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Short communication

Bacteriophages as Biocontrol Agent in Food Safety

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Abstract

Food contamination with pathogens has been continual and significant problem worldwide. These pathogens causes foodborne diseases that lead to deleterious effect to the health of consumer and the financial losses to food manufacturer. The emergence of foodborne pathogen is further complicated with the infection from multiple antibiotic resistance food pathogens and the formation of biofilm on food processing equipment as well as utensils that cause the process of decontamination become more challenging. Bacteriophage is recognized for its effectiveness as biocontrol against food pathogen and relatively harmless to man. Bacteriophage can be applied at any point of the food chain such as phage therapy of livestock, biosanitizer (disinfectant) in the food processing environment, and biocontrol (additives) of food products. Bacteriophage is an obligate parasite of bacterial and due to its host specificity and binding capability, bacteriophage appear as a more advantageous decontamination agent compared to other chemical intervention which might introduce chemical hazard. Various studies have shown the effectiveness of bacteriophage in combating food-borne pathogens and insignificant risk of development bacteriophage resistant bacteria. However the efficacy of the bacteriophages varies depending on the source of isolation, the type of food, surface materials and food matrixes. Thus, it is noteworthy to get the insight of current microbiological hazard in food and the potential use of bacteriophages as biocontrol agent.

Keywords: *Bacteriophage; pathogens; biofilm; biocontrol; food safety.*

1. Introduction

The potential of bacteriophage was forgotten since its first bactericidal discovery in 1896 due to the introduction of antibiotics which show good therapeutic efficacy and easy to administer [1, 2]. Recently, there are renewed interests in bacteriophage activities against microorganisms owing to its specificity against microbial species, relatively harmless to man, availability in environment and food [3, 4]. The increase of antibiotic resistant microbial strains, emergence of super bug together with limited and slow discovery of new antibiotics have expedite the call for alternative approach in combating these pathogens.

In 1971, six "genera" phages were issued in the first report of International Committee on Taxanomy of Viruses (ICTV) which included T-even phages, I, lipid phage M2, the fX group, "filamentous phage" and "ribophage group" thus this was regarded as the beginning of phage classification [5]. Recently, phages are classified into two major orders which are Caudovirales and Ligamenvirales, five families, nine subfamilies, 145 genera and 684 species. Siphoviridae (linear dsDNA), Myoviridae (linear dsDNA) Podoviridae (linear dsDNA) and Inoviridae (circular ssDNA) are common and widespread in nature [6, 7].

Most of the bacteriophages (> 90%) consist of large and double stranded DNA genomes which is located in icosahedral heads with varying lengths of tails. Moreover, these bacteriophages are categorized into three major groups as follows: (i) myoviridae (long, rigid, contractile tails), (ii) Siphoviridae (Long, flexible, non-contractile tails), (iii) Podoviridae (short, non-contractile tails) and the rests is variable which may have DNA or RNA genomes [8].

2. Bacteriophage as a Biocontrol against Foodborne Pathogens

There are basically four important criteria for selecting specific phages as pre-harvest and post-harvest biosanitation treatment: (i) phage cycle (lytic cycle is favorable), (ii) host range (broad host range is favorable), (iii) exploitable elements (can be used in phage cocktails and lysin application is favourable), (iv) temperature stability and effective range (wide range or within range of host pathogen is favourable) [9].

The polyvalent bacteriophages (with broadcast host range) is the best virulent phages as they can eliminate many species within a bacterial genus [10]. The mode of action of bacteriophages on pathogens or host bacterial was described as follows: (i) penetration (occurs a very short time after attachment of the virus to the receptor of the virus to the receptor of the target cell in the cell membrane), (ii) uncoating (virus capsid is removed and the genome is released as nucleoprotein complex, (iii) genome replication and gene expression (depend on the nature of genetic materials and determine the virulence of bacteriophages such as acute, chronic, persistent or latent), (iv) assembly (collect the necessary components to form mature virion, (v) maturation (virus become infectious), (vi) release (the infected cells breaks and release the virus for lytic bacteriophage; budding process for enveloped bacteriophages) [11].

Table 1: Bacteriophage can be applied as passive or active treatment to combat pathogens in food [12].

