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The Use of Bacteriophages for Food Safety

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The use of bacteriophages for food safety

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The search for natural biocontrol agents that allow the production of foods that are safe for human consumption and do not impact the taste, texture, and nutritional quality of the food, is a constant challenge for diverse food industries worldwide, particularly as the human population continues to rise globally, and multiple antibiotic resistance in pathogenic bacteria is increasingly prevalent. Bacteriophages (phages), the naturally occurring predators of bacteria, are harmless to humans and animals and are ubiquitous in the environment — and as such, have been recognised as promising antimicrobial agents to help control specific bacterial pathogens in food production. This short review details recent developments in relation to phage biocontrol in food, highlighting both their applicability for enhancing microbial safety and also the challenges within this area of food biotechnology. It also highlights developments in the use of phages for pathogen detection.

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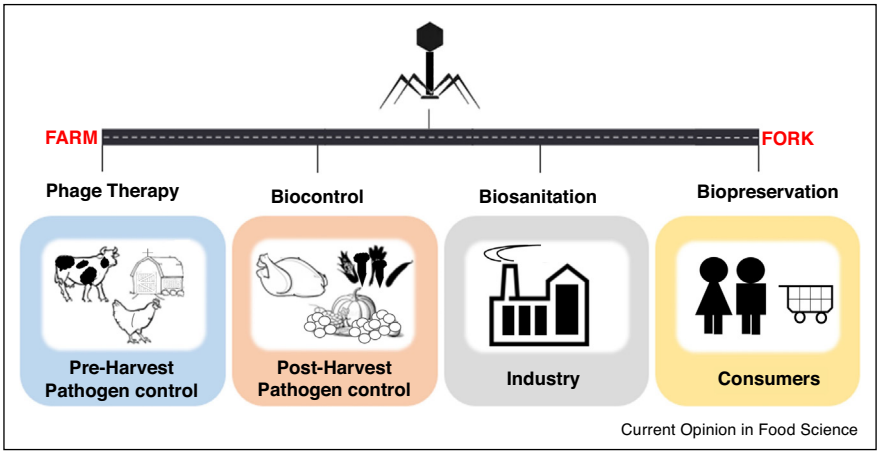
Introduction

Food sustainability and safety are challenges that continue to dominate food industries worldwide. The shift in Western countries towards consuming foods that are produced by natural means adds significant pressure to produce foods that are safe, natural, free from chemical preservatives, and are of acceptable quality to meet consumer demands [1]. The global population is growing at an increasing rate on an annual basis and according to the UNDESA, 2019, the population is expected to increase to 9.7 billion by 2050, and 11.2 billion by 2100. This will result in a proportional increase in demand for food globally, placing continued pressure on the industry to comply with regulations associated with food safety and international trade. The WHO (2020) estimates that globally, approximately 600 million people experience serious foodborne illness annually (requiring hospitalisation) resulting in up to 420,000 deaths. The financial impact of this, due to direct healthcare costs and indirect loss of productivity costs is estimated to be in excess of $110 billion. The health and its socioeconomic impacts are difficult to ascertain precisely, but it is clear food safety efforts warrant continued developments to curb the level of foodborne illness that occurs every year [2]. The food production sector has had to continually participate in efforts to prevent infectious diseases and the issues that surround antibiotic resistance in human pathogens originating in food animals. Irrespective of the many advances in technological methods for the detection and elimination of foodborne pathogens at each stage of the food production process, in good manufacturing practices, in quality control and hygiene, changes in animal husbandry and agronomic processes, microbial safety problems are still prevalent. In addition, the restricted use of certain antibiotics during food animal production, coupled with the lack of development of new antimicrobials has put further strain on the food production sector and as such, there is a need for development of alternative antibacterial approaches at production level to maintain safety standards, control foodborne pathogens and limit their negative impact on the food industry and on human health.

