

Bacteriophages and their applications

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Abstract

Bacteriophages are ubiquitous in our world, mainly in the oceans, soil, the water and food we consume. They can be used efficiently in modern biotechnology, as well as alternatives to antibiotics for many antibiotic resistant bacterial strains. Phages can be used as vehicles for vaccines both DNA and protein, for the detection of pathogenic bacterial strain, as bio-control agents in agriculture and food industry. This review outlines the properties as well as the influence of different external physical and chemical factors like temperature and acidity on phage persistence. A better understanding of the complex problem of phage sensitivity to external factors may be useful for other researchers working with phages. Furthermore, the applications of bacteriophages were described in this paper as well.

1. Introduction

Bacteriophages or phages are natural enemies of bacteria and hence man friends to fight bacterial infections. In fact, bacteriophages were first discovered dates back to the second decade of the 20th century (Twort, 1915; Goodridge and Abedon, 2003) and their bactericidal activity was first reported by Ernest Hankin in 1896 (Sulakvelidze *et al.*, 2001). Accordingly, phages are ten times more numerous in the environment than bacteria, with an estimated number of 10³² bacteriophages (Brüssow and Kutter, 2005). Based on their self-replicating and self-limiting, bacteriophages offer a great advantage over antibiotics as the amplification continues at the site of infection as long as the hosts are present. Currently, over 5500 different bacteriophages, having genetic information encoded by

double-stranded DNA, single-stranded DNA, double-stranded RNA and single-stranded RNA, have been discovered (Ackermann, 2007). The most abundant group of lytic bacteriophages belong to tailed dsDNA viruses (*Myoviridae*, *Podoviridae* and *Siphoviridae*), order of *Caudovirales* (Wittebole *et al.*, 2014). Important commercial bacteriophage preparations available are Agriphage™, EcoShield™, ListShield™, Listex™ P100 and Salmonellex™ (Madhusudana Rao and Lalitha, 2015). The outstanding attributes of bacteriophages for treating zoonotic pathogens are their short generation time, non-toxicity, high specificity to the bacterial host, and they do not affect the viability of other flora in the environment (Hudson *et al.*, 2013; Rong *et al.*, 2014; Jamal *et al.*, 2015). Thus, intensive research efforts have been focused on the prevalence of bacteriophages from different sources in order to accelerate or to expand their

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applications in various fields. For instance, bacteriophages have been isolated from different types of food products, including chicken meat, ground beef, roast turkey breast, ruminant-based foods, cheese, oil, raw skim milk, common carp and marine fish, sardine, clam, mussels, shellfish, shrimp, and vegetables (Sulakvelidze and Barrow, 2005). In light of the increasing interest in bacteriophages, studies were undertaken not only to enumerate and to evaluate their lysis efficacy, but also to enhance our immediate understanding of this virulence bacteriophage. For these purposes, bacteriophages need to be purified and characterized. Upon methodological approaches, bacteriophages can be purified by polyethylene glycol (PEG) precipitation (Han *et al.*, 2014) or cesium chloride (CsCl) gradients centrifugation (Wong *et al.*, 2014), and characterized in terms of morphology, genomic DNA, proteomic and biological measurements of phage-host infections (Yang *et al.*, 2010; Sun *et al.*, 2012; Tiwari *et al.*, 2013). Work on characterization and recent applications were reviewed in this paper.

2. Classification

In 1973, the International Committee on Taxonomy of Viruses (ICTV) was formed to authorize and organize the taxonomic classification of and the nomenclatures of viruses. Importantly, classification is mainly used for the following purposes in order to identify novel bacteriophages, to detect the relationships among bacteriophages, to maintain bacteriophage databases and collections. Besides, it can be used for identification of either bacteriophages with therapeutic and industrial applications, or harmful bacteriophages in biotechnology and fermentation industry for control and eradication purpose (Kutter and Sulakvelidze, 2005).

