Mini-review

Received: 22 March 2013 Revised: 28 April 2013 Accepted article published: 13 May 2013 Published online in Wiley Online Library: 17 June 2013

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6222

Using lytic bacteriophages to eliminate or significantly reduce contamination of food by foodborne bacterial pathogens

Alexander Sulakvelidze*

Abstract

Bacteriophages (also called 'phages') are viruses that kill bacteria. They are arguably the oldest (3 billion years old, by some estimates) and most ubiquitous (total number estimated to be 10^{30} – 10^{32}) known organisms on Earth. Phages play a key role in maintaining microbial balance in every ecosystem where bacteria exist, and they are part of the normal microflora of all fresh, unprocessed foods. Interest in various practical applications of bacteriophages has been gaining momentum recently, with perhaps the most attention focused on using them to improve food safety. That approach, called 'phage biocontrol', typically includes three main types of applications: (i) using phages to treat domesticated livestock in order to reduce their intestinal colonization with, and shedding of, specific bacterial pathogens; (ii) treatments for decontaminating inanimate surfaces in food-processing facilities and other food establishments, so that foods processed on those surfaces are not cross-contaminated with the targeted pathogens; and (iii) post-harvest treatments involving direct applications of phages onto the harvested foods. This mini-review primarily focuses on the last type of intervention, which has been gaining the most momentum recently. Indeed, the results of recent studies dealing with improving food safety, and several recent regulatory approvals of various commercial phage preparations developed for post-harvest food safety applications, strongly support the idea that lytic phages may provide a safe, environmentally-friendly, and effective approach for significantly reducing contamination of various foods with foodborne bacterial pathogens. However, some important technical and nontechnical problems may need to be addressed before phage biocontrol protocols can become an integral part of routine food safety intervention strategies implemented by food industries in the USA.

Keywords: bacteriophages; phages; food safety; foodborne illness

INTRODUCTION

Bacteriophages are bacterial viruses discovered independently by Frederick Twort and Felix d'Herelle during the early 20th century.[1] The name, coined by d'Herelle, originates from *bacteriophage* or 'bacteria-eater' (from the Greek *phago* meaning *to eat* or *devour*).[2] Because of their remarkable antibacterial potency, phages were used to prevent and treat human infections (a clinical approach commonly called 'bacteriophage therapy' or 'phage therapy') almost immediately after their discovery.[3,4] However, clinical use of phages declined with the discovery and increased use of antibiotics during the 1940s and 1950s, and because of several other factors.[4–6] Conversely, interest in the practical applications of bacteriophages has been growing recently, and their potential applications are increasingly being examined for various purposes ranging from improving food safety to preventing and treating bacterial diseases, particularly those caused by multi-drug-resistant bacterial pathogens.

The recently increased interest in phage biocontrol protocols for improving food safety has occurred for several reasons, the most important of which may be increased customer and regulatory pressures to ensure food safety while reducing the use of environmentally harsh chemical sanitizers and disinfectants. Indeed, poor food safety is a very significant problem worldwide. For example, the USA's Centers for Disease Control and Prevention estimates that each year about one in six Americans (*ca* 48 million people) get sick, 128 000 are hospitalized, and 3000 die of foodborne diseases.[7] According to a recent estimate,[8] the cost of illnesses caused by 14 major foodborne pathogens is *ca* $14 billion per year in the USA, *ca* 90% of which is caused by the five most common causes of foodborne diseases. Of these, *Salmonella enterica* is the most common etiologic agent ($3.3 billion), followed by *Campylobacter* spp. ($1.7 billion), *Listeria monocytogenes* ($2.6 billion), *Toxoplasma gondii* ($3 billion), and norovirus ($2 billion). Diseases caused by *Salmonella* spp. also constitute a very significant risk outside the USA; for example, they have been estimated[9] to cause *ca* 93.8 million illnesses globally, and *ca* 155,000 deaths, each year. Therefore, food processors worldwide implement various approaches to ensure the safety of the foods they produce. Currently, the conventional pathogen decontamination protocols in food-processing facilities focus primarily on using chemicals, physical disruption techniques, and irradiation to remove a broad spectrum of foodborne bacterial pathogens from those facilities and from the foods produced in them.[10–12] However, no single approach is 100% effective,

* *Correspondence to: Alexander Sulakvelidze, Intralytix, Inc., The Columbus Center, Baltimore, MD 21202, USA. E-mail: asulakvelidze@intralytix.com*

Intralytix, Inc., The Columbus Center, Baltimore, MD 21202, USA

and the above-mentioned approaches also have some significant drawbacks, including their ability to corrode food-processing equipment, the toxic effects of chemical residues, and their ability to damage the organoleptic properties of some foods. These broad-spectrum approaches also kill potentially beneficial bacteria that are important components of foods. In contrast, although bacteriophages have their own limitations,[13–16] they do offer an environmentally safe, noncorrosive, and effective modality for eliminating or significantly reducing the levels of their specifically targeted bacterial pathogens in various foods, without a deleterious impact on the organoleptic properties, and without disrupting the normal and often beneficial microflora, of those foods.

FOOD SAFETY-RELATED APPLICATIONS OF BACTERIOPHAGES

Contamination of foods with foodborne bacterial pathogens may be reduced by three main types of phage treatments: (i) treating domesticated livestock with phages; (ii) treatments for decontaminating inanimate surfaces in food-processing facilities and other food establishments; and (iii) post-harvest treatments involving direct applications of phages onto the harvested foods. This mini-review briefly reviews the first two approaches before focusing on the last type of intervention, which currently seems to be the one gaining the most momentum.

Using phages to prevent or significantly reduce colonization of domesticated livestock with pathogenic bacteria

This approach involves, as the name implies, administering phages to live 'food animals' before they are processed for meat. The proposed rationale for this approach is that phages may be used to prevent and/or significantly reduce colonization of livestock with pathogenic bacteria that may contaminate their meat during the animals' slaughter and subsequent carcass processing. In other words, the phages are administered in order to reduce shedding of specific foodborne bacterial pathogens and thus reduce the risk of subsequent contamination of food products containing the animals' meat. Administering phages to domesticated animals, for the purpose of preventing or treating bacterial diseases, is commonly called 'phage therapy' and it is not reviewed here. However, several publications[17] and other reviews[13] dealing with that subject are available.

