Introduction To Bacteria

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SECTION 1

Anatomy of Bacteria

Bacteria are classified as prokaryotic cells—they lack membrane-bound nuclei and other types of specialized intracellular structures. Bacteria are exceedingly diverse in form, occurring naturally in one of three basic shapes: spherical, rodlike, and curved. These categories are themselves divided into more precise designations. Bacteria are very small, with the average bacterium measuring approximately 2 micrometers in length.

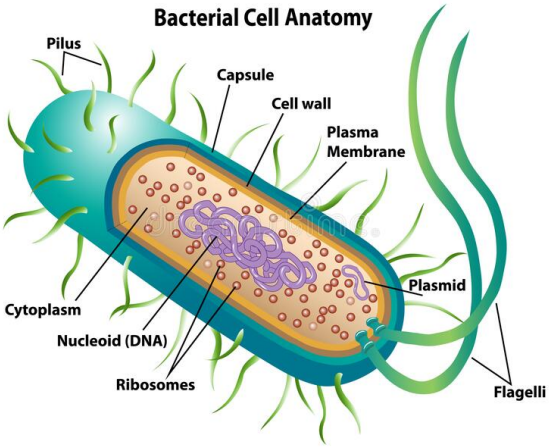

Figure 1: Buckley, By: Gabe, et al. "Prokaryotic Cell - Definition, Examples & Structure." *Biology Dictionary*, 6 Nov. 2020, https://biologydictionary.net/prokaryotic-cell/.

Each cell consists of many smaller components that contribute to its overall functionality. The bacterium obtains its structure from a rigid polymer layer called the **cell wall**, which serves to both maintain the cell's shape and protect its internals from damage. Within the cell wall is the **cytoplasmic membrane**. This flexible layer modulates the

flow of important materials between the inside and outside of the cell. The cytoplasmic membrane, like the cell wall, also provides protection for the cell's interior from the environment. Inside, the majority of the bacterium consists of a gel-like substance known as the **cytoplasm**, in which the cell's organelles are suspended.

Many species of bacteria have distinctive structures that occur on the outside surface of the cell. For example, some are covered in **pili**, hair-like appendages that assist the bacterium in attaching to other surfaces. Each pilus has a shaft composed of various protein units, and at the end is an adhesive tip whose shape corresponds to a receptor on a host cell. Other surface structures include **flagelli**, which are similarly hair-like and allow bacteria to propel themselves in a "swimming" motion, and the **capsule**, a gelatinous secretion that prevents desiccation and defends against phagocytes.

1.1 Cell Wall and Membrane

The cell wall is the barrier that separates the inside of the cell from its surroundings. However, it has many other functions as well, such as helping the cell maintain its shape and protecting the genetic information inside the cell. It is made out of different things depending on the organism and type of cell. In plants, fibers and cellulose are its main components. Cell walls on bacteria are made of **peptidoglycan**, which is less commonly called murein. It is a polymer of amino acids. Peptidoglycan is not a component of eukaryotic cells, or cells that have a nucleus.

In **gram-positive** bacteria, the cell wall is made up of several layers of peptidoglycan, making it more durable and strong. Peptidoglycan is also porous, which means that it has holes, and can therefore let microscopic particles and molecules in. However, in **gram-negative** bacteria, **lipopolysaccharides**, or LPS, is the outermost layer and encapsulates the peptidoglycan layer. It serves as a defense mechanism from outside threats, especially in pathogenic bacteria. LPS as an endotoxin can decrease the effectiveness or kill antibiotics such as penicillin. Cell walls not only protect the cell and defend against any threats, but it also helps regulate key processes happening within the cell, such as **turgor pressure**. Turgor pressure is the pressure that the atmosphere exerts on a cell, and it is primarily generated by the selectively permeable membrane letting in water from outside of the cell.

The cell membrane is an extra layer beneath the cell wall in a bacterial cell; however, animal cells do not possess cell walls and therefore have the cell membrane as the outermost layer. Cell membranes are much more flexible and delicate, and although it also makes up the cell's shape and structure, it is more round. The cell wall is composed of carbohydrates, proteins, and lipids, and is selectively permeable. Therefore, it helps control what is entering and exiting the cell. Being a semipermeable membrane, it allows only certain objects to enter through–mostly small objects. Semipermeable membranes are composed of two layers of phospholipid, known collectively as the lipid bi-

layer. Large objects such as other cells, ions, and nutrients cannot enter through the gaps in the phospholipid layer, which ensures that the inner systems of the cell are not disrupted.

Phospholipids will automatically form a bilayer by itself in water. Examples of this are in micelles, isolated aggregates of phospholipids in water. The hydrophobic ends, which repel water, face in towards one another, while the hydrophilic head faces outwards. This is due to the hydrophilic heads of phospholipids being polar, and because water is polar, they have favorable intermolecular interactions that cause them to be attracted together. By default, the hydrophobic heads will be in the middle of the bilayer and avoid water.

The lipid bilayer has transmembrane proteins, which can communicate extracellular signals and act as a gate for materials. There are two types of membrane proteins–integral and peripheral. Integral, or intrinsic, proteins do not leave the membrane and are permanently attached by different types of forces, such as hydrophobic or electrostatic attraction.

1.2 Nucleoid

The **nucleoid** is an irregularly-shaped region in a prokaryotic cell which contains the **genophore**, or the genetic information for the cell. Bacteria is a type of prokaryotic cell, so it contains a nucleoid. In eukaryotic cells, the genetic information is contained in the nucleus. The difference between these two is that the nucleoid does not have a membrane surrounding it, while the nucleus does. Instead, the nucleoid is attached to the cell membrane and directly touches the cell's cytoplasm, a liquid that fills the inside of a cell (this will be defined in more detail later in the chapter).

The nucleoid is composed of approximately 60% of DNA, along with some RNA and protein. A single cell may contain multiple copies of DNA, obtained from DNA replication. Prokaryotic DNA is a bit different than eukaryotic DNA, as in it is contained in small, circular molecules known as **plasmids**. This DNA is also double-stranded, like eukaryotic DNA. In most bacteria, DNA is negatively **supercoiled**. Supercoiling means the double-helix shape of DNA has undergone additional twisting or is undertwisted. It is measured by a property of DNA known as the **linking number** (Lk).

Some proteins are also contained in the nucleoid, along with the DNA, including **nucleoid-associated proteins (NAPs)**. These proteins help in the process of nucleoid condensation, where genetic material is wound up and compressed into the nucleoid. They bind to the DNA and structurally change it in order to make sure it fits into the cell. The most common types of these proteins include HU, (heat-unstable proteins), Dps (DNA-binding protein from starved cells), Lrp (leucine-responsive regulatory protein), H-NS (histone-like nucleoid structuring protein), IHF (integration host factor), and Fis (fac-

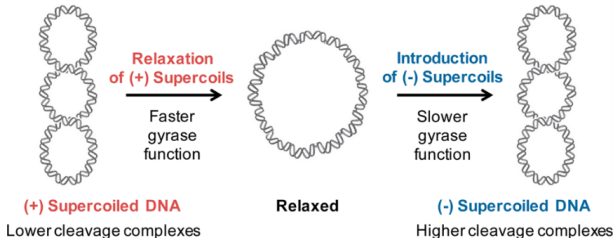

Figure 2: Ashley, Rachel E., et al. "Recognition of DNA Supercoil Geometry by Mycobacterium Tuberculosis Gyrase." Biochemistry, vol. 56, no. 40, 2017, pp. 5440–5448., https://doi.org/10.1021/acs.biochem.7b00681.

tor for inversion stimulation). The amount and type of NAPs expressed throughout the nucleoid changes throughout the growth of the DNA as the proteins all have different functions relating to cellular processes. Some proteins help out with DNA transcription, while others aid DNA replication, and other types assist with DNA repair.