	Passive Treatment	Active Treatment
Mechanism of infection	- Primary infection (lysis from without)	- Secondary infection (replication happen within host bacterial cells)
Disadvantages	- Much more bacteriophages are required	- Phage infection might be prevented by viscous material or inert bacteria
Advantages	- Address the issue of natural resistance due to retriction enzymes in the host bacteria - Can target broad range of host bacterial due to shared attachment antigen between some bacterial taxa	- Small dose of bacteriophages required for infection

Bacteriophage can be used as a biocontrol for food because it can control pathogen of food and prevent spoilage of foods in order to extend shelf life of food [13]. For example, Li et al. [14] had demonstrated 10^6 pfu ml^{-1} of Spp001 bacteriophage was capable to control efficiently *Shewanella putrefaciens* (spoilage bacterium) in chilled fish fillet up to 14 days. The result showed the total viable counts and *S. putrefacienns* was reduced by 3.6 and 3.2 log cfu/g respectively within 4 to 6 days.

Broiler is the main reservoir for *Campylobacter* spp. that cause campylobacteriosis, a type of gastroenteritis in man. Study showed a phage cocktail applied for one day in the drinking water, the counts of *Campylobacter* was significantly reduced by > 3.2 CFU/g cecal content during slaughtering. Thus, phage application after slaughtering will help to prevent cross contamination of food is possible [15]. Besides, *Listeria monocytogenes* always caused listeriosis which is known to be a major foodborne in United States and another study demonstrated that ListShield ™ can be an effective biocontrol agent to reduce *L. monoctogenes* in ready-to-eat food such as lettuce, cheese and smoked salmon by 91, 82 and 90%, respectively without altering the organoleptic properties of foods [16]. Spricigo et al [17] had demonstrated the effectiveness of bacteriophage cocktail in combating *Salmonella* Typhimurium and *S.* Enteritidis in chicken as *Salmonella* is the second frequent reported foodborne disease. The result showed *S.* Typhimurium and *S.* Enteritidis on chicken breast meat reduced significantly (2.2 and 0.9 $\log_{10}$ cfu/g, respectively) after dipped in bacteriophage solution for 5 minutes and refrigerated at 4°C for 7 days. It is known that *E. coli* contamination is always associated with fresh produce and Sharma et al. [18] had demonstrated the effectiveness of bacteriophage in reducing contamination of fresh cut cantaloupes and lettuce by *E. coli* O157:H7.

2.1. Mechanism of Phage Infection on Bacteria

Adsorption is the key factor for host-specificity infection and protein receptors is responsible for the role of adsorption and initiation of infection. There are distinctive structures of protein receptors between Gram-positive and Gram-negative bacteria which divided into five classes of protein receptors. Then, the receptors sites are typically recognized by somatic phages such as the members from families of Myoviridae, Siphoviridae, Podoviridae and Microviridae [19]. In order for phage infection to happen, binding between tail fiber and protein receptor of host bacteria is important which will lead to change in orientation of baseplate through transmitted signal [20]. A typical bacteriophage contained an icosahedral capsid, a baseplate (situated at distal end) connected with contractile sheath (for infecting host) and tail fibers (binding with protein receptor) [21].

Phage penetration is a stage where nuclei acid injected into the host cell and this mechanism are specific for individual phage, besides, this process is influenced by electrochemical membrane potential, ATP molecule and enzymatic splitting of peptidoglycan layer [22]. For example, bacteriophage Ø29 apply a push-pull mechanism during penetration in which during the push mechanism 65% of genome is being pushed into the host cell due to the pressure developed in viral capsid while during the pull mechanism, p17 (viral early proteins that facilitate DNA replication later) pulls the remaining DNA into the host cell [23].

Before the replication of viral DNA in host cell, the phosphodiester bond between the neighbouring base on each strand is broken before the hydrogen bonds between complementary DNA strands is disrupted followed by melting of the terminal hydrogen bonds of double-stranded DNA resulting in a single stranded DNA templates that can be formed for the subsequent step of replication mechanism which are different for individual phage [24].