Bacteriophages are classified into 13 families based on the nature of their encapsidated nucleic acid and their virion morphology (Hanlon, 2007). There are 9 families with dsDNA: *Corticoviridae* (icosahedra capsid with lipid layer), *Fuselloviridae* (pleomorphic, envelope, lipids, no capsid), *Lipothrixviridae* (enveloped filaments, lipids), *Myoviridae* (contractile tail), *Plasmaviridae* (pleomorphic, envelope, lipids, no capsid), *Podoviridae* (short, non-contractile tail), *Rudiviridae* (helical rods), *Siphoviridae* (long, non-contractile tail) and *Tectiviridae* (icosahedral capsid with inner lipoprotein vesicle) (Figure 1). *Inoviridae* (rod-shaped with helical symmetry) and *Microviridae* (icosahedral capsid) exhibited in ssDNA. Two families with dsRNA and ssRNA are *Cystoviridae* (enveloped, icosahedral capsid, lipids) and *Leviviridae* (quasi-icosahedral capsid), respectively. Ackermann (2007) reported that more than 96% of all bacteriophages are tailed phages, which

belong to the order of the *Caudovirales*. Besides, their properties are extremely varied, such as their dimensions and fine structure, DNA content and composition, nature of constitute proteins, serology, host range, and physiology (Kutter and Sulakvelidze, 2005). The three main families comprising the *Caudovirales* with 60% of the characterized phages are *Siphoviridae*, 25% are *Myoviridae* and 15% are *Podoviridae* (Ackermann and Dubow, 1987; Kutter and Sulakvelidze, 2005; Lopes *et al.*, 2014). However, only 3-4% of the studied phages (polyhedral, filamentous, and pleomorphic) belong to 10 families, where some of which are very small (Ackermann, 2007).

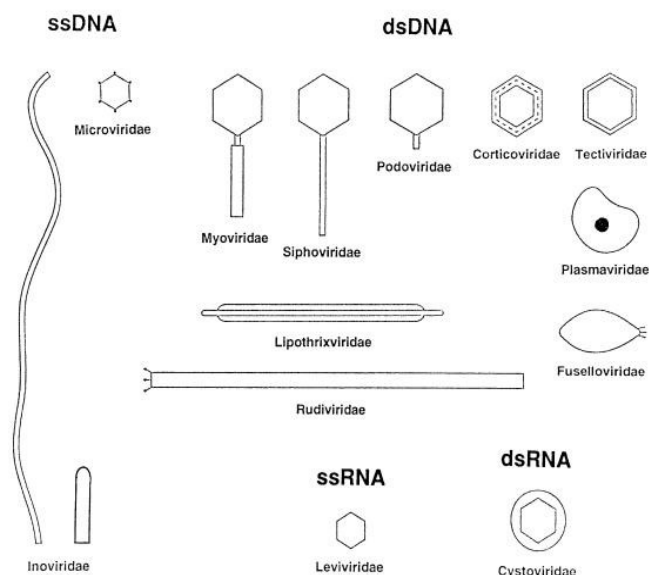


Figure 1. Bacteriophages morphotypes (Source: Ackermann and Dubow, 1987)

3. Life cycles

Since bacteriophages lack an own metabolism, they completely rely on the energy and protein biosynthetic machinery of their bacterial hosts to reproduce. Therefore, bacteriophages infect bacteria and can propagate in two possible ways; lytic life cycle and lysogenic life cycle. It is known that virulent phages go through lytic cycle, where firstly attach to the target host by binding to specific cell wall receptors such as peptidoglycan, lipopolysaccharide, oligosaccharide and teichoic acid (Lenski, 1988; Weinbauer, 2004; Elbreki *et al.*, 2014). This binding specificity might occur at the cell capsule, flagella and/or conjugative pili (Hanlon, 2007). Thereafter, phages will inject its genome into the host bacteria, leaving the viral capsid outside of the cell. Then, the viral DNA is transcribed by host's RNA polymerase, leading to redirect the bacteria's metabolic machinery to produce more phages. Finally, new phages will assemble in abundance, and phage progeny are released upon bacterial cell lysis. According to Kutter

