Phage Therapy in the Food Industry

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Abstract

Despite advances in modern technologies, the food industry is continuously challenged with the threat of microbial contamination. The overuse of antibiotics has further escalated this problem, resulting in the increasing emergence of antibiotic-resistant foodborne pathogens. Efforts to develop new methods for controlling microbial contamination in food and the food processing environment are extremely important. Accordingly, bacteriophages (phages) and their derivatives have emerged as novel, viable, and safe options for the prevention, treatment, and/or eradication of these contaminants in a range of foods and food processing environments. Whole phages, modified phages, and their derivatives are discussed in terms of current uses and future potential as antimicrobials in the traditional farm-to-fork context, encompassing areas such as primary production, postharvest processing, biosanitation, and biodetection. The review also presents some safety concerns to ensure safe and effective exploitation of bacteriophages in the future.

INTRODUCTION

Food is a fundamental requirement of everyday life, but in all too many instances, contamination with pathogenic bacteria can result in illness and even death. According to the Centers for Disease Control and Prevention (CDC), it was estimated in 2011 that approximately 48 million cases of food poisoning occur each year in the United States alone, of which 128,000 result in hospitalization and 3,000 in fatalities (CDC 2011). Known pathogens account for an estimated 9.4 million of these illnesses, 55,961 hospitalizations, and 1,351 deaths (Scallan et al. 2011). Statistical data on the worldwide incidence of foodborne disease are fragmented, mainly focusing on specific pathogens or their rate of occurrence in particular countries. As a consequence of this, the true measure of foodborne illness on a global scale is unattainable. Despite the development of effective modern technologies in an effort to control microbial contamination, food safety is continuously challenged as a direct result of food market globalization.

In addition to microbial contamination posing a threat to health and well-being, it also impacts greatly the food industry in terms of food spoilage. The latter is a complex metabolic process that results in undesirable changes to the sensory characteristics of food, making it unacceptable for human consumption (Doyle 2007). Microbial spoilage is by far the most common type of food spoilage encountered. Even with current preservation methods and good manufacturing practices, quality control, and hygiene, an estimated 25% of the total food produced every year is lost due to microbial damage (Nat. Res. Counc. Food Nutr. Board 1985, Razzaghi-Abyaneh & Shams-Ghahfarokhi 2011).

The financial burden of microbial contamination on human health and the food processing industry is enormous, costing the global economy billions of dollars every year. Food manufacturers are continuously challenged by consumer expectations for products that are pathogen free and of acceptable quality without the use of artificial preservatives. Given that the food and beverage industry was valued at $5.7 trillion USD worldwide in 2008 (Int. Messag. Access Protoc. 2010) and is expected to increase in value by 3.5% to $7 trillion USD by 2014 (**http://www.businessvibes.com**), we must exploit natural antimicrobials in an effort to reduce contamination and help sustain this global industry.

The natural biotherapeutic potential of bacteriophages is well recognized throughout the world. In 2006, a major milestone in Western world phage history was achieved with the approval of the first phage-based product (ListShield^{TM}) to control *Listeria monocytogenes* in meat and poultry products (Bren 2007). Since then, other phage products have also been approved for use as biotherapeutics in food (Sulakvelidze 2011). These advances in phage biocontrol highlight their potential in controlling additional food pathogens and spoilage organisms and also confirm that the use of phages is acceptable to the food industry. This review attempts to showcase recent advances in phage research in the area of phages as biocontrol agents against food pathogens and food spoilage organisms, highlighting their suitability for use in the food industry.

FOODBORNE PATHOGENS

Food is the primary route of transmission for more than 200 known diseases, of which many are attributed to bacteria (Oliver et al. 2005). The leading bacterial foodborne pathogens of concern are *Salmonella*, *Campylobacter*, pathogenic varieties of *Escherichia coli*, and *Listeria monocytogenes*, each of which can be associated with serious gastrointestinal infection (Schlundt 2002). Several others are also important and have become targets for phage-mediated biocontrol in the scientific literature (see **Table 1**).

Table 1 Bacterial food pathogens and their properties[a]

Pathogen	Symptoms	Source of infection	Mechanism of action
Salmonella enterica	Salmonellosis: common gastroenteritis, enteric fever, bacteremia	Contaminated meats of animal origin and fruits and vegetables	Bacteria colonize the small intestine and colon. Invading cells may produce cytotoxins, inhibiting protein synthesis in the host. An acute inflammatory response is stimulated, resulting in ulceration and subsequent scarring of the intestines (Giannella 1996, Plym & Wierup 2006).
Campylobacter species	Campylobacteriosis: severe acute gastroenteritis, linked to the development of Guillain-Barré syndrome	Consumption of undercooked/raw meat, pasteurized milk, vegetables, and environmental water	*Campylobacter* species invade the epithelial cells of the intestine, leading to mucosal damage and inflammation (Everest et al. 1993, Konkel et al. 1992). Infection at low levels can result in full campylobacteriosis. Lipid A from the LPS of some *Campylobacter* species is involved in systemic infection, leading to sepsis and shock (Moran 1996).
E. coli O157:H7	Diarrhea, severe hemorrhagic colitis, hemorrhagic uremic syndrome	Consumption of contaminated undercooked foods of bovine origin	This organism has an extremely low infectious dose (10 cells) (Kaper 1998). It produces outer membrane proteins called intimins, which induce intestinal mucosa A/E lesions in the gut (Adu-Bobie et al. 1998). The organism also produces Shiga toxins involved in the development of hemolytic uremic syndrome and acute renal failure (Mohawk et al. 2010).
Listeria monocytogenes	Listeriosis: nausea, vomiting, abortion, fetal death, septicemia, meningitis	Primarily through consumption of RTE foods	This organism induces its own uptake by enterocytes/M cells in the small intestine. In immunocompromised individuals, *Listeria* multiplies in macrophages and is transported to various organs where it penetrates the blood-brain and placental barriers (Cossart & Lecuit 1998, Kuhn & Goebel 1999, Renzoni et al. 1999). *Listeria* is phytotrophic and is able to persist and proliferate at low temperatures, low pH, and high salt concentrations (Guenther et al. 2009).
Cronobacter sakazakii	Necrotizing enterocolitis, meningitis, and bacteremia in neonates and infants	Contaminated powdered infant formula (infection in infants)	The organism attaches to the endothelial cells of the GI tract; infiltrates, persists and replicates within macrophages; and can penetrate the blood-brain barrier (Townsend et al. 2007, Pagotto et al. 2008, Li et al. 2010). It can persist for long periods in unfavorable environmental conditions such as desiccation and osmotic stress.
Mycobacterium avium subsp. *paratuberculosis* (Map)	Implicated as a causative agent of Crohn's disease in humans	Consumption of contaminated dairy products such as milk	The exact mechanism of how Map causes disease in humans is unclear but it is thought that consumption of contaminated dairy products such as milk is a possible source of infection for humans (Grant et al. 1998, 2002; Donaghy et al. 2004).
Clostridium perfringens	Gas gangrene and necrotic enteritis in humans and animals	Consumption of contaminated poultry products	The organism colonizes the gut, producing toxins and degrading enzymes, to break down host tissue, utilizing amino acids that the bacterium itself is unable to produce (Shimizu et al. 2001). The organism can also suppress the growth of normal flora, leading to an increase in the rate of disease progression (Feng et al. 2010, Stanley et al. 2012).

