LAB 9: THE FUNGI, PART 1 - THE YEASTS

INTRODUCTION

Fungi are eukaryotic organisms and include the yeasts, molds, and fleshy fungi. Yeasts are microscopic, unicellular fungi; molds are multinucleated, filamentous fungi (such as mildews, rusts, and common household molds); the fleshy fungi include mushrooms and puffballs.

Review of Prokaryotic and Eukaryotic Cells from Unit 1.

All fungi are **chemoheterotrophs,** requiring organic compounds for both an energy and carbon source, which obtain nutrients by absorbing them from their environment. Most live off of decaying organic material and are termed **saprophytes**. Some are **parasitic**, getting their nutrients from living plants or animals.

The study of fungi is termed **mycology** and the diseases caused by fungi are called mycotic infections or **mycoses**.

In general, fungi are **beneficial** to humans. They are involved in the decay of dead plants and animals (resulting in the recycling of nutrients in nature), the manufacturing of various industrial and food products, the production of many common antibiotics, and may be eaten themselves for food. Some fungi, however, damage wood and fabrics, spoil foods, and cause a variety of plant and animal diseases, including human infections.

YEASTS

DISCUSSION

Yeasts are unicellular, oval or spherical fungi which increase in number asexually by a process termed budding (see Fig. 1). A bud forms on the outer surface of a parent cell, the nucleus divides with one nucleus entering the forming bud, and cell wall material is laid down between the parent cell and the bud. Usually the bud breaks away to become a new daughter cell but sometimes, as in the case of the yeast *Candida*, the buds remain attached forming fragile branching filaments called hyphae (see Fig. 10). Because of their unicellular and microscopic nature, **yeast colonies appear similar to bacterial colonies** on solid media. It should be noted that certain dimorphic fungi (see Lab 10) are able to grow as a yeast or as a mold, depending on growth conditions.

- Scanning electron micrograph of *Saccharomyces*; courtesy of Dennis Kunkel's Microscopy.
- Transmission electron micrograph of *Candida albicans* (see Fig. 3).
- Movie of *Saccharomyces cerevisiae* reproducing by budding. Movie of Growth and Division of Budding Yeast (*Saccharomyces cerevisiae*) . © Phillip Meaden, author. Licensed for use, ASM MicrobeLibrary.

Yeasts are facultative anaerobes and can therefore obtain energy by both aerobic respiration and anaerobic fermentation. The vast majority of yeasts are nonpathogenic and some are of great value in industrial fermentations. For example, *Saccharomyces* species are used for both baking and brewing.

The yeast ***Candida*** is **normal flora** of the gastrointestinal tract and is also frequently found on the skin and on the mucous membranes of the mouth and vagina. *Candida* is normally held in check in the body by:

1. normal immune defenses, and
2. normal flora bacteria.

However, *Candida* may become an opportunistic pathogen and overgrow an area of colonization if the host becomes immunosuppressed or is given broad-spectrum antibiotics that destroy the normal bacterial flora. (Since *Candida* is eukaryotic, antibiotics used against prokaryotic bacteria do not affect it.)

Any infection caused by the yeast *Candida* is termed **candidiasis**. The most common forms of candidiases are oral mucocutaneous candidiasis (**thrush;** see Fig. 7A), **vaginitis** (see Fig. 7B), **balantitis** (infection of the penis), **onychomycosis** (infection of the nails), and **dermatitis** (diaper rash and other infections of moist skin). In addition, *Candida* can cause urinary tract infections. However, antibiotic therapy, cytotoxic and immunosuppressive drugs, and immunosuppressive diseases such as diabetes, leukemias, and AIDS can enable *Candida* to cause severe opportunistic systemic infections involving the skin, lungs, heart, and other organs. In fact, *Candida* now accounts for 10% of the cases of septicemia. Candidiasis of the esophagus, trachea, bronchi, or lungs, in conjunction with a positive HIV antibody test, is one of the indicator diseases for AIDS.

