



Application of Bacteriophages in Food Industry: A Review

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Abstract

Every year microbial origin foodborne illnesses put an enormous effect on human health and food processing industry. Thus, public health and economic incentives are required to develop novel approaches for managing microbial contamination in foods. Bacteriophages are one of the several antimicrobial due to possessing potent and highly specific antibacterial activity., phages may provide an all-natural, non-toxic, and effective means for reducing or eliminating bacterial pathogens. Bacteriophages used for direct food applications have already been approved. This review has focused on the application of bacteriophages at various stages from farm to fork, all-inclusive areas such as primary production on the farm, bio-sanitation, food spoilage, processing, and packaging. Use of bacteriophages represents an emerging “Green” technology that can help to improve food safety and enhance the economy of the country, India.

Keywords: Antimicrobials, Bacteriophage, Food Contamination, Food Safety, Phages



Introduction

Globally foodborne illnesses are considered a human health problem as they encompass a wide spectrum of illnesses worldwide (Firestone *et al.*, 2020). Maximum (30%) death from foodborne illnesses is among under 5-year age children (WHO, 2020; CDC, 2020). After the implementation of GMP and HACCP practices, there are 48 million, 1.28 lakhs, and 3000 people get sick, hospitalized, and die every year respectively (CDC, 2020). The microbial contamination is putting an enormous financial burden on human health and the food processing industry, costing the global economy a huge amount (Endersen *et al.*, 2014). Foodborne illness estimated to cost more than \$15.6 billion each year (CDC, 2020).

In India, bacteria rank second (mycotoxins being first) concerning a number of foodborne illnesses cases among total infected persons during 1980-2009. These are expected to rise from 100 million in 2011 to 150-177 million in 2030 (Whitworth, 2018). Further, foodborne illness cost India about \$28 billion or ~0.5% of GDP every year (World Bank, 2018). The analysis shows that investment in food safety can bring a very good impact on the Indian economy. Considering the available facts and figures, the government must continue with the food safety drive across the nation with its various initiatives and makes India a safer place in terms of food safety and hygiene.

The emergence of multi-drug resistant (MDR) pathogens has increased the pressure on health authorities. Based on analysis one can tell that the use of antibiotics in food animals, is considered as the main source of bacterial resistance. India is the world's largest consumer of antibiotics (Kumar *et al.*, 2020). Effective control measures to reduce food-borne outbreaks and stop the spread of resistant bacteria are needed. In food market globalization many effective novel technologies have come into role but still, food safety issues are continuously challenging areas (Endersen *et al.*, 2014). The lytic bacteriophages are great alternatives for different kinds of antibiotics to combat bacterial infections. Among the many alternative medicine strategy, bacteriophages are strong candidates to act against the many foodborne pathogens. Bacteriophages are emerging as a “Green” technology to deal with the issues in food industries (Sulakvelidze, 2016). The present review focused on the application of bacteriophages in the food industry and how to combat economic losses due to foodborne illness in India.

What are Bacteriophages?

These are viruses of prokaryotes widespread in all habitats where their hosts are located (Gutierrez *et al.*, 2016). Bacteriophages are classified based on their shape, size, and kind of nucleic acids. Bacteriophages belong to order *Caudovirales* (tailed- bacteriophages) (Ackermann and Prangishvili, 2012) and considered as a ubiquitous organism present in natural and man-made environment (de Melo *et al.*, 2018). Phages are having strong antimicrobial properties along with this they are safer and more specific and effective against MDR bacteria as compare to conventional agents (Fernandez *et al.*, 2018).

Bacteriophages have two types of life cycles i.e. lytic and lysogenic life cycle (Sulakvelidze and Kutter, 2004). In Lysogenic cycle, phage genome is entered into the host genome and undergoes replication along with host chromosomes and in lytic cycle phages lyse the host bacteria and thus, its antimicrobial activities are linked to lytic phage (Gutierrez *et al.*, 2016). Due to the effective results, this technique is rapidly used in several fields such as agriculture, veterinary medicine, food safety, and wastewater treatment (Jassim *et al.*, 2016; Carvalho *et al.*, 2017). Along with the antimicrobial effect against pathogenic bacteria, phages can provide an effective alternative to traditional disinfectants (Hernandez *et al.*, 2011). Due to surface decontamination property, phages can also be applied as food bio preservatives. Thus, phage preparations against *Listeria monocytogenes* (Listshield TM), *Salmonella enterica* (SalmFresh TM), and *Escherichia coli* (Ecoshield TM) have received Generally Recognized as Safe (GRAS) designation by the FDA for direct application to food and are commercially available.

