Control of food-borne pathogens growth using bacteriophage

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Bacteriophages (also called 'phage') were first time isolated in early 1900's. Until late 1930s, they were used to treat infectious diseases in different parts of the world. Improper use of phages and understandable formulations of phage particles reduced the use of phage therapy association with discovering the antibiotics. In recent years, antibiotic resistance is widespread in the world. Also, an increasing resistance to multiple antibiotics has been noted. Phage therapy has again taken into consideration due to the alarming spread of antibiotic resistance. The phage treatment is a new and effective hurdle to control food-borne pathogens including *Listeria monocytogenes, Campylobacter jejuni, Escherichia coli* O157: H7, *Staphylococcus aureus*, and *Salmonella* spp. Food-borne diseases are among the most serious and costly public health concerns worldwide. In despite of good manufacturing, quality control, and hygiene-safety concepts, food-borne illnesses still increased over the past decade. Among food preservation technologies such as physical treatments (heat, pressure, UV, and pulsed light) or chemical sanitizers, phage application has been gained particular interest. One advantage of the phages is that bacterial populations can be controlled selectively in the complex food systems. Phages can be very host specific and also are common in the environment and present in many foods. Phage treatments on food to control of pathogens are to extend shelf-life, to enhance hygienic quality, minimizing the impact on the nutritional and organoleptic properties of perishable food products as they are natural, non-toxic and specific to the bacterial species they infect. Many studies have described the phage biocontrol of these pathogens in a range of foods. This review is a summary of the phage-based strategies for control of food-borne pathogens.

Keywords: biocontrol; food-borne pathogens; phage

1. Introduction

Bacteriophages (also called 'phage') are viruses that specifically infect bacteria. They can be found in every environment where their bacterial hosts are present. They were first discovered independently by Frederick Twort in 1915 and by Felix d'Herelle in 1917 [1, 2, 3]. The global phage population is estimated to be more than 10^{30} [4]. A phage can only attach to a bacterial cell with a specific cell surface receptor. Once attaching to a susceptible cell, phage may undergo one of the two different life cycles (virulent or temperent) [5]. While virulent or lytic phage will enter the cell and then hundreds of copies of the original phage are released, temperate or lysogenic phages do not lyse the host cell and their DNA integrates into the genome of the bacterial cell. Temperate phages can undergo lytic cycles and pick up bacterial host genes including virulence genes and antibiotic resistance genes, so they transfer them to a new bacterial host. For this reason, temperate phages are not selected for phage therapy. Due to their remarkable antibacterial activity, virulent phages have been used as biological tools in humans, animals and plants, particularly against multidrug-resistant bacteria [6, 7].

The food industry has interest in finding alternative approaches to inactivate bacterial pathogens. The use of phage to control pathogens in foods has emerged as a promising tool for food safety in all stages of the food production chain (from farm to folk). Phages may be suitable: i) to prevent or reduce colonization and diseases in livestock (phage therapy), ii) to decontaminate carcasses and other raw products, iii) to disinfect equipment and contact surfaces (phage biosanitation and biocontrol), iv) to extend the shelf life of perishable manufactured foods as natural preservatives (biopreservation), and v) to improve strains used for production, and strain typing. Phages should also be considered in hurdle technology in combination with different preservation methods [8]. In comparison to biocontrol, application of phages for improvement and selection of industrial strains has been poorly described [9].

Phage therapy was first developed early in the last century and showed much promise. One of the most important problems in the phage therapy is the emergence of resistant bacterial strains. These bacteria have always been perceived as a potential obstacle. Most phage resistant cells exhibit changes in their membrane components responsible for specific phage binding. Alteration or deletion of phage receptors from the cell surface protects bacteria from phage attack [10]. In addition, phage preparation can readily be modified in response to changes in bacterial pathogen populations [11]. Development of bacterial resistance and the modification of phage preparations can be managed by using phage cocktails with different combinations of phages that target different receptors on the host bacteria [12].

Phages have been isolated from a wide range of foods like ground beef, pork, chicken and other meat products, chilled and frozen crabmeat, fermented dairy products like cheese and yogurt, and from lettuce and mushrooms. Briefly, phages can be considered a part of the natural microflora of foods [13].

In this review, we aimed to discuss phage application and summarize the current literature on phage based biocontrol to reduce food-borne pathogens in food. In addition, we focused on reducing *Listeria monocytogenes,*

Campylobacter jejuni, Escherichia coli O157:H7, *Salmonella* spp. and *Staphylococcus aureus* on food via phage application.

