Prokaryotes

Strand Life at the Molecular and Cellular Level

Topic Investigating prokaryotes

Primary SOL BIO.3 The student will investigate and understand relationships between cell structure and function. Key concepts include
- b) characteristics of prokaryotic and eukaryotic cells.

Related SOL BIO.1 The student will demonstrate an understanding of scientific reasoning, logic, and the nature of science by planning and conducting investigations in which
- a) observations of living organisms are recorded in the lab and in the field;
- b) hypotheses are formulated based on direct observations and information from scientific literature;
- c) variables are defined and investigations are designed to test hypotheses;
- e) conclusions are formed based on recorded quantitative and qualitative data;
- f) sources of error inherent in experimental design are identified and discussed;
- h) chemicals and equipment are used in a safe manner;
- i) appropriate technology, including computers, graphing calculators, and probeware, is used for gathering and analyzing data, communicating results, modeling concepts, and simulating experimental conditions
- m) current applications of biological concepts are used.

BIO.4 The student will investigate and understand life functions of Archaea, Bacteria, and Eukarya. Key concepts include
- a) comparison of their metabolic activities;
- c) how the structures and functions vary among and within the Eukarya kingdoms of protists, fungi, plants, and animals, including humans.

Background Information

Prokaryotes are very simple single-celled life forms. The typical prokaryotic cell includes a circular piece of free-floating DNA called a “nucleoid,” ribosomes, a cell membrane and cell wall, and perhaps a flagellum or other motility apparatus. This is very different from the complex, membrane-bound organelles and defined nucleus of the eukaryotic cell.

Fossils of prokaryotes have been found that are 3.5 billion years old, making today’s prokaryotes descendants of the oldest living inhabitants of Earth. Prokaryotes have evolved with an amazing ability to adapt. They are ubiquitous, found in every conceivable environment. They are found in the deepest of the deep sea trenches, producing their own food from the sulfur vents via chemosynthesis. They survive the highest mountain lakes, the driest deserts, low (0) pH environments, and high pH environments in soda lakes. The prokaryotes that are survivors of these extreme conditions are called “extremophiles.” Scientists who have studied the genomes of these extremophiles have placed them in a domain or kingdom all their own—the Archaea.

Other prokaryotes have been placed in the domain or kingdom Eubacteria (or Bacteria, in some textbooks). These are the bacteria that are commonly found in human environments. Some are beneficial, enabling production of foods such as yogurt and sauerkraut, while others are deadly, like *Clostridium botulinum,* which causes botulism, and *Bacillus anthrasis,* which causes anthrax. Most other bacteria simply exist in all human environments, filling their ecological niche as decomposers.

Bacteria are ubiquitous: they are found in all microenvironments. Some microenvironments may have more bacteria—a bacterial load—than others. Warm, moist environments may have more bacteria than areas that are cold and/or dry. Environments with a rich supply of carbohydrates and protein may also have more bacteria. School buildings have an abundance of microenvironments where large bacterial loads may be found.

Materials

- Modeling clay in four colors
- Rubber gloves
- Prepared slides of eubacteria (optional)
- Microscopes, at least one of which has oil immersion (optional)
- Sterile test tubes with stoppers
- Deionized sterile water
- Sterile agar plates (If sterile agar plates are not cost effective or available, plain gelatin can be used with either petri dishes or ice cube trays. Prepare the gelatin as directed, and fill the petri dishes or ice cube trays half full. Allow gelatin to solidify. These will not be sterile, but the validity of the data, as well as other organisms such as mold that may be seen can be discussed.)
- Sterile cotton swabs
- Sterile micropipettes
- Sealable plastic bags
- Incubator or warm area for incubating plates
- Hand lenses and/or dissecting scopes
- Biohazard bag
- Autoclave
- Incubating Prokaryotes handout (attached)

Vocabulary

Archaea, autoclave, biohazard, capsule, cilia, Eubacteria, flagella, pili, prokaryote, sterile

Student/Teacher Actions (what students and teachers should be doing to facilitate learning)

Prior to engaging in this lesson, students should complete the lesson entitled "The Parts of an Experiment: Introduction to Inquiry and the Scientific Process" in order to gain more experience in designing experiments and writing lab reports. That lesson also includes generic differentiation strategies for general inquiry lessons.

1. Review the characteristics of a prokaryotic cell, using a labeled diagram. Allow students to reference the diagram as they create prokaryote models, using modeling clay.