The natural specificity of bacteriophages (phages) to infect and kill their target bacteria, in addition to the fact that they are ubiquitous in the environment, and are harmless to humans and animals, makes them valuable candidates for use in both the detection and the control of pathogens at each stage of the food production process from farm-to-fork (Figure 1). In recent years, a number of phage-based products have gone into commercial use to control some of the leading foodborne pathogens including *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* serovars [3,4••]. This development is quite promising and it highlights the industry's confidence in the efficacy and safety of phage-based preparations, having Generally Recognised as Safe (GRAS) status for use in controlling harmful pathogens in the food industry and many commercial phage companies have FDA approval for their food safety products including Intralytix, Micreos Food Safety, FINK TEC GmbH, Passport Food Safety Solutions and Phagelux. This review will illustrate the recent advances in phage biocontrol with respect to the food sector.

Figure 1



Phage application in food safety at each stage of the farm to fork process.

Phage biocontrol at farm level

Food safety begins at the farm level during crop and livestock production. Over the years, the agriculture environment has relied heavily on the use of antibiotics for growth promotion, and for disease prevention and control, and indeed, the level of antibiotics used in this sector is the largest proportion worldwide. As such, imprudent and overuse of antibiotics in that sector threatens their efficacy for pathogen control due to the emergence and proliferation of multidrug resistance in bacteria [5]. Drug resistant bacteria account for over 700,000 deaths worldwide each year, and if resistance trends and the lack of development of new antibiotics continues, this number is expected to increase to 10 million by 2050 [6]. In light of this, phages and their derivatives are increasingly being recognised as viable complimentary approaches for use in food safety at various stages of the production process. In the light of the brevity of this article, the reader is also directed to recent comprehensive reviews depicting the significant developments in both pre-harvest and post-harvest production of food such as O'Sullivan *et al.* [7•] and Vikram *et al.* [8••]. In addition to highlighting the significance of the commercial phage products, this article summarises the most recent experimental work on phages aimed at food safety.

Phage biocontrol and sanitation at the pre-harvest stage of production

Nowadays, farm animals are housed in large numbers, often in confined conditions which promotes proliferation of infectious disease agents among livestock. As such, many animals act as reservoirs for different zoonotic bacterial pathogens that enter the food chain resulting in human illnesses and deaths. Investigations aimed at controlling bacterial pathogens in various farm animals using phages have been described in detail by Vikram *et al.* recently [8••], a review article where the extensive spectrum of phage products on the market or possibly near market is described comprehensively. In this section, recent advances in the application of phages for biocontrol of clinically relevant pathogens at the pre-harvest stage of food production will be discussed.

Poultry

The two principle pathogens associated with poultry are *Campylobacter* and *Salmonella*. In the context of the former, the reader is directed to a comprehensive review by Ushanov *et al.* [9••]. Specific studies on this area include a report by Chinivasagam *et al.* [10] who demonstrated the use of phage cocktails to control *Campylobacter* in broiler chickens, following the birds from farm to the processing plant. Phage cocktails were selected to target *C. jejuni* and *C. coli*. These phages were administered to 47-day old birds for 24 hour immediately before slaughter. Researchers found that in general, the cocktails were effective at reducing intestinal *Campylobacter* levels in the market ready broilers. However, a few birds exhibited high cecal *Campylobacter* counts coupled with low phage titres, and as such the authors suggested an increased treatment time beyond 24 hour to ensure successive phage replication for biocontrol of *Campylobacter in vivo* [10]. Richards *et al.* [11] conducted a study in broiler chickens to determine the efficacy of a two-phage cocktail against *C. jejuni*. Results revealed a significant (2.4 $\log_{10}$ CFU/g) reduction in caecal counts of the bacterium two days post-treatment. In contrast to the broad bactericidal effects of antibiotics on the gut microbiome at large, these researchers also determined that following phage administration with predation on *C. jejuni* in the broiler chickens, the microbiota of the chickens remained unaffected. This paper, in addition to highlighting the *Campylobacter* control problem, reassures the user of the specificity of phages for