and Sulakvelidze (2005), bacteriophage lytic enzymes (endolysins or lysins) play an important role in breaking down the bacterial cell wall structure (peptidoglycan) during the final stage of the lytic cycle. In addition, some phages with filamentous morphology are capable to escape from the host cell via cell wall extrusion without causing destruction of the host (Hanlon, 2007). In lysogenic cycle, temperate phages insert its nucleic acid (the prophage) in the host genes, where it remains silent for extended periods without any metabolic consequences for the host cell (Elbreki *et al.*, 2014). This prophage genome is vertically transmitted along with the whole bacterial chromosome to its progeny until the lytic cycle is induced (Weinbauer, 2004). Previously, Ranquet *et al.* (2005) have shown that a temperate phage ‘Mu’ can switch between lysogeny and lytic growth under the influence of high temperature and stationary phase.

Apart from the two life cycles, Ackermann and Dubow (1987) proposed that a unique condition referred as pseudolysogeny (unstable carrier-state), where phage genomes act like a prophage regardless either virulent or temperate phage might occur. Under stressful conditions, phage genomes coexist in an unstable relationship with bacterial cells for extended periods, which mainly resulted from a low energy state affected by several factors like at low temperatures in the bacterium, concentration of nutrients accessible or the presence of antibiotics in the bacterial cell (Ripp and Miller, 1997). Thus, the metabolism of the bacterium is slow and generation times increased. Nonetheless, if unfavorable conditions are removed or the nutrients are provided to the bacterium, the pseudolysogens resolve and enter into either lysogenic cycle or lytic cycle (Ripp and Miller, 1998).

4. Enumeration, concentration and purification of bacteriophages

The main requirement for developing “bacteriophage technology” for practical purpose is the ability to quantitate viable bacteriophages accurately and reproducibly. Plaque assays is a simple enumeration procedure, where several dilutions of the phage preparation are gently mixed with a permissive host bacterium in molten agar and dispersed evenly on the surface of the agar medium. After incubation, a clear circular area of lysed cells known as plaque will develop in a confluent lawn of host bacterium on the agar medium (Anderson *et al.*, 2011). Lately, the double agar overlay or soft agar overlay methods of plaque assay are most commonly used approach for determining phage titers. This technique has been used by researchers with *Bacillus* (Shin *et al.*, 2011), *Campylobacter* (Premarathne *et al.*, 2017), *Escherichia* (Cormier and

Janes, 2014), *Klebsiella* (Kęsik-Szeloch *et al.*, 2013), *Salmonella* (Thung *et al.*, 2017), *Staphylococcus* (Li and Zhang, 2014), *Vibrio* (Peng *et al.*, 2014) and others. Furthermore, phage particles can also be enumerated by other methods such as electron microscopy or fluorescent microscopy (Table 1).

Table 1. Methods for enumeration of bacteriophages

Technique	Method features
Plaque assay	<ul style="list-style-type: none"> • Good detection limit • Total counts
Transmission electron microscopy (TEM)	<ul style="list-style-type: none"> • Morphological characterization • Total counts
Epifluorescence microscopy	<ul style="list-style-type: none"> • No detection limit for environmental samples • Relatively rapid • High precision

(Source: Weinbauer, 2004)

The concentration of infectious particles is prerequisites for structural and functional characterization of bacteriophages. Methods used to concentrate phages are filter-adsorption-elution, flocculation and ultracentrifugation. Additionally, filtration through 0.2-0.4 μm pore-size filters followed by ultrafiltration was applied to avoid losses of infectivity. For example, Jun *et al.* (2013) had employed 0.45 μm membrane filter followed by centrifugation to increase the titer of bacteriophage pSF-1, which infects *Shigella flexneri*. Bourdin *et al.* (2014) reported that phage lysates of T4-like *Escherichia coli* phages were concentrated by different centrifugation and through 0.22 μm membrane filter without titer loss. In some cases, PEG precipitation is applied in the concentration of phage particles procedure. It was used to prepare high titers of *Salmonella*-specific phages by Bao *et al.* (2011), Wong *et al.* (2014), Bao *et al.* (2015) and other researchers.

