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Biocontrol Effect of Lytic Bacteriophages against Various Foodborne Diseases

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Foodborne disease is one of the major causes of hospitalization and death around the world. Many advance antimicrobial techniques, food sanitation techniques are present nowadays but still Foodborne diseases are become more serious day by day. Some traditional well known antimicrobial methods including chemical treatment, pasteurization, high pressure processing, and irradiation are some popular techniques to control bacteria causing Foodborne diseases but they have several drawbacks like high cost, machine and processing equipment damage, damage nutritive value and organoleptic properties of foods and more importantly adverse effect on health. In this situation most promising and safe technique is biocontrol method. The interest for natural antimicrobial agent has exhibited due to consumer awareness towards the use of chemical based pathogen control methods or preservatives in food processing sectors. Use of bacteriophage is one of the most useful and promising natural biocontrol methods that targets specific strains of bacteria and kill the specific bacterial cell (or inhibit bacterial cell count). Bacteriophages can control foodborne disease outbreaks and ensure food safety by four different stages including therapy, biocontrol, biosanitation, and preservation. Bacteriophages are easily available in the environment and can be used safely in various foods ranging from fresh fruits, perishable animal product, and vegetables to ready-to-eat food products for bacterial decontamination. Approved commercial bacteriophages are also available to ensure food safety. bacteriophage biocontrol is recently recognized as an alternative method to reducing pathogenic bacteria from foods naturally and secure food safety. This review work is a brief overview of current bacteriophage related work in the field of foodborne diseases and food safety.

Keywords : Biological preservation , Bacteriophages , Food born disease & Biocontrol.

Consumption of contaminated food stuffs during any step of pre harvest, post harvest, storage, delivery and consumption process cause food-borne diseases. Wide range of pathogenic microorganisms like virus (4%), bacteria (66%), fungi, parasites (4%) and microorganism derived toxins and some harmful chemicals (26%) are some main causes of food borne diseases. Currently bacteria causing foodborne disease is the most prevalent public health problem globally. Bacteria contributes two third of food borne disease including 250 types of different diseases. 31 pathogens have been detected that resulting foodborne diseases, but among them some bacterial pathogens like *Salmonella* species, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter* species, *Escherichia coli* are most common. Animal food products including meat,

egg, milk, fish and their derived food products are the main carriers of foodborne infections because their high nutritional value(high lipid and protein content) is very appropriate for bacterial growth. Use of contaminated water for food processing, preparation, cleaning purposes, inadequate storage conditions, inappropriate food handling practices, active infectious food workers and cross-contamination are some common favorable conditions for easy spreading of food borne infection. According to WHO contaminated foods affects near about one out of ten people worldwide around the world resulting in more than 420000 deaths every year and loss of 33 million healthy life years (DALYs). General consequences of foodborne diseases are diarrhea, stomach upset, stomach cramps, nausea, vomiting. Symptoms may visible after hours or days after consumption of contaminated foods. Symptoms like high fever (over 102°F), bloody stools, dehydration, frequent vomiting and diarrhea (present more than 3 days) are visible during severe condition. Some time severe case can cause death also. Foodborne disease is one of the major public health concerns which are not only prevalent in lower middle income countries but also widely observe across the developed countries (WHO, 2018). Under 5 years usually carry almost 40% of the foodborne disease along with 125 000 deaths every year. Every year 550 million people shows diarrheal diseases with 230 000 deaths due to contaminated foods. Nutrition, food safety and food security are directly inter related to each other, long term or frequent onset of foodborne disease condition leads to malnutrition which particularly risky for vulnerable groups such as infants, young children, sick, elderly person(WHO, 2020). Vulnerable groups of the community like older people, infant, children, pregnant women, older people, and those people with immune deficiency, diabetes, renal problems, liver problems, organ transplant, HIV/AIDS, cancer are more prone to develop foodborne diseases. According to Centre for Disease Control and Prevention every year foodborne infection affects 48 million people across America with 128000 hospitalization and 3000 deaths (CDC Foodborne germs, 2020) (CDC symptoms, 2020) (Todd, 2020). "Hurdle approach" that is combination of several physical and antimicrobial methods build the new focus area of food processing industry due its negligible amount of impact on the nutritional value and organoleptic properties of food materials. Physical technologies that use high thermal processing methods can damage the quality of the foods. Bacteriophage application is one of the effective techniques against foodborne pathogens. Recently biocontrol approach by using lytic bacteriophage based treatment has gained public interest because of its wide availability, mode of action, specificity against foodborne pathogens, minimal to no adverse effect on organoleptic properties of foods. Lytic bacteriophage not only effective approach to control foodborne pathogens but also bacteriopahge has efficacy to control biofilm and spoilage forming microorganisms (Abdelhamid *et al.,* 2020). Frequent or long term use of chemical control methods, chemical preservatives, sanitizers against foodborne disease causing pathogens have adverse effect not only on human health but also on organoleptic properties and consequently develop resistant bacteria. Nowadays conscious consumers only prefer organic foods without any chemical treatment and biocontrol is a novel significant approach as it is a natural method to control harmful bacterial infection and may overcome many hurdles or disadvantages of ongoing food processing and preservation technologies. Biocontrol method use biological factors against various microorganism mainly pathogenic bacteria. Usually bacteriophage treatments used for decontamination at three stages, those are Pre-harvest stage to control, surface decontamination in food processing industries and other food establishment during processing and storage and lastly post-harvest stage through direct application of bacteriophage treatment against foodborne pathogens. Some bacteriophage based FDA approved commercial products are EcoShieldTM, ListShieldTM, and SalmoFreshTM that are used as food additives. bacteriophage based products like PLSV-1TM and INT-401TM used in veterinary purpose. United States Environmental Protection Agency (EPA) approved ListShieldTM is used against *Listeria monocytogenes* for surfaces decontamination in food-processing industries. *Salmonella enterica* specific SalmoFreshTM is also consider as a safe approach (GRAS= Generally Regarded as Safe). Bacteriophage based commercial formulations like Listex P100 TM, EcoShieldTM, ListShieldTM

are now successfully used in dairy, meat, farm, and marine products (Ngene *et al.,* 2020). Bacteriophage based biological control strategy is more stable and long lasting compared to physical or chemical controls techniques. Isolation processes of bacteriophage are less expensive and relative easy than any other chemical, physical and other biological control measures. Bacteriophage application as a biocontrol agent is harmless and safe to environment and human beings and animals.

