

Title	Overcoming barriers to phage application in food and feed
Authors	Lewis, Rhea; Hill, Colin
Publication date	2019-11-11
Original Citation	Lewis, R. and Hill, C. (2020) 'Overcoming barriers to phage application in food and feed', <i>Current Opinion in Biotechnology</i> , 61, pp. 38-44. doi: 10.1016/j.copbio.2019.09.018
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.copbio.2019.09.018
Rights	©2019 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). - http://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2023-09-25 17:30:49
Item downloaded from	https://hdl.handle.net/10468/9340



ELSEVIER



Overcoming barriers to phage application in food and feed

Rhea Lewis^{1,2} and Colin Hill^{1,2}

Bacteriophages (phages) can play a useful role as narrow spectrum antimicrobials in food safety and in food production. Consumer attitudes towards traditional additives have led to a search for natural, potentially clean label, alternatives. At the same time, the rise in antimicrobial resistance has created a need for alternative antimicrobials for disease prevention and treatment in animal husbandry. Phages represent a viable option for both of these applications. We highlight important barriers which should be considered to improve the chance of a positive outcome when using phages in food and food production. These include the feasibility of adding high concentrations of phages, the physico-chemical properties of the food or target, how and when phages are applied, and which phages are chosen.

Addresses

¹ APC Microbiome Ireland, University College Cork, Cork T12 YT20, Ireland

² School of Microbiology, University College Cork, Cork T12 YN60, Ireland

Corresponding author: Hill, Colin (c.hill@ucc.ie)

Current Opinion in Biotechnology 2020, 61:38–44

This review comes from a themed issue on **Food biotechnology**

Edited by **Mark A Blenner** and **Jan Peter van Pijkeren**

<https://doi.org/10.1016/j.copbio.2019.09.018>

0958-1669/© 2019 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Bacteriophages (phages) are bacterial viruses and use bacteria to produce new phages. Phages can replicate using lytic or lysogenic lifecycles. Lytic phages replicate by attaching to a host cell, injecting phage nucleic acid, replicating the phage nucleic acid, assembling proteins, packaging nucleic acid with head and tail proteins, and lysing the host cell to release new phages [1]. Lysogenic phages integrate into the host genome and replicate along with the host. Interest is growing in the use of lytic phages as biocontrol agents of foodborne pathogens in food and food production [2]. Phages can be used in a number of ways in food and feed and can also be applied as preharvest or post-harvest interventions. Recently there has been a change in consumer attitudes towards how food should be processed and preserved with rising interest in

the concept of ‘natural foods’ and ‘naturalness’ [3]. The importance of consumers’ perception should not be overlooked as it has a significant effect on willingness to buy a product and can even affect how individuals enjoy and experience that product [4]. The importance of this changing consumer attitude has led to the introduction of the term ‘clean label’; these products aim to have simple ingredient lists free from ‘chemical-sounding’ terms and negatively perceived ingredients [5]. It has been found that consumers are more willing to accept additives from natural sources than the chemical additives that have been used for decades. This rise in clean label food has increased the demand for new methods to ensure food safety. Another reason for introducing phages is the problem of antimicrobial resistance. A WHO report on antimicrobial resistance drew attention to the contribution of antibiotic use in animals to antimicrobial resistance in general [6]. Antimicrobial resistance has been termed a One Health problem and requires an integrative approach targeting the environment and the health of humans and animals [7]. Historically the antibiotics used in treating livestock have been the same as, or closely related to, those used in human medicine and the use of long-term subtherapeutic doses of antibiotics, as they have been used in livestock as growth promoters, can increase the risk of emergence of antibiotic resistance.

Phage concentration

The success of phages for use in food and feed depends on the overcoming of a number of barriers. The number of phages used is of great importance; in general, the higher the concentration of phages the more significant the reductions in target bacteria. Bai *et al.* [8] found that a phage cocktail at multiplicity of infections (MOIs) of 10^3 and 10^4 significantly reduced the *Salmonella* Typhimurium load of cucumbers and lettuce. The cocktail at an MOI of 10^4 led to a greater and more sustained reduction. Phage at MOIs of 1, 10 and 10^2 significantly reduced *S. Typhimurium* on lettuce with the reductions increasing as the phage concentration increased [9]. This was also evident using SalmoFreshTM, an FDA approved *Salmonella* lytic bacteriophage preparation, on chicken fillets [10]. SalmoFreshTM applied at MOIs of 10^2 and 10^3 both significantly reduced a combination of *S. Typhimurium*, *S. Heidelberg* and *S. Enteritidis* at an initial concentration of 3 log CFU/g on chicken fillets. SalmoFreshTM at an MOI of 10^3 was significantly better than phage at an MOI of 10^2 . In other examples, a single phage was used to control *Escherichia coli* on raw and cooked beef [11], while ShigaShieldTM reduced Shigella