The function of a nucleoid is crucial for the survival of the cell. It is where DNA replication and transcription takes place, so it controls the function of every other part of the cell, like the nucleus.

1.3 Plasmids

As mentioned in the previous section, the majority of a bacteria's genetic information is stored within the nucleoid, the dense region containing the bacterial DNA and RNA. However, floating alongside the nucleoid in the cytoplasm are small, circular DNA segments, known as plasmids. These rings contain genetic information that is separate from the chromosomal DNA, contained in the nucleoid, and are often found naturally occurring in many types of bacteria, archaea, and even eukaryotes (cells with membrane-bound organelles). Though small individually, the vast number of plasmids present within an organism can result in up to 25% of genetic information being contained within plasmids.

The contained information in plasmids is not vital for the survival of the cell; yet, these small DNA molecules provide an additional source of genes, potentially providing a selective advantage for the bacteria. Plasmids typically carry half a dozen to several hundred genes, some of which encode for proteins responsible for antibiotic resistance, **green fluorescent protein** (GFP), or essentially any **transgene** that can be engineered into the plasmid.

Plasmids are an integral tool in molecular biology and genetic engineering because of their versatility and ability to be modified, with applications in introducing new genes into bacterial cells, producing numerous copies of a specific gene, and studying the process of DNA replication.

A characteristic trait of plasmids is their ability to undergo an independent replication

process because they are autonomous genetic elements. Despite being located directly adjacent to the chromosomal DNA in the cytoplasm of a bacterium, the replication of plasmids is controlled by one of several alternative mechanisms.

One such mechanism is the theta-type mechanism, which is typically used in the prototypical circular plasmids found in gram-negative bacteria. The process always begins at a specific DNA sequence located on the plasmid, known as the origin of replication (ori). From this point onward, the double-stranded plasmid is unraveled to produce two separate single strands, connected at up to two replication forks. The interconnected plasmids form a structure similar to the Greek letter theta (θ), hence the name. As the process proceeds, the two single strands serve as a base template for the synthesis of the two new strands of DNA. The resulting two plasmids should both contain one original DNA strand and one newly synthesized stand, and thus, the process is considered **semi-conservative**. The other plasmid replication mechanisms will not be elaborated on, but it is important to note that all such methods of replication are independent of the replication for the chromosomal DNA.

Some plasmids have the ability to be transferred between similar bacterial species through a process called **conjugation**. This is a type of **horizontal gene transfer**, which is the movement of genetic information between individuals. organisms. The process of conjugation allows bacteria to be introduced to new genes from the surrounding population, which they previously did not possess as a permanent part of its genome.

During plasmid conjugation, the bacterium carrying a F-factor**F-factor** (fertility factor) is considered the donor, while the other bacterium lacking said F-factor is the recipient. When the two bacteria are within close proximity, the donor extends a pilus, or a small, thin tubule to attach to the recipient bacteria. The plasmid is then able to be nicked to transfer a single strand of DNA, which can be replicated by both bacteria to produce a fully functioning plasmid. Since the bacteria are directly contacting cell-to-cell and transferring genetic information, the process of conjugation can be considered the bacterial form of sexual reproduction.

1.4 Flagella

The flagella refers to a tail-like structure present in all forms of bacteria regardless of gram-positivity. This structure is key in the rapid-movement of certain bacteria comparative to their microscopic sizes. Made from a type of **microtubule**, cytoskeletal elements which support the structure of the cell and engage in cell motility, known as flagellin, flagella are also responsible for taxis, direct movements in response to external stimuli. Microtubules in the flagella arrange themselves helically, allowing for greater flexibility in structure and the ability to change shape. These stimuli include chemical and physical ones, and can be towards or away from a stimuli depending on the situation.

The flagella consists of three main components: the filament, hook, and basal body. The **filament** is the primary extension of the flagella, projecting from the bacterial cell. Its ability to move is not inherent, but is a result of the hook and the effects of **proton-pumping** which occurs in the **electron transport chain** (ETC) in cellular respiration. Protons are pumped out of the cell which creates a higher concentration of protons on the outside of the bacterial cell. Due to this greater concentration on the outside, protons diffuse inwards to counteract this gradient. This movement provides the force for the **hook** to rotate, causing the filament to do so as well and propel the bacterial cell, thus in ways similar to a corkscrew rather than a tail.

Finally, the **basal body** is the microtubule organizing center of the bacteria, made from **centrioles** and responsible for attaching the filament and hook to the bacterial cell. Nested within the cell membrane similar to transport proteins as per the **fluid-mosaic model**, it contains various rings and rods which allow it to function as a motor.

Flagella are not limited to singular, isolated locations, and variations in amount and distribution contribute to the wide diversity in types of bacteria. Polar bacteria describe bacteria containing one or more flagella in a single spot. Meanwhile, peritrichous bacteria refer to bacteria with flagella occupying multiple areas.

1.5 Cytoplasm

The **cytoplasm** is known as a gelatinous liquid within cells that are made from organic molecules such as water and salts. The cytoplasm essentially acts as separation between all the organelles inside of the cell. Organelles such as the **mitochondria**, nucleus, and **lysosomes** all reside in this substance.

The main function of the cytoplasm is to keep the components of the cell intact and protect from any damage. Because the cytoplasm is a gelatinous liquid in the interior of the cell, it is also liable for giving the cell its shape. A popular comparison to the structure of a cell is a water balloon. The cell membrane acts as the actual balloon while the cytoplasm is like the water inside of the balloon, thus creating the shape of the structure.

Because the cytoplasm houses the organelles that let the cell function, the processes of replication, growth, and expansion take place in the cytoplasm. The organelles that are contained are also called cytoplasmic organelles. In some cells, “protoplasm” is more commonly used, this refers to the nucleus and cytoplasm. Along with cellular processes, the cytoplasm can also transfer materials, like hormones throughout the cell which eventually dissolves into cellular waste.

The cytoplasm is composed of two parts known as the **endoplasm** and **ectoplasm**. The endoplasm is the area which contains the organelles, while the ectoplasm is the gelati-

nous section of cytoplasm. In prokaryotic cells, the cytoplasm contains all the contents of the cell. However, in eukaryotic cells, the cytoplasm consists of the cytosol (aqueous part of cytoplasm), organelles, and nutrients.

Cyclosis is the process in which particles are transferred and moved within the cell so that they can be used for function. Some examples of this are the movement of proteins and hormones.

SECTION 2

Types of Bacteria

2.1 Classification Hierarchy

All living organisms are classified using **taxonomy**, which is a system used to group similar species and groups of species together. It communicates how organisms are biologically related to each other, from either similar living conditions or evolution. The eight levels in the classification hierarchy are domain, kingdom, phylum, class, order, family, genus, and species; domain is the most general term, while species is the most specific term. Organisms in the same species can breed and produce offspring.

There are three domains, or general broad categories of living organisms: Archaea, Bacteria, and Eukarya. Eukarya has organisms that consist of eukaryotic cells, or cells with a nucleus. Archaea and Bacteria are both unicellular prokaryotic organisms, which means that they consist of one cell that lacks a nucleus. Contrary to popular belief, however, organisms in Archaea are significantly different from those in Bacteria.

Before archaea were discovered, bacteria were considered to be the only prokaryotes and the two terms were used interchangeably. However, Carl Woese, an American microbiologist, found that a group of bacteria he was using for research exhibited different traits. He proposed that a new domain, archaea, be made because the new type of cell was so different. A key feature of bacteria is that they may be pathogenic, or disease-causing. However, archaea are not. The two domains of organisms also have significantly different chemical and genetic makeups.

