

# ***Serratia* Exposed to UV Radiation Show Increase In Survival Capabilities**

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## **I. Introduction**

Mutations caused by UV light are typically due to the creation of pyrimidine dimers and single or double stranded breaks. These mutations cause cells to activate repair systems, but will also most likely cause the cell's death. Some mutations may result in changes in color due to changes in the prodigiosin production sequence. More commonly the mutations result in death, so increased amounts of UV radiation will most likely correspond with increased cell death. This was the theory we tested in the group part of this experiment. Specific proteins must be turned on to help repair the DNA structure after mutations occur. These proteins may be the reason for some cells' abilities to survive radiation, so our independent experiment researched these surviving cells to see if they were better adapted to the radiation. If mechanisms of repair for UV damage require certain genes to be turned, then bacteria that have already activated this mechanism should be more able to survive further exposure to radiation.

## **II. Materials and Methods**

Materials:

- *S. marcescens* from UV500 and no UV
- 72 plates of peptone-glycerol
- 8 flasks of peptone-glycerol broth
- 24 petri dishes
- Microfuge and sterile microfuge tubes
- Tinfoil
- 16- 9.9mL sterile saline tubes
- 56- 4.5mL sterile saline tubes
- 1mL and .1mL pipets
- Loops and sterile toothpicks
- Bunsen burners and strikers
- Glass spreaders and alcohol

## Methods:

In part 2, we decided to test whether the bacteria that survived an initial exposure to UV500 were better able to survive than those that were not exposed to UV. In order to do this, we used the colonies from part 1 to make overnight cultures that had been exposed to UV previously. We transferred 1.5mL of the overnight culture into a microcentrifuge tube. Then we placed it in the microfuge at full speed for 15 seconds. Then we poured off the liquid and added another 1.5mL of sterile saline. Then we vortexed it and transferred 1mL of solution to a petri dish. We then exposed the sample to the 0, 300, and 500 J/m<sup>2</sup>. For each time we exposed the sample, we placed the petri dish's contents in a microcentrifuge tube and proceeded to make dilutions to plate. We assumed the concentration of cells was 10<sup>9</sup>/mL and diluted each dosage less than the one before. For example, we plated UV500 exposed *Serratia* with a dilution of 10<sup>0</sup> and 10<sup>1</sup>, UV300 with a dilution of 10<sup>1</sup>, 10<sup>2</sup>, and 10<sup>3</sup>, etc.

We allowed these to grow for about 48 hours, which turned out to have a much higher concentration than we had anticipated. Because of the underestimate of the cell density, the UV 500 produced plates that had far too many colonies. We retried the experiment using one of our samples. This time we estimate slightly higher cell concentrations and were able to obtain cell/mL counts for exposure to UV 300, UV500, and no UV.

### III. Results

In part 1, we found that the percent survivors decreased consistently down to an asymptote of zero (Figure 1). This also corresponded with the data that the rest of the class obtained. We only used data from one other group because the other two groups had incomplete or inaccurate results. We found two color mutants in part 1, but this was not distributed throughout the experiment so we were not able to make calculations.

In part 2, we compared the results of re-exposing *Serratia* to UV 500. It turned out that the bacteria were able to survive slightly better after they had been previously exposed to high levels of UV (Figure 2). The bacteria also maintained

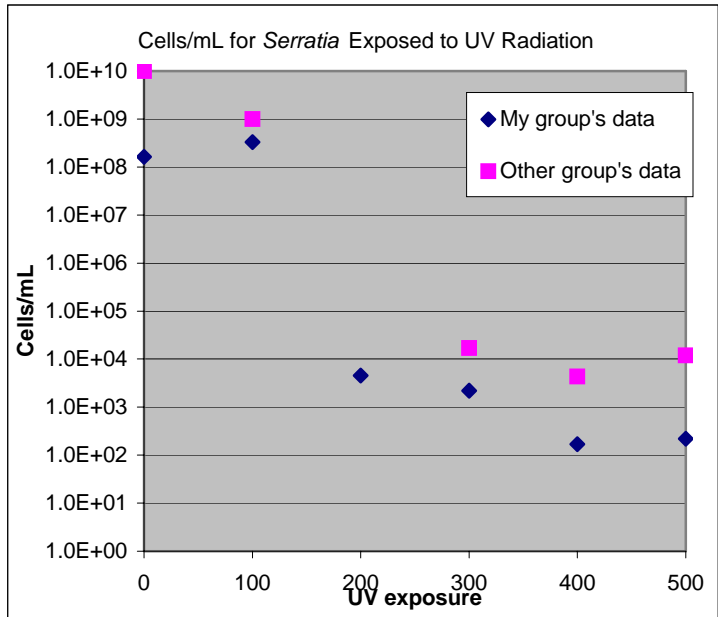


Figure 1. *Serratia* cell concentration decreases with increased exposure to UV. We exposed a stock sample of bacteria to UV in order to analyze mutations.

