

Simple Staining of Microbes

Introduction: Many classrooms have microscopes with only 40X objectives that do not generally allow students to see microorganisms very well. The two microorganisms that will be stained in this lab are unusually large and can be seen under the microscope with a 40X objective.

Objective: To stain and observe the yeast *Saccharomyces cerevisiae* and the bacterium *Bacillus megaterium*.

Materials (per experiment or group of students):

- yeast suspension (about 1 ml)
- *Bacillus megaterium* suspension (about 1 ml)
- microscope slide
- inoculating loop or dropper
- crystal violet stain with dropper
- Bunsen burner
- paper towels
- microscope with 40X objective
- marking pen
- clothespin or clamp to hold microscope slide

Procedure:

1. Label each end of a microscope slide with Y for yeast or B for *Bacillus*.
2. Using a small dropper or an inoculating loop, remove a small droplet of each culture from the tube and place on the microscope slide.
3. Holding the slide with a clothespin or clamp, gently heat the slide in the Bunsen burner until the droplets have evaporated and the slide is warm.
4. Cool the slide for a minute.
5. Gently flood the slide with crystal violet so that the cells are under the stain.
6. Leave the stain on the slide for about 1 minute.
7. Rinse the slide with water.
8. Blot the slide dry with a paper towel.
9. Observe the microorganisms under the microscope using a 40X objective. (Note: you do not need a cover slip for the slide.)

Results: The yeast will appear as round or oval cells that are dark blue or purple in color. Some may appear to have smaller cells attached to the larger cells. These are called buds. The buds are new daughter cells produced by the original mother yeast cell.

The *Bacillus* cells are rod-shaped and are also purple. You may also see small purple cells with a clear center. These cells are called spores. Spores are very resistant to heat, desiccation and ultraviolet light. A spore will germinate into the rod-shaped cells that you see that are called vegetative cells.

Related activity: See the laboratory called "Isolating *Bacillus* from Soil" for a lab activity to isolate a spore-forming strain of *Bacillus*.

Teacher Instructions

In advance:

1. Order crystal violet and *Bacillus megaterium*.
2. Prepare a few nutrient agar plates according to instructions on package. Pour plates of about 30 ml/plate.
3. Streak the culture of *Bacillus megaterium* on 2-3 nutrient agar plates. Incubate at room temperature or 30° until there is good growth. This should be done about a week before class to encourage spore formation.
4. Purchase baker's yeast at the grocery store.

The day of class:

1. Prepare the yeast culture by suspending about 1 gram of baker's yeast in 200 ml water. Aliquot the suspension into small tubes (about 1 ml per tube) for the students. The suspension should not be dense!
2. Prepare a suspension of *Bacillus megaterium* cells by scraping a loopful of cells from the surface of a plate or slant of a culture of *Bacillus megaterium* into about 5 ml water. Vortex vigorously to make a uniform suspension. Aliquot the suspension into small tubes for the students. The suspension should not be dense! (Note: older cultures are more likely to contain spores.)

Contributed by:

Teresa Thiel, Ph.D.
Department of Biology
University of Missouri-St. Louis
St. Louis, MO 63121
USA