2.2 Phylogenetic Trees & Theories

The term "phylogeny" was first coined by German biologist, Ernst Haeckel, who wrote about his proposed evolutionary theories in his book, Generelle Morphologie der Organismen. At the time of its publication, Haeckel's theories about phylogenetics were widely accepted for its novelty, namely his statement: "ontogeny recapitulates phylogeny." Haeckel believed strongly that ontogeny, the morphological developments of a single organism within its lifetime, follows and reflects the same patterns exhibited in phylogeny, or the evolutionary lineage of many species. Despite his theories being later disproven in the 20th century, Haeckel's writings and usage of phylogeny brought about a new field of evolutionary study, phylogenetics.

Phylogenetics seeks to study the evolutionary history of a specific taxonomy, defined as a group of genetically related organisms, through the analysis of inherited genetic traits. Prior to the invention of modern computational techniques, biologists relied heavily on homologous traits, or the physical features that appear similarly across species, to infer the relationships between organisms. These methodologies relied heavily on scientific inference and judgment as to the similarity of two traits, and thus, were prone to heavy error and disputes. As techniques for molecular data collection emerged, the full genomes of species could be efficiently modeled, allowing for the precise tracking of specific genes. As a result, phylogenetics shifted towards the mathematical modeling of genomes to reconstruct evolutionary histories and lineages.

The most common representation of the data from a phylogenetic analysis is through a **phylogenetic tree**, an evolutionary diagram consisting of a complex branching network to demonstrate genealogical relationships. The branches on the phylogenetic tree converge to form individual nodes, each of which represents an operational taxonomic unit (OTU) or a group of genetically similar species. Referring to the diagram below, each OTU is represented by a capital letter. The branches that connect nodes show a direct connection between the two OTUs and the pathways of evolution from a species to a descendent. The length of the branch provides insight into the time frame for the evolution process or the number of genetic changes that occur, with onger branches connecting more genetically different OTUs.

Phylogenetic trees can be classified as rooted or unrooted, depending on the directional flow of the model. Rooted phylogenetic trees (fig Ai) show the direct evolutionary path of all OTUs from a common ancestor, termed the root. There is a clear flow over time horizontally, with the more recent species located towards the upper and/or right end of the tree. Since a rooted phylogenetic tree traces back the evolutionary history to a single common ancestor, these trees require more data to construct. Alternatively, unrooted phylogenetic trees (fig Aii) specify the genetic and physiological relationships between

OTUs, but fail to show a direction of evolution.

The groupings of OTUs within a phylogenetic tree can be distinguished into monophyletic, paraphyletic, and polyphyletic taxons. A monophyletic taxon, alternatively named a clade, is composed of the most recent common ancestor and all the descendants of that particular common ancestor. The taxonomic ranks mentioned in Section 2.1 are almost always classified as monophyletic, because all the genetically closely related species are grouped together within one hierarchical group. A paraphyletic taxon is shrunken from the monophyletic taxon to include the most recent common ancestor and only some of the descendants, though all of the OTUs are still genetically closely related. A polyphyletic taxon groups together descendents from multiple different evolutionary lineages, thus effectively eliminating the common ancestor. All the OTUs within the group may inherit a similar trait, but the grouping is still considered artificial because the common ancestor is not present.

Incorporating molecular data has become an integral part of computational phylogenetics and many new algorithmic methods have been developed to construct the phylogenetic trees. A few of the simpler methods will be elaborated on below.

Distance based methods use a coding algorithm to determine the smallest difference between two species, depending on either molecular or morphological characteristics. The most common example is through a comparison of the nucleotide sequences of two different species, through which the genetic differences can be calculated. The algorithm can then use these calculated values to construct a phylogenetic tree with the branch lengths of best fit.

Parsimony methods measure the number of evolutionary changes or steps, namely DNA mutations, required to change from one OTU to the subsequent OTU. The algorithm prefers evolutionary lineages with the fewest evolutionary changes, hence the name parsimony.

2.3 Gram Strain Test

Gram Staining is used to test out which kinds of bacteria are found in a suspected area. In other words, it helps diagnose harmful bacteria that can lead to serious health problems. Gram stain tests differentiate bacteria by “gram-negative” and “gram positive” classifications. Gram-negative typically indicates that the bacteria have a thin **peptidoglycan/murein** layer, a polymer of sugars and amino acids that form a plasma membrane outside of most bacteria. Gram negative bacteria also have a thick outer membrane that resists antibiotics. The outer membrane is an asymmetrical lipid bilayer, composed of phospholipids (which prevents accumulation of fats in the liver) and gly-

colipids (maintain stability of the cell membrane). Gram-negative bacteria tend to be the cause of pneumococcal and streptococcal infections (respiratory). On the other hand, gram positive bacteria have a thicker peptidoglycan layer, and no outer membrane. Gram-positive bacteria tend to be the cause of bloodstream infections and other infections transmitted by bodily fluids.

2.4 Classification by Morphology

Bacteria can also be classified by shape due to microscopic technology and the outer layer properties in bacteria. Due to the rigid cell wall and capsule, a thick and sticky layer of polysaccharide and protein surrounding the bacteria, the bacteria is well defined and can be observed underneath a microscope. While bacteria naturally form a diverse range of patterns and shapes, the three most commonly classified shapes are understood to be spherical, curved, and rodlike.

Spherical bacteria, known as *coccus*, has its own genus. It comes from the Greek noun "cóccos," which means berry, because several of these bacteria together look like circular fruits. Even the shape of cocci bacteria depends on features such as its bacterial wall. Gram-positive cocci, which have thick peptidoglycan layers, are different sizes, depending how individual species' cell division occurs. There are multiple planes that the bacteria may divide in; if bacteria multiply in multiple planes, then its shape will be three-dimensional. Streptococcus, or "twisted berry," form in long chains of bacteria because cell division occurs in the same plane. Sarcina bacterial cells are formed in a cube-like shape because they are formed by cell divisions in three planes. They adhere to each other, mostly in groups of eight. However, some cocci divide irregularly and form a structure that resembles more of a clump than a geometric shape. For example, *Staphylococcus aureus* form clusters.

Coccoid-shaped bacteria are thought to have evolved from rod-shaped bacteria that no longer elongated, but still survived well. This is also called the degenerate form.

Bacilli are rod-shaped bacteria. In fact, it is thought to be one of the first shapes of bacteria to exist. The cylindrical shape is extremely common not only in bacteria, but also in other organisms such as fungi, which is because the rod shape may have certain evolutionary advantages that help the bacteria survive. For one, cell division is much faster and more effective in a rod shape. Chromosomes must be separated far enough apart during division for the septum to avoid splitting them. Minimal effort is required to grow and maintain the rod structure, as it only grows in one dimension. Furthermore, the ratio between the cell's volume and surface area is kept constant with rod-shaped bacteria, allowing it to be more adaptive and maintain consistency with its environment. Spherical bacteria do not have this advantage.

Bacilli divides from the center point outwards. It splits mid cell into two identical daugh-

ter cells. Streptobacilli is the term for bacilli arranged in a line or chain, and it is formed when bacteria divide on one plane. Palisades form in multiple directions because the bacteria bends at points of divisions. A few Palisades resemble a crooked fence when zoomed in, but its general pattern is more random than Streptobacilli. Coccobacilli are a combination of spherical and rod-like bacteria because they look like short rods that are cut off. They have oval-shaped bodies that form in clusters. Diplobacilli is when two bacteria are conjoined after division.

Spirilla, or curved bacteria, resemble a corkscrew or spiral. These types of bacteria are sometimes flexible and able to move, but most commonly rigid. Vibrio are spiral bacteria with one curve, and they look like skinny beans. Spirilla and Spirochetes look very similar, but Spirilla are rigid while Spirochetes are very flexible. Furthermore, Spirochetes are able to move on their own.

