

Bacteriophage: Biocontrol agents for food borne diseases

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ARTICLE DETAILS

Article History

Published Online: 10 October 2018

Keywords

Bacteriophage, food borne pathogen, phage therapy, bio-control agents

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ABSTRACT

Bacteriophages are the viruses that infect the bacterial cells and play an important role in shaping and maintaining the microbial diversity on planet earth. The ability of phages to infect a specific type of host has opened a new paradigm for exploring their applications in various areas including food, medicine, agriculture etc. Food borne pathogens is a global threat for the food industry and also for the human health. A large number of disease outbreaks all over the world are related to food contamination and various international agencies has published large amount of data showing the risk posed by food borne pathogens to humans. Despite a number of advances in preservation techniques, health risks related to food contamination by pathogenic microorganisms is still a big issue. Therefore, designing an innovative and biological method is required to control food borne pathogens including pathogenic bacteria. Phage therapy is one such method to detect and control food borne pathogens which offers several advantages over other methods including a specific targeting of the microorganism and their use in single or as cocktail against several pathogenic microorganisms such as *E. coli* O157:H7, *Salmonella* sp., *Campylobacter jejuni*, *Listeria monocytogenes*. Several studies have been reported suggesting the use of phages as innovative biocontrol agents where phages can either directly be used in the food product or their purified endolysin is applied to control food borne pathogens.

1. Introduction

Food borne pathogens and related outbreaks has become a major concern for the food industries in the modern world. With increasing number of outbreaks related to food borne illness a huge challenge is lying in front of the food industries to develop or modify the methods for food preservation so that a good quality food can be provided to the consumer. Annually about 9.4 million cases related to food borne illness have been reported in the United States of America (Scallan et al., 2011). In addition to food borne illness due to contamination of food with pathogens a huge amount of food material is also lost causing a big impact on the economy of the state or country (Sarhan and Azzazy., 2015).

According to CDC, major food borne pathogens contaminating the food materials worldwide include *Listeria monocytogenes*, *Campylobacter*, *Salmonella* spp, *Clostridium perfringens*, *Shigella* spp. *Streptococcus* spp. (Scallan et al., 2011).

Several preservation techniques are being used by the industry to preserve food products and control the growth of pathogenic microorganisms so that a safe and healthy food can be provided to the consumer. Despite technological advances and a number of efforts by government agencies for spreading awareness related to food borne pathogens and food safety, contamination of food products with pathogenic organisms and outbreaks related to food borne illness is still a matter of great concern. For control of food borne pathogens a novel, effective and safer method has to be developed. One such novel method is the use of bacteriophages to control food borne pathogens and prevent contamination of food products and reduce the number of outbreaks related to food borne illness. Bacteriophages are highly target specific in their mode of action and robust in nature.

1.1 Bacteriophages and their general characteristics

Bacteriophages are the viruses that infect a specific type of bacterial cell. They show great diversity and abundance in the biological world (Penades et al., 2015). Majorly distinguished on the basis of the life cycle they follow for their replication and propagation after they infect their specific bacterial cell. Bacteriophages use bacterial cellular machinery and can adopt either of the two types of life cycle:

- Lytic cycle
- Lysogenic cycle

1.1.1 Lytic cycle: It involves the infection of bacterial cell and resulting in its complete lysis releasing a large number of virus particles for further infection of other cells. Bacteriophages attaches to the target bacterial cell through specific cell surface receptors present on bacterial cell. Generally, bacteriophages use lipopolysaccharides present on the cell wall of bacteria as receptors for their attachment to the target cell. Enzymatic action helps in penetration of the cell membrane and injection of viral genome into the target cell.

