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Current advancements in applications of bacteriophages in food microbiology: A review

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Abstract: Using bacteriophages (phages) to target and neutralize food-borne pathogens offers an innovative approach to improving safety and quality of various foods. In place of traditional antibiotics and chemical preservatives, this novel approach applies phages as biocontrol agents against common bacterial pathogens. This review comprehensively compiles the latest literature, the historical background of phage therapy, its current uses in food safety and preservation, consumer acceptance and regulatory issues, and potential future applications of phage-based interventions in the food industry. Although phages show great promise in lowering foodborne illness rates and prolonging food product shelf life, their actual application depends on overcoming obstacles like phage resistance and maintaining regulatory compliance. To fully explore the advantages of phages in enhancing food safety, this work advocates for a multidisciplinary approach and emphasizes the necessity for additional research to maximize phage therapy in food systems.

1. Introduction

Our world is driven by microbes, and it has been made possible by bacteria and archaea that were able to moderate the harsh environments that existed in the atmosphere at the beginning of the Earth and made it favorable for the growth of eukaryotic forms of life (Cowan et al., 2024). Enzymes encoded by bacteria and archaea play a crucial role in the carbon and nitrogen cycles, catalyzing all the major processes involved in global biogeochemical cycling (Staley et al., 2003). They also produce over half of the oxygen in the Earth's atmosphere. Prokaryotic cells are more common than eukaryotic cells in humans and animals, where they help with critical survival functions like nourishment and defense. Phages have long been overlooked, but it is now widely acknowledged that they are essential to the biology of bacteria, which have a great impact on the environment around them. The environment may include water, air, and any place where they can get nutrition (Lin et al., 2003). To understand the role of phages in the environment and specifically in the food sector, we first need to get a hold of what bacteriophages are, how they were discovered, and what their mechanism of living is (Simmonds 2018).

Phages, or bacteriophages, as they are more commonly called, are a broad class of viruses that only infect bacteria. Early in the 20th century, Frederick Twort in 1915 and Félix d'Herelle in 1917 separately discovered the bacteriophages (Clokie et al., 2011). New directions in microbiology, biotechnology, and, more recently, food science have been made possible by their discovery.

Phages are acknowledged as the most prevalent biological agents on Earth and are found everywhere in the environment. Phages are categorized according to their morphology, which includes the structure of their head and tail, as well as the type of nucleic acid they contain, which can be either single- or double-stranded DNA or RNA. Their size, appearance, and genetic structure exhibit remarkable diversity. All, however, are made up of a nucleic acid genome covered in a capsid protein shell that has been encoded with phages, protecting genetic information, and facilitating its transfer to the subsequent host cell (Louten 2016).

Phage therapy was once investigated for its ability to treat bacterial infections, but once antibiotics became available, interest in it decreased. However, the emergence of antibiotic resistance has piqued researchers'

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interest in phages once more, not only for therapeutic but also for food microbiological applications (Kasman and Porter, 2022).

For a long time, mankind has encountered problems regarding food, like spoilage and contamination. Foodborne infections are a major worldwide problem that arises from the consumption of contaminated food. Certain pathogenic bacteria and fungi are often involved in such cases. Ensuring food safety and quality is largely dependent on food microbiology. It makes use of several tactics to improve food preservation methods, find, manage, and eradicate harmful bacteria, and increase the shelf life of food items (Lorenzo et al., 2018).

One important characteristic that draws phages to food microbiology applications is their host-specificity to bacteria. Antimicrobial phages, in contrast to broad-spectrum antibiotics, can target specific bacteria without upsetting the beneficial microbiome. The development of tailored therapies against foodborne pathogens is made possible by the specificity resulting from the recognition mechanisms between the bacterial cell surfaces and phage surfaces (Dicks and Vermeulen, 2024). Moreover, the diversity of phages is like the diversity of bacteria, which means that there is a phage that can infect almost any species of bacterium. This enormous diversity provides a wealth of resources that can be used for a variety of food safety and preservation applications. Molecular biology and genetics advancements have made it easier to identify, characterize, and manipulate phages to serve specific purposes, making them an effective weapon against foodborne diseases (Jo et al., 2023). Given their potential, phages' biology and ecology must be understood if food microbiology is to successfully use them. To fully utilize phages, scientists are still investigating the intricate relationships that exist between them, their bacterial hosts, and the habitats in which they reside. This entails not just focusing on certain dangerous bacteria but also being aware of how applying phages to food products may affect the larger microbial ecology (Elfadadny et al., 2023).

