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Bacteriophage solutions as processing aids in beef production: An essential hurdle to reduce *E. coli* O157:H7 and the "Big Six" serotypes.

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Abstract

The research presented herein demonstrates the applications of bacteriophage solutions against pathogenic *E. coli* on fresh beef. The first chapter demonstrates the effects of a novel seven-bacteriophage (Mello-Shebs 1 phages) cocktail application against the seven USDA adulterant Shiga Toxin-producing *E. coli* (STEC) O157:H7 and the "Big Six" in vitro and on beef trim before grinding. The in vitro killing efficiency of each phage against the strain used for isolation ranged from 96.2% to 99.9%. On beef, inoculated and treated samples were held for 30 min or 6 h and at 7°C or 25°C before grinding to simulate refrigeration and ambient air temperatures. Both phage application ($P \leq 0.0001$) and temperature ($P = 0.0451$) had significant effects on STEC reduction. After 30 minutes of lysing time, phage application led to a significant 0.76 Log at 7°C and 0.81 Log reduction at 25°C. This study shows that the application of a seven-bacteriophage cocktail that target STEC O157:H7 and 'Big Six' serotypes are effective in reducing STEC on ground beef.

The research presented in chapter two demonstrates the effects of a commercially available bacteriophage cocktail compared to lactic acid and peroxyacetic acid in reducing *E. coli* O157:H7 on fresh beef surface when kept under vacuum or aerobic conditions simulating a High Event Period, a period of high *E. coli* rates in beef trim. During which, beef subprimals are typically removed from vacuum and treated with antimicrobials, then re-packaged and later tested to verify the absence of STEC. Bacterial loads were evaluated 30 min and 6 h after antimicrobial applications. Under

aerobic conditions for 30 min and 6 h, phage reduced STEC in beef by approximately 1.4 Log CFU/cm^2 whereas organic acids resulted in approximately 0.5 Log CFU/cm^2 reduction. Under vacuum conditions for 30 min, bacteriophage significantly reduced STEC by 1 Log CFU/cm^2, while no significant effects were observed from organic acid treatment. Under vacuum conditions for 6 h, bacteriophage reduced STEC loads by 1.4 Log CFU/cm^2, lactic acid reduced by 0.6 Log CFU/cm^2, and no significant effects were observed with peroxyacetic acid application.

The research presented in chapter three demonstrates the effects of multiple antimicrobial treatments against STEC O157:H7 and the "Big Six" on fresh beef surface: a novel seven-bacteriophage cocktail (P), peroxyacetic acid (PAA), ultraviolet light (UV), and acidified chlorite (ASC) each alone and in combinations of two under simulated High Event Period scenarios. All treatment combinations that included phage significantly decreased STEC populations ($P<0.0001$). Under vacuum conditions, Phage + PAA resulted in the lowest STEC load (1.49 Log CFU/cm^2 reduction), followed by Phage, Phage + UV, Phage + ASC, Phage + PAA and UV + ASC, respectively. Under aerobic conditions, P + UV provided the greatest reduction (1.46 Log CFU/cm^2), followed by Phage + PAA, P, Phage + ASC, UV+ASC, and PAA, respectively. When analyzing contrasts, treatments with phage significantly decreased STEC loads when compared to other treatments ($P<0.0001$) and control ($P<0.0001$). Overall, bacteriophage application has been demonstrated to have a high efficacy in reducing STEC loads on beef alone or with other antimicrobial treatments during a simulated industry application.

Overall, bacteriophage applications targeting STEC provided an efficient control against all adulterant serotypes. It is clear that organic acids commonly used during meat processing are not efficient in controlling STEC. Bacteriophages emerged as an effective antimicrobial that can be applied in different processing steps and during different production scenarios. Currently, according to the United States Department of Agriculture (USDA), bacteriophage solutions allowed to be used in meat processing have Generally Recognized as Safe (GRAS) status only against O157:H7. This research showed that phages can also lower the “Big Six” serotypes. Therefore, when producing phage cocktails that successfully target the “Big Six”, biotechnology companies must request to the Food and Drug Administration (FDA) GRAS status for solutions not only targeting STEC O157:H7, but also the O145, O121, O111, O103, O45, and O26 serotypes.

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Introduction

Foodborne illness will affect 1 in 6 people in the United States every year, despite the numerous antimicrobial interventions used to reduce contamination of food products, particularly those of animal origin. In the United States, ground beef is the most widely consumed beef product at an estimated 42% of all beef cuts (Davis & Lin, 2005). Ground beef is also known by its associations with foodborne diseases caused by Shiga Toxin-producing *E. coli* (STEC) and *Salmonella* (Scallan et al., 2011). These pathogens are found within the intestines of animal hosts as well as within their environment. There is a high inherent risk of cross-contamination from animals to their raw food products during processing, requiring the need for food producers to have robust and effective food safety systems to ensure they produce safe food for the public.

Escherichia coli is known to be present on cattle hides before the slaughter process, unfortunately hide removal can create opportunity for cross-contamination from hide to carcass (Arthur et al., 2007). This is likely a main mechanism of transfer of enteric organisms, such as *E. coli*, from the gastrointestinal tract to fresh beef products. The contamination of carcasses can produce contaminated whole cuts (primals, subprimals, steaks) and contaminated trim. Trim from hundreds of carcasses can make up one lot of trim, with many lots combined for ground beef. The USDA does mandate sampling of Shiga Toxin-producing *E. coli* on beef carcasses and in beef trim, but

even the best sampling plans do not ensure identification or complete absence of pathogens.

In order to maintain the safety of our food there needs to be continuous efforts in improving antimicrobial food safety interventions. The inseparability of microorganisms from food animals and the environment will always pose a risk for contaminating final food products. From upstream preventative measures to direct sanitizing applications, antimicrobial interventions are extensively used in order to combat the presence of foodborne bacteria, viruses, and parasites that cause food poisoning (Marriott, Schilling, & Gravani, 2018). The recent acceptance of bacterial viruses (bacteriophages) as a natural method for food biocontrol has continued to show promise for their efficacy and versatility and could be the next advancement for food safety systems.

The continuous advancement of food safety interventions is essential to decrease and eliminate foodborne pathogens and to mitigate the burden of foodborne illnesses. The objectives within the presented research are to 1) Evaluate the efficacy of reducing all seven STEC serotypes in ground beef with a cocktail of seven environmentally isolated bacteriophages, 2) Evaluate the efficacy of a commercial bacteriophage preparation on beef surface under a High Event Period scenario compared to other commonly used antimicrobial solutions, and 3) Determine the optimal treatment of STEC during a simulated High Event Period between organic acids PAA and ASC, UV light, and a 7-bacteriophage cocktail alone and in combinations.

Review of Literature

Foodborne diseases

Foodborne illness affects 1 in 6 people in the United States every year and remains a major cause of hospitalization and death worldwide despite many advances in food sanitation techniques and pathogen surveillance (Moye, Woolston, & Sulakvelidze, 2018). The Centers for Disease Control and Prevention (CDC) estimates that every year 48 million people become ill, 128,000 are hospitalized, and 3,000 die from foodborne diseases in the United States (CDC, 2020a). There are 31 major foodborne pathogens that are acquired in the United States, the top five illness-causing microorganisms are norovirus (58%), followed by nontyphoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%), *Campylobacter* spp. (9%), and *Staphylococcus aureus* (3%). The five leading causes of hospitalization were nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), *Toxoplasma gondii* (8%), and *E. coli* O157:H7 (4%). The five leading causes of death were nontyphoidal *Salmonella* spp. (28%), *T. gondii* (24%), *Listeria monocytogenes* (19%), norovirus (11%), and *Campylobacter* spp. (6%) (Scallan et al., 2011). In 2013, foodborne illnesses imposed over $15.5 billion of economic burden onto the public, with 90% of the burden caused by only five pathogens. *Salmonella* nontyphoidal spp. poses the greatest economic burden at an estimated $3.66 Billion, roughly 25% of the total burden by foodborne illnesses (Hoffmann, Maculloch, & Batz, 2015).

Escherichia coli

Escherichia coli are motile, gram negative, rod shaped bacteria belonging to the *Enterobacteriaceae* family. They are facultative anaerobes that make up a part of our gut microbiota as commensal bacterium living symbiotically within mammals such as humans, cattle, and deer. Most strains of *E. coli* are harmless; however, many strains have acquired DNA that code for virulence factors such as enterotoxins, invasins, or antibiotic resistance genes that allow them to cause severe gastrointestinal, urinary, or meningeal infections (Nataro & Kaper, 1998). Acquisition of new DNA is by horizontal gene transfer from extracellular DNA (transformation), other bacteria (conjugation), or bacteriophages (transduction). There are six well-known *E. coli* pathotypes that have acquired virulence factors and cause their own diseases: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Nataro & Kaper, 1998).

The EHEC pathotype belong to the same group as verotoxigenic *E. coli* (VTEC) also known as Shiga toxin-producing *E. coli* (STEC), which are characterized by adhering to the intestinal walls of the colon and distal ilium (Nataro & Kaper, 1998; Phillips et al., 2000). Virulence factors found in this pathotype include the locus of enterocyte effacement, shiga toxins, and the pO157 plasmid, among others (Nataro & Kaper, 1998). The locus of enterocyte effacement (LEE) pathogenicity island in the chromosomal DNA (cDNA) codes for a type III secretion system (T3SS), secretory effector proteins, intimin, and Translocated intimin receptor (Tir) that are partially responsible for the attaching-

and-effacing (A/E) histopathology (Jarvis & Kaper, 1996; Mcdaniel, Jarvis, Donnenberg, & Kaper, 1995; Perna et al., 1998). Intimin is an adhesin encoded by the *eae* gene whose product is expressed on the bacterial cell surface. Intimin binds strongly to Tir (Translocated intimin receptor) which is inserted into the host enterocyte membrane by the bacterial T3SS, which also translocates many other bacterial proteins into the cytoplasm (Stevens, Galyov, & Stevens, 2006). The Shiga toxin produced by STEC is an AB_5 toxin coded by either *stx1* or *stx2* genes in the cDNA. The *stx1* is identical to the *Shigella dysenteriae* I gene (Nakao & Takeda, 2000; O'Brien et al., 1992) and *stx1* and *stx2* have 55-58% sequence identity (Jackson, Neill, O'Brien, Holmes, & Newland, 1987), while *stx2* has been shown to be 400 times more toxic (Siegler et al., 2003; Tesh et al., 1993). The secreted toxin is endocytosed into the host cell where it is able to cleave the 60S ribosomal subunit, inhibiting protein synthesis. All clinical isolates of *E. coli* O157:H7 possess a pO157 plasmid while most non-O157 EHEC isolates possess a similar plasmid (Hales, Hart, Batt, & Saunders, 1992; Lim, Yoon, & Hovde, 2010). The pO157 plasmid is a highly conserved F-like plasmid with many insertion sequences indicating that the genetic fragments are likely from many different origins (Lim et al., 2010). Some virulence factors contained within pO157 include hemolysins, type II secretion system, adhesins, and many functional enzymes (Brunder, Schmidt, & Karch, 1996, 1997; Lathem et al., 2002; Schmidt, Henkel, & Karch, 1997; Schmidt, Karch, & Beutin, 1994; Tatsuno et al., 2001). Such genes

could influence pathogenesis of the bacterial hosts by increasing abilities of adherence, colonization, and acid resistance (Lim et al., 2010).

Strains of *E. coli* within a pathotype are differentiated by serotyping, which evaluates lipopolysaccharides (LPS), a major component of cell wall structures. Serotyping identifies the unique combination of LPS structure, position, stereochemistry, and linkage of this highly variable region of the cell membrane (Liu et al., 2010; Stenutz, Weintraub, & Widmalm, 2006). The three components used for serotyping are the LPS O-antigen, flagellar H-antigen, and the capsule K-antigen. There are 183 identified O groups and 53 H groups (Joensen, Tetzschner, Iguchi, Aarestrup, & Scheutz, 2015) with O157:H7 being the most notorious. Other serotypes within the EHEC pathotype include O20, O25, O26, O44, O91, O111, O114, O126, O127, O128, O142, and O158 (Nataro & Kaper, 1998). Other notorious serotypes are those known to cause foodborne illness include STEC O145, O121, O111, O104:H4, O103, O45, and O26 whose infections can range from very mild to life-threatening (CDC, 2018). In 2014, there were 4,437 culture confirmed STEC infections (43.2% due to O157, 56.5% due to non-O157) and over $298 million in medical care costs in the US (CDC, 2017; USDA-ERS, 2014). Common symptoms of STEC infections include diarrhea, most often bloody, abdominal cramps, and vomiting with the possible development of Hemolytic Uremic Syndrome (HUS), a type of kidney failure that develops in about 5-10% of STEC O157 infections that can be life-long or fatal (CDC, 2016). The infectious dose (ID) of STEC is very low with estimates of fewer than 700 cells to 10-100 cells (ID_{50}= < 100 cells) that

can cause illness (Armstrong, Hollingsworth, & Morris, 1996; FDA, 2012; Griffin & Tauxe, 1991; Tuttle et al., 1999).

In 1994, the USDA deemed *E. coli* O157:H7 an adulterant in ground beef after a multistate outbreak from undercooked hamburger patties served at a national fast food chain that resulted in over 500 illnesses and four deaths (CDC, 1993). Years later in 2012, six more serotypes referred to as the "Bix Six" were declared adulterants by the USDA after causing 64% of the STEC foodborne illnesses (USDA-FSIS, 2012b). From 2010 to 2018, there were 8,802 STEC infections caused by O157 (48.6%), O26 (17.8%), O103 (15.2%), O111 (10.5%), O121 (3.9%), O145 (2.5%), and O45 (1.5%) (CDC, 2020b). The implementation of the USDA's "Zero Tolerance" policy caused many food safety interventions to be established to eliminate the presence of foodborne *E. coli* in beef products before being sold to consumers (USDA-FSIS, 2012a). The USDA regulates sampling frequencies for the seven adulterant STEC serogroups and mandates that no STEC positive products are sold into commerce but are instead able to be sold to a cooker for further processing at a reduced profit.

Routes of contamination

It is known that *E. coli* can colonize the colorectal junction of cattle where it can be excreted in manure potentially contaminating the environment, their hides, and the hides of other cattle (Burnens et al., 1995; Callaway et al., 2008; Clarke, 1994; Griffin & Tauxe, 1991; Hancock et al., 1994; Wells et al., 1991). The

presence of STEC on cattle hides has been shown to be a major source of bacterial contamination of beef carcasses due to direct or cross contamination during harvesting and processing, particularly at dehiding and evisceration steps (Arthur et al., 2007, 2008, 2004, 2010; Bacon et al., 2000; Bell, 1997; Brichta-Harhay et al., 2008; Byrne, Bolton, Sheridan, McDowell, & Blair, 2000; McEvoy et al., 2000; Nou et al., 2003; Rhoades, Duffy, & Koutsoumanis, 2009). Evaluating the prevalence of *E. coli* within a beef harvesting facility, Barkocy-Gallagher et al. (2003) recovered *E. coli* O157:H7 from 5.9% of fecal samples, 60.6% of hide samples, and 26.7% of carcasses sampled before the pre-evisceration wash and from 1.2% of post-intervention carcasses at chilling at low levels of <3.0 cells per 100 cm^2. Slaughter facilities must implement extra preventative measures and increase sampling during warmer months (spring to mid-autumn) which have shown to have a higher incidence of *E. coli* (Barkocy-Gallagher et al., 2001; USDA-FSIS, 2014, 2017). Barkocy-Gallagher et al. (2003) also observed that non-O157 STEC *stx*-positive isolates were detected by PCR in fecal (34.3%), hides (92.0%), pre-evisceration carcasses (96.6%), and post-evisceration carcasses (16.2%) with about 4% of post-intervention carcasses having ≥3.0 cells per 100 cm^2 of non-O157 STEC and *stx*-harboring cells. Bacon et al. (2000) also demonstrated that the cattle hides can have approximately 5-7 Log_{10} CFU/100 cm^2 of *E. coli* prior to the slaughter process, with hide removal resulting in 3-5 Log_{10} CFU/100 cm^2 on the carcass, and 0.9 Log_{10} CFU/100 cm^2 on the post-chilling carcass. Arthur et al. (2004) observed a positive relationship between contamination level of hides and pre-evisceration carcass. Not only are individual carcasses at risk for contamination, but contamination can also be

disseminated throughout fabrication lines, food contact surfaces, equipment, and other beef products by cross contamination during poor harvesting and dressing procedures (Yang, He, Badoni, Tran, & Wang, 2017). As a result, it was found that the most prevalent serogroups in beef trimmings at a midwestern plant were: O121(17.9%), O45 (8.6%), and O111 (8.6%), while beef trimmings from another plant were positive for O103 (31%), O121 (24.6%), and O45 (21.8%) (Kanankege et al., 2017). The Zero Tolerance policy mandates that no trace of the adulterant serogroups should be detected in fresh beef, however, a realistic and feasible sampling plan cannot ensure the complete absence of a specific organism from all products (Huffman, 2006; ICMSF, 2002).

Food Safety interventions

The implementation of food safety regulations and interventions by industry and regulatory agencies has shown to reduce the overall number of retail food associated illnesses (Pollari et al., 2017). All antimicrobial interventions applied onto meat and poultry products are reviewed and approved by the Food Safety Inspection Service (FSIS) and listed in FSIS Directive 7120.1 (USDA-FSIS, 2019). There are many interventions that are effective in reducing microorganisms that can be implemented pre-harvest, during lairage, during harvest, and post-harvest (Wheeler, Kalchayanand, & Bosilevac, 2014). The best adopted pre-harvest interventions are probiotic feed supplementation and application of phages onto cattle hides, with vaccines against *E. coli* O157

also being well researched (Callaway, 2010; Dean-Nystrom, Gansheroff, Mills, Moon, & O'brien, 2002; Fingermann et al., 2018; Judge, Mason, & O'brien, 2004; Rabinovitz et al., 2012; Smith, 2014; Snedeker, Campbell, & Sargeant, 2012; Wheeler et al., 2014; Wolf et al., 2012). In lairage, phage products for hide sprays applied prior to entering the slaughter facility have become commercially available (Brashears & Chaves, 2017). During harvest, a multi-hurdle approach combining washing and sanitizing solutions has been effective in reducing bacterial populations on carcasses as long as the bacterial load does not exceed the capacity of the interventions (Koohmaraie et al., 2007). The most common physical interventions during harvest include steam-vacuuming (hocks and initial hide cuts), knife trimming (visible contamination), and water rinsing (Dorsa, Cutter, & Siragusa, 1996, 1997). Chemical interventions used during and after harvest mainly consist of organic acids with acetic, citric, and lactic acids being some of the most common agents (Belk, 2001). Other antimicrobial compounds used during or after harvest include oxidizing agents such as peroxyacetic acid (PAA), acidified sodium chlorite (ASC), ozone, or hypobromous acid (Wheeler et al., 2014).

