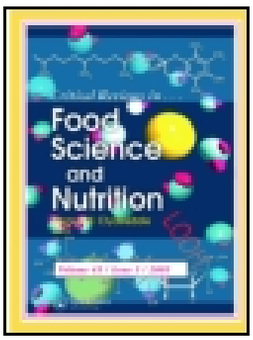

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Use of Encapsulated Bacteriophages to Enhance Farm to Fork Food Safety

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Abstract:

Bacteriophages have been successfully applied to control the growth of pathogens in foods and to reduce the colonisation and shedding of pathogens by food animals. They are set to play a dominant role in food safety in the future. However, many food processing operations and the microenvironments in food animals' guts inactivate phages and reduce their infectivity. Encapsulation technologies have been used successfully to protect phages against extreme environments and have been shown to preserve their activity and enable their release in targeted

environments. A number of encapsulation technologies have shown potential for use with bacteriophages. This review discusses the current state of knowledge about the use of encapsulation technologies with bacteriophages to control pathogens in foods and food animals.

Keywords Bacteriophages, microencapsulation, food safety, phage therapy, pathogen control

INTRODUCTION

Bacteriophages

Bacteriophages (phages) are natural predators of bacteria and are viruses that can only infect bacteria. In terms of size, a bacteriophage is approximately 50 times smaller than a bacterial cell (20–200 nm). They are ubiquitous in the environment (soil and water) and in a number of food products (Rohwer and Edwards, 2002; Kutter and Sulakvelidze, 2005). They are predominantly present in oceans and counts range from 10^7 to 10^8 phage particles per millilitre in coastal seas and non-polluted water. The estimated phage population in the world is around 10^{31} particles, which makes them the most abundant living objects on our planet (Rohwer, 2003). In other words, there are ten phage particles for each bacterial cell on earth. Bacteriophages are specific to one or a limited number of bacterial groups. Normally they are named after the bacterial group, strain, or species that they can kill. For example, the phages that infect the bacterium *Escherichia coli* are called coliphages. Specific phage examples are presented in Table 1. Bacteriophage applications have been demonstrated in health therapy, the biocontrol of pathogens in foods, the sanitation of processing plants and hygiene in hospitals.

Bacteriophage therapy – an alternative to antibiotics

Bacteriophage therapy offers a viable alternative to conventional antibiotic treatments for bacterial infections (Górski et al., 2003; Reardon, 2014). Therefore, advocates of phage therapy use strong arguments for it as an alternative to antibiotic use in agriculture. Recently, many multi-resistant bacterial strains have emerged to a range of antibiotics. In reality, antibiotic

resistant pathogens are posing a threat to public health worldwide. For example, antibiotic-resistant infection-related hospitalisations increased approximately 2.5-fold in the USA from 1997 to 2006 (Mainous et al., 2011). The situation is now becoming alarming and the scientific community is trying to solve this issue. Phage therapy has several advantages over antibiotic use, and one of the key features of phages is their specificity, as the selected phages do not harm the useful bacteria that live in and on the human body. In contrast, antibiotics also attack harmless bacteria and the normal microflora of living organisms. Furthermore, phages also kill bacteria that are resistant to antibiotics. A study conducted in 1983 involved several hundred patients with suppurative bacterial infections, and the majority of infections were drug-resistant; this study showed that phage therapy achieved a 92.4% overall success rate (Slopek et al., 1987). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prevalent pathogens and causes diseases in humans and farm animals (Adegoke and Okoh, 2014; Cuny and Witte, 2013). Several reports suggested that phage therapy can be an effective way to reduce intestinal colonisation by resistant pathogens (including MRSA) in food animals (Alisky et al., 1998; Sulakvelidze and Barrow, 2005). Phage therapy is a potential means to control MRSA as an alternative to antibiotics.

Potential applications and related issues in improving food safety

In food animal production, the occurrence of multi-drug resistant strains of foodborne pathogens has become a major problem due to the use of antibiotics as growth promoters (Sulakvelidze et al., 2001; Boerlin, 2010; CDC, 2013). The EU has banned the prophylactic use of antibiotics for

animal production, and the rest of the world is under intense pressure to take serious measures to find alternatives to the use of antibiotics (USFDA, 2006; Cogliani et al., 2011). This means there is a need and increased interest in developing alternatives to the conventional antibiotics used in food animal production. Bacteriophage therapy offers a possible alternative (Atterbury et al., 2007; Goodridge, 2010), and several phage strains, such as phage K, phage LS2a, phage MSa and phage øMR11 have been tested for the prevention and/or treatment of animal infections caused by MRSA (O'Flaherty et al., 2005; Wills et al., 2005; Capparelli et al., 2007). Therefore, there is a huge potential to exploit phage therapy to control intestinal bacterial pathogens in animals.