Considering the distinct variability characteristic of bacteriophages such as size, density, nucleic acid content and resistance mechanical stressors (Thurber *et al.*, 2009), the choice of purification methods are known to influence the degree of specific phages purity. Centrifugation in CsCl gradients is ideal for purifying large amounts of phage, where the contaminant like bacterial lipopolysaccharide (i.e. endotoxin) can be removed from the phage preparations, resulting highly purified yields (Carlson, 2005). Kleiner *et al.* (2015) had used CsCl density gradient centrifugation for virus-like particles (VLP) purification and illustrated the significance of phage density among the samples. Previously, CsCl density gradient ultracentrifugation was widely used in bacteriophage purification such as *Acinetobacter baumannii* phage ΦAB2 (Lin *et al.*, 2010),

Bacillus cereus phage JBP901 (Shin et al., 2011), *Salmonella enterica* serovar Typhimurium phage Φ st1 (Wong et al., 2014) and *Staphylococcus aureus* phage SPW (Li and Zhang, 2014).

5. Properties of bacteriophages

Bacteriophages are differentiated on the bases of their morphology. Thus, electron microscopy is often the easiest and fastest way for phage identification, as well as to assign an unknown phage to a family (Ackermann, 2007). Akhtar et al. (2014) had employed TEM to characterize a collection of *Salmonella enterica* lytic bacteriophages isolated from animal feces and sewage samples. The phages in the collection were mostly *Siphoviridae* and *Myoviridae*. Meanwhile, Pope et al. (2007) had used cryo-electron microscopy to characterize a purified cyanophage Syn5 which host-specific to *Synechococcus* WH8109 strain. They reported that the capsid has a single 'horn', a novel fibrous structure protruding from the opposing end of the capsid from the tail of the virion.

On the other hand, phage structural proteins are most commonly analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). This is partly because one can describe the bands not just in terms of their relative mobility to each other, but also in terms of their molecular size. For the identification of *Salmonella* Enteritidis phage PVP-SE1 structural proteins, twenty-one protein bands with molecular masses ranging from 14,000 Da to 94,000 Da were estimated by SDS-PAGE (Santos et al., 2011). A CsCl-purified bacteriophage vB_SenS-Ent1, host-specific to *Salmonella* Enteritidis was isolated from swine effluent, which consisted of nine polypeptide chains with molecular weight between 9,000 Da and 90,000 Da (Turner et al., 2012). In another study, two structural proteins of a novel *Vibrio* bacteriophage SIO-2, designated as the major capsid protein and the tape measure protein were estimated to be 29,000 Da and 137,000 Da by SDS-PAGE, respectively (Baudoux et al., 2012). Besides, Han et al. (2013) have found that a *Myoviridae* phage SAH-1 possessed several structural proteins; mainly the major capsid and tail sheath proteins were predicted at 60,000 Da and 78,000 Da by SDS-PAGE, respectively.

It is well known that bacteriophages are strictly host-specific, which infects only one bacterial species or one serotype within a species (Ackermann et al., 1978; Bielke et al., 2007; Koskella and Meaden, 2013). However, not all bacteriophages are host-specific. Particularly, wide host range bacteriophages would be expected to be advantaged relative to phages with narrow

host range, since a wide host range allows a phage to utilize one prey species when another is not available, or even utilize several simultaneously. For instance, phage SEA2 (Akhtar et al., 2014), phage Bc431v3 (El-Arabi et al., 2013) and phage JG004 (Garbe et al., 2011) were reported as broad host range bacteriophage against *Salmonella enterica* serovars, *Bacillus* species and *Pseudomonas aeruginosa* mutant strains, respectively. Turki et al. (2012) had isolated two bacteriophages, *Salmonella* Zanzibar phage sww275 and *Salmonella* Anatum phage sww65, which lytic on more than ten *Salmonella* strains, and were able to infect *Escherichia coli* and *Citrobacter* isolates. Interestingly, four *Escherichia coli* bacteriophages isolated from Lake Michigan showed lysis against *Arthrobacter*, *Chryseobacterium*, *Microbacterium* and *Pseudomonas aeruginosa*, exhibiting a width of host range capacity (Malki et al., 2015).

