Bacterial gene exchange and mobile genetic elements

Professor, I read that bacteria reproduce by making copies of themselves. How come they aren't all the same?

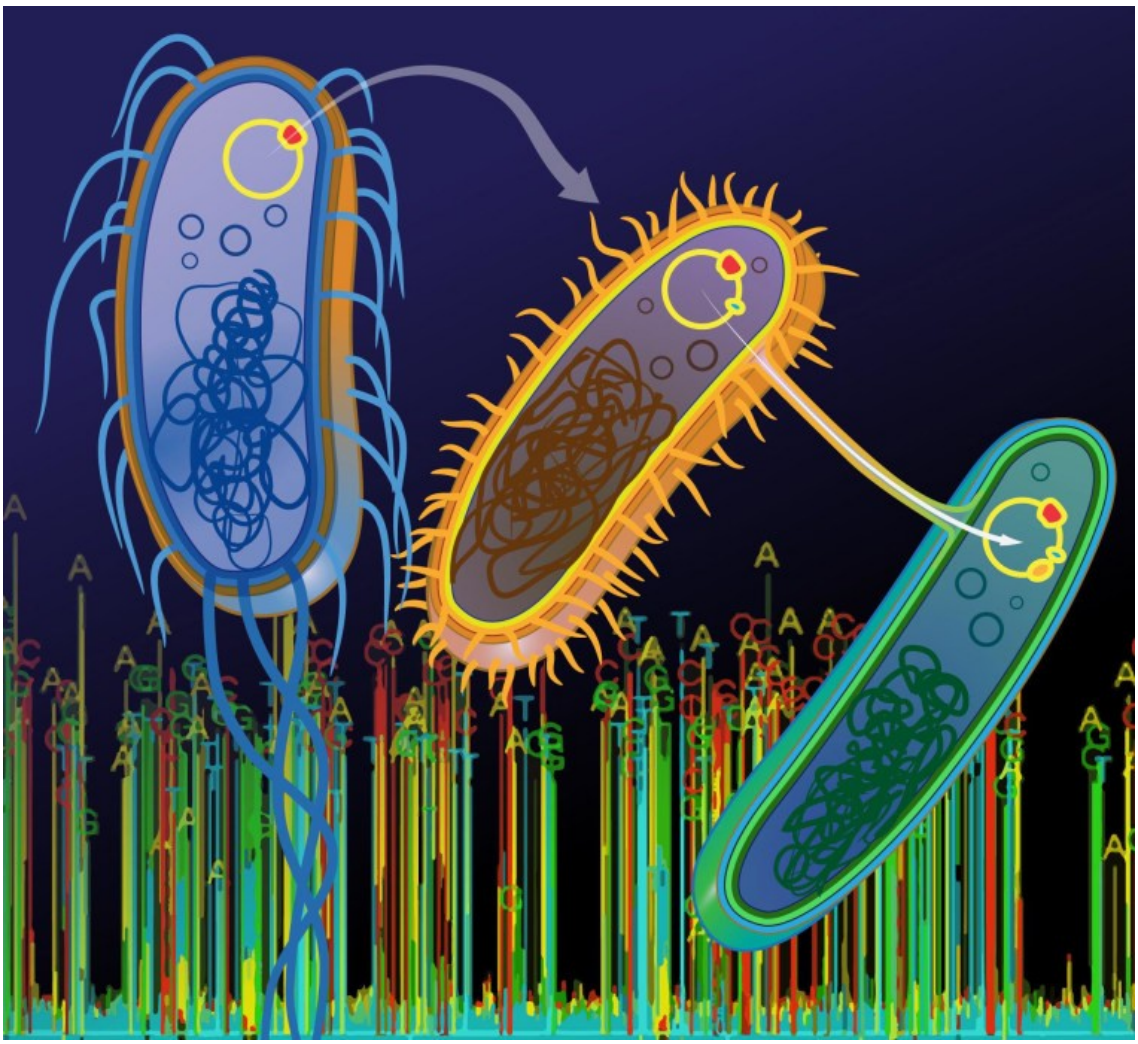

Image courtesy of Darryl Leja, NHGRI/NIH

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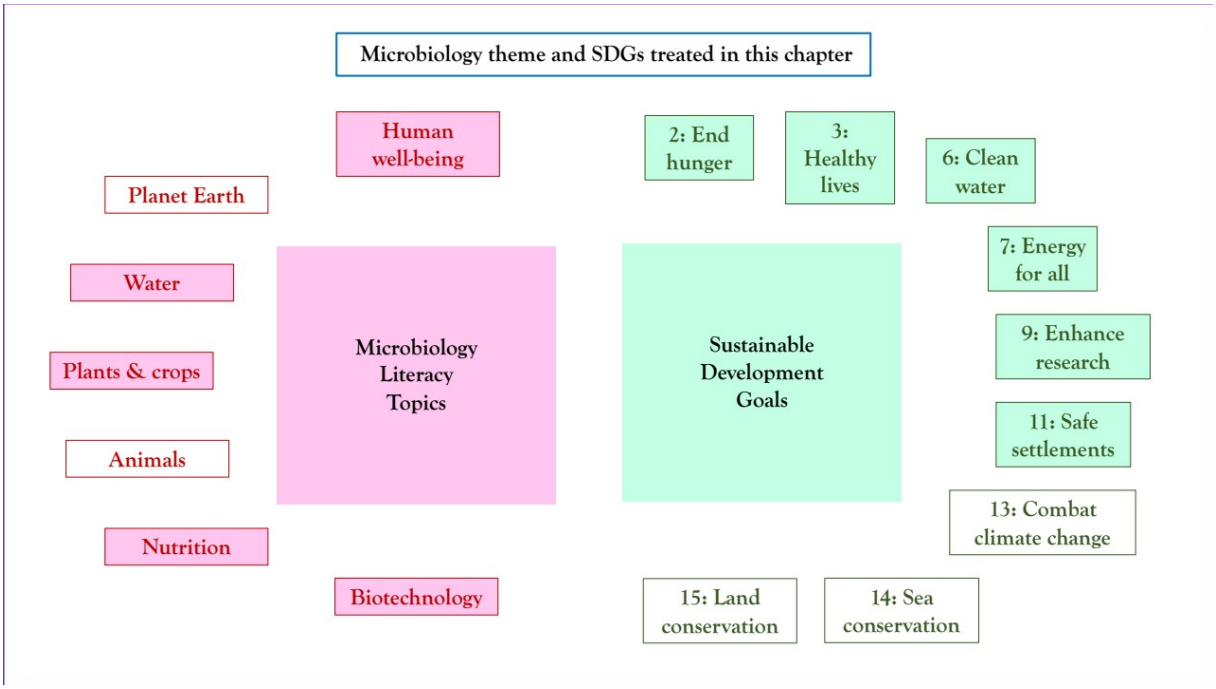
Storyline

Bacteria are simple, small organisms and do not have a sexual reproduction cycle in the way that humans and other types of organisms do. Instead, they reproduce by dividing into two identical or nearly identical copies of the original cell - that is, clones. Like any organism on earth, small changes to bacterial genomes – mutations – occur over time, allowing for gradual evolution of species. However, bacteria possess another ability to evolve that only microorganisms have - the ability to exchange genes between individuals of the same or different species! Imagine if we could give a piece of our genome to our dog or get one from a friend. This gene sharing process is called "horizontal gene transfer" and is almost like human sex but not quite! In humans, sex is both for gene exchange and for making more humans. In bacteria, these are separate events. Mixed bacterial communities share genes easily and frequently, allowing rapid adaptation to environmental challenges and opportunities. This sharing of genes by bacteria has both helpful and harmful consequences for humans. Let's learn about the microbiology of bacterial gene sharing and all the ways it affects our society!

The Microbiology and Societal Context

The microbiology: bacterial genetics and exchange of genetic information; plasmids and other mobile genetic elements; antibiotic resistance; virulence factors; food safety; genetic engineering; GMOs; environmental remediation; biofuel production. *Sustainability issues:* food security; health; water management; sustainable energy; research and innovation; human settlements.



Bacterial gene exchange: The Microbiology

