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Review- Bacteriophages in Food Preservation

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ABSTRACT

Foodborne illnesses are a major cause of morbidity and mortality worldwide. The World Health Organization estimates that globally, diarrhea diseases alone (a majority of which are caused by foodborne pathogens) kill 1.9 million children per year. Food is a fundamental requirement of everyday life, but in all too many instances, contamination with pathogenic bacteria can result in illness and even death. Microbial spoilage is by far the most common type of food spoilage encountered. Even with current preservation methods and good manufacturing practices, quality control, and hygiene, an estimated 25% of the total food produced every year is lost due to microbial damage. Bacteriophages (phages) are bacterial viruses that only infect and multiply within their specific hosts, disrupt bacterial metabolism, and cause the bacterium to lyse. The initial trials of phage therapy produced varied results which have been attributed in part to the limited scientific rigour applied to the experiments, including the failure to conduct double-blind trials which were not standard practice at the time, as well as poor understanding of the biological nature of phage and lysogeny. Bacteriophage it is one of the method for to infect the bacteria and this will not affect the taste, texture, smell and color of food.

Key words: *Bacteriophages, Food Preservation, Foodborne Pathogens, WHO.*

INTRODUCTION

Foodborne illnesses are a major cause of morbidity and mortality worldwide. The World Health Organization estimates that globally, diarrhea diseases alone (a majority of which are caused by foodborne pathogens) kill 1.9 million children per year. Foodborne diseases do not only occur in developing countries, in the United States of America for example, it is estimated that foodborne diseases result in 76 million illnesses, 325,000 hospitalizations and 5,000 deaths each year. Considerable effort has been directed towards the control of the major bacterial food-borne pathogens. However, this has had little impact on addressing the problem in many countries. This is because the effectiveness of the intervention initiatives has been obscured by other changing factors. These changing factors may be associated with the pathogens, their hosts (humans) or political, economic and environmental factors. Environmental challenges have caused food-borne bacterial pathogens to evolve and the susceptibility of human population to infections are also changing due to declining acquired immunity and increased numbers of immune compromised individuals.

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There has been a continuous increase in several foodborne diseases caused by bacterial pathogens such as *Salmonella*, *Campylobacter*, *Escherichia coli* and *Listeria* despite the employment of modern technologies to inactivate these pathogens in food. These pathogens come into contact with foods during harvest or slaughtering, processing, storage and packaging. Physical treatments such as UV light, high pressure, dry heat and steam are viable strategies of reducing pathogenic bacteria in raw products. These methods have been considered because the use of antibiotics has been restricted over the years due to the risk of antibiotic-resistant bacteria entering the human food chain and causing negative impact on human antimicrobial treatment. However, physical methods of reduction of microbial load in raw foods have been known to negatively impact the organoleptic properties of the products hence reducing their acceptability. There has thus been an increasing need to develop novel strategies to reduce bacterial pathogens in foods and still satisfy consumer demand for minimally processed foods with low concentrations of chemical preservatives. Bacteriophages (phages) have found use as natural antimicrobials that can be used in controlling bacterial pathogens in foods and food processing environments[1].

Food is a fundamental requirement of everyday life, but in all too many instances, contamination with pathogenic bacteria can result in illness and even death. According to the Centers for Disease Control and Prevention (CDC), it was estimated in 2011 that approximately 48 million cases of food poisoning occur each year in the United States alone, of which 128,000 result in hospitalization and 3,000 in fatalities (CDC 2011). Known pathogens account for an estimated 9.4 million of these illnesses, 55,961 hospitalizations and 1,351 deaths[20].

