Genetic Modification of E. coli

Byung Sik Yun

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Professor: Josh Cannon

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Abstract

E. coli is a common kind of bacteria that lives in the intestines of animals and people. Various types of E. coli, such as ETEC and EPEC, are becoming increasingly resistant to available antimicrobial agents. One of the most significant technologies used to kill E. coli is UV light. UV light is often used to clean medical equipments and to disinfect water and food products. This paper will examine the mutations of E. coli and whether UV irradiation can cause E. coli to mutate and to develop antibiotic resistance.

Genetic Modification of E. coli

First identified in 1885 by German bacteriologist Theodor Escherich, Escherichia coli (E. coli) is a common kind of bacteria that lives in the intestines of animals and people. E. coli O157:H7, one serogroup of the bacteria, has caused illness and major disease outbreaks in the United States. The Centers for Disease Control and Prevention (CDC) estimates 73,000 cases of infection with E. coli O157:H7 and 61 deaths occur in the U.S. every year. Although most types of E. coli are harmless and live in the intestines of healthy humans and animals, the O157:H7 type produces a powerful toxin that can cause severe illness (Oregon Health Services, 1998).

Origin and Cause

E. coli (0157:H7) was first associated with a sporadic case of hemorrhagic colitis in 1975 (Giusti, 2005) and was first recognized as a cause of illness in 1982, during an outbreak of bloody diarrhea traced to undercooked hamburgers. Particularly dangerous types of E. coli, such as E. coli O157:H7, produce one or more kinds of toxins called Shiga toxins. Shiga toxins can severely damage the lining of your intestines and kidneys. E. coli O157:H7 is spread by eating undercooked meat (usually hamburger), drinking unpasteurized (raw) milk or juice, by swallowing polluted water (drinking water, pool water, rivers, lakes), or by eating hard-to-clean raw vegetables (like sprouts or lettuce) contaminated with the bacteria (Oregon Heath Services, 1998). E. coli can be spread to playmates by toddlers who are not toilet-trained or by adults who do not wash their hands carefully after changing diapers. Children can pass the bacteria in their stool to another person for 2 weeks after they have gotten well from an E. coli O157:H7 illness (CDC, 2006).

Symptoms and Treatment

Infections can be very mild, even asymptomatic, or very severe, even fatal. E. coli O157:H7 toxin can damage the lining of intestines and cause symptoms such as acute gastroenteritis, diarrhea, bloody diarrhea, hemorrhagic colitis, abdominal cramps, vomiting, and mild fever (NIAID, 2007). In some persons, mostly young children, the infection can also cause hemolytic uremic syndrome, a kind of kidney failure. About 2% to 7% of reported infections lead to this problem. In the United States, hemolytic uremic syndrome is the principle cause of acute kidney failure in children, and most cases of hemolytic uremic syndrome are caused by E. coli O157:H7 (Oregon Health Services, 1998). Up to 10% of cases develop severe complications, including the hemolytic uremic syndrome, with a 5% case fatality (Phillips, etal, 2000).

Most people infected with E. coli O157:H7 get better within 5 to 10 days without treatment. Unfortunately, there is no treatment that has been found to be effective against E. coli O157:H7, and it is possible that some antibiotics may increase the risk of problems. Anti-diarrheal agents should also be avoided. Some patients become dehydrated and may need IV fluids (Oregon Health Services, 1998).

Increasing Resistance

The antibiotic sensitivities of different strains of E. coli vary widely. Antibiotics that may be used to treat E. coli infection include amoxicillin, cephalosporin, ciprofloxacin, and the amino glycosides (Wikipedia, 2008). Antibiotic Resistance is a developing problem: various types of E. coli, such as ETEC and EPEC, are increasingly resistant to available antimicrobial agents (CDC, 2005). Some of this is due to overuse of antibiotics in humans, but some of it is due to the use of antibiotics as growth promoters in animal foods.

