Physical Methods of Control: Heat

The successful man lengthens his stride when he discovers that the signpost has deceived him; the failure looks for a place to sit down.

JOHN R. ROGERS

Objectives

After completing this exercise, you should be able to:

1. Compare the bactericidal effectiveness of dry heat and moist heat.
2. Evaluate the heat tolerance of microbes.
3. Define and provide a use for each of the following: incineration, hot-air oven, pasteurization, boiling, and autoclaving.

Background

The use of extreme temperature to control the growth of microbes is widely employed. Generally, if heat is applied, microbes are killed; if cold temperatures are used, microbial growth is inhibited.

Bacteria exhibit different tolerances to the application of heat. Heat sensitivity is genetically determined and is partially reflected in the *optimal* growth ranges, which are **psychrophilic** (about 15°C), **psychrotrophic** (20°C to 30°C), **mesophilic** (25°C to 40°C), **thermophilic** (45°C to 65°C), **hyperthermophilic** (about 80°C or higher), and by the presence of heat-resistant endospores (Figure 22.1). Overall, bacteria are more heat resistant than most other forms of life. Heat sensitivity of organisms can be affected by container size, cell density, moisture content, pH, and medium composition.

Heat can be applied as dry or moist heat. **Dry heat,** such as that in hot-air ovens or incineration (for example, flaming loops), denatures enzymes, dehydrates microbes, and kills by oxidation effects. A standard application of dry heat in a hot-air oven is 170°C for 2 hours. The heat of hot air is not readily transferred to a cooler body such as a microbial cell. Moisture transfers heat energy to the microbial cell more efficiently than dry air, resulting in the denaturation of enzymes. **Moist heat** methods include pasteurization, boiling, and autoclaving. In **pasteurization** the temperature is maintained at 63°C for 30 minutes or 72°C for 15 seconds to kill designated organisms that are pathogenic or cause spoilage. **Boiling** (100°C) for 10 minutes will kill

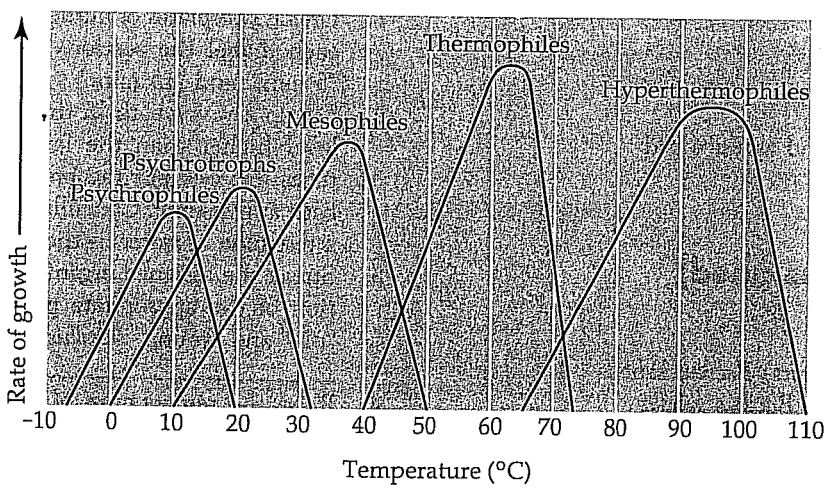


Figure 22.1

Typical growth responses of different types of microorganisms to temperature.

Table 22.1

Relationship Between Pressure and Temperature of Steam

Pressure (pounds per square inch, psi, in excess of atmospheric pressure)	Temperature (°C)
0 psi	100°C
5 psi	110°C
10 psi	116°C
15 psi	121°C
20 psi	126°C
30 psi	135°C

Source: G. J. Tortora, B. R. Funke, and C. L. Case. *Microbiology: An Introduction*, 9th ed. San Francisco, CA: Benjamin Cummings, 2007.

vegetative bacterial cells; however, endospores are not inactivated. The most effective method of moist heat sterilization is **autoclaving,** the use of steam under pressure. Increased pressure raises the boiling point of water and produces steam with a higher temperature (Table 22.1). Standard conditions for autoclaving are 15 psi, at 121°C, for 15 minutes. This is usually sufficient to kill endospores and render materials sterile.

