The use of phages for the removal of biofilms in the food industry

E. Unal Turhan[1,*], S. Kalkan[2], Z. Erginkaya[3]

[1] Kadirli Applied Sciences School, Department of Food Technology, Osmaniye Korkut Ata University, 80760, Kadirli, Osmaniye, Turkey
[2] Faculty of Engineering, Department of Food Engineering, Giresun University, 28200 Giresun, Turkey
[3] Faculty of Agriculture, Department of Food Engineering, Cukurova University, 01300 Adana, Turkey
* Corresponding author: E. UNAL TURHAN (PhD), e-posta: emelunalturhan@gmail.com

Biofilms are defined as complex multicellular microbial communities attached to the surface or embedded in the bacterial extracellular polymeric substances. Control of the biofilms has a major concern in the food processing plants because biofilms are generally more resistant to antimicrobial agents than planktonic cells and causes many problems in food industry. Various biofilm control strategies have been applied for prevention of biofilm formation in food processing environments. Recently, the interest in biopreservation of food led to the development of new natural antimicrobial compounds from different origins. Phages as novel biocontrol strategies have a unique potential in terms of the removal of microbial biofilms in the food chain. Especially, the emergence of antibiotic-resistant strains has raised a growing concern in application of phages. The use of phages as antimicrobial agents offers an alternative to chemical preservatives and enhances microbiological safety. Phages for food safety have been basically used at reduction of colonization in living animals or final food products, disinfection of food contact surfaces and equipment, prevention of contamination during food processing and proliferation of pathogenic bacteria during food storage period along the food chain. This chapter highlights the importance of microbial biofilms in food safety control and provides an overview of applications of phages for the removal of biofilms in the food industry.

Keywords Biofilm; Phages; Bacteriophages; Biopreservation; Food Industry

1. Introduction

Biofilms is highly important risk factors in the food industry due to the potential contamination of food products with pathogenic and spoilage microorganisms. Food spoilage and deterioration by biofilm contamination results in huge economic losses and deficiency of food quality and safety [1]. The majority of bacteria are able to adhere and to form biofilms and survive for a long time depending on the microorganism and the environmental conditions. Because bacteria in biofilms are resistant against environmental stress and common disinfectants, detection and elimination of bacterial biofilms in a food processing environment is extremely difficult [2, 3]. Due to the increased resistance of biofilms to conventional disinfection processes, new control strategies were detected to control biofilm [4]. Among alternative food preservation technologies, particular attention has been paid to biopreservation to extent the shelf-life and to enhance the hygienic quality, minimizing the impact on the nutritional and sensorial properties of perishable food products. Biopreservation rationally exploits the antimicrobial potential of naturally occurring microorganisms in food and/or their metabolites with a long history of safe use. In this sense, bacteriophage and their associated enzymes (endolysins) have been suggested as alternative novel agents to conventional methods such as chemical and physical treatments [5, 6, 7].

Bacteriophages are bacterial viruses with host specificity and lysis activities. Phages or their endolysins have been useful as natural biocontrol agents against food-borne pathogens without any harm to human cells, indicating their safety [8, 9, 10]. Bacteriophages have been used at each stage of the farm-to-fork in food chain [11]. The effectiveness of bacteriophages in controlling foodborne pathogens and spoilage microorganisms has led to the development of different phage products approved by USFDA and USDA. The majority of these products are to be used in farm animals or animal products such as carcasses, meats and also in agricultural and horticultural products. Treatment with specific phages in the food industry can prevent the decay of products and the spread of bacterial diseases and ultimately promote safe environments in animal and plant food production, processing, and handling [12]. Phages also have the ability to lyse bacteria present in a biofilm and so phage therapy as new biofilm control strategy was suggested [8]. Antimicrobial resistance in biofilms poses a significant hurdle to eliminating biofilms with conventional antimicrobial substances, but phages also overcame this resistance problem [13]. A phage biocontrol strategy for biofilm elimination can be an acceptable, more natural alternative to traditional approaches to food safety and preservation [14]. Until now, various phage and phage endolysin applications provided inactivation effect on some microorganisms and their biofilms including *E.coli* O157:H7, *Bacillus* spp., *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., *Staphylococcus* spp., *Cronobacter* spp., *Vibrio* spp., *Clostridium* spp., *Campylobacter jejuni* and *Pseudomonas* spp. [9, 14, 15].

