Review paper
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THE ROLE OF BACTERIOPHAGES IN THE FOOD INDUSTRY: TWO SIDES OF THE MEDALLION

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Abstract

Bacteriophages, other commonly used name phages, are viruses that infect bacteria and use them as their hosts. They are the most numerous living organisms known in the world. Since bacteriophages' target is bacteria, they are harmless to humans, animals and plants. The question to be asked for phages is "is it beneficial or harmful for the food industry?" It is possible to answer the question with both "yes" and "no". This review focuses on answers of these two questions.

Bacteriophages are one of the alternative biopreservation methods food safety. Advantage of phages is that they do not have any negative effects such as resistance problem like the antibiotics and antimicrobials develop. Since phages are host-specific viruses, there are studies showing that the use of selected phage species is successful both in inactivation of pathogens present in the food matrix and in reducing the microbial load in food processing areas. There are studies on phage applications in the protection of farm animals' health. On the other hand, bacteriophages in the food industry have some technological disadvantages. It is undesirable for the production environment to be contaminated with phages, especially in enterprises producing fermented foods. Especially when starter cultures are infected by phages in the production of cheese, yoghurt and butter acid formation stops an undesirable taste and aroma can be seen and to the development of pathogenic microorganisms during ripening may occur.

As a conclusion, studies on the use of bacteriophages as a biopreservation method under controlled conditions in the food industry are promising. It is important that it is an alternative to antimicrobials, especially to which bacteria are resistant. On the other hand, it is necessary to take various precautions against phage contamination that will harm the starter cultures.

***Key words**: Food industry, Bioprotection, Bacteriophage, Phages.*

1. Introduction

Bacteriophages (other popular name - phages) are viruses which have an exceptional ability to kill the bacteria they infect [1]. In order to understand the mission of the bacteriophages on this world, we have to focus on the brief definition of bacteriophages. It is reported that those viruses represent the most abundant biological entities on Earth [2]. The population of phages is estimated at around 10^{31} in the biosphere and significantly limits the number of bacteria in various ecosystems [3]. Bacteriophages, which are widely found in nature, are abundant in salt water, fresh water, soil, plants, and animals where their bacterial hosts are found [4]. Phages proliferate by hijacking the biosynthetic pathways of their hosts, bacteria [5]. Phages first bind to a specific receptor on the host cell surface. This property makes them host-special parasitic microorganisms [6]. This binding is highly specific and gives some advantages and disadvantages for their persistence in food industry. The development and proliferation of phages vary according to environmental conditions. However, despite these conditions, phages develop much faster than bacteria [7, 8].

It was first reported by bacteriologist Ernest Hanbury Hankin in 1896 when filtered water from the Ganga and Jamuna rivers of India who found that they have antibacterial activity against *Vibrio cholera* [9, 10, and 11]. Then, in 1915, the existence of phages was assured as a result of the findings based on the observations of the bacteriologist Frederick Twort while trying to reproduce the Vaccinia virus, and two years later, during the work of the French-Canadian microbiologist Felix d'Herelle. Knowledge about the morphology of phages increased with the discovery of the electron microscope in 1940. Studies have emphasized that phages can be natural agents, especially in the fight against infectious diseases. Phage therapy has been used in many countries since ancient times [5, 12].

If the structure of bacteriophages is examined, we can see that the DNA and RNA genomes of bacteriophages are encapsulated in a protein capsid. The important details of its morphological structure can be summarized as follows: the head or capsid exhibits a distinctive three-dimensional structure, usually icosahedral, consisting of structural proteins and nucleic acid (DNA or RNA) as a carrier of genetic information. Tail is consisting of a tail tube surrounded by a spiral shrinkable sheath. Both the tail tube and the helical sleeve are attached to the dome-shaped baseplate. The tail fibers, which are six in number, are folded under the baseplate and are made entirely of proteins responsible for identifying specific molecules on the surface of the host bacterium. The baseplate is a hexagonal dome-like structure made up of 15 different proteins called host cell or receptor binding proteins (Figure 1).