Microbial spoilage is by far the most common type of food spoilage encountered. Even with current preservation methods and good manufacturing practices, quality control, and hygiene, an estimated 25% of the total food produced every year is lost due to microbial damage. The financial burden of microbial contamination on human health and the food processing industry is enormous, costing the global economy billions of dollars every year. Food manufacturers are continuously challenged by consumer expectations for products that are pathogen free and of acceptable quality without the use of artificial preservatives**.**

Fluctuating environmental conditions have caused evolution of many foodborne bacterial pathogens, these pathogens come into contact with foods at different levels. Human population is getting more susceptible to diseases, emergence of new antibiotic-resistant bacterial strains is a new challenge to face, So, there is need for development of novel strategies to reduce bacterial pathogens in foods[16].

IMPORTANT BACTERIAL FOOD PATHOGENS

Pathogen	Symptoms	Source of infection
Salmonella enterica	Salmonellosis: common gastroenteritis, enteric fever, bacteremia	Contaminated meats of animal origin and fruits and vegetables
Campylobacter species	Campylobacteriosis: severe acute gastroenteritis, linked to the development of Guillain-Barré syndrome	Consumption of undercooked/raw meat, pasteurized milk, vegetables, and environmental water
E. coli O157:H7	Diarrhea, severe hemorrhagic colitis, hemorrhagic uremic syndrome	Consumption of contaminated undercooked foods of bovine origin
Listeria monocytogenes	Listeriosis: nausea, vomiting, abortion, fetal death, septicemia, meningitis	Primarily through consumption of RTE foods

Cronobacter sakazakii	Necrotizing enterocolitis, meningitis, and bacteremia in neonates and infants	Contaminated powdered infant formula (infection in infants)
Mycobacterium vium subsp.	Implicated as a causative agent of Crohn's disease in humans	Consumption of contaminated dairy products such as milk
Clostridium perfringens	Gas gangrene and necrotic enteritis in humans and	Consumption of contaminated poultry products.
Staphylococcus aureus	Gastroenteritis	Consumption of contaminated meat, poultry and dairy products
Shigella	Shigellosis: bloody diarrhea, fever, stomach cramps, inflammatory enteritis	Consumption of contaminated water, vegetables milk, dairy, poultry

Bacteriophage discovery

Bacteriophages (phages) are bacterial viruses that only infect and multiply within their specific hosts, disrupt bacterial metabolism, and cause the bacterium to lyse. They were discovered a long time ago but the history of bacteriophage discovery has been the subject of lengthy debates. Ernest Hankin, a British bacteriologist, reported in 1896 that the waters of the Ganges and Jumna rivers in India had marked antibacterial action against *Vibrio cholerae* and that ingestion of the water of these rivers prevented spread of cholera epidemic[12].

He suggested that an unidentified substance (which passed through fine porcelain filters and was heat labile) was responsible for this phenomenon. A similar observation was made 2 years later by the Russian bacteriologist Gamaleya, while working with *Bacillus subtilis.* However, their findings were not explored further until in 1915 Frederick Twort, a British pathologist, and a French-Canadian bacteriologist Felix d'Herelle working at the Pasteur Institute in Paris independently reported in 1917 isolating filterable entities that could destroy bacterial cultures and produce small clear areas on bacterial lawns. D'Herelle called them "bacteriophages"—bacteria eaters[1].

LIFE CYCLE

There are 5 stages

Adsorption
Penetration or injection
Latent period
Maturation or morphogenesis
Lysis or release