2. Provide four colors of clay to each student, and tell students that each prokaryote model should include DNA, ribosomes, cell membrane, cell wall, flagella, and cilia. Direct each student to decide which color will represent which macromolecule in their model and to make a key.
3. Direct students to construct prokaryote models out of the clay and to highlight the four macromolecules as they build their models.
4. Once the models are complete, assess each one, and have students correct them, as needed. Then, have students draw their prokaryote models in their notebooks and color their drawings according to their color keys.
5. Optional: Allow students to examine slides of eubacteria under multiple magnifications and document their observations.
6. Direct students to identify five locations at school that may have large numbers of bacteria— i.e., large bacterial loads—and to rank these locations according to bacteria count, location 1 being the one most likely to have the highest number of bacteria. Have students explain their reasoning in writing, using words such as *damp, warm, sweaty, nutrients (food) available, not cleaned, sanitized,* and *special*.
7. Direct students to design and set up a control for the experiment. Have them also create a data table to record observations.
8. Distribute copies of the attached Incubating Prokaryotes handout, and review the precautions and procedure for collecting specimens, inoculating plates with specimens, and disposing of plates.
9. Have students collect their specimens and inoculate their plates, following the procedures on the handout and observing all precautions.
10. After incubating the plates for 48 hours, review the precautions again, and direct students to observe and assess the colonies formed, using hand lenses or dissecting scopes.
11. Once all observations are made and all data recorded, have students place all plates in a bio hazard bag. Autoclave entire biohazard bag at 15 lb./in.^{2} (15 psi) pressure at a temperature of 121°C for 20 minutes. Alternatively, take the bag to a medical center to deposit with sharps (needles), or dispose of the materials according to the directions of your school division's chemical hygiene plan.
12. Discuss with students the validity of their experiments and results in terms of qualitative and quantitative data.

Assessment

- **Questions**
  - Which plate had the most bacterial colonies?
  - Which plate had the least?
  - How did the actual results compare with the predicted results?
  - Why was a control necessary?
  - Were there any unexpected results? If so, explain.
  - From the results, where is the highest number of microorganisms (largest bacterial load) found in the school? Why?

  - What is the effect of hot water, soap, detergent, or sanitizer on the number of microorganisms? (See results of samples taken from the clinic, cafeteria, or restrooms.)
- **Journal/Writing Prompts**
  - Discuss why sanitation is so important in the world today.
  - Discuss the overuse of antibiotics and the rise of antibiotic-resistant bacteria such as Methicillin-resistant Staphylococcus aureus (MRSA).
- **Other**
  - Have students design an experiment to test the effect of a sanitizer on the cultures grown in this experiment.
  - Have students create a photo essay, slide show, or poster presentation of their observations. This may be assessed for content, identification, and microscope technique. Since a mixed flora is expected (except for the *Nostoc*, sp.), a variety of documented observations should be included.

Extensions and Connections (for all students)

- Have students investigate use of bacterial cultures as fuel cells, using REDOX reactions.
- Have students explore an interesting nanotechnology application, such as Atomic Force Microscopy (AFM) technology, applied to the study of bacterial adherence.
- Have students examine bacterial roles in bioremediation or recombinant DNA technologies.
- If culture facilities are available (e.g., incubators, agar plates or slants, or liquid media and culture dishes) and students can use them *safely,* have them conduct extensive surveys. *(CAUTION: May be hazardous at this level.)*

Strategies for Differentiation

- Have students create a model of a prokaryotic cell, using a plastic bottle as the basic cell structure and materials of their own choosing for the other parts. For example, glitter could be added to the bottle for ribosomes and yarn used for the flagella.

Incubating Prokaryotes

Collecting specimens at sampling locations

1. Label your test tubes 1 through 5.
2. Take a stopper, a cotton swab, and a test tube half filled with deionized sterile water to one of the locations selected.
3. Put on rubber gloves. *(CAUTION: Microorganisms [bacteria] should always be treated with caution. Wear gloves during collection and observations of prokaryotes.)*
4. Dip the cotton swab into the water in the test tube.
5. Roll the wet cotton swab over the sampling location surface.
6. Put the cotton swab into the test tube.
7. Put the stopper in the test tube
8. Label the test tube with the location.
9. Repeat the process for the other four test tubes, going to other locations.

Inoculating plates in the classroom after sampling

1. Record initials, date, and locations of samplings on agar plates.
2. Shake a stoppered test tube 25 times to mix the sample and water thoroughly.
3. Wearing gloves, remove the stopper from the tube. *(CAUTION: Microorganisms [bacteria] should always be treated with caution. Wear gloves during collection and observations of prokaryotes.)*
4. With a micropipette, withdraw 1 mL of the water from the tube.
5. With the agar plate or petri dish on a flat surface, dispense the 1 mL sample onto the middle of the plate. Place the cover back on the plate/dish. *(CAUTION: When plating samples, do not touch the swab plates with your fingers.)*
6. Repeat the process for each of the other samples.
7. Place plates in separate plastic bags, and seal the bags *completely.*
8. Carefully dispose of all plate-preparation materials, according to teacher directions. Clean work areas, place micropipettes and swabs in bleach solution, and wash hands in hot, soapy water.
9. Incubate plates with clear side up for 48 ± 2 hours at 35°C. *(CAUTION: Leave the bags sealed before, during, and after incubation time! Do not remove plates from the bags.)*
10. After incubation, observe and assess the colonies of prokaryotes formed, using a hand lens or dissecting scope.