Overview of Bacteriophage Biology

Bacteriophages, also called phages (bacteria eaters) are bacterial viruses which infect and replicate inside bacterial host cells only. Bacteriophage has the ability to kill the specific host bacteria by infecting the host cell. In 1915 William Twort first discoved bacteriophages. After that in 1917 Felix d'Herelle stated that bacteriophage bacteria killing potentiality. Near about 96% of total bacteriophages are comes under tailed phages only and rest of the part comes under non-tailed category (Garvey, 2020). They are abundantly present on earth in different size, genomic structure, and morphology with number of 10^{31}. According to Committee on the Taxonomy of Viruses (ICTV) 19 phage families are present till the date. Bacteriophages are come under families of *Myoviridae*, being *Myoviridae*, *Podoviridae, Siphoviridae*, *Microviridae, Inoviridae*, and two more are *Ackermannviridae* and *Herelleviridae (*recently described*)*. Single or double-stranded DNA or RNA can be present inside the bacteriophage genome inside the capsid protein (Sieiro *et al.,* 2020). Bacteriophages are host specific and infect only bacteriophage specific bacterial host cells by attaching to specific host. Bacteriophage follows generally two replication cycle, one is lytic cycle and another is lysogenic cycle. In case of lytic cycle bacteriophage attaches to a specific bacterial host cell and insert the bacteriophage genome into the host cell that convert host cell genome into viral genome in a rapid way and then bacteriophage replicate to produce multiple copies. Finally newly formed bacteriophage copies destroy the host cell to come out from the host cell and infect other specific host cells. This type of bacteriophage also called virulent phage. In the lysogenic cycle the bacteriophage also attaches host bacterium but cannot lyse the host cells and become a part of host chromosome. Bacteriophage that follows lysogenic replication cycle called temperate bacteriophage (Kasman *et al.,* 2020).

Advantages of Bacteriophage

Bacteriophage can infect and lyse only specific bacterial species, due to this kind of specificity and narrow spectrum of antibacterial activity bacteriophage have negligible or no effect on normal microflora compare to antibiotics wide host range. Bacteriophages are self replicating. Bacteriophages are widely available in the environment and isolation processes of bacteriophages are normally easy and relative inexpensive than other biocontrol process or antibiotic therapies. It is also effective against multi drug resistant pathogens and can be used in combination with antibiotics or instead of antibiotic therapy. Different host specific bacteriophages can be combined to make cocktails, to increase host range, address pathogen diversity and emergence of resistance bacteria. Due to weak immunogens bacteriophages cannot stimulate functions of antibody and inflammatory response in human body (Nikolich *et al.,* 2020). Bacteriophage cocktails are effective against wide host range of pathogenic bacteria and may overcome the risks related to bacteroophage resistant microorganisms (Hudson *et al.,* 2005). Bacteriophage encoded lysins enzymes, which also known as antibacterial agents can be actively used in food processing and food preservation industries to reduce pathogenic microorganisms and ensure food safety. Lysin in purified protein form can be used directly to the food products or feed. Lysins destroy bacterial peptidoglycan layer at the final stage of bacteriophage replication process to release newly form bacterophages from host cell. Lysin enzyme is very effective against gram-positive bacteria because this enzyme attacks peptidoglycan layer of bacterial cell wall. Though lysin has narrow host range but lytic bacteriophage produced lysin enzyme is a novel concept to reduce antibiotic resistant bacteria. Lysin based biocontrol treatment can also used against pathogen produced biofilms and for rapid pathogen detection (Chang, 2020) (Kazi *et al.,* 2016). Bacteriophages are not harmful for humans as well as animals. Bacteriophages don't have any adverse or toxic effect on eukaryotic cells. There is no effect of bacteriophages on the sensory properties of food products. In post harvest stage bacteriophage therapy can be given at the

time of food processing and packing to reduce pathogen contamination. Bacteriophages can also used in biosanitization process to kill biofilm producing pathogenic bacteria from the e surface of the equipments. In case of biopreservation, bacteriophage based therapy may directly added to food stuffs to inhibit spoilage causing bacteria and extend the expiry date of food (Po³aska *et al.,* 2019). Activity of bacteriophage largely depends on several factor, one of the important factors is the food matrix structure because physicochemical properties of food matrix can alter phage treatment. Another major factor is multiplicity of infection (MOI) that is the ratio of the total phages number to the total bacterial cells. Multiplicity of infection shows probability of interaction between pahe and host cell. External environmental conditions such as pH and temperature can also influence the activity of bacterophage. Extreme values of pH and temperature (high pH, low pH, and high temperatures) can inactivate bacteriophages activity (García *et al.,* 2019). Salt concentration of the solution may alter the bacteriophage stability under certain external environmental conditions and destroy bacteriophage activity because high salt concentration of the solution affects electrostatic pressure in the viral capsid through which genetic information of bacteriophage transfer into the host cell. Dehydrated or dry food materials may hamper bacteriophage diffusion to infect pathogens. Bacteriophage application in liquid samples can increase diffusion of bacteriophage and enhance the susceptibility of bacteriophage to attatch and incect the host microorganisms due high flow of the fluid and bacterial motility (Hudson *et al.,* 2005).

