

Lab 10: Effects of UV Light Exposure on Bacteria

Introduction

Mutations are a heritable change in the base sequence of DNA. We learned previously that some mutations can be neutral or beneficial to an organism, but most are actually harmful because the mutation will often result in the loss of an important cellular function. Mutations occur naturally in bacteria at a rate $10^{-7} - 10^{-8}$ per base pair during one round of replication. In the presence of a **mutagen**, however this rate can increase dramatically. Mutagens can be in the form of a chemical, such as nicotine, or in the form of electromagnetic radiation.

There are two forms of electromagnetic radiation that are mutagenic; ionizing radiation and non-ionizing radiation. **Ionizing radiation**, such as x-rays or gamma radiation carries enough energy to remove electrons from molecules in a cell. When electrons are removed from molecules, ions called free radicals are formed. Free radicals can damage most other molecules in a cell, such as DNA or RNA, by oxidizing them. **Non-ionizing radiation**, such as ultraviolet (UV) light, exerts its mutagenic effect by exciting electrons in molecules. The excitation of electrons in DNA molecules often results in the formation of extra bonds between adjacent pyrimidines (specifically thymine) in DNA. When two pyrimidines are bound together in this way, it is called a **pyrimidine dimer**. These dimers often change the shape of the DNA in the cell and can cause problems during replication. The cell often tries to repair pyrimidine dimers before replication, but the repair mechanism can also lead to mutations as well.

Electromagnetic radiation can be differentiated according to its wavelength, frequency and/or energy. Conventionally, as the wavelength of the radiation decreases the energy emitted by the radiation increases. For instance, ionizing radiation has a wavelength that is typically less than 1 nanometer and energy that is greater than 100 eV (electron Volts). Nonionizing radiation has a wavelength that is between 100 and 390 nm and energy that is only a few to 100 eV. Because of this, ionizing radiation is considered more dangerous than non-ionizing radiation. For instance, clothing can block UV light, but it takes a lead vest to block x-rays.

Both ionizing and nonionizing radiation are used to control the growth of microorganisms in clinical settings, the food industry and in laboratories. Since ionizing radiation has more energy, it can penetrate cells and endospores very easily and quickly. It is used to sterilize medical supplies and some food products you eat. Only some forms of nonionizing radiation is useful for controlling microbial growth. There are 3 general types of UV light; UVa, UVb and UVc. Each of these has a different wavelength. UVa and UVb have a longer wavelength than UVc; therefore, they have less energy and cannot penetrate cells as well. While they are still dangerous, they are not considered germicidal because they produce only a small effect on most microbes. If cells are exposed to UVc long enough then it can penetrate and kill them. UVc is used as a germicidal agent in surgery wards, restaurants and it is used to treat air flowing through air conditioning ducts in many hospitals and clinics.

Lab Procedure overview:

Everybody will be assigned a number and each number will correspond to a particular culture and UV exposure time (see table 1). Each person will inoculate some media with their assigned culture and will keep their media plate open. An index card will be placed onto $\frac{1}{2}$ of the plate and the plate will be placed under UV light for the assigned amount of time (see figure 1). After UV exposure the plate will be closed and incubated for 24 hours.

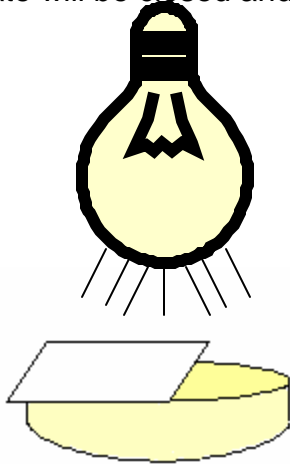


Figure 1. An open media plate culture is exposed to UV light plate. An index card is covering $\frac{1}{2}$ of the plate.

Pre-lab Questions

Answer the following questions before you begin:

1. Which culture should be able to survive the longest exposure to UV (SA or BS)? Why?

2. Do you expect to see a difference between the growth on the side of the plate that was under the index card and the side that was open? If so, explain what you expect to see.