their target bacteria [11]. Considerable commercial opportunities still exist for phage products for *Campylobacter* control. In the context of *Salmonella*, a study by Vaz *et al.* [12] determined the effect of the timing of therapy using a phage cocktail containing three lytic phages against *S.* Enteritidis in broilers. The birds were challenged with *S.* Enteritidis on the day of hatch. Following bacterial inoculation, they received both early (6–10 days) and late (31–35 days) phage treatments. The researchers found that while both *in vivo* trials displayed a significant reduction in intestinal *S.* Enteritidis counts when compared to the control group, they reported higher efficacy with the late phage application, where a 1.08 log CFU/g reduction (from a starting concentration of ~4.44 $\log_{10}$ CFU/g) was observed, and showed that multiple treatments could further enhance the overall ability of the cocktail to control intestinal *S.* Enteritidis colonisation in poultry [12]. Clavijo *et al.* [13] assessed the efficacy of SalmoFREE®, a recently patented phage preparation for use against *Salmonella*. The phage product was administered on three time points (day 18,27,35) during the production cycle of broiler chickens on a commercial farm via their drinking water. *Salmonella* detection by cloacal swabbing before and after phage treatment revealed that the phage product was successful at controlling *Salmonella*. Treated broiler houses dropped *Salmonella* counts to 0% on day 34 in comparison to the control broiler houses where the bacterium was still detected. In addition, Salmo FREE® had no negative effect on the birds themselves or any of the standardised production parameters used [13]. From the progress in the area, the use of phages in poultry shows considerable promise.

Cattle

Intralytics Inc., one of the top companies involved in the commercialisation of phage preparations for use against leading foodborne pathogens, including *Salmonella*, *E. coil*, *Listeria*, and *Shigella* species, recently commercialised Ecolicide PX^{TM} which targets and significantly reduces *E. coli* O157:H7 contamination on the hides of live animals [8••]. In addition to this, a more recent study by Tolen *et al.* [14] evaluated a different commercial prototype phage cocktail (from Passport Food Safety Solutions, Inc., USA) against *Escherichia coli* O157, and other (non O157) Shiga-Toxigenic *E. coli* on cattle hide and found that while the cocktail in general reduced *E. coli* counts by 0.4−0.7 $\log^{10}$ CFU/cm^2 on pieces of cattle hide, there was variation in susceptibility of the different *E. coli* serotypes to the specific phages in the mix used [14]. This suggests that while there is considerable merit in their recent study, further research is needed, for example to establish broader host-range phage mixes and effective application approaches for commercialisation. In a similar recent study in 2017, Arthur *et al.* [15] assessed the application of phages against *E. coli* O157:H7 on cattle hides and carcasses while in holding in the lairage at beef processing plants. In this study, cattle lots were administered the phage preparation via spray at the entrance to the holding area, in two separate processing plants. Hide and carcass samples were retrieved and tested for *E. coli* O157:H7 counts. Results in their case demonstrated a slight reduction in counts for both sample types when compared to the untreated controls and was judged to be not significant, showing that further research would be needed to optimise their approach [15]. These later reports re-emphasise the challenges in developing effective phage products for control of EHEC in cattle.

Slurry fertiliser

Grygorcewicz *et al.* [16] looked at the potential of phages to reduce the level of *Salmonella* present in pig slurry, a fertiliser whose wide-spread use on soil or pre-harvest food crops increases the transfer of harmful microorganisms. Phage sall_v01 was observed to reduce *Salmonella* Enteritidis counts by 3.8 log CFU/mL (from a starting concentration of ~5.55 Log CFU/mL) in the slurry and as such, the technology was judged by the authors to have potential to help reduce transmission of *Salmonella* in agriculture [16]. This is also backed up by older literature on the topic cited by these authors.

Phage biocontrol at the food processing stage

Increased consumer awareness of potential adverse effects of chemical food preservatives has resulted in preferences for naturally produced foods that are minimally processed, without chemical preservatives but that are still safe to eat. Satisfying these preferences does raise issues for shelf-life and safety. In this context, phages offer a natural means of selectively eliminating several dangerous bacterial pathogens and do not pose any risk to humans or animals. They are abundant in the environment, naturally found in food materials and water, and also make up a significant portion of the human microbiome [17]. Many phage preparations have gained regulatory acceptance, in both the US and EU, for use in controlling some of the leading bacterial pathogens in food products [4••]. In recent years, significant advances have been documented in the literature as reviewed very recently [8••] highlighting the applicability of phages for naturally improving food safety.

