**Controlling Microorganism**

**Background:** The uncontrolled growth of microorganisms can cause many problems for us humans. In the case of microorganisms growing in our bodies, these often-unwelcome guests can cause minor irritations to life threatening disease. Certain microorganisms growing in our food can lead to the spoilage of the food, gastrointestinal upsets and in some cases even death.

Contaminated water too can be a big problem. Microorganisms growing in our general environment can lead to problems such as unpleasant odors, discoloration of surfaces, deterioration of fabrics and even the destruction of our homes.

In order to keep these unwanted organisms in control, there are a number of strategies one can employ. As you are already well aware, temperature extremes, high or low pH, high salt concentration, the presence of chemical agents, antibiotics, etc… all may have antimicrobial activity, but all are not equally acceptable for every application. For example 10% chlorine bleach is very effective at killing microorganisms on inert surfaces like shower stalls, but it is not appropriate to be used to flush out wounds or to be given by IV to treat infections.

In this lab we will explore a wide variety of physical and chemical means to control the growth of microorganisms.

**Salting:** Bacterial cells hate too much salt! While this statement might seem a little silly, it is nonetheless true.

Bacteria generally prefer to live in a **hypotonic** environment – an environment, which has less salt than within the cell itself. If too much salt is present, water will leak out of the

bacterial cell causing it to shrivel up and stop reproducing. This information is actually useful! The “salty” nature of your skin prevents its colonization by many groups of bacteria. Similarly, preserving fish and meat by salting is a common practice. In this experiment you will look at the effects of salt on the growth of several different bacteria.

# Materials:

1. cultures of *E. coli, S. aureus, P. aeruginosa, S.faecalis*
2. 1 plate of each of the following media:
   1. TSA
   2. TSA + 10% NaCl

# Protocol:

1. obtain the necessary plates as described in the **Materials** section and label them appropriately.
2. divide the plates into 4 equal sections by drawing lines on the bottom of the plates. Label one section with the name of each bacterium.
3. aseptically remove a loopful of bacteria from a culture and streak onto the medium in the appropriately labeled area.
4. once all the plates have been inoculated, place the plates into the 37oC incubator overnight.

**Temperature:** Some like it hot, but others don’t. Temperature has a dramatic effect on the growth and viability of bacteria. Some organisms grow best at low temperatures like those found in your refrigerator, while others can survive boiling hot springs. In this section of the lab we investigate the

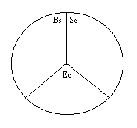
effects of high temperatures on the viability of several different bacteria.

# Materials:

1. cultures of *E. coli, S. aureus, B. cereus* marked 100oC
2. TSA plate
3. beaker with water
4. hotplate

# Protocol:

1. obtain the necessary plates and cultures as described in the **Materials** section.
2. divide the plate into 3 equal sections by drawing lines on the bottom of the plates. Label the plate with the name of one of the three bacteria and the temperature. See figure 1.
3. Place a beaker with water in it on the hotplate and turn on the heat. Bring the water to a boil.
4. Place the tubes containing the cultures into the water. **BE SURE YOU DO THIS AFTER YOU HAVE COMPLETED ALL PARTS OF THE LAB.**
5. At 10 minutes remove the tubes, aseptically remove a loopful of culture, streak it onto the appropriate area of the labeled TSA plates.
6. Once the plates have been inoculated, place the plates into the 37oC incubator overnight.



*Figure 1.*

**Ultraviolet Radiation:** Sunlight is key to life on Earth. It keeps us warm and, ultimately, provides us with food through photosynthesis, but it does, however, have a few drawbacks. Too much time in the sun can be hazardous to your health. Invisible ultraviolet light will bombard your skin and, if overdone, can damage the DNA inside the delicate skin cells. The alterations to the DNA in the damaged cells can cause these cells can run amuck, dividing and invading surrounding tissue. This uncontrolled cell growth is sometimes referred to as skin cancer. Similarly, when bacteria are exposed to UV light their DNA is damaged. This damage can lead to mutations and even the death of the organisms. Today you will study the effects of ultraviolet light on bacterial growth.

