

## **A. Fungi Imperfecti/imperfect fungi**

A class of fungi for which a sexually reproductive stage of the life cycle has not been found. It consists of fungi ranging from **yeast and yeast like organisms, dimorphic fungi and dermatophytes** A heterogenous phylum of fungi which lack a sexual phase, or of which the sexual phase is not known.

This is big class of all those fungi which have not shown sexual reproduction or whose sexual stages are all undiscovered. When sexual stage is known that fungi is transferred to ascomycetes Ex- *Aspergillus* and *Penicillium* were previously in fungi imperfecti but now they are in Ascomycetes.

Most pathogenic fungi belong to fungi Imperfecti class. Ex- *Cryptococcus*, *Candida*, *Histoplasma*, *Epidermophyton*, *Trichophyton* and *Microsporum*.

According to morphological classification *Cryptococcus* is yeast, *candida* is yeast like, *histoplasma* is dimorphic and *epidermophyton* & *microsporum* all mycelial fungi but all are coming under fungi Imperfecti in Systematic Classification.

### **Common pathogenic fungi are: -**

- *Cryptococcus* spp. (Yeast and Yeast like fungi)
- *Candida* spp. (Yeast and Yeast like fungi)
- *Blastomyces*
- *Histoplasma*
- *Sporotrichum*
- *Coccidioides* (Dimorphic fungi)
- *Dermatophytes* (*Microsporum*, *Trichophyton*, *Epidermophyton*)

### **Yeasts**

Yeasts are fungi that grow as single cells (**Unicellular**), producing daughter cells either by **budding** (the budding yeasts) or by **binary fission** (the fission yeasts).

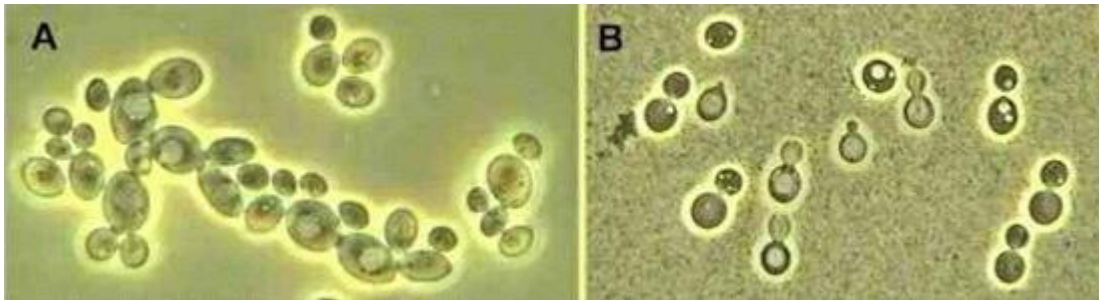
They differ from most fungi, which grow as thread-like hyphae. But this distinction is not a fundamental one, because some fungi can alternate between a yeast phase and a hyphal phase, depending on environmental conditions. Such fungi are termed dimorphic (with two shapes).

- **Certain Yeast produces mycelium and certain species of yeast may produces Pseudomycelium**

Here we consider several examples of yeasts and dimorphic fungi:

- The genus *Cryptococcus*, which includes *C. neoformans*, a pathogen of humans and animals

- The dimorphic fungus *Candida albicans* which can be a significant pathogen of humans and animals



**Budding and formation of conidia and arthrospores** are the usual methods of Asexual reproduction. However certain yeast forms spores and the spore that develops from mother cells called **Blastospores**.

Some species of yeast produces pseudomycelium and mycelium and forms **Arthrospores** or certain species of yeast reproduce sexually by forming **Ascospores**.

