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REVIEW

Bacteriophage biocontrol of foodborne pathogens

Mustafa Kazi[1] **· Uday S. Annapure**[1]

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Abstract Bacteriophages are viruses that only infect bacterial cells. Phages are categorized based on the type of their life cycle, the lytic cycle cause lysis of the bacterium with the release of multiple phage particles where as in lysogenic phase the phage DNA is incorporated into the bacterial genome. Lysogeny does not result in lysis of the host. Lytic phages have several potential applications in the food industry as biocontrol agents, biopreservatives and as tools for detecting pathogens. They have also been proposed as alternatives to antibiotics in animal health. Two unique features of phage relevant for food safety are that they are harmless to mammalian cells and high host specificity, keeping the natural microbiota undisturbed. However, the recent approval of bacteriophages as food additives has opened the discussion about 'edible viruses'. This article reviews in detail the application of phages for the control of foodborne pathogens in a process known as "biocontrol".

Keywords Bacteriophage · Biocontrol · Biosanitation · Lysin · Lysogeny · Phage therapy

✉ Uday S. Annapure
udayannapure@gmail.com

[1] Department of Food Engineering and Technology, Institute of Chemical Technology, Nathalal Parekh Marg, Matunga, Mumbai 400 019, India

Introduction

Microorganisms may be beneficial or harmful to us, and we are constantly fighting with the harmful microorganisms to keep them away. One battle is in the food chain against spoilage and poisoning bacteria. Many technologies have been devised to combat bacteria, many a times at the cost of food quality. Heat treatments are associated with deterioration of organoleptic properties, extensive use of sanitizers have led to the development of resistant bacteria, chemical preservatives have negative effect not only on sensory parameters but also on health as many of them are carcinogenic. In addition, these strategies are not infallible which is proved by continuous rise in the number of food borne diseases and increasing loss in food production. *Listeria* and others have an enormous impact on public health (DuPont 2007). On the other hand most of these strategies cannot be applied to fresh fruits, vegetables and ready to eat products. Hence there is a need for new strategies that fulfill consumer demand for minimally processed foods with fewer chemical preservatives.

Novel technologies like radiation processing, plasma processing, high pressure processing, pulsed electric field and ultrasound are expensive but may be the answer. Another promising approach is the use of natural antagonist towards bacteria to control bacterial contamination in food in a process called "biocontrol" which may tackle the drawbacks of current processing and preservation technology and is likely to be acceptable to consumers.

Bacteriophages are obligate parasites of bacteria, using the resources of the bacterial cell to replicate. They are typically highly specific, often being restricted to particular strains within a single bacterial species. However, some bacteriophages have a relatively broad host range, infecting multiple species within a genus and can even infect members of other genera closely related to their normal host. Bacteriophages

will multiply when (and only when) their specific bacterial host is present, allowing the use of extremely low input doses (Monk et al. 2010).

This review attempts to describe bacteriophage as biocontrol agents and to review biocontrol strategies for major foodborne pathogens. It will also give an overview of other strategies based on bacteriophage to enhance food safety like phage therapy, biopreservation, biosanitation and use of phage lysin as an alternate to phage application.

Bacteriophage biology

Phages are the largest group of viruses, utilizing species in the Bacteria and Archaebacteria as hosts. Measuring between 20 and 200 nm (Ackermann and DuBow 1987), they are the most abundant form of life on the planet with estimated 10^{31}phages in the biosphere (Kutter and Sulakvelidze 2005).

Phages may be roughly categorized by shape into tailed, polyhedral (icosahedral or quasiicosahedral bodies), filamentous, and pleomorphic phages (Ackermann and Prangishvili 2012). In tailed phages the tail fibers contain proteins that recognize molecules on the surface of bacterial cell walls, which provide the ability to attach only to host cells (Ackermann 2009; Guttman et al. 2005). Most tailed phages are stable in the pH range from 5 to 9 and are inactivated by heating at 60 °C for 30 min (Ackermann 2007).