2. Application of phage in food system

The ideal phages for use as biocontrol agents on pathogenic bacteria must meet some criteria before being considered as suitable candidates. These are: i) their ability to infect specific bacterial target cells and their ability to infect eukaryotic cells, ii) phages generally do not cross bacterial species or genus barrier, and therefore do not affect desirable microorganisms commonly present in foods, gastrointestinal tract or the normal bacterial microbiota, iii) Producing progeny phage without the capacity to integrate into the bacterial genome or transduce bacterial genes from one cell to another, iv) not including virulence genes and antibiotic resistance genes, v) having a broad host range, vi) determined the complete genome sequence of phages, vi) phages being stable over storage and application, vii) phages being amenable to scale up for commercial production, viii) oral feeding studies show no adverse effect, ix) the generally recognized as safe (GRAS) approval for use in foods [13, 14, 15, 16]. Phages should also possess physical characteristics. The stability of phages in foods is important because they may need to be stable under physiochemical conditions of the food to which they are applied. These conditions should be food pH, ion content and osmolarity, thermo tolerance, visible and UV light, osmotic shock, pressure, and processing environments [17].

Inoculum size, timing, phage host range, phage adsorption rate, burst size and density of target bacteria are key elements in the success of phage therapy against food-borne pathogens. There are two ways of bacterial reduction of phages. Passive reduction refers to the reduction of bacteria by the initial phage dose. Therefore, it implies a high number of applied viruses per bacterial cell. In contrast, active reduction can take place with a lower initial dose when phages reach sufficient numbers for bacterial reduction by replication. There are some challenges of phage application on pathogen bacteria. Firstly, a threshold density of bacteria is necessary. It is hard to estimate the time when threshold levels are met. Another challenge is that phages are highly specific for the certain host. Very few phages are able to infect different species, and the host range of most of them includes just a number of strains of one bacterial species [12, 18, 19].

Most phage application studies on food have focused on main emergent food-borne pathogens, such as *L. monocytogenes, C. jejuni, E. coli* O157:H7, *S. aureus*, and *Salmonella* spp. in meats, fruits, vegetables, dairy products and ready-to-eat foods. Contaminating bacteria can get access to food during slaughtering, milking, fermentation, processing, storage or packaging [20]. Bacterial reduction from 0.9 to 6.8 $\log_{10}$ CFU or even their complete elimination in many studies was reported [1]. Phage treatment may also help prevent an incidence of food-borne diseases, reducing food processing (e.g. temperature application) and use of chemical additives (e.g. sulphite and nitrate) [8].

Phage biocontrol has been shown to be more effective in liquid foods than in solid foods [8, 21]. In liquids, even a very small initial number of phages can cause completely lysis of the bacteria in a relatively short time [22]. On the other hand, on solid foods diffusion is limited. Also a greater concentration of phages may be necessary to achieve the same result as in liquid foods. As a conclusion, the concentration of the phages must be sufficiently high to enable contact and subsequent infection, even when bacteria are present at very low numbers. However, there are also some other problems to inhibit food-borne pathogens via phages. The most relevant is the food-matrix [22, 23]. This is a decisive parameter which physically limits the distribution of phage particles in order to reach all targeted bacteria. Moreover, targeted bacteria may be embedded within the rather complex food matrix, thereby shielding them from phage particles. On these grounds, a greater biocontrol effect may be achieved by modifying phage application [21, 23]. It has been suggested that phages can be added by dipping or spraying or as a liquid to a large volume of food. These methods may not be ideal, as they could be wasteful and lead to the potential inactivation of the phage particles. Moreover, when phages are added directly to a batch of food, two major problems may be occurred: 1) the dilution of phages, and 2) the evaluation of bacterial resistance. To overcome of these problems, the addiction of large numbers and volumes of phages using phage cocktails and the regular disinfection of the equipment using effective protocols might help. In addition, immobilized phage may also help to solve these problems [24].

Although phages represent a novel approach, there are no reports of their industrial use to improve safety, even if this "new, ecological and safety" technology may be cheaper than older technologies, since phages can be isolated from the environment and are self-replicating entities [23]. There are several phage preparations commercialized and marketed so far, such as ListShield™ LMP102 (Intralytix, USA), EcoShields™ (Intralytix, USA), SalmoFresh™ (Intralytix, USA), and Listex™ P100 (Micreos Food Safety, The Netherlands) [22]. The first formal approval of a phage–based preparation developed for food safety came during August 2006, when The Food and Drug Administration (FDA) cleared ListShield™ LMP102 for use an antimicrobial agent against *L. monocytogenes* contamination of ready to eat foods [25]. ListShield™ LMP-102 containing a cocktail of six phages is also used for surfaces in food production facilities [26]. Shortly after that approval, during October 2006, a single phage containing preparation (designated Listex™ P100) was approved by FDA for another *L. monocytogenes*-specific phage [4, 25, 27, 28]. The FDA issued a 'no abjection' letter for GRAS designation for both of two commercial anti-listerial phages. Also afterwards, in February 2013, SalmoFresh™ was also approved by as GRAS to control *Salmonella enterica* by the FDA and US

Department of Agriculture (USDA) [28]. Finally, EcoShield™ (ECP-100) is a FDA-cleared commercial phage cocktail of three bacteriophages. It is used to eliminate or reduce food contamination of *E. coli* O157:H7 [3].