The most common *Candida* species causing human infections is *C. albicans*, causing 50-60% of all *Candida* infections. *Candida glabrata* is second, causing 15-20% of *Candida* infections; *Candida parapsilosis* is third, responsible for 10-20%.

Candida is said to be **dimorphic**, that is it **has two different growth forms**. It can **grow as an oval, budding yeast**, but under certain culture conditions, the **budding yeast may elongate and remain attached producing filament-like structures called pseudohyphae**. *C. albicans* may also **produce true hyphae** similar to molds. In

this case long, branching filaments lacking complete septa form. The pseudohyphae and hyphae help the yeast to invade deeper tissues after it colonizes the epithelium. Asexual spores called **blastoconidia** (blastospores) develop in clusters along the hyphae, often at the points of branching. Under certain growth conditions, thick-walled survival spores called **chlamydoconidia** (chlamydospores) may also form at the tips or as a part of the hyphae (see Fig. 2A and Fig. 2B)

A lesser known but often more serious pathogenic yeast is **C*ryptococcus neoformans***. Like many fungi, this yeast can also reproduce sexually and the name given to the sexual form of the yeast is ***Filobasidiella neoformans***. It appears as an oval yeast 5-6 µm in diameter, forms buds with a thin neck, and is surrounded by a thick **capsule.** It does not produce pseudohyphae and chlamydospores. The capsule enables the yeast to **resist phagocytic engulfment**. The yeast is dimorphic. In its sexual form, as well as in its asexual form under certain conditions, it can produce a hyphal form.

Cryptococcus infections are usually mild or subclinical but, when symptomatic, usually begin in the lungs after inhalation of the yeast in dried bird feces. It is typically associated with pigeon and chicken droppings and soil contaminated with these droppings. *Cryptococcus*, found in soil, actively grows in the bird feces but does not grow in the bird itself. Usually the infection does not proceed beyond this pulmonary stage. However, in an immunosuppressed host it may spread through the blood to the meninges and other body areas, often causing cryptococcal meningoencephalitis. Any disease by this yeast is usually called **cryptococcosis**.

Dissemination of the pulmonary infection can result in severe and often fatal **cryptococcal meningoencephalitis**. Cutaneous and visceral infections are also found. Although exposure to the organism is probably common, large outbreaks are rare, indicating that an immunosuppressed host is usually required for the development of severe disease. Extrapulmonary cryptococcosis, in conjunction with a positive HIV antibody test, is another indicator disease for AIDS. People with AIDS-associated cryptococcal infections account for 80%-90% of all patients with cryptococcosis.

Cryptococcus can be identified by preparing an India ink or nigrosin negative stain of suspected sputum or cerebral spinal fluid in which the encapsulated, budding, oval yeast cells (see Fig. 4A) may be seen. It can be isolated on Saboraud Dextrose agar and identified by biochemical testing. Direct and indirect serological tests (discussed in Labs 17 & 18) may also be used in diagnosis.

Pneumocystis jiroveci, (formerly called *Pneumocystis carinii*), causes an often-lethal disease called ***Pneumocystis* pneumonia (PCP)**. It is seen **almost exclusively in highly immunosuppressed individuals** such as those with AIDS, late stage malignancies, or leukemias. PCP and a positive HIV-antibody test is one of the more common indicators of AIDS.

P. jiroveci can be found in **3 distinct morphologic stages**:

- The trophozoite **(trophic form)**, a haploid amoeboid form 1-4 µm in diameter that replicates by mitosis and binary fission. The trophic forms are irregular shaped and often appears in clusters.
- A **precystic form** or early cyst. Haploid trophic forms conjugate and produce a diploid precyst form or sporocyte.
- The precyst form matures into a **cyst form**, which contains several intracystic bodies or spores are 5-8 µm in diameter. It has been postulated that in formation of the cyst form (late phase cyst), the zygote undergoes meiosis and subsequent mitosis to typically produce eight haploid ascospores (sporozoites) See Fig. 9. As the haploid ascospores are released the cysts often collapse forming crescent-shaped bodies (see Fig. 5). *P. jiroveci* is usually transmitted by inhalation of the cyst form. Released ascospores then develop into replicating trophic forms that attach to the wall of the alveoli and replicate to fill the alveoli.
    - Proposed life cycle for *Pneumocystis jiroveci*; from dpd.cdc.gov

