



## Application of Phage for Biocontrol of *Salmonella* Species in Food Systems

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ARTICLE INFO	ABSTRACT
<p><i>Review Article</i></p> <p>Received : 16/06/2020 Accepted : 21/09/2020</p> <p><b>Keywords:</b> <i>Salmonella</i> Biocontrol Phage Food Food Safety</p>	<p>The studies on phage applications that provide successful results in biocontrol of foodborne pathogens and offer an environmentally friendly approach have been increasing today. Phages are viruses that can infect and kill the specific target bacterial cell. <i>Salmonella</i> is one of the most important pathogenic microorganisms that leading causes of food-borne illnesses called salmonellosis. Meat products especially chicken meat, fresh eggs, dairy products, ready-to-eat foods, seafood products and all kinds of contaminated food can be cause of salmonellosis. In this review, the phage application studies to control of <i>Salmonella</i> in food systems were summarized taking into account the research studied in recent years.</p>

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 8(10): 2214-2221, 2020

## Gıda Sistemlerinde *Salmonella*'nın Biyokontrolü İçin Faj Uygulamaları

MAKALE BİLGİSİ	ÖZ
<p><i>Derleme Makalesi</i></p> <p>Geliş : 16/06/2020 Kabul : 21/09/2020</p> <p><b>Anahtar Kelimeler:</b> <i>Salmonella</i> Biyokontrol Faj Gıda Gıda Güvenliği</p>	<p>Gıda kaynaklı patojenlerin biyokontrolünde başarılı sonuçlar veren ve çevre dostu bir yaklaşım sunan faj uygulamaları ile ilgili, çalışmalar günümüzde giderek artmaktadır. Fajlar, spesifik hedef bakteri hücrelerini enfekte edebilen ve öldürebilen virüslerdir. <i>Salmonella</i>, salmonelloz adı verilen gıda kaynaklı hastalıklara yol açan en önemli patojenik mikroorganizmalardan biridir. Et ürünleri, özellikle tavuk eti, taze yumurta, süt ürünleri, tüketime hazır gıdalar, deniz ürünleri ve her türlü kontamine gıda salmonelloza neden olabilir. Bu derlemede, gıda sistemlerinde <i>Salmonella</i>'nın kontrolüne yönelik faj uygulama çalışmaları, son yıllarda yapılan güncel araştırmalar dikkate alınarak özetlenmiştir.</p>

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## Introduction

The additives and heat treatments used in the food industry are significant in terms of safe food production. However, the technological processes lead to loss of nutrients, vitamins and color loss in food, whereas the additives may also have negative effects on human health. Therefore, consumers demand for safe food products, which close to natural as possible, have no chemical additives, and are also less processed is increasing today (Şanlıbaba and Uymaz, 2015). Thus, the newly developed food preservation technologies have adopted the “farm to fork” approach. So, alternative food preservation methods such as biocontrol increase the shelf life and safety of the food, and also show minimum unfavorable effects on the sensory properties and nutritional value of perishable foods. The bacteriocin and bacteriophage called phage treatments (Garcia et al., 2010; Şanlıbaba and Uymaz, 2015) are natural bio-preservation. The use of phage in biocontrol assay to control foodborne pathogens is a promising and environmentally friendly application (Gouvea et al. 2015, Thung et al. 2017).

Phages are suitable for disease prevention in livestock (phage therapy), equipment and surface disinfection (also entitled phage bio-sanitation), decontamination of raw food products such as carcass, fresh fruits, and vegetables. Also, another role of phages for controlling of food safety is the use of phage in the detection of foodborne pathogens (Martínez et al. 2019). Phages are viruses that specifically infect to bacteria and also multiply in it (Choińska-Pulit et al. 2015), so they definitely need a specific host bacterial cell to survive and replicate.

They are host-specific, and do not infect unrelated cells or bacteria (Pereira et al. 2016). Phages are divided into two groups according to their lifestyle, as *i*) virulent and *ii*) temperate phages. Virulent phages follow a lytic cycle, which means that they proliferate in the bacterial cell, lyse the cell and subsequently release the phage progeny. On the other hand, temperate phages can enter the lysogenic cycle where their DNA combines with the bacterial genome and become prophage. As long as the environmental conditions for the bacteria are suitable, phage continues to exist ineffectively. With changes in environmental conditions such as the depletion of food sources, phage in prophage form can return to a virulent form and initiate the lytic cycle and finally lyse the bacterial cell (Luo et al. 2012, Cadieux et al. 2018). Phages that have the lytic cycle show antimicrobial activity against their target bacteria. Phages, however, should not be affected by the physicochemical properties of foods such as pH, temperature, water activity, preservative components. In addition, they must be resistant to adverse conditions in the storage process. It is expected to maintain the activity of phage in the gastrointestinal tract, where have highly acidic environment and enzymes after being taken into the body with food. There are some studies on microencapsulation applications to protect phages from all these adverse conditions (Choińska-Pulit et al. 2015, Ergin et al. 2017).

The most common foodborne infections have caused by *Salmonella*, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Campylobacter* (Schmelcher and Loessner, 2014). There are some chemical, physical and biological methods commonly used to control

*Salmonella* spp., which including irradiation, high-frequency heating, steam pasteurization, chlorine, organic acids, trisodium phosphate, ozone, plant extracts, essential oils and also antibiotics. However, chemical residues can have some negative adverse impact on the quality of the foods (Huang et al. 2018, Duc et al. 2020). Moreover, antimicrobial agents like antibiotics have also been commonly used to treat salmonellosis infections in animals, which leading cause multidrug-resistant *Salmonella* strains in recent years. Concern about antibiotic residues in animal origin foods has increased steadily (Vaz et al. 2020). Therefore, the phages can offer a friendly approach as an alternative to antibiotics and also chemical agents for effectively control of *Salmonella* (Thung et al. 2017). The use of phages against to foodborne pathogens in foods has some advantages. These are high specificity, self-replication, lack of unfavorable sensory effects, and non-toxicity to humans (Yıldırım et al. 2018, Wong et al. 2020). The first report of using of phages against *Salmonella* serovar was dated back to early 20<sup>th</sup> century, and then, phages have effectively used to control various diseases caused by *Salmonella* (Hooton et al. 2011, Zinno et al. 2014, Thung et al. 2017, Huang et al. 2018, Zhang et al. 2019, Wong et al. 2020).