(*Continued*)

Table 1 (*Continued*)

Pathogen	Symptoms	Source of infection	Mechanism of action
Staphylococcus aureus	Gastroenteritis	Consumption of contaminated meat, poultry, and dairy products	The organism produces toxins that can penetrate the intestinal lining of the gut and triggers both local and systemic immune responses, ultimately resulting in the formation of lesions in the stomach and GI tract (Kent 1966, Shupp et al. 2002).
Shigella	Shigellosis: bloody diarrhea, fever, stomach cramps, inflammatory enteritis	Consumption of contaminated water, vegetables, milk, dairy, poultry	Often referred to as bacterial dysentery, the organism is highly infectious, with as little as 10 cells able to result in disease (DuPont et al. 1989). Infection is established in the large intestine, triggering its own uptake by M cells invading the epithelial cells, eliciting a strong inflammatory response (Sansonetti et al. 1996). The organism can also produce enterotoxins responsible for vascular lesions in the colon, kidney, and central nervous system (Cherla et al. 2003).

[a]The table is limited to those that have been subjected to phage-based biocontrol strategies.
Abbreviations: A/E, attachment and effacement; GI, gastrointestinal; LPS, lipopolysaccharides; RTE, ready to eat.

BACTERIOPHAGES AS THERAPEUTICS

History

The first characterization of bacteriophages (phages) dates back to 1917 to the work of Felix d'Herelle (Fruciano & Bourne 2007); earlier, Ernest Hankin, Nikolay Gamaleya, and Frederick Twort were recognized (in 1896, 1898, and 1915, respectively) for their independent observations of the bactericidal effects of these bacterial viruses. Throughout the 1920s, d'Herelle published extensive work on phage biology and was accredited for helping establish the International Bacteriophage Institute in Tbilisi, Georgia in 1923 (Sulakvelidze 2001). Bacteriophages initially appeared to offer great potential as frontline therapeutics against infectious disease in the pre-antibiotic era and were employed in many countries up until World War II (Fruciano & Bourne 2007). However, due to a relatively limited understanding of phage properties and a poor understanding of the underlying causes of many human diseases, many applications of phage preparations as therapeutics were inappropriate. This resulted in variable outcomes for phage therapy with many medical practitioners becoming skeptical of their benefit, particularly with the advent of antibiotics in the 1940s (Harper & Morales 2012). With the increasing reliance on antibiotics after World War II, the use of phages as therapeutics largely ceased, with the exception of the former Soviet Union and Poland, where extensive research and development continued (Slopek et al. 1987, Sulakvelidze 2001). Due to the widespread problem of antibiotic resistance coupled with the paucity of new antibacterial drugs, interest has been renewed in exploiting bacteriophages as a realistic option for treating antibiotic-resistant bacterial infections and for the control of problematic bacteria in many other areas including food (Clark & March 2006, O'Mahony et al. 2011a).

Biology

Bacteriophages are bacterial viruses that infect bacterial cells with high specificity, and in the case of lytic phages, they disrupt and lyse their host cells, resulting in cell death (**Figure 1**). Typically, the life cycles of bacteriophages can be classified broadly into two categories, lytic (virulent) and lysogenic (temperate phage) cycles (Kutter & Sulakvelidze 2005). In the lytic cycle, phages adsorb

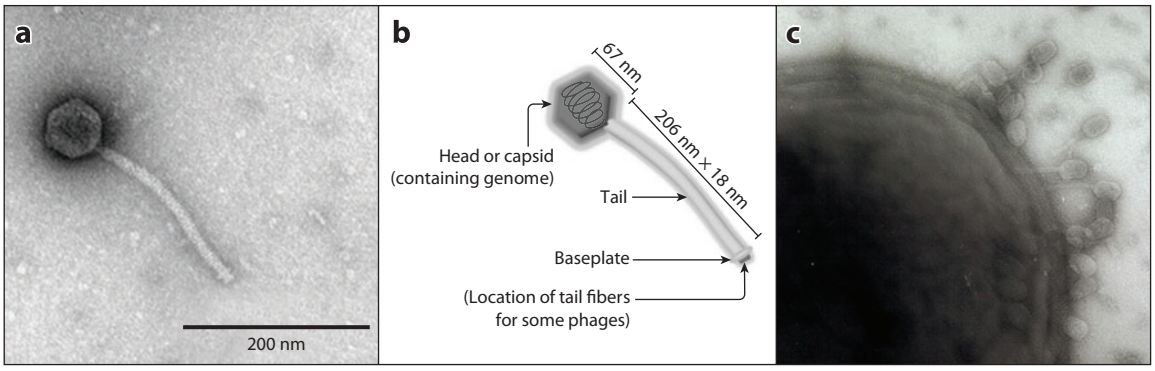

Figure 1

(*a*) Electron micrograph of a typical bacteriophage specific for the genus *Mycobacterium*. (*b*) Diagram showing the main phage components with dimensions. (*c*) Bacteriophages adsorbing to a bacterial cell surface, the initial stage of the phage replication cycle.

to specific receptor sites on the surface of the host cell, which is followed by subsequent irreversible attachment. Ability to recognize and attach to receptor molecules on the cell surface largely dictates the host range of a bacteriophage. Tail penetration through cell walls degraded enzymatically drives insertion of phage DNA into the cytoplasm of the host. Once inside the cell, specific enzymes encoded by the phage genome are synthesized to divert the host cell's DNA and protein synthesis toward the generation of new phage particles. These include structural phage proteins and enzymes necessary for cell lysis and progeny phage release. The phage-encoded structural components of the phage are assembled to form new phage particles, and the newly replicated phage genomes are packaged into the phage heads. At a precise time at the end of the phage cycle, phage-encoded holins form pores in the cell membrane allowing phage-encoded peptidoglycan hydrolases (lysins) access to the peptidoglycan. This results in rapid cell destruction with the release of progeny phage, which then proceeds to infect neighboring susceptible cells (Young & Blasi 1995, Kropinski 2006). A phenomenon known as "lysis from without" can also result in lysis of neighboring cells without phage replication and progeny release. Lysis from without occurs as a result of high multiplicities of infections (>100) and a change in the membrane potential of the cell brought about by excess phage adhesion. This process is driven by cell wall–degrading enzymes located at the tail tip of many bacteriophages (Abedon 2011).

Temperate phages, in addition to being capable of undergoing the lytic life cycle, have the ability to persist as a prophage within the genome of their host bacteria (Oyaski & Hatfull 1992). A copy of the phage genome is stably maintained in a repressed state within the host genome and replicates in concert with the host chromosome. The prophage may enter the lytic cycle following induction by an environmental stimulus resulting in lysis of the host cell (Stella et al. 2009). Temperate phages are generally avoided for direct use as therapeutics because they may mediate transduction of genetic material from one bacterial cell to another. Indeed, they may also transmit genes that increase the virulence of the host in a process known as lysogenic conversion. As a result of their replication cycle, they do not kill all of the bacteria that they infect. Moreover, a bacterial cell harboring a prophage within its genome becomes immune to infection by the same or closely related phages, a process known as superinfection immunity (Wagner & Waldor 2002, Deresinski 2009, Gill & Hyman 2010). In contrast, virulent phages have the ability to replicate exponentially on a bacterial culture and can rapidly eliminate bacteria regardless of their antibiotic resistance profiles. This makes virulent phages very appealing candidates for use as biotherapeutic agents.