Thermal decontamination methods are commonly used and have shown to be very effective (Olson & Nottingham, 1980). Steam vacuuming, hot, pressurized water sprays, and steam pasteurization are very common thermal interventions with nearly all large U.S. beef processing plants have automated hot water wash cabinets for pre-evisceration and final carcass washes (Wheeler et al., 2014). Spraying hot water (85 °C) for 15 s at 15 psi has shown to reduce all seven STEC serotypes between 3.2 and 4.2 Log CFU/cm^2 on inoculated beef surfaces (Kalchayanand et al., 2012). Even though thermal

treatments can cause the meat to have a cooked or whitened appearance, but the discoloration lessens after chilling (Castillo, Hardin, Acuff, & Dickson, 2002). Non-thermal interventions do not affect quality attributes such as color, making them a good alternative to thermal interventions and chemical interventions. The beef industry uses electron beams (e-beams), cold atmospheric plasma, UV light, UV and ozone combination, and high-pressure processing (Wheeler et al., 2014). Electron beams utilize streams of electrons to damage microbial DNA, inhibiting replication (Silindir & Özer, 2009). UV light, like e beams, damages microbial DNA causing replication inhibition. Cold atmospheric plasma is a weak ionized gas that generates reactive molecules like atoms, ions, and radicals that damage the cell wall (Laroussi, 2005; Stoffels, Sakiyama, & Graves, 2008). Lastly, high pressure processing is able to decontaminate food throughout instead of only surface decontamination while in packaging, however, it does cause discoloration (Gross & Jaenicke, 1994; Wheeler et al., 2014). No single intervention results in complete sterilization, making a multi-hurdle approach necessary to produce safe food and to maximize reduction of foodborne microorganisms (Wheeler et al., 2014).

Organic Acids

Weak acidic solutions such as lactic acid or acetic acid diffuse across the bacterial cell wall and dissociate in the cytoplasm, disrupting the chemiosmotic functions of the cell membrane, and the transmembrane proton gradient

causing the cytoplasmic pH to decrease (Baldry & Fraser, 1988; Leaper, 1984; Liberti & Notarnicola, 1999). The efficacy of these chemical interventions has been demonstrated with high reductions (Harris, Miller, Loneragan, & Brashears, 2006; Laury et al., 2009; Zhao, Zhao, & Doyle, 2009), but lower or inconsistent efficacy has also been observed (Bosilevac, Nou, Barkocy-Gallagher, Arthur, & Koohmaraie, 2006; King et al., 2005; Yeh, de Moura, Van Den Broek, & de Mello, 2018). These interventions are advantageous due to their cost, availability, and simple application (Mataragas, Skandamis, & Drosinos, 2008). However, their potential decrease in efficacy has been demonstrated with the development of acid resistant bacterial pathogens when exposed to sub-lethal applications (Bearson, Bearson, & Foster, 1997; Brown, Ross, McMeekin, & Nichols, 1997; Capita, Riesco-Peláez, Alonso-Hernando, & Alonso-Calleja, 2014; Cheng, Yu, & Chou, 2003; Foster & Hall, 1990; Goodson & Rowbury, 1989; Leyer, Wang, & Johnson, 1995; Wesche, Gurtler, Marks, & Ryser, 2009).

Acid Resistance

E. coli O157:H7 outbreaks associated with acidic foods such as apple cider and fermented sausage have brought attention to the ability of *E. coli* to survive in foods previously believed to be preserved by low pH (Leyer et al., 1995; Tsai & Ingham, 1997). Furthermore, acid resistance has also been described as having a cross-protection effect against heat, salt, and irradiation preservation of foods (Buchanan & Edelson, 1999; Cheng & Kaspar, 1998; Garren, Harrison, & Russelv, 1998) and that acid tolerance of *E. coli* O157:H7 is sustained or even enhanced within refrigerated storage (Cheng &

Kaspar, 1998; Clavero & Beuchat, 1996; Lin et al., 1996; Miller & Kaspar, 1994). There are two main acid tolerance mechanisms that EHEC are known to have: a pH-dependent system (acid-induced) involving arginine decarboxylase and/or glutamate decarboxylase (Hersh, Farooq, Barstad, Blankenhorn, & Slonczewski, 1996; Lin et al., 1996) and a pH-independent system (general stress protection system) regulated by σ^{38} encoded by *rpoS* (RNA polymerase, sigma) (Cheville, Arnold, Buchrieser, Cheng, & Kaspar, 1996; Kolter, 1993; Matin, Auger, Blum, & Schultz, 1989). *rpoS* regulates the transcription of many proteins, some of which have protective functions that could increase bacterial survival (Loewen & Triggs, 1984; Sak, Eisenstark, & Touati, 1989). Interruption of rpoS significantly reduces the acid tolerance of *E. coli* O157:H7, *Salmonella typhimurium*, and *Shigella flexneri* (Cheville et al., 1996; Lee, Slonczewski, & Foster, 1994; Small, Blankenhorn, Welty, Zinser, & Slonczewski, 1994). Gorden & Small (1993) found that 80% of tested *E. coli* isolates were considered acid resistant and that the acid resistance mechanism can be triggered at pH 5.0, which allows for up to nearly 1,000-fold enhanced survival of *E. coli* 0157:H7 at a second exposure (Brudzinski & Harrison, 1998). Some non-O157:H7 serotypes (O26, O45, O104) have been shown to be more acid resistant than O157 (Kim, Breidt, Fratamico, & Oh, 2015). Acid resistance may enhance virulence of pathogenic strains (Arnold & Kaspar, 1995; Gorden & Small, 1993; O'Driscoll, Gahan, & Hill, 1996) which increases the need for effective, multi-hurdle decontamination efforts for animal products.

Ultraviolet Light

Ultraviolet (UV) light is a common method for disinfection used in many different fields. Ultraviolet light is approved for use by the U.S. Food and Drug Administration (FDA) to decontaminate food, food contact surfaces, juices, and water because of its non-thermal and environmentally friendly nature (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; FDA, 2000; Sommers, Sites, & Musgrove, 2010). UV light applied at 250-270 nm is considered germicidal UV-C with 254 nm the most common wavelength used for disinfection, compared to UV-A (315-400 nm) and UV-B (280-315 nm) that have limited microbial properties (Bintsis et al., 2000; Guerrero-Beltrán & Barbosa-Cánovas, 2004). The antimicrobial ability of UV-C light is based on the ability for the light to be absorbed by the DNA of microorganisms to cause pyrimidine dimerization through a thiamine and cytosine cross-linkage which cannot be replicated, leading to cell death (Escalona, Aguayo, Martínez-Hernández, & Artés, 2010; Guerrero-Beltrán & Barbosa-Cánovas, 2004; Hijnen, Beerendonk, & Medema, 2006; Sastry, Datta, & Worobo, 2000; Snowball & Hornsey, 1988). The amount of DNA damage depends on the UV absorption characteristics, the target micro-organism's resistance to UV exposure, and the applied UV dose (Ngadi, Smith, & Cayouette, 2003).

Bacteriophage (phage)

Bacteriophages are bacterial viruses, discovered independently by Fredrick Twort in 1915 and Felix D' Herelle in 1917, but it was D'Herelle that coined the term "bacteriophage", from Greek *phagein* meaning "to devour" (Kutter, Kuhl, Alavidze, & Bs,

2013). Phages are nature's most abundant microorganism with over 10^{30} on Earth, outnumbering bacteria 10 to 1 (Chibani-Chennoufi, Bruttin, Dillmann, & Brüssow, 2004; Wommack & Colwell, 2000). They are obligate intracellular parasites, which are organisms that require a host cell to replicate. Phages were researched in the early 20th century for their antibacterial properties to be used for bacterial infections, however, trouble creating phage therapies and the introduction of Penicillin in 1928 led to the near disappearance of phage therapy in Western countries (Kortright, Chan, Koff, & Turner, 2019). In recent time, the development of antibiotic resistant bacteria and 'superbugs' along with the decline in research and development of new antibiotics has raised a global alarm for new and alternative methods to combat life threatening resistant infections that kill over 35,000 people each year in the United States alone (CDC, 2019).

Tailed phages belong to the order *Caudovirales*, that are then divided into three families based on the tail morphology: *Myoviridae* (long contractile tail), *Siphoviridae* (long non contractile tail), and *Podoviridae* (short non contractile tail) (Ackermann, 2003). *E. coli* infecting bacteriophages, known as T4 phages belonging to the *Myoviridae* family, are relatively large viruses with complex viral structures at about 90 nm wide and 200 nm long with around 168 kbp size double stranded DNA genome stored inside their icosahedral capsid (Fuller, Raymer, Kottadiel, Rao, & Smith, 2007; Miller et al., 2003; Petrov et al., 2006). Connected to the capsid is the collar region that has short whiskers called whisker antigen control, followed by the long contractile tail consisting of a

hallow tail tube covered by a contracting tail sheath. The tail is connected to the complex baseplate at the end that has a set of six short and six long tail fibers (Cerritelli, Wall, Simon, Conway, & Steven, 1996; Coombs & Eiserling, 1977).

Mechanism of Infection

There are two mechanisms of infection used by phages: the lytic cycle and the lysogenic cycle. The lysogenic cycle is typically seen in temperate phages which can integrate their DNA into the host genome and can remain there for multiple replication cycles as a prophage, but can also undergo the lytic cycle (Echols, 1972). *Escherichia coli* infecting T4 phages are strictly lytic, replicating only by the lytic cycle. Briefly, the lytic begins by 1) attachment of the long and short tail fibers to the surface of a bacterial cell via receptor specificity 2) tail sheath contraction and tail tube penetration occur in a syringe-like motion that injects phage DNA into host cytoplasm 3) replication and synthesis of phage genome and proteins using host mechanisms 4) newly synthesized phage proteins and DNA assemble into new virions 5) virion release via host cell lysis (Fineran, Petty, & Salmond, 2009).

Phage attachment is initiated by the reversible binding of at least three long tail fibers to cell surface structures via receptor specificity determined by protein gp37, a 14 amino acid residue region on the tail fibers (Heller, 1992; Montag, Hashemolhosseini, & Henning, 1990). Tail fibers bind to bacterial structures including proteins, lipopolysaccharides (LPS), teichoic acids, pili, and flagella, with the most important being OmpC porins and the O-chain on LPS (Heller, 1992; Rakhuba, Kolomiets, Szwajcer-Dey, &

Novik, 2010; Yu & Mizushima, 1982). *E. coli* and *Salmonella* have been shown to share 98% of OmpC amino acid sequence identity and immunogenic structure (Singh, Singh, Williams, Jones, & Abdullah, 1995; Singh, Upshaw, Abdullah, Singh, & Klebba, 1992). Bacteriophages are able to recognize similar core structures of LPS of related bacteria since they are relatively conserved across gram-negative species allowing for a potentially broad host range (Rakhuba et al., 2010). Long tail fiber binding causes a conformational change in the baseplate from a hexagonal dome-shaped structure to a six-point star that allows the short tail fibers to unfold and bind irreversibly to the host cell (Le et al., 2013; Yap & Rossmann, 2014). Short tail fiber binding causes the tail sheath to undergo a conformational change causing it to contract to about one-third of its original length while extending the hollow tail tube underneath, in a syringe-like motion, through the outer membrane (Kostyuchenko et al., 2005; Yap & Rossmann, 2014). To assist with tail tube penetration and cell wall lysis, the lysozyme gp5 protein on the spike-form structure at the tip of the tail dissolves the outer membrane before dissociating into the periplasmic space (Hu, Margolin, Molineux, & Liu, 2015; Kanamaru, Ishiwata, Suzuki, Rossmann, & Arisaka, 2005; Kanamaru et al., 2002). After gp5 dissociates, gp27 is able to contact the inner membrane to form a transmembrane channel, allowing the phage genome to be adsorbed directly into the host cytoplasm (Hu et al., 2015; Kanamaru et al., 2002). Adsorption of phage DNA into the host cell has been shown to be faster at higher bacterial growth rates which is positively correlated to cell size and

directly proportional to total cell surface area (Hadas, Einav, Fishov, & Zaritsky, 1997; Rabinovitch, Fishov, Hadas, Einav, & Zaritsky, 2002). In conditions where there is a high number of phages per bacterial cell (>100 phages per cell), a phenomenon called 'lysis from without' occurs where the cumulative effects of gp5 on the cell wall cause sufficient damage and quick lysis without the need for phage replication (Abedon, 2011). Upon DNA adsorption into the host cytoplasm, replication and translation begin using hijacked cytosolic machinery such as ribosomes until virion proteins are assembled and the host cell wall lyses via lysosomal activity, releasing new phages that are able to begin the cycle on to new and susceptible bacteria (Kao & McClain, 1980). Host lysis can release 10 to 400 new phages depending on host factors such as growth rate, rates of synthesis and assembly of phage components, and by lysis time which seem to depend on the content of the protein-synthesizing system and cellular dimensions (Hadas et al., 1997).

Phage Therapy as Biocontrol in Food

Phages have been studied for their antibacterial ability in the medical field, and recently, have been approved for a clinical trial investigating their use as an intravenous therapy against multi-drug resistant bacteria (LaFee & Buschman, 2019). Phages have been well studied in other environments and have been used in fields such as aquaculture (Nakai & Park, 2002; Oliveira, Castilho, Cunha, & Pereira, 2012), dairy (Brüssow, 2001), and agriculture (Svircev, Roach, & Castle, 2018) due to their ability to lyse only targeted bacteria without disrupting other non-targeted microbiota (Carlton,

1999; Meaden & Koskella, 2013; Sulakvelidze, Alavidze, & Morris, 2001). Since the approval of List-Shield™ by the FDA in 2006 to target *L. monocytogenes* on ready-to-eat (RTE) foods, there have been many commercial phage products including Ecoshield™ and Listshield™ by Intralyix, Inc that target *E. coli* and *L. monocytogenes* respectively and Listex™ and PhageGuard S™ are products of Micreos Food Safety, Inc that target *Listeria* and *Salmonella* for direct food application (Bren, 2007; Kazi & Annapure, 2016). Phage use in food safety has been well investigated for reducing foodborne pathogens including *E. coli*, *Salmonella*, and *Listeria*. Phages are viewed as a natural alternative to traditional antimicrobial approaches to food safety and preservation, and have many advantages as well as notable disadvantages (Goodridge & Abedon, 2003; Greer, 2005). Research is still needed to thoroughly understand the mechanisms of phage resistance that can be acquired by the hosts and the development of methods to overcome phage resistance. Although the results of the studies appear to be encouraging, they should be interpreted with caution (Kazi & Annapure, 2016; Mahony, McAuliffe, Ross, & van Sinderen, 2011). Using bacteriophages for specific pathogen biocontrol offers an additional hurdle for robust food safety management systems (Endersen et al., 2014).

Conclusion

There are many food safety interventions that can be used to improve the safety of our food supply. With the continued evolution of pathogens, our

antimicrobial interventions also need to evolve and adapt to address the changing demands. New and alternative interventions must be introduced in order to maintain an effective food safety system. Good hygienic slaughtering practices reduce fecal contamination on carcasses but does not guarantee the absence of STEC from products. The only effective method of eliminating STEC from foods is to introduce antimicrobial treatments in a multi-hurdle system (Wheeler et al., 2014). New interventions such as bacteriophages have been studied for their potential as antibacterial agents showing promise to combat acid resistant and virulent pathogens such as Shiga Toxin-producing *E. coli*.

References

Abedon, S. T. (2011). Lysis from without. *Bacteriophage*, *1*(1), 46–49. https://doi.org/10.4161/bact.1.1.13980

Ackermann, H.-W. (2003). Bacteriophage observations and evolution. *Research in Microbiology*, *154*, 245–251. https://doi.org/10.1016/S0923-2508(03)00067-6

Armstrong, G. L., Hollingsworth, J., & Morris, J. G. (1996). Emerging foodborne pathogens: Escherichia coli O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiologic Reviews*, *18*(1), 29–51. https://doi.org/10.1093/oxfordjournals.epirev.a017914

Arnold, K. W., & Kaspar, C. W. (1995). Starvation- and stationary-phase-induced acid tolerance in Escherichia coli O157:H7. *Applied and Environmental Microbiology*, *61*(5), 2037–2039.

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Guerini, M. N., Kalchayanand, N., Shackelford, S. D., … Koohmaraie, M. (2007). Transportation and Lairage Environment Effects on Prevalence, Numbers, and Diversity of Escherichia coli O157:H7 on Hides and Carcasses of Beef Cattle at Processing. *Journal of Food Protection*, *70*(2), 280–286. Retrieved from http://www.cdc.gov/

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., King, D. A., Shackelford, S. D., … Koohmaraie, M. (2008). Source tracking of Escherichia coli O157:H7 and Salmonella contamination in the lairage environment at commercial U.S. beef processing plants and identification of an effective intervention. *Journal of Food Protection*, *71*(9), 1752–1760. https://doi.org/10.4315/0362-028X-71.9.1752

Arthur, T. M., Bosilevac, J. M., Nou, X., Shackelford, S. D., Wheeler, T. L., Kent, M. P., … Koohmaraie, M. (2004). Escherichia coli O157 Prevalence and Enumeration of Aerobic Bacteria, Enterobacteriaceae, and Escherichia coli O157 at Various Steps in Commercial Beef Processing Plants. In *Journal of Food Protection* (Vol. 67). Retrieved from http://vm.cfsan.fda.gov/;ebam/bam-a2.html

Arthur, T. M., Brichta-Harhay, D. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2010). Super shedding of Escherichia coli O157:H7 by cattle and the impact on beef carcass contamination. *Meat Science*, *86*(1), 32–37. https://doi.org/10.1016/j.meatsci.2010.04.019

Bacon, R. T., Belk, K. E., Sofos, J. N., Clayton, R. P., Reagan, J. O., & Smith, G. C. (2000). Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *Journal of Food Protection*, *63*(8), 1080–1086. https://doi.org/10.4315/0362-028X-63.8.1080

Baldry, M. G. C., & Fraser, J. A. L. (1988). Disinfection with peroxygens. In *Industrial biocides* (pp. 91–116). New York, NY, USA: John Wiley & Sons.

Barkocy-Gallagher, G. A., Arthur, T. M., Rivera-Betancourt, M., Nou, X., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2003). Seasonal Prevalence of Shiga Toxin-Producing Escherichia coli, Including O157:H7 and Non-O157 Serotypes, and Salmonella in Commercial Beef Processing Plants. *Journal of Food Protection*, *66*(11), 1978–1986. https://doi.org/10.4315/0362-028X-66.11.1978

Barkocy-Gallagher, G. A., Arthur, T. M., Siragusa, G. R., Keen, J. E., Elder, R. O., Laegreid, W. W., & Koohmaraie, M. (2001). Genotypic Analyses of Escherichia coli O157:H7 and O157 Nonmotile Isolates Recovered from Beef Cattle and Carcasses at Processing Plants in the Midwestern States of the United States. *Applied and Environmental Microbiology*, *67*(9), 3810–3818. https://doi.org/10.1128/AEM.67.9.3810-3818.2001

Bearson, S., Bearson, B., & Foster, J. W. (1997). Acid stress responses in enterobacteria. *FEMS Microbiology Letters*, *147*(2), 173–180. https://doi.org/10.1016/S0378-1097(96)00503-4

Belk, K. (2001). Beef contamination technologies. *National Cattlemen's Beef Association*. Centennial, CO: National Cattlemen's Beef Association, Research and Technical Services.

Bell, R. G. (1997). Distribution and sources of microbial contamination on beef carcasses. *Journal of Applied Microbiology*, *82*, 292–300. https://doi.org/10.1046/j.1365-2672.1997.00356.x

Bintsis, T., Litopoulou-Tzanetaki, E., & Robinson, R. K. (2000). Existing and potential applications of ultraviolet light in the food industry - a critical review. *Journal of the Science of Food and Agriculture*, *80*(6), 637–645. https://doi.org/10.1002/(SICI)1097-0010(20000501)80:6<637::AID-JSFA603>3.0.CO;2-1

Bosilevac, J. M., Nou, X., Barkocy-Gallagher, G. A., Arthur, T. M., & Koohmaraie, M. (2006). Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and Enterobacteriaceae and reduce the prevalence of Escherichia coli O157:H7 on preevisceration beef carcasses. *Journal of Food Protection*, *69*(8), 1808–1813. https://doi.org/10.4315/0362-028X-69.8.1808

Brashears, M. M., & Chaves, B. D. (2017). The diversity of beef safety: A global reason to strengthen our current systems. *Meat Science*, *132*, 59–71. https://doi.org/10.1016/j.meatsci.2017.03.015

Bren, L. (2007, January 1). Bacteria-eating virus approved as food additive. *FDA Consumer*, Vol. 41, pp. 20–22.