## *Salmonella* Species

Karl Joseph Eberth and Rudolf Virchow studied *Salmonella* firstly during the early 19<sup>th</sup> century and recognized the organism from the abdominal lymph nodes and the spleen. Then, Gaffky isolated the bacillus, which caused typhoid fever. Then *Salmonella* was discovered and isolated from intestines of pigs with classical swine fever called hog cholera by Theobald Smith and Daniel Elmer Salmon, in 1885. Later, the bacterial strain was named as *Salmonella* by Daniel Elmer Salmon, who was an American pathologist (Eng et al. 2015, Jajere 2019). Today, the nomenclature of the genus *Salmonella* is confusing, controversial, and still being developed (Eng et al. 2015). The genus *Salmonella* is classified into two broad species named *S. bongori* and *S. enterica* based on differences in their 16S rDNA sequence analyses, according to National Center for Biotechnology Information (NCBI) (Anonymous, 2020a). *S. enterica* can further be divide into six subspecies on the basis of differences in their biochemical properties and genomic relatedness. *S. enterica* subspecies have designated with roman numerals; (I) *S. enterica* subsp. *Enterica*, (II) *S. enterica* subsp. *Salamae*, (IIIa) *S. enterica* subsp. *Arizonae*, (IIIb) *S. enterica* subs. *Diarizonae*, (IV) *S. enterica* subsp. *Houtenae*, and (VI) *S. enterica* subsp. *Indica*. However, *S. bongori* is composed of twenty-two little-studied subspecies as they have mainly connected with cold-blooded animals, and their infections are very uncommon (Lamas et al. 2018)., *S. enterica* subsp. *Enterica* is the most commonly isolated subspecies, predominantly found associated with mammals among all of the *Salmonella* subspecies, and causes 99% of *Salmonella* infections in both human and warm-blooded animals. However, the other five subspecies and *S. bongori* are commonly isolated from either environment or cold-blooded animals (Eng et

al. 2015, Lamas et al. 2018). Moreover, more than 2600 serovars have been described to date for *Salmonella*, and all of these serovars belong to *S. enterica* species (Chen et al. 2013, Jajere 2019). However, less than 100 serovars causes serious illnesses in both humans and animals (Pulido-Landinez, 2019). *S. Enteritidis* and *S. Typhimurium* are the most commonly determined serovars in the incidence of Salmonellosis, which is one of the most common foodborne diseases in worldwide and significantly damages the economy of the country (Mukhopadhyay and Ramaswamy 2012, Dalyan Cilo et al. 2015). In terms of isolating of serovars, different serovars can be isolated from different settlements of the world. *S. Infantis* can be isolated from many parts of the world, whereas *S. Newport* is generally found in Europe, Latin America and North America. Moreover, *S. Hadar* is found in Europe, and *S. Virchow* is mostly in Asia, Europe and Oceanic countries. Lastly *S. Agona* is in North America, Latin America and Europe. The isolated serovars differ by region, but there are no significant differences between those isolated from countries within the same region (Hendriksen et al. 2011).

The genus *Salmonella*, a member of the *Enterobacteriaceae* group, is one of the main bacteria causing foodborne diseases worldwide (Mostafa et al. 2016). *Salmonella* bacteria are Gram-negative, non-sporulating, motile with peritrichous flagella, capsule-free, catalase positive, oxidase negative, rod-shaped bacilli (LeLievre et al. 2019). Poultry, milk, dairy products, raw or undercooked red meat, fruits, vegetables, egg products and contaminated water can be contaminated with *Salmonella*. Moreover, the contamination of *Salmonella* takes place also through contact between foods and animal or human feces (Mostafa et al. 2016). *Salmonella* can be transmitted to fruits and vegetables through the environment or by using fertilizing and irrigation water, because of the contaminating of feces into the environment and water sources.

Ready-to-eat foods (RTE) can also contaminated with *Salmonella* because of cross contamination issues (Sánchez-Vargas et al. 2011). Salmonellosis occurs when consuming contaminated foods with an average *Salmonella* concentration at the level of  $10^5$  CFU/g or CFU/mL (Lee et al. 2015). The range of infection in different host varieties depends on the virulence factors of the bacteria, the host-resistant capability, and the immune system (Gómez-Baltazar et al. 2019). Commonly, *S. Enteritidis*, *S. Typhimurium*, *S. Virchow*, *S. Hadar*, *S. Infantis*, and *S. Hiedelberg* are responsible for gastroenteritis cases in humans (Gülener, 2015). The disease symptoms are nausea, vomiting, enteric fever, diarrhea, septicemia, abdominal pain, stomach cramps, or bacteremia. It has also been reported that there are some findings such as reactive arteritis after infection (Grygorcewicz et al. 2017, Heredia and Garcia 2018). Acute gastroenteritis in the childhood is one of the most important symptoms of salmonellosis and causes child mortality in undeveloped countries (Sanchez-Vargaz et al. 2011). Besides, *Salmonella* serovars can cause typhoid and paratyphoid, which are an endemic disease in Turkey whilewidespread all over the world in humans (Gülener, 2015). Most people recover without treatment from *Salmonella* infection, whereas the others such as children

under five years, infants, adults aged 65 and older, people with a weakened immune system are at increased risk of *Salmonella* infection (Pulido-Landinez 2019). According to the Centers for Disease Control and Prevention (CDC) reports, about 1.35 million *Salmonella* infections are detected in the United States every year., Moreover, as a result of *Salmonella* infections, it was observed that 26.500 people hospitalizations and 420 deaths (Anonymous 2020b). A 94.625 confirmed cases of salmonellosis in humans and 126 deaths occurred in EU in 2015 (Lamas et al. 2018), whereas nearly one of three foodborne outbreaks was caused by *Salmonella* in the EU in 2018 based on the reports by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) (Anonymous 2020c).