The development of new disinfection products, non-toxic to humans and friendly to the environment has good prospects for the future. Bacteriophage-based disinfectants fulfill all the requirements regarding effectiveness and safety [16].

2. Biofilm formation: A Food Safety Concern

Biofilm is the sessile form of microbial life, characterized by adhesion of microorganisms to biotic or abiotic surfaces, with consequent extracellular production of polymeric substances [17]. In nature, many species of bacteria, fungi, protozoa and algae can form biofilms. However, bacteria have received the most attention and it is estimated that 99% of the bacteria present in natural and pathogenic ecosystems can grow in these metabolically integrated communities [18].

Biofilm formation involves (1)initial attachment of planktonic cells to the surface, (2) production of extracellular polymeric matrix (EPS), (3) micro colony formation and secretion of chemical signals, (4) maturation of biofilm structure and (5) dispersion of cells [2, 19, 20]. Bacterial adhesion to the surface constitutes the first and essential step of the biofilm formation. After attachment, the cells start to replicate into micro colonies. Reversible adhesion becomes irreversible mainly through the secretion of exopolymeric substances that form the biofilm matrix. The extracellular matrix consists of a mixture of polymeric compounds such as polysaccharides, proteins, nucleic acids, and lipids. These substances allow bacteria to stick to surfaces and to each other. At this stage, the process of biofilm maturation begins in order to create a mature biofilm in which the cells are encased in an extracellular matrix complete with a complex structure with water channels. Such a matrix acts as a scaffold for the stabilization of the three-dimensional biofilm structure [20, 21].

Microbial adhesion and biofilms by pathogenic and spoilage microorganism are of great importance for the food industry and occur on a variety of food contact surfaces. The transition from planktonic growth to biofilm occurs in response to environmental changes [22]. Biofilms constitute a protective mechanism of microbial growth that allows many bacteria to survive unfavorable environmental conditions [11]. Therefore, they continue to pose concerns to food manufacturers as the presence of biofilms may lead to food spoilage, economical losses, reduced shelf life of products or transmission of pathogens [2, 18]. Biofilm formation has been documented in many different industrial environments [11]. Biofilms mainly cause problems in the dairy, meat, poultry, seafood, and vegetable processing industries. Biofilms form both on processing environment surfaces and on food itself [16]. The common sources involved in biofilm accumulation are floors, waste water pipes, bends in pipes, rubber seals, conveyor belts, stainless steel surfaces, glasses, air, people, cleaning systems, improperly cleaned and sanitized equipment etc. [17].

The main microorganisms caused the problems of biofilm in food industry are "*Salmonella, E.coli* O157:H7*, L.monocytogenes, Shigella, Bacillus cereus, Clostridium perfringens* and *Yersinia* biofilms" for fresh produce industry, "*L. monocytogenes*, thermoduric *Streptococci, E. coli, S. aureus* and *Bacillus* species biofilms" for dairy industry, "*Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio alginolyticus L. monocytogenes, Salmonella* spp., *Bacillus* spp., *Aeromonas hydrophila*, and *Pseudomonas* spp." for fish processing industry, "*Salmonella typhimurium, E. coli, E. faecalis, Campylobacter jejuni* biofilms" for poultry industry, "*E.coli* O157:H7, *L. monocytogenes* and *Acinetobacter calcoaceticus* biofilms" for meat industry and "*L. monocytogenes* and *E.coli* O157:H7 biofilm" for ready-to-eat foods [2, 16, 19].

2.1. Recent biofilm control strategies

Biofilms of microorganisms are able up to 1,000-fold more resistant to disinfectants and biocides than planktonic cells of these microorganisms and this characteristic may be associated with aspects related to the structure and physiology of biofilms, such as reduced diffusion, anaerobic growth, physiological changes due to reduced growth rates and the production of enzymes that degrade antimicrobial substances [2]. The elimination of biofilms is a very difficult task because many factors affect detachment. The main mechanism of biofilm resistance to antimicrobial agents is the failure of an agent to penetrate the full depth of the biofilm [23]. The purpose of cleaning is to remove residual materials that may interfere with the sanitation procedure, because the presence of organic material on the biofilms significantly reduces the log kill. This reinforces the importance of adequate cleaning before the use of sanitizers [17].