The phage life cycle can be one of two types, the productive or virulent cycle and the temperate or lysogenic cycle (Ackermann and Prangishvili 2012; Guttman et al. 2005). According to this, phages are classified as lytic (virulent) or lysogenic (temperate).

Lytic phages infect bacterial cells causing inhibition of host metabolism and subverting it to the production of phage progeny. The lytic cycle results in the lysis of the bacterium accompanied by the release of multiple phage particles. The new progeny phages produced by the host bacterium spread to infect other cells. The time for the whole cycle is usually within 1–2 h and the number of phage produced depends upon the phage type (Guttman et al. 2005).

Some phages infect cells and incorporate their nucleic acid into the genome of the host cell or exist as an episomal element, leading to a permanent association as a prophage with the cell and all its progeny. During lysogeny, phages neither produce virions nor lyse bacteria. These phages are called temperate, and the cells that harbor a prophage are known as lysogenic. The lysogenic relationship between a temperate phage and its host bacterium provides a safe home to the temperate phage genome, blocks replication of non-virulent homologous phages, and has the potential to alter the phenotype of the host cell (Gill and Abedon 2003).

Characteristics of phage for food application

Phages intended for use in food should be strongly lytic. Their host range should cover all epidemiologically important strains of the target microorganism. They should also display minimum transduction, a process wherein host DNA is packaged into phage heads, rather than phage DNA (Ikeda and Tomizawa 1965). Phages selected should be stable within the intended use environment (Gill and Abedon 2003). In addition to all these phages should also have a broad host range, however this limitation can be mitigated using phage cocktails (McIntyre et al. 2007).

Influence of phage and host concentration on biocontrol

Historically, most research on phage biocontrol has been done in liquids and usually with a high concentration of pure target bacteria (Hagens and Loessner 2010). In liquid environments, thermal motion-driven particle diffusion and mixing due to either fluid flow or active swimming (bacterial motility) increase the likelihood of phages to encounter and infect susceptible host bacteria (Murray and Jackson 1992). When it comes to food applications, there are two major obstacles. First, a significant portion of targeted foods is solid rather than liquid in nature. Second, bacterial contamination would likely occur at very low numbers due to the expected high hygiene standards in place (Hagens and Loessner 2010). This problem can be overcome by inundating the food environment with overwhelming number of phages thereby increasing the chances of phage - target bacteria interaction (threshold of approximately 1×10^8 PFU/ml). In other words, low numbers of phages are unlikely to infect low numbers of bacteria simply because phages and bacteria are unlikely to come into contact with each other. The bacterial host concentration is not a limiting factor if the critical concentration of phage numbers is reached and is able to cover the entire available surface of the targeted food matrix (Hagens and Loessner 2010). Experimental verification of this claim has been achieved when a Salmonella phage (P7) was incubated with its respective host at 24 °C for up to 2 h in Luria-Bertani (LB) broth at varying ratios of phage and host cell concentrations, and the surviving host cells were counted (Bigwood et al. 2009). It was observed that inactivation of Salmonella by P7 seemed to be independent of the host concentration, with nearly complete inactivation occurring at a phage concentration of around 5×10^8 PFU/ml. This was again supported by studies on the control of spoilage bacteria on meat surfaces, which suggest that phages can be effective biocontrol agents when the population of host cells is as low as 46 CFU/cm^2 (Greer 1988). Hence, the requirement of a minimum bacterial density as a

prerequisite for successful phage biocontrol was not accepted (Kasman et al. 2002).

The results of phage-mediated inactivation of food borne pathogens in some reports using high phage concentrations may be due to lysis from without (Delbruck 1940). Lysis from without occurs when host cells to which numerous phage particles are adsorbed are inactivated rapidly in the absence of phage replication. In *E. coli* phage T4, this "lysis from without phenomenon" is mediated by a lysozyme on the baseplate (Abedon 1999). It occurs when more than 100 phages are adsorbed on a bacterial cell, which is followed by swelling and bulging of the membrane within 5–10 min after adsorption. Finally, this results in the formation of holes in the cell wall, through which cytoplasmic contents may escape (Tarahovsky et al. 1994).