Upon infecting its host bacteria, most of the virulent phages act optimally at different ratio of multiplicity of infection (MOI). For example, the optimal MOI of a virulent bacteriophage KSL-1 specific for *Pseudomonas fluorescens* was determined to be 0.001 (Sun et al., 2012). Li and Zhang (2014) had isolated a lytic *Staphylococcus aureus* bacteriophage SPW from wastewater of cleaning dairy cattle udders with the optimal MOI of 0.01. On the contrary, a lower MOI ratio of 0.0001 was reported for bacteriophage AB1 of *Acinetobacter baumannii* (Yang et al., 2010). Recently, higher ratio of 0.1 was considered as the optimal MOI for *Escherichia* phage CICC 80001 (Xu et al., 2016) and *Salmonella* Typhimurium phage Φ st1 (Wong et al., 2014). Meanwhile, the proliferation rate of bacteriophages always depends on their latent period and burst size along with the number of infected cells. Abedon and Culler (2007) showed that an optimal latent period would improve phage fitness and an increase in burst size might affect the plaque sizes. It has also been demonstrated that latent period and burst size are influenced by the host, the medium compositions, the incubation temperature and the specific growth rate (Carey-Smith et al., 2006). Studies on the proliferation of bacteriophages have been reported in different latent periods and burst sizes (Table 2).

Most of the phages generally appear to be active over a broad pH range, from acidic to alkaline. Zhang et al. (2015) have reported a lytic phage of *Lactobacillus casei* isolated from Chinese sauerkraut was stable between pH 4.0 and 11.0. A novel cold-active bacteriophage VNPH-1 specific for *Aeromonas sobria* was comparatively stable at pH 5.0 to 10.0 and the optimum phage infectivity occurred at pH 9.0 (Ji et al., 2015). Similarly, *Salmonella*-infecting bacteriophages

include phage PA13076 (Bao *et al.*, 2015), phage SE2 (Tiwari *et al.*, 2013), phage PSPu-95 and phage PSPu-4-116 (Bao *et al.*, 2011), were relatively stable within the pH range of 4.0 to 10.0. However, recent studies by Hu *et al.* (2016) on thirteen lytic distinct *Pseudomonas* bacteriophages reported that all phages were active over a very narrow pH range, from 6.0 to 7.0.

Table 2. Latent period and burst size of bacteriophages

Phage	Latent period (min)	Burst size (PFU/cell)	Reference
SE2	10	155	Tiwari <i>et al.</i> (2013)
CICC 80001	10	198	Xu <i>et al.</i> (2016)
φPA-HF17	10	200	Han <i>et al.</i> (2014)
AB1	18	409	Yang <i>et al.</i> (2010)
PSPu-4-116	20	86	Bao <i>et al.</i> (2011)
SAH-1	20	100	Han <i>et al.</i> (2013)
MJ1	21	300	Jamal <i>et al.</i> (2015)
JBP901	27	100	Shin <i>et al.</i> (2011)
JG004	31	13	Garbe <i>et al.</i> (2011)
Φst1	40	22	Wong <i>et al.</i> (2014)
FGCSSa1	50	139	Carey-Smith <i>et al.</i> (2006)
Lcb	75	16	Zhang <i>et al.</i> (2015)
Bc431v3	80	318	El-Arabi <i>et al.</i> (2013)
KSL-1	90	52	Sun <i>et al.</i> (2012)
Φ22	110	55	Pringsulaka <i>et al.</i> (2011)

