# Improving Food Safety with Genomics

It happens to all of us: for no apparent reason, we suddenly get sick. The symptoms include diarrhea, vomiting, cramps, nausea and fever. The consequences can range from missing a day or two of school or work to hospitalization or even death. It's often called "food poisoning" yet the cause is not really poison but rather an infectious organism that we ingest through contaminated food or drink. Foodborne illnesses affect us all, but the impacts are most severe for children, the elderly and other people whose immune systems are not strong enough to fight off the infection. Such illnesses are more common than you might think; one in eight Canadians experiences a case of foodborne illness each year, amounting to about 4 million cases annually[1].
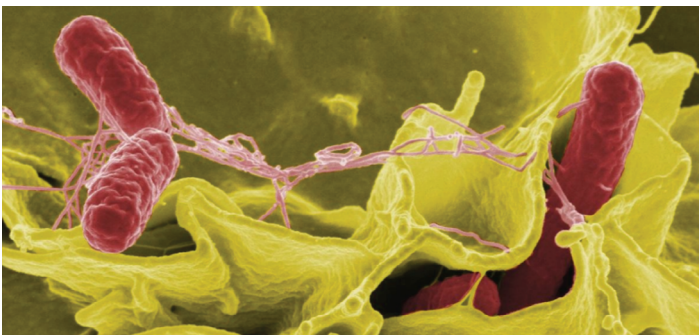
Cells of *Salmonella* typhimurium *(red) shown in this colour-enhanced scanning electron micrograph, are about 0.7 to 1.5 micrometres in diamater and from 2 to 5 micrometres in length. Salmonella is one of the most common causes of foodborne illnesses. (Photo credit: United States National Institute of Allergy and Infectious Diseases)*

Most of the time, preventing foodborne illnesses is a matter of hygiene. For example, if a piece of uncooked chicken is contaminated with Salmonella, and the same food preparation equipment is used for both preparing chicken and for salad, there is a risk that some of the bacteria will stick to the cutting board and the knife, thus contaminating the salad; this is called cross-contamination. When workers who handle food, whether in a grocery store, a restaurant or a food processing plant do not practice proper handwashing procedures, they can pass on any infectious agents they may carry to people who eat the food they have prepared.

In some cases, foodborne illnesses can cause large-scale and sometimes tragic, events. In 2008, 22 people died after an outbreak of *Listeria* in an Ontario meat-packing plant[3]. Ten years earlier, 805 Canadians, mainly elementary school students, were sickened by cheese contaminated with *Salmonella* in ready-to-eat processed food[4]. More recently, in the fall of 2012 an *E. coli* outbreak at an XL Foods slaughter plant in Brooks, Alberta infected 18 people and prompted the largest beef recall in Canadian history[5].

Genomics - the branch of science concerned with manipulating and mapping DNA - plays an important role in tracking the source of outbreaks like these. Tools like DNA fingerprinting enable researchers to identify the strain of bacteria responsible for a particular illness and determine whether it's the same strain that is causing other cases of the illness. Genomics-based testing can also be used to prevent future outbreaks by enabling rapid identification of pathogens in food and stopping the distribution of these products before they reach the consumer. Finally, genomics can further our fundamental understanding of why some strains are so deadly and others are not, leading the way toward vaccines, antibiotics and other disease-fighting strategies.

How it works

An organism's genome is the 'blueprint of life,' the complete set of instructions for making its cells, cellular components (e.g. flagella, whip-like appendages used for swimming) and any molecules it may produce. In a single-celled bacteria, the genome is encoded in the form of DNA. DNA is a long chain molecule made up of four possible types of building blocks: organic molecules named adenine (A), cytosine (C), guanine (G) and thymine (T). Thus, the genome can be thought of as a book written in a code that contains four possible letters: A, C, G and T. The study of genomics has enabled scientists to learn some of the words in this code, for example, sequences that describe how to make a particular protein molecule are called genes. These genes influence traits of the bacteria or virus; allowing them to digest a certain form of sugar, or resist attack by a certain antimicrobial drug.

Unlike plants and animals, microbes have incredible genetic diversity. While your genome is more than 99 per cent identical to every other member of your species, a given strain of *E. coli* typically has only about 20 per cent of its genome in common with all other *E. coli*[6]. Thus, individual strains can differ wildly in the traits they possess, which is why identifying the strain is so important. Identifying a given strain by its DNA sequence also helps track the origin and spread of a given outbreak.

