Bacteriophages in food safety

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Bacteriophages (phages) are naturally occurring viruses of bacteria that only infect and multiply within their specific bacterial hosts [1, 2]. Their host specificity is generally restricted at strain level, species level, or, more rarely, at the genus or class level. This specificity has allowed for directed targeting and killing of pathogenic bacteria using phages in what is referred to as phage therapy [2]. Phage therapy against infectious bacterial diseases was proposed and implemented shortly after the discovery of phages with partial success. However, the advent of antibiotics put an end to phage therapy research in many countries [3].

Globally the use of phages as therapeutic and biocontrol agents to decontaminate animals, plants, and food products is receiving renewed attention. The risk of antibiotic-resistant bacteria entering the human food chain and the recent legislation restricting the use of antibiotics as growth promoters in animal production have been the driving forces for the development of alternative methods of pathogen control such as the use of phages.

Phages can be used to control pathogens in food at all stages of production in the classic "Farm-to-fork" continuum in the human food chain [4]. In pre-harvest control of foodborne pathogens, phage therapy can be used to prevent as well as reduce colonization and disease. Phages can also serve as biocontrol agents or biosanitizers in the post-harvest control of foodborne pathogens in meat, fresh produce and processed foods. Finally, the use of phages has also been proposed as biopreservatives in the extension of the shelf-life of manufactured foods.

Despite the relative success reported in the several studies documenting the use of phages as biocontrol agents in food, several considerations have to be taken into account in determining the desirable properties of phages to be used as biocontrol agents. Here, we discuss these properties in detail and review the current literature on phage based biocontrol and biosanitization strategies used to reduce foodborne pathogens in food.

Keywords bacteriophage; biocontrol; food safety; foodborne pathogens

1. Introduction

Foodborne illnesses are a major cause of morbidity and mortality worldwide. The World Health Organization estimates that globally, diarrhoeal diseases alone (a majority of which are caused by foodborne pathogens) kill 1.9 million children per year [5]. Foodborne diseases do not only occur in developing countries, in the United States of America for example, it is estimated that foodborne diseases result in 76 million illnesses, 325,000 hospitalizations and 5,000 deaths each year [6]. Considerable effort has been directed towards the control of the major bacterial food-borne pathogens. However, this has had little impact on addressing the problem in many countries. This is because the effectiveness of the intervention initiatives has been obscured by other changing factors. These changing factors may be associated with the pathogens, their hosts (humans) or political, economic and environmental factors. Environmental challenges have caused food-borne bacterial pathogens to evolve and the susceptibility of human population to infections are also changing due to declining acquired immunity and increased numbers of immunocompromised individuals [7].

There has been a continuous increase in several foodborne diseases caused by bacterial pathogens such as *Salmonella*, *Campylobacter*, *Escherichia coli* and *Listeria* despite the employment of modern technologies to inactivate these pathogens in food. These pathogens come into contact with foods during harvest or slaughtering, processing, storage and packaging. Physical treatments such as UV light, high pressure, dry heat and steam are viable strategies of reducing pathogenic bacteria in raw products. These methods have been considered because the use of antibiotics has been restricted over the years due to the risk of antibiotic-resistant bacteria entering the human food chain and causing negative impact on human antimicrobial treatment. However, physical methods of reduction of microbial load in raw foods have been known to negatively impact the organoleptic properties of the products hence reducing their acceptability. There has thus been an increasing need to develop novel strategies to reduce bacterial pathogens in foods and still satisfy consumer demand for minimally processed foods with low concentrations of chemical preservatives [4].

Bacteriophages (phages) have found use as natural antimicrobials that can be used in controlling bacterial pathogens in foods and food processing environments. Hagens and Loessner [8] listed the nine desirable properties of phages for use as biocontrol agents in foods. We hereby discuss in detail these properties and how they influence the use of phages as biocontrol agents in different foods. Current literature available on phage-based biocontrol and biosanitization strategies used to reduce foodborne pathogens at different stages of the "farm-to-fork" continuum will also be discussed.

2. Bacteriophage research; historical overview

Evidence concerning the existence of phages can be traced to reports dating as far back as the late nineteenth century [9]. However, the actual discovery of these agents of bacterial death is credited to pioneering work by Félix d'Hérelle, and Fredrick W. Twort [1, 2]. The particulate nature of phages remained a controversial topic until they were directly visualized by electron microscopy in 1940 [10, 11]. Interest in the biological nature of phages was overshadowed by the attention to their therapeutic potential. The observation by d'Hérelle's that phage titres increased in stool samples from dysentery patients led to a large following on the commercial and medicinal possibilities of phages. A note authored by Bruynoghe and Maisin [12], was the first report on the therapeutic use of phages. Other subsequent documented trials of phage application in human medicine, in animals and agribusiness are reviewed in Kutter and Sulakvelidze [3]. The initial enthusiasm in embracing phage therapy was confronted with critical scepticism which led to its subsequent abandonment in some parts of the world. The main cause of concern on the therapeutic use of phages arose from the fact that the biological nature of bacteriophages was poorly understood and the lack of standards for purity and potency of phages in remedies made it impossible to compare results from successful as well as failed studies published at the time [13, 14).

The documentation of therapeutic properties of penicillin [15] heralded the "antibiotic era" which was characterized by subsequent discovery and extensive use of many different antibiotics. Antibiotics were found to be more favourable, than phages, for use in clinical settings owing to their broader specificity. Due to these developments, many western phage researchers shifted their focus from medicine to the fundamental nature of phages, thereby laying ground for the nascence of the disciplines of molecular biology and microbial genetics. Phages contributed to our understanding of bacterial cellular processes and also to the development of a of important genetic and biochemical tools [15, 16].

In recent years, there has been an increased interest in phage therapy due to the emergence of an alarming number of antibiotic-resistant bacteria. This is evident from the upsurge of research papers and review articles on this aspect of phage research [3]. By and large the interest in phage research has been renewed by three recent trends: (i) phages have potential use against antibiotic-resistant bacterial pathogens, (ii) they influence cycling of organic matter [17, 18] and (iii) they have a major impact on bacterial gene evolution thereby contributing in the evolution, physiology and pathogenicity of their hosts [19, 20].

3. Basic phage biology: Lytic versus temperate phages

Bacteriophages, just like all other viruses, are obligate parasites. They are ubiquitous in nature and form the most numerically abundant biological entities on earth with an estimated population of about 10^{30} to 10^{32} phage particles - this makes them key contributors in the regulation of the microbial balance in every ecosystem [21, 22].

Phages can be divided into two main classes based on their life cycle: lytic or temperate. Virulent phages can only multiply by means of a lytic life cycle which leads exclusively to host cell lysis and the release of progeny phage particles. On the other hand, infection by temperate phages may occur via two alternate pathways: (i) the lytic pathway whereby infection leads to propagation and lysis of the host cell, or (ii) lysogenization in which the phage lytic functions are repressed and its genome coexists in a stable form within its host. In the latter case, the phage genome assumes a quiescent state; either integrated into the bacterial chromosome (as a prophage) or remains separate, as a 'plasmid'. The prophages are replicated and transmitted to daughter cells upon cell division. Host cells harbouring the prophages are capable of coming out of the quiescent state and enter the lytic pathway (Fig. 1).