Applications of Bacteriophages in the Food Industry

1. Control of Zoonotic Bacteria in Primary Production

It will be more effective to kill the bacteria in the primary production system like on-farm. Farm animals are seemed to be reservoirs for many pathogenic bacterial such as *Listeria monocytogenes*, *Campylobacter jejuni*, *Escherichia coli* and *Salmonella enterica spp.* and they are mainly responsible for most of the human infection. The bacteriophages can be used to kill or reduce bacterial infection on-farm itself. Furthermore, several studies have

evaluated the efficacy of phages in animals to control zoonotic bacteria which increases the threat for public health (Richards, 2014; Carvalho *et al.*, 2017; Doss *et al.*, 2017; Wernicki *et al.*, 2017). Despite the promising result of phages against the bacteria's in farm animals, there are some drawbacks like phages can get inactivated in the gut and its removal from circulatory system following oral or parental route. To overcome these drawbacks several researchers are working on the phage encapsulation to overcome acidic conditions. Samtlebe *et al.* (2016) reported improved survival of phages in acidic conditions through encapsulation. *Lactococcus* phage P008 was encapsulated in three different matrix materials like alginate, alginate-whey-protein, and gelled milk protein and investigated its *in vitro* stability at acidic pH 2.0 for different time period (Samtlebe *et al.*, 2016). Sillankorva *et al.* (2012) evaluated the cocktail of two O157- specific lytic phages KH1 and SH1 against *E. coli* O157:H7 in mouse model and reported effectiveness in reducing the intestinal *E. coli* load. Similarly, the *in vitro* effect of microencapsulated bacteriophages Felix O1 with alginate/CaCO₃ capsules against *Salmonella spp.* was investigated and observed microencapsulation facilitated the delivery of phages (Ma *et al.*, 2008; Polaska and Sokolowska, 2019).

2. Phages as a Biocontrol Agent on Farm Level

In farm animals, phages could be used as biocontrol agents to reduce the economic burden caused by bacterial diseases (Abedon *et al.*, 2017, Vincent *et al.*, 2017). Many phages cocktails are nowadays under trial in animal models to prevent the infection (Cooper, 2016, Buttmer *et al.*, 2017). As a biocontrol agent, phages have a broad host range and can sustain in adverse environmental conditions. Use of phages to control foodborne pathogens in swine is also reported. Goodridge and Bisha (2011) examined a single *Salmonella* specific phage Felix 01 in three-week-old pigs orally and intramuscularly against *Salmonella* Typhimurium and reported a significant reduction in the amount of *S. Typhimurium* in tonsil and cecum contents from sacrificed pigs on comparing with control treatment. Similarly, several studies through which pathogenic bacteria were bio controlled using phages have been proven in the primary production of cattle and sheep. The fecal concentration of *E. coli* was effectively reduced through phage DC22 evaluated against *E. coli* O157:H7 in experimentally diseased sheep (Bach *et al.*, 2003). For reference several studies are enlisted in the Table 1.

Table 1: Bacteriophages and their applications against different pathogens

S. No.	Area of Application	Name of Phages	Pathogen	References
1	Control of zoonotic bacteria in Primary Production	Felix O1	<i>Salmonella spp.</i>	Ma <i>et al.</i> , 2008
		Cocktail phages KH1 and SH1 against	<i>E. coli</i> O157:H7	Sillankorva <i>et al.</i> , 2012
		<i>Lactococcus</i> phage P008	Human gut microbiota	Samtlebe <i>et al.</i> , 2016
		Felix O1 with alginate/CaCO ₃ capsules	<i>Salmonella spp.</i>	Polaska and Sokolowska, 2019
2	Phages as a biocontrol agent on farm level	Phage DC22	<i>E. coli</i> O157:H7	Bach <i>et al.</i> , 2003
		Cocktail of Phages	<i>E. coli</i> O157:H7	Callaway <i>et al.</i> , 2003
		Felix 01	<i>S. Typhimurium</i>	Goodridge and Bisha, 2011
3	Decontamination of surface sanitization	Phage Guard Listex	<i>L. monocytogenes</i>	Hernandez <i>et al.</i> , 2011
		Phage Guard S	<i>Salmonella enterica spp.</i>	Hernandez <i>et al.</i> , 2011
4	Food processing and packaging	Listex P100	<i>L. monocytogenes</i>	Iacumin <i>et al.</i> , 2016
		Georgian bacteriophage cocktails	MDR <i>E. coli</i>	Gundogdu <i>et al.</i> , 2016
		Phage mix derived from family <i>Myoviridae</i> , <i>Siphoviridae</i> , <i>Podoviridae</i>	<i>Staphylococcus aureus</i>	Elhaddad <i>et al.</i> , 2016
		Bacteriophages S16 and Felix-O1a (FO1a)	<i>Salmonella spp.</i>	Yeh <i>et al.</i> , 2017
		ShigaShield™	<i>Shigella sonnei</i>	Soffer <i>et al.</i> , 2017
5	Food spoilage and bio sanitization	Polyvalent phage isolated from a potato	<i>Streptomyces-</i>	McKenna <i>et al.</i> , 2001
		Bacteriophages	<i>Xanthomonas campestris</i> pv. <i>Vesicatoria</i>	Obradovic <i>et al.</i> , 2004
6	Bacteriophages against bacterial biofilms	Phage KH1	<i>E. coli</i> O157:H7	Sharma <i>et al.</i> , 2005
		Phage CP8 and CP30	<i>C. jejuni</i>	Siringan <i>et al.</i> , 2011
		Phage P100	<i>L. monocytogenes</i>	Montañez-Izquierdo <i>et al.</i> , 2012
		Phage K	<i>S. aureus</i>	Alves <i>et al.</i> , 2014