Brichta-Harhay, D. M., Guerini, M. N., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., … Koohmaraie, M. (2008). Salmonella and Escherichia coli O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: An evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Applied and Environmental Microbiology*, *74*(20), 6289–6297. https://doi.org/10.1128/AEM.00700-08

Brown, J. L., Ross, T., McMeekin, T. A., & Nichols, P. D. (1997). Acid habituation of Escherichia coli and the potential role of cyclopropane fatty acids in low pH tolerance. *International Journal of Food Microbiology*, *37*(2–3), 163–173. https://doi.org/10.1016/S0168-1605(97)00068-8

Brudzinski, L., & Harrison, M. A. (1998). Influence of Incubation Conditions on Survival and Acid Tolerance Response of Escherichia coli 0157: H7 and Non-0157:H7 Isolates Exposed to Acetic Acid. *Journal of Food Protection*, *61*(5), 542–546. Retrieved from http://meridian.allenpress.com/jfp/article-pdf/61/5/542/1671173/0362-028x-61_5_542.pdf

Brunder, W., Schmidt, H., & Karch, H. (1996). KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic Escherichia coli O157:H7. *Microbiology*, *142*(11), 3305–3315. https://doi.org/10.1099/13500872-142-11-3305

Brunder, W., Schmidt, H., & Karch, H. (1997). EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. *Molecular Microbiology*, *24*(4), 767–778. https://doi.org/10.1046/j.1365-2958.1997.3871751.x

Brüssow, H. (2001). *PHAGES OF DAIRY BACTERIA*. Retrieved from www.annualreviews.org

Buchanan, R. L., & Edelson, S. G. (1999). Effect of pH-dependent, stationary phase acid resistance on the thermal tolerance of Escherichia coil O157:H7. *Food Microbiology*, *16*(5), 447–458. https://doi.org/10.1006/fmic.1998.0260

Burnens, A. P., Frey, A., Lior, H., & Nicolet, J. (1995). Prevalence and Clinical Significance of Vero-cytotoxin-producing Escherichia coli (VTEC) isolated from Cattle in Herds with and without Calf Diarrhoea. *Journal of Veterinary Medicine, Series B*, *42*(1–10), 311–318. https://doi.org/10.1111/j.1439-0450.1995.tb00715.x

Byrne, C. M., Bolton, D. J., Sheridan, J. J., McDowell, D. A., & Blair, I. S. (2000). The effects of preslaughter washing on the reduction of Escherichia coli O157:H7 transfer from cattle hides to carcasses during slaughter. *Letters in Applied Microbiology*, *30*, 142–145. https://doi.org/10.1046/j.1472-765x.2000.00689.x

Callaway, T. R. (2010). *Pre-Harvest Control of E. coli O157:H7*. Retrieved from https://www.beefresearch.org/CMDocs/BeefResearch/Safety_Fact_Sheets/Pre_Harvest_Control_of E_coli.pdf

Callaway, T. R., Edrington, T. S., Brabban, A. D., Anderson, R. C., Rossman, M. L., Engler, M. J., … Nisbet, D. J. (2008). Bacteriophage isolated from feedlot cattle can reduce Escherichia coli O157:H7 populations in ruminant gastrointestinal tracts. *Foodborne Pathogens and Disease*, *5*(2), 183–191. https://doi.org/10.1089/fpd.2007.0057

Capita, R., Riesco-Peláez, F., Alonso-Hernando, A., & Alonso-Calleja, C. (2014). Exposure of Escherichia coli ATCC 12806 to Sublethal Concentrations of Food-Grade Biocides Influences Its Ability To Form Biofilm, Resistance to Antimicrobials, and Ultrastructure. *Applied and Environmental Microbiology*, *80*(4), 1268–1280. https://doi.org/10.1128/AEM.02283-13

Carlton, R. M. (1999). Phage therapy: Past history and future prospects. *Archivum Immunologiae et Therapiae Experimentalis*, *47*(5), 267–274.

Castillo, A., Hardin, M., Acuff, B., & Dickson, J. (2002). Reduction of microbial contaminants on carcasses. In V. K. Juneja & J. N. Sofos (Eds.), *Control of Foodborne Microorganisms* (pp. 372–403). Retrieved from https://www.taylorfrancis.com/books/e/9780429175282/chapters/10.1201/b16945-32

CDC. (1993). Update: multistate outbreak of Escherichia coli O157:H7 infections from hamburgers--western United States, 1992-1993. *MMWR. Morbidity and Mortality Weekly Report*, *42*(14), 258–263. https://doi.org/10.1001/jama.1993.03500170024007

CDC. (2016). Escherichia coli Factsheet. Retrieved from https://www.cdc.gov/ecoli/pdfs/CDC-E.-coli-Factsheet.pdf.

CDC. (2017). National Shiga toxin-producing Escherichia coli (STEC) Surveillance Annual Report, 2014. Retrieved from https://www.cdc.gov/nationalsurveillance/pdfs/STEC-2014-REPORT_508c.pdf

CDC. (2018). General information of E. coli. Retrieved from https://www.cdc.gov/ecoli/general/index.html

CDC. (2019). *Antibiotic Resistance Threats in the United States, 2019*. https://doi.org/10.15620/cdc:82532

CDC. (2020a). Foodborne Germs and Illnesses. Retrieved April 12, 2020, from Centers for Disease Control and Prevention website: https://www.cdc.gov/foodsafety/foodborne-germs.html

CDC. (2020b). FoodNet: Pathogen Surveillance Tool. Retrieved February 4, 2020, from Atlanta, Georgia: U.S. Department of Health and Human Services. website: https://www.cdc.gov/foodnet/foodnet-fast.html

Cerritelli, M. E., Wall, J. S., Simon, M. N., Conway, J. F., & Steven, A. C. (1996). Stoichiometry and Domainal Organization of the Long Tail-fiber of Bacteriophage T4: A Hinged Viral Adhesin The long-tail fibers (LTFs) form part of bacteriophage T4's apparatus for. *Journal of*

Molecular Biology, *260*, 767–780.
Cheng, C.-M., & Kaspar, C. W. (1998). Growth and processing conditions affecting acid tolerance in Escherichia coli O157:H7. *Food Microbiology*, *15*, 157–166.
Cheng, H. Y., Yu, R. C., & Chou, C. C. (2003). Increased acid tolerance of Escherichia coli O157:H7 as affected by acid adaptation time and conditions of acid challenge. *Food Research International*, *36*(1), 49–56. https://doi.org/10.1016/S0963-9969(02)00107-2
Cheville, A. M., Arnold, K. W., Buchrieser, C., Cheng, C. M., & Kaspar, C. W. (1996). rpoS regulation of acid, heat, and salt tolerance in Escherichia coli O157:H7. *Applied and Environmental Microbiology*, *62*(5), 1822–1824. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8633882
Chibani-Chennoufi, S., Bruttin, A., Dillmann, M.-L., & Brüssow, H. (2004). Phage-Host Interaction: an Ecological Perspective. *JOURNAL OF BACTERIOLOGY*, *186*(12), 3677–3686. https://doi.org/10.1128/JB.186.12.3677-3686.2004
Clarke, R. C., & RC. (1994). Verocytotoxin-producing Escherichia coli (VTEC) in the food chain : Preharvest and processing perspectives. *Recent Advances in Verocytotoxin-Producing Escherichia Coli Infections*.
Clavero, M. R. S., & Beuchat, L. R. (1996). Survival of Escherichia coli O157:H7 in broth as influenced by pH, a-w, and temperature, and suitability of media for its enumeration. *Abstracts of the General Meeting of the American Society for Microbiol.*, *96*(8), 370.
Coombs, D. H., & Eiserling, F. A. (1977). Studies on the Structure, Protein Composition and Assembly of the Neck of Bacteriophage T4. *Journal of Molecular Biology*, *116*, 376406.
Davis, C. G., & Lin, B. H. (2005). Factors affecting US beef consumption. US Department of Agriculture, Economic Research Service.
Dean-Nystrom, E. A., Gansheroff, L. J., Mills, M., Moon, H. W., & O'brien, A. D. (2002). Vaccination of Pregnant Dams with Intimin O157 Protects Suckling Piglets from Escherichia coli O157:H7 Infection. *Inf*, *70*(5), 2414–2418. https://doi.org/10.1128/IAI.70.5.2414-2418.2002
Dorsa, W. J., Cutter, C. N., & Siragusa, G. R. (1996). Effectiveness of a steam-vacuum sanitizer for reducing Escherichia coli O157:H7 inoculated to beef carcass surface tissue. *Letters in Applied Microbiology*, *23*(1), 61–63. https://doi.org/10.1111/j.1472-765X.1996.tb00029.x
Dorsa, W. J., Cutter, C. N., & Siragusa, G. R. (1997). Effects of Acetic Acid, Lactic Acid and Trisodium Phosphate on the Microflora of Refrigerated Beef Carcass Surface Tissue Inoculated with Escherichia coIi0157:H7, Listeria innocua, and Clostridium sporogenes t. *Journal of Food Protection*, *60*(6), 619–624. Retrieved from http://meridian.allenpress.com/jfp/article-pdf/60/6/619/1666450/0362-028x-60_6_619.pdf
Echols, H. (1972). Developmental Pathways for the Temperate Phage: Lysis VS Lysogeny. *Annual Reviews Genetics*, *6*, 157–190. Retrieved from www.annualreviews.org
Endersen, L., O'mahony, J., Hill, C., Ross, R. P., Mcauliffe, O., & Coffey, A. (2014). Phage Therapy in the Food Industry. *Annual Review of Food Science and Technology*, *5*, 327–349. https://doi.org/10.1146/annurev-food-030713-092415
Escalona, V. H., Aguayo, E., Martínez-Hernández, G. B., & Artés, F. (2010). UV-C doses to reduce pathogen and spoilage bacterial growth in vitro and in baby spinach. *Postharvest Biology and Technology*, *56*(3), 223–231. https://doi.org/10.1016/j.postharvbio.2010.01.008
FDA. (2000). Irradiation in the production, processing and handling of food. , Pub. L. No. 21 CFR Part 179, Federal Register.

FDA. (2012). *Foodborne Pathogenic Microorganisms and Natural Toxins Handbook, 2ed edition*. Retrieved from https://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf

Fineran, P. C., Petty, N. K., & Salmond, G. P. C. (2009). Transduction: Host DNA Transfer by Bacteriophages. In *Encyclopedia of Microbiology* (pp. 666–679). San Diego, CA: Elsevier Academic Press.

Fingermann, M., ıa Avila, L., Bel en De Marco, M., Azquez, L. V, ıo Nicol as Di Biase, D., Ver onica, A. M., … Luis erez Qui, J. P. (2018). OMV-based vaccine formulations against Shiga toxin producing Escherichia coli strains are both protective in mice and immunogenic in calves. *Human Vaccines & Immunotherapeutics*, *18*(9), 2208–2213. https://doi.org/10.1080/21645515.2018.1490381

Foster, J. W., & Hall, H. K. (1990). Adaptive acidification tolerance response of Salmonella typhimurium. *Journal of Bacteriology*, *172*(2), 771–778. https://doi.org/10.1128/JB.172.2.771-778.1990

Fuller, D. N., Raymer, D. M., Kottadiel, V. I., Rao, V. B., & Smith, D. E. (2007). Single phage T4 DNA packaging motors exhibit large force generation, high velocity, and dynamic variability. *PNAS*, *104*(43), 16868–16873. Retrieved from www.pnas.orgcgidoi10.1073pnas.0704008104

Garren, D. M., Harrison, M. A., & Russelv, S. M. (1998). Acid Tolerance and Acid Shock Response of Escherichia coli 0157:H7 and Non-0157:H7 Isolates Provide Cross Protection to Sodium Lactate and Sodium Chloride. *Journal of Food Protection*, *61*(2), 158–161. https://doi.org/10.4315/0362-028X-61.2.158

Goodridge, L., & Abedon, S. T. (2003). Bacteriophage biocontrol and bioprocessing: Application of phage therapy to industry. *SIM News*, *53*(6), 254–262.

Goodson, M., & Rowbury, R. J. (1989). Habituation to normally lethal acidity by prior growth of Escherichia coli at a sub-lethal acid pH value. *Letters in Applied Microbiology*, *8*(2), 77–79. https://doi.org/10.1111/j.1472-765X.1989.tb00227.x

Gorden, J., & Small, P. L. C. (1993). Acid Resistance in Enteric Bacteria. *Infection and Immunity*, *61*(1), 364–367. Retrieved from http://iai.asm.org/

Greer, G. G. (2005). Bacteriophage control of foodborne bacteria. *Journal of Food Protection*, *68*(5), 1102–1111. https://doi.org/10.4315/0362-028X-68.5.1102

Griffin, P. M., & Tauxe, R. V. (1991). The Epidemiology of Infections Caused by Escherichia coli O157:H7, Other Enterohemorrhagic E. coli, and the Associated Hemolytic Uremic Syndrome. *Epidemiologic Reviews*, *13*(1), 60–98. https://doi.org/10.1093/oxfordjournals.epirev.a036079

Gross, M., & Jaenicke, R. (1994). Proteins under pressure. The influence of high hydrostatic pressure on structure, function and assembly of proteins and protein complexes. *European Journal of Biochemistry*, *221*(2), 617–630. https://doi.org/10.1111/j.1432-1033.1994.tb18774.x

Guerrero-Beltrán, J. A., & Barbosa-Cánovas, G. V. (2004). Review: Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, *10*(3), 137–147. https://doi.org/10.1177/1082013204044359

Hadas, H., Einav, M., Fishov, I., & Zaritsky, A. (1997). Bacteriophage T4 development depends on the physiology of its host Escherichia coli. *Microbiology*, *143*(1), 179–185. https://doi.org/10.1099/00221287-143-1-179

Hales, B. A., Hart, C. A., Batt, R. M., & Saunders, J. R. (1992). The large plasmids found in

enterohemorrhagic and enteropathogenic Escherichia coli constitute a related series of transfer-defective Inc F-IIA replicons. *Plasmid*, *28*(3), 183–193. https://doi.org/10.1016/0147-619X(92)90050-K

Hancock, D. D., Besser, T. E., Kinsel, M. L., Tarr, P. I., Rice, D. H., & Paros, M. G. (1994). The prevalence of Escherichia coli O157.H7 in dairy and beef cattle in Washington State. *Epidemiol. Infect*, 207. https://doi.org/10.1017/S0950268800051633

Harris, K., Miller, M. F., Loneragan, G. H., & Brashears, M. M. (2006). Validation of the use of organic acids and acidified sodium chlorite to reduce Escherichia coli O157 and Salmonella typhimurium in beef trim and ground beef in a simulated processing environment. *Journal of Food Protection*, *69*(8), 1802–1807. https://doi.org/10.4315/0362-028X-69.8.1802

Heller, K. J. (1992). Molecular interaction between bacteriophage and the gram-negative cell envelope. *Archives of Microbiology*, *158*(4), 235–248. https://doi.org/10.1007/BF00245239

Hersh, B. M., Farooq, F. T., Barstad, D. N., Blankenhorn, D. L., & Slonczewski, J. L. (1996). A Glutamate-Dependent Acid Resistance Gene in Escherichia coli. *Journal of Bacteriology*, *178*(13), 3978–3981.

Hijnen, W. A. M., Beerendonk, E. F., & Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research*, *40*, 3–22. https://doi.org/10.1016/j.watres.2005.10.030

Hoffmann, S., Maculloch, B., & Batz, M. (2015). *United States Department of Agriculture Economic Burden of Major Foodborne Illnesses Acquired in the United States*. Retrieved from www.ers.usda.gov

Hu, B., Margolin, W., Molineux, I. J., & Liu, J. (2015). Structural remodeling of bacteriophage T4 and host membranes during infection initiation. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(35), E4919–E4928. https://doi.org/10.1073/pnas.1501064112

Huffman, R. (2006). Control of E. coli O157:H7 in Beef Production FSIS Public Meeting on Control of Salmonella in Poultry Products Control of Control of E. coli E. coli O157:H7 in Beef Production O157:H7 in Beef Production FSIS Public Meeting on Control of FSIS Public Meeting on Control of Salmonella Salmonella in in Poultry Products Poultry Products Overview of Main Points Overview of Main Points. *American Meat Institute Foundation*. Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/dc5826dc-99dc-481a-9281-4af15e3cc034/Non-0157_STEC_Huffman.pdf?MOD=AJPERES

ICMSF. (2002). Selection of cases and attributes plans. In *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management* (1st ed., pp. 145–165). Retrieved from https://www.springer.com/gp/book/9781461352211

Jackson, M. P., Neill, R. J., O'Brien, A. D., Holmes, R. K., & Newland, J. W. (1987). Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli* 933. *FEMS Microbiology Letters*, *44*(1), 109–114. https://doi.org/10.1111/j.1574-6968.1987.tb02252.x

Jarvis, K. G., & Kaper, J. B. (1996). Secretion of extracellular proteins by enterohemorrhagic Escherichia coli via a putative type III secretion system. *Infection and Immunity*, *64*(11), 4826–4829. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8890245

Joensen, K. G., Tetzschner, A. M. M., Iguchi, A., Aarestrup, F. M., & Scheutz, F. (2015). Rapid and easy in silico serotyping of Escherichia coli isolates by use of whole-genome sequencing data. *Journal of Clinical Microbiology*, *53*(8), 2410–2426. https://doi.org/10.1128/JCM.00008-15

Judge, N. A., Mason, H. S., & O'brien, A. D. (2004). Plant Cell-Based Intimin Vaccine Given Orally to Mice Primed with Intimin Reduces Time of Escherichia coli O157:H7 Shedding in Feces. *Infection and Immunity*, *72*(1), 168–175. https://doi.org/10.1128/IAI.72.1.168-175.2004

Kalchayanand, N., Arthur, T. M., Bosilevac, J. M., Schmidt, J. W., Wang, R., Shackelford, S. D., & Wheeler, T. L. (2012). Evaluation of commonly used antimicrobial interventions for fresh beef inoculated with shiga toxin-producing escherichia coli serotypes O26, O45, O103, O111, O121, O145, and O157:H73. *Journal of Food Protection*, *75*(7), 1207–1212. https://doi.org/10.4315/0362-028X.JFP-11-531

Kanamaru, S., Ishiwata, Y., Suzuki, T., Rossmann, M. G., & Arisaka, F. (2005). Control of bacteriophage T4 tail lysozyme activity during the infection process. *Journal of Molecular Biology*, *346*(4), 1013–1020. https://doi.org/10.1016/j.jmb.2004.12.042

Kanamaru, S., Lehman, P. G., Kostyuchenko, V. A., Chipman, P. R., Mesyanzhinov, V. V., Arisaka, F., & Rossmann, M. G. (2002). Structure of the cell-puncturing device of bacteriophage T4. *Nature*, *415*(6871), 553–557. Retrieved from http://www.nature.com

Kanankege, K. S. T., Anklam, K. S., Fick, C. M., Kulow, M. J., Kaspar, C. W., Ingham, B. H., … Opfer, D. €. (2017). Evaluating the efficacy of beef slaughter line interventions by quantifying the six major non-O157 Shiga toxin producing Escherichia coli serogroups using real-time multiplex PCR. *Food Microbiology*, *63*, 228–238. https://doi.org/10.1016/j.fm.2016.11.023

