

System for the Detection of E. Coli O157: H7 in a Food Processing Company

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Abstract

After recent E. coli scares in the United States, a detection system for E. coli and other bacteria in food products before they reach the consumer is key for food safety. We have created an effective detection system for food companies, particularly a tomato packing company. Lab experiments using real-time PCR machines that amplified DNA of E. coli cells were performed and determined whether there was contamination. Other detection methods such as biosensors and sandwich ELISA (Enzyme-Linked ImmunoSorbent Assay) were also studied and then applied to our detection system. Our detection method will provide food companies with multiple options for E. coli detection that fits their own requirements.

Introduction

In 2006, North America experienced an E. coli outbreak of the food born O157: H7 strain. It occurred in two phases; the first phase in September with contaminated fresh spinach and the second in the November and December, this time, carried by packaged iceberg lettuce.

The first spinach outbreak was traced back to plants bagged and grown in San Benito County, California. The E. coli was thought to have originated from

irrigation water contaminated with cattle and deer feces. By October of that year, 199 people had been infected, three of who died and another 31 who suffered kidney failure. The FDA eventually called for all fresh spinach products to be removed from the market in hopes of caging the virus.

In December of 2006, a second outbreak of E. coli was found in Taco Bell restaurants in four states. 71 people were affected and 52 tested positive for the same strain of E. coli, which was traced back to the lettuce used in the majority of the restaurant's products. After investigation, farms in Central Valley, California were found to be the source of the infected lettuce.

Since the series of outbreaks in 2006, many legislative proposals have emerged to require stricter food production, processing, handling, as well as other various measures in a national attempt to improve food safety. The impact of the E. coli outbreaks makes it evident that a method to detect E. coli in food, before it is released to the consumers, is necessary.

After visiting a local food packer, we used the company as a case study and have designed the major components of an E. coli detection system. Such a company would benefit from a microorganism detection system to prevent any loss if they were to accidentally purchase infected tomatoes. The system developed for E. coli detection describes where and when a

detection machine would be placed during the food processing process as well as other possibly more efficient E. coli detection methods that could be further studied in the future.

Background

E. Coli O157:H7

Escherichia coli O157:H7 is one strain of the bacterium *Escherichia coli*, commonly known as *E. coli*.^[7] Even though most strains of *E. coli* are not detrimental, O157:H7 can be particularly harmful to humans and it is the leading cause of food borne illnesses. It was first documented in 1982 as a powerful bacterium when contaminated hamburgers throughout the United States were infected, causing several people severe cases of bloody diarrhea. According to 1999 estimates, each year, in the US alone, 73,000 cases of *E. coli* infection occur, 61 eventually leading to death.

E. coli is commonly found in the intestines of all animals, including humans. Normally, *E. coli* functions in the body by suppressing the growth of harmful bacterial species and by synthesizing vitamins. The *E. coli* serotype O157:H7 produces large quantities of strong toxins called verotoxin or shiga-like toxin that cause severe damage to the lining of the intestine.

E. Coli O157:H7 Location and Transportation

The *E. coli* bacteria can be found in the intestines of many animals, especially in farms and zoos. The bacteria can contaminate the ground, feed bins, and fur of the animals. Meat

can also become contaminated during slaughter through direct contact. Also, bacterium present in cows can be transmitted through their milk. Some of these animals, like cattle, swine, and deer, do not have any receptors for the disease, allowing them to go unaffected. Instead, they act as carriers, giving no visible warnings to humans consuming their products. This strain of *E. coli* can also be found in filth flies on cattle farms, houseflies, and on wounded fruit transmitted by fruit flies. Eating undercooked contaminated meat can also cause infection since the bacteria were not killed in the heating process. However, meat is not the only way for humans to get *E. coli*. One can get it from the consumption of lettuce, spinach, sprouts, unpasteurized milk or juice, and by drinking contaminated water. Also humans can pass it on to each other due to inadequate hygiene.

E. Coli O157:H7 Infection and Symptoms

Once infected with *E. coli*, one can get bloody diarrhea, abdominal cramps, and/or kidney failure. This can occur two to eight days within infection. However, an infection can sometimes cause no symptoms and be resolved in as little as five to ten days. Most people who are infected recover without the help of antibiotics, as there are currently no antibiotics available for the illness caused by *E. coli* contamination. In rare cases, it can cause hemolytic uremic syndrome (HUS). HUS is a condition that destroys the red blood cells and eventually leads to kidney failure. If someone were to develop HUS, blood transfusions and kidney dialysis are required for optimal safety. HUS can also lead to long term effects such as

blindness, paralysis, kidney failure, and possible removal of one's bowels.

