MYCOLOGY - CHAPTER THREE

YEASTS

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Figure 1
Brewer's yeast (also known as Baker's yeast) with bud and bud scars (*Saccharomyces cerevisiae*). © Dennis Kunkel Microscopy, Inc. Used with permission

Yeasts are single-celled budding organisms (figure 1). They do not produce mycelia. The colonies are usually visible on the plates in 24 to 48 hours. Their soft, moist colonies resemble bacterial cultures rather than molds. There are many species of yeasts that can be pathogenic for humans. We shall only discuss the three most significant species:

- *Candida albicans*
- *Cryptococcus neoformans*
- *Crytococcus gattii*

CANDIDIASIS *(Candida albicans)*

There are more than 20 species of the genus *Candida* that cause disease. The infections caused by all species of *Candida* are called candidiasis.

Candida albicans (figure 2 and 3) is an endogenous organism and the most common species in human infections. It can be found in 40 to 80% of normal human beings. It is present in the mouth (figure 4), gut, and vagina. It may be present as a commensal or a pathogenic organism.

- Candidiasis of the mouth (oropharyngeal candidiasis) is called thrush which is seen as white patches on the mucosa of the mouth including the tongue. The affected area can become inflamed and may cause difficulty in swallowing. Cracking and inflammation may occur around the mouth. This is referred to as oral cheilitis. Oral candidiasis may spread to the esophagus (esophagitis). Although most people harbor Candida species, oral candidiasis is typically found in immuno-compromised individuals. These include people infected with HIV (9 to 31% of AIDS patients) or who have received immunosuppressive drugs for cancer chemotherapy (20%) and organ transplantation. Other factors that have been associated with oral candidiasis are diabetes, certain dentures and the use of corticosteroids.
  CDC estimates that between 5 and 7% of neonates develop oral candidiasis
  Untreated oral candidiasis can lead to serious invasive disease.
  Treatment for the oral form consists of clotrimazole tablets or a nystatin suspension. If this is not effective, especially in the case of esophageal involvement, fluconazole or itraconazole. Some forms are resistant to these drugs in which case amphotericin B may be used.

- Genital or vulvovaginal candidiasis (yeast infection) cause genital itching, a burning sensation and vaginal discharge in females. In men, the penis may have an itching rash. This is rare in men but most women will have at least once episode of vulvovaginal candidiasis. Women are more at risk of the infection if they are:
  Pregnant

Diabetic
Use broad spectrum antibiotics
Use corticosteroids

- Invasive candidiasis (candidemia) is a serious disease when Candida, which is normally on the skin or the gastro-intestinal tract, enters the bloodstream where it can disseminate to other organs. Symptoms include fever and chills that do not respond to anti-bacterial agents.
  These are often nosocomial infections of people who:
  have a central venous catheter
  are immunosuppressed
  take broad-spectrum antibiotics
  show neutropenia
  are on hemodialysis
  have diabetes
  There are about 46,000 cases of candidemia each year in the United States and the disease shows high rates of morbidity and mortality. In hospital conditions with already sick people, mortality due to candidemia may be as high as 19-24%. Diagnosis of candidemia is by culture of the organism from blood.


Figure 2
Candida albicans - yeast and hyphae stages. A yeast-like fungus commonly occuring on human skin, in the upper respiratory, alimentary & female genital tracts. This fungus has a dimorphic life cycle with yeast and hyphal stages. The yeast produces hyphae (strands) and pseudohyphae. The pseudohyphae can give rise to yeast cells by apical or lateral budding. Causes candidiasis which includes thrush (an infection of the mouth and vagina) and vulvo-vaginitis.

Infections with *Candida* usually occur when a patient has some alteration in cellular immunity, normal flora or normal physiology. Patients with decreased cellular immunity have decreased resistance to fungal infections. Prolonged antibiotic or steroid therapy destroys the balance of normal flora in the intestine allowing the endogenous *Candida* to overcome the host. Invasive procedures, such as cardiac surgery and indwelling catheters, produce alterations in host physiology and some of these patients develop *Candida* infections. Although it most frequently infects the skin and mucosae, *Candida* can cause pneumonia, septicemia or endocarditis in the immuno-compromised patient.