2.1 Phage to control *Esherichia coli* O157:H7 contamination

E. coli serogroup O157:H7 known to cause bloody diarrhea and hemolytic uremic syndrome in humans was identified in 1983 [10, 29]. It is a highly virulent food-borne pathogen with an infective dose of ~ 100 cells in humans [30]. Outbreaks have been attributed to food, water, and person-to-person and direct fecal contact. They can be isolated from many foods of animal origin [21]. More than 60 phages specific *E. coli* O157:H7 have been reported. The commercial EcoShield™ is based on ECP-100 and contains a phage concentration of at least 10^{11} PFU/mL [31]. This phage preparation was approval by FDA as commercial phage cocktail of three bacteriophages to eliminate or reduce food contamination of *E. coli* O157:H7 [3].

Phages DT1 and DT6, either alone or mixed in a cocktail, were evaluated for their efficiency to inhibit the growth of *E. coli* strains during milk fermentation by Tomat et al. [16]. In absence of phages, bacterial strain reached 4-6 $\log_{10}$ CFU/mL at 5-6 h. *E. coli* DH5α and O157:H7 STEC strains were rapidly and completely inactivated by phage DT1, while 0157:H7 STEC was completely inactivated either by DT1 or by DT6 after 8 h. By the way, pH values evolved falling to 4.5 at 8 h and 4.0 at the end of the fermentation process. All phage titres excluding the phage cocktail constant or increased slightly throughout the first 8 h, with a subsequent decrease between 8 and 24 h. The authors have said that the low pH and accumulated lactic acid might be related the partial phage inactivation.

Mclean et al. [21] also studied that three phages were investigated for their ability to inhibit the growth of three strains of *E. coli* in ultra-high-temperature treated and raw bovine milk. A cocktail of the three phages completely inhibited *E. coli* ATCC 25922, *E. coli* O5: H- and *E. coli* O127:H6 in UHT milk at 25 °C. Similar results were obtained from raw milk. No re-growth of bacterial strains was observed in any of the phage treated milk samples. Under refrigeration temperatures, the phage cocktails inhibited all three strains of *E. coli* to below the level of detection within 24 h of incubation. Furthermore, phage titers did not fluctuate by more than 1 $\log_{10}$ PFU/mL. The phage cocktail containing two phages eliminated ATCC 25922 and O127:H6 in raw milk within 3 h and 6 h of incubation, respectively, at 25 °C and in refrigerated samples. Furthermore, phage titers did not fluctuate by more than 0.5 $\log_{10}$ PFU/mL. In contrast, the phage cocktails completely inhibited O5: H- in UHT milk at both temperatures. In raw milk, this phage cocktails initially inhibited growth of O5: H- but re-growth occurred for 9 h at 25 °C. The researchers have demonstrated that differences in milk composition (proteins or oil globules), microbiota, and lower target cell and phage concentrations are effective on phages against O5: H-.

Tomat et al. [23] evaluated that the reduction of viable cells of *E. coli* O157:H7, and enteropathogenic *E. coli* strains on meat after exposure to phage applications at 5 °C and 24 °C for 3, 6, and 24 h. Two phages (DT1 and DT6) were selected. When enteropathogenic *E. coli* and O157:H7 strains were tested, viable cell reduction of 0.67 $\log_{10}$ and 0.77 $\log_{10}$ after 3 h incubation and 0.80 $\log_{10}$ and 1.15 $\log_{10}$ after 6 h. They implied that higher reductions were observed at higher temperature, probably due to the active growth of bacteria allowing an efficient phage replication. In addition, the phage cocktail at a higher number of different phages was able to of further reduce viable cell counts of O157:H7 than individual phages on meat products.

The ECP-100 was examined for its ability to reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef by Abuladze et al. [27]. Treatments (5 min) of the contaminated hard matrices with the ECP-100 preparation containing different concentrations of phages (10^{10}, 10^{9}, 10^{8} PFU/mL) resulted in significant reduction of 99.99%, 98%, and 94%, respectively. Samples of broccoli, tomato, and spinach, and also ground beef samples were contaminated with 710, 650, 14000 and 3400 CFU of O157:H7/g, respectively. The observed reduction ranged from 94% (at 120 h post-treatment tomato samples) to 100% (at 24 h post-treatment of spinach samples). The authors found that naturally occurring phages may be useful for reducing contamination of various food samples by O157:H7.

O'Flynn et al. [29] were identified that two distinct lytic phages and a cocktail of three phages were evaluated to lyse the E. coli O157:H7. Phages resulted in a 5 $\log_{10}$ unit reduction of pathogen numbers in 1 h at 37 °C. In an initial meat trial experiment, the phage cocktail completely eliminated O157:H7 from the beef surface. Similarly, single phage application caused a 3 $\log_{10}$ unit reduction in the number of viable cells within 2 h at 37 °C. When incubated at 37 °C for all phages when they applied individually or as a cocktail, the culture started to grow again within 2 to 3 h. But, this re-growth was not observed for incubation at 30 °C.