Bacteriophage biocontrol of foodborne pathogens

There has been much success to control pathogens using bacteriophage. Table 1 summarizes a list of studies on bacteriophage biocontrol of foodborne pathogens. However, this list is not exhaustive.

All postharvest interventions to control *E. coli O157:H7* have been successful. During a recent evaluation of the control of *E. coli* O157:H7 in broths, O'Flynn et al. (2004) reported that phages could eliminate the bacteria at temperatures of 30 or 37 °C where the organism was growing but could not lyse the cells in the absence of growth at 12 °C. Therefore it can be said that the replication of *E. coli* phage requires the metabolic processes associated with host cell growth.

Studies on reduction of *Campylobacter* contamination from chicken skin, raw and cooked beef have also been successful. The lysis from without was seen to be the mechanism of *Campylobacter* reduction on chicken skin as a 10^7 concentration of phage caused a 2-log CFU reduction where as 10^5 and 10^3 failed to reduce CFU.

All *Salmonella* phages reported have been able to decrease the number of viable cells present on raw meats, processed and ready-to-eat foods, and fresh products. Furthermore, the combined treatment of phage and *Enterobacter asburiae*, a strain exhibiting antagonistic activity against Salmonella, to control this pathogen on tomatoes, mung bean sprouts, and alfalfa seeds, represents a highly promising, chemical-free approach.

Phage and nisin combination used to control *Listeria* in ground beef revealed to be ineffective. This strategy had a synergistic effect once added to melon and apple resulting in an improved reduction of *Listeria* compared to phage or nisin alone. The efficacy of phage-nisin mixture was however significantly reduced in apples on the account of a decline of phage numbers possibly due to low pH. Other studies have used phage P100, which was highly effective in inhibiting Listeria growth.

Post harvest applications in pasteurized milk show that the use of combined phage treatments with nisin and high hydrostatic pressure could synergistically be used to reduce *Staphylococcal* contamination (Martınez et al. 2008; Tabla et al. 2012). Inactivation of *S. aureus* has also been accomplished in both fresh and hard-type cheeses using a phage cocktail during cheese manufacturing (Bueno et al. 2012).

Besides the above five pathogens, several other foodborne pathogens are responsible for illnesses, hospitalizations, and deaths, such as *Clostridium spp*., *Shigella spp*., *Vibrio spp.* and *Cronobacter sakazakii.* Only few studies have been done to control them using phage biocontrol.

Phage therapy

This is a strategy where phage treatment is applied preharvest during plant and animal growth to reduce the probability of plant or animal disease and to prevent the contamination of human pathogens in the food produced.

Many studies aimed at assessing the ability of phage to eliminate bacterial pathogens from food of plant origin and to control plant diseases have been carried out. Several promising trials have been carried out to control plant diseases such as bacterial blotch, bacterial spot, and fire blight in cultivated mushrooms, tomato, and apple. One of the earliest use of phage therapy to control bacterial spots in stone fruits was done by Civerolo and Kiel in 1969. He observed 42 % reduction of *Xanthomonas arboricola pv. pruni* on peach leaves when phage were applied prior to infection. Although phage application after infection had no effect. In another study by Ravensdale et al. (2007), *Pectobacterium carotovorum* load on Calla lily was reduced by phages. The phages however, were inactivated by fertilizer solutions. There have been many such successful interventions, but it should be remembered that the application of phages in an open field is associated with some difficulties such as uncontrolled environmental factors including temperature, sun exposure and humidity, uneven phage distribution and allocation, and rapid inactivation of the applied phages (Maura and Debarbieux 2011).