2.2. Lytic and Lysogenic Bacteriophages

There are two possible life cycle encountered by bacteriophages: the lysogenic and lytic. After adsorption of bacteriophage onto the surface of host bacterial, the lytic bacteriophage control the biochemical machinery of the host cell immediately for replication of virions and the cycles will be ended by destroying the host cell. For the temperate bacteriophage (lysogenic cycle) incorporate their genome into the chromosome of the host cell and it will remain there in dormant condition. However, the lysogenic bacteriophage is possible to be initiated into lytic cycle when it is exposed to particular stimuli [25]. Besides, bacteriophages might encounter another two life cycles known as pseudolysogenic and chronic life cycle [26].

For pseudolysogenic, it is a stage where the bacteriophage was carried along with the host bacteria and express itself neither lysogeny nor lytic phages and this scenario was discovered when cell lysis by phages delayed during host's starvation [27]. The benefits for this type of life cycle is that the bacterial host can be a protective layer for the phages as the DNA of phages can be shielded from harsh condition and pseudolysogen can prevent themselves being dependent on the host's DNA and thus chances for induction of prophages would be higher [27, 28].

In contrast, the chronic life cycle of bacteriophages happened when phages can be maintained and persistent in rich nutrient condition and the progeny are constantly budded off the cell or passed down to daughter cells asymmetrically after division and this situation also known as host-resistance mechanism [29, 30].

2.3. Persistence and Survival of Bacteriophages in Food

In the late 1970s, viruses were described as abundant substances in the sea and a study discovered that one milliliter of sea water contains millions of virus by using transmission electron microscopy [31, 32, 33].

The salt concentration of the solution is one of the factors that affect the stability or prevalence of the bacteriophage in certain environment, for example, when Salmonelex™ bacteriophages were transferred to filter water could render inactivation of the bacteriophages because the electrostatic pressure in the viral capsid which is used to inject genetic information into the host is affected by the salt concentration [34].

Temperature also plays a vital role in the attachment, penetration, multiplication and latent period of phages [35]. Study discovered low temperature (15^{o}C) impaired investigation on the multiplication of *Aeromonas hydrophila* phages isolated from a river water at high temperature. It was found high temperature phages have a very long latent period and reduced adsorption when investigation being done at low temperature [36]. Bacteriophage should work best in the food matrices with the incubation temperature which achieved more with their optimum growth temperature, as study demonstrated application of bacteriophage VPp1 in depuration of oysters and the result showed that at 16^{o}C, *V. parahaemolyticus* in oysters achieved greater reduction (2-3 log CFU/g) compared that in 12^{o}C (1-2 log CFU/g) [37].

Lu et al. [38] investigated bacteriophage from in vegetable fermentation (acidic condition) and the results showed that the phages isolates were very stable after fermentation of 60 days (pH < 3.5) and the phages were found to be members of Myoviridae or Siphoviridae family. pH can be a factor that might affect the effectiveness of bacteriophage in the food matrix as study reported that bacteriophage (Listex P100) was unable to reduce the population of *Listeria monocytogenes* in the apple juice (acidic condition) and moreover, the phage titer was reduced over the incubation period [39].

Moisture in food is another factor that determine the persistence of bacteriophage particularly in food. Dry cooked meat samples treated with phages was found not significantly different from untreated group. It was thought dry food samples limit the diffusion of phages in the samples to infect the microorganism [40].

Detergent (chemical) is always considered included for disinfection in food production and it is a major continuous process, however, addition of SDS reduced phage infectivity of P100 by 1-2 $\log_{10}$ unit with 1 day [41]. A study showed that even 1 % of SDS imposed a negative impact on the infectivity, survival and replication of Vibrio bacteriophages [42].

2.4. Phage Bacteriolytic Enzymes

Bacteriophages are equipped with various virion-associated carbohydrate active enzymes (polysaccharide depolymerases and lysins) which are useful to degrade carbohydrate barriers of host cell after successful receptor recognition and adsorption to the cell surface of host bacteria [43]. Phage-encoded enzymes (lysin) is used to digest the cell wall of host bacterial for releasing phage progeny and the enzymes was discovered as effective antibacterials particularly Gram-positive bacteria [44]. Basically, phage-encoded enzymes also known as enzybiotic can be classified into two classes which are endolysins and virion associated hydrolase [45]. An effective endolysin should able to perform two basic functions: (i) substrate recognition, (ii) enzymatic hydrolysis and their action is regulated by holins (hydrophobic protein encoded by phage). Besides, the cell wall binding domains of endolysins is involved for the substrate specificities as the N-terminal target the possible bond in peptidoglycan network in order to achieve sufficient substrate affinity while C-terminal perform the enzymes on the substrate [46].