There are two different methods of measuring heat effectiveness. **Thermal death time (TDT)** is the length of time required to kill all bacteria in a liquid culture at a given temperature. The less common **thermal death point (TDP)** is the temperature required to kill all bacteria in a liquid culture in 10 minutes.

Materials

Petri plates containing nutrient agar (2)

Thermometer

Empty tube

Beaker

Hot plate or tripod and asbestos pad

Ice

Cultures (as assigned)

Group A:

Old (48 to 72 hours) *Bacillus subtilis*
Young (24 hours) *Bacillus subtilis*

Group B:

Staphylococcus epidermidis
Escherichia coli

Group C:

Young (24 hours) *Bacillus subtilis*
Escherichia coli

Group D:

Mold (*Penicillium*) spore suspension
Old (48 to 72 hours) *Bacillus subtilis*

Demonstration

Autoclaved and dry-heated soil

Techniques Required

Inoculating loop technique, Exercise 10

Aseptic technique, Exercise 10

Plate streaking, Exercise 11

Graphing, Appendix D

Procedure

Each pair of students is assigned two cultures and a temperature.

Group	Group
A: 63°C _____	A: 72°C _____
B: 63°C _____	B: 72°C _____
C: 63°C _____	C: 72°C _____
D: 63°C _____	D: 72°C _____

You can share beakers of water as long as the effect of the same temperature is being evaluated.

1. Divide two plates of nutrient agar into five sections each. Label the sections "0," "30 sec," "2 min," "5 min," and "15 min."
2. Set up a water bath in the beaker, with the water level higher than the level of the broth in the tubes. Do not put the broth tubes into the water bath at this time. Carefully put the thermometer in a test tube of water in the bath.
3. Streak the assigned organisms on the "0" time section of the appropriate plate. Why are we using "old" and "young" *Bacillus* cultures? _____
4. Raise the temperature of the bath to the desired temperature and maintain that temperature. Use ice to adjust the temperature. Why was 63°C selected as one of the temperatures? _____

5. Place the broth tubes of your organism into the bath when the temperature is at the desired point. After 30 seconds, remove the tubes, resuspend the culture, streak a loopful on the corresponding sections, and return the tubes to the water bath. Repeat at 2, 5, and 15 minutes. What is the longest time period that any microbe is exposed to heat?

6. When you are done, clean the beaker and return the materials. Incubate the plates, inverted, at 35°C until the next lab period. Record your results and the results for the other organisms tested: (−) = no growth, (+) = minimum growth, (2+) = moderate growth, (3+) = heavy growth, and (4+) = maximum growth.

7. Examine the demonstration plates and record your observations. (Refer to Color Plate V.3.) Collect results from your classmates to complete the data table in your Laboratory Report.

Exercise 22

LABORATORY REPORT

Physical Methods of Control: Heat

NAME _____

DATE _____

LAB SECTION _____

Purpose

_____

_____

_____

Data

Record growth on a scale from (−) to (4+).