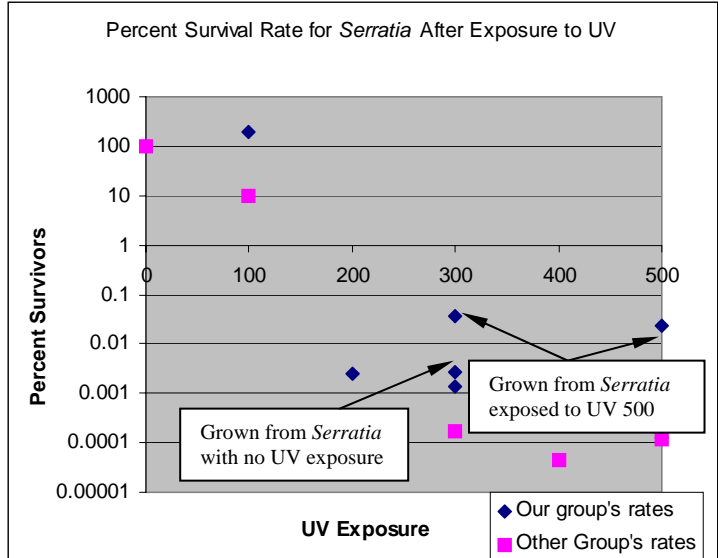


Figure 2. Comparison of percent survival of *Serratia* after UV exposure. We found the percent survivors using a control group exposed to no UV. This also contrasts the re-exposed *Serratia* survival rate.

However, the bacteria that were allowed to grow in our second experiment had a much higher initial concentration. This was contradicted by the fact that these bacteria actually had a lower survival rate than the ones that had been previously exposed to UV radiation (Figure 2).

This survival rate was based on a combination of data from our partially failed attempt at part 2 and the second, more successful attempt. The bacteria that were not exposed to UV before the experiment grew much faster than the bacteria exposed to UV 500 previously (Table 1).

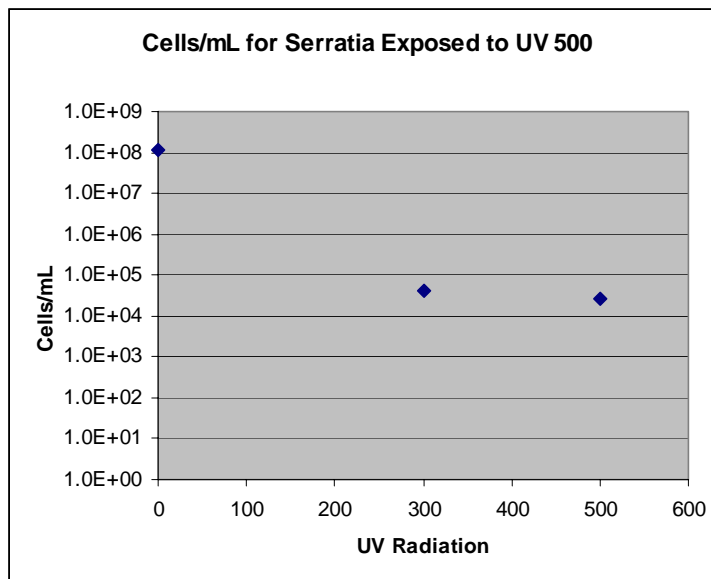


Figure 3. Concentration of *Serratia* after UV exposure. These bacteria were exposed to UV 500 in the original experiment and then to varying level in the second experiment.

**Table 1. Comparison of growth rates of *Serratia***

Test Tube	Original UV Exposure <sup>c</sup>	Fd	Colonies <sup>b</sup>	Cells/mL
1	None <sup>a</sup>	1.0E+06	157	(1.57+/- .12)*10 <sup>9</sup>
2	none	1.0E+07	433	(4.33+/- .21)*10 <sup>10</sup>
3	none	1.0E+07	259	(2.59+/- .16)*10 <sup>10</sup>
4	none	1.0E+07	924	(9.24+/- .3)*10 <sup>10</sup>
1	500	1.0E+05	(220/206)	(6.3+/- .3)*10 <sup>7</sup>
2	500	1.0E+06	(156/157)	(1.6+/- .08)*10 <sup>9</sup>
3	500	1.0E+07	352	(3.52+/- .13)*10 <sup>10</sup>
4	500	1.0E+07	153	(1.53+/- .09)*10 <sup>10</sup>