Sharma et al. [32] determined that the efficacy a mixture of three O157:H7 specific phages (ECP-100) in reducing the number of viable *E. coli* O157:H7 on contaminated fresh cut iceberg lettuce and cantaloupe. Bacterial strain was spot inoculated on lettuce pieces with a population of 3.76 $\log_{10}$ CFU/ cm^2, allowed to dry, and then sprayed ECP-100 to deliver 7.98 $\log_{10}$ PFU/cm^2 to lettuce stored for 2 days at 4 °C. Spraying reduced the number of viable of O157:H7 on fresh cut lettuce. Furthermore, bactericidal effect occurred very quickly after spraying. Cut pieces of cantaloupe were also spot inoculated with O157:H7 (4.55 $\log_{10}$ CFU/mL) and treated with ECP-100 (6.69 $\log_{10}$ PFU/mL) and then stored at 4 °C or 20 °C for up to 7 days. Populations of O157:H7 on lettuce treated with ECP-100 on 0, 1, and 2 days (0.72, <0.22, and 0.58 $\log_{10}$ CFU/cm^2 of lettuce) were significantly lower than those treated with the control (2.64, 1.79, and 2.22 $\log_{10}$ CFU/cm^2), respectively. Populations on cut cantaloupes treated with ECP-100 on days 2, 5, and 7 (0.77,

1.28, and 0.96 $\log_{10}$ CFU/mL) and then stored at 4 °C were significantly lower than those treated with the control (3.34, 3.23, and 4.09 $\log_{10}$ CFU/mL), respectively.

2.2 Phage to control *Salmonella* contamination

Salmonella is a gram negative, rod-shaped bacterium. Salmonellosis is one of the most commonly reported zoonotic diseases in many countries [13]. Animal-derived food, including eggs, egg products and raw or undercooked meats are the principal source of infection. Vegetables products are also been reported [1]. Although more than 2500 serovars of *Salmonella* enterica have been identified, the human infections are caused by a limited number of serovars *S.* enteritidis, *S.* newport and *S.* typhimurium belong to the most common serovars [33, 34]. According to the most recent report, approximately 1.4 million infections per year are estimated, resulting in 400-600 deaths. Therefore, the development and evaluation of new strategies for the control of *Salmonella* are urgently needed [13, 35]. Apart from the traditional use of heat and chemicals, many emerging technologies, phages were reported to be successful against *Salmonella* [33, 35]. Among the phages to inhibit *Salmonella enterica*, SalmoFresh™ and SalmoLyse™ were approved by as GRAS by the FDA and US Department of Agriculture (USDA) [28]. SalmoFresh™ is prepared with a cocktail of naturally occurring lytic phages and inhibited *Salmonella* including strains belonging to the most common pathogenic serotypes: Typhimurium, Enteriditis, Heidelberg, Newport, Hadar, Kentuck and Thomson. SalmoLyse™ is a reformulated phage cocktail derived from SalmoFresh™ in which two of six phages in the original cocktail have been replaced [31]. Most of the many studies have focused on the use of phage to reduce carriage of *Salmonella* in poultry rather than as a food additive [36].

Galarce et al. [1] evaluated the effectiveness of five phages applied as a cocktail to reduce the counts of *S. enterica* serotype enteritidis (SE) in the two types of processed meat products. Each sample was contaminated with SE, treated with a phage cocktail and then incubated for ten days at 18 °C and 4 °C. A significant reduction in bacteria was obtained on days 3, 6 and 10 incubated at 18 °C (from 0.48 to 2.12 $\log_{10}$ CFU/g) and at 4 °C (from 0.23 to 2.06 $\log_{10}$ CFU/g). They implied that the bacterial reduction obtained in their study was lower using the same phage cocktail in fresh chicken and turkey breast. Industrial processing of the food, changes the chemical composition, moisture, water activity and pH, bacterial growth and phage activity are responsible for these differences.

Guenther et al. [13] evaluated the reduction of *S.* Typhimurium in different ready-to eat-foods by virulent phage FO1-E2. Samples were inoculated with 10^3 CFU/g *Salmonella* cells and treated with 10^8 PFU/g phage and incubated for 6 days at 8 °C or 15 °C. At 8 °C, no viable cells remained following phage application, corresponding to a more than 3 $\log_{10}$ unit reduction. At 15 °C, application of phage lowered *S.* Typhimurium counts by 5 $\log_{10}$ units on turkey deli meat and in chocolate milk and by 3 $\log_{10}$ units on hot dogs and in seafood. They observed that phage resistant *Salmonella* strains appeared at the end of the incubation period. They stressed the environmental condition with an incubation temperature of 15 °C.

Hungaro et al. [34] studied that five phages with inoculating in chicken skin used to reduce *S.* enteritidis at 37 °C and 25 °C. The chicken skin sections exhibited natural contamination of approximately 10^5 CFU/cm^2 of *S.* enteritidis. All five phages were able to reduce *S.* enteritidis growth at 10^9 PFU/mL in both temperatures evaluated. The phage cocktail at 10^9 PFU/mL applied to chicken skin for 30 min achieved significantly reduction in *S.* enteritidis counts. When *S.* enteritidis was challenged with phages at 10^3 PFU/mL and 10^6 PFU/mL, no reduction in bacterial growth were observed. They demonstrated that bacterial growth inhibition was clearly phage concentration-dependent.