There are several strategies for controlling biofilms such as physical and/or mechanical removal, chemical removal, and the use of antimicrobials, sanitizers, or disinfectants [5].Some of these strategies are application of sodium hypochlorite, hydrogen peroxide, ozone, peracetic acide, clean-in-place, ultrasonication, electrolyzed water technology, enzymes (subtilisins, lysostaphin, bacteriophage lysins, amylases, alginate lyase, hydrogen peroxide-producing enzymes, hydrogen peroxide-responsive enzymes, anti-quorum sensing enzymes), phages, surfactants, biosurfactants, nanoparticle, plant extract, bacteriocin, essential oil, hurdle technology and other antibiofilm agents such as capsular polysaccharides, molsidomine, diethylamine nonoate diethylammonium, a catheter lock solution, chitosan, terpinen, povidone iodine (PVP), gallium, lactoferrin, xylitol and dispersin B [5, 19, 22, 24, 25]. These strategies can be classed into two major groups. The aim of the first one is to prevent bacterial adhesion and biofilm formation with either surface property modification or antimicrobial surface coating. The second one aims to eradicate/disrupt formed biofilms using antimicrobial agents, physical forces, enzymes, phages, etc. [21].

As known, biofilms are more resistant or tolerant to antibiotics and disinfectants and more difficult to remove mechanically when compared to planktonic cell [22]. The most common disinfectants used by food industries (acidic compounds, aldehyde-based biocides, caustic products; chlorine, hydrogen peroxide, iodine, isothiazolinones, ozone, peracetic acid, phenolics, biguanidines, surfactants halogens, and quaternary ammonium compounds) is not enough to remove the biofilms [2]. Some of the concerns with the use of these traditional chemicals include development of resistance by microbial cells and cross-reactivity of these chemicals to plant equipment surfaces. In this regard, various innovative antibiofilm approaches have been detected, but it is difficult to reliably compare all these strategies. Some of the emerging novel approaches, such as natural substances, bacteriophages, quorum quenching, nanotechnology, bacteriocin and various enzymes are promising and may help to find antibiofilm strategies that are superior to the conventional ones. Moreover, a combination of these novel techniques with conventional methods (antibiotics, disinfectants, and physical methods) is expected to solve the "biofilm problem" in the near future [5].

Recently, there is a general trend toward the use of bacteriophages [26]. In this sense, applications of bacteriophage in food industry were developed to overcome the problems related to microbial biofilms, multidrug resistance and current disinfectants [4, 24]. Phage therapy is based on the use of lytic phages that also can potentially destroy the integrity of biofilm EPS matrix by enzymatic mechanism. The use of bacteriophages in food processing environment has been suggested as a good option to kill undesirable bacteria in biofilms [2].

3. The importance of Phages in Food Industry

Bacteriophages or phages are the most abundant microorganisms in the environment (10^{31} particles) and widely spread including foods of various origins [7]. They are present in water, effluent, soil or any environment else that supports the growth of bacteria. There are many reports detailing the isolation of phages from various foods, suggesting that phages are normal inhabitants of food ecosystems [27]. Phages were first discovered in the early 1900s and soon after, the concept of phage therapy was conceived. Phage therapy involves the application of bacterial viruses to eliminate pathogenic bacteria [8].

Bacteriophages have both positive and negative effects as regards food industry. For example they represent a serious threat in the dairy industry, causing losses in the production of cheese and fermented products. However, they are also seen as positive agents, replacing antibiotics in the control of pathogens. Two main applications have been developed to prevent food contamination and/or to treat bacterial infections in animals or plants [28].

Bacteriophages are bacterial viruses that infect bacterial cells with high specificity. In natural environments, phages infect or interact with bacteria in different ways for their survival [11]. Depending on their life style, phages are divided into virulent and temperate phages. Virulent phages strictly follow a lytic cycle whereby they multiply within the bacterial cell to finally lyse the cell to release the phage progeny. By contrast, temperate phages may enter the lysogenic cycle by inserting their DNA into the bacterial chromosome (prophage) where it replicates as part of the host genome until it may be induced to enter the lytic cycle. This last lytic step is precisely where the phage antimicrobial activity resides [7]. After a phage reach a suitable host surface, it is adsorbed onto the cell surface receptors of the host. The absorbed phage starts its infection process by injecting the phage DNA into the cell and then phages follow either a lytic or lysogenic life cycle inside the host. Lytic phages are preferred for eliminating pathogens from food [27, 29].