Promising antimicrobials that can be utilized to prevent food contamination include some phage proteins. Endolysins—lytic proteins that break down the peptidoglycan in the bacterial cell wall at the end of the lytic cycle—are sometimes referred to as enzybiotics and have been researched as a novel antibacterial tactic since the year 2000. The basis for this use is the utilization of these proteins as explosions, which, when supplied exogenously, will cause cell lysis by breaking down the cell walls from the outside (Nazir et al. 2023). Endolysins from phages that infect both Gram-positive and Gram-negative bacteria exhibit a modular structure made up of a cell wall binding domain (CBD) and one or more enzymatic catalytic domains. With the use of domain shuffling and this structure, new chimeric proteins can be created that often exhibit enhanced lytic activity in comparison to their "parent" proteins (Rendueles et al., 2022).

This review comprehensively compiles the latest literature, the historical background of phage therapy, its current uses in food safety and preservation, consumer acceptance and regulatory issues, and potential future applications of phage-based interventions in the food industry. Although phages show great promise in lowering foodborne illness rates and prolonging food product shelf life, their actual use depends on overcoming obstacles like phage resistance and maintaining regulatory compliance. To fully explore the advantages of phages in enhancing food safety, this work advocates for a multidisciplinary approach and emphasizes the necessity for additional research to maximize phage therapy in food systems.

2. Bacteriophage Applications in Food Microbiology

2.1 Biocontrol Agents in the Production of Food

Ensuring microbiological safety in food production has undergone a paradigm shift with the use of bacteriophages as biocontrol agents. Their use is widespread in the food business, mainly directed against harmful microorganisms without compromising the food items' sensory qualities. Applications for phages that target pathogenic food-borne bacteria are currently being used as a post-harvest biocontrol strategy as well as for the cleaning and treatment of live farm animals. The US Food and Drug Administration (FDA) has authorized multiple phage products that target Salmonella sp., Escherichia coli, and Listeria sp. for this use (Ushanov et al., 2020).

2.2 The Dairy Industry

The use of bacteriophages has been focused on the dairy industry, especially against Listeria monocytogenes, a microbe of great concern because it can grow at temperatures below refrigeration. To reduce Listeria on cheese surfaces and in dairy processing environments, phage preparations have been created and used; they have shown effectiveness without affecting the organoleptic qualities of the cheese (Połaska and Sokołowska 2019). This focused strategy adds another degree of security to current sanitation and hygiene practices.

2.3 Meat and Poultry

Bacteriophages are used in meat and poultry processing as a component of decontamination techniques to combat two common foodborne pathogens linked to these products: *Salmonella spp*. and *Campylobacter jejuni*. There are, of course, many other pathogens in meat and poultry but these two stand out as the most prevalent. To lessen the bacterial load on carcasses during processing, phage sprays and washes are used. Studies have shown that these methods significantly reduce pathogen levels, which lowers the risk of contamination (Atterbury et al., 2003).

2.4 Seafood

Vibrio species, especially *Vibrio cholera*, are endemic to coastal habitats as they prefer a saline environment. Phage applications against such species benefit the seafood sector. Phage treatments have been studied for usage in seafood products and aquaculture settings, and they have the potential to increase safety without having a negative effect on the marine ecology.

The development and well-being of shrimp larvae may be impacted by the microflora associated with their larval phases. Certain bacteria, like the luminous *Vibrio harveyi*, can be fatal. Since most antibiotics are no longer permitted for use in aquaculture, alternative methods of controlling bacterial infections are required. After additional analysis, it was discovered that two bacteriophages, which are members of the Siphoviridae family, effectively decreased the number of *V. harveyi* bacteria in the biofilm that was produced on the surface of high-density polyethylene (HDPE) (Karunasagar et al., 2007).

One major factor contributing to foodborne outbreaks is processed foods. Foods like sausages, deli meats, cheeses, and powdered milk have been connected to outbreaks of hemolytic uremic syndrome, listeriosis, salmonellosis, and hemorrhagic colitis. Another application of phages as biocontrol agents is the management of foodborne pathogens in various foods, demonstrating the variety of foods that phages may be used to treat and the adaptability of phages as antimicrobial agents (Goodridge and Bisha, 2011).