1.1.2 Lysogenic cycle: It involves the infection of bacterial cell with a virus and resulting in the incorporation of viral genome into bacterial genome resulting in a prophage. Bacteriophages following such kind of life cycle fall under the category of temperate phages. Control of lysogeny is under a gene called *cI* gene. The gene product suppresses the expression of all the other bacteriophage genes except itself resulting in the inactivation of lytic cycle. Conversion of lysogenic cycle into lytic cycle

2. Bacteriophage cocktails

Bacteriophage cocktails are a mixture of bacteriophages target specific for a particular bacterium. A number of US Food and Drug Administration (FDA) approved bacteriophage cocktails are commercially available. ListShield was the first Food and Drug Administration (FDA) Approved bacteriophage cocktail to be used against *Listeria monocytogenes* in food products in 2006 (Bai et al., 2016). Another bacteriophage cocktail targeting *Listeria monocytogenes* is Listex™P100. Commercially available bacteriophage cocktails being used against food borne pathogens are listed below in table 1

Table 1

Commercially available bacteriophage cocktails and their target food borne pathogens

| S.No. | Bacteriophage Cocktail | Number of phages | Targeted food borne pathogen |
|-------|------------------------|-------------------------|-------------------------------|
| 1. | ListShield | Mixture of six phages | <i>Listeria monocytogenes</i> |
| 2. | Listex™P100 | Single phage mixture | <i>Listeria monocytogenes</i> |
| 3. | Ecoshield | Mixture of three phages | <i>E.coli</i> O157:H7 |
| 4. | Salmofresh | Mixture of six phages | <i>Salmonella</i> |
| 5. | ECP-100 | Mixture of three phages | <i>Salmonella Enteritidis</i> |

All over the world countries are investing their mind and money preparing these bacteriophage mixtures to be used in food products including USA, UK, Georgia, Germany, India, Australia, Canada, Israel, Ireland, Portugal, South Korea and Netherlands (Bai et al., 2016; Endersen et al., 2014). The worldwide production of bacteriophage cocktails show the potential of this method in controlling food borne pathogens without posing any threats to the human health. With reference to Endersen et al., (2014) the Table 2 given below shows some of the countries and companies located there, producing bacteriophage cocktails approved by US Food and Drug Administration (FDA) and other government agencies.

Table 2

Companies producing bacteriophage cocktails and respective countries

| S.No. | Countries | Companies preparing bacteriophage cocktails |
|-------|--------------------------|---|
| 1. | United States of America | Intralix, Gangagen Inc. |
| 2. | United Kingdom | Novolytics |
| 3. | Georgia | Biopharm Ltd. |
| 4. | India | Gangagen Biotechnologies Pvt. Ltd. |
| 5. | Germany | Hexal Genentech |
| 6. | Australia | Special Phage Services Pty, Ltd. |

2.1 Effect of bacteriophage cocktails against common food borne pathogens

Salmonella spp.: In a study conducted by D.A. Spricigo et al., (2013) the effect of bacteriophage cocktail against *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis was analysed. Food products such as chicken breasts, packaged lettuce, fresh eggs experimentally contaminated with *Salmonella* spp. were treated with bacteriophage cocktail comprising bacteriophage UAB_Phi20, UAB_Phi78 and UAB_Phi87 in the ratio 1:1:1. Bacteriophage cocktail was very much effective in reducing the *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis contamination in all the food products.

These bacteriophages target a broad range of bacterial species (Bardina et al., 2012). Bacteriophage cocktails have been very effective in reducing *Salmonella* Enteritidis levels on melons. Experimentally contaminated fresh cut honey dew melons were treated with SCPLX-1, a mixture of four bacteriophages and stored at 5° C and 10° C for 3 days. After treatment a significant reduction was observed in the levels of *Salmonella* Enteritidis (Sharma., 2013; Leverentz et al., 2001).

In another study conducted by Wang et al., (2017) *Salmonella* specific lytic bacteriophage was used check the growth of *Salmonella* in fresh chilled pork. Not only the levels of *Salmonella* but other parameters including sensory characteristics and shelf life of were also analysed. Results showed there was no adverse effect on sensory characteristics shelf life of fresh chilled pork in addition to a significant decline in the levels of *Salmonella* showing the potential of bacteriophages as good food preservative.

2.1.1 Campylobacter: *Campylobacter* is one of the major foodborne pathogens contaminating a large variety of food products, resulting in food borne related illness. In humans *Campylobacter* causes campylobacteriosis. Major sources of *Campylobacter* transmission in humans is by consuming contaminated food products including raw milk and undercooked chicken (Joana Silva and Paula Teixeira., 2015). *Campylobacter* sp. are commonly present in the gut region of animals such as sheep, pigs and chickens and can get transferred to humans upon the consumption of meat from these infected animals as food products (Carvalho et al., 2012). Species specifically contaminating the food products are *Campylobacter jejuni* and *Campylobacter coli*.