Encapsulation may improve phage efficacy and broaden its application

The demonstrated role of bacteriophages to control foodborne pathogens in food products shows a viable role for them in food safety applications. However, food processing conditions, such as the use of high temperatures or antimicrobial agents, could limit phage activity and/or performance. Encapsulation is an excellent technique to provide phages with much needed protection against such environmental factors.

This article will review the development of the application of phage technology in phage therapy and food safety enhancement and also discuss the future development of encapsulation techniques.

APPLICATIONS OF BACTERIOPHAGES IN IMPROVING FOOD SAFETY

Phages possess many unique characteristics that make them ideal candidates to improve safety throughout the food supply chain. Table 2 describes the functions of phages relevant to potential applications in food safety from the farm to the fork.

Animal production to promote growth

Phage therapy is the therapeutic use of bacteriophages to treat pathogenic bacterial infections in humans or animals. It has several potential applications in human medicine as well as in dentistry, veterinary science and agriculture (McAuliffe et al., 2007). Phage therapy can be used as a means of reducing intestinal colonisation by resistant pathogens using an oral delivery method (Sulakvelidze and Barrow, 2005). One of the major applications of phage therapy is its use in animals intended for consumption that act as reservoirs of pathogens.

Phage therapy has been recognised as a powerful technology holding tremendous potential to combat increasingly dangerous bacterial infections (Keen, 2012). Researchers are optimistic about the future of this technology and continue to explore the science behind phage therapy. Phage therapy has been demonstrated as extremely effective at treating a number of bacterial infections in controlled animal studies (Smith et al., 1983; Biswas et al., 2002; Hawkins et al., 2010). Bacteriophage Felix O1, which showed a broad host range within the genus *Salmonella* was, therefore, considered as an excellent candidate to treat *Salmonella* infections in animals (Whichard et al., 2003). A study in which three to four week-old pigs were inoculated with *Salmonella* bacteria, and then immediately administered the anti-salmonella phage cocktail,

showed a 99.0 to 99.9% reduction in *Salmonella* colonisation in the tonsils and parts of the small and large intestines (Wall et al., 2010). Loc Carrillo et al. (2007) showed that phage treatment of *C. jejuni*-colonized broiler chickens reduced *Campylobacter* counts by between 0.5 and 5 $\log_{10}$ CFU/g of caecal contents, compared to the untreated controls, within five days of administration. However, these reductions were found to be dependent on the phage-*Campylobacter* combination, the dose of phage applied and the time elapsed after their administration. Several other studies have also shown the potential of phages to reduce *Campylobacter* in the broiler guts and on chicken skins (Carvalho et al., 2010; Kittler et al., 2013; Hammerl et al., 2014). It is important to note that phage survival may be affected by various environmental factors in animal digestive systems (Smith et al., 1987). When orally administered, the viability of phages may decline rapidly due to the acidic conditions and the presence of bile and digestive enzymes (Chibani-Chennoufi et al., 2004; Joerger, 2002). The use of encapsulated phages offers an improvement in their ability to infect bacterial cells in the animal gastrointestinal (GI) tract (Ma et al., 2008; Wang and Sabour, 2010). Applications of encapsulation in phage therapy are discussed in a later section.

Reducing pathogen carriage before slaughter

Bacteriophages are also effective in reducing bacterial loads in food materials (such as carcasses and other raw products) and on equipment and contact surfaces. They have the ability to act as natural preservatives in foods to extend their shelf life. Several reports demonstrated the use of phages against zoonotic pathogens in live animals and on food surfaces to improve food safety of

products (Barrow et al., 1998; Hudson et al., 2010; Smith and Huggins, 1983). In brief, bacteriophage technology can be used as a food safety tool at different stages of food production, i.e. at the animal production stage, to reduce the introduction and spread of pathogens into distribution food chains, or in the final product to minimise contamination.

It is reasonable to assume that reducing the carriage of pathogens in food animals would help to minimise the likelihood of bacterial contamination in the food supply chain. Bacteriophages can be used to eradicate undesirable and pathogenic microorganisms during food production and throughout the supply chain. In this way, bacteriophages are increasingly becoming a useful extension of the instruments that food companies deploy to control food safety issues and, ultimately, phages are expected to become an industry standard (Warmerdam, 2006). The application of phage therapy has been described as a successful approach for reducing microbial loads to control spoilage bacteria and human pathogens during the postharvest storage of foods and supply under a variety of environmental conditions (Greer, 2005). Some of the examples available in the literature showed pre-slaughter phage treatment of food animals reduced salmonellosis in chickens, enteropathogenic *E. coli* infections in calves, piglets, and lambs, and *E. coli* O157:H7 shedding by beef cattle. Further research activities are in progress to develop this technology to produce foods with low microbial loads; thus, helping to improve food safety.

