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They Make Probiotics for My Home? Analyzing Microbe-Based Household Cleaners

Overview

You can now purchase probiotic household cleaners that claim to contain living microbes. These microbes are supposed to help clean your home by breaking down dirt and they may prevent more harmful microbes from being able to live on these surfaces. However, companies don't always identify what microbes they've added to their cleaners, so consumers don't fully know what they are buying. In this lab lesson, students will be microbe detectives and will *grow*, *see*, and *sequence* the microbes from a readily available probiotic cleaner. They will first grow and isolate the microbes from the probiotic cleaner, see and characterize the microbes using light microscopy and/or SEM, and use DNA sequencing of the 16s rRNA gene to determine the identity of the microbes in the cleaner. This is a multi-lab activity and can be used as a capstone project for a microbiology course or, alternatively, as the basis for an undergraduate research project.

Key Search Words

Microbes, microbiome, bacteria, probiotic, built environment, microscope, SEM, Gram stain, 16s rRNA, DNA sequencing, microbiology, hands-on, laboratory

Learning Objectives

Learning objectives or outcomes are what students are expected to know after completing the lesson.

- At the end of this lesson, students will be able to define microbiome of the built environment and describe two ways that probiotic bacteria impact the environmental microbiome.
- At the end of this lesson, students will be able to distinguish bacterial images produced by a scanning electron microscope from those produced by a compound light microscope.
- At the end of this lesson, students will be able to perform an isolation streak plate to isolate individual bacteria from a mixture.
- At the end of this lesson, students will be able to prepare a bacterial sample for viewing using SEM.
- At the end of this lesson, students will be able to prepare a Gram-stained bacterial sample and view it using light microscopy.
- At the end of this lesson, students will be able to explain and perform the steps required for 16s rRNA sequencing of a DNA sample.

Curriculum Alignment

The activities of this lesson address the following statements from the ASM Curriculum Guidelines for Undergraduate Microbiology:

- Students should be able to properly prepare, view, and analyze specimens using microscopy.
- Students should be able to apply appropriate microbiological, molecular, serological, and bioinformatics methods to isolate and differentiate microorganisms.
- The health of the environment and all organisms (microbes, plants, humans, other animals) are closely linked and interdependent, as described by the One Health paradigm.
- Humans leverage microbes and their products to address problems and improve quality of life.

Classroom time required

To complete all components of this lesson will require time across several labs (3-4 at a minimum), including overnight incubation time for the microbes to grow. However, most individual activities can be completed in 30 minutes-two hours.

If faculty must prepare their own materials for these activities, there will be additional preparation time needed prior to *each* activity (0.5-1hr/activity).

Additionally, the activities are intended to be somewhat modular (grow, see, sequence), so not all activities are required, and faculty can select the activities that fit their lab schedules and that are appropriate for their students. Recommended module combinations: Grow+See+Sequence, Grow+See, Grow+Sequence, or just the Grow module on its own.

Grow the Microbes

- This is the shortest activity to complete and should take no longer than 30 minutes for most students depending on students' prior experience growing (culturing) microbes on solid media.
- The microbes will need to be incubated overnight (and ideally for a full 24-hour period) before there will be sufficient growth for easy viewing by the students and for use in the subsequent microscopy and sequencing activities.

See the Microbes

- Light microscopy: Slide preparation, Gram-staining, and light microscopy will take one-two hours depending on students' prior experience preparing microscopy slides.
- SEM microscopy: Sample preparation for SEM can be relatively quick and require about 30 minutes for students to apply the microbial sample to the SEM disk (wafer). There is additional hands-off time needed to let the sample dry (ideally overnight in a laminar flow hood if available).

Sequence the Microbes

- 16s rRNA sequencing: Preparing samples for 16s rRNA sequencing will require one-two hours on at least two separate lab days. There is additional hands-off time after the first lab day for the PCR to run.
- The PCR samples will need to be sent to an external lab/company for 16s rRNA sequencing. It usually takes several days to receive the sequencing results

Materials & Technology

Materials are listed as follows as if each student is doing the activity individually unless stated differently. Adjust materials as needed if students will work in pairs or groups.

