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Bacteriophages and their application in food safety

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Keywords

antimicrobial, bacteriophage, biocontrol, food, pathogen.

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2008/0785: received 8 May 2008, revised and accepted 26 June 2008

doi:10.1111/j.1472-765X.2008.02458.x

Abstract

In recent years it has become widely recognized that bacteriophages have several potential applications in the food industry. They have been proposed as alternatives to antibiotics in animal health, as biopreservatives in food and as tools for detecting pathogenic bacteria throughout the food chain. Bacteriophages are viruses that only infect and lyse bacterial cells. Consequently, they display two unique features relevant in and suitable for food safety. Namely, their safe use as they are harmless to mammalian cells and their high host specificity that allows proper starter performance in fermented products and keeps the natural microbiota undisturbed. However, the recent approval of bacteriophages as food additives has opened the discussion about 'edible viruses'. In this review, we examine the promising uses of phages for the control of foodborne pathogens and the drawbacks on which more research is needed to further exploit these biological entities.

Introduction

The current technologies employed to inactivate bacterial pathogens in foods are not infallible, as proved by the continuous increase in several foodborne diseases caused by pathogens, such as *Salmonella*, *Campylobacter*, *Escherichia coli*, *Listeria* and others that have an enormous impact on public health (DuPont 2007). Contaminating bacteria can get access to food during slaughtering, milking, fermentation, processing, storage or packaging. Over the last few years, a number of strategies to minimize the microbial load of raw products have been explored as the use of antibiotics is restricted due to the negative impact on human antimicrobial therapies. Problems of acceptability and deterioration of the organoleptic properties have been described after physical treatments such as steam, dry heat and UV light. Moreover, the extensive use of sanitizers has led to the development of resistant bacteria rendering these procedures less effective. On the other hand, some approaches often used in the food industry to reduce contamination by foodborne pathogens cannot be directly applied to fresh fruits, vegetables and ready-to-eat products. Hence, despite recent advances to avoid transmission of bacterial pathogens throughout the food chain, novel strategies are still required to fulfil

consumer demands for minimally processed foods with fewer chemical preservatives.

Recently, research on phage molecular biology has fuelled multiple biotechnological applications in very diverse fields including nanotechnology, vaccine development, therapeutic delivery, bacterial detection systems, novel antimicrobials against antibiotic-resistant bacteria, etc. Another promising field of application is the use of phages as natural antimicrobials in food to inhibit undesirable bacteria, which is likely to be acceptable to consumers (Hagens and Loessner 2007; Strauch *et al.* 2007). This review aims to provide an insight into how phages can be exploited to generate antibacterial agents to enhance microbial food safety.

Antimicrobial activity of phages: past and present

Bacteriophages are viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse. They were discovered by Ernest Hankin (1896) and Frederick Twort (1915) who described their antibacterial activity. However, Felix d'Herelle (1919) was probably the first scientist who used bacteriophages as a therapy to treat severe dysentery. At that time, several companies then actively started up the

commercial production of phages against various bacterial pathogens for human use. However, phage production was quickly displaced by the discovery of antibiotics in most of the Western world. Nevertheless, phage therapy is still an on-going practice in Eastern Europe and countries from the former Soviet Union. Several institutions in these countries have been involved in phage therapy research and production, with activities centralized at the Eliava Institute of Bacteriophage, Microbiology and Virology (Tbilisi, Georgia, <http://www.evergreen.edu/phage/home.htm>) and the Hirszfeld Institute of Immunology and Experimental Therapy (Wroclaw, Poland). Their work in this field has recently been extensively reviewed (Kutter and Sulakvelidze 2005).

The current threat of antibiotic-resistant bacteria has renewed the interest in exploring bacteriophages as biocontrol agents in Western countries (Matsuzaki *et al.* 2005; Sulakvelidze and Kutter 2005). In fact, some products based on bacteriophages are already commercially available ('PhageBioderm', 'Bacteriophagum Intestinalis Liquidum', 'Pyobacteriophagum Liquidum'). Additionally, some care centres are particularly specialized in phage therapy (for example, Southwest Regional Wound Care Centre, Texas, <http://www.woundcarecenter.net>).