Although research about endolysin in food application is still at early stage, the capability of endolysin to kill zoonotic and foodborne pathogens has been reported in a few studies. Moreover, there is no sign of resistance to endolysins has been reported [47]. Bacteriolytic enzyme had been applied in the food industry to control microorganism. For instance, the purified endolysin gene (lysH5) was able to destroy *Staphylococcus aureus* growing in pasteurized milk and resulted in the removal of *S. aureus* after 4 hours of incubation at 37^{o}C [48]. Besides, Zimmer et al [49] had discovered that *Clostridium perfringens* bacteriophage Φ3626 which consist of holing gene and endolysin gene that able to lyse all 48 tested strains of *C. perfringens*. However, the effects of the released enzymes towards food environment and intestines of poultry required further study as *C. perfringens* is a common anaerobic spore forming pathogens in food and feed [49].

2.5. In Vivo Safety and Efficacy Studies of Bacteriophages

It is indeed there were some challenges or difficulties faced by early phage study such as insufficient scientific proof of efficacy, narrow host range, impurity of phage preparation, phage resistance and phage inactivation [50]. Recently, there were studies demonstrated some measures to address these issues. In-vitro testing results on bacteriophages will normally further evaluated with in vivo testing in order to provide sufficient data on the efficacy of bacteriophages. A study on bacteriophage used to treat strain of *Pseudomonas aeruginosa* has tested nine bacteriophages using a mouse lung infection model. Seven out of nine bacteriophages showed correlation between in-vitro and in-vivo activities [51]. Besides, some studies have been done for discovery and development of polyvalent bacteriophages which are capable to infect more than one host such as demonstrated by Parra and Robeson [52]. They isolated novel bacteriophages that targeted *Salmonella enterica* serovar Choleraesuis which are capable of infecting other strains of *E. coli* and *S. enterica* [52].

Besides, encapsulation was developed as an ideal protection phages in order to improve the viability of bacteriophages and delivery to the intestinal tract [53]. For example, a study about oral delivery systems for encapsulated bacteriophages targeted at *Escherichia coli* O157:H7 in feedlot cattle showed that phage encapsulated with methacrylate polymer experienced recovery after released from encapsulation. In contrast, non-encapsulated phages experienced total loss of activity which may deactivated due to acidic condition through intestinal tract [54].

Sarhan and Azazi [25] stated that safety and efficacy are the two critical factors that needed to be standardized during bacteriophage manufacturing even though the phages have been applied internally and externally on human in the past until now otherwise it may lead to negative impact on phage therapy. Efficacy of bacteriophage can be enhanced by incorporating it on positively-charged cellulose membrane in order to retain phages near to the treated surface and prevent phage wastage, for example, when immobilized phage cocktail applied o ready-to-eat packed meat, *L. monocytogenes* reduced to undetectable limit at 4^{o}C after one day [55].

When comes to safety, the bacteriophages are non-pathogenic, nevertheless, they are capable of stimulating immune response such as antibody production or induction of interferon in the human host [56]. Besides, lysis of bacteria by bacteriophages will lead to presence of exotoxins and pyrogens and therefore efficient purification during manufacturing is important to remove the crude lysates of the host bacteria [25, 57]. The characteristics of a strong antibacterial efficacy included high adsorption rate towards targeted bacteria, large burst size and short generation time [26].

3. Microbial Biofilm Formation

Coughlan et al. [58] reported that several steps are required for formation of biofilm, namely attachment, cell-to-cell adhesion, expansion, maturation and dispersion. During attachment, pili and flagella are important in aiding the attachment of the individual bacterial cells to the surface [59]. Subsequently, the development of biofilm structure is initiated by passive attachment of bacteria to solid surface with weak bonds (van der waals forces, electrostatic forces, hydrophobic interactions) and gradually irreversible attachment with strong bonds take place. Then the accumulation cells lead to production of extracellular polymeric substances [58]. The life cycle of biofilm is ended when the adhered cells revert back to planktonic condition in order to search additional attachment site or removed by chemical means (passive attachment)

[60]. In fact, the surface hydrophobicity and surface properties of the cell such as the presence of extracellular filamentous appendages, flagella and pili playing important roles in affecting the extent of microbial attachment in order to form a mature biofilm on food-contact surface [61]. Finally, a classic mature biofilm should be in a clump of cells of three-dimensional structure supported by extracellular matrix and separated from other microcolonies by channels that facilitates waste removal and diffusion of required nutrients and oxygen [62].