Bacteriophage Biocontrol against Foodborne Pathogens

Salmonella

Salmonella is gram-negative, hydrogen sulfide producing, motile bacteria from *Enterobacteriaceae* family. *Salmonella* is one of the leading causes of food poisoning across the world. Current taxonomy classified *Salmonella* into two species, one is *Salmonella enterica* and another one is *Salmonella bongori*. Serovars *typhi* and *paratyphi* cause typhoid and paratyphoid fever (enteric fever) and remaining *Salmonella enterica* serovars are nontyphoidal Salmonella (NTS) (Ajmera *et al.,* 2020). *Salmonella* is a ubiquitous microorganism which can tolerate water and dry weather for several months. Salmonellosis occurred due to *Salmonella* infection which causes abdominal pain, fever, diarrhoea, nausea and vomiting after 6–72 hours of consumption of contaminated foods. Foods from animal origin including meat, eggs, poultry, milk, and also vegetables are prone to become infected by *Salmonella*. As per World Health Organization Salmonella is one of the major causes of the diarrhoeal diseases out of the four key global causes (WHO, 2018). According to centre for disease control and prevention (CDC, 2020) *Salmonella* infection cause near about 1.35 million infection cases, 26,500 hospitalizations, and 420 deaths per year in the United States (CDC, 2020). Huang et al. (2018) collected samples for bacteriophage isolation from sewage near the river, wastewater treatment plant, chicken and pigs' feces farm ditch near the lake. They isolated bacteriophage by using host strain *Salmonella* strain ATCC 13076. They isolated wide amount of bacteriophages only from domestic sewage, poultry sources. They selected bacteriophage LPSE1 due to wide spectrum of lytic effect and applied to several ready to eat (RTE) food stuffs to prevent *Salmonella*. They confirmed the lytic effect of bacteriophage LPSE1 on *Salmonella* Enteritidis-ATCC13076 treated milk, sausage, and lettuce. At 28°C bacteriophage LPSE1 treated milk sample showed approximately 1.44 $\log_{10}$ CFU/mL and 2.37 $\log_{10}$ CFU/mL reduction of *Salmonella* concentration at MOI of 1 and 100. At 28°C bacteriophage LPSE1 reduced 0.52 $\log_{10}$ bacterial count at an MOI of 1 and at 4°C bacteriophage treatment decreased 0.49 $\log_{10}$ *Salmonella* count at MOI of 100from sausage samples. bacteriophage LPSE1 preparation successfully reduced *Salmonella* load by 2.02 $\log_{10}$. Incubation of LPSE1 on lettuce reduced recoverable *Salmonella* by 1.45 $\log_{10}$, 1.71 $\log_{10}$ and 2.02 $\log_{10}$, CFU/mL at an MOI of 100, 10, and 1, respectively compared to the negative control. Islam et al. (2020) selected environmentally sourced water samples to isolate bacteriophages and isolated 42 phages against *Salmonella enterica* host strain. Finally they selected only three *Salmonella*-specific bacteriophage named LPSTLL, LPST94 and LPST153 due to broad antibacterial spectrum. Phage cocktail prepared by three bacteriophages (LPSTLL, LPST94 and LPST153) in 1:1:1 ratio. A significant decrease

was observed in *Salmonella* with a viable count of 3 $\log_{10}$ CFU in milk and chicken breast at either 25 °C or 4 °C. Phage cocktail composed of 1:1:1 mixture of phage LPSTLL, LPST94 and LPST153 to evaluate its biological control effect against *Salmonella* infected milk and chicken breast. Milk and chicken breast samples were infected by either *Salmonella typhimurium* (ATCC 14028) or *Salmonella* culture mixture (*Salmonella typhimurium* ATCC 14028 and *Salmonella enteritidis* ATCC 13076) culture at 3 $\log_{10}$ CFU/mL concentration then bacteriophage cocktail preparation were added to samples and kept it at 4 °C and 25 °C to observe the change in bacterial load. Study stated that this bacteriophage cocktail preparation completely reduced *Salmonella* load from milk and chicken breast sample at both 4 °C and 25 °C. Islam et al. (2020) used *Salmonella enterica* (UK-1, ATCC 13311) strain for bacteriophages isolation. Among 40 *Salmonella* lytic bacteriophage they selected only bacteriophage LPST94 because this bacteriophage had highest bacteria lysis capability and broadest range. They experimented biological control activity of bacteriophage LPST94 in non-typhoidal *Salmonella* infected (3 $\log_{10}$ CFU/mL) milk, apple juice, chicken breast, and lettuce. Study revealed that bacteriophage LPST94 based preparation almost completely destroyed *Salmonella* count at both 4 °C (decreased by 3 $\log_{10}$ CFU/ml) and 25 °C (decreased by upto 2.56 $\log_{10}$ CFU/ml) within 48 hours. Study found bacteriophage LPST94 as promising biological control agents to prevent *Salmonella* causing infection in various food matrices. Kim et al., (2020) isolated four lytic bacteriophages by using *Salmonella enteritidis* from river connected to duck farm. They showed bacteriophage were from Myoviridae and Siphoviridae family. Isolated bacteriophages showed wide range of lytic effect against *Salmonella enteritidis* (11 strains), *Salmonella typhimurium* (11 strains), *Salmonella paratyphi* (1strain) and *Salmonella* typhi (1strain). Bacteriophage cocktail made from these four isolate bacteriophages and tested the antibacterial activity to reduce *Salmonella enteritidis*. Study showed bacteriophage cocktail preparation significantly decreased *Salmonella enteritidis* cell count in *Salmonella enteritidis* infected raw chicken breast samples ($P < 0.05$) at 4 °C for 7 days. They concluded that bacteriophage treatment could be a potential antibacterial agent against Salmonella spp. Another study conducted by Nabil *et al. (2018) confirmed efficiency of Salmonella lytic bacteriophages in poultry industry. They used isolated Salmonella typhimurium* and *Salmonella enteritidis* from diseased broiler chickens for further production of Bacteriophages. They choose environmental sewage samples for bacteriophage isolation. Study result suggested that oral administration of bacteriophage based treatment with five successive doses reduced *Salmonella enteritidis* and *Salmonella typhimurium* infection in cecum of broiler chicks within short time span.

