being enriched by nitrogenous substrates
- Occur worldwide, most cases in USA - Mississippi
- Intracellular pathogen
- Histoplasmosis-initiated by inhalation of conidia
 - conidia develop into yeast cells and replicate in alveolar macrophages
 - disseminate to reticuloendothelial tissue (liver, spleen, lymph nodes)

Histoplasma capsulatum

Diagnostic Laboratory Tests:

- microscopic examination
- culture
- serology
- skin test

Treatment: itraconazole or amphotericin B

II Phylum *Basidiomycota* (Basidiomycetes)

- Sexual reproduction: club-shaped basidium with four progeny basidiospores

Genus *Cryptococcus*

- *C. neoformans*: 4 serotypes (A-D), possess large polysacharide capsules
- Occurs worldwide in nature (dry pigeon feces)
- **Cryptococcosis** – following inhalation of dry yeast cells (primary **pulmonary** infection)
- **Neurotropic** -typically migrate to the central nervous system (meningoencephalitis)
- **Dissemination** in skin, adrenals, bone...
- Occurs in immunocompetent persons but more often in patients with **AIDS** and other immunosuppressive conitions

Cryptococcus neoformans

Antigenic structure:

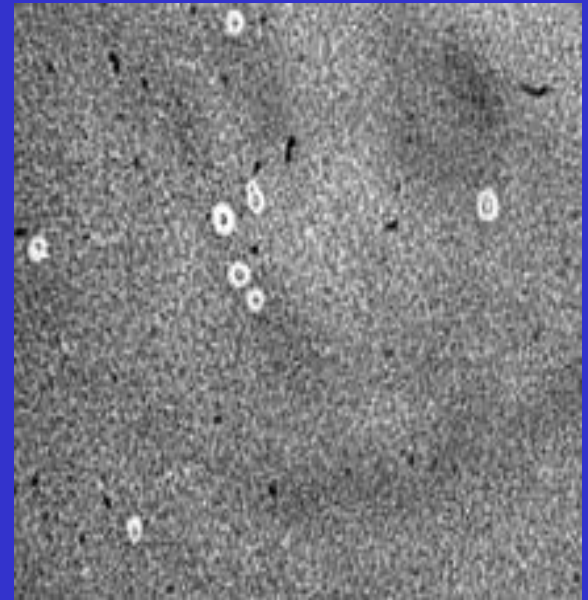
- capsular polysaccharide

Diagnostic Laboratory Tests

- specimens: CSF, tissue, blood...

1. Microscopic Examination

- Wet mounts or India ink
(spherical, budding yeast cells,
5-10 μ m in diameter, surrounded
by a thick nonstaining capsula
in specimens)



C. neoformans

2. Culture - whitish, mucoid colonies within 2-3 days, on most media at room temperature; urease positive

3. Serology

Tests for capsular antigen- latex slide agglutination tests or enzyme immunoassay

C. neoformans

Treatment

- amphotericin B (+ flucytosine)
- HIV +: flukonazol (CNS)

Malassezia furfur

- lipophilic yeasts, part of the cutaneous microbiota
- Pityriasis Versicolor is a chronic, superficial infection of the cornified skin (chronic, macular patches of discolored skin)
- Contributor to seborrheic dermatitis



Malassezia furfur

Diagnostic test:

direct microscopic examination of scrapings of infected skin (with 10-20% potassium hydroxide or calcofluor white)

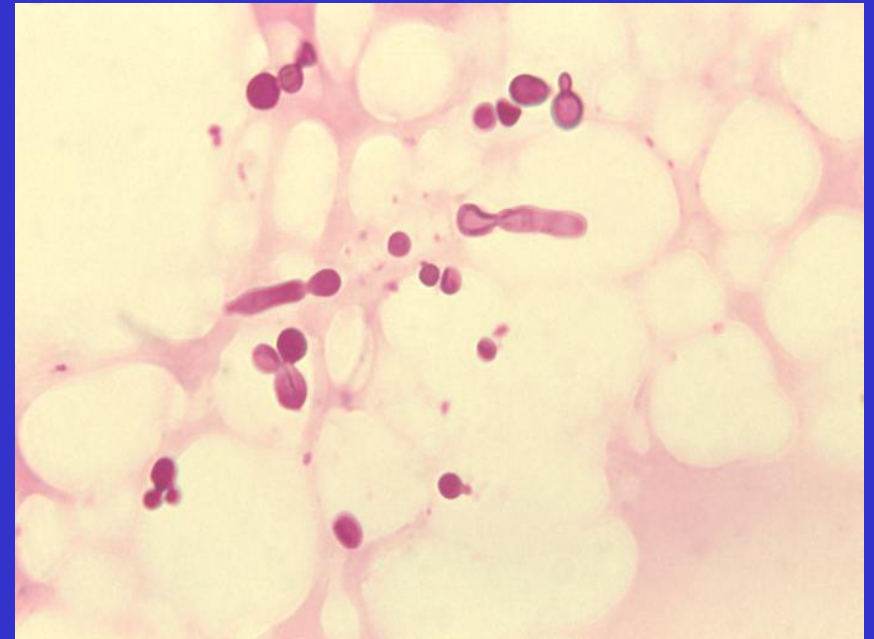


Figure. Short, unbranched hyphae and spherical cells.

Malassezia furfur

- **Treatment:**
 - Azoles (topical or oral)
 - Selenium sulfide