3. Decontamination of Surface Sanitization

Surface bacteria like *Pseudomonas spp.*, *Enterobacteriaceae* and *Acinetobacter spp* are few of the important sources of food contamination. Surface bacteria like *Stenotrophomonas maltophilia* have been reported as a major source

of antimicrobial resistance emergence (Hernandez *et al.*, 2011). Phages can be used as an attractive alternative to traditional disinfectants. Several authorities have quoted that bacteriophages are harmless but it is not clear whether they can protect against food re-contamination (Fernandez *et al.*, 2018). Indeed, at present two such products against *L. monocytogenes* (PhageGuardListex) and *Salmonella enterica* spp. (PhageGuard S) are available.

4. Food Processing and Packaging

Most of the bacterial contamination can be identified at the food product processing and packaging time (Moye *et al.*, 2018). This contamination can be reduced through treatment with commercial phage cocktails (de Melo *et al.*, 2018). Many studies to investigate the use of bacteriophage to kill the pathogenic bacteria present on food products like *L. monocytogenes* (Iacumin *et al.*, 2016), *Escherichia coli* (Gundogdu *et al.*, 2016), *Salmonella* spp. (Yeh *et al.*, 2017), *Shigella sonnei* (Soffer *et al.*, 2017) and *Staphylococcus aureus* (Elhaddad *et al.*, 2016), etc. are reported. Use of phage endolysins and depolymerases to reduce number of bacterial pathogens has also been reported (Pires *et al.*, 2016). Addition of these enzymes can prevent contamination while processing and packaging of food.

5. Food Spoilage and Bio Sanitation

Preservation of food has always been an essential part of food production. Bacteriophages have been suggested as natural food preservatives (Bai *et al.*, 2016). The fruits and vegetables are perishable foods and get spoiled easily by many of the spoilage bacteria. The phage can prevent fruit spoilage and result in an increased amount of income in fruit market. Several examples where food spoilage was successfully controlled through use of phages like tomato bacterial spot caused by *Xanthomonas campestris* pv. *Vesicatoria* (Obradovic *et al.*, 2004), *Streptomyces*-infected seed potato tubers were assessed by McKenna *et al.* (2001). Above examples is evidence that bacteriophages have emerged as promising way for bio sanitization in the food industry.

6. Bacteriophages Against Bacterial Biofilms

Nowadays, biofilm production is a major concern in food industries which causing most of the troubles while handling foods (Myszka and Czaczyk, 2011). The complex community of bacteria attached to a surface and surrounded an extracellular polymeric material. Which are resistant to most of cleaning and disinfecting agents making them most difficult to remove from surfaces. Because of this resistance, it is having high impact on human health. To overcome this, one has to promote the development of different approaches to control or remove biofilm formation. Phages effectively eliminating biofilm formation in food industry have also been reported (Gutierrez *et al.*, 2016). Few phages evaluated against various biofilm produce by pathogenic bacteria are enlisted in Table 1. The phage proteins have created an entirely new aspect of biofilm biocontrol and are worthy of more extensive research (Kumar *et al.*, 2020).

Precautions to be Taken in Usages of Bacteriophages in Food Industry

However, there are certain disadvantages of bacteriophages application-

1. Natural resistance can be developed in pathogenic bacteria against bacteriophages.
2. These can act as a vector for horizontal gene transfer, may transfer virulence and antibiotic resistance genes (ARGs) among bacteria, as well as allergy-causing proteins.
3. There are many safety issues during its use as a therapeutic agent *viz.*, poor pharmacokinetics, that is, poor absorption, distribution, and survival in situ.

Conclusion

Taken together, we conclude that bacteriophages can be considered as an emerging strategy to tackle the increased foodborne illnesses which are causing huge economic losses to the food industry. These phages offer compelling advantages over more conventional agents including specificity, safety, and effectiveness against MDR bacteria. The lytic bacteriophages are great alternatives for different kinds of antibiotics to combat bacterial infections. The application of bacteriophages can be encouraged to enhance food safety and hygiene, ultimately developing the economy of the country.

Conflict of Interests

There is no conflict of interest.

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