Kao, S. H., & McClain, W. H. (1980). Baseplate protein of bacteriophage T4 with both structural and lytic functions. *Journal of Virology*, *34*(1), 95–103. https://doi.org/10.1128/JVI.34.1.95-103.1980

Kazi, M., & Annapure, U. S. (2016). Bacteriophage biocontrol of foodborne pathogens. *Journal of Food Science and Technology*, *53*(3), 1355–1362. https://doi.org/10.1007/s13197-015-1996-8

Kim, G. H., Breidt, F., Fratamico, P., & Oh, D. H. (2015). Acid Resistance and Molecular Characterization of Escherichia coli O157: H7 and Different Non-O157 Shiga Toxin-Producing E. coli Serogroups. *Journal of Food Science*, *80*(10), M2257–M2264. https://doi.org/10.1111/1750-3841.12996

King, D. A., Lucia, L. M., Castillo, A., Acuff, G. R., Harris, K. B., & Savell, J. W. (2005). Evaluation of peroxyacetic acid as a post-chilling intervention for control of Escherichia coli O157:H7 and Salmonella Typhimurium on beef carcass surfaces. *Meat Science*, *69*(3), 401–407. https://doi.org/10.1016/j.meatsci.2004.08.010

Kolter, R. (1993). The Stationary Phase of the Bacterial Life Cycle. *Annual Review of Microbiology*, *47*(1), 855–874. https://doi.org/10.1146/annurev.micro.47.1.855

Koohmaraie, M., Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., Shackelford, S. D., … Hruska, R. L. (2007). Interventions to reduce/eliminate Escherichia coli O157:H7 in ground beef. *Meat Science*, *77*, 90–96. https://doi.org/10.1016/j.meatsci.2007.04.004

Kortright, K. E., Chan, B. K., Koff, J. L., & Turner, P. E. (2019). Phage Therapy: A Renewed Approach to Combat Antibiotic-Resistant Bacteria. *Cell Host and Microbe*, *25*(2), 219–232. https://doi.org/10.1016/j.chom.2019.01.014

Kostyuchenko, V. A., Chipman, P. R., Leiman, P. G., Arisaka, F., Mesyanzhinov, V. V., & Rossmann, M. G. (2005). The tail structure of bacteriophage T4 and its mechanism of contraction. *Nature Structural and Molecular Biology*, *12*(9), 810–813. https://doi.org/10.1038/nsmb975

Kutter, E., Kuhl, S. J., Alavidze, Z., & Bs, B. G. B. (2013). Phage Therapy : Bacteriophages as

Natural , Self-limiting Antibiotics. In M. T. Pizzorno & M. T. Murray (Eds.), *Textbook of Natural Medicine* (Fourth, pp. 945–956). https://doi.org/10.1016/B978-1-4377-2333-5.00112-7

LaFee, S., & Buschman, H. (2019). With OK From FDA, UC San Diego Researchers Prepare to Launch Novel Phage Study. *UC San Diego Health*. Retrieved from https://health.ucsd.edu/news/releases/Pages/2019-01-08-FDA-okays-uc-san-diego-to-launch-novel-phage-study.aspx

Laroussi, M. (2005). Low Temperature Plasma-Based Sterilization: Overview and State-of-the-Art. *Plasma Processes and Polymers*, *2*(5), 391–400. https://doi.org/10.1002/ppap.200400078

Lathem, W. W., Grys, T. E., Witowski, S. E., Torres, A. G., Kaper, J. B., Tarr, P. I., & Welch, R. A. (2002). StcE, a metalloprotease secreted by Escherichia coli O157:H7, specifically cleaves C1 esterase inhibitor. *Molecular Microbiology*, *45*(2), 277–288. https://doi.org/10.1046/j.1365-2958.2002.02997.x

Laury, A. M., Alvarado, M. V., Nace, G., Alvarado, C. Z., Brooks, J. C., Echeverry, A., & Brashears, M. M. (2009). Validation of a lactic acid- and citric acid-based antimicrobial product for the reduction of Escherichia coli O157:H7 and Salmonella on beef tips and whole chicken carcasses. *Journal of Food Protection*, *72*(10), 2208–2211. https://doi.org/10.4315/0362-028X-72.10.2208

Le, S., He, X., Tan, Y., Huang, G., Zhang, L., Lux, R., … Hu, F. (2013). Mapping the Tail Fiber as the Receptor Binding Protein Responsible for Differential Host Specificity of Pseudomonas aeruginosa Bacteriophages PaP1 and JG004. *PloS ONE*, *8*(7), e68562. https://doi.org/10.1371/journal.pone.0068562

Leaper, S. (1984). Influence of temperature on the synergistic sporicidal effect of peracetic acid plus hydrogen peroxide on Bacillus subtilis SA22 (NCA 72-52). *Food Microbiology*, *1*(3), 199–203. https://doi.org/10.1016/0740-0020(84)90034-0

Lee, I. S., Slonczewski, J. L., & Foster, J. W. (1994). A Low-pH-Inducible, Stationary-Phase Acid Tolerance Response in Salmonella typhimurium. *Journal of Bacteriology*, *176*(5), 1422–1426.

Leyer, G. J., Wang, L. L., & Johnson, E. A. (1995). Acid adaptation of Escherichia coli O157:H7 increases survival in acidic foods. *Applied and Environmental Microbiology*, *61*(10), 3752–3755. https://doi.org/10.1128/AEM.61.10.3752-3755.1995

Liberti, L., & Notarnicola, M. (1999). Advanced treatment for municipal wastewater reuse in agriculture. *Water Science and Technology*, *40*(4–5), 235–245. https://doi.org/10.1080/01919510008547217

Lim, J. Y., Yoon, J. W., & Hovde, C. J. (2010). A brief overview of Escherichia coli O157:H7 and its plasmid O157. *Journal of Microbiology and Biotechnology*, *20*(1), 1–10. https://doi.org/10.4014/jmb.0908.08007

Lin, J., Smith, M. P., Chapin, K. C., Suk Baik, H., Bennett, G. N., & Foster, J. W. (1996). Mechanisms of Acid Resistance in Enterohemorrhagic Escherichia coli. *Applied and Environmental Microbiology*, *62*(9), 3094–3100. Retrieved from http://aem.asm.org/

Liu, B., Knirel, Y. A., Feng, L., Perepelov, A. V, Senchenkova, ya N., Wang, Q., … Wang, L. (2010). *Structure and genetics of Shigella O antigens*. https://doi.org/10.1111/j.1574-6976.2010.00224.x

Loewen, P. C., & Triggs, B. L. (1984). Genetic Mapping of katF, a Locus That with katE Affects the Synthesis of a Second Catalase Species in Escherichia coli. In *Journal of Bacteriology,*

160(2), 668-675.

Mahony, J., McAuliffe, O., Ross, R. P., & van Sinderen, D. (2011). Bacteriophages as biocontrol agents of food pathogens. *Current Opinion in Biotechnology*, *22*(2), 157–163. https://doi.org/10.1016/j.copbio.2010.10.008

Mataragas, M., Skandamis, P. N., & Drosinos, E. H. (2008). Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations. *International Journal of Food Microbiology*, *126*, 1–12. https://doi.org/10.1016/j.ijfoodmicro.2008.05.014

Matin, A., Auger, E. A., Blum, P. H., & Schultz, E. (1989). Genetic Basis of Starvation Survival in Nondifferentiating Bacteria. *Annual Review of Microbiology*, *43*, 293–316. Retrieved from www.annualreviews.org

Mcdaniel, T. K., Jarvis, K. G., Donnenberg, M. S., & Kaper, J. B. (1995). A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(5), 1664–1668. https://doi.org/10.1073/pnas.92.5.1664

McEvoy, J. M., Doherty, A. M., Finnerty, M., Sheridan, J. J., McGuire, L., Blair, I. S., … Harrington, D. (2000). The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Letters in Applied Microbiology*, *30*(5), 390–395. https://doi.org/10.1046/j.1472-765x.2000.00739.x

Meaden, S., & Koskella, B. (2013). Exploring the risks of phage application in the environment. Front Microbiol 4: 358. *Frontiers in Microbiolofy*, *4*(358).

Miller, E. S., Heidelberg, J. F., Eisen, J. A., Nelson, W. C., Durkin, A. S., Ciecko, A., … Fraser, C. M. (2003). Complete Genome Sequence of the Broad-Host-Range Vibriophage KVP40: Comparative Genomics of a T4-Related Bacteriophage Downloaded from. *Journal of Bacteriology*, *185*(17), 5220–5233. https://doi.org/10.1128/JB.185.17.5220-5233.2003

Miller, L. G., & Kaspar, C. W. (1994). Escherichia coli O157:H7 acid tolerance and survival in apple cider. *Journal of Food Protection*, *57*(6), 460–464. https://doi.org/10.4315/0362-028X-57.6.460

Montag, D., Hashemolhosseini, S., & Henning, U. (1990). Receptor-recognizing proteins of T-even type bacteriophages. The receptor-recognizing area of proteins 37 of phages T4 TuIa and TuIb. *Journal of Molecular Biology*, *216*(2), 327–334. https://doi.org/10.1016/S0022-2836(05)80324-9

Moye, Z. D., Woolston, J., & Sulakvelidze, A. (2018). Bacteriophage Applications for Food Production and Processing. *Viruses*, *10*(205). https://doi.org/10.3390/v10040205

Nakai, T., & Park, C. (2002). Bacteriophage therapy of infectious diseases in aquaculture. In *Research in Microbiology*, *153*, 13-18. https://doi.org/10.1016/S0923-2508(01)01280-3

Nakao, H., & Takeda, T. (2000). Escherichia coli Shiga toxin. *Journal of Natural Toxins*, *9*(3), 299–313. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10994531

Nataro, J. P., & Kaper, J. B. (1998). *Diarrheagenic Escherichia coli* (Vol. 11).

Ngadi, M., Smith, J. P., & Cayouette, B. (2003). Kinetics of ultraviolet light inactivation ofEscherichia coli O157:H7 in liquid foods. *Journal of the Science of Food and Agriculture*, *83*(15), 1551–1555. https://doi.org/10.1002/jsfa.1577

Nou, X., Rivera-Betancourt, M., Bosilevac, J. M., Wheeler, T. L., Shackelford, S. D., Gwartney, B. L., … Koohmaraie, M. (2003). Effect of Chemical Dehairing on the Prevalence of Escherichia coli O157:H7 and the Levels of Aerobic Bacteria and Enterobacteriaceae on Carcasses in a Commercial Beef Processing Plant. *Journal of Food Protection*, *66*(11), 2005–2009. https://doi.org/10.4315/0362-028X-66.11.2005

O'Brien, A. D., Tesh, V. L., Donohue-Rolfe, A., Jackson, M. P., Olsnes, S., Sandvig, K., … Keusch, G. T. (1992). Shiga toxin: Biochemistry, genetics, mode of action, and role in pathogenesis. *Current Topics in Microbiology and Immunology*, Vol. 180, pp. 65–94. https://doi.org/10.1007/978-3-642-77238-2_4

O'Driscoll, B., Gahan, C. G. M., & Hill, C. (1996). Adaptive acid tolerance response in Listeria monocytogenes: Isolation of an acid-tolerant mutant which demonstrates increased virulence. *Applied and Environmental Microbiology*, *62*(5), 1693–1698. https://doi.org/10.1128/aem.62.5.1693-1698.1996

Oliveira, J., Castilho, F., Cunha, A., & Pereira, M. J. (2012). Bacteriophage therapy as a bacterial control strategy in aquaculture. *Aquaculture International*, *20*(5), 879–910. https://doi.org/10.1007/s10499-012-9515-7

Olson, J., & Nottingham, P. (1980). *Temperature in microbial ecology of food*. ICMSF Academic Pres, *New York*.

Perna, N. T., Mayhew, G. F., Gyo¨, G., Po´sfai, G., Po´sfai, P., Elliott, S., … Blattner, F. R. (1998). Molecular Evolution of a Pathogenicity Island from Enterohemorrhagic Escherichia coli O157:H7. *Infection and Immunity*, *66*(8), 3810–3817.

Petrov, V. M., Nolan, J. M., Bertrand, C., Levy, D., Desplats, C., Krisch, H. M., … Nolan, J. M. (2006). Plasticity of the Gene Functions for DNA Replication in the T4-like Phages. *Journal of Molecular Biology*, *361*, 46–68. https://doi.org/10.1016/j.jmb.2006.05.071

Phillips, A. D., Navabpour, S., Hicks, S., Dougan, G., Wallis, T., & Frankel, G. (2000). Enterohaemorrhagic Escherichia coli O157:H7 target Peyer's patches in humans and cause attaching/effacing lesions in both human and bovine intestine. *Gut*, *47*(3), 377–381. https://doi.org/10.1136/gut.47.3.377

Pollari, F., Christidis, T., Pintar, K. D. M., Nesbitt, A., Farber, J., Lavoie, M.-C., … Johnson, R. P. (2017). *Evidence for the benefits of food chain interventions on E. coli O157:H7/NM prevalence in retail ground beef and human disease incidence: A success story*. https://doi.org/10.17269/CJPH.108.5655

Rabinovitch, A., Fishov, I., Hadas, H., Einav, M., & Zaritsky, A. (2002). Bacteriophage T4 development in Escherichia coli is growth rate dependent. *Journal of Theoretical Biology*, *216*(1), 1–4. https://doi.org/10.1006/jtbi.2002.2543

Rabinovitz, B. C., Gerhardt, E., Tironi Farinati, C., Abdala, A., Galarza, R., Vilte, D. A., … Mercado, E. C. (2012). Vaccination of pregnant cows with EspA, EspB, γ-intimin, and Shiga toxin 2 proteins from Escherichia coli O157: H7 induces high levels of specific colostral antibodies that are transferred to newborn calves. *Journal of Dairy Science*, *95*(6), 3318–3326. https://doi.org/10.3168/jds.2011-5093

Rakhuba, D. V., Kolomiets, E. I., Szwajcer-Dey, E., & Novik, G. I. (2010). Bacteriophage receptors, mechanisms of phage adsorption, and penetration into host cell. *Polish Journal of Microbiology*, *59*(3), 145–155.

Rhoades, J. R., Duffy, G., & Koutsoumanis, K. (2009). Prevalence and concentration of verocytotoxigenic Escherichia coli, Salmonella enterica and Listeria monocytogenes in the beef production chain: A review. *Food Microbiology*, *26*(4), 357–376. https://doi.org/10.1016/j.fm.2008.10.012

Sak, B. D., Eisenstark, A., & Touati, D. (1989). Exonuclease III and the Catalase Hydroperoxidase II in Escherichia coli are Both Regulated by the katF Gene Product. *Proc. Natl. Acad. Sci.*, *86*(9), 3271–3275.

Sastry, S. K., Datta, A. K., & Worobo, R. W. (2000). Ultraviolet Light. *Journal of Food Science -*

Supplement, *65*(8), 90–92. https://doi.org/10.1111/j.1750-3841.2000.tb00623.x

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., … Griffin, P. M. (2011). Foodborne illness acquired in the United States-Major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15. https://doi.org/10.3201/eid1701.P11101

Schmidt, H., Henkel, B., & Karch, H. (1997). A gene cluster closely related to type II secretion pathway operons of Gramnegative bacteria is located on the large plasmid of enterohemorrhagic Escherichia coli O157 strains. *FEMS Microbiology Letters*, *148*, 265–272. https://doi.org/10.1111/j.1574-6968.1997.tb10299.x

Schmidt, H., Karch, H., & Beutin, L. (1994). The large-sized plasmids of enterohemorrhagic *Escherichia coli* O157 strains encode hemolysins which are presumably members of the *E. coli* Î±-hemolysin family. *FEMS Microbiology Letters*, *117*(2), 189–196. https://doi.org/10.1111/j.1574-6968.1994.tb06763.x

Siegler, R. L., Obrig, T. G., Pysher, T. J., Tesh, V. L., Denkers, N. D., & Taylor, F. B. (2003). Response to Shiga toxin 1 and 2 in a baboon model of hemolytic uremic syndrome. *Pediatric Nephrology*, *18*(2), 92–96. https://doi.org/10.1007/s00467-002-1035-7

Silindir, M., & Özer, A. Y. (2009). Sterilization Methods and the Comparison of E-Beam Sterilization with Gamma Radiation Sterilization. *Journal of Pharm. Sci*, *34*, 43–53.

Singh, S. P., Singh, S. R., Williams, Y. U., Jones, L., & Abdullah, T. (1995). Antigenic Determinants of the OmpC Porin from Salmonella typhimurium. *Infection and Immunity*, *63*(12), 4600–4605. https://doi.org/10.1128/IAI.63.12.4600-4605.1995

Singh, S. P., Upshaw, Y., Abdullah, T., Singh, S. R., & Klebba, P. E. (1992). Structural Relatedness of Enteric Bacterial Porins Assessed with Monoclonal Antibodies to Salmonella typhimurium OmpD and OmpC. *Journal of Bacteriology*, *174*(6), 1965–1973. https://doi.org/10.1128/JB.174.6.1965-1973.1992

Small, P., Blankenhorn, D., Welty, D., Zinser, E., & Slonczewski, J. L. (1994). Acid and Base Resistance in Escherichia coli and Shigella flexneri: Role of rpoS and Growth pH. *Journal of Bacteriology*, *176*(6), 1729–1737.

Smith, D. R. (2014). Vaccination of Cattle against Escherichia coli O157:H7. In *Microbiology Spectrum* (Vol. 2, pp. 505–529). https://doi.org/10.1128/microbiolspec.ehec-0006-2013

Snedeker, K. G., Campbell, M., & Sargeant, J. M. (2012). A Systematic Review of Vaccinations to Reduce the Shedding of Escherichia coli O157 in the Faeces of Domestic Ruminants. *Zoonoses and Public Health*, *59*(2), 126–138. https://doi.org/10.1111/j.1863-2378.2011.01426.x

Snowball, M., & Hornsey, I. (1988). Purification of water supplies using ultraviolet light. In R. Robinson & U. Barking (Eds.), *Developments in food Moicrbiology* (pp. 171–191). Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201302076036

Sommers, C. H., Sites, J. E., & Musgrove, M. (2010). Ultraviolet light (254 nm) inactivation of pathogens on foods and stainless steel surfaces. *Journal of Food Safety*, *30*(2), 470–479. https://doi.org/10.1111/j.1745-4565.2010.00220.x

Stenutz, R., Weintraub, A., & Widmalm, G. (2006). The structures of Escherichia coli O-polysaccharide antigens. *FEMS Microbiology Reviews*, *30*(3), 382–403. https://doi.org/10.1111/j.1574-6976.2006.00016.x

Stevens, J. M., Galyov, E. E., & Stevens, M. P. (2006). Actin-dependent movement of bacterial pathogens. *Nature Reviews Microbiology*, *4*(2), 91–101. https://doi.org/10.1038/nrmicro1320

Stoffels, E., Sakiyama, Y., & Graves, D. B. (2008). Cold atmospheric plasma: Charged species and

their interactions with cells and tissues. *IEEE Transactions on Plasma Science*, *36*(4 PART 2), 1441–1457. https://doi.org/10.1109/TPS.2008.2001084

Sulakvelidze, Alexander, Alavidze, Z., & Morris, J. G. (2001). *Bacteriophage Therapy*. *45*(3), 649–659. https://doi.org/10.1128/AAC.45.3.649-659.2001

Svircev, A., Roach, D., & Castle, A. (2018). Framing the future with bacteriophages in agriculture. *Viruses*, *10*(5), 1–13. https://doi.org/10.3390/v10050218

Tatsuno, I., Horie, M., Abe, H., Miki, T., Makino, K., Shinagawa, H., … Sasakawa, C. (2001). toxB gene on pO157 of enterohemorrhagic Escherichia coli O157:H7 is required for full epithelial cell adherence phenotype. *Infection and Immunity*, *69*(11), 6660–6669. https://doi.org/10.1128/IAI.69.11.6660-6669.2001

Tesh, V. L., Burris, J. A., Owens, J. W., Gordon, V. M., Wadolkowski, E. A., O'brien, A. D., … O'brien, A. D. (1993). Comparison of the Relative Toxicities of Shiga-Like Toxins Type I and Type II for Mice. *Infection AND Immunity*, *61*(8), 3392–3402.