<sup>a</sup> Does not include natural exposure to light in classroom

<sup>b</sup> Only plated once because of lack of supplies late in the day

<sup>c</sup> Each test tube sample from different plate of *Serratia*

#### IV. Discussion

From the first part of this experiment, it was easy to see that UV radiation mutated the DNA of *Serratia* enough to cause a certain percent of the cells to die. It was also clear that fewer survived as the amount of radiation increased. I thought that it would be really interesting to know if these survivors of the high UV doses were actually more capable of survival than bacteria that had not been previously exposed to excessive radiation. If mechanisms of repair for UV damage require certain genes to be turned, then bacteria that have already activated this mechanism should be more able to survive further exposure to radiation. This led me to design our experiment mainly testing to see if bacteria exposed to UV500 were, in fact, able to proliferate more effectively in additional harsh conditions.

In the first part of the experiment we were supposed to find out what kinds of effects the UV-induced mutations would have on the *Serratia*. This attempt led to one conclusion: the vast majority of mutations resulted in the death of the bacterium rather than a change in pigmentation. Though we did find two mutants, this seemed a rather insignificant number because they could have been contaminants. This part of experiment also showed that despite our uncharacteristic spike in the *Serratia* concentration at UV100, the concentration is directly proportional to the amount of UV exposure. This experiment also showed a 10% survival rate at approximately UV125. We would never be able to predict a survival rate of 0% because the graph reaches an asymptote at zero.

We found that the bacteria that were not originally exposed to significant amounts of UV were able to grow better than bacteria exposed to UV 500. Conversely, these same bacteria had a lower survival rate as they were exposed to the UV radiation. This seems to show that the bacteria re-exposed to high levels of UV are more able to survive. Because of underestimates in

the amount of bacteria in our overnight broths and the time constraints of the block plan, we would need substantially more data to concretely conclude this. Our experiment, when compared to the results from part 1, did seem to demonstrate this a little more conclusively. The re-exposure to UV500 allowed the *Serratia* to have a higher percent survival rate than the bacteria that were not previously exposed. The ability of the *Serratia* not exposed to excessive UV radiation to grow faster may have to do with the bacteria's not having to switch from mechanisms of repair to mechanisms of replication. The *Serratia* previously exposed to UV500 were probably able to utilize survival mechanisms such as those described in an article by Ying-Hsiu et al. (1998). These mechanisms include photo reactivation and most importantly the SOS response. The SOS response is activated by DNA damage and inhibition of DNA replication. This response may have occurred in the *Serratia* that were able to withstand original exposure to UV500 and may have redirected energy needed for rapid and effective cell division.

The fact that the *Serratia* actually seemed to develop an increased tolerance for UV-damage is rather thought provoking. I would be interested to see if the level of initial UV exposure had an effect on the theoretical resistance. Obviously, a little more data would need to be collected to substantiate this claim. So, I would also like to see if this claim would hold true if I repeated the experiment using the re-exposed *Serratia* sample again. Overall, this data shows some trends that could show an increase of resistance to UV-damage. I also believe that this may be a very limited phenomenon if it is indeed caused by the shift to SOS activated proteins.

## V. Calculations

**Table 2. *Serratia* mutations resulting from re-exposure to UV**

UV Exposure	Original UV Exposure <sup>a</sup>	Fd	Colonies <sup>b</sup>	Cells/mL	Percent Survivors
0	none	10 <sup>6</sup>	TMTC		
0	none	10 <sup>7</sup>	TMTC		
0	none	10 <sup>8</sup>	TMTC		
0	500	10 <sup>5</sup>	111	(1.11+/-0.1)*10 <sup>8</sup>	100%
0	500	10 <sup>6</sup>	50		
0	500	10 <sup>7</sup>	14		
300	none	10 <sup>2</sup>	TMTC		
300	none	10 <sup>3</sup>	120	(1.2+/-0.1)*10 <sup>6</sup>	2.77*10 <sup>-3</sup> % <sup>c</sup>
300	none	10 <sup>4</sup>	8		
300	500	10 <sup>1</sup>	TMTC		
300	500	10 <sup>2</sup>	40	(4+/-0.6)*10 <sup>4</sup>	3.6*10 <sup>-2</sup> %
300	500	10 <sup>3</sup>	6		
500	none	10 <sup>0</sup>	TMTC		
500	none	10 <sup>1</sup>	TMTC		
500	none	10 <sup>2</sup>	TMTC		
500	500	10 <sup>0</sup>	TMTC		
500	500	10 <sup>1</sup>	257	(2.57+/-0.2)*10 <sup>4</sup>	2.31*10 <sup>-2</sup> %