3.1. Biofilm Formation in Food Industry

The environment factors such as temperature, nutrient availability, types of contact surfaces, pH and humidity are the factors that affect the formation of biofilm [63]. The density of biofilm formation by *S. aureus* S3 on polypropylene and stainless steel surfaces in vegetable-based broth is greater at 28^{o}C than at 7^{o}C [64]. The food contact surface is an important source of contamination in the food processing industry as it is the place for the pathogens to survive and form biofilm, for example, *Salmonella* Weltevreden enter through the poultry meat form a biofilm with greater cell density on plastic (3.4 x 10^7 cfu/cm^2) than cement (1.57 x 10^6 cfu/cm^2) and steel (3x 10^5 cfu/cm^2) [65]. Besides, Rode et al. [66] had demonstrated that *Staphylococcus aureus* showed highest biofilm formation in the presence of 5% glucose and sodium chloride instead of individual effects of glucose and sodium chloride. Besides, quorum sensing is another important factor for regulating biofilm formation by which cell-to-cell communication will only be activated when counts of bacteria achieved particular threshold and then lead to release of signal molecules that cause bacterial cells to release toxins or form biofilm [67].

Formation of biofilm in food processing line really require a more effective and comprehensive sanitation measure due to its persistence in the environment. For example, Dourou et al. [68] had demonstrated that even after 168 hour of cold storage (4^{o}C) the counts of attached cells (formed by *E. coli* O157:H7) to the HDPE coupons which suspended in ground beef increased from 3.7 to 4.1 log CFU/cm^2. The resistance of biofilm towards the sanitizer could be dependent on the strains of adhered pathogens, for example, *E. coli* O157:H7 had been showed to produce curli that render it greater resistance to chlorine treatment compared with non-pathogenic *E. coli* and thus it resulted no significant reduction on biofilm development by *E. coli* O157:H7 during treatment of chlorine for an additional 4 min [69].

3.2. Bacteriophages Activity Against Food Pathogens Biofilm

Microorganisms can be viewed as simple creatures, however, it is possible or capable to integrate itself in order to synthesize a new morphological structure for surviving in harsh environment and this phenomena can be indicated as attachment of communities of microorganisms to a surface (biofilm) [70]. Formation of biofilm lead to production of a matrix of exopolymeric substances such as polysaccharides, proteins DNA and lipids which enable the persistence of pathogenic bacterial in a given environment as the biofilm protects them from common sanitation processes [58].

The physiological and structural characteristics of biofilm would affect the resistance towards disinfectant and biocides is difficult to penetrate into the multiple layers of cells with complex structure of biofilm and thus hampering the efficacy of disinfection [71]. Then, the ability of matured bacterial biofilm to resist antibiotic concentration could reach 1000 times greater than planktonic cells [72]. A study has demonstrated that *C. perfringens* biofilm can form an effective protection towards disinfectants commonly used on farms and in food processing condition such as quaternary ammonium chloride, potassium monopersulfate solution, sodium hypochlorite solution, hydrogen peroxide solution and glutaraldehyde-based disinfectant [73]. However, bacteriophages show a greater ability to target biofilm due to some properties such as they able to produce enzymes that facilitate degradation of extracellular matrix, infect persister cells, remaining dormant within biofilm and re-activate when they become metabolically active [74]. Depolymerase released by bacteriophage able to degrade extracellular polymeric substance formed on the biofilm and allow bacteriophage to diffuse deeper and lyse the bacterial cells [75]. Kelly et al. [76] had demonstrated the efficacy of bacteriophage in preventing formation of biofilm, for example, there was no sign of growing for *S. aureus* Xen29 when inoculated with the phage cocktail, however, biofilm appeared without phage treatment.