1. ***How do bacteria store their genetic information?*** Bacteria are their own domain on the tree of life and are different from plants, animals, and fungi in many ways. They are always single cells, they do not have most of the specialized compartments found in cells of other organisms, and their genetic material is not surrounded by a nuclear membrane. With a few exceptions, they have a single, circular chromosome, which they carry one copy of at a time. Bacteria can also have a variety of small, independently-replicating, circular DNA units called plasmids. Plasmids can be picked up from the environment, and some can transfer themselves between bacteria by a process called conjugation, or mating. This mechanism of acquiring genes from other, even unrelated, bacteria is called "horizontal gene transfer" (HGT). In the receiving bacteria these plasmids can then be passed on to their descendants (known as "vertical gene transfer" or vertical inheritance). Plasmids carry a diverse array of genes, usually including one or more that are necessary or helpful for the survival of their bacterial host, sometimes in particular environments. These helpful traits could include virulence factors, antibiotic or heavy metal resistance, defense mechanisms, or the ability to utilize certain kinds of nutrients or other chemicals (including some that are toxic to humans). In this way, exchanging plasmids is a successful strategy for survival in changing environments. It should be noted that although this chapter focuses solely on bacteria, microbes from another domain on the tree of life, called archaea, share many of the genetic features of bacteria.

2. ***Plasmids as "selfish elements".*** Although plasmids can help bacteria, they are generally thought of as selfish elements that exist only to proliferate themselves. They often have a negative impact on their host cell's fitness. Since they cannot replicate outside of a bacterial cell, they need a way to ensure that they can persist inside of one. Encoding helpful traits is one way to do this, but plasmids can also behave more maliciously by forcing bacteria to maintain them. Some plasmids carry what is known as an addiction system. They encode both a toxin and its antitoxin and produce both molecules inside the bacteria. The antitoxin is broken down more quickly than the toxin. If the plasmid is lost, the toxin remains in the host but no more antitoxin can be made, resulting in disabling or death of the host. Since not all plasmids carry this kind of system, many eventually get lost from a bacterial population when they no longer provide a benefit or are unable to efficiently replicate in their host cell.