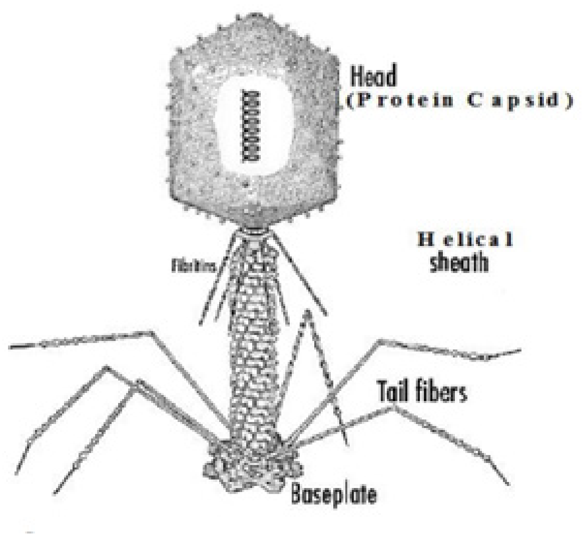

Figure 1. Image of phage fragments [5]

In general, and with simple definition of their life cycle, the phage genome is injected into the host bacterium, the phage genome is replicated, and structural components are produced, after which the lysis of the host cell is occurred, followed by the release of hundreds of progeny phages to the environment. This process can occur in as little as 15 minutes. The progeny phage can then continue the cycle by infecting other bacterial cells [6]. Bacteriophages appear in two types according to their lifestyles, lytic and lysogenic. Lytic phages take over the bacterial host and direct the production of new virions, lysing the cell once and releasing the viral generation [4]. Lysogenic phages incorporate their genetic material and remain inactive in the host cell. They reproduce inside the host until the lytic cycle ends. Lytic phages show rapid DNA fragmentation in the host bacterial cell. New phages are then produced within the bacterial cell using the synthetic machinery of the host. Lysogenic phages, in contrast, integrate into the host genome (prophage) or exist as plasmids within the host cell (Figure 2).

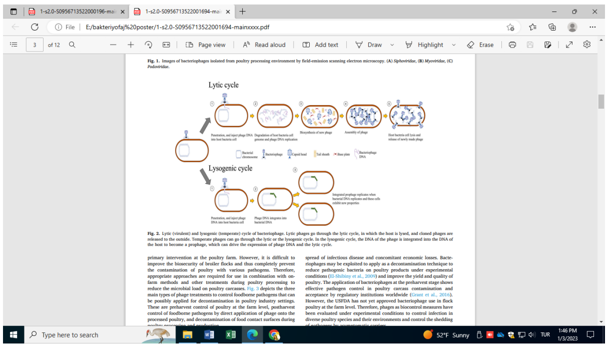

Figure 2. Lytic and lysogenic phage [8]

Since bacteriophages' target is bacteria, they are harmless to humans, animals and plants. The question to be asked for phages is "is it beneficial or harmful for the food industry?" It is possible to answer the question with both "yes" and "no". This review focuses on answers of these two questions.

2. Are bacteriophages enemy or friend? Two sides of the medallion

2.1 The friendly face of the phages

In recent years, the use of bacteriophages has emerged as a treatment method in western countries [13]. The first studies on the use of bacteriophages in humans appear in the applications of D'Herelle for dysentery and plague diseases. It has been reported that *Clostrdium difficile* infections are treated by the phages extracted from healthy human feces [14]. Phages are also suitable to be used against infections caused by bacteria that are resistant to multiple drugs. Current bacteriophage preparations generally focus on *Pseudomonas aeruginosa*, *Acinetobacter baumanni*, *Klebsiella pneumoniae* and *Staphylococcus aures* [15]. Dedrick et al., [16], reported that phages highly effective in cystic fibrosis disease caused by *P. aeruginosa* and *Mycobacterium abscessus*. Bao *et al.*, [17], reported that a 63-year-old patient with recurrent urinary tract infection caused by sulfamethoxazoleresistant *Klebsiella pneumoniae* recovered after being treated with phage cocktail therapy. Petrovic Fabijan *et al.*, [18], reported that 13 patients severely infected with *Staphylococcus aureus* were treated with the phage preparation AB-SA01, which not only effectively controlled the infection but also did not show any reaction. Weber Dabrowska *et al.*, [19], reported that they used the cocktail they prepared from phages with topical application in the treatment of burns. Markoishvili *et al.*, [20], applied phages to bandages for use in the treatment of patients with chronic ulcers and reported that it had a significant effect in the treatment of their wounds. Brown *et al.*, [21], isolated 10 phages from human skin and proved that these phages have lytic activity against acne. Bruttin and Brussow [22], and Sarker *et al.,* [23], used *Escherichia coli*-T4 phage to treat acute bacterial infectious diarrhea in adults and children. Vahedi *et al.*, [24], compared the therapeutic effects of phages and ciprofloxacin in a mouse model infected with enteropathogenic *Escherichia coli* (EPEC) and found that phage treatment could not only reduce EPEC content *in vivo*, but also restore normal growth of mice. Wang *et al.*, [25], reported that phage applications are effective in *Pseudomonas* bacteremia, which has developed resistance to antibiotics. Fukuda *et al.*, [26], reported that they obtained positive results with the application of phages to the eye in the form of drops in a case of keratitis caused by *Pseudomonas* spp. Kusradze *et al.*, [27], and Regeimbal *et al.*, [28], reported that bacteriophage application had positive results in wound infections caused by *A. baumannii* in mice.