Bacteria's **morphology** affects how well they can survive in harsh environments. By the process of natural selection, the most optimal species and shapes of bacteria have survived due to their high fitness and versatility. Large bacteria have high fitness because they have control over their location, as well as being able to fight larger organisms that harm them. For instance, the bacteria *Epulopiscium* is so large that it can be seen with the human eye, without a microscope or any magnifying techniques. However, it still manages to survive. The bacteria's membrane and cell wall help to keep invaders out and protect the interior from harsh conditions, such as stomach acid or soil.

2.5 Classification by Nutritional Modes and Respiration

Aside from morphology, bacteria can also be classified based on differing nutritional modes which are characteristic of groups of bacteria. Bacteria differ in nutritional modes primarily based on their source of energy and where they obtain carbon. These broadly include autotrophs, which synthesize their food using simple, inorganic compounds and sources, and heterotrophs, which on the contrary, consume organic compounds.

Autotrophs are further divided into *photoautotrophs* and *chemoautotrophs*, involved in the synthesis of energy using the inorganic resources available to them. Photoautotrophs are an example of this, and harness light energy and carbon sources such as carbon dioxide (CO_2) and bicarbonate (HCO_3^-) in order to replenish their respective energy needs. Photoautotrophic bacteria such as **cyanobacteria** are often photosynthetic and like plants, have metabolic systems which allow them to harness light and inorganic carbon sources to generate molecules for fuel. However, unlike plants, cyanobacteria and other photoautotrophic bacteria do not contain chloroplasts, a prokaryotic property, and thus rely on photosynthetic pigment in their membrane (i.e chlorophyll). Photoautotrophic bacteria can often be found arranged in filaments, length chains for synchronized photosynthetic action aside from underlying respiratory limitations, discussed in Section 2.7. Chemoautotrophs, meanwhile, utilize inorganic compounds such

as iron ions and nitrogen for nutrition. These bacteria can be found in limited quantities along the filament in specialized cells called **heterocysts**, which solely fixate nitrogen (discussed later) unlike the collective photosynthesis observed in photoautotrophic bacteria in the filament. Heterocysts heavily restrict oxygen entry through a thick cell wall, but cell junctions are present which allow the heterocyst to transfer fixated nitrogen to neighboring cells.

Chemoautotrophic bacteria are heavily studied due to their utilization of **nitrogen fixation**, a special form of metabolism unique to them which allows chemoautotrophic bacteria to obtain nitrogen through a flexible, diverse range of sources. Cyanobacteria, an example discussed earlier, converts nitrogen gas in the air to ammonia (NH_3) in the "fixation" step. Nitrogen is important in chemoautotrophic bacteria and bacteria of any nutritional mode in general due to nitrogen's vital role as a component of amino acids and organic molecules. It should be noted that while bacteria can be classified through various "modes", these are often extremely flexible. Chemoautotrophs, for instance, still utilize light and water to synthesize organic molecules.

Heterotrophs, meanwhile, are divided into *photoheterotrophs* and *chemoheterotrophs*. Photoheterotrophs are bacteria which obtain energy to synthesize items such as ATP through light, but rely on multiple organic compounds as a source for constructing structures within the bacteria. A common source of misidentification of photoheterotrophic bacteria is confusion with photoautotrophic bacteria. While both utilize light as an energy source, they can be distinguished by how they obtain carbon and organic molecules, with photoautotrophic bacteria often fixing carbon molecules from various inorganic molecules while photoheterotrophic bacteria obtain their compounds through organic means. Chemoheterotrophs are similar, obtaining carbon and organic molecules through organic sources (etc. animal carcasses), but they rely on organic compounds for energy synthesis instead of light.

Heterotrophs are unique in their aggregation into **biofilms**, surface-coating colonies which engage in cooperative metabolism. Bacterial cells engaged in these structures secrete signaling molecules which attract other bacterial cells. These cells collectively produce a slimy layer consisting of polysaccharides and proteins which covers the entirety of the biofilm. Channels similar to the cell junctions in a filament allow nutrients to be transported into the bacterial cells, while excess molecules and waste can be disposed of.

Finally, bacterial metabolism and nutritional modes are heavily affected by the form of respiration utilized by the specific bacteria. Obligate Aerobes are bacteria which require oxygen gas (O_2) for cellular respiration and are unable to survive without it. Obligate Anaerobes, inversely, cannot survive in aerobic conditions and are poisoned by oxygen. In order to curb these limitations, obligate anaerobes obtain energy through fermentation and other anaerobic means. The use of inorganic ions in chemoautotrophs, such

as nitrate ions (NO_3^-) as mentioned previously, demonstrates this as these ions emulate the role of oxygen in the electron transport chain, a stage in cellular respiration involving electron transfers. Facultative Anaerobes are a third grouping, referring to bacteria which can use oxygen if present, but can also carry out similar processes that obligate anaerobes to conduct in anaerobic conditions.

SECTION 3

Functions of Bacteria

3.1 Bacteria in the Body

Looking at certain brands of yogurt, there are advertisements about "helping gut health" which refers to the bacteria present in the human body's gut. Bacteria, a form of microbe or microorganism, is involved with and is a vital part of important processes in the body, contrary to popular belief that bacteria are inherently and only harmful.

There are numerous types of bacteria in and on the body that are referred to as the human microbiota. These bacteria are on the human body from birth, where the colonization of bacteria such as Lactobacilli (bacteria that help digest milk and lactose) occurs during delivery. Additional bacteria come from diet and further interactions with the environment.

Bacteria can be found everywhere on the body, but the most prominent examples that get more focus include bacteria on the skin, in the nose, throat, mouth, vagina, and especially the gut. There are around ten times more bacteria than the number of cells in the body (for reference, the human body cell count goes into the trillions).

These bacteria are involved with important functions of the human body. The usage of the bacteria can be broken into general categories: degradation of food, nutrient availability, and defense against pathogens.

Degradation of food, mainly **carbohydrates**, is done through a type of enzyme found in-

side bacteria known as CAZymes or “Carbohydrate-active enzymes.” Different bacterias that degrade different compounds such as simple carbohydrates versus complex carbohydrates have and require different CAZymes. Bacteria and the enzyme break down the carbohydrates into simple sugars which are turned into ATP (Adenosine triphosphate) which carries energy through cells. This is how humans gain energy and is highly important to life.

In order to degrade a wide range of food in the human diet, there are numerous kinds of bacteria inside the human body. Not only do bacteria break up food from large pieces to more manageable, smaller pieces, but they also produce vitamins that are necessary for the body for growth and development. Gut bacteria produce Vitamin K and B groups including biotin, riboflavin, pantothenic acid, etc... These vitamins are important for bacterial metabolism, as cross-feeding (here, living off the products of another kind of bacteria) between gut microbes is essential for growth. Likely, a large proportion of the microbially produced vitamins are used by non-vitamin producing bacteria. There is also evidence for other uses such as in metabolism in mammals, as low vitamin K levels in rats led to hemorrhages.

Other than for degradation of food, CAZymes are prominent in the vagina where CAZymes combat against more simple sugars that attempt to build biofilms. Biofilms are bacteria held together by a mucus-like carbohydrate background that sticks to a surface. It can cause **Bacterial Vaginosis** (BV) which is a genital tract infection in females during their reproductive years able to cause serious health issues. Bacteria are able to protect the body from toxins, pathogens, and harmful intruders. Bacteria can break down toxins, such as those in the intestines, and promote the repair of damaged tissue. Without the helpful bacteria in the body, there is a lack of defense against harmful bacteria.

Due to the importance of microbes in the human body, there has been a different approach to studying microbiota. It is a top-down approach called “-omics.” For example, metagenomics looks into the possible expressed genes. The -omics approach is often complemented with a mathematical model such as agent-based modeling, and topological analysis. It also focuses on hypothesis generation, not hypothesis testing. E very -omics approach is a different view of the complex world of studying microbiota.