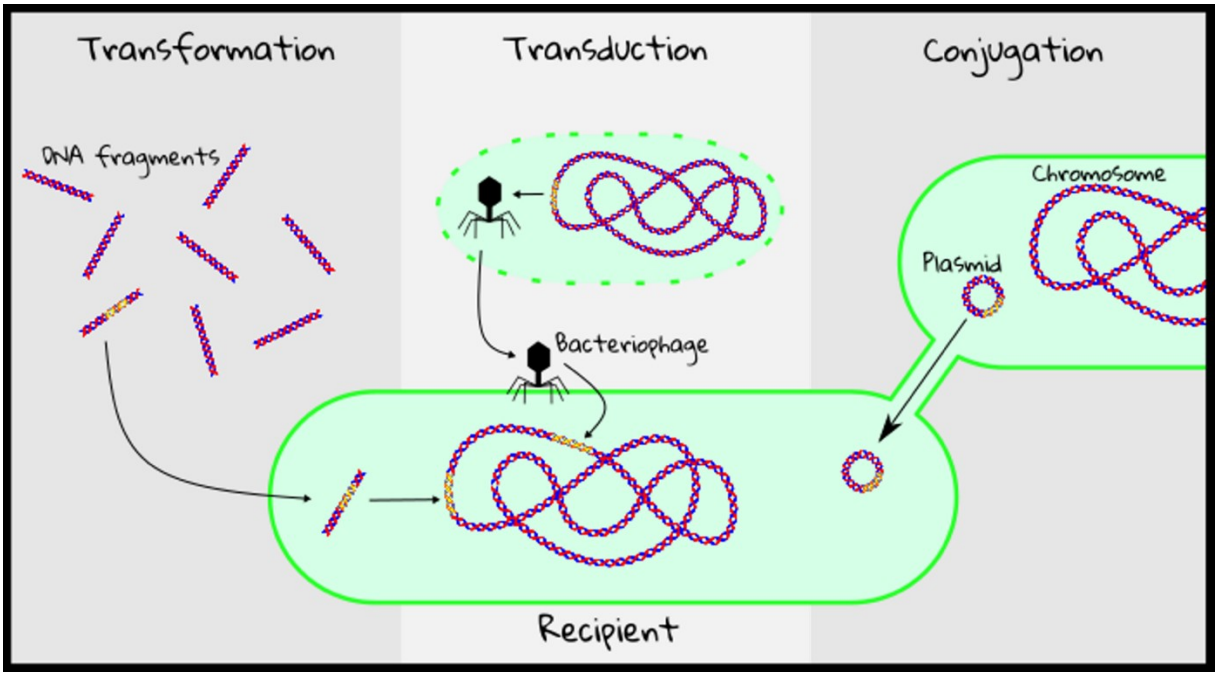
3. ***How do bacteria share genetic information?*** There are a number of ways bacteria can exchange genetic information through HGT, among which three have been studied the most. We will focus on conjugation throughout most of our topics but will also go over the other two, transformation and transduction.

   a. Transformation: some bacteria are naturally capable of transporting DNA found in the environment into their cytoplasm, and potentially incorporating this DNA into their genome. This is a complicated process and requires specific conditions, but the acquired DNA sequences can lead to great advantages if they encode any genes for beneficial traits. Transformation has been adopted as a common laboratory procedure for genetic engineering, discussed more below.

   b. Transduction: occurs when viruses that infect bacteria (called bacteriophages) carry DNA from one bacterium to another. When bacteriophages are packaging up their own

DNA within a bacterial cell, they sometimes include bacterial genome segments or even whole plasmids in the package. These bacterial or plasmid DNA molecules are then injected into the next bacterial cell infected by the bacteriophage. Although transduction is rarer than transformation and involves potentially harmful bacteriophages, it has a major advantage in that the DNA being transferred is not exposed to extracellular environments where it could be quickly degraded.

c. Conjugation: the process most like human mating, where bacteria exchange DNA using direct cell-to-cell contact. One bacterium, called the donor cell, assembles a thin tube-like structure called a sex pilus (also called a conjugation pilus or just a pilus). The pilus attaches to a second bacterium, called the recipient. One or more plasmids are then copied and transferred from the donor to the recipient. Occasionally, some of the host's chromosomal genes are carried along with a plasmid. Some plasmids, called conjugative plasmids, carry genes encoding the machinery needed to assemble a sex pilus but other, often smaller, plasmids do not. These non-conjugative plasmids must rely on conjugative plasmids to initiate conjugation and then tag along with them for the ride into the next bacterium. Once a recipient cell receives a conjugative plasmid, it too can be a donor cell.



Types of HGT in bacteria include transformation, transduction, and conjugation. The yellow sections on the DNA fragments represent functional genes that could be passed around to different kinds of bacteria by way of these HGT mechanisms.

4. ***Are plasmids the only way genes can move around independently of the chromosome?*** Plasmids are one of only a few genetic elements that can replicate and maintain themselves separately from the chromosome, but there are many other types of mobile genetic elements, or MGEs (see Table 1 for a list of MGEs found in bacteria and some of their characteristics). MGEs make up a large portion of the genome of plants and animals too – even humans! In fact, it is estimated that up to 50% of the human genome is made up of MGEs,

many of which can no longer actively move around (these are called endogenous retroelements).

Depending on the type, a bacterial MGE will carry the genes required to move itself around between chromosomes, plasmids, and/or bacterial cells. Most MGEs also carry additional genes, called accessory genes, that fall under the categories listed above for plasmids (virulence factors, antibiotic resistance, metabolizing certain kinds of nutrients or other chemicals, or bacteriocins). Plasmids and other MGEs make up a large network of genetic elements that make most bacterial genomes quite dynamic, with plenty of gain and loss of genetic material even over short periods of time! We'll mostly be talking about plasmids but keep in mind that other MGEs exist, and they can be important too.