Escherichia coli

Escherichia coli (*E. coli*) is a facultative anaerobic, Gram-negative, rod-shaped bacteria. Some of the strains (enterohemorrhagic *Escherichia coli*) produced Shiga toxins that lead to hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC) in humans. Examples of human pathogenic enterohemorrhagic *Escherichia coli* serotypes are O26:H11, O111:H8, O91:H21, O157:NM, and O157:H7 (Lim *et al.,* 2010). *Escherichia coli* O157: H7 that produce Shiga toxin is one of the harmful food and waterborne pathogen in humans and causes hemorrhagic colitis, diarrhea, and hemolytic-uremic syndrome (HUS), alimentary tract infection, abdominal cramps. *Escherichia coli* O157: H7 can be transmitted due to contaminated, undercooked food consumption, via fecal-oral route. In the United States every year near about 63,000 hemorrhagic colitis cases occur by *Escherichia coli* O157: H7 infection and 10 out of 14 sub-region studies conducted by world health organization showed the incidence rate of *E. coli* infection was approximately 2.8 million cases per year across the globe (Ameer et al., 2020).

One study conducted by Lu et al. (2015) showed the potentiality of a bacteriophage as a biocontrol agent in various foods against *Escherichia coli* O157: H7. They isolated a new *Escherichia coli* O157:H7 specific bacteriophage Ö241 by using industrial cucumber fermentation as sample. Study show bacteriophage Ö241 was from *Myoviridae* family and effectively lyse 48 different strains of *Escherichia coli* O157:H7. Bacteriophage showed significant lysis activity at MOI of 10, 3, and 0.3. Bacteriophage reduced 3.5 log of bacterial cell count after one hour of

incubation and 4.5 log reduction after two hour. Bacteriophage specificity towards *Escherichia coli* O157: H7 and high pH and salinity tolerance activity make this bacteriophage very effective biocontrol agent. Lee et al. (2020) conducted an experimental study to evaluate *Escherichia coli* O157: H7 lytic bacteriophage. They collected slaughterhouse samples for *Escherichia coli* O157: H7 specific bacteriophage which belonged to *Myoviridae* family (*Rb49virus* genus, subfamily *Tevenvirinae*) and named the bacteriophage (KFS-EC). This bacteriophage KFS-EC effective against 60 bacterial strains of *Escherichia coli* O157: H7. Study also detected that there was no pathogenicity and lysogenic property present in bacteriophages KFS-EC gene confirm its safe application. This bacteriophage had stability at pH 3-11, 20 °C-50 °C, biocides (0.1% peracetic acid, 0.1% citric acid, and 1% citric acid) and organic solvents including ethanol and chloroform. Bacteriophage (KFS-EC) effectively decreased inhibit *Escherichia coli* O157: H7 for 8 hours at MOI of 0.01 and retained its stability upto 12 weeks storage period at both 4 °C and 22 °C. Lee et al. (2016) took *Escherichia coli* O157: H7and *Shigella flexneri* for bacteriophage isolation because this two are well known food-borne pathogens that can cause food poisoning at low infectious doses also. They isolated bacteriophage HY01 (Myoviridae family) by using swine fecal sample which was effective against both pathogenic *Escherichia coli* O157: H7and *Shigella flexneri*. Study selected edible cabbage for bacteriophage HY01 food applications and infected by two strains of *Escherichia coli* O157: H7 (ATCC 43895 and ATCC 43890). More than 2 log reductions of bacterial load within 2 h of incubation were visible after bacteriophage treatment. Study concluded that due to presence of two different host specific tail genes bacteriophage HY01 were able to reduce *Escherichia coli* O157: H7as well as *Shigella flexneri* E. at the same time and it could be a new preservation or biocontrol agent against *Escherichia coli* O157: H7and *Shigella flexneri* pathogens. Hong et al. (2014) chose three *Escherichia coli* O157: H7 specific bacteriophage from bacteriophage library to prepare bacteriophage cocktail. Out of three two bacteriophage were from *Myoviridae* family and another one was under *Siphoviridae* family. For experiment bacteriophage cocktail preparation was added to *Escherichia coli* O157: H7 infected (10^7cfu at MOI of 1) ground beef, cheese slices and spinach leaves. 1.97 log10 cfu/mL, 0.56 log10 cfu/mL and 0.48 log10 cfu/mL bacterial count reduced ($P < 0.05$) from infected ground beef after 24 hours bacteriophage cocktail application at 24 °C, 46 °C and 4 °C respectively. In case of spinach sample bacteriophage treatment decreased ($P < 0.05$) bacterial count by 3.28, 2.88, and 2.77 log10 cfu/mL after 24, 48, and 72 h respectively at room temperature but bacteriophage was not effective against contaminated cheese. Son et al. (2018) used two types of *Escherichia coli* O157:H7 that produce Shiga toxin (STEC) and extended-spectrum beta-lactamase (ESBL) for bacteriophage isolation from bovine intestine. Isolated bacteriophage PE37 belonged to *Myoviridae* family. Bacteriophage PE37 was very effective against STEC O157:H7 and showed 4.9 and 2.6 log CFU/mL log reduction of bacterial count after 6 hours at 25 and 8 °C respectively in broth medium. After 24 hours of adding bacteriophage on STEC O157:H7 contaminated raw beef showed significant reduction of bacterial load 0.9 and 2.3 log CFU/piece at 8 and 25 °C respectively. Again bacteriophage potentiality checked against mixture of STEC O157:H7 and ESBLEC contaminated raw beef. Experiment revealed that after 24 hours of treatment the bacteriophage reduced 1.4 and 1.0 log CFU/piece at 25 and 8 °C, respectively.

