Homegrown Bioterrorism

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Topics in Biology

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Abstract

With the ever constant threat of bioterrorism lurking in our world today, many forms of bacteria can be mutated to become weapons of mass destruction. With *E. coli* being one of the most studied types of bacteria, it is readily available to anyone who knows where to look and what to do. In this experiment, ultraviolet light was used to induce mutations to develop a strain of *E. coli* that is highly tolerant to streptomycin to ultimately raise the category strength of the bacteria. The experiment was a partial success due to the fact that there was a reduction in the zone of clearance from the original diameter of 20mm to 16mm.

Background:

Many people talk about the threat of bioterrorism, but what is it really? Bioterrorism is the intentional use of viruses, bacteria, or other germs to cause sickness or death in people, animals, or plants. A person does not have to directly use harmful agents against humans; they could do it through another medium. For example, a person could infect a large population of cows with an infectious disease, which would then either kill the cow or the cow could be killed for beef, thus passing on the disease. Agents that can be used for bioterrorism are ranked into three different categories: A, B, and C. Category A agents are organisms or toxins that pose the highest threat to national or public security. Category A agents are easily spread and have the potential for high death rates. Category B agents are somewhat easy to spread and can cause moderate illness. Category C agents are promising agents that could be genetically customized to become bioterrorist weapons. The classification of certain organisms can change as new methods are developed to avert an attack or to make an accessible agent more lethal (Zubay, 2005).

When a permanent change in the DNA sequence of a gene occurs, it is called a mutation. When a mutation occurs in a cell's DNA, it can change how the cell acts. The reason this takes place is because the DNA contains genes which house the instructions needed for a cell to function properly. If the instructions encoded on the genes are changed, then the cell may be confused as to how it should react to its environment. Mutations can be inherited when parents pass down mutations that previously happened to them. Mutations can be acquired from mistakes when cells divide or from harmful environmental forces (Genetic Science Learning Center, 2010). The four types of mutations are substitution, insertion, deletion, and frameshift. A substitution type of mutation exchanges one base for another. An insertion mutation happens when extra base pairs are added to the DNA strand. A deletion mutation occurs when a section of DNA is lost or erased. When the base pairs are matched incorrectly

within the DNA strand, a frameshift mutation occurs (Understanding Evolution, 2010). The mutation that the E. coli bacteria experienced was the substitution type.

Ultraviolet light was used to induce the said mutations in *E. coli*. Ultraviolet light is present from the 10-400nm band on the electromagnetic spectrum. That part of the spectrum is then divided into three regions: UVA, UVB, and UVC. The UVA region is the 100-280nm area on the electromagnetic spectrum. The UVB region exists in the 280-315nm area, while the UVC region is on the 315-400nm section of the electromagnetic spectrum. The UVC region was used in this experiment to induce mutations in the *E. coli* bacteria. Ultraviolet light kills bacteria by piercing through the cell to damage the cell's DNA. The DNA is damaged by ultraviolet light because it shakes around the electrons, which disrupt the chemical bonds that hold the DNA together. Bacteria have the ability to repair their DNA, but if the bacterium is exposed to the ultraviolet light for too long the damage may be too great to repair. Short exposure to UV light is not enough to kill a large amount of bacteria, but it can cause lasting DNA mutations in some. The reason it can cause mutation is because when the cell tries to repair its DNA, it can make mistakes and match the wrong base pairs together.

In this experiment, artificial selection was used as a possible catalyst for the creation of a strain of *E. coli* that is highly tolerant to streptomycin. Artificial selection is the process of altering the characteristics of an organism through artificial means. Artificial selection is very similar to natural selection except for the fact that in artificial selection, humans decide which organisms pass on their traits to a further generation. While in natural selection, the environment decides which individual has its traits passed on to its offspring. (Gibson & Gibson, 2009)

Antibiotic resistance refers to the resistance exhibited by a microorganism to a drug. When a microorganism has the capacity to live through prolonged exposure to an antibiotic drug, that microorganism is considered to have a resistance. As of 1998, approximately 90 percent of

Staphylococcus aureus strains were partially or completely resistant to penicillin and other similar antibiotics. Antibiotic resistance is on the rise because of the nonchalant use of antibiotics by doctors to treat patients (Harrison, 1998).

The drug being used in my experiment is streptomycin. Streptomycin is a bacterial antibiotic that works by inhibiting typical protein synthesis. The streptomycin binds to the S12 protein of the 30S subunit of the bacterial ribosome, which prevents the initiation of protein synthesis and leads to the death of microbial cells. To determine whether a type of bacteria is susceptible or resistant to streptomycin, bacteriologists interpret the zone diameter around a streptomycin disk in relation to the bacteria. The zone of clearance or zone diameter is the area where no bacteria are growing. For a 10 mcg streptomycin disk, a susceptible type of bacteria has a zone diameter of fifteen millimeters or greater. A resistant type of bacteria would have a zone diameter of ten millimeters or under. The area in between ten and fifteen millimeters is an intermediate area. *Escherichia coli* typically have a zone diameter of twelve to twenty millimeters (WebMD, 2008). In this experiment the gradual shrinking of the zone diameter showed that the *Escherichia coli* were becoming tolerant of the streptomycin.