2.5 Fruits and Vegetables

More and more foodborne illness incidents are being linked to fresh fruits and vegetables. For instance, at least 554 foodborne outbreaks linked to vegetables occurred between 1990 and 2003; these outbreaks caused around 28,000 hospitalizations and many fatalities. The need for improved interventions to prevent the presence of foodborne pathogens in raw foods is highlighted by the recent epidemic of a pathogenic isolate of *E. coli* related to bean sprouts grown on an organic farm in Germany. Phage biocontrol is a great option in organic farming settings because organic farming procedures require the use of only natural antimicrobials in production. To determine whether phages could be utilized to control Salmonella in sprouting seeds, Pao and colleagues carried out numerous experiments. In this study, two bacteriophages with distinct host ranges were isolated and characterized by the researchers. It was then demonstrated that a combination of the two phages led to a 1.50 log reduction in the quantity of Salmonella in the soaking water used to soak broccoli seeds (Pao et al., 2004).

3. Bio-preservation

Beyond biocontrol, bacteriophages are used in bio-preservation to inhibit spoilage organisms and prolong food products' shelf lives. This application is especially important for products that certain bacterial strains can cause to deteriorate. Given the prevalence of phages in the environment, phage-based bio-preservatives are regarded as natural and are in line with consumer expectations for "clean label" food items. The dual functioning of phages in improving food safety and decreasing food waste, contributing to sustainable food production systems, is highlighted by their usage in bio-preservation.

4. Foodborne Pathogen Detection

Phage-based detection techniques, which provide quick, accurate, and sensitive identification of foodborne pathogens, are a novel application in food microbiology. By using phages that have been designed to emit a detectable signal upon the infection of their target, these techniques take advantage of the selectivity of phages for their host bacterium. For example, reporter phages have been created that, when they enter a bacterial cell, express fluorescent, or bioluminescent markers. This makes it possible to monitor the presence of pathogens in food samples in real time (Goodridge and Bisha, 2011). This technology has the potential to improve food safety by making it possible to quickly identify instances of contamination and then take appropriate action.

5. Use of Phage Therapy in Animal Husbandry

Phage therapy is used in animal husbandry procedures in addition to direct food product interventions. One method to lower the occurrence of foodborne pathogens at the source is to treat livestock with phages, either as a preventive approach or to treat illnesses that already exist. Antibiotic resistance concerns can be addressed by lowering the use of antibiotics in animal husbandry using this technique. Phage therapy has been shown to be effective in treating bacterial infections in cattle, pigs, and poultry in this field of study, suggesting that it is a feasible option for enhancing animal health and food safety (Smith and Huggins, 2003).

6. Phage Application's Challenges and Constraints in Food Microbiology

Although promising, the use of bacteriophages in food microbiology is not without obstacles and restrictions. For phage technology to be successfully incorporated into methods for food safety and preservation, certain issues must be resolved.

6.1 Acceptance by Authorities and Consumers

A major obstacle to the extensive integration of phage technology in the food sector is the state of regulations and customer acceptability. It is the responsibility of regulatory organizations across the globe to guarantee that any novel intervention in food safety does not pose hazards to public health. Phage product approval procedures can be lengthy out and complicated, as they vary greatly between states. As a result, food producers may be cautious and the implementation of phage-based therapies may be delayed (Hagens and Loessner, 2010). A further significant aspect is acceptance by consumers. The deliberate placement of phages into food products may cause customers who are not familiar with the concept to become concerned, even though phages are naturally occurring and have been consumed for as long as people have eaten food. To alleviate consumer concerns, educational initiatives are required to convey the safety, effectiveness, and natural occurrence of phages.

6.2 Resistance to Phages

For millions of years, bacteria have coevolved with phages, creating defense systems against phage infection. The efficiency of phage-based therapies is significantly challenged by the evolution of bacterial strains that are resistant to phages. Many pathways can lead to resistance, including bacterial defense systems like CRISPR-Cas being activated or the bacterial cell surface being altered to hinder phage attachment (Labrie et al., 2010). Phage cocktails, which are collections of several phages that target the same pathogen, have been proposed to combat resistance. Although these cocktails potentially lessen the chance that resistance will develop, they also make the formulation, control, and use of phage products more difficult.