Element type	Replication independent of chromosome	Transfer between bacterial cells	Properties
Plasmids	Yes	Yes	Wide range of sizes; can carry many genes with a variety of functions. Can move between bacterial cells
Integrative Conjugative Elements	No	Yes	Typically found on chromosome; can cut themselves out and transfer between bacterial cells during conjugation, but cannot remain in this extrachromosomal form
Transposons	No	No	Known as "jumping genes", they are prevalent in all organisms. In bacteria, can move between chromosomes, plasmids, and bacteriophages. Have multiple mechanisms of transposition including "copy-paste" or "cut-and-paste" using recombination sites, or copy-paste by way of an RNA intermediate.
Gene cassettes	No	Yes	A gene with a recombination site that allows it to move between genomic regions called integrons. Integrons can contain multiple gene cassettes, which often carry antibiotic resistance genes.
Group 1 introns	No	No	Non-coding regions of tRNA, rRNA, or protein-coding genes that can cut themselves out of RNA after transcription. Some are considered MGEs because they carry genes to help them spread to similar regions of the genome.
Insertion sequences	No	No	Smallest and most numerous transposable elements; move within and between chromosomes; can affect gene expression by activating or deactivating genes; encode only genes for their own transposition
Viral agents*	Yes	Yes	Includes viruses, viroids, proviruses, and endogenous retroviruses. Infect hosts and replicate inside of them. Can integrate into host chromosomes and pop out again. Viruses that infect bacteria are called bacteriophages.

MGEs that can be found in bacteria.

**Some scientists consider viruses to be "non-cellular life", because they exhibit some characteristics of living organisms. Others consider them to be MGEs, because they are essentially genetic molecules that transfer between hosts and replicate inside of them.*

5. ***Can MGEs make us sick?*** While antibiotic resistance genes (ARGs) transferred horizontally between bacteria can be helpful for the bacteria, they mean bad news for humans. Some bacteria are pathogens, meaning they can cause disease in humans, other animals, or plants. When pathogenic bacteria acquire resistance to a particular antibiotic, we can no longer treat the infection with that antibiotic. Bacteria can quickly accumulate multiple ARGs, making infections very difficult and sometimes impossible to treat. This happens when plasmids move around between bacteria, taking and leaving resistance genes by exchanging smaller MGEs like

transposons back and forth with bacterial chromosomes and other plasmids. You may have heard the term "superbugs", referring to multi-drug resistant (MDR) bacteria often associated with hospital-acquired infections. Plasmids can also carry "virulence factors", which help bacteria infect and damage plant or animal tissue, and turn a bacterium initially causing only mild infection into a nasty pathogen.

a. *Hospital acquired infections.* Unfortunately, hospitals are fertile ground for breeding MDR bacteria. Without even knowing it, patients bring in diverse kinds of bacteria that can meet each other and exchange genes in the hospital environment. Even with proper isolation and disinfection techniques, bacteria can persist in places like sink drains where they are free to mix and mingle their resistance genes. These MDR bacteria are dangerous to everyone but particularly to hospital patients who may be less able to fight off the infection because they are already sick. The Centers for Disease Control and Prevention estimated in a 2019 report that over 2.8 million antibiotic-resistant infections occur each year in the USA, and at least 35,000 of these result in death. Not only is the death toll high, the estimated cost for treatment of these infections each year is close to $5 billion.

b. *Overuse of antibiotics is likely to contribute to the spread of resistant bacteria.* Exposure to antibiotics (as well as some non-antibiotic pharmaceuticals and other chemicals) is commonly thought to provide selection for antibiotic resistant bacteria and increase the spread of ARGs. Repeated, persistent, and low-dose exposures provide the strongest selective pressures to develop resistance, and the overuse of antibiotics all over the globe may be responsible for much of this exposure. One problem is antibiotics being improperly prescribed to treat non-bacterial infections such as the common cold or the flu. However, it is an even bigger issue in agriculture and aquaculture , where antibiotics are given to healthy animals to make them grow bigger and faster. While this practice has been banned from the EU since 2006, countries like US and China only recently undertook regulatory actions to restrict the use of antibiotics as growth promoters.

Antibiotic-resistant bacteria that grow in farm animals can get into humans through handling or eating improperly cooked food, or by direct contact with the animals or their environment. The animals' feces can contaminate drinking or swimming water, or water used to irrigate crops. The drug-resistant *Salmonella* infection breakouts we often hear about lately are a major concern for public health and are responsible for up to 100,000 food-borne illnesses annually (along with another 1.1 million non-resistant *Salmonella* infections). These infections disproportionately affect kids under five years old.