Fresh produce sprays

Eating fresh or uncooked contaminated food causes a large number of foodborne illness cases around the world each year. One common way to solve this issue was to wash foods with

solutions of various antibacterial chemicals; however, the extensive use of chemical sanitisers has led to various resistances in bacteria and also adversely affected the environment. With the advantages of being natural predators of bacteria and having effective bactericidal activity against specific bacteria strains, the inactivation of foodborne pathogens through spraying or inoculating free phages into foods has been attracting increasing interest (Abuladze et al., 2008; Bigot et al., 2011). Several published reports have demonstrated the effectiveness of phages in controlling: *Salmonella* in ready-to-eat (RTE) foods (Guenther et al., 2012), cheddar cheese (Modi et al., 2001), sprout seeds (Pao et al., 2004), chicken frankfurters (Whichard et al., 2003); *L. monocytogenes* in RTE foods (Bigot et al., 2011; Guenther et al., 2009); and *E. coli* O157:H7 in tomatoes, spinach, broccoli, and ground beef (Abuladze et al., 2008). These examples clearly indicate the potential of bacteriophage applications to enhance the food safety of fresh produce.

Food processing and storage

Bacterial contamination during processing or storage was considered a major cause of food poisoning outbreaks worldwide. The application of bacteriophages is a potential alternative to the preservatives and toxic chemicals used in the food processing industry. Compared to classical antimicrobial agents, phages are very specific to the bacterial cells of the host. Two of the most commonly discussed advantages are that phages will not harm the normal microbiology of a food system and that there will be no disturbance of the commensal gut flora of the consumer (Ly-Chatain, 2014). Therefore, their existence in food products is not considered a concern to human health, in contrast to conventional antibiotics and antimicrobials. Moreover, phages only

replicate as long as the targeted bacterium is present and so are naturally self-limiting (Ceyssens and Lavigne, 2010; Connerton and Connerton, 2005).

The use of bacteriophages as bio-control agents is, currently, considered to be one of the best options to control pathogens in food. Applications of bacteriophage technology have been shown to extend the storage life and/or improve the safety of fruits, dairy products, chicken and red meats. Some specific examples of meat products safety improvement are given in Table 3. Guenther et al. (2009) showed that virulent broad-host-range phages, such as A511 and P100, were effective for the specific biocontrol of *L. monocytogenes* in contamination-sensitive RTE foods. The authors used a comprehensive set of experiments to evaluate the control of *L. monocytogenes* strains Scott A (serovar 4b) and WSLC 1001 (serovar 1/2a) in different RTE foods, which commonly carried the pathogen. It was found that *L. monocytogenes* counts rapidly dropped below the level of direct detection in liquid foods (chocolate milk and mozzarella cheese brine) and reduced by up to 5 $\log_{10}$ CFU units on solid foods (hot dogs, sliced turkey meat, smoked salmon, seafood, sliced cabbage and lettuce leaves). Several other authors also showed the efficacy of bacteriophages in biocontrol of pathogens in food products (Gill et al., 2010).

Detection of foodborne pathogens

Bacteriophages also possessed certain characteristics that can be used in the microbiological testing of the pathogens in food samples. Bacteriophages have high target cell specificity, inherent signal-amplifying properties, have easy and inexpensive production, and are robust

(Schmelcher and Loessner, 2014), which make them ideal tools for diagnostic assays. The lytic multiplication cycle of a phage particle (from the initial recognition of the host cell to the final lysis event) can be used in several ways for the purpose of bacterial detection. Moreover, phage-derived affinity molecules, such as cell wall binding domains and receptor binding proteins, can also be useful in developing detection assays.

ENCAPSULATION TECHNOLOGY TO ENHANCE PHAGE EFFICACY

It has been clearly demonstrated that bacteriophages would be very useful to improve food safety from farm to fork, either through phage therapy or the control of pathogens in foods. However, there are challenges to the use of bacteriophages; for example, inactivation by environmental factors is one of the technological barriers at present. Bacteriophages are known to be sensitive to low pH and the bile salt they commonly face in the GI tract of animals during phage therapy applications. Low or high temperatures or exposure to chemical agents in food processing could compromise the performance of the phages against the target pathogen. Bacteriophages need to be protected against potential harsh conditions for their successful applications to improve food safety. Encapsulated phage particles have better tolerance to environmental stress conditions. Therefore, encapsulation technology has a key role to play in order to exploit the potential of bacteriophages in food safety as a management approach in the years ahead.

Encapsulation was a technology of packaging solids, liquids, or gaseous materials in small particles that release their contents at controlled rates and under specific conditions.

Encapsulation technologies have been developed mostly for the target delivery of bioactive components (including microbial cells or particles) into the human body to protect the sensitive encapsulated substances from inactivation in the stomach and release them through the various sections of the intestines. Microencapsulation of bacteriophages is a relatively new area of research. There are only a limited number of reports available in the literature on this topic.

Figure 1 shows how microencapsulation could help to protect microbial cells from different kinds of life threatening conditions during their application. We should note that these mechanisms of protections through encapsulation are not well-documented and the limited research based on evidence that is available to date. In addition, protection mechanisms for phages are yet to be validated and explained properly.