levels in smoked salmon and yogurt [12**]. In both cases the reduction was concentration dependent with the greatest reduction occurring at the highest phage titre tested. Using Listex™ P100, a commercially available phage against *Listeria monocytogenes*, a greater reduction was seen in tuna using an MOI of 10^2 than an MOI of 0.1 [13]. The greatest effect was seen with a lower starting concentration of *L. monocytogenes* combined with a higher concentration of Listex™ P100. However, phage concentration is not a definitive indicator of a positive outcome. A low titre phage cocktail decreased chickens' mortality and morbidity in a natural outbreak of Avian Pathogenic *E. coli* (APEC) but not in experimentally infected chickens [14]. This could have been due to the non-synchronous nature of the natural outbreak. In a natural outbreak all of the cases would be not at the same point in their infection cycle and the low titre phage cocktail may have controlled the infection at the early stages and interrupted the transmission. This would not have occurred in the experimental infection as all of the infections occurred at the same time.

Feasibility of adding high phage concentrations

The need for high numbers of phages for successful phage treatment brings up the question of the feasibility of generating phages at high enough titres to deploy in food or feed. *Salmonella* on post-chill chicken carcasses has been suggested as a target as numbers do not usually exceed 3 log CFU/g [15]. The inoculation of 25 g samples of turkey fillet with 500 μ l of a 10^9 PFU/ml bacteriophage preparation was sufficient to significantly reduce *S. Heidelberg* over a seven day period and represented a reasonable volume of phage preparation to be applied. In a study of the application of Finalyse™ hidewash, $\sim 3 \times 10^{10}$ phage/head of cattle in one gallon of water was used [16]. This was insufficient to reduce *E. coli* O157:H7 contamination of cattle hides and carcasses but given the one gallon quantity needed it may be difficult and cost prohibitive to increase the titre used. Recombinantly expressed endolysins isolated from phages could possibly be used in place of whole phages to avoid this issue [17], though once again the economic viability of this solution would have to be investigated. Issues with GMO legislative approval and consumer acceptance may limit the use of endolysins in food safety [18].

Properties of the target matrix

The properties of the targeted food or animal in question can be a barrier to phage success. The target matrix can affect the efficiency of phages. In a study of a single phage to reduce *S. Typhimurium* in whole milk, skimmed milk, energy drink, apple juice, and liquid egg the smallest effect of the phage was seen in liquid egg [19]. Phage titre increased in all foods except for liquid egg, in which a decrease occurred. The highly viscous matrix of egg limiting diffusion and homogeneous distribution of the phage particles was suggested as a reason for this reduced

activity and the decrease in phage numbers. Similarly when looking at a phage cocktail to reduce *S. Enteritidis* in milk, cabbage, and chicken breast the greatest effect was seen in milk [20]. Again it was proposed that the liquid allowed greater diffusion of the phages. Using a single phage it was necessary to use an MOI of 10^5 to reduce *S. Typhimurium* load in liquid egg and fruit juice while an MOI of 10^7 was required to give a similar reduction in cooked beef and chicken [21]. Salmonex™, a commercially available phage cocktail, was effective in reducing *Salmonella* on chicken [22]. Salmonex™ diluted in tap water was more effective than in filtered tap water with reduced calcium, magnesium, and sodium. Temperature can also affect the activity of phages. The antibacterial activity of ListShield™, a commercially available phage against *L. monocytogenes*, was reduced at 12°C compared to 4°C [23]. When using phages to reduce *Staphylococcus aureus* during the manufacturing of cheese it was observed that phage titre decreased with decreasing pH [24]. Antacids can be given before or in combination with phages in animal application to improve the survival of phages in the gut by increasing pH [25,26].