Phage treatment of food-producing animals reduces the probability of contamination of the resulting food products during processing. Risk assessment models indicate that a 1 and 2-log reduction in the number of pathogens shed in feces of the slaughtered animal could reduce the risks to the consumers by 45 and 75 %, respectively. For example, it is estimated that a reduction of 2 log on the *Campylobacter* loads in poultry intestines is sufficient to diminish 30 fold the incidence of campylobacteriosis associated with consumption of chicken meals (Rosenquist et al. 2003). Carvalho et al. (2010) administered campylobacter phage to poultry by oral gavage

Table 1 Bacteriophage biocontrol of foodborne pathogens

Target pathogen	Description and result of the study	Reference
E. coli	100 % reduction in CFU within an hour of addition of phage DT1 and DT6 in milk during milk fermentation.	Tomat et al. 2013
	Spraying of phage cocktail on spinach blades resulted in a 4.5 log reduction of CFU after 2 h of phage addition	Patel et al. 2011
	No survivors detectable on spinach and lettuce leaves after 10 min. of phage addition combined with cinnamaldehyde treatment	Viazis et al. 2011
	Significant reduction in CFU on lettuce and cantaloupe after 2 days of spraying with phage cocktail (ECP-100)	Sharma et al. 2009
	Phage cocktail e11/2, e4/1c, pp01 applied on meat surface resulted in eradication of *E.coli* in seven of nine samples	O'Flynn et al. 2004
Campylobacter	Phage Φ29C when applied on top of chicken skin at MOI (multiplicity of infection) 1 caused less than 1 log reduction in CFU; MOI 100–1000 caused 2 log reduction in CFU	El-Shibiny et al. 2009
	Phage Cj6 was applied on top of raw and cooked beef, largest reductions were recorded at high host cell densities over a period of 8 days and incubation at 51 °C	Bigwood et al. 2009
	Phage Φ2 applied on top of chicken skin at a conc. of 10^7 PFU/ml caused 2 log reduction whereas 10^5 and 10^3 PFU/ml failed to reduce CFU count	Wagenaar et al. 2005
Salmonella	Salmonella phage F01-E2 when added to turkey deli meats and chocolate milk resulted in 5 log reduction of CFU and a 3 log reduction when applied to hot dogs	Guenther et al. 2012
	More than 99 % reduction in CFU on meat skin treated with phage cocktail PC1 at MOI 10 or above and temp 4 °C for 96 h	Hooton et al. 2011
	Combined biocontrol of phage cocktail with *Enterobacter asburiae* suppressed pathogen growth on mung beans and alfa alfa seeds	Ye et al. 2010
	Reduction of *S. javiana* in tomatoes when treated with phage and *E. asburiae* combination, although major suppressing activity was attributed to anatagonistic effect of *E. asburiae*	Ye et al. 2009
	Reduction of 3–4 log CFU in raw and cocked beef at 5 °C and 6 log CFU at 24 °C when treated with phage P7	Bigwood et al. 2009
	Phage cocktail caused significant reduction on fresh cut melons but not on apples. The result maybe explained as phage particles were inactivated due to low pH on apple surface	Leverentz et al. 2001
	No survival during 89 days in pasteurized cheeses containing phage SJ2 (MOI 10^4)	Modi et al. 2001
L. monocytogenes	Reduction of CFU by 2.5 log at 30 °C in RTE chicken. At 5 °C, regrowth was prevented over 21 days	Bigot et al. 2011
	In red smear cheese phage A511 applied on the surface caused CFU to decrease by 3 logs after 22 days. Repeated application of A511 further delayed re-growth	Guenther and Loessner 2011
	Reduction in CFU on catfish and salmon fillet upon surface application of phage P100	Soni et al. 2010
	Rapid 1 log reduction of CFU. 2 log reduction after 14 to 28 days of storage on cooked ham surface treated with Phage P100	Holck and Berg 2009
	Complete eradication of CFU on red smear soft cheese during rind washing with phage P100	Carlton et al. 2005
	Spraying melon pieces with phage cocktail after 1 h of listeria challenge reduced CFU by 6.8 log units after 7 days of storage	Leverentz et al. 2004
	Phage cocktail caused a CFU reduction of 2.0 to 4.6 log in melons and only 0.4 log in apples. Phage + nisin reduced CFU by 5.7 log in melons and 2.3 log in apple	Leverentz et al. 2003
	Phage-nisin mixture was effective in broth but not in buffer or on raw beef	Dykes and Moorhead 2002
Staph. aureus	Phage cocktail added to pasteurized milk challenged with *S. aureus,* led to reduction of *S. aureus* to undetectable levels after 6 h in fresh cheese and continuous reductions in hard cheese. In curd a reduction of 4.64 log CFU per g was obtained compared with control	Bueno et al. 2012
	Combination of HPP (high pressure processing) and phage resulted in *S. aureus* elimination in pasteurized milk within the 48 h regardless of the initial contamination level (1×10^6or 1×10^4 CFU per mL)	Tabla et al. 2012
	Phage cocktail (Φ88 and Φ35) along with nisin application decreased *S. aureus* by 1 log unit more than phage or nisin applied alone (24 h at 37 °C) in pasteurized milk	Martınez et al. 2008
	Lysis was inhibited when phage K was added to raw milk whey. This might be due to adsorption of whey proteins on S. *aureus* cells inhibited phage attachment	Gill et al. 2006
	Adsorption of phage K was reduced in raw milk	O'Flaherty et al. 2005
Cronobacter sakazakii	A cocktail composed of five phages prevented the growth of 35 of 40 test strains tested in 45 experimentally contaminated infant formula. Also, a dose of 10^8 PFU/mL 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	Zuber et al. 2008
	In experimentally contaminated infant formula phage concentration of 10^9 PFU/ml was the most effective and able to completely eradicate the target organism	Kim et al. 2007