Reports showed that the viability of bacteriophages is greatly affected by temperature (Yang *et al.*, 2010; Baudoux *et al.*, 2012; Keşik-Szeloch *et al.*, 2013; Wong *et al.*, 2014), which specifically influencing in their attachment, penetration, multiplication, and the length of the latent period (Jończyk *et al.*, 2011). Most bacteriophages act optimally at temperatures between 30 -50°C (Bao *et al.*, 2015; Ji *et al.*, 2015; Zhang *et al.*, 2015). Bacteriophages were also found to be active over the temperature range of 4-50°C (Jun *et al.*, 2013) and 4-60°C (Easwaran *et al.*, 2015) for *Shigella flexneri* phage pSf-1 and *Escherichia coli* phage Sw1, respectively. Significant inactivates of phages were observed at 70°C for *Siphoviridae* (Lee *et al.*, 2013), *Myoviridae* (Jamal *et al.*, 2015) and *Podoviridae* (Augustine *et al.*, 2013). On the contrary, *Escherichia coli* O157:H7 phage BECP2 (Lee and Park, 2015), *Pseudomonas aeruginosa* phage φPA-HF17 (Han *et al.*, 2014), and *Weissella cibaria* phage Φ22 (Pringsulaka *et al.*, 2011) showed heat resistance at 70°C.

6. Application of bacteriophages

Nowadays, bacteriophages have contributed a lot to the field of molecular biology and biotechnology, and numerous applications have already been developed. The wide applications of phages in agriculture, clinical use, diagnosis of the disease, veterinary bio-control and also food safety have been studied extensively (Heringa *et al.*, 2010; Larbanoix *et al.*, 2011; Bardina *et al.*, 2012; Jaiswal *et al.*, 2013; Zinno *et al.*, 2014; Liu *et al.*, 2015; Lone *et al.*, 2016).

6.1 Therapeutic agents

Bacteriophages have been extensively used to combat various bacterial infections after their discovery in the early 20th century. For example, d'Herelle had employed bacteriophages as therapeutic agents in 1929 (Madhusudana Rao and Lalitha, 2015). Although the use of bacteriophages as therapeutic agents subsequently declined in the West, however, the emerging of various antibiotic-resistant bacteria have prompted the Western world to revive the interest of phage therapy in recent times (Jaiswal *et al.*, 2013). In addition, phage therapy has been used in animals, plants, and humans with different degree of success (Haq *et al.*, 2012). Several potential beneficial effects of phage therapy are: (i) narrow antibacterial spectrum allowing preservation of the existing microbiome, (ii) activity against both Gram-positive and Gram-negative bacteria, (iii) less importance of side effects, (iv) extensive distribution upon systemic administration, (v) possible effect on the inflammatory response, (vi) improved efficacy as compared with antibiotics, and (vii) cost-effectiveness (Wittebole *et al.*, 2014).

6.2 Detection of bacterial pathogens

The specificity of the interaction of a phage with its host cell immediately lends itself to methods for the identification of bacteria, in particular, the pathogens. One of the first uses of phage was in typing schemes, where a panel of phages with different lytic spectra is used to discriminate between different isolates of a bacterial species or genus, according to their ability to infect the isolate and form plaques. There are several methods can be used for pathogenic bacteria detection. For example, the use of bacteriophages which able to deliver reporter genes (e.g., *luxAB*) (Thouand *et al.*, 2008) or using green fluorescent protein (Piuri *et al.*, 2009) that would express after infection of bacteria. Besides, bacteriophages covalently attached with a fluorescent dye to their coats can be employed for the detection of specific adsorption (Goodridge *et al.*, 1999). In certain cases, detection of other released components, which include adenosine triphosphate (ATP), adenylate kinase (AK) and β-D-galactosidase after the specific lysis