General Supplies

- PPE (disposable gloves (nitrile), goggles, lab coats)
- Sharpies or similar lab marker
- Lab tape
- Biohazard bags
- Racks for holding test tubes/Falcon tubes and microcentrifuge tubes
- Electronic device to view the sequencing results (students can likely view this on their phone or laptop)

Grow the Microbes (Initial isolation of microbes from cleaner)

- Mrs. Meyers Probiotic Multi-Surface Cleaner Concentrate divided into tubes (5ml/student is plenty)
- Sterile cotton swabs (individually wrapped or bulk wrapped is fine) (1-2 swabs/student)
- Sterile test tubes or sterile disposable 15ml conical ("Falcon") tubes (1 tube/student)
- Growth media (LB agar or tryptic soy agar prepared petri plates) (1 plate/student)

Grow the Microbes (Isolation plating of microbes to new agar)

- Sterile disposable inoculating loops (individually wrapped or bulk wrapped is fine) (1-2/student)
- Growth media (LB agar or tryptic soy agar prepared petri plates) (1-2 plates/student)
- Each student should be given their agar plate from the first *Grow the Microbe* activity. There should be growth on the plate (as colonies or large patches). If a student's plate has no growth, they can use microbes from another student's plate to complete this activity.

See the Microbes (Gram-stain, light microscopy)

- Each student should be given their agar plate from the *Grow the Microbe* isolation plate. There should be individual colonies (small circles) of growth on the plate. If a student's plate has no growth, they can use microbes from another student's plate to complete this activity.
- Sterile disposable inoculating loops (1-2/student)
- Microscope slides (1-2 slides/student)

- Water (sterile, if available)
- Compound light microscopes (1 microscope/student or student pair) (microscopes should have at least a 40/60X objective)
- Lens paper
- Gram stain kits (1 kit/3-4 students)
- Waste container to collect the Gram stain reagents if staining is done at the lab bench.

See the Microbes (SEM)

- Each student should be given their agar plate from the *Grow the Microbe* isolation plate. There should be individual colonies (small circles) of growth on the plate. If a student's plate has no growth, they can use microbes from another student's plate to complete this activity.
- Tube of sterile *liquid* growth media (LB or tryptic soy broth) (TS broth can be purchased from Carolina Bio already in tubes) (1 tube of broth/sample prepped for SEM)
- Sterile disposable inoculating loops (1 loop/sample prepped for SEM)
- 1ml plastic transfer pipette (1 loop/sample prepped for SEM)
- SEM "wafer" (usually supplied by the facility performing the SEM)
- Optional: laminar flow hood (chemical hood or biosafety cabinet) to help dry samples
- Empty petri dishes or other containers to transport SEM wafers to SEM facility

Sequence the Microbes

- Each student should be given their agar plate from the *Grow the Microbe* isolation plate. There should be individual colonies (small circles) of growth on the plate. If a student's plate has no growth, they can use a microbial colony from another student's plate to complete this activity.
- Tube of sterile *liquid* growth media (LB or tryptic soy broth) (TS broth can be purchased from Carolina Bio already in tubes) (1 tube of broth/student or group doing each DNA extraction)
- Sterile disposable inoculating loops (1 loop/student or group doing each DNA extraction)
- Micropipettes (p20, p200, p1000) and tips (1 set of micropipettes/student pair or lab bench)
- Sterile water (ideally, DNAse/RNAse free water)
- Genomic DNA extraction kit (Important: The kit must be for the extraction of genomic DNA and not plasmid DNA)
    - Example kits: Qiagen QIAamp DNA Mini Kit, Zymo Research Quick-DNA Miniprep Kit
    - Select the number of "preps" in the kit based on the number of students/groups that will perform the DNA extraction.
- Sequencing facility should be selected that can complete 16s rRNA sequencing and return a report of the results.
    - Example: Zymo Research Targeted Amplicon Sequencing using the V3V4 primers for $75/sample
    - These companies will often do free consultations to help make sure you order the correct sequencing.