# Materials:

1. cultures of *S. aureus, E. coli* and *B. cereus*
2. ultraviolet lamp (254 nm)
3. 3” by 5” index card
4. 3 TSA plates

# Protocol:

1. aseptically place one of the sterile swabs into a culture of bacteria. Gently, twirl the swab to remove excess culture medium.
2. take swab with the bacteria on it and swab the appropriately labeled plate. The goal here is to evenly distribute the bacteria over the surface of the medium so that they will grow as a continuous lawn.
3. remove the lid from **ONE PLATE** and cover ½ of the plate with an index card.
4. turn on a UV lamp and place the plate under lamp for a predetermined amount of time.

# NOTE: DO NOT look directly into the UV lamp!!!

1. turn off the UV lamp, remove the index card and replace the lid of the plate.
2. Repeat steps 3 – 5 for the rest of your plates.
3. once all the plates have been exposed to the UV light, place the plates into the 37oC incubator overnight.

**Chemical Antiseptics and Disinfectants:** The major difference between antiseptics and disinfectants is that antiseptics can be used on the surfaces of the human body whereas disinfectants usually cannot. Even though these two classes of antimicrobial agents have different applications, they both are very helpful in limiting the spread of disease.

Disinfectants help limit the growth of microorganisms on common surfaces such as sinks, toilets, bathtubs, kitchen counters, medical utensils, etc…, places where potential infectious agents may hangout. Antiseptics help to limit the growth of microorganisms on the surface of the body and hence reduce the chances of infection by potential pathogens that may be lurking there.

The proper use of these agents has greatly reduced infection rates and saved many lives. We will be looking at two common chemicals, iodine, which is used as an antiseptic, and chlorine bleach, which is used as a disinfectant.

***At the discretion of the instructors other disinfectants and antiseptics maybe used*** Be sure to record what disinfectant you were given and the time you allowed it be in contact with the bacteria.

# Materials:

1. cultures of *P. aeruginosa, E. coli*
2. 2 TSA plates
3. 2 sterile 10ul loops
4. 2 tubes containing 1 ml of disinfectant or antiseptic

# Protocol:

1. aseptically place one of the sterile loop into a culture of bacteria.
2. take loop full of the bacteria and place it into one of the tubes of disinfectant. Mark the tube with the name of the organism
3. repeat the process with the other organism.
4. allow theloops to remain in the tube for ten minutes
5. after the ten minutes are up, remove the loops from the tubes and inoculate a TSA plate using the semi-quantitative streak method. The instructor will demonstrate.
6. repeat steps 5 of the process with the other loop.
7. once you have inoculate the plates, place the plates into the 37oC incubator overnight.

# Day Two Salting

1. observe the plates for growth and record your results in table 1.

# Table 1 - Salting

|  |  |  |
| --- | --- | --- |
| **Organism** | **No Salt** | **10% Salt** |
| *E. coli,* |  |  |
| *S. aureus,* |  |  |
| *P. aeruginosa,* |  |  |
| *S.faecalis* |  |  |

*record a + for little growth*

*++ for moderate growth*

*+++ for heavy growth*

*--- for no growth*

# Temperature

* 1. observe the plates for growth and record your results in table 2.

# Table 2 – Temperature

|  |  |
| --- | --- |
|  | **Temperature** |
| **Organism** | **100 C**  **100oC** |
| ***E. coli*** |  |
| *S. aureus* |  |
| ***B. cereus*** |  |

*record a + for little growth*

*++ for moderate growth*

*+++ for heavy growth*

*--- for no growth*

# Ultraviolet Radiation

1) observe the plates for growth and record you results in table 3.

# Table 3 – Ultraviolet Radiation

|  |  |  |
| --- | --- | --- |
| **Organism** | **Uncovered** | **Covered** |
| *E. coli* |  |  |
| *S. aureus* |  |  |
| *B. cereus* |  |  |

*record a + for little growth*

*++ for moderate growth*

*+++ for heavy growth*

*--- for no growth*

# Chemical Antiseptics and Disinfectants

1. observe the tubes for growth
2. record your results in table 4.

# Table 4 – Chemical Antiseptics and Disinfectants

**NOTES:**

**Questions:**

1. Which organism(s) were the most resistant to salt?
2. Did all the organisms respond to the elevations in temperature in the same fashion?
3. Did you notice any differences in the response of the organisms to UV light?
4. After looking at the class data for the different disinfectants, which disinfectant was most effective? Which was least effective? They all of the disinfectants kill both bacteria?

|  |  |  |
| --- | --- | --- |
|  | **Chemical Agent** | |
| **Organism** |  |  |
| *E. coli* |  |  |
| *P. aeruginosa* |  |  |