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Bacteriophages as biocontrol agents for foodborne diseases

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Abstract
Bacteriophages (phages) discovered during the early 20th century can find a novel use as an effective approach for the control of foodborne bacterial pathogens. Phages are natural enemies of bacteria, infect and multiply within their specific host bacterial species and are stable under varied pH and temperature conditions. Various types of phage treatments have been used to reduce contamination of foods with foodborne bacterial pathogens, facilitating pre-harvest and post-harvest control of foodborne pathogens and also the decontamination of inanimate surfaces in food-processing facilities and other food establishments. Several companies have developed phage based products for their applications in food safety that have been approved by FDA, United States Environmental Protection Agency (EPA) and other such agencies, for their usage in food/feed additives. Phage therapy in biocontrol of food borne pathogens is proving to be beneficial for food safety and public health. However, further studies are required to explore the emergence of phage resistant mutants, concentration and mode of phage application to pave way for phage biocontrol protocols to become an integral part of routine food safety intervention strategies that food industries can follow.

Keywords: Bacteriophage, biocontrol, pathogens, bacteria, foodborne

1. Introduction
Food borne diseases are of major concern globally, with two-third of outbreaks because of bacterial origin [1, 2]. The World Health Organization (WHO) estimates that diarrhoeal diseases alone (majority of which are caused by food borne pathogens) are responsible for around 1.9 million children deaths every year globally. Considerable efforts have been directed towards the control of major bacterial food-borne pathogens but there has been a continuous increase in several food borne diseases caused by these pathogens viz., *Salmonella*, *Campylobacter*, *Escherichia coli*, *Listeria* etc. These pathogens find their way into food during harvest/slaughtering, processing, storage and packaging. Many technologies have been employed to combat bacteria, many a times at the cost of food quality, for example heat treatments are associated with deterioration of organoleptic properties, extensive use of sanitizers have led to the development of resistant bacteria, chemical preservatives have negative effect not only on sensory parameters but also on health as many of them are carcinogenic. The consumers prefer organic food which is free from all sorts of chemicals and addition of these prevents the temptation to buy these products. The dilemma is that in absence of a quality food control, there would be a continuous rise in the number of food borne diseases and increasing loss in food production. Hence, there is a need for new strategies that fulfill consumer demand and ensure food safety. One such promising approach is the use of a natural antagonist towards bacteria to control bacterial contamination in foods by way of a process called as biocontrol which may tackle the drawbacks of current processing and preservation technologies and is likely to be acceptable to consumers.
One of the recent upcoming methods of biocontrol is the use of Bacteriophages (phages) as an effective approach for the control of foodborne bacterial pathogens. These phages are natural enemies of bacteria which are commonly found in wastewater and soil [3, 4]. The bacteriophages were discovered by Frederick Twort and Felixd' Herelle during the early 20th century [5]. These are viruses that infect and multiply within their specific host bacterial species and are stable under varied pH and temperature conditions [6]. Phages are the most ubiquitous known organisms on earth and play a key role in maintaining microbial balance in every ecosystem wherever bacteria exist. Further, they are highly specific for their host cell as their

adsorption and entry are mediated by specific receptors present on host cells (e.g., carbohydrates, proteins, lipo-polysaccharides etc.).

2. Life cycle of phages

Phages exhibit one of the two types of life cycle, lytic and lysogenic [7]. The lytic phages cause rapid lysis and death of the host bacterial cell, whereas temperate or lysogenic phages spend part of their life cycle in a quiescent state called prophage. The concept of fighting bacterial pathogens in foods using specific lytic phages is known from many years [8]. The phages are also part of the normal microflora of all fresh and unprocessed foods. The principle in the use of lytic phages is that they specifically adsorb to their host bacterium, multiply and cause its lysis, thus preventing spoilage of food by micro-organisms. Recently, the applications of phages in food safety are gaining success for several reasons, the most important of which is increased customer and regulatory pressures to ensure food safety while reducing the use of harsh chemical sanitizers and disinfectants besides these methods providing a safe, environment friendly and effective approach for significantly reducing contamination of various foods with bacterial pathogens borne by various foods [9, 10, 4].