One reason antibiotics are used so freely in agriculture is that many are available without a prescription and are labeled by their manufacturers as acceptable for use in animal production. Fortunately, some companies are starting to remove these labels so that a veterinarian would have to prescribe them for use, and some countries are putting laws into effect that limit the use of antibiotics for non-therapeutic purposes. If we aren't careful about regulating and managing the use of antibiotics, bacteria will develop resistance to any new antibiotic available and it will quickly be rendered ineffective.

c. *Wastewater treatment plants (WWTPs).* WWTPs are another place where bacteria have the opportunity to exchange genetic information including ARGs. Bacteria from the human gut, both friendly and pathogenic, meet distantly related environmental bacteria washed into the wastewater collection system. The presence of antibiotics and other selective chemicals may promote the selection of bacteria carrying plasmids. While not all plasmids can survive in

such a broad range of host bacteria, those that are able to may be spread to new environments on the receiving end of water exiting the treatment plant and eventually work their way back to humans and animals. This creates a loop that can contribute to the overall spread of antibiotic resistance.

d. *Food safety.* Some bacteria can colonize our food products and make us sick, especially if the food is not handled or cooked properly. The bad bacteria that cause foodborne illnesses can do so in a variety of ways, like by making toxins that they secrete into our bodies or by directly colonizing our cells and doing damage to them. Plasmids can increase the odds of humans ingesting these bad bacteria by spreading genes that make the bacteria more tolerant to treatments we commonly use to kill them, such as heat, salt, acid, and even disinfectants!

e. *Don't spoil my beer!* Humans have been imbibing alcoholic beverages similar to beer for thousands of years. Tasty and rich in nutrients, these beverages were also a hostile environment for microorganisms, making them safer to drink than nearby water supplies that could be contaminated by animal waste. Today, breweries produce beer in great quantities across the globe. Unfortunately, some types of bacteria have evolved to survive in beer and cause it to spoil. Craft beers that are not filtered or pasteurized are particularly susceptible to spoilage. The bacteria in beer use a variety of mechanisms to survive in and spoil it, some of which can be carried on plasmids and shared between different species. For example, genes allowing bacteria to tolerate the hops used in beer brewing are found on plasmids.

f. *Plasmids in space?* As if astronauts didn't have enough to worry about, plasmid-borne antibiotic resistance, virulence, and other environmental adaptation genes may pose an additional threat to their health. The International Space Station (ISS) is in some ways similar to a hospital, in that its inhabitants have stressed immune systems (here due to cosmic radiation and microgravity, instead of illness) and it is a closed environment where each astronaut and each load of supplies or equipment brings a diverse group of microorganisms on board. Sampling of bacteria from the ISS found a variety of commensal (friendly) and pathogenic species. Most of these bacteria harbored conjugative plasmids and had resistance to one or more antibiotics. Research on bacteria in microgravity environments shows that they require higher levels of antibiotics to be killed and they develop antibiotic resistance more quickly than those on Earth. Other genes of concern carried on plasmids include those for metal resistance (including silver, which is used to disinfect the ISS potable water supply), increased UV-C resistance, and metabolism or degradation of unusual substances. These can make bacteria more resilient and more resistant to disinfection methods.

6. ***Can MGEs be our friends?*** As it turns out, MGEs and HGT can be quite useful in biotechnology for research, medicine, and other applications. About 70 years ago, it was discovered that DNA carries genetic information, and soon after that the first plasmids were identified and isolated. Ever since then, researchers have been manipulating plasmid DNA to use as a genetic engineering tool. Plasmids are relatively easy to work with, as they are stable and it is easy to modify them to add, remove, or change genetic information. And, crucially, since they are agents of gene transfer, we can use them to transfer genes of choice including those we manipulate, to organisms of choice. Research labs use harmless bacteria that multiply rapidly to produce many copies of their plasmid to clone the DNA sequences that they are interested in. Plasmids used for this purpose are called "cloning vectors" or just "vectors". Additionally, plasmids can be designed to recombine with host chromosomal DNA, swapping out or adding

in segments of DNA at specific target sites. Bacteria can also be stimulated to produce large amounts of proteins encoded by genes on plasmids. By studying natural HGT mechanisms, researchers developed modified versions of these processes that can be used to get cloning vectors or other plasmids into cells. Many bacteria and other microbes can be transformed, that is they will take up plasmids from the environment, when exposed to certain chemicals or given an electric shock (the latter method is called electroporation). Conjugation can be used if the plasmid of interest is already in a bacterial cell and the genes required to produce conjugation machinery are present. It is even possible to get plant or animal cells to take up plasmids, although this can be a bit more difficult. In the case of plants, scientists can literally shoot plasmids into cells with a device called a "gene gun" to get them through the tough plant cell walls. It is also possible to use certain viruses or bacteria to get plasmids into plant cells, one example of which is discussed below. Transduction and transformation are both used with animal cells, although these cells are not as forgiving as bacterial cells and transforming them may cause undesired results. Plus, most methods for transformation are not possible when the cells are part of a living organism and not in a petri dish in the lab! Although HGT into organisms other than bacteria has proven challenging, this technology is advancing rapidly. Who knows what will be possible in the future?

