Bacteriophage: An Underutilized Bacterial Combatant

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Introduction

Viral infections are commonly known to occur in humans, but what most people do not know is that viral infections are just as common in bacteria [1]. Bacterial viruses are termed "bacteriophage" or "phage" for short and can be found in any environment: soil, water, snow, food products, and even sediments. Viruses are generally 1000X smaller than their bacterial hosts and can possess a wide variety of different genotypic and phenotypic characteristics. Such diversity includes morphology (size, shape, and structure), the number and type of open reading frames (translational portions of the genome), host range, and mode of infection. Phage differ from animal viruses in a few distinct aspects. Phage attach directly to the bacterial cell wall, inject their viral nucleic acids into the cytoplasm, and become synthesized by bacterial components. In contrast, most animal viruses have to bind to a cell membrane protein, become engulfed by the mammalian cell, outer protective layer digested by enzymes, and genetic material finally synthesized in order to cause infection [2]. Bacteriophage were first discovered in 1915 by F. W. Twort and have yet to be utilized to their full potential over 100 years later [3].

Bacteriophage Characteristics and their Detection

Bacteriophage harbor a few unique physical and genetic characteristics. Their physical characteristics (phage morphology) can be separated into 5 categories: icosahedral, hexagonal, spherical, tailed, and filamentous [1]. The length of the phage tail varies among phage types as some have no tail, some possess a short tail, and others have a tail twice the length of the capsid head. Phage morphology and tail formation dictate their functional capabilities. Genetically, genome types are variable and may consist of either double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), or single-stranded RNA (ssRNA) which ultimately determine how the phage will function in the host, and what particular mode of infection they harbor. In contrast, viruses that infect humans are typically single-stranded RNA (ssRNA) that function as messenger RNA (mRNA) and can be directly translated into a polyprotein [4].

Another unique characteristic is that some phage can infect multiple species of bacteria while others are more species-specific [1]. This is termed 'host range' and can be of great importance when trying to find a way to combat multi-drug resistant infections. Due to the ease with which phage can acquire genes from bacterial hosts, phage can evolve to infect a wide variety of bacteria; advantageous to the phage, disadvantageous to the bacterial hosts. The mode of infection differs among phage types as they can be either lytic, lysogenic, or latent [1]. Before entering either the lytic cycle or lysogenic cycle, all phage must first exhibit the latent stage in which the phage attaches to the cell surface, penetrates the membrane, and incorporates its DNA into the host genome. Lytic phage replicate inside of the bacterial host before lysing (breaking open) the cell, releasing more phage into the environment. Lysogenic phage incorporate their DNA into the bacterial genome in order to have the bacteria, unknowingly, replicate the phage genetical material numerous times. This allows the phage to have its genome carried within the bacterial progeny, an advantage to the phage itself. However, lysogenic phages can, at times, become virulent due to the protection they obtain from "hiding" inside the bacterial cells and going undetected. This could deem problematic as this is a similar mechanism to how MRSA (multi-resistant *Staphylococcus aureus*) infects: *S. aureus* "hides out" inside immune cells, going undetected by the immune system, and ultimately rendering antibiotics useless. This gives MRSA time to replicate before the immune cell undergoes autophagy, releasing the MRSA into the bloodstream.

One method used by scientists for detecting phage is by observing plaque formations within agar [5]. Plaques are formed when phage lyse bacterial cells, spread to neighboring bacterial cells, and lyse again [6]. The lysing of the neighboring cells forms a circular, clear "lawn" that is visible to the human eye and is known as a plaque. Plaques can be counted to determine how infectious a phage is to the host and to help determine host range. Plaque formation is dictated and influenced by the phage genome, rate of

adsorption (attachment), time spent inside of the host cells, and phage morphology [5]. Depending on the phage genome, different genes could be present that ultimately affect the size of the plaque. Adsorption rate impacts plaque size as well because as the phage is slowly adsorbed into the bacteria, the phage have more time to reproduce before lysing the cell. This phenomenon leads to a higher concentration of phage within the plaque formations. The density of phage inside the host cell dictates the formation of the plaque. In a study done by Gallet, Kannoly, and Wang, it was found that "the higher the adsorption rate then the lower the phage concentration within the plaques."

