Advance Journal of Food Science and Technology 16(SPL): 92-101, 2018
DOI:10.19026/ajfst.16.5942
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: September 14, 2017 Accepted: January 25, 2018 Published: November 10, 2018

Research Article

Bio-Sanitization Using Specific Bacteriophages to Control *Escherichia coli* O157:H7 in Cherry Tomatoes

[1]M.E.S. Lopez, [2]M.T.P. Gontijo, [3]L.S. Batalha and [3]R.C.S. Mendonça
[1]Departamento de Ingeniería de Alimentos, Universidad de Córdoba, Montería 230002, Colombia
[2]Departamento de Microbiologia, Universidade Federal de Viçosa, Avenida Peter Henry Rolfs s/n Campus Universitário, Viçosa 36570000,
[3]Departamento de Tecnologia de Alimentos, Universidade Federal de Viçosa, Avenida Peter Henry Rolfs s/n Campus Universitário, Viçosa 36570000, Brazil

Abstract: This study aimed to evaluate the use of bacteriophages as "bio-sanitizers" to prevent and to control *E. coli* O157:H7 growth in cherry tomatoes. Phages with specificity towards this pathogen were propagated, titrated and characterized through transmission electron microscopy and it was also determined the feasibility of the isolated phages after the sanitization process for further application on the tomatoes. Furthermore, the *in vitro* behavior of *E. coli* O157:H7 in the presence or absence of the viral particles and the lytic spectrum of the isolated phages were also determined. Moreover, the action of a pool of bacteriophages and some chemical sanitizers commonly used in the food industry were compared in terms of the growth of *E. coli* O157:H7 and their potential as life extending agents was evaluated for tomatoes stored at room temperature. Bacteriophages reached high concentrations, ranging from 10^9 to 10^{14} PFU/mL and were predominantly specific for the *Escherichia* genus, showing no lytic activity for *Salmonella*, *Pseudomonas*, *Enterobacter* and *Enterococcus*. Bacteriophages were classified within the *Myoviridae* family and presented viability after the sanitization processes with sodium dichloroisocyanurate and hydrogen peroxide. There was no significant difference ($p>0.05$) between the sanitizing action of the pool of bacteriophages and the chemical sanitizers in the growth of *E. coli* O157:H7. Through *in vitro* assays, we found that bacteriophages substantially reduce microbial growth and, thus, showed potential as biological sanitizers.

Keywords: Bacteriophages, biocontrol, bio-sanitizer, cherry tomatoes, *E. coli* O157:H7

INTRODUCTION

In the last few years, the intake of fresh and organic foods associated with nutritional and health benefits have increased around the world (Rana and Paul, 2017). Read-to-eat products consumption have grown exponentially over the years (Kim and Min, 2017) and among the fruit varieties, tomatoes are the fourth mostly highly consumed vegetable in the USA, behind only of potatoes, lettuce and onions (USDA, 2016) and the cherry-type represents a great part of the intake. The concern of the consumer regarding tomatoes consumption is related to some compounds associated with the improvement of health, such as carotenoids (lycopene) and phenolic compounds with antioxidant activity (Cichon *et al*., 2017; Stajčić *et al*., 2015).

However, the number of outbreaks and contaminations associated with Ready-to-Eat (RTE) and minimally processed fruits and vegetables have increased (Campos *et al*., 2013; Cerna-Cortes *et al*., 2015; De Silva Felício *et al*., 2015). One of the main causative agents of these outbreaks in North America is *E. coli* O157:H7 (Boggione *et al*., 2017; CDC, 2017; Lopez *et al*., 2017). The infections caused by this pathogen are mainly associated with the intake of fresh and ready-to-eat foods (Kotzekidou, 2013), salads (Chitarra *et al*., 2014), spinach (Alam *et al*., 2014), carrots, melons and pineapples (Abadias *et al*., 2012) and tomatoes (Deering *et al*., 2015).

The incubation period of *E. coli* O157:H7 after the ingestion of the contaminated food is between three to four days and the majority of patient present watery diarrhea and abdominal pains as the main symptoms (Majowicz *et al*., 2014), those are symptoms associated with the production of Shiga toxins (Berry and Wells, 2010; Lim *et al*., 2010). Around 80 to 90% of the cases, the diarrhea becomes bloody (hemolytic colitis) after

Corresponding Author: M.E.S. Lopez, Food Engineer Department, Universidad de Córdoba, Montería, Colombia, Tel.: +573104690204

two to three days of illness. The hospitalization of the patient is necessary in 23 to 47% of the symptomatic patients. Moreover, this pathogen is associated with hemolytic uremic syndrome and neural dysfunctions, which can be deadly in severe cases (Lim *et al*., 2010).

Vegetables and several legumes are often eaten raw without additional cooking. The decontamination strategy that has been adopted by the majority of industries and suppliers of ready-to-eat foods is limited to chemical sanitization. Chlorine-based sanitizers are highly used due to their fast action, easy application, effective reduction of the microbial load (from 1 to 3.15 log CFU/g) and complete dissociation in water (Ramos *et al*., 2013). Despite the fact that the USDA approves the use of chloride sanitizers for the National Organic Program (NOP), in the last few years, there has been an increase in concerns about the use of precursors such as organic chloramines. Organic chloramines are harmful to humans and have a high carcinogenic potential (D'Acunzo *et al*., 2012; Van Haute *et al*., 2013). These problems and the requirements for a green stamp on ready-to-eat products make it necessary to research and apply new technologies in the industry.