a. *Plasmids can treat or even prevent disease.* The first use of plasmids in medicine was for production of human insulin, a very simple protein, in bacteria. This was a very important advance because insulin had previously been obtained from pig pancreases and, not being exactly the same as human insulin, was not as good as our own insulin. As biotechnology advanced, more complicated proteins and other biological molecules could be made from plasmids in both bacterial and animal cells. The idea of gene therapy to correct genetic defects sparked much interest in using HGT-based methods. Using gene therapy to replace, fix, or regulate genes that cause disease turned out to be quite possible and there are already some FDA-approved gene therapy drugs on the market. Most of the drugs in use and in testing are actually modified viruses called viral vectors, but plasmids are still important to this system as they are used in the production of these viral vectors. Using plasmids themselves for gene therapy is more difficult because they do not naturally infect cells like viruses do. However, they offer many potential benefits over viral delivery such as reduced chance of triggering unwanted immune responses or causing cancerous cells to develop. A recent advance in plasmid gene therapy research enabled researchers edit the genomes inside of cultured, living mammalian cells! Another clinical use of plasmids is for vaccines. One or a few genes from a pathogen are inserted into a plasmid so those genes can be expressed in the body, triggering an immune response to that pathogen without actually having to inject the whole thing into the body. This is a similar concept to the new mRNA vaccines developed to prevent SARS-CoV2 infection. Both plasmid DNA and mRNA vaccines are cheap, easy, fast, and safe to produce, and easy to modify. They can even be used to treat non-infectious diseases like Alzheimer's, and to create personalized vaccines to train someone's immune system to recognize and attack a cancerous tumor in their body. Plasmid DNA is very stable, a property that is very useful when vaccines are needed in areas where refrigeration is difficult. Just like with gene therapy though, delivery of the plasmid DNA to target cells is not very efficient and immune responses are weak. However, plasmid vaccine technologies are still under development and continue to improve.

b. *Fighting plasmids with plasmids*. Researchers are developing a way to stop resistance plasmids from spreading in human gut bacteria by "curing" them using probiotics carrying engineered plasmids. The probiotics would be ingested and be able to conjugate with resident bacteria in the gut to pass on their curing plasmid. This curing plasmid will displace the resistance plasmids in the gut bacteria and provide the antitoxins for addiction systems used by the resistance plasmids.

c. *Keeping infants safe and healthy.* Probiotics have been used to improve the intestinal health of infants and protect them from infections by bad bacteria that can cause illnesses. The probiotics added to dairy products have been improved by conjugation with other bacteria that naturally live in the human gut. The plasmids transferred into these probiotics have genes for metabolizing casein and lactose, and also to increase production of lactic acid. Metabolism of casein and lactose means that these probiotics can now live in the dairy product (so they don't need to be added separately) and break down otherwise non-digestible components of breast milk in the infant's gut. Increased lactic acid production lowers the pH in the intestines and makes it more difficult for pathogenic bacteria to live there and make the infant sick.

d. *Give GMOs a chance.* When we hear about GMOs as crops, it just means plants that have had their genomes directly modified by genetic engineering, rather than over time as a result of selective breeding (which humans have been doing for millennia). You might be surprised to learn that plasmids are involved in GMO development. As mentioned above, it can be very difficult to get plasmids into plant cells, and only a few plasmids are known to replicate in plants. Currently, we have a good system for working with plants that relies on a bacteria called *Agrobacterium*. *Agrobacterium* naturally transfers a plasmid to plant cells (this method is more commonly used now than the "gene gun" described above). This plasmid, called the Ti plasmid (Ti = tumor-inducing) inserts a piece of itself into the plant's genome where it is replicated and expressed, causing a disease characterized by abnormal cell growth. By inserting an extra gene into this Ti plasmid, after removal of genes specifying tumor induction, scientists were able to create cultured petunia cells that were the first transgenic plants. These plants were created in the early 1980s, and though the applications have broadened widely since then, the concept is still the same. Generally, genetic modifications in plants are used to increase crop yield or improve nutritional value. No genetically engineered crops on the market have been modified to be incredibly large (that is, the "Franken-food" you may have seen in the news). GMOs can have a beneficial impact on society when and if they are proven safe for human consumption. For example, the product Golden Rice is a modified variety of rice that was created to help the hundreds of thousands of young children who die or go blind every year due to vitamin A deficiency. Golden Rice synthesizes pro-vitamin A, which can be converted to vitamin A in humans. Most of the children who suffer from vitamin A deficiency live in areas where rice is a staple crop. Studies have shown that Golden Rice and other the other commercially available GMOs are safe for consumption. However, because of unsubstantiated fears, Golden Rice has not yet been widely grown, to the detriment of all the children who suffer from vitamin A deficiency. There is an urgent need for evidence-based policies.

e. *Delicious plasmids.* Strains of *Lactobacillus* can ferment carbohydrates into lactic acid and are used for making fermented dairy products, such as yogurt and cheese. They are responsible for fermented vegetable foods like kimchi and sauerkraut, and for sourdough bread too! Plasmids naturally found in *Lactobacillus* encode traits affecting the flavor, texture, and aroma of these fermented foods, and some of these have been purposefully introduced during production of dairy products. Other plasmid-encoded traits can affect the rate of lactic acid production, infer the ability to utilize casein (as mentioned above with probiotics), allow *Lactobacillus* to survive better in the human gut, and help keep other unwanted species of bacteria in under control.

f. *Plasmids can help the environment.* A technique called bioremediation can help clean up pollution. Bioremediation means utilizing plants, fungi, or bacteria to break down environmental contaminants into harmless substances. Bacteria are particularly useful for breaking down organic compounds like solvents, petroleum derivatives, and chlorinated compounds (including chloroethenes, chloroethanes, and polychlorinated benzenes, which are are some of the worst contributors to soil pollution all over the world). Introducing new bacteria to a polluted environment is not always an effective method of bioremediation, as they may not survive or disperse into their surroundings very well. A plasmid-based bioremediation solution could help with this. Donor bacteria with plasmids encoding genes for degradation of organic compounds are introduced to the site, so that they can conjugate with well-established native bacteria. That way, even if the donor bacteria do not survive, their plasmids will live on. Interestingly and conveniently, many genes involved in contaminant degradation pathways are naturally found on various types of MGEs.