Application method of phages

The method of phage application can also be a barrier to a positive outcome. Although dipping and spraying are common methods for phage application, they can have negative effects [27]. Dipping and spraying can release phage particles into the environment, dipping liquid can be a source of cross contamination and spraying equipment may not be available in the processing environment. Different methods can be used to suit the situation or to expand the conditions of activity of the phages. Microencapsulated phages significantly reduced *E. coli* on tomatoes and maintained the reduction for five days [28]. Microencapsulation reduced the UV sensitivity of the phages and increased the survival of phages at pH 3–7 and extreme temperatures. Free phages, paper impregnated with phages, and encapsulated phages all immediately reduced *E. coli* on alfalfa seeds and sprouts [27]. After five days free phages and encapsulated phages had a significant effect compared to untreated controls. This can be case dependent as in the same study free phages reduced *L. monocytogenes* by 3 log CFU/g in cantaloupes while encapsulated phages reduced numbers by only 1 log CFU/g over the same period. This was suggested to be due to how the bacteria attached to different food matrices represented by cantaloupe and alfalfa sprouts. A phage cocktail applied to chicken feed was as effective as phage introduced by crop gavage and represents a much easier mode of inoculation [25]. Phages can also be applied in more unexpected ways such as in the depuration of bivalves where phages were added to the depuration tank water [29]. Longer treatment time was required in control tanks to obtain comparable reductions to those achieved when using phage tanks (Table 1).

Table 1

The application of phages to eradicate target microbes. In the case of ListShield™ against *L. monocytogenes* in Spanish dry cured ham bacterial numbers increased after treatment at 12°C but decreased after treatment at 4°C, this increase was represented by a (+) [23]

Phage cocktail	Target organism	Food matrix	MOI	Application method	Bacterial reduction	Reference					
BSPM4, BSP101, BSP22A	<i>S. Typhimurium</i>	Iceberg lettuce	10 ³ 10 ⁴	Applied to surface	1.1–1.9 log CFU/cm ² 2.8–3.9 log CFU/cm ²	[8**]					
		Cucumber	10 ³ 10 ⁴		0.7–1.2 log CFU/cm ² 2.5–2.8 log CFU/cm ²						
LPST10	<i>S. Typhimurium</i>	Lettuce	1 10 10 ²	Applied to surface	0.7–1.7 log ₁₀ CFU/cm ² 1.1–1.7 log ₁₀ CFU/cm ² 1.9–2.7 log ₁₀ CFU/cm ²	[9]					
			SalmoFresh™		<i>S. Typhimurium</i> , <i>S. Heidelberg</i> , <i>S. Enteritidis</i>		Chicken breast fillets	10 ² 10 ³	Applied to surface	0.6 log CFU/g 1.1 log CFU/g	[10]
								FAHEc1		<i>E. coli</i>	
Cooked beef	10 ² 10 ⁴	Spraying	1 log CFU/cm ² 5 log CFU/cm ²	[12**]							
ShigaShield™	<i>Shigella sonnei</i>		Smoked salmon		2.25 × 10 ² 2.25 × 10 ³ 2.25 × 10 ⁴	Spraying	0.16 log CFU/g 0.50 log CFU/g 1.098 log CFU/g	[12**]			
		Yogurt		4.5 × 10 ² 4.5 × 10 ³ 4.5 × 10 ⁴	Mixed in to food		0.07 log CFU/g 0.26 log CFU/g 1.01 log CFU/g				
				Listex™ P100			<i>L. monocytogenes</i>		Tuna sashimi	0.1 10 ² 10 ³ 10 ⁶	Applied to surface
phi F78E	Avian pathogenic <i>E. coli</i>	Experimentally infected chickens Naturally infected chickens	30		Oral gavage and spraying Oral gavage and spraying	No reduction in morbidity or mortality Decreased mortality to below 0.5% in no more than three weeks		[14]			
			SalmoFresh™ Finalyse™		<i>S. Heidelberg</i> <i>E. coli</i>	Turkey breast				10 ⁴	
Live cattle	~3 × 10 ¹⁰ phage/ head of cattle in one gallon of water. Looked at prevalence in cattle not bacterial numbers.	Applied to hide surface		6.6 % reduction in prevalence		[16]					
P22	<i>S. Typhimurium</i>	Whole milk	10 ⁸	Mixed in to liquid	4.45 log CFU/ml	[19]					
		Skimmed milk			4.32 log CFU/ml						
		Energy drink			2.09 log CFU/ml						
		Apple juice			2.06 log CFU/ml						
		Liquid egg			0.96 log CFU/ml						
PA13076, PC2184	<i>S. Enteritidis</i>	Milk	10 ⁴	Applied to surface	4 log CFU/sample	[20]					
		Cabbage		Applied to surface	3.86 log CFU/sample						
		Chicken breast		Mixed in to liquid	2.5 log CFU/sample						

