2022

ENG 300: The Efficacy of Bacteriophage & Lysin Antimicrobials in Industrial & Commercial Settings

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English 300: Writing in the Disciplines

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February 24, 2022

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Bacteria are ubiquitous. They are found in every environment, and their brilliant evolution has made them our most imposing adversary. The development of antibiotics in the early 20th century changed how we mitigate bacterial infection and contamination. Though effective, antibiotics cannot be used in every situation, especially in industrial settings where it is both dangerous to the consumer and ineffective over time (contaminating bacteria can develop antibiotic resistance). A new alternative lies within the power of bacteria's natural adversary- Bacteriophage. Bacteriophages are viruses that only infect bacteria, and they do not interact with eukaryotic cells. Because bacteriophages have a limited host range, it is possible to isolate bacteriophages to combat non-indigenous bacteria. The enzymes bacteriophages synthesize to kill their host bacterium, called lysins, are specific to their host range, so it may be possible to use the enzymes alone as an antimicrobial agent. The use of bacteriophages and their lysins to create novel antimicrobials is a promising area of research and development in the biology community.

Hospitals are host to an incredibly diverse microbiome. The flux of patients with different flora makes the building, and the people within, an epicenter for infectious diseases. In fact, almost 15% of hospital patients are clinically impaired by a hospital-acquired infection (HAI) (D'Accolti et al., 2018). Normal sanitizer and antimicrobials are often ineffective in eliminating pathogens from hard surfaces. Aggressive microbes often develop multi-drug resistance when left to mutate inside immunocompromised patients. A group of researchers in the UK hypothesized in their journal article, "Efficient removal of hospital pathogens from hard surfaces by a combined use of bacteriophages and probiotics: Potential as sanitizing agents" , that

bacteriophages could be used to decontaminate hard non-porous surfaces (glass, ceramics, plastic, etc). The researchers grew cultures of *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* and spread the broth onto the test surface. When tested after just an hour, the colony forming units had been reduced by 90±8% when compared to the controls (D'Accolti et al., 2018). What was even more noteworthy was the increase in activity over time. Because bacteriophages replicate at an exponential rate (1 bacteriophage infection can produce >100 new bacteriophages), bacteriophage decontaminants will keep working after the initial round of infection. When coupled with probiotics, the bacteriophage solution was able to eliminate 99% of contaminants after 15 days (D'Accolti et al., 2018). The extended period at which bacteriophages are capable of mediating infection is of great interest to the poultry industry.

The inability to track the conditions of products is a shortcoming of the poultry industry. Though the product may leave the facility without contamination, any change in temperature conditions during transportation may allow common pathogens like *Salmonella enteritidis* (SE) to grow within the tissue. In "Use of a specific bacteriophage treatment to reduce Salmonella in poultry products", Researchers at the University of Arkansas attempted to reduce the repeated SE contamination of poultry carcasses in a processing facility. They isolated 72 SE bacteriophages from municipal wastewater to select those SE was most susceptible to. They ran multiple trials where a culture of SE was spread onto the poultry carcass and suspensions of various concentrations of bacteriophage were allowed to coat the carcass. When recovered after 3 hours, the SE was reduced up to 100% when the bacteriophage concentration was greater than 10^{10} virions (Higgins et al., 2005).

The use of bacteriophage antimicrobials in hospitals and commercial food industries has

exposed the benefits and potential drawbacks of its implementation. Both studies conclude the antimicrobials were most effective when the concentrations of bacteriophage were very high. There is no current evidence that suggests the highest possible concentrations should not be used (Higgins et al., 2005). However, when high titer lysates ($>10^{10}$ virions) are stored, the proteins of the viral particles begin to degrade due to the close proximity and reduce the titer of the lysate. While relative host specificity is beneficial, the presence of different strains of the same species may limit the effectiveness of a singular phage. To combat this, "phage cocktails" containing bacteriophages with overlapping host ranges should be used (Higgins et al., 2005). This ensures that the target pathogen is infected. While the pathogen may be infected, there is no control over the lifecycle of the bacteriophage once it has entered the host (D'Accolti et al., 2018). The goal is to only select lytic bacteriophages: bacteriophages that do not integrate into the genome and lie dormant. However, there is a chance that a "lytic" bacteriophage may integrate into the host genome. This protects the host from all future bacteriophage infections and may produce a strain of bacteria that are more virulent and resistant to the experimental antimicrobial (Higgins et al., 2005). This uncertainty of infection has led some researchers to consider using bacteriophage enzymes instead of full viral particles.

