Exercise 10
(Ed. Fall 2010)
The Effects of Temperature on Bacteria
Or why we Pasteurize food

INTRODUCTION:
Student Learning Objectives: After completing this exercise students will:
1. Demonstrate the effects of temperature on the growth of selected bacteria.
2. Describe the effects of an increase in temperature on bacterial survival.
3. Demonstrate the differences between spore forming and non-spore forming bacteria with regard to survival under variable temperatures.
4. Identify the mechanisms by which temperature kills microorganisms.

Activities for today:
- Expose *S. marcescens* and *B. subtilis* to specific temperatures and evaluate their survival.

Materials
Work in groups of 4 per table. This is a group activity. Each group needs the following:

Broth cultures of *S. marcescens* and *Bacillus subtilis* in flasks
Nutrient Agar Plates (2 plates)
Sterile tubes with closures (7 tubes)
Sterile pipettes
Timer (Wrist watch)
500 ml beaker (for water bath)
Thermometer

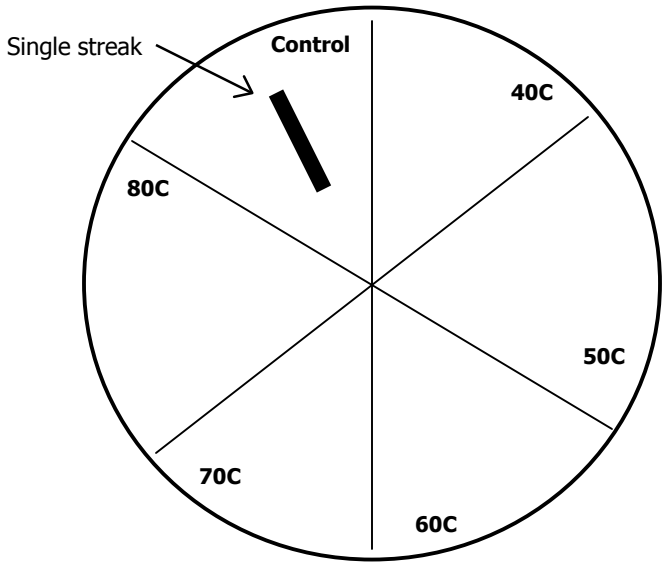
Determining the effects of temperature on bacterial survival
Temperature is used to control the growth of bacteria in various settings. Autoclaving at high temperature and pressure kills viruses, bacteria, fungi as well as their spores, resulting in sterilization. In the process of canning, the same result is usaully obtained by heating the food or fluid in the can under similar conditions. However, cooking food does not always sterilize it, but do you need to sterilize food completely to eliminate the hazards of food poisoning? Is killing the pathogens enough? In this exercise, you will work with your labmates to investigate the temperatures at which bacteria can be inhibited or killed. Teamwork is important in this exercise, as it will save you time and effort.

Procedure:

1. Using a marker, label 6 sterile tubes with closures with the following information; Table #, species (*S. marcescens*, *B. subtilis*), Control, 40C, 50C, 60C, 70C, and 80C.
2. Using a marker, draw six sectors on the (outside) bottom of 2 nutrient agar plates, and label them based on the above information.
3. Place the beaker on the hotplate with about 300 ml of water. Place a tube with about 10 ml of water (non-sterile) in the beaker, and place the thermometer in that tube to check the temperature of the water.

(each table will be given a designated temperature at which to maintain the water)

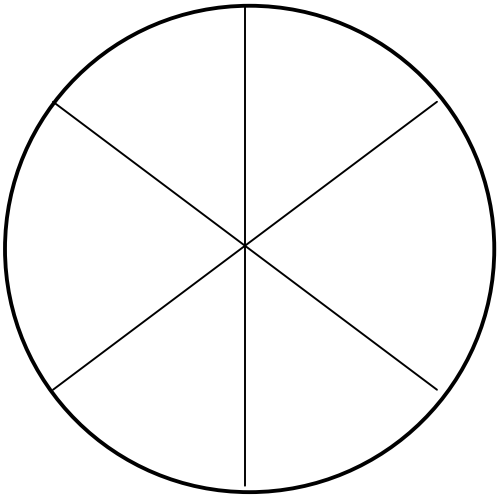
4. Using the aseptic technique, dispense 2 ml of *S. marcescens* broth culture provided in the flask into the 6 sterile tubes with closures. Do the same for *B. subtilis*
5. Take the tubes to the table with the designated temperature and place them in the water bath for 10 minutes. Remove the tubes, wipe dry with a paper towel.
6. Using your inoculating loop, inoculate the plate sectors according to the temperature, with a single streak as shown in the diagram below.
7. Incubate the plates at 30 C for 24 – 48 hours. Observe for growth and record your findings in the table below.



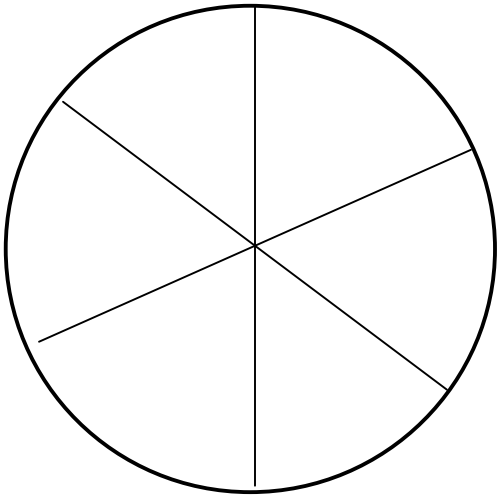
Results

	C	40	50	60	70	80
S. marcescens						
B. subtilis						

S. marcescens



B. subtilis



General Questions

1. How did the temperature affect the growth of *S. marcescens*? B. subtilis?

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2. Compared to *S. marcescens*, were the higher temperatures more effective or less effective at inhibiting *B. subtilis*? Why do you believe this occurred?

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3. Define Pasteurization. Is pasteurized Vitamin D milk sterile? Why?

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4. Can boiling at 100 C alone be used to sterilize media or canned food? Why?

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5. How do high temperatures kill microorgnansims?

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6. Are there any microorganisms or infectious agents that can survive high temperatures? If so identify them and find how they can do so.

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