g. *Biofuels* Plasmids have been used to introduce genes to help optimize microbial synthesis of chemicals used as biofuels. The first example of this was a strain of *E. coli* supplied with plasmid-borne genes to chew up cellulose (insoluble plant fiber) and produce biodiesel fuel. Some types of alcohols used as biofuels (branched, intermediate chain alcohols like isobutanol) can also be produced by bacteria. The environment needed for production of these biofuel alcohols is harsh and only certain kinds of bacteria can live there, but these bacteria do not naturally produce the right kinds of alcohols! Fortunately, strains that can survive in production environments can be transformed with plasmids engineered to carry genes for enzymes that will metabolize biofuel pathway intermediates to the final alcohol products. This process is a type of fermentation and is currently being improved to make it an energy efficient method for production of biofuels.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 2: End Hunger.** One of the ways in which plasmids are helping the End Hunger goal is through their use in creating genetically modified crops that contain favorable characteristics, such as resistance to pests or increased nutritional value. Traits such as resistance to pests are frequently found in other plants. When such a trait is discovered, a plasmid can be used to isolate the gene of interest. The plasmid can subsequently be used to express that trait in crops, resulting in crops resistant to pests or carrying other desirable traits.

Plasmids can also cause problems related to hunger, as they can spread traits that make bacteria more resistant to being killed or increase the chances of bacteria being able to make us sick. In areas where it is already difficult to store and prepare food safely, these plasmid-borne traits are of even more concern.

- **Goal 3: Ensure healthy lives and promote well-being for all at all ages.** Plasmids both positively and negatively affect goal 3. In the positive light, plasmids are commonly used for medical purposes such as production of medicines, enhancing probiotics, gene therapy, diagnostics, and DNA vaccines. Overall, plasmids represent a versatile approach for addressing diseases that affect the wellbeing of humans. In the negative aspect, plasmids play an important role in the spread of antibiotic resistance. Some of the genes that confer antibiotic resistance are frequently carried on plasmids or other mobile genetic elements. Due to their mobility and ease of transfer to other bacteria, plasmids and other mobile genetic elements are important players in the increase in worldwide antibiotic resistant infections.
- **Goal 6: Ensure availability and sustainable management of water and sanitation for all.** Research on the use of microbes in bioremediation efforts, such as cleaning up the environment and recycling industrial wastewater into reusable water is well underway. Using bacterial conjugation, we can transfer plasmids with useful remediation genes into bacteria that are indigenous to specific environments. This practice can minimize disruptions to ecosystems. Plasmid-borne genes useful for efficiently cleaning up environmental, wastewater, or drinking water contamination, include those encoding pathways for breaking down pollutants or removing heavy metals. Unfortunately, plasmids can also be responsible for the transfer and spread of traits that are harmful to humans, which is of particular concern in places where human and/or animal waste accumulate.
- **Goal 7: Ensure access to affordable, reliable, sustainable and modern energy.** One of the most important challenges we face today is the generation of renewable energy to replace fossil fuels. Biofuels made by bacteria could be a viable source of clean energy once production methods are made more efficient. By finding the right combination of bacteria and plasmids carrying genes for biofuel synthesis pathways, we can enhance efficiency and work towards large scale production of these biofuels. Plasmids can also be used to deal with byproducts of biofuel production. With biodiesel, for example, surplus glycerol is generated as a byproduct of the chemical process. Using bacteria with plasmids encoding ethanol-producing enzymes and acetyltransferases, this surplus glycerol can be used for further biodiesel production.
- **Goal 9: Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation (enhance scientific research).** Using bacteria as biological workhorses has become common practice over the past 50 years. We now have a good understanding of how bacteria transfer MGEs to each other and have harnessed these mechanisms for research and industrial processes. Being able to direct the transfer of genes between bacteria has allowed us to expand our experiments with their use for medical, agricultural, civil engineering, clean energy, food science, and bioremediation purposes. Gene transfer is particularly useful for making these processes more efficient and possible on a larger scale. Many innovations in bioengineering have already been made but there is a whole world of possibilities of what we can do using MGEs.
- —**Goal 11: Make cities and human settlements inclusive, safe, resilient, and sustainable.** Changes in land use, urbanization, climate, and demographics are affecting interactions

between humans, animals, and the environment. These changing interactions provide new opportunities for bacteria from different environments to exchange genes by HGT. In the case of MGEs encoding traits like virulence factors or antibiotic resistance, these new gene exchange networks can be detrimental to human health as they could accelerate the emergence of pathogens resistant to antimicrobials. Without efforts to curb the horizontal spread of antibiotic resistance genes, current antibiotics will continue to lose their effectiveness and future antibiotics will be rendered ineffective soon after they become available.

Potential Implications for Decisions

1. Individual

a. Keeping yourself and your family safe from antibiotic-resistant infections.

b. How individuals can help curb the spread of antibiotic resistance and antibiotic-resistant pathogens.

c. Career options in bioengineering research and development.

2. Community policies

a. Helping to prevent the spread of antibiotic resistance within the community.

b. Identifying reservoirs of antibiotic resistant pathogens in the local environment.

c. Costs associated with horizontal gene transfer – affecting local health care systems, agriculture

d. Increasing awareness and availability of biofuels in the community.