BACTERIOPHAGES AND THEIR DERIVATIVES AS BIOCONTROL AGENTS IN THE FOOD INDUSTRY

There is great potential for the use of phages as natural antibacterials to control food pathogens and spoilage organisms at the pre- and postharvest stages of production. Indeed, the need to control microbial contamination during the manufacture of food is reinforced by the negative economic impact of both pathogenic and food spoilage organisms. Bacteriophages are suitable for use at each stage of the farm-to-fork continuum: from the decontamination of livestock, to sanitation of equipment and contact surfaces on farms and in industry, to biocontrol in raw meats and fresh produce, and also as natural preservatives in foods to extend product shelf life. Using either whole phages or their derivatives, many researchers have demonstrated phage biocontrol in food, often with promising results. Many studies have been conducted in both preharvest (farm animals) and postharvest (meat, fresh and packaged foods) environments, and they control for a broad spectrum of some of the major and emerging foodborne pathogens, including *Salmonella*, *Listeria*, *Campylobacter*, and *Escherichia*. Goodridge & Bisha (2011) reviewed recently the major intervention studies describing phage biocontrol of foodborne pathogens pre- and postharvest. Additional studies demonstrating the biocontrol potential of phages against *Mycobacteria* and *Cronobacter sakazakii* have been described by Endersen et al. (2012) and Kim et al. (2007), respectively.

Control of Foodborne Pathogens in Primary Production

Farm animals are reservoirs for many important bacterial pathogens such as *Listeria*, *Campylobacter*, *Escherichia*, and *Salmonella* and are thus responsible for many human infections. For instance, Fey et al. (2000) reported a case where a multidrug-resistant strain of *Salmonella* was isolated from a 12-year-old boy in Nebraska suffering from salmonellosis. He had acquired it from direct contact with infected cattle on the family farm. Controlling pathogens on the farm is a complex process, and for many years the main line of defense in disease prevention and treatment has been antibiotics. However, controlling pathogens in the farm environment has become increasingly difficult due to the overuse of antibiotics in animal feed and the concomitant increase in the prevalence of antibiotic resistance. In the 1950s, the US Food and Drug Administration (FDA) approved the use of common antibiotics in animal feeds to improve the efficiency of livestock and poultry production. Although the mechanism by which antibiotics improve the productivity of livestock is unclear, it is thought to enhance feed utilization by the animals (Walsh et al. 2007). As much as 20% of feed per pound of weight gained in pigs is saved as a direct result of antibiotics (Cromwell 2002). It is agreed that many of the antibiotic-resistant bacterial pathogens associated with human and animal disease emerged as a direct result of the overuse of antibiotics in animal feed. However, this did not deter the feed industries, as improved production and reduced costs greatly outweighed the risk of creating harmful superbugs impervious to antibiotic treatment (Nat. Res. Counc. Agric. Board 1956). In Europe today, the use of antibiotics for growth-promoting purposes is prohibited, whereas in the United States, it is still acceptable (Allen et al. 2013). A total of 17 different classes of antibiotics are approved for use in feed, many of which are used to treat human disease. The resulting increase in antibiotic resistance has given rise to challenges in finding effective antimicrobials to treat animal and human infections and to control the levels of pathogens associated with food animals and materials. The potential benefits of bacteriophages to control and eradicate pathogenic bacteria in food-producing animals on the farm have been evaluated in a variety of studies, many using in vivo models in poultry, swine, sheep, and cattle.

Phage applications in poultry. Poultry is a major reservoir for two of the world's most prominent food pathogens, *Salmonella* and *Campylobacter*, which are responsible for causing salmonellosis

and campylobacterosis in humans. Much work has been published on the applications of phages to control foodborne pathogens in poultry (Connerton et al. 2004, Higgins et al. 2005, Sillankorva et al. 2010). The colonization of *Campylobacter jejuni* in broiler chickens is common and is difficult to prevent or contain. Carrillo et al. (2005) investigated the biotherapeutic effect of phages with the intent of controlling the spread of the organism throughout the entire flock. Two phages were selected and administered at different phage-strain combinations to 25-day-old broiler chickens experimentally colonized with *Campylobacter* isolates. A reduction in the *Campylobacter* counts was observed in cecal contents of phage-treated birds when compared to the control group. Wagenaar et al. (2005) carried out a similar study investigating the potential use of phages to control *C. jejuni* colonization in broiler chickens. They performed preventative and treatment experiments using single phages and phage mixtures. Single-phage prevention experiments resulted in colonization delay with an initial 2-log reduction. However, *C. jejuni* cell numbers were comparable to the control group within a week. Combination-phage treatment experiments resulted in a 3-log reduction that stabilized after five days to a level that was 1-log lower than the control group. Generally, experiments that combined numerous phages gave rise to a notable decrease in the level of *Campylobacter* found in the caeca of the treated chickens, indicating an increase in bactericidal activity. El-Shibiny et al. (2009) published a report that also demonstrated the efficacy of phages in reducing the levels of both *C. jejuni* and *C. coli* in the caeca of *Campylobacter*-colonized chickens. Berchieri et al. (1991) demonstrated the biocontrol ability of bacteriophages against *Salmonella* Typhimurium in chickens. Single-phage administration over seven days did not result in a decrease in *Salmonella* shedding. However, a significant reduction in *Salmonella* shedding was observed when a mixture of two phages was used over a 21-day period. Borie et al. (2008) found that the use of a mixture of three phages administered 24 h following infection with *Salmonella* Enteritidis via aerosol spray or in drinking water was effective in reducing the incidence of *S.* Enteritidis in chickens over a 20-day period. Recently, Bardina et al. (2012) evaluated the potential of phage mixtures as biotherapeutics against *S.* Typhimurium and to reduce the populations of the bacteria in the intestines of mice and chickens. In the absence of phage, the *S.* Typhimurium infection in the mice resulted in death. A 50% increase in the survival rate of the mice was achieved when the phage mixture was administered preinfection and readministered at 6 h, 24 h, and 30 h postinfection. For the chickens, *Salmonella* counts fell in cecum contents following multiple administrations of phage mixtures pre- and postinfection. This study highlights the importance of administering the phage prior to infection and the need for continued administration to achieve significant protection. Lim et al. (2012) evaluated the efficacy of using a single virulent bacteriophage ϕCJ07 to inhibit the growth of *S.* Enteritidis in the intestinal tracts of infected chickens and investigated its biotherapeutic effect in preventing horizontal transmission of the organism to uninfected cohabitating chicks. One-day-old chicks infected with 5×10^7 CFU/bird of *S.* Enteritidis were in contact with uninoculated chicks with which they were cohabiting. All birds were administered a bacteriophage preparation at 10^5, 10^7, and 10^9 PFU/g of feed for 21 days following introduction of the bacteria. The researchers found that all phage treatments resulted in a notable decrease in intestinal colonization of *S.* Enteritidis in the infected birds and also in the contact chicks at weeks 1, 2, and 3 compared with the control group. Regarding the phage titers used in the study, statistically significant reductions ($p < 0.05$) in colonization were seen when bacteriophage concentrations were used at 10^7 and 10^9 PFU/g. Seventy percent of the contact chickens treated with 10^9 PFU/g of bacteriophage ϕCJ07 had no detectable levels of intestinal *Salmonella* at the end of the three-week trial, indicating the potential of bacteriophages to prevent horizontal transmission of the organism in chickens. These studies clearly demonstrate the potential of phages, and in particular mixtures, for the biocontrol of pathogenic bacteria in primary production.