In the selection of suitable phages for treatment; attention should be paid to criteria such as phage specificity, efficacy and side effects. The use of phage cocktails is preferred in treatments. Since bacteriophages are in protein structure, they are affected by high temperature, pH, organic substances and physical effects. Phages to be used in treatments can be administered enterally, topically, inhaled or injectable. Approximately 10^8 bacteriophages/mL are required for successful bacterial reduction [29, 30, and 31].

In addition to the use of phages as an alternative treatment method against antibiotics in human and veterinary medicine, it is also used as a bioprotection method in the food industry. This is the other friendly side of phages. Today, natural measures for maintaining food safety are becoming more and more popular. Bacteriophages have also taken their place in the list of bioprotection methods applied for this purpose. One of the main reasons they are preferred is that they are highly specific and usually only infect one type of bacteria. Thus, they do not harm the natural commensal microbiota in the gastrointestinal tract of humans and animals. No adverse or toxic effects on eukaryotic cells have been observed with the use of bacteriophages. They do not make any changes in the sensory properties of food products [31, 33]. Bacteriophages are used in three main areas in the food industry to ensure food safety: production, bioconservation and biosanitization [33]. For this purpose, phages can be used during the growth of plants or animals or in the pre-harvest stage of production. Phages can also be applied during food processing and packaging to decontaminate the potential pathogens. Phages are applied to prevent and reduce biofilms on the surface of equipment. In biological preservation, bacteriophages can also be added directly to food products to extend the expiration date of foods [32, 34]. The application of phages in the food chain also has several advantages can be summarized as follows: firstly, bacteriophages are highly specific and usually can infect only one species or one type of bacteria. Thus, the natural commensal microbiota in the gastrointestinal tract of humans and animals is not destroyed. No adverse or toxic effect on eukaryotic cells has been observed with the use of bacteriophages. They are ubiquitous and do not change the sensory properties of food products. Bacteriophages are highly resistant to the stress created during food processing so they can keep their ability to protect food even after the food is processed [3, 32, and 33].

Various control measures are implemented in food production, including good manufacturing practices (GMP) and hazard analysis control points (HACCP) [35]. Despite all the precautions taken, according to the European Food Safety Authority (EFSA) 2020 report, it has been reported that 1,319 people infected with *Campylobacter* spp. infection in 17 countries became ill. In addition, 3,686 people had salmonellosis, 694 people had listeriosis, and 17 patients died. One death occurred out of 208 cases was caused by Shiga toxin secreted by *E. coli* (STEC), a foodborne pathogen [36]. Those are only the examples of the most important foodborne infection pathogens. On the other hand, the development of resistance to antibiotics in bacteria due to uncontrolled use of antibiotics is another important problem in terms of food safety [37]. Hence, scientific studies on phages that can be used as biocontrol agents in food production continue depending on those reasons. The advantage of bacteriophages over antimicrobials is that they are specific for certain species on the microbiota. Antimicrobials used to control the microbiota on food can cause undesirable taste, odor and texture on the product. For this reason, phages are considered as an alternative protection method [38].