Listeria monocytogenes

Listeria monocytogenes is Gram-positive, rod-shaped, oxidase negative, catalase positive and facultative anaerobic bacterium. Consumption of *Listeria monocytogenes* contaminated foods can cause mild to severe gastroenteritis diarrhoea, fever, flu-like illness, abdominal pain, vomiting. *Listeria monocytogenes* can survive under different food preservation conditions including high acidity, salinity, refrigeration temperatures *which could be a serious issue* for the food industry. Raw food ingredients like raw fish, seafood, milk, egg, meat, uncooked vegetables, ready to eat food products are some common carrier for *Listeria monocytogenes*. Bacteria can be also contaminated after food processing methods (Kawacka *et al.,* 2020) (Rogalla *et al.,* 2020)**.** Lee et al. (2017) isolated two bacteriophages LMP1 and LMP7 (*Siphoviridae* family) from chicken feces by using *Listeria monocytogenes*

as host strain. Study proved lytic activity of two bacteriophages against *Listeria monocytogenes* ATCC 15313, 7644, 19115 and 19114. At both 10°C and 30°C bacteriophage LMP1 and LMP7 reduced the bacterial growth effectively, but in case of *Listeria monocytogenes* ATCC 19114 bacteriophage LMP1 was more efficient than LMP1 LMP7. *Listeria monocytogenes growth was reduced more by bacteriophage* LMP7 than *bacteriophage* LMP1. *Listeria monocytogenes* ATCC 7644 *contaminated milk sample was taken for bacteriophage treatment. Experiment cleared lytic activity of both bacteriophage* LMP1 and LMP7 on bacterial growth at 4°C. This study concluded that their isolate novel bacteriophage LMP1 and LMP7 had ability to reduce bacterial load of refrigerated products. Guenther et al. (2009) applied kytic bacteriophage A511 and P100 to control the growth of *Listeria monocytogenes strains*. *Study used Listeria monocytogenes* Scott A (serovar 4b) and WSLC 1001 (serovar 1/2a) for ready-to-eat (RTE) foods bacterial contamination (1×10^3 CFU/g). Bacteriophage A511 and P100 applied (3×10^6 to 3×10^8 PFU/g) to contaminated for samples and kept it for 6 days at 6°C. Study showed significant degradation level of bacterial count from contaminated liquid food including mozzarella cheese brine, chocolate milk after bacteriophage application and up to 5 log reduction were detected for contaminated solid foods including sliced turkey meat, hot dogs, smoked salmon, sliced turkey meat, seafood, lettuce leaves and sliced cabbage. Bacteriophage application with high dose showed more promising effect. Silva et al. (2014) conducted another study to test the lytic activity of bacteriophage P100 against mixture o *Listeria monocytogenes* 1/2a and Scott A. Minas Frescal and Coalho cheeses were considered as appropriate media for this experiment. Bacteriophage P100 (8.3×10^7 PFU/g) added to *Listeria monocytogenes* 1/2a and Scott A contaminated food samples (approximately 10^5 cfu/g) and immediately analyzed to detect lysis activity. Experiment revealed that after 30minutes of bacteriopahe treatment effectively decreased ($p < 0.05$) 2.3 log units and 2.1 log units *Listeria monocytogenes* in Minas Frescal cheese and Coalho cheese respectively but after seven days refrigeration period bacteriophage P100 became ineffective and increase (approximately one log) in bacterial cell count was observed. Soni et al. (2010) evaluated *Listeria monocytogenes* specific U.S. Department of Agriculture's Food Safety and U.S. Food and Drug Administration approved bacteriophage Listex P100 (phage P100). Surface of the fresh catfish fillet samples were inoculated by two strains of *Listeria monocytogenes* (approximately 4.3 $\log^{10}$ CFU/g) that were 1/2a and 4b. It was proved that potentiality of the bacteriophage P100 influenced by phage-host interaction time and bacteriophage dose. Bacteriophage P100 decreased 1.4-2.0 $\log^{10}$CFU/g and 1.7-2.1 $\log^{10}$CFU/g viable cell count at 4°C and 22°C respectively. 30 minutes bacteriophage and host contact time was sufficient for more than 1 $\log^{10}$ CFU/g reduction of bacterial growth rate on the surface of the catfish fillet. Bacteriophage P100 stability and lytic activity were maintained on catfish fillet samples for more than 10 days at both 4°C and 10°C . Aprea et al. (2018) used 12 *Listeria monocytogenes* strains and and three *Campylobacter jejuni* strains for bacteriophage isolation. *Listeria monocytogenes* specific bacteriophages were purified from Italian blue cheese plants drain and *Campylobacter jejuni* specific bacteriophages from fresh chicken stool sample. Study evaluated *Listeria monocytogenes* and *Campylobacter jejuni* specific bacteriophages against antimicrobial resistance bacteria that are major public health problem nowadays. Study showed activity of bacteriophages as alternative strategy for antibiotic resistance pathogens in the field of human and veterinary medicine. They found one interesting thing that *Campylobacter jejuni*12662 strain again regain its sensitivity towards antibiotic drugs (tetracycline, ciprofloxacin, nalidixic acid) after bacteriophage treatment. They confirmed various bacteriopage advantages such as self-replicating activity, safety levels, easy availability, inexpensive and high speficity towards target bacteria compare to antibiotic therapy [5].