Besides phage therapy, the use of bacteriophages as antimicrobial agents and tools for detecting pathogens in feed and foodstuffs is also expanding with several companies having been created recently (Table 1). Fields of application comprise of water and food safety, agriculture and animal health. An example is OmniLytics, Inc. that gained US Environmental Protection Agency approval for the use of its product AgriPhage against plant pathogenic bacteria. In food manufacturing industry, EBI Food Safety recently marketed Listex™ P100 for controlling *Listeria* in meat and cheese products (Carlton *et al.* 2005). In August 2006, the US Food and Drug Administration (FDA) approved the use of a phage preparation targeting *Liste-*

Table 2 Main advantages of bacteriophages as biocontrol tools in food safety

| | |
|------------------------------------|---|
| History of safe use | Ubiquitous in nature including food ecosystems |
| | Natural commensals of humans and animals |
| | Extensive clinical use in Eastern Europe |
| Highly active and specific | No adverse effects on the intestinal microbiota |
| | Innocuous to mammalian cells |
| | Autoreplicative |
| | Can be active against biofilms |
| Genetically amenable | |
| Versatile use along the food chain | Phage therapy, biosanitation, biopreservation |
| Tools for detecting pathogens | |
| Source of potent antimicrobials | Endolysins and other peptidoglycan hydrolases |

ria, LMP 102 (Intralytix, Inc.), in ready-to-eat meat and poultry products.

Bacteriophages for biocontrol of pathogens in food

Bacteriophage-based biocontrol measurements have a great potential to enhance microbiological safety based, namely, on their long history of safe use, relatively easy handling and their high and specific antimicrobial activity (Table 2).

The concept of combating pathogens in food by means of phages can be addressed at all stages of production in the classic 'farm to fork' approach throughout the entire food chain (Fig. 1). Bacteriophages are suitable (i) to prevent or reduce colonization and diseases in livestock (phage therapy), (ii) to decontaminate carcasses and other raw products, such as fresh fruit and vegetables, and to disinfect equipment and contact surfaces (phage biosanitation and biocontrol) and (iii) to extend the shelf life of perishable manufactured foods as natural preservatives

| Company | Country | Website |
|-------------------------------|-------------|---|
| Exponential Biotherapies Inc. | EEUU | http://www.expobio.com/ |
| Gangagen | EEUU | http://www.gangagen.com/ |
| Intralytix | EEUU | http://www.intralytix.com/ |
| Omnilytics | EEUU | http://www.phage.com/home5.html |
| Phage Biotech | Israel | http://www.phage-biotech.com/ |
| Hexal Genentech | Germany | http://www.hexal-gentech.com/index.html |
| Novolytics | UK | http://www.novolytics.co.uk/ |
| Biophage Inc. | Canada | http://www.biophagepharma.net/index.html |
| Biopharm Pharmaceuticals | Georgia | http://www.biopharmservices.com/Pharma.aspx |
| EBI Food Safety | Netherlands | http://www.ebifoodsafety.com |

Table 1 Companies involved in bacteriophage application

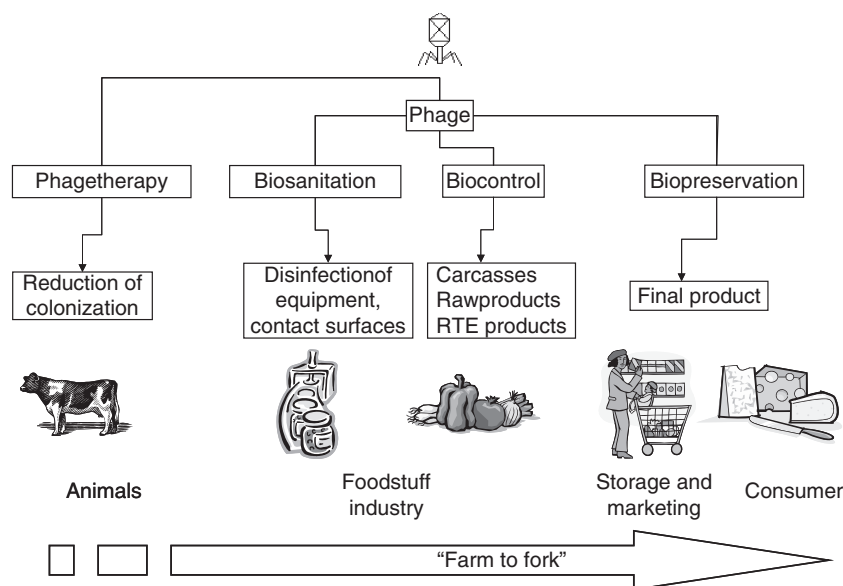


Figure 1 Examples of phage application along the food chain.