In biopsies from lung tissue or in tracheobronchial aspirates, both a **trophic form** about 1-4 µm in diameter with a distinct nucleus and a **cyst form** between 5-8 µm in diameter with 6-8 intracystic bodies (ascospores) can be seen.

When viewing cysts of *P. jiroveci* in lung tissue after utilizing the Gomori methenamine silver stain method, the walls of the cysts are stained black and often appear crescent shaped or like crushed ping-pong balls. The intracystic bodies are not visible with this stain.

- *P. jiroveci* cysts from bronchoalveolar lavage (see Fig. 5)
- *P. jiroveci* cysts from the lungs (see Fig. 9)

Malassezia globosa

Malassezia globosa is a dimorphic yeast that is the most frequent cause of a superficial skin infection called **tinea versicolor** that commonly appears as a hypopigmentation or hyperpigmentation of the infected skin. *M. globosa* is also the most common cause of **dandruff** and **seborrheic dermatitis**. The yeast is naturally found on the skin.

For a decription of **antifungal agents used to treat fungal infections**, see section IIE: Chemotherapeutic Control of Fungi in you lecture E-text.

Medscape articles on infections associated with organisms mentioned in this lab exercise. Registration to access this website is free.

- *Candida albicans*
- *Cryptococcus neoformans*
- *Pneumocystis jeroveci*
- tinea versicolor

Today we will use three agars to grow our yeast: Saboraud Dextrose agar (SDA), Mycosel agar, and Rice Extract agar. **Saboraud Dextrose agar (SDA)** is an agar similar to trypticase soy agar but with a higher sugar concentration and a lower pH, both of which **inhibit bacterial growth but promote fungal growth**. SDA, therefore, is said to be **selective for fungi**.

Another medium, **Mycosel agar,** contains chloramphenicol to **inhibit bacteria** and cycloheximide to **inhibit most saprophytic fungi**. Mycosel agar, therefore, is said to be **selective for pathogenic fungi**.

Rice Extract agar with polysorbate 80 **stimulates the formation of hyphae, blastoconidia, and chlamydoconidia** (see Fig. 2A and Fig. 2B), structures unique to *C. albicans*, and may be used in its identification. The speciation of *Candida* is based on sugar fermentation patterns.

Concept map for Lab 9

Return to Menu for Lab 9

MATERIALS

Coverslips, alcohol, forceps, and one plate each of Saboraud Dextrose agar, Mycosel agar, and Rice Extract agar.

ORGANISMS

Trypticase Soy broth cultures of *Candida albicans* and *Saccharomyces cerevisiae*.

PROCEDURE (to be done in pairs)

1. With a wax marker, divide a **Saboraud Dextrose agar** and a **Mycosel agar** plate in half. Using a sterile **swab**, inoculate one half of each plate with *C. albicans* and the other half with *S. cerevisiae*. Incubate the two plates **upside down and stacked in the petri plate holder on the shelf of the 37°C incubator corresponding to your lab section** until the next lab period.

2. Using a sterile **swab**, streak two straight lines of *C. albicans* into a plate of **Rice Extract agar plate**. Pick up a **glass coverslip** with forceps, dip the coverslip in alcohol, and ignite with the flame of your gas burner. Let the coverslip cool for a few seconds and place it over a portion of the streak line so that the plate can be observed directly under the microscope after incubation. Repeat for the second steak line and incubate the plate **upside down at room temperature** until the next lab period.