Safety

The microbes in the cleaner are assumed to be safe to handle. However, since their identity is unknown, standard microbiological personal protective equipment (PPE) should be worn by the students while performing all parts of this lesson. This should include lab coats and gloves. Goggles are also recommended.

Lab benches should be cleaned before and after lab activities with an appropriate disinfectant (70% ethanol, Conflikt, or similar).

Students should also be reminded to wear gloves for the entire duration of the DNA extraction procedure. This provides protection to the students but, also importantly, protects the DNA from enzymes present on the skin that can cause the DNA to be degraded (which will interfere with the sequencing process).

Students (and teachers) should always wash their hands after completing the lab activities and before leaving the lab, even if gloves have been worn the entire time.

Teacher Preparation for Activity

Grow the Microbes (Initial isolation of microbes from cleaner)

- Before Lab:
    - There is no advanced prep required for this lab other than collecting the needed supplies.
    - Optional: Remove the agar plates from the fridge a couple of hours before use. The plates are easier to handle and write on when warmed to room temperature.
    - Optional: The faculty can dilute the cleaner in advance instead of having the students do this individually.
- After Lab:
    - After 24 hours or overnight, place the agar plates with microbial growth in the fridge until the next lab period.

Grow the Microbes (Isolation plating of microbes to new agar)

- Before Lab:
  - There is no advanced prep required for this lab other than collecting the needed supplies.
  - Optional: Remove the microbial plates from the **second** *Grow the Microbes* activity from the fridge a couple of hours before use. The plates are easier to handle and write on when warmed to room temperature.
- After Lab:
  - The agar plates from the first *Grow the Microbes* activity can be stored in the fridge in case students need to re-use these plates.
  - After 24 hours or overnight, place the new agar plates with isolated microbial growth (colonies) in the fridge until the next lab period.

See the Microbes (Gram-stain, light microscopy)

- Before Lab:
  - There is no advanced prep required for this lab other than collecting the needed supplies.
  - Optional: Remove the microbial plates from the **second** *Grow the Microbes* activity from the fridge a couple of hours before use. (Ideally these plates have individual colonies of growth.) The plates are easier to handle and write on when warmed to room temperature.
- After Lab:
  - The agar plates from the **second** *Grow the Microbes* activity can be stored in the fridge in case students need to re-use these plates.
  - If Gram stain reagents were collected at the students' benches, these should be disposed of appropriately.
    - Small amounts can be flushed down the sink with water. Large amounts are usually collected as chemical waste. Consult your EHS office if appropriate.

See the Microbes (SEM)

- Before Lab:
  - There is no advanced prep required for this lab other than collecting the needed supplies.
  - Optional: Add 5-10ml liquid broth into sterile tubes if prepared broth tubes were not purchased.
  - Optional: Remove the microbial plates from the **second** *Grow the Microbes* activity from the fridge a couple of hours before use. (Ideally these plates have individual colonies of growth.) The plates are easier to handle and write on when warmed to room temperature.
- After Lab:
  - The agar plates from the **second** *Grow the Microbes* activity can be stored in the fridge in case students need to re-use these plates.
  - After overnight incubation, place the culture tubes in the fridge until the next lab period.
  - After drying the SEM wafers overnight, collect the wafers in an empty petri dish or other container to transport them to the SEM facility. A small piece of plain tape on the bottom of the wafer will hold it in place in the container.