The bacterium that was used in this experiment was *Escherichia coli*. *Escherichia coli* are gram-negative bacteria that do not require oxygen to live. If a bacterium is gram-negative, it has a thin layer of peptidoglycan in its cell wall and also has an external membrane. *E. coli* is the best known of all cellular organisms. All facets of this organism, which includes structure, biochemical functions, and genetics, have all been studied in-depth. Each *Escherichia coli* bacterium has five to ten flagella which allow the cell to move around and one circular chromosome (Lederberg, 2000). *Escherichia coli*'s genome has 4,639, 221 base pairs, which code for approximately 4,288 different proteins (Blattner, 2009).

Materials and Methods:

The materials that were used for the experiment are as follows: A streptomycin resistant strain of *E. coli*, a regular strain of *E. coli*, nutrient agar plates, a UV hood, an incubator, a burner, inoculating loops, glass vials, and a flame hood.

Using sterile technique, two vials of luria broth were inoculated with *E. coli* and labeled properly. One vial was inoculated with a regular strain, while another vial was inoculated with a streptomycin resistant strain (see figure 1). The two vials were then put in an incubator, set at 37° Celsius, for 24 hours.

Figure 1 - Luria broth inoculated with *E. coli*

Three premade NA plates were divided into sections and labeled properly. Two of the plates were split into six sections, while the remaining plate, the control plate, was divided in half. The two vials were then taken out of the incubator and the regular strain of E. coli was spread onto the NA plates that were split into six sections. Those two plates were then exposed to one minute and thirty seconds of ultraviolet light. After their exposure, six 10 mcg streptomycin disks were placed onto each plate, one in each section (see figure 2). The regular and streptomycin resistant strains of E. coli were spread onto the remaining plate, one in each half. One 10 mcg streptomycin disk was placed in each half where the bacteria were spread (see figure 3). The three plates were then put into an incubator for 24 hours to grow.

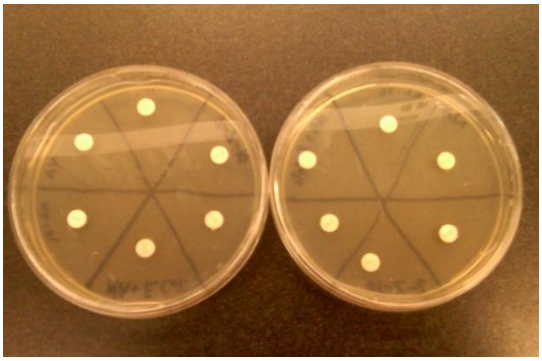

Figure 2 - NA plates with regular E. coli and streptomycin disks

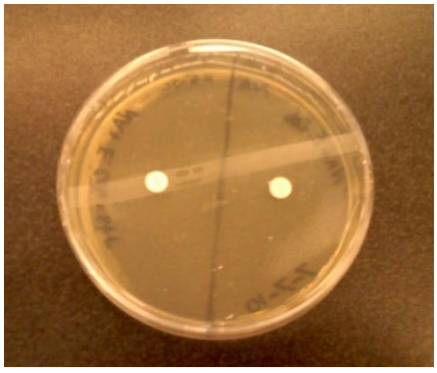

Figure 3 - Control plate with E. coli and streptomycin disks

The two NA plates with the streptomycin disks were removed from the incubator and were observed. The zone of clearance for the regular E. coli strain was averaging at a 20 mm diameter and a 10 mm radius (see figure 4). A colony was selected off of each plate to inoculate in luria broth based off of its proximity to the streptomycin disk. From Sample 1, a colony was selected that was 8 mm from the streptomycin disk. From Sample 2, a colony was selected that was 7 mm from the streptomycin disk. The two colonies that were selected were each put into a vial of luria broth and were put into the incubator for 24 hours.

Figure 4 - NA plates with E. coli and zones of clearance at 20 mm. diameters.

Two premade NA plates were each divided into six sections and labeled. The vials of selected colonies were removed from the incubator and each was spread onto a separate plate. The plate that had the 8 mm sample of E. coli was exposed to another minute and thirty seconds of ultraviolet light to see if multiple exposures to ultraviolet light had a great affect than one single exposure. The other plate, which had the 7 mm sample of E. coli spread onto it, was not exposed to the ultraviolet light. After the exposure, both plates had a single 10 mcg streptomycin disk placed into each of their six sections. The plates were then left at room temperature for 48 hours.