Bacteriophages are viruses with lytic activity towards bacterial cell, these entities are able to infect, replicate and lysate the host microorganism. They are ubiquitous viruses that have an important function of the regulation of microbial populations in several ecosystems due to their ability of infect bacteria (Golkar *et al*., 2014; Pardini *et al*., 2017; Hungaro *et al*., 2014). Some studies demonstrated the efficiency of bacteriophages in the control of several pathogens in swine (Albino *et al*., 2014), poultry (Hungaro *et al*., 2013), chicken (Zampara *et al*., 2017), processing surfaces (Hosseinidoust *et al*., 2014) and on fresh fruits (Oliveira *et al*., 2014).

The contamination of cherry tomatoes by *E. coli* O157:H7 can occur before or after harvesting (Jung *et al*., 2014). Due to the effectiveness in the reduction of pathogens, the ease of application, self-replication, self-limitation and no interference in the physical, chemical or and sensory characteristics of the product, bacteriophages are a potential bio-sanitizer that could be used in the food industry (Nobrega *et al*., 2015). In this way, this study aimed to use bacteriophages specific for *E. coli* O157:H7 to control this pathogen and evaluate its potential to decontaminate cherry tomatoes in comparison with chemical agents (sanitizers).

MATERIALS AND METHODS

Bacteria and bacteriophage species: To perform these studies, *E. coli* O157:H7 (ATCC 43895) was used as the host in the propagation process of the lytic bacteriophages and to induce the contamination of the cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*). The used bacteriophages and bacteria are present in Table 1 and 2, respectively.

Propagation of bacteriophages: Bacteriophages were propagated according to the methodology adapted from Kudva *et al*. (1999), while adding to the Petri plaques with positive lyses 10 mL of SM buffer (0.1 mol/L NaCl (Vetec, RJ, Brazil), 0.01 mol/L $MgSO_4.7H_2O$ (Chemco, SP, Brazil), 0.05 mol/L Tris-Cl (Sigma Aldrich, Germany), pH = 7.5). The plaques were incubated at 37°C, while being agitated at 100 rpm by using a shaker (SL-221, SOLAB, Brazil) for 24 h. After this period, an aliquot from the plate was removed and centrifuged at 13,000 g (Sigma 3K30) for 5 min. Next, the suspension was filtered through

Table 1: Bacteriophages propagated in *E. coli* O157:H7

Phage code	Origin (Brazil)	Host isolation	Titer log PFU/mL
UFV-BPFA1	Wastewater from chicken processing plant	*E. coli* ATCC 11229	11.0±0.1
UFV-BPFC1	Exudate from chicken carcasses		10.6±0.5
UFV-AREG1*	Stable wastewater		12.0±0.4

*: Phage genome available on Genbank, under accession number KX009778.3 (Lopez *et al*., 2016)

Table 2: Evaluation of specificity of different bacteriophages specific for *E. coli* O157:H7 in relation to different types of *Salmonella*, *Escherichia coli* and *Enterobacter*

		Bacteriophages			
Bacteria	ATCC	UFV-BPFA1	UFV-BPFC1	UFV-AREG1	Pool
Salmonella enteritidis	13076	-	-	-	-
Salmonella typhi	6539	-	-	-	-
Salmonella cholerasius	10708	-	-	-	-
E. coli	11229	+	+	+	+
E. coli O111ab	CDC O11ab	+	+	+	+
E. coli	23229	+	+	+	+
E. coli O86:H35	CDC O86:H35	-	-	-	-
Pseudomonas aeruginosa	25619	-	-	-	-
Pseudomonas fluorescens	NCTC 10038	-	-	-	-
Enterococcus faecium	6569	-	-	-	-
Enterobacter aerogenes	6538	-	-	-	-

-: Result of negative lyse; +: Result of positive lyse

a cellulose acetate membrane (Sartorius Stedim Biotech, Germany) with 0.22-µm pores. The filtrate was stored and kept under refrigeration for further analysis.

Titration of bacteriophages: To determine the concentration of bacteriophages in the suspension, SM buffer was used to perform decimal dilutions in order to obtain the count of Plaques Forming Units (PFU) between 10 and 100 PFU/mL. An aliquot of 500 µL of *E. coli* O157:H7 cultivated in broth BHI (Brain Heart Infusion-HIMEDIA, India) at 37°C for 24 h was mixed in 5 mL of semisolid BHI agar (0.7%). To the semisolid agar containing the bacteria, 100 µL of serial dilutions of bacteriophage suspension were added and poured onto agar based plates of BHI (HIMEDIA-India), the lyse plaques were analyzed after an incubation period of 15 to 18 h at 37°C.

Evaluation of the lytic spectrum of bacteriophages: With the aim of verifying whether the selected bacteriophages would be able to control the growth of *E. coli* O157:H7 and other bacteria lineages, the lytic activity of phages was evaluated for different bacteria (Table 2) via the surface micro-drop technique adapted from Hungaro *et al*. (2013), where the phages were suspended in the title of 10^8 PFU/mL. The plaques were incubated for 24 h at the optimum temperature for target microorganism growth. The specificity of phages was confirmed by visualization of halos on the plaque surface.

Evaluation of the morphology of bacteriophages: The morphologic analysis of bacteriophages was performed according to the methodology adapted from Oliveira *et al*. (2009). An aliquot of 1 mL of the suspension of 10^{14} PFU/mL was centrifuged at 26,000 g (HETTICH-Zentrifugen Mikro 200R) for 60 min. The sediment was washed with 0.1 mol/L ammonium acetate solution (Vetec, RJ, Brazil) and, again, was centrifuged at 26,000 g for 60 min. The sediment was re-suspended in 1 mL of distilled water and was filtered through a cellulose acetate membrane with 0.22-µm pores. A volume of 8 µL of this suspension was placed on the surface of an electron microscopy grid coated with formvar resin (KOCH Electron Microscopy Ltda, Brazil). A drop of 2% (w/v) uracil acetate water solution (Sigma Aldrich, Germany) was placed on the screen surface for 15 sec, after which it was washed with distilled water and dried at ambient temperature for 24 h. Next, the observation was performed by using a transmission electron microscopy at 80 kV, in the Núcleo de Microscopia e Microanálise (NMM) of the Universidade Federal de Viçosa.