Phage applications in swine. Relatively few studies have been reported demonstrating the use of phages to control foodborne pathogens in swine (Goodridge & Bisha 2011). Wall et al. (2010) demonstrated the effects of an anti-*Salmonella* phage mixture on reducing colonization of *S.* Typhimurium in pigs during transport and holding prior to slaughter. Preliminary studies were carried out in three–four-week-old pigs. A phage suspension was administered via oral gavage and resulted in a significant reduction in ileal, cecal, and tonsil *Salmonella* titers. Similarly, with market-ready pigs, phages achieved a 95% reduction in cecal contamination and a 90% reduction in ileal contamination. The researchers then investigated the efficacy of administering a microencapsulated phage mixture in pig feed to control *S.* Typhimurium and compared their results to previous administrations of oral gavage. They found that the pigs that were fed the microencapsulated phages directly shed less *S.* Typhimurium when compared to the gavage and control groups. They also observed a 1-log reduction in concentrations of *S.* Typhimurium in ileal and cecal contents with respect to the control pigs (Saez et al. 2011). In a similar study, Callaway et al. (2011) developed a phage mixture and evaluated its ability to reduce and control *Salmonella* in swine. Each pig was inoculated by oral gavage with 10^{10} CFU/pig of *S.* Typhimurium, and fecal samples were collected every 24 h over the course of a 96-h period. At 24 h and 48 h, pigs were treated with a 10^{9}-PFU/ml phage suspension. Pigs were euthanized after 96 h. A reduction in the levels of *S.* Typhimurium in both cecal and rectal contents was observed, but a greater reduction ($p < 0.05$) was observed in the rectal contents.

Phage applications in cattle. The main route of *E. coli* O157:H7 transmission to humans is through foods of bovine origin. Many in vivo studies have been conducted in an effort to control colonization of *E. coli* O157:H7 in cattle (Goodridge & Bisha 2011). Rozema et al. (2009) evaluated the efficacy of both oral and rectal administration of *E. coli* O157-specific bacteriophages in an effort to reduce fecal shedding of *E. coli* O157:H7 in experimentally inoculated cattle. Shedding was monitored over 83 days for oral, rectal, and oral-plus-rectal administration and control experiments following multiple treatments of an O157-specific bacteriophage mixture. A trend toward reduction in shedding was observed in orally treated cattle, whereas rectal and oral-plus-rectal-administered groups did not result in a significant reduction in shedding when compared to the control group. The authors concluded that continuous administration of phages is needed to effectively control *E. coli* O157:H7 shedding in feedlot cattle. Rivas et al. (2010) examined the effects of two bacteriophages, e11/2 and e4/1c, against *E. coli* O157:H7 in cattle. There was no notable decrease in the level of *E. coli* O157:H7 shedding in experimentally inoculated cattle when compared to the control. However, when phages e11/2 and e4/1c were challenged individually against *E. coli* O157:H7 in an ex vivo rumen model, cell numbers were significantly reduced within 1 h and 2 h, respectively. These phages also demonstrated strong potential in reducing *E. coli* O157:H7 on cattle hide (Coffey et al. 2011). Nevertheless, additional research is required to improve the in vivo biotherapeutic effects of phages. Stanford et al. (2010) developed a method using a mix of four encapsulated bacteriophages to target and control *E. coli* O157:H7 in cattle. Encapsulated phages proved more resistant to the low-pH environment of the stomachs of cattle. A recovery of 13.6% was obtained following exposure to pH 3.0 for 20 min, whereas for untreated phages, a complete loss of activity was observed. Cattle were inoculated with 10^{11} CFU of nalidixic acid–resistant *E. coli* O157:H7 on day 0 and treated with the encapsulated phage cocktail on days −1, 1, 3, 6, and 8 of the challenge. The encapsulated phages were administered orally at 10^{10} PFU per animal per day to one treatment group, while the other treatment group received the encapsulated phages top-dressed on their feed at 10^{11} PFU per animal per day. Shedding was monitored for 10 weeks, and it was found that the encapsulated phages did not bring about a decrease in the shedding of nalidixic acid–resistant *E. coli* O157:H7 overall. However, it was evident that orally administered encapsulated

phages were successful at reducing the level of shedding for 14 days ($p < 0.1$). It was concluded that a greater understanding of the phage-*E. coli* O157:H7 ecology is necessary before phage therapy can be considered a viable option for controlling the organism in feedlot cattle (Stanford et al. 2010).

Phage applications in sheep. Ovine ruminants are also considered to be a significant reservoir of *E. coli* O157:H7, as reviewed by Goodridge & Bisha (2011). Bach et al. (2009) assessed the potential use of bacteriophages as a means of reducing levels of *E. coli* O157:H7 in experimentally inoculated sheep. Sheep were inoculated with a mixture of four 10^9 CFU nalidixic acid–resistant strains of *E. coli* O157:H7 on day 0 of the challenge. A cocktail of three bacteriophages at a concentration of 10^{11} PFU was administered on days −2, −1, 0, 6, and 7 of the trial. After collecting fecal samples on 14 occasions over the 21-day challenge, it was evident that oral administration of bacteriophages reduced shedding ($p < 0.05$) of *E. coli* O157:H7. Researchers also noted a rapid decline in the number of bacteriophages over the 21 days and considered an alternative delivery system that would protect the phages during passage through the intestinal tract. Raya et al. (2011) carried out trials using bacteriophages in an attempt to reduce *E. coli* O157:H7 naturally resident in the intestines of sheep. Results revealed that a mixture of two phages (CEV2 and CEV1–10^{11} PFU) administered once orally was effective at reducing cell numbers from cecum and rectum contents in comparison to the control group. Sheep naturally carrying CEV2 phage showed the greatest reduction in *E. coli* O157:H7 in cecum and rectum contents.

Although the above studies illustrate the possible use of bacteriophages to control foodborne pathogens in food-producing animals, many challenges still remain. These are more likely to be overcome as a result of a better understanding of the phage-bacteria interactions in the gut and within the complex alimentary system of live animals (Mills et al. 2013) and may possibly require more sophisticated experimental designs.