3. Methods to control foodborne pathogens

There are three main types of phage treatments used to reduce contamination of foods with foodborne bacterial pathogens:

- Pre-harvest control of foodborne pathogens in food producing livestock and poultry
- Decontamination of inanimate surfaces in food-processing facilities and other food establishments
- Post-harvest control of foodborne pathogens by direct applications of phages onto the harvested/processed foods

3.1 Pre-harvest control of food borne pathogens in food producing livestock and poultry

Phage therapy has an effective pre-harvest application in controlling foodborne pathogens of animals and birds [11, 12]. This approach is based on the principle that phages may be used to prevent and/or significantly reduce colonization of pathogenic bacteria in livestock animals and birds. In other words, the phages are administered in order to reduce shedding of specific foodborne bacterial pathogens and thus reduce the risk of subsequent contamination of food products containing the animals' or birds' products

3.1.1 Control of food-borne pathogens in animals: Phage based interventions have been aimed at controlling *E. coli* serotype O157:H7 in cattle and other ruminants whose gut contents and fecal material on hide may contaminate meat during slaughter [13]. This pathogen causes several food borne disease manifestations including diarrhea, hemorrhagic colitis, haemolytic uremic syndrome and thrombotic thrombocytopenic purpura[14]. Animals that shed high levels of this pathogen may pose great risk of contamination to the food chain if presented for slaughter. Therefore, phage-based approaches to reduce fecal shedding of this pathogen have been designed to limit both the duration of shedding and concentration of *E. coli* O157 in the bovine gastrointestinal tract.

Salmonella sp. is also one of the principal causes of food-borne diseases causing diarrhoea worldwide and is commonly found in chickens, turkey, pigs etc. Wall *et al.* (2010) [15] investigated the efficacy of phage mixture in reducing *S. typhimurium* colonization of pigs during their transportation and prior to being slaughtered. The researchers administered phages orally and in microencapsulated form that led to efficient reduction in *Salmonella typhimurium* contamination of ileum, cecum and tonsils of pigs. Also, the researchers reported that the microencapsulated phages were more efficient than the orally administered phage mixture.

Listeria monocytogenes is also one the important food-borne pathogen responsible for causing the deadly disease listeriosis. It is an opportunistic pathogen and is commonly associated with fresh and ready to eat products contaminated via infected humans, equipment or factory environment.

3.1.2 Control of food-borne pathogens in poultry: The phage therapy applications to control foodborne pathogens in live animals have also been conducted in poultry, since they and egg products are important sources of the human pathogens. Poultry has been recognized as a major reservoir of two most important food borne pathogens, *Salmonella* sp. and *Campylobacter* spp [16].

Fiorentin *et al.* (2005) [17] isolated a cocktail of phages from free range chickens and used them to reduce the concentration of *Salmonella enterica* serovar *enteritidis* Phage Type 4 (PT4) in the ceca of broilers. Five days post phage treatment, the concentration of *S. enteritidis* PT4 per gram of cecal content in the phage-treated group was reduced by 3.5 log units and samples collected up to 25 days after treatment revealed that the treated birds still had lower Colony Forming Units (CFU) of *S. enteritidis* PT4 per gram of cecal content compared with untreated broilers.

Borie *et al*. (2008) [18] administered a mixture of three phages to chickens 24 h post-infection with *Salmonella enteritidis* by aerosol spray or oral administration (in drinking water). The results indicated that the phage therapy was effective in reducing *S. enteritidis* in chickens over a period of 20 days.

Lim *et al.* (2012) [12] measured the effectiveness of phages to control *S. enteritidis* infection in 1 day old chicks. The study reported that Bacteriophage ΦCJ07 in 10^9 PFU dosage as a feed additive significantly lowers (70%) bacterial counts in cecal tissue and proposed their usage in control of *Salmonella* infections in poultry and the incidence of food poisoning cases caused by this bacteria.

Hungaro *et al*. 2013[19] suggested that, bacteriophages can be applied as an aerosol spray for reducing the horizontal transfer of *Salmonella* sp. in poultry during transportation of eggs from incubators to hatchers.

Loc Carrillo *et al.* (2005)[20] studied the effect of phage administration in controlling *Campylobacter* sp. infection of broiler chickens and observed an efficient reduction in bacteria in cecal contents of phage treated birds. A similar study was conducted by Wagenaar *et al*. (2005) [21] for controlling *C. jejuni* colonization in broiler chickens using single phage and phage mixtures. The experiments using phage mixture provided a notable decrease of *Campylobacter* levels in the caeca of the treated chickens compared to the single phage treated chickens.

Carvalho *et al*. (2010) [22] tested a phage cocktail consisting of three phages for the control of *C. coli* and *C. jejuni* through two routes of phage administration (i.e., by oral lavage and in feed). The phage cocktail was able to reduce the titre of both *C. coli* and *C. jejuni* in faeces by approximately 2 $\log_{10}$ CFU/g when administered by oral lavage and in feed. This reduction persisted throughout the experimental period and neither pathogen regained their former numbers. Further, it was observed that administration of phage in feed to *Campylobacter* infected chicks leads to an earlier and more sustainable reduction of *Campylobacter* compared to the oral lavage.

Since the above studies indicate the possibility of using phages to control bacterial colonization in cattle and poultry, more studies need to be conducted to determine adequate phage dose, number of doses (a single dose vs. continuous dosing), standardized methods of phage delivery (water or feed delivery vs. rectal delivery) and the economics of phage therapy in food producing animals and birds.