Feasibility of bacteriophages in sanitizer solutions: The selected phages for the biocontrol of *E. coli* O157:H7 were evaluated for their feasibility in sanitizer solutions while aiming to evaluate *E. coli* resistance after sanitization processes. This evaluation was performed according to the methodology adapted from Olofsson *et al.* (1998) and Adams (1959) in which 9 mL of sodium dichloroisocyanurate (200 mg/L), peracetic acid (80 mg/L) and hydrogen peroxide (60 mg/L) were utilized. In the sanitizer solutions, 1 mL of the mixture of phages was added to the concentration of 10^9 PFU/mL. Thus, the feasibility was determined after 15 min of interaction between phages by counting the number of halos or plaques in cultures of *E. coli* O157:H7 as described above.

Comparison of the action of sanitizers and phages on *E. coli* O157:H7: Cherry tomatoes were decontaminated by gamma irradiation at 4 kGy for 2 h in order to reduce the maximum initial contamination by using gamma irradiation equipment Nordion, IR214, GB 127 from the Nuclear Technology Development Center (CDTN) of the Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil. The tomatoes were inoculated with 10^5 CFU/mL of *E. coli* O157:H7 by immersion (five minutes) in 1 L of bacteria suspension. Samples were submerged in sanitizer solutions of hydrogen peroxide (NIPPO-LAT 2000-Nippon Chemical) 58.1±5.2 mg/L, sodium dichloroisocyanurate (NIPPO-CLOR-Nippon Chemical) 219±13.2 mg/L, peracetic acid (NIPPO-LAT 2000AP-Nippon Chemical) 91±17.2 mg/L and the pool of bacteriophages (10^9 PFU/mL) for 15 min. Any neutralizing agent was added to test solutions following the methodology described by Beuchat (1992). The action of the sanitizer solutions and the phage suspension were determined by counting *E. coli* after plating them in EMB agar (Eosin Methylene Blue, HIMEDIA, India).

***In vitro* growth of *E. coli* O157:H7 in the presence of bacteriophages:** Tubes of 10 mL tubes of BHI broth were inoculated with 1% (v/v) of *E. coli* and incubated at 37°C up to DO: 0.5 to 600 nm. At this point, suspensions of each bacteriophage (10^8 PFU/mL) were added to previously incubated cultures. The activity of bacteriophages was determined by absorbance in the spectrophotometer (600 nm) at intervals of 1 min until the constant results.

Evaluation of shelf life after phage bio-sanitization: Microbiological analyses were performed at times 0, 3, 6, 9 and 12 days, respectively after storage at a temperature of 25°C (BOD, Solab-SL200) after the phase bio-sanitization process. Twenty-five grams of tomato were transferred to sterilized bags and were homogenized in 225 mL of peptone water solution (Difco, Sparks, USA) 0.1% for 2 min in a Stomacher (Marconi, MA440, Brazil). For the study of *E. coli*

O157:H7 present in the samples, selective seeding in EMB agar (Himedia, India) and Mac Conkey agar (Himedia, India) were performed at 37°C for 24 h.

Statistical analyses: All experiments were conducted in triplicate with three repetitions, the effect of treatment with phages and sanitizers on *E. coli* O157:H7 was analyzed by ANOVA and Tukey test at a 5% level of significance. The mean *E. coli* O157:H7 counts during the evaluation of shelf life were evaluated by the use of regression. All statistical analyses were performed using the Minitab16® Statistical Software.

RESULTS AND DISCUSSION

Propagation and titration of bacteriophages: Bacteriophages showed specificity for *E. coli* O157:H7. The presence of lyse plaques or phage halos on the agar surface indicates that phages have a lytic profile. Bacteriophages were propagated in *E. coli* O157:H7 (ATCC 43895) as the host and reached concentrations between 10^9 and 10^{12} PFU/mL according to the titration process (Table 1). High concentrations of bacteriophages can be explained by conditions that are favorable for microorganism growth and consequently phage growth, because bacteriophages have an important role as regulators of microbial growth in high density bacterial population (Miller and Day, 2008) and in conditions that are favorable for the interaction between bacteria and the infection process.

Lytic spectrum: The selected bacteriophages presented lytic activities against more than one *E. coli* strain (Table 2). None of the selected bacteriophages presented lytic activity against *Pseudomonas*, *Salmonella*, or *Enterobacter aerogenes*. Bacteriophages in this study are highly specific for the *E. coli*, which suggests a large advantage for the application of these bacteriophages for phage therapy and the control of these pathogens in foods because of its exclusive action against *E. coli* O157:H7, avoiding the interference with other strains that can be beneficial for animals, humans, or plants. However, a lack of specificity in relation to genus is also a disadvantage in the use of bacteriophages for bio control of more than one different pathogen, which can be replaced by the use of cocktails or a mix of phages.

Niu *et al*. (2012) found a high specificity for *E. coli* isolating the bacteriophage AKFV33, thus showing that the ability to be highly specific for *E. coli* without specificity for other microorganisms can guarantee, up to a certain point, efficiency and security in the therapeutic use of these bacteriophages against the *Escherichia* genus. However, Callaway *et al*. (2008) demonstrated different results in the specificity of isolated bacteriophages for *E. coli,* which presented with lytic activities on *Salmonella typhimurium*. Hungaro *et al*. (2013) isolated bacteriophages that were specific for *Salmonella enteritidis* and they presented lytic activity against *E. coli.*

The similarity between surfaces structures of bacteria from the same genus can contribute to the identical profile of specificity, which can belong to the same group or lineage of bacteriophages, although their source of isolation was different (Niu *et al*., 2012). The selectivity of phages also allows the elaboration of a pool of bacteriophages are able to act on the *E. coli* lineage; the goal of this study was to focus on increasing the efficiency of the decontamination process or biocontrol while also reducing the probability of host resistance.