Certain yeast produces arial spores called **Ballistospores**. But there are certain group of yeast do not produce ascospores and ballistospores and reproduce exclusively by vegetative process, this group of fungi called as fungi imperfecti

The Pathogenic yeast:

1. *Candida spp.*
2. *Cryptococcus spp.*
3. *Malassezia spp.*

### 1. Candidiasis: -

Genus: *Candida*:

- C. albicans*
- C. tropicalis*
- C. krusei*

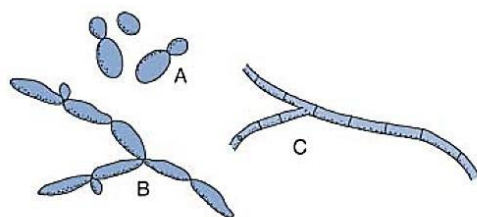
### **Habitat**

- All *Candida* occur as **saprophytes** as commensal of the **oral, gastro-intestinal and genital tract of many species of animals and humans.**
- *Candida* species commonly present in the **crop of birds**
- *C. albicans* infection -  
**Moniliasis, Candidiasis or Candidosis, Thrush/Sour crop: in poultry**

### **Morphology:**

- Budding cell appears as G+ve cell. Pseudohyphae (Yeast cell repeatedly divide and form chain like structure) and serve as spore and form Chlamydospores (resting spore)

**Figure 45.1** Three forms of the polymorphic yeast *Candida albicans*: budding yeast cell (A); pseudohypha (B); true septate hypha (C).



*Candida* species

### Disease

Host (s)	Disease (s)
Chicken, turkey, pigeon, ducklings	<b>Crop mycosis or Avian moniliasis</b>
Swine	Stomach ulcers and cutaneous candidiasis
Puppies, kittens, calves and foals	Mycotic stomatitis and enteritis
Mares and bitches	Genital tract infection
Cattle	Mastitis and Abortion
Dogs	Otitis

### Pathogenesis:

The candida infection is common in individuals undergoes prolonged antibiotic therapy, it occurs as cutaneous and mucous membrane infection

**Thrush/Candidiosis** in poultry: young birds are more susceptible to digestive tract infection. Older birds are less susceptible because they overcome infection. In chronic infection the **crop wall is thicken** and covered by corrugated membrane of yellowish-gray necrotic material resembling **Turkis Towel** like appearance.

### Diagnosis

#### Specimens

Scrapings from lesions, centrifuged milk samples, biopsy or tissue samples in 10% formalin for histopathology.

#### Based on morphology

- Direct Microscopic examination of clinical material or colonies grown on agar medium shows masses of budding cell with fragment of mycelium.
- **Gram-stained smears** - purple-blue yeast cell
- **PAS-haematoxylin** (or) methanamine silver stains, the *C. albicans* appear as thin-walled oval, budding yeast cells and/or in the form of pseudohyphae.

#### Based on isolation and identification

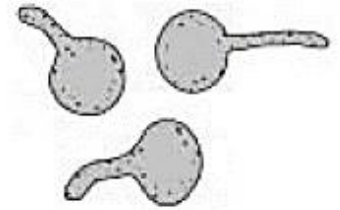
- **Blood agar or SDA** without inhibitors can used. Other media like Malt agar, Raulins media and Corn meal agar can be used for isolation

**On SDA agar at 30°C shows budding cell and pseudomycelium**

- **BiGGy agar (Bismuth-sulphite-glucose- glycine- yeast agar):** Most bacterial contaminants are inhibited by the Bismuth sulphite. *C. albicans* and *C. tropicalis* strongly reduce the Bismuth sulphite to Bismuth sulphide
- *C. albicans* gives **smooth, circular, brownish colonies** and no color diffusion into the surrounding medium
- Animal inoculation test: Rabbit and mice are susceptible to candida infection

**Germ tube or serum tube test**

- A small inoculum from an isolated colony is suspended in 0.5 ml of sheep, bovine, rabbit or human serum and incubated at 37°C for 2-3 hrs.
- A drop of the preparation is examined under phase contrast or high objective of the light microscope.
- Small, thin-walled tubes will be seen projecting from some of the yeast cells. This is characteristic of *C. albicans*.