There are several methods to fight *Salmonella*. This bacterium is sensitive to thermal treatment, so it can be easily inactivated by this process. Besides, some chemicals such as chlorine, iodine and hydrogen peroxide are also used against *Salmonella*. Ozone, UV radiation, plasma discharge, ultrasound, electrolyzed oxidized water, high pressure carbon dioxide, and bacteriophage are the novel emerging technologies used for inactivating *Salmonella*. Among these technologies, the phage application is hopeful method against *Salmonella* (Mukhopadhyay and Ramaswamy 2012).

### Phages of *Salmonella* Species

The phage activity was firstly discovered based on the antimicrobial activity against *Vibrio cholerae* in the Ganges and Yamuna rivers in India by English bacteriologist Ernest Hankin in 1896. Then Frederick Twort described the glassy transformation of *Micrococcus* colonies by an agent, and used the definition of “the agent that kills bacteria by infecting” in 1915, and also suggested that this antibacterial effect may also be caused by a virus. Later, Felix d’Herelle working at the Pasteur Institute, was the first person to introduce the antimicrobial feature of the phage to the world using the term phage, which means bacteria-eater in 1917 (Gündoğdu and Ulu-Kılıç 2018, Uğur 2018).

Phages using for biocontrol of pathogens in food industry must have some criteria before being considered as suitable candidates. Phages are; *i*) infect specific bacteria, *ii*) generally do not cross bacterial species or genus barrier, *iii*) do not affect desirable microorganisms commonly present in foods, *iv*) do not affect gastrointestinal tract or the normal bacterial microbiota in human, *v*) must be virulent, *vi*) not include virulence genes, *vii*) not include antibiotic resistance genes, *viii*) have a wide host range, *ix*) determined the complete genome sequence of phages, *x*) being stable over storage and food application, *xi*) oral feeding studies show no adverse effect, *xii*) being amenable to scale up for commercial production, *xiii*) phage genome sequences should be defined, and *xiv*) have the generally recognized as safe (GRAS) approval for use in foods (Şanlıbaba and Uymaz Tezel 2017).

Phages can be isolated from many sources, such as digestive tract of humans and animals, foods, soil, water, sewage, feces, and other ecological habitats (LeLievre et al. 2019). Phage application significantly reduced the

population of the foodborne pathogen bacteria, ranging from 0.3 to 5.9 log CFU (Jorguera et al. 2015). There are several phage preparations commercialized and marketed. ListShield™ LMP102 (Intralytix, USA), and Listex™ P100 (Microes Food Safety, The Netherlands) are used against *Listeria monocytogenes*, while EcoShields™ (Intralytix, USA) and EcoShield™ (ECP-100) are suitable for against *Escherichia coli* O157:H7 (Şanlıbaba and Uymaz Tezel 2017). Moreover, there are also several commercial *Salmonella* phage products (LeLievre et al. 2019). Firstly, SalmoFresh™ (Intralytix, USA) targets *S. Enterica*. It is defined as GRAS by the FDA for direct applications to poultry, fish and shellfish, fresh and processed fruits and vegetables. SalmoFresh consists of a mixture of six individual lytic phages to provide effective protection against pathogenic *S. enterica* cells such as Typhimurium, Enteritidis, Heidelberg, Newport, Hadar, Kentucky, Thompson, Georgia, Agona, Grampian, Senftenberg, Alachua, Infantis, Reading, and Schwarzengrund. However, SalmoFresh is not able to eliminate the growth of Paratyphi B strains in spot-test analyses (Huang et al. 2018). This phage product does not affect the general composition, taste, aroma, or color of foods. It listed in OMRI (Organic Material Review Institute) and certificated both Halal and Kosher. In addition, it is found FSIS-listed known as safe and suitable for use in the production of poultry products as a processing aid with no labeling requirements. SalmoFresh™ is accepted as a processing aid in Canada and Israel, and can be applied directly on food surfaces by spraying method. The diluted working solution is applied by spraying at a concentration of 1-4 mL per pound of food product in direct applications (Zhang et al. 2019). Secondly, Salmoex™ (Microes BV, The Netherlands) is the second phage products for food safety, after an earlier approval of Listex™. It is accepted and certified as organic in Australia and New Zealand. Salmoex contains two specific phages as S 16 and FO1a. These phage particles are misted onto meat and poultry surface and kill *Salmonella* without any sensory effects of food (Sukumaran et al. 2015). Thirdly, Biotector™ S1 (Cheil Jedang Corporation, Republic of Korea) is particularly efficient to control *S. Gallinarum* and *S. Pullorum* responsible for fowl typhoid and pullorum disease, respectively. It is used as a feed additive against challenge with *S. Gallinarum* in broiler breeders and also developed to replace antibiotics in animal feed. Biotector™ S4 is also other the Biotector phage product (additives in swine feed), which could specifically control *S. Typhimurium* (Nobrega et al. 2016, Huang et al. 2018). Fourthly, BacWash™ (Omnilytics, USA) could directly spread onto living animals, and hides of livestock (LeLievre et al. 2019). Fifthly, Armament™ was also approved by USDA (United States Department of Agriculture)'s Food Safety and Inspection Services for application as spray mist or wash on the feathers of live poultry before slaughter to decrease pathogen transfer to meat (Huang et al. 2018). Sixthly, SalmoPro™ (Phagelux, Canada) consists of a mixture of equal concentrations of two different *Salmonella*-specific lytic phages called as monophage(s). The current SalmoPro is a liquid made up of equal parts of two monophages as BP-63 and BP-12 Triumvirate. Each of these monophages is specifically effective against a wide

host range of *S. Enterica* serovars (Nobrega et al. 2016). Finally, PhageGuard S™ produced in the Netherlands is reported to be effective on all serovars of *Salmonella*, and also is approved by the USDA and defined as GRAS by the FDA (American Food and Drug Administration). Moreover, this phage preparation is certified both Halal and Kosher, and also OMRI listed. PhageGuard S provides a 1-3 log reduction in pathogens without causing a change in the sensory properties of the food, dissolve fat, and corrosive to equipment used. This phage preparation can be used after slaughter or in the later stages of the poultry process in many countries such as Canada, Australia, and Israel (Anonymous 2020d).