2.1.2 Listeria monocytogenes: *Listeria monocytogenes* is a common and major food borne pathogen causing severe clinical manifestations including listeriosis. It is a pathogenic Gram positive bacterium infecting food products at various stages of food processing. In a recent study carried out by M.N. Perera et al., (2015) the effectiveness of bacteriophages against *Listeria monocytogenes* contaminated ready to eat food products including lettuce, smoked salmon, cheese was evaluated. All the food products were experimentally contaminated with *Listeria monocytogenes* and treated with commercially available bacteriophage cocktail ListShield™ which is a mixture of six bacteriophages. After treatment a considerable reduction in the number of *Listeria monocytogenes* was observed. The organoleptic properties were also not affected by application of bacteriophage cocktail.

Treating the food products with bacteriophage in combination with chemical antimicrobials such as potassium lactate and sodium diacetate can be beneficial as chemical antimicrobials can also reduce *Listeria monocytogenes* number and help bacteriophage to control the food borne pathogens. To experimentally check this, **A. Chibeu et al., (2013)** evaluated the effectiveness of Listex™P100, Food and drug administration (FDA) approved commercially available bacteriophage cocktail against *Listeria monocytogenes* in ready to eat products including roasted beef and turkey both with and without chemical antimicrobials. Sliced roasted beef and turkey were first contaminated with *Listeria monocytogenes*. Thereafter, slices of the food product were treated and incubated with Listex™P100 firstly without any addition of chemical antimicrobial, secondly in combination with chemical antimicrobials potassium lactate and sodium diacetate and observed at regular time intervals. After incubation, reduction of *Listeria monocytogenes* was observed in slices of turkey and roasted beef treated only with Listex™P100. In comparison to this, a significant reduction in the levels of *Listeria monocytogenes* was seen in the food product treated with the combination of Listex™P100 and chemical antimicrobials. Control of food borne pathogens and making the food products much safer for consumers can be made much more effective if the food products are treated with chemical antimicrobials in combination with Listex™P100.

Fresh cut honey dew melons experimentally contaminated with *Listeria* strains were treated with target specific bacteriophage cocktails. LMO-102 and LMP-103 which are mixture of six and fourteen *Listeria* specific phages respectively. Bacteriophage cocktails were applied onto fresh cut honey dew melons and stored at 10°C for 7 days. Results showed a significant reduction in the levels of *Listeria monocytogenes* (**Sharma., 2013; Leverentz et al., 2003**).

2.1.3 E.coli O157:H7: *E.coli* O157:H7 is a shiga toxin producing leading foodborne pathogen which causes severe disorders in humans including diarrhoea and hemorrhagic colitis (**Tomat et al., 2013**). A number of outbreaks related to food borne illness in recent years are due to contamination of food products by *E.coli* O157:H7 (**Ferguson et al., 2013; Zaczek et al., 2015**). Over the years, large number of physical and chemical methods was developed to decontaminate the food product from *E.coli* O157:H7. These methods are effective but have certain disadvantages such as variable efficiency and their adverse effect on sensory characteristics and nutritive quality of the food product. Chemical methods can affect the flavour and texture of food and at the same time physical methods like radiations can also affect the texture and nutritive quality of food (**Carter et al., 2012**). Recently, a more natural and modern method is being explored to decontaminate the food product and prevent it from *E.coli* O157:H7 contamination using target specific bacteriophages. Studies have shown that various food products contaminated with food borne pathogens when treated for a fixed incubation period with target specific bacteriophages reduces the number of pathogen significantly. **Carter et al., (2012)** reported a significant reduction in the number of *E.Coli* O157:H7 in beef and lettuce. After treating the food product with specific bacteriophages for 5 minutes bacterial levels were reduced by >94% in beef and 87% in lettuce. Several commercially prepared bacteriophage cocktails