Tsai, Y. W., & Ingham, S. C. (1997). Survival of Escherichia coil O157:H7 and Salmonella spp. in Acidic condiments. *Journal of Food Protection*, *60*(7), 751–755. https://doi.org/10.4315/0362-028X-60.7.751

Tuttle, J., Gomez, T., Doyle, M. P., Wells, J. G., Zhao, T., Tauxe, R. V., & Griffin, P. M. (1999). Lessons from a large outbreak of Escherichia coli O157:H7 infections: Insights into the infectious dose and method of widespread contamination of hamburger patties. *Epidemiology and Infection*, *122*(2), 185–192. https://doi.org/10.1017/S0950268898001976

USDA-ERS. (2014). *Cost estimates of foodborne illnesses*. Retrieved from https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx#48446

USDA-FSIS. (2012a). *United States Department of Agriculture - Food Safety Inspection Service, Shiga toxin-producing Escherichia coli in certain raw beef products*. Retrieved from https://www.federalregister.gov/documents/2012/05/31/2012-13283/shiga-toxin-producing-escherichia-coli-in-certain-raw-beef-products

USDA-FSIS. (2012b). United States Department of Agriculture - Food Safety Inspection Service, USDA Targeting Six Additional Strains of E.coli in Raw Beef Trim Starting Monday | USDA. Retrieved March 23, 2020, from https://www.usda.gov/media/press-releases/2012/05/31/usda-targeting-six-additional-strains-ecoli-raw-beef-trim-starting

USDA-FSIS. (2014). *United States Department of Agriculture - Food Safety Inspection Service, Compliance Guideline for Establishments Sampling Beef Trimmings for Shiga Toxin-Producing Escherichia coli (STEC) Organisms or Virulence Markers*. Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/e0f06d97-9026-4e1e-a0c2-1ac60b836fa6/Compliance_Guide_Est_Sampling_STEC_0512.pdf?MOD=AJPERES

USDA-FSIS. (2017). *United States Department of Agriculture - Food Safety Inspection Service, Compliance Guideline for Minimizing the Risk of Shiga Toxin-Producing Escherichia coli (STEC) in Raw Beef (including Veal) Processing Operations 2017 Compliance Guideline*.

USDA-FSIS. (2019). *United States Department of Agriculture - Food Safety Inspection Service, Safe and Suitable Ingredients used in the Production of Meat, Poultry, and Egg Products*. Retrieved from https://www.fsis.usda.gov/wps/portal/fsis/topics/regulations/directives/7000-series/safe-suitable-ingredients-related-document

Wells, J. G., Shipman, L. D., Greene, K. D., Sowers, E. G., Green, J. H., Cameron, D. N., …

Wachsmuth, I. K. (1991). Isolation of Escherichia coli serotype O157:H7 and other Shiga-like-toxin-producing E. coli from dairy cattle. *Journal of Clinical Microbiology*, *29*(5), 985–989. https://doi.org/10.1128/jcm.29.5.985-989.1991

Wesche, A. M., Gurtler, J. B., Marks, B. P., & Ryser, E. T. (2009). Stress, Sublethal Injury, Resuscitation, and Virulence of Bacterial Foodborne Pathogens. *Journal of Food Protection*, *72*(5), 1121–1138.

Wheeler, T. L., Kalchayanand, N., & Bosilevac, J. M. (2014). Pre- and post-harvest interventions to reduce pathogen contamination in the U.S. beef industry. *Meat Science*, *98*(3), 372–382. https://doi.org/10.1016/j.meatsci.2014.06.026

Wolf, M. J., Miller, M. F., Parks, A. R., Loneragan, G. H., Garmyn, A. J., Thompson, L. D., … Brashears, M. M. (2012). Validation comparing the effectiveness of a lactic acid dip with a lactic acid spray for reducing Escherichia coli O157:H7, Salmonella, and Non-O157 shiga toxigenic Escherichia coli on beef trim and ground beef. *Journal of Food Protection*, *75*(11), 1968–1973. https://doi.org/10.4315/0362-028X.JFP-12-038

Wommack, K. E., & Colwell, R. R. (2000). Virioplankton: Viruses in Aquatic Ecosystems. *Microbiology and Molecular Biology Reviews*, *64*(1), 69–114. https://doi.org/10.1128/mmbr.64.1.69-114.2000

Yang, X., He, A., Badoni, M., Tran, F., & Wang, H. (2017). Mapping sources of contamination of Escherichia coli on beef in the fabrication facility of a commercial beef packing plant. *Food Control*, *75*, 153–159. https://doi.org/10.1016/j.foodcont.2016.12.004

Yap, M. L., & Rossmann, M. G. (2014). Structure and function of bacteriophage T4. *Future Microbiology*, *9*(12), 1319–1337. https://doi.org/10.2217/fmb.14.91

Yeh, Y., de Moura, F. H., Van Den Broek, K., & de Mello, A. S. (2018a). Effect of ultraviolet light, organic acids, and bacteriophage on Salmonella populations in ground beef. *Meat Science*, *139*, 44–48. https://doi.org/10.1016/j.meatsci.2018.01.007

Yu, F., & Mizushima, S. (1982). Roles of lipopolysaccharide and outer membrane protein ompc of Escherichia coli K-12 in the receptor function for bacteriophage T4. *Journal of Bacteriology*, *151*(2), 718–722.

Zhao, T., Zhao, P., & Doyle, M. P. (2009). Inactivation of Salmonella and Escherichia coli O157:H7 on Lettuce and Poultry Skin by Combinations of Levulinic Acid and Sodium Dodecyl Sulfate. *Journal of Food Protection*, *72*(5), 928–936. https://doi.org/10.4315/0362-028X-72.5.928

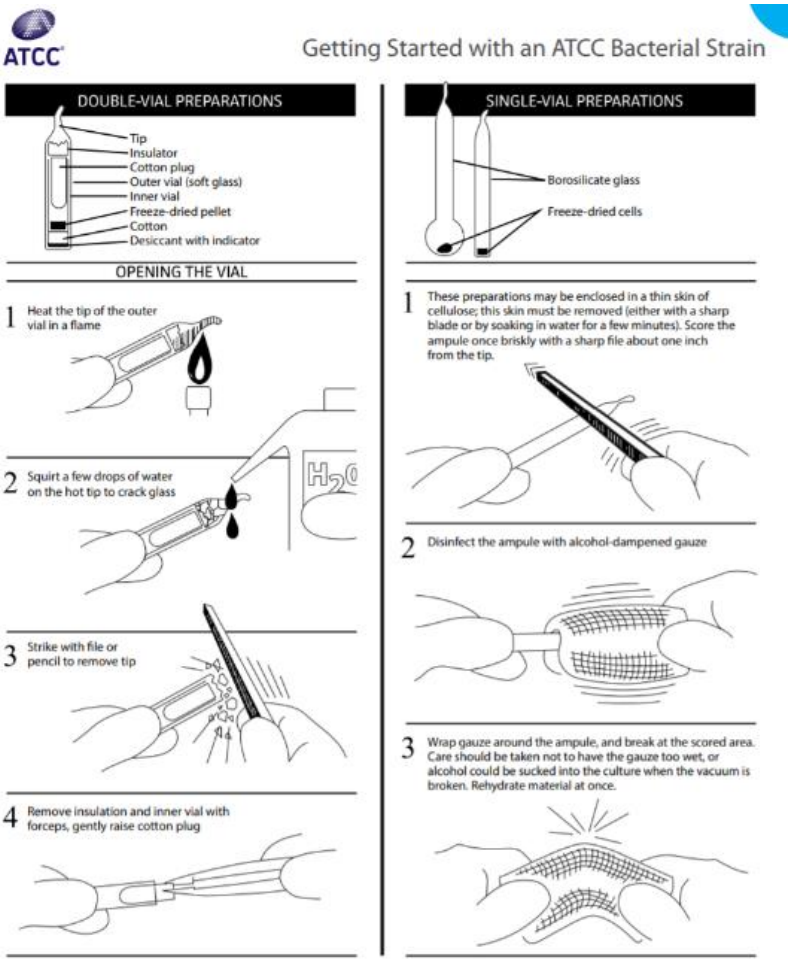
Appendix I. Safe Minimum Cooking Temperatures Charts (adapted from FoodSafety.gov)

Follow the guidelines below for minimum cooking temperatures and rest time for meat, poultry, seafood, and other cooked foods. Be sure to use a food thermometer to check whether meat has reached a safe internal temperature that is hot enough to kill harmful germs that cause food poisoning.

Food	Type	Internal Temperature (°F)
Ground meat and meat mixtures	Beef, pork, veal, lamb	160
	Turkey, chicken	165
Fresh beef, veal, lamb	Steaks, roasts, chops Rest time: 3 minutes	145
Poultry	All Poultry (breasts, whole bird, legs, thighs, wings, ground poultry, giblets, and stuffing)	165
Pork and ham	Fresh pork, including fresh ham Rest time: 3 minutes	145
	Precooked ham (to reheat) Note: Reheat cooked hams packaged in USDA-inspected plants to 140°F	165
Eggs and egg dishes	Eggs	Cook until yolk and white are firm
	Egg dishes (such as frittata, quiche)	160
Leftovers and casseroles	Leftovers and casseroles	165
Seafood	Fish with fins	145 or cook until flesh is opaque and separates easily with a fork
	Shrimp, lobster, crab, and scallops	Cook until flesh is pearly or white, and opaque
	Clams, oysters, mussels	Cook until shells open during cooking

Date Last Reviewed April 12, 2019

Appendix II. Getting Started with an ATCC Bacterial Strain (ATCC, 2015)



CHAPTER ONE

Application of MS bacteriophages on trimmings reduce O157:H7 and the "Big Six" STEC in ground beef

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Abstract

According the United States Food and Drug Administration (FDA) agency, bacteriophage solutions targeting only the serotype O157:H7 are Generally Recognized as Safe (GRAS) to control STEC during beef processing. However, outbreaks involving the “Big Six” STEC increased the industry concern about those serotypes. In this study, we evaluated the efficacy of Mello-Shebs (MS) bacteriophages targeting the “Big Six” O26, O45, O103, O111, O121, and O145 STEC. The in vitro killing efficiency of phages isolated for each specific serotype varied from 96.2% to 99.9%. When applied to contaminated trim, a 90% reduction of all STEC was observed in ground beef. Therefore, bacteriophage applications may provide an additional barrier against the “Big Six” STEC in robust food safety systems. Results of this research provide support documentation to the FDA to extend GRAS status for bacteriophages as processing aids against all adulterant STEC.

Introduction

Shiga-toxin *E. coli* (STEC) continues to be a major public health concern due to the severity of its foodborne infections that range from very mild to life-threatening (CDC, 2018). In 2014, there were 4,437 culture-confirmed STEC infections (43.2% due to O157, 56.5% due to non-O157) and caused more than $298 million in medical care costs in the United States (CDC, 2017; USDA-ERS, 2014). Animal products, especially ground beef, have demonstrated to be a common source of contamination of STEC (CDC, 2016). Previous research showed that STEC can be transferred to beef carcasses from hides during the harvesting process and persist on fresh beef after fabrication (Ferens & Hovde, 2011; Arthur et al., 2010; Bell, 1997; Byrne, Bolton, Sheridan, McDowell, & Blair, 2000; McEvoy et al., 2000).

The United States Department of Agriculture (USDA) deemed STEC O157:H7 adulterant in ground beef in 1994 after a multistate outbreak from undercooked hamburger from a fast food chain. In 2012, six more serotypes including STEC O145, O121, O111, O103, O45, and O26 (the "Big Six") were also deemed adulterant due to their association with foodborne outbreaks (USDA-FSIS, 2012). According to the USDA-Food Safety Inspection Service (FSIS), beef trim tested positive for STEC still can be used for human consumption, but it must be further processed into heat-treated products to eliminate bacteria (USDA-FSIS, 2014). Positive-tested trim is sold at discounted price, leading to economic losses for the industry. Therefore, due to human health and economic impacts, regulatory agencies developed processing policies whereas private

companies implemented food safety interventions to decrease the incidence of STEC in beef products. In the United States, large beef processing facilities commonly use hot water carcass washing during harvest and applications of acidic solutions during harvest and fabrication as antimicrobial interventions (Wheeler, Kalchayanand, & Bosilevac, 2014). Although the effectiveness of acid solutions as a food safety intervention against *E. coli* is supported by some research (Harris et al., 2006; Laury et al., 2009; Schmidt et al., 2014; Wolf et al., 2012; Zhao, Zhao, & Doyle, 2009), several studies have shown that *E. coli* may become resistant to low pH after exposure to acid stress (Anderson & Marshall, 1989; Cheng et al., 2003; Kim et al., 2015; Leyer et al., 1995; Samelis, Sofos, Kendall, & Smith, 2005; Smulders & Greer, 1998). Therefore, there is an immediate need for developing more efficient aids to decontaminate beef.

Bacteriophage (phage) solutions are known as bacteria-specific interventions that can be applied on food products (Atterbury, Connerton, Dodd, Rees, & Connerton, 2003; Endersen et al., 2014; Mahony et al., 2011) Recently, phage applications were reported to significantly decrease *Salmonella* contamination in ground meats (Grant, Parveen, Schwarz, Hashem, & Vimini, 2017; Jorquera et al., 2015; Sharma, Dhakal, & Nannapaneni, 2015; Yeh et al., 2017). When in contact with a bacteria cell, phages attach to specific cell structures to introduce its genome into the bacteria. The lysis of the host is achieved by phage replication and release (Lindberg, 1973; Montag et al., 1990; Rakhuba et al., 2010; Yu & Mizushima, 1982) In the US, the Food Safety and

Inspection Service (FSIS) Directive 7120.1 lists bacteriophage solutions as a processing aid to control only one STEC serotype (O157:H7) (USDA-FSIS, 2019). The objectives of this study were: i) to demonstrate that phages can decrease "Big Six" contamination in beef; and ii) provide technical evidence to the FDA to extend GRAS status for bacteriophages as processing aids against all adulterant STEC.

Materials and methods

E. coli strains and inoculum preparation

Strains used in this study included *E. coli* O26 (ATCC® BAA-2196™), O45 (ATCC® BAA-2193™), O103 (ATCC® BAA-2215™), O111 (ATCC® BAA-2440™), O121 (ATCC® BAA-2219™), O145 (ATCC® BAA-2192™), and O157:H7 (ATCC® 35150™). Frozen stocks from each strain were briefly thawed and a loopfull of culture was aseptically streaked onto 1.6% agar LB agar plates in duplicate and incubated for 24 h at 37°C to ensure cultures were live and viable. For each strain, a single isolated colony was transferred into independent sterile tubes containing 10 mL sterile LB broth and incubated at 37°C overnight with shaking (200 rpm). To prepare the inoculum, 15 mL of LB broth was inoculated with 750 µL of an individual culture and incubated with shaking at 37°C until culture absorbance was measured to be between 0.5 and 0.6 OD_{600}. Once each strain achieved this absorbance, equal amounts of the cultures were combined while on ice to avoid overgrowth. This *E. coli* cocktail was then diluted with sterile 0.1% Buffered Peptone Water (BPW) to create a 25% culture cocktail solution for inoculation (approximately 1.1 x 10^8 CFU/mL).

Bacteriophage preparation and stock titer

Solutions of bacteriophages Mello-Shebs 1 (MS1) 026, MS1 045, MS1 103, MS1 111, MS1 121, MS1 145, and MS1 157 were obtained directly from the University of Nevada, Reno - Food Safety Microbiology Laboratory Library. The concentration of phage solutions was determined by the double-layer agar methodology described by Adams (1959) and modified by Yeh et al. (2017). In short, 100 µL of 10-fold diluted phage and 100 µL of Log-phase *E. coli* culture was added into four mL of molten LB agar (0.6% agar) tempered at 45°C. Solution was mixed by inverting twice then evenly distributed onto hard LB agar (1.6%) plates until solidified. Duplicated plates were incubated at room temperature overnight for plaque enumeration. Phage concentration of individual solutions and the cocktail were determined to be 10^8 PFU/mL.

In vitro bacteriophage killing efficiency

Killing efficiency of the seven bacteriophages was determined by plating each phage against each of the seven STEC serotypes. Briefly, 100 µL of diluted overnight cultures were plated in quadruplicate onto 1.6% LB agar followed by 100 µL of each phage solution. Plates were allowed to fully dry before being inverted and incubated overnight at 37°C. Resulting colonies were counted and compared to quadruplicate controls plated without phage.

Sample preparation and treatment design

A 15 kg batch of beef trim (80% lean) was sourced from a USDA-inspected beef processing facility, screened for background *E. coli* and frozen until all analyses could be done. Upon thawing, 100 g aliquots of beef trim were randomly assigned to 8 treatments: Control 7°C 30 min, Control 7°C 6 h, Control 25°C 30 min, Control 25°C 6 h, Phage 7°C 30 min, Phage 7°C 6 h, Phage 25°C 30 min, and Phage 25°C 6 h. Control inoculated samples (Control) were treated with BPW, and Phage samples were treated with the phage cocktail. The experiment was designed as a Completely Randomized Design and the following model was used: $Y_{ij}=\mu + \alpha_i + \varepsilon_{ij}$, where Y_{ij} was STEC count, μ was the overall mean across the treatments, and α_i was the effect of treatment from the grand mean specific to the 8 treatment levels. The model errors (ε_{ij}) were assumed to be random and independent of each other.

Beef trim inoculation, bacteriophage application, bacterial enumeration

Trim samples were inoculated with a cocktail comprising of all STEC strains (O26, O45, O103, O111, O121, O145, and O157:H7) to yield in a contamination level of about 5 Log CFU/g in the ground product. A volume of 1 mL of the inoculum was applied by dripping to each side of trim samples, for a total of 2 mL for each 100 g sample. After 30 minutes at 7°C, samples were treated with 2 mL of bacteriophage cocktail solution (10^8 PFU/mL of MS1 026, MS1 045, MS1 103, MS1 111, MS1 121, MS1 145, and MS1 157 phages) or with 2 mL of BPW. One (1) mL was distributed on each side of the trim. Subsequently, samples were covered and held for either 30 min or 6 h while at 25°C or

7°C. After holding, samples were aseptically ground using a tabletop electric grinder (model 33-0201-w, Weston China). A 25g aliquot of each sample was collected in a sterile sampling bag and masticated with 225ml of BPW for two minutes at 230 rpm. For *E. coli* enumeration, 10 mL of the masticated homogenate was centrifuged at 10,000 x *g* for 6 minutes and the supernatant discarded to avoid plating phages. The pellet was resuspended with 10 mL of BPW, vortexed, serial diluted in BPW, and 100 µL was plated onto LB agar in duplicate. The plates were inverted and incubated at 37°C for 24 h and the resulting colonies counted (CFU/g).