3. National Policies

a. Being good stewards of antibiotics by not overusing or overprescribing them.

b. The role of government in regulating use of antibiotics.

c. Allocating funding for study of horizontal gene transfer in microbes and disease management. Basic science research can lead to novel ways of preventing the spread of "bad" genes like those for antibiotic resistance. Translational research can develop new methods to address antibiotic resistant pathogens in clinical settings, such as new antibiotics, non-antibiotic therapeutics, and vaccines.

d. Funding to develop and encourage alternative practices in agriculture to reduce overuse of antibiotics.

e. Funding to accelerate development of biofuels and bioremediation technologies.

Pupil Participation

1. Class discussion/Pupil stakeholder awareness

a. What are plasmids? Are they parasites or are they part of bacteria? Do they exist to help bacteria or are they "selfish" genetic elements?

b. What if humans and other animals could share DNA as easily as bacteria? What traits would you want from your friends, or even from a lizard or fish?

c. What is happening in the image on the first page?

d. What happens if antibiotics lose their effectiveness due to the spread of antibiotic resistance between bacteria?

e. What are other alternatives to prevent or treat infections besides antibiotics?
f. What can you do to help reduce the spread of antibiotic resistance genes?
g. Besides the health of humans during space travel, what are some additional concerns about bacteria on spaceships becoming more resistant to conditions in space or other non-Earth environments (like other planets!)?
h. What are some potential benefits and concerns of genetically modified crops like Golden Rice?

The Evidence Base, Further Reading and Teaching Aids

https://www.cdc.gov/drugresistance/index.html
https://journals.asm.org/doi/10.1128/microbiolspec.PLAS-0022-2014#b16
https://www.nature.com/articles/hdy201024
https://www.statnews.com/2018/02/20/antibiotic-resistance-bacterial-sex/

Glossary and abbreviations used

Bacteriocins – proteins made by bacteria that negatively affect similar bacteria.
Bacteriophage – a virus that infects bacteria.
Commensal – bacteria thought of as "friendly" that naturally live in a human or other organism and do not cause any harm.
Conjugation – a mechanism of HGT. It is the most direct and efficient method for bacteria to exchange genetic information. There is direct cell-to-cell contact between bacteria when one "donor" bacterium builds a sex pilus, which attaches to a second "recipient" bacteria. Plasmids and other integrative conjugative elements can pass through the sex pilus, and the recipient bacteria is now called a "transconjugant".
Conjugative plasmid – a plasmid encoding the machinery it needs to build a sex pilus and transfer itself to other bacteria.
DNA cloning – making many copies of a particular piece of DNA. Plasmids can be used for this by inserting that piece of DNA into the plasmid, where it will be replicated in bacteria along with the plasmid.
DNA recombination – when DNA segments are rearranged to form new combinations, usually by breaking and re-joining the DNA. Genetic material can be recombined within an organism or exchanged between organisms.
Electroporation – using a quick electric shock to create temporary pores in bacterial cell membranes so bacteria can take up DNA from their surroundings.
Endogenous retroelements – retroviruses that have permanently integrated into a host genome. These elements can be active (genes are intact and can be used to create their encoded products) or inactive, and are passed down to offspring by vertical gene transfer.
Gene expression – turning information carried by a gene into a product such as a protein. Expression of a gene will create an observable characteristic in an organism, for example, hair color.
Horizontal gene transfer (HGT) – also called lateral gene transfer. Occurs when genetic information is moved around between organisms (not including information being passed from parent to offspring, see vertical gene transfer); usually referring to bacteria. The bacteria do not

need to be closely related to share genes. The main mechanisms of HGT are transformation, transduction, and conjugation. HGT plays a major role in the evolution of bacteria.
ISS – International Space Station
MDR – Multidrug resistant
Pathogen – a microorganism that can make you sick (also known as a germ!). Most microbes in your body are harmless, but some can become pathogens under certain circumstances, including being the recipient of a virulence factor by HGT.
Probiotics – "good" bacteria that may have health benefits when consumed. Probiotics are considered to be safe, but there is not much evidence that their health benefits are real.
Selection – environmental pressure that forces an organism to maintain a specific trait. For example, the presence of antibiotics makes bacteria keep their antibiotic resistance genes. Additionally, bacteria which have received specific genes by HGT can be selected for by applying environmental pressure, killing off any bacteria that don't have that gene. This type of selection occurs naturally and is also used commonly in the laboratory.
Transduction – a type of HGT where DNA is moved between cells by viruses. In nature, viruses may accidentally pick up host cell genetic information and bring it with them to the next cells they infect. In the laboratory, viral vectors can be engineered to insert desired information into target cells.
Transformation – a type of HGT where cells take up new genetic information from their surroundings (you can think of this as loose DNA present in the environment) through their cell membrane. In a laboratory, cells can be coaxed to take in DNA from their environment by using electroporation or exposing them to certain chemicals.
Transgenic – an organism that has an artificially modified genome containing DNA sequences not naturally found in that organism. GMO crops are transgenic plants that have been modified to contain desirable genes from other types of plants.
Vertical gene transfer – passing genetic information down from parent to offspring. In bacteria, this is a clonal process, meaning that the two daughter cells have the same genetic information as the mother cell.
Virulence factors – products made by microorganisms (like bacteria) that help the microorganism but make its host sick. The most well-known example in bacterial pathogens is probably toxins, but other virulence factors can help the bacteria attach to host tissue, avoid the host immune system, or even destroy parts of the host immune system.
WWTP – Wastewater Treatment Plant