Other Pathogens

Rasool et al. (2016) collected sewage water for *Staphylococcus aureus* strain specific bacteriophages isolation and evaluated its antibacterial activity on Methicillin resistant *Staphylococcus aureus* (MRSA). Study stated that bacteriophage pq/48 activity started after 30 minutes incubation period and highest

lytic activity was identified after three to six hours of bacteriophage treatment. Bacteriophage based treatment was able to reduce *Staphylococcus aureus* associated symptoms like poor healing, inflammatory signs, and abscess formation. Bacteriophage treated groups showed decreased amount of *Staphylococcus aureus* cell count in wound swab collection after 48 hours compared to bacterial control group. Study concluded that the novel bacteriophage pq/48 had antibacterial effect and can be used as a bio-control agent against *Staphylococcus aureus* associated infections. Nasser et al. (2019) experimented regarding one of the common resistant bacteria methicillin-resistant *Staphylococcus aureus* (MRSA) that is not affected by Staphylococcal infections specific antibiotic therapies. Study isolated *Staphylococcus aureus* host strain specific bacteriopahe from livestock and sewage and confirmed its lytic activity by plaque assays and double layer agar method. Study confirmed bacteriopahe activity against clinical methicillin-resistant *Staphylococcus aureus* (MRSA) and suggested its application in the field of medicines and microbiology without any harmful adverse effect on human cell. Tan et al. (2020) isolated bacteriophages were isolated from sewage sample by suing *staphylococcus aureus* as host bacteria. From ten isolated bacteriophages two lytic bacteriophages namely namely ÖNUSA-10(*Siphoviridae* family) and ÖNUSA-1(*Myoviridae* family) were selected for further study due to their wide host spectrum against >80% of experimentally tested Methicillin-resistant Staphylococcus aureus (MRSA) and Methicillin susceptible Staphylococcus aureus (MSSA). Spot test confirmed the activity against hospital isolated 36 MSSA and 25 MRSA. This study also suggested the use of bacteriophage against MRSA due to its lytic potentiality and stability under physiologic temperature and pH. Furuta et al. (2017) selected *Campylobacter jejuni* for bacteriophage isolation and used meats samples (liver and skin) from market for bacteriophage isolation. Out of the isolated 26 bacteriophage only bacteriophage PHC10 showed large lytic range which is near about 67.4% of the 46 experimentally tested *Campylobacter jejuni* strains. 6-12 hour of bacteriophage PHC10 treatment decreased 1-3 log of bacterial cell growth at 42!. Finally study stated the effectiveness of *Campylobacter jejuni* lytic bacteriophage in food industry as a biocontrol agent. [4]

Bacteriophage biocontrol of Biofilm forming Pathogens

One or many bacterial strains can accumulate and form a complex ecosystem (cement-like matrix) of microorganisms called biofilms that is protected by an extracellular matrix. Biofilms may form in natural, clinical setups as well as industrial environments wherever suitable microorganisms, nutrient for cell growth and nourishment and water are present. Extracellular matrixes nature depends upon biofilm forming species and environment of the food manufacturing site. Extracellular matrix contains polysaccharides including protein, cellulose, exogenous DNA which is helpful for biofilm persistence in food industry as well as medical industry. Bacteria and fungi are generally responsible for biofilm formation process. Different types of bacterial species facilitate the attachment of biofilm to the surface and show disinfectant resistance characteristics. Hard surfaces like food storage, processing, transport sites, equipments in food industries, perishable foods (meat. vegetables, fruits, bones etc.), stainless steel, glass, polyethylene, wood, rubber, polypropylene etc. are most common sites for bacterial biofilm formation. Biofilm shows strong mechanical, antibacterial, chemical (antimicrobials, chemicals and disinfectants), UV light, antibiotics, host immune response, and other external stresses resistant characteristics and form very rapidly in suitable environment. The very first step towards biofilm production is surface conditioning and the reversible binding of biofim producing viable cells to that particular surface and the next step is irreversible binding of cells to surface and colony formation that leads to formation of tridimensional biofilm structure. Proteases and lipases secreted from biofilm producing microorganisms which can alter organoleptic properties of food stuffs and cause serious problem in various food industries like dairy industry. Biofilm associated with various food-borne illnesses due to infection or intoxication when they contaminated with food stuffs (Galié *et al.,* 2018). *Bacillus cereus* (cause diarrhea and vomiting), *Listeria monocytogenes*, enterotoxigenic and enterohemorrhagic strains of *Escherichia coli* (which may include strains), enteric toxin