The focus on CAZymes once mentioned before is in the **metagenomics** approach. Metagenomics is the analysis of DNA directly from an environmental sample, without obtaining a pure culture. It is used to find differences in composition in disease states such as inflammatory bowel disease, obesity, and diabetes compared to healthy individuals. It is used more commonly in studying gut health than anything as seen in studies in CAZymes. Other categories include metatranscriptomics, metaproteomics, and metabonomics/metabolomic.

3.2 Bacteria as Treatment

Bacteriotherapy, or the utilization of bacteria to treat disease, is a relatively new feat, chiefly occurring in the synthesis of new gut microbiomes. These microbiomes are complex and depend on bacteria for proper operation and perpetual maintenance of the treated gastrointestinal system in the body.

Bacteria used for such purposes either naturally exhibit beneficial properties, in the case of probiotics, or are genetically engineered to produce the necessary therapeutic effects. Almost all of these bacteria treatments are utilized in treating cancer or digestive diseases/disorders.

Scientific interest in bacterial therapy as a viable means of treatment has arisen most prominently in cancer research. Various strains and species of bacteria have been identified as tumor-homing, able to colonize the tumor microenvironment and deliver targeted therapeutic agents to inhibit cancerous growth.

The efficacy of bacterial solutions, unlike other forms of gastrointestinal cancer therapy, is not contingent upon the genetic composition of the tumor. Treatments such as chemotherapy target the reproduction cycle of cancer cell DNA and deliberately damage cancer DNA to prevent the cancer from reproducing and spreading in the patient's body. The danger of damaging this cancer DNA is that normal body DNA is also affected by exposure to chemotherapy radiation. This exposure can lead to serious adverse effects in the body, such as permanent nerve damage, cardiovascular issues, and more long-lasting symptoms. Normal body DNA can also mutate during this operation (due to production of artificial gaps in the DNA sequence by radiation) and this mutation can be fatal and can possibly cause developmental disorders in young patients. Even though more serious adverse effects are relatively rare, the risk of such symptoms (serious or not) can be greatly reduced by utilizing bacteriotherapy in gastrointestinal cancer treatment.

Recent advances in bacterial bioengineering have further magnified the potency of bacteriotherapy while minimizing adverse effects. However, bacteriotherapy is not as effective as more established techniques such as the ones described, as the treatment is still being developed as increasing knowledge and research in advanced genetic engineering advances. Nevertheless, this rapidly innovating branch of treatment therapy remains hugely promising for future developments in the field.

3.3 Bacteria in Nature

Bacteria play important roles in natural ecosystems, serving as producers and decomposers. As producers, they generate phosphorus and nitrogen. Phosphorus is incredibly important to ecosystems because it is crucial to cell development, transferring energy

inside organisms, and molecules that hold energy. Similarly, nitrogen is also vital to ecosystems since it is an element in amino acids, which make up proteins, DNA, and RNA. Without either phosphorus or nitrogen, plant growth will be inhibited, making both crucial to plant soil. Since bacteria produces both, bacteria proves its necessity in ecosystems.

One of bacteria's crucial roles in large ecosystems and nature is **decomposition**. They help clean and maintain areas by decomposing dead organisms into simpler compounds in order for the environment to reabsorb and reuptake into the cycle. This ability allows the environment to maintain stability and provides conversion services for various molecular structures that are initially unusable by organisms into more utilizable forms.

In processes like nitrogen fixation, bacteria are able to take nitrogen from the atmosphere and convert it into ammonia, NH_3.

Nitrogen often is a major **limiting nutrient** and reactant in nature, meaning that the amount of end products (such as ammonia) from natural reaction largely relies on the amount of nitrogen at the beginning. With a higher volume of ammonia, more plants can grow, and they will grow faster. This reactionary dependency on nitrogen makes bacteria's role of molecular conversion all the more important.

Plants are better able to access nitrogen from ammonia than from the atmosphere, so bacteria play a key role in facilitating the process of plants extracting nitrogen essential to their growth. Some bacteria are able to process forms of phosphorus and convert it into a form that makes it easier for plants to consume and grow, especially in marine areas. Examples of these bacteria include Pseudomonas aeruginosa, Agrobacterium spp., and Bacillus circulans.

Later as plants and animals die, they leave nitrogenous compounds that bacteria decompose back into ammonia for later use. Microbes are essential for the nitrogen cycle: converting nitrogen from the atmosphere to ammonia which are to be consumed by organisms, and decomposing nitrogenous compounds from the corpses of organisms back into ammonia which can be used later for the plants.

SECTION 4

Bacteria as a Pathogen

4.1 What is a pathogen?

What actually constitutes a **pathogen** is more complex than one might think, but before discussion of why a pathogen has not been truly defined, here is a baseline definition. Pathogens are organisms that can cause disease. Pathogens can generally be split into five types: viruses, bacteria, fungi, protozoa, and worms. Even under those types, there are subtypes.

More prominent examples of pathogens include *Human immunodeficiency virus* (HIV), a retrovirus under RNA viruses; *Chlamydia trachomatis* which is a bacterial pathogen; *Schistosoma* a blood/liver worm pathogen.

One can notice the "can" in the definition makes it more loose than expected. The first thing to notice is that organisms of the same species can be both pathogenic and non-pathogenic at the same time. The prominent example is being modified in a lab to not be able to cause the disease or to weaken it. The *attenuated* microbes are seen in live vaccines, such as the chickenpox vaccine, that use a weakened version of the pathogen so the body is able to "learn and store" information on the pathogen if it ever shows again.

Furthermore, the body and its immune system cannot recognize between the bacteria and microbes that normally reside in the body (known as **commensal bacteria**) and outside pathogenic bacteria. The immune system has methods to make sure commen-

sal is behaving correctly so there is no harm done to the body. The distinction is made when there is harm done. Although the body has systems to keep outside pathogens away, such as mucus membranes, there is no distinction of pathogen/nonpathogens. Merely a control of microbial growth and invasion.

Then, does whether or not pathogens cause sickness depend on the host? As in if the pathogen is able to do harm to the host, it is considered a pathogen? Well, it would make sense, but the ability to do harm has changed a lot over the years, especially with new medical technologies. New developments in the medical world have both caused pathogens to be less effective towards the host's body due to increased immunity and the host became more vulnerable to microbes that were once seen as nonpathogenic.

In the former, if the host is immune, the pathogen has not done damage and cannot be considered a pathogen in that case because no disease (which is "harmful deviation" from normal functions) has occurred.

On the other side, examples of the latter include: immunosuppressive therapies, organ transplantation, joint replacement, implantable devices and permanent catheters. These new technologies have improved countless people's lives, but they can also disrupt the "normal" state of the human body. For example, antibiotics change the composition of the body's microbiota which can cause the body to be more susceptible to other damages or disease.

4.2 How do bacteria cause disease?

Bacterial infections are one of the three main causes of disease, even though less than 1% of bacteria cause diseases. Eyes, mouth, nose, and urogenital openings are common areas that bacteria pass through to infect the body. They can also infect humans through breaks in the skin barrier, like wounds, since it is a passage from outside into the human body.

Diseases can spread through direct or indirect contact. Bacteria can spread if direct contact is made with body fluids, infected skins, and mucous membranes. The nose and mouth have mucous membranes, which is part of why they are susceptible to diseases. Sexually transmitted infections, like chlamydia, gonorrhea, and syphillis, are examples of bacterial diseases that can be spread through direct contact with bodily fluids. Bacteria spreads via indirect contact when an infected person leaves a microbe on common surfaces such as tabletops, counters, doorknobs, faucets, handles, etc., and an uninfected individual then touches that surface and touches either their eye, mouth, or nose.

Contaminated food and water can also carry bacteria into the digestive system that lead to disease. Common bacteria in contaminated food and water are Escherichia coli (E. coli) and salmonella. Raw vegetables, undercooked ground beef, and raw dairy products

like cheese and milk are often the sources of E. coli, and raw or undercooked chicken, various meats, vegetables, and fruits can be the source of salmonella.