The establishment of infection with *Candida* species appears to be a property of the host - not the organism. The more debilitated the host, the more invasive the disease.

The clinical material to be sent to the lab depends on the presentation of the disease: Such material may include blood cultures, vaginal discharge, urine, feces, nail clippings or material from cutaneous or mucocutaneous lesions.

Candida is a polymorphic yeast, i.e., yeast cells, hyphae and pseudohyphae are produced. It has been shown that *Candida* needs a transcription repressor to maintain the yeast form. This ability to assume various forms may be related to the pathogenicity of this organism. The yeast form is 10 to 12 microns in diameter, gram positive, and it grows overnight on most bacterial and fungal media. It also produces germ tubes (figure 9 and 10), and pseudohyphae (figure 6 and 7) may be formed from budding yeast cells that remain attached to each other. Spores may be formed on the pseudomycelium. These are called chlamydospores and they can be used to identify different species of *Candida*. Some mycologists think that the pseudomycelial form represents a more invasive form of the organism. The species are identified by biochemical reactions. The organism occurs world-wide.

The drugs of choice for systemic infection are itraconazole and fluconazole. If an artificial heart valve or in-dwelling catheter becomes infected, it must be replaced. Drug therapy alone will not suppress the organism if the foreign body remains in the host. This resistance is due to biofilms which we will discuss later.

CANDIDA SPECIES

Candida species (other than *albicans*) account for an increasing number of nosocomial infections. Speciation is important because there is significant antibiotic resistance among the different species.

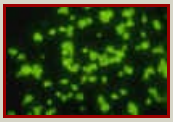


Figure 3
Oval budding yeast cells of Candida albicans. Fluorescent antibody stain.
CDC/Maxine Jalbert, Dr. Leo Kaufman. lek1@cdc.gov


Figure 4
Oral thrush. CDC


Figure 5
Gross pathology of rabbit kidney lesions due to experimental *Candida albicans* infection. Rabbit was cortisone-treated. CDC


Figure 6
Sputum smear from patient with pulmonary candidiasis. Gram stain. CDC

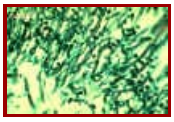


Figure 7
Histopathology of *Candida albicans* infection. Methenamine silver stain. Pseudohyphae and true hyphae. ASCP


Figure 8
Histopathology of Candida esophagitis. Methenamine silver stain (digitally colorized). CDC


Figure 9
Candida albicans showing germ tubes. Calcofluor white stain in peptone medium. Germ tube production is a diagnostic feature of C. albicans. CDC/Mercy Hospital, Toledo, OH/Dr. Brian Harrington


Figure 10
Candida albicans showing germ tube production in serum. Gram stain.
CDC/Dr. Lucille K. Georg


Figure 11
Gram-stain of vaginal smear showing *Candida albicans* epithelial cells and many gram-negative rods. (1,000X oil) © Danny L. Wiedbrauk, Warde Medical Laboratories, Ann Arbor, Michigan and The MicrobeLibrary

CRYPTOCOCCOSIS

Cryptococcus neoformans

Cryptococcosis manifests itself most commonly as meningitis but in recent years many cases of pulmonary disease have been recognized. Most infections are, however, asymptomatic.

C. neoformans is a very distinctive yeast. The cells, which are spherical and 3 to 7 microns in diameter (figure 12), produce buds that characteristically are narrow-based and the organism is surrounded by a polysaccharide capsule (figure 14).


Figure 12
Encapsulated pathogenic yeast fungus (*Cryptococcus neoformans*). A yeast-like fungus that reprouces by budding. A acidic mucopolysaccharide capsule completely encloses the fungus. It can cause the disease called cryptococcosis; especially in immune deficient humans, such as in patients with HIV / AIDS. The infection may cause meningitis in the lungs, skin or other body regions. The most common clinical form is

There is evidence that the capsule may suppress T-cell function and can be considered a virulence factor. *C. neoformans* also produces an enzyme called phenoloxidase which appears to be another virulence factor.