3.2 Decontamination of inanimate surfaces in food-processing facilities and other food establishments

The second approach (also called 'Phage bio-sanitation') involves using phages to improve food safety by decontaminating various inanimate surfaces in household kitchens, food processing facilities and other food establishments, so that the foods contacting those surfaces are less likely to become contaminated with food borne bacterial pathogens [23]. Although most of the food borne bacterial pathogens are inactivated during cooking, some of them may survive on the surfaces on which the foods were processed before cooking and other foods which are ready-to eat may come in contact with those surfaces, get contaminated and lead to food borne diseases[24,25]. Similarly, food borne bacteria may persist on various surfaces in food-processing facilities and contaminate foods that are being processed or packaged in those facilities. Use of bacteriophages can be a natural and non-toxic alternative to eliminate or reduce levels of food borne bacterial pathogens on various hard surfaces commonly used in food-processing facilities and in kitchens. In food industry, biofilms are found on the surfaces of equipment used, for example, in the food handling, storage, or processing, especially on the surfaces that are not easy to clean or to sanitize. Roy *et al.* (1993) [26] studied the effectiveness of different phages to remove *Listeria* from stainless steel and polypropylene surfaces. They found that phage treatment alone was able to achieve approximately a decrease in cell number. In another study Montanez-Izquierdo *et al.* (2012) [27] evaluated *Listeria* phage P100 to control biofilm formation by *L. monocytogenes* on stainless steel surfaces and found a mean reduction of 5.29 log CFU/cm^2. Apart from controlling *Listeria* biofilms, *Campylobacter* biofilms were successfully removed from the surface of glass [28] and growth of *E. coli* O157:H7 was controlled using phage mixture BEC8 on stainless steel and ceramic tiles [29]. Use of phage for bio sanitation is promising although very challenging due to the diversity of bacteria found in different environments [30].

3.3 Post-harvest control of foodborne pathogens by direct application of phages onto the harvested/processed foods

The outbreaks of food borne illness associated with the consumption of raw and processed foods emphasize the need for an effective natural antimicrobial agent which can be directly applied to the food to kill pathogenic micro-organisms. US Food and Drug Administration (FDA) has mentioned a few bacterial pathogens viz., *Staphylococcus aureus, Shigella* sp., *E. coli, Salmonella* sp. and *Listeria monocytogenes* that can be found in fruits, vegetables, fresh cut products that make salads, dairy and meat products. The bacteriophages are naturally present in all fresh and non-processed foods, including fresh ground beef, fresh fruits and vegetables, raw skim milk, cheese and frozen mixed vegetables and thus, is a natural way of preventing bacterial contamination in foods [29, 31]. The approach of using phages involves directly applying them to the food surfaces to eradicate or significantly reduce the number of specifically targeted food borne bacterial pathogens contaminating the foods. Alternatively, the concept of using phage biocontrol protocols involving the direct application of phages to various foods is essentially based on using a microorganism that may already be present in those foods and simply applying the appropriate number of appropriate phages at appropriate location. Thus, if a food is contaminated with a bacterial pathogen that is the host for the lytic phages applied to the food's surface, the phages should eliminate or significantly reduce the contamination, thereby making the food safe for consumption without deleterious effects on its normal, beneficial microflora and organoleptic qualities.

3.3.1 Application of phage lysin in food

Lysins are enzymes produced by lytic phages, which play role in the degradation of the bacterial cell wall through targeting its various peptidoglycan bonds to allow the newly formed progeny phages to be released from the host cell [32]. Since lysin enzymes attacks the cell wall peptidoglycan, they are highly effective against Gram-positive bacteria when added externally and may be used as biocontrol agents to enhance food safety [33]. Lysins generally have a narrow spectrum activity restricted to its host species [34]. Lysin can be added as a purified protein directly to food or feed.

4. Commercially available phage products

Several companies have developed phage based products for their applications in food safety. Intralytix Inc., a USA based company has developed three phage based products viz., ListShieldTM, EcoShieldTM and SalmoFreshTM that has been approved by FDA for their usage in food additives[35]. Besides this, they have two phage preparations for veterinary applications: PLSV-1TM and INT-401TM. Further, ListShieldTM has been approved by United States Environmental Protection Agency (EPA) for its usage in reducing *L. monocytogenes* contamination of inanimate surfaces in food-processing facilities. Also, SalmoFreshTM which is specific against *Salmonella enterica* has been given a GRAS (Generally Regarded as Safe) designation in 2013.

5. Conclusion

The possibility of using phage therapy in biocontrol of food borne pathogens is highly beneficial for food safety and public health. However, more rigorous studies are required concerning the emergence of phage resistant mutants, concentration and mode of phage application, depending on the types of foods used so that phage biocontrol protocols can become an integral part of routine food safety intervention strategies implemented by food industries.

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