(biopreservation). Bacteriophages should also be considered in hurdle technology in combination with different preservation methods (Leverentz *et al.* 2003; Martínez *et al.* 2008). Several examples of phage application throughout the food chain are outlined below and summarized in table 3.

Bacteriophage to control *E. coli* O157:H7 contamination

The emergence of pathogens, such as *E. coli* O157:H7, remains a continuous public health threat because its ingestion at concentrations as low as 10–100 cells may

result in potent toxin exposure. Ruminants comprise the principal reservoir for this strain and contamination of animal products occurs during milking or slaughtering. Phage treatment aims to reduce pathogen contamination prior to slaughtering (Table 3). After the oral administration of phage CEV1, a 2-log-unit reduction in intestinal *E. coli* O157:H7 was achieved within 2 days in sheep (Raya *et al.* 2006). However, orally administered phage KH1 was not effective. When a combination of phages KHI and SH1 were administered rectally to cattle and phages were also maintained at 10^6 PFU ml⁻¹ in the drinking water, significantly lower cell numbers were observed (Sheng *et al.* 2006).

Table 3 Bacteriophages application in food safety and reported effects

| Pathogen | Reported effect | References |
|---------------------------------|---|---|
| <i>Escherichia coli</i> O157:H7 | Intestinal reduction by oral administration (sheep) | Raya <i>et al.</i> 2006 |
| | Reduction by rectal administration and in drinking water (cattle) | Sheng <i>et al.</i> 2006 |
| | Elimination from the beef meat surface | O'Flynn <i>et al.</i> 2004 |
| <i>Salmonella</i> | Lower contamination but not complete elimination (chickens) | Sklar and Joerger 2001; Fiorentin <i>et al.</i> 2005 |
| | More active in Cheddar made of pasteurized milk than raw milk | Modi <i>et al.</i> 2001 |
| | Phage inactivation on apple but not on melon | Leverentz <i>et al.</i> 2001 |
| | Reduction in chicken frankfurters | Whichard <i>et al.</i> 2003 |
| <i>Campylobacter</i> | Significant host inactivation at 5°C in cooked and raw beef | Bigwood <i>et al.</i> 2008 |
| | Decreased counts in cecal content (broilers) | Wagenaar <i>et al.</i> 2005 |
| | Lower viable counts over a 5-day period (broilers) | Loc Carrillo <i>et al.</i> 2005 |
| <i>Listeria monocytogenes</i> | Decontamination of chicken skin | Atterbury <i>et al.</i> 2003; Goode <i>et al.</i> 2003 |
| | Eradication on sliced apples and melon. Synergy with nisin | Leverentz <i>et al.</i> 2003 |
| | No combined phage-nisin action in beef at 4°C | Dykes and Moorhead 2002 |
| <i>Enterobacter sakazakii</i> | Eradication on surface-ripened red-smear soft cheese | Carlton <i>et al.</i> 2005 |
| | Growth arrest in reconstituted infant formula | Kim <i>et al.</i> 2007b |
| <i>Staphylococcus aureus</i> | Phage K inactivation in the mammary gland | Gill <i>et al.</i> 2006a |
| | Phage K inactivation in raw milk | Gill <i>et al.</i> 2006b; O'Flaherty <i>et al.</i> 2005 |
| | Inhibition in acid and enzymatic curds by phages of dairy origin | García <i>et al.</i> 2007 |

Phages were also applied to the meat surface to avoid pathogen development. A mixture of three different phages was applied to beef contaminated with 10^3 CFU g^{-1} *E. coli* O157:H7. In most of the samples, no viable cells could be recovered after storage at 37°C (O'Flynn *et al.* 2004).