***E. Coli* O157:H7 Detection**

E. coli can quickly be detected by sampling the fecal matter of a possible carrier or victim. Testing contaminated material for *E. coli* and withholding that material, before releasing it to the market is one way of preventing human infection and illness. On a personal level, one should always cook beef thoroughly, drink pasteurized juices and milk, wash fruits and vegetables under running water, and practice good hygiene habits to prevent from getting infected.

Radiant Energy and Spectrophotometers

Radiant energy is a substance in the form of electromagnetic waves.^[9] It exists in many types, from low energy radio waves to high-energy gamma rays. The visible light is also a component of radiant energy. Within the spectrum, each wavelength corresponds to a different energy form. The electromagnetic wavelength is inversely proportional to its frequency and energy: the lower the frequency of the wave, the higher the wavelength and vice versa. Visible light is between 380 nanometers and 740 nanometers. Our eyes detect red, blue and green lights the best, since the cone cells in our eyes best respond to these three colors. The color that is not absorbed by an object determines what color the object appears to be. If an object absorbs all wavelengths of light except for the orange wavelength, the object appears orange. Also, if an object reflects more of a certain wavelength, it absorbs the color at the opposite end of

the spectrum more strongly. A blue object absorbs orange light more than any other wavelengths of light.

One biological law takes advantage of the many aspects unique to radiant energy. The Beer-Lambert Law relates the absorbance of any solution to its concentration. If you were to plot the points of several known concentrations along the axis of their respective absorbencies, a standard curve will begin to form. The equation for this line is represented by rules expressed in the Beer-Lambert Law; it allows for unknown concentrations to be discerned based on their absorbency readings, which are constructed using spectrophotometers.

Spectrophotometers use the principles of light passing through opaque liquids to determine the absorbency of a sample. They are used to analyze the radiant energy emitted, or reflected, by an object. This is applied in *E. coli* detection using PCR machines. A gene expressing fluorescence binds to the *E. coli* DNA during the PCR process. Later, the light from the fluorescence is detected by a spectrophotometer. The more light that is detected, the greater amounts of DNA, and therefore *E. coli* there is in a sample.

Polymerase Chain Reaction (PCR) Machines

In the mid 1980s, scientists successfully created the first PCR machine which could control polymerase chain reactions.^[5] PCR is a technique used for isolating and amplifying a fragment of DNA by enzymatic replication. Because PCR is an *in vitro* technique, it can be performed without restrictions on any form of DNA.

PCR requires several basic components which include a DNA template with the fragment of DNA needed to be amplified, 3' and 5' primers, DNA polymerase, deoxynucleotides, buffer solution for optimum chemical environment, and various ions for PCR-mediated DNA mutagenesis. The PCR reaction is then carried out in small tubes placed in a thermal cycler.

The samples are then cooled down to their original temperatures so that the new DNA strains completely connect, thus, successfully creating one 'synthetic' single-strain for every 'natural' one. The process is then repeated, each time doubling the number of double-strained DNA. A scientist pre-programs the machine to do the process for a certain number of cycles, depending on how much DNA they need.

Today, there are many different PCR methods; of them, real-time PCR is one of the most effective and timely. The machine is able to give readings as it amplifies the DNA using photocells to detect the dye's fluorescence, which is an indicator of how much DNA has been created. Using real-time PCR, it can take up to an hour for DNA to be amplified. PCR machines allow for more secure analyses and faster experiments, giving scientists higher productivity. The problem, however, with real-time PCR is that it amplifies DNA in dead cells as well as alive ones and gives rise to complex interpretations and legal questions.

The new system that the USDA has adopted to detect the presence of E. coli in foods is the BAX System. This system uses PCR technology for rapid amplification and fluorescent detection. This method can be used as a quick

method for accurate detecting for a wide variety of foods. The BAX uses PCR to amplify a specific fragment of DNA that is stable and unaffected by growth environment. By using a genetic sequence specific to the organism that is present, the system then uses fluorescent detection to analyze PCR product for positive or negative results.

