

Name \_\_\_\_\_

Period \_\_\_\_\_

Biology

Date \_\_\_\_\_

## LAB \_\_\_\_\_. LACTIC ACID FERMENTATION

(aka Making Yogurt)

### INTRODUCTION

Yogurt is a fermented milk product which originated in Turkey. It is made by adding to milk a bacterial culture which is a mixture of two species — *Lactobacillus bulgaricus* (or *Lactobacillus acidophilus*) and *Streptococcus thermophilus*. The bacteria grow on the milk sugar, or **lactose**. In the process of digesting the milk, the bacteria make an acid — **lactic acid** — which curdles the milk protein, making the whole mixture thick. The lactic acid lowers the pH of the milk and therefore gives the yogurt its tartness. Since the milk is partially digested, yogurt is a mild food to eat because it is easily digestible. In addition, the bacteria that live in the yogurt are some of the beneficial species that normally live in your intestines. Because of this, people often eat yogurt after they have taken antibiotics (pills) for an infection. When you take antibiotics, the medicine not only kills off the disease-causing bacteria in your infection, it also kills off the beneficial bacteria in your intestines. So eating yogurt will allow you to replenish the beneficial bacteria in your digestive system and help to decrease the digestive upset that often comes from taking antibiotics.

In this lab, we will make yogurt to review some of the beneficial, and necessary, jobs that bacteria do in this world. We will also review some of the techniques for working with bacteria and some of the conditions that bacteria need to grow.

Several factors are crucial for successful yogurt making:

- a. good sterile technique (i.e., proper cleansing and heat treatment of glassware, and keeping out unwanted bacteria)
- b. proper incubation temperature. *Lactobacillus* is killed if exposed to temperatures over 55°C (130°F), and does not grow well below 37°C (98°F). We will incubate at 50°C, a temperature on the high side of its preferred growth temperature (122°F), a temperature which inhibits the growth of pathogenic bacteria. (Note that many recipes call for cooler temperatures than this. We find the results less dependable when incubation temperatures are lower.)
- c. protection of the starter from contamination. Do not open the starter (either plain yogurt, or 8 oz starter from the previous yogurt batch) until you are ready to make the next batch.

Yogurt is preserved by its acidity which inhibits the growth of decomposing or pathogenic bacteria. With lids intact, this yogurt will keep at least a month or two in the refrigerator. After that time, especially if your refrigerator is on the "warm" side, a layer of non-pathogenic white mold may form on the top. Merely lift off the mold with a fork, discard, and use the yogurt for cooking.

Baked goods will rise well when yogurt is used, due to its acidity. Use yogurt as part or all of the liquid in cakes, waffles, pancakes and muffins, and cut down on the amount of baking powder. The thickness of yogurt helps to hold up the baking batter.

Yogurt is an excellent dish by itself, but is valuable in its many other uses .

**INSTRUCTIONS**

1. **STERILIZE STORAGE JARS**: Sterilize jars and lids which will be used to make the yogurt. Place in a large pot and cover with boiling water. Leave for 5 minutes.
2. **STERILIZE MILK**: Use a pot with a thick bottom to **scald** the milk. Scalding is bringing milk *almost* to a boil. Add milk to the pot. You may use whole, 2% or skimmed milk. Warm the milk over a medium fire (not so hot that it burns on the bottom). Heat until the temperature of the milk is 85°-90°C (185-195°F). It is not necessary to boil, and do not let boil over...what a mess!
3. We will pour the milk into separate jars at this so that each group can add their flavoring and sweetness. Each group will get one cup of milk.
4. **COOL MILK**: Let your jar of milk cool down. Keep the jar covered during this process. You may speed the cooling process by placing the jar in a pan of clean cold water to cool it down. Cool the milk to 50°C to 55°C (122°-130°F). Remove your jar of cooled milk from the cooling bath.
5. **INOCULATE**: We will use uncontaminated yogurt (it will say “live cultures” on the label) as a **starter** — our source of bacteria. Place two spoons full of yogurt into your jar and blend. Stir very well to thoroughly distribute the yogurt starter. Cover immediately with sterile tops. Tighten well.
6. **INCUBATE**: Choose one of these methods:
  - Warm a gallon of fresh clean water to 55°C, pour into a clean cooler. Place in a warm location. Carefully set the jars of inoculated milk in the water so the bottom of the lids are above the water. Check to see that the water in the cooler does not fall below 50°C (122°F). Close the cooler, place in warm place and let sit undisturbed for three hours. If the starter was active and the temperature correct, the yogurt will have gelled
  - Pre-warm oven to 200 degrees F and turn off. Use an oven thermometer to monitor temperature — do not let it drop below 100°F. Turn oven on for short periods during incubation to maintain a temperature of 108°F to 112°F.
  - Nestle several cardboard boxes inside each other, placing crushed newspapers between each box.
  - A simple way to incubate a small amount of yogurt is to pour the yogurt mixture into a wide-mouth thermos and cover with a tight lid. When the yogurt is ready, loosen the thermos lid before storing it in the refrigerator so the yogurt can cool rapidly.
  - Set filled container(s) of yogurt on a towel-covered heating pad set on medium heat in a sheltered corner on a kitchen counter. Cover the jars with several towels.



**SUMMARY QUESTIONS**

1. Why did we initially heat the milk to 90°C? (Hint: What does this have to do with Louis Pasteur?)

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2. Why did we put the yogurt jars in boiling water?

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3. Why did we cool the milk to 50°C before putting in the yogurt culture?

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4. What is in the yogurt culture? Why are we adding it to the warm milk?

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5. Why did we keep the milk at 50°C overnight? What is going on biologically in the yogurt jars?

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6. What happened to the pH of the milk as it turned to yogurt? Why did this change occur?

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7. Why does the yogurt thicken? \_\_\_\_\_

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8. Why does this process of making yogurt preserve milk?

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9. Design an experiment to show that this process is due to live bacteria in the yogurt culture.