One of the largest fermentation industries in the United States is the production of Ethanol. This process is extensive, requiring months of fermentation, leaving plenty of room for contaminating bacteria to take over. The primary culprit of contamination is Lactic Acid Bacteria, specifically Lactobacillus species (Roach et al., 2013). These bacteria are well adapted to the environment of fermentation (high ethanol, low pH, and low oxygen conditions). To control the contaminants, prophylactic supplements of penicillin are often added at each step in the fermentation process. While effective, the repeated use of antibiotics causes chronic

Lactobacillus contamination because individuals in the population can mutate to develop resistance. A safer alternative is the addition of bacteriophage lytic enzymes, referred to as lysins, to the fermentation process. During a normal phage infection cycle, lysins are expressed at the peak of infection, weakening the peptidoglycan (PG) cell wall until the cell ruptures and the virion escape into the environment. When lysins are purified, they may be able to perform exolytic activity: the degradation of the PG cell wall without infecting the cell. The authors of "Bacteriophage-encoded lytic enzymes control growth of contaminating Lactobacillus found in fuel ethanol fermentations" were able to identify 2 lysins, LysA and Lysga, that eliminated 100% of the streptococci LAB and 80% of the staphylococci LAB. Another lysin, λSa2, was able to diminish the notorious *L. fermentum* by a factor of 1.5 log10. Furthermore, these lysins were resistant to the high pH and ethanol concentrations of fermentation and still showed high efficiency (Roach et al., 2013).

The authors of "Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme" isolated a PG lysin from streptococci bacteriophage C1 and tested its effectiveness at reducing upper respiratory infection in mice. The lysin was purified and confirmed through chromatography. To confirm its function, the bulge of the membrane and subsequently rupture of the cell was observed must be observed using an electron microscope (Nelson et al., 2001). Mice, infected with a strain of streptococci, were given a dose of the lysin. All the mice that were given the lysin showed no signs of active infection after 24 hours. It was determined through further assays that 10 ng of the lysin could sterilize a culture of 10^7 streptococci cells within 5 seconds. However, eradication of the pathogen from the mucosa did not always indicate complete eradication of the infection, so this revealed the possibility of rebound infections (Nelson et al., 2001).

The applications of bacteriophage lysins may be more universal than the use of entire bacteriophage particles. The lysins, unlike the full bacteriophage complex, are easily inactivated. When experimentally used in the ethanol fermentation process, the lysins were denatured during the distillation process, preventing any impact on the microbiome of the animals fed the by-products of the ethanol production (Roach et al., 2013). Like bacteriophages, lysins are very host-specific. They only target the PG cell wall of their specific bacterial host, and, because enzymes cannot integrate into the genome of their host, there is no concern over the development of lysin resistance (Nelson et al., 2001). Finally, bacteriophage lysins work much faster than bacteriophage since they work from the outside of the cell. They are able to bypass the entire infection and growth stage of viral replication and skip straight the degradation of the cell wall. These qualities make lysins much better suited for applications involving the short-term eradication of pathogens: treating contamination, mediating infection, and use as a disinfectant.

Though lysins may prove to be more effective in short-term applications, bacteriophage will still be needed when long-term management of a large microbiome is necessary. An example could be the control of water pollution. Blooms of cyanobacteria and biofilm colonies are huge sources of cyanotoxins, which are detrimental to animals and have led to incredible economic losses (Ji et al., 2020). Treating water with antibiotics is not an option, for the antibiotics would undoubtedly end up in runoff water and then ingested by animals. In an experimental trial detailed in "Bacteriophages in water pollution control: Advantages and limitations", researchers added a cocktail of bioengineered bacteriophages (modified to overproduce an enzyme that depolymerizes cell walls) to a wastewater treatment plant dealing with heavy biofilm contamination. When the water was recovered, 99.97% of biofilm was reduced. In this scenario, lysins would not be applicable since they have no means of sustained

replication. Bacteriophages will remain stable in an aqueous environment for long periods, replicating and actively fending off the production of biofilms (Ji et al., 2020).

It is evident bacteriophage and their lysins have the potential to change the way we combat pathogenic bacteria. Their antimicrobial properties are the key to combating antibiotic resistant infections and preventing multidrug resistance. As our understanding of bacteriophages has been furthered, there have been successful attempts to recombineer bacteriophage genomes. It was hinted at in the previous paragraph: the next step of bacteriophage treatment is the modification of their genomes to increase efficiency. This is the future of personalized medicine and therapeutics. The development and implementation of genome editing will be a feat of incredible magnitude in disease treatment and the mitigation of contamination.

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