Alternative E. coli Detection Methods

Biosensors utilize many techniques such as optical and electrochemical to detect their target sample ^[4]. In optical biosensors, it utilizes the property of gold and other materials that absorb laser light, which produce electron waves on the gold surface. This only occurs at a certain angle and wavelength so that the target sample produces a measurable signal. In electrochemical biosensors, enzymatic catalysis of reactions produces ions. A target sample reacts on the active electrode surface and the ions that are produced give the reference electrode a signal, indicating that the target is present.

In this case, the biosensor is a both optical and electrochemical biosensor that detects E. coli bacteria suspended in solution. The optical side uses a microscopic layer of gold, which reflects light at certain frequencies, triggering the detection. This is a polymer coated, open circuit based on immunoassays. The bacteria attach to the antibodies, creating a biological sensing surface. The change in the impedance that is caused by the attachment of the bacteria can be measured and the number of bacteria can be found. The electrochemical and physical changes that occur trigger the sensor and thereby detect the presence of the E. coli.

Biosensors are easy to use, very portable, and reagentless. They also produce results extremely quickly. The downside of biosensors is that they have difficulty detecting low levels of bacteria content. Since only 10 cells are necessary for *E. coli* infection to occur, biosensors do not have the ability to ensure food safety. The long incubation time of the bacteria, the many steps that it takes to prepare the sample for detection, and the interference on the biological interface are some challenges that biosensors face.

ELISA is another viable option to detect *E. coli* in samples^[8]. It uses antibodies that are cultured to seek out *E. coli* O157:H7, ensuring accuracy in detection numbers. One method in particular is sandwich ELISA, in which two antibodies are used to detect the antigen. One antibody is bound to the bottom of the plate well and captures the antigens by attaching them to the antibody. A 2nd antibody attaches itself to the antigen, creating the sandwich. An enzyme that attaches to the 2nd antibody is added as well as a color-changing substrate to detect the amount of antigen in the plate well.

ELISA is useful because it is both fast and accurate. Unlike other methods, sandwich ELISA does not need purified samples to work with which saves both time and money. However, ELISA is dependent on having the specific antibody for the particular strain of bacteria that you are trying to detect to ensure correct bonding. Also, both antibodies must be purified, and must not inhibit the binding ability of each other.

Experimental Design

Preparation of *E. coli* DNA from Pure Culture

10 milliliters of *stx1* and *stx2* were cultured in a LB (Lysogeny Broth) medium.^[2] These cells were then placed in a centrifuge for 5 minutes. A centrifuge spins samples at high speeds, applying forces perpendicular to its axis. This separates the different types of matter in the sample by mass, sending the denser matter towards the bottom. After the centrifuge, the cells are transferred to Eppendorf tubes and resuspended with Tris-EDTA, along with lysozyme. The mixture is then incubated at 37°C for 30 min, and then at 65°C for another 30 min. After the incubation, chloroform is added to the solution to precipitate the DNA. It is then placed in a centrifuge again, and transferred to a different Eppendorf tube. RNaseA is also added to digest the *E. coli* RNA. After the addition, it is incubated a third time at 37°C for 5 minutes. Three molar sodium acetates were added to the mixture, again, the mixture was placed in the centrifuge. This separates the DNA in the mixture and a spectrophotometer was used to measure the concentration. The DNA is then diluted and run on a 1% agarose gel that is stained with ethidium bromide. After the gel electrophoresis is complete, it can be viewed by an UV-Doc.

Inoculation, Isolation, and Detection *E. coli* O157:H7 using Real-Time PCR

The detection of *E. coli* is key in the production and transportation of foods in order to avoid incidents similar to the outbreaks of 2006. In a previous

experiment, real-time PCR was used for the detection of *E. coli* in food samples.

A simple description of the procedure commonly used to detect *E. coli* contamination would begin with suspected samples. Pure samples were inoculated with *E. coli* into water, lettuce, ground beef, and apple juice. Then, samples of the inoculated materials were extracted. After the extraction, the samples were heated and then placed in a centrifuge. The bacterial DNA and RNA were isolated and then mixed with primers for seven O157:H7 genes: Shiga-toxin 1 (*stx1*), Shiga-toxin 2 (*stx2*), attaching and effacing protein intimin (*eaeA*), flagellar H7 antigen (*fliC*), hemolysin (*flyA*), somatic antigen O157 (*rfbE*), and 16S rRNA (*16S*). The primers were mixed with SYBR Green Master Mix (containing dNTP, buffer, SYBR Green dye, and DNA polymerase), forward primer, and reverse primer. After the addition of the mix, the samples were once again placed in a centrifuge, which spun the samples at high speeds. The mixtures were then pipetted into a 96 well plate and then put in a PCR machine and cycled at 1 x 50°C for 2 min, 1 x 95°C for 10 min, and 40 x 95°C for 15 sec, and 60°C for 1 min, taking a total of 2 hrs and 15 min (40 cycles).