The timing of the lysis stage determines how long the phage has to reproduce inside the cell beforehand [5]. Gallet et. al. found that there was a linear relationship between lysis time and burst size (plaque formation). Phage with a low adsorption rate spend more time externally to the bacterial cells, therefore, by the time the phage DNA is inserted into the cell, there is less time for the virus to cause infection. The physiology of the bacterial cells themselves could have changed by the time the phage infects, resulting in a smaller plaque size. The less time the phage have to diffuse out of the lysed cell, the smaller the plaque. Phage morphology and architecture also determine the plaque size and formation after lysis. The longer the sheath and legs of the phage, the smaller the plaque size. This is due to the ability of the virus to diffuse through the agar layer when forming a plaque. A smaller virion would lead to a larger plaque due to the ease at which it can diffuse through the matrix of the agar.

Phage Classification

As of 2011, approximately 750 different phage types have been successfully isolated and fully sequenced, with about 5,000 phage types yet to be elucidated or identified [7]. There are two types of comparative analyses that allow for a better understanding of a specific phage: viral metagenomics and prophage mining. Viral metagenomics is the process by which phage are harvested in large numbers and sequenced at random. This allows for large numbers of phage samples to be examined together from a specific environment and compared. Utilizing the mining method entails comparing phage genomic sequences against portions of a complete bacterial genome that harbor areas of prophage sequences. To attempt to isolate an individual phage genome, it is best to extract DNA from individual plaques following host-range experiments.

Most interestingly, bacteriophage can possess either DNA or RNA genomes, however, the vast majority possess linear double-stranded DNA genomes (dsDNA) and a tail (sheath): Order *Caudovirales* [7, Figure 1]. The dsDNA of this phage order is generally comprised of 55%-70% G+C. Most of the order *Caudovirales* fit into the *Siphoviridae* family (55%) possessing dsDNA and flexible non-contractile sheaths, while others make up either the *Myoviridae* (25%) family who possess contractile sheaths, or the *Podoviridae* (20%) family that exhibit short, stubby sheaths. The structure of the phage sheath has been found to reflect the phage genome, mode of infection, assembly, and maturation [8]. It has been determined that the long non-contractile tails of the *Siphoviridae* were assembled first then added to the head, whereas the short non-contractile tails were assembled after the phage heads. The similarity of the phage types in this order is currently being described as a result of horizontal gene transfer among phage. Other, less common phage families include *Tectiviridae* (lipid-containing), *Corticoviridae* (lipid-containing, circular genome), *Plasmaviridae* (enveloped), *Lipothrixviridae* (rod-shaped), *Rudiviridae* (non-enveloped), and *Fuselloviridae* (non-enveloped, lemon-shaped) [9]. These are the current, known and classified phage families but more remain undiscovered.

The *Myoviridae* family is comprised of many species of phage: T4, T2, P1, P2, Mu, *Bacillus* phage SP01, and *Halobacterium* phage ϕH [10]. Phage in this family are characterized as having an elongated head (90-110nm in diameter) and a long tail to match (100-120nm in length). The species share about 50-70% of their genomes to one another, making characterization of unknown phages difficult. Meanwhile, the *Siphoviridae* family is comprised of phage T1, T5, *Mycobacterium* L5, *Lactococcus* c2, λ, and ΨM1 [9]. These phages are characterized by having a smaller head (60nm in diameter) and longer sheath (70-90nm in length). What is most interesting is that the *Myoviridae* and *Siphoviridae* families are the only phage known to infect both bacteria and archaea. It is hypothesized that this is due to their contractile tails and the evolution of phages from

prokaryotes [11,12]. The lesser known family, *Podoviridae*, is comprised of phage T7, enterobacteria phage P22, and *Bacillus* phage ϕ29 [9]. *Podoviridae* are characterized as having a head of about 60nm in diameter and a short tail with maximum length of about 20nm.

Conclusions

Bacteriophage have been studied for years but have yet to be utilized to their full potential. Phage can be beneficial to both the food industry and pharmaceutical industry. Phage with a wide host range can be used to combat multiple bacterial infections, while phage who are selective to a certain bacterial host can be used against one particular infection. Additionally, when antibiotics are rendered useless, phage could be the answer. Phage can more easily bind to and attack bacterial cells. Phage could also be used in combination with antibiotics to fully rid a patient of an infection. Phage do not target mammalian cells, therefore, they may also be a safer therapeutic.