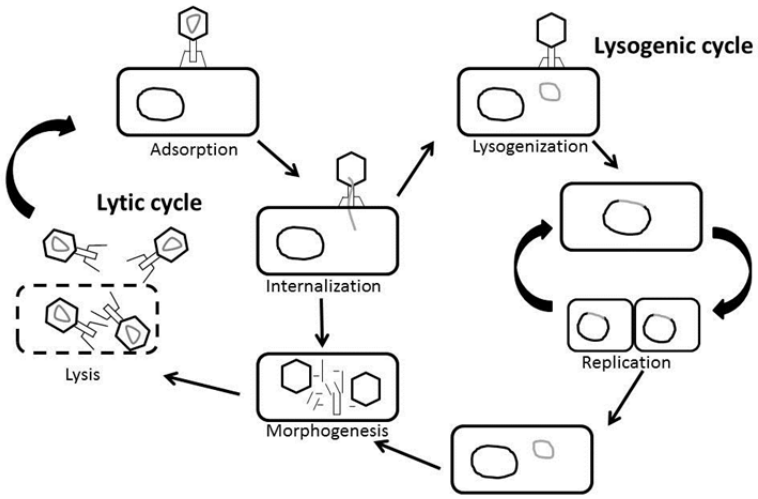

Fig. 1 Lytic and temperate bacteriophage replication cycles. The bacteriophage recognizes the receptors on the host cell membrane surface and adsorbs using its tail and tail fiber proteins. This is then followed by internalization of the phage genome after which the cycle proceeds to either the lysogenic or lytic replication cycles.

3.1. Lytic cycle of phages

3.1.1. Adsorption

The first step in a bacteriophage lytic cycle (Fig. 1) is the interaction between the phage and specific binding site (receptor) on the cell surface. The presence or absence of attachment moieties is a determinant of phage host range. Phages of Gram-negative bacteria may interact with components of the lipopolysaccharides (LPS, endotoxin), with outer membrane proteins (OMP) or may have complex adhesins that recognize multiple receptors [23-25]. Phages of Gram-positive bacteria recognize one or several molecules e.g. teichoic acid which are embedded in the layers of peptidoglycan that form the outer wall of these bacteria.

The infection of Gram-negative bacteria by tailed phages has been observed to follow almost the same general scheme: the tail fibres make first contact with the cell surface. This binding is followed by specific and irreversible binding of one of the proteins located at the tip of the tail to a surface membrane component. Phage binding triggers conformational changes that are transmitted along the tail to the connector allowing its opening and release of the viral genome from the capsid.

The concentration and spatial orientation of the receptor molecule are critical for phage recognition and adsorption. External factors such as requirement of specific cofactors e.g. divalent cations and the bacterial host physiological state also play a key role on the adsorption process [26]. Loss of some surface molecules used by phages as receptors may result in bacterial loss of important functions or lack of competitiveness. This is especially the case if the surface molecules are essential for the bacterial cell growth under particular environmental conditions.

Even though bacteria may develop resistance through alteration or loss of receptors, phages can acquire compensating adaptation through mutations that may recognize the changed cell surface component or enable them to bind to a different receptor.

3.1.2. Penetration

Each phage has its own unique system of transferring its genome into the host cell; however, a general mechanism involves the tail tip which possesses an enzyme for penetrating the peptidoglycan layer to release DNA into the cell [27]. For some phages, entry of DNA is dependent on host cellular biochemistry as has been demonstrated in T7 [28].

3.1.3. Replication of phage genome

Phages overcome susceptibility to exonucleases and restriction enzymes by circularizing their genomes, inhibition of host nucleases or use of modified nucleotides in their DNA [29, 30].

The recognition of phage promoters by the host RNA polymerase leads to transcription of early genes whose products play the role of protecting the phage genome and convert the host environment appropriately for the needs of the phage [31]. In certain cases, phage proteins may be injected into the host cell with the phage genome [32]. Replication of phage genome commences upon establishment of optimal host environment conditions. Lytic phages have been found to often encode their own replication mechanism as opposed to the case in temperate and small lytic phages which depend on the host cell for their replication [33].

3.1.4. Morphogenesis and cell lysis

Morphogenesis involves the initial assembly of new phage heads and capsids (or protein shells called procapsids) involving complex interactions between scaffolding proteins and the major head structural proteins. This process precedes the packaging of replicated and concatemized phage genomes [33]. The timing of the lysis of host bacteria is tightly controlled [34] with most tailed phages using a two component lysin-holin system. The lysin is an enzyme that cleaves key bonds in the peptidoglycan and the holin is a protein that assembles pores in the inner membrane to allow the timely access of the peptidoglycan by the lysin [33]. Lysis of the host cell and the concomitant release of progeny phages forms the final stage in the lytic cycle (Fig. 1).

4. Desirable properties of phages used as biocontrol agents in foods

Bacteriophage-mediated applications in the "farm-to-fork" continuum can be found in i) phage therapy through the prevention or reduction of colonization and diseases in livestock; ii) phage biosanitation, in the decontamination of carcasses and other raw products, such as fresh fruit and vegetables, and disinfection of equipment and contact surfaces; and, iii) phage biopreservation in the extension of the shelf life of perishable manufactured foods [4]. In their use to improve of food safety, virulent phages are applied onto foods that may be contaminated with bacterial pathogens, leading to the elimination of the latter. Through the reduction in pathogen numbers, the phage makes the food safe for consumption.

In their review, Hagens and Loessner [8] listed some of the desirable properties of phages that are meant for use as biocontrol agents in foods. We herein highlight these properties and discuss them in detail in order of significance with regards to their relevance in selecting a suitable phage for biocontrol in foods.

4.1. Phage should be strictly lytic

Only virulent phages should be used as biocontrol agents in foods. Temperate phages are not desirable because they may contain within their genome particular genes that may change the phenotype of their bacterial host (lysogenic conversion genes: LCG). Phage conversion may result in phage-encoded genes converting their bacterial host from a non-pathogenic strain to virulent strain. Through lysogenic conversion, temperate phages have played a major role in the evolution of new human foodborne pathogens. Virulence genes in several foodborne pathogen species are commonly found in bacteriophages and bacteriophage remnants. Examples include: Shiga toxin 1 from phages H-19B and VT2-sakai in bacterial hosts *Escherichia coli*, Cholera toxin (*ctxAB*) from phage CTXϕ in bacterial host *Vibrio cholera* [35-38].

When selecting virulent phages for biocontrol in food, clear plaque-forming phages should be chosen. In addition, in order to confirm that a particular phage is strictly lytic, its DNA should be sequenced and the genome carefully screened for the presence of integrase and lysogeny-related genes.

4.2. Phage should have a broad host range

Host specificity is generally found at strain level, species level, or, more rarely, genus level or class level. Specificity allows for directed targeting of pathogenic bacteria using phages. The high specificity of phages makes them ideal biocontrol agents as they do not result in the eradication of useful microbiota like many other antimicrobials. A phage with a broad host range capable of infecting many strains of the target species and/or genus is desirable for biocontrol applications in food [16]. An example of such a phage is the commercially available anti-*Listeria* phage preparation LISTEX™P100 (produced by MICREOS Food safety, Inc, Wageningen, The Netherlands) which is able to infect and kill a majority of *Listeria monocytogenes* strains, and has found application as an antimicrobial in a number of ready-to-eat (RTE) products and raw fish [39-43]. Other examples of broad host range phages are Felix O1 [44-46] and φS1 [47] which lyse almost all *Salmonella* serotypes and a broad range of fluorescent pseudomonads, respectively.