Four phage treatments were used against *Salmonella* from poultry carcass rinse samples by Higgins et al. [37]. In the first two experiments, the highest concentration of phage (10^{10} PFU/mL) applied significantly reduced *S.* enteritidis recovery between 50 and 100% as compared with the control rinse water sample. In the experiments 3 and 4, broiler carcasses were inoculated with 31CFU/g *S.* enteritidis, sprayed with selected concentrations of phage. Similarly results were found that application of 10^8 or 10^{10} PFU/mL phage reduced the frequency of pathogen counts. They suggest that the treatment with large number of phage is desirable.

The effectiveness of a phage cocktail was determined in four different food matrices (pig skin, chicken breast, fresh eggs and packaged lettuce) contaminated with *S.* typhimurium and *S.*enteritidis by Spricigo et al. [38]. A significant bacterial reduction (>4 and 2 $\log_{10}$/cm^2) was obtained in pig skin sprayed with the phage cocktail and then incubated at 33 °C for 3 and 6 h. These times were chosen because both of them are the duration of the pre-slaughter withdrawal period. Chicken breast were dipped for 5 min in a solution containing the phage cocktail and then maintained for 7 days at 4 °C. By day 7 of treatment, *S.* typhimurium concentration was 2.2 $\log_{10}$ CFU/g lower than the initial concentration whereas the reduction in *S.* enteritidis counts was greatest on day 5 post-treatment. Only a minor reduction of the bacterial concentration (0.9 $\log_{10}$ CFU/cm^2 in the concentration of both *Salmonella* serovars) was achieved in fresh eggs sprayed with the phage cocktail and then incubated at 25 °C for 2 h. Likewise, there was a reduction of the *Salmonella* concentration in lettuce. The cocktail was more effective in *S.* typhimurium, with a decrease of 3.4 and 3.9 $\log_{10}$ CFU/g after 30 and 60 min than in *S.* enteritidis, in which a significant reduction of 1.9 and 2.2 $\log_{10}$ CFU/g after 30 and 60 min of treatment respectively was observed.

Wong et al. [39] showed that inoculation of 10^{12} PFU/mL of the phage in the chickens challenged with 10^{10} CFU/mL of *S.* typhimurium was able to reduce the *S.* typhimurium. The *Salmonella* count reduced to 2.9 log10 CFU/mL within 6 h of post-challenge. Zinno et al., (2014) focused on the use of phage P22 to eradicate *S.* typhimurium

in different foods. Bacterial growth was monitored for 24 and 48 h at 4 °C. The cell loads at the initial time of in different treated foods did not show significant differences. On the contrary, significant difference in the mean value of viable count at 24 and 48 h was shown. When 10^4 CFU/g host inoculums were used, 2 - 3 $\log_{10}$ bacterial inactivations were detected in all food matrices after 48 h. Their results also showed that in liquid foods there was a significant reduction of the bacterial population after 48 h, while in other foods the cell loads were not affected. They emphasized that phage application was not enough to inactivate the entire population of pathogen bacteria. So, combination of phage with other natural antimicrobials such as bacteriocins or essential oils should be encouraged. In addition, their results indicated that phage ability against the pathogen bacteria is more effective in liquid foods than the solid.

2.3 Phage to control *Listeria monocytogenes* contamination

Listeria is ubiquitously found in the environment, and transmission usually occurs via contaminated food and water [22, 31]. Among the *Listeria* species, *L. monocytogenes* are the greatest concern, owing to grow at low temperatures, survive in high salt environments and produce biofilms. Because of these properties, it is a high risk organism for food production [40]. Listeriosis is associated with mortality rates up 30%. To date, more than 500 *Listeria* phages have been isolated and characterized. Phage Listex™ P100 has received GRAS status by FDA/USDA for use in food materials. P100 was isolated from a sewage effluent sample of a dairy processing plant in Germany [41]. In addition, the phage preparation LMP-102 has been commercialized as ListShield™ LMP-102 containing a cocktail of six phages. It has been shown to be effective against 170 different strains of *L. monocytogenes,* reducing significantly from 10 to 1000 fold [31]. It was also approved FAD and USDA for application on food and surfaces in food production facilities as microbial pesticide [42].