At the present time, the value of treating live animals with phages in order to improve food safety is somewhat controversial because studies examining the efficacy of that approach have yielded somewhat discordant results. For example, as early as the middle 1920s, Topley *et al.*[18] reported that oral administration of *Salmonella*-specific phages did not reduce the number of salmonellae shed by experimentally infected mice. A similar observation with *Salmonella* in chickens was reported recently by Hurley *et al.*[19] Also, Bach *et al.*[20] found that a phage possessing strong lytic activity against a challenge strain of *Escherichia coli* O157:H7 did not significantly reduce the shedding of the bacterium by experimentally challenged sheep. On the other hand, several other publications reported that phages significantly reduced shedding of bacterial pathogens from phage-treated live animals. For example, Niu *et al.*[21] reported that fecal shedding of *E. coli* O157:H7 was reduced if penned cattle harbored *E. coli* O157:H7-specific phages. Similarly positive results were published by other investigators[22–29] who observed that oral administration of phages significantly reduced their targeted bacterium's shedding from the treated animals. If the latter laboratory data are reproduced in real-life food-processing settings, the public heath implications of such phage biocontrol protocols could be significant. For example, according to one modeling study,[30] a 2-log reduction in the *Campylobacter* loads in poultry intestines may result in a 30-fold reduction in the incidence of campylobacteriosis associated with consumption of meals containing chicken meat. However, even if the approach is effective, its practical implementation may face some significant challenges, ranging from identifying the optimal route for phage administration to cost considerations. Also, exposing phages to complex environments, such as chicken houses and cattle barns, is likely to select for phage-resistant mutants, which may hinder the long-term efficacy of the approach. Finally, the regulatory requirements may be more onerous than those for post-harvest interventions, and they may vary depending on the marketing claims made for the phage-based products. For more information on the topic, several reviews are available.[13,15,16,31]

Using phages to decontaminate inanimate surfaces

The second broad approach (sometimes called 'phage biosanitation') for using phages to improve food safety is to use them to decontaminate various inanimate surfaces in household kitchens, food processing facilities, and other food establishments, so that the foods contacting those surfaces are less likely to become contaminated with foodborne bacterial pathogens. Indeed, appropriate cleaning and decontamination of food-contact surfaces is important for preventing foodborne bacterial diseases.[32] Although most, if not all, foodborne bacterial pathogens are inactivated when foods are properly cooked, some of the contaminating bacteria may survive on the surfaces on which the foods were processed before cooking. Therefore, if other foods come into contact with those surfaces, the bacteria may contaminate them and cause foodborne disease, particularly if the foods are ready-to-eat foods that are not cooked before consumption.[33,34] Similarly, foodborne bacteria may persist on various surfaces in food-processing facilities and contaminate foods that are being processed or packaged in those facilities. To address this problem, food processors commonly use various chemicals to remove a broad spectrum of bacteria from various surfaces in their facilities.[12] However, although chemical sanitizers and disinfectants may be effective, they also may have significant drawbacks not relevant for phage preparations, such as corrosion of equipment, toxic effects of chemical residues, and damage to the organoleptic properties of foods. Thus one of the potential applications of bacteriophages is to use them to eliminate or reduce levels of foodborne bacterial pathogens on various hard surfaces commonly used in food-processing facilities and in home kitchens.[31,35]

The results of recent studies suggest that lytic phages can significantly reduce contamination of various hard surfaces (e.g. gypsum board, stainless steel, glass) with various bacterial pathogens, including *Listeria monocytogenes*, [36,37] *E. coli* O157:H7,[38–40] and *Yersinia pestis*.[41] Also, at least one bacteriophage preparation – ListShield™ – has been registered, by the United States Environmental Protection Agency (EPA registration number 74234-1), as a 'microbial pesticide' suitable for significantly reducing *L. monocytogenes* contamination of food-processing plants and food-handling establishments. Because of the specificity of phages, the EPA's registration mandates that ListShield™ must be

used as part of an overall sanitation and cleaning protocol rather than in a 'stand-alone' protocol. Furthermore, because most chemical sanitizers inactivate phages, an appropriate time period must be allowed to pass before treating phage-treated surfaces with chemical sanitizers. For example, if surfaces are treated with ListShield™, at least 5 min must elapse before treating the surfaces with chemical sanitizers. However, despite those limitations, if the efficacy reported in the above-referenced published studies can be reproduced in food-handling and processing facilities, a phage cocktail-based approach may help to reduce the immediate levels of targeted foodborne bacterial pathogens in various food-processing establishments. On a more hypothetical level, phage-based preparations might be useful for eliciting a long-term reduction in the contamination of food-processing facilities with specific bacterial pathogens of concern, as explained in the following paragraph.

At the present time, various chemical sanitizers or disinfectants are routinely used during attempts to produce bacteria-free environments in food-processing and packaging facilities.[12] However, such environments are unsustainable because they tend to become rapidly repopulated by various microorganisms, potentially including the pathogenic bacteria that were the targets of the original, chemical-based sanitation protocol. Therefore, routinely using a phage preparation that only kills a specific foodborne bacterial pathogen of concern may provide a subtle selective pressure that may, over time, make it increasingly difficult for that pathogen to re-establish itself in those environmental niches. The idea of such a phage-mediated 'eco-management' approach is purely hypothetical at the present time, and additional long-term studies are required to confirm or refute its validity.

Post-harvest interventions applying phages to the surfaces of various foods

The third approach of using phages to improve food safety is to apply them to the surfaces of the foods, an approach designed to eradicate or significantly reduce the number of specifically targeted foodborne bacterial pathogens contaminating the foods. Phages are naturally present in all fresh and non-processed foods, including fresh ground beef, fresh fruits and vegetables, raw skim milk, cheese, and frozen mixed vegetables.[13,14,42] Thus the concept of using phage biocontrol protocols involving the direct application of phages to various foods is essentially based on using a microorganism that may already be present in those foods, and simply applying the appropriate number of appropriate phages to the appropriate location. Thus, if a food is contaminated with a bacterial pathogen that is the host for the lytic phages applied to the food's surface, the phages should eliminate or significantly reduce the contamination, thereby making the food safe to consume without deleterious effects on its normal, beneficial microflora and organoleptic qualities.

The results of a growing number of published studies support the idea that directly applying appropriate lytic phages to the surfaces of various foods often significantly reduces their contamination with various foodborne bacterial pathogens. The efficacy of phage treatment appears to vary depending on the types of foods used, the levels of contaminating bacteria, the phage concentrations applied, etc. The treatment outcomes ranged from ineffective (e.g. in one study[43] listeriophages combined with nisin were unable to reduce *L. monocytogenes* concentrations in raw beef samples) to complete eradication of the targeted pathogens.[13] Most of the publications focus on determining the impact of phages on such common foodborne pathogens as *L. monocytogenes* and *Salmonella*, although their

Figure 1. Examples of commercially available phage preparations for food safety applications. Commercial packaging of Listex™ P100 (left) and ListShield™ (right). Both preparations target *L. monocytogenes* in various foods.

impact on other, less-common foodborne bacterial pathogens, e.g. *Enterobacter sakazakii* (now *Cronobacter sakazakii*)[44,45] and *Staphylococcus aureus*,[46,47] also has been reported. Interestingly, several studies[48–50] found that phages were effective in reducing the levels of bacterial pathogens in various foods at storage temperatures ranging from 5 to 20 °C, even though many foodborne bacteria do not grow well at refrigeration temperatures. Some of the relevant studies are summarized in Table 1, and they are discussed in more detail in several recent review articles.[13,14,16,31,35,51–53] Furthermore, the FDA and USDA recently approved several commercial phage-based products for direct food applications (Figure 1), as discussed in more detail in the 'Regulatory Approvals' section of this mini-review.

