Is That Really Bacteria Living in My Yogurt?

https://www.sciencebuddies.org/science-fair-projects/project-ideas/FoodSci_p072/cooking-food-science/bacteria-living-in-yogurt (http://www.sciencebuddies.org/science-fair-projects/project-ideas/FoodSci_p072/cooking-food-science/bacteria-living-in-yogurt)

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Experimental Procedure

Working with Biological Agents

For health and safety reasons, science fairs regulate what kinds of biological materials can be used in science fair projects. You should check with your science fair's Scientific Review Committee before starting this experiment to make sure your science fair project complies with all local rules. Many science fairs follow Intel® International Science and Engineering Fair (ISEF) regulations. For more information, visit these Science Buddies pages: Project Involving Potentially Hazardous Biological Agents (http://www.sciencebuddies.org/science-fair-projects/competitions/biological-agents-regulations) and Scientific Review Committee (http://www.sciencebuddies.org/science-fair-projects/competitions/scientific-review-committee-src). You can also visit the webpage ISEF Rules & Guidelines (https://www.societyforscience.org/isef/international-rules/) directly.

Bacteria are everywhere. They live within the human gut, all around us in the air, on surfaces around our homes and outdoors, and basically in every corner of our environment. We come in contact with bacteria on a daily basis. So to make sure your science project shows whether live bacteria are in the yogurt, you need to avoid growing bacteria from other places. Many of the steps are about how to be extra careful and clean, so you do not end up growing these other bacteria.

Culturing Yogurt Bacteria in an Anaerobic Chamber

1. Make sure the Pyrex® bowl, cutting board, and medicine droppers are clean.
2. If you are using a wooden cutting board, have an adult help you cut a piece of plastic wrap large enough to cover the surface of the cutting board. Then tape the edges of the plastic wrap around the cutting board, covering its surface.
3. Put on disposable gloves to avoid getting any bacteria from your hands on the agar plates.
4. Moisten a paper towel with some 70% isopropyl rubbing alcohol. Wipe down the cutting board (or plastic wrap on top of the cutting board) with the rubbing alcohol-moistened paper towel.
    a. Wiping rubbing alcohol on something helps to **sterilize** it, or kill bacteria.
    b. This will help prevent bacteria that are not from the yogurt from growing on your plates.
5. Place four agar plates on the cutting board.
    a. Be sure not to open the plates when handling them. Opening the plates can cause bacteria from the air to float into the plates and grow there.
6. Use the permanent marker to label the bottom of each plate "1," "2,"3" or "4". (The "bottom" is the side filled with agar.)
7. Suck up rubbing alcohol into one of the medicine droppers. Hold the rubbing alcohol in the dropper for 10 seconds. Then squirt the rubbing alcohol onto a clean paper towel. Rub the outside of the dropper with the paper towel.
    a. Set the dropper down but propped up so that the tip is not touching anything. You can use the moist paper towel to prop it up, as shown in Figure 2.
    b. Repeat this step with the second dropper so that you have two sterilized droppers.
    c. If either dropper does not look dry, wave it around a little for ten or more seconds until it looks dry.

Figure 2. When you have sterilized a medicine dropper, prop it up somewhere, such as on the moist paper towel (folded on itself), to prevent its tip from touching anything.

8. Open a fresh yogurt container.
9. Using one of the sterilized droppers, mix the top part of the yogurt a little and then suck up a lot of yogurt, filling most of the dropper. Do not let the dropper's tip touch anything other than the yogurt
10. One at a time, open the agar plates labeled 1, 2, and 3, and add two drops of yogurt to each plate. Add the drops into the center of the plates, one drop on top of the other. Close each plate before moving on to the next one. **Do not** add yougurt to plate 4.
11. Use the second dropper to suck up distilled water. Add two drops of distilled water to the center of each plate, one drop on top of the other, just as you did with the yogurt. This time include plate 4. Plate 4 will be a *control*, to make sure that you are not growing bacteria from the distilled water.
12. Use clean swabs to spread the yogurt and distilled water around the plates in zigzags. Use a new swab for each plate.
13. Apply even pressure with the swab, trying not to puncture the agar.
14. Evenly spread and distribute the yogurt and water drops, leaving no large clumps.
15. Do not set the lids down on anything when you open the plates. Because of this you may want a helper to open the plates for you. If you have a helper do this, make sure they put disposable gloves on too.
16. Prepare each plate one at a time. When you have finished with one plate, quickly put its lid back on. Try not to spend too much time on a plate, because leaving its lid open could cause bacteria that is not from the yogurt to grow on the plate.
17. When you have finished spreading the yogurt on each plate, make sure to replace the lids on the plates and let them sit in the middle of the cutting board.
    a. Letting the plates sit for a few minutes will help the yogurt attach to the agar.
18. Prepare the anaerobic chamber by lining the clean edge of the Pyrex bowl with a thick coil of Play-Doh®, as shown in Figure 3.
    a. You can stretch one coil out to cover more of the edge so long as each part of the edge has some Play-Doh. Try to distribute the Play-Doh evenly on the bowl's edge.