Table 1 (Continued)

Phage cocktail	Target organism	Food matrix	MOI	Application method	Bacterial reduction	Reference
SE07	<i>S. Enteritidis</i>	Liquid egg	10^5	Mixed in to liquid	1.96 log CFU/ml	[21]
		Fruit juice	10^5	Mixed in to liquid	2.06 log CFU/ml	
		Beef	10^7	Spraying	2.03 log CFU/ml	
		Chicken	10^7	Spraying	2.18 log CFU/ml	
Salmonalex™	<i>S. Newport</i> , <i>S. Typhimurium</i> , <i>S. Heidelberg</i> , <i>S. Enteritidis</i>	Skinless chicken legs and thighs	10^3	Applied to surface in tap water Applied to surface in filtered water	0.39 log CFU/cm ² 0.23 log CFU/cm ²	[22]
ListShield™	<i>L. monocytogenes</i>	Spanish dry cured ham	10^2	Applied to surface 4 °C	1.5 log CFU/cm ²	[23]
			10^2	Applied to surface 12 °C	+1.5 log CFU/cm ²	
			10^3	Applied to surface 4 °C	2 log CFU/cm ²	
			10^3	Applied to surface 12 °C	+3 log CFU/cm ²	
vB_SauS-phi-IPLA35, vB_SauS-phi-SauS-IPLA88)	<i>S. aureus</i>	Fresh cheese	10^4	Applied to surface 4 °C	3.5 log CFU/cm ²	[24]
			6	Added during cheese manufacture	3.83 log CFU/g	
phiCcoIBB35, phiCcoIBB37, phiCcoIBB12	<i>C. jejuni</i>	Live chickens	15	In feed Oral gavage	1.96 log CFU/g 1.69 log CFU/g	[25]
CP68, CP14	<i>C. jejuni</i>	Live chickens	10	Oral gavage	3 log CFU/g	[26]
LinM-AG8, LmoM-AG13, LmoM-AG20	<i>L. monocytogenes</i>	Cantaloupe	10^5 10 - 10^2	Applied to surface Encapsulated phage	3 log CFU/g 1 log CFU/g	[27]
EcoM-HG2, EcoM-HG7, EcoM-HG8	<i>E. coli</i>	Alfalfa seeds and sprouts	10^4 10 - 10^2	Applied to surface Encapsulated phage	1.5 log CFU/g 1.3 log CFU/g	
FJLA23, FKP26, FC119, FE142	<i>E. coli</i>	Tomatoes	N/A 10^4	Impregnated paper Spraying microencapsulated phage	0.6 log CFU/g 2.5 log CFU/tomato	[28]
phT4A, ECA2	<i>E. coli</i>	Cockles	1	Added to depuration tank water	0.6 log CFU/g	[29]
SalmoFresh™	<i>S. Newport</i>	Cucumbers	7.9×10^4	Sprayed before slicing Sprayed unsliced	1 log CFU/sample 1.83 log CFU/sample	[30]
Neptra, Lelidair, Nobby, Slant, Gaspode, Momine	<i>Pectobacterium atrosepticum</i>	Potatoes	10^2	Phage wash	Reduction in disease incidence 61.3%	[31]
DT6	<i>E. coli</i>	Milk	2.4×10^4	Added during milk fermentation	1.1 log CFU/ml	[36]
wksI3	<i>S. Enteritidis</i>	Chicken skin	5×10^3	Spraying	2.43 log CFU/cm ²	[39]
Team1, P68, LH1-MUT	<i>S. aureus</i>	Cheddar cheese	150	Added during cheese manufacture	2 log CFU/ml	[41]

When phages should be applied

Phage interventions must be carried out at the correct stage of processing for a positive outcome and to avoid reintroduction of bacteria. For example, cucumbers that were sprayed with SalmoFresh™ and then sliced did not show a significant reduction in *S. Newport* while unsliced cucumbers did show a significant reduction [30]. It is believed that insufficient numbers of phages were transferred by cutting to achieve a significant reduction. A cocktail of six phages reduced the symptoms of *Pectobacterium atrosepticum* soft rot in potato tubers significantly [31]. However, phage alone increased the disease severity in uninfected control tubers. This was suggested to be caused by the presence of enzymes or metabolites from phage production. This could limit the use of phages as a prophylactic treatment but not as an intervention as the cost to uninfected tubers could outweigh the benefit of reduction of symptoms in uninfected tubers. Regarding the issue of reinfection pigs can become infected with *Salmonella* after just two hours in a contaminated abattoir environment so would need to be treated at the correct time to avoid this [32]. To reduce *Campylobacter* in chicken, interventions should be performed at multiple stages as *Campylobacter* can re-enter the food due to its ubiquitous nature on farms [33].