Morphological characterization of bacteriophages: The three selected bacteriophages for the biocontrol of *E. coli* O157:H7 in the decontamination of cherry tomatoes presented with a similar morphology (Fig. 1).

The analysis by transmission electron microscopy showed that the 4 isolated bacteriophages presented a similar morphology: rigid icosahedral head and non-contractile long tail with a size between 100 and 200 nm. In according with morphology, the results suggested these phages can be classified in the *Caudovirales* order and in the *Myoviridae* family.

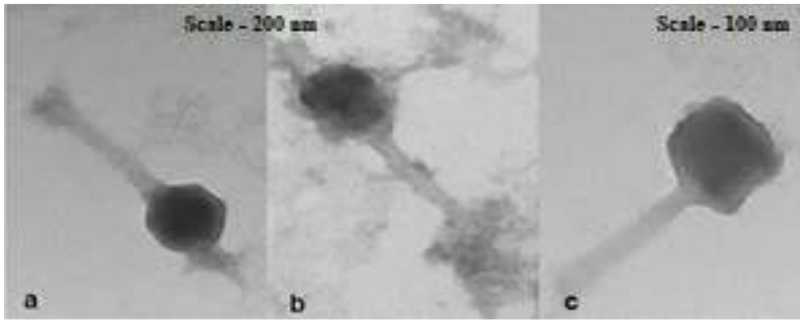

Fig. 1: Photomicrography of the isolated bacteriophages specific for *E. coli* O157:H7 characterized by transmission electron microscopy (TEM) 80 Kv, increased 85,000X. Morphological characteristics: rigid icosahedral head and long tail (a) UFV-BPFA1: Tail- 115.83×22.01 nm; Head- 61.80×57.00 nm, (b) UFV-BPFC1: Tail- 130.92×19.43 nm; Head- 88.46×68.85 nm, (c) UFV-AREG1: Tail- 106.31×19.59 nm; Head- 67.30×64.29 nm (Lopez *et al*., 2016)

Table 3: Logarithm of PFU/mL of the pool of bacteriophages submitted to different sanitizing solutions used in the food industry after 15 min of contact

Phage	Control	Sodium dichloroisocyanurate (219±13.2 mg/L)	Peracetic acid (91±17.2 mg/L)	Hydrogen peroxide (58.1±5.2 mg/L)
UFV-BPFA1	11.3±0.2	10.3±0.2	-	7.8±0.4
UFV-BPFC1	10.8±0.1	10.3±0.4	-	7.5±0.1
UFV-AREG1	11.1±0.1	10.0±0.4	-	9.8±0.1
Pool	13.4±0.1	10.3±0.1	-	9.4±0.3

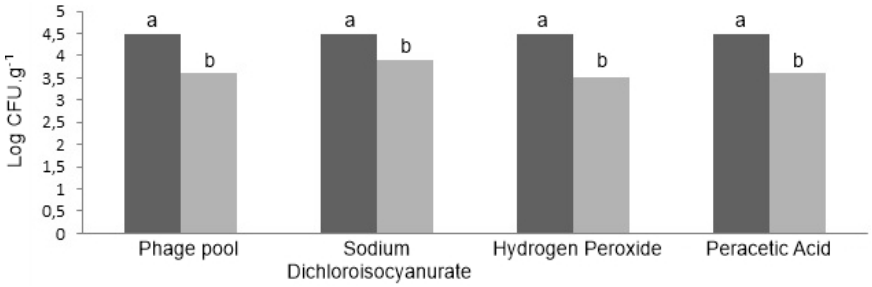

Fig. 2: *E. coli* O157:H7 count in cherry tomatoes in the control treatment (darker bars) and after treatment with different sanitizing solutions or the phage pool at 25°C (lighter bars)
Means represented by *E. coli* O157:H7 count (log CFU/g); Means with the same letter at each combination of control/treatment indicates no significant different (p>0.05)

Bacteriophages are subdivided into 13 families, of which *Myoviridae*, *Podoviridade* and *Siphoviridae* are the most common families for pathogen biocontrol, once they belong to the *Caudovirales* order (Ackermann, 1998; Fokine and Rossmann, 2014; Hungaro *et al*., 2013).

Feasibility of bacteriophages after the sanitization process: The sensitivity of the three bacteriophages and also its pool in different sanitizer solutions can be observed in Table 3. Generally, the least aggressive sanitizer for phages was sodium dichloroisocyanurate. The bacteriophage UFV-AREG1 presented the greatest observed resistance towards hydrogen peroxide. The maximum reduction observed by the action of the sanitizers was evident in the pool of bacteriophages, of 3.1 and 4 log PFU/mL for sodium dichloroisocyanurate and hydrogen peroxide, respectively. For peracetic acid, bacteriophages lost their feasibility when submitted to the solution for a time of 15 min.

Hayes *et al*. (2017) tested the efficiency of the cleaning protocol of common sanitizers against *Lactococcus lactis* phages. It was tested benzalkonium chloride, polyvinylpyrrolidone-iodine, hydrogen peroxide, ethanol, isopropanol, sodium percarbonate, sodium dichloroisocyanurate and sodium chlorite in combinations. Benzalkonium chloride was the most effective pure compound, resulting in a complete elimination after 30 min. On the other hand, hydrogen peroxide presented low effective virucidal effect, as observed in our study.