producing *Staphylococcus aureus Salmonella enterica* are some common biofilm producing common human pathogens that can cause several serious health consequences along with financial losses. Biofilm formation in clinical sectors may result several serious health problems and chronic infectious diseases (Abebe *et al.,* 2020). Islam et al. (2019) used environmentally sourced water samples to get bacteriophages and ultimately isolated 42 phages against *Salmonella enterica* host strain. They selected only three *Salmonella*-specific bacteriophage named LPSTLL, LPST94 and LPST153 due to broad antibacterial spectrum. Phage cocktail made by three bacteriophages (LPSTLL, LPST94 and LPST153) in 1:1:1 mixture. Study tested bacteriophage cocktail against biofilm of either *Salmonella typhimurium* (ATCC 14028) or a mixture of *Salmonella* (*Salmonella typhimurium* ATCC 14028 and *Salmonella enteritidis* ATCC 13076). They took stainless steel surface and 96-well microplate for this experiment and evaluated the bacteriopahge activity at 30°C. Study found significant biofilm reducing activity of bacteriophage in both 96-well microplate assay (44–63%) and stainless steel surface (5.23 to 6.42 $\log_{10}$). Islam et al. (2020) collected water sample from lake for bacteriophage isolation to detect antibacterial potentiality of bacteriophages to control *Salmonella* infection in various food industry. Study selected *Salmonella typhimurium* ATCC 13311 strain specific bacteriophage LPST153. *Salmonella typhimurium* ATCC 13311 producing biofilms on 96-well microplates were used to detect bacteriophage activity. Colorimetric method confirmed bacteriophage LPST153 application at 7 $\log_{10}$ and 8 $\log_{10}$ PFU/mL reduced ($p < 0.01$) viable bacterial cell count 35% and 45% respectively. Another experimental technique performed to obsereved bacteriopahge activity on existing biofilm. This experiment showed bacteriophage LPST153 application at 7 $\log_{10}$ and 8 $\log_{10}$ PFU/mL inhibited ($p < 0.01$) *Salmonella* biofilm approx 25% and 31%, respectively. LPST153 was also able to inhibit the formation of biofilms and it had the ability to reduce and kill bacteria from inside, including existing biofilms. Rizzo et al. (2020) detected *Salmonella* Gallinarum (SG) causing fowl typhoid is one of the leading cause of economic losses in poultry industry. Again Multidrug resistance (MDR) and biofilm producing capacity of *Salmonella* Gallinarum make this strain more dangerous. Study assessed killing activity of two *Salmonella* lytic novel bacteriophages namely *Salmonella* phages UPF_BP1 and UPF_BP2 against biofilm and Multidrug resistant *Salmonella* Gallinarum strains. Study showed 85% of *Salmonella* Gallinarum strains were lysed by at least one phage and 74% were destroyed by both phages. According to this study bacteriophage UPF_BP1 and UPF_BP2 could be a successful biological control agent to decrease fowl typhoid outbreaks. Kosznik-Kwaœnicka et al. (2020) took *Salmonella enterica* forming biofilm inhibiting bacteriophages including bacteriophage vB_SenM-1, bacteriophage vB_SenM-2, and bacteriophage vB_SenS-3 and tested its efficiency level. They selected *Salmonella typhimurium* 12, *Salmonella typhimurium* 13, *Salmonella enteritidis* 64 and *Salmonella enteritidis* 1392 for their experiment. Study found 47%–99% reduction of biofilm forming bacterial cells. A decrease in the biofilm mass was evident after treatment by phages vB_SenM-1, vB_SenM-2, and vB_SenS-3. They also noticed change within four selected *Salmonella enterica* serotypes producing biofilm mass after 4 hours of bacteriopahge treatment at 25°C, 37°C, and 42 °C. Milho et al. (2019) used bacteriophages to control 24 hours old single-species produced biofilms. They applied bacteriophage Daica against strains of *Escherichia coli* (EC 434 and EC 515) and bacteriophage ö135 on strains of *Salmonella enteritidis* (SE Ex2 and SE 269). Study further mixed biofilms of EC 434 + SE Ex2 to check the biofilm inhibiting of bacteriophage cocktail preparation. Study showed a positive lytic effect of bacteriophage cocktail activity after 8 hours of incubation period with reduction of 1.15 Log_{10} of EC 434 and 0.88 Log_{10} of SE Ex2 bacterial cell count within biofilm. Garcia et al. (2017) found the biofilm producing ability of 58 different strains of *Salmonella spp*. among 123 tested samples. Out of all biofilm forming *Salmonella* strains *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella Heidelberg*, *Salmonella Kentucky*, *Salmonella senftenberg*, and *Salmonella mbandaka*, were selected for their highest biofilm producing efficiency.study showed that stainless materials and glass materials were more susceptible and favored biofilm production ($P<0.05$) but PVC surface was

not suitable surface for biofilm formation. Study also isolated bacteriophages from sewage to test the Salmonella spp lytic activity and selected bacteriophage 4.3, bacteriophage 4.8, bacteriophage 5.7 from total ten isolated bacteriophages due to their lytic effect on most of the selected bacteria. Study also evaluated bacteriophages activity towards Salmonella spp. biofilms on the surface of PVC, glass and stainless steel materials and showed that bacteriophages were more effective against 9 hours old biofilms on glass surface and infect highest number of bacterial cells than others. Jiang et al. (2020) isolated bacteriophage WX (*Siphoviridae* family) from pig slaughter house. Optimal multiplicity of infection (MOI) of this bacteriophage was 0.01 and lysed most of the virulent clinical strains of hospital isolated *Staphylococcus aureus*. Bacteriophage WX had better antibiofilm activity when bacterial concentration was relatively high, but in low bacterial load Streptomycin showed better antibiofilm effect than bacteriophage treatment. Mixture of both Bacteriophage WX and streptomycin showed more significant antibiofilm effect compared to single use. Highest lytic activity of bacteriophage WX was observed after 1 hour of bacteriophage treatment at 42!, and started to lose its activity at 60 ! and further inactivated at 90 !. Bumunang et al. (2020) evaluated Shiga toxin-producing *Escherichia coli* produced biofilm reducing activity of *Escherichia coli* specific bacteriophage SA21RB (isolated from cattle feces) on stainless-steel materials. They selected bacteriophage SA21RB due to its lytic effect on *Escherichia coli* O154:H10 and *Escherichia coli* O113:H21 and upgrade de-polymerase activity. Three hours of bacteriophage SA21RB treatment (10^{13} PFU/mL) on 24-h-old biofilms on stainless steel surface decreased (p dd 0.05) viable bacterial cells by 2.5 and 2.1 $\log_{10}$ CFU/cm^2 for *Escherichia coli* O113:H21 and *Escherichia coli* O154:H10, respectively.

Bacteriophage in Food Biopreservation

Bacteriophages can be excellent biopreservative in food manufacturing and food processing industries due to its ability to extend the shelf life of the food ingredients as well as kill spoilage causing bacteria. Bacteriophages can be active and destroy pathogenic bacteria even under low temperature (lower than 1 °C also). Various bacteriophge related studies proved the lytic effect of bcteriophages. One of them was bacteriophage against *Brocothrix thermosphacta* to inhibit the growth of spoilage bacteria *Brocothrix thermosphacta* within pork adipose tissue and increase self life the food. Another study found bcteriophage could extend shelf life of the food stuffs from 4 days to 8days without any spoilage. Bacteriophage produced lysin enzymes were also used as biopreservatives due to its role in bacterial degradation by targeting peptidoglycan bonds in cell wall. lysin enzymes are highly effective to control Gram-positive bacteria because it mainly target peptidoglycan bonds and ensure food safety associated health issues (Kazi *et al.,* 2016). Due to presence of natural antibacterial compound bacteriophage based biocontrol approach have gained greatest attention recently in the field of biopreservation. One biopreservation related study showed bacteriophage application decreased viable cell count of *Pseudomonas fragi* WY contaminated (more than 103 CFU/ml) refrigerated milk at 7 °C for 72 h. They selected *pseudomonads* because it is a well known spoilage bacteria in milk, milkproducts,meat products and can alter organoleptic properties of foods by proteolytic proteolytic and lipolytic enzymes (Pérez *et al.,* 2016). *Pseudomonas fluorescens* is one of the major food spoilage organisms which can form biofilm also. , usually found in the form of biofilms. Sillankorva et al. (2008) showed lytic bacteriophge could remove *Pseudomonas fluorescens* biomass by 63 and 91% but the lysis activity was depending upon surrounding conditions of the biofilm and age of the biofilm. Bacteriophage ÕIBB-PF7A was isolated from sewage treatment plant by using *Pseudomonas fluorescens* as host bacteria. Study selected only Bacteriophage ÕIBB-PF7A due to its capacity to infect wide range of dairy industry isolated *Pseudomonas fluorescens* bacteria.[Bacteriophage ÕIBB-PF7A was more effective against newly formed biofilm after short term incubation. Deasy et al. (2011) used bacteriophage therapy to maintain beer quality from certain *Lactobacillus* spoilage mainly *Lactobacillus plantarum* and *Lactobacillus brevis*. They isolated a new virulent *Lactobacillus brevis* specific bacteriophage to control beer spoilage. Isolated bacteriophage SA-C12 was stable in beer and efficiently controlled spoilage