The ecological niche of *C. neoformans* is pigeon and chicken droppings. However, although this organism can be easily recovered from pigeon droppings, a direct epidemiological link has yet to be established between exposure to pigeon droppings and a specific human infection. Infection and disease production is probably a property of the host -- not the organism. The source of human infection is not clear. This organism is ubiquitous, especially in areas such as abandoned buildings contaminated with pigeon droppings.

The portal of entry is the respiratory system. Evidence is developing which indicates that the initial exposure may be many years prior to the manifestation of disease. The organism can be sequestered for this time. Infection may be subacute or chronic. The highly fatal meningoencephalitis caused by *C. neoformans* has a prolonged evolution of several months. The patients symptoms may begin with vision problems and headache, which then progress to delirium, nuchal rigidity leading to coma and death unless the physician is thinking about cryptococcus and does a spinal tap for diagnosis and institutes aggressive therapy. The CSF is examined for its characteristic chemistry (elevated protein and decreased glucose), cells (usually monocytes), and evidence of the organism.

meningoencephalitis. It is caused by inhaling the fungus found in soil that has been contaminated by pigeon droppings.

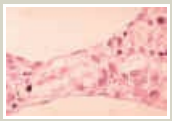


Figure 13
Cryptococcosis of lung in patient with AIDS. Histopathology of lung shows widened alveolar septum containing a few inflammatory cells and numerous yeasts of *Cryptococcus neoformans*
CDC/Dr. Edwin P. Ewing, Jr. epe1@cdc.gov

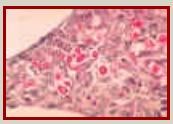


Figure 14
Cryptococcosis of lung in patient with AIDS. Mucicarmine stain. Histopathology of lung shows widened alveolar septum containing a few inflammatory cells and numerous yeasts of *Cryptococcus neoformans.* The inner layer of the yeast capsule stains red. CDC/Dr. Edwin P. Ewing, Jr. epe1@cdc.gov


Figure 14a
Life cycle of *Cryptococcus gatti.*
CDC

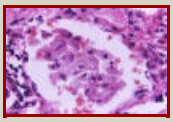


Figure 14b
A lung lesion tissue specimen, with morphology associated with the disease cryptococcosis due to the infiltration of Cryptococcus. sp. fungal organisms.
CDC


Figure 14c
Global burden of HIV-related cryptococcal meningitis
CDC: Adapted from BJ Park et al., AIDS 2009;23:525-530

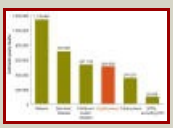


Figure 14d

The latter is measured by the visual demonstration of the organism (India Ink preparation, figure 15) or by a serologic assay for the antigen of *C. neoformans*. The India Ink test, which demonstrates the capsule of this yeast, is supplemented by the latex agglutination test for antigen which is more sensitive and more specific. The Latex Agglutination test measures antigen, not antibody. A decreasing titer indicates a good prognosis, while an increasing titer has a poor prognosis. When you consider Cryptococcosis, think of Capsules and CNS disease.

In addition to causing meningitis, *C. neoformans* may also infect lungs (figure 16) and skin. The disease in the lungs and skin is characterized by the formation of a granulomatous reaction with giant cells. As with other fungal diseases, there has been an increase in the recognition of pulmonary infection. The yeast may also form a mass in the mediastinum called a cryptococcoma.

The clinical material sent to the lab is CSF, biopsy material, and urine (for some unexplained reason the organism can be isolated from the urine in both the CNS and systemic infections). This organism will grow overnight on bacterial or fungal media at 37 C. but growth is a little slower at room temperature. In culture, the organism grows as creamy, white, mucoid (because of the capsule) colonies. Growth in culture is usually visible in 24 to 48 hours. As the culture ages, it turns brown due to a melanin produced by the phenoloxidase.