A BRIEF OVERVIEW OF THE COMPANIES INVOLVED WITH DEVELOPING AND COMMERCIALIZING PHAGE-BASED PREPARATIONS FOR POST-HARVEST FOOD SAFETY APPLICATIONS

The companies involved with developing and commercializing phage preparations for food safety applications have been briefly reviewed previously.[31,35] However, since that review, some of the companies have gone out of business, or redirected their focus, or changed their names. Therefore, an updated list of the 'phage companies' is presented in Table 2. Overall, the number of companies involved with the development and commercialization of phage-based preparations for post-harvest food safety applications is still very small. However, some of them recently reported entering into various licensing and partnership agreements with multinational large corporations. For example, Omnilytics has entered into a collaborative research and licensing agreement with Elanco, a division of Eli Lilly and Company (NYSE: LLY), to develop and market phage-based products active against various foodborne bacterial pathogens, including *E. coli* O157:H7 and *Salmonella* (http://www.omnilytics.com/news/news020.html). These types of partnerships with large corporations are likely to accelerate the introduction of phage-based products into various food industries and result in benefits to the industries and the consumers of their products.

Table 1. Some studies of post-harvest phage biocontrol interventions (direct food applications)

Targeted bacterium	Comments /study summary	Ref.
Escherichia coli O157:H7	A mixture of three phages applied to the surfaces of beef contaminated with the test bacterium (10^3 CFU g^{-1}) eradicated the bacterium in most of the treated specimens incubated at 37 °C	62
	EcoShield™ (a commercially available preparation composed of three lytic bacteriophages specific for *E. coli* O157:H7) significantly ($P < 0.05$) reduced the test bacterium concentration in tomatoes, spinach, broccoli, and ground beef. The reductions ranged from 94% (at *ca* 120 h post-treatment of the tomato specimens) to 100% (at *ca* 24 h post-treatment of the spinach specimens)	38
	EcoShield™ significantly reduced the concentration of the test bacterium on lettuce and cut cantaloupe	63
	EcoShield™ significantly reduced the test bacterium concentration in experimentally contaminated beef by ≥94% and in lettuce by *ca* 87%. However, the one-time application of EcoShield™ did not protect the foods from recontamination with the test bacterium	59
Listeria monocytogenes	A phage cocktail (LMP-102, later renamed to ListShield™, a commercially available preparation composed of six lytic bacteriophages specific for *L. monocytogenes*) significantly reduced the test bacterium levels by 2.0–4.6 log units on melons and by 0.4 log units on apples. Combined treatment with the phage cocktail and nisin significantly reduced bacterial counts, by 5.7 and 2.3 log units, on cut melon and apples, respectively. The observed reductions were approximately the same irrespective of whether the phages were applied by spraying or pipetting onto the pieces of cut fruit	56
	LMP-102 was most effective and its efficacy was phage concentration dependent when administered 1, 0.5, or 0 h before contamination with the test bacterium. It reduced the bacterium concentration by up to 6.8 log units after 7 days' storage	57
	Listeria phage P100 (a component of Listex™, a single phage-containing commercial phage preparation), applied to the surfaces of ripened red-smear soft cheese during rind washings, significantly reduced test bacterium levels in treated cheese specimens by at least 3.5 log units. Evidence of phage-resistant mutants was not detected in the *Listeria* isolates recovered from specimens	64
	Listeria phages A511 and P100 rapidly reduced bacterial counts, in a concentration-dependent manner, to undetectable levels in chocolate milk and mozzarella cheese brine stored at 6 °C. Also, they reduced bacterial counts, by up to 5 log units, on various solid foods (hot dogs, sliced turkey meat, smoked salmon, seafood, sliced cabbage, and lettuce leaves)	65
	Listex™ significantly reduced test bacterium concentrations in raw catfish fillets by 1.4–2.0 log CFU g^{-1} at 4 °C, by 1.7–2.1 log CFU g^{-1} at 10 °C, and by 1.6–2.3 log CFU g^{-1} at room temperature (22 °C). The reductions in test bacterium concentrations were maintained during storage for 10 days at both refrigerated temperatures	66
	Listex™ applied to raw salmon fillets (*ca* 10^8 PFU g^{-1}) significantly reduced test bacterium levels (after storage at either 4 or 22 °C) by 1.8, 2.5, and 3.5 log CFU g^{-1} from their initial loads of 2, 3, and 4.5 log CFU g^{-1}	67
	Listeria phage A511 significantly reduced the levels of test bacterium in white mold cheese and in washed-rind cheese with a red-smear surface. The results of studies performed with low initial levels of contamination (10–10^2 CFU cm^{-2} of cheese) revealed that the phage treatment reduced viable counts to below the limit of detection, which corresponded to more than a 6 log reduction in the control cheeses' initial levels of contamination	68
	A *Listeria* phage morphologically similar to phage A511 significantly reduced test bacterium levels in vacuum-packed, ready-to-eat chicken breast rolls by 2.5 log CFU cm^{-2} at 30 °C. Also, storage at 5 °C prevented bacterial regrowth for >21 days post-treatment with the phage	69
Salmonella spp.	This published study was the first one that examined the value of phage biocontrol for post-harvest food applications. The authors observed that a phage cocktail reduced *Salmonella* concentrations, by *ca* 3.5 log units, on honeydew melon slices stored at 5 and 10 °C, and by *ca* 2.5 log units on slices stored at 20 °C. The phages did not significantly reduce *Salmonella* contamination of apple slices, presumably because of their rapid inactivation by the apples' acidic pH of 4.2	50
	Lytic bacteriophages applied to chicken skin experimentally contaminated with *Salmonella* Enteritidis (the phage:bacteria ratio was 100:1 or 1000:1) rapidly reduced the number of viable bacteria by *ca* 2 log units after storage for 48 h. Also, the phages eradicated low levels of contamination	49
	Phage Felix-O1 significantly reduced *Salmonella typhimurium* contamination of chicken frankfurters by 1.8–2.1 log units	70
	Phage PHL 4 significantly reduced the frequency of *Salmonella* recovery from experimentally contaminated broiler carcasses	71
	Phage P7 significantly reduced *Salmonella typhimurium* levels (by 2–3 log units at 5 °C and by >5.9 log units at 24 °C) in raw and cooked beef. Efficacy increased when the phage:bacteria ratio was increased to 10 000:1 and host density was high (*ca* 10 000 CFU cm^{-2}). Phage-resistant mutants were not detected in surviving colonies of the test bacterium	72

Table 1. (*Continued*)