are approved by different regulatory authorities for example ListShield™, Listex P-100™ and EcoShield™ (**Sulakvelidze, 2010; Carter et al., 2012**). These bacteriophage cocktails are a mixture of several lytic bacteriophages specific for a particular food borne pathogens. In as study conducted by **Carter et al., (2012)**, effect of EcoShield™ against *E.coli* O157:H7 in beef was evaluated. EcoShield™ is a FDA approved, commercially available bacteriophage cocktail consisting of three lytic phages namely ECML-4, ECML-117, ECML- 134. In this, beef steak artificially contaminated with *E.coli* O157:H7 was treated with EcoShield™ at 4°C for a time period of 5, 15, 30, 60, 120 and 1440 mins and the level of *E.coli* O157:H7 were recorded. A significant reduction of 94% to 98 % was recorded at all time periods.

Study done by **Jina Seo., (2016)** shows the inhibiting effect of bacteriophages against *E.coli* O157:H7 in different meat products like beef, pork and chicken meat. Specific bacteriophage strain BPEC019 was used in experimentally contaminated meat products mentioned above.

All the research studies mentioned above and much of the scientific literature available shows that the use of bacteriophages against food borne pathogens including *E.coli* O157:H7 is very much effective, safe and holds immense potential in the food industry.

3. Phage endolysin for control of food borne pathogens

Endolysins are peptidoglycan lytic enzymes that attack and degrade peptidoglycan layer of the bacterial cell wall. Endolysins are very active and specific hydrolases coded by bacteriophages during the end stage of their life cycle. These enzymes lyse the bacterial cell wall also termed as bacteriolysis and releasing the newly synthesized viral progeny. This excellent property of phage endolysin to lyse bacterial cell proves to be a very novel approach towards using them as new antimicrobial agents and to control food borne pathogens (**Oliveria et al., 2012**).

3.1 Mode of action

Once the bacteriophages replicate inside the bacterial host cell and new viral particles are synthesized the next step is the release of new viral particles from the bacterial host cell. For their release the cell lysis must take place and the rigid cell wall must be degraded. Bacterial cell wall is a structure which maintains the cell's structural integrity. Bacterial cell wall consists of a rigid, polymeric compound known as peptidoglycan. It is mainly comprised of N-acetylglucosamine and N-acetylmuramic acid bounded by β -glycosidic bond. In addition to these, several amino acids are also present including D-glutamic acid, D alanine, and meso-2,6-diaminopimelic acid. Peptidoglycan layer is much thicker in Gram- positive bacteria in comparison to Gram-negative bacteria. Peptidoglycan layer not only provides rigidity but also protect the cells from various degradative enzymes. Bacteriophages perform endolysin based enzymatic degradation of the cell wall. Endolysins are the enzymes that degrade peptidoglycan layer of a bacterial cell. For this to happen, endolysin needs a protein Holin which helps endolysin to cross the cytoplasmic membrane and reach the murein layer and perform its degradation (**Oliveria et al., 2012; Wang et al., 2000**). Endolysin act upon both Gram positive and Gram negative bacterial cell walls. In Gram positive bacterial cells, phage endolysin don't need holin protein to degrade the cell

wall as they lack protecting outer membrane which prevents the peptidoglycan layer from being accessible to the enzyme (Oliveria et al., 2014; Schmelcher et al., 2016).

4. Advantages of phage therapy

Phage therapy has several advantages over the use of antibiotics and other methods in controlling food borne pathogens. Phages are highly specific and very effective in lysing the target pathogen, preventing dysbiosis, that is, without altering the normal flora and thus minimizing the risk of super-infection. The specificity of phages also enables their use in the control of pathogenic bacteria in foods since they will not harm useful bacteria, like starter cultures. Moreover, phages do not affect eukaryotic cells, or cause adverse side effects as revealed through their extensive clinical use. Further, phages provide an added advantage of simple and low-cost production

and relatively high storage stability under different environmental conditions

5. Conclusion

The specific mode of action and wide range of applicability of bacteriophages makes them a very valuable food industry tool. The above-mentioned studies have shown that the use of either single species of bacteriophage or a cocktail is very efficient in targeting and reducing or killing the food borne pathogens. Bacteriophage cocktails are being produced commercially but safety concern of phages is still one area where more extensive *in vitro* studies are required so that the technology can be employed directly for human consumption worldwide.

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