EFFECTS OF UV IRRADIATION ON MICROORGANISMS

I. OBJECTIVES

- To learn the effects of UV irradiation on different bacterial species.
- To learn if there is a correlation between the length of exposure to UV irradiation and the number of bacteria killed.

II. INTRODUCTION

Light is a form of energy that travels in rhythmic waves. The distance between the crest of two waves is called the wavelength and is expressed in metric units. The light spectrum covers a wide range of rays with different wavelengths. At one extreme are the gamma rays with wavelengths of less than a nanometer (1 nm=10^{-9} meter) while at the other extreme are the radio waves with wavelengths of more than a kilometer. The most important part of the light spectrum is a narrow band called the visible light (380-750 nm). These wavelengths, taken together, are seen as homogeneous white light, but if passed through a prism, we see that the component wavelengths (in nm) are actually perceived as the colors of the visible spectrum.

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        violet   indigo      blue      green        yellow    orange        red          far red
  UV- - --|-------------|--------------|--------------|--------------|--------------|--------------|------------|- IR
        400            450            500            550            600            650            700
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Light wavelengths between 100 and 400 nm are considered ultraviolet (UV). Wavelengths below 100 nm are extremely hazardous (i.e.; ionizing radiation such as gamma rays and X-rays). Beyond 800 nm, the radiation is in the form of infrared (or heat rays), microwaves and radio waves.

UV irradiation is commonly used for surface sterilization of materials that would otherwise be damaged by high heat (e.g., autoclaving) or hard to sterilize by other methods (e.g. operating rooms). Although its rays have a very low power of penetration and are not as harmful as the rays of lower wavelengths, they are still quite powerful mutagens. Their main effect on biological systems stems from (1) production of a large number of free radicals that destabilize macromolecules and (2) the fact that they are absorbed by the DNA molecules in the cells and cause alterations that are harmful to cell survival.

In humans, skin cancer is the most common effect of UV-induced mutations observed. The interaction of the UV with skin cell DNA causes unrestricted growth of some skin cells that ultimately produce a tumor. Although skin cancer is the most common type of cancer, its prevention is rather easy. Protecting the skin from sun rays which contain UV irradiation by wearing proper clothing or sun screen lotions will definitely inhibit skin cancer.

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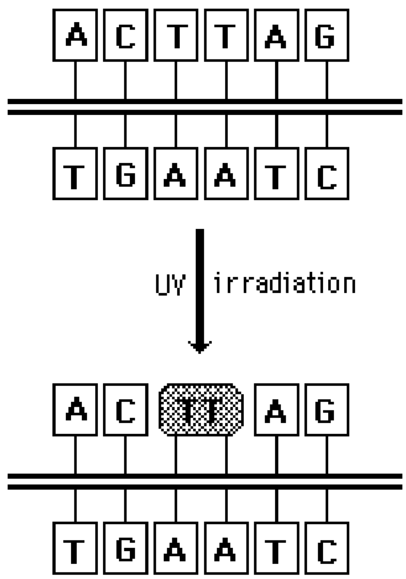
To understand the mutagenic effects of UV irradiation on a molecular level, it is a well-known fact that short wavelength rays (such as UV) interact with water molecules in the cell to produce free radicals (-OH). Such free radicals lack an electron and attack other molecules such as cell proteins or DNA to rob them of an electron. Many free radicals may retrieve electrons from the same large cell molecule and cause changes in the molecule in a way that makes its role useless.

The most common change in the DNA molecule brought about by UV irradiation and consequently by the free radicals occur at locations on the molecule where two thymine (T) bases occur adjacent to each other. UV irradiation causes the two T bases to fuse to each other on the same strand. Such structures are called thymine dimers and cause a distortion in the shape of DNA.



Thus, when it is next time for DNA replication, a wrong base may be incorporated at the thymine dimer position on the strand being synthesized. This would constitute a site of mutation and if it involves a protein that plays a role in cell survival, it may be lethal.

III. LABORATORY SUPPLIES

Serratia marcescens grown O/N	5 ml/table
Bacillus subtilis grown O/N	5 ml/table
UV lamp	1/lab
BHI plates	4/group
Half-lids	1/lab
Sterile droppers	2/group
Sterile swabs	2/group
Goggles	4/lab

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IV. PROCEDURE (The students at each tableside will work together as a group.)

1. Obtain 4 BHI plates and label each with your group initials. Draw a line dividing each plate into two equal halves on the bottom of each plate.