Some antibiotics work by binding to the cell walls of bacteria. Resistant bacteria change their cell walls, so the antibiotics cannot attach, or produce enzymes to disable the antibiotics. Other antibiotics target the ribosomes of bacteria. Resistant bacteria evolve slight changes in their ribosomes so that the antibiotics cannot bind to them (Fergus, 2006).

Antibiotic-resistant E. coli may pass on the genes responsible for antibiotic resistance to other species of bacteria. E. coli often carry multidrug resistant plasmids and under stress readily transfer those plasmids to other species. E. coli is a frequent member of biofilms, where many species of bacteria exist in close proximity to each other. This mixing of species allows E. coli strains to accept and transfer plasmids to and from other bacteria. Thus, E. coli is an important reservoir of transferable antibiotic resistance (Wikipedia, 2008).

Mutation

Biologists and researchers have been debating when, why, and how bacteria mutate or increase their mutation rate. Some argue that mutations are caused by the bacteria's natural adaptation to their environment. Others argue that mutations are induced by artificial means such as exposure to electron beam radiation and UV light, or selection pressure imposed by the use of antibiotics.

Natural Mutation

Biologists Foster and Layton (2003) have found that, as E. coli cells begin to starve, the bacteria quadruple their expression of DNA Polymerase IV, a mutation-causing enzyme. Polymerase IV provides the bacterium with new properties that help them elicit from difficulty by, for instance, giving them the ability to use other food sources for growth. Foster and Layton have shown that a very common situation in nature (starvation) is enough to cause E. coli to activate Polymerase IV's gene. Sometimes, these mutations give rise to genes with new functions, endowing offspring bacteria with new properties, such as antibiotic resistance (Indiana University, 2003).

Induced Mutation

Levanduski and Jaczynski have found that the decimal reduction time (the time required at a certain temperature to kill 90% of the organisms being studied) for E. coli in ground beef increases due to repetitive exposure to electron beam at sub-lethal levels (Levanduski, Jaczynski, 2007). Their data demonstrated that electron beam can efficiently inactivate E. coli in food products; however, E. coli develops increased resistance to electron beam if the same populations are repetitively subjected to the process. (Levanduski, Jaczynski, 2007).

Researchers from the University of Maryland and Pennsylvania State University have examined E. coli collected from humans, cattle, and food to understand the prevalence of antimicrobial resistance among these organisms. Their data suggested that selection pressure imposed by the use of antibiotics (including testracycline derivatives, sulfa drugs, cephalosprins, and penicillins), whether therapeutically in human and veterinary medicine or as medical procedure in the animal production environment, is a key driving force in the selection of antimicrobial resistance in E. coli (C. Schroeder, etal, 2001).

In the experiment conducted by Biologists Eichenbaum and Livneh (1998), a new mutagenesis assay system based on the phage434 cI gene (virus that can infect bacterial cells) was used to investigate the effect of UV light on the intermolecular transposition of IS 10. IS 10 can increase the fitness of Escherichia coli, assisting in increasing cell survival under hostile environmental conditions (UV light). In the study, it was observed that UV irradiation of

Escherichia coli cells stimulates intermolecular transposition of IS 10, which results in increased survival and mutation rates of E. coli under UV light exposure (Eichenbaum, Livneh, 1998).

Figure 1. Cell survival %

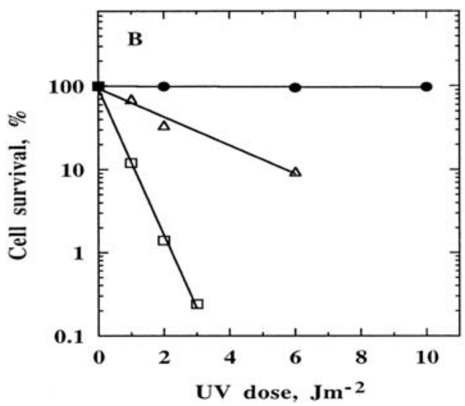


Figure 1 indicates that, as the UV dose increases, almost all of the E. coli strain transposed with IS 10 gene survives the exposure, while the two non-mutated strains begin to die out.