Statistical analysis

The experiment was replicated 5 times with 2 repetitions per treatment (n=80 total e.u., n=10 e.u. per treatment). Data was analyzed using the GLIMMIX procedure of SAS (® 9.3 package, SAS Institute, Inc., USA). Contrast analyses were also performed to evaluate single effects of bacteriophage application, and lysing temperature and time. When significance ($P \leq 0.05$) was detected, means separations and significance for main treatments were performed by using the LSMEANS and DIFF function statements.

Results

The results of killing efficiency of each bacteriophage against all STEC serotypes is demonstrated in Table 1. When looking to effects of serotype-

specific phage against it respective STEC (gray highlighted diagonal in the table), phages demonstrated at least 96.2% of killing efficiency (MS1 103 against STEC O103). The highest killing efficiency (99.9%) was observed for MS1 026 and MS1 157. Overall, when tested against their non-specific STEC serotypes, killing efficiency of MS1 phages varied from 34.1% (MS1 103 against STEC O111) to 99.8% (MS1 157 against STEC O26).

Effects of the phage cocktail application on contaminated beef trim and resulting STEC populations after grinding are presented in Figure 1. Overall, bacteriophage application on trim significantly decreased STEC populations in ground beef in all temperature and lysing time scenarios. Reductions from 4.68^{AB} to 3.87^{DE}, 5.12^{A} to 3.82^{DE}, 4.74^{AB} to 3.98^{DC}, and 4.39^{BC} to 3.40^{E} Log CFU/g, were observed for 25°C 30 min, 25°C 6 h, 7°C 30 min, and 7°C 6 h, respectively. Reductions in Log are numerically presented in Table 2. When analyzing contrasts, there was no effect of lysing time (P = 0.2797), but a significant effect of lysing temperature was observed (P = 0.0497).

Discussion

Bacteriophages MS1 exerted high killing efficiency in vitro against their respective serotypes (above 1 Log). All MS1 bacteriophages were isolated from sewage as described by Mirzaei, Eriksson, Kasuga, Haggård-Ljungquist, & Nilsson (2014), Hudson et al. (2013), Carey-Smith, Billington, Cornelius, Hudson, & Heinemann (2006), Hooton, Atterbury, & Connerton (2011), and Pereira et al. (2016) by using the same STEC serotypes applied on beef trim in this study. The ability for each phage to lyse other non-specific STEC serotypes is possible associated to its genetic similarity. Although complete characterization of MS1 phages is still currently in progress, genomic data

deposited at GenBank® showed that MS1 phages have similar nucleotide sequences when compared to other *E. coli* phages. The MS1 phages were isolated using the same strain (*E. coli*) but different serotypes (O26, O45, O103, O111, O121, O145, and O157:H7), which explains the similarity and possible differences in their genome. Bacteriophages targeting the same strain usually show similar nucleotide sequences, however, genomic differences that make them more efficient against different serotypes is possibly associated to the ability of phages to move between serotypes in the environment and acquire different host genomic material, which potentially broadens their host range (Bergman, Fineran, Petty, & Salmond, 2019).

To our knowledge this is the only study that evaluates the efficacy of a 7-bacteriophage cocktail to reduce STEC in ground beef. The use of a phage cocktail prevents the emergence and phage resistance mutants as the different phages are able to bind to different host cell surface receptors adding to the versatility and overall effectiveness of a bacteriophage solution (Sulakvelidze & Barrow, 2005). The ability for each phage to lyse every STEC strain that it was not isolated against, increases the efficacy of a cocktail that targets all 7 adulterant serotypes that may be present on beef products, rather than a single serotype. There are many reported *E. coli* targeting bacteriophage cocktails, but none contain more than six individual phages (Carter et al., 2012; Coffey et al., 2011; Greer, 2005; Magnone, Marek, Sulakvelidze, & Senecal, 2013; O'Flynn et al., 2004; Tomat et al., 2018; Viazis, Akhtar, Feirtag, & Diez-Gonzalez, 2011). A commercially available bacteriophage preparation with 3 phages was evaluated by

(Carter et al., 2012) against *E. coli* O157:H7 and demonstrated over 94% and 87% reduction on beef and lettuce, respectively. O'Flynn et al. (2004) showed that a three-phage cocktail targeting STEC O157:H7 completely eliminated bacteria cells on beef surface after 1 h at 37°C.

Regarding lysing temperature, Tomat et al. (2018) evaluated a six-phage cocktail against four strains of *E. coli*, an enteropathogenic *E. coli* (EPEC) O157, DH5α, and two STEC (O157:H7 and O18) and demonstrated that a phage cocktail can maintain low *E. coli* contamination levels on meat surface at 4°C, but not at 24°C or 37°C when regrowth was observed. Previously, Leverentz et al. (2001) demonstrated that phage efficacy was independent of temperature (5°C, 10°C, 20°C) against *Salmonella* on fresh fruit. Conversely, the majority of research reports, including this study, showed that bacteriophage applications have shown to have higher efficiency at higher temperatures, particularly at 24°C or at optimal bacteria growth range (37°C) (Bigwood, Hudson, Billington, Carey-Smith, & Heinemann, 2008; Hudson et al., 2013; Hudson, Billington, Wilson, & On, 2015; Liu et al., 2015; Sharma, Dhakal, & Nannapaneni, 2015; Spricigo et al., 2013). Optimal phage activity at higher temperatures is possibly related to the metabolism of the host and its effect on the lytic cycle. Hadas et al. (1997) described how phage adsorption depends on the growth rate of host *E. coli* cells which seems to be dependent on nutrient availability, the rate of the protein synthesizing system, and cellular dimensions. Additionally, Sillankorva et al. (2004) suggested that the optimal growth temperature for *E. coli* ranges between 37-42°C, whereas while no growth is usually observed at refrigeration temperatures, 5-8°C (Buchanan & Klawitter,

1992; Buchanan, Stahl, & Whiting, 1989; Gonthier, Guérin-Faublée, Tilly, & Delignette-Muller, 2001; Nauta & Dufrenne, 1999; Rabinovitch et al., 2002; Sillankorva et al., 2004). However, studies performed by our team (Yeh et al., 2017; Yeh et al., 2018) are in agreement with previous research that reported significant bacteria reduction when phages were applied at low temperatures (Fiorentin, Júnior, Suínos, & Author, 2005; Guenther, Herzig, Fieseler, Klumpp, & Loessner, 2012). This was possible because bacteria thrive in cold temperatures by adopting a cold tolerance basal metabolism, allowing the cell to remain alive (Sengupta & Chattopadhyay, 2013). Authors then hypothesized that even in conditions where bacteria adopt this type of metabolism, phages are still able to perform their lytic cycle. In this study, the contrast analysis revealed significant differences (P = 0.0497) between treatments at 7 and 25°C, suggesting that temperature speeds up the lyse of the host. A similar trend was suggested by Liu et al. (2015), who evaluated 3 temperatures including 4°C, 22°C and 37°C and demonstrated that phage applied at higher temperatures took less time to achieve the same level of *E. coli* O157 reduction compared to reduction rates observed under refrigeration temperatures (3.2 Log CFU/cm^2 reduction within 3 h at 37°C vs 144 h at 4°C).

Similar to temperature, previous studies have also demonstrated the effects of time during phage lysing activity. Carter et al. (2012) tested different lysing times for a phage cocktail against *E. coli* O157:H7 on beef from 5 min to 24 h and revealed that there was no significant bacteria reduction with longer

phage contact time. Sharma, Dhakal, & Nannapaneni (2015) also showed no effect of time in reducing *Salmonella* counts on raw turkey with phage application after 2, 12, or 24 h. Similarly, our team previously tested bacteriophage applications against *Salmonella* holding red meat products at 4°C for 6 and 18 h, and no effect of time was observed (Yeh et al., 2017). In this study, after we applied the phage cocktail on contaminated trim, samples were held for 30 min or 6 h prior to grinding. The contrast analysis showed no effects of lysing time on ground beef either held for 30 min or 6 h ($P = 0.2797$). However, previous research showed that longer lysing times may lead to higher bacteria reduction. Seo et al., 2016 showed a gradual decrease in *E. coli* O157:H7 populations overtime with the application of bacteriophage, being reduced from 5.09 Log CFU/cm^2 to 1.37 Log CFU/ cm^2 at 4 h and being completely inhibited after 8 h. In similar fashion, Spricigo, Bardina, Cortés, and Llagostera et al. (2013) tested phage applications on chicken breast contaminated with *Salmonella* at 4°C and observed a 1.6 Log CFU/g reduction after one day and 2.2 after 7 days. Conversely, Yang, Sadekuzzaman, and Ha (2017), demonstrated an initial 0.8 Log CFU/g reduction of *Listeria* when applying phages on contaminated chicken breast. However, when lysing time was extended to 72 h, *Listeria* gradually regrew achieving similar values reported for contaminated samples at 0 h.

Phage DNA adsorption occurs faster at higher bacterial growth rates, and lysing time is limited by the rate of protein synthesis that is dependent on the host protein systems (Hadas et al., 1997). Previous research showed that host inactivation begins within the first few hours after phage application, even though phages are able to

complete the first lytic cycle ending in the release of new phages in 20-40 minutes depending on the MOI (Atterbury et al., 2003; Campbell, 2003; Guosheng et al., 2003; Leverentz et al., 2001; Rabinovitch et al., 2002). Overall although phages are efficient in decreasing bacteria populations in refrigerated temperatures, it seems that in higher temperatures, higher reductions may be achieved.

Conclusion

According to the FSIS Directive 7120.1, only phage solutions targeting *E. coli* O157:H7 are allowed to be used as a processing aid during meat production. In this study, we demonstrated that bacteriophages are also effective against the "Big Six" serotypes. This study provides evidence to the FDA and to biotechnology companies that bacteriophages targeting all adulterant STEC, not only O157:H7. Therefore, future FDA requests to obtain GRAS status for phages targeting the "Big Six" must be considered. In addition, results of this research demonstrated that bacteriophage applications reduce STEC loads when applied in warm and cold temperatures, suggesting that phage solutions may be successfully used in different processing points from harvest and fabrication.

References

Adams, M. H. (1959). Methods of Study of Bacterial Viruses. In *Bacteriophages* (1st ed.). Retrieved from https://www.biodiversitylibrary.org/item/30293

Anderson, M. E., & Marshall, R. T. (1989). Interaction of Concentration and Temperature of Acetic Acid Solution on Reduction of Various Species of Microorganisms on Beef Surfaces. *Journal of Food Protection*, *52*(5), 312–315.

Arthur, T. M., Brichta-Harhay, D. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2010). Super shedding of Escherichia coli O157:H7 by cattle and the impact on beef carcass contamination. *Meat Science*, *86*(1), 32–37. https://doi.org/10.1016/j.meatsci.2010.04.019

Atterbury, R. J., Connerton, P. L., Dodd, C. E. R., Rees, C. E. D., & Connerton, I. F. (2003). Application of Host-Specific Bacteriophages to the Surface of Chicken Skin Leads to a Reduction in Recovery of Campylobacter jejuni. *Applied and Environmental Microbiology*, *69*(10), 6302–6306. https://doi.org/10.1128/AEM.69.10.6302-6306.2003

Bell, R. G. (1997). Distribution and sources of microbial contamination on beef carcasses. *Journal of Applied Microbiology*, *82*, 292–300. https://doi.org/10.1046/j.1365-2672.1997.00356.x

Bergman, J. M., Fineran, P. C., Petty, N. K., & Salmond, G. P. C. (2019). Transduction: The Transfer of Host DNA by Bacteriophages. In *Reference Module in Biomedical Sciences* (4th ed.). https://doi.org/10.1016/b978-0-12-801238-3.11350-9

Bigwood, T., Hudson, J. A., Billington, C., Carey-Smith, G. V., & Heinemann, J. A. (2008). Phage inactivation of foodborne pathogens on cooked and raw meat. *Food Microbiology*, *25*(2), 400–406. https://doi.org/10.1016/j.fm.2007.11.003

Buchanan, R. L., & Klawitter, L. A. (1992). The effect of incubation temperature, initial pH, and sodium chloride on the growth kinetics of Escherichia coli O157:H7. *Food Microbiology*, *9*(3), 185–196. https://doi.org/10.1016/0740-0020(92)80046-7

Buchanan, R. L., Stahl, H. G., & Whiting, & R. C. (1989). Effects and Interactions of Temperature, pH, Atmosphere, Sodium Chloride, and Sodium Nitrite on the Growth of Listeria monocytogenes. *Journal of Food Protection*, *52*(12), 844–851. https://doi.org/10.1016/0168-1605(91)90039-R

Byrne, C. M., Bolton, D. J., Sheridan, J. J., McDowell, D. A., & Blair, I. S. (2000). The effects of preslaughter washing on the reduction of Escherichia coli O157:H7 transfer from cattle hides to carcasses during slaughter. *Letters in Applied Microbiology*, *30*, 142–145. https://doi.org/10.1046/j.1472-765x.2000.00689.x

Campbell, A. (2003). The future of bacteriophage biology. *Nature Reviews Genetics*, *4*(6), 471–477. https://doi.org/10.1038/nrg1089

Carey-Smith, G. V, Billington, C., Cornelius, A. J., Hudson, J. A., & Heinemann, J. A. (2006). Isolation and characterization of bacteriophages infecting Salmonella spp. *FEMS Microbiol Letters*, *248*(2006), 182–186. https://doi.org/10.1111/j.1574-6968.2006.00217.x

Carter, C. D., Parks, A., Abuladze, T., Li, M., Woolston, J., Magnone, J., … Sulakvelidze, A. (2012). Bacteriophage cocktail significantly reduces Escherichia coli O157. *Bacteriophage*, *2*(3), 178–185. https://doi.org/10.4161/bact.22825

CDC. (2016). Escherichia coli Factsheet. Retrieved from https://www.cdc.gov/ecoli/pdfs/CDC-E.-coli-Factsheet.pdf.

CDC. (2017). National Shiga toxin-producing Escherichia coli (STEC) Surveillance Annual Report, 2014. Retrieved from https://www.cdc.gov/nationalsurveillance/pdfs/STEC-2014-REPORT_508c.pdf

CDC. (2018). General information of E. coli. Retrieved from https://www.cdc.gov/ecoli/general/index.html

Cheng, H. Y., Yu, R. C., & Chou, C. C. (2003). Increased acid tolerance of Escherichia coli O157:H7 as affected by acid adaptation time and conditions of acid challenge. *Food Research International*, *36*(1), 49–56. https://doi.org/10.1016/S0963-9969(02)00107-2

Coffey, B., Rivas, L., Duffy, G., Coffey, A., Ross, R. P., & McAuliffe, O. (2011). Assessment of Escherichia coli O157:H7-specific bacteriophages e11/2 and e4/1c in model broth and hide environments. *International Journal of Food Microbiology*, *147*(3), 188–194. https://doi.org/10.1016/j.ijfoodmicro.2011.04.001

Endersen, L., O'mahony, J., Hill, C., Ross, R. P., Mcauliffe, O., & Coffey, A. (2014). Phage Therapy in the Food Industry. *Annual Review of Food Science and Technology*, *5*, 327–349. https://doi.org/10.1146/annurev-food-030713-092415

Ferens, W. A., & Hovde, C. J. (2011). Escherichia coli O157:H7: Animal reservoir and sources of human infection. *Foodborne Pathogens and Disease*, *8*(4), 465–487. https://doi.org/10.1089/fpd.2010.0673

Fiorentin, L., Júnior, B., Suínos, E., & Author, A. (2005). Brazilian Journal of Poultry Science Revista Brasileira de Ciência Avícola Use of Lytic Bacteriophages to Reduce Salmonella Enteritidis in Experimentally Contaminated Chicken Cuts. Brazillian Journal of Poultry Science, 7(4), 255–260.

Gonthier, A., Guérin-Faublée, V., Tilly, B., & Delignette-Muller, M. L. (2001). Optimal growth temperature of O157 and non-O157 Escherichia coli strains. *Letters in Applied Microbiology*, *33*(5), 352–356. https://doi.org/10.1046/j.1472-765X.2001.01010.x

Grant, A., Parveen, S., Schwarz, J., Hashem, F., & Vimini, B. (2017). Reduction of Salmonella in ground chicken using a bacteriophage. *Poultry Science*, *96*(8), 2845–2852. https://doi.org/10.3382/ps/pex062

Greer, G. G. (2005). Bacteriophage control of foodborne bacteria. *Journal of Food Protection*, *68*(5), 1102–1111. https://doi.org/10.4315/0362-028X-68.5.1102

Guenther, S., Herzig, O., Fieseler, L., Klumpp, J., & Loessner, M. J. (2012). Biocontrol of Salmonella Typhimurium in RTE foods with the virulent bacteriophage FO1-E2. *International Journal of Food Microbiology*, *154*, 66–72. https://doi.org/10.1016/j.ijfoodmicro.2011.12.023

Guosheng, L., Yi, L., Xiangdong, C., Peng, L., Ping, S., & Songsheng, Q. (2003). Study on interaction between T4 phage and Escherichia coli B by microcalorimetric method. *Journal of Virological Methods*, *112*(1–2), 137–143. https://doi.org/10.1016/S0166-0934(03)00214-3

Hadas, H., Einav, M., Fishov, I., & Zaritsky, A. (1997). Bacteriophage T4 development depends on the physiology of its host Escherichia coli. *Microbiology*, *143*(1), 179–185. https://doi.org/10.1099/00221287-143-1-179

Harris, K., Miller, M. F., Loneragan, G. H., & Brashears, M. M. (2006). Validation of the use of organic acids and acidified sodium chlorite to reduce Escherichia coli O157 and Salmonella typhimurium in beef trim and ground beef in a simulated processing environment. *Journal of Food Protection*, *69*(8), 1802–1807. https://doi.org/10.4315/0362-028X-69.8.1802

Hooton, S. P. T., Atterbury, R. J., & Connerton, I. F. (2011). Application of a bacteriophage cocktail to reduce Salmonella Typhimurium U288 contamination on pig skin. *International Journal of Food Microbiology*, *151*(2), 157–163. https://doi.org/10.1016/j.ijfoodmicro.2011.08.015

Hudson, J. A., Billington, C., Cornelius, A. J., Wilson, T., On, S. L. W., Premaratne, A., & King, N. J. (2013). Use of a bacteriophage to inactivate Escherichia coli O157: H7 on beef. *Food Microbiology*, *36*(1), 14–21. https://doi.org/10.1016/j.fm.2013.03.006

Hudson, J. A., Billington, C., Wilson, T., & On, S. L. W. (2015). Effect of phage and host concentration on the inactivation of Escherichia coli O157:H7 on cooked and raw beef. *Food Science and Technology International*, *21*(2), 104–109. https://doi.org/10.1177/1082013213513031

Jorquera, D., Navarro, C., Rojas, V., Turra, G., Robeson, J., & Borie, C. (2015). The use of a bacteriophage cocktail as a biocontrol measure to reduce Salmonella enterica serovar Enteritidis contamination in ground meat and goat cheese. *Biocontrol Science and Technology*, *25*(8), 970–974. https://doi.org/10.1080/09583157.2015.1018815

Kim, G. H., Breidt, F., Fratamico, P., & Oh, D. H. (2015). Acid Resistance and Molecular Characterization of Escherichia coli O157: H7 and Different Non-O157 Shiga Toxin-Producing E. coli Serogroups. *Journal of Food Science*, *80*(10), M2257–M2264. https://doi.org/10.1111/1750-3841.12996

Laury, A. M., Alvarado, M. V., Nace, G., Alvarado, C. Z., Brooks, J. C., Echeverry, A., & Brashears, M. M. (2009). Validation of a lactic acid- and citric acid-based antimicrobial product for the reduction of Escherichia coli O157:H7 and Salmonella on beef tips and whole chicken carcasses. *Journal of Food Protection*, *72*(10), 2208–2211. https://doi.org/10.4315/0362-028X-72.10.2208

Leverentz, B., Conway, W. S., Alavidze, Z., Janisiewicz, W. J., Fuchs, Y., Camp, M. J., … Sulakvelidze, A. (2001). Examination of Bacteriophage as a Biocontrol Method for Salmonella on Fresh-Cut Fruit: A Model Study. *Journal of Food Protection*, *64*(8), 1116–1121.