Postharvest Control of Foodborne Pathogens

Since the regulatory acceptance of the first phage-based product, ListShield™, approved for use in controlling *Listeria* in meats and poultry products, the search to develop new phage-based technologies for pathogen control in postharvest foods has increased. Intervention strategies using phages to control foodborne pathogens in postharvest foods have been demonstrated by many and have had better success than those reported in preharvest pathogen control. Increased efficacy in postharvest foods may be due to the fact that phages are not subject to the complex ever-changing microenvironments found within living animals. Phage-based decontamination of postharvest foods that do not undergo processing to kill bacterial pathogens before consumption is a worthwhile endeavor. Such foods include meat carcasses, fresh fruit, vegetables, processed ready-to-eat (RTE) foods, powdered infant formula, and pasteurized milk.

Meat. Research on phage biocontrol in the area of meat includes experiments by Atterbury and colleagues (2003), where phages were used to control *C. jejuni* contamination on the surface of chicken skin. The application of phages resulted in a 1–1.3-log reduction in *C. jejuni* within 24 h. An increase in biocontrol efficiency was obtained when phage treatment was combined with the freezing of chicken skin at −20°C. Hungaro et al. (2013) used a bacteriophage mixture of chemical agents (i.e., dichloroisocyanurate, peroxyacetic acid, lactic acid) to reduce *S.* Enteritidis on chicken skin. Similar results were observed for both treatments where a 1-log CFU/cm^2 reduction in *S.* Enteritidis was observed. They concluded that bacteriophages could thus be used as alternative biosanitizers for this pathogen on poultry carcasses in an industrial setting. Anti-*Salmonella* phages were also evaluated on pig skin by Hooten et al. (2011). In this case, a phage mixture against *S.* Typhimurium was shown to reduce cell numbers to undetectable levels when applied to the

skin at a multiplicity of infection of 10 or higher. These results support the application of phages to control *Salmonella* contamination of pig skin postslaughter. O'Flynn et al. (2004) evaluated the use of a three-phage mixture to reduce *E. coli* O157:H7 on beef. Eighteen pieces of beef were inoculated with 10^3 CFU rifampicin-resistant *E. coli* O157:H7. Nine pieces were then treated with the phage. Following a 1-h incubation, all samples were enriched in brain-heart infusion broth for a further 2 h. No detectable levels of *E. coli* were present on seven of the nine phage-treated meats. Of the two that were positive, counts of less than 10 CFU/ml were obtained in comparison to counts of 10^5 CFU/ml in the control pieces. Immobilization of phages onto a modified cellulose membrane was shown to be effective by Anany et al. (2011), who targeted *L. monocytogenes* and *E. coli* O157:H7 in RTE and raw meat. These researchers showed that the immobilized cocktail of phages was active and capable of reducing the titer in excess of 1-log for both pathogens at a range of different temperatures. The immobilization method is based on charge, allowing the phage heads (net negative charge) to bind to the cellulose membranes (net positive charge) and thus leaving the tails free to capture and kill the contaminating bacteria. These researchers suggested that this method would help reduce excessive phage waste and would maximize their potential by positioning the phage directly at the meat surface.

Fresh fruits and vegetables. Leverentz et al. (2001) examined the biocontrol potential of bacteriophages against *Salmonella* on fresh-cut fruit. The phage mixture was successful at reducing counts of the bacterium on fresh-cut melons by 3.5-logs when stored at 5°C and 10°C, and by 2.5-logs when stored at 20°C. These bacteriophages were also compared to chemical sanitizers on honeydew melon slices, and it was found that the phages mediated a greater reduction of *Salmonella* than did the chemicals. However, when the same experiment was applied to apple slices, no significant decrease in *Salmonella* counts was observed, but a reduction in phage titer did occur. The authors suggested that the acidic pH of the apples may have inactivated the phages and therefore, the use of an acid-tolerant phage mutant may be necessary to control *Salmonella* on apples. In another study, Leverentz et al. (2003) demonstrated the synergistic effect of using a bacteriophage mixture with nisin to control *L. monocytogenes* on fresh-cut melon and apple slices. They found that the combination of both antimicrobials (bacteriophage spray + nisin) was better for reducing *L. monocytogenes* populations on the fruits than either treatment alone. In a similar synergy study, Viazis et al. (2010) assessed the antimicrobial ability of a bacteriophage mixture alone and in combination with an essential *trans*-cinnameldehyde oil on different *E. coli* O157:H7 strains contaminating lettuce and spinach leaves. Their results showed that when the *E. coli* was present at low levels (10^4 CFU/ml), the use of the phage or oil individually was successful at inhibiting the growth of *E. coli* O157:H7 on both leaves after 24 h at 23°C and 37°C. When the *E. coli* was present on the leaves at a higher level (10^6 CFU/ml), a decrease in efficiency of both antimicrobials was observed. However, when phages were combined with oil, complete inactivation of *E. coli* O157:H7 was achieved. This further supports the use of phages in combination with other food-grade antimicrobials for the reduction of *E. coli* O157:H7. Recently, Boyacioglu et al. (2013) reported the improved effect of an anti-*E. coli* O157:H7 bacteriophage mixture when used with modified atmosphere packaging of fresh-cut leafy greens.

Processed foods. Several foodborne outbreaks have been associated with various processed foods, including a variety of RTE meals, cheese, milk, and powdered infant formula. Modi et al. (2001) assessed the biotherapeutic ability of a single bacteriophage SJ2 against *S.* Enteritidis during the manufacture, ripening, and storage of cheddar cheese made from raw and pasteurized milk. They found that after 89 days storage at 8°C, *Salmonella* was not detected in pasteurized milk cheese. The raw milk cheese contained *Salmonella* counts of 50 CFU/g after 99 days storage at 8°C. Nevertheless, a reduction in cell numbers was observed in comparison to the control where