Studies have reported that the W25 phage they tested on *S. typhimurium* reduced the microbial load by log 2.19 [39, 40]. Zhang *et al.*, [41], reported a $\log^5$

reduction in *Salmonella* spp. with the phage they applied in raw lettuce samples. It has been reported that there was log^{1,2} and log^{1,3} decrease in chicken breast and turkey breast meat for *Salmonella* spp., respectively [42, 43]. The application period of phages are also important in reducing the microbial load. Grant *et al.*, [44], applied the cocktail application containing Fo1a and S16 phages to chicken meats for 30 minutes and 8 hours. They reported that while the presence of *Salmonella* spp. decreased by log$^{0.4}$ in the samples applied for 30 minutes, it decreased by log$^{0.7}$ in the samples applied for 8 hours. On the other hand, as it is reported, phages can maintain their activity even in a wide temperature range. Due to the natural flora of beef, *E. coli* contaminations are frequently seen. For this purpose, FAHEc1 phage was used in carcass meats and it was reported *E. coli* load was decreased approximately log^{2} [45]. Abuladze *et al.*, [46], reported that they reduced the level of *E. coli* O157:H7 by log^{1,2} in the study they conducted with 3 different phage cocktails in minced meat samples. O'Flynn *et al.*, [47], reported that they reduced the level of *E. coli* O157:H7 in beef by 77% by using a phage cocktail. In a study on UHT and raw milk, *E. coli* ATCC strain was experimentally inoculated. It has been reported that phages applied despite storage conditions at 4 ^{0}C and 25 ^{0}C completely inhibit *E. coli* bacteria [48]. In the studies conducted for *Shigella*, they reported a decrease in log1 level for the samples of melon, lettuce, yoghurt, canned beef, smoked salmon, and chicken breast [49]. There are many research studies on *Listeria monocytogenes* which is a worldwide common foodborne pathogen. In a study, a decrease of log^{2} and log$^{0.4}$ was observed as a result of phage cocktail application to melon and apple slices, respectively, while a higher decrease (log^{5,7} and log^{2,3}) was reported as a result of phage application combination with nisin [50]. In a study on ripened cheese, *L. monocytogenes* decreased by log$^{3.5}$ with a single phage application. It was determined that *L. monocytogenes* isolated from the same cheese after phage application did not form a resistance against phages [51]. It has been reported that *L. monocytogenes* level is completely inhibited at 6 ^{0}C in experimentally contaminated mozzarella cheese brine. In the same study, it was reported that there was a log5 decrease in smoked salmon, mixed seafood, sausage and sliced turkey meat [52]. It has been reported that a single phage application to fish fillets results in a log$^{1.4}$ - 2.0 cfu/g reduction in 4 ^{0}C and no growth of *L. monocytogenes* was observed after 10 days of storage [53].

It is known that phages are also used to suppress the undesired microflora in food products. For this reason, it has been reported that the vB_SflS-SF001 phage provides a log^{2} reduction in the number of *S. flexneri* in raw and cooked chicken breast meats. This result shows that phage control and biocontrol in foods can be effective. In another study, the combination of vB_SflS-SF001 and vB_SsoSISF002 phages was tested in raw and cooked chicken breasts, and it was reported that they were more effective in reducing the number of *Shigella* when used together [54]. In addition, bacteriophages are used as one of the components of biosensors for the rapid detection of pathogens in food [3].

Phage therapy is a promising alternative in antimicrobial treatment especially against drug resistance. On the other hand, some disadvantages were being discussed when the phages were firstly introduced as alternative treatment method. These were the low survival of phages in the acidic environment (e.g., the human stomach) and the presence of endotoxins in the applied phage cocktails. Phage immunogenicity refers to the generation of specific antibodies against bacteriophage antigens, which is a challenge in phage therapy, mainly due to its effects on the pharmacokinetics and the possible side effects of phages [55].