The organism is a round, single cell, yeast surrounded by a capsule. Identification is based on physiological reactions. Pathologists use a mucicarmine stain, which stains the capsule, to identify the organism in tissue sections (Figure 14). There is usually little or no inflammatory response. The Direct Fluorescent Antibody test identifies the organism in culture or tissue section specifically, by causing the yeast cell wall to stain green. To test the patient's serum there are three serologic tests: The Indirect Fluorescent Antibody test, the Tube Agglutination test for antibody, and the Latex Agglutination test for antigen. The latex agglutination test can be used as a prognostic test. As the patient improves, the serum antigen titer will also decrease.

The geographical distribution of this organism is world-wide and, like other yeast infections, is most commonly seen in immunocompromized patients. There are about 1 million cases of crypotococcal meningitis each year, mostly among HIV-infected AIDS patients, resulting in 625,000 deaths. Most cases are in sub-Saharan Africa (720,000) with 133,000 in southeast Asia and 70,000 in the Americas (figures 14 c and d).

The drugs of choice to treat *cryptococcus* infection are amphotericin B and 5-Fluorocytosine (5-FC). 5-FC is an oral drug. If it is given as the only treatment, there are relapses so most physicians use both drugs simultaneously. These two drugs are synergistic, and thus, their association is advantageous.

Cryptococcus gattii

This is a newly recognized pathogenic species of *Cryptococcus*. It is found in soil and in association with several species of trees in tropical and sub-tropical regions of the world but has recently been found in the western United States and western Canada. The patient acquires the infection as a result of the inhalation of fungal spores which lodge in the lungs. Here, the spores transform into dividing yeast cells and disseminate to other parts of the body via the bloodstream, sometimes inside macrophages. There have been about 100 United States cases of *Crytococcus gattii* infections in the period from 2004 to 2011 and almost all of them have been in the Pacific Northwest. In western Canada, 218 cases were reported between 1999 and 2007.The appearance of symptoms usually occurs several months (average six to seven) after breathing in the spores but in some cases, several years pass before symptoms are observed. The disease is not contagious.

Infection of the lungs is accompanied by

- malaise (fever and headache)
- a cough and shortness of breath
- chest pain

The fungus can spread to the nervous system, including the brain where is causes meningoencephalitis. According to the CDC, there is a long latent time (two to fourteen months) between exposure and the manifestation of symptoms. Cryptococcal meningitis symptoms include:

- Fever

Causes of death in sub-Saharan Africa, excluding HIV/AIDS
CDC: Adapted from BJ Park et al., AIDS 2009;23:525-530

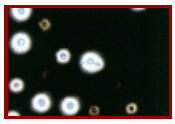
- Headache
- Neck pain
- Nausea
- Light sensitivity
- Confusion

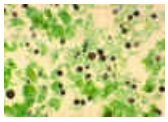
The presence of *Cryptococcus gatti* can lead to the growth of cryptococcomas in various parts of the body.

As with other fungal infections, people at most risk include those with compromised immune systems (AIDS and cancer patients and people on immunosuppressive therapy).

Identification of a *Cryptococcus gattii* infection is by microscopy (Figure 14b) after growth in the laboratory. To distinguish *Cryptococcus gattii* from *Cryptococcus neoformans*, the organisms are grown on canavanine-glycine-bromthymol blue agar. Only *Cryptococcus gattii* turns this blue.

The patient requires treatment by anti-fungal agents for up to six months. In severe cases, amphotericin B, often in combination with flucytosine, is recommended. In milder cases, fluconazole or itraconazole is used.

 Figure 15
C. neoformans: India Ink preparation
Dr Arthur DiSalvo

 Figure 16
Cryptococcosis of lung in patient with AIDS. Methenamine silver stain. Histopathology of lung shows numerous extracellular yeasts of *Cryptococcus neoformans* within analveolar space. Yeasts show narrow-base budding and characteristic variation in size. CDC/Dr. Edwin P. Ewing, Jr. epe1@cdc.gov

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This page last changed on Sunday, February 07, 2016
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