The two plates were observed after the 48 hour period. The plate with the 7 mm strain of *E. coli* had growth on all of the plate except for 10 mm around the streptomycin disk (see figure 5). The plate that was exposed for another round of UV light was inconclusive because all of the bacteria were killed

except for a couple colonies growing on the plate (see figure 6). Two colonies were selected off the 7mm plate and inoculated into two separate vials. The two vials were left in an incubator for 24 hours.

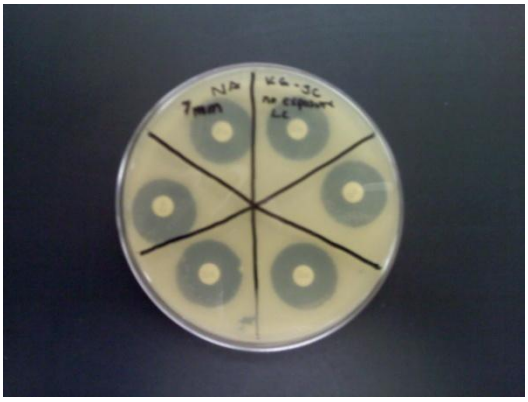

Figure 5 - NA plate with growth 10 mm from streptomycin disk.

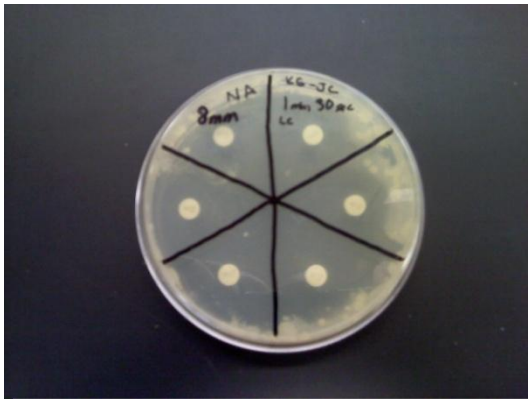

Figure 6 - NA plate exposed to another round of UV light.

The two vials of selected colonies were each spread onto a properly labeled NA plate. Each plate was given a round of UV light exposure, however, the amount of time differed. One plate was left under the UV light for one minute and thirty seconds, while another plate was left for three minutes. Six streptomycin disks were placed on each plate and the plates were put into an incubator for 24 hours.

The two NA plates were removed from the incubator and observed. The plate that was exposed to three minutes of UV light had mostly lethal mutations, but the plate that was exposed for a minute and thirty seconds had growth. The plate with growth had a zone of clearance of 16mm (see figure 7). All bacterial specimens that were used in this experiment were put in a biohazard bag to be autoclaved.

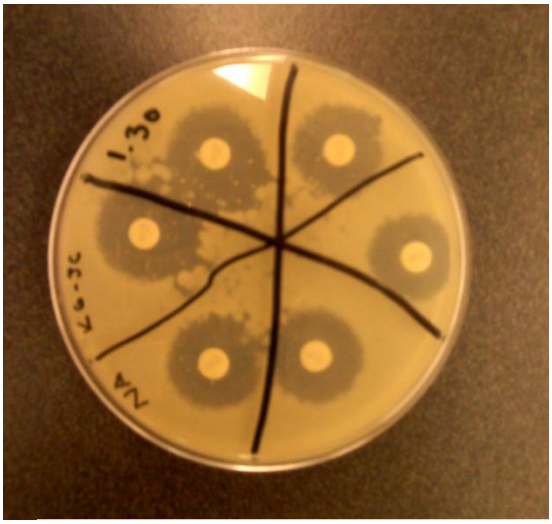

Figure 7 - NA plate with zone of clearance of 16mm.

Results

After the final round of ultraviolet light exposure, the zone of clearance was reduced from the original diameter of 20mm to the new diameter of 16mm. This occurred from a combination of past mutations caused by other rounds of exposure and the final round of exposure to the ultraviolet light. Overall, the UV light mutated the DNA of the *E. coli* and gave the bacteria a slightly higher tolerance to streptomycin.

Conclusion

The results of the experiment proved that UV light could be used to make a regular strain of *E. coli* highly tolerant to streptomycin and raise the category strength given more time. The mutation that the UV light can cause is random, so if someone had the time to expose E. coli to UV light, a highly tolerant strain could be created. All the materials used in this experiment can be purchased from the internet or from any regular grocery or retail store. For example, a small UV light can be purchased from the internet for a relatively inexpensive price. The *E. coli* used in the experiment can be isolated from any human or animal body. Any person with a credit card could become a bioterrorist with the right motivation. All the resources are available; all they have to do is look and put in the time to work with the bacteria.

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