Figure 2. Mutation frequency

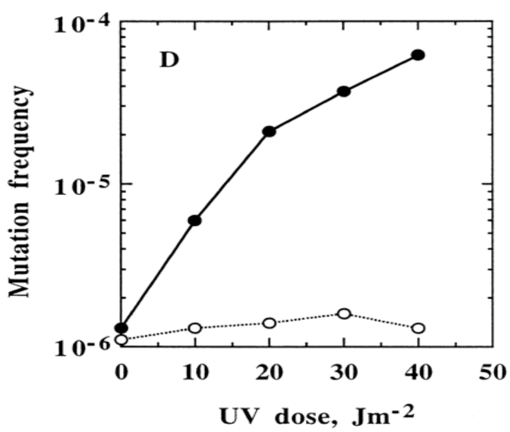


Figure 2 indicates that, as the UV dose increases, E. coli strain transposed with IS 10 gene develops a rapid increase in mutation frequency, while the non-mutated strain only develops a small increase in mutation frequency that eventually drops.

(Eichenbaum, Livneh, 1998) http://www.genetics.org/cgi/content/full/149/3/1173/F1

Use of UV Light and Mutations

UV light is used for disinfection of water and food products and for killing pathogens in human blood products or bacteria in waste treatment systems (Gradbner, 1998). It can be used to prolong the shelf life of food products or to reduce health hazards associated with certain products by reducing the number of viable microorganisms in meat, poultry, and seafood (EUFIC, 2000). UV light kills bacterial cells by damaging their DNA. It causes the nucleic acids in DNA to break their bonds and pair incorrectly. This causes the DNA to either be unreadable or misread, which in turn causes the bacteria to be unable to carry out their normal functions or to reproduce (YahooAnswers, 2008).

However, the UV radiation may cause mutations in the DNA of the bacteria. When the DNA molecules absorb the UV light, the two most vulnerable nucleotides in DNA - cytosine and thymine - can change base-pairing properties (Wikipedia, 2008).

Antimicrobial and UV light use in therapy and growth promotion in chicken production has been associated with the emergence and dissemination of antimicrobial-resistant bacteria. Researchers at the Johns Hopkins University School of Medicine conducted a study to measure the relative risk for colonization with antimicrobial-resistant E. coli among poultry workers compared with community referents (Price, etal, 2007). Their data suggested that the poultry workers had 32 times the possibility of carrying gentamicin-resistant (a commonly used antibiotic) E. coli compared with community referents. The poultry workers were also at significantly increased risk of carrying multidrug-resistant E. coli (Price, etal, 2007). The results indicated that exposure to antimicrobial-resistant E. coli from live-animal contact in chicken industry may be an important route of entry for antimicrobial-resistant E.coli into the community (Price, etal, 2007).

Research Question/ Problem

The central problem of this study was can UV irradiation mutate normal Escherichia coli strains to develop resistance to antibiotics?

In this experiment, standard E. coli strains were exposed to UV light for different amounts of time (0, 15, 30, 60, and 300 seconds) to observe whether the exposure to UV light would transform E. coli strains to develop resistance to ampicillin, an antibiotic.

Methods

Materials used in this experiment included standard E. coli strains (no antibiotic resistance), ampicillin-resistant E. coli strain, LB (Luria-Bertani), ampicillin discs, parafilm, UV light source, and equipments needed to carry out aseptic technique.

First, as a contol of the experiment, LB & ampicillin plates were flooded with ampicillin-resistant strains of E. coli using aseptic technique. At the same time, LB plates without ampicillin were flooded with standard strains of E. coli using aseptic technique. Each plate was exposed to UV light source for indicated amounts of time (0, 15, 30, 60, and 300 seconds). In order to test the development of antibiotic resistance, ampicillin discs were placed on each of the LB plates. Lastly, plates were sealed with parafilm and put in the incubator at 37 °C for 24 hours.