Sequence the Microbes

- Before Lab:
  - This lab typically requires the largest amount of advanced preparation, so plan accordingly.
  - It is recommended to set aside 1-2 hours to divide the reagents of the DNA extraction kit into individual quantities needed by each student/group.
  - It's often necessary to provide students a small excess in volume than what is stated in the procedure to account for pipetting errors.
  - Optional: Add 5-10ml liquid broth into sterile tubes if prepared broth tubes were not purchased.
  - Optional: Remove the microbial plates from the **second** *Grow the Microbes* activity from the fridge a couple of hours before use. (Ideally these plates have individual colonies of growth.) The plates are easier to handle and write on when warmed to room temperature.
  - **Day before lab:** If not done by the student, prepare liquid cultures of the microbial samples. Pick a colony from the agar plates from Lab 3 of the *Grow the Microbes* activity. It is important that you pick a colony that is isolated from any other growth to make sure the culture contains only one type of microbe.
- After Lab:
  - Leftover liquid cultures can be autoclaved and disposed of using proper biosafety procedures. If no autoclave is available, the liquid cultures can be carefully poured into a beaker of 10% bleach and allowed to sit for at least 10 minutes before pouring down the drain.
  - Leftover student tubes of reagents from the DNA extraction procedure can be disposed of in biohazard disposal.
  - Any remaining extraction reagents in the original bottles will stay good for several weeks/months if stored as described in the kit instructions.

Student Preparation for Activity

If this activity is being used as a capstone project, students should review the concepts connected to each of the lesson activities. This would include:

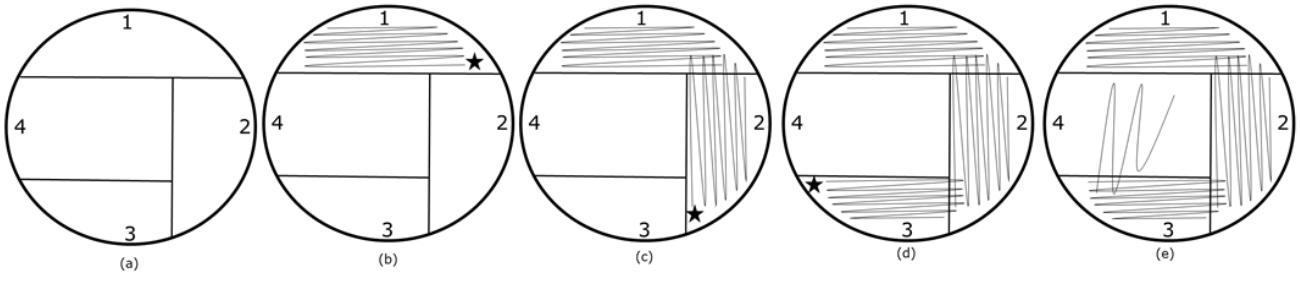
- Reviewing metric measurements for volume (mL and uL) and conversion of cups/gallons into metric units.
- Reviewing the goal of isolation streak plating and how to perform this procedure.
- Reviewing the Gram stain and the differences between Gram + and Gram – bacteria.
- Reviewing the differences between light and electron microscopy.
- Reviewing how 16s rRNA is used to help identify prokaryotic organisms.
- Reviewing the process of DNA isolation and DNA sequencing.

If any of these concepts have not already been covered in lecture or lab, it is recommended that the teacher do an overview of the topic or skill prior to beginning the activity.