S. Enteritidis had reached a final concentration of 10^3 CFU/g. In a similar study, Guenther et al. (2012) evaluated the efficacy of phage F01-E2 in reducing *S.* Typhimurium in a variety of RTE foods. Foods were inoculated with 10^3 CFU *Salmonella* cells and then treated with 3×10^8 PFU/g for six days. Phage-treated foods incubated at 8°C resulted in complete eradication of *Salmonella* in all cases. When the trials were performed at 15°C, a 1-log to 6-log reduction in the level of *S.* Typhimurium was achieved for all foods with the exception of egg yolk. In this case, a decrease in *Salmonella* was observed after two days but cell numbers were similar to those of the control after six days. In another study, Guenther et al. (2009) assessed the efficacy of an anti-*L. monocytogenes* bacteriophage mixture in a variety of RTE foods. It was established that a bacteriophage mixture was able to reduce bacterial counts by up to 5 logs on solid foods, whereas in liquid foods, bacterial counts dropped below the limit of detection, highlighting the suitability of phages to control *Listeria* in RTE foods. In addition, Bigot et al. (2011) demonstrated the ability of a single bacteriophage, FWLLm1, to limit the growth of *L. monocytogenes* in RTE poultry. A reduction in the growth of the bacteria was observed over 24 h at 30°C and 5°C. The greatest reduction was observed when a lower starting inoculum of 10^2 CFU cm^{-2} was treated with 10^6 PFU cm^{-2} of phages (Bigot et al. 2011). Zhang et al. (2013) demonstrated the efficacy of single phages and phage cocktails against different species of *Shigella* in RTE spiced chicken. The food was artificially contaminated with individual species or a mixture (*S. flexneri* 2a, *S. dysenteriae*, and *S. sonnei*) at 3×10^4 CFU/g. The *Shigella* spp. were challenged with individual phages or the phage cocktail at a concentration of 1×10^8 PFU/g or 3×10^8 PFU/g and incubated at 37°C for 72 h. These researchers found that although the individual phages were effective in reducing cell numbers, the phage cocktail was more effective, as it resulted in complete elimination of *Shigella* spp. from the contaminated foods after 72 h. A study focused on infant formula was conducted by Kim et al. (2007), who used two bacteriophages separately (ESP 732-1 and ESP 1–3) to control *Cronobacter sakazakii* in reconstituted infant formula at a range of different temperatures. The results revealed that phage ESP 732-1 was successful at eradicating the organism at 12°C, 24°C, and 37°C, and phage ESP 1–3 was successful at 24°C; complete eradication was not achieved at the other two temperatures. This suggests that a combination of phages should be used to eliminate this pathogen. Although Zuber et al. (2008) demonstrated the antibacterial efficacy of a five-phage mixture against *C. sakazakii* in broth, these phages were not applied in infant formula. In another study, Endersen et al. (2012) assessed the antibacterial potential of six mycobacteriophages, both individually and as a phage mixture, to inhibit the growth of *Mycobacterium smegmatis* in reconstituted skim milk. The effect of temperature and pH on phage infectivity was established, and it was found that the phages were relatively heat stable up to 72°C for 15 minutes and generally retained infectivity at pH 4 to pH 10. Overall, the single-phage experiments results in a 6- to 7-log reduction in cell numbers over 96 h at 37°C in reconstituted skim milk, whereas a mixture of all six phages applied over the same time period and temperature resulted in complete elimination of *M. smegmatis*. Given that these phages were also capable of lysing *Mycobacterium avium* subsp. *paratuberculosis* (L. Endersen, J. O'Mahony & A. Coffey, unpublished observation), their ability to remain viable after incubation at 72°C for 15 minutes is significant in the context of application for control of this important pathogen, which has been isolated from pasteurized milk.

Phage-Derived Enzymes: Potential Biocidal Agents in Food Materials

Although few studies on the application of phage-derived enzymes in food have been reported in comparison to their use in human and veterinary medicine, their potential as biocontrol agents in the food industry is increasingly becoming a focus of research interest (O'Flaherty et al. 2009, Coffey et al. 2010, Garcia et al. 2010, Shakeeba et al. 2010, Callewaert et al. 2011).

Phage-derived peptidoglycan hydrolases. Endolysins (lysins) are peptidoglycan-degrading enzymes produced by bacteriophages during the terminal stage of their lytic life cycle (Young 1992). They are particularly effective when applied exogenously to Gram-positive bacteria, resulting in immediate bacteriolysis. Lysins are generally modular in structure, displaying an N-terminal catalytic domain and a C-terminal binding domain, and are categorized into four different groups depending on their cleavage site: (*a*) N-aceytlmuramidases (lysozymes), (*b*) N-acetyl-β-D-glucosaminidases (glycosidases), (*c*) N-acetylmuramoyl-L-alanine amidases, and (*d*) L-alanoyl-D-glutamate endopeptidases and interpeptide bridge-specific endopeptidases (Loessner 2005). Lysozymes and glycosidases hydrolyze the β-1–4 glycosidic bond in the sugar moiety of the cell wall. Amidases cleave the amide bond between the peptide and sugar moieties of the cell-wall peptidoglycan, and the endopeptidases are responsible for breaking the peptide bond of the bacterial cell wall (Loessner 2005). The bactericidal activity of endolysins has been demonstrated in several food-related applications. Gaeng et al. (2000) engineered a *Lactococcus lactis* dairy starter strain to secrete the anti-*Listeria* endolysin Ply511, which is active against *L. monocytogenes*. The authors proposed that this strain could be used to control *L. monocytogenes* in cheese production and in other milk fermentations. In another study, Zhang et al. (2012) evaluated the potential of the *Listeria* endolysin LysZ5 to eliminate *L. monocytogenes* in soya milk and observed a 4-log CFU/ml reduction after incubation at 4°C for 3 h. These authors suggested that LysZ5 could potentially be used to control this important pathogen in RTE foods. In the area of the foodborne pathogen *Clostridium perfringens*, Zimmer et al. (2002) showed that the *Clostridium* endolysin Ply3626 was active against all 48 *C. perfringens* strains tested and proposed an application for the control of *C. perfingens* in poultry. In the area of enterotoxin-producing *Staphylococcus aureus*, a study by Obeso et al. (2008) showed that the staphylococcal phage endolysin LysH5 completely eliminated the pathogen in pasteurized milk following a 4-h incubation at 37°C; however, they did not explore its activity in raw milk, where staphylococci are prevalent (as distinct from pasteurized milk). In a study by Rodríguez-Rubio et al. (2013), staphylococcal elimination in pasteurized milk was also effective. Significantly, experiments on raw milk were included, and the authors found that when a starting concentration of 10^3 CFU/ml *S. aureus* was challenged with the phage endolysin CHAPSH3b in raw milk, the lysin was effective in inhibiting the growth of the organism for ~30 minutes at 37°C. At room temperature, cell numbers increased but were kept below the control counts by ~1-log at both temperatures after 2 h. In a study on porcine skin decontamination, Fenton et al. (2013) demonstrated the potential of the bacteriophage-derived peptidase $CHAP_K$ to control *S. aureus*. Following the application of $CHAP_K$, 99% of cells were eliminated from pig skin in 30 minutes, indicating the potential application of endolysins for use in animal and carcass sanitation prior to slaughter and processing. In the area of *Salmonella* biocontrol, Waseh et al. (2010) conducted studies with a purified truncated phage tailspike endoglycosidase (P22sTsp) from the bacteriophage P22 targeting *S.* Typhimurium. Oral administration of P22sTsp to *S.* Typhimurium–infected chickens resulted in a significant reduction of the pathogen in the gut and prevented further penetration into internal organs. The authors suggested that the ability of tailspike proteins to resist the acid-induced denaturation and digestion by proteases in the gastrointestinal tract make them attractive for use as oral therapeutics against bacterial Gram-negative pathogens in food-producing animals.

Food Spoilage and Biosanitation

Food preservation has always been a necessary part of food production. Regardless of current preservation techniques and the fact that increased research has led to a greater understanding of how microbial food spoilage occurs, large quantities of foods produced globally each year are lost

to microbial spoilage (Gram et al. 2002). In the area of fruits and vegetables, Obradovic et al. (2004) demonstrated in field experiments the successful use of phages to control tomato bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria*. The application of six different species-specific phages at 10^{10} PFU/ml significantly reduced the bacterial spot severity in tomatoes artificially contaminated with 10^8-CFU *X. campestris* pv. *vesicatoria* by aerosolized spray. The authors found that the phage-treated fruit resulted in an increased amount of marketable produce in comparison to the control fruits. In a similar study, Flaherty et al. (2000) found that bacteriophages decreased the severity of the *X. campestris* disease by 17.5% in the autumn of 1997 and 16.8% in the autumn of 1998 when compared to the untreated control. *Streptomyces scabies* is a common contaminant of seed potato, and the efficacy of a polyvalent phage to sterilize *Streptomyces*-infected seed potato tubers was assessed by McKenna et al. (2001). Artificially infected seeds were bathed in a 10^9-PFU/ml phage suspension for 24 h, and it was found that the number of scab lesions on the mother tubers treated with the phage was significantly reduced ($p < 0.05$) compared with the controls. Also, there was a notable decrease ($p < 0.05$) in lesions on progeny tubers resulting from phage treatment. It is evident from the above examples that bacteriophages have a promising role to play as biosanitizing agents in the food industry.