When the morphological structure of *Salmonella* phage is examined, it has an icosahedral protein capsid and capsomers. The capsid thickness is about 60 nm and generally in the range of 34-160 nm (Uğur, 2018). They are generally members of the *Myoviridae* (with contractile tails), *Siphoviridae* (with long noncontractile tails) and *Podoviridae* (with short noncontractile tail) families (Choińska-Pulit et al. 2015, Thung et al. 2017). *Salmonella* specific phages have been isolated from a broad range of foods such as fermented dairy products like cheese and yogurt, retail beef, buffalo, poultry, pork and other meat products, chilled and frozen crabmeat, lettuce and mushrooms, various wastes, animal feces and sewage water (Guenther et al. 2012, Huang et al. 2018). Phage stability is an important parameter to evaluate the effectiveness of biocontrol applications. Phages isolated from food matrices show higher stability in various animal origin foods than from sewage (Robeson et al. 2014).

To date, there are numerous *Salmonella* specific phage isolated and characterized worldwide (Lee et al. 2011, Bao et al. 2015, Li et al. 2016, Thung et al. 2017, Ata 2018, Huang et al. 2018, Khan et al. 2018, Yildirim et al. 2018, Bao et al. 2019, El-Dougdoug et al. 2019) and these were summarized in Table 1.

### Application of Phage for Biocontrol of *Salmonella*

The use of lytic phage appears as a promising approach for improving food safety. Many works have been published about controlling *Salmonella* serovar in food systems and food processing environments (LeLievre et al. 2019). There are several factors, which can negatively affect phage effectiveness in biocontrol assays. For example, food matrix such as proteins and fat globules have an impact on the accessibility of target bacteria to phage. Besides, a major problem of the phage application is the phage resistant bacteria. This problem can be eliminated by using of phage cocktails or applying phage rotation systems. The other factor is the antimicrobial activity displayed by the phages in the laboratory conditions. The causes of this problem can be the presence of inhibitory compounds, change of temperature and pH value, decrease of the possibility of collision between the host and phage, microbial load, forming a mechanical barrier to the phage receptor regions.

Another factor is the phage titer used for food application. The highest titer of phages should be used in biocontrol studies as much as possible. If there are how much more phages used in the biocontrol assays, the higher the probability that phage will capture the target bacteria.

Table 1. *Salmonella* Phages

Phage	Host	Isolation Source	Reference
SH17 SH18 SH19	<i>S. Typhimurium</i> WT	Sewage	Hooton et al. 2011
SPN3US CJ07	<i>S. Enterica</i> <i>S. Enteritidis</i>	Chicken sewage Sewage	Lee et al. 2011 Lim et al.2012
vB_SenM-PA13076 (PA 13076) vB_SenM-PC2184 (PC 2184) STP4-a SE07	<i>S. Enteritidis</i> ATCC13076 <i>S. Enteritidis</i> CVCC2184 <i>S. Typhimurium</i> ATCC 14028 <i>S. Enteritidis</i>	Chicken sewage Chicken sewage Sewage Retail chicken meat	Bao et al. 2015 Li et al. 2016 Thung et al. 2017
Af1-Ka  Af3-Ka	<i>S. Enteritidis</i>  <i>S. Enteritidis</i>	Commercial broiler facilities Commercial broiler facilities	Ata 2018
LPSE1 Sal-PE	<i>S. Enteritidis</i> ATCC 13076 <i>S. Enteritidis</i>	Ready-to-eat foods Egg	Huang et al. 2018 Khan et al. 2018
ST-Phage-4 SE-Phage-14  ST-Phage-21 SE-Phage-24	<i>S. Typhimurium</i> LT2 SR II <i>S. Enteritidis</i> DMC8 <i>S. Typhimurium</i> Wild type 14028 <i>S. Enteritidis</i> DMC22	Rivers Sewage Food processing waste water Fisheries waste water	Yıldırım et al. 2018
vB_SpuM_SP116	<i>S. Pullarum</i> SPu 116	Chicken feces	Bao et al. 2019
vB_SnwM_CGG4-1 vB_SnwM_CGG4-2 vB_SnwM_CGG3-1 vB_SnwM_CGG3-2	<i>S. Newport</i>	Sewage water samples	El-DougDoug et al. 2019

The last one is the chemical composition of the food matrix in biocontrol studies. Among these factors, food factors are significant to play decisive role in between phage and target bacteria (Garcia-Anaya et al., 2020). The application of phages as a cocktail rather than individual studies to control of bacterial population could increase the reduction in the number of bacteria in biocontrol studies (Wong et al. 2020). Moreover, previous researches (Hooton et al. 2011, Zinno et al. 2014, Thung et al. 2017, Zhang et al. 2019, Wong et al. 2020) have shown that if phage application time increases in food for controlling of pathogen bacteria, the antimicrobial effect of the phage against that strains decreases. For this reason, it is necessary to choose the most suitable incubation time according to the phage-type in that assays (Thung et al. 2017).