Additionally, bacteriophages may release polysaccharide depolymerase enzymes, which bind to the capsular material of bacterial cells, during infection. Use of these phage enzymes represents a natural, highly specific, and non-toxic approach to controlling microorganisms [3].

3.1 Phages based biocontrol in Food Industry

New approaches to improve food safety by inactivating pathogens and spoilage microorganisms were employed. Bacteriophages provide an attractive alternative since phages have great potential for use as natural antibacterial to control food pathogens and spoilage organisms at the pre-harvest (farm animals) and post-harvest (meat, fresh and packaged foods) stages of food production [11, 30]. Especially, phages have received much attention as a possible alternative to antibiotics in the treatment of antibiotic-resistant bacterial infections [31]. A major step forward, in phage-related biocontrol, occured in 2006 when the FDA approved, under generally recognised as safe (GRAS) criteria, two phage cocktails, namely ListShield (produced by Intralytix) and Listex (produced by MICREOS formerly EBI) for the prevention of Listeria contamination on ready-to-eat foods. In 2009 the European Food and Safety Authority (EFSA) approved phage-related preparations as additives to organic foods and in August 2012 the regulatory body Food Standards Australia and New Zealand (FSANZ) approved that phages could be used as a food processing aid [8].

Natural lytic bacteriophages has been approved in the category of generally recognized as safe (GRAS) by United States Food and Drug Administration (FDA) [29]. The applications of phage were reported to inhibit bacteria such as *S. aureus, E. coli, Salmonella, L. monocytogenes* in food [11, 31]. There are several phage preparations commercialized, such as ListShield and EcoShield (Intralytix,Inc.,USA), Agriphage (Omnilytics,Inc.,USA) and Listex P100 (Micreos Food Safety, Formerly EBI Food Safety, The Netherlands).The approval of using phage product Listex P100 in food products by FDA and USDA provides the impetus to further investigate phage applications [32].

The stability of phages in foods are the other important issue about biocontrol. Phages should be stable under the physiochemical conditions (pH tolerance, thermos tolerance, processing ingredients or environment, visible and UV light, osmotic shock and pressure and water activity) of the food to which they are applied [27].

The use of phages to promote food safety can be basically done at four different stages along the food chain. Phage applications are phage therapy (Reduction of colonization in living animals during primary production), biopreservation (Prevention of contamination and pathogen proliferation on foods during storage and marketing of the final products), biocontrol (Reduction of colonization of foods at industry food processing) and biosanitation (Disinfection of food contact surfaces and equipments) [7, 12]. For example, phages have also been applied to control the growth of pathogen and spoilage microorganisms such as *Listeria monocytogenes*, *Salmonella* spp., *S. aureus, Campylobacter jejuni, Pseudomonas* spp. and *Brochothrix thermosphacta* in a variety of foods such as fruit, milk, dairy products, poultry, meat and meat products [14].

In addition to the use of bacteriophages for combating pathogens or spoilage microorganisms in food, applications of bacteriophages encoded endolysins along the food has good antimicrobial potential. [7]. Bacteriophage endolysins (peptidoglycan hydrolases) have been seen a new class of antimicrobial agents useful for controlling bacterial infection or other unwanted contaminations in various fields, particularly in the light of the worldwide increasing frequency of drug-resistant pathogens. The use of endolysins in foods displayed lytic activity against foodborne pathogens or food spoilage bacteria in vitro (*S.aureus, S. epidermis, Clostridium tyrobutyricum, C. sporogenes, C. acetobutylicum, C. perfringens, B. cereus, B. subtilis, Listeria monocytogenes, Pseudomonas aeruginosa, E. coli, Salmonella, Cronobacter sakazakii* and some other Gram- negative bacteria) [15].