Encapsulation of phages for use in animal production

Encapsulation of phages has been successfully shown to assist in achieving the objective of phage therapy; for example oral delivery, avoiding gastric acid and enzymatic deactivation. The technologies used nowadays to encapsulate phages mainly consisted of two categories: encapsulate in alginate-based beads for oral delivery; and immobilise in membrane matrices or electrospun fibres for food packaging. A brief description of microencapsulation techniques for the encapsulation of phages is given in Table 4.

Alginate is a naturally-derived polysaccharide extracted from various species of algae and is composed of β-D-mannuronic and α-L-guluronic acids (Burgain et al., 2011). The gelation of the alginate polymers is triggered by cross-linking between the carboxylate anions of guluronic acid and calcium ions. This mild gel forming characteristic has made it the most widely used material for the encapsulation of molecules and cells over many years. However, due to its inferior

physical properties, the alginate hydrogel was unstable in the presence of non-gelling cations, such as sodium and magnesium ions. Hence, one popular way to overcome this defect was to incorporate or coat the alginate hydrogels with various polymers, such as chitosan, whey protein, cellulose acetate phthalate, xanthan gum, starch, and gelatin, to enhance the strength of the gel structure. This has already been widely used to protect probiotic bacteria during *in vitro* simulated gastrointestinal digestion (Anal and Singh, 2007). Similar to the probiotic cells, phages are also very sensitive to low pH conditions and need to be protected by encapsulation in order to achieve the desirable therapeutic effect.

To the best of our knowledge, the first study to encapsulate phages in alginate based beads was reported in our laboratory by Ma et al. (2008) who encapsulated phage Felix O1 into chitosan coated alginate beads to protect the phages during *in vitro* digestion. However, only partial protection of bacteriophages against gastric acidity was provided when tested in simulated gastric fluid (SGF) at pH 2.0 and the release time of the encapsulated phages into simulated intestinal fluid (SIF) at pH 6.8 was a little slow , at approximately 5 h for the complete release. With the addition of $CaCO_3$ into the alginate network, the acid resistance of the encapsulated phages to SGF was enhanced, however, no improvement in release rate was observed when presented in SIF (Ma et al., 2012). The incorporation of $CaCO_3$ would provide a buffering effect against hydrogen ions penetrating to the microcapsule core and the newly solubilised calcium ions may also interact with the carboxyl group on the alginate molecule, which further enhanced the Ca-alginate gel networks in the SGF. However, when the incubation medium changed from SGF to SIF, the free Ca^{2+} dissociated from $CaCO_3$ still exist within the gel network and would

thus inhibit the swelling of the alginate gel, resulting in a slow release rate. *In vivo* tests on one to three week old chicks revealed that the encapsulated phage in the alginate-$CaCO_3$ microcapsules could not completely be released during the transition through the GI tract (manuscript in preparation) and a large proportion of live phages within the alginate matrix were detected in the faeces. For the purpose of solving this issue, incorporating whey protein, a by-product of cheese making available at low cost, with alginate gel to encapsulate phage Felix O,1 was developed (Tang et al., 2013). As whey protein hydrogel swelled readily at pH above its isoelectric point (pI~5.1) and can be degraded by pancreatic enzymes present in SIF, the addition of whey protein would markedly accelerate the release of encapsulated phage into SIF. Meanwhile, as the denatured whey protein has abundant exposed hydrophobic surfaces that would retard direct contact of acid to phages, the survival of the encapsulated phages in SGF was well maintained at pH 2.0 for 2 h. Whey protein-alginate microcapsules also provided good protective and release properties to phage K, which suggested that the formula of incorporating whey protein into alginate matrix would be a potential candidate for the oral delivery of other similar phages (Tang et al., 2015). A recent animal trial has demonstrated that phages encapsulated in alginate-whey protein microspheres were more effective in reducing *Salmonella* counts in the intestines of young chickens compared to un-encapsulated phages.

Other phage microencapsulation technologies, using methoxylated pectin (Dini et al., 2012) or methacrylate polymer (Eudragit® S100) (Stanford, et al., 2010), have also been developed and showed potential to protect phage from *in vitro* digestion. Pectin is an inexpensive, non-toxic polysaccharide extracted from plant cell walls. Unlike the gel forming character of alginate,

methoxylated pectins are able to form rigid gels by the action of multivalent cations crosslinking with the galacturonic acid moieties. Due to the hydrophobic motifs present in the methoxylated pectins, the diffusion rate of H^+ in aqueous environments penetrating through the physical barriers of polymer matrix was retarded compared to that of the alginate network which has a hydrophilic matrix and, thus, this resulted in a better protection effect when present in acid conditions (Dini et al., 2012). Eudragit is a commercial product that has been widely used in the pharmaceutical industry. Its unique property that it only dissolves at a specific pH and makes the desired drug release material to release the drug at the right site, at the right time or over a desired period of time. Through using Eudragit as the matrix material, spray drying technology can be employed to produce phage microcapsules (Stanford et al., 2010). With the advantages of a continuous and rapid process with a low cost and high reproducibility, spray drying is already widely used in the microencapsulation area and is suitable for large-scale industrial production. Encapsulation of phages using spray drying would greatly increase the efficiency and reduce the cost of the process and make it possible for large scale industrial production. However, exposure to heat during spray drying has been shown to reduce phage activity. Researchers in our group and others are actively seeking solutions to improve the survival of phages after spray drying. Further work with encapsulated phages through spray drying may be of great interest and formulas that are suitable to be incorporated should be developed.