Aside from direct and indirect contact and contaminated food and water, bacterial diseases can also be spread through airborne transmission. Microbes, contained in dust particles or evaporated droplets, can be left in air for uninfected people to ingest. One notorious bacterial disease that became airborne was the bubonic plague. The bacteria, *Yersinia pestis*, eventually became airborne and wiped millions of Europe's population in the 14th century.

After making a successful entry into the body, bacteria can then cause disease. One method is by releasing toxins that then cause disease. These bacteria-released toxins inhibit normal cellular functions and interfere with the ability of processes that cells would otherwise would be able to carry out. There are two types of toxins: endotoxins and exotoxins. Endotoxins are the lipid elements of lipopolysaccharides (often secreted by Gram-negative bacteria) that are part of the outer membrane of bacteria, and exotoxins are formed inside pathogenic bacteria (secreted by Gram-positive and sometimes Gram-negative bacteria) that enter into host cells through many steps.

Bacteria reproduce in the body with instructions from its single molecule of DNA. Through **binary fission**, when one bacteria cell splits into two identical bacterial cells, bacteria are able to reproduce and spread within the body. Sometimes this process does not always run smoothly, and mutations occur, which actually helps the bacteria. These mutations increase the diversity of the bacteria populations, increasing its ability to resist drugs and adapt to various environments.

Other forms of bacteria are able to cause disease by invading and damaging cells. Some bacteria, like intracellular pathogens, will not survive without invading cells, so they reproduce and cause disease by invading and damaging cells. Some bacteria enter cells through phagocytosis, a process in which macrophages in a cell ingest and eliminate particles bigger than a certain size and foreign items. Certain bacteria have learned to survive past this process, which allows them to then damage the cell once it has successfully entered the cell.

4.3 Bacterial Diseases

Tuberculosis (TB) is a bacteria disease caused by *Mycobacterium tuberculosis*. TB is able to attack all parts of the body but the most prominent is the lungs. When it comes to Tuberculosis diseases, there are two forms: Latent TB infection (LTBI) and TB disease. Latent TB infection is true to its name with the bacteria being "hidden or concealed" inside the body without any symptoms. LTBI patients also usually cannot spread the bacteria to others, have a positive TB test, may develop TB disease if they do not receive treatment for latent TB infection. TB disease is more serious with the immune system

being unable to stop the growth of TB bacteria. Common symptoms of TB disease include: pain in the chest, coughing up blood or sputum, weakness or fatigue, chills. TB is airborne or spreads from one person to another through the air. It is usually only spread when the infection is in the lungs or throat, instead of the kidneys or brain.

Pneumonia is an infection of the lungs caused by a multitude of factors, involving bacteria, viruses, or other fungi. Potentially caused by the ongoing COVID-19 pandemic, pneumonia involves patients' lungs filling up with pus (purulent material), which causes difficulty breathing, fever, chills, and coughing.

Although the symptoms are usually quite mild, it's primarily important for these high risk groups to see a doctor:

- Adults who are older than 65 and children younger than 2 years old
- People with underlying health conditions or those who have a weakened immune system

In addition, pneumonia often has four main stages, especially for seniors.

1. Congestion. The lungs become heavy and congested, leading to coughing, fatigue, and rapid breathing.
2. Red Hepatization. Red blood cells enter the lungs to give them a red appearance, leading to extreme fatigue and low levels of oxygen in the blood.
3. Gray Hepatization. Red blood cells eventually disintegrate, giving the lungs a grayish color. However, the immune cells remain.
4. Resolution. As the immune cells rid the bodies of infection, the symptoms slowly relieve.

Cholera is a diarrheal disease caused by the bacteria Vibrio cholerae. The primary way that it infects its host is through water and food, so epidemics of cholera happen in areas with underdeveloped water systems or street vended food.

Although Cholera is not a serious disease with a high mortality rate, some patients are severely affected with symptoms such as cramps, vomiting, diarrhea, and extreme thirst. If the thirst, or dehydration, is left untreated, it could lead to kidney failure, shock, coma, and eventual death. Rehydration therapy, including use of IVs and electrolytes, is used on the majority of Cholera patients, as they lose much of their water through diarrhea and vomiting. However, when the disease and symptoms persist, antibiotic treatment, or medicines that kill bacteria, could also be prescribed in order to shorten the length of the illness.

Because Cholera is spread mainly through feces and unclean water, it is relatively easy

to contain and prevent from becoming a pandemic. Boiling water or using chlorine products before using water to wash hands, prepare food, and brush teeth will kill the bacteria and reduce its spreading. Additionally, using latrines or bleach to disinfect feces in the absence of clean sewer systems will eliminate its primary method of spreading.

Meningitis is an umbrella term for a disease which typically causes the inflammation of the cerebrospinal fluid and membranes surrounding the brain and spinal cord. Meningitis is induced by a wide variety of different **vectors**, making it an extremely versatile disease. It can be spread by bacteria, viruses, fungi, parasites, amoebas, and even via non-organic means, such as a side-effect of certain pharmaceuticals, head injury, and cancer.

The most common way to contract meningitis is through viral infection. Mainly caused by a class of viruses known as **enteroviruses**, viruses which induce meningitis are often spread through transmission of bodily fluid ranging from mucus, feces, and saliva. Although the most common, viral meningitis infections are often quite mild and the condition itself serves as symptoms for other contracted diseases.

Another common way of contracting meningitis is through bacteria, which enter the bloodstream often in similar ways to viruses, although also through a lack of sanitary practices and the unintentional consumption of bacteria carrying meningitis. Bacterial Meningitis can also be induced via direct contact with the **meninges**, a three layered membrane surrounding the brain and spinal cord, consisting of the dura mater (closest to the skull), the arachnoid mater, and the pia mater (closest to the brain). This is possible via ear/sinus infections, skull fractures, and through surgical procedures with a lack of hygiene consideration. **Streptococcus Pneumoniae** is currently the most common bacteria to cause meningitis in the United States, but more commonly causes pneumonia or ear infections in children instead.

Other less common ways to obtain meningitis include fungi and parasites. Fungal meningitis is extremely rare and is similar to acute meningitis caused by bacteria, only transmitted from vector to human and not between humans. Sources for fungal transmission include through the **spores** of fungi and through decay in nature. Fungal meningitis is often mild, and only prominently impacts those with other immune deficiencies. Similarly, parasitic meningitis cannot be spread between humans and is only contracted from humans who consume animals containing those parasites, which include **tapeworms** and **malaria**. **Amoebas** extremely rarely transmit meningitis in fresh water but when they do, it can be life-threatening.

Common symptoms for meningitis include high fever, a stiff neck, an extreme headache, flu-like symptoms, concentrational difficulties, and rashes (in bacterial infections). Meningitis onset can span only a few hours or through a couple of days. In complications often involving chronic forms of meningitis especially in infants and young children, seizures

and permanent neurological damage can occur. Other complications due to meningitis include hearing loss, memory issues, brain damage, kidney failure, and most severely, death.

Certain individuals are far more susceptible to the severe effects of meningitis, which include individuals below the age of 20, even more so for children under 5, high density residential sites, and a complicated immune system as a result of conditions such as AIDS or through lifestyle patterns like alcoholism. Pregnant Women are also susceptible to contracting **listeriosis** which can cause meningitis as well as miscarriages, stillborns, and premature birth.

Various vaccines are recommended by the **Center of Disease Control (CDC)**. Haemophilus Influenzae Type B (Hib) vaccine is one example of a vaccine given to infants, often administered in 2 month old babies. Adults who have conditions which increase meningitis risk, which includes sickle cell anemics and AIDS patients, are also recommended to take the vaccine. Pneumococcal conjugate vaccine (PCV13) is another meningitis vaccine administered regularly to those under the age of 2. Meningococcal conjugate vaccine is given to teenagers between the age of 13-15 and an additional shot is given at 16-18. Finally, the Pneumococcal polysaccharide vaccine (PPSV23) is recommended to individuals over the age of 65. Various antibiotic treatments are also available for bacterial meningitis.