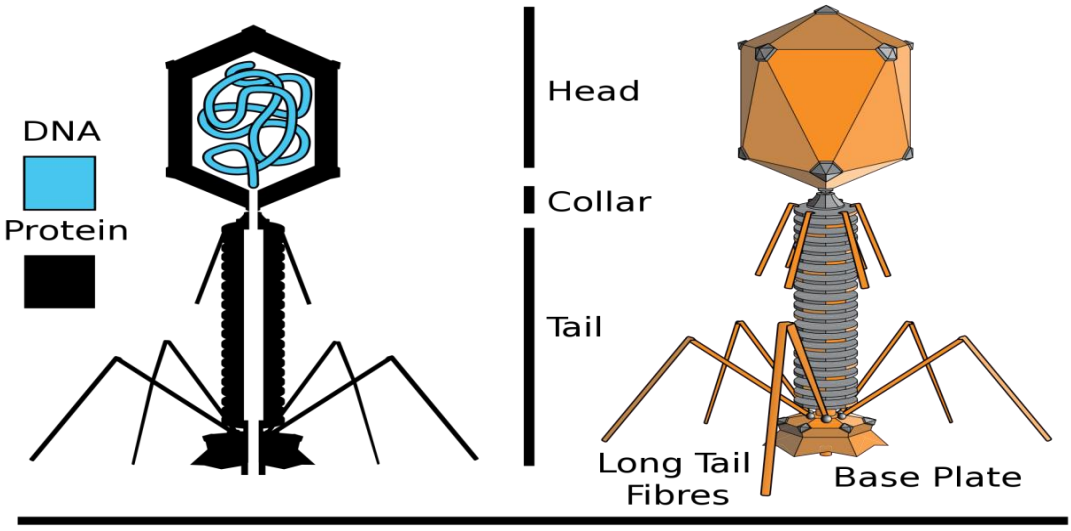

Fig. 1: Morphology and structure

1. Adsorption:

Infection with tailed phages starts when the specialized adsorption structures, such as fibers or spikes, bind to the specific surface molecules on their target bacteria. The nature of the bacterial receptor varies for different bacteria; they may be located on the cell wall, flagella, pili, capsules, or the plasma membrane. The environment is an important factor in the adsorption process and some cofactors may be required to enhance the adsorption.

2. Penetration or injection:

Phage nucleic acid enters the host and the shell remains outside. Mechanisms of nucleic acid injection are different for phages of different morphology. When the sheathed tailed phages (e.g., T4 phage) contact with the host outer membrane receptors, conformational changes in the phage structure are initiated that lead to the contraction of the tail sheath, which in turn forces the hollow inner tube into the cell. The short-tail fibers help to anchor the distal portion of the tail and baseplate to the cell surface receptors. At this time, the whole tail structure shrinks and widens, bringing the internal pin-like tube in contact with the outer membrane of the bacterial cell and phage enzymes located on the tail tip degrade the bacterial cell wall. As the tail tube punctures the outer and inner membranes of the cell, the viral genome is injected through the tail tube into the host cell's cytoplasm.