3. Observe the following demonstrations:

   a. Direct stain of *Saccharomyces cerevisiae* (see Fig. 1)

   b. Direct stain of *Candida albicans* (see Fig. 6)

   c. Oral smear from a person with thrush (see fig. 7A)

   d. Lung tissue infected with *Candida albicans* (see Fig. 8)

   e. India ink preparation of *Cryptococcus neoformans* (see Fig. 4B)

   f. Cyst form of *Pneumocystis jiroveci* from lung tissue (see Fig. 5)

Return to Menu for Lab 9

RESULTS

1. In the table below, describe the appearance of *Candida albicans* and *Saccharomyces cerevisiae* on Saboraud Dextrose agar.

Also in the table below, describe the appearance of *Candida albicans* and *Saccharomyces cerevisiae* on Mycosel agar.

Yeast	SDA	Mycosel agar
Candida albicans		
Saccharomyces cerevisiae		

2. Remove the lid of the Rice Extract agar plate and put the plate on the stage of the microscope. Using your **yellow-striped 10X objective**, observe an area under the coverslip that appears "fuzzy" to the naked eye. **Reduce the light** by moving the iris diaphragm lever almost all the way to the right. Raise the stage all the way up using the **coarse focus** (large knob) and then lower the stage **using the coarse focus** until the yeast comes into focus. Draw the hyphae, blastoconidia, and chlamydoconidia. See lab 1 for focusing instructions using the 10X objective.

Candida albicans producing hyphae, blastoconidia, and chlamydoconidia on Rice Extract agar

3. Observe and make drawings of the demonstration yeast slides.

 Direct stain of *Saccharomyces cerevisiae*	 Direct stain of *Candida albicans*	 *Candida albicans* from thrush specimen

 *Candida albicans* in lung tissue	 India ink preparation of *Cryptococcus neoformans*	 *Pneumocystis jiroveci* cyst in lung tissue

Return to Menu for Lab 9

PERFORMANCE OBJECTIVES FOR LAB 9

After completing this lab, the student will be able to perform the following objectives:

INTRODUCTION

1. Define mycology and mycosis.
2. State three ways fungi may be beneficial to humans and three ways they may be harmful.

DISCUSSION

1. Describe the typical appearance of a yeast cell and its usual mode of reproduction.

2. Describe yeasts in terms of their oxygen requirements.

3. State two ways the yeast *Saccharomyces* is beneficial to humans.

4. Name three yeasts that commonly infect humans.

5. Name four common forms of candidiasis.

6. Describe two conditions that may enable *Candida* to cause severe opportunistic systemic infections.

7. Describe pseudohyphae, hyphae, blastoconidia (blastospores), and chlamydoconidia (chlamydospores).

8. State the usefulness of Saboraud Dextrose agar, Mycosel agar, and Rice Extract agar.

9. State how *Cryptococcus neoformans* is transmitted to humans, where in the body it normally infects, and possible complications.

10. State the primary method of identifying *Cryptococcus neoformans* when causing cryptococcal meningoencephalitis.

11. State what disease is caused by *Pneumocystis jiroveci* and indicate several predisposing conditions a person is normally seen to have before they contract the disease.

12. Name an infection caused by *Malassezia globosa*.

RESULTS

1. Describe the appearance of *Saccharomyces cerevisiae* and *Candida albicans* on Saboraud Dextrose agar and on Mycosel agar. 2. When given a plate of Mycosel agar showing yeast-like growth and a plate of Rice Extract agar showing hyphae, blastosconidia (blastospores), and chlamydoconidia (chlamydospores), identify the organism as *Candida albicans*. 3. Recognize the following observed microscopically:

a. *Saccharomyces cerevisiae* and *Candida albicans* as yeasts in a direct stain preparation
b. A positive specimen for thrush by the presence of budding *Candida albicans*
c. *Cryptococcus neoformans* in an India ink preparation

d. *Pneumocystis jiroveci* in lung tissue

Return to Menu for Lab 9

SELF-QUIZ

Self-quiz

Answers

Return to Menu for Lab 9

Lab Manual Table of Contents