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Bacteriophages: A Potential Mitigation Strategy for Pathogenic Bacteria in Food[1]

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This fact sheet provides a brief introduction to bacteriophages and their potential for mitigating foodborne pathogens. It is intended for a general audience, including Extension agents.

Bacteriophages (or "phages"), discovered in 1915 by William Twort, are a group of viruses that infect bacteria and are not known to cause illness in humans. The name bacteriophage translates to "bacteria eater." Bacteriophages are the most abundant biological entities in the environment, to the extent that millions of them can be found in a drop of seawater. They exist in varying sizes, though even the largest of them needs an electron microscope to be viewed (Clokie and Kropinski 2009; Dion, Oechslin, and Moineau 2020).

Bacteriophages have a similar life cycle when compared to other viruses. They undergo the same basic stages of infection:

1. Attachment to the host cell
2. Entry
3. Multiplication
4. Exit (usually leading to the death of the host cell)

This process is called the "infection cycle" and is how viruses have managed to survive for billions of years. Despite their small sizes, usually less than a ten-thousandth of a millimeter, their existence has had a profound impact on the life on our planet (Clokie et al. 2011). Their long journey through evolution has allowed bacteriophages to become very sophisticated bacterial hunters. For example, their surface is equipped with molecular structures that attack a particular bacterium. Researchers utilize this unique feature to harness bacteriophages as biological alternatives to antibiotics against pathogenic bacteria in water, food, and even humans (Dion, Oechslin, and Moineau 2020; O'Sullivan et al. 2019; Kakasis and Panitsa 2019).

Pathogenic bacteria such as *Salmonella*, *Escherichia coli*, *Listeria*, and *Vibrio* have been responsible for illnesses in humans through contact with or ingestion of contaminated food or water. Infections from antibiotic-resistant bacteria take thousands of lives annually in the United States due to their ability to circumvent conventional treatment with antibiotics (CDC 2021). Bacteriophage therapy of infected individuals has proven to be an effective alternative to antibiotic treatment, particularly against multi-drug-resistant pathogens (Kakasis and Panitsa 2019).

The initial step in developing bacteriophages for use against bacterial pathogens begins with their isolation from the environment (known as a "wild-type") or construction in

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the laboratory through genetic engineering. While bacteriophages are abundant in the environment, the isolation of wild-type strains requires a substantial effort to provide a suitable host and environment so that they can be handled in the laboratory. Recent advancements in genetics have allowed scientists to incorporate unique characteristics into wild-type bacteriophages. For example, modifying molecular structures on the surface of bacteriophages may enable them to attack a different or a wider range of bacterial strains, improving their antibacterial efficacy (Chen et al. 2019; Moye, Woolston, and Sulakvelidze 2018). Regardless, bacteriophages are intended to target only a specific group of bacteria so that, once applied, they would be ineffective against harmless or beneficial bacterial.

The practical applications of bacteriophage may require a single or a combination of different types known as a "cocktail." The use of a cocktail is beneficial when used to attack a wide range of bacteria or when more than one strain of a particular pathogen is present (Perera et al. 2015; Carter et al. 2012). For use in food products, application methods vary depending on the purpose. These methods include dipping a food product in a bacteriophage-containing solution, adding to aquatic animal rearing water, or applying as a coating or spray on the food or food-contact surfaces (Carter et al. 2012; Viazis et al. 2011; Woolston et al. 2013; Teplitski, Wright, and Lorca 2009). Bacteriophages can also be administered orally to animals during production to prevent the transmission of naturally occurring pathogens that can cause illness in humans (Gambino and Brondsted 2021).

Table 1 summarizes a representative list of FDA-approved bacteriophage-based products that have been developed to improve the microbial safety of food. Many commercial bacteriophage products are considered GRAS, or "Generally Recognized as Safe." The granting of GRAS status means the foods containing these bacteriophages are safe and not subjected to premarket approval by FDA. Other bacteriophage-based antibacterial products are approved for nonfood applications. For example, AgriPhage™ is approved by the Environmental Protection Agency (EPA) for treating crops such as tomatoes, peppers, apples, and citrus to prevent and control harmful plant pathogens (EPA 2006).

When developing a new bacteriophage-based antibacterial treatment, many factors need to be carefully optimized. Dosage, environmental stability, purification techniques, contact time, application method, characteristics of the food being treated, and storage conditions must all be considered. During the production, the purity and integrity of bacteriophages must be maintained to guarantee a desired antibacterial efficacy. Bacteriophages may lose recognition of the bacterial host naturally over time and become ineffective, or the bacterial host itself may become resistant by acquiring antibacteriophage defense mechanisms. Once bacteriophages are applied to food, it is crucial to assess their antibacterial activity over an extended period of time in case of a potential recontamination event (Moye, Woolston, and Sulakvelidze 2018).

Some regulatory challenges still exist for getting bacteriophages more widely accepted for food use. The European Food Safety Authority and Medicines Agency have assessed the antibacterial efficacy for some FDA-approved products (such as the anti-*Listeria* product Listex™ P100); however, they have not proposed any processes for the approval of the bacteriophage-based food biopreservatives (Moye, Woolston, and Sulakvelidze 2018; EFSA Panel on Biological Hazards (BIOHAZ) 2016). Promoting the commercialization of bacteriophages as food additives requires a collaborative effort by stakeholders and regulatory agencies while considering the consumers' acceptability. Overall, there is solid evidence on the effectiveness of bacteriophages to reduce the risks of human exposure to pathogens. Bacteriophage-based food biopreservatives do not seem to alter the sensory and quality attributes of food and do not cause illness in humans if ingested via food. Therefore, they may serve as an alternative to artificial chemical preservatives and help reduce the risks of antibacterial resistance through the food supply chain (O'Sullivan et al. 2019; Moye, Woolston, and Sulakvelidze 2018; Perera et al. 2015).