Soni et al. [4] studied that the influence of phage dose, phage contact time, and storage temperature on the listericidal activity of phage P100 in reducing *L. monocytogenes* loads on the surface of fresh channel catfish fillet. The fresh catfish fillet samples inoculated with ~ 4.3 $\log_{10}$ CFU/g of a two serotype mix of *L. monocytogenes* cells and then surface treated with phage P100. The effect of phage P100 concentrations of 10^3, 10^5, and 10^7 PFU/g against *L. monocytogenes* on catfish fillet pieces within 2 h at room temperature. P100 treatment of 10^7 PFU/g resulted in an average of 1.6 $\log_{10}$ CFU/g reduction in *L. monocytogenes* counts. At density of 10^5 PFU/g of phage, there was a slight reduction of 0.4 $\log_{10}$ CFU/g reduction in *L. monocytogenes* loads. But, there was no reduction in *L. monocytogenes* counts at 10^3 PFU/g of phage P100 dose. The reduction in *L. monocytogenes* counts with the phage P100 dose of $2x10^7$ PFU/g (7.3 $\log_{10}$ PFU/g) was 1.4-2.0 $\log_{10}$ CFU/g at 4 °C, 1.7-2.1 $\log_{10}$ CFU/g at 10 °C, and 1.6-2.3 $\log_{10}$ CFU/g at 22 °C on raw catfish fillet. The phage contact time of 30 min was adequate to yield greater than 1 $\log_{10}$ CFU/g reduction in *L. monocytogenes*, whereas 15 min contact time with phage yielded less than 1 $\log_{10}$ CFU/g reduction of *L. monocytogenes* loads on catfish fillet. The authors suggested that P100 titer was stable on catfish fillet samples, and the cell counts were still maintained over 10 day shelf life at 4 °C or 10 °C by P100 treatment.

Oliveira et al. [22] investigated that the efficacy of phage P100 to control *L. monocytogenes* growth on melon, pear, and apple products (juices and slices) stored at 10 °C. Phage treatment was effective on melon followed by pear, but no effect on apple products was observed. Reduction of about 1.50 and 1.00 $\log_{10}$ CFU/plug for melon and pear slices were found. In juices, higher reductions were obtained in melon (8.00 $\log_{10}$ CFU/mL) followed by pear (2.10 $\log_{10}$/mL) after 8 days of storage. The bacterial cells in apple juice was unaffected by phage treatments. Consequently, phage treatment decreases *L. monocytogenes* growth on fruit slices and juices. However, these reductions were greater in fruit juices than fruit slices. The authors said that the pH differences may be a major factor contributing to the differences in the bacterial populations on the fruit slices and juices. In addition, phages are seemingly immobilized after addition to solid surface. Therefore, they could not come into contact with the surviving bacteria through limited diffusion.

Bigot et al. [43] were aimed to control of *L. monocytogenes* on ready to eat foods. First of all, broth assays showed that A511 phages added at $5.2x10^7$ PFU/mL prevented the growth of *L. monocytogenes* (10^8 CFU/mL) at 30 ° for 7 h. In the presence of phages, there was a reduction of cell concentration after four to five hours of incubation, but re-growth occurred after 24 h. At the same temperature, but on the surface of vacuum-packed ready to eat chicken breast roll, there was an immediate 2.5 $\log_{10}$ CFU/cm^2 reduction in pathogen concentration following addition of phages and then re-growth. However, when similar experiments were performed at 5 °C, there was an initial 1.5 $\log_{10}$ CFU/cm^2 reduction in host cell concentration. In addition, re-growth of bacterial cell was prevented over 21 days incubation.

Guenther and Loessner [44] showed that two different soft-ripened cheese models (white mold and red-smear) were established in the laboratory to evaluate the potential of A511 phage for controlling *L. monocytogenes* on the cheese surfaces. The surfaces of cheese were inoculated with 10^1-10^3 CFU/cm^2 bacterial cells. Phage was applied at defined time points at thereafter, in single or repeated treatments, at 10^8 or 10^9 PFU/cm^2. With 10^3 CFU/cm^2 of bacterial cell concentrations and a single dose of A511 (10^8 PFU/cm^2) on white-mold cheese samples, viable counts dropped 2.5 logs at the end of the 21 day ripening period. Repeated phage application did not further inhibit the bacteria. A single higher dose (10^9 PFU/cm^2) was found to be more effective. On red-smear cheese ripened for 22 days, *Listeria* counts were down by more than 3 logs. Repeated application of A511 further delayed re-growth of *Listeria*, but did not affect bacterial counts after 22 days. With a lower initial *Listeria* contamination, viable counts dropped below the limit of detection.

Rossi et al. [45] investigated that *L. monocytogenes* 1/2a was inoculated in Brazilian fresh sausage ($2.1x10^4$ CFU/g) with a phage P100 added thereafter ($3x10^7$ PFU/g). Samples were analyzed immediately and then stored at 4 °C for 10 days. P100 reduced bacterial cell counts by 2.5 $\log_{10}$ units at both 0 and 10 days. In spite of this, the bacterial cells increased over the 10 day storage. This result demonstrated the psychotropic characteristic of the bacteria.

Soni et al. [46] analyzed that phage P100 was evaluated against *L. monocytogenes* cold growth in queso fresco cheese. When 9 $\log_{10}$ CFU/mL of stationary phase cells of *L. monocytogenes* exposed to P100, there was a 3 to 5 $\log_{10}$ CFU/mL reduction after 24 h at 4 °C in vitro study. In cheese samples, the bacterial population increased from the initial 3.5 $\log_{10}$ CFU/cm^2 to 7.7 $\log_{10}$ CFU/cm^2 in 28 days at 4 °C. Treatment with 7.8 $\log_{10}$ PFU/cm^2 of phage P100 showed strong listericidal effect initially by reducing *L. monocytogenes* counts by 2 to 3.5-4 $\log_{10}$ CFU/cm^2.