Phage selection

There are a number of disadvantages associated with the use of phages but this barrier can be overcome by careful selection of phages. The emergence of phage resistant bacteria is a risk [34]. To decrease the issue of resistance treatments can be rotated or cocktails of phages can be used instead of single phages. Phages should only be applied when there is no risk of treated bacteria being reintroduced into the processing environment and causing issues with resistance. The highly specific nature of phages to their host has historically been viewed as a disadvantage in that they may only infect a limited number of strains [35]. This view is changing as it is recognised that their specificity limits negative effects to the surrounding bacterial community. For example, it would be possible to use phages to reduce enteropathogenic or Shiga toxinogenic *E. coli* during milk fermentation without compromising the performance of starter cultures [36]. Cocktails of multiple phages can also be used to increase the number of strains targeted [37]. Phages can carry virulence genes or antibiotic resistance genes but this can be largely avoided by using lytic rather than lysogenic phages [38]. There are also concerns about the immunogenicity of phages and the cytotoxicity which could result by lysis of target bacteria. Phages have been found to cause no adverse reactions in rats and mice suggesting they are safe for human use [39,40]. Phages against *S. aureus* in cheddar manufacture did not increase enterotoxin production [41]. Phages are constantly encountered by humans since they are found naturally in the gut of humans [42], on their skin [43], in animals [44], in sewage treatment systems [45], and during the breakdown of food fermentations [46] among others.

Conclusion

Phages show promise for use in controlling bacterial pathogens as additives from natural sources, which may be more readily accepted by consumers than traditional additives, and also in the growing fight against antibiotic resistance. Phages may only reduce bacterial populations in food and not eliminate them completely, but this is not a serious issue. Criteria are put in place by groups, such as the European Union, for acceptable levels of pathogens in food depending on the pathogen, food, and intended consumer with some required to be absent and some acceptable at low levels [47]. There is zero tolerance for *Salmonella* in foods such as pre-cut vegetables, *L. monocytogenes* in food for infants and for medical purposes, and *E. coli* O157:H7 on sprouts. Phages reduced *S. Typhimurium* in whole milk and skimmed milk to undetectable, and therefore authorised, levels [19]. Phages reduced *L. monocytogenes* to undetectable levels on tuna and Spanish dry cured ham [13,23]. Coagulase-positive staphylococci are permitted in cheeses during manufacture up to 10⁴ CFU/g. Phages have been successful in reducing *S. aureus* in cheddar cheese to safe levels [41].

Bacteria use a number of methods to defend against phage infection at various points in the infection process [48]. Bacteria can inhibit phage adsorption by altering or blocking receptors. Superinfection exclusion occurs when a prophage causes the expression of proteins which stops phage injection. Restriction modification methylates host DNA and cleaves invading unmethylated DNA. Bacteria using the CRISPR/Cas system (clustered, regularly interspaced, short palindromic repeat) integrate small fragments of invading DNA and cleave DNA with this sequence. Abortive infection limits the spread of the phage by the death of infected host cells. Care must be taken in the application of phages and their use to ensure they achieve a favourable outcome before they become a widely used and accepted aid in food processing. Phage concentration, the feasibility of adding a high concentration of phages, the properties of the food or animal to be treated, how phages are applied, when they are applied, and what phages are used are all barriers which must be overcome when designing a process using phages for decontamination or bacterial control.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Sulakvelidze A, Alavidze Z, Morris JG Jr: **Bacteriophage therapy**. *Antimicrob Agents Chemother* 2001, **45**:649-659.
2. Stone E, Campbell K, Grant I, McAulliffe O: **Understanding and exploiting phage-host interactions**. *Viruses* 2019, **11**:567.

