This lesson plan was created by a teacher participating in the Research Experiences for Teachers program from the Precision Microbiome Engineering Research Center. Are you interested in spending part of your summer in a lab getting paid to do microbiome research and create lesson plans?

Learn more here: https://premier-microbiome.org/for-teachers-ret/

Lesson Plan by April Hester

Title: Microbes in the School House

Overview:
In this lesson series, students will delve into the fascinating world of microbes that inhabit our built environments. Through hands-on activities, discussions, research and critical thinking, students will gain a deeper understanding of the diversity of microbes, their roles (both beneficial and harmful), and their prevalence in everyday surroundings. The goal is to spark curiosity about the microscopic world and its impact on our lives.

Key Search Words:
Microbes, built environment, bacteria, fungi, viruses, microbiome

Learning Objectives:
North Carolina Standard Course of Study Objectives:
LS.Bio.3.3 Use models to illustrate how cellular respiration [aerobic and anaerobic] transforms chemical energy into ATP.
LS.Bio.9.1 Analyze and interpret data to summarize how various factors such as geographic isolation, pesticide resistance, antibiotic resistance can influence natural selection.
LS.Bio.10.2 Use models (including dichotomous keys, scientific nomenclature, cladograms, phylogenetic trees) to identify
organisms and exemplify relationships.

Curriculum Alignment:
Standards:
LS.Bio.3 Analyze the relationship between biochemical processes and energy use.
- Focusing on aerobic and anerobic

LS.Bio.9 Understand natural selection as a mechanism for biological evolution.
- Focusing pesticide resistance and antibiotic resistance

LS.Bio.10 Analyze evolutionary relationships among organisms.
- Focusing on identifying organisms

Classroom time required:
Approximately 2 weeks (10 class sessions of 50 minutes each).

Materials & Technology:

Materials:

Gloves
Petri dishes
Disposable cotton swabs
Disposable plastic pipets
Screwcap test tube
Deionized water
Gram stain kits
Microscope slide
Cover slips
Petroleum jelly
Hydrogen peroxide
Paper towels
Toothpicks
Sharpie
Paper
Pencil/pen

Technology:

Microscopes
Chromebook

Safety

Review safety rules and expectations with students.
Students need to always keep gloves on.
Students need to wash hands immediately after removing gloves.
NO EATING/DRINKING.
NO APPLYING MAKEUP.

Teacher Preparation for Activity:

Pre-teach key vocabulary words related to microbes and the built environment.
Run all experiments to make sure they work.
Gather all materials and prepare the student's lab space in advance.

Student Preparation for Activity:

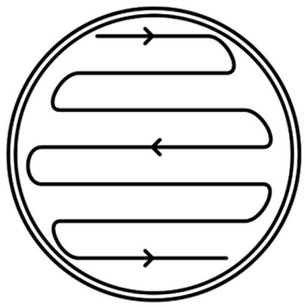
- Student will need to read ALL Instructions before starting the activities.
- Groups need to decide on which surface they want to sample in the school, Sampling should not take longer than 5-7 minutes.

Procedure:

Groups of 3 or 4 students, Maximum of 4 students.

Obtain Samples:

1. Each group will have 2 petri dishes (1 for building surface sample, 1 for human surface sample).
2. Using disposable cotton swabs, dip swab in deionized water and then swab the surface.
3. Lightly rub the swab across the petri dish in a zig zag motion. Make sure to not talk or breathe over the petri dishes, want to decrease the possibility of contamination.
4. Parafilm the plates.
5. Incubate for 24-48 hours.



zig zag streak
streaking pattern

zig zag streak
pattern of bacterial growth

Isolations:

- Once the petri dishes have had a chance to incubate and grow colonies, now it is time to isolate the colonies.
- Students need to open the petri dish and decide which colony they would like to isolate. Again, make sure to not talk or breathe on the petri dish while it is open.

1. Take a disposal cotton swab, dip swab into deionized water and lightly graze over the colony that is being isolated.
2. Perform the zig zag method and incubate (Steps 3-5 as above).

Gram Staining

Prepare the slide:

1. On a microscope slide add 5µL of deionized water.
2. Choose a colony on the petri dish, swab a colony with a toothpick and carefully smear in the water on the microscope slide. The mixture will be a very thin layer and not be seen with the naked eye.
3. Let dry for approximately 10 minutes.
4. (Optional) After drying pass the microscope slide over a lighter flame to seal in the bacteria.