Organism	Temperature/Time									
	63°C					72°C				
	0	30 sec	2 min	5 min	15 min	0	30 sec	2 min	5 min	15 min
Old *Bacillus subtilis*										
Young *Bacillus subtilis*										
Staphylococcus epidermidis										
Escherichia coli										
Mold (*Penicillium*) spores										

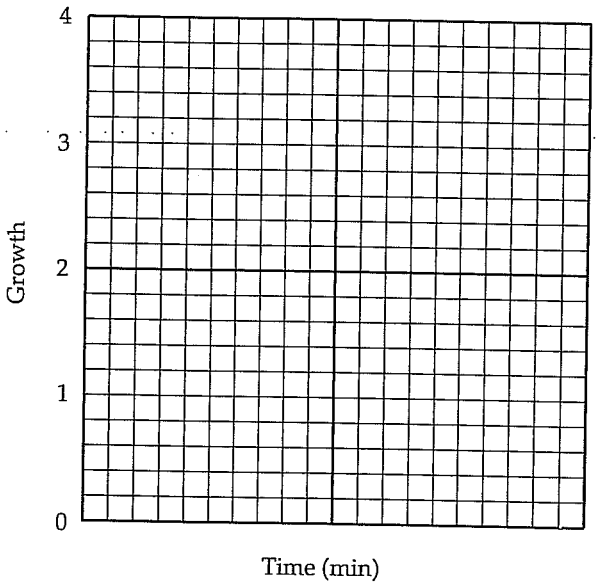
Demonstration Plates

	Control	Autoclaved	Dry-Heated
Number of colonies			
Number of different colonies			

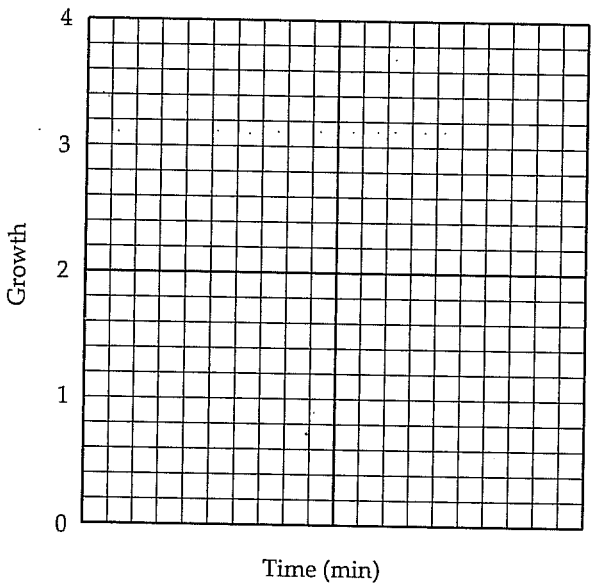
Use a computer graphing application to graph the effect of heating on each organism or draw your graphs below.

Graph your cultures at ____°C.

Organism _____



Organism _____



Conclusions

_____

_____

_____

_____

Questions

1. Compare the heat sensitivity of fungal spores to that of bacterial endospores. _____

_____

2. Compare the effectiveness of autoclaving and dry heat. _____

_____

_____

_____

3. Give an example of an application (use) of thermal death time. _____

_____

4. In the exercise, was the thermal death time or thermal death point determined? _____

_____

5. Give an example of a nonlaboratory use of each of the following methods to control microbial growth:

   a. Incineration: _____

   b. Pasteurization: _____

   c. Autoclaving: _____

6. Define pasteurization. What is the purpose of pasteurization? _____

_____

_____

_____

Critical Thinking

1. Explain why fungi and *Bacillus* sometimes grow better after heat treatment.

2. The decimal reduction time (DRT) is the time it takes to kill 90% of cells present. Assume that a DRT value for autoclaving a culture is 1.5 minutes. How long would it take to kill all the cells if 10^6 cells were present? What would happen if you stopped the heating process at 9 minutes?

3. Indicators are used in autoclaving to ensure that sterilization is complete. One type of chemical indicator turns color when it has reached a specific temperature; the other type turns color when it has reached a specified temperature and been exposed to steam. Which type of indicator should be used?

4. A biological indicator used in autoclaving is a vial containing 10^9 *Geobacillus stearothermophilus* cells that is placed in the autoclave with the material to be sterilized. After autoclaving, the vial is incubated and examined for growth. Why is this species used as opposed to *E. coli* or *G. subtilis*?