A cocktail of phages with different host spectra can also be used in an effort to achieve broader host range and also to overcome of host resistance due to for example altering of receptor by host bacteria. In some cases, the host has to lose its viability altogether so as to gain resistance against all the phages in the cocktail. An example of a commercially available phage cocktail preparation is ListShield™ (produced by Intralytix, Baltimore, U.S.A.): a six-phage cocktail that lyses a large number of *L. monocytogenes* strains with various serotypes [48].

4.3. Determine the complete genome sequence of phages

The complete and annotated genome sequence of phages intended for use as a biocontrol agent in food will provide information on whether or not the phage encodes any proteins that presents a potential health risk. The phages will also be screened for the presence of laterally transferable virulence and antimicrobial resistance determinants on the basis of homologies with known virulence and resistance genes available in GenBank. As mentioned in section 4.1, information

from the genomic sequences will confirm whether or not the phages are strictly lytic by ruling out the presence of integrase and lysogeny-related genes. Carlton *et al*. [39], sequenced phage P100 as part of the process of determining its safety for use as a biocontrol agent against *L. monocytogenes* in various food products.

4.4. Lack of transduction of bacterial DNA

In generalized transduction, any gene within the infected host bacterium (donor) can be transferred to the new host bacterium (recipient) by either a lytic or temperate phage. However, lytic phages display negligible rates of transduction. In the process of generalized transduction, a few defective phage particles (transducing particles) are produced when fragments of degraded host genome are accidentally packaged by phage head assembly system. The transducing particles cannot initiate normal infection (as they lack viral DNA), but can deliver the host bacterial DNA to a recipient bacterium's cytoplasm. If the bacterial DNA delivered by the phage recombines with the recipient bacterium DNA and the genes expressed, it may result in change in the recipient bacterium's phenotype [38].

Specialized transduction is more efficient than generalized transduction. It is carried out solely by temperate phages. The phage incorporates its genome into the same point in the bacterial genome. Upon induction, the integrated prophage excises from the host genome. However, in case of imprecise excision, there may be co-packaging of adjacent sections of host genome in the daughter phages. These host-gene-encoding phages (transducing particles) have the potential to integrate discrete bacterial genetic material along with phage genome into new host's genome.

E. coli are example of bacterial species whereby temperate phages transfer the toxin *stx* genes to new hosts and therefore creating new types of Shiga toxin-producing *E. coli* [49-52]. Thus in addition to screening for undesirable genes (e. g., bacterial virulence genes) by genome sequencing, evaluation of candidate phages for use as biocontrol in foods there is need to include testing for transduction [53].

4.5. Phage should be propagated on non-pathogenic host

If the host employed for propagation is non-pathogenic or even having a Generally Recognized as Safe (GRAS) status, then the phenomenon of generalized transduction is unproblematic. In addition, a non-pathogenic host renders large scale propagation and purification of the phage easier.

4.6. Oral feeding studies of phages should show no adverse effects

All available evidence indicates that oral consumption of phages (even at high levels) is entirely harmless to humans. Safety studies of *Listeria* phage P100, in which rats were fed high doses of phages with no measurable effects compared to control group [39]. A study with *E. coli* phages both in mice and human volunteers showed no significant effects on test subjects [54, 55].

4.7. Phage preparation should be stable over storage and application

Physiochemical conditions (e.g., pH and water activity) of the food to which the phages are applied may affect the stability of phages. It is important that the phages remain stable under these conditions for them to be successful in the biocontrol of foodborne pathogens on the foods to which they are applied. Most phages are stable at pH range between 4 and 10 [56]. A study aimed at controlling *L. monocytogenes* on fruit found that the titre of a phage cocktail remained stable for a longer period on melon slices but declined rapidly on apple slices which have a lower pH [48]. The effect of other physiochemical factors such as temperature, visible and UV light, osmotic shock and pressure are reviewed in detail in [57].

4.8. Phage should be amenable to scale up for commercial production

Several factors come into play when phage have to be produced at industrial scale e.g. using a continuous flow reactor vessel whereby conditions of temperature, culture solution composition and resident time determine whether the phage will replicate and increase in concentration during production [58]. More importantly factors that influence the rate and efficiency of phage adsorption to host bacteria such as temperature, pressure, concentration of phage and bacteria are critical to achieve large scale replication. Phages which have poor adsorption properties [59] may pose a challenge in batch culture production.

5. Phage biocontrol of food-borne pathogens

5.1. *Escherichia coli*

Even though a majority of the members of the species *Escherichia coli* are commensals and can be found in the gastrointestinal tracts of animals and humans, there exist pathogenic strains that cause enteric disease in humans and/or animals. These include Verocytotoxin-producing *E. coli* (VTEC) also known as Shiga toxin-producing *E. coli* (STEC),

enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli*, and enteroaggregative *E. coli* [60]. Majority of VTEC infections in humans are associated with serotype O157:H7 [61]. Phages may be used as biocontrol agents against infection by *E. coli* O157:H7 which typically occurs through ingestion of contaminated food or water, through direct contact with animals, or via person-to-person transmission. Some of the recent pre- and postharvest phage research targeting *E. coli* are discussed herein.

5.1.1. Pre-harvest *E. coli* O157:H7 control

In the pre-harvest approach to control *E. coli* O157:H7, the aim is to treat live food animals with phages and thus reduce the *E. coli* O157:H7 populations before entering the food chain. Where possible, phages can be used in conjunction with other methods to create a multiple barriers.

Bach *et al* [62] assessed the ability of phage to control *E. coli* O157:H7 in sheep by giving oral treatment of phage DC22 to lambs experimentally infected with the bacteria. The single dose of phage DC22 did not clear the pathogen even though it had been shown to be effective in an artificial rumen system. In another study, sheep receiving a single oral dose of phage CEV1 showed a 2-log reduction in intestinal *E. coli* O157:H7 within 2 days compared to levels in the controls [63]. The same research group did another phage therapy trial against *E. coli* O157:H7 in sheep using a cocktail of phages CEV1 and CEV2. The cocktail when administered once orally to the sheep was more effective in removing resident *E. coli* O157:H7 than CEV1 alone [64].

As the primary site of *E. coli* colonization in cattle is the recto-anal junction (RAJ), Sheng *et al*. [65], investigated the ability of phages to eliminate the bacteria in steers by applying the phages directly to the rectoanal junction mucosa. During the same period, a concentration of 10^6 PFU/ml of the phage was also maintained in the cattle's drinking water. This strategy achieved reduced numbers of *E. coli* O157:H7 among phage treated steers compared to control steers. A similar study [66] comparing oral and rectal administration of *E. coli* O157:H7-specific phage cocktail to steers found that oral treatment with the phages was more effective than rectal and rectal-oral administered phage for controlling the fecal shedding of the bacteria.

5.1.2. Post-harvest *E. coli* O157:H7 control

In a study by O'Flynn *et al.* [67], a cocktail of three lytic phages, e11/2, e4/1 and pp01 were evaluated for their ability to reduce *E. coli* O157:H7 on experimentally contaminated meat surface. The phages were able to completely eliminate the bacteria in seven out of the nine samples tested. Abuladze *et al*. [68] carried out a study to determine whether the treatment with *E. coli* O157:H7-specific phage cocktail significantly reduces the bacteria on experimentally contaminated surfaces and various foods. There was significant reduction of *E. coli* O157:H7 recovered from glass cover slips and gypsum board surfaces. The phage cocktail also significantly reduced number of viable *E. coli* O157:H7 on tomato, spinach, broccoli and ground beef.