**Prevention and control:**

- Nystatin ointment and Amphotericin-B: for Cutaneous candidiasis
- Copper sulphate dilution in drinking water is effective for control and prophylactic measure.
- Iodine
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**2. Cryptococcosis**

- Cryptococcosis - **European blastomycosis or Torulosis**
- Subacute or chronic mycotic infection of man and various species of animals involving the CNS, the respiratory system and eye
- **Encapsulated yeast** – *Cryptococcus neoformans*
- *C. neoformans* - pathogenic for animals and humans

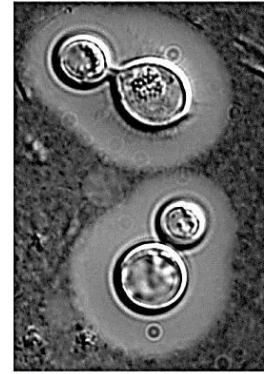
Host (s)	Disease (s)/Pathogenicity
Dogs and cats	<b>Subcutaneous and nasal granulomas</b> <b>Meningitis:</b> Causes disease of CNS where there is inco-ordination, circling, blindness and locomotory disturbances can be observe.
Horses	Nasal granuloma
Cattle	Cryptococcal <b>mastitis</b>
Human	Cryptococcal <b>meningitis</b>

**Morphology**

- *C. neoformans* - fungi imperfecti.
- *C. neoformans* is a spherical to oval, thin walled, budding yeast
- Cells are surrounded by a **mucoïd/gelatinous polysaccharide capsule** that varies in thickness, but in animal tissues usually very large, the width of the capsule exceeding the d.m. of the parent cell
- Yeast can be stained by **Gram staining** (taking violet stain) and **indian ink**

### Habitat

- Skin, mucous membrane and intestinal tract of normal animals and birds
- Faeces of birds, particularly pigeons and soil contaminated by avian excreta
- Organisms are concentrated in pigeon faeces due to their high content of creatinine
- Creatinine inhibits many other microorganisms but can be utilized by *C. neoformans*
- Survive in pigeon droppings for more than a year
- *C. neoformans* has a worldwide distribution
- Bird seed agar used for selective medium for *C. neoformans*



### Cultural character:

- On SDA agar, between 25°C to 37°C it produces **Slimy brownish creamy colony**
- It can be grown on Blood and malt agar
- Do not ferment sugar but can utilize Urea (urease +ve)

### Diagnosis:

- Based on clinical sign and history
- Demonstration of yeast in spinal fluid of affected animals: centrifuge CSF and prepare slide from sediment by adding 1 drop of **Nigrosin dye** (1%) and see under microscope. **The Yeast observe round to oval with budding cells having refractile gelatinous capsule.**
- Cultural isolation on SDA agar (**Slimy brownish creamy colony**)
- Can be observed by immunofluorescence

### 3. Malassezia:

This is lipophilic yeast that reproduces by unipolar budding. It occurs on the oily area of skin and ears of dogs, cat and some other animals. In some cases of otitis externa in dogs the yeast is appear in large number and produce pathogenic lesions.

Species: *Malassazia pachydermatis*

Disease: **Canine seborrheic dermatitis:** Yeast infection causes excessive sebaceous secretion

**Otitis externa:** Yeast produces proteolytic enzymes result in damage to mucosa of the ear canal which causes inflammatory changes and inflammatory exudate and necrotic debris accumulate in canal

### Habitat:

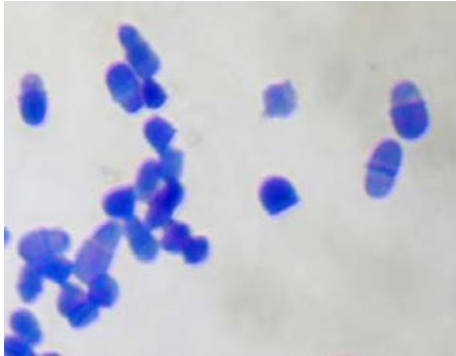
- It can be found on skin of mammals and birds (Area rich in sebaceous gland)
- Anal region, external ear canal, lips and interdigital skin of dogs

## Morphology:

- Bottle shaped cells of yeast, **monopolar budding** on a broad base with the formation of collarette.

### Genus *Malassezia*

Many species are recognized; only *M. pachydermatis* is of veterinary importance



### *Malassezia pachydermatis* - Foot-print shaped yeast

Monopolar budding on a broad base, with the formation of a prominent collarette, is a characteristic of this yeast.

## Cultural character:

- Unique budding pattern (bottle shaped cells)
- Grown on SDA agar without lipid supplementation

## Diagnosis:

- History and Clinical sign on skin and ear
- Yeast cell from the exudates is being demonstrate using methylene blue staining
- Based on cultural characteristics on SDA medium (Grow without lipid suppl.)