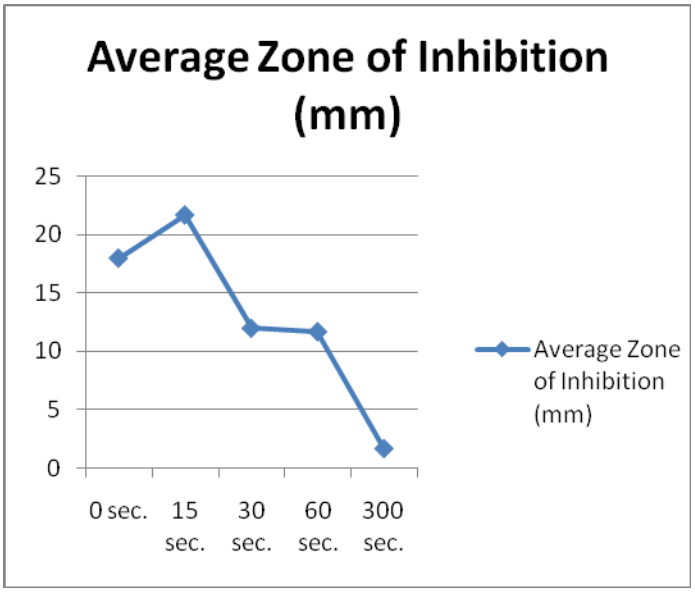
Results

The exposure to UV light resulted in the mutations of E. coli strains. The average zone of inhibition, measured by averaging the nearest distances from the center of each ampicillin disc to E. coli strain, was inversely proportional to UV exposure time (Figures 3 and 4). None of the E. coli in the 0-second plate showed resistance to ampicillin. However, one-third of the E. coli strains in 15-second, 30-second, and 60-second plates developed antibiotic resistance, indicating mutation rate of roughly 33%. This mutation rate was doubled (to 66%) in the E. coli strain that was exposed to UV light for 300 seconds (5 minutes); two-thirds of the E. coli strain in the 300-second plate developed antibiotic resistance.

Figure 3. Table Average zone of inhibition

Plate (UV Exposure Time)	Average Zone of Inhibition (mm)
LB Agar + E. coli (0 seconds)	18
LB Agar + E. coli (15 seconds)	21.7
LB Agar + E. coli (30 seconds)	12
LB Agar + E. coli (60 seconds)	11.7
LB Agar + E. coli (300 seconds)	1.7

Figure 4. Graph: Average zone of inhibition



Conclusions/ Recommendations/ Extensions

First recognized in 1982, E. coli causes 73,000 infections and 61 deaths in the U.S. every year. There are several antibiotics that may be used to treat E. coli infection. However, recently, it has been observed that various types of E. coli are becoming increasingly resistant to available antimicrobial agents.

Biologists and researchers have been studying why, when, and how these mutations are induced. For instance, Foster and Layton have found that starvation causes E. coli to quadruple their expression of DNA Pol IV, a mutation-causing enzyme that gives the bacteria ability to elicit from difficulty. Levanduski and Jaczynski have found that E. coli develops increased resistance to electron beam when repetitively subjected to it. Researchers from the University of Maryland and Penn. State University have found that the selection of pressure imposed by the use of antibiotics is a key driving force in the selection of antimicrobial resistance in E. coli. Lastly, Eichenbaum and Livneh have found that UV irradiation stimulates intermolecular transposition of IS 10 which increases the fitness of E. coli.

In this study, strains of E. coli were tested under UV radiation for different amounts of time. The results confirmed that this UV irradiation had caused mutations in the strains of E. coli tested. One-third of the strains exposed to UV radiation for sixty seconds and under developed antibiotic resistance. Further, this mutation rate of 33% was doubled in the strain exposed to UV radiation for five minutes.