Listeriosis outbreaks due to *L. monocytogenes* is a challenge for seafood processing environment owing to its ability to form biofilm, grows at room temperature and ability to survive up to 10% (w/v) sodium chloride [77, 78]. The study showed that the three phages (LiMN4L, LiMN4p and LiMN17) can control or reduce dislodged biofilm cells of *L. monocytogenes* strain on stainless steel soiled with fish proteins [77]. Besides, it is possible for initiation and formation of biofilm in the gastrointestinal tract of animals besides water pipes or other equipment in the food industry that lead to cross contamination in the processing line. However, bacteriophage was proved to have the ability to disperse the bacteria in biofilm, for example, bacteriophage treatment on *C. jejuni* formed on the glass resulted in the reduction of viable counts by 1-3 log CFU/cm^2 after 24 hours [79].

4. Advantages and Limitations of Bacteriophages

Bacteriophage can be applied mainly as a preharvest and postharvest strategy as it can target the pathogen carried by livestocks and reduce pathogens on the products [80]. Sometimes, within the complex biodiversity of intestinal tract of animal, the efficacy of bacteriophage treatment might be reduced due to some factors such as non-specific binding with the food particles in the intestinal tracts and inactivation of phages by the acidic environment [54, 80, 81].

However, Bardina et al. [82] had demonstrated that bacteriophages (*Salmonella*-specific bacteriophages) able to withstand the acidic condition (pH 2) for 30 min of incubation and suggested that bacteriophages are able to resist condition of gastrointestinal passage. Table 2 summarizes the advantages and limitations of bacteriophages usage in food industry.

Sometimes, when comes to application, not all the host bacterial cell bind to the bacteriophage and the failure might be due to spraying method as a course spray cannot ensure a better coverage of food contact surface compared with fine sprayer. Moreover, sometimes, it might need a very high concentrations of bacteriophage to eradicate the contaminants thoroughly, therefore, it impose a financial burden to the thin profit margin food manufacturer [50].

Table 2: Advantages and limitations of bacteriophages applications in the food industry.

Advantages	Limitations
Autodosing (Highly specific and replication of phages corresponded with host) [83]	Total removal of pathogens in the intestinal tract of livestocks is hard to be achieved [80]
Application of phages can be at various points of food processing lines as it harmless to human and animals [84, 85].	Technical problem might happen during application of bacteriophages on various foods [50].
Higher efficacy when cocktail of phages is applied [86].	Not economical for thin-profit margin company [50].
Effective biocontrol by ensuring food safety and prolong the shelf life using phage immobilization on food packaging material [87].	Market acceptance [50].

Another non-technical problem or limitation regarding phage application are market acceptance by the food producers and consumers as food manufacturers might not willing to absorb the significant margin cost for utilization of bacteriophage as a food biocontrol, besides, consumers are still not confident to the intake of 'viruses' when consuming fresh produce sprayed with bacteriophage [50].

5. Phage Cocktail

The two main functions of phage cocktails are creating broad host-range for bacteriophage and reduce the risk for emergence of phage-resistant bacterial mutants [88]. A well proposed cocktail of bacteriophage should reduce the risk for development of bacteriophage insensitive mutants because during onset of lytic infection (adsorption process), the phages able to utilize different receptor molecules on the host bacteria and bacteria in food matrices are not permissive for replication during lytic infection [85]. There are some selected studied cocktail bacteriophages on different food matrices as shown in Table 3.

6. Application of Bacteriophages in Food

As an antimicrobial agent, phages act differently or even better when compared with antibiotics which are non-specific, lead to antimicrobial resistance and they imposed a negative effect in preharvest stage of food chain [93]. A country has to bear with the cost of illness, loss of quality-adjusted life years (QALY) and loss of productivity due to foodborne illness. Based on a study, the 14 foodborne pathogens examined in United States imposed a cost of $14 billion and 90% of the cost of illness and QALY loss are due to the top five pathogens (*Salmonella*, *Taxoplasma*, *Listeria*, norovirus and *Campylobacter*) [94]. In developed countries, the food-borne outbreaks are mostly associated with campylobateriosis caused by *C. jejuni* [93].

Application of bacteriophage can be applied from the farm to the fork. One of the example is phage treatment on the pre-slaughtered food animals in order to reduce salmonellosis in chickens and enteropathogenic *E. coli* infections in cattle [95]. Besides, the fresh produce sprays has been gaining the interest as study showed that the treatment of bacteriophage (Listex P100) on melon was effective and reductions of bacteria were obtained after 8 days of storage at 10^{o}C [96]. Besides, another study stated that cruciferous vegetables are associated with bacterial blight due to phytopathogen *Pseudomonas cannabina* pv alisalensis which is the causal agent which rendered the crop unmarketable.

