I-LEARN TEACHING RESOURCES

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Exploring Bacterial Diversity through Hand-printing & Bacterial Art

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Abstract: Bacteria are present in a wide variety of environments, ranging from deep ocean floor to volcanoes. Based on their niche, bacteria can differ in their physiologies. This lesson is designed to make the students aware of the presence of bacteria in their surroundings. This lesson also aims to demonstrate the diversity in bacterial species using colorful bacteria. The bacteria used in this lesson require specific temperatures to grow and develop their color. By the end of this lesson, students will be able to visualize the different colored bacteria on the plate.

Supporting File: PowerPoint presentation included.

Lesson Description:

Grade Level: Grade 6-12

Estimated Time for Completing Activity: Two 45-minute class periods and one 15-minute class periods.

- Day 1: Background Knowledge and Activity 1 (preparing LB agar plate)
- Day 2: Activity 2 (Bacterial Handprints) and Activity 3 (Bacterial Art)
- Day 3: Results, Conclusion and Discussions

Student learning objectives:

- Students will be aware of the presence of different micro-organisms in the surroundings.
- Students will learn the techniques to culture bacteria in a laboratory setup.
- Students will create their artwork using colorful bacteria
- Students will learn the importance of optimal temperature required for the growth of the different bacteria.

Students will learn to critically think how the genotype influences the phenotype of the organism and how the surroundings affect the phenotype as well.

South Dakota State Science Standards:

- MS-LS1-1: Plan and carry out an investigation to provide evidence that living things are made of cells; either one cell or many different types and numbers of cells. (SEP: 3; DCI: LS1.A; CCC: Scale/Prop., Technology)
- MS-LS1-5: Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms. (SEP: 6; DCI: LS1.B; CCC: Cause/Effect)

Standards Link: https://doe.sd.gov/contentstandards/documents/sdSciStnd.pdf)

Oceti Sakowin Essential Understandings and Standards:

- ***ESSENTIAL UNDERSTANDING 1***: Essential Understanding 1: The original land base and natural resources of the Oceti Sakowin [oh-CHEH-tee shaw-KOH-we] were under communal stewardship prior to immigrant settlement. Oceti Sakowin have a distinct and unique interrelationship with the environment that is essential to South Dakota.
- Standard 1.3 – Demonstrate understanding of the interrelationships of Oceti Sakowin people, places, and environments within in all tribal lands in South Dakota. *Standard*: Students can identify and explain how a tribal government manages the ecosystem and its natural gifts.
- Standards Link: https://indianeducation.sd.gov/documents/OcetiSakowinEUS.pdf

Day 1

Activity 1: **LB Agar media preparation, sterilization and plate pouring**

Background

Lysogeny broth (LB) is a nutritionally rich medium that is often used to grow bacteria such as *Escherichia coli* (abbreviated as *E. coli*). LB Agar is the agar form of the medium which is made by the combination of LB broth with Agar (a gelatinous substance extracted from sea weeds, used in many food products) in a specific proportion. To inhibit the growth of unwanted bacteria, contamination, in the media after preparation, the media is generally sterilized before use. The most used technique to sterilize media is Autoclaving. Autoclave is an instrument which can generate high heat and high pressure. The very high temperature and pressure destroys the bacterial cells and its spores. However, autoclaves are difficult to set up in some laboratories because of space or financial constraints. Therefore, a second method of sterilization, which is the microwave method is an easier alternative in places which do not have autoclave. Microwave uses high heat to prevent the growth of unwanted micro-organisms in the media.

Materials required

- Measuring cup or a graduated cylinder (A),
- Autoclavable/ microwaveable glass bottle with cap (B; can be purchased from Fisher, Media bottles with cap- 500 ml (Cat. No. FB800500);
- Autoclave Gloves (C; Fisher Cat. No. 19-013586) or
- Oven mitts (D).

A.

B.

C.

D.

- Weighing Boat(s) (E; Fisher Cat. No. 08-732-114)
- Distilled Water (DI H2O) or purified water such as MilliQ purified Water (Alternatively, tap water will work fine for E. coli)
- LB/agar media (Purchase from Fisher, Cat No. J75851A1)
- Ampicillin sodium salt (Purchased from: Thermo Scientific Chemicals; Catalogue number: AAJ6380706); Microwave ; Scale/balance
- Bunsen burner/Alcohol burner/ Spirit lamps
- Glass/plastic petri-plates
- Incubator

E.