Encapsulation for reducing contamination during processing, packaging and storage

Major benefits to the use encapsulated phages include prevention from harsh environmental conditions, such as thermal damage and dehydration during food processing and storage. Using phages to control spoilage bacteria and pathogens in foods has received increasing interest during the last few years and some successful studies have been reported. Typically, phages are simply sprayed on to the surface of the food. For example, phages have been used to control *Salmonella* infections in cheddar cheese (Modi et al., 2001), turkey deli meat (Guenther et al., 2012), chicken frankfurters (Whichard et al., 2003), chicken skin (Pao et al., 2004), swine (Wall et al., 2010) and *Listeria monocytogenes* growth in ready-to-eat chicken breast rolls (Bigot et al., 2011). However, these methods may not be ideal, as they could be wasteful and lead to potential inactivation of the phage particles as a consequence of the inclusion of other materials within the wash fluid, such as residues of sanitizers used to clean the surfaces of processing areas. Moreover, if the phage-containing fluids themselves contained nutrients that supported bacterial growth, then the potential for the bacterial evolution for phage resistance exists (Anany et al., 2011). In order to expand the use of phages to control harmful bacteria and pathogens in foods, there is a need to encapsulate phages for improved stability and release in foods for delivery. However, so far, few studies have been reported that apply encapsulated phages to prevent foods from spoilage by bacteria and pathogens (Table 5).

Bacteriophages can be coated on the packaging material or directly sprayed onto the food surface or incorporated into the food matrix. The phages encapsulated in edible film formulations showed stability for more than five weeks under ambient conditions. This encapsulation approach allowed a burst release pattern over three to five hours in water. Release measurements also showed that encapsulated phages could be released upon contact with food material and

could maintain their activity for an extended period of time (The Free Library, 2014). An immobilisation method of phages through the electrostatic interaction between the phage and cellulose membrane was reported by Anany et al. (2011). As the phage head exhibited an overall net negative charge and the tails carried a net positive charge, it would be likely to attach to positively charged modified cellulose membrane surfaces and, hence, leave the tails free to capture and lyse bacteria. Cocktails of phages active against *Listeria* or *Escherichia coli* immobilised on these membranes were shown to effectively control the growth of *L. monocytogenes* and *E. coli* O157:H7 in ready-to-eat and raw meat, respectively, under different storage temperatures and packaging conditions (Anany et al., 2011). Similarly, encapsulation of phages was also achieved in whey protein films as pathogen-specific antimicrobial food packaging materials (Vonasek et al., 2014). With excellent mechanical properties and oxygen barrier properties (Janjarasskul and Krochta, 2010). this technique has already been used to incorporate diverse antimicrobial agents (Pintado et al., 2010), whey protein film is an ideal edible food packaging material. An antimicrobial study showed that incorporating phage T4 into whey protein films resulted in an approximate 2 log CFU decrease of *E. coli* BL21 from the initial inoculation level while the negative control showed an increase of 3 $\log_{10}$ CFU/g, which meant a total 5 $\log_{10}$ CFU/g reduction in microbial count with respect to the negative control (Vonasek et al., 2014). These results demonstrated that with the use of phages immobilised on whey protein film, the growth rate of microbes can be reduced and the level of microbes can be effectively controlled below the initial inoculum level. Recently, phages have also been encapsulated into poly (ethylene oxide)/cellulose diacetate fibres using electrospinning technology (Korehei and Kadla, 2014). Electrospun nanofibrous mats have large surface areas,

with good dimensional stability and are relatively easy to produce, so electrospun biopolymer fibres are considered as a reliable carrier/delivery matrix for many bioactive agents (Andrady, 2008; Sorrentino et al., 2007). Through using the blends of hydrophilic/hydrophobic poly(ethylene oxide) (PEO)/cellulose diacetate (CDA) at the same ratio as the encapsulating material, the desired release rate can be achieved in storage media buffer (pH 7.5) (Korehei and Kadla, 2014). This controlled release property of the PEO-CDA electrospun fibres makes it a potential food packaging material in the control of food pathogens.