Processing is also a critical stage for food industry and efficacy of decontamination by bacteriophage is promising. For example, during tumbling the risk of contamination and internalization of pathogen is higher, however, when 10^8 PFU/ml of phage applied on trim surface had reduced *Salmonella* by 1 log CFU/g and the higher the concentration of bacteriophage applied, the greater the effect of decontamination [97]. In fact, concentration and timing of phage application are very important in order to maximize the efficiency of bacteriophage treatment in food industry, for example, Leverentz et al. [98] had demonstrated that viable counts of *L. monocytogenes* could be reduced to undetectable limit on day 0 when 10^8 log PFU/ml of phage was applied there and when phage applied as soon as 1 hour before contamination (at the time of fruit cutting).

6.1. Milk and Dairy Products

In the past, presence of phage always regarded as a stumbling block for fermentation process in dairy industry, however, intensive researches have been done to improve the quality of dairy products, while also recognized the beneficial properties of bacteriophages in dairy products [99]. The presence of bacteriophages has caused a major problem during fermentation process of dairy products namely dead vats and slow vats. Dead vats means the milk is not wholly acidified due to loss of starter culture activity by phage infection while slow vats means fermentation period is longer and quality of final product is downgraded due to impairment of the starter culture activity by phage infection [99]. However, bacteriophage found in dead vats can eliminate pathogenic bacteria while slow vats it encodes lysis ability of lactococcus strain in cheese ripening.

There are several critical points in dairy processing line where the bacteriophage can be applied as milk and its related products can be contaminated with undesirable bacteria from collection to consumption. Even though pasteurization impose an impact on the efficacy of bacteriophage treatment, effectiveness of bacteriophage at eliminating or reducing pathogenic bacteria in dairy industry has been proven [100]. As an evidence, antimicrobial activity of staphylococcal phage towards staphylococcal strains that caused mastitis in livestocks and contamination of raw milk is effective in some milk samples as cocktail of phages reduced the staphylococcal counts significantly after 4 h of incubation [3].

Bacteriophage can act as allies of dairy products in the context of infecting milk-borne pathogens without contributing to antibiotic resistance [101]. There are many entry points for pathogenic microorganism into the dairy products along the food chain: (i) raw milk production (pathogenic microorganisms carried by the cattle, farmer, milking equipment and storage containers), (ii) pasteurization failure and (iii) improper storage during distribution to consumer (temperature abuse) [101, 102]. Here, bacteriophage can be applied as phage therapy for infected animals in the farm, disinfectant for farmers and food handlers in the industry and biocontrol of final product such as cheese and milk as they are harmless to humans, animals and environment without causing damage of processing equipment and affect the organoleptic properties of food [101, 103]. For example, when bacteriophages and bacteriocins performed infection together on *Listeria monocytogenes* in milk sample, the bacterial counts were reduced to undetectable limits during storage of day 4 at 4^{o}C for 10 days. In addition, the result suggested that it create lower risks of resistance development among the pathogens [104].

Table 3: Efficacy of phage cocktail application against foodborne pathogens.

Phage Cocktail	Targeted Pathogens	Types of foods	Results	References
EcoShield TM	Pathogenic strains of *E. coli* O157:H7	Beef, Lettuce	Reduced level of bacterium in beef by ≥94% while in lettuce by 87 % after 5 min	[89]
PC1	*Salmonella* spp	Pig skin	At MOI≥10, bacterium reduced to undetectable limits (≥90% reduction)	[90]
ΦSH17, ΦSH18, ΦSH19	*Salmonella* Typhimurium U288 (multidrug-resistant)		Able to lyse various *Salmonella* enterica serovars	
CHOED phage	*Vibrio anguillarum* *V. ordalii*	Atlantic Salmon	When phage added to the tank, counts of *Vibrio* bacteria reduced from 2.9 x 10^4 CFU/ml to undetectable limits by day 3-post infection	[91]
			When 10 PFU of phage per bacterial cell added, there was 0 mortality by 20 days post infection	
SF-A2	*Salmonella dysenteriae*	Ready-to-eat	*S. dydenteteriae* and *S. sonnei* were reduced to unde-	[92]