3. Román S, Sánchez-Siles LM, Siegrist M: **The importance of food naturalness for consumers: results of a systematic review.** *Trends Food Sci Technol* 2017, **67**:44-57.
 4. Naanwaab C, Yeboah O-A, Ofori Kyei F, Sulakvelidze A, Goktepe I: **Evaluation of consumers' perception and willingness to pay for bacteriophage treated fresh produce.** *Bacteriophage* 2014, **4**:e979662.
 5. Aschemann-Witzel J, Varela P, Peschel AO: **Consumers' categorization of food ingredients: Do consumers perceive them as 'clean label' producers expect? An exploration with projective mapping.** *Food Qual Preference* 2019, **71**:117-128
- The study explored consumers' perception and categorisation of food ingredients. Unknown ingredients were perceived negatively and there was a preference for natural ingredients.
6. World Health Organisation: *Antibacterial Agents in Clinical Development an Analysis of the Antibacterial Clinical Development Pipeline, Including Tuberculosis.* . reference number: WHO/EMP/IAU/2017.1 WHO; 2017 <http://apps.who.int/iris/bitstream/handle/10665/258965/WHO-EMP-IAU-252017.258911-eng.pdf?sequence=258961>.
 7. Walsh TR: **A one-health approach to antimicrobial resistance.** *Nat Microbiol* 2018, **3**:854-855.
 8. Bai J, Jeon B, Ryu S: **Effective inhibition of *Salmonella* Typhimurium in fresh produce by a phage cocktail targeting multiple host receptors.** *Food Microbiol* 2019, **77**:52-60
- The authors isolated and developed a phage cocktail effective against *Salmonella* species and highlighted the importance of choosing phages free from genes involved in lysogeny and virulence factor production.
9. Huang C, Shi J, Ma W, Li Z, Wang J, Li J, Wang X: **Isolation, characterization, and application of a novel specific *Salmonella* bacteriophage in different food matrices.** *Food Res Int* 2018, **111**:631-641
- A phage was tested in a range of foods, temperatures and concentrations. Greater bacterial reduction by phage occurred at higher temperatures as bacteria were more actively growing.
10. Sukumaran AT, Nannapaneni R, Kiess A, Sharma CS: **Reduction of *Salmonella* on chicken meat and chicken skin by combined or sequential application of lytic bacteriophage with chemical antimicrobials.** *Int J Food Microbiol* 2015, **207**:8-15.
 11. Hudson J, Billington C, Wilson T, On S: **Effect of phage and host concentration on the inactivation of *Escherichia coli* O157:H7 on cooked and raw beef.** *Food Sci Technol Int* 2015, **21**:104-109.
 12. Soffer N, Woolston J, Li M, Das C, Sulakvelidze A: **Bacteriophage preparation lytic for *Shigella* significantly reduces *Shigella sonnei* contamination in various foods.** *PLoS One* 2017, **12**:e0175256
- This study looked at the use of ShigaShield™, a commercially available phage cocktail, in deli meat, smoked salmon, precooked chicken, lettuce, melon and yogurt at various concentrations. virulent and/or toxic genes) present. No toxin genes, virulence genes, repressor genes, integrases, recombinases or any bacterial gene listed in the US Code for Federal Regulations (40 CFR §725.421) were detected in the genomes of the phages.
13. Migueis S, Saraiva C, Esteves A: **Efficacy of LISTEX P100 at different concentrations for reduction of *Listeria monocytogenes* inoculated in sashimi.** *J Food Prot* 2017, **80**:2094-2098.
 14. Oliveira A, Sereno R, Azeredo J: **In vivo efficiency evaluation of a phage cocktail in controlling severe colibacillosis in confined conditions and experimental poultry houses.** *Vet Microbiol* 2010, **146**:303-308.
 15. Sharma CS, Dhakal J, Nannapaneni R: **Efficacy of lytic bacteriophage preparation in reducing *Salmonella* in vitro, on Turkey breast cutlets, and on ground Turkey.** *J Food Prot* 2015, **78**:1357-1362.
 16. Arthur TM, Kalchayanand N, Agga GE, Wheeler TL, Koohmaraie M: **Evaluation of bacteriophage application to cattle in lairage at beef processing plants to reduce *Escherichia coli* O157:H7 prevalence on hides and carcasses.** *Foodborne Pathog Dis* 2017, **14**:17-22.