Table 3. Bacteriophages used for bio-control in various foods

Target species	Treated food	Bacteriophage	Reference
<i>Bacillus cereus</i>	Fermented food	JBP901	Shin et al. (2011)
<i>Campylobacter jejuni</i>	Raw and cooked beef meat	Cj6	Bigwood et al. (2008)
<i>Escherichia coli</i> O157:H7	Beef meat	FAHEc1	Hudson et al. (2013)
	Turkey breast and raw beef meat	EcoM-AG10	Anany et al. (2011)
<i>Listeria monocytogenes</i>	Turkey breast and raw beef meat	LmoM-AG13 and LmoM-AG20	Anany et al. (2011)
	RTE foods	A511 and P100	Guenther et al. (2009)
<i>Salmonella enterica</i>	Chicken breast, cabbage and milk	PA13076 and PC2184	Bao et al. (2015)
	RTE foods	FO1-E2	Guenther et al. (2012)
<i>Staphylococcus aureus</i>	Fresh and hard-type cheese	phi-IPLA35 and phi-IPLA88	Bueno et al. (2012)
<i>Vibrio parahaemolyticus</i>	Oysters	VPP1	Rong et al. (2014)

of bacteria can also be used (Guzmán Luna et al., 2009; Lee et al., 2011; Chen et al., 2015).

On the other hand, bacteriophage amplification assays attributed an attractive alternative method to detect pathogenic bacteria, which retained the inherent biological specificity of the phage for its target but utilized an assay end-point that was not dependent upon a genetically modified virion (Stewart et al., 1998; Schmelcher and Loessner, 2014). This technique has the advantage of detecting only viable cells. Therefore, it has most extensively been used for the detection of *Campylobacter*, *Escherichia coli*, *Listeria*, *Mycobacterium tuberculosis*, *Pseudomonas*, and *Salmonella* (Stewart et al., 1998; Pai et al., 2005; Oliveira et al., 2012).

6.3 As bio-control agents in food industry

Food-borne illnesses of microbial origin are serious food safety problems worldwide. With the current concern over the emerging of antibiotic-resistant foodborne pathogens resulted from the abuse and misuse of antibiotics, bacteriophage-based bio-control may serve as an alternative antimicrobial, which represents an economically viable field in the food industry (Henry and Debarbieux, 2012; Tan et al., 2014). Many studies reported success in decreasing the bacterial load (for *Campylobacter*, *Listeria*, *Salmonella*, and others) in a variety of foods (Table 3).

Several systems have already been approved by authorities with the “generally recognized as safe” (GRAS) status for use on food products such as ListShield™ (Intralytix) or LISTEX™ (Microcos) for the control of *Listeria monocytogenes* (both FDA- and USDA-approved), EcoShield™ (FDA-cleared) targeting *Escherichia coli* O157:H7 and SALMONELEX™ against *Salmonella* (Henry and Debarbieux, 2012). Importantly, the efficacy of bacteriophages in food depends on the structure and chemical composition of the different food items, as well as sufficient diffusion ability of the phage particles, is necessary (Guenther et al., 2009). Furthermore, phage effectiveness is also

influenced by pH and activity on a solid substrate or biofilm, the emergence of resistant bacteria mutants, and the relative numbers of phages and host required to allow replication (Hudson et al., 2005; Oliveira et al., 2015).

The use of immobilized phage as packaging material to maintain the safety of fresh produce is a novel concept that could allow the controlled release of bacteriophage particles into the food and reduce bacteriophage waste during food treatment using spraying (Lone et al., 2016). Currently, bacteriophage cocktails were introduced into prototypes of packaging materials using different techniques: i) immobilizing on positively charged modified cellulose membranes, ii) impregnating paper with bacteriophage suspension, and iii) encapsulating in alginate beads followed by application of beads onto the paper, in order to enhance the safety of fresh produce and RTE meat (Lone et al., 2016). Interventions using bacteriophages can be performed at different or even multiple points in the food processing facility or throughout the food chain to enhance the control process. It has been suggested that *Salmonella* bacteriophages can be added by dipping or spraying or as a liquid to different food matrices (Spricigo et al., 2013; Zinno et al., 2014; Bao et al., 2015; Sukumaran et al., 2015).

Conflict of Interest

The authors declare no conflict of interest.

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