References

Carter, C. D., A. Parks, T. Abuladze, M. Li, J. Woolston, J. Magnone, A. Senecal, A. M. Kropinski, and A. Sulakvelidze. 2012. "Bacteriophage Cocktail Significantly Reduces *Escherichia coli* O157: H7 Contamination of Lettuce and Beef, but Does Not Protect against Recontamination." *Bacteriophage* 2 (3): 178–185.

CDC. 2021. "Antibiotic/Antimicrobial Resistance (AR/AMR)." Accessed November 22, 2021. https://www.cdc.gov/drugresistance/

Chen, Y., H. Batra, J. Dong, C. Chen, V. B. Rao, and P. Tao. 2019. "Genetic Engineering of Bacteriophages against Infectious Diseases." *Front Microbiol* 10:954.

Clokie, M. R. J., and A. M. Kropinski. 2009. *Bacteriophages - Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions.* Springer. p. 13.

Clokie, M. R., A. D. Millard, A. V. Letarov, and S. Heaphy. 2011. "Phages in Nature." *Bacteriophage* 1 (1): 31–45.

Dion, M. B., F. Oechslin, and S. Moineau. 2020. "Phage Diversity, Genomics and Phylogeny." *Nature Reviews Microbiology* 18 (3): 125–138.

EFSA Panel on Biological Hazards (BIOHAZ). 2016. "Evaluation of the Safety and Efficacy of Listex™ P100 for Reduction of Pathogens on Different Ready-to-Eat (RTE) Food Products." *EFSA Journal* 14 (8): 4565.

EPA. 2006. "*Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato* specific Bacteriophages." https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006449_12-Sep-06.pdf

Gambino, M., and L. Brondsted. 2021. "Looking into the Future of Phage-Based Control of Zoonotic Pathogens in Food and Animal Production." *Current Opinion in Biotechnology* 68:96–103.

Intralytix. 2021. "Intralytix Receives FDA Regulatory Clearance for Phage-Based Campylobacter Technology." Press Release. http://www.intralytix.com/index.php?page=news&id=106&fbclid=IwAR1ezYbrajWS3q6s2cb54j1_U9bGdZ8eznvvI2ik-75CtDcapHwhGVHzN2g

Kakasis, A., and G. Panitsa. 2019. "Bacteriophage Therapy as an Alternative Treatment for Human Infections. A Comprehensive Review." *Int J Antimicrob Ag* 53 (1): 16–21.

Kawacka, I., A. Olejnik-Schmidt, M. Schmidt, and A. Sip. 2020. "Effectiveness of Phage-Based Inhibition of *Listeria* Monocytogenes in Food Products and Food Processing Environments." *Microorganisms* 8 (11).

Moye, Z. D., J. Woolston, and A. Sulakvelidze. 2018. "Bacteriophage Applications for Food Production and Processing." *Viruses* 10 (4).

O'Sullivan, L., D. Bolton, O. McAuliffe, and A. Coffey. 2019. "Bacteriophages in Food Applications: From Foe to Friend." *Annu Rev Food Sci Technol* 10:151–172.

Perera, M. N., T. Abuladze, M. R. Li, J. Woolston, and A. Sulakvelidze. 2015. "Bacteriophage Cocktail Significantly Reduces or Eliminates *Listeria* Monocytogenes Contamination on Lettuce, Apples, Cheese, Smoked Salmon and Frozen Foods." *Food Microbiology* 52:42–48.

Teplitski, M., A. C. Wright, and G. Lorca. 2009. "Biological Approaches for Controlling Shellfish-Associated Pathogens." *Curr Opin Biotechnol* 20 (2): 185–90.

Viazis, S., M. Akhtar, J. Feirtag, and F. Diez-Gonzalez. 2011. "Reduction of *Escherichia coli* O157:H7 Viability on Hard Surfaces by Treatment with a Bacteriophage Mixture." *International Journal of Food Microbiology* 145 (1): 37–42.

Woolston, J., A. R. Parks, T. Abuladze, B. Anderson, M. Li, C. Carter, L. F. Hanna, S. Heyse, D. Charbonneau, and A. Sulakvelidze. 2013. "Bacteriophages Lytic for *Salmonella* Rapidly Reduce *Salmonella* Contamination on Glass and Stainless Steel Surfaces." *Bacteriophage* 3 (3): e25697.

Table 1. Examples of FDA-approved bacteriophage-related products.

Product	Company	Application	Target	References
ListShield™	Intralytix, Inc. USA	Ready-to-eat foods	*Listeria monocytogenes*	Perera et al. 2015
EcoShield™	Intralytix, Inc. USA	Ground beef, fruits, vegetables, etc.	*E. coli* O157:H7	Carter et al. 2012
SalmoFresh™	Intralytix, Inc. USA	Poultry, fish and shellfish, and fresh and processed fruits and vegetables	Pathogenic *Salmonella*	Woolston et al. 2013
CampyShield™	Intralytix, Inc. USA	Raw red meat and raw poultry		Intralytix 2021
PhageGuard Listex™	Micreos Food Safety B.V., Netherlands	Seafood, ready-to-eat meats	All *Listeria* strains	Kawacka et al. 2020