Table 1 (continued)

Target pathogen	Description and result of the study	Reference
Shigellae	Single phages or a phage cocktail were used to treat meat contaminated with either individual *Shigella spp.* (1×10^4 CFU/g) or a mixture of *Shigellae* (*S. flexneri 2a, S. dysenteriae* and *S. sonnei*, at a total concentration of 3×10^4 CFU/g). 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 to eradication	Zhang et al. 2013

and reduced levels of *C. coli* and *C. jejuni* in feces by 2 log CFU/g. Studies have also been conducted on cattle to reduce fecal shedding of *E. coli* O157:H7 (Sheng et al. 2006). Many other trials have been conducted with success for reducing intestinal colonization and fecal shedding of *E. coli*, *Salmonella* and *Campylobacter* (Greer 2005).

Biosanitation

In food industry, biofilms are found on the surfaces of equipment used, for example, in the food handling, storage, or processing, especially on the surfaces that are not easy to clean or to sanitize.

Roy et al. (1993) studied the effectiveness of different phages to remove *Listeria* from stainless steel and polypropylene surfaces. They found that phage treatment alone was able to achieve approximately a 3-log cycle decrease in cell number. In another study Montanez-Izquierdo et al. (2012) evaluated *Listeria* phage P100 to control biofilm formation by *L. monocytogenes* on stainless steel surfaces and found a mean reduction of 5.29 log CFU/cm^2. Apart from controlling *Listeria* biofilms, *Campylobacter* biofilms were successfully removed from the surface of glass (Siringan et al. 2011) and growth of *E. coli O157:H7* was controlled using phage mixture BEC8 on stainless steel and ceramic tiles (Viazis et al. 2011). Use of phage for biosanitation is promising although very challenging due to the diversity of bacteria found in different environments (Sillankorva et al. 2012).