Procedure

Grow the Microbe

- Lab 1: Initial Isolation of Microbes from Cleaner
    - Dilute the probiotic cleaner in water according to the directions on the bottle.
        - *Teacher Tip:* A small volume (5-10ml) of cleaner is needed, so adjust the final volumes of the cleaner and water down or make a single diluted stock for use by all students.
    - Label the bottom of an agar plate with the following: Initials, Date, Sample Source
    - Dip a sterile swab into the diluted cleaner and gently swab the surface of the agar plate in a zig zag motion to cover the entire plate surface.
    - Dispose of the swab in biohazard waste.
    - Incubate the agar plates *upside down* for 18-24hr at room temperature.
    - Agar plates should be stored *upside down* in the refrigerator until the next lab.
        - *Teacher Tip:* Incubating and storing the plates upside down prevents condensation from collecting on the plate surface.
- Lab 2: Isolation Plating of Microbes to New Agar
    - Examine the agar plate from *Grow the Microbe* Lab 1 for microbial growth.
        - *Teacher Tip*: If a student's plate has no microbial growth, they should collect a sample from the plate of a classmate to complete the activity for Lab 2.
    - Label the bottom of a *new* agar plate with the following: Initials, Date, Sample Source
    - Use a disposable inoculating loop to collect a small amount of microbial growth from the agar plate from Lab 1.
    - Prepare an "isolation streak plate" according to the image below using the microbial sample on the inoculating loop.
        - *Teacher Tip*: Drawing the lines shown in (a) onto the bottom of the agar plate can be a helpful guide for students.
        - *Teacher Tip*: If sufficient loops are available, students can use a new loop for each section (as indicated by the stars in the image).
    - Dispose of the inoculating loops in biohazard waste.
    - Incubate the agar plates *upside down* for 18-24hr at room temperature.
        - Agar plates should be stored *upside down* in the refrigerator until the next lab.



- Lab 3: Examination of Isolation Plates
    - Examine the isolation plates from *Grow the Microbe* Lab 2 for microbial growth.
        - If the procedure was performed correctly, individual colonies of growth should be visible across the surface of the agar plate.
        - *Teacher Tip*: If a student's plate has no microbial growth or growth without individual colonies, students can redo their isolation plate or collect a colony from a classmate's plate if needed for the *See the Microbe* or *Sequence the Microbe* procedures.

See the Microbes (Gram-stain, light microscopy)

Students will need to complete Labs 1-3 from the Grow the Microbe activity before completing this activity.

- Lab 1:
  - Collect the agar plate from *Grow the Microbe* Lab 3 containing individual colonies.
    - *Teacher Tip:* If the probiotic cleaner has more than one type of microbe, colonies with different appearances may be visible on the agar plates. Based on time and resources, students can make slides and view all the different microbes on their plates or examine just one microbe.
  - Microbial Slide Preparation
    - Add a small drop of water to the middle of a microscope slide.
      - *Teacher Tip:* A large amount of water on the slide will take a long time to air dry.
    - Collect a single colony from the agar plate from *Grow the Microbe* Lab 3 and mix it with the water on the slide.
    - Spread the sample out to make a thin layer across the slide surface.
    - Let the slide air dry for 10-20 minutes until the sample is completely dry.
    - Pass the slide briefly through the flame of a Bunsen burner.
    - The slide is ready to be Gram-stained but can also be stored and stained during a later lab.
  - Gram Stain Procedure
    - Add crystal violet to the slide. Wait one minute.
    - Rinse the slide with deionized water until it rinses clear.
    - Add Gram's Iodine to the slide. Wait one minute.
    - Rinse the slide with deionized water until it rinses clear.
    - Add decolorizer to the slide until it rinses clear. **This will only take 5-8 seconds.**
    - Rinse the slide with deionized water.
    - Add safranin to the slide. Wait one minute.
    - Rinse the slide with deionized water until it rinses clear.
    - Gently dry the slide with bibulous paper or with a piece of lens paper. Be careful not to wipe off the sample.
      - *Teacher Tip:* Gram stain reagents stain clothing and skin. These reagents should be handled carefully. Perform the procedure over the sink or over a waste container at the lab bench.
  - Light Microscopy
    - Examine the slide using a light microscope. Cell shapes should be clear at 40X magnification but 100X magnification will provide the clearest view of the cell shapes and arrangements.
    - Slides can be placed in biohazard waste or glass waste after viewing.

See the Microbes (SEM)

Students will need to complete Labs 1-3 from the Grow the Microbe activity before completing this activity.