Gonorrhea is a disease transmitted by sexual contact which infects both men and women, and is caused by the *Neisseria gonorrhoeae* bacteria. It is primarily characterized by inflammation in the urethra or genital tract.

The incubation period is usually from 3-5 days from first contact. Common symptoms, in both men and women, include increased genital discharge, painful urination, and pain/swelling in the genital area. If the bacteria comes into contact with other portions of the body, pain in the rectum, eyes, throat and joints may also be symptoms of the disease.

When left untreated for a long period of time, gonorrhea can have major effects on the body. If the bacteria spreads to the uterus and fallopian tubes, it causes PID or pelvic inflammatory disease, which can lead to infertility or future pregnancy complications. Additionally, the disease may be transmitted during childbirth if the mother is infected. Infected babies most commonly experience symptoms in the eyes. In men, the bacteria can cause inflammation in a specific tube near the testicles known as epididymis, causing epididymitis. Without treatment, this causes infertility.

Prevention is similar to any other sexually transmitted disease. Using protection during sexual activies, limiting the number of sexual partners, and making sure you and your partner are both tested and disease-free are all recommended prevention meth-

ods. Treatment includes an implementation of dual drug therapy, due to strains of the bacteria resistant to usual antibiotics emerging in the past 50 years. The identity of the drugs used in the treatment depends mainly on the area it was acquired, as different drug-resistant strains exist in different locations.

Syphilis refers to a typically sexually-transmitted bacterial disease which typically result in an initial, painless sore, followed by rashes. However, unlike many of the previous bacterial diseases mentioned, there are little severe effects until decades after infection where subsequent health effects appear.

This bacterial infection is divided into various stages of onset, differing in severity of symptoms and effects. The first stage, primary syphilis, involves the formation of sores around the genitals and other mentioned areas. These sores are round in shape but typically do not cause pain when touched. Secondary syphilis onset often occurs around a month after initial spread, and can sometimes be mild enough to evade detection. Symptoms include skin rash, fever, and swollen lymph nodes. Finally, Tertiary syphilis can occur *years* after initial contact, often resulting in severe medical conditions related to the brain, heart, and lungs.

Tertiary syphilis, in particular, is the cause for the greatest medical attention due to the severe, negative health impacts that it causes. In particular, **neurosyphilis** is a form of syphilis which affects the central nervous system. This can result in extreme headaches, muscle weakness and motor issues, and dementia-like symptoms. Similarly, ocular syphilis and otosyphilis affect eye and ear function respectively.

Syphilis is only transmitted through direct contact with a syphilis sore known as a **chancre**, typically located on the genitals, lips/mouth, or in the anus/rectum. The bacteria carried in these sores almost always induce syphilis via any form of sexual activity. A less common, yet possible method of transmission involves its spread from a mother to their unborn child. Syphilis cannot be spread from nonhuman items (i.e toilet seats) that may have potentially come into contact with other syphilis-infected individuals.

Syphilis can be tested using blood tests known as **treponemal tests**, which verify the presence of antibodies specific to syphilis bacteria. Thankfully, syphilis can also be cured using various antibiotics including benzathine penicillin G which primarily treats patients with primary or secondary syphilis. However, these antibiotics are unable to reverse tertiary bodily changes and effects, giving greater urgency to regular testing and early detection.

Antibiotics are known as medicines that fend off infections caused by different bacterias in organisms, however, they cannot be used to cure viral infections such as the flu or common cold. The immune system is usually able to fend off against bacteria before it multiplies and causes symptoms, but when the bacteria is excessive, that is when antibi-

otics are mainly utilized. As antibiotics become more commonly used, some bacteria have become more resistant, leading to old antibiotics such as penicillin becoming less useful. Antibiotics are separated into two groups that include bacteriostatic and bactericidal antibiotics. Bacteriostatics simply halt bacteria from multiplying while bactericidal antibiotics (such as penicillin), kill the bacteria. Antibiotics may also be prescribed to prevent infections from occurring such as before a surgery. New antibiotics are still being produced today in order to defend against infections and to fix certain problems such as side effects, which include, rashes, diarrhea, and nausea.

SECTION 5

Bacteria in Synthetic Biology

5.1 History of Synthetic Biology

François Jacob and Jacques Monod made one of the most significant discoveries of synthetic biology in 1963. By studying genetic mechanisms, they published a series of hypotheses that theorized how DNA and RNA are transferred to explain cell division. It was known as the **replicon hypothesis** for how chromosomes are separated. Accurate theories on how DNA is replicated led to the birth of synthetic biology and developing methods to alter genetic information.

In 1985, Kary B. Mullis invented the **polymerase chain reaction**, or PCR, which allowed millions of copies of DNA to be replicated from a small sample. Commonly used today in fields such as medicine, forensics, and paleontology, PCR revolutionized the field of biology and synthetic biology by allowing genetic analysis to be applied to a broader range of situations and fields. Over the course of several decades, scientists have improved the efficiency and yield of PCR; for instance, fresh enzymes were added at each stage of the process before the discovery of Taq polymerase, which is stable in a wider range of temperatures.

5.2 What is SynBio?

Synthetic biology is a field that involves the altering of organisms for the purpose of giving them new uses. A related field called "**systems biology**" focuses on the study of the purpose and function of basic components of single processing mechanisms, feedback loops and other processes. Synthetic biology builds on that research, but it includes the design and construction of biological components into engineered systems with a specified purpose.

This research has many applications, ranging from allowing an organism to express a new function or feature to allowing for sustainable mass-scale industrial manufacturing. For example, golden rice is a variety of rice modified to produce **beta-carotene**, which is metabolized into vitamin A in the human body. Beta-carotene is a pigment that is usually not found in rice. As vitamin A supports visual and immune health, the inclusion of beta-carotene into rice, which is a staple food in many cultures, promotes our well-being.

Figure 3: Courtesy www.openwebdesign.org / Modified: Jorge Mayer. *Golden Rice Project*, https://www.goldenrice.org/Content2-How/how.php.

The **engineering approach** is the use of a systematic approach to solving a problem. It includes identifying the problem, conducting background research, planning and designing a solution, and developing and finally constructing the product. A key feature of 21st-century synthetic biology is its application of the engineering approach to the modification of organisms. Using this approach, scientists can harness the techniques described in the following sections to most effectively reach their goal.

5.3 Techniques

5.3.1 DNA Synthesis

To understand why DNA synthesis is important to synthetic biology, it is best to first understand how DNA synthesis works.

DNA synthesis, which can be natural or artificial, is dependent on Crick and Watson's discovery of the double helix structure of DNA. The initially double-stranded DNA is separated by **helicase**, an enzyme that breaks the hydrogen bonds that hold the complementary bases (A and T, G and C) together. Then, single-stranded DNA binding proteins (SSB proteins) bind to the DNA, preventing the strands from joining again. An enzyme pinpoints the origin of replication with a primer.

DNA polymerase, which are the enzymes that carry out the physical act of replication, functions in the 5' to 3' direction, meaning that it must synthesize DNA strands from different directions. It does so by adding nucleotides towards the direction of the replication fork (leading strand). Nucleotides are added in chunks, which creates **Okazaki fragments**, or sequences that are discontinuous.

The new DNA strands are created using a polymerase enzyme called **telomerase**, which catalyzes telomere sequences—repetitive nucleotides at each end of a **chromatid**, or a tail of a chromosome—at the ends of the DNA. The telomere functions to protect the end of the chromosome from deterioration or from fusion with neighboring chromosomes.