Targeted bacterium	Comments /study summary	Ref.
	Phages specific for *Salmonella* Enteritidis decreased that test bacterium's concentration, by 1–2 log units, in raw and pasteurized cheeses made from milk to which the phages were added. In contrast, the level of contamination in cheeses made from milk to which the phages were not added increased by *ca* 1 log unit	48
	Treatment with phage FO1-E2 (*ca* 3×10^8 PFU g^{-1} of food) eradicated (i.e. totally eliminated) *Salmonella typhimurium* from various ready-to-eat foods stored at 8 °C. At 15 °C, the treatment significantly reduced viable counts, by 5 log units, on turkey deli meat and in chocolate milk, and by 3 log units on hot dogs and in seafood	73
Campylobacter jejuni	Treatment with the *C. jejuni*-specific phage Cj6 significantly reduced the levels of test bacterium in cooked meat and in raw meat. The reduction was highest (>4 log units) when the phage:bacteria ratio was increased to 10 000:1 and host density was high (*ca* 10 000 CFU cm^{-2}). In some instances, primarily where the initial bacterial contamination was low (i.e. *ca* 100 CFU cm^{-2}), the reduction was not statistically significant	72
	C. jejuni phages significantly reduced test bacterium concentration on experimentally contaminated chicken skin by *ca* 2 log units, when the phage:bacteria ratio was 100:1 and 1000:1	49
	Applying *Campylobacter*-specific bacteriophages to the surface of chicken skin inoculated with *Campylobacter* significantly reduced the number of viable bacteria, by *ca* 1 log CFU, in contaminated skin specimens stored at 4 °C	58
Cronobacter sakazakii	Phages suppressed the growth of *Enterobacter sakazakii* (now *Cronobacter sakazakii*) in infant formula stored at 24 and 37 °C. Also, the highest phage concentration tested (10^9 PFU mL^{-1}) eradicated the bacterium from the formula	44
	A cocktail composed of five phages prevented the growth of 35 of 40 test strains tested in experimentally contaminated infant formula. Also, a dose of 10^8 PFU mL^{-1} eradicated the test strains from a liquid culture medium contaminated with both high and low concentrations (10^6 and 10^2 CFU mL^{-1}) of the bacterial cells	45
Shigella spp.	Single phages or a phage cocktail were used to treat ready-to-eat, spiced specimens of chicken meat contaminated with either individual *Shigella* spp. (*ca* 1×10^4 CFU g^{-1}) or a mixture of shigellae (*S. flexneri* 2a, *S. dysenteriae* and *S. sonnei*, at a total concentration of *ca* 3×10^4 CFU g^{-1}). Treatment with the phage cocktail was more effective than treatment with a single phage-containing preparation. However, in all instances, the phage preparations elicited a significant reduction in viable counts, ranging from 2 log units g^{-1} to eradication	74
Staphylococcus aureus	A cocktail composed of two phages (Φ88 and Φ35) eradicated *S. aureus* from experimentally contaminated whole milk at 37 °C. Also, the test bacterium exposed to the phages was not detected in acid curd after 4 h of incubation at 25 °C, and it was eradicated from renneted curd within 1 h of incubation at 30 °C	47

REGULATORY APPROVALS

Bacteriophage-based products designed to improve food safety must be cleared by pertinent regulatory agencies before they can be used in post-harvest food-processing facilities. Such preparations can be regulated by various regulatory strategies, as illustrated by the following examples of several recent approvals. The first formal 'approval' of a phage-based preparation developed for food safety applications came during August 2006, when the FDA cleared ListShield™ (formerly known as 'LMP-102'), a multivalent (i.e. containing several phages) phage product designed to reduce or eliminate *L. monocytogenes* contamination in ready-to-eat poultry and beef products (21 CFR §172.785).[54] Shortly after that approval, during October 2006, the FDA issued a 'no objection' letter for the GRAS (Generally Recognized As Safe) designation for a single phage-containing preparation (designated Listex™) that also was active against *L. monocytogenes* (GRN #000198). More recently, during February 2011, the FDA cleared EcoShield™ (formerly known as 'ECP-100'), a multivalent phage preparation designed to reduce contamination of ground beef with *E. coli* O157:H7, as a food contact substance (FCN #1018). Finally, in February 2013, the FDA issued a 'no objection' letter for the GRAS designation for another multivalent phage preparation called SalmoFresh™, which was designed to kill *Salmonella enterica*, particularly strains belonging to the most common/highly pathogenic serotypes Typhimurium, Enteritidis, Heidelberg, Newport, Hadar, Kentucky, and Thompson, Georgia, Agona, Grampian, Senftenberg, Alachua, Infantis, Reading, and Schwarzengrund (GRN #000435). Thus, in the USA, phage-based products developed for food safety applications may be commercialized by pursuing any of the above three regulatory paths. Brief descriptions of the paths and their respective pros and cons are presented below.

Food additive (FA)

A phage-based product may be regulated as an FA by having a filed Food Additive Petition (FAP) cleared by the FDA. Intralytix, Inc. pursued that route for the first phage-based food safety product ListShield™, which was cleared by the FDA in August of 2006 (Figure 1). A food additive approval is the most complex and, perhaps, the most prestigious FDA clearance because it implies that the FDA and the USDA reviewed the product's portfolio and concurred that it is both safe and effective. Also, if the regulation is properly worded, it may permit using new phages to update a phage cocktail without having to file a new FAP with the FDA (for more information concerning that subject, see the 'Updating of lytic phage-based products' section). On the downside, since an FAP is relatively complex to assemble, it is also the most expensive to prepare and takes the longest time to be approved.

Table 2. Companies that are developing phage-based preparations for food safety applications (in alphabetical order)