Sharma *et al.* [69] also carried out a study to assess the efficacy of a *E.* coli O157:H7-specific phage cocktail (ECP-100) in reducing the bacteria on contaminated fresh-cut iceberg lettuce and cantaloupe. There was a significant reduction of the *E.* coli O157:H7 populations on both lettuce and cantaloupe for samples stored at 4 °C, when compared to the controls.

In a recent study by Carter *et al*. [70], the commercially available phage preparation, EcoShield™, significantly reduced the levels of *E. coli* O157:H7 in experimentally contaminated beef and lettuce. However, the one-time application of EcoShield™ did not protect the foods from contamination with *E. coli* O157:H7. This demonstrated that even though EcoShield™ was effective in reducing *E. coli* O157:H7 contamination on beef and lettuce, it does not protect against potential later contamination, for example, due to unsanitary post processing handling of the foods.

In a study to determine the effect of the *E. coli* O157:H7-specific phage cocktail, BEC8, on the *E. coli* O157:H7 strains applied on materials used in food processing surfaces, the phage cocktail was effective within an hour against low levels of the bacteria at above room temperature on all the surfaces tested [71].

Phages in combination with the essential oil *trans*-cinnamaldehyde (TC) have also been assayed for their efficacy in reducing *E. coli* O157:H7 on lettuce and spinach [72]. It was found that the phage cocktail-TC combination was highly effective on both leafy greens.

5.2. *Salmonella*

Salmonella spp. are considered one of the major causes of zoonotic diseases worldwide. This is because they can colonise a wide range of hosts including all major livestock species, eventually producing contaminated meat and other food products [73]. In the USA, more than 50% of salmonellosis is caused by only three *Salmonella enterica* serovars; *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Newport [74]. *Salmonella* Typhimurium causes non-typhoid salmonellosis, a disease characterized by abdominal pain, nausea, diarrhea and even life threatening infections [75]. About 40,000 cases of non-typhoid salmonellosis per year are reported in the USA [74]. An increase in salmonellosis in the developed world has been due to *Salmonella* Enteritidis, which is linked to eggs and poultry [73].

5.2.1. Pre-harvest *Salmonella* control

One of the most common *in vivo* models for the study of the therapeutic value of phages is the treatment of *Salmonella*-infected laboratory animals. One of the recent studies in pre-harvest *Salmonella* phage applications is by Atterbury *et al*. [76] who selected three *Salmonella* phages with the broadest host ranges from a collection of 232 for phage therapy in broiler chicken experimentally colonized with *Salmonella*. Orally administered phage φ25 and φ151 resulted in a reduction of 2.19 log and 4.2 log, respectively of *Salmonella.* Andreatti *et al.*[77] evaluated the efficacy of a cocktail of four different bacteriophages obtained from commercial broiler houses (CB4Ø) and 45 bacteriophages from a municipal wastewater treatment plant (WT45Ø) to reduce *Salmonella* Enteritidis in experimentally infected chicks. The study found that the chicks treated both cloacally and orally with the phage cocktail had significant reduction of *Salmonella* Enteritidis in the cecal tonsils at 24 h as compared with untreated controls, but no significant differences were observed at 48 h following treatment. In another study aimed at using phages to reduce *Salmonella* Enteritidis colonization in chicken, a cocktail of three phages (BP1, BP2, and BP3) was administered by either coarse spray or in drinking water prior to *Salmonella* Enteritidis challenge. Phage delivery both by coarse spray and drinking water reduced the intestinal *Salmonella* Enteritidis colonization [78].

In an experiment with swine, treatment with the anti-*Salmonella* phage cocktail significantly reduced cecal *Salmonella* concentrations (95%) while also reducing ileal Salmonella concentrations (90%) [79]. In another study with weaned pigs, orally delivered phage cocktail significantly reduced the bacterial CFU in the rectum [80].

5.2.2. Post-harvest *Salmonella* control

Anti-*Salmonella* phages have been investigated for use as biocontrol agents to reduce *Salmonella* contamination on fresh produce (e.g. fresh-cut melon, apple, sprouts and tomatoes), meat, ready-to-eat foods, cheese and milk [81-86]. Modi *et al.* [81] investigated the ability of *Salmonella* Enteritidis to survive in the presence of phage, SJ2, during manufacture, ripening, and storage of Cheddar cheese produced from raw and pasteurized milk. This bacterium survived in raw milk and pasteurized milk cheese without phage but did not survive in pasteurized milk cheese after 89 days in the presence of phage. Leverentz *et al*. [82] examined the effect of lytic, *Salmonella*-specific phages on reducing *Salmonella* in experimentally contaminated fresh-cut melons and apples stored at various temperatures. The phage cocktail reduced *Salmonella* populations on honeydew melon slices but did not significantly reduce *Salmonella* populations on the apple slices. A study to investigate whether there was any difference in efficacy of wild type phage Felix O1 and its clear plaque producing variant (PL) in suppressing *Salmonella* Typhimurium DT104 was performed on chicken frankfurters by Wichard *et al*. [83]. Suppression levels of 1.8 and 2.1 log units were achieved with wild type Felix O1 and LP, respectively.

In a study to evaluate the potential for using bacteriophages to control *Salmonella* in sprouting seeds, phage A reduced *Salmonella* CFU by 1.37 log on mustard seeds and a mixture of phage-A and phage-B caused a 1.50 log suppression of *Salmonella* growth in the soaking water of broccoli seeds [84]. Higgins *et al*. [85] inoculated broiler carcasses with *Salmonella* Enteritidis and then sprayed with selected concentrations of phage PHL 4. The samples were then rinsed for *Salmonella* Enteritidis enrichment and isolation. Phage PHL 4 treatments reduced the frequency of *Salmonella* Enteritidis recovery as compared with controls.

Guenther *et al*. [87] evaluated the reduction of *Salmonella* Typhimurium in different RTE foods by broad host range, virulent phage FO1-E2. At 8 °C, the phage FO1-E2 application resulted in more than 3 log unit reduction leading to no viable cells. At 15 °C, application of phage lowered *Salmonella* Typhimurium counts by 5 log units on turkey deli meat and in chocolate milk, and by 3 logs on hot dogs.

5.3. *Campylobacter*

Campylobacter is a genus of gram-negative, spiral, motile, and microaerophilic bacteria, whose species have emerged over the last three decades as significant clinical pathogens. Acute bacterial enteritis due to these organisms is particularly of human public health concern. Two species, *C. jejuni* and *C. coli*, are responsible for most of these gastrointestinal-related infections [88]. Phages have been used successfully as pre and post-harvest interventions to reduce *Campylobacter* contamination [89].