Protocol:

1. Mixing

a) Measure the appropriate amount of dry LB/agar powder (written on the bottle) on a scale (weighing balance).
   - Grams (g) required per liter (L) depends on the manufacturer.
   - The LB/agar we use requires 35 g/L, equivalent to 3.5 g/100 ml.

b) Add the measured LB Agar powder to a glass bottle and then add the appropriate amount of DI water or MilliQ water using a graduate cylinder.
   - We will make 200 ml, which requires 7 g dry LB/agar powder (~. This volume will vary depending upon the number of plates required. One plate can contain approximately 25mL of the LB agar media.

c) Put the cap on and shake the bottle to mix.

d) Leave space in the top of the bottle, so the bottle is not under pressure during heating
   - Do NOT fill the bottles to their maximum capacity (the bottle may crack due to pressure)
   - 1000 ml bottle should have no more than 800 ml of media
   - 500 ml bottle should have no more than 400 ml of media

e) Loosen the cap before the next step
   - The cap is loosened so that when heated, the bottle is not under pressure (pressure can cause the bottle to crack)

2. Two options: use the (a) autoclave (best) or (b) microwave

(a) Autoclave
   - Before autoclaving, loosen the cap a little on the bottle because the bottle will be under extreme pressure and high heat, which might cause it to crack and break.
   - Autoclave on liquid settings for 15 minutes following autoclave directions.
   - Be sure to follow all autoclave rules described by your facility and autoclave manufacturer.
   - The liquid and bottle will be hot after autoclaving. Wear autoclave gloves to hold the bottle.
   - Skip to Step 3.

(b) Microwave method
   - Before placing the bottle in the microwave, make sure the cap is loose to vent the pressure created inside the bottle.
   - The bottle can crack under high pressure.

- Microwave the bottle in intervals of 20 seconds until the liquid starts to boil.
- After the liquid starts boiling, with autoclave gloves, swirl the bottle in between to dissolve any remaining agar.
- The liquid and bottle will be hot. Wear autoclave gloves and mix slowly.
- Any particulates should be completely dissolved.
- Bring the liquid to boil 3-5 times.

3. Remove the agar from the microwave, and let it cool at room temperature.

- The bottle can cool at room temperature but be careful not to let it solidify. It requires approximately 15-20 min for the media in the bottle to cool down in the room temperature (25℃). To make sure that the media is cool enough to pour, touch the body of the bottle. If it is too hot to touch, let it cool for some more time. When the bottle is cool enough to be touched proceed to step 4.

4. Turn on the burner and proceed the following steps near the flame to avoid contamination.

5. After cooling, add non-autoclavable or non-microwavable reagents such as antibiotics to the media.

- The plates must be poured immediately after adding these reagents.
- If the media is too hot, it may inactivate or harm reagents such as antibiotics.

6. Pouring and Storing Plates

- Having the flame on can minimize contamination.
- Place desired number of Petri dishes on the benchtop.
- Pour a thin layer of LB Agar (20-25 ml) into each plate.

7. Let plates cool until solid (~30 minutes), then flip to avoid dripping condensation on the agar.

- One plate usually take 30-45min to solidify. However, if kept in stacks, the plates take longer time to solidify. Therefore, it is recommended to make the agar plates a day before the actual experiment.

8. Store plates in plastic bags in the fridge with your name, date made, and the name of antibiotics.

- Antibiotics can lose their activity if the plates are kept outside.
- LB Agar plates can dry out if the plates are not put in a plastic wrap.
- In most cases, it is best to use the plates within a month.

Assessment Day-1:

- Diagnostic Assessment – Monitor students preparing LB agar and LB + Amp agar plates.
- Formative Assessment -
- End of Chapter Exam Questions

- What is the term used to group the millions of prokaryotes that live on and within your biome?
  a) Microbiome
  b) Human microscopic environment
  c) Bacterial biome
  d) Prokaryote

- Using your knowledge of bacterial cells and enzyme function, explain why certain bacteria used in the bacterial art activity did not develop colors under certain incubation temperatures.

- Certain ingredients in soap are added because they can dissolve lipids. Why are these ingredients helpful in removing bacterial cells from your hands when washing?

Day 2

Activity 2: To demonstrate the presence of bacteria in the surroundings by bacterial hand printings

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Bacterial Handprints

Growing bacteria around you in plates

Background

Washing hands is one of the most important means of preventing the spread of infection.
In this simple activity, students discover the importance of good hand hygiene by comparing two agar plates of their handprints – one before washing, and one after.

Materials

1. Premade LB/agar plate
2. Marker pen
3. Hand soap, water, and paper towels

Protocol

1. Using a marker pen, label the two plates ‘before’ and ‘after’ on the underside of each plate, along with your name and date.
2. Place the agar plates on the table (the correct way up) and remove the lid.
3. Press your fingers firmly onto the agar (see figure below) without damaging the agar and then remove them.