After amplification, the presence of *E. coli* O157:H7 was detected to be 1.5×10^2 to 1.5×10^3 CFU (colony forming units) per milliliter. Using the TaqMan probe for *stx2*, viable *E. coli* O157:H7 was detected with a sensitivity of 15 to 1.5×10^2 CFU per milliliter.

Both DNA and RNA should be used as templates for the presence of *E. coli* because, while DNA is present in both alive and dead *E. coli* cells, RNA is only present in *E. coli* cells that are alive. By using RNA to track the number

of *E. coli* cells present, only the number of alive cells are counted, and thus giving a more accurate number. Unfortunately, this particular experiment had a time limit that required for faster detection, thus, the PCR process only detected DNA, not RNA.

Results and Analysis

For the DNA standard concentrations for all seven genes, we used 5, 0.5, 0.005 and 0.00005 ng of pure *E. coli*, each representing 1×10^9 , 1×10^8 , 1×10^6 , 1×10^4 bacterial cells, respectively. For every 10^{th} dilution, the cycle threshold was about 3.3 cycles apart, until it hit an amplification peak. These results fit the standard curves that would be expected for the cell representation.

Two columns were given to each sample for added accuracy, with all seven genes represented in each row. For the water control, the cycle thresholds were 26-32.0 and 28.5-29.5, 27.5-29.5 for ground beef (Figure 1), 28.0-30.0 and 28.0-31.0, for lettuce and 26.5-29.0 for apple juice. By comparison to the standard curves, the average of cycle threshold correlates to about 0.005 ng DNA, or 1×10^6 cells.

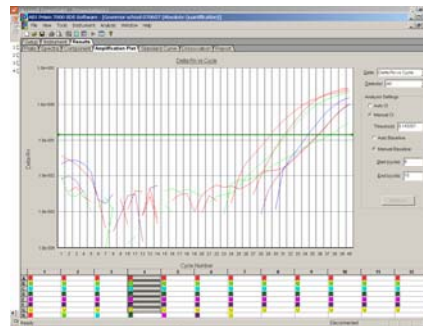


Figure 1

This experiment shows what a scientist could expect when amplifying *E. coli* DNA using PCR. With further

analysis one can discover how long amplification may take depending on the host sample. In this case, ground beef took the longest to reach its cycle threshold, because the fatty tissue within the beef hinders DNA extraction, thus decreasing the initial concentration of DNA.

Also, a scientist can determine just how much DNA can be expected. We began with one μl of DNA, about 100 cells. After PCR, the DNA samples were raised to 0.005 ng, or 1×10^6 cells. A basic equation can be derived from these results for a general expectance rate.

E Coli Detection System Case Study

Procedure of Violet Packing LLC

The farms that supply Violet Packing with their tomatoes use heavy machinery during the picking process. A harvester shakes the tomato plant making the tomatoes fall into the holding container. The tomatoes are then transported down a line and reach a set of electronic fingers. The fingers (Figure 2) use colorimeters, where the photocells read the color of the tomato and automatically reject the ones that don't fit the color specifications.



Figure 2

After the tomatoes go through the photocell, the harvester then transports the properly colored tomatoes to the truck moving along side it. The tomatoes in the truck are then hand sorted for any additional imperfections.

Once the tomatoes are delivered to the packing company, they are further examined by inspectors to see if the tomatoes comply with the standards of the company and USDA. For example, if live insects are found in the batch, the entire load is then rejected, since most food borne illnesses spread rapidly. The tomatoes are then separated by grade (quality) and are thoroughly washed. Afterwards, they are placed in open kettles and water presses (see figure 3) to be cooked for 15-20 minutes at 200°F .



Figure 3

After the cooking procedure is complete, the cooked tomato is then canned and packaged to deliver to customers.