However, to expand the use of encapsulated phage into food products, there is still a need to develop new novel encapsulation strategies to improve stability and release for delivery in different food systems. For example, in the majority of previous studies, the phage cocktail was typically sprayed on the surface of a meat product to prevent the growth of pathogens. This method of application did not guarantee uniform phage distribution and restricted the access of the phage to *L. monocytogenes*, and negatively affected the appearance of the product. It is, therefore, required to develop an alternative method of anti-*Listeria* phage application, such as including it in the raw meat before stuffing it into fibrous casings and exposing it to heat (cooking) or high pressure during the preparation; however, phages are sensitive to heat and high pressure. So, without protection, phages may not survive the cooking or high pressures. Microencapsulation, hence, has shown potential to protect the phage from damage caused by various food processing procedures. It was expected that bacteriophages can be similarly protected with appropriate encapsulation formulation. The challenge was that the formulation should not only protect the encapsulated bacteriophage, but also release the bacteriophage to the

meat matrix when bacteria were present. Thus, further study on the formulation of the phage microcapsules should be undertaken to protect the phage from the harsh conditions that applied during meat processing.

Encapsulation of phages for sanitation and pathogen detection

Another emerging application of bacteriophage technology was the use of phages as active agents in hygiene and sanitation products. This is a key element of food safety management that ensures high level hygiene and sanitation in processing facilities. Discussions about bacteriophage-based antibacterial solutions give us new directions to design innovative hygiene and sanitation products for industrial applications (food and meat processing plants, hospital sanitation, etc). A T7-like phage, phage ϕIBB-PF7A, was found to be a potential candidate as a sanitation agent for controlling the prevalence of spoilage caused by *Pseudomonas fluorescens* strains in dairy and food-related environments (Sillankorva et al., 2008). Lack of evidence-based research is a major limitation at present. Microencapsulation technology could be a viable approach to develop stable and effective products with controlled release and improved infectivity of antimicrobial products containing phages.

Future applications of encapsulated phages in agriculture and food

Applications of encapsulated bacteriophages in agricultural and food are severely limited due to the lack of technological developments. Bacteriophages are highly susceptible to denaturation and degradation under several environmental conditions; therefore effective methods are urgently needed for bacteriophage delivery through encapsulation onto diverse surfaces and in

the food matrix to maintain their stability and infectivity. Encapsulated bacteriophages could be successfully employed in many important areas in food safety:

- Fresh produce and surface disinfection
- Hygiene products
- Pathogen detection methods
- Biosensor integrating phages with biomaterials
- Biocontrol materials that facilitate pathogen dispersal in particular settings

CONCLUSIONS

Bacteriophage technology could improve food safety through reducing infections in farm animals and minimising microbial loads in the food supply chain (phage therapy), biocontrol of foodborne pathogens in food products or act as sanitising agents. Encapsulation could improve the efficacy of bacteriophages by providing them protection against harsh environmental conditions, i.e. acidity and the high bile contents in the GI tract, or heat or chemical agents, during the manufacture of processed food products. Both emerging technologies have huge potential to grow in parallel to improve food safety in the near future. Much research is needed to develop encapsulation formulations targeting for diversified applications along the food supply chain.

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Table 1. Common bacteriophages and phage preparation against major pathogenic bacteria

	Target bacteria	**Specificity/Features**	**Application**	**Reference**
Phage K	*Staph. aurues*	A polyvalent phage that is effective against human and veterinary strains	Phage therapy Bio-control	Tang et al., 2015
Felix O1	*Salmonella*	A *Salmonella*-specific bacteriophage	Phage therapy Bio-control Detection method Animal production and processing	Ma et al., 2008
SP6	*Salmonella*	-	Administered to chicken to combat salmonelosis	Hurley et al., 2008
A511	*L.*	A dsDNA virus. It	Bio-control	Guenther et

	monocytogenes	consists of a head and a tail separated by a neck		al., 2009
LMP-102	*L. monocytogenes*	First regulated as food additive	Bio-control	Bren, 2007
Cj6	*Campylobacter*	Poor host range	Food decontamination	Wong et al., 2004
29C	*Campylobacter*	-	Infected chicken skin	Goode et al., 2003
e11/2	*E. coli* O157:H7	-	Meat surfaces	O'Flynn et al., 2004
ECP-100	*E. coli* O157:H7	-	Hard surface foods	Abuladze et al., 2008
ϕIBB-PF7A	*P. fluorescens*	Fast and efficient in lysing	Sanitation in dairy and food related environments	Sillankorva et al., 2008
LISTEX™	*L. monocytogenes*	Anti-listeria phage preparation	Bio-control Bio-preservation	-

ListShield™	*L. monocytogenes*	Effective under a wide range of food storage conditions (2°C-42°C)	Contamination in foods and food processing facilities.	-

Table 2. Properties of phages relevant to food safety applications in supply chain

Application	**Functions**	**Advantages**
Farms	Reduction of foodborne pathogen on farm settings Decrease in pathogenic levels in farm animals Reduction of pathogen colonization on foods Reduction of biofilm formation Enhance preservation quality Increase product quality Effective against antibiotic-resistant bacteria	High specificity to target Self-replication and self-limiting Relatively cheap and easy to isolate and propagate
Distribution and Transportation	Disinfection of food contact surfaces Potential bio-sanitation agents for equipment	Low toxicity
Processing, Preservation and	Disinfection of food processing surfaces	Low inherent toxicity