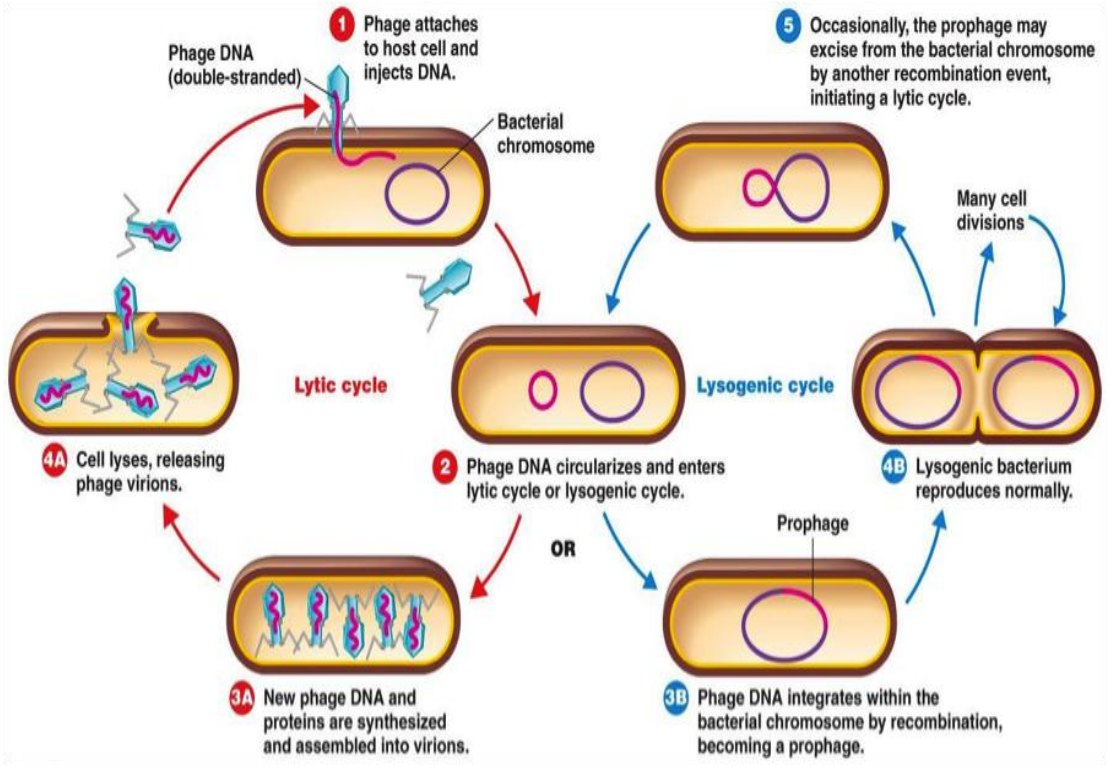

Fig. 2: LIFE CYCLE

In sheathless tailed phages (e.g., phage 1), the tail sheath does not contract during DNA injection. However, filamentous DNA phages of E. coli (e.g., phage M13) seem to enter the cell by being drawn into the inner membrane of the cell envelope while being uncoated; the DNA is released intracellular as the coat protein dissociates into subunits which remain in the membrane[9].

1. Latent period

Immediately after the entry of the viral genome, the expres- sion of early proteins begins, which

are needed to replicate the phage genome and to modify the cellular machinery so that the synthetic capacity of the cell is diverted to phage reproduction. During this stage, the synthesis of a number of copies of the phage DNA occurs. Each of these copies can then be used for transcription and translation of a second set of proteins, called the late proteins that make up the capsomeres and the various components of the tail assembly. Lysozyme is also a late protein that will be packaged in the tail of the phage to be used to escape from the host cell during the last step of the replication process[9].

2. Maturation or morphogenesis

The period during which the new phage components are assembled into virions. Assembly can occur spontaneously or with the help of specific enzymes. The DNA is packaged into preassembled protein shells called procapsids. In most phages, their assembly involves complex interactions between a specific scaffolding protein and the major head structural proteins. In tailed phages, the head and tails are assem- bled by separate pathways and are joined together after DNA encapsidation[9].

3. Lysis or release.

Phages are liberated by lysis. Specific enzymes hydrolyse the cell wall from inside, liberating infectious phages that are capable of starting the cycle over again and infecting new susceptible host cells[1,9]. The number of phages produced depends on the phage type and the physiology of the host cell. The tailed phages use two enzymes for the lysis of the host cell: lysin—an enzyme capable of degrading the cell wall peptidoglycan and holin— an enzyme that assembles pores in the inner membrane to let the lysin reach the peptidoglycan layer. These enzymes disrupt the cell membrane and cell wall causing the cell to burst, and phages are released into the surrounding medium. The tailless phages encode a variety of single lysis-precipitating proteins that sabotage the host peptidoglycan-processing enzymes by different modes of action[5]. The number of new phages released per infected cell is called the "burst size" shown in **Fig.2**.

Where we can find phages?

- In human and animal intestines
- In the soil
- Running water
- Effluent outlets
- Sewage from corpses

APPLICATION OF PHAGES

- Therapy
- Sanitation
- Bio control
- Preservation

1. Phage therapy

- The bactericidal nature of virulent phage sparked interest in their potential for use as antimicrobial agents following their discovery in the early 20th century.
- Phage therapy refers to the application of virulent bacteriophages to treat bacterial infections in humans or animals.
- The initial trials of phage therapy produced varied results which have been attributed in part to the limited scientific rigour applied to the experiments, including the failure to conduct double-blind trials which were not standard practice at the time, as well as poor understanding of the biological nature of phage and lysogeny.
- Lysogenic phage are now known to be unsuitable for phage therapy, due to their ability to confer virulence and antibiotic resistance to bacterial hosts via phage-mediated gene transfer and their ability to remain in a dormant prophage state. Examples of virulence factors encoded by bacteriophage genes include the toxins produced by *Corynebacterium diphtheriae*, *Vibrio cholerae* and *Salmonella enterica*.