Leyer, G. J., Wang, L. L., & Johnson, E. A. (1995). Acid adaptation of Escherichia coli O157:H7 increases survival in acidic foods. *Applied and Environmental Microbiology*, *61*(10), 3752–3755. https://doi.org/10.1128/AEM.61.10.3752-3755.1995

Lindberg, A. A. (1973). Bacteriophage Receptors. *Annual Reviews in Microbiology*, *27*(1), 205–241. Retrieved from www.annualreviews.org

Liu, H., Niu, Y. D., Meng, R., Wang, J., Li, J., Johnson, R. P., … Stanford, K. (2015). Control of Escherichia coli O157 on beef at 37, 22 and 4°C by T5-, T1-, T4-and O1-like bacteriophages. *Food Microbiology*, *51*, 69–73. https://doi.org/10.1016/j.fm.2015.05.001

Magnone, J. P., Marek, P. J., Sulakvelidze, A., & Senecal, A. G. (2013). Additive approach for inactivation of Escherichia coli O157:H7, Salmonella, and Shigella spp. on contaminated fresh fruits and vegetables using bacteriophage cocktail and produce wash. *Journal of Food Protection*, *76*(8), 1336–1341. https://doi.org/10.4315/0362-028X.JFP-12-517

Mahony, J., McAuliffe, O., Ross, R. P., & van Sinderen, D. (2011). Bacteriophages as biocontrol agents of food pathogens. *Current Opinion in Biotechnology*, *22*(2), 157–163. https://doi.org/10.1016/j.copbio.2010.10.008

McEvoy, J. M., Doherty, A. M., Finnerty, M., Sheridan, J. J., McGuire, L., Blair, I. S., … Harrington, D. (2000). The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Letters in Applied Microbiology*, *30*(5), 390–395. https://doi.org/10.1046/j.1472-765x.2000.00739.x

Mirzaei, M. K., Eriksson, H., Kasuga, K., Haggård-Ljungquist, E., & Nilsson, A. S. (2014). Genomic, proteomic, morphological, and phylogenetic analyses of vB-EcoP-SU10, a podoviridae phage with C3 morphology. *PLoS ONE*, *9*(12), 1–19.

https://doi.org/10.1371/journal.pone.0116294

Montag, D., Hashemolhosseini, S., & Henning, U. (1990). Receptor-recognizing proteins of T-even type bacteriophages. The receptor-recognizing area of proteins 37 of phages T4 TuIa and TuIb. *Journal of Molecular Biology*, *216*(2), 327–334. https://doi.org/10.1016/S0022-2836(05)80324-9

Nauta, M. J., & Dufrenne, J. B. (1999). Variability in Growth Characteristics of Different E. coli O157 : H7 Isolates, and its Implications for Predictive Microbiology. In *Quantitative Microbiology* (Vol. 1).

O'Flynn, G., Ross, R. P., Fitzgerald, G. F., & Coffey, A. (2004). Evaluation of a cocktail of three bacteriophages for biocontrol of Escherichia coli O157:H7. *Applied and Environmental Microbiology*, *70*(6), 3417–3424. https://doi.org/10.1128/AEM.70.6.3417-3424.2004

Pereira, C., Moreirinha, C., Lewicka, M., Almeida, P., Clemente, C., Cunha, Â., … Almeida, A. (2016). Bacteriophages with potential to inactivate Salmonella Typhimurium: Use of single phage suspensions and phage cocktails. *Virus Research*, *220*, 179–192. https://doi.org/10.1016/j.virusres.2016.04.020

Rabinovitch, A., Fishov, I., Hadas, H., Einav, M., & Zaritsky, A. (2002). Bacteriophage T4 development in Escherichia coli is growth rate dependent. *Journal of Theoretical Biology*, *216*(1), 1–4. https://doi.org/10.1006/jtbi.2002.2543

Rakhuba, D. V., Kolomiets, E. I., Szwajcer-Dey, E., & Novik, G. I. (2010). Bacteriophage receptors, mechanisms of phage adsorption, and penetration into host cell. *Polish Journal of Microbiology*, *59*(3), 145–155.

Samelis, J., Sofos, J. N., Kendall, P. A., & Smith, G. C. (2005). Survival or growth of Escherichia coli O157:H7 in a model system of fresh meat decontamination runoff waste fluids and its resistance to subsequent lactic acid stress. *Applied and Environmental Microbiology*, *71*(10), 6228–6234. https://doi.org/10.1128/AEM.71.10.6228-6234.2005

Schmidt, J. W., Bosilevac, J. M., Kalchayanand, N., Wang, R., Wheeler, T. L., & Koohmaraie, M. (2014). Immersion in antimicrobial solutions reduces Salmonella enterica and shiga toxin-producing Escherichia coli on beef cheek meat. *Journal of Food Protection*, *77*(4), 538–548. https://doi.org/10.4315/0362-028X.JFP-13-300

Sengupta, D., & Chattopadhyay, M. K. (2013). Metabolism in bacteria at low temperature: A recent report. Journal of Bioscience, 38(2), 409–412. https://doi.org/10.1007/s12038-013

Seo, J., Seo, D. J., Oh, H., Jeon, S. B., Oh, M.-H., & Choi, C. (2016). Inhibiting the Growth of Escherichia coli O157:H7 in Beef, Pork, and Chicken Meat using a Bacteriophage. *Korean Journal for Food Science of Animal Resources*, *36*(2), 186–193. https://doi.org/10.5851/kosfa.2016.36.2.186

Sharma, C. S., Dhakal, J., & Nannapaneni, R. (2015). Efficacy of lytic bacteriophage preparation in reducing Salmonella in vitro, on Turkey breast cutlets, and on ground Turkey. *Journal of Food Protection*, *78*(7), 1357–1362. https://doi.org/10.4315/0362-028X.JFP-14-585

Sillankorva, S., Oliveira, R., Vieira, M. J., Sutherland, I., & Azeredo, J. (2004). Pseudomonas fluorescens infection by bacteriophage US1: the influence of temperature, host growth phase and media. *FEMS Microbiology Letters*, *241*, 13–20. https://doi.org/10.1016/j.femsle.2004.06.058

Smulders, F. J. M., & Greer, G. G. (1998). Integrating microbial decontamination with organic acids in HACCP programmes for muscle foods: Prospects and controversies. *International Journal of Food Microbiology*, *44*(3), 149–169. https://doi.org/10.1016/S0168-1605(98)00123-8

Spricigo, D. A., Bardina, C., Cortés, P., & Llagostera, M. (2013). Use of a bacteriophage cocktail to control Salmonella in food and the food industry. *International Journal of Food Microbiology*, *165*(2), 169–174. https://doi.org/10.1016/j.ijfoodmicro.2013.05.009

Sulakvelidze, A., & Barrow, P. (2005). Phage therapy in animals and agribusiness. In *Bacteriophages: Biology and Applications* (pp. 335–380). Boca Raton, FL: CRC Press.

Tomat, D., Casabonne, C., Aquili, V., Balagué, C., & Quiberoni, A. (2018). Evaluation of a novel cocktail of six lytic bacteriophages against Shiga toxin-producing Escherichia coli in broth, milk and meat. *Food Microbiology*, *76*, 434–442. https://doi.org/10.1016/j.fm.2018.07.006

USDA-ERS. (2014). *Cost estimates of foodborne illnesses*. Retrieved from https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx#48446

USDA-FSIS. (2012). *United States Department of Agriculture - Food Safety Inspection Service, Shiga toxin-producing Escherichia coli in certain raw beef products*. Retrieved from https://www.federalregister.gov/documents/2012/05/31/2012-13283/shiga-toxin-producing-escherichia-coli-in-certain-raw-beef-products

USDA-FSIS. (2014). *United States Department of Agriculture - Food Safety Inspection Service, Compliance Guideline for Establishments Sampling Beef Trimmings for Shiga Toxin-Producing Escherichia coli (STEC) Organisms or Virulence Markers*. Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/e0f06d97-9026-4e1e-a0c2-1ac60b836fa6/Compliance_Guide_Est_Sampling_STEC_0512.pdf?MOD=AJPERES

USDA-FSIS. (2019). *United States Department of Agriculture - Food Safety Inspection Service, Safe and Suitable Ingredients used in the Production of Meat, Poultry, and Egg Products*. Retrieved from https://www.fsis.usda.gov/wps/portal/fsis/topics/regulations/directives/7000-series/safe-suitable-ingredients-related-document

Viazis, S., Akhtar, M., Feirtag, J., & Diez-Gonzalez, F. (2011). Reduction of Escherichia coli O157:H7 viability on hard surfaces by treatment with a bacteriophage mixture. *International Journal of Food Microbiology*, *145*(1), 37–42. https://doi.org/10.1016/j.ijfoodmicro.2010.11.021

Wheeler, T. L., Kalchayanand, N., & Bosilevac, J. M. (2014). Pre- and post-harvest interventions to reduce pathogen contamination in the U.S. beef industry. *Meat Science*, *98*(3), 372–382. https://doi.org/10.1016/j.meatsci.2014.06.026

Wolf, M. J., Miller, M. F., Parks, A. R., Loneragan, G. H., Garmyn, A. J., Thompson, L. D., … Brashears, M. M. (2012). Validation comparing the effectiveness of a lactic acid dip with a lactic acid spray for reducing Escherichia coli O157:H7, Salmonella, and Non-O157 shiga toxigenic Escherichia coli on beef trim and ground beef. *Journal of Food Protection*, *75*(11), 1968–1973. https://doi.org/10.4315/0362-028X.JFP-12-038

Yang, S., Sadekuzzaman, M., & Ha, S. Do. (2017). Reduction of Listeria monocytogenes on chicken breasts by combined treatment with UV-C light and bacteriophage ListShield. *LWT - Food Science and Technology*, *86*, 193–200. https://doi.org/10.1016/j.lwt.2017.07.060

Yeh, Y., de Moura, F. H., Van Den Broek, K., & de Mello, A. S. (2018). Effect of ultraviolet light, organic acids, and bacteriophage on Salmonella populations in ground beef. *Meat Science*, *139*, 44–48. https://doi.org/10.1016/j.meatsci.2018.01.007

Yeh, Y., Purushothaman, P., Gupta, N., Ragnone, M., Verma, S. C., & de Mello, A. S. (2017). Bacteriophage application on red meats and poultry: Effects on Salmonella population in final ground products. *Meat Science*, *127*, 30–34.

https://doi.org/10.1016/j.meatsci.2017.01.001

Yu, F., & Mizushima, S. (1982). Roles of lipopolysaccharide and outer membrane protein ompc of Escherichia coli K-12 in the receptor function for bacteriophage T4. *Journal of Bacteriology*, *151*(2), 718–722.

Zhao, T., Zhao, P., & Doyle, M. P. (2009). Inactivation of Salmonella and Escherichia coli O157:H7 on Lettuce and Poultry Skin by Combinations of Levulinic Acid and Sodium Dodecyl Sulfate. *Journal of Food Protection*, *72*(5), 928–936. https://doi.org/10.4315/0362-028X-72.5.928

Table 1. Lysing efficiency[1] of individual bacteriophages MS1-O157:H7, MS1-O145, MS1-O121, MS1-O111, MS1-O103, MS1-O45, and MS1-O26 against STEC O157:H7 (ATCC® 35150™), O26 (ATCC® BAA-2196™), O45 (ATCC® BAA-2193™), O103 (ATCC® BAA-2215™), O111 (ATCC® BAA-2440™), O121 (ATCC® BAA-2219™), and O145 (ATCC® BAA-2192™) in vitro.

	Bacteriophage						
STEC serotype	**MS1-O157:H7**	**MS1-O145**	**MS1-O121**	**MS1-O111**	**MS1-O103**	**MS1-O45**	**MS1-O26**
O157:H7 (ATCC 35150)	99.9%	96.5%	99.1%	50.9%	45.8%	54.9%	77.2%
O145 (ATCC BAA-2192)	98.8%	99.6%	97.1%	89.0%	84.0%	62.8%	96.7%
O121 (ATCC BAA-2219)	87.0%	88.0%	96.6%	85.6%	79.1%	73.4%	58.5%
O111 (ATCC BAA-2440)	98.9%	99.9%	99.3%	99.4%	34.1%	82.2%	67.3%
O103 (ATCC BAA-2215)	99.7%	97.4%	79.7%	97.0%	96.2%	55.6%	93.7%
O45 (ATCC BAA-2193)	99.4%	96.2%	88.5%	93.3%	95.8%	98.6%	68.4%
O26 (ATCC BAA-2196)	99.8%	96.1%	95.6%	97.6%	80.7%	58.7%	99.9%

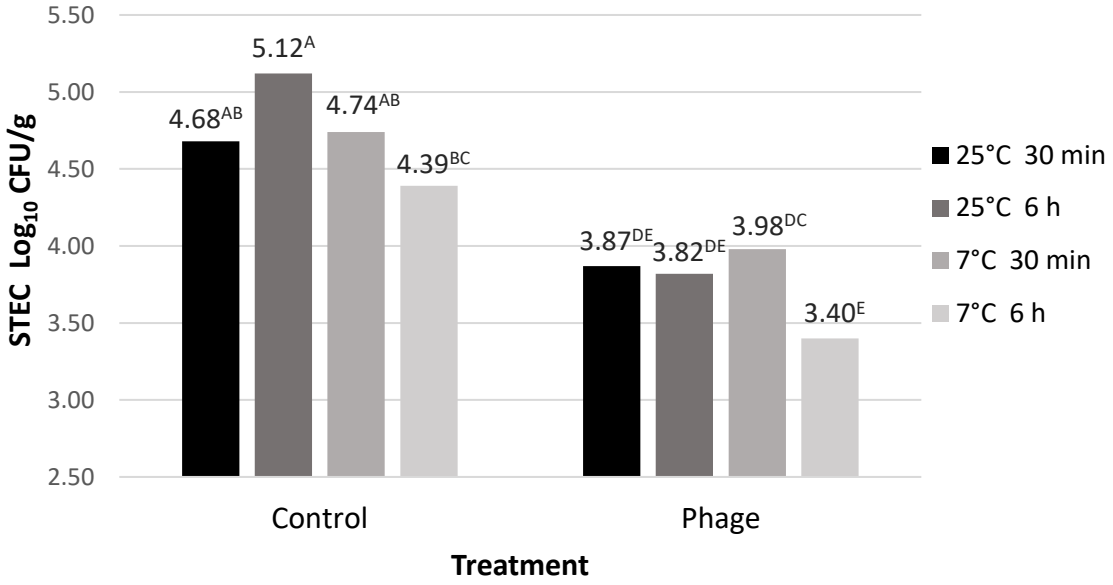
[1] In vitro lysing efficiency of individual phages based on the average CFU of 4 LB agar plates treated with phage versus inoculated.

Table 2. Means of Shiga Toxin *E. coli* contamination level (Log CFU/g) strains in ground beef following application of control or bacteriophage cocktail preparation MS1-O26, MS1O-45, MS1-O103, MS1-O111, MS1-O121, MS1-O145, and MS1-O157) at two lysing times 30 min and 6 h at 7°C or 25°C on beef trim prior to grinding.

Holding Time	**Temperature (°C)**	**Control***	**Bacteriophage application**	**Reduction (Log_{10} CFU/g)**
30 min	7	5.09	4.34	0.76
	25	5.03	4.22	0.81
6 h	7	4.74	3.76	0.98
	25	5.48	4.18	1.30

* No bacteriophage applied.

Figure 1. Least Square Means of Shiga Toxin *E. coli* populations (Log CFU/g) in ground beef following control and phage applications on beef trim with fixed effects of time (30 min, 6 h) and temperature (7° C, 25° C). Std error = 0.2477, $P < 0.0001$. Contrasts: Phage vs Control, $P < 0.0001$; 7°C vs 25°C, $P = 0.0497$; and 30 min vs 6 h, $P = 0.2797$.



Appendix III. Phage sewage isolation and purification

Raw sewage was acquired from Reno Municipal sewer wastewater treatment facility from both north and south sources. Aseptically, 40 mL of sewage (equal parts of both sources) were combined in sterile Erlenmeyer flasks and inoculated with 5 mL of overnight pure culture of a single strain of Shiga Toxin-producing *E. coli* (O157:H7, O145, O121, O111, O103, O45, O26). Flasks were covered and incubated with shaking (230 rpm) overnight at 37°C.

Subsequently, 10 mL of solution was centrifuged at 5,000 x g for 20 minutes and the supernatant was filtered with a 0.45 µM syringe filter twice. Soft agar (appendix IV) was performed on the resulting lysate. Plaques were evaluated visually, and a single, well-isolated, wide, and clear plaque was extracted from each serotype agar plate and placed in 1 mL of sterile Buffered Peptone Water (BPW) with one drop of chloroform before vortexing.

These isolated phages were amplified by combining 10 mL of sterile Luria Broth (LB) with 1 mL of overnight pure culture and 1 mL of the isolated phage extract and incubating overnight with shaking at 37°C. Subsequently, soft agar (appendix IV) assay was preformed and a single, well-isolated, wide, and clear plaque was extracted from each serotype agar plate and placed into 1 mL of BPW and vortexed. This phage solution was amplified into an isolated working phage solution.

Appendix IV. Double-layer Agar Assay Adams (1959), modified by Yeh et al. (2017)

1. Prepare 0.6% soft agar (1 g Bacteriological agar and 4 g Luria Broth, Miller per 200 mL deionized water)
2. Sterilize the mixture in autoclave at 121 °C for at least 15 minutes.
3. Allow the solution to cool in a water bath tempered at 45°C.
4. Equilibrate 50 mL conical tubes in the water bath prior to aseptically adding 4 mL of the soft agar into each tube.
5. Add 100 µL of Log phase or overnight bacteria into the tube followed by 100 µL of serially diluted phage to achieve ~100 plaques per plate
6. Invert twice and pour slowly onto a pre-made 1.6% LB agar plate and spread soft agar across surface of plate and allow to solidify
7. Incubate overnight (not inverted) at 37°C for plaque enumeration

CHAPTER TWO

Efficiency of bacteriophage and organic acids in decreasing STEC O157:H7 populations in beef kept under vacuum and aerobic conditions: A simulated High Event Period scenario

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Abstract

During High Event Periods, beef subprimals are usually removed from vacuum and treated with antimicrobials. After re-packaging, subprimals are tested to verify the absence of STEC. In this study, bacteriophage and organic acids were applied on fresh beef surface contaminated with STEC O157:H7 to evaluate the efficiency of industry practices. Beef samples inoculated with STEC were treated with bacteriophage, lactic acid, and peroxyacetic acid and kept under vacuum or aerobic conditions. STEC loads were evaluated 30 min and 6 h after antimicrobial applications. Under aerobic conditions for 30 min and 6 h, phage reduced STEC in beef by approximately 1.4 Log CFU/cm^2 whereas organic acids led to approximately 0.5 Log CFU/cm^2 reduction. Under vacuum conditions for 30 min, bacteriophage significantly reduced STEC by 1 Log CFU/cm^2, while no significant effects were observed in samples treated with organic acids. Under vacuum conditions for 6 h, bacteriophage reduced STEC loads by 1.4 Log CFU/cm^2, lactic acid reduced by 0.6 Log CFU/cm^2, and no significant effects were observed with peroxyacetic acid application.