Company	Web address	Location	Products and regulatory strategy
GangaGen, Inc.	www.gangagen.com	Bangalore, India; Newark, CA, USA	The company, which was founded (as GangaGen Biotechnologies Pvt. Ltd) in India during September 2000, initially utilized an *in vivo* intervention strategy for developing food safety products. However, at the present time, it appears to focus on developing human therapeutic applications. Its main candidate product is recombinant protein P128 for the topical prevention and treatment of staphylococcal skin infections
Intralytix, Inc.	www.intralytix.com	Baltimore, MD, USA	Intralytix (founded during 1998) is the first company to receive a food additive approval from the FDA, for ListShield™ during 2006. It also received EPA approval (during 2008) for using the same product to clean inanimate surfaces in food-processing facilities. The Company's 2nd food safety product – EcoShield™, which is specific for *E. coli* O157:H7 – received FDA clearance, as an FCS, during 2011. During February 2013, the company announced that it had received a GRAS designation for its 3rd food safety product – SalmoFresh™, which is specific for *Salmonella enterica* in various foods
Micreos Food Safety	www.micreosfoodsafety.com	Wageningen, Netherlands	The company is a spin-off of EBI Food Safety, which was, in turn, a spin-off of Exponential Biotherapies. The latter was the first 'phage therapy' company founded (during the early 1990s) in the USA. Micreos has one product on the market – Listex™ P100 – which is specific for *L. monocytogenes* in various foods. Listex™ P100 was the first phage-based product to receive a GRAS designation (during 2006) for food safety applications. The company recently announced the development of its 2nd product – Salmonelex – which is specific for *Salmonella* in foods. According to the company's website, the Dutch Medicine Evaluation Board has issued a Temporary Use Exemption for using Salmonelex, presumably in the Netherlands
Novolytics, Ltd.	www.novolytics.co.uk	Warrington, UK	Novolytics is a spin-off formed during 2002 by the University of Warwick. Initially, the company was interested in developing phage products for disinfecting food equipment; however, it appears to have shifted its focus to the development of therapeutic phage products for clinical applications. The company uses the uncommon approach of including temperate phages in its lytic phage-containing preparations. At the present time, it does not have any food safety products on the market
OmniLytics, Inc.	www.omnilytics.com	Sandy, UT, USA	OmniLytics (formerly known as AgriPhi, Inc.) developed AgriPhage™ (the first phage-based product to receive EPA approval, during 2005) to control bacterial spot. At the present time, the company appears to be focusing on the development of pre-harvest, rather than post-harvest, applications for phages. The company also has a collaborative research and license agreement with Elanco (a division of Eli Lilly and Company – NYSE: LLY), to develop and market phage products active against various foodborne bacterial pathogens, including *E. coli* O157:H7 and *Salmonella*. In addition, its phage-based product Finalyse™ that targets *E. coli* O157:H7 on hides is currently being distributed by Elanco
Phage Biotech, Ltd.	www.phage-biotech.com	Rehovot, Israel	Phage Biotech (incorporated during March 2000) is a contract development company specializing in tailored phage biocontrol approaches for various applications. The company also serves as the distributor for Intralytix's food safety products in Israel

Note: The above list includes most known 'phage companies' involved with developing phage-based products for food safety applications. The list is not meant to be comprehensive; i.e. to include all of the companies that utilize phages or phage-derived proteins for human therapeutic, diagnostic, or other non-food safety applications. It also does not include multiproduct companies that are involved with phage-based product marketing and distribution as part of their broader portfolio, but are not phage product-development companies at their core. Some of the above-noted company-related information was obtained from the companies' websites and have not been independently verified.

Food contact substance (FCS)

A phage-based product may be regulated as an FCS by having a filed Food Contact Notification (FCN) cleared by the FDA. Intralytix, Inc. pursued that route for EcoShield™, which the FDA cleared as 'an antimicrobial agent applied to red meat parts and trim prior to grinding' in 2011. Assembling and filing an FCN is relatively simple. Also, because an FCS is only present in incidental/very small amounts which do not provide a persistent technical effect, it is considered to be a 'processing aid' and its approval automatically assumes no labeling requirements; i.e. a food treated with an FCS does not need to list the FCS on its label. However, in order to ensure incidental phage levels, the FCN approval route for a phage-based product may only be pursued when the product is added to foods requiring further processing (e.g. grinding). Also, phage substitutions may not be permitted without filing a new FCN. The main reason for pursuing an FCS designation is the 'no labeling' requirement that is inherent with its approval. However, the types of foods which may be processed with an FCS are limited, and the absence of a labeling requirement is also possible with other regulatory approvals, such as the GRAS.

Generally recognized as safe (GRAS)

A phage-based product may be regulated as a GRAS product by having a filed GRAS Notification cleared by the FDA. EBI Food Safety (now called Micreos) pursued that route for Listex™, which the FDA affirmed in 2006 (GRN #000198) (Figure 1), and Intralytix pursued that route for SalmoFresh™, which the FDA affirmed in 2013 (GRN #000435). A GRAS Notification is relatively simple to assemble and file; it can be used for diverse applications for phage-based products (including the treatment of ready-to-eat foods), and the regulatory approval process after submission is a relatively short 180 days. As with many other 'FDA approvals', the GRAS designation is not an approval *per se*. Rather, the FDA reviews the information supplied by the petitioner, as well as other data and information available to the Agency, and simply states that it has 'no questions' about the petitioner's conclusion that the substance under review is GRAS. Although the USDA is also involved in the review process, primarily to determine the efficacy and suitability of using the phage-based product to treat beef, poultry and eggs, as well as the safe conditions of use, GRAS 'approval' does not imply that either agency agrees that the GRAS product is effective. It merely indicates that the FDA and USDA have no further questions about its safety and efficacy at a given time. Food processors are not legally required to wait for the FDA's affirmation of the GRAS status, and they can use the phage product (or any other GRAS product) in their facility if they agree with the manufacturer's self-determination that the product is GRAS. However, most food processors prefer to wait for a 'no objection' letter from the FDA before they use GRAS products in their foods. The GRAS route is an attractive strategy for getting various phage-based food safety products to market quickly and in the most economical way, and that regulatory designation is consistent with the idea that lytic bacteriophages are naturally occurring, safe, and environmentally friendly antibacterial agents. Thus it is likely that increasing numbers of new phage-based products for post-harvest food safety applications will be entering the marketplace under the GRAS designation.

Updating of lytic phage-based products

One of the most handicapping factors for all antimicrobials is that bacteria eventually find ways to protect themselves against their actions; i.e. resistant mutants emerge. The emergence of phage-resistant mutants has been proposed as a potentially significant obstacle to ensuring the long-term efficacy of various bacteriophage products; however, various strategies may be used to minimize that risk.[13] For example, phage cocktails/mixtures may be used when multiple phages in the cocktail kill the same bacterial strain. That strategy increases the number of genetic mutations that must occur in the sensitive wild-type strain before a mutant strain becomes resistant to all of the phages comprising the cocktail, and it therefore reduces the likelihood of resistance emerging against the cocktail. Another approach involves using bacteriophages at 'epidemiological endpoints' in the food-processing chain, in order to minimize the selective pressure exerted by phages.[13,49] For example, one could spray an appropriate anti-*Salmonella* phage cocktail onto processed chicken carcasses before they are packaged and shipped, instead of trying to reduce the prevalence of *Salmonella* in chicken houses by spraying them with the phage cocktail. Despite those two strategies, it is likely that phage resistance will emerge eventually against lytic phage preparations developed for food safety applications, just as many multi-antibiotic-resistant mutants have emerged recently. However, as explained below, the ability to update lytic phage-based products offers an intriguing opportunity to maintain their efficacy despite the eventual emergence of phage-resistant mutants.