2. Phage biocontrol

- A growing focus of phage-related research is in the area of 'phage biocontrol', which is the term used to describe the intentional application of hostspecific virulent phages to

environmental settings for the purpose of controlling pathogenic or spoilage bacteria.

- Phage biocontrol applications have been investigated for areas as diverse as food safety, agriculture, aquaculture and wastewater treatment. The following sections will review the research in these areas, with a primary focus on phage biocontrol in the food industry.

3. Phage biocontrol in the food industry

- Foodborne illness is a major cause of morbidity and mortality worldwide. Food production methods are constantly evolving to accommodate growing populations and consumer demands. Globalisation has ensured that consumers are able to access traditionally 'seasonal' produce all year round, as well as increasing the availability of imported exotic foods. In addition to these benefits, globalisation and mass production of food have contributed to the increased risk of food contamination and the potential for large-scale foodborne illness outbreaks.

4. Biosanitation

Food preservation has always been a necessary part of food production. Regardless of current preservation techniques and the fact that increased research has led to a greater understanding of how microbial food spoilage occurs, large quantities of foods produced globally each year are lost to microbial spoilage[10]. In the area of fruits and vegetables[17] demonstrated in field experiments the successful use of phages to control tomato bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria.* The application of six different species-specific phages at 10^{10} PFU/ml significantly reduced the bacterial spot severity in tomatoes artificially contaminated with 10^8 -CFU *X. campestris* pv. *vesicatoria* by aerosolized spray. The authors found that the phage-treated fruit resulted in an increased amount of marketable produce in comparison to the control fruits. In a similar study, Flaherty *et al.*[6] found that bacteriophages decreased the severity of the *X. campestris* disease by 17.5% in the autumn of 1997 and 16.8% in the autumn of 1998 when compared to the untreated control. *Streptomyces scabies* is a common contaminant of seed potato, and the efficacy of a polyvalent phage to sterilize *Streptomyces*-infected seed potato tubers was assessed by McKenna *et al.*[14]. Artificially infected seeds were bathed in a 10^9 -PFU/ml phage suspension for 24 h, and it was found that the number of scab lesions on the mother tubers treated with the phage was significantly reduced ($p < 0.05$) compared with the controls. Also, there was a notable decrease ($p < 0.05$) in lesions on progeny tubers resulting from phage treatment. It is evident from the above examples that bacteriophages have a promising role to play as biosanitizing agents in the food industry.

Criteria for phage biocontrol in foods

Phage bio control has been done in liquids and usually with a high concentration of pure target bacteria[11]. In liquid environments, thermal motion-driven particle diffusion and mixing due to either fluid flow or active swimming (bacterial motility) increase the likelihood of phages to encounter and infect susceptible host bacteria.

When it comes to food applications, one might face two major obstacles. First, a significant portion of targeted foods is solid rather than liquid in nature. Second, bacterial contam- ination would likely occur at very low numbers due to the expected high hygiene standards in place[11]. So, a high number of phages is required (threshold of approximately 1 X 10^8 plaque-forming units (PFU)/ml) to ensure sufficiently rapid contact with and infection of the few targeted bacterial cells present.

In other words, low numbers of phages are unlikely to infect low numbers of bacteria simply because phages and bacteria are unlikely to come into contact with each other. The bacterial host concentration is not a limiting factor if the critical concentration of phage numbers is reached and is able to cover the entire available surface of the targeted food matrix[13].