5.3.1. Pre-harvest *Campylobacter* control

Bacteriophage therapy is one possible means by which this colonization of chicken by *C. jejuni* could be controlled, thus limiting its entry the human food chain. Loc Carrillo *et al*. [90], orally administered phages, CP8 and CP34, to 25-day-old broiler chickens experimentally colonized with the *C. jejuni* broiler isolates. Phage treatment of *C. jejuni*-colonized birds resulted in *Campylobacter* counts falling between 0.5 and 5 log CFU/g of cecal contents compared to untreated controls over a 5-day period post administration. Phages have also been tested to establish their effectiveness in the control of *C. jejuni* colonization in young broilers, either as a preventive or a therapeutic measure. In such a study, Wagenaar *et al*. [91] established that the phages caused an initial 3 log decline in *C. jejuni* counts in the therapeutic group and in the prevention group, colonization of *C. jejuni* was delayed. El-Shibiny *et al.* [92],

administered phage CP220 to both *C. jejuni*- and *C. coli*-colonized broiler chickens. A 2-log 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 PFU oral dose of CP220. In another phage therapy study, a phage cocktail administered by oral gavage and in feed to broiler chickens was able to reduce the titre of both *C. coli* and *C. jejuni* in faeces by approximately 2 log cfu/g [93].

5.3.2. Post-harvest *Campylobacter* control

Chicken skin pieces inoculated with approximately 10^4 CFU/cm^2 of *C. jejuni* strain C222 and treated with phage 12673 at an approximate density of 10^6 PFU/cm^2 reduced the recovered *Campylobacter* counts by approximately 95% [94]. In another study to determine the efficacy of phages to reduce the number of recoverable *C. jejuni* on artificially contaminated chicken skin, phage ϕ2 was found more effective in reducing the recoverable *C. jejuni* in frozen chicken and at a high concentration of the phage (10^7 PFU). The researchers in the study recommended combining freezing and phage treatment to ensure further falls in *Campylobacter* prevalence on broiler carcasses by [95].

5.4. *Listeria monocytogenes*

Listeria monocytogenes is an opportunistic pathogen that has become a major foodborne pathogen of public health concern with a high mortality rate in at risk individuals such as pregnant women, neonates, immunocompromised individuals and the elderly [96]. This bacterium has the ability to grow at a wide range of temperatures (0.4 °C to 45 °C) and pH values (4 to 9.6) enabling it to persist within food processing environments for long periods [97, 98].

Phage research into the control of *L. monocytogenes* has focussed on post-harvest applications, some of which we discuss here.

5.4.1. Post-harvest *Listeria* control

Leverentz *et al.* [48] examined the effect of *L. monocytogenes*-specific phages on *L. monocytogenes* populations in artificially contaminated fresh-cut melons and apples. The phage mixture reduced *L. monocytogenes* CFU reduction by 2.0 to 4.6 log units on honeydew melons and by only 0.4 log units on apples. The phage mixture in combination with nisin reduced *L. monocytogenes* populations by up to 5.7 log units on honeydew melon slices and by up to 2.3 log units on apple slices. It was suggested that a possible reduced efficacy of the phage on the apple slices may be the low pH on the cut surface of the apple.

Carlton *et al.* [39] determined the efficacy of phage P100 to reduce *Listeria* contamination on surface-ripened red-smear soft cheese. Cheeses were contaminated with low concentrations of *L. monocytogenes* at the beginning of the ripening period, and P100 was applied to the surface during the rind washings. A significant reduction of at least 3.5 logs or a complete eradication of *Listeria* viable counts was obtained. By using phage P100 in addition to protective cultures in a multiple hurdle approach, Holck and Berg [99] were able to overcome outgrowth of surviving *L. monocytogenes* cells on ham after phage P100 treatment. Guenther *et al.* [100] carried out a series of elegant experiments to evaluate the efficacy of the virulent, broad-host-range phages A511 and P100 for control of *L. monocytogenes* in different ready-to-eat foods In liquid foods (chocolate milk and mozzarella cheese brine), the bacterial CFU rapidly dropped below the level of direct detection. On solid foods (hot dogs, sliced turkey meat, smoked salmon, seafood, sliced cabbage, and lettuce leaves), phages reduced bacterial CFU by up to 5 log units. Phage P100 has also been found to be effective in inhibition of *L. monocytogenes* on raw salmon fillet and able to reduce CFU on catfish fillet [42. 43].

6. Bacteriophages in education

Bacteriophages are the ideal tool for training young molecular biologists/virologists in manipulative skills and in genomics, bioinformatics, and proteomics; and, in evolutionary biology. As a result they have attracted considerable academic interest, since one can go from enrichment [101] and isolation [102-104] to fully annotated genome within a few weeks. Furthermore, if host pathogenesis is a problem, there are hosts such as *E. coli* K12, *Bacillus subtilis* and *Mycobacterium smegmatis* which are essentially nonpathogenic. In the case of rapid growing bacterial hosts, phage titrations can be set up in the morning and the phage plaques counted in the afternoon.

Perfect examples of this are the Howard Hughes Medical Institute's Science Education Alliance program funded projects: Phage Hunters Advancing Genomics and Evolutionary Science (PHAGES) and Phage Hunters Integrating Research and Education (PHIRE). In the United States approximately 60 institutions are involved in in these programs resulting in the full characterization of hundreds of *M. smegmatis* phages [105]. Other institutions in the North America have instituted smaller programs in characterizing *Burkholderia* (Texas A&M University) and *Bacillus* phages (University of Guelph). In Europe the German Collection of Microorganisms and Cell Cultures (DSMZ) is partnering with universities on Project Phage Trapper (https://www.dsmz.de/research/microorganisms/projects/phage-trapper-project.html). In all of these cases association with a sequencing centre, permitting inexpensive sequencing is essential. Most of the latter will, by request, assemble the full sequence, but the submitter should carefully check the final

assembly for orientation and frameshifts prior to annotation. For those who want to assemble their own primary sequence data information a number of software packages can be found [106, 107]. Several software companies, such as DNADragon (http://www.sequentix.de), offer fully functional free trials on their assembly software.

There are some excellent free packages for genome annotation available: three of these are Artemis (http://www.sanger.ac.uk/resources/software/artemis/), MyRAST (http://blog.theseed.org/servers/installation/distribution-of-the-seed-server-packages.html) and Unipro UGENE (http://ugene.unipro.ru/). The Internet also provides additional free resources for full characterization of genes, proteins and tRNAs, as well as tools for discovering promoters and terminators. A good place to start is Online Analysis Tools (http://molbiol-tools.ca).

Their small sized genomes permit the rapid acquisition of publishable data plus offer opportunities in the area of industrialization through potential development of (a) useful vectors for genetic manipulations in natural hosts rather than *E. coli* and (b) novel enzymes for biotechnological or recombinant DNA processes.