Bacteriophages have co-evolved with their host bacteria for >3 billion years, as part of the ongoing process of natural co-evolution.[55] As a result, various ecological niches will always contain some lytic phages active against mutant strains that are resistant to many other phages capable of lysing the mutants' parental wild-type strains. Thus it should be possible to respond successfully to the emergence of resistant bacterial strains, and thereby maintain the efficacy of commercial phage-based products, by updating the products with new lytic phages isolated from appropriate ecological niches. The approach of updating phage preparations is technically feasible, as demonstrated by its success when commonly used to update therapeutic phage preparations in the former Soviet Union (reviewed in more detail in [4]). However, whether or not that approach can be implemented in the USA will largely depend on the degree of flexibility its regulatory agencies are willing to offer to phage product manufacturers. In that regard, a recent, positive development was the FDA's flexibility during its clearance of ListShield™. The Agency's clearance allows the original version of that phage cocktail to be updated with new lytic phages if and when necessary, if the updating process meets the requirements set forth in the relevant regulation (21 CFR § 172.785), including two logical and technically feasible requirements: (i) the new phages must meet the same stringent safety and efficacy criteria as do the product's original phages; and (ii) the manufacturing process and all quality control protocols approved for the original ListShield™ product must be strictly adhered to for all of the new phages. As of the present time, such updates have not yet been required and implemented, so it remains to be seen how well the process functions during real-life situations.

PROBLEMS INTERFERING WITH THE ACCEPTANCE OF PHAGE-MEDIATED BIOCONTROL FOR POST-HARVEST FOOD SAFETY APPLICATIONS

Despite the safety and ubiquity of lytic phages, their acceptance by modern food-processing industries has been relatively slow. In addition to the possibility that the companies comprising those industries may be overly cautious about adopting a novel technology, there are several other potential problems that may be interfering with the wide acceptance of phage biocontrol strategies.[31] The problems may be either technical (e.g. identifying optimal methods for industrial applications, incorporating phage treatment in the existing hazard analysis and critical control points protocols, and the need to use phages in high concentrations for optimal efficacy), or nontechnical (e.g. the efficacy of phage treatment *versus* the cost to the food processor, consumer acceptance, etc.), and one example of each type is briefly discussed below.

A technical problem: efficacy of phage preparations in various foods

Lytic bacteriophages are very effective in lysing their specific bacterial targets (i.e. their bacterial host cells). However, although phages can significantly reduce the levels of the targeted bacterial pathogens in various foods, they may not always eradicate (i.e. totally eliminate) those contaminants unless very high concentrations of phages are used.[35,38,56–59] The underlying reason for this phenomenon is not clear, but it simply may be due to the fact that not all of a phage's host cells are 'found' by phage particles when a phage suspension is sprayed onto foods. Indeed, using a fine spray rather then a coarse spray for a local phage treatment (to ensure an even and thorough coverage of a food's surface) usually increases the treatment's efficacy (A Sulakvelidze, unpublished data). Also, Hudson *et al.*[60] have suggested that moist foods may require fewer phages to achieve a significant reduction in the levels of their targeted bacterial pathogens than do drier foods, presumably because the water in the former increases the chance of phages coming into contact with their targeted bacterial hosts. Theoretically, one might be able to spray foods with highly concentrated phage preparations capable of eliminating *all* of the specifically targeted contaminants; however, using such preparations may not be economically feasible, especially for those food companies that operate on thin profit margins. However, even when the targeted bacterium is not totally eliminated from the foods (very few, if any, other technologies are capable of achieving 100% eradication), a significant reduction in foodborne bacterial contamination is still likely to render the food safer to eat. For example, and to put this into a broader perspective, the FDA and USDA's FSIS jointly authored a risk assessment study during 2003 entitled 'Quantitative assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods' (http://www.fda.gov/downloads/food/scienceresearch/researchareas/riskassessmentsafetyassessment/ucm197330.pdf). The publication estimated the relative risks of eating various foods contaminated with *L. monocytogenes*, and it modeled a series of 'what if' scenarios, including one in which reductions in deli meat contamination would affect the mortality rate of elderly people. According to that analysis, a 10-fold reduction and 100-fold reduction in pre-retail contamination with *L. monocytogenes* would reduce the mortality rate by *ca* 50% and 74%, respectively. Several studies have reported that phages reduced the levels of their targeted pathogens in various foods by >10-fold (reviewed in more detail elsewhere[13,16]). Thus, assuming that those laboratory data are reproducible in commercial food-processing facilities, industry-wide implementation of phage biocontrol protocols – even if they do not eradicate (i.e. totally eliminate) the targeted foodborne pathogens in foods – may yield significant improvements in food safety and public health.

A nontechnical problem: market acceptance

Using phages to improve food safety is a relatively novel approach; therefore, how widely it will be used by various food industries will depend on 'market acceptance'. The latter term encompasses a fairly broad range of issues, from consumer acceptance to the cost of the phage preparations to the food industry. For example, if using phages to improve the safety of a food increases its cost by a significant margin (e.g. by >1.5 or 2 cents per pound of poultry), producers may be reluctant to absorb the price increase or to pass it on to their customers. However, even if raising the price is feasible, customer acceptance may still be a challenge, especially if the food is labeled as being phage-treated. The general public may be reluctant to accept the idea that phages (i.e. viruses) are intentionally being sprayed onto some of the foods they eat, until they understand that they are consuming naturally occurring phages every time they eat fresh, unprocessed foods during their lives.[14,61] Correspondingly, until consumers feel confident about the safety and desirability of 'edible viruses',[35] food processors may be reluctant to use phages because of their uncertainty about consumers' reactions. Therefore, a well-designed and scientifically sound education campaign regarding phages' ubiquity and nontoxic nature can go a long way toward educating the consumer about the safety and desirability of a phage-based biocontrol approach.

REFERENCES

1 Duckworth DH, 'Who discovered bacteriophage?' *Bacteriol Rev* **40**:793–802 (1976).
2 Summers WC, *Bacteriophage Discovered: Felix d'Herelle and the Origins of Molecular Biology*. Yale University Press, New Haven, CT, pp. 47–59 (1999).
3 Summers WC, Bacteriophage therapy. *Annu Rev Microbiol* **55**:437–451 (2001).
4 Sulakvelidze A and Kutter E, Bacteriophage therapy in humans, in *Bacteriophages: Biology and Application*, ed. by Kutter E and Sulakvelidze A. CRC Press, Boca Raton, FL, pp. 381–436 (2005).
5 Sulakvelidze A, Alavidze Z and Morris JG Jr, Bacteriophage therapy. *Antimicrob Agents Chemother* **45**:649–659 (2001).
6 Summers WC, The strange history of phage therapy. *Bacteriophage* **2**:130–133 (2012).
7 Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, *et al,* Food-related illness and death in the United States. *Emerg Infect Dis* **5**:607–625 (1999).
8 Hoffmann S, Batz MB and Morris JG Jr, Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. *J Food Prot* **75**:1292–1302 (2012).
9 Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, *et al,* The global burden of nontyphoidal *Salmonella gastroenteritis*. *Clin Infect Dis* **50**:882–889 (2010).
10 Gomez-Lopez VM, *Decontamination of Fresh and Minimally Processed Produce*. Wiley, Hoboken, NJ, 2012.
11 Hui YH, *Food Plant Sanitation*. CRC Press, Boca Raton, FL, 2003.
12 Maukonen J, Matto J, Wirtanen G, Raaska L, Mattila-Sandholm T and Saarela M, Methodologies for the characterization of microbes in industrial environments: a review. *J Ind Microbiol Biotechnol* **30**:327–356 (2003).