- Lab 1:
  - Collect the agar plate from *Grow the Microbe* Lab 3 containing individual colonies.
    - *Teacher Tip:* If the probiotic cleaner has more than one type of microbe, colonies with different appearances may be visible on the agar plates. Due to the cost of SEM, it is recommended that only one-two samples of each different microbe are prepared SEM analysis.
  - Broth Culture Preparation
    - Label a tube of sterile broth with the following: Initials, Date, Sample Source
    - Use a disposable inoculating loop to collect an individual colony from the agar plate from *Grow the Microbe* Lab 3.
    - Gently swirl the inoculating loop into a tube containing sterile broth.
    - Close the tube, leaving the cap slightly loose to allow oxygen to enter the tube.
    - Dispose of the inoculating loops in biohazard waste.
    - Incubate the broth cultures for 18-24hr at room temperature.
      - *Teacher Tip:* Students can prepare their broth cultures on Lab 3 of the *Grow the Microbe* procedure when they examine their isolation plates for colonies. Alternatively, broth cultures can be prepared by the instructor the day before lab.
- Lab 2:
  - SEM Sample Preparation
    - Place an SEM wafer in a small petri dish or other container for easier holding and transport.
    - Using a (1ml) transfer pipette, collect a small volume of the liquid culture from the tube.
    - Apply a small drop of the culture directly onto the surface of the SEM wafer.
    - Allow the sample on the SEM wafer to thoroughly dry.
      - *Teacher Tip*: Allow samples to dry overnight on the benchtop or in a laminar flow hood.
    - Transport or send the samples to the SEM facility for observation and image collection.

Sequence the Microbes (SEM)

Students will need to complete Labs 1-3 from the Grow the Microbe activity before completing this activity.

- Lab 1:
    - Collect the agar plate from *Grow the Microbe* Lab 3 containing individual colonies.
        - *Teacher Tip:* If the probiotic cleaner has more than one type of microbe, colonies with different appearances may be visible on the agar plates. Each student should select only one colony to use for the DNA extraction procedure.
    - Broth Culture Preparation
        - Label a tube of sterile broth with the following: Initials, Date, Sample Source
        - Use a disposable inoculating loop to collect an individual colony from the agar plate from *Grow the Microbe* Lab 3.
        - Gently swirl the inoculating loop into a tube containing sterile broth.
        - Close the tube, leaving the cap slightly loose to allow oxygen to enter the tube.
        - Dispose of the inoculating loops in biohazard waste.
        - Incubate the broth cultures for 18-24hr at room temperature.
            - *Teacher Tip:* Students can prepare their broth cultures on Lab 3 of the *Grow the Microbe* procedure when they examine their isolation plates for colonies. Alternatively, broth cultures can be prepared by the instructor the day before lab.
- Lab 2:
    - DNA Extraction Procedure
        - Follow the procedure provided in the DNA extraction kit to isolation DNA from the microbial cells.
            - *Teacher Tip:* DNA extraction procedures commonly have several wash steps to clean the DNA for future use. It can be easy to accidentally discard the DNA instead of the wash liquid. Caution students to read the directions carefully and follow the procedure slowly to help avoid mistakes.
            - *Teacher Tip*: Each DNA extraction kit usually highlights where the procedure can be stopped if the procedure can't be completed in a single lab session.
- Lab 3:
    - 16s rRNA Sequencing Procedure
        - Prepare the DNA samples as recommended by the sequencing facility selected for sequencing.
        - Ship DNA samples to sequencing facility.
            - *Teacher Tip:* The sequencing facility should provide instructions on how to prepare and ship the DNA samples. Follow their instructions to ensure the best chance for the sequencing process to work correctly.