Bacteriophage to control *Salmonella* contamination

Salmonella can be isolated from numerous animal species and is the major cause of food poisoning. Phage cocktails reduced the *Salmonella enteritidis* average faecal counts by 0.3–1.3 log units, but the pathogen was not completely eradicated even when more than 10^7 PFU of phage were present per gram of cecal content (Sklar and Joerger 2001). More recently, artificially infected broilers treated orally with high numbers of bacteriophages (10^{11} PFU) (Fiorentin *et al.* 2005). Although the bacteria were not eradicated from the birds, both studies showed that phage treatment would decrease levels of the pathogen bacteria entering the poultry production line.

The activity of the *Salmonella* phage SJ2 was tested in cheddar cheese manufacturing (Modi *et al.* 2001). In the presence of phages (MOI 10^4), *Salmonella* did not survive in the pasteurized cheeses after 89 days, whereas about 50 CFU ml^{-1} were still viable in raw milk cheeses.

Salmonella phage cocktails have been also assayed on fruits. Phage numbers remained relatively stable on melon and gave a significant reduction of target bacteria. On the contrary, a quick decline of infective phage particles was observed on apples due to the lower pH of this fruit (Levrentz *et al.* 2001). In another study, *Salmonella* phage Felix-O1 was tested in biocontrol experiments with *Salmonella typhimurium* on chicken frankfurters contaminated with 300 CFU. Bacterial count reductions of 1.8 and 2.1 log units were achieved (Whichard *et al.* 2003). Phages infecting *Salm. typhimurium* PT160 and *Campylobacter jejuni* were added at a low or high (10 or 10^4) MOI to either low or high (<100 or 10^4 cm^{-2}) densities of host bacteria inoculated onto raw and cooked beef. Significant host inactivation of the order of 2–3 log units at 5°C and >5.9 log units at 24°C was achieved (Bigwood *et al.* 2008).

Bacteriophage to control *Campylobacter* contamination

The colonization of broiler chickens by the enteric pathogen *Camp. jejuni* is widespread and difficult to prevent. Oral infection with this pathogen has become the most common cause of foodborne disease in industrialized countries. The presence of phages negatively correlated with the levels of *Campylobacter* in cecal contents indicating that some degree of biocontrol may occur (Atterbury

et al. 2005). Bacteriophages have been effective to decrease *Campylobacter* counts recovered from cecal contents, thereby lowering the risk of cross contamination during slaughtering (Loc Carrillo *et al.* 2005; Wagenaar *et al.* 2005). Significant decreases have been reported, although total clearance of the host could not be achieved by phage treatments.

Other authors have shown the efficacy of bacteriophage F2 or several lytic bacteriophages applied on artificially contaminated chicken skin (Atterbury *et al.* 2003). The antibacterial phage activity was detected at 4°C and –20°C using a very high MOI (MOI 10^5). At room temperature, lower MOIs of around 100 were already effective and more than 95% of the target cells were killed (Goode *et al.* 2003).

Bacteriophage to control *Listeria monocytogenes* contamination

Foodborne infection due to *L. monocytogenes* is often associated with either fresh or minimally processed foods, such as dairy products or salads, or with processed foods which are stored at low temperatures. Listeriosis usually has a low incidence. However, due to its high mortality rate, up to 30%, *Listeria* is considered as a relevant pathogen. Levrentz *et al.* (2003) evaluated a phage cocktail to control *Listeria* on fresh, cut apples and melon in combination with the bacteriocin nisin. Phage population rapidly declined on sliced apple likely due to the low pH. However, eradication of the pathogen was achieved in both fruits. Besides, the phages were shown to work synergistically with the bacteriocin. Similarly, a synergistic effect was seen in broth cultures but a phage-nisin mixture was not effective on ground beef stored at 4°C (Dykes and Moorhead 2002). Hence, phage activity should be optimized for each food type on a case-by-case basis.

Recently, a commercial product named Listex P100 was approved by the FDA as a food biopreservative and granted as GRAS (Generally Recognised As Safe) (Federal Register: August 18, 2006. Volume 71, Number 160, pp. 47729–47732). This product is based on the virulent phage P100 (Carlton *et al.* 2005) and, depending on dosage and treatment, a complete eradication of target cells was achieved. A bacteriophage-based preparation (LMP 102™) of six bacteriophages isolated from the environment has also been developed as an additive for ready-to-eat foods (Lang 2006).