6.3 Dynamics of Phage-Bacteria Interaction

The complex interactions that exist between phages and their bacterial hosts can affect how well phage application works in food systems. Phage interventions can be successful or unsuccessful depending on several factors, including the phage's host range, the timing of treatment, and environmental factors like pH and temperature. The process of optimizing these parameters for every food product and pathogen is intricate and demands a great deal of knowledge and testing.

Furthermore, it is important to consider the possibility that phages may transfer genetic material, particularly genes that confer antibiotic resistance, between bacteria. Even if the risk is usually regarded as minimal, particularly when it comes to lytic phages, this emphasizes the necessity of thorough screening and phage selection for food applications (Touchon et al., 2017).

7. Prospects and Advancements in Phage Applications for the Future

It appears that bacteriophage use in food microbiology will continue to grow and increase in the future. With more investigation and advancement, it will be possible to overcome present constraints and fully utilize phages to transform food safety and quality. This progressive outlook includes several important points of emphasis.

7.1 Progress in Phage Engineering

Enhancing efficiency, safety, and specificity of phages for food applications can be achieved through new potential presented by synthetic biology and genetic engineering. Engineered phages can be made to carry enzymes or antimicrobial peptides that increase their antibacterial activity, target pathogens more efficiently, and reduce the emergence of resistance. Advances in CRISPR-Cas technology offer a mechanism to disarm potentially dangerous bacteria of virulence factors or antibiotic resistance genes, in addition to customizing phages for uses. This allows for a more comprehensive approach to food safety (Citorik et al., 2014).

7.2 Combining Food Safety Measures

Phage therapy, when combined with current food safety protocols, offers a comprehensive solution to microbiological control. Synergistic effects can be obtained by mixing phages with other antimicrobial treatments, such as organic acids, essential oils, or high-pressure processing, which will reduce contamination in food products more effectively. To maintain the long-term effectiveness of phage applications in the food sector, this integrative technique can also aid in preventing the emergence of phage resistance within target bacteria (Gutiérrez et al., 2017).

7.3 Improvement in the Food Packaging Sector

Using bacteriophages in food packaging materials is an innovative way to guarantee food safety all the way through the supply chain. Phage-infused edible films and coatings can offer a persistent antibacterial presence, actively lowering the possibility of contamination during transportation and storage. The field of phage-impregnated packaging research is investigating diverse delivery methods, such as microencapsulation, that will protect phages and regulate their release, guaranteeing their effectiveness throughout the food product's shelf life (Scarpellini et al., 2015).

7.4 Government and Public Acceptance

For phage technology to be successful in the future, efforts must be made to improve public acceptance of the technology and expedite regulatory processes. Consumer worries may be reduced by educational programs that emphasize safety of phages, natural occurrence, and function in preserving microbial balance. Furthermore, the approval of phage-based products will be accelerated by the establishment of precise, scientifically grounded regulatory frameworks, which will stimulate investment and innovation in this area.

7.5 Perspectives on Environmental and Global Health

Use of phages use in food microbiology also brings up more general environmental and global health implications. Phages are in line with the ideas of sustainable agriculture and food production, providing a healthy substitute for chemical preservatives and antibiotics. By offering a competitive substitute for antibiotics in animal husbandry and aquaculture, their function in fighting antibiotic-resistant bacteria advances global health initiatives in addition to enhancing food safety.

8. Conclusions

Modern scientific research and ancient biological interactions come together in the research of bacteriophages in food microbiology. Bacteriophages occupy a special place in the connection between environmental sustainability, public health, and food safety, as this subject continues to develop. Chemical preservatives and broad-spectrum antibiotics lack the nuanced approach to microbial control that comes from their capacity to selectively target pathogenic bacteria while protecting beneficial microbiota. Phage therapy is relevant in current food systems because it can reduce dependency on the use of chemicals, which is in line with consumer demand for natural and minimally processed foods. To sum up, the investigation into bacteriophages holds great potential for creating safer and healthier food systems. A new age in microbial control is being characterized by its focused action against pathogens, conformity with current food safety regulations, and potential for innovation in food processing and packaging. Phage integration into food microbiology will probably become a key component of contemporary food safety strategies such as research and regulatory frameworks change. This will provide a long-term substitute for conventional antimicrobials and aid in the worldwide fight against food-borne diseases.

Author's contribution

Samra Munir conceptualized and wrote the manuscript and other authors drafted and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Competing Interest

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