Phages effectively used to control of the *Salmonella* contamination has been researched in various food samples so far (Guenther et al. 2012, Zinno et al. 2014, Bao et al. 2015, Thung et al. 2017, Wong et al. 2020). In these studies, more than 2 log unit reduction of *Salmonella* have been detected. For example, Guenther et al. (2012) tested the potential and effectiveness of phage FO1-E2 on the control of *S. Typhimurium* in different ready-to-eat foods including seafood (cooked and chilled cocktail of shrimps, squid and shellfish), hot dogs (wiener sausages), cooked and sliced turkey breast, pasteurized egg yolk and chocolate milk (whole milk with cocoa and sugar added). *S. Typhimurium* was experimentally contaminated into these food samples at the rate of  $10^3$  CFU/g or CFU/mL, and then also treated with  $3 \times 10^8$  PFU/mL phage FO1-E2 at 8°C or 15°C for 6 days. The viable number of *Salmonella* in foods stored at 8°C decreased significantly,

and no viable cells remained. *S. Typhimurium* counts were dramatically decreased by 5 log units in chocolate milk and on turkey deli meat samples at 15°C, whereas the reduction of the bacterium was found by 3 log units on hot dogs and seafood. The other study was done by Zinno et al. (2014) who studied that phage P22 was applied to various food samples including liquid eggs, energy drinks, whole and skimmed milk, apple juice, chicken breast and chicken mince with *S. Typhimurium* for 24 and 48 h at 4 °C. When  $10^4$  CFU/g host inoculum was used in biocontrol assays, reduction of the bacteria was approximately 2-3 log cycles compared to phage-free controls in all food matrices after 48 h at 4 °C. Moreover, the reduction rate of wild strains belonging to the serotypes Typhimurium, Enteritidis, Derby Give, Newport, Muenchen, and Muenster were also investigated using P22 phage in this study. Only isolates of *S. Typhimurium* as well as *S. Derby* and *S. Enteritidis* was inhibited by the presence of P22 phage. The authors emphasized in this study that food matrices either liquid or solid did not seem to affect the phage ability of *Salmonella* infection compared to similar tests performed *in vitro*. The effectiveness of phage PA 13076 and PC 2184 on pasteurized whole milk, chicken breast, and Chinese cabbage was investigated by Bao et al. (2015). All of the samples were firstly contaminated by  $10^4$  CFU/gr, either individual *S. Enteritidis* or a mixture of these strains (ATCC 13076 and CVCC 2184), and then, inoculated with an individual phage or a two-phage cocktail at  $10^8$  PFU/mL. These mixtures were incubated at 4°C or 25°C for 5 h. In all food samples except for Chinese cabbage, the inhibitory effect of phage and phage cocktail application at 4°C was found to be better than at 25°C. The reduction of viable *Salmonella* count in all tested samples was detected

in the range of 1.5-4.0 log CFU. The authors especially noted that the phage cocktail application was more effective than either single phage against pathogen bacteria. Thung et al. (2017) also demonstrated that applying SE07 lytic *Salmonella* phage in different retail food samples, which include fruit juice, liquid egg, beef and chicken meat experimentally contaminated with *S. Enteritidis*. Liquid foods including fruit juice, and liquid egg were contaminated with *S. Enteritidis* at a rate of approximately  $10^4$  CFU/mL and phage suspension ( $10^{11}$  PFU/mL). They showed that a significant reduction of viable *S. Enteritidis* (approximately 2 log cycles) in both samples at 4°C and 48 h. Moreover, within the same period, cell loads were reduced by 2.1 and 2.0 log cycles on the phage treated sliced beef and chicken meat samples, respectively. Another study was also reported by Huang et al. (2018) who determined that the reduction of *S. Enteritidis* ATCC 13076 in ready-to-eat foods (milk, lettuce and sausage) using LPSE1 phage. Phage LPSE1 application at 28°C in milk samples was reduced recoverable *S. Enteritidis* by approximately 1.44 log<sub>10</sub> CFU/mL and 2.37 log CFU/mL at MOI of 1 and 100, respectively, compared to untreated samples. Similarly, in sausage samples upon administration of LPSE1, *Salmonella* count decreased 0.52 log at MOI of 1. On the other hand, the bacterium count decreased 0.49 log<sub>10</sub> at 4°C at MOI of 100. On lettuce samples incubated with LPSE1, viable cell number reduced by 2.02 log<sub>10</sub>, 1.71 log<sub>10</sub>, and 1.45 log<sub>10</sub> CFU/mL at MOI of 1, 10, and 100, respectively. Taş (2018) examined that the lytic activity of 4 different StypH-phages against *S. Typhimurium* in the form of individual and cocktail in UHT milk during storage at either refrigerator or room temperatures. UHT milk samples contaminated with *S. Typhimurium* at the level of  $10^3$  CFU/mL were treated with STypH-phages at  $10^9$  PFU/mL level, and then stored at refrigerator or room temperature. When the STypH-phages were applied at the level of  $10^9$  PFU/mL in both forms to UHT milk samples contaminated with *S. Typhimurium* at the level of  $10^3$  CFU/mL, the viable cells number of their host bacteria in all samples kept at refrigerated or room temperatures decreased to undetectable level during storage. The effectiveness of a commercial SalmoFresh™ phage cocktail to reduce *Salmonella* on the surface of lettuce, and mung bean sprouts at different temperatures and storage times was determined by Zhang et al. (2019). SalmoFresh™ was applied to the surface of samples that were spot-inoculated with a five *Salmonella* strain mixture (Newport, Braenderup, Typhimurium, Kentucky, and Heidelberg). After the treatments with phage mixture ( $10^8$  PFU/mL) either spraying or immersion for 5 h at 25°C, spraying SalmoFresh™ onto lettuce and sprouts reduced *Salmonella* counts by 0.76 and 0.83 log<sub>10</sub> CFU/g respectively, and application of immersion of phage was decreased *Salmonella* by 2.43 and 2.16 log<sub>10</sub> CFU/g on lettuce and sprouts, respectively. The authors especially emphasized that immersion of produce in a phage solution was better at killing *Salmonella* than spraying. Wong et al. (2020) who studied that seven *S. Enterica* strains (S3, S200, S203, S2, S193, S194, and S195) at a rate of approximately  $10^5$  CFU/mL were singly inoculated on the surface of Romaine lettuce leaf and cantaloupe tissues treated with a phage cocktail including of equal proportions of five lytic

phages at the density of approximately  $2.5 \times 10^8$  PFU/cm<sup>2</sup> at 8°C for 24 h. While population of S3, S200 and S203 on Romaine lettuce leaf were reduced by up to 4 log CFU/cm<sup>2</sup>, reduction of viable cells S2, S193, and S194 were lower, ranging between approximately 1 and 2 log CFU/cm<sup>2</sup>. On the other hand, on cantaloupe tissues, populations of S3, S200 and S203 were reduced approximately 2-3 log CFU/cm<sup>2</sup>, those of S2, S193 and S194 were decrease by approximately 0.5-1.5 log CFU/cm<sup>2</sup>. The authors implied that magnitude of the effect of the phage applications was strain dependent.