Bacteriophage to control *Enterobacter sakazakii* contamination

Enterobacter sakazakii is an uncommon, but often fatal, invasive pathogen that causes bloodstream and central

nervous system infections. A recent study has addressed the issue of *Ent. sakazakii* in reconstituted infant formula milk (Kim *et al.* 2007a). Newly isolated phages against this pathogen were able to effectively suppress growth in prepared infant formula, both at 24 and 37°C. The highest phage concentration tested (10^9 PFU ml⁻¹) was the most effective and able to completely eradicate the target organism (Kim *et al.* 2007b).

Bacteriophage to control *Staphylococcus aureus* contamination

Staphylococcus aureus often causes food poisoning and is also a major etiologic agent in opportunistic and nosocomial infections. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins preformed in the food matrices (Le Loir *et al.* 2003). Mastitis caused by *Staph. aureus* is a major concern to the dairy industry and the most important source of milk contamination by this pathogen. The ability of the lytic *Staph. aureus* bacteriophage K to eliminate bovine *Staph. aureus* intramammary infection during lactation was evaluated, but there was significant degradation or inactivation of the infused phage within the gland (Gill *et al.* 2006a). Phage K inactivation was also reported in raw milk, likely due to the adsorption of whey proteins to the cell surface that interfere with phage attachment (O'Flaherty *et al.* 2005; Gill *et al.* 2006b). However, a cocktail of two lytic phages of dairy origin added at MOI 10² successfully inhibited *Staph. aureus* in acid and enzymatic curd manufacturing processes (García *et al.* 2007).

Conclusions and future prospects

The use of biological control measurements such as bacteriophage biocontrol seems to be a promising alternative for the management of food contamination as the use of chemical preservatives becomes restricted. Future developments involve safety and technological issues as well as expanding the antimicrobial skills of bacteriophages (Table 4).

Bacteriophages may act as vectors of undesirable traits (virulence and antibiotic-resistance genes) and temperate phages mediate lysogenic conversion that have raised safety concerns. Recent advance in genomics and phylogenetic studies make it possible to understand gene flow among phages and hosts and potentially harmful bacteriophages could be avoided or re-designed without undesirable traits and lacking any gene dissemination systems. As an example, bacteriophages could be genetically engineered to block phage replication once the host has been killed (Hagens *et al.* 2004). This would prevent the release of large numbers of phages in a particular environment.

Table 4 Future challenges of phage-based biopreservation

| Challenge | Action |
|--------------------------------------|---|
| Lack of undesirable traits | Better knowledge of gene flow phage-host Genomics Blocking gene dissemination systems |
| Large-scale safer production systems | Use of nonvirulent hosts |
| Enhance activity in food systems | Modelling phage behaviour Case-by-case study Same environment as phage source Better knowledge of phage-host physiology |
| Expanding host range | Use of phage mixtures Engineering tail fibre genes |
| New phage-derived antimicrobials | Endolysins Peptidoglycan hydrolases Inhibitors of host metabolism |

Technological challenges to strengthen the future use of bacteriophages as biopreservatives will require the establishment of safe large scale production processes. It is advisable to develop nonvirulent, genetically well-characterized bacteria as hosts in phage propagation. On the other hand, the antimicrobial activity of phages observed in laboratory conditions could be greatly reduced in food systems. Limiting factors are reduced diffusion rates that decrease the chance of host-phage collisions, the microbial load which might also act as a mechanical barrier by providing unspecific phage binding sites and other adverse factors such as temperature, pH and inhibitory compounds. As is the case of any food biopreservatives, bacteriophage efficacy in food should be evaluated on a case-by-case basis. To guarantee proper phage performance, it is wise to isolate phages from the same niche/habitat, as those phages will probably be better adapted to replicate and survive in those conditions. Bacterial fitness and physiological status has a clear influence on the phage infection rate. Phages that selectively bind to the host and replicate in different physiological states should be chosen.