The undeniable antimicrobial properties of phages coupled with the decrease in antibiotic efficacy, the steep decline in the number of pharmaceutical companies investing in new pipeline drugs and consumer demands for the production of foods that are free from pathogens and synthetic chemicals has encouraged numerous companies to invest in the production of phage-based products [11]. Phages offer advantages as biocontrol agents for several reasons: (i)high specificity to target their host determined by bacterial cell wall receptors, leaving untouched the remaining microbiota, aproperty that favors phages over other antimicrobials that can cause microbiota collateral damage; (ii) self-replication and self-limiting, meaning that low or single dosages will multiply as long as there is still a host threshold present, multiplying their overall antimicrobial impact; (iii) as bacteria develop phage defense mechanisms for their survival, phages continuously adapt to these altered host systems; (iv) low inherent toxicity, since they consist mostly of nucleic acids and proteins; (v) phages are relatively cheap and easy to isolate and propagate but may become time consuming when considering the development of a highly virulent, broad-spectrum, and nontransducing phage; (vi) they can generally withstand food processing environmental stresses (including food physiochemical conditions); (vii) they have proved to have prolonged shelf life. Phages are readily abundant in foods and have been isolated from a wide variety of raw products, processed food, fermented products and seafood [12, 14]. Some of the drawbacks of biopreservation with phages are a limited host range, the requirement for threshold numbers of the bacterial targets, phage-resistant mutants, and the potential for the transduction of undesirable characteristics from one bacterial strain to another [14].

Some applications about phage based biocontrol in food Industry was represented in table 1.

Table 1. Some applications about phage based biocontrol in food Industry

Biocontrol agent	Scope of Application	Target microorganisms	Efficacy of treatment	References
Bacteriophage Listex P100	Melon, pear and apple products (juices and slices)	*L. monocytogenes*	1-1.50 log cfu/plug reduction for fruit slices and 2-8 log cfu/ml for fruit juices	[32]
Lytic bacteriophages	Pig skin, chicken breasts, fresh eggs and packaged lettuce	*Salmonella enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis	~2-4 log units reduction	[33]
Lytic bacteriophage mixture	Leafy green vegetables	*Escherichia coli* O157:H7	~1-3 log cfu/leaf reduction	[34]
Lytic bacteriophages	Fresh-cut fruits and vegetables	*L. monocytogenes*	2-4.6 log units reduction	[35]
Bacteriophage LISTEXP100	Raw salmon fillet tissue	*Listeria monocytogenes*	0.5-3.5 log cfu/g reduction	[36]
Lytic bacteriophage, Salmofresh™	Glass and stainless steel surfaces	*Salmonella*	>99% elimination rate	[37]

3.2 Use of Phages as antimicrobial agent for the removal of biofilms

As known since their discovery, bacteriophages have been extensively studied and have been used for a variety of practical applications such as for phage therapy, biocontrol of food borne pathogens, and detection of pathogenic bacteria. Recently, bacteriophages (or phages) have been used for the reduction of bacterial biofilms [38].The use of phages as antimicrobial agent for the removal of biofilms may provide a natural, highly specific, nontoxic, and feasible approach for controlling several microorganisms involved in biofilm formation [17]. The combination of phages with other antibacterial agents also showed interesting outcomes [21]. Phage-based detergents, such as phage particles, endolysin, and phage-borne glycanase, are known as bio-cleaners and serve as a natural option to overcome the problem with BFs in the food industry [3, 15].

The treatment of biofilms using phages is a complex process and only strictly lytic phages should be used. When phages come into contact with biofilms, further interactions occur, depending on the susceptibility of the biofilm cells to the phage and to the availability of receptor sites [4]. When host bacteria are included in a biofilm, the biofilm matrix can constitute a first physical barrier to the phage. To solve this problem, some phages possess polysaccharide depolymerases which are specific hydrolytic enzymes that can use polysaccharides or polysaccharides derivatives as substrate [16].

The recent interest in phage therapy as an alternative to traditional antimicrobial has fostered the use of phages in multiple applications. Like in phage infection of planktonic cells, there are several essential steps that need to occur. The first and crucial step in phage infection is the adsorption of phages to the receptors of the target bacteria. The EPS matrix of biofilms can constitute a problem for phages during infection of bacteriophages against a host cell. Because these capsular EPS matrix delay or prevent phages from gaining access to receptors on the cell wall of each infected bacterium. However phages possess the potential ability of producing polysaccharide degrading enzymes. Thus, phage associated enzymes provides the removal of biofilms by destroying the associated capsular EPS [3, 16, 18].

Some applications about use of phages as antimicrobial agent for the removal of biofilms were summarized in Table 2.