## Conclusion

Foodborne diseases are responsible for high levels of morbidity and mortality in the general population in the world. Today, many studies involving phage for the biocontrol of foodborne pathogens have been undertaken with promising outcomes. Researches on the biocontrol of *Salmonella* species in a number of foods have generally produced positive results. However, most of them have focused on the use of phage to reduce carriage of *Salmonella* in poultry rather than as a food additive, so additional studies are necessary to isolate different *Salmonella* phages and to determine their potential for biocontrol studies in food systems.

## References

- Anonymous, 2020a. Available from: <https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi> Access Date: June 13, 2020.
- Anonymous, 2020b. Available from: <https://www.cdc.gov/salmonella/index.html> Access Date: June 13, 2020.
- Anonymous, 2020c. Available from: <https://www.efsa.europa.eu/en/topics/topic/salmonella> Access Date: June 11, 2020.
- Anonymous, 2020d. Available from: <https://phageguard.com/salmonella-solution/> Access Date: June 1, 2020.
- Ata Z. 2018. Türkiye’de Sık Rastlanan *Salmonella* Enteritidis Serovarlarına Spesifik Bakteriyofajların İzolasyonu. Etlik Veteriner Mikrobiyoloji Dergisi, 29 (2): 136-142.
- Bao H, Zhang P, Zhang H, Zhou Y, Zhang L, Wang R. 2015. Bio-Control of *Salmonella* Enteritidis in Foods Using Bacteriophages. Viruses, 7: 4836-4853. <https://doi.org/10.3390/v7082847>
- Bao H, Shahin K, Zhang Q, Zhang H, Wang Z, Zhou Y, Zhang X, Zhu S, Stefan S, Wang R. 2019. Morphologic and genomic characterization of a broad host range *Salmonella enterica* serovar Pullorum lytic phage vB\_SpuM\_SP116. Microbial Pathogenesis, 136:103659. <https://doi.org/10.1016/j.micpath.2019.103659>
- Cadioux B, Colavecchio A, Jeukens J, Freschi L, Emond-Rheault JG, Kukavica-Ibrulj I, Levesque RC, Bekal S, Chandler JC, Coleman SM, Bisha B, Goodridge LD. 2018. Prophage induction reduces Shiga toxin producing *Escherichia coli* (STEC) and *Salmonella enterica* on tomatoes and spinach: A model study. Food Control, 89: 250-259. <https://doi.org/10.1016/j.foodcont.2018.02.001>
- Chen HM, Wang Y, Su LH, Chiu CH. 2013. Nontyphoid *Salmonella* Infection: Microbiology, Clinical Features and Antimicrobial Therapy. Pediatrics and Neonatology, 54: 147-152. <https://doi.org/10.1016/j.pedneo.2013.01.010>
- Choińska-Pulit A, Mituła P, Śliwka P, Łaba W, Skaradzińska A. 2015. Bacteriophage encapsulation: Trends and potential applications. Trends in Food Science & Technology, 45(2): 212-221. <https://doi.org/10.1016/j.tifs.2015.07.001>