Biofilms. Biofilm formation has been documented in many different industrial environments. They constitute a protective mechanism of microbial growth that allows many bacteria to survive unfavorable environmental conditions (Simoes et al. 2008). Doolittle et al. (1995) demonstrated the ability of bacteriophage $T4D^+$ to disrupt, infect, and multiply within the exopolymeric matrix of an *E. coli* 3000 XIII biofilm on the surface of polyvinylchloride coupons. In another study, Ferreira et al. (2011) assessed the antibacterial effect of a bacteriophage mixture against *S.* Enteritidis biofilms of different ages (4 days, 8 days, and 12 days cultivation) on stainless steel coupons. The bacteriophage mixture was effective in reducing biofilms of all ages, with the most significant decrease observed on the eight-day-old biofilms when treated with 10^7-PFU/ml bacteriophages for 35 minutes. In a similar study, Sillankorva et al. (2004) showed that anti-*Pseudomonas fluorescens* bacteriophages were successful at eliminating biofilms in the early stages of development. Five-day-old biofilms treated with bacteriophages resulted in an 80% removal of the attached cells under optimal conditions. In the area of *L. monocytogenes* biofilms, Soni & Nannapaneni (2010) demonstrated the biosanitizing effects of bacteriophage P100 against 21-L *monocytogenes* biofilms representing 13 different serotypes. A reduction in biofilm was observed across a wide range of *L. monocytogenes* strains with a 3.5–5.5 log/cm^2 decrease in *L. monocytogenes* cells achieved following the application of bacteriophage P100 to established biofilms (Soni & Nannapaneni 2010). *S. aureus* is a major biofilm producer, causing problems in many areas, including food-processing equipment. The biocontrol potential of a mixture of bacteriophage K and six modified derivatives to prevent and treat *S. aureus* biofilms was investigated by Kelly et al. (2012). Results suggested that the phage cocktail was effective in inhibiting biofilm formation after 48 h. Bacteriophage treatment of established biofilms resulted in a significant reduction in biomass after 72 h when compared to treatment for 24–48 h; as such, the authors proposed that phage efficacy is dependent on the duration of exposure. Fenton et al. (2013) used a phage lysin, $CHAP_K$ (which was originally cloned from the genome of phage K), to successfully disrupt staphylococcal biofilms. In this case, the authors reported biofilm elimination within 4 h following lysin application.

Biosensors

The rapid and early detection of foodborne pathogens is central to the food industry maintaining safe pathogen-free foods. The search for novel biological recognition elements to detect pathogens

in food and production environments is ongoing. Bacteriological methods for pathogen detection, although highly accurate, are relatively cumbersome and time consuming and not feasible for on-site use due to the need for extensive sample preparation and incubation. Advances in phage-based biosensors for foodborne pathogen detection have recently been reviewed by Singh et al. (2013), and the following are some notable examples. Arya et al. (2011) used surface plasmon resonance (the transducer) combined with chemically immobilized bacteriophage T4 (the specific receptor) for the detection of *E. coli* K12 and found that the maximum bacterium capture was obtained when a titer of 1.5×10^{11} PFU/ml of phage T4 was used for immobilization onto gold surfaces. The lower and higher limits of detection ranged from 7×10^2 to 7×10^8 CFU/ml, highlighting the usefulness of this bacteriophage-based bioassay. In another study, Blasco et al. (1998) developed an assay for the rapid detection of *E. coli* or *Salmonella* using bacteriophages as probes to bind and lyse the bacterial cells, which released an indirect bioluminescent marker, adenylate kinase. These researchers reported that less that 10^3 CFU/100 μl could be detected in the sample after 1 h. A different approach exploiting biotinylated phages conjugated to streptavidin-coated quantum-dot complexes for bacterial detection was demonstrated by Edgar et al. (2006), and this method also proved to be very sensitive, detecting as few as ten cells per ml in experimental samples. In another study focusing on the detection of *Listeria*, Loessner et al. (1996) constructed a luciferase reporter phage for the rapid detection of viable *L. monocytogenes* cells. The sensitivity of the assay was as low as 10^2 to 10^3 CFU/ml, following a 2-h incubation postinfection. Luminescence was detected using a single-tube luminometer. The same research group exploited a *Listeria* phage endolysin-associated cell wall–binding domain in a magnetic separation technique to successfully capture and detect *L. monocytogenes* in food (Kretzer et al. 2007). The paramagnetic beads were coated with a recombinant cell wall–binding domain, which specifically recognized and bound the peptidoglycan of *L. monocytogenes*, capturing more than 90% of *L. monocytogenes* cells from artificially contaminated samples within 20–40 minutes. In addition, the same research group used this method to successfully capture *L. monocytogenes* from artificially contaminated raw milk (Walcher et al. 2010). Loessner et al. (2002) and Eugster et al. (2011) fused different endolysin-associated cell wall–binding domains to green fluorescent protein, which permitted the detection and visualization of many different serovars of *L. monocytogenes*.

SAFETY CONCERNS

Bacteriophages and their derivatives offer great potential for use as agents for pathogen biocontrol and pathogen detection in foods, and the evidence suggests that the advantages far outweigh the disadvantages (**Table 2**). To date, there is no evidence that phages exhibit harmful effects on humans or animals (O'Mahony et al. 2011b). They are abundant in the environment and are consumed unwittingly on a daily basis via our food and water. In addition, the results of numerous animal studies provide convincing evidence as to the safety of bacteriophages as biotherapeutics. Carlton et al. (2005) conducted oral toxicity studies in albino rats, monitoring them for eight days following five consecutive days of administrations of a 5×10^{11} PFU/ml phage preparation. They concluded that the phages have no effect on rodent behavior or physical appearance. Bruttin & Brussow (2005) administered *E. coli* phage T4 to a group of volunteers at low (10^3 PFU/ml) and high (10^5 PFU/ml) doses without any adverse effects. The principal safety requirement is that phages that are to be used as antibacterials must be confirmed by genome sequencing to be non-temperate (exclusively virulent) phages. The sequencing approach ensures that the genome is free of genes encoding bacterial virulence factors and also free of genetic elements, which facilitate gene transfer among bacteria. Temperate phages, which enter the lysogenic cycle, play an important role in bacterial evolution