13 Sulakvelidze A and Barrow P, Phage therapy in animals and agribusiness, in *Bacteriophages: Biology and Applications*, ed. by Kutter E and Sulakvelidze A. CRC Press, Boca Raton, FL, pp. 335–380 (2005).
14 Greer GG, Bacteriophage control of foodborne bacteria. *J Food Prot* **68**:1102–1111 (2005).
15 Goodridge L, Application of bacteriophages to control pathogens in food animal production, in *Bacteriophages in the Control of food- and Waterborne Pathogens*, ed. by Sabour PM and Griffiths MW. ASM Press, Washington, DC, pp. 61–77 (2010).
16 Goodridge LD and Bisha B, Phage-based biocontrol strategies to reduce foodborne pathogens in foods. *Bacteriophage* **1**:130–137 (2011).
17 Miller RW, Skinner EJ, Sulakvelidze A, Mathis GF and Hofacre CL, Bacteriophage therapy for control of necrotic enteritis of broiler chickens experimentally infected with *Clostridium perfringens*. *Avian Dis* **54**:33–40 (2010).
18 Topley WWC and Wilson J, Further observations of the role of the Twort–d'Herelle phenomenon in the epidemic spread of murine typhoid. *J Hyg* **24**:295–300 (1925).
19 Hurley A, Maurer JJ and Lee MD, Using bacteriophages to modulate *Salmonella* colonization of the chicken's gastrointestinal tract: lessons learned from *in silico* and *in vivo* modeling. *Avian Dis* **52**:599–607 (2008).
20 Bach SJ, McAllister TA, Veira DM, Gannon VPJ and Holley RA, Effect of bacteriophage DC22 on *Escherichia coli* O157:H7 in an artificial rumen system (Rusitec) and inoculated sheep. *Anim Res* **52**:89–101 (2003).
21 Niu YD, McAllister TA, Xu Y, Johnson RP, Stephens TP and Stanford K, Prevalence and impact of bacteriophages on the presence of *E. coli* O157:H7 in feedlot cattle and their environment. *Appl Environ Microbiol* **75**:1271–1278 (2009).
22 Smith HW and Huggins MB, Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *J Gen Microbiol* **129**:2659–2675 (1983).
23 Smith HW, Huggins MB and Shaw KM, Factors influencing the survival and multiplication of bacteriophages in calves and in their environment. *J Gen Microbiol* **133**:1127–1135 (1987).
24 Smith HW, Huggins MB and Shaw KM, The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. *J Gen Microbiol* **133**:1111–1126 (1987).
25 Berchieri A Jr, Lovell MA and Barrow PA, The activity in the chicken alimentary tract of bacteriophages lytic for *Salmonella typhimurium*. *Res Microbiol* **142**:541–549 (1991).
26 Raya R, Callaway T, Edrington T, Dyen M, Droleskey R, Kutter E, *et al*, (eds), *In vitro* and *in vivo* studies using phages isolated from sheep to reduce population levels of *Escherichia coli* O157:H7 in ruminants, in *103rd General Meeting of the American Society for Microbiology*, American Society for Microbiology, Washington, DC (2003).
27 Jordan D, McEwen SA, Lammerding AM, McNab WB and Wilson JB, Pre-slaughter control of *Escherichia coli* O157 in beef cattle: a simulation study. *Prev Vet Med* **41**:55–74 (1999).
28 Sheng H, Knecht HJ, Kudva IT and Hovde CJ, Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants. *Appl Environ Microbiol* **72**:5359–5366 (2006).
29 Loc Carrillo C, Atterbury RJ, el-Shibiny A, Connerton PL, Dillon E, Scott A, *et al,* Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Appl Environ Microbiol* **71**:6554–6563 (2005).
30 Rosenquist H, Nielsen NL, Sommer HM, Norrung B and Christensen BB, Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. *Int J Food Microbiol* **83**:87–103 (2003).
31 Sulakvelidze A and Pasternack G, Industrial and regulatory issues in bacteriophage applications in food production and processing, in *Bacteriophages in the Control of Food- and Waterborne Pathogens*, ed. by Sabour PM and Griffiths MW. ASM Press, Washington, DC, pp. 297–326 (2010).
32 Gibson H, Taylor JH, Hall KE and Holah JT, Effectiveness of cleaning techniques used in the food industry in terms of the removal of bacterial biofilms. *J Appl Microbiol* **87**:41–48 (1999).
33 Bloomfield SF and Scott E, Cross-contamination and infection in the domestic environment and the role of chemical disinfectants. *J Appl Microbiol* **83**:1–9 (1997).
34 Kusumaningrum HD, Riboldi G, Hazeleger WC and Beumer RR, Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. *Int J Food Microbiol* **85**:227–236 (2003).
35 Garcia P, Martinez B, Obeso JM and Rodriguez A, Bacteriophages and their application in food safety. *Lett Appl Microbiol* **47**:479–485 (2008).
36 Hibma AM, Jassim SA and Griffiths MW, Infection and removal of L-forms of *Listeria monocytogenes* with bred bacteriophage. *Int J Food Microbiol* **34**:197–207 (1997).
37 Roy B, Ackermann HW, Pandian S, Picard G and Goulet J, Biological inactivation of adhering *Listeria monocytogenes* by listeriaphages and a quaternary ammonium compound. *Appl Environ Microbiol* **59**:2914–2917 (1993).
38 Abuladze T, Li M, Menetrez MY, Dean T, Senecal A and Sulakvelidze A, Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef by *Escherichia coli* O157:H7. *Appl Environ Microbiol* **74**:6230–6238 (2008).
39 Viazis S, Akhtar M, Feirtag J and Diez-Gonzalez F, Reduction of *Escherichia coli* O157:H7 viability on hard surfaces by treatment with a bacteriophage mixture. *Int J Food Microbiol* **145**:37–42 (2010).
40 Patel J, Sharma M, Millner P, Calaway T and Singh M, Inactivation of *Escherichia coli* O157:H7 attached to spinach harvester blade using bacteriophage. *Foodborne Pathog Dis* **8**:541–546 (2011).
41 Rashid MH, Revazishvili T, Dean T, Butani A, Verratti K, Bishop-Lilly KA, *et al,* A *Yersinia pestis*-specific, lytic phage preparation significantly reduces viable *Y. pestis* on various hard surfaces experimentally contaminated with the bacterium. *Bacteriophage* **2**:168–177 (2012).
42 Hudson JA, Billington C, Carey-Smith G and Greening G, Bacteriophages as biocontrol agents in food. *J Food Prot* **68**:426–437 (2005).
43 Dykes GA and Moorhead SM, Combined antimicrobial effect of nisin and a listeriophage against *Listeria monocytogenes* in broth but not in buffer or on raw beef. *Int J Food Microbiol* **73**:71–81 (2002).
44 Kim KP, Klumpp J and Loessner MJ, *Enterobacter sakazakii* bacteriophages can prevent bacterial growth in reconstituted infant formula. *Int J Food Microbiol* **115**:195–203 (2007).
45 Zuber S, Boissin-Delaporte C, Michot L, Iversen C, Diep B, Brussow H, *et al,* Decreasing *Enterobacter sakazakii* (Cronobacter spp.) food contamination level with bacteriophages: prospects and problems. *Microb Biotechnol* **1**:532–543 (2008).
46 Garcia P, Madera C, Martinez B, Rodriguez A and Evaristo Suarez J, Prevalence of bacteriophages infecting *Staphylococcus aureus* in dairy samples and their potential as biocontrol agents. *J Dairy Sci* **92**:3019–30126 (2009).
47 Garcia P, Madera C, Martinez B and Rodriguez A, Biocontrol of *Staphylococcus aureus* in curd manufacturing processes using bacteriophages. *Int Dairy J* **17**:1232–1239 (2007).
48 Modi R, Hirvi Y, Hill A and Griffiths MW, Effect of phage on survival of *Salmonella* Enteritidis during manufacture and storage of cheddar cheese made from raw and pasteurized milk. *J Food Prot* **64**:927–933 (2001).
49 Goode D, Allen VM and Barrow PA, Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. *Appl Environ Microbiol* **69**:5032–5036 (2003).
50 Leverentz B, Conway WS, Alavidze Z, Janisiewicz WJ, Fuchs Y, Camp MJ, *et al,* Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: a model study. *J Food Prot* **64**:1116–1121 (2001).
51 Coffey B, Mills S, Coffey A, McAuliffe A and Ross RP, Phage and their lysins as biocontrol agents for food safety applications. *Annu Rev Food Sci Technol* **1**:449–468 (2010).
52 Sillankorva SM, Oliveira H and Azeredo J, Bacteriophages and their role in food safety. *Int J Microbiol* **2012**:article 863945 (2012).
53 Mahony J, McAuliffe O, Ross RP and Sinderen D, Bacteriophages as biocontrol agents of food pathogens. *Curr Opin Biotechnol* **22**:157–163 (2011).
54 Bren L, Bacteria-eating virus approved as food additive. *FDA Consum* **41**:20–22 (2007).
55 Lenski RE, Coevolution of bacteria and phage: are there endless cycles of bacterial defenses and phage counterdefenses? *J Theor Biol* **108**:319–325 (1984).
56 Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M, *et al,* Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Appl Environ Microbiol* **69**:4519–4526 (2003).
57 Leverentz B, Conway WS, Janisiewicz W and Camp MJ, Optimizing concentration and timing of a phage spray application to reduce *Listeria monocytogenes* on honeydew melon tissue. *J Food Prot* **67**:1682–1686 (2004).