Biopreservation

Biopreservation is the use of bacteriophage as a preservative in perishable manufactured foods to extend its shelf life. Phages are excellent as food biopreservation agents since they are reported to lyse their hosts at temperatures as low as 1 °C (Greer 1982, 1988) limiting the growth of pathogenic and spoilage bacteria on even refrigerated foods (specially psychrotrophic bacteria).

The role of phages in fish and red meats have been recognized for some time. However, their role as food preservatives has been explored only recently. Research on fishes (Delisle and Levin 1969) led to the discovery of phages in fish fillets that were active against psychrophilic spoilage pseudomonads and *Shewanella putrefaciens* of marine origin. However, those phages were evaluated only from the perspective of strain differentiation by means of a phage-typing scheme, and there has been no further work to examine them as biopreservation agents in fishes.

Preservative effects of *Pseudomonas* phages in raw chilled beef have also been examined. The retail shelf life of raw chilled beef was extended significantly after *Pseudomonas* specific lytic phage application (Greer 1988). However, when similar work was carried out using naturally contaminated beef samples and *Pseudomonas* phage mixture, the shelf life was not significantly affected (Greer 2005). This may be due to the narrow specificity of the used phages that were unable to infect all the spoilage bacteria present.

Another attempt has been made to investigate the ability of *Brocothrix thermosphacta* lytic phage to control the growth of its host and extend the shelf life of pork adipose tissue (Greer 2005). It was found that bacterial counts were reduced after 2 days of storage at both 2 and 6 °C but the growth of phage-sensitive and resistant strains were detected after this period. However, phage treatment extended the shelf life from 4 days in the control samples to at least 8 days.

Application of phage lysin in food

Lysins are enzymes produced by lytic phages, which play role in the degradation of the bacterial cell wall through targeting its various peptidoglycan bonds to allow the newly formed progeny phages to be released from the host cell (Borysowski et al. 2006). Because lysin enzymes attacks the cell wall peptidoglycan, they are highly effective against Gram-positive bacteria when added externally and may be used as biocontrol agents to enhance food safety (Fischetti 2008). Lysins generally have a narrow spectrum activity restricted to its host species. An exception is an enterococcal phage lysin that not only lyses enterococci but also *Streptococcus pyogenes*, group *B streptococci*, and *S. aureus*, making it one of the broadest acting lysins identified so far (Yoong et al. 2004).

Lysin can be added as a purified protein directly to food or feed. For example the growth of *Staphylococcus aureus* in pasteurized milk was controlled by addition of purified lysin at 37 °C (Obeso et al. 2008). Forty-eight strains of *Clostridium perfringens* were lysed by murein hydrolase (lysin) enzyme that is produced by *C. perfringens* phage j3626 (Zimmer et al. 2002). Another technique of using lyisn is via lysin- secreting recombinant bacteria (Borysowski et al. 2006). This was demonstrated in the case of recombinant *Lactococcus lactis* cells containing listerial lysin encoding genes to lyse *L. monocytogenes* in the surrounding medium (Gaeng et al. 2000). This study also showed that the expression of functional lysin by *L. lactis* was detected in the presence of lactose that is used in milk fermentation. These promising results suggested the possibility of using these recombinant starter *lactococcal* cultures to selectively protect dairy products against *L. monocytogenes* contamination.

Similar approaches for lysin application were investigated to control the growth of phytopathogenic bacteria. It was shown that when recombinant lysozyme of *Erwinia amylovora* phage Ea1h was applied on immature pears after inoculation with *E. amylovora*, disease symptoms such as ooze formation and necrosis were retarded or inhibited (Kim et al. 2004). Alternatively, transgenic plants able to produce lysin enzyme at the intercellular spaces of the plant to kill bacteria at a very early stage of infection could be developed (During et al. 1993).