Table 2. Some applications about use of phages as antimicrobial agent for the removal of biofilms

Biocontrol agent	Scope of Application	Target microbial biofilm	Efficacy of treatment	References
Phage enzyme	In vitro	*Klebsiella*	Elimination rate of 80%	[3]
Lytic bacteriophages	Milk	*S. aureus*	6 log units reduction	[30]
Lytic phages	Stainless steel surfaces, in seafood processing plant	*L. monocytogenes*	~2-3 log units reduction	[29]
Lytic coliphages	Lettuce, cabbage, meat and egg	*E. coli*	>3 log units reduction	[39]
Phage mixture	Milk	*E. coli* O157:H7	2-6 log units reduction	[38]
Lytic phage, phage F S1	In vitro	*Pseudomonas fluorescens*	A biomass reduction of ~85%	[40]
Phage P100	Stainless steel surfaces	*Listeria monocytogenes*	A mean reduction of 5.29 log cfu/cm^2	[41]
Bacteriophage (EFDG1)	In vitro	*E. faecalis*	A 5 log reduction	[42]
Bacteriophage-derived peptidase, CHAPK	In vitro	*Staphylococcus aureus*	A 4 log drop	[43]
Endolysin LysH5	In vitro	*Staphylococcus aureus* and *Staphylococcus epidermidis*	1–3 log units reduction	[44]
Engineered enzymatic phage	In vitro	*E. coli*	~99.997% removal	[13]
Lytic bacteriophage	Stainless steel	*Escherichia coli* O157:H7	1-2 log units reduction	[45]
Bacteriophages CP8 and CP30	In vitro	*Campylobacter jejuni*	1-3 log cfu/cm^2 reductions	[46]

4. Conclusion

Microbial biofilms are ubiquitous in nature and an important reservoir of microbial contamination of food products. Contamination of biofilm in food processing plant industry may threaten quality and safety of food products and result in food-borne disease and economic losses. Therefore, biofilm prevention and control is the mandatory in food industry. However, biofilms are more resistant to traditional antimicrobial agents than planktonic cells and so new antibacterial agents new biofilm prevention and control strategies are urgently needed. In this sense, much effort should be invested in research on novel antibacterial agents for the elimination of biofilms. Bacteriophages are already in use in agricultural, food safety and diagnostic applications, demonstrating the utility and viability of such approaches. The application of bacteriophages represents a promising, safe, environmentally friendly and chemical-free alternative to the use of chemicals/sanitizers on produce. Numerous researcher focused on bacteriophages or bacteriophages endolysins and applications in food industry has increased considerably over the past decade. Additionally, the emergence and rapid spread of multi drug-resistant bacterial pathogens which makes it necessary to identify new treatment options led to a trend towards use of bacteriophages or bacteriophages endolysins.