<sup>a</sup> Does not include natural exposure to light in classroom

<sup>b</sup> Only plated once because of lack of supplies late in the day

<sup>c</sup> Uses data from the experiment in Table 1 in order to obtain percent survivors

**Table 3. Mutations of *Serratia* due to ascending UV exposure**

UV Exposure	Fd	Colonies (Plate 1/Plate 2)	Mutants	Cells/mL	Percent Survivors
0	10 <sup>7</sup>	(1/1)			
0	10 <sup>6</sup>	(15/12)			
0	10 <sup>5</sup>	(155/177)		(1.64+/-0.09)*10 <sup>8</sup>	100
100	10 <sup>6</sup>	(37/29)		(3.3+/-0.4)*10 <sup>8</sup>	201 <sup>a</sup>
100	10 <sup>5</sup>	TMTC			
100	10 <sup>4</sup>	TMTC			
200	10 <sup>4</sup>	(0/0)			
200	10 <sup>3</sup>	(0/0)			
200	10 <sup>2</sup>	(4/5)		(4.5+/-1.5)*10 <sup>3</sup>	2.44*10 <sup>-3</sup>
300	10 <sup>3</sup>	(0/0)			
300	10 <sup>2</sup>	(3/3)			
300	10	(26/18)		(2.2+/-0.3)*10 <sup>3</sup>	1.34*10 <sup>-3</sup>
400	10 <sup>2</sup>	(1/0)			

400	10	(4/1)			
400	1	(20/14)	(1/1) <sup>b</sup>	(1.7+/-0.3)*10 <sup>2</sup>	4.15*10 <sup>-4</sup>
500	10	(3/1)			
500	1	(18/25)		(2.2+/-0.3)*10 <sup>2</sup>	1.28*10 <sup>-4</sup>

<sup>a</sup> Abnormal result may be due to measuring error

<sup>b</sup> May result from contamination or may be very rare

## 1. Calculating Cells/mL of *Serratia*

### Step 1

I selected the only one of our plates that had the most viable colonies. For example the UV500 plate with 18 and 25 colonies from Table 3.

### Step 2 Calculating Percent Error

$$\% \text{ error} = (100 * \sqrt{43}) / 43 = 15.2\%$$

### Step 3 Average number of bacteria

$$\text{Avg} = 43 / 2 = 21.5 \text{ colonies per } .1\text{mL}$$

### Step 4 Bacteria per mL solution

$$(21.5 \text{ colonies} / .1\text{mL}) * 10^0 = 215 \text{ cells/mL}$$

### Step 5 Actual Error

$$\underline{215 \pm 33 \text{ cells/mL}}$$

I repeated this procedure for all of the results from Tables 1, 2, and 3. I also used data from other students to obtain these results from the rest of the classes data.

## 2. Percent Survivors

I found the percent survivor rate by dividing the number of cells/mL for each radiation dosage by the number of cells/mL found in the control which was exposed to no UV.

$$\text{Percent survivor} = (215 \text{ cells/mL}) / (1.64 * 10^8 \text{ cells/mL}) = 1.28 * 10^{-4}\%$$

## VI. Acknowledgements

First and foremost, I'd like to take Professor Lostroh, Andy, Delane, Colorado College and everyone else who helped to set up and produce this lab. Secondly, I'd like to thank my wonderful and intelligent group members from Part 1: Aissa, Martha, and Joslyn. I'd also like to

thank Melissa, my lab partner from Part 2. I also appreciate the helpful information from the rest of the class.

## VII. References

Ying-Hsiu L., A. Cheng, and T.V. Wang. 1998. Involvement of *recF*, *recO*, and *recR* Genes in UV-Radiation Mutagenesis of *Escherichia coli*. *J Bacteriol* **180**:1766-1770.

Abstract:

The *recF*, *recO*, and *recR* genes were originally identified as those affecting the RecF pathway of recombination in *Escherichia coli* cells. Several lines of evidence suggest that the *recF*, *recO*, and *recR* genes function at the same step of recombination and postreplication repair. In this work, we report that null mutations in *recF*, *recO*, or *recR* greatly reduce UV-radiation mutagenesis (UVM) in an assay for reversion from a  $\text{Trp}^-$  (*trpE65*) to a  $\text{Trp}^+$  phenotypes. Introduction of the defective *lexA51* mutation [*lexA51(Def)*] and/or *UmuD'* into *recF*, *recO*, and *recR* mutants failed to restore normal UVM in the mutants. On the other hand, the presence of *recA2020*, a suppressor mutation for *recF*, *recO*, and *recR* mutations, restored normal UVM in *recF*, *recO*, and *recR* mutants. These results indicate an involvement of the *recF*, *recO*, and *recR* genes and their products in UVM, possibly by affecting the third role of RecA in UVM.