SD-11	*S. sonnei*	spiced chicken	tectable limit after 72 h of incubation
SS-92	*S. flexneii*		

6.2. Meat Products

It is known that livestocks are always contaminated with *Salmonella*, *Campylobacter* and *E. coli* O157:H7 and therefore application of bacteriophage is an approach to prevent colonization of pathogenic bacteria into the food processing line as bacteriophage are usually administrated to the live animals before being slaughtered and processed [80]. Based on animal study, the application of bacteriophage (CEV1) on the sheep with ~ 10^{10} CFU of novobiocin/nalidixic acid resistant *E. coli* O157:H7 EDL 933 and the result showed that 933 strain levels were reduced by 2 -3 log units in the phage treated sheep [105].

In 2006, phage had been approved as an additive by US Food and Drug Administration (FDA) in meat and poultry products such as hot dogs and lunch meats through spraying without exceeding 1 ml per 500 cm^2 as these ready-to-eat meat product might not undergo further cooking after purchased by consumers and therefore there is higher contamination risk by *L. monocytogenes* [106]. When the isolated phages (phage cocktails) were mixed with *S.* Enteritidis and *S.* Typhimurium on the chicken breast sample, the viability of both bacterial strain reduced significantly after 2 h incubation at 8^oC [107]. Besides, when $2.5x10^7$ PFU/cm^2 of phages was added to the chicken breast roll, the concentration of *L. monocytogenes* reduced immediately by 2.5 log_{10} CFU/cm^2 at 30^oC [108].There was a study demonstrated during processing (tumbling) of red meat and poultry, bacteriophage cocktail (F01a) was able to reduce 1 and 0.8 log CFU/g of *Salmonella* in ground beef and ground pork respectively under processing temperature [97]. Thus these study showed that phages able to combat pathogens in a ready-to-eat meat products.

6.3. Fresh Produce

Contamination of leafy vegetables with bacterial pathogens can happen as early during production while *E. coli* and *Salmonella* are the pathogens that often associated with the contamination during production [109]. For example, water used for irrigation, animal manure and feral animals are the reasons for the contamination of the leafy vegetables [110].

However, current common treatments (heat treatment, irradiation and chemical sanitizers) are not the ideal options for de
contamination of fresh produce, one of the main reasons is they are not necessary killing the targeted pathogenic bacteria. In addition, heat treatment is not suitable as the fresh produce will be cooked and the appearance or nutritional contents of the fresh produce will be affected while chemical sanitation is not environmental friendly and not favorable by society in preference of organic foods [111, 112, 113]. Therefore, bacteriophage can be an ideal natural intervention for decontamination of fresh produce as bacteriophages actually always present on food without extensive processing such as fresh produce and fermented food [114].

There were many types of fresh produce such as melon, sprouts, tomatoes and serrano peppers and the main reason for these outbreaks happened in United States is due to the ability of *Salmonella* to internalize into vegetables and fruits [115]. Since the foodborne outbreaks associated with fresh produce is raising concerns of everyone, Magnone et al [116] discovered that when lytic bacteriophage cocktail combined with levulinic acid produce wash, it resulted more than 4.0 log reductions in pathogens for the inoculated samples (broccoli, cantaloupe and strawberries). As an evidence, salmonella counts on broccoli reduced significantly from 6.49 log CFU/g to 1.94 log CFU/g for 5 min treatment.

7. Conclusion and Future Prospect

The numerous studies from the past to date have shown bacteriophage can be an alternative biopreservative for food product, biosanitation and biocontrol agent for food processing line and phage-therapy for livestocks. However, there are some studies demonstrated the limitations of bacteriophage efficacy in food matrices. Several factors known to affect the phage-host system include the adopted methods of phage administration, control methods, and bacteriophage concentration. Besides, some phage studies had demonstrated emergence of phage-resistant bacterial strain, however, the effect is not significant as caused by antibiotics treatment and the risk of resistant actually can be reduced or prevented by using polyvalent bacteriophage or cocktail bacteriophage. This review shows bacteriophage is a promising alternative as antimicrobial agent and there are still many studies need to be done so that it can be used at full potential to control foodborne pathogens to ensure safe food for consumption from farm to fork.

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