References

[1] Houdt RV, Michiels CW. Biofilm formation and the food industry, a focus on the Bacterial outer surface. Journal of Applied Microbiology. 2010; 109: 1117–1131.
[2] Winkelströter LK, Teixeira FBR, Silva EP, Alves LF, Martinis ECPD. Unraveling microbial biofilms of importance for food microbiology. Microb Ecol 2014; 68:35–46.
[3] Chai Z, Wang J, Tao S, Mou H. Application of bacteriophage-borne enzyme combined with chlorine dioxide on controlling bacterial biofilm. LWT-Food Science and Technology. 2014; 59:1159-1165
[4] Simoes M, Simoes LC, Vieira MJ. A review of current and emergent biofilm control strategies. 2010; 43: 573–583.
[5] Sadekuzzaman M, Yang S, Mizan MFR, Ha SD. Current and recent advanced strategies for combating biofilms. Comprehensive Reviews in Food Science and Food Safety. 2015; 14:491-509.
[6] Tait K, Skillmanand LC, Sutherland IW. The efficacy of bacteriophage as a method of biofilm eradication. Biofouling. 2002; 18(4):305–311
[7] Garcia P, Rodriguez L, Rodriguez A, Martinez B. Food biopreservation: promising strategies using bacteriocins, bacteriophages and endolysins. Trends in Food Science & Technology. 2010; 21:373-382.
[8] Keary R, McAuliffe O, Ross RP, Hill C, O'Mahony J, Coffey A. Bacteriophages and their endolysins for control of pathogenic bacteria. Microbial pathogens and strategies for combatting them: science, technology and education. A. Mendez-Vilas Ed., Formatex 2013, p:1028-1040.
[9] Bai J, Kim YT, Ryu S, Lee JH. Biocontrol and rapid detection of food-borne pathogens using bacteriophages and endolysins. Frontiers in Microbiology. 2016; 7(474):1-15.
[10] Strydom A, Witthuhn CR. *Listeria monocytogenes*: A target for bacteriophage biocontrol. Comprehensive Reviews in Food Science and Food Safety. 2015; 14:694-704.
[11] Endersen L, O'Mahony J, Hill C, Ross RP, McAuliffe O, Coffey A. Phage Therapy in the food industry. The Annual Review of Food Science and Technology. 2014; 5:327–49.
[12] Sillankorva SM, Oliveira H, Azeredo J. Bacteriophages and Their Role in Food Safety. International Journal of Microbiology. 2012; Article ID 863945, 13 pages, doi:10.1155/2012/863945
[13] Lu TK, Collins JJ. Dispersing biofilms with engineered enzymatic bacteriophage. PNAS. 2007; 104(27): 11197–11202
[14] Greer GG. Bacteriophage control of foodborne bacteria. Journal of Food Protection. 2005; 68(5):1102–1111.
[15] Schmelcher M, Loessner MJ. Bacteriophage endolysins: applications for food safety. Current Opinion in Biotechnology. 2016; 37:76–87.
[16] Gutierrez D, Rubio LR, Martinez B, Rodriguez A, Garcia P. Bacteriophages as weapons against bacterial biofilms in the food industry. Frontiers in Microbiology. 2016; 7(825):1-15.
[17] Ciccio PD, Conter M, Zanardi E, Ghidini S, Vergara A, Paludi D, Festino AR, Ianieri A. *Listeria monocytogenes*: biofilms in food processing. Ital. J. Food Sci. 2012; 24: 203-213.
[18] Sillankorva SM. Use of bacteriophages to control biofilms. Dissertation thesis for the PhD degree in Chemical and Biological Engineering, University of Minho, Braga. 2008; 179p.
[19] Srey S, Jahid IK, Ha SD. Biofilm formation in food industries: A food safety concern. Food Control. 2013; 31:572-585.
[20] Cappitelli F, Polo A, Villa F. Biofilm Formation in food processing environments is still poorly understood and controlled. Food Eng Rev. 2014; 6:29–42.
[21] Abdallah M, Benoliel C, Drider D, Dhulster P, Chihib NE. Arch Microbiol. 2014; 196:453–472.
[22] Aswathanarayan JB, Vittal RR. Microbial biofilms and their control by various antimicrobial strategies. Microbial pathogens and strategies for combatting them: science, technology and education. A. Mendez-Vilas Ed., Formatex 2013, p:124-133.
[23] Cortes ME, Bonilla JC, Sinisterra RD. Biofilm formation, control and novel strategies for eradication. Sicence against microbial pathogens: communicating current research and technological advances. A. Mendez-Vilas Ed., Formatex 2011, p:896-905.
[24] Thallinger B, Prasetyo EN, Nyanhongo GS, M. Guebitz GM. Antimicrobial enzymes: An emerging strategy to fight microbes and microbial biofilms. Biotechnolgy Journal. 2013; 8:97–109.
[25] Valeriano C, Oliveira LC, Carvalho SM, Cardoso MG, Alves E, Piccoli RH. The sanitizing action of essential oil-based solutions against *Salmonella enterica* serotype Enteritidis S64 biofilm formation on AISI304 stainless steel. Food Control. 2012; 25:673-677.