Thus, it has been observed that UV irradiation can transmute E. coli. However, what do these results mean and what kind of impacts will they have on industries and community? UV light is an important technique currently being used by food industries to disinfect water and food products/ equipments and to reduce health hazards associated with certain products by reducing the number of bacteria in meat, poultry, and seafood. Yet, UV irradiation may increase the danger of infection by mutating the bacteria. The continued use of UV light may alter easily treated bacteria into non-treatable, resistant bacteria. Application of UV technology in domestic, industrial, and medical areas may not be reliable. Dishwashing machines and vacuum cleaners with UV technology, as well as food products and medical equipments treated with UV radiation, may cause a wide spread of antibiotic resistant, mutated bacteria among consumers and patients.

At the same time, some aspects of this study remain unanswered and require further research. For instance, can UV irradiation cause mutations not only in E. coli, but also in other types of bacteria? Will longer period of UV irradiation result in greater mutation rate, or is there a limit to the amount of UV irradiation the bacteria can survive? UV light, a significant source of technology for disinfection of food products and medical equipments, has been proven to be competent for causing hazardous mutations in bacteria. Should scientists develop a new, safer technology, or should we keep employing UV technology with more awareness and care?

As UV technology remains the most imperative and frequently applied source for disinfection, the risk to global community, imposed by the possible mutations and the increased resistance of bacteria, persists. Further study must be carried out for the development of potential alternatives and solutions to this problem.

References

Adaptive mutation is common in E. coli, say IU researchers. (2003, October 23). Indiana University, Retrieved July 8, 2008, from http://newsinfo.iu.edu/news/page/normal/1160.html

Basic Summary for Escherichia coli O157:H7. (2007, September 7). Retrieved July 8, 2008, from CureResearch.com Web site: http://www.cureresearch.com/e/escherichia_coli_o157_h7/basics.htm

Causes of Escherichia coli O157:H7. (2007, September 7). Retrieved July 8, 2008, from CureResearch.com Web site: http://www.cureresearch.com/e/escherichia_coli_o157_h7/causes.htm

Diarrheagenic Escherichia coli. (2005, October 6). Retrieved July 8, 2008, from Centers for Disease Control and Prevention Web site: http://www.cdc.gov/ncidod/dbmd/diseaseinfo/diarrecoli_t.htm

E. coli O157:H7 fact sheet. (1998, November). Retrieved July 08, 2008, from Oregon Health Services Web site: http://www.oregon.gov/DHS/ph/acd/diseases/ecoli/facts.shtml#symptoms

Escherichia coli O157:H7 General: DBMD . (2007, September 7). Retrieved July 8, 2008, from CureResearch.com Web site: http://www.cureresearch.com/artic/escherichia_coli_o157_h7_general_dbmd.htm

E. coli: Overview. (2007, February 27). Retrieved July 8, 2008, from National Institute of Allergy and Infectious Diseases Web site: http://www3.niaid.nih.gov/healthscience/healthtopics/ecoli/Overview.htm

E. coli: Cause. (2007, February 27). Retrieved July 8, 2008, from National Institute of Allergy and Infectious Diseases Web site: http://www3.niaid.nih.gov/healthscience/healthtopics/ecoli/Cause.htm

E. coli: Transmission. (2007, February 27). Retrieved July 8, 2008, from National Institute of Allergy and Infectious Diseases Web site:

http://www3.niaid.nih.gov/healthscience/healthtopics/ecoli/Transmission.htm

E. coli: Symptoms. (2007, February 27). Retrieved July 8, 2008, from National Institute of Allergy and Infectious Diseases Web site: http://www3.niaid.nih.gov/healthscience/healthtopics/ecoli/Symptoms.htm

E. coli: Treatment. (2007, February 27). Retrieved July 8, 2008, from National Institute of Allergy and Infectious Diseases Web site: http://www3.niaid.nih.gov/healthscience/healthtopics/ecoli/Treatment.htm