- Dalyan Cilo B, Özmerdiven GE, Efe K, Güleşen R, Levent B, Ağca H, Sınırtaş M, Özakin C. 2015. Güney Marmara Bölgesinde İzole Edilen *Salmonella* Serotiplerinin Dağılımı ve Antibiyotik Duyarlılıklar. *Türk Mikrobiyoloji Cemiyeti Dergisi*, 45(3): 122-127. <https://doi.org/10.5222/TMCD.2015.122>
- Duc HM, Son HM, Yi HPS, Sato J, Ngan PH, Masuda Y, Honjoh K, Miyamoto T. 2020. Isolation, characterization and application of a polyvalent phage capable of controlling *Salmonella* and *Escherichia coli* O157:H7 in different food matrixes. *Food Research International*, 131: 108977. <https://doi.org/10.1016/j.foodres.2020.108977>
- El-Dougdoug NK, Cucic S, Abdelhamid AG, Brovko L, Kropinski AM, Griffiths MW, Anany H. 2019. Control of *Salmonella* Newport on cherry tomato using a cocktail of lytic bacteriophages. *International Journal of Food Microbiology*, 293: 60-71. <https://doi.org/10.1016/j.ijfoodmicro.2019.01.003>
- Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG, Lee LH. 2015. *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science*, 8(3): 284-293. <https://doi.org/10.1080/21553769.2015.1051243>
- Ergin F, Yıldız G, Çomak Göçer EM, Küçükçetin A. 2017. Bakteriyofajların Antibakteriyel Ajan Olarak Kullanımı. *Akademik Gıda*, 15(2): 172-181. <https://doi.org/10.24323/akademik-gida.333674>
- Garcia P, Rodriguez L, Rodriguez A, Martinez B. 2010. Food biopreservation: Promising strategies using bacteriocins, bacteriophages and endolysins. *Trends in Food Science & Technology*, 21: 373-382. <https://doi.org/10.1016/j.tifs.2010.04.010>
- Garcia-Anaya MC, Sepulveda DR, Saenz-Mendoza AI, Rios-Velasco C, Zamudio-Flores P, Acosta-Muniz CH. 2020. Phages as biocontrol agents in dairy products. *Trends in Food Science and Technology*, 95: 10-20. <https://doi.org/10.1016/j.tifs.2019.10.006>
- Gómez-Baltazar A, Vázquez-Garcidueñas MS, Larsen J, Kuk-Soberanis ME, Vázquez-Marrufo G. 2019. Comparative stress response to food preservation conditions of ST19 and ST213 genotypes of *Salmonella enterica* serotype Typhimurium. *Food microbiology*, 82: 303-315. <https://doi.org/10.1016/j.fm.2019.03.010>
- Gouvea DM, Mendonça RCS, Soto L, Cruz RS. 2015. Acetate cellulose film with bacteriophages for potential antimicrobial use in food packaging. *LWT-Food Science and Technology*, 63: 85-91. <https://doi.org/10.1016/j.lwt.2015.03.014>
- Grygorcewicz B, Grudziński M, Wasak A, Augustyniak A, Pietruszka A, Nawrotek P. 2017. Bacteriophage-mediated reduction of *Salmonella* Enteritidis in swine slurry. *Applied Soil Ecology*, 119: 179-182. <https://doi.org/10.1016/j.apsoil.2017.06.020>
- Guenther S, Herzig O, Fieseler L, Klumpp J, Loessner MJ. 2012. Biocontrol of *Salmonella* Typhimurium in RTE foods with the virulent bacteriophage FO1-E2. *International Journal of Food Microbiology*, 154: 66-72. <https://doi.org/10.1016/j.ijfoodmicro.2011.12.023>
- Gülener, M. 2015. Farklı Kaynaklardan İzole Edilen *Salmonella* Suşlarında Virulans Faktörlerinin Genotipik Olarak İncelenmesi. Yüksek Lisans Tezi, İstanbul Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı, İstanbul, Türkiye.
- Gündoğdu A, Ulu-Kılıç A. 2018. Bakteriyofaj Terapisi: Unutulmuş Bir Şifa Kaynağı. *Klimik Dergisi* 31(2): 78-87. <https://doi.org/10.5152/kd.2018.22>
- Hendriksen RS, Vieira AR, Karlsmose S, Lo Fo Wong DM, Jensen AB, Wegener HC, Aarestrup FM. 2011. Global monitoring of *Salmonella* serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: Results of quality assured laboratories from 2001 to 2007. *Foodborne Pathogens and Disease*, 8(8): 887-900. <https://doi.org/10.1089/fpd.2010.0787>
- Heredia N, Garcia S. 2018. Animals as sources of food-borne pathogens: A review. *Animal Nutrition*, 4: 250-255. <https://doi.org/10.1016/j.aninu.2018.04.006>
- Hooton PTS, Atterbury RJ, Connerton IF. 2011. Application of a bacteriophage cocktail to reduce *Salmonella* Typhimurium U288 contamination on pig skin. *International Journal of Food Microbiology*, 151: 157-163. <https://doi.org/10.1016/j.ijfoodmicro.2011.08.015>
- Huang C, Virk SM, Shi J, Zhou Y, Willas SP, Morsy MK, Abdelnabby HE, Liu J, Wang X, Li J. 2018. Isolation, Characterization and Application of Bacteriophage LPSE1 against *Salmonella enterica* in Ready to Eat (RTE) Foods. *Frontiers in Microbiology*, 9, 1046: 1-11. <https://doi.org/10.3389/fmicb.2018.01046>
- Jajere SM. 2019. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Veterinary World*, 12(4): 504-521. <https://doi.org/10.14202/vetworld.2019.504-521>
- Jorguera D, Navarro C, Rojas V, Turra G, Robeson J, Borie C. 2015. The use of a bacteriophage cocktail as a biocontrol measure to reduce *Salmonella enterica* serovar Enteritidis contamination in ground meat and goat cheese. *Biocontrol Science and Technology*, 25(8): 970-974. <https://doi.org/10.1080/09583157.2015.1018815>
- Khan AS, Ashik MR, Rahman SR. 2018. Isolation of Bacteriophage from Eggs and Use of Excreta as Biocontrol Agent in Controlling Eggs Related Salmonellosis. *Bangladesh Journal of Microbiology*, 35(1): 37-44.
- Lamas A, Miranda JM, Regal P, Vazquez B, Franco CM, Cepeda A. 2018. A comprehensive review of non-enterica subspecies of *Salmonella enterica*. *Microbiological Research*, 206: 60-73. <https://doi.org/10.1016/j.micres.2017.09.010>
- Lee JH, Shin H, Kim H, Ryu S. 2011. Complete genome sequence of *Salmonella* bacteriophage SPN3US. *Journal of Virology*, 85(24):13470-13471. <https://doi.org/10.1128/JVI.06344-11>
- Lee KM, Runyon M, Herrman TJ, Phillips R, Hsieh J. 2015. Review of *Salmonella* detection and identification methods: Aspects of rapid emergency response and food safety. *Food Control*, 47: 264-276. <https://doi.org/10.1016/j.foodcont.2014.07.011>
- LeLievre V, Besnar A, Schlusshuber M, Desmases N, Dalmaso M. 2019. Phages for biocontrol in foods: What opportunities for *Salmonella* sp. control along the dairy food chain? *Food Microbiology*, 78: 89-98. <https://doi.org/10.1016/j.fm.2018.10.009>
- Li M, Li M, Lin H, Wang J, Jin Y, Han F. 2016. Characterization of the novel T4-like *Salmonella enterica* bacteriophage STP4-a and its endolysin. *Archives in Virology*, 161(2):377-384. <https://doi.org/10.1007/s00705-015-2647-0>
- Lim TH, Kim MS, Lee DH, Lee YN, Park JK, Young HN, Lee HJ, Yang SY, Cho YW, Lee JB, Park SY, Choi IS, Song CS. 2012. Use of bacteriophages for biological control of *Salmonella* Enteritidis infection in chicken. *Research in Veterinary Science*, 93: 1173-1178. <https://doi.org/10.1016/j.rvsc.2012.06.004>
- Luo Z, Simmons CW, VanderGheynst JS, Nitin N. 2012. Quantitative real time measurements of bacteria-bacteriophages interactions in fresh lettuce leaves. *Journal of Food Engineering*, 111(1): 176-185. <https://doi.org/10.1016/j.jfoodeng.2011.12.025>
- Martínez B, García P, Rodríguez A. 2019. Swapping the roles of bacteriocins and bacteriophages in food biotechnology. *Current Opinion in Biotechnology*, 56: 1-6. <https://doi.org/10.1016/j.copbio.2018.07.007>
- Mostafa MM, Nassef M, Badr A. 2016. Computational determination of the effects of 2220virulent *Escherichia coli* and *Salmonella* bacteriophages on human gut. *Computer Methods and Programs in Biomedicine*, 135: 27-35. <https://doi.org/10.1016/j.cmpb.2016.07.018>