The simplest method for identifying a strain is genotyping, a kind of DNA fingerprinting that can be used to compare how closely related strains are to each other. It is carried out by a technique known as pulsed-field gel electrophoresis, or PFGE. First, a culture of bacteria is grown from a sample; this could be from a sick patient or from a food product. Next, DNA is extracted from this culture and special enzymes are used to cut it into pieces. These enzymes cut the DNA only when they encounter a particular sequence; for example, an enzyme might recognize the sequence AGCT, and anytime it encounters that sequence,

Most common causes of foodborne illnesses in Canada[2]

The microorganisms responsible for foodborne illnesses are generally either bacteria or viruses. Bacteria are single-celled microscopic living organisms that can be found in our intestines. Most of them are harmless to us, in fact some are even beneficial and help us digest food. Viruses are even smaller than bacteria; they survive by invading a host cell and taking over its machinery in order to make more copies of themselves.

| Bacterium	Sources	Symptoms
Campylobacter	raw or undercooked poultry, beef, pork and lamb, raw eggs, unpasteurized (raw) milk,, unpasteurized milk products, raw vegetables, shellfish	diarrhea (blood or mucus), abdominal pain, nausea, vomiting, malaise, fever
Clostridium perfringens	thick soups, stews, raw meat, poultry and beef, meat products, gravies, dried or pre-cooked foods, cooked beans, meat pies	abdominal bloating, pain and cramps, increased gas, diarrhea (profuse and watery), nausea, loss of appetite and weight loss, muscle aches, fatigue
Listeria	raw or contaminated milk, soft cheeses and ready-to-eat meats such as hot dogs, pâté and deli meats	abdominal bloating, pain and cramps increased gas, diarrhea (profuse and watery), nausea, loss of appetite and weight loss, muscle aches, fatigue
Salmonella	unpasteurized dairy products, such as raw milk and raw cheese, and cream-filled desserts and toppings; raw fruit and vegetables (especially sprouts and cantaloupes) and their juices. Homemade products such as salad dressings, hollandaise sauce, mayonnaise, ice cream, cookie dough, tiramisu, and frostings	diarrhea, fever, abdominal cramps, nausea, and vomiting
Escherichia coli (E. coli)	improperly cooked beef, raw fruits and uncooked vegetables, including sprouts, untreated drinking water, unpasteurized (raw) milk and (raw) milk products, including raw milk cheese, unpasteurized apple juice/cider, and direct contact with animals at petting zoos or farms.	severe stomach cramps; diarrhea (often watery and may develop into bloody); vomiting; and fever (generally not very high - usually less than 38.5˚C/101˚F)
Clostridium botulinum	improperly prepared home-canned, low-acid foods (for example, corn,, beets, spaghetti sauce); improperly stored low acid fruit juices (for example, carrot juice); leftover baked potatoes stored in aluminium foil; and honey, which has been linked to cases of infantile botulism and should not be fed to infants under one year of age	fatigue, weakness and dizziness; blurred or double vision; dryness in the mouth, throat and nose and difficulty in swallowing and speaking; headache; nausea, vomiting, abdominal pain and, less commonly, diarrhea; and paralysis that starts in the shoulders and arms and moves down the body

it would cut between the G and C. Thus, the amplified DNA is 'digested' into a series of longer and shorter fragments, the exact pattern of which is unique to that particular strain, acting as a kind of fingerprint.

In order to visualize the fragment pattern, the fragments of DNA loaded into a gel, which is a big flat sheet of a gelatin-like substance. An electric current is then run through the gel, which causes the DNA fragments (which are negatively charged) to slowly migrate through the gel toward the positive electrode. The longer fragments encounter more resistance and move more slowly, while the shorter fragments slip easily through the gel and get further. The electric current pulses through three different directions; this helps to separate the larger fragments more efficiently.

After a set amount of time, the current is turned off, and the result is a series of bands that represents a unique

'fingerprint' for the strain of bacteria. Researchers can then compare visually if an unidentified strain in a outbreak is similar to a known strain. The PulseNet Canada network, a surveillance system of foodborne diseases which includes public health laboratories from each province and two federal laboratories, has created a database of DNA fingerprints from disease-causing bacterial strains. This helps quickly identify outbreaks of organisms such as *E. coli*, *Salmonella* and *Campylobacter*[7].