2. Divide the plates into 2 groups. Label one group with S for *Serratia* and the others with B for *Bacillus*. Further, add the designation 10 or 20 seconds for the exposure to UV light as shown in the table below:

Plate Designation	Treatments: Bacteria		Treatments: UV Exposure	
	Serratia	*Bacillus*	10 sec	20 sec
S-10	√		√	
S-20	√			√
B-10		√	√	
B-20		√		√

3. Dip a sterile swab into the overnight-grown *Serratia* broth culture and inoculate the S-10 plate by streaking. Rotate the plate 90 degrees and re-swab. Repeat for the S-20 plate.

4. In a similar manner to the step above, swab the B-10 and B-20 plates with the *Bacillus* culture.

5. UV RAYS ARE HARMFUL. WEAR PROTECTIVE GLOVES AND GOGGLES. UV irradiation may cause serious skin burns. Do not look at the lamp directly.

6. Prepare each plate for UV exposure by taking its lid off and replacing it with a "half-lid". Match the half-lid with the line on the bottom of the plate. Turn the lamp on and wait 5 seconds for it to warm up. Place the plate under the UV lamp and expose it for exactly 10 or 20 seconds based on the plate designation. Remove the plate and replace the half-lid with its original lid.

7. Make sure to turn the lamp off at the end of your experiment. Place the plates inverted at room temperature for 2 days. Note that wild type *Serratia marcescens* produces pink colonies at 30°C or below but white colonies at over 30°C.

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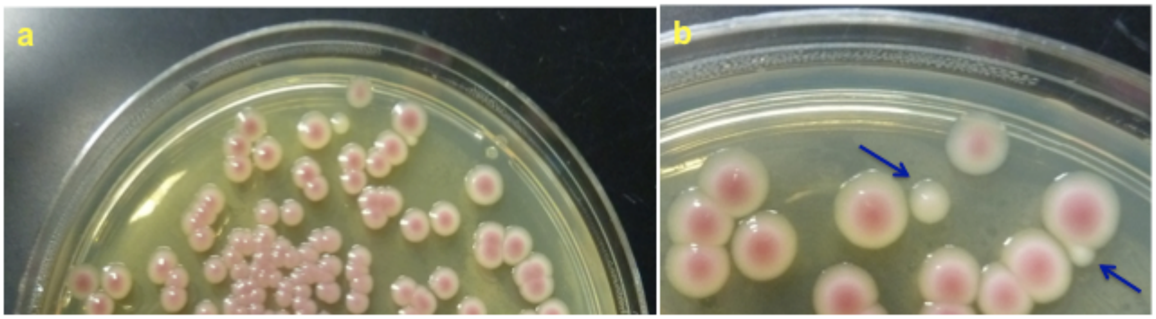
Figures below show (a) a plate of *Serratia marcescens* after UV irradiation and part of the same plate enlarged (b). Arrows show two possible mutants.

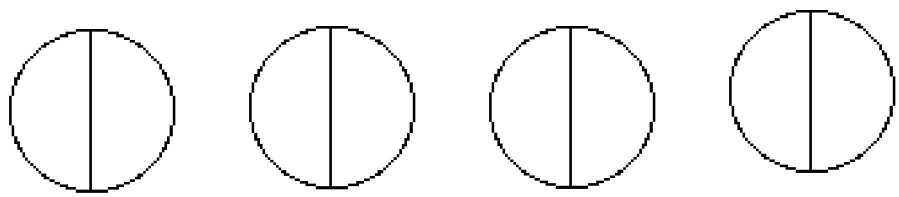


Results of the effects of UV irradiation on bacteria

NAME ___________________________ DATE ____________ GROUP NAME ________

PARTNER(S) ____

1. Draw the growth on the following plates:



	S-10	S-20	B-10	B-20
No. of colonies on the radiated section	________	_________	________	________
No. of white colonies*	________	_________		

* A colony is considered white if its center is not pink.

2. Did you observe any white colonies in UV-treated *Serratia* plates? How could white colonies originate if the plates were incubated at room temperature (which is far less than 30°C)?

3. Compare the effect of UV radiation on the two species you used. Which one is more sensitive? Could you give a biological reason for the differences you observe between the two species?

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4. We saw in the lab that a short duration of UV radiation could kill a large number of the cells. We also saw in the Pure Culture lab that a very large number of bacteria existed in the soil. (If we had sampled other environments outside in nature, we would have found plenty of bacteria.) Based on these two pieces of information, explain whether bacteria could survive under sunshine? Give a logical reason.

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