 17. Fan J, Zeng Z, Mai K, Yang Y, Feng J, Bai Y, Sun B, Xie Q, Tong Y, Ma J: **Preliminary treatment of bovine mastitis caused by *Staphylococcus aureus*, with *trx*-SA1, recombinant endolysin of *S. aureus* bacteriophage IME-SA1.** *Vet Microbiol* 2016, **191**:65-71.
 18. Misiou O, van Nassau TJ, Lenz CA, Vogel RF: **The preservation of *Listeria*-critical foods by a combination of endolysin and high hydrostatic pressure.** *Int J Food Microbiol* 2018, **266**:355-362.
 19. Zinno P, Devirgiliis C, Ercolini D, Ongeng D, Mauriello G: **Bacteriophage P22 to challenge *Salmonella* in foods.** *Int J Food Microbiol* 2014, **191**:69-74.
 20. Bao H, Zhang P, Zhang H, Zhou Y, Zhang L, Wang R: **Bio-control of *Salmonella* Enteritidis in foods using bacteriophages.** *Viruses* 2015, **7**:4836-4853.
 21. Thung TY, Krishanthi Jayarukshi Kumari Premaratne JM, San Chang W, Loo YY, Chin YZ, Kuan CH, Tan CW, Basri DF, Jasimah Wan Mohamed Radzi CW, Radu S: **Use of a lytic bacteriophage to control *Salmonella* Enteritidis in retail food.** *LWT* 2017, **78**:222-225.
 22. Grant AQ, Parveen S, Schwarz J, Hashem F, Vimini B: **Reduction of *Salmonella* in ground chicken using a bacteriophage.** *Poult Sci* 2017, **96**:2845-2852.
 23. Gutiérrez D, Rodríguez-Rubio L, Fernández L, Martínez B, Rodríguez A, García P: **Applicability of commercial phage-based products against *Listeria monocytogenes* for improvement of food safety in Spanish dry-cured ham and food contact surfaces.** *Food Control* 2017, **73**:1474-1482.
 24. Bueno E, García P, Martínez B, Rodríguez A: **Phage inactivation of *Staphylococcus aureus* in fresh and hard-type cheeses.** *Int J Food Microbiol* 2012, **158**:23-27.
 25. Carvalho CM, Gannon BW, Halfhide DE, Santos SB, Hayes CM, Roe JM, Azeredo J: **The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens.** *BMC Microbiol* 2010, **10**:232.
 26. Hammerl JA, Jäckel C, Alter T, Janzcyk P, Stingl K, Knüver MT, Hertwig S: **Reduction of *Campylobacter jejuni* in broiler chicken by successive application of Group II and Group III phages.** *PLoS One* 2014, **9**:e114785.
 27. Lone A, Anany H, Hakeem M, Aguis L, Avdjian A-C, Bouget M, Atashi A, Brovko L, Rochefort D, Griffiths MW: **Development of prototypes of bioactive packaging materials based on immobilized bacteriophages for control of growth of bacterial pathogens in foods.** *Int J Food Microbiol* 2016, **217**:49-58.
 28. Ramirez K, Cazarez-Montoya C, Lopez-Moreno HS, Castro-del Campo N: **Bacteriophage cocktail for biocontrol of *Escherichia coli* O157:H7: stability and potential allergenicity study.** *PLoS One* 2018, **13**:e0195023.
 29. Pereira C, Moreirinha C, Teles L, Rocha RJM, Calado R, Romalde JL, Nunes ML, Almeida A: **Application of phage therapy during bivalve depuration improves *Escherichia coli* decontamination.** *Food Microbiol* 2017, **61**:102-112.
 30. Sharma M, Dashiell G, Handy ET, East C, Reynnells R, White C, Nyarko E, Micallef S, Hashem F, Millner PD: **Survival of *Salmonella* newport on whole and fresh-cut cucumbers treated with lytic bacteriophages.** *J Food Prot* 2017, **80**:668-673.
 31. Carstens AB, Djurhuus AM, Kot W, Hansen LH: **A novel six-phage cocktail reduces *Pectobacterium atrosepticum* soft rot infection in potato tubers under simulated storage conditions.** *FEMS Microbiol Lett* 2019, **366**
- A phage cocktail was developed against *Pectobacterium atrosepticum*, the causative agent of potato soft rot, and was effective but had a negative effect on uninfected controls.
32. Walia K, Argüello H, Lynch H, Grant J, Leonard FC, Lawlor PG, Gardiner GE, Duffy G: **The efficacy of different cleaning and disinfection procedures to reduce *Salmonella* and *Enterobacteriaceae* in the lairage environment of a pig abattoir.** *Int J Food Microbiol* 2017, **246**:64-71.
 33. Fischer S, Kittler S, Klein G, Glünder G: **Impact of a single phage and a phage cocktail application in broilers on reduction of**