DNA fingerprinting can trace the spread of particular strains and identify new strains when they arise, but there are other genomic techniques that can help fight foodborne illnesses. One of these is polymerase chain reaction (PCR) technology, which targets a particular segment of a genome and copies that segment millions of times. 'Amplifying' parts of the genome in this way it allows researchers to pick up on even tiny amounts of contaminating bacteria, as little as a
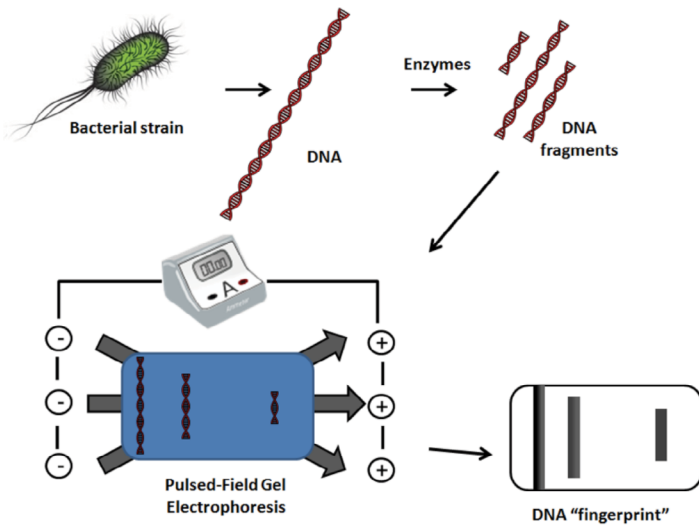
Above: Bacteria are fingerprinted by extracting their DNA and using enzymes to cut it into tiny pieces. The pattern of long and short pieces is unique to each bacterial strain. These pieces are then loaded into a gel and move through it by means of an electric current; small pieces encounter less resistance and move further through the gel. The electric current switches between three different directions in order to better separate the larger DNA fragments.

Below: This image from the gel shows the unique fingerprint obtained by pulsed-field gel electrophoresis for various strains of E. coli*. (Credit: United States Centres for Disease control and prevention, via Wikimedia Commons)*
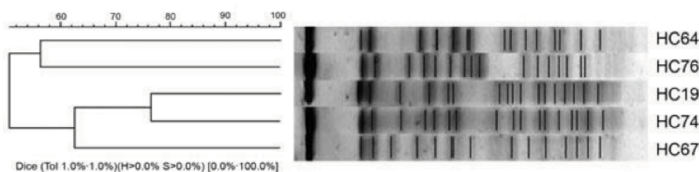
few cells. This means that contaminated samples (e.g. food products) can be tested directly, instead of having to grow a culture of the bacteria in the lab first; this makes the test faster. By using amplification to target a gene or sequence that is unique to a particular bacterium, scientists can quickly detect whether that species or strain is present or not. A variation of PCR called reverse transcriptase PCR (RT-PCR) looks not at DNA but RNA, which is only produced when a gene is expressed, i.e. turned on. Thus, RT-PCR tells you what genes an organism is using at a given point in time, rather than which genes are simply present in the sample. This can be used to identify genes responsible for specific actions, such as producing toxins or resisting antibiotics.

Finally, technology exists to sequence of the entire genome of a bacterium; as of 2012, 3144 complete bacterial genomes had been sequenced[9]. This process is more expensive and time-consuming than those listed above, but it is crucial in order to understand why some bacterial strains cause deadly disease while others - even different strains of the same species - are relatively harmless. Uncovering the genetic basis of a strain's disease-causing capabilities can help researchers develop strategies to fight infections, from vaccines to new antibiotics.

Current research in genomics for food safety

Shiga toxin-producing Escherichia coli (E. coli)

E. coli is a bacterium that lives in the digestive tracts of most animals - including humans - and can be shed in their feces. Many strains of *E. coli* are benign but a family of strains known as *E. coli* O157 produces toxins that can cause a serious and sometimes fatal gastrointestinal illness

2008 Canadian *listeriosis* outbreak
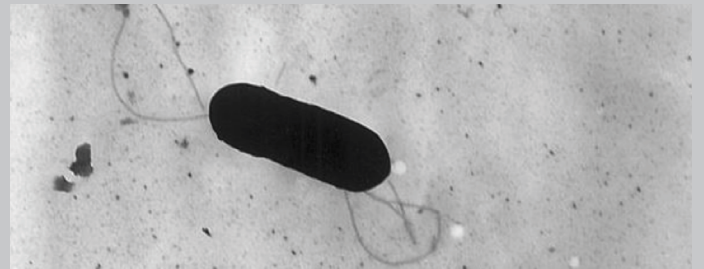
Listeria *is a rod-shaped bacterium about 0.5 micrometres wide and 1.5 micrometres long. It can cause a foodborne illness called* listeriosis *that can turn deadly in people with weakened immune systems (Photo credit: US Centres for Disease Control and Prevention, via Wikimedia Commons)*

The 2008 outbreak of listeriosis from cold cuts was among the deadliest in Canadian history[3,8]. In mild cases of *listeriosis*, the bacteria remains in the digestive tract and symptoms such as diarrhea and nausea last only a few days. However, if the bacterium invades the bloodstream and central nervous system, it can cause confusion, loss of balance, convulsions and even death. After the ingestion of contaminated food, the disease can take up to 70 days to develop.