Table 2 Advantages and possible disadvantages of phage therapy

Whole bacteriophages		Bacteriophage endolysins	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Highly specific, natural, low-cost, bactericidal agents that impart minimal disruption to normal microflora if applied to animals	Narrow host range, meaning that a mixture of several phages is usually required	Rapid bactericidal effect	The outer membrane of Gram-negative bacteria hinders unmodified endolysins reaching the peptidoglycan.
Ability to replicate at the site of infection, facilitating the bacterial-killing process	Knowledge of phage biology often required by end users	Endolysin resistance has never been reported.	Endolysins are generally available as recombinant proteins, resulting in higher production costs than phages.
Do not affect the taste, texture, smell and color of food	Potential for negative consumer perception regarding the use of the terminology "viruses" or "virulent phages" in foods	Endolysins target prokaryotic peptidoglycan and are harmless to humans, animals and plants	Potential limited shelf life of endolysins may limit their application.
Phages are abundant in the natural environment and are harmless to humans, animals, and plants.		Like bacteriophages, endolysins are generally genus specific, causing minimal interference with commensal flora.	
Unlike antibiotics, phages and their derivatives are active against dividing and nondividing cells.			
Effective in elimination of biofilms			
Phages also have applications as delivery vehicles and pathogen detection systems.			

and have been shown to transmit genetic determinants for potent virulence factors among bacteria. A key example is the shiga-converting phages, which are responsible for the spread of *stx* genes in *E. coli* O157 (Schmidt 2001, Herold et al. 2004). Another interesting example of gene transfer was reported by Chen & Novick (2009). In this case, a transducing staphylococcal phage was shown to be responsible for the transfer of a pathogenicity island between *S. aureus* and *L. monocytogenes*. Although it is generally recognized that phage-mediated genetic transfer frequently occurs among species within a bacterial genus, this study documented the transfer of genetic material across distinct genera. Another requirement is that phage products must guarantee complete elimination of all bacterial members within a specific pathogenic bacterial grouping, e.g., the hemorrhagic *E. coli* group. Thus, it is vital that phage products be composed of lytic phage mixes in which the phages each have a sufficiently broad host spectrum. There have also been reports that lytic phages can select for mucoid phenotypes in *Pseudomonas fluorescens* (Scanlan & Buckling 2011). Although the precise mechanistic basis of this coevolution was not identified, it does suggest that the response of bacteria to phage attack should be considered when designing a phage strategy for clinical or food settings.

CURRENT EXPLOITATION OF PHAGES AS BIOCONTROL AGENTS IN THE FOOD INDUSTRY

The undeniable antimicrobial properties of phages coupled with (*a*) the decrease in antibiotic efficacy, (*b*) the steep decline in the number of pharmaceutical companies investing in new pipeline drugs, and (*c*) consumer demands for the production of foods that are free from pathogens and synthetic chemicals has encouraged numerous companies to invest in the production of phage-based products. In the context of food safety, the bacteriophage-based product Agriphage™, produced by OmniLytics Inc. to treat bacterial spot disease on crops, was the first phage-based product formally approved for use in agriculture by the US regulatory agencies (US Environ. Prot. Agency 2005). A year later, in 2006, approval was granted for the first food safety–related phage preparation ListShield™ (LMP-102™), from Intralytix Inc., to control *L. monocytogenes* in RTE foods composed of meat and poultry products in the United States (Bren 2007). This event marked the first time phages were considered generally recognized as safe (GRAS) by the FDA. Since then, ListShield™ has been registered for use as an organic food additive in Europe and has also received approval by Food Standards Australia & New Zealand (Hodgson 2013). Numerous other phage-based products have been approved for use; for instance, another anti-*Listeria* phage preparation, Listex P100, developed by EBI Food Safety, the Netherlands, has been approved for use as a processing aid in all foods susceptible to *L. monocytogenes* contamination (Hagens & Offerhaus 2008). In 2007, the FDA approved the use of anti-*E. coli* and anti-*Salmonella* phage-based preparations, produced by OmniLytics Inc., to decontaminate live animals prior to slaughter (Garcia et al. 2010). EcoShield™ (Intralytix Inc.) received regulatory approval in 2011 for use against *E. coli* O157:H7 on red meat before being ground into hamburgers, eliminating 95–100% of contaminants (Sillankorva et al. 2012). In February 2013, SalmoFresh™ (Intralytix Inc.) received regulatory approval for use in eliminating *Salmonella* in poultry products and other foods (**http://www.intralytix.com**). Elanco Food Solutions together with OmniLytics Inc. produced two phage products to reduce contamination in meats and poultry prior to processing, namely Finalyse, which targets *E. coli* O157:H7 and is currently available for use as a hide spray on cattle prior to slaughter; Armament, also commercially available for use, targets *Salmonella* on poultry (Goodridge & Bisha 2011). BioTector, developed by CJ CheilJedang (Seoul, South Korea), is the first phage-based product to replace antibiotics in animal feed, controlling *Salmonella* species responsible for causing fowl typhoid and pullorum disease (Monk et al. 2010).

FOOD FOR THOUGHT: THE FUTURE OF PHAGE THERAPY IN THE FOOD INDUSTRY

Phage therapy historically fell into disuse with the advent of antibiotics. It is now back on the agenda, with increasing belief in its efficacy as a natural, effective biotherapeutic. This has resulted from the improved understanding of phage biology and also an appreciation of their technical limitations. The application of phage technology is further strengthened by the number of companies worldwide investing in the production of phage-based products for use in biocontrol, decontamination, sanitation, and detection in the food industry (**Table 3**). Phages and their derivatives show tremendous promise as potential means to control and eradicate pathogenic bacteria in pre- and postharvest foods, as biopreservatives against food spoilage organisms, as sanitizing agents on farms and in industrial settings, and as biorecognition devices to detect harmful pathogens in foods. Commercially exploiting bacteriophages to minimize the economic burden of microbial contamination in foods and food processing environments is worthy of strong and continued consideration.

Table 3 Worldwide distribution of companies exploiting phages for therapeutic applications

Company	Location	Company	Location
Intralytix	Maryland, USA	Phage Therapy Center	Tbilisi, Georgia
OmniLytics Inc.	Utah, USA	Special Phage Services Pty, Ltd.	New South Wales, Australia
Elanco Food Solutions	Illinois, USA	Gangagen Biotechnologies PVT Ltd.	Bangalore, India
EBI Food Safety	Wageningen, the Netherlands	Phage Biotech Ltd.	Rehovot, Israel
CJ CheilJedang Corporation	Seoul, Korea	Hexal Genentech	Holzkirchen, Germany
Phage Works	Fermoy, Ireland	Innophage	Porto, Portugal
BigDNA	Edinburgh, UK	Viridax	Florida, USA
Blaze Venture Technologies	Hertfordshire, UK	Gangagen Inc.	California, USA
Phico	Cambridge, UK	Phage International	California, USA
AmpliPhi Biosciences Corporation	Bedfordshire, UK	New Horizons Diagnostics	Maryland, USA
Novolytics	Coventry, UK	Neurophage Pharmaceuticals	Massachusetts, USA
JSC Biochimpharm	Tbilisi, Georgia	Targanta Therapeutics	Massachusetts, USA
Biopharm Ltd.	Tbilisi, Georgia	Biophage Pharma Inc.	Montreal, Canada

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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