Escherichia coli. (2008, March 27). Retrieved July 8, 2008, from Centers for Disease Control and Prevention Web site: http://www.cdc.gov/nczved/dfbmd/disease_listing/stec_gi.html

Why/how does UV light kill bacteria and viruses? (2008, April). Retrieved July 9, 2008, from Yahoo!Answers Web site: http://answers.yahoo.com/question/index?qid=20080313215355AAbvhBT

Escherichia coli. (2008). In Wikipedia [Web]. Wikimedia Foundation, Inc.. Retrieved July 8, 2008, from http://en.wikipedia.org/wiki/E_coli#Antibiotic_therapy_and_resistance

Mutation. (2008). In *Wikipedia* [Web]. Wikimedia Foundation, Inc.. Retrieved July 9, 2008, from http://en.wikipedia.org/wiki/Mutation#Adaptive_mutation

Eichenbaum, Z, & Livneh, Z (1998, July). UV Light Induces IS10 Transposition in Escherichia coli. Genetics Society of America, Retrieved July 8, 2008, from http://www.genetics.org/cgi/content/full/149/3/1173#MATERIALS_AND_METHODS.com

Fergus, Charles (2006, November 16). Probing Question: Why are some strains of E. coli resistant to antibiotics?. Research Penn State, Retrieved July 8, 2008, from http://www.rps.psu.edu/probing/ecoli.html

Graebner, L (1998, December 4). UCD sheds new light on anti-bacteria technology. Sacramento Business Journal , Retrieved July 9, 2008, from http://sacramento.bizjournals.com/sacramento/stories/1998/12/07/story5.html

Giusti, D (2005, March 16). E. coli's Inidious Spread. Marler Clark LLP, PS, Retrieved July 8, 2008, from http://www.ecoliblog.com/2005/03/articles/e-coli-watch/e-colis-insidious-spread/

Lay , A (2000, September). How is UV light being used in food processing technology?. EUFIC, Retrieved July 9, 2008, from http://www.eufic.org/page/en/faqid/uv-light-used-food- processing-technology/

Levanduski, L, & Jaczynski, J (2007). Increased resistance of Escherichia coli O157:H7 to electron beam following repetitive irradiation at sub-lethal doses.. PubMed, Retrieved July 8, 2008, from http://www.ncbi.nlm.nih.gov/sites/entrez.

Phillips, A (2000). Enterohaemorrhagic Escherichia coli O157:H7 target Peyer's patches in humans and cause attaching/effacing lesions in both human and bovine intestine. PubMed, Retrieved July 8, 2008, from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1728033

Price, L, Graham, J, Lackey, L, Roess, A, Vailes, R, & Silbergeld, E (2007, December). Elevated Risk of Carrying Gentamicin-Resistant Escherichia coli among U.S. Poultry Workers . Environmental Health Perspectives, 115, Retrieved July 9, 2008, from http://www.ehponline.org/docs/2007/10191/abstract.html.

Questions&Answers: Sickness caused by E. coli. (2006, December 10). Retrieved July 8, 2008, from Centers for Disease Control and Prevention Web site: http://www.cdc.gov/ecoli/qa_ecoli_sickness.htm

Schroeder, C, Zhao, C, DebRoy, C, Torcolini, J, Zhao, S, White, D, Wagner, D, McDermott, P, Walker, R, & Meng, J (2001). Antimicrobial Resistance of Escherichia coli O157 Isolated from Humans, Cattle, Swine, and Food . Applied and Evironmental Microbiology, Retrieved July 8, 2008, from http://aem.asm.org/cgi/content/abstract/68/2/576.

Symptoms of Escherichia coli O157:H7. (2007, September 7). Retrieved July 8, 2008, from

CureResearch.com Web site:
http://www.cureresearch.com/e/escherichia_coli_o157_h7/symptoms.htm

Treatments for Escherichia coli O157:H7. (2007, September 7). Retrieved July 8, 2008, from CureResearch.com Web site: http://www.cureresearch.com/e/escherichia_coli_o157_h7/treatments.htm