- Mukhopadhyay S, Ramaswamy R. 2012. Application of emerging technologies to control *Salmonella* in foods: A review. Food Research International, 45: 666-677. <https://doi.org/10.1016/j.foodres.2011.05.016>
- Nobrega F, Costa A, Santos J, Siliakus, MF, van Lent JWM, Kengen SWM, Azeredo J, Kluskens LD. 2016. Genetically manipulated phages with improved pH resistance for oral administration in veterinary medicine. Scientific Report, 6: 39235. <https://doi.org/10.1038/srep39235>
- Pereira C, Moreirinha C, Rocha RJ, Calado R, Romalde JL, Nunes ML, Almeida A. 2016. Application of bacteriophages during depuration reduces the load of *Salmonella* Typhimurium in cockles. Food Research International, 90: 73-84. <https://doi.org/10.1016/j.foodres.2016.10.031>
- Pulido-Landinez M. 2019. Food safety-*Salmonella* update in broilers. Animal Feed Science and Technology, 250: 53-58. <https://doi.org/10.1016/j.anifeedsci.2019.01.008>
- Robeson J, Turra G, Huber K, Borie C. 2014. A note on stability in food matrices of *Salmonella enterica* serovar Enteritidis controlling bacteriophages. Electronic Journal of Biotechnology, 17: 189-191. <https://doi.org/10.1016/j.ejbt.2014.06.001>
- Sánchez-Vargas FM, Abu-El-Haija MA, Gómez-Duarte OG. 2011. *Salmonella* infections: An update on epidemiology, management, and prevention. Travel medicine and infectious disease, 9(6): 263-277. <https://doi.org/10.1016/j.tmaid.2011.11.001>
- Şanlıbaba P, Uymaz B. 2015. Gıdalarda *Listeria monocytogenes*'in Biyokontrolünde Faj Uygulaması. Akademik Gıda, 13(1): 81-88.
- Şanlıbaba P, Tezel Uymaz B. 2017. Control of Food-Borne Pathogens Growth Using Bacteriophage", Science within Food: Up-to-date Advances on Research and Educational Ideas, Ed: Méndez-Vilas, A., Formatex Research Center, page: 53-61, Spain. ISBN-13 Collection: 978-84-947512-1-9.
- Schmelcher M, Loessner MJ. 2014. Application of bacteriophages for detection of foodborne pathogens. Bacteriophage, 4(2): e28137. <https://doi.org/10.4161/bact.28137>
- Sukumaran AT, Nannapaneni R, Kiess A, Sharma CS. 2015. Reduction of *Salmonella* on chicken breast fillets stored under aerobic or modified atmosphere packaging by the application of lytic bacteriophage preparation SalmoFresh™. Poultry Science, 95: 668-675. <https://doi.org/10.3382/ps/pev332>
- Taş B. 2018. *Salmonella* Typhimurium ve *Escherichia coli* O157:H7'nin Sütte Gelişimi Üzerine Bakteriyofajların Etkisi. Yüksek Lisans Tezi, Niğde Ömer Halisdemir Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği Anabilim Dalı, Niğde, Türkiye.
- Thung TY, Premarathne JMKJK, San Chang W, Loo YY, Chin YZ, Kuan CH, Tan CW, Basri DF, Radzi CWJWM, Radu S. 2017. Use of a lytic bacteriophage to control *Salmonella* Enteritidis in retail food. LWT, 78: 222-225. <https://doi.org/10.1016/j.lwt.2016.12.044>
- Uğur E. 2018. Çiğ Sütten Yapılan Beyaz Peynirde Ticari Faj Preparatlarıyla Patojen Bakterilerin Kontrolü. Yüksek Lisans Tezi, Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, Gıda Mühendisliği Anabilim Dalı, Isparta, Türkiye.
- Vaz CSL, Voss-Rech D, Alves L, Coldebella A, Brentano L, Trevisol IM. 2020. Effect of time therapy with wild-type lytic bacteriophages on the reduction of *Salmonella* Enteritidis In broiler chickens. Veterinary Microbiology, 240: 108527. <https://doi.org/10.1016/j.vetmic.2019.108527>
- Wong CWY, Delaquis P, Goodridge L, Levesque RC, Fong K, Wang S. 2020. Inactivation of *Salmonella enterica* on post-harvest cantaloupe and lettuce by a lytic bacteriophage cocktail. Current Research in Food Science, 2: 25-32. <https://doi.org/10.1016/j.crfs.2019.11.004>
- Yıldırım Z, Sakın T, Çoban F. 2018. Isolation of Lytic Bacteriophages Infecting *Salmonella* Typhimurium and *Salmonella* Enteritidis. Acta Biologica Hungarica, 69:350-369 (2018). <https://doi.org/10.1556/018.68.2018.3.10>
- Zhang X, Niu YD, Nan Y, Stanford K, Holley R, McAllister T, Narvaez-Bravo C. 2019. SalmoFresh effectiveness in controlling *Salmonella* on romaine lettuce, mung bean sprouts and seeds. International Journal of Food Microbiology, 305: 108250. <https://doi.org/10.1016/j.ijfoodmicro.2019.108250>
- Zinno P, Devirgiliis C, Ercolini D, Ongeng D, Mauriello G. 2014. Bacteriophage P22 to challenge *Salmonella* in foods. International Journal of Food Microbiology, 191: 69-74. <https://doi.org/10.1016/j.ijfoodmicro.2014.08.037>