

Cultivation of Bacteria from Commercial Yogurt

Introduction: Yogurt is produced by the fermentation of milk. Two of the bacteria found in yogurt are *Lactobacillus bulgaricus* and *Lactococcus thermophilus*. These are lactose fermenting bacteria that produce lactic acid from the lactose in milk. The lactic acid they produce gives yogurt its sour taste. These bacteria are anaerobes: they grow best in the absence of oxygen. They are also thermophiles: they grow best at fairly high temperatures, about 40-42° C.

Objective: To cultivate *Lactobacillus bulgaricus* and *Lactococcus thermophilus* from yogurt on agar plates.

Safety: The Bunsen burner is a fire hazard. Hair and sleeves must be kept well away from the flame. Students should know where fire extinguishers are and how to use them.

Materials - per group

- sample of yogurt (best results are obtained with very fresh Yoplait Custard Style yogurt; Pevely yogurt also works well)
- MRS plate
- inoculating loop
- Bunsen burner
- marking pen

Per class

- airtight wide-mouth screw cap jar (gallon-size screw cap jars from restaurant)
- small votive candle
- parafilm
- black construction paper
- flashlights
- magnifying glass with at least 6X power
- incubator (37° C)
- Drierite

Procedure - Day 1

1. Label the bottom of the MRS agar plate with your group number or initials and the date.
2. Sterilize the inoculating loop and allow it to cool for about 30 seconds.
3. Use the quadrant streak technique as described below to inoculate the yogurt on the plate:
 - a) Remove a small amount of yogurt with the inoculating loop and gently streak it over a quarter of the plate using a gentle back and forth motion.
 - b) Flame the loop again and allow it to cool. Going back to the edge of the area that you just streaked, extend the streaks into the second quarter of the plate.
 - c) Flame the loop again and allow it to cool. Going back to the area that you just streaked, extend the streaks into the third quarter of the plate.
 - d) Flame the loop again and allow it to cool. Going back to the area that you just streaked, extend the streaks into the fourth quarter of the plate.
4. Give your plate to your teacher and watch as the plates are put in the CO₂ jar by your teacher as described below.
 - a) Pour about a quarter to a half cup of drierite in the bottom of a wide-mouth screw cap jar and place the inoculated plates on top of the drierite, upside down. The drierite absorbs excess water from the fresh plates.
 - b) Place a lighted candle (fixed to the bottom of a petri dish with molten wax) atop the plates in the jar. Allow the candle to burn as you seal the jar.
 - c) Stretch Parafilm over the mouth of the jar and place the lid over the parafilm. The candle will eventually burn out.
5. Incubate the plates at 37-42° C for 2-3 days.

Procedure - Day 2 (2-3 days after day 1) - Observation of colonies

1. Remove plates from jar and observe colonies as described below:
2. Place the plate on the black paper. Remove the lid from the plate and shine the flashlight on the colonies at a 30° angle from the surface.
3. Bring the 6X magnifying glass down close to the plate and look at the colonies.
4. There should be predominantly two types of colonies: *Lactobacillus bulgaricus* colonies are wide and flat. They appear to have a crystalline structure, and when touched with a toothpick they are sticky. *Lactococcus thermophilus* colonies are smaller and slightly yellowish. They are not sticky but seem to dissolve when touched with a toothpick and they will probably greatly outnumber the larger *Lactobacillus* colonies.

Optional - Staining the cells in the colonies (may be done later)

Additional materials required

- microscopes with at least 40X objective
- microscope slides
- marking pens
- toothpicks
- forceps or clothespins to hold slide
- Bunsen burner
- 250 ml beaker
- Crystal Violet stain
- paper towels

Procedure

1. Prepare a glass slide by labeling one end "B" for *Lactobacillus* and the other end "C" for *Lactococcus*.
2. Place a small droplet of water next to each label.
3. Using a separate toothpick for each colony, gently touch one colony of each type with the tip of the toothpick (see description above to help you identify the colony type for each organism).
4. Gently rub the cells off the tip of the toothpick into the droplet of water. Spread the droplet with the bacteria from the colony over an area a little smaller than the size of a dime.
5. Using forceps or a clothespin, hold the slide and gently pass it through the flame of a Bunsen burner several times until the droplets of water have evaporated and the slide is hot. Do not hold the slide in the flame - just pass it through the flame.
6. Place the slide on an empty 250 ml beaker, with the side containing the cells facing upward.
7. Using a dropper, place several drops of Crystal Violet on the slide until it is just covered with the stain.
8. Leave the stain on the slide for about 30 seconds.
9. Gently but completely rinse the stain off the slide, preferably with slowly running water. This can also be done by dipping the slide into several changes of water in a beaker.
10. Place the slide on a paper towel and gently blot most of the water off the slide. Do not rub the slide or the cells will come off.
11. Observe the cells for each colony type under the microscope. While 100X oil is best for a detailed view, the morphology can be seen at 40X.
12. The *Lactococcus* cells are small and round and tend to form short chains. The *Lactobacillus* cells are long, slender rods, that are sometimes curved.

Teacher Instructions

Preparation of the MRS plates

Per liter (makes about 35 plates)

MRS broth powder 55 g

Agar 15 g

1. Place all dry ingredients into a 2 liter Erlenmeyer flask. Add 1 liter of distilled water and place the flask on a stirrer or mix the ingredients with a glass stirring rod. Cover the flask with aluminum foil, autoclave for 20 min. and cool to 50-55° C.
2. Pour the medium into sterile petri dishes (about 30 ml per plate) and allow the agar to solidify (about 30-60 min).
3. **The plates must be used within 1-2 hours or stored in a CO₂ jar very soon (30-60 min) after they have hardened. If they are exposed to air for long, the plates absorb oxygen and the bacteria will not grow.**

To store the plates:

1. Pour about a quarter to a half-cup of drierite in the bottom of a wide-mouth screw cap jar and place the plates on top of the drierite, upside down. The drierite absorbs excess water from the fresh plates.
2. Place a lighted candle (fixed to the bottom of a petri dish with molten wax) atop the plates in the jar. Allow the candle to burn as you seal the jar.
3. Stretch Parafilm over the mouth of the jar and place the lid over the parafilm. The candle will eventually burn out.
4. The plates can be stored for several days, but longer storage increases the risk of contamination.

Contributed by:

Teresa Thiel

Department of Biology

University of Missouri – St. Louis

© 1999 Science in the Real World: Microbes in Action

This material may be duplicated by teachers for use in the classroom. Any other use is prohibited.