The absence of bacterial resistance against lysin is considered as a major advantage of using phage lysins (Fischetti 2010), as the bacterial cell would have to modify the structure of its cell wall to avoid enzymatic action. It was found that exposing bacteria to a particular lysin for 40 reproductive cycles did not give any resistant strains (Fischetti 2010). However, the production of lysin is expensive and, moreover, they are relatively unstable large proteins that are prone to proteolysis and lose its activity in some foods (Coffey et al. 2010).

Advantages of bacteriophage biocontrol

There are many advantages of phages over traditional antimicrobials such as antibiotics and sanitizers.

They have a history of safe use, as they are bacterial viruses, infection of mammalian cells is unlikely. All available evidence indicates that their oral consumption is entirely harmless to humans as they represent a normal component of an everyday diet. Oral toxicity tests on rats that were given phages against *Listeria monocytogenes* at a dose of 2×10^{12} PFU/kg body weight/day showed no signs of abnormality with regards to histological changes, morbidity, or mortality (Carlton et al. 2005). Similar results were found in a human study with *E. coli* T4 phages that were added to drinking water (Bruttin and Brussow 2005). Individuals with HIV and other immunodeficiency diseases and healthy volunteers have also been intravenously injected with purified phages (e.g., FX174) without any apparent side effects (Atterbury 2009). Indeed, early phage therapy pioneers demonstrated safety by ingesting preparations themselves. The phages used were not only administered orally or superficially, but also were injected intramuscularly, intravenously, and even into the pericardium and carotid artery without any adverse effect being observed.

Phages are highly active and specific against their host with no adverse effects on the intestinal microbiota. Bacteriophages are auto-replicative, hence when bacterial contamination is high, low concentrations of phage can get the desired pathogen reduction. Phage production is relatively simple and has high storage stability under different environmental conditions.

Drawbacks of using phage for food preservation include limited host range, risk for the development of resistant mutants, and the potential for the transduction of virulent characters from one bacterial strain to another. Yet these are not very significant as overcoming them is simple. Phage cocktail can be used when multiple strains of host are present. Spontaneously occurring phage-resistant mutants are not likely to significantly influence treatment efficacy and the complex phage resistance mechanisms common in bacteria can be overcome by screening for broad host range phages and/or use of phage cocktails (Hagens and Loessner 2010). From another perspective, it has been noted that a phage-resistant strain of *E. coli O157:H7* had a smaller, more coccoid cellular morphology than the parental strain and it reverted to phage sensitivity within 50 generations (O'Flynn et al. 2004). Likewise, phage-resistant mutant strains of *Salmonella Enteritidis* lost the O-polysaccharide layer, which is required for phage adsorption, and as a result became avirulent (Santander and Robeson 2007). Finally transduction of virulence can be prevented by choosing phages which show low transduction frequencies. Another disadvantage is that most current research on their efficacy have involved experiments with artificially inoculated foods that do not necessarily reflect the real commercial environments where phages will be applied.

Conclusion

Bacteriophages were created by nature to combat bacteria around us. We have manipulated these bacterial viruses to control and detect bacterial pathogens in food as well as in medicine and veterinary. Research is still needed to thoroughly understand the mechanisms of phage resistance acquired by the hosts and methods to overcome phage resistance.

Although the results of the studies appear to be encouraging, they should be interpreted with caution. For instance,

some phage studies have proven that phages are inefficient in reducing their host, such as biocontrol on apple slices. Also, several authors have reported the emergence of phage-resistant phenotypes, but this has not significantly affected the results of the phage trials.

Application and commercialization of phage based technologies have begun and still have a long way to go. To name a few products, Ecoshield™ and Listshield™ are phage preparations by Intralyix, Inc that target *E. coli* and *L. monocytogenes* respectively and Listex™ and salmonlex™ are products of Micreos food safety that are active against *Listeria* and *Salmonella.*

Although phages are and will be present forever in foods, the consumer's perception of adding viruses to foods will, arguably, be the most critical hurdle to be overcome in order for phages to be used widely for biocontrol of bacterial pathogens in food (Strauch et al. 2007).

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