Determining the Influence of Genotypic Mutation of Phenotypes of Bacteria and Its Progeny

Uma Knaven
02/09/19
Brooke Washburn BIOL-2511
Lab Partner: Emily Vandenberg

Introduction:

The bacteria *Serratia marcescens* produces an enzyme called prodigiosin. When grown at room temperature, the enzyme produces a bright red color that causes red colonies. When grown at 37^{o}C, the enzyme is denatured, and the bacteria display a cream color. This indicates that pigment-less bacteria are naturally cream colored and shows a phenotypic change in the bacteria. A phenotypic change is a change in the displayed traits of an organism, which may be caused by the environment, like temperature. A genotypic change can also change the phenotype and is due to a change in the organism's DNA. Pigment-less bacteria can be produced through a mutation in the gene, or a genotypic change, that codes for the prodigiosin enzyme. The mutation can be caused by UV light, which creates thymine dimers. Thymine dimers are bonds formed between adjacent thymine bases in DNA, which can be repaired using photorepair. In photorepair, bacteria can use light to repair the damage. However, this reparation can cause mutations, or permanent genetic changes to the bacterial DNA. This can lead to several possible outcomes. A high dosage of UV radiation could completely kill an entire plate of bacteria. If the bacteria do survive, the mutations caused by the UV radiation could eventually kill it and no colonies would form. If a mutation occurs in the gene that codes for the enzyme that causes a red pigment, photorepair can fully repair the DNA and the bacteria can reproduce as normal to make red colonies. However, if photorepair does not repair the mutation properly, pigment production can be inhibited, causing cream colored bacteria. If the bacteria pass on the mutated gene, cream colonies will be formed, and the mutation can be determined to be heritable. Further studies have been done to show evolutionary benefits of a mutated pigment gene. They suggest cream-colored bacteria are more resistant to further UV radiation than red bacteria (Hanks et al. 1971). It is hypothesized that if the bacteria are subjected to a high UV dosage and incubated in the dark, permanent but heritable damage will cause colonies of cream-colored bacteria to form. If the bacteria are subjected to a high UV dosage but then incubated in the light, the bacteria will photorepair and produce colonies of red-colored bacteria. Bacteria subjected to low or no UV radiation will only produce red colonies, regardless of incubation in light or dark (Shibai et al 2017).

Materials and Methods:

Part 1: Plating Bacteria

Petri dishes with agar were labeled for each UV dosage and whether incubation was in the dark or light; 6 were used. *S. marcescens* was then plated onto each plate using the streak method. The streak method was used to obtain individual colonies with a known single bacteria source. Proper sterilization technique was used to limit contamination. To do this, an inoculating loop was sterilized in a Bunsen burner, and then dipped into the sample of *Serratia marcescens.* The bacteria were streaked across the plate 4 times, with sterilization between each streak. One control plate was set to incubate in the light and the other in the dark, while each other plate was exposed to a high or low dose of UV radiation with a crosslinker. The high dosage was dosed at 80 μJ/cm^2 x100 while the low dosage was dosed at 5 μJ/cm^2 x100. One high dose and one low dose were incubated in light, with the other two in the dark. The plates were incubated at about 25^{o}C until colonies appeared, and then stored at 4^{o}C until the next part of the experiment.

Part 2: First Observation of Bacterial Growth

The plates from Part 1 were examined. One cream colored colony from the high dosage in light and one cream colored colony from the low dosage in light plates were selected and replated using the streak method and proper sterilization techniques again. The low dosage colony was taken from another group's plates, since the original low dosage plate did not contain a white colony. These were again incubated in the light at 25^{o}C. Two colonies from the original control plate were replated, with one incubated at 25^{o}C and one incubated at 37^{o}C. A total of 4 plates were used. The plates were incubated again until colonies grew and then stored at 4^{o}C until the next part of the experiment.

Part 3: Final Observations

The plates were examined.

