Exercise 9
(Ed. Fall 2010)
The Effects of Ultraviolet Light on Bacteria

INTRODUCTION:
Student Learning Objectives: After completing this exercise students will:

1. Demonstrate the effects of Ultraviolet light on the growth of selected bacteria.
2. Describe the relationship between time of exposure and bactericidal effects.
3. Identify the different ways in which radiation is used to inhibit or kill microorganisms in the medical and food industry.

Activities for today:

- Streaking agar plates for confluent growth.
- Exposing the plates to UV light for different time periods.

Materials

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

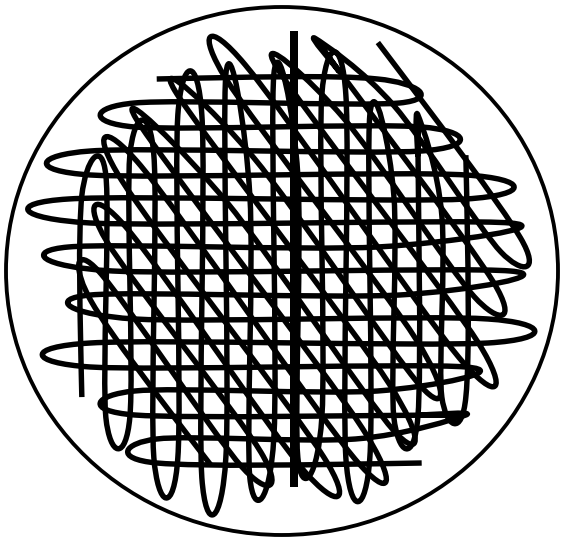
Broth cultures of *Serratia marcescens* and *Bacillus subtilis*
Nutrient Agar Plates (8 plates)
Index cards
Timer (Wrist watch)
Sterile cotton swabs
UV light source (wavelength~ 265)

Determining the effects of UV light exposure on bacteria

Ultraviolet light is often used in different ways to inhibit the growth or kill bacteria on exposed surfaces as well as in the air. Ventilation systems in airplanes and hospitals often employ some form of UV light to kill airborne organisms. UV light affects the DNA structure in chromosomes, causing irreversible mutations that prevent bacteria from multiplying. In this exercise, you will determine the bactericidal effects of UV light based on exposure time using two species of bacteria.

Procedure:

1. Using the aseptic technique, dip a sterile cotton swab into the broth culture of Serratia marcescens, squeeze the excess broth on the side of the tube, and make a single streak across the middle of the nutrient agar plate.
2. Using the same swab, streak for confluent growth by spreading the initial streak across the agar, as if you were painting the surface with a brush.
3. Turn the plate a quarter turn and continue to streak the surface perpendicular to the previous streak using the same swab.
4. Turn the plate one eighth of a turn and streak one more time across the whole plate. By now, your streaks should have covered the surface of the plate in all directions, to obtain a lawn of growth, also known as confluent growth.

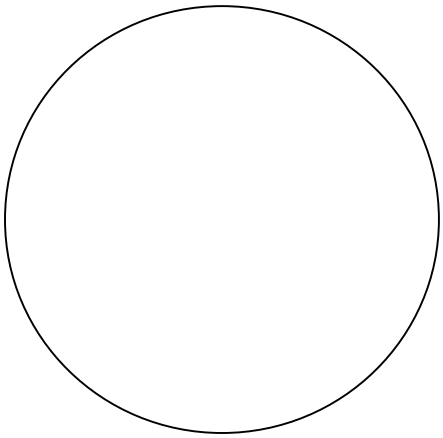


Streaking pattern using a cotton swab to obtain confluent growth

5. Repeat the procedure for three more plates of *S. marcescens*, and for 4 plates of *Bacillus subtilis*.
6. Label your plates with the name of the bacterium, and the following time periods: 1 minute, 5 minutes, 10 minutes, and Control.
7. Place the agar plates labeled 1 minute in the bacteriological hood facing up.
8. Obtain the index cards with the letter "E" cut out of the center, remove the lids of the plates and place the card on top of the plate covering it.
9. Turn on the UV light source for exactly one minute. Turn it off and remove the cards.
10. Replace the lids on the plates and incubate at 30 C for 24 – 48 hours.
11. Observe for growth or inhibition of bacteria.
12. Repeat the procedure for the 5 minute, and 10 minute exposure times.
13. Do not expose the Control plates to any UV light. Incubate all plates at 30 C for 24 – 48 hours. Record your findings below.

Draw the growth on the agar plates below. Note the growth or inhibition patterns.