2.2 The unfriendly face of the phages

With the use of starter culture in fermentation process of the foods especially in the dairy industry, the desired quality in the products can be consistently achieved. However, after a while, the starter culture may not achieve the desired fermentation and the products deteriorated. It has been reported that the reason for this is phages that inhibit the activity of starter cultures and kill bacteria. The bacteriophage problem, which has attracted attention in the dairy industry, first appeared in New Zealand and Australia in the early 20th century. Since the pure cultures to be used for cheese production do not create the necessary acidity in the milk and accordingly, the desired taste and aroma do not occur in the cheeses, the focus is on the culture used. As a result of the investigations, it was determined that the development of bacteria in pure culture is stopped in a short time and their cell membranes are destroyed. So the fermentation is stopped or slowed down. Bacteriophages have been pointed out as the reason for this [56]. On the other hand, fermentation failure can be reduced by using effective disinfectants-peracetic acid and sodium hypochlorite are one of the most efficient biocides tested on LAB-specific phages. Efficiency of ozone treatment and UV light irradiation on phages is still unclear. Also, a simple and important method that is still being used. This is starter/strain rotation. Genetic engineering methods that allow the construction of bacteriophage-resistant strains of LAB are also promising [3].

Raw milk is shown to be the main source of contamination by bacteriophages in dairy industry.

It has been reported that there are 10^1 - 10^4 phages per mL of raw milk in farms [57]. Various studies have reported that phages can survive at the pasteurization temperature [58]. In addition to raw milk, phage contamination can occur in dairy farms by aerosol route. Especially personnel movements and equipment are important factors in the contamination of phages. Bacteriophage problems occur in the dairy industry, especially in the production of cheese, yoghurt and butter. It is possible to encounter phage contamination in dairy farms. In a study, it was reported that 64% of dairy products have different types of phage contamination [59]. With phage contamination, starter cultures cannot develop, and the desired quality of the product is not achieved and the development of unwanted microorganisms can be seen [29]. Serious problems arise especially in yogurt technology. When the yoghurt bacteria *Lb. bulgaricus* and *S. thermophilus* are infected with phages, the ratio of bacteria in yogurt changes, the acid increase stops, and a product devoid of taste and aroma emerges. Depending on the rate of bacteriophage effect, the incubation period is prolonged and yoghurt production is not possible with the increase in the number. In butter manufacturing, *S. cremoris*, *S. diacetylactis* and *L. citrovorum* bacteria are generally used for the ripening of the cream and the formation of its aroma. In the butter where bacteriophage contamination occurs, the desired flavor does not occur and a malty taste is formed. This creates a serious economic loss for producers [60].

Phage contamination is an inevitable threat in dairy farms and should be brought under control with proper planning. Some of the measures to be taken are; culture rotation, direct starter culture inoculation, use of phage-resistant cultures, and careful handling or disposal of whey. Facility design is also very important for phage control. There should be no contact between the raw material milk and waste whey. Some studies have shown that there may be aerosol contamination during the pouring of milk into tankers. For this reason, it has been reported that the milk intake area should be separate from the other parts of the facility. In addition, the starter culture preparation room should be separated from the production area [61].

Phages are not affected by pasteurization at low degrees. Studies have reported that some phage species become inactive in 10 minutes at 65°C, and some in 12 minutes at 75 ^{0}C [29]. It persists in a wide pH range upon the activity of phages. They can maintain their activities even between pH 2.5 - 11.8. For these reasons, if milk containing phage comes to the enterprise, there will be a high rate of phage in the whey. Considering these factors, after the production is completed, the rooms should be sprayed with chlorine, the equipment should be cleaned and disinfected, 200 - 300 ppm/L chlorinated water, and 100 ppm/L ammonium compounds should be used for the disinfection of the pipes. In addition, milk stone accumulations on tools and equipment should be prevented [62]. During the preparation of starter cultures, contamination may occur from the air and from unclean containers. For this reason, great attention should be paid to hygiene in the starter culture preparation rooms. The rooms where the cultures are prepared must be away from the production areas and aseptic conditions must be provided during preparation. It has been reported that hygienic conditions and culture rotation are more effective in combating bacteriophage [60]. Another method to combat bacteriophages is to prevent phages from growing in culture. Phages need mineral salts and especially calcium for their growth [63].

3. Conclusions

- Bacteriophages have a friendly face which provides beneficial effects for human being in two ways. These are alternative antimicrobial therapy in human and animal diseases and have a promising alternative property to antibiotic resistance. And the other way supporting us is their bio preservation effect in food industry against foodborne pathogens and spoilage bacteria.
- On the other phages may also show their unfriendly face in food industry by infecting the bacteria used as starter culture and causing to stop the fermentation process. Some principle preventative methods were introduced to come this problem over and the researchers are still working on it.

4. Referances

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