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References

[1] Twort FW. An investigation on the nature of ultra-microscopic viruses. *Lancet.* 1915; II :1241–1243.
[2] d'Herelle, F. Sur un microbe invisible antagoniste des bacilles dysentériques. *Comptes rendus de l'Académie des Sciences*. 1917; 165: 373–375.
[3] Kutter E, Sulakvelidze A. Introduction. In: Kutter E, Sulakvelidze A, ed. *Bacteriophages, biology and applications*. Boca Raton: CRC press; 2006: 1-4.
[4] García P, Martínez B, Obeso, JM, Rodríguez A. Bacteriophages and their application in food safety. *Letters in Applied Microbiology*. 2008; 47:479-485.
[5] WHO. WHO Initiative to estimate the global burden of foodborne diseases. 2008.
[6] Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV. Food-related illness and death in the United States. *Emerging Infectious Diseases*.1999; 5: 607-25.
[7] Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, Opsteegh M, Langelaar M, Threfall J, Scheutz F, van der Gieessen J, Kruse H. Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*. 2010; 139(Suppl 1), S3–S15.
[8] Hagens S, Loessner MJ. Bacteriophage for biocontrol of foodborne pathogens: calculations and considerations. *Current Pharmaceutical Biotechnology*. 2010; 11:58-68.
[9] Hankin EH. L'action bactericide des eaux de la Jumna et du Gange sur le vibrion du cholera. *Annales de l'Institut Pasteur Microbiology.* 1896; 10: 511.
[10] Pfankuch E, Kauche GA. Isolierung und übermikroskopische abbildung eines bakteriophagen. *Naturwissenshaften.* 1940; 28: 46.
[11] Ruska H. Die sichtbarmachung der bakteriophagen lyse im ubermikroskop. *Naturwissenschaften*. 1940; 28: 45.
[12] Bruynoghe R, Maisin J. Essais de thérapeutique au moyen du bactériophage du Staphylocoque. *J Compt Rend Soc Biol.* 1921; 85: 1120-1121.
[13] Eaton MD, Bayne-Jones S. Bacteriophage therapy. Review of the principles and results of the use of bacteriophage in the treatment of infections. *Journal of American Medical Association.* 1934; 23: 1769-1939.
[14] Summers WC. Bacteriophage therapy. *Annual Reviews in Microbiology.* 2001; 55: 437-451.
[15] Chain E, Florey HW, Gardner AD, Heatley NG, Jennings MA, Orr-Ewing J, Sanders AG. Penicillin as chemotherapeutic agent. *Lancet.* 1940; 2: 226.
[16] Kropinski AM. 2006. Phage therapy-everything old is new again. *Canadian Journal of Infectious Diseases and Medical Microbiology*. 17: 297–306.
[17] Fuhrman JA. Marine viruses and their biogeochemical and ecological effects. *Nature*. 1999; 399: 541-548.
[18] Canchaya C, Proux C, Fournous G, Bruttin A, Brussow H. Prophage genomics. *Microbiology and Molecular Biology Reviews*. 2003; 67: 238-276.
[19] Dobrindt U, Reidl J. Pathogenicity islands and phage conversion: evolutionary aspects of bacterial pathogenesis. *International Journal of Medical Microbiology*. 2000; 290: 519-527.
[20] Boyd EF, Davis BM, Hochhut B. Bacteriophage-bacteriophage interactions in the evolution of pathogenic bacteria. *Trends in Microbiology*. 2001; 9: 137-144.
[21] Bergh O, Borsheim KY, Bratbak G, Heldal M. High abundance of viruses found in aquatic environments. *Nature.* 1989; 340: 467–468.
[22] Brussow H, Kutter E. Phage ecology. In: Kutter E, Sulakvelidze A, eds. Bacteriophages, biology and applications. Boca Raton; CRC press; 2005: 129-164.
[23] Yokota S, Hayadhi T, Matasumoto H. Identification of the lipopolysaccharide core region as receptor site for cytotoxin-converting phage, phi CTX, of *Pseudomonas aeruginosa*. *Journal of Bacteriology*. 1994; 176: 5262-5269.
[24] Tetart F, Desplats C, Krisch HM. Genome plasticity in the distal tail fiber locus of T-even bacteriophage: Recombination between conserved motifs swaps adhesion specificity. *Journal of Molecular Biology.* 1998; 282: 543-556.
[25] Wang J, Hofnung M, and Charbit A. The C-terminal portion of the tail fiber protein of bacteriophage lambda is responsible for binding to LamB, its receptor at the surface of *Escherichia coli* K-12. *Journal of Bacteriology*. 2000; 182: 508–512.
[26] Guttman B, Raya P, Kutter E. Basic phage biology. In: Kutter E, Sulakvelidze A, eds. Bacteriophages, biology and applications. Boca Raton; CRC press; 2005: 29-66.

[27] Molineux I J. No syringes please, ejection of phage T7 from virion is enzyme driven. *Molecular Microbiology*. 2001. 40: 1-8.
[28] Moffatt BA, Studier F W. Entry of bacteriophage T7 DNA into the cell and escape from host restriction. *Journal of Bacteriology*. 1988; 170: 2095-2105.
[29] Arber W, Dussoix D. Host specificity of DNA produced by *Escherichia coli.* I. Host controlled modification of bacteriophage λ. *Journal of Molecular Biology*. 1962; 5: 18-36.
[30] Dussoix D, Arber W. Host specificity of DNA produced by *Escherichia coli.* II. Control over acceptance of DNA from infecting phage λ. *Journal of Molecular Biology*. 1962; 5: 37-49.
[31] Duckworth DH. Biological activity of bacteriophage ghosts and "take-over" of host functions by bacteriophage. *Bacteriological Reviews*. 1970; 34: 344-363.
[32] Kutter E, Guttman B, Carlson K. Transition from phage to host metabolism after T4 infection. In: Karam, JD, ed. Molecular biology of Bacteriophage T4. Washington, D.C .; American Society of Microbiology; 1994: 343-346.
[33] Kutter E, Raya P, Carlson K. Molecular mechanisms of phage infection In: Kutter E, Sulakvelidze A, eds. Bacteriophages, biology and applications. Boca Raton; CRC press; 2005: 165-222.
[34] Wang IN, Deaton J, Young R. Sizing the holing lesion with an endolysin-beta-galactosidase fusion. *Journal of Bacteriology*. 2003; 185: 779-787.
[35] De Grandis S, Ginsberg J, Toone M, Climie S, Friesen J, Brunton J. Nucleotide sequence and promoter mapping of the *Escherichia coli* Shiga-like toxin operon of bacteriophage H-19B. *Journal of Bacteriology*. 1987; 169: 4313-4319.
[36] Makino K, Yokoyama K, Kubota Y, Yutsudo CH, Kimura S, Kurokawa K, Ishii K, Hattori M, Tatsuno I, Abe H, Iida T, Yamamoto K, Onishi M, Hayashi T, Yasunaga T, Honda T, Sasakawa C, Shinagawa H. Complete nucleotide sequence of the prophage VT2-Sakai carrying the verotoxin 2 genes of the enterohemorrhagic *Escherichia coli* O157:H7 derived from the Sakai outbreak. *Genes and Genetic systems*. 1999; 74: 227-239.
[37] Faruque SM, Albert MJ, Mekalanos, JJ. Epidemiology, Genetics, and Ecology of Toxigenic *Vibrio cholerae*. *Microbiology and Molecular Biology Reviews*.1998; 62: 1301-1314.
[38] Brabban AD, Hite E, Callaway, TR. Evolution of Foodborne Pathogens via Temperate Bacteriophage-Mediated Gene Transfer. *Foodborne Pathogens and Disease*. 2005; 2: 287-303.
[39] Carlton RM, Noordman WH, Biswas B, de Meester ED, Loessner MJ. Bacteriophage P100 for control of Listeria monocytogenes in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. *Regulatory Toxicology and Pharmacology*. 2005; 43: 301-312.
[40] Guenther S, Huwyler D, Richard S, Loessner MJ. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. Applied Environtal Microbiology. 2009; 75: 93-100.
[41] Guenther S, Loessner MJ. Bacteriophage biocontrol of *Listeria monocytogenes* on soft ripened white mold and red-smear cheeses. *Bacteriophage*. 2011; 1: 94-100.
[42] Soni KA, Nannapaneni R. Bacteriophage significantly reduces *Listeria monocytogenes* on raw salmon fillet tissue. *Journal of Food Protection*. 2010; 73: 32-38.
[43] Soni KA, Nannapaneni R, Hagens S. Reduction of *Listeria monocytogenes* on the surface of fresh channel catfish fillets by bacteriophage Listex P100. *Foodborne Pathogens and Disease*. 2010; 7: 427-434.
[44] Hirsh DC, Martin LD. Rapid detection of *Salmonella* spp. by using Felix-O1 bacteriophage and high-performance liquid chromatography. *Applied Environmental Microbiology*. 1983; 45: 260-264.
[45] Kallings LO. Sensitivity of various *Salmonella* strains to felix 0-1 phage. *Acta pathologica et microbiologica Scandinavica*. 1967; 70: 446-454.
[46] van der Walt ML, Steyn HC. The biochemical differentiation between Salmonella and Citrobacter. *The Onderstepoort journal of veterinary research*. 1989; 56:263-269.
[47] Kelln A, Warren A. Isolation and properties of a bacteriophage lytic for a wide range of pseudomonads. *Canadian Journal of Microbiology*. 1971; 17: 677-682.
[48] Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M, Saftner R, Sulakvelidze A. Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Applied Environtal Microbiology*. 2003; 69: 4519-4526.
[49] Acheson DW, Reidl J, Zhang X, Keusch GT, Mekalanos JJ, Waldor M K. In vivo transduction with shiga toxin 1–encoding phage. *Infection and immunity*. 1998; 66: 4496-4498.
[50] Herold S, Karch H, Schmidt H. Shiga toxin-encoding bacteriophages-genomes in motion. *International journal of medical microbiology*. 2004; 294:115–121.
[51] Schmidt H, Bielaszewska M, Karch H. Transduction of enteric *Escherichia coli* isolates with a derivative of Shiga toxin 2–encoding bacteriophage phi3538 isolated from Escherichia coli O157:H7. *Applied and Environmental Microbiology*. 1999; 65: 3855-3861.
[52] Toth I, Schmidt H, Dow M, Malik A, Oswald E, Nagy B. Transduction of porcine enteropathogenic *Escherichia coli* with a derivative of a shiga toxin 2-encoding bacteriophage in a porcine ligated ileal loop system. *Applied and Environmental Microbiology*. 2003; 69: 7242-7247.
[53] Waddell TE, Kropinski AM, Mazzocco A, Franklin K, Johnson RP. Generalized transduction by lytic bacteriophage. In: Clokie M, Kropinski A M eds. Bacteriophages: Methods and Protocols. Totawa, NJ; Humana Press; 2008: 293-303.
[54] Chibani-Chennoufi S, Sidoti J, Bruttin A, Kutter E, Sarker S, Brussow H. In vitro and in vivo bacteriolytic activities of *Escherichia coli* phages: Implications for phage therapy. *Antimicrobial Agents and Chemotherapy*. 2004; 48: 2558-2569.
[55] Bruttin A, Brussow H. Human volunteers receiving *Escherichia coli* phage T4 orally: A safety test of phage therapy. *Antimicrobial Agents and Chemotherapy*. 2005; 49: 2874-2878.
[56] Adams, MH. Bacteriophages. New York, NY: Interscience Publishers. 1959.
[57] Hudson JA, Billington C, Carey-Smith G, Greening G. Bacteriophages as biocontrol agents in food. Journal of food protection. 2005; 68: 426-437.
[58] Baldwin D, Summer NS. Process for continuous production of bacteriophage. U.S. Patent Application 13/175,511.