58 Atterbury RJ, Connerton PL, Dodd CE, Rees CE and Connerton IF, Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. *Appl Environ Microbiol* **69**:6302–6306 (2003).
59 Carter C, Parks A, Abuladze T, Li M, Woolfson J, Magnone J, *et al,* Bacteriophage cocktail significantly reduces *Escherichia coli* O157:H7 contamination of lettuce and beef, but does not protect against recontamination. *Bacteriophage* **2**:178–185 (2012).
60 Hudson JA, McIntyre L and Billington C, Application of bacteriophages to control pathogenic and spoilage bacteria in food processing and distribution, in *Bacteriophages in the Control of Food- and Waterborne Pathogens*, ed. by Sabour PM and Griffiths MW. ASM Press, Washington, DC (2010), pp. 119–135.
61 Sulakvelidze A, Safety by nature: potential bacteriophage applications. *Microbe* **6**:122–126 (2011).
62 O'Flynn G, Ross RP, Fitzgerald GF and Coffey A, Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. *Appl Environ Microbiol* **70**:3417–3424 (2004).
63 Sharma M, Patel JR, Conway WS, Ferguson S and Sulakvelidze A, Effectiveness of bacteriophages in reducing *Escherichia coli* O157:H7 on fresh-cut cantaloupes and lettucet. *J Food Prot* **72**:1481–1485 (2009).
64 Carlton RM, Noordman WH, Biswas B, de Meester ED and Loessner MJ, Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. *Regul Toxicol Pharmacol* **43**:301–312 (2005).
65 Guenther S, Huwyler D, Richard S and Loessner MJ, Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. *Appl Environ Microbiol* **75**:93–100 (2009).
66 Soni KA, Nannapaneni R and Hagens S, Reduction of *Listeria monocytogenes* on the surface of fresh channel catfish fillets by bacteriophage Listex P100. *Foodborne Pathog Dis* **7**:427–434 (2010).
67 Soni KA and Nannapaneni R, Bacteriophage significantly reduces *Listeria monocytogenes* on raw salmon fillet tissue. *J Food Prot* **73**:32–38 (2010).
68 Guenther S and Loessner MJ, Bacteriophage biocontrol of *Listeria monocytogenes* on soft ripened white mold and red-smear cheeses. *Bacteriophage* **1**:94–100 (2011).
69 Bigot B, Lee WJ, McIntyre L, Wilson T, Hudson JA, Billington C, *et al,* Control of *Listeria monocytogenes* growth in a ready-to-eat poultry product using a bacteriophage. *Food Microbiol* **28**:1448–1452 (2011).
70 Whichard JM, Sriranganathan N and Pierson FW, Suppression of *Salmonella* growth by wild-type and large-plaque variants of bacteriophage Felix O1 in liquid culture and on chicken frankfurters. *J Food Prot* **66**:220–225.
71 Higgins JP, Higgins SE, Guenther KL, Huff W, Donoghue AM, Donoghue DJ, *et al,* Use of a specific bacteriophage treatment to reduce *Salmonella* in poultry products. *Poult Sci* **84**:1141–1145 (2005).
72 Bigwood T, Hudson JA, Billington C, Carey-Smith GV and Heinemann JA, Phage inactivation of foodborne pathogens on cooked and raw meat. *Food Microbiol* **25**:400–406.
73 Guenther S, Herzig O, Fieseler L, Klumpp J and Loessner MJ, Biocontrol of *Salmonella typhimurium* in RTE foods with the virulent bacteriophage FO1-E2. *Int J Food Microbiol* **1**:66–72 (2012).
74 Zhang H, Wang R and Bao H, Phage inactivation of foodborne *Shigella* on ready-to-eat spiced chicken. *Poult Sci* **92**:211–217 (2013).

