Exercise 34

EXERCISE 34

Fungi: Yeasts

Objectives

After completing this exercise, you should be able to:

1. Culture and identify yeasts.
2. Differentiate between yeasts and bacteria.

Background

Fungi possess eukaryotic cells and can exist as unicellular or multicellular organisms. They are chemoheterotrophic and obtain nutrients by absorbing dissolved organic material through their cell walls and plasma membranes. Fungi (with the exception of yeasts) are aerobic. Unicellular yeasts, multicellular molds, and macroscopic species such as mushrooms are included in the Kingdom Fungi. Compared to bacteria, fungi generally grow better in more acidic conditions and tolerate higher osmotic pressure and lower moisture. They are larger than bacteria, with more cellular and morphologic detail. In contrast to bacterial characterization, primary characteristics, such as morphology and cellular detail, are used to identify fungi, with little attention given to secondary characteristics, such as metabolism and antigenic composition. Fungi are structurally more complex than bacteria but are less diverse metabolically.

Yeasts are nonfilamentous, unicellular fungi that are typically spherical or oval in shape. Yeasts are widely distributed in nature and frequently found on fruits and leaves as a white, powdery coating. When budding yeasts reproduce asexually, cell division is uneven. A new cell forms as a protuberance (bud) from the parent cell (Figure 34.1a). In some instances, when buds fail to detach themselves, a short chain of cells called a **pseudohypha** forms (Figure 34.1b). When yeasts reproduce sexually, they may produce one of several types of sexual spores. The type of sexual spore produced by a species of yeast is used to classify the yeast to a phylum. Metabolic activities are also used to identify genera of yeasts.

Yeasts are facultative anaerobes. Their metabolic activities are used in many industrial fermentation processes. Yeasts are used to prepare many foods, including bread, and beverages, such as wine and beer.

In the laboratory, **Sabouraud agar,** a selective medium, is commonly used to isolate yeast. Sabouraud agar has very simple nutrients (glucose and peptone) and a low pH, which inhibits the growth of most other organisms. Many of the techniques useful in working with bacteria can be applied to yeasts.

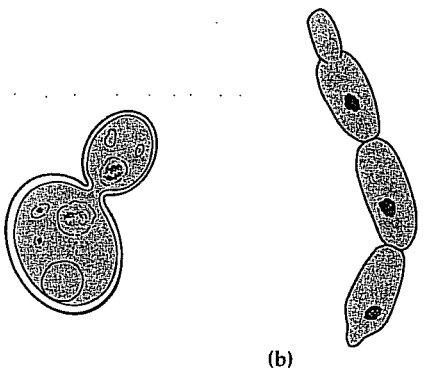

Figure 34.1

Budding yeasts. **(a)** A bud forming from a parent cell. **(b)** Pseudohyphae are short chains of cells formed by some yeasts.

Materials

Glucose fermentation tubes (2)

Sucrose fermentation tubes (2)

Petri plates containing Sabouraud agar (2)

Bottle containing glucose–yeast extract broth

Sterile cotton swab

Coverslip

Test tube

Balloon

Fruit or leaves

Methylene blue (second period)

Cultures (as assigned)

Baker's yeast

Rhodotorula rubra

Candida albicans

Saccharomyces cerevisiae

Techniques Required

Compound light microscopy, Exercise 1

Colony morphology, Exercise 9

Wet-mount technique, Exercise 2

Plate streaking, Exercise 11

Fermentation tests, Exercise 14

Procedure

Yeasts

1. Gently suspend a pinch of baker's yeast in a small amount of lukewarm water in a test tube, creating a milky solution.
2. Each pair of students will use one of the yeast cultures and the suspension of baker's yeast.
   a. Divide one Sabouraud agar plate in half. Streak one-half with a known yeast culture and the other half with the baker's yeast suspension.
   b. Inoculate each organism into a glucose fermentation tube and a sucrose fermentation tube.
3. Incubate all media at 35°C until growth is seen.
4. Make a wet mount of each culture by using a small drop of methylene blue. Record your observations.
5. After the yeasts have grown, record your results. Examine cultures of the yeasts you did not culture, and record pertinent results. (See Color Plate IV.1.)

Yeast Isolation

1. Cut the fruit or leaves into small pieces. Place them in the bottle of glucose–yeast extract broth. Cover the mouth of the bottle with a balloon. Incubate the bottle at room temperature until growth has occurred. Record the appearance of the broth after incubation. Was gas produced? _____
2. Divide a Sabouraud agar plate in half. Each partner inoculates half of the medium, following either procedure a or procedure b.
   a. Swab the surface of your tongue with a sterile swab. Inoculate one-half of the agar surface with the swab. Discard the swab in the disinfectant. Why will few bacteria grow on this medium? _____
   b. Using a sterile inoculating loop, streak one-half of the agar surface with a loopful of broth from the bottle just prepared in step 1. Replace the balloon.
3. Incubate the plate, inverted, at room temperature until growth has occurred. Prepare wet mounts with methylene blue from different-appearing colonies. Record your results.

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Fungi: Yeasts

LABORATORY REPORT

NAME _____

DATE _____

LAB SECTION _____

Purpose

_____

_____

_____

Data

Yeasts

Fermentation tubes:

Organism	Glucose			Sucrose		
	Acid	Gas	Fermentation	Acid	Gas	Fermentation
Rhodotorula rubra						
Candida albicans						
Saccharomyces cerevisiae						
Baker's yeast						

Sabouraud agar plates:

Organism	Draw a Typical Colony	Wet Mount
Rhodotorula rubra	Color: _____	_____ ×
Candida albicans	Color: _____	_____ ×

Organism	Draw a Typical Colony	Wet Mount
Saccharomyces cerevisiae	Color: _____	_____ ×
Baker's yeast	Color: _____	_____ ×

Yeast Isolation

Plants used: _____

Describe the appearance of the glucose broth after _____ days' incubation. _____

_____

Was gas produced? _____

Sabouraud agar:

Area Sampled	Colony Appearance	Color	Size	Wet Mount
Tongue:				
Bottle:				

Any bacteria seen? _____

If so, which colonies? _____

Questions

1. Could you identify the genus of baker's yeast? _____

2. Did you culture yeast from your mouth? _____ From the plants? _____ How do you know? _____

3. What was the purpose of the balloon on the glucose–yeast extract broth bottle? _____

4. Define the term *yeast*. _____

5. Compare and contrast yeast and bacteria regarding their appearance both on solid media and under the microscope.

6. Why are yeast colonies larger than bacterial colonies? _____