Characteristics for its application in food preservation

1. **Phage should be strictly lytic:**

- Only virulent phages should be used as biocontrol agents in foods.
- Temperate phages are not desirable because they may contain within their genome particular genes that may change the phenotype of their bacterial host (lysogenic conversion genes: LCG).
- Phage conversion may result in phage-encoded genes converting their bacterial host from a non-pathogenic strain to virulent strain. Through lysogenic conversion, temperate phages have played a major role in the evolution of new human foodborne pathogens.
- Virulence genes in several foodborne pathogen species are commonly found in bacteriophages and bacteriophage remnants.

2. **Phage should have a broad host range**
    - The high specificity of phages makes them ideal biocontrol agents as they do not result in the eradication of useful microbiota like many other antimicrobials.
    - A phage with a broad host range capable of infecting many strains of the target species and/or genus is desirable for biocontrol applications in food.
3. **Phages should have known genome sequences**

    The complete and annotated genome sequence of phages intended for use as a biocontrol agent in food will provide information on whether or not the phage encodes any proteins that presents a potential health risk.

4. **oral feeding studies of phages should show no adverse effects**
    - All available evidence indicates that oral consumption of phages (even at high levels) is entirely harmless to humans.
    - Safety studies of *Listeria* phage P100, in which rats were fed high doses of phages with no measurable effects compared to control group [39]. A study with *E. coli* phages both in mice and human volunteers showed no significant effects on test subjects
5. **Phage preparation should be stable over storage and application**
    - Physiochemical conditions (e.g., pH and water activity) of the food to which the phages are applied may affect the stability of phages.
    - It is important that the phages remain stable under these conditions for them to be successful in the biocontrol of foodborne pathogens on the foods to which they are applied. Most phages are stable at pH range between 4 and 10.
6. **Phage should be propagated on non-pathogenic host**
        - If the host employed for propagation is non-pathogenic or even having a Generally Recognized as Safe (GRAS) status, then the phenomenon of generalized transduction is unproblematic.
        - In addition, a non-pathogenic host renders large scale propagation and purification of the phage easier..

Immobilization of phages by:

- Encapsulation and incorporation into a capsule or tablet

Phage applications in meat

- Bacteriophages can be used for controlling pathogens on meat and poultry carcasses in an industrial setup.
- Phages were used to control

Camplylobacter jejuni contamination on the surface of chicken skin. The application resulted in a 1–1.3-log reduction in *Camplylobacter jejuni* within 24 hours[2].

- O'Flynn *et al.*[7] evaluated the use of a three-phage mixture to reduce *E. coli* O157:H7 on beef. It was also effective in eliminating the pathogen.

Phage applications on fresh fruits and vegetables

- Fresh fruits and vegetables have become responsible for many foodborne illness.
- Between 1990 and 2003, there were at least 554 foodborne outbreaks associated with vegetables, and these outbreaks resulted in approximately 28,000 illnesses and several deaths.
- Furthermore, organic farming practices suggests use of natural antimicrobial agents in production, which makes phages an excellent choice as a biocontrol approach.

Phage applications on processed foods

- Several foodborne outbreaks have been associated with various processed foods, including a variety of ready to eat foods, cheese and milk.
- Modi *et al*.[15] assessed the ability of a single bacteriophage SJ2 against *Salmonella enterica* during the manufacture, ripening, and storage of cheddar cheese made from raw and pasteurized milk. They found the complete absence of pathogen after 89 days storage at 8˚C.

Advantages and possible disadvantages of phage application in foods

Advantages	Disadvantage
Highly specific, natural, low-cost, minimal disruption to normal micro flora if applied to animals	Narrow host range
Ability to replicate at the site of infection	Negative consumer perception regarding the use of the terminology "viruses" or "virulent phages" in foods
Do not affect the taste, texture, smell and color of food	Required large number of target bacteria.
Effective in elimination of biofilms	Phage resistant bacterial mutants.
Used as delivery vehicles and pathogen detection systems.	
Cost effective method	

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