[59] Chibeu A, Ceyssens PJ, Hertveldt K, Volckaert G, Cornelis P, Matthijs S, Lavigne R. The adsorption of *Pseudomonas aeruginosa* bacteriophage φKMV is dependent on expression regulation of type IV pili genes. *FEMS microbiology letters*. 2009; 296: 210-218.
[60] J.P. Nataro, J.B. Kaper. Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*. 1998; 11; 142-201.
[61] Karmali MA, Gannon V, Sargeant JM. Verocytotoxin-producing *Escherichia coli* (VTEC). *Vetinary Microbiology*. 2010; 140: 360-370.
[62] Bach SJ, McAllister TA, Veira DM, Gannon VPJ, Holley RA. Effect of bacteriophage DC22 on Escherichia coli O157:H7 in an artificial rumen system (Rusitec) and inoculated sheep. *Animal Research*. 2003; 52:89-101.
[63] Raya RR, Varey P, Oot RA, Dyen MR, Callaway TR, Edrington TS, Kutter EM, Brabban AD. Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep. *Applied and Environmental Microbiology*. 2006;72(9):6405–6410.
[64] Raya RR, Oot RA, Moore-Maley B,Wieland S, Callaway TR, Kutter EM, Brabban AD. Naturally resident and exogenously applied T4-like and T5-like bacteriophages can reduce *Escherichia coli* O157:H7 levels in sheep guts. *Bacteriophage*. 2011; 1: 15-24.
[65] Sheng H, Knecht HJ, Kudva IT, Hovde CJ. Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants. *Applied and Environmental Microbiology*. 2006; 72: 5359-5366.
[66] Rozema EA, Stephens TP, Bach SJ, Okine EK, Johnson RP, Stanford K, McAllister TA. Oral and rectal administration of bacteriophages for control of *Escherichia coli* O157:H7 in feedlot cattle. *Journal of Food Protection.* 2009; 72: 241-250.
[67] O'Flynn G, Ross RP, Fitzgerald GF, Coffey A. Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. *Applied and Environmental Microbiology*, 2004; 70: 3417-3424.
[68] Abuladze T, Li M, Menetrez MY, Dean T, Senecal A, Sulakvelidze A. Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef by *Escherichia coli* O157:H7. *Applied and Environmental Microbiology*. 2008; 74: 6230-6238.
[69] Sharma M, Patel JR, Conway WS, Ferguson S, Sulakvelidze A. Effectiveness of bacteriophages in reducing *Escherichia coli* O157:H7 on fresh-cut cantaloupes and lettuce. *Journal of Food Protection*. 2009; 72: 1481-1485.
[70] Carter CD, Parks A, Abuladze T, Li M, Woolston J, Magnone J, Senecal A, Kropinski AM, Sulakvelidze A. Bacteriophage cocktail significantly reduces *Escherichia coli* O157: H7 contamination of lettuce and beef, but does not protect against recontamination. *Bacteriophage*. 2012;2: 178-185.
[71] Viazis S, Akhtar M, Feirtag J, Diez-Gonzalez F. Reduction of *Escherichia coli* O157:H7 viability on hard surfaces by treatment with a bacteriophage mixture. *International Journal of Food Microbiology.* 2011;**145**(1):37-42.
[72] Viazis S, Akhtar M, Feirtag J, Diez-Gonzalez F. Reduction of *Escherichia coli* O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and trans-cinnamaldehyde. *Food Microbiology*. 2011; 28:149-57.
[73] Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, Opsteegh M, Langelaar M, Threfall J, Scheutz F, van der Giessen J, Kruse H. Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*. 2010; 139 Suppl 1:S3-S15.
[74] CDC. *Salmonella* surveillance: Annual summary, 2004. *Centers for Disease Control and Prevention*. 2005.
[75] Darwin KH, Miller VL. Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. *Clinical Microbiology Reviews*. 1999; 12: 405-428.
[76] Atterbury RJ, van Bergen MAP, Ortiz F, Lovell MA, Harris JA, De Boer A, Wagenaar JA, Allen VM, Barrow PA. Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. *Applied and Environmental Microbiology*. 2007; 73: 4543-4549.
[77] Andreatti Filho RL, Higgins JP, Higgins SE, Gaona G, Wolfenden AD, Tellez G, Hargis BM.. Ability of bacteriophages isolated from different sources to reduce *Salmonella* enterica serovar Enteritidis in vitro and in vivo. *Poultry Science*. 2007; 86: 1904-1909.
[78] Borie C, Albala I, Sànchez P, Sánchez ML, Ramírez S, Navarro C, Morales MA, Retamales AJ, Robeson J. Bacteriophage treatment reduces *Salmonella* colonization of infected chickens. *Avian Diseases*. 2008; 52: 64-67.
[79] Wall SK, Zhang J, Rostagno MH, Ebner PD. Phage therapy to reduce preprocessing *Salmonella* infections in market-weight swine. *Applied and Environmental Microbiology*. 2010; 76: 48-53.
[80] Callaway TR, Edrington TS, Brabban A, Kutter B, Karriker L, Stahl C, Wagstrom E, Anderson R, Poole TL, Genovese K, Krueger N, Harvey R, Nisbet DJ. Evaluation of phage treatment as a strategy to reduce *Salmonella* populations in growing swine. Foodborne Pathogens and Disease. 2011; 8: 261-266.
[81] Modi R, Hirvi Y, Hill A, Griffiths MW. Effect of phage on survival of *Salmonella* Enteritidis during manufacture and storage of Cheddar cheese made from raw and pasteurized milk. *Journal of Food Protection*. 2001; 64: 927-933.
[82] Leverentz B, Conway WS, Alavidze Z, Janisiewicz WJ, Fuchs Y, Camp MJ, Chighladze E, Sulakvelidze A. Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: a model study. *Journal of Food Protection*. 2001; 64: 1116-1121.
[83] Whichard JM, Sriranganathan N, Pierson FW. Suppression of *Salmonella* growth by wild-type and large-plaque variants of bacteriophage Felix O1 in liquid culture and on chicken frankfurters. *Journal of Food Protection*. 2003; 66: 220-225.
[84] Pao S, Randolph SP, Westbrook EW, Shen H. Use of bacteriophages to control *Salmonella* in experimentally contaminated sprout seeds. *Journal of Food Science*. 2004; 69: M127-M130.
[85] Higgins JP, Higgins SE, Guenther KL, Huff W, Donoghue AM, Donoghue DJ, Hargis BM. Use of a specific bacteriophage treatment to reduce *Salmonella* in poultry products. *Poultry Science*. 2005; 84: 1141-1145.
[86] Hooton SPT, Atterbury RJ, Connerton IF. Application of a bacteriophage cocktail to reduce *Salmonella* Typhimurium U288 contamination on pig skin. *International Journal of Food Microbiology*. 2011; 151: 157-163.
[87] Guenther S, Herzig O, Fieseler L, Klumpp J, Loessner MJ. Biocontrol of *Salmonella* Typhimurium in RTE foods with the virulent bacteriophage FO1-E2. *International Journal of Food Microbiology*. 2012;154: 66-72.