Results:

The below images were taken after week 2 and week 3. After plating, treating, and incubating the bacteria, observations were made. The *Serratia marcescens* were treated with a high UV dose of 80 μJ/cm^{2}x100 or a low UV dose of 5 μJ/cm^{2}x100. They were then incubated in the light or dark to regrow. Bacteria subjected to more UV grew fewer and smaller colonies than the control. All colonies remained red, except the plate incubated at 37^{o}C, which turned white.

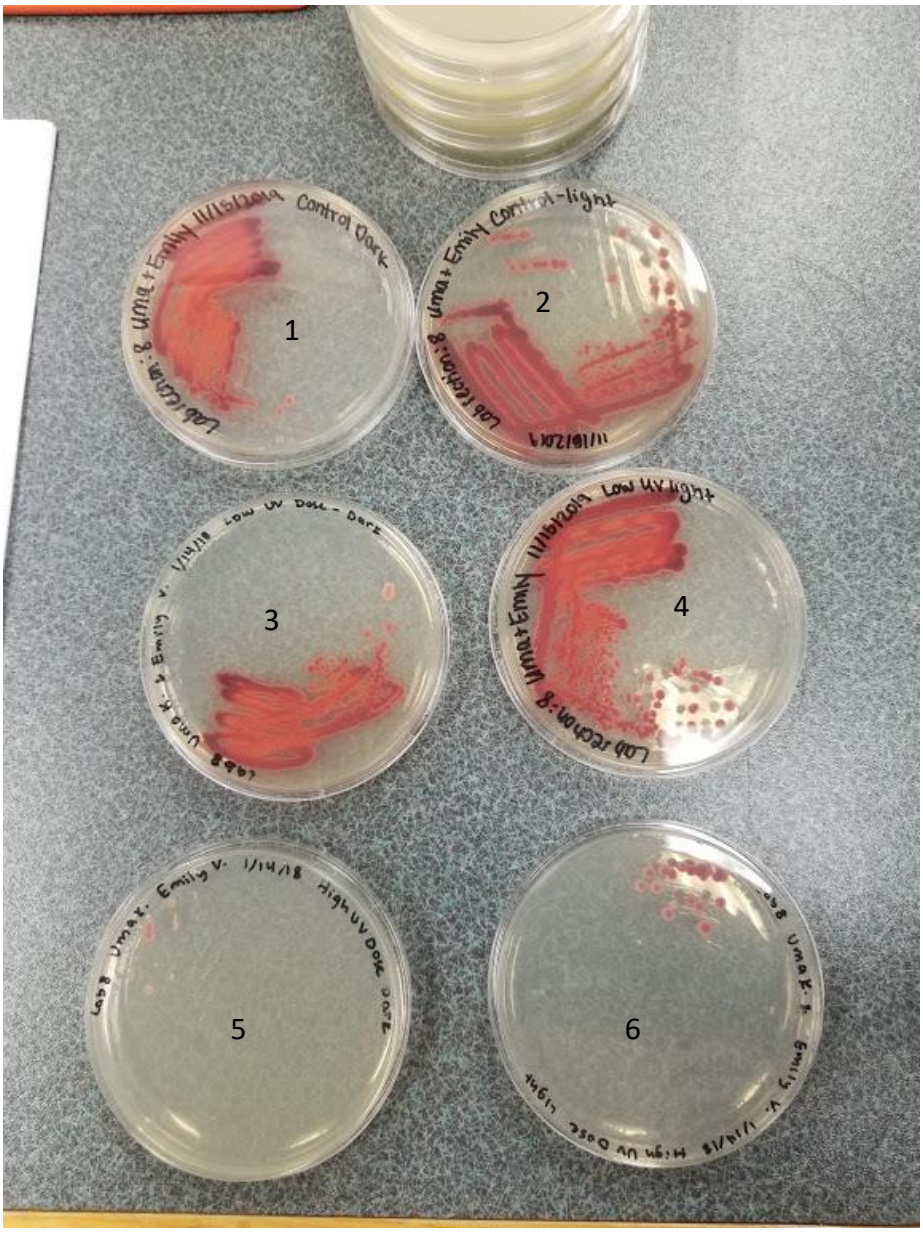

Figure 1. Colonies of *Serratia marcescens* exposed to varying levels of UV light

The above figure shows the results after Part 1 of the experiment. The *S. marcescens* were plated with the streak method and grown in various conditions at 25°C. Plates 1 and 2 were not exposed to UV light as control, while plates 3, 4, 5, and 6 were. Plates exposed to UV light grew bacteria more poorly than the control plates. Plates 1, 3, and 5 were grown in the dark, while plates 2, 4, and 6 were grown in the light. Plates grown in the light grew colonies better than if grown in the dark. All plates produced red bacteria.