Storage	Enhance antimicrobial activity of food package. Limit growth of spoilage bacteria and extend shelf-life of manufactured foods	Ability to tolerate environmental stresses Excellent bio-preservation agents
Detection of pathogens	Specifically detect target cells Distinguish between living and dead cells	Inherent signal-amplifying properties Easy and inexpensive production Robustness

Table 3. Examples of meat products safety improvement through phage technology

Product	**Phage type**	**Pathogen reduction/shelf life increase**	**References**
Chicken frankfurters	Felix O1	*Sal. enterica* serovar *Typhimurium* DT104/ Suppression levels of 1.8 to 2.1 log units were achieved.	Whichard et al., 2003
Beef steaks	Phage C35	*Pseudomonas* spp. (spoilage control)/ 1- to 2 log reduction in the level of bacterial contamination	Greer, 1986
Beef steaks	Phages e11/2 and e4/1c	*E. coli* O157:H7/ 5log reduction of pathogen numbers in 1 h at 37°C	O'Flynn et al., 2004
Vacuum-packed beef	Listeriophage LH7	*L. monocytogenes/* reduced bacterial cells in combination with nisin	Dykes and Moorhead, 2002
Ready-to-eat foods	Phages A511 and P100	*L. monocytogenes/* reduced bacterial counts by up to 5 log units.	Guenther et al., 2009
Cooked and raw meat	Phage P7 and Phage cj6	*Sal. typhimurium* PT160 and *C. jejuni/* reduction of 2–3 $\log_{10}$ cm^{-2} at 5 °C	Bigwood et al., 2008

		and >5.9 $\log_{10}$ cm^{-2} at 24 °C	
Different foods including chicken breast and chicken mince	Phage P22	*Salmonella enterica* serovar *Typhimurium/* reduction of 2-3 log cycles after 48h at 4°C	Zinno et al., 2014

Table 4. Improvement in animal phage therapy through encapsulation technology

Encapsulation technique	**Material**	**Outcome/impact**	**References**
Microencapsulation through extrusion method (Felix O1)	Chitosan-Alginate microspheres	Encapsulated phage Felix O1had 2.58 log units decrease at pH 2.4 after 2 h. Free phages not detectable after 5 min exposure to pH 3.7 or less. Improvement in delivery technology of bacteriophages in oral therapeutic applications.	Ma et al., 2008
Microencapsulation through spray drying (Phage rV5, wV7, wV8, wv11)	Methacrylate polymer (Eudragit S100) microspheres	Encapsulated phage remained viable after exposed to pH 3.0 for 20 min and released well after adjusting to pH 7.2. However, no significant effect was found to reduce the shedding of *E. coli* O157:H7 in fecal grab and hide swab samples with the feeding of encapsulated phages. Oral delivery of phages in cattle inoculated with *E. coli* O157:H7.	Stanford et al., 2010

Microencapsulation (Phage K)	Calcium carbonate-incorporated alginate microspheres	Encapsulated phage K had 0.17 log units reduction at pH 2.5 after 2 h. Free phage K lost viability after exposure to pH 2.5 for 2.5 min. Improved efficacy of encapsulated phage K in animal gut applications.	Ma et al., 2012
Microencapsulation through extrusion method (Phage CA933P)	Alginate or pectin microspheres with or without coating of pectin or guar gum	Emulsified pectin beads showed the highest encapsulation efficiency and highest survival of phage CA933P at pH 1.6 after 30 min. Free phage lost viability after exposure to pH 2.5 for 10 min in the presence of 0.5 mg mL^{-1} pepsin. Oral delivery of phages in bovine intestine.	Dini et al., 2012
Microencapsulation through extrusion method (Felix O1)	Alginate-whey protein microspheres	Encapsulated Felix O1remained viable in simulated gastric fluid (pH 2.5) after 2 h incubation. Free phages were inactivated within minutes. Oral applications of phages in farm animals such as chicken.	Tang et al., 2013
Microencapsulatio	Alginate-whey	Encapsulated phage had less than 1%	Tang et al.,

n through extrusion-calcium induced gelation method (Phage K)	protein microspheres with maltose protectant	loss on wet microsphere basis. Viable numbers of phage K remained unchanged in simulated gastric fluid (pH 2.5) after 2 h incubation. Free phages became inactivated within a minute. Alginate-whey protein microspheres of phage K a potential oral delivery technology.	2015

Table 5. Examples of encapsulated phages applications in food products to control pathogens