2.4 Phage to control *Campylobacter jejuni* contamination

Campylobacter was first described in 1880 by Theodore Escheric. These bacteria are curved, S-shaped or spiral gram negative rods, which can only multiply in warm blooded animals such as poultry, pigs, cattle and wild birds. *C. jejuni, C. coli* and *C. lari* are the most frequently reported in *Campylobacter* species [47]. In the developed world, *C. jejuni* is a leading cause of food-borne gastrointestinal disease [48]. Human infections arise from uncooked poultry meat, hand-to-mouth transfer in the kitchen, and cross-contamination of other foods [19]. A significant traceable source of human infection is poultry. Cooking is the key to eliminating the risk of *Campylobacter* enteritis from poultry dishes. To safely cook such dishes, critical core temperature of 68-70 °C must be reached and held for periods as long as 45 min which can result in unacceptable sensory characteristics. The use of organic acid was found to cause a colour change or bleaching of the lever surface [49]. More useful mechanisms to control *Campylobacter* is the use of host-specific phage [50]. *Campylobacter* phages have been isolated from several different sources such as sewage, pig and poultry manure, abattoir effluents, broiler chickens and retail poultry [51]. From the intestinal content of poultry, purified phages may be transferred to the surface of poultry meat, at slaughter. They are able to survive for more than 10 days and naturally present on foods for human consumption. *Campylobacter* specific phages offer the prospect of a sustainable measure to reduce the numbers of *Campylobacter* colonization in poultry [52]. This is promising and results have indicated *Campylobacter* reductions of up to three $\log_{10}$ units have been achieved by phage application [53]. To date, there are more than 170 phages of *Campylobacter* species reported [36]. There are only a few reports on phage biocontrol against *Campylobacter*, with all the studies being conducted on poultry [51].

El-Shibiny et al. [18] also studied the effect of phage CP220 on *C. jejuni* and *C. coli* colonized broiler chickens. A 2 $\log_{10}$ CFU/g decline in cecal *Campylobacter* counts was observed after 48 h in birds colonized with *C. jejuni* HPC5 and administered with a single 7 $\log_{10}$ PFU oral dose of CP220. To achieve a similar reduction in *Campylobacter* reduction in *C. coli* OR12-colonized birds, a 9 $\log_{10}$ PFU dose of CP220 was required.

Kittler et al. [19] used to a phage cocktail of *Campylobacter* reduction on three commercial broiler farms. One day after phage application, *Campylobacter* counts were reduced under detected limit (<50 CFU/g) in fecal samples. At slaughter, a significant reduction of >$\log_{10}$ 3.2 CFU/g cecal content was detected. They also revealed that maximum reduction of *Campylobacter* at the slaughterhouse might be achieved by phage application 1 to 4 days prior to slaughter.

Fischer et al. [47] inoculated commercial broilers with 10^4 CFU/bird of a *C. jejuni* field strain. Then, each group of birds was treated with 10^7 PFU/bird of a single phage or four phage cocktails. Finally, they demonstrated that the *Campylobacter* load was permanently reduced by the phage cocktail as well as by the single page. The reduction was reached a maximum of $\log_{10}$ 2.8 CFU/g cecal contents.

Firlieyanti et al. [49] showed that the application of broad host range phages (8 $\log_{10}$ PFU/g) to liver homogenates containing *C. jejuni* strains of diverse origin at 4 °C resulted in modest but significant reductions in the viable counts ranging from 0.2 to 0.7 $\log_{10}$ CFU/g.

Atterbury et al. [50] observed that the enumeration of campylobacters from chicken ceca in the presence of phage (mean $\log_{10}$ 5.1 CFU/g) was associated with a significant reduction in numbers compared to samples with *Campylobacter* alone (mean $\log_{10}$ 6.9 CFU/g). The detection limit was determined as $\log_{10}$ 2.0 PFU/g *Campylobacter* CFU/g of cecal contents and similarly for phage $\log_{10}$ 2.0 PFU/g of cecal contents.

Carvalho et al. [51] studied the efficacy of a phage cocktail in reducing the levels of colonization by both *C. coli* and *C. jejuni* in broiler birds. They showed that approximately 2 $\log_{10}$ CFU/g reduction of both *Campylobacter* strains was occurred. They also implied a 30 fold reduction in the incidence of campylobacteriosis associated with consumption of chicken meals could be leaded according to mathematical models.

Atterbury et al. [54] determined the efficacy of phages to reduce the number of recoverable *C. jejuni* on artificially contaminated chicken skin. A high concentration of the phage (10^7 PFU) was found more effective in reducing the recoverable *C. jejuni* in frozen chicken. The researchers in the study recommended combining freezing and phage treatment are ensured further falls in *Campylobacter* prevalence on broiler carcasses.