causing *Lactobacillus* growth in beer. Result of the study confirmed the ability of bacteriophages in the field of biopreservation in various food industries [3].

Bacteriophage based treatment as biopreservatives is quite popular nowadays. It can successfully used in many food industries to prevent bacteria causing infection outbreaks. For example bacteriophage can be used in cheddar production, and chicken frankfurters to prevent common *Salmonella causing infection, in curd producing industries against Staphylococcus aureus, to prevent Listeria monocytogenes* growth during semi-hard and acid coagulated cheeses manufacturing. Bcteriophages are also used in reconstituted infant formula milk to eradicate *Enterobacter sakazakii* pathogen. Some FDA approved commercial bacteriophge preparation including *Listex* and *LMP 102* may used for decontamination of ready-to-eat meat products and animals prior to slaughtering (Singh *et al.,* 2018).

Bacteriophage Biocontrol of Antimicrobial resistant Bacteria

Antibiotic resistant pathogen is one of the serious health issues recently across the world because of antibiotic over dose, frequent consumption of antibiotics, misuse. Microorganisms become antibiotic resistant by chromosomal mutations or resistant gene. Microorganism can modify or alter common phenotypes, target binding sites, cell permeability ability, enzyme inactivation, exhibit antibiotic efflux, which are common strategies to become resistant to various antibiotics (Wang *et al.,* 2020). Recently in clinical sectors many antibiotics (b-lactam/b-lactamase, colistin, carbapenems, aminoglycoside etc.) are restricted to use because of multidrug resistant (MDR), pandrug-resistant (PDR), extensively drug-resistant (XDR) nature and toxicity level. Survey in United Nations predicted that approx 10 million persons will expire due to extensively contaminating antibiotic resistant harmful pathogens by 2050. Long term use of antibiotics in human and veterinary medicines may result not only antibiotic resistant but also cause dysbiosis in natural human microbiota including gut microbiota, and associated infections (invasive or local candidiasis). Therefore some alternative strategies are requires to overcome this emerging antibiotic associated problems. Application of bacteriophage based therapy is one of the most promising ways to resolve this problem. Bacteriophages can significantly decrease the growth of pathogenic bacteria after attachment to the host strain. Bacteriophages are not harmful for eukaryotic cells due to absence of receptors for eukaryotic cells. For several reasons such as easy availability, inexpensive, not required multiple doses like antibiotics, self replicating nature, now adverse effects towards human health, stability under various pH, harsh environments and temperature bacteriopahge can be a potential and successful alternative against various antibiotic resistant bacteria causing infections (Taati et al., 2020). Jung et al. (2017) evaluated bacteriopahe activity to inhibit (more than 5 log reduction) *Salmonella typhimurium* KCCM 40253, *Salmonella typhimurium* ATCC 19585, ciprofoxacin-induced antibiotic-resistant *Salmonella typhimurium* ATCC 19585 cell count. This study suggested a developing bacteriophage based control strategy against multidrug-resistant pathogens including resistant *Salmonella typhimurium*. Ding et al. (2020) explained the antibacterial properties of various lytic bacteriophages and bacteriophage extracted endolysins to reduce bacterial infection. Endolysin is produced from bacteriophage that is one kind of protein. This article showed antibacterial property of newly isolated endolysin LysSE24 against 23 different kind of multidrug-resistant *Salmonella* spp. Another study revealed the antibacterial function of globular endolysin that had broader lytic host range including multidrug-resistant *Salmonella* and Gram-negative pathogens. Peng et al. (2020) discovered *Klebsiella pneumoniae* lytic new bacteriophage namely vB_ KleS-HSE3 (*Siphoviridae* family)from hospital sewage sample. Study focused on *Klebsiella pneumoniae* pathogen due to its multidrug-resistance ability. They observed lytic activity and high stability of bacteriophage vB_KleS-HSE3 against broad range of antibiotic resistant *Klebsiella pneumoniae.*

CONCLUSION

Food safety is the major issue in the world recently. Foodborne diseases are constant and emerging threats among vulnerable groups including, especially for individuals with weaker

immune systems, e.g., children, elderly, and pregnant women. Though some hurdles remain, biocontrol methods using bacteriophage is become widely accepted across the globe as promising, and safe biocontrol method to decrease bacterial contamination in foods. Bacteriophage application can prevent bacterial food contamination by targeting particular pathogenic strains during food production, processing and storage at different time period. Bacteriophage preparation can be used by spraying on food products, applying to animals, birds before slaughtering, direct applying to food contact surfaces in food processing units and during post harvest time. Commercial bacteriophage preparations are recently available and safe for human consumption. Consequently, phage will be the most demanding next-generation biocontrol agent and rapid pathogen detection tool to control the outbreaks of various harmful foodborne diseases.

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