Differentiation

- Scaffolded Learning
    - Have students read the lab procedure prior to the day's lab.
    - Assign a short pre-lab quiz or assignment to review important procedures or concepts for the day's lab.
    - Assign a short post-lab quiz or assignment to review results or data analysis after completing the lab.
    - If procedures are new to the students, simplified images of the procedure (for instance, the isolation plating image) can be projected as a reference as the students complete the procedure.
- Lab Logistics
    - Have students work in pairs or groups if supplies are not available for individual work or if students don't have prior experience with microbiology procedures.
    - Many general microbiology labs should be able to grow microbes from the probiotic cleaner and Gram stain and visualize the microbes with light microscopy.
        - These procedures will not allow the students to identify the species of microbes in the cleaners.
        - Students should be able identify the general group of microbes present if sequencing is not an option and could research possible microbes with the growth and microscopy data collected.

Assessment/Check for Understanding

Possible assessment types:

- Students can collect data and results across all three lessons and then submit their findings (and the potential identity of their microbe) in a lab report style write-up.
- Students can be given shorter "pre-lab" or "post-lab" quizzes or question sets for each lesson activity to specifically review the content for that lesson. (For instance, a post-lab assignment could be assigned after students complete both *Grow the Microbe* activities, and then again after the other two activities.)
- If the faculty wants to emphasize the procedures, students can be evaluated on a "skill's test" in which they perform a particular lab skill (such as Gram staining a slide and viewing it under the microscope).
- Students could be asked to give a short presentation of their results or to give a short presentation on an extension topic (environmental microbiomes, probiotics, etc.). These presentations could be before the lesson activities to introduce important concepts or could be at the end of the lesson activities to help summarize the

concepts or have students thinking “out” and reflecting on additional applications for the lesson procedures or additional ways to potentially engineer the built environment.

Possible topics or questions to have students address in an assessment:

- If students are successful in identifying the microbes in the cleaner, have them explore why these specific microbes would be intentionally spread around the home. How are they the same or different from microbes that we try to eliminate from our homes?
- Have students discuss why 16s rRNA sequencing is used to help determine the identity of prokaryotes. Additionally, students could discuss why 16s rRNA is not used for the evaluation of eukaryotes.
- Have students discuss the difference between pathogenic and nonpathogenic microbes and how each group should be managed in the built environment.
- If emphasizing the activity procedures, have students arrange the steps of a procedure in the correct order (correctly order the steps of the Gram stain, correctly order the steps needed to sequence a DNA sample).
- Provide students “incorrect” results (for example, an image of a poorly done isolation plate) and have students identify mistakes that could produce the given results and recommendations for fixing the mistake.

Required resources

There are no required external websites or programs needed to complete the activities in this lesson.

Supplemental resources

The American Society for Microbiology (ASM) has a website with a lot of protocols for basic microbiology techniques: https://asm.org/browse-by-content-type/protocols . This is a useful resource for additional background and procedural information for many of the techniques in this lesson (isolation streak plates, Gram stains, etc.).

Author comments

I tried to write the lesson plan where the main procedures are separated into individual modules. Since the entire set of procedures will take several labs and may require resources that aren’t available or appropriate for every student group, faculty can choose the modules that work for their lab or course. However, the *See the Microbe* and *Sequence the Microbe* protocols both require microbial samples that have been grown from the probiotic cleaner, so faculty should generally start with the *Grow the Microbe* protocol first.

While this lesson was written with the goal of identifying and studying the microbes in a probiotic cleaner, other starting samples could possibly be used. Depending on the focus of the lab and interests of the students, this lesson could also be used to explore:

- Probiotic tablets (taken as a supplement)
- Probiotic cosmetics
- Microbe-containing foods (fermented foods, yogurts, etc.)

If one of these other microbe-containing samples is used, it is recommended that the faculty try to grow the microbes from the product prior to beginning this lesson to verify that the microbes grow. Some of the microbes in these other products may need different types of growth media or different temperatures for growth than what is listed in this lesson plan.

Sources

https://mrsmeyers.com/products/probiotic-multi-surface-concentrate-lemon-verbena?variant=50673217470738

Orlando, Anthony (2025). The Good Microbes Around Me [Unpublished manuscript]. Wake Tech Community College BIO275, Raleigh.

Appendices

Please attach any student handouts (with answer keys if available) to the end of your lesson.