[88] Moore JE, Corcoran D, Dooley JS, Fanning S, Lucey B, Matsuda M, McDowell DA, Mégraud F, Millar BC, O'Mahony R, O'Riordan L, O'Rourke M, Rao JR, Rooney PJ, Sails A, Whyte P. *Campylobacter*. *Veterinary Research*. 2005; 36: 351-382.
[89] Carvalho C, Santos SB, Kropinski AM, Ferreira EC, Azeredo J. Phages as therapeutic tools to control major foodborne pathogens: *Campylobacter* and *Salmonella*. In: Ipek Kurtboke, ed. *Bacteriophages*. Rijeka: Croatia; 2012: 179-214.
[90] Carrillo CL, Atterbury RJ, El-Shibiny A, Connerton PL, Dillon E, Scott A, Connerton IF. Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Applied and Environmental Microbiology*. 2005; 71: 6554-6563.
[91] Wagenaar JA, Bergen MAPV, Mueller MA, Wassenaar TM, Carlton RM. Phage therapy reduces *Campylobacter jejuni* colonization in broilers. *Veterinary Microbiology*. 2005; 109: 275-283.
[92] El-Shibiny A, Scott A, Timms A, Metawea Y, Connerton P, Connerton I. Application of a group II campylobacter bacteriophage to reduce strains of *Campylobacter jejuni* and campylobacter coli colonizing broiler chickens. *Journal of Food Protection*. 2009; 72: 733-740.
[93] Carvalho CM, Gannon BW, Halfhide DE, Santos SB, Hayes CM, Roe JM, Azeredo J. The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of Campylobacter coli and *Campylobacter jejuni* in chickens. *BMC Microbiology*. 2010; 10, article 232.
[94] Goode D, Allen VM, Barrow PA. Reduction of experimental Salmonella and Campylobacter contamination of chicken skin by application of lytic bacteriophages. *Applied and Environmental Microbiology*. 2003; 69: 5032-5036.
[95] Atterbury RJ, Connerton PL, Dodd CER, Rees CED, Connerton IF. Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. *Applied and Environmental Microbiology*. 2003; 69: 6302-6306.
[96] Farber JM, Peterkin PI. *Listeria monocytogenes*, a foodborne pathogen. *Microbiological Reviews*. 1991; 55: 476-511.
[97] Walker SJ, Archer P, Banks JG. Growth of *Listeria monocytogenes* at refrigeration temperatures. *Journal of Applied Bacteriology*. 1990; 68: 157-162.
[98] Gandhi M, Chikindas ML. Listeria: A foodborne pathogen that knows how to survive. *International Journal of Food Microbiology*. 2007; 113: 1-15.
[99] Holck A, Berg J. Inhibition of *Listeria monocytogenes* in cooked ham by virulent bacteriophages and protective cultures. Applied Environmental Microbiology. 2009; 75: 6944-6946.
[100] Guenther S, Huwyler D, Richard S, Loessner MJ. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. Applied Environmental Microbiology. 2009; 75: 93-100.
[101] Van Twest R, Kropinski AM. Bacteriophage enrichment from water and soil. *Methods in Molecular Biology*. 2009; 501:15-21.
[102] Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages using the small drop plaque assay system. *Methods in Molecular Biology*. 2009; 501: 81-5.
[103] Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages by the direct plating plaque assay. *Methods in Molecular Biology*. 2009; 501: 77-80.
[104] Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages by double agar overlay plaque assay. *Methods in Molecular Biology*. 2009; 501: 69-76.
[105] Hatfull GF; Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science Program; KwaZulu-Natal Research Institute for Tuberculosis and HIV Mycobacterial Genetics Course Students; Phage Hunters Integrating Research and Education Program. Complete genome sequences of 138 mycobacteriophages. *Journal of Virology*. 2012; 86: 2382-2384.
[106] Miller JR, Koren S, Sutton G. Assembly algorithms for next-generation sequencing data. *Genomics*. 2010; 95: 315-327.
[107] Zhang W, Chen J, Yang Y, Tang Y, Shang J, Shen B. A practical comparison of de novo genome assembly software tools for next-generation sequencing technologies. PLoS One. 2011; 6: e17915.