Figure 3. Thickly coil Play-Doh around the edges of the Pyrex bowl, as shown here.

18. Stack the plates and flip them so that they are upside down (with the agar on the top of each plate).
19. Arrange a tea light and the plates on the cutting board so that the bowl can cover them when it is upside down.
    a. The tea light can go on top of the stack of plates or to the side of them.
20. Ask an adult to light the tea light.
21. Put the bowl upside down on top of the plates and the lit tea light, as shown in Figure 4.
    a. Press down hard on the bowl so that the Play-Doh seals the bowl's edges on the cutting board.
    b. If the bowl is completely sealed, you have just created an anaerobic chamber. The tea light flame should consume any remaining oxygen inside of the sealed chamber. Once the oxygen is all gone, the flame will burn out, since fire needs oxygen to burn.

Figure 4. Stack the plates you prepared upside down on top of the cutting board, set the tea light candle next to the plates (the candle is on the left of the plates in this picture), light the tea light, and then flip the Pyrex bowl upside down on top of the plates and tea light. Press down hard on the bowl to seal the bowl's edges. The candle should go out (as it has here) within a minute.

22. Watch the tea light. It should go out within about one minute of sealing the anaerobic chamber.
    a. If the tea light stays lit for more than two minutes, check to make sure that the anaerobic chamber is sealed. Add more Play-Doh around the edge of the bowl if needed.
23. Leave the plates in a warm room, ideally between 22 to 27 degrees Celsius (about 72 to 80 degrees Fahrenheit), for six days.
    a. After the plates have been in the anaerobic chamber for two days, check the Play-Doh on the anaerobic

chamber. If there are large cracks in the Play-Doh, as shown in Figure 5, carefully remove the Pyrex bowl and add fresh Play-Doh to seal the cracks.

    i. Ask an adult to help you re-light the candle and seal the bowl on top of the plates and candle again, pushing down hard on the bowl to seal it with the Play-Doh.

b. Continue checking the anaerobic chamber daily to see if large cracks in the Play-Doh develop. If they do, repair them as described in part a of this step.