Figure 2. Regrowth of *Serratia marcescens* from intitial colonies

The above figure shows the regrowth of bacterial colonies from the initial plates shown in Figure 1. The control in light plate, the high UV dose in light plate, and the low UV dose in light plate were replated with the streak method and reincubated in the light at 25°C. The control plate and high UV dose were taken from plates 2 and 6 in Figure 1. The low UV dose was taken

from another group's plate. All plates produced red bacteria. The bacteria initially dosed with high UV grew more poorly than the control and the low UV dosed plate.

Figure 3. *Serratia marcescens* grown at 37°C

The above figure shows bacteria not dosed with UV light and grown at 37°C in the light. The bacteria produced little to no pigment. This plate functioned as a control.

Discussion:

The hypothesis of this experiment was not supported. It was expected that bacteria submitted to high levels of UV radiation would sustain damage to their genetic makeup that would subsequently lead to the growth of cream-colored colonies because heritable damage occurred. However, all bacteria grown during the experiment were red, even after a high dose of UV radiation. This could be due to photorepair, though only bacteria incubated in the light could use this process. The bacteria grown in the dark also continued to produce red pigment, meaning photorepair was not what caused the red pigmentation. Most likely, the UV light simply did not affect the gene encoding the red enzyme. Because there was no phenotypic change during the incubation of our UV treated bacteria, there is no evidence that a genetic

mutation occurred. However, other groups in this laboratory did have the production of cream-colored bacteria after UV treatment, so there is evidence that UV treatment could cause a genotypic change.

While no cream-colored colonies formed, there was a difference in the number of colonies grown based on UV doses. The high dosed bacteria grew fewer colonies than the low dose bacteria, likely because the high UV radiation killed the bacteria.

Studying mutations in model organisms is important for understanding the effects of mutations in humans. By understanding the effects of mutations in bacteria and how they can come about, the influence of mutations and how they come about can be studied in humans. Model organisms reproduce quickly and in large numbers, making the study of genotypic and phenotypic changes easier and more obvious.

An organism's environment can influence its genetic make-up. If *Serratia marcescens* is exposed to high UV radiation in its environment, then a mutation or death can occur. While some mutations don't help the bacteria, it's possible that the inhibition of the red pigment makes the bacteria more resistant to further UV damage, thus giving it an evolutionary advantage (Hanks et al. 1971). Bacteria are not the only organisms influenced by their environment; a famous example is Darwin's Galapagos finches. Each species of finch adapted to its island environment based on food availability and predators. Certain genes were naturally selected for during evolution, leading to genetic and phenotypic changes (Earthwatch 2018).

In conclusion, the study of *Serratia marcescens* can demonstrate many things about phenotypic and genotypic change. The use of model organisms helps show not only the effect of the environment and genetics on phenotypes, but how this may be applied to humans. The study of mutations in bacteria can be applied to mutations in humans, and through the ease of short generation times and the production of many progeny, research

References:

Darwin's Finches and Natural Selection in the Galapagos. [Internet] Earthwatch Institute; [cited 2019 Feb 12]. Available from https://earthwatch.org/Expeditions/Darwins-Finches-and-Natural-Selection-in-the-Galapagos

Hanks AR, Mroz E. 1971. Ultraviolet Radiation Sensitivity of White Mutants and Red Wild-Type Serratia marcescens. Radiat Res 48(2):312–318.

Shibai A, Takahashi Y, Ishizawa Y, Motooka D, Nakamura S, Ying B-W, Tsuru S. 2017. Mutation accumulation under UV radiation in Escherichia coli. Sci Rep-UK 7(1).