Phage biocontrol at the post-harvest stage of production

Foods are nutritionally rich environments that, depending on levels of preservatives employed, can facilitate the survival and growth of many bacterial pathogens. Intervention strategies using phages to control pathogens in post-harvest food materials have been documented by many [8••] and the literature shows that the use of carefully selected phages can reduce the presence of specific harmful pathogens.

Meat

Among the recent studies in meat systems, Vikram *et al.* [18•] performed a detailed examination of the Intralytix phage cocktail EcoShield PX™, specifically targeting Shiga toxin-producing *E. coli*. Their study demonstrated the efficacy of these phages at reducing the pathogen (at 3.0 log CFU/g) in eight different food products, including beef chuck roast, ground beef, chicken breast, cooked chicken, salmon, cheese, cantaloupe, and romaine lettuce. Significant reductions ($P < 0.05$) of *E. coli* O157:H7 were observed in 97% of foods tested when the phages were applied at 5×10^6 and 1×10^7 PFU/g. In commercially sold beef chunks where typical levels of *E. coli* (1–10 CFU/10 g) were encountered, a ≥80% reduction in counts of the pathogen were observed [18•].

In another study, Kim *et al.* [19] evaluated the efficacy of four *Salmonella* phages with activity against serovars Enteritidis, Typhimurium, Paratyphi A, San Diego, and Typhi in chicken breast meat. Following phage challenge experiments at 4°C, data revealed a range of CFU reduction figures in *Salmonella* counts ($P < 0.05$) during cold storage of the meat, but generally indicating good antibacterial potential for the phage employed [19]. In a similar study conducted at 8°C, Duc *et al.* [20] demonstrated the benefit of using a five-phage cocktail to control *Salmonella* on chicken breast samples in reducing both *S.* Enteritidis and *S.* Typhimurium on the breast meat, also observing a statistically significant reduction ($P < 0.05$) of viable counts by 1.41 and 1.86 log CFU/piece, respectively [20]. These results compare very well with those observed by the commercially available SalmoFresh™ (produced by Intralytix Inc.) where up to a 1.5 log reduction in *Salmonella* counts was observed following application to contaminated chicken breasts [8••,21]. Tomat *et al.* [22] conducted trials in beef meat, where they evaluated the efficacy of a phage cocktail against the clinically relevant Enteropathogenic and two Shiga-toxigenic *E. coli* strains and observed the positive impact of the phage cocktail at controlling these pathogens, and compared their data from similar challenges in broth and sterile milk. However, they did note that while results were good when experiments were performed at 24°C and 37°C, they were less so when performed at 4°C [22]. However, the commercially available EcoShield PX™ (produced by Intralytix Inc.) demonstrated superior results, where this preparation was capable of reducing *E. coli* O157:H7 levels by as much as 97% on a variety of food products [18•]. In another study, Zampara *et al.* [23•] assessed the efficacy of a two phage cocktail against *C. jejuni* in chicken meat at 5°C. They found that the phage preparation was capable of reducing *C. jejuni* by a 0.73 logs (from a starting of 10^4 CFU/mL) on the contaminated chicken skin and concluded that while sufficient proof of principle was obtained following execution of their experiments, a thorough understanding of phage–host interactions are a necessary prerequisite. Insights into the necessary interactions between phages and their host during refrigeration are important considerations for biocontrol strategies targeting *C. jejuni* [23•].

Fruit and vegetable foods

Liu *et al.* [24] evaluated the potential of an anti-*Salmonella* phage LSE7621 for biocontrol of the pathogen on lettuce and found that *Salmonella* counts were reduced by 0.86 $\log^{10}$ CFU/mL, at an MOI of 100, and 1.02 $\log^{10}$ CFU/mL, at an MOI of 1, within six hour. In addition, following similar challenge experiments in tofu (coagulated soy milk), 3.55 $\log^{10}$ CFU/mL (MOI = 100) and 1.86 $\log^{10}$ CFU/mL (MOI = 1) reductions in *Salmonella* counts within four hour were observed [24]. In a similar study conducted by Wong *et al.* [25], a five-component phage cocktail was used to control seven *S. enterica* strains from four different serovars, Enteritidis, Newport, Javiana, and Thompson, following inoculation onto romaine lettuce leaves and cantaloupe. The phage cocktail was applied to the food samples 24 hour before inoculating with the bacteria. The results varied considerably between the different *Salmonella* targets and essentially highlighted that while the phages had potential for *Salmonella* biocontrol, success was strain dependant [25].