Andrade *et al*. (2008) stated that peracetic acid has an action spectrum on gram-positive bacteria, filamentous fungi and yeast, viruses and bacterial spores, a fact that can explain the loss of the feasibility of phages. However, these results did not prevent the use of bacteriophages and sanitizers, which together were resistant of the sanitization processes in the food industry. Although, the phage resistance to sanitization can represent severe problems for the food industry, as like the dairy industry where many fermentation processes are developed with lactic bacteria, the bacterial growth can be affected by bacteriophages due to the role as a regulator of microbial growth (Marcó *et al*., 2012).

Comparison of action of sanitizers and bacteriophages on *E. coli* O157:H7 in the decontamination of tomatoes: The treatment of tomatoes with gamma irradiation (four kGy) for two hours was enough to eliminate the natural microbiota of the tomatoes, once it was not possible to detect the presence of microorganisms in the microbiologic analysis. The count of viable cells of *E. coli* O157:H7 observed in tomatoes from the control treatment was close to the inoculum limit of 10^4 CFU/g. The decontamination treatment of tomatoes with the sodium dichloroisocyanurate solution reduced the count of *E. coli* O157:H7, on average, by 0.57 log CFU/g in comparison to the control treatment. A reduction of approximately 0.98 log CFU/g was observed when tomatoes were treated with hydrogen peroxide. The action of peracetic acid resulted in reductions of 0.9 log CFU/g. The treatment of cherry tomatoes with a pool of bacteriophages resulted in a reduction of approximately 0.95 log CFU/g in the population of *E. coli* O157:H7 (Fig. 2).

The efficiency of the use of bacteriophages in the reduction of microorganism contamination of foods depends on several factors such as concentration, contact time, temperature, pH and matrix of food and it must be evaluated for each type of food and processing condition (Drulis-Kawa *et al*., 2012; Guenther *et al*., 2009).

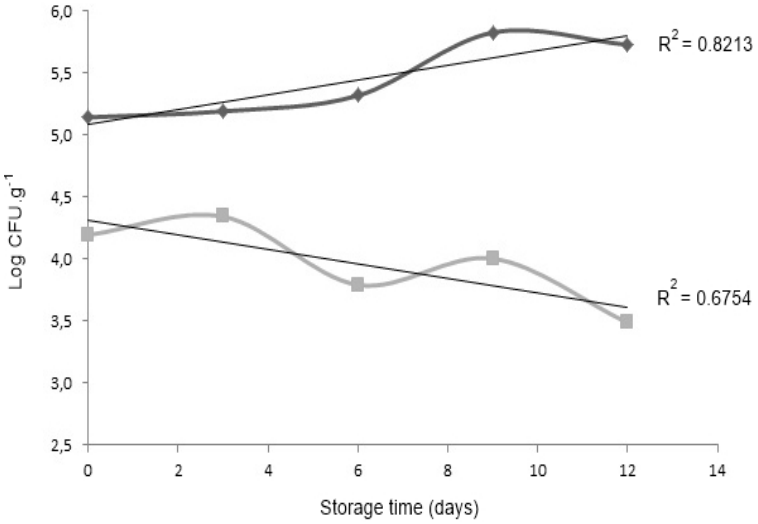

Fig. 3: Logarithm of the number of *E. coli* O157:H7 inoculated in cherry tomatoes as positive control (darker lines) and treated with bacteriophages (lighter lines) as a function of storage time

As previously observed, any treatment succeeded in the total elimination of the initial inoculum in a desirable level, once the minimum dose of infection of *E. coli* O157:H7 oscillates between 2 and 2,000 cells, demonstrated by their resistant to acidic substances (Law, 2000; Lim *et al*., 2010; Nguyen and Sperandio, 2012) and the possibility of acquiring disease is relatively high. Moreover, due to the fact that microorganisms have developed resistance to some substances such as sanitizers and antibiotics, an increased concentration of active ingredients becomes necessary to obtain better results. These increases can lead to sanitizers reacting with organic matter present in the majority of foods, thereby decreasing its concentration and producing harmful substances to humans and consequently leading to a reduction in its efficiency or activity (Davidson and Harrison, 2002; Nguyen and Yuk, 2013).

The results presented in this study are promising for the use of phage therapy in the preventive bio control of fruits and vegetables because in addition to matching the efficiency of sanitizers, bacteriophages offer an economic, healthy and reliable alternative for pathogen bio control. The use of bacteriophages is an interesting tool to combat bacterial resistance to sanitizers and antibiotics (Endersen *et al*., 2014).

Evaluation of the action of bacteriophages as a function of storage time: The behavior of the pool of bacteriophages was observed in the biocontrol of *E. coli* O157:H7 for a 12 days period when using cherry tomatoes at a temperature of 25°C with an initial contact time of 15 min to simulate industrial sanitization conditions (Fig. 3). The results show that the pool of bacteriophages reduced the number of *E. coli* O157:H7 cells as a function of time, which was not observed in the control treatment. The deterioration of tomatoes started in the 7th day, showing exudate, which probably favored the multiplication of microorganisms in the control treatment and, consequently, of bacteriophages in this treatment. The initial inoculum of *E. coli* O157:H7 in the tomatoes was approximately 10^5 CFU/g and the addition of a mix of bacteriophages at a concentration of 10^9 PFU/mL allowed the elimination of approximately two logarithmic cycles during 12 days of storage at a storage temperature of 25°C.

The findings of *E. coli* O157:H7 in cherry tomatoes were low compared to phage removal in liquid of aqueous systems. Bacteriophages adhered to the surface of tomatoes after the drying process at ambient temperatures, thus becoming frozen and avoiding interactions with bacteria; consequently, there was a minimum logarithmic reduction in the number of microorganisms, which restricts the application on a solid food matrices. This finding is corroborated by Guenther *et al*. (2009), which observed a reduction of up to 2 Log PFU/mL when bacteriophages specific for *L. monocytogenes* were applied in plant products stored at 20°C for 6 days and attributed these results to the immobilization of the bacteriophages by the food matrix.