Encapsulation technique	**Material**	**Outcome/impact**	**References**
Microencapsulation through electrostatic adsorption method (Phage T4)	Modified cellulose membrane	95 % of phages were captured in modified membranes. The number of inoculated *E. coli* O157:H7 on raw beef was reduced below the detection limit (10 CFU/mL) with the phage immobilized membrane after 12 days under aerobic condition at 4 ℃. Application of immobilized phage to control food borne pathogens on meat surfaces.	Anany et al., 2011
Microencapsulation through film forming method (Phage T4)	Whey protein film	With the application of phage encapsulated whey protein film, a total of 5 log CFU reduction in microbial count of *E. coli* BL 21 was presented compared with that in negative control sample. Application of encapsulated phages in pathogen specific	Vonasek et al., 2014

		antimicrobial packaging materials.	
Microencapsulation through electrospun method (Phage T4)	Electrospun poly(ethylene oxide)/cellulose diacetate fiber	Phage T4 was completely encapsulated during the process. The encapsulated phages were controlled released during 60 min in storage media buffer (pH 7.5) by blending poly(ethylene oxide) and cellulose diacetate. Application of encapsulated phages in food packaging material.	Korehei et al., 2014

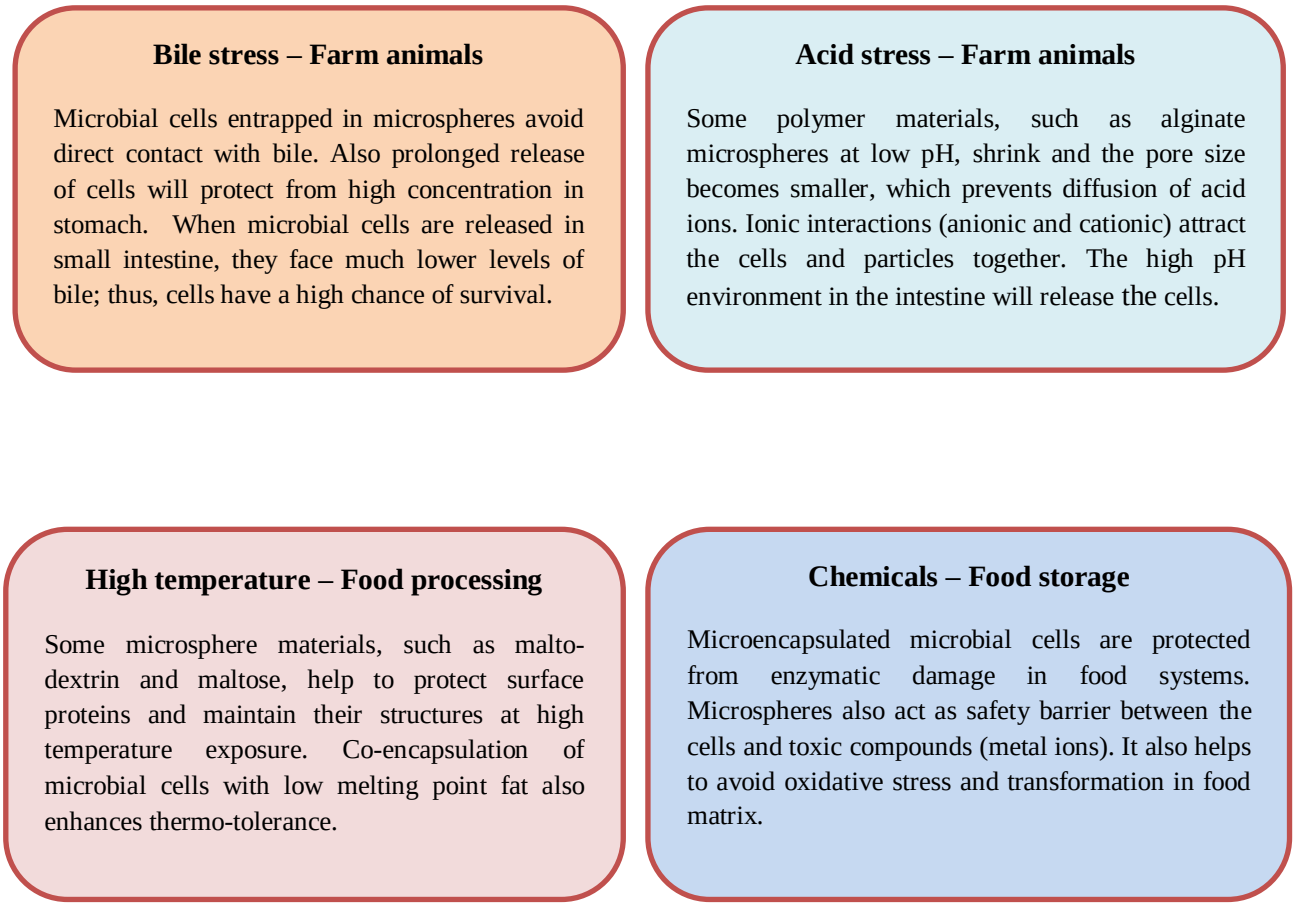

Figure 1. Ways encapsulation offers protection to microorganisms against different stress conditions. Bile and acid stresses are important for phage therapy (applications in animal farming) while high temperature and chemicals agents are relevant to food processing and storage applications.