In the food industry, bacteriophage could be used when pasteurization and antibiotics fail to rid the product of bacteria. Bacteria can form a protective outer layer (biofilm) that antibiotics and high temperatures fail to penetrate. When the environment is right for bacteria to disperse from the biofilm, phage can be used as a final protective measure for food products. With Science and Technology rapidly advancing, now is the time to begin adding phage to the list of more commonly used biological control agents.

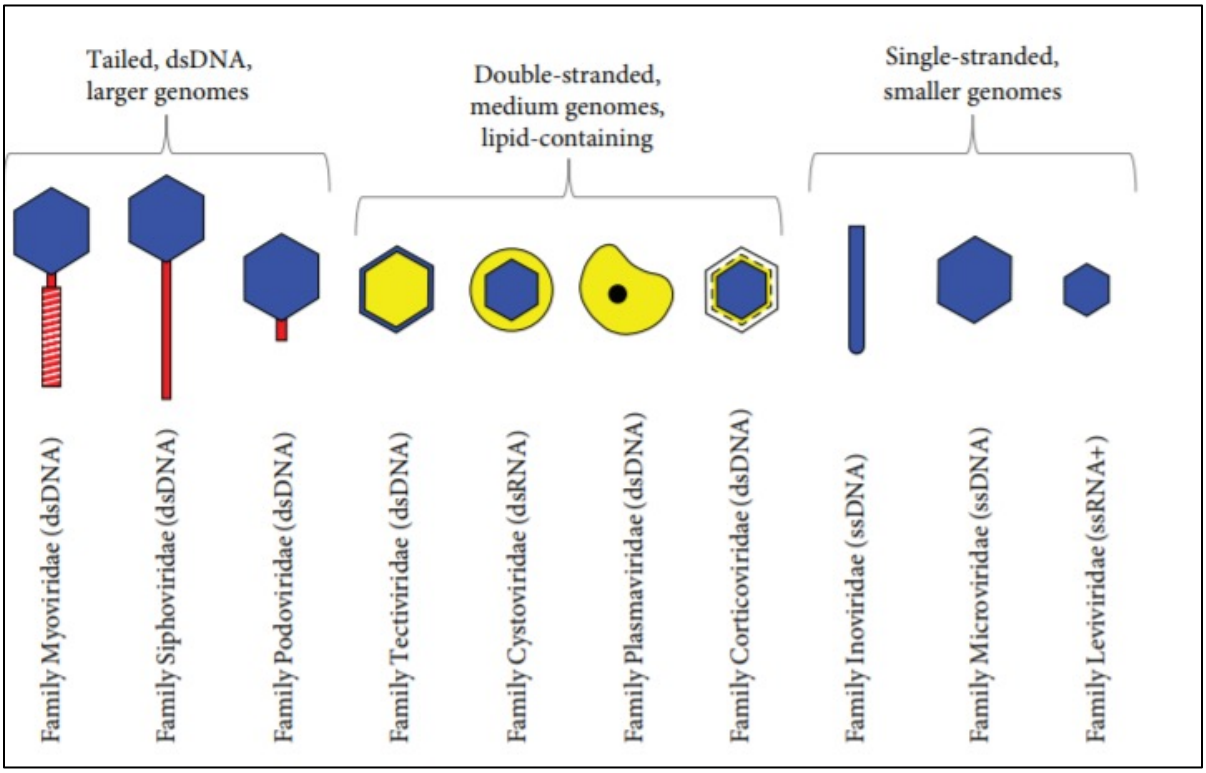

Figure 1. **Bacteriophage Familial Identification [7] Phage are classified into 13 distinct families, 3 of which have been grouped together into the Order *Caudovirales* [7]. *Caudovirales* make up the vast majority of phages (roughly 96%) that have been reported [13]. They are comprised of the families *Myoviridae*, *Siphoviridae*, and *Podoviridae*. The other 10 phage families make up only 4% of reported and classified phage.**

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