• Cell Biology Practical 2 — Cell Types: Prokaryote (Bacterium) vs Eukaryote (Yeast)

Context and Objectives

This practical follows the lecture on the classification of the kingdoms and domains of life and the introduction to the nucleus and cell organelles. It offers the chance to directly observe, under the light microscope, a representative of the domain Bacteria (lactic acid bacteria from yogurt) and a representative of the domain Eukarya (baker's yeast, Saccharomyces cerevisiae).

Learning objectives:

- Observe the morphology of prokaryotic cells and unicellular eukaryotic cells under the light microscope.
- Compare, based on direct observations, the structural criteria that distinguish the two cell types (size, presence of a distinct nucleus, organelles).
- Demonstrate, through a simple experiment, a metabolic activity specific to the eukaryotic cell (fermentation) and discuss its link with the presence of intracellular compartments.

Part 1 — Observation of Prokaryotic Cells (Lactic Acid Bacteria from Yogurt)

Plain yogurt naturally contains two species of lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*), which are easy to obtain and safe to handle in a practical session.

Materials: plain unsweetened yogurt, light microscope (x100 oil-immersion objective if available, otherwise x40), slides and coverslips, toothpick or improvised sampling loop (fine toothpick), diluted methylene blue, water.

Protocol

1. Take a very small amount of yogurt (tip of a toothpick) and spread it in a very thin layer on a clean slide.
2. Let it air-dry for a few minutes (do not heat).
3. Place a drop of diluted methylene blue on the smear, leave for 1 minute, then rinse gently with water and let dry.
4. Observe at x400 magnification, then at x1000 with oil immersion if the microscope allows it: locate the elongated rods (*Lactobacillus*) and the spherical cells grouped in short chains (*Streptococcus*).

To record in the lab report: a labeled drawing showing the very small size of the bacteria (on the order of a micrometer) compared with the field of view, and the absence of distinguishable internal structures at this magnification.

Part 2 — Observation of Eukaryotic Cells (Baker's Yeast)

Materials: fresh or dry baker's yeast, lukewarm slightly sweetened water, light microscope, slides and coverslips, diluted methylene blue (or Lugol's iodine), dropper.

Protocol

1. Rehydrate a small amount of yeast in lukewarm sweetened water for 10 to 15 minutes to restart its activity.
2. Place a drop of the yeast suspension on a slide, add a drop of diluted methylene blue, and cover with a coverslip.
3. Observe at x400 magnification: locate the ovoid cells, which are clearly larger than the bacteria from Part 1, and look for cells in the process of budding (a small outgrowth attached to a mother cell).
4. In one field of view, count the proportion of single cells and budding cells.

To record in the lab report: a labeled drawing of the yeast cell (wall, cytoplasm, bud) and a visual estimate of the size ratio between a yeast cell and a bacterium observed in Part 1.

Part 3 — Metabolic Activity of Yeast: Fermentation

This experiment illustrates a complex metabolic activity made possible by eukaryotic cell organization, and allows a macroscopic observation without a microscope.

Materials: a small empty plastic bottle, baker's yeast, lukewarm water, granulated sugar, a balloon.

Protocol

1. Pour about 100 mL of lukewarm water, one tablespoon of sugar and one packet (or one teaspoon) of yeast into the bottle; mix.
2. Quickly fit the deflated balloon over the neck of the bottle, without inflating it by hand.
3. Place the bottle in a warm spot and observe every 10 minutes for 30 to 45 minutes.
4. Note the progressive inflation of the balloon, due to the production of carbon dioxide (CO_2) by the fermentation of sugar.

Recommended control: prepare in parallel a bottle without yeast (water + sugar only) or without sugar (water + yeast only): the balloon should not inflate, which confirms that it is indeed the metabolic activity of living yeast that is responsible for the release of gas.

Expected interpretation: the yeast, a eukaryotic cell, breaks down sugar in the absence of sufficient oxygen (alcoholic fermentation) and produces CO_2 and ethanol. This organized metabolic activity illustrates the greater functional complexity of the eukaryotic cell compared with the prokaryotic cell, in connection with the presence of compartments and specialized enzymes.

Structural Comparison: Prokaryote vs Eukaryote

Criterion	Bacterium (prokaryote)	Yeast (eukaryote)
Approximate size	1 to 5 µm	5 to 10 µm (clearly larger)
Nucleus	Absent (DNA free in the nucleoid)	Present, surrounded by a nuclear envelope
Membrane-bound organelles	Absent	Present (mitochondria, endoplasmic reticulum, vacuole, etc.)
Cell wall	Peptidoglycan	Chitin and glucans
Mode of reproduction	Binary fission	Budding (or fission, depending on the species)
Domain (classification)	Bacteria	Eukarya (kingdom Fungi)

This table can be completed by students directly from their observations in Parts 1 and 2, in particular the size and mode of reproduction rows, which are visible without additional equipment.

Materials, Organization, Safety and Assessment

Summary of Materials (Low-Cost)

Material	Part(s)	Remark
Plain yogurt	1	Common, inexpensive product
Baker's yeast	2, 3	Fresh or dry, available at grocery stores
Diluted methylene blue	1, 2	A small quantity is enough for a whole class
Light microscope	1, 2	Share equipment among pairs/groups of three; x1000 oil immersion is useful but not essential
Plastic bottle + balloon	3	Recycled materials
Sugar, lukewarm water	2, 3	Already available

No Gram stain reagents, centrifuge or sterile culture equipment are needed: all observations are made from common food products.

Practical Organization

- Work in pairs or groups of three; rotate between microscopes if the number is limited.
- Start Part 3 (fermentation) at the very beginning of the session, so that it progresses while the microscope observations of Parts 1 and 2 are being made.

Safety Instructions

- Methylene blue stains clothing and skin heavily: handle with care, wearing gloves if necessary.
- Handle slides and coverslips carefully (sharp edges).

Lab Report Questions

1. What difference in size did you observe between the bacterium and the yeast? Is this difference consistent with what was covered in lecture about prokaryotes and eukaryotes?
2. Why is it not possible to distinguish a distinct nucleus in the bacterium observed in Part 1?
3. What does the budding observed in yeast indicate about its mode of reproduction?
4. Why does the balloon not inflate (or only slightly) in the control bottle without yeast or without sugar?
5. How does the fermentation experiment illustrate a functional difference between prokaryotic and eukaryotic cells?