3. Some cells have a means of protecting themselves against radiation. Can you name a few structures that might help protect a cell from UV light?

Protocol

Supplies to get (per/student):

- ? 1 nutrient agar (NA) plate
- ? 1 sterile swab
- ? 1 assigned culture (the culture that corresponds to your assigned number—see table 1).
- ? 1 index card

Culture	UV Exposure time							
	5 seconds	15 seconds	30 seconds	1 minute	5 minutes	10 minutes	15 minutes	15 minutes Petri dish lid on
SA	1, 17	3	5, 19	7, 21	9, 23	11, 25	13	15
BS	2, 18	4	6, 20	8, 22	10, 24	12, 26	14	16

Table 1. Culture and UV exposure times.

Day one instructions.

1. Use Table 1 to determine your culture and exposure time. Get your supplies.
2. Label your plate: initials, culture, exposure time and date.
3. Dip the swab in your assigned culture. Use the swab to smear the culture all over the entire plate (see figure 2).
4. Keep the Petri dish open and place an index card on half of the exposed agar. Place the plate under a UV light (in the hood) and leave it there for the assigned amount of time. **WARNING: Do not look directly into the UV light as it can cause damage to your eyes. Do not expose your direct skin to the UV either. Wear gloves.**
5. Remove the plate from the UV, remove the card, and cover the plate with the Petri dish lid.
6. Incubate at 37 degrees C (upside down) for 24 hours.

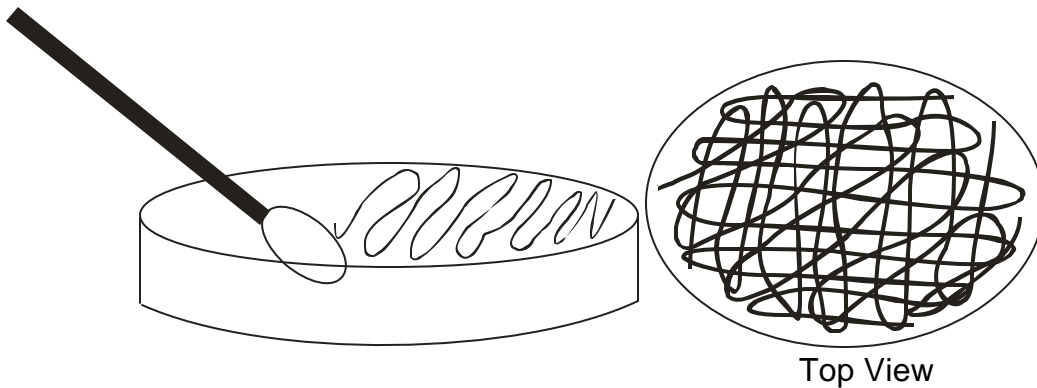


Figure 2. Swab the culture in all directions on the agar.

Day two instructions.

1. Observe your exposure plate. Determine which side of the plate was exposed to UV light.
2. Count the colonies on the side of the plate that was exposed to UV and record the number in the data table. If there are too many colonies to count and the colonies are on top of one another than just put TNTC.
3. Record the data from the rest of the class. We will gather data together and talk about the plates.
4. Answer the questions in the lab report.

Name _____ Lab Instructor _____

Lab Report

1. Can UV penetrate paper? How about a Petri dish lid?
2. How many colonies did you have on the side of the plate that was exposed to UV light?

Data Table

After the class counts their colonies record the average class data in the table.

Culture	UV Exposure time							
	5 seconds	15 seconds	30 seconds	1 minute	5 minutes	10 minutes	15 minutes	15 minutes Petri dish lid on
SA								
BS								

3. Did one organism survive exposure to UV longer than another? Why or why not?
4. Would you use UV light to sterilize a syringe packaged in a protective film? Why or why not?
5. Ionizing radiation is used to treat a number of fresh fruits and vegetables before they are sold to customers. Why would it be a problem to use UV for this?