- Campylobacter jejuni* and development of resistance.** *PLoS One* 2013, **8**:e78543.
34. Guenther S, Herzig O, Fieseler L, Klumpp J, Loessner MJ: **Biocontrol of *Salmonella* Typhimurium in RTE foods with the virulent bacteriophage FO1-E2.** *Int J Food Microbiol* 2012, **154**:66-72.
 35. Cieplak T, Soffer N, Sulakvelidze A, Nielsen DS: **A bacteriophage cocktail targeting *Escherichia coli* reduces *E. coli* in simulated gut conditions, while preserving a non-targeted representative commensal normal microbiota.** *Gut Microbes* 2018, **9**:391-399.
 36. Tomat D, Mercanti D, Balague C, Quiberoni A: **Phage biocontrol of enteropathogenic and Shiga toxin-producing *Escherichia coli* during milk fermentation.** *Lett Appl Microbiol* 2013, **57**:3-10.
 37. Costa P, Pereira C, Gomes ATPC, Almeida A: **Efficiency of single phage suspensions and phage cocktail in the inactivation of *Escherichia coli* and *Salmonella* Typhimurium: an in vitro preliminary study.** *Microorganisms* 2019, **7**:94
- Although about hospital-acquired infections the outcome can be applied to food settings. A phage cocktail was more effective against *S. Typhimurium* than a single phage. It was determined that cocktails could be used as broad spectrum agents before a target was identified.
38. Cui Z, Guo X, Dong K, Zhang Y, Li Q, Zhu Y, Zeng L, Tang R, Li L: **Safety assessment of *Staphylococcus* phages of the family *Myoviridae* based on complete genome sequences.** *Sci Rep* 2017, **7**:41259.
 39. Kang H-W, Kim J-W, Jung T-S, Woo G-J: **wksl3, a new biocontrol agent for *Salmonella enterica* Serovars Enteritidis and Typhimurium in foods: characterization, application, sequence analysis, and oral acute toxicity study.** *Appl Environ Microbiol* 2013, **79**:1956-1968.
 40. EFSA Panel on Biological Hazards: **Evaluation of the safety and efficacy of Listex™ P100 for reduction of pathogens on different ready-to-eat (RTE) food products.** *EFSA J* 2016, **14**: e04565.
 41. El Haddad L, Roy J-P, Khalil GE, St-Gelais D, Champagne CP, Labrie S, Moineau S: **Efficacy of two *Staphylococcus aureus* phage cocktails in cheese production.** *Int J Food Microbiol* 2016, **217**:7-13.
 42. Shkoporov A, Khokhlova EV, Fitzgerald CB, Stockdale SR, Draper LA, Ross RP, Hill C: **ΦCrAss001 represents the most abundant bacteriophage family in the human gut and infects *Bacteroides intestinalis*.** *Nat Commun* 2018, **9**:4781.
 43. van Zyl LJ, Abrahams Y, Stander EA, Kirby-McCollough B, Jourdain R, Clavaud C, Breton L, Trindade M: **Novel phages of healthy skin metaviromes from South Africa.** *Sci Rep* 2018, **8**:12265.
 44. Morozova V, Kozlova Y, Shedko E, Babkin I, Kurilshikov A, Bokovaya O, Bardashova A, Yunusova A, Tikunov A, Tupikin A *et al.*: **Isolation and characterization of a group of new *Proteus* bacteriophages.** *Arch Virol* 2018, **163**:2189-2197.
 45. Fernandez-Cassi X, Timoneda N, Martínez-Puchol S, Rusiñol M, Rodríguez-Manzano J, Figuerola N, Bofill-Mas S, Abril JF, Girones R: **Metagenomics for the study of viruses in urban sewage as a tool for public health surveillance.** *Sci Total Environ* 2018, **618**:870-880.
 46. Halter MC, Zahn JA: **Characterization of a novel lytic bacteriophage from an industrial *Escherichia coli* fermentation process and elimination of virulence using a heterologous CRISPR-Cas9 system.** *J Ind Microbiol Biotechnol* 2018, **45**:153-163.
 47. European Commission: **COMMISSION REGULATION (EC) No 2073/2005.** 2005.
 48. Azam AH, Tanji Y: **Bacteriophage-host arm race: an update on the mechanism of phage resistance in bacteria and revenge of the phage with the perspective for phage therapy.** *Appl Microbiol Biotechnol* 2019, **103**:2121-2131.