Vulnerabilities in the System

There are several points within their system where E. coli can get into the fruit. There are also several places where testing can be done for the E. coli. E. coli can first enter the system in the

farms during the growing and harvesting stage. Another entry point is during open air cooking process since the vessels are not closed. In general, because the entire process is held in open air, there is chance of infection any time, from contaminated water or trucks that have contamination due to previous tainted transports.

Design

When the tomatoes are being picked, care should be taken to pick out the tomatoes that are damaged or open, since cracked fruit provides a site for flies carrying *E. coli* to land and transmit the bacteria. Additionally, the farmers should grow the type of hybrid tomatoes with firmer skins to initially attempt to prevent cracking.

Apart from the standards of the USDA, we suggest that a small sample of tomatoes should be taken aside from each batch and put through a detection process that utilizes cameras. A treated silicon chip is combined with a digital camera to identify the *E. coli*^[3]. The *E. coli* are stuck to the silicon chip, coated with an *E. coli* protein Translocated Intimin Receptor (TIR), which reflects a laser light that is pointed toward the chip. The laser light then becomes visible to the digital camera, which can be instantly identified.

Our second line of detection would be implemented after the tomatoes have gone through the open air cooking. After the cooking processes is complete, a sample from each kettle is taken and then sent through the *E. coli* isolation system mentioned above in order to detect any possible traces of *E. coli* the batch. Though *E. coli* dies at 160°F, the toxins produced by the bacteria do not disintegrate until

thousands of degrees Fahrenheit. Fortunately, shiga-toxins 1 and 2 can be detected using the Shiga Toxin *E. coli* (STEC) Microplate Assay^[6]. Using those two techniques, not only can any surviving *E. coli* bacteria be detected, but the toxins that the bacteria produce can also be identified. If any sort of contamination is found, the entire batch would then have to be rejected and discontinued from further processing.

Design Analysis

The detection system that we outlined is specific to tomatoes and the Violet Packing plant. Other food companies that deal with products like meat or apple juice may need to modify or use different detection methods depending on the needs of the company. Cost may be another factor, especially for smaller companies. Technology like the silicon chip and digital camera would be expensive since it is a new and advanced technique. Small companies like this might only want to use PCR when they are detecting *E. coli*, since PCR is a relatively low cost method.

Conclusion

Due to recent current scares in lettuce and spinach, it is evident that an effective detection method for *E. coli* is necessary for the health of the consumers. In this project, we set out to devise a system of *E. coli* detection for food packaging plants. Doing this, we studied various methods for *E. coli* detection in foods, their lab procedure, and how food-processing companies can use them to prevent *E. coli* from getting to the consumers. These various methods include real-time PCR, biosensors, and sandwich ELISA.

After various lab experiments with PCR and with research on biosensors and sandwich ELISA, we then developed a partial system for a tomato packaging plant to detect E. coli using methods of PCR. This system can then be integrated into other already developed detection systems we researched. The alternative E. coli detection methods that were discussed can also be implemented after further testing that proves what is best for the needs of a particular food company.

Future Work

In our current detection method, real-time PCR is used as the process for E. coli detection. The sensitivity of PCR machines is undoubtedly higher than that of other detecting methods. However, the accuracy varies since the DNA of dead cells is also recorded as hits of possible of E. coli, even though the cell is dead and is not producing any toxins. We believe that in future experiments, the RNA, which has the same life span as the cell, should amplified in the DNA, so that there is a more accurate count of viable E. coli cells.

Though PCR is the conventional method of detecting E. coli, there are other alternatives that may provide better results. If we had more time, other possibilities like alternative E. coli detection methods could be tested and their efficiency and accuracy compared against that of PCR. We would inoculate a sample of ground beef with a known amount of E. coli and then run the sample of beef through PCR, biosensors, and ELISA. By looking at the results, one can determine the best method to use for E. coli detection in food.

Another set of experiments that could be performed would be to test the

best method to detect E. coli in certain types of food. Because E. coli does not limit itself to one type of food, different methods might need to be used depending on the kind of food that is tested, whether it is meat, fresh produce, or apple juice. For example, it is difficult to amplify DNA of ground beef due to the presence of fatty tissue. Due to this situation, real-time PCR is not the best method to use. One could use a biosensor or an ELISA, which does not need purified substances.^[1] This way, every kind of packing plant could have its own customized E. coli detection process and techniques that are tailored to the type of food that they deal with.

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