Using Temperature to Control the Growth of Bacteria

Introduction

Heat is commonly used to decontaminate inanimate objects or to control the growth of microorganisms in food. When the temperature of the water surrounding the cell wall and/or plasma membrane of microbial cells is increased above the maximum temperature for growth, the macromolecules found in these structures begin to deteriorate and the cells lyse open. Heat disrupts the lipid bi-layer of the plasma membrane, denatures proteins and reduces the integrity of the peptidoglycan layer. Different species of microorganisms vary in their ability to tolerate heat. For example, Gram negative bacteria lyse at lower temperatures than Gram positive bacteria in general. Endospores are destroyed at much higher temperatures than vegetative cells; therefore, endospore producing bacterial populations can withstand greater amounts of heat than non-endospore producing bacteria.

When heat is used to sterilize an object the temperature is typically elevated to 100 degrees C or above for a sufficient amount of time to destroy all of the vegetative cells, endospores and other dormant structures. An autoclave is a device that can effectively sterilize solid objects and liquids. An autoclave, which works similarly to a pressure cooker, heats water in an airtight vessel. A large amount of steam is generated and the pressure inside the vessel increases to approximately 15 psi. The elevation of the pressure in the vessel allows the steam to reach a temperature of 120 degrees C without evaporating. Most microorganisms and endospores are completely destroyed within 15 minutes when subjected to this extremely high temperature and elevated pressure.

Most people think that the food items they eat are sterile, but in fact they are not sterile. When food items and beverages are packaged they are typically treated with chemicals, heat or radiation to reduce the number of microorganisms contaminating them. When food is heated to a specific temperature for a period that will eliminate most of the contaminating microbes, the process is called pasteurization. While pasteurized food is not sterile, there are relatively few microorganisms remaining, which prevent diseases from being transmitted by the food and limits food spoilage. For example, milk is pasteurized at 71 °C for 15 seconds and it can be maintained in a refrigerator without spoiling for several weeks. If a food item is heated for too long or at a temperature that is too high then the flavor and texture of the food item is typically altered; therefore, it is very difficult to sterilize food using heat without making it inedible. When food scientists determine the optimum temperature for pasteurization it is necessary for them to determine the thermal death time (TDT) and the thermal death point (TDP) for various organisms that are likely to contaminate food. The TDT is the time it takes to destroy a suspension of cells or endospores at a specific temperature and the TDP is the temperature at which a suspension of cells/spores is destroyed within 10 minutes.

During this laboratory activity students will explore the process of pasteurization and will calculate the TDT of three different species of bacteria (E. coli, S. aureus and B. subtilis). Students will
expose these three cultures to two different temperatures (71 °C and 100 °C) and determine their thermal death times at each temperature.

Protocol

1. You will work in groups of 4 on this activity. Obtain 1 EC broth culture, 1 BS broth culture and 1 SA broth culture for your group.
2. Obtain 3 nutrient agar plates
3. Your instructor will assign your group a temperature to use for this experiment.
4. Label your 3 cultures with a group name and the proper temperature and label your 3 plates as follows: organism (each plate gets a separate organism), group name, and temperature.
5. Divide your plates up into 5 sections (see figure to right). Label each section as follows (0, 15 sec., 2 min., 5 min., 15 min.).
6. Use your loop to inoculate the section labeled 0 with the appropriate culture on the 3 plates (BS goes on one, EC on another and SA on another).
7. Put your 3 cultures in the appropriate water bath (100 or 71 degrees C) and start timing. After 15 seconds remove the cultures and inoculate the section labeled 15 of each plate with the appropriate culture.
8. Put the 3 cultures back in the water bath and start timing again. After 1 minute and 45 seconds remove them and inoculate in the 2 min. section.
9. Put them back in the water bath and start timing. After 3 minutes remove and inoculate the 5 min. section.
10. Put them back in the bath and time for 10 minutes. After 10 then inoculate the 15 min. section of the plate.
11. Place the plate at 37 degrees C and put the cultures in the biohazard rack.