The behavior of data obtained from the treatment with bacteriophages follows an exponential regression with R^2 = 0.6754, which can be explained by the ecological relationship between phage and bacteria and which decreases its potential of infection when bacteria is at a low concentration in the environment (Boggione *et al*., 2017; Pereira *et al*., 2017). This somehow guarantees the perpetuation of its species, because its replication depends exclusively on the concentration of the host in the environment (Hungaro *et al*., 2013).

***In vitro* growth of *E. coli* O157:H7 in the presence of bacteriophages:** The effect was evident on the growth of *E. coli* O157:H7 after the addition of bacteriophages

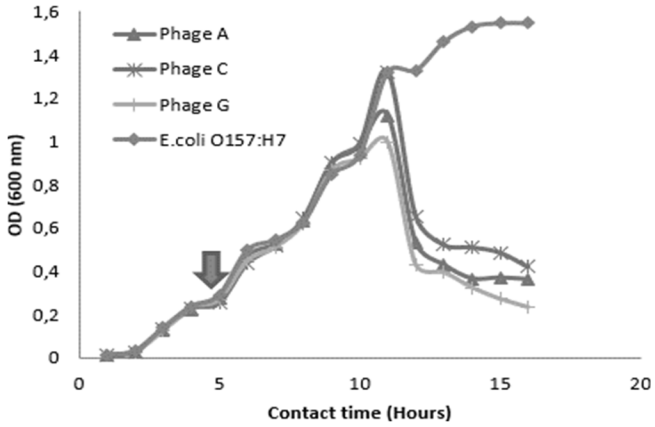

Fig. 4: *In vitro* growth of *E. coli* O157:H7 in the absence (positive control) or in the presence of the isolated bacteriophages The arrow indicates the point of addition of each bacteriophage solution (around 5 h)

at a concentration of 10^8 PFU/mL (Fig. 4), as the reduction in the OD indicates cell lysis ant the release of new viral particles, marking the beginning of new infection cycles occurred (Endersen *et al*., 2014).

Bacteriophages can naturally reduce the absorbance up to approximately 1.3 units. This fact is a natural and spontaneous process that makes bacteriophages a fascinating tool as specific bio-sanitizers for *E. coli* O157:H7. Biocontrol is based on a lytic process, so once it is identified, the bacteria that is responsible for a particular infection combats it naturally by using a specific bacteriophage (Boggione *et al*., 2017; Oliveira *et al*., 2014; Hungaro *et al*., 2013). This interest is based on certain features such as the high multiplication ability of bacteriophages, an absence of toxicity, specificity for bacterial hosts and its abundance in nature (Denes and Wiedmann, 2014; Endersen *et al*., 2014; Hungaro *et al*., 2013).

As observed in the Fig. 4, bacteria are more sensitive to infection during the growth deceleration phase (nutrient stress) and, at this point, the absorbance decreased, thereby indicating that the bacterial population was high enough to guarantee the meeting between bacteria and bacteriophage and a consequent infection. In the food industry, there are many stressors that microorganisms face and these hostilities can be the key for the implementation of new barrier technologies that complement the use of bacteriophages. The use of bacteriophages in the biological control of pathogens has great potential to increase the food microbiological security based on its long history of secure use, relatively easy manipulation and its high specific antimicrobial activity (Boggione *et al*., 2017; Endersen *et al*., 2014; Hungaro *et al*., 2013).

CONCLUSION

The obtained results that with the pool of bacteriophages in the biocontrol of *E. coli* O157:H7 in tomatoes was similar to those presented for the evaluated sanitizers; therefore, the pool of bacteriophages could be used as bio-sanitizers as a complement technology to control this pathogenic microorganism. The feasibility of bacteriophages in sanitizer solutions supports the possibility of a combination of these technologies as a bio-conservation tool in order to reduce contamination by pathogens and to considerably increase the shelf life of these products so they are economic, efficient and considered secure.

CONFLICT OF INTEREST

Authors declare no conflict of interests of any nature.

REFERENCES

Abadias, M., I. Alegre, M. Oliveira, R. Altisent and I. Viñas, 2012. Growth potential of *Escherichia coli* O157:H7 on fresh-cut fruits (melon and pineapple) and vegetables (carrot and escarole) stored under different conditions. Food Control, 27(1): 37-44.

Ackermann, H.W., 1998. Tailed bacteriophages: The order *Caudovirales*. Adv. Virus Res., 51: 135-201.

Adams, M.H., 1959. Bacteriophages. Interscience, New York, pp: 450-451.

Alam, M., C. Ahlström, S. Burleigh, C. Olsson, S. Ahrné, M. El-Mogy *et al*., 2014. Prevalence of *Escherichia coli* O157:H7 on spinach and rocket as affected by inoculum and time to harvest. Sci. Hortic-Amsterdam, 165: 235-241.

Albino, L.A., M.H. Rostagno, H.M. Húngaro and R.C. Mendonça, 2014. Isolation, characterization and application of bacteriophages for *Salmonella* spp. biocontrol in pigs. Foodborne Pathog. Dis., 11(8): 602-609.

Andrade, N.J., C.L. Pinto, O. Pinto and M.S. Rosado, 2008. Controle da higienização na indústria de alimentos. In: Andrade, N.J. (Ed.), Higiene na indústria de alimentos: Avaliação e controle da adesão e formação de biofilmes bacterianos. Varela, São Paulo, Brasil, pp: 185-221.

Berry, E.D. and J.E. Wells, 2010. *Escherichia coli* O157:H7: Recent advances in research on occurrence, transmission and control in cattle and the production environment. Adv. Food Nutr. Res., 60: 67-117.

Beuchat, L.R., 1992. Surface disinfection of raw produce. Dairy, Food Environ. Sanit., 12(1): 6-9.