Artificial gene synthesis, on the other hand, does not require the same methods with a parent strand of DNA, meaning that it is possible to create any sequence of any size or length to be synthesized. There are two main steps to gene synthesis, the first of which includes solid-phase DNA synthesis or DNA printing.

The main method of DNA synthesis in synthetic biology is known as **oligonucleotide synthesis**. Oligonucleotides are "short single strands of synthetic DNA or RNA" that are built from using building blocks called **nucleoside phosphoramidites**. Phosphoramidites, which have the structure (RO)2PNR2 are derived from protected nucleosides and are **monoamide** (one amide group: NR'R") of a phosphite diester. In this process, one phosphoramidite is added at a time in the 3' to 5' direction, which is in the backward direction to the synthesis mentioned above.

The longer the sequence is artificially synthesized, the more likely that there will be mistakes, so the current practical limit is about 200 base pairs. Longer sequences can be synthesized in parallel on gene chips. A gene chip, also known as a biochip, is a "collection of microscopic DNA spots attached to a solid surface." These gene chips measure the expression of many genes. Each spot contains picomoles (10-12 moles) of a DNA sequence, known as oligos (or probes or reporters).

Artificial gene synthesis has also led to the creation of "unnatural base pairs" (UBPs), which deviate from the usual four nucleotides seen in DNA. These UBPs were named d5SICS and dNaM. What makes them special is that these nucleotides had hydrophobic bases and two aromatic rings ("unsaturated ring[s] of atoms with a/many double or

triple bonds that is stabilized by an interaction of the bonds forming the ring[s]") that form a (d5SICS–dNaM) base pair in DNA. These were synthesized by Floyd Romesberg, who is a chemical biologist at the Scripps Research Institute in San Diego, California.

DNA printing is used to make DNA sequences that have specific biological functions. Due to certain limitations, DNA printing is unable to be used for making multi-gene circuits or synthetic chromosomes or genomes; instead, they are used for joining parts. There are three main groups of DNA printing: endonuclease-mediated assembly, site-specific recombination, and long-overlap-based assembly.

Endonuclease-mediated assembly uses enzymes that target specific sections of DNA that can be used in DNA assembly. These enzymes are known as restriction enzymes, which recognize and "cleave" at recognition sites. One main assembly that uses this method is BioBricks by Tom Knight in 2003. It has become a form of "base" for other DNA assemblies. Some assemblies are type IIs restriction endonuclease assemblies. Other than restriction enzymes, scientists use phage (a type of enzyme) integrases site-specific recombination (recombination between two DNA recognition sequences). Other forms include: Golden Gate cloning, plasmid design and assembly, MoClo and Golden Braid.

5.3.2 Modularity

Modules in the field of synthetic biology refers to the methodological segmentation of biological systems into smaller, connected ones for easier engineerability; the ability to replicate and apply biotechnological and engineering system frameworks into these systems; and an alternative solution to studying systems individually for greater depth of understanding. Module segmentation fits into a broader topological field known as network biology.

Within network biology, systems are composed at an elementary level to nodes and edges. Nodes refer to the individual components that make up the participants or involved structures within the system. Edges, meanwhile, are the interactions between these nodes and how different nodes within a system are related to each other. The ability for semi-autonomous/autonomous modules to function collaboratively as a unit is known as modularity. Scientists continue to study this phenomenon in organisms such as bacteria in order to extend upon existing understanding of evolutionary development, anatomical and physiological morphology, and relationships between different modules.

An introductory example of modularity within bacterial systems can be observed from a genetic standpoint. The expression of genes and the production of certain proteins is attributed to a complex, yet interconnected system of regulatory and dynamic parts. Genes, promoters, transcription factors, enhancers, and terminators are all examples of

nodes within this genetic module. Meanwhile, genetic processes that occur as a result of these nodes such as DNA replication, the Central Dogma, and gene expression constitute the edges.

Another example of modularity within bacterial systems is plasmid interactions and connections. Unlike the previous example, mechanisms such as horizontal gene transfer primarily involve a singular type of node known as the plasmid—small, circular genetic structures within bacteria—whilst their respective interactions serve as edges. Horizontal gene transfer demonstrates that modularity, at times, can be self-mediated within a system and does not require third-party, external structures to assist in modulation.

The hierarchical nature of network biology also extends to a far more macroscopic level, involving the modulation of individual bacteria (nodes) connected by far more observable edges. These often incorporate a far more nuanced understanding of each module's respective environments and its role in influencing the edges formed between bacteria. One notable example of these forms of macroscopic modules come in symbiotic associations with other organisms. *Gut microbiota*, for example, involve microbes in the small intestine and cecum, among other organs, working together in order to support digestion. Despite its relative broadness in level of hierarchy compared to the previous examples of gene expression and horizontal gene transfer, these smaller modules still occur in a broader module. This is crucial to understand, as although modules do exist and cooperate with specific others in nature, modules are ultimately determined by human observation and are not in reality mutually exclusive to other hierarchical levels.

A strong understanding of modularity can be applied in a biotechnology context. For instance, BioBricks, small genetic parts which contain various manipulatable DNA sequences, are used to serve as a basis for the manipulation and designation of a specific form of expression. By altering any component which is a node—in this instance, the DNA sequence itself—we are thus able to compound these changes onto the edges, which in turn can affect the outcome (in this case, protein synthesis). Biosensors, a tool used in biotechnology, are devices which incorporate modified nodes into new contexts through differences in edge formation and interaction. A microbe, most commonly bacteria, has its genome modified, which allows it to serve in detection purposes.

Finally, modularity and the various, interconnected modules of all levels of hierarchy within network biology can be understood through an evolutionary lens. The property of evolvability refers to whether changes within modules can proceed based on its compounding effects, as discussed previously. Current literature most commonly agrees that aside from dramatic genetic changes within a population, changes in modular dynamics are constrained to ones which do not severely alter the module's overlying premise.

5.3.3 Modeling

Modeling in synthetic biology uses data to create biological circuits that are analyzed based on their behavior, serving as a tool to observe how a network will act when altered in specific ways. New biological devices are thus able to be formed, guiding experimentation. Some popular gene circuits that have been made include **logic gates**, **oscillators**, and **bistable switches**. Scientists are also exploring possible applications to biofuels and gene therapies. Models are also commonly used in gene expression, which is when the instructions in DNA make working products such as protein. These particular models illustrate details at a molecular level as well as interactions in translation, transcription, induction, transport, and regulation.

5.4 Looking Ahead

As synthetic biology advances, it has gradually provided solutions to medicine, national security, and many other issues, such as the production of food and energy.

Synthetic biology has allowed us to produce molecules that hold potential for various applications. In regards to national security, the MIT-Broad Institute Foundry was able to engineer key molecular compounds on demand for the U.S. Department of Defense, demonstrating the progress of synthetic biology and the ability of scientists to potentially create molecules that are in short supply. However, more research is currently being done on engineering molecules related to medicine, since scientists are trying to engineer these molecules to perform otherwise-unnatural processes.

Although there remains some difficulty in producing certain medicinal molecules, synthetic biological developments in medicine have already been made. One of the most notable developments is the **chimeric antigen receptor (CAR)** technology, which has greatly advanced healthcare by helping cancer patients.

CAR technology helps cancer patients by teaching those patients' T cells, which are key components in the immune system responsible for attacking and disposing of hostile substances in the body. To use CAR technology, a patient's blood sample is taken, and their white blood cells, along with T cells, are separated from the blood. The blood is returned to the patient, and the T-cells are then taken to a lab or manufacturing facility where they are engineered to attack cancer cells. Then, the newly engineered T cells are placed back into a patient's bloodstream, where they will eventually attack cancer cells.

There is also research on developing vectors capable of delivering drugs to specific tissues; this will be especially useful in making vaccines more effective. If this technology is able to be implemented into foods for vaccines, vaccines will be better mass-distributed in the event of an epidemic.

SECTION 5

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