[26] Desai MA, Soni KA, Nannapaneni R, Schilling MW, Silva JL. Reduction of *Listeria monocytogenes* biofilms on stainless steel and polystyrene surfaces by essential oils. Journal of Food Protection. 2012; 75(7):1332–1337.
[27] Hudson JA, Billington C, Smith GC, Greening G. Bacteriophages as biocontrol agents in food. Journal of Food Protection. 2005; 68(2):426–437.
[28] Maura D, Debarbieux L. Appl. Microbiol. Biotechnol. Bacteriophages as twenty-first century antibacterial tools for food and medicine. Appl Microbiol Biotechnol. 2011; 90:851–859.
[29] Arachchi GJG. A study of natural lytic *Listeria* phages with decontaminating properties for use in seafood processing plants. PhD thesis in Food Biotechnology, Institute of Food Food, Nutrition and Human Health, Massey University, Auckland, New Zealand, 2013, 199p.
[30] Garcia P, Madera C, Martinez B, Rodriguez A. Biocontrol of *Staphylococcus aureus* in curd manufacturing processes using bacteriophages. International Dairy Journal. 2007; 17:1232–1239.
[31] Jo A, Ding T, Ahn J. Synergistic antimicrobial activity of bacteriophages and antibiotics against *Staphylococcus aureus*. Food Sci. Biotechnol. 2016; 25(3): 935-940
[32] Oliveira M, Vinas A, Colas P, Anguera M, Usall J, Abadias M. Effectiveness of a bacteriophage in *reducing Listeria monocytogenes* on fresh-cut fruits and fruit juices. Food Microbiology. 2014; 38:137-142.
[33] Spricigo DA, Bardina C, Cortés P, Llagostera M. Use of a bacteriophage cocktail to control *Salmonella* in food and the food industry. International Journal of Food Microbiology. 2013; 165:169–174.
[34] Viazis S, Akhtar M, Feirtag J, Diez-Gonzalez F. Reduction of *Escherichia coli* O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and trans-cinnamaldehyde. Food Microbiology. 2011; 28:149-157.
[35] Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M, Saftner R, Sulakvelidze A. Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. Applied and Environmental Microbiology.2003; 69(8):4519–4526.
[36] Soni KA, Nannapaneni R. Bacteriophage significantly reduces *Listeria monocytogenes* on raw salmon fillet tissue. Journal of Food Protection. 2010; 73(1):32–38.
[37] Woolston J, Parks AR, Abuladze T, Anderson B, Li M, Carter C, Hanna LF, Heyse S, Charbonneau D, Sulakvelidze A. Bacteriophages lytic for *Salmonella* rapidly reduce *Salmonella* contamination on glass and stainless steel surfaces. Bacteriophage. 2017; 3(3), 1-6.
[38] Lee YD, Park JH. Characterization and application of phages isolated from sewage for reduction of *Escherichia coli* O157:H7 in biofilm. LWT-Food Science and Technology. 2015; 60:571-577.
[39] Jassim SAA, Abdulamir AS, Bakar FA. Novel phage-based bio-processing of pathogenic *Escherichia coli* and its biofilms. World J Microbiol Biotechnol. 2012; 28:47–60.
[40] Sillankorva S, Oliveira R, Vieira MJ, Sutherland IW, Azeredo J. Bacteriophage F S1 infection of *Pseudomonas fluorescens* planktonic cells versus biofilms. Biofouling. 2004; 20(3):133–138.
[41] Montañez-Izquierdo VY, Salas-Vázquez, DI, Rodríguez-Jerez JJ. Use of epifluorescence microscopy to assess the effectiveness of phage P100 in controlling *Listeria monocytogenes* biofilms on stainless steel surfaces. Food Control. 2012; 23:470-477.
[42] Khalifa L, Brosh Y, Gelman D, Coppenhagen-Glazer S, Beyth S, Poradosu-Cohen R, Que YA, Beyth N, Hazan R. Targeting *Enterococcus faecalis* biofilms with phage therapy. Applied and Environmental Microbiology. 2015; 81(8):2696-2705
[43] Fenton M, Keary R, McAuliffe O, Ross RP, O'Mahony J, Coffey A. Bacteriophage-derived peptidase CHAPK eliminates and prevents *Staphylococcal* biofilms. International Journal of Microbiology. 2013; ArticleID625341, 8 pages.
[44] Gutierrez D, Ruas-Madiedo P, Martinez B, Rodriguez A, Garcia P. Effective Removal of *Staphylococcal* Biofilms by the Endolysin LysH5. PLOS ONE. 2014; 9(9):1-8.
[45] Sharma M, Ryuand JH, Beuchat LR. Inactivation of *Escherichia coli* O157:H7 in biofilm on stainless steel by treatment with an alkaline cleaner and a bacteriophage. Journal of Applied Microbiology. 2005; 99:449–459.
[46] Siringan P, Connerton PL, Payne JHR, Connerton IF. Bacteriophage-mediated dispersal of *Campylobacter jejuni* biofilms. Applied and Environmental Microbiology. 2011; 77(10):3320–3326.