Processed foods

Considerable successful phage work has been conducted over the years on the generation of specific FDA-approved phage products targeting various key pathogens as reviewed very recently [4••,7•,8••]. These include, *E. coli* O157:H7, *Salmonella* spp., *Shigella* spp., and *Listeria monocytogenes*. Some recent additional studies are mentioned below, which further validate the use of phages for food safety. Zhou *et al.* [26] isolated an anti-*Listeria* SH3-3 phage from a food processing plant and determined biocontrol efficacy against *L. monocytogenes* in both salmon and orange juice [26]. Thung *et al.* [27] demonstrated the biocontrol potential of an anti-*Salmonella* Enteritidis phage SE07 in different types of retail food, including fruit juice, fresh eggs, beef and chicken and found that following a 48 hour challenge at 4°C, a 2-log reduction of the bacterium was achieved in fruit juice and fresh eggs. Similar results were observed in the meat products [27]. All of the work mentioned above strongly support the continued development of broad-host-range phage products in the light of diverse potential applications.

Enzybiotics

Although the application of endolysins to control the growth of pathogenic microorganisms in food is a relatively new concept, it has gained increasing attention in recent years. The reader is directed to two excellent reviews on the topic which comprehensively discuss the many developments in the area [28••,29•]. Endolysins are phage-encoded enzymes that are produced at the end stage of their lytic life cycle. Their function is to cleave

Figure 2

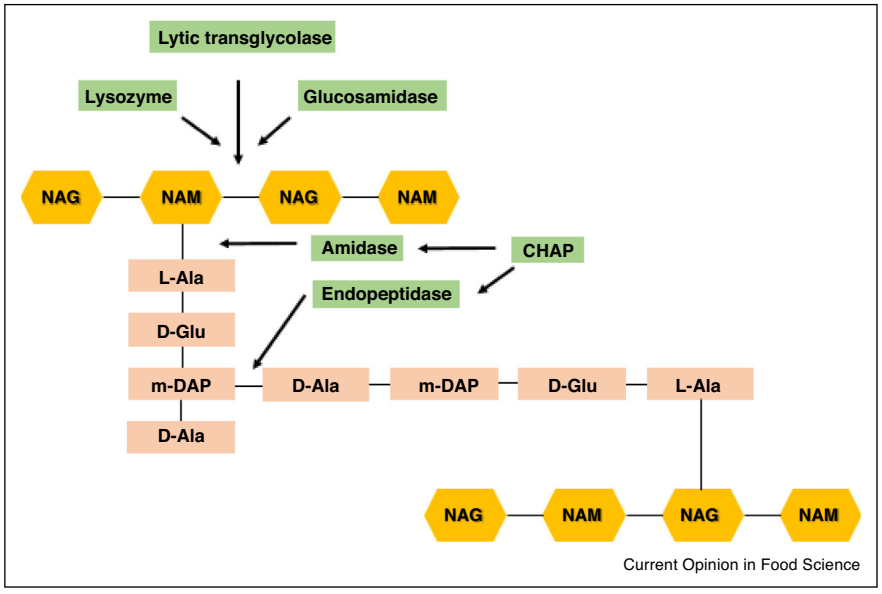


Diagram of different endolysin/ectolysin catalytic domain cleavage sites within the bacterial cell wall peptidoglycan. The peptidoglycan is composed of repeating reducing sugar units, *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) linked by β (1–4) glycosidic bonds, which are cross-linked via an interpeptide bridge between the meso-diaminopimelic acid (m-DAP) and D-Alanine (D-Ala) residues of adjacent tetrapeptide chains. The chains also contain L-Alanine (L-Ala) and D-glutamic acid (D-Glu). The different catalytic domains include glucosamidase, lytic transglycolase, lysozyme (targetting the bond between NAM and NAG), amidase (targetting the bond between m-DAP and NAM), endopeptidase (targetting the bond between m-DAP and L-Ala) and CHAP (can have the function of both amidase and endopeptidase).