Carrillo et al. [55] determined that CP8 and CP34 were applied at different dosages, to 25-day-old broiler chickens experimentally colonized with the *C. jejuni* broiler isolates. Phage treatment of *C. jejuni* resulted in *Campylobacter* counts falling between 0.5 and 5 $\log_{10}$ CFU/g of cecal contents compared to untreated controls over a 5-day period post administration. The optimum dose for phage therapy was reported to be 7 $\log_{10}$ PFU, with the higher (9 $\log_{10}$ PFU) and lower doses (5 $\log_{10}$ PFU) of phage being generally less effective.

2.5 Phage to control *Staphylococcus aureus* contamination

S. aureus has been responsible for outbreaks associated with milk and dairy products. In addition, particularly these strains can able to produce heat stable enterotoxins [56]. This strain is dependable for the 1-9 % outbreaks [57]. Biocontrol of staphylococci has focused considerably on the prevention and treatment of mastitis and improvement of animal health [40].

Bueno et al. [56] studied that two *S. aureus* lytic phages were evaluated for their potential as biocontrol agents against this pathogenic bacteria in both fresh and hard-type cheeses. Pasteurized milk was contaminated with pathogen (about 10^6 CFU/mL) and a cocktail of the lytic phages (about 10^6 PFU/mL) was also added. In both types of cheeses, the presence of phages resulted in a notorious decrease of S. aureus viable counts during curdling. In fresh cheeses, a reduction of 3.83 $\log_{10}$CFU/g of *S. aureus* in 3h and viable cells were under the detection limits after 6 h. At the end of the curdling process, the pathogen strain was undetected and no re-growth was occurred during the cold storage. In hard cheeses, the presence of phages resulted in a continuous reduction of *S. aureus* counts. In curd, viable counts of *S. aureus* were reduced 4.64 $\log_{10}$ CFU/g. The authors also emphasized that starter strains were not affected by the presence of phages in the cheese making processes and cheese maintained their expected physic-chemical properties.

Garcia et al. [57] determined the ability of specific phages of S. aureus growth in curd manufacturing processes. A cocktail of two lytic phages produced a complete elimination of 3 x 10^6 CFU/mL of the pathogen in ultra high temperature whole milk at 37 °C. Furthermore, the frequency of emergence of phage insensitive mutants was reduced up to 200-fold in the presence of phage cocktail. In acid curd, the pathogen was not detected after 4 h of incubation at 25 °C, whereas pathogen clearance was achieved within 1 h of incubation at 30 °C in renneted curd. The authors indicated that even though the phage cocktail was partially inactivated by low pH, it was able to completely eradicate viable S. aureus cells in curd made of heavily contaminated milk.

Haddad et al. [58] selected two anti S. aureus phage cocktails, each containing three phages. The use of these phages did not trigger over production of S. aureus enterotoxin C. Cocktail 1 was first tested against *S. aureus* on Cheddar cheese and the latter inoculated at 10^6 CFU/mL of milk. At the initial milk maturation and coagulation steps, no significant bacterial reduction was observed. Reduction of 1 and 2 $\log_{10}$ units of the staphylococcal counts were observed from the initial step until the coagulation step. At the coagulation step, the whey removal and the cheddaring step helped increase phage efficiency and dropped the staphylococcal concentration by 1.3 and 4 $\log_{10}$ units per g of curd. After a 14 day ripening, all cheeses with added phages did not containing *S. aureus* cells. For the cocktail 2, a significant decrease in the concentration of pathogen began at maturation. Similarly to the phage cocktail 1, no cells were detected above the limit of detection of 100 CFU/mL in all cheeses with the added phage cocktail 2 after 14 days.

Tabla et al. [59] researched a cocktail of two phages performance against *S. aureus* in milk by high hydrostatic pressure (HHP) treatments. Four hundred MPa was found to be most suitable pressure to be used in combination with these phages. Two different levels of bacterial initial contamination (10^4 and 10^6 CFU/mL) were tested, and a synergistic effect between HHP and phages was observed in both cases. The combine treatment was able to reduce the initial pathogen contamination below the detection limit. The authors particularly emphasized that phages can be regarded as a valuable hurdle on minimally processed food.

3. Conclusion

Recently, there has been an increased interest in application of phage as an alternative antimicrobial chemotherapy in various fields such as human infections, food safety, agriculture and veterinary sciences. The scientific literatures demonstrate that phages have been suggested as an alternative approach to effectively reduce the amount of food-borne pathogens in food safety. It is necessary to determine the criteria for application of phages in the control of pathogen bacteria in food and therefore, all newly isolated phages with the potential application in antibacterial therapy should be characterized in detail.

Latest developments in genome sequencing and bioinformatic technologies have allowed a large number of phage studies to be carried out at the genomic level. These studies are provided further opportunities for applications in novel biocontrol agents and phage therapy. The results of such studies appear to be encouraging. But research is still needed to thoroughly because there are many questions that remain to be answered through the collection of scientific data such as phage-bacteria interactions and ecology, phage efficacy, under different environment conditions of food producing, and phage resistance.

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