There are several strategies to improve and expand the antimicrobial activity of phages. Comparative genomics of phage tail fibre genes involved in the recognition of specific host receptors will lead to approaches to expand the host range. A detailed analysis of the bacterial receptors will also help to understand and predict the development of bacteria insensitive mutants often due to the loss or mutation of these molecules. Both approaches will enhance the use of phages as antimicrobials as well as bacterial detection systems (Rees and Dodd 2006). New phage-derived antibacterial strategies based on phage-encoded proteins that commit cellular metabolism to

phage proliferation, such as endolysins, are currently being evaluated (Liu *et al.* 2004; Loessner 2005).

Despite the fact that current research is basically at the experimental stage, the increasing number of publications and emerging companies in this area are paving the way for the future of phage-based biopreservation technologies. Landmarks for the next step towards the establishment of expanded commercial applications will be influenced by the accurate knowledge of bacteriophage biology to allow consumers to feel confident about the safety of 'edible viruses'.

References

- Atterbury, R.J., Connerton, P.L., Dodd, C.E.R., Rees, C.E.D. and Connerton, I.F. (2003) 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.
- Atterbury, R.J., Dillon, E., Swift, C., Connerton, P.L., Frost, J.A., Dodd, C.E., Rees, C.E. and Connerton, I.F. (2005) Correlation of *Campylobacter* bacteriophage with reduced presence of hosts in broiler chicken ceca. *Appl Environ Microbiol* **71**, 4885–4887.
- Bigwood, T., Hudson, J.A., Billington, C., Carey-Smith, G.V. and Heinemann, J.A. (2008) Phage inactivation of foodborne pathogens on cooked and raw meat. *Food Microbiol* **25**, 400–406.
- Carlton, R.M., Noordman, W.H., Biswas, B., de Meester, E.D. and Loessner, M.J. (2005) Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. *Regul Toxicol Pharmacol* **43**, 301–312.
- DuPont, H.L. (2007) The growing threat of foodborne bacterial enteropathogens of animal origin. *Clin Infect Dis* **45**, 1353–1361.
- Dykes, G.A. and Moorhead, S.M. (2002) 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.
- Fiorentin, L., Vieira, N.D. and Barioni, W. Jr (2005) Oral treatment with bacteriophages reduces the concentration of *Salmonella Enteritidis* PT4 in caecal contents of broilers. *Avian Pathol* **34**, 258–263.
- García, P., Madera, C., Martínez, B. and Rodríguez, A. (2007) Biocontrol of *Staphylococcus aureus* in curd manufacturing processes using bacteriophages. *Int Dairy J* **17**, 1232–1239.
- Gill, J.J., Pacan, J.C., Carson, M.E., Leslie, K.E., Griffiths, M.W. and Sabour, P.M. (2006a) Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical *Staphylococcus aureus* mastitis in lactating dairy cattle. *Antimicrob Agents Chemother* **50**, 2912–2918.
- Gill, J.J., Sabour, P.M., Leslie, K.E. and Griffiths, M.W. (2006b) Bovine whey proteins inhibit the interaction of *Staphylococcus aureus* and bacteriophage K. *J Appl Microbiol* **101**, 377–386.
- Goode, D., Allen, V.M. and Barrow, P.A. (2003) Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. *Appl Environ Microbiol* **69**, 5032–5036.
- Hagens, S. and Loessner, M.J. (2007) Application of bacteriophages for detection and control of foodborne pathogens. *Appl Microbiol Biotechnol* **76**, 513–519.
- Hagens, S., Habel, A., von Ahsen, U., von Gabain, A. and Bläsi, U. (2004) Therapy of experimental pseudomonas infections with a nonreplicating genetically modified phage. *Antimicrob Agents Chemother* **48**, 3817–3822.
- Kim, K., Jang, S.S., Kim, S.K., Park, J.H., Heu, S. and Ryu, S. (2007a) Prevalence and genetic diversity of *Enterobacter sakazakii* in ingredients of infant foods. *Int J Food Microbiol* **122**, 196–203.
- Kim, K.P., Klumpp, J. and Loessner, M.J. (2007b) *Enterobacter sakazakii* bacteriophages can prevent bacterial growth in reconstituted infant formula. *Int J Food Microbiol* **115**, 195–203.
- Kutter, E. and Sulakvelidze, A. (2005) *Bacteriophages Biology and Applications*. Boca Raton, FL: CRC Press.
- Lang, L. (2006) FDA approves use of bacteriophages to be added to meat and poultry products. *Gastroenterology* **131**, 1370.
- Le Loir, Y., Baron, F. and Gautier, M. (2003) *Staphylococcus aureus* and food poisoning. *Genet Mol Res* **31**, 63–76.
- Leverentz, B., Conway, W.S., Alavidze, Z., Janisiewicz, W.J., Fuchs, Y., Camp, M.J., Chighladze, E. and Sulakvelidze, A. (2001) Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: a model study. *J Food Prot* **64**, 1116–1121.
- Leverentz, B., Conway, W.S., Camp, M.J., Janisiewicz, W.J., Abuladze, T., Yang, M., Saftner, R. and Sulakvelidze, A. (2003) Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Appl Environ Microbiol* **69**, 4519–4526.
- Liu, J., Dehbi, M., Moeck, G., Arhin, F., Bauda, P., Bergeron, D., Callejo, M., Ferretti, V. *et al.* (2004) Antimicrobial drug discovery through bacteriophage genomics. *Nat Biotechnol* **22**, 185–191.
- Loc Carrillo, C., Atterbury, R.J., el-Shibiny, A., Connerton, P.L., Dillon, E., Scott, A. and Connerton, I.F. (2005) Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Appl Environ Microbiol* **71**, 6554–6563.
- Loessner, M.J. (2005) Bacteriophage endolysins – current state of research and applications. *Curr Opin Microbiol* **8**, 480–487.
- Martínez, B., Obeso, J.M., Rodríguez, A. and García, P. (2008) Nisin-bacteriophage crossresistance in *Staphylococcus aureus*. *Int J Food Microbiol* **122**, 253–258.
- Matsuzaki, S., Rashel, M., Uchiyama, J., Sakurai, S., Ujihara, T., Kuroda, M., Ikeuchi, M., Tani, T. *et al.* (2005)

- Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases. *J Infect Chemother* **11**, 211–219.
- Modi, R., Hirvi, Y., Hill, A. and Griffiths, M.W. (2001) 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.
- O’Flaherty, S., Coffey, A., Meaney, W.J., Fitzgerald, G.F. and Ross, R.P. (2005) Inhibition of bacteriophage K proliferation on *Staphylococcus aureus* in raw bovine milk. *Lett Appl Microbiol* **41**, 274–279.
- O’Flynn, G., Ross, R.P., Fitzgerald, G.F. and Coffey, A. (2004) Evaluation of a cocktail of three bacteriophages for bio-control of *Escherichia coli* O157:H7. *Appl Environ Microbiol* **70**, 3417–3421.
- Raya, R.R., Varey, P., Oot, R.A., Dyen, M.R., Callaway, T.R., Edrington, T.S., Kutter, E.M. and Brabban, A.D. (2006) Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157:H7 levels in sheep. *Appl Environ Microbiol* **72**, 6405–6410.
- Rees, C.E.D. and Dodd, C.E.R. (2006) Phage for rapid detection and control of bacterial pathogens in food. *Adv Appl Microbiol* **59**, 159–186.
- Sheng, H., Knecht, H.J., Kudva, I.T. and Hovde, C.J. (2006) Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants. *Appl Environ Microbiol* **72**, 5359–5366.
- Sklar, I.B. and Joerger, R.D. (2001) Attempts to utilize bacteriophages to combat *Salmonella enterica* serovar Enteritidis in chickens. *J Food Safety* **21**, 15–29.
- Strauch, E., Hammerl, J.A. and Hertwig, S. (2007) Bacteriophages: new tools for safer food? *J Verbr Lebensm* **2**, 138–143.
- Sulakvelidze, A. and Kutter, E. (2005). Bacteriophage therapy in humans. In *Bacteriophages: Biology and Application* ed. Kutter, E. and Sulakvelidze, A. pp. 381–436. Boca Raton, FL: CRC Press.
- Wagenaar, J.A., van Bergen, M.A., Mueller, M.A., Wassenaar, T.M. and Carlton, R.M. (2005) Phage therapy reduces *Campylobacter jejuni* colonization in broilers. *Vet Microbiol* **109**, 275–283.
- Whichard, J.M., Sriranganathan, N. and Pierson, F.W. (2003) 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.