Serratia marcescens



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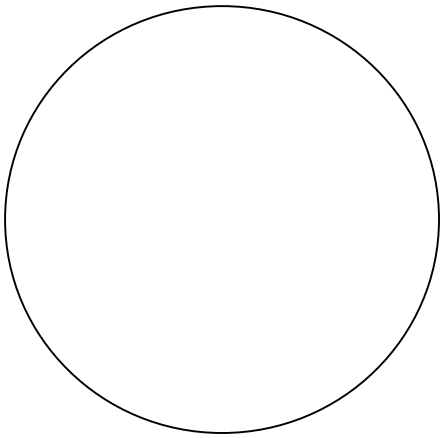

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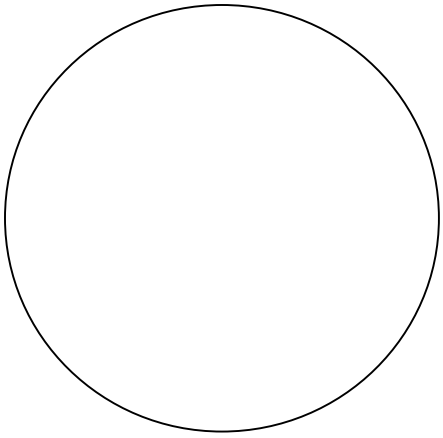

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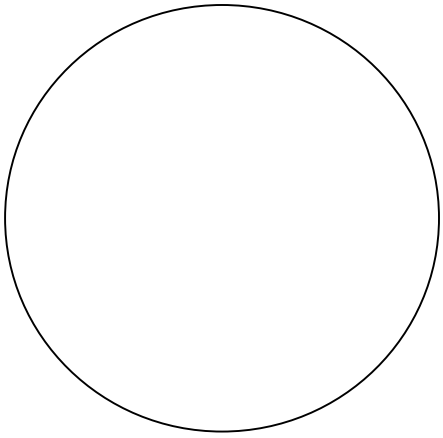

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1. How did the UV light affect the growth of this species?

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2. What is the minimum exposure time required to inhibit the growth of S. marcescens?

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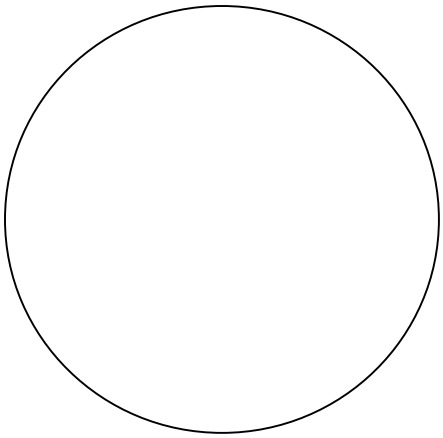
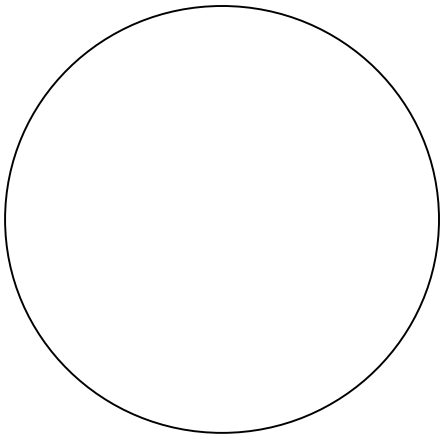
Bacillus subtilis





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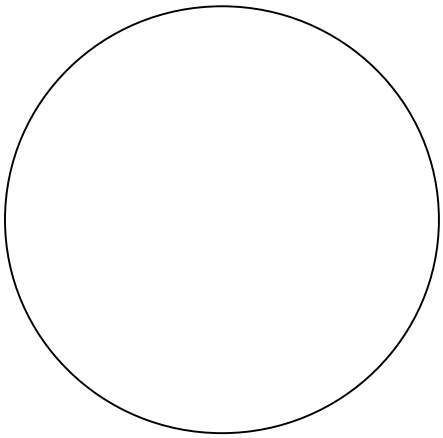

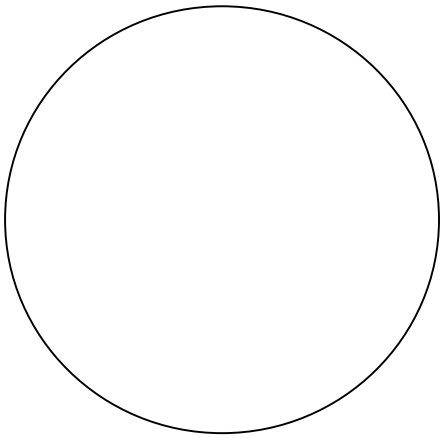

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1. How did the UV light affect the growth of this species?

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

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General Questions

1. What was the purpose of using control plates?

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2. Can UV light be used to sterilize media? Why?

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3. How does UV light damage the DNA of orgnansims?

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4. Gamma radiation (ionizing radiation) is also used for sterilization. What are the advantages of using this over UV light? Describe the some of the uses of gamma rays.

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5. Are there any bacteria that can repair damage to their DNA caused by radiation?

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