4. Clean your hands using soap and water. Dry them well using paper towels.
5. Repeat the process for the agar plate marked ‘after’, pressing your fingers firmly onto the agar without damaging the agar.
6. Wash your hands after touching the agar.
7. Leave both plates in a safe area of the classroom to incubate at room temperature (e.g., 25ºC) or 37 ºC incubator for 24-48 hours, or until you can see some bacterial colonies.

Results and discussion

When the agar plates are ready, students can compare the 'before' and 'after' handprints. The following questions and tasks can encourage them to analyze the results:

1. What do you observe on your agar plates? What are the dots? Are there different types of dots (e.g., different colors and shapes)?

2. Is there a difference in bacterial growth between the 'before' and 'after' handprints? What does this show?

3. Count the dots on each plate to estimate the number of bacterial colonies. If there are too many dots to count, make an estimate for the whole agar plate based on one section. Record the result of bacterial colonies before and after wash and find out the percentage change
   - Percent change = [(Colony No. before wash - Colony No. after wash)/ Colony No. before wash) × 100%].

Name/ID	**Before wash**	**After wash**	**Percent change**
Example	145	140	3.5%

4. Compare your agar plates to those of other students in your class. Why do some plates have more growth than others?

5. What does this tell you about your hand hygiene or the hygiene of your peers?

Activity 3: Exploring Bacterial Diversity Through Art

Diversity of Life
Bacteria Art

Background

When most people discuss the diversity of life, they mainly consider the different plant and animal species they are more likely to see in their daily lives. However, most of the living organisms on Earth are prokaryotes. Prokaryotes are single-celled organisms that do not have a nucleus, and most are often so small that they cannot be seen with a naked eye. Both archaea and bacteria are prokaryotes, but we will only focus on bacteria in this module. Bacteria exist nearly everywhere on Earth and are critical to all ecosystems. Though some are harmful, many are beneficial to humans and the environment. As a result, we have made great use of these organisms; they are important in food production, pharmaceuticals, chemical production, water sewage treatment, and many more. Bacteria are incredibly diverse and display numerous phenotypes and functions. In this module, we will visualize different colorful bacteria and study the morphology of the colonies formed by these bacteria. This module will also help us understand the optimum growth temperature required by these bacteria and the effect of temperature on the phenotypes of the bacteria.

Materials

- Premade LB Agar plate with antibiotic

- Sterile loops or sterile wooden applicator sticks or flat toothpicks

- Bunsen burners

- Different bacterial cultures

List of colorful bacteria available

Name	Pigment color	Optimum growth temperature (˚C)
Stigmatella aurantiaca	Yellow or yellowish orange	30-37
Pseudomonas aeruginosa	Greenish	37
Serratia marcescens	Red	27-30
Micrococcus luteus	Yellow	25-30
Chromatobacterium violaceum	Violet	30-37

Protocol

1. Sterilize the inoculum loop by holding the loop in the flame until it is red hot. Ensure that the loop has cooled down by touching it on the agar plate (30 seconds to minutes).
   - Sterile sticks or pre-sterilized loops can be purchased to avoid using the flame

2. Dip the inoculum loop into the tube with bacterial culture and transfer the bacterial culture onto the end of the sterile inoculating loop.

3. Use the loop like a pen to draw on the surface of the agar plate. You can draw various shapes, write your name, or even sketch a whole scene.

4. When required, collect more bacteria on the loop. This is usually every time you have finished drawing a line. If you use more than one type of bacteria to create different colors or colony shapes in your drawing, sterilize the previous loop or use a new sterile inoculating loop for each culture.

5. Once finished, place the lid onto the plate and incubate at room temperature or 30 ˚C or 37˚C for 24–72 hours to allow the artwork to develop. The plates have to be incubated upside down in order to prevent condensation on the lid and compromise the culture.

Observation Table

	24 hours		48 hours	
Bacteria	30̊ C	37̊ C	30̊ C	37̊ C
Stigmatella aurantiaca				
Pseudomonas aeruginosa				
Serratia marcescens				
Micrococcus luteus				
Chromatobacterium violaceum				

- Assessment Day-2:
    - Diagnostic Assessment – Venn Diagram Discussion Sheet (Top 1/2 of Diagram)
    - Formative Assessment – Think-Pair-Share Discussion over Venn Diagram Note Sheet
    - Summative Assessment – Venn Diagram Discussion Sheet (Bottom ½ of Diagram).

- Supplementary File includes PowerPoint presentation.

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