Staining procedure:

1. Cover slide with Crystal Violet Reagent for one minute.
2. Rinse slide with deionized or tap water.
3. Cover slide with Iodine Reagent for one minute.
4. Gently rinse the slide with deionized or tap water and allow to drain.
5. Tilt the slide and flood the slide with a few drops of Deionizer until no violet color runs off. This will usually take 10 seconds or less. DO NOT OVER DECOLORIZE.
6. Rinse slide gently with deionized or tap water.
7. Cover slide with Safranin Advanced Counterstain or Basic Fuchsin counterstain for one minute.
8. Rinse slide gently with deionized or tap water. The rinse water in this step should be slightly pink. DO NOT OVER WASH.
9. Allow slide to drain and air dry or gently dry with paper towel.
10. Examine slide under microscope (preferably oil immersion lens, 1,000X magnification).

Note: Use the staining procedure that comes with the Gram Staining kit being used. Use caution so that slides are not over decolorized, causing gram-positive bacteria to appear gram-negative.

Aerobic vs Anaerobic

1. Use a toothpick to pick a colony from the petri dish.

2. Smear the colony on to a microscope slide or an empty petri dish (no agar should be on this dish).
3. Add 2 to 4 drops of 3% Hydrogen Peroxide (H_2O_2).
4. Look for bubble, can be seen with the naked eye or under the microscope.

- **Bubbles = Aerobic (Oxygen)**
- **No Bubbles = Anerobic (No Oxygen)**

Motility Test

Preparing slide:

1. Use a toothpick to pick a colony from the petri dish.
2. Smear the colony into the well of the microscope single concave slide.
3. Add 1 drop of deionized water into the well of the microscope single concave slide.
4. Use a toothpick to obtain some petroleum jelly and smear it around the top rim of the concave well.

Microscope use:

1. Place a microscope slide cover on top of the petroleum jelly, this should help hold the slide cover in place.
2. Place microscope slide under the microscope starting a 10X magnification to find the edge of the water drop. Increase magnification to 40X to see the bacteria within the water drop, you may be able to see motility at this magnification. If not, you can increase the magnification to 100X and add immersion oil.

Differentiation

- Pre-teach key vocabulary words related to microbes and the built environment.
- Have visual aids available.
- Verbalize instructions as well as breakdown instructions into small steps.
- Pair students with learning disabilities with supportive peers for group activities and projects.
- Create mixed-ability groups to encourage cooperative learning and peer tutoring.
- Provide frequent check-ins with each group to gauge comprehension and provide opportunities for questions.

Assessment/Check for Understanding

Here are critical thinking questions students could answer to demonstrate understanding of the lab:

1. **What methods did you use to collect and identify the microbes in your sample?**
    - Discuss the techniques and tools used for sampling, staining, and observing the microbes.

2. **What characteristics did you observe that helped you classify the bacteria (e.g., gram-positive or gram-negative, motile or non-motile)?**
    - Explain the visual and behavioral traits observed under the microscope and how they informed your classification.

3. **Based on the observed characteristics, what type of bacteria do you hypothesize you have found?**
    - Deduce the likely identity of the bacteria based on gram staining results, motility, and oxygen requirements.

4. **Why do you think this particular type of bacteria is found in the location where you collected your sample (e.g., bathroom sink, stairway railing, student desk)?**
    - Analyze the environmental conditions and human activities in the sampled location that may contribute to the presence of the identified bacteria.

5. **How do the characteristics of the bacteria you identified influence its survival and production in the sampled environment?**
    - Discuss the ecological and physiological traits that enable the bacteria to thrive in specific built environments.

6. **What are the potential implications of the presence of this type of bacteria in the sampled location for human health and hygiene? What interventions could be done to prevent the spread of pathogens?**
    - Evaluate the impact of the identified bacteria on indoor air quality, hygiene, and potential health risks.

7. **What further tests or experiments could you conduct to confirm the identity of the bacteria and understand its behavior better?**
    - Propose additional methods or experiments, such as biochemical tests, DNA sequencing, or environmental monitoring, to verify your findings and expand your understanding.

Sources

Hartline, R. (2023, February 20). *1.8: Plating on Petri plates for isolation*. LibreTexts Biology. https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_Laboratory_Manual_(Hartline)/01%3A_Labs/1.08%3A_Plating_on_Petri_Plates_for_Isolation