Boggione, D.M.G., L.S. Batalha, M.T.P. Gontijo, M.E.S. Lopez, A.V.N.C. Teixeira, I.J.B. Santos and R.C.S. Mendonça, 2017. Evaluation of microencapsulation of the UFV-AREG1 bacteriophage in alginate-Ca microcapsules using microfluidic devices. Colloid. Surface B, 158: 182-189.

Callaway, T.R., T.S. Edrington, A.D. Brabban, R.C. Anderson, M.L. Rossman *et al*., 2008. Bacteriophage isolated from feedlot cattle can reduce *Escherichia coli* O157:H7 populations in ruminant gastrointestinal tracts. Foodborne Pathog. Dis., 5(2): 183-191.

Campos, J., J. Mourão, N. Pestana, L. Peixe, C. Novais and P. Antunes, 2013. Microbiological quality of ready-to-eat salads: An underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. Int.J. Food Microbiol., 166(3): 464-470.

CDC, 2017. Centers for Disease Control and Prevention. Escherichia coli. Retrieved form:< https://www.cdc.gov/ecoli/index.html> Acesso em: 26 de julho de 2017.

Cerna-Cortes, J.F., N. Leon-Montes, A.L. Cortes-Cueto, L.P. Salas-Rangel, A.C. Helguera-Repetto *et al*., 2015. Microbiological quality of ready-to-eat vegetables collected in Mexico City: Occurrence of aerobic-mesophilic bacteria, fecal coliforms and potentially pathogenic nontuberculous mycobacteria. BioMed Res. Int., 2015: 9.

Chitarra, W., L. Decastelli, A. Garibaldi and M.L. Gullino, 2014. Potential uptake of *Escherichia coli* O157:H7 and *Listeria monocytogenes* from growth substrate into leaves of salad plants and basil grown in soil irrigated with contaminated water. Int. J. Food Microbiol., 189: 139-145.

Cichon, M.J., K.M. Riedl and S.J. Schwartz, 2017. A metabolomic evaluation of the phytochemical composition of tomato juices being used in human clinical trials. Food Chem., 228 : 270-278.

D'Acunzo, F., A. Del Cimmuto, L. Marinelli, C. Aurigemma and M. De Giusti, 2012. Ready-to-eat vegetables production with low-level water chlorination. An evaluation of water quality and of its impact on end products. Ann. dell'Istituto Super. Sanità, 48(2): 151-160.

Davidson, P.M. and M.A. Harrison, 2002. Resistance and adaptation to food antimicrobials, sanitizers and other process controls. Food Technol., 56(11): 69-78.

De Silva Felício, M.T., T. Hald, E. Liebana, A. Allende, M. Hugas, C. Nguyen-The *et al*., 2015. Risk ranking of pathogens in ready-to-eat unprocessed foods of non-animal origin (FoNAO) in the EU: Initial evaluation using outbreak data (2007-2011). Int. J. Food Microbiol., 195: 9-19.

Deering, A.J., D.R. Jack, R.E. Pruitt and L.J. Mauer, 2015. Movement of Salmonella serovar Typhimurium and E. coli O157:H7 to ripe tomato fruit following various routes of contamination. Microorganisms, 3(4): 809-825.

Denes, T. and M. Wiedmann, 2014. Environmental responses and phage susceptibility in foodborne pathogens: Implications for improving applications in food safety. Curr. Opin. Biotech., 26: 45-49.

Drulis-Kawa, Z., G. Majkowska-Skrobek, B. Maciejewska, A.S. Delattre and R. Lavigne, 2012. Learning from bacteriophages - advantages and limitations of phage and phage-encoded protein applications. Curr. Protein Pept. Sci., 13(8): 699-722.

Endersen, L., J. O'Mahony, C. Hill, R.P. Ross, O. McAuliffe and A. Coffey, 2014. Phage therapy in the food industry. Ann. Rev. Food Sci. Technol., 5: 327-349.

Fokine, A. and M.G. Rossmann, 2014. Molecular architecture of tailed double-stranded DNA phages. Bacteriophage, 4(2): e28281.

Golkar, Z., O. Bagasra and D.G. Pace, 2014. Bacteriophage therapy: A potential solution for the antibiotic resistance crisis. J. Infect. Dev. Ctries, 8(2): 129-136.

Guenther, S., D. Huwyler, S. Richard and M.J. Loessner, 2009. Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. Appl. Environ. Microbiol., 75(1): 93-100.

Hayes, S., J. Murphy, J. Mahony, G.A. Lugli, M. Ventura, J.P. Noben, C.M.A.P. Franz, H. Neve, A. Nauta and D. Van Sinderen, 2017. Biocidal inactivation of *Lactococcus lactis* bacteriophages: efficacy and targets of commonly used sanitizers. Front. Microbiol., 8: 107.

Hosseinidoust, Z., A.L.J. Olsson and N. Tufenkji, 2014. Going viral: Designing bioactive surfaces with bacteriophage. Colloid. Surface B, 124: 2-16.

Hungaro, H.M., R.C.S. Mendonça, D.M. Gouvêa, M.C.D. Vanetti and C.L.O. Pinto, 2013. Use of bacteriophages to reduce *Salmonella* in chicken skin in comparison with chemical agents. Food Res. Int., 52(1): 75-81.

Hungaro, H.M., M.E.S. Lopez, L.A.A. Albino and R.C.S. Mendonça, 2014. Bacteriophage: The Viruses Infecting Bacteria and Their Multiple Applications. In: Reference Module in Earth Systems and Environmental Sciences, Elsevier, USA, pp: 1-12.

Jung, Y., H. Jang and K.R. Matthews, 2014. Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. Microb. Biotechnol., 7(6): 517-527.