The first signs that something was wrong started in June 2008 and late July when Ontario public health officials noticed higher rates of reported cases of *listeriosis*. At the beginning of August, Health Canada used DNA fingerprinting methods, including pulsed-field gel electrophoresis (PFGE) to confirm that a sandwich in a nursing home in Toronto was contaminated with *Listeria*. By mid-August, DNA samples had confirmed that a Maple Leaf Foods plant in Toronto was the source of the outbreak and the company began to recall its contaminated products. A federal investigation was launched in the aftermath of the outbreak, and recommendations made by investigator Sheila Weatherill's report were adopted by the federal government in September 2009. For example, the federal government has updated meat-handling manuals and hired more food safety inspectors.

in humans. Many cows carry *E. coli* O157 in their gut, and although it does not cause disease for them, it can make its way into humans through their meat or dairy products. Canadian researchers have developed a vaccine against *E. coli* 0157 [10], but there has been debate over whether or not reducing levels of *E. coli* O157 in cows would actually prevent its spread into humans, and thus whether vaccination is worth the expense.

By analysing the genome of *E. coli* O157, scientists have determined that in the presence of certain genes host animals tend to shed much higher quantities of the bacteria than normal. Researchers have used genomics to show that has shown that these so-called "super-shedding" genes are also present in the strains of *E. coli* O157 that cause outbreaks in humans; this finding supports the use of vaccination in cows[11].
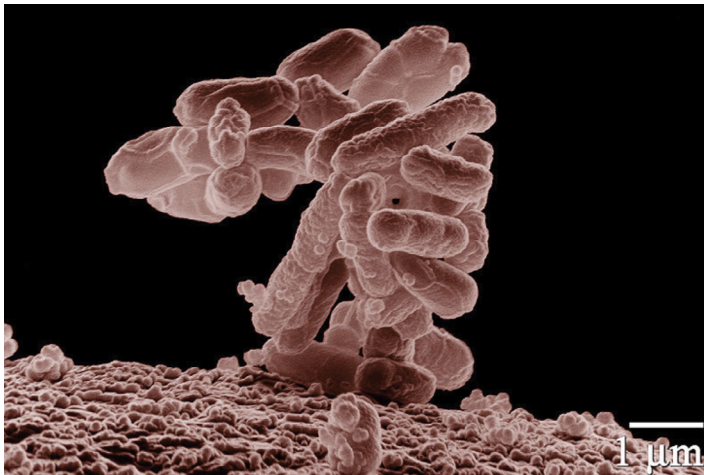
This scanning electron micrograph shows a cluster of E. coli cells, which live in the guts of most mammals. Some strains of E. coli are benign, but others, like E. coli O157, produce toxins and cause gastrointestinal illness in humans. (Credit: United States Department of Agriculture, via Wikimedia Commons)

Probiotic control of disease-causing microbes

Another way to control disease-causing organisms is to encourage the growth of 'good bacteria' - also known as probiotics - in the intestines of animals. These beneficial organisms compete with the toxic bugs for food, but they can also produce compounds that reduce the disease causing ability of the infectious agent. Researchers at the University of Guelph have used genomic techniques like RT-PCR to show that when certain beneficial strains are introduced into the guts of animals like pigs, they actually cause a reduction in the expression of genes used by bad organisms to produce toxins or colonize the intestines[12]. Feeding these probiotics to livestock could help them keep their own guts healthy and reduce the risk to humans at the same time.

Faster detection and tracking

As mentioned, amplifying sections of the genome that are specific to a given species of bacterium can provide a rapid test for the presence or absence of that strain. A team at Université Laval and the Canadian Food Inspection Agency are looking to speed up in *E. coli* O157 detection using a form of PCR that targets the genes they use to produce their harmful toxins. Their goal is to be able to identify as few as 10 cells present in 325 g of ground or trim beef in less than 8 hours[13].

On the *Listeria* side, a team from the University of Alberta and from the Public Health Agency of Canada is using whole-genome sequencing technology to develop a database of Listeria monocytogenes strains[14]. They will then search this database for genes associated with those *Listeria* strains that cause disease. Knowing these, they then can use amplification-based techniques to look for these specific markers to quickly check for the presence or absence of dangerous strains in samples of meat or other food products. Simple tests based on this technique could be installed on site, for example, in meat-packing plants. So far, the database contains over 200 different Listeria monocytogenes strains.

Conclusion

We all have a role to play in food safety. Food producers, processors and consumers can use techniques such as handwashing and proper labelling and sterilization of equipment to reduce the spread of harmful organisms. But genomics provides the forensics tools we need to fully understand how a given outbreak started, knowledge that is crucial to learning from our mistakes and preventing future outbreaks. They also offer insight in the underlying reasons why one strain is more harmful and virulent than another, and can point the way toward new vaccines, new antibiotics and other new strategies - such as probiotics - to fight against infections. In this way, genomics can help everyone enjoy safer food.

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