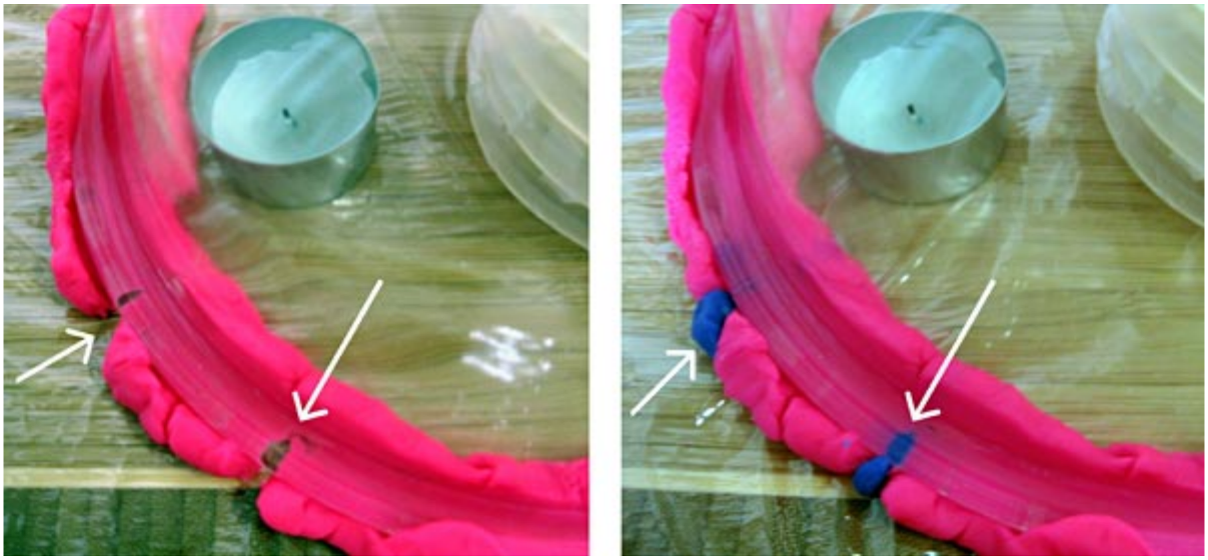

Figure 5. Check the anaerobic chamber after two days. If there are large cracks in the Play-Doh (as shown on the left, with the white arrows pointing to cracks), then carefully remove the Pyrex bowl and add fresh Play-Doh to seal the cracks, as shown on the right (using fresh blue Play-Doh, as the arrows point out).

24. After the plates have been in the anaerobic chamber for six days, carefully remove the Pyrex bowl and take a look at the agar plates.
    a. In your lab notebook, make a data table like Table 2.
    b. Do you see anything on the plates, such as small spots, that could be bacteria colonies? If so, do some of the spots look different from others (in shape, texture, or color), possibly indicating that there are different types of bacteria colonies? Do some plates have more spots than others, or are all three plates similar? Write your answers and other observations in the data table in your lab notebook.
    c. Plate 4 should have had only distilled water on it, with no yogurt. Do you see anything on plate 4 that could be bacteria colonies? If so, does it look like this plate has more or fewer bacteria colonies than the plates with yogurt? What does this tell you? Record your observations. *Hint:* Re-read step 13c.
    d. *Tip:* If there is a lot of moisture on the inside of the lid making it difficult for you to see any potential bacteria colonies, you can hold the plate vertically (so it is not flat) and tap one end on a hard surface several times to make the moisture roll off of the lid.
    e. If you have a camera, take some pictures of your plates for your lab notebook or your Science Fair Project Display Boards (http://www.sciencebuddies.org/science-fair-projects/science-fair/science-fair-project-display-boards).
        i. Putting the plates on a black background, such as a sheet of black construction paper, may help make your results more visible.
25. What do your results tell you about whether yogurt has living organisms in it?
26. If you are doing this experiment as a science fair project, repeat the procedure two more times. Scientists always repeat their experiments to make sure their findings are true and repeatable.

Agar Plate	Are Bacteria Colonies Visible?	Are There Different Types of Bacteria Colonies?	Other Observations
#1			
#2			
#3			
#4			

Table 2. Record your results and observations in your lab notebook in a data table like this one.

Bacterial Safety

Bacteria are all around us in our daily lives and the vast majority of them are not harmful. However, for maximum safety, all bacterial cultures should always be treated as potential hazards. This means that proper handling, cleanup, and disposal are necessary. Below are a few important safety reminders.

- Keep your nose and mouth away from tubes, pipettes, or other tools that come in contact with bacterial cultures, in order to avoid ingesting or inhaling any bacteria.
- Make sure to wash your hands thoroughly after handling bacteria.
- **Proper Disposal of Bacterial Cultures**
    - Bacterial cultures, plates, and disposables that are used to manipulate the bacteria should be soaked in a 10% bleach solution (1 part bleach to 9 parts water) for 1–2 hours.
    - Use caution when handling the bleach, as it can ruin your clothes if spilled, and any disinfectant can be harmful if splashed in your eyes.
    - After bleach treatment is completed, these items can be placed in your normal household garbage.
- **Cleaning Your Work Area**
    - At the end of your experiment, use a disinfectant, such as 70% ethanol, a 10% bleach solution, or a commercial antibacterial kitchen/bath cleaning solution, to thoroughly clean any surfaces you have used.
    - Be aware of the possible hazards of disinfectants and use them carefully.