Kim, J.H. and S.C. Min, 2017. Microwave-powered cold plasma treatment for improving microbiological safety of cherry tomato against *Salmonella*. Postharvest Biol. Tec., 127: 21-26.

Kotzekidou, P., 2013. Microbiological examination of ready-to-eat foods and ready-to-bake frozen pastries from university canteens. Food Microbiol., 34(2): 337-343.

Kudva, I.T., S. Jelacic, P.I. Tarr, P. Youderian and C.J. Hovde, 1999. Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. Appl. Environ. Microbiol., 65(9): 3767-3773.

Law, D., 2000. Virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli*. J. Appl. Microbiol., 88(5): 729-745.

Lim, J.Y., J. Yoon and C.J. Hovde, 2010. A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. J. Microbiol. Biotechn., 20(1): 5-14.

Lopez, M.E.S., L.S. Batalha, P.M.P. Vidigal, L.A.A. Albino, D.M.G. Boggione *et al*., 2016. Genome sequence of the enterohemorrhagic *Escherichia coli* bacteriophage UFV-AREG1. Genome Announc., 4(5): e00412-16.

Lopez, M.E.S., M.T.P. Gontijo, D.M.G. Boggione, L.A.A. Albino, L.S. Batalha and R.C.S. Mendonça, 2017. Microbiological Contamination in Foods and Beverages: Consequences and Alternatives in the Era of Microbial Resistance. In: Grumezescu, A. and A.M. Holban (Eds.), Microbial Contamination and Food Degradation. 1st Edn., Chapter: 3, Elsevier, pp: 472.

Majowicz, S.E., E. Scallan, A. Jones-Bitton, J.M. Sargeant, J. Stapleton, F.J. Angulo, D.H. Yeung and M.D. Kirk, 2014. Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: A systematic review and knowledge synthesis. Foodborne Pathog. Dis., 11(6): 447-455.

Marcó, M.B., S. Moineau and A. Quiberoni, 2012. Bacteriophages and dairy fermentations. Bacteriophage, 2(3): 149-158.

Miller, R.V. and M.J. Day, 2008. Contribution of Lysogeny, Pseudolysogeny and Starvation to Phage Ecology. In: Abedon, S.T. (Ed.), Bacteriophage Ecology: Population Growth, Evolution and Impact of Bacterial Viruses. Chapter 5, Cambridge University Press, Cambridge, pp: 114-143.

Nguyen, H.D.N. and H.G. Yuk, 2013. Changes in resistance of *Salmonella* Typhimurium biofilms formed under various conditions to industrial sanitizers. Food Control, 29(1): 236-240.

Nguyen, Y. and V. Sperandio, 2012. Enterohemorrhagic *E. coli* (EHEC) pathogenesis. Front. Cell. Infect. Mi., 2: 90.

Niu, Y.D., K. Stanford, A.M. Kropinski, H.W. Ackermann, R.P. Johnson *et al*., 2012. Genomic, proteomic and physiological characterization of a T5-like bacteriophage for control of Shiga toxin-producing Escherichia coli O157:H7. PLoS One, 7(4): e34585.

Nobrega, F.L., A.R. Costa, L.D. Kluskens and J. Azeredo, 2015. Revisiting phage therapy: New applications for old resources. Trends Microbiol., 23(4): 185-191.

Oliveira, A., S. Sillankorva, R. Quinta, A. Henriques, R. Sereno and J. Azeredo, 2009. Isolation and characterization of bacteriophages for avian pathogenic *E. coli* strains. J. Appl. Microbiol., 106(6): 1919-1927.

Oliveira, M., I. Vinas, P. Colas, M. Anguera, J. Usall and M. Abadias, 2014. Effectiveness of a bacteriophage in reducing *Listeria monocytogenes* on fresh-cut fruits and fruit juices. Food Microbiol., 38: 137-142.

Olofsson, L., J. Ankarloo and I.A. Nicholls, 1998. Phage viability in organic media: Insights into phage stability. J. Mol. Recogn, 11: 91-93.

Pardini, G.M.T., B.L. Silva and L.M.E. Soto, 2017. Bacteriophage genome sequencing: A new alternative to understand biochemical interactions between prokaryotic cells and phages. J. Microb. Biochem. Technol., 9: 169-173.

Pereira, C., C. Moreirinha, L. Teles, R.J. Rocha, R. Calado, J.L. Romalde, M.L. Nunes and A. Almeida, 2017. Application of phage therapy during bivalve depuration improves *Escherichia coli* decontamination. Food Microbiol., 61: 102-112.

Ramos, B., F.A. Miller, T.R. Brandão, P. Teixeira and C.L.M. Silva, 2013. Fresh fruits and vegetables-an overview on applied methodologies to improve its quality and safety. Innov. Food Sci. Emerg., 20: 1-15.

Rana, J. and J. Paul, 2017. Consumer behavior and purchase intention for organic food: A review and research agenda. J. Retail. Consum. Serv., 38: 157-165.

Stajčić, S., G. Ćetković, J. Čanadanović-Brunet, S. Djilas, A. Mandić and D. Četojević-Simin, 2015. Tomato waste: Carotenoids content, antioxidant and cell growth activities. Food Chem., 172: 225-232.

USDA (United States Department of Agriculture), 2016. Tomatoes. Retrieved from: https://www.ers.usda.gov/topics/crops/vegetables-pulses/tomatoes.aspx.

Van Haute, S., I. Sampers, K. Holvoet and M. Uyttendaele, 2013. Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. Appl. Environ. Microbiol., 79(9): 2850-2861.

Zampara, A., M.C.H. Sørensen, A. Elsser-Gravesen and L. Brøndsted, 2017. Significance of phage-host interactions for biocontrol of *Campylobacter jejuni* in food. Food Control, 73: 1169-1175.