the peptidoglycan of the bacterial cell wall, resulting in the cell bursting and subsequent release of progeny phages (Figure 2). As such, endolysins are particularly effective when used against Gram-positive pathogens due to the absence of an outer cell membrane thus allowing the endolysins direct access to the peptidoglycan for degradation (unlike Gram-negatives). Nevertheless, significant advances have also been made in the use of endolysins specific for Gram-negative pathogens (http://www.lysando.com), including *Campylobacter* spp., *Pseudomonas aeruginosa*, *Salmonella* spp., *Escherichia coli*, *Vibrio* spp., *Neisseria gonorrhoeae*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* [30]. Recent advances in the evaluation the bactericidal activity of endolysins have been demonstrated in several other food applications. Liu *et al.* [31] assessed the efficacy of the endolysins LysWL59, and LysLW60, to control *S.* Typhimurium on lettuce. The researchers found that both endolysins displayed broad lytic activity when used against chloroform-treated Gram-negative bacteria. However, LysWL59 demonstrated greater stability and thus was used in the food trial. When LysWL59 was used in combination with an outer membrane permeabilising agent EDTA (0.5 mmoL/L) to expose the peptidoglycan, it resulted in a 93.03% decrease of *S.* Typhimurium on lettuce. While these results are promising, methods to allow this enzyme to work without the application of the outer membrane destabiliser, would need to be explored [31]. In another study, van Nassau *et al.* [32] evaluated a combined control using endolysins and high pressure to inactivate *L. monocytogenes* in ready-to-eat food products, including soft cheese and smoked fish. Results demonstrated a 5.5 log CFU reduction, compared to 0.2–0.3 log reduction when either treatment was used individually. The authors concluded that the endolysins substantially increased the anti-bactericidal effect of high pressure, enabling inactivation of bacterial cells at much lower pressure levels [32]. In another combination treatment study, Chang *et al.* [33] evaluated the synergistic effect of LysSA97 and carvacrol oil for biocontrol of *Staphylococcus aureus* in foods, including milk and beef and similarly found that when the treatments were used in isolation, an average of 0.8 ± 0.2 and 1.0 ± 0.0 log CFU/mL of *S. aureus* was observed. However, when both compounds were applied together, an average of a 4.5 ± 0.2 log CFU/mL reduction in *S. aureus* in the foods tested was observed. The authors did note that the synergistic activity observed appeared to be influenced by the lipid content of the foods [33]. In addition to endolysins, other phage related enzymes associated with peptidoglycan degradation are also gaining attention in recent years. Virion-associated peptidoglycan hydrolases (VAPGHs) or ectolysins, are also becoming a new focus of attention, and their biocontrol potential has been recently demonstrated [34•].

Exploitation of phages for food pathogen detection

This article is primarily concerned with biocontrol, nevertheless another area where phages contribute is in pathogen detection in foods. This topic has seen pioneering work conducted by groups such as those of Martin Loessner in Zurich, and Catherine Rees in Nottingham. Developments in the field were comprehensively reviewed relatively recently [35••] and included pathogen detection systems-based on phage-mediated release of specific bacterial cytoplasmic markers such as adenylate kinase, ATP and β-D-galactosidase. Phage amplification assays are also discussed as developed to detect a variety of food-borne pathogens and in particular the slow-growing *Mycobacterium avium* using the FastPlaqueTB assay. These methods, whether enhanced by bioluminescence or immunomagnetic separation have had remarkable pathogen detection sensitivities. Genetically engineered reporter phages is also discussed, in particular the US-based company Sample6, which developed the Sample6 Bioillumination Platform™, which enabled highly sensitive (single cell) in-plant detection of foodborne pathogens without enrichment and generates results within a few hours. Reporter phages engineered to carry the luciferase gene for bacterial detection are particularly sensitive because of the bioluminescent signal they generate when transcribed by a bacterial target and at this stage, and such systems have been adapted for a wide range of bacterial targets. Other reporter phages have incorporated genes for green fluorescent protein or β-galactosidase allowing detection of colorimetric, fluorescent, or luminescent signals when the target bacterial pathogen is present in a food material. Another area that has seen considerable success is the use of labelled phage particles or phage components such as endolysin cell binding domains or receptor binding proteins of phages [35••]. In a later review by Foddai and Grant, some more recent phage-based detection methods are discussed although their specific application in food systems is not described [36•]. A recent review on engineered phage-based biosensors by Aliakbar-Ahovan *et al.* [37••] covers recent developments. One recent example by Wisuthiphaet *et al.* [38] used an engineered an phage T7-ALP expressing alkaline phosphatase to detect *E. coli* in beverage samples with a sensitivity of 100 CFU/g in six hour [38]. Another study by Zhang *et al.* [39] describes a reporter phage containing luciferase NanoLuc, which was able to detect *E. coli* 0157:H7, at a sensitivity of about 5 CFU/mL, in a food sample within nine hour. Another NanoLuc phage T7-based method described by Hinkley *et al.* [40] was designed for the detection of *E. coli* in water with a sensitivity of less than 10 CFU/mL. In conclusion, the area of pathogen detection using phages is technologically very diverse and has shown considerable promise in food safety.

Advantages and challenges of using phages

The fact that phages are currently being used as biocontrol agents in sectors of food production [4••,7•,8••] proves their merit as efficacious complimentary approaches for controlling specific harmful pathogens in food in many circumstances. The fact that they have GRAS status supports their safety for food applications, and indeed, there is no known negative side effect of using virulent phages towards humans or animals. Phages are natural, and low cost to produce [41••,42]. While an appropriate propagating host must be selected to ensure endotoxin and virulence factor contamination of the preparation does not occur, commercialisation timeframe is less stringent than what might be required for human therapeutic applications [43]. In addition, phages are highly specific for their target bacterial host and as such have no significant impact on consumers' resident microflora. Phages also do not impact the sensory and quality characteristics of food [44••]. Unlike chemical biocides or antibiotics that have the capacity to leach into food produce and persist in the environment, phages (albeit harmless anyway) persist in high numbers for a short time without their host [45]. Commercial phage products are 100% natural and non-GMO. They're generally Kosher, Halal and permitted in organic foods, with several officially certified as such [4••,8••,44••].

In general there are two principal technological challenges in phage-mediated biocontrol in food. Firstly, the components of the phage product must have a host range broad enough to kill all members within the target pathogenic genus or species. Secondly, the phages need to be applied such that the particles physically come in contact with all or most of the target bacterial cells in order to work. Additional considerations are the natural ability of bacteria to develop phage resistance following repeated exposure to phages, and the potential emergence of phage-unrelated members of a target bacterial genus/species. The use of phage products with multiple broad-host-range phage components addresses this issue along with our ability to update and/or replace phage components over time if necessary, in response to the evolving epidemiology of food pathogens. It is also important that users of phage products in the food sector understand that individual products do not ensure full safety of foods if the foods are contaminated by a different foodborne pathogen (e.g. a pathogen not targeted by the phage product applied to the food).

Concluding remarks

Bacteriophages represent a class of natural antibacterial agents that over the years have demonstrated considerable promise for their biocontrol properties against several leading and emerging pathogens in food. The commercial successes of phage products for pathogen biocontrol in the food industry is relatively recent, with the first phage-based product (ListShield™) gaining regulatory approval

for use to control *L. monocytogenes* in meat and poultry products in 2006. Since then, many phage companies have received FDA approval for their food safety products including FINK TEC GmbH, Intralytix, Micreos Food Safety, Passport Food Safety Solutions and Phagelux [9••] and it is certain that many more will follow. This short review has highlighted recent advances in the area and it is evident from the literature that important research work is ongoing, some of which highlights the challenge in developing reliable commercial phage products for food applications. The body of knowledge accumulated to date has led to an improved understanding of phage biology, phage–host interactions, and their technical limitations, thus contributing to a better understanding of the specific considerations that need to be taken into account when utilising phages for biocontrol of pathogenic bacteria in food to ensure appropriate application for maximum effect.

Conflict of interest statement

The authors declare that there is no conflict of interest in the writing of this review.

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- •• of outstanding interest

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