Keywords: Bacteriophage, PAA, lactic acid, high event period, STEC, E. coli O157:H7, beef

Introduction

In the United States, the production interval when beef processing establishments experience an elevated rate of STEC contamination in trimmings is referred to as a "High Event Period" (HEP). In large operations, contamination of trimmings is assessed by sampling combos using the N60 or N60 plus methods (USDA-FSIS, 2015). More recently, novel methodologies using a manual sampling device (MSD) and a continuous sampling device (CSD) have been also been implemented to sample combos (Wheeler & Arthur, 2018). The United States Department of Agriculture (USDA) – Food Safety Inspection Service (FSIS) considers an establishment's process to be within a HEP if the percent of positive samples within a specified production interval is 5% or greater (USDA-FSIS, 2014). A HEP may indicate a localized out-of-control event or a systemic breakdown of slaughter dressing operations that led to contamination of beef carcass components including primals, subprimals, and trimmings (USDA-FSIS, 2014). Previous studies have identified that hides are the major source of contamination of carcasses during processing (Arthur et al., 2008; Bell, 1997; Byrne, Bolton, Sheridan, McDowell, & Blair, 2000; McEvoy et al., 2000). During hide removal, bacteria on hides can be transferred onto the carcass (Barkocy-Gallager et al., 2003; Nou et al., 2003) whereas contamination of trim and primals depend on incoming bacterial loads and the ability of establishments to prevent cross contamination between hides and carcasses during dressing

procedures (Brichta-Harhay et al., 2008). Although a decrease of STEC loads is achieved by implementing good dressing procedures and antimicrobial applications during slaughter (Brashers and Chavez, 2017), Yang, He, Badoni, Tran, and Wang (2017) showed that 62.2% of the *E. coli* found in meat are shared during processing and fabrication by mesh gloves, conveyor belts, and cutting tables. Therefore, if not eliminated or reduced by interventions during slaughter, higher STEC loads may be disseminated during fabrication to contaminate trimmings and primals, and lead to higher rates of positive STEC trim samples.

According to the USDA-FSIS, while STEC positive combos must be diverted to further processing to be heat treated, beef primals and subprimals produced from the same carcasses that generated positive trimmings must be tested before entering commerce (USDA-FSIS, 2014). After experiencing a HEP, establishments rework subprimals associated with contaminated trimmings by (1) removing them from vacuum sealed bags, (2) treating with antimicrobials, and (3) repackaging. Subsequently, in order to ensure that subprimals are not contaminated, the pieces are (4) removed from vacuum sealed bags again, and (5) the meat surface is swabbed to test for STEC contamination. Industry personnel estimate that the time spent from steps 1 to 4 may vary from 30 min to 6 h depending on processing speed and production volume.

In the United States, application of organic acids to decontaminate meat surfaces is the most common intervention used to decrease bacteria loads and eliminate STEC (Wheeler, Kalchayanand, & Bosilevac, 2014). Advantages of using those acids include availability, cost, and simplicity of usage (Mataragas, Skandamis, &

Drosinos, 2008). However, previous studies demonstrated that *E. coli* strains may develop resistance to commonly used organic acids including lactic acid (Smulders & Greer, 1998; Dickson & Kunduru, 1995; Anderson & Marshall, 1989). In this study, we evaluated the killing efficiency of organic acids including peroxyacetic and lactic acids and a bacteriophage solution on fresh beef contaminated with STEC, under vacuum and aerobic conditions, by simulating steps and time intervals where primals and subprimals are reworked and retreated after a HEP.

Materials and Methods

E. coli strains and inoculum preparation

Four strains of *E. coli* O157:H7 were used in this study to ensure that interventions were tested against different genotypes. Strains included ATCC® 35150™, ATCC® 43895™, ATCC® 43894™, and NCTC 13128. All ATCC strains were *stx*1 and *stx*2 positives whereas the NCTC strain was negative for both genes. ATCC strains were recovered by following the ATCC bacterial culture guidelines (ATCC, 2015) whereas the strain NCTC 13128 was directly recovered from a glycerol stock by streaking the content of the micro tube onto LB agar plates. Briefly, strains were thawed and a loop full of each stock was aseptically streaked on to 1.6% LB agar plates in duplicate. After incubating overnight at 37°C, one isolated colony was aseptically added to 10 mL of sterile LB broth and incubated overnight at 37°C with shaking (200 rpm). Subsequently, 1 mL was then transferred into a tube containing LB broth and cultured overnight at

37°C. After approximately 16 h, final absorbance was verified at 600 nm (OD_{600}) and CFU concentration per mL was estimated by using the Agilent *E. coli* cell culture biocalculator (AGILENT, 2019). Cultures were individually diluted in 0.1% sterile buffered peptone water (BPW) to achieve $1x10^7$ CFU/ml. The final inoculum was prepared by combining equal amounts of all four individual *E. coli* strains.

Bacteriophage solution titer and killing efficiency

The commercial preparation PhageGuard E™ containing two bacteriophages (EP75 and EP335) was provided by MICREOS Food Safety, Inc. (Wageningen, The Netherlands). Bacteriophage EP75 is a Vi1virus, that belongs to the *Caudovirale* order, *Ackermannviridae* family, and subfamily *Cvivirinae* whereas the bacteriophage EP 335 is a Phieco32virus that belongs to *Caudovirale* order and *Podoviridae* family (Van Mierlo et al., 2019). Phage stock concentrations was determined by following the double-layer agar method with some modifications (Adams, 1959). Briefly, the PhageGuard E™ stock solution was serially diluted in sterile potable water and added into Luria Broth (LB) soft agar (0.6% agar) tempered at 45°C after inoculation with individual fresh-log phase *E. coli* cultures. Tubes were vortexed twice and distributed onto the lawn of hard LB hard agar plates (1.6%) in duplicate. The soft agar was allowed to solidify at room temperature and subsequently, plates were inverted and incubated overnight at 37 °C. Concentration of the PhageGuard E™ was determined to be $2x10^{11}$ PFU/ml.

Killing efficiency of PhageGuard E™ was determined by testing the stock solution against individual *E. coli* strains. A volume of 100 µl of diluted overnight *E. coli* cultures

were spread onto LB agar plates. After the plates dried, the same volume of stock bacteriophage solution was applied onto the lawn of the plates. Plates were allowed to dry and were inverted and incubated overnight at 37 °C. Killing efficiency was determined in quadruplicate. Bacteriophage was not applied onto control plates.

Sample preparation and experimental design

Beef Rose Meat (m. *cutaneous trunci*, IMPS 194) (USDA-AMS, 2014) was procured from a USDA-inspected facility and transported under refrigeration (4 °C) to the University of Nevada, Reno's Meat Quality Laboratory. Upon arrival, aliquots from individual coolers (n = 4, approximately 18 kg each) were screened for generic *E. coli*. A total of 160 samples measuring 10 x 10 cm^2 were subsampled, fabricated from rose meat pieces, and assigned to a completely randomized design (CRD). Fixed effects included antimicrobial treatment (CI = Control Inoculated, PGE = Bacteriophage-treated, PAA = peroxyacetic acid-treated, and LA= lactic acid-treated), packaging atmosphere (V=under vacuum and NV=wrapped with O_2 permeable film), and lysing time (30 min and 6 h).

Sample inoculation, antimicrobial applications, and bacterial enumeration

Beef samples were inoculated with a cocktail containing all four *E. coli* strains at $1x10^7$ CFU/ml. A volume of 500 µl of the inoculum was uniformly pipetted on meat and distributed throughout the whole surface area of the sample using a sterile plastic spreader. In order to simulate contamination of

beef trimmings and beef cuts in a HEP scenario, samples were kept under exposure of O_2 (NV) or in vacuum conditions (V). All samples were kept under refrigeration prior inoculation and during the trial (5±2°C). Samples wrapped with O_2 permeable film (NV) were: (1) inoculated, (2) wrapped with film and stored for 17-24 h, (3) removed from package and treated with antimicrobials (PGE, PAA, or LA), (4) wrapped with film and stored for 30 min or 6h, and (5) removed from package and swabbed for bacteria enumeration. Vacuum packaged samples were processed in similar fashion. Samples were (1) inoculated, (2) vacuum packaged (model VMC10OP, VacPak-It, Lititz, PA, United States) and stored for 17-24 h, (3) removed from package and treated with antimicrobials (PGE, PAA, or LA), (4) vacuum packaged and stored for 30 min or 6h, and (5) removed from package and swabbed for bacteria enumeration. All antimicrobials were applied at a volume of 500 µl by pipetting and distributing the solution throughout the sample surface. PhageGuard E™ was diluted in potable water as recommended by the manufacturer and applied at a concentration of $1x10^8$ PFU/ml. Lactic acid solution (2-hydroxypropionic acid, 87.5–88.5%, w/w) was applied at 5% at 50°C, whereas PAA (Ethaneperoxoic acid, stabilized, < 43%) was applied at 400 ppm at room temperature. Control inoculated samples were treated with BPW. For bacteria enumeration, all surface area was swabbed (QS1000, Hygiena, Camarillo, CA, United States) and the swab homogenate was vortexed, serially diluted, and 100 µL was spread-plated onto LB agar plates in duplicate. Plates were inverted and incubated overnight at 37°C, and *E. coli* colonies were enumerated (CFU/cm^2). Differently than our previous experiments (Yeh et al., 2017; Yeh, de Moura, Van Den Broek & de Mello, 2018), the homogenate obtained

by swabbing the beef samples was not treated to eliminate possible residual phages or organic acids before plating. Our goal was to mimic real industry scenarios where phage separation and organic acid neutralization are not practiced by laboratories after receiving samples from processing facilities.

Statistical analysis

The experiment was conducted as a CRD and was replicated 5 times whereas 1 replication had 1 repetition, 1 replication had 3 repetitions, and 3 replications had 2 repetitions (n=160 total e.u., n=10 per fixed effects combination). The following model was used: $Y_{ij} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$, where Y_{ij} was *E. coli* count, μ was the overall mean across the treatments included in the experiment, α_i was the effect of antimicrobial from the grand mean specific to the *i* levels (CI, PGE, PAA, and LA), β_j was the effect of lysing time from the overall mean specific to *k* levels (30 min and 6 h), and γ_k was the effect of packaging atmosphere from the overall mean specific to *j* levels (V and NV). All interactions and respective errors were also added in the model. In addition, we identified two nested models of interest within packaging atmosphere (γ). Therefore, data was also analyzed separately as a CRD for V and NV and the following model was used for both: $Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijl}$. Data were analyzed using the GLIMMIX procedure of SAS® 9.3 package (SAS Institute, Inc., USA) as a 4 x 2 x 2 factorial for the full model and 4 x 2 for each nested

model. When significance ($P \leq 0.05$) was indicated by ANOVA, means separations were performed by using the LSMEANS and DIFF function statements.

Results

Killing efficiency of PhageGuard E™ is shown in table 1. Bacteriophages EP75 and EP335 reduced the growth of the strains ATCC® 35150™, ATCC® 43895™, ATCC® 43894™, and NCTC 13128 by approximately 98.2%, 97.2%, 96.7%, and 98.3%, respectively. A three-way interaction (P=0.035) was observed when evaluating the full model including the three fixed effects (antimicrobial treatment, packaging atmosphere, and lysing time). However, when looking at individual effects, antimicrobial treatment and packaging atmosphere were highly significant (P<0.0001) whereas lysing time did not individually drive the three-way interaction (P=0.226) (table 2).

Results of the nested model evaluating samples kept under vacuum are shown in figure 1. Control inoculated samples (CI) treated with BPW yielded 2.07 and 2.37 Log CFU/cm^2 after 30 min and 6 h, respectively. The ANOVA showed an interaction between antimicrobial treatment and lysing time (P=0.006). When samples were treated with PGE, 1.07 and 1.41 Log reductions were observed for lysing time of 30 min and 6h, respectively. Within lysing time of 30 min, no statistical difference was observed when comparing CI, LA, and PAA, whereas PGE applications significantly reduced bacterial load. Overall, samples treated with PGE yielded the lowest bacterial load when compared to CI and organic acids treatments.

Results for samples kept under aerobic conditions are shown in table 3. For this model, no interaction between antimicrobial treatment and lysing time was observed (P=0.986). Individual effects of lysing time were also not significant (P=0.432). However, the effects of antimicrobial treatment were statistically significant at P<0.0001. Under aerobic conditions, samples treated with organic acids showed lower *E. coli* counts when compared to CI samples. Lactic acid applications reduced bacteria loads by 0.47 Log CFU/cm^2 whereas PAA reduced 0.53 Log CFU/cm^2 when compared to CI samples. Bacteriophage applications led to the optimal reduction, lowering bacteria counts by 1.41 Log CFU/cm^2 when compared to CI samples. Therefore, in both packaging atmosphere conditions (V and NV), bacteriophage applications showed improved control of *E. coli*, when compared to LA and PAA.

Discussion

The killing efficiency of the bacteriophage solution against the 4 strains was at least 96.7%. Both *caudovirales* phages were previously described by Mierlo et al. (2019) as STEC-specific phages with genome size of >50 nucleotides. The EP 75 shares its nucleotide identity of 96% with the *Escherichia* phage Phaxl, 92%, is homologous to *Escherichia* phage ECML-4, and have at least 91% nucleotide identity with other *Salmonella* phages. The bacteriophage EP 335 has nucleotide identity of 96% with the *Escherichia* phage KBNP1711 and 85.47% with *Escherichia* phage NJO1. The EP 335 is also homologous to two more *Escherichia* phages and to the vB_EcoP_SU10, a

bacteriophage that can infect a wide range of *E. coli* strains (Mirzaei, Erikson, Kasuga, Haggård- Ljungquist, and Nilsson, 2014). Bacteriophages move between different host species and acquire different genetic material, which possibly, broadens their host range.

To our knowledge, this study is the first to compare the effects of bacteriophage versus organic acid solutions on the reduction of *E. coli* contamination in vacuum and aerobically packaged beef. After a HEP, beef processors usually segregate subprimals corresponding to STEC positive trim combos to be treated with antimicrobials. These subprimals are only allowed to enter the commerce after tests are negative for STEC, otherwise they must be diverted to further processing as positive trimmings. In the United States, LA and PAA are two of the most common organic acids used to decontaminate beef carcasses and subprimals. Peroxyacetic acid is a combination of hydrogen peroxide and acetic acid that when diluted in water is capable to disrupt chemiostic functions of the membrane and inhibit bacterial biochemical metabolism (Baldry & Fraser, 1988; Leaper, 1984; Liberti & Notarnicola, 1999). The mode of action of LA is based on the disruption of cell regulation, which requires the bacterium to use additional amount of energy to maintain its internal optimal pH in order to avoid acid stress damage on cellular mechanisms (Desriac et al., 2013; Foster, 1995).

The efficacy of both organic acids has been reported by several authors, whose results showed inconsistent reductions varying from no reduction to 3.5 Log. Regarding PAA, Penney et al. (2007) evaluated spray applications at 180 ppm on beef and veal carcass surfaces and observed a 3.5 Log reduction of nonpathogenic *E. coli* O157:H7.

Ellebracht et al. (2005) detected no more than 1.2 Log reduction when dipping beef trimmings in PAA solutions at 200, 500, and 1000 ppm. Both studies used only one *E. coli* O157:H7 strain (Penney et al. used strain NZRCC 3614 whereas Ellebracht et al. used a pathogenic rifampicin-resistant strain) and tested the efficiency of PAA using different application methods. Based on results presented in both studies, it was expected to observe optimal reduction when samples were submerged in PAA solutions due to a better distribution of the antimicrobial on trimmings. Conversely, Penney et al. observed lower counts of *E. coli* counts when compared to results presented by Ellebracht et al. In addition, King et al. (2005), using the same strain as used by Ellebracht et al., did not observe a significant reduction of *E. coli* when PAA was applied on contaminated carcass surfaces.

For years, LA has been widely used in the United States to decontaminate beef carcasses and subprimals (Fouladkhah, Geonaras, Yang & Sofos, 2013). Bosilevac, Nou, Barkocy-Gallagher, Arthur and Koohmaraie (2006) showed a 1 Log reduction of *Enterobacteriaceae* counts when applying a solution of 2% LA at 42°C on carcasses. Castillo et al. (2001) tested the efficiency of LA on the same *E. coli* strain used by Ellebracht et al. and showed that a solution of 4% led to a 2-log reduction when a post chill spray was applied onto outside rounds. In addition, Pittman et al. (2012) tested the effectiveness of LA on five strains of STEC O157:H7 and achieved a 1.6 Log reduction of bacterial loads when applying LA at 5% under vacuum conditions with lysing time of 24 h. and in the same study, an additional 1.2 Log reduction was observed after a second application of LA after beef samples were removed from vacuum. However, Uytendaele

et al. (2001) showed no reduction of two (n=2) different *E. coli* strains (an acid resistant - II/45/4, and an acid sensitive - IX/8/16) when LA at 2% was applied onto fresh beef surface.

In our study, when simulating a rework procedure of subprimals after a HEP (treating with antimicrobials and re-vacuum packaging the meat), PAA did not significantly reduce bacteria loads (for 30 min, CI=2.07^{A} vs PAA=1.80^{A}; and for 6 h, CI=2.37^{A} vs PAA=2.30^{A} Log CFU/cm^2). No reduction was also observed when samples were treated with LA for 30 min under vacuum conditions (CI=2.07^{A} vs LA=$2.12^{A\ Log}$ CFU/cm^2). However, when lysing time was extended to 6 h, LA led to a significant 0.65 Log CFU/cm^2 reduction in *E. coli* counts. Overall, *E. coli* O157:H7 strains have been reported to be able to self-adapt in acid conditions, including two strains tested in this research (Tsai & Ingham, 1997). Although Pittman et al. (2012) showed a large and significant reduction (1.6 Log) of one strain (ATCC 43895) combined with other strains in a cocktail, our results showed a small reduction when compared to bacteriophage applications. When the bacteriophage solution was applied, optimal *E. coli* reductions were observed when compared to both LA and PAA (for 30 min, CI=2.07^{A} vs BA=1.00^{B}; and for 6 h, CI=2.37^{A} vs BA=$0.96^{C)}$.

Under aerobic conditions, lysing time did not affect *E. coli* counts. Although both organic acids significantly lowered contamination, the lowest counts were observed when beef was treated with PGE (CI=2.70^{A}, LA=2.23^{B}, PAA=2.17^{B}, and BA=1.29^{C}). Better activity of organic acids under aerobic conditions when compared to vacuum may be associated to different bacterial metabolisms. In vacuum conditions, *E. coli* metabolism

possibly shifts from aerobic to anaerobic due to low availability or absence of O_2 (Unden, Becker, Bongaerts, Schirawski & Six, 1994). Although vacuum packaging does not remove all O_2 from the package (Kelly, Cruz-Romero, Kerry & Papkovsky, 2018), *E. coli* strains possibly adopt a less effective metabolism alternating from aerobic to anaerobic when growing in low O_2 conditions as indicative of being a facultative anaerobe. It is known that anaerobic metabolism and fermentation is less efficient than aerobic metabolism (Wulffen, RecogNice-Team, Sawodny & Feuer, 2016), therefore, under aerobic conditions (NV), bacteria express a higher metabolic rate. As previously mentioned, the effects of both organic acids used in this research are directly dependent on bacterial metabolism, which in optimal aerobic conditions, could lead to a better bacterial load reduction when compared to the absence of antimicrobial effects observed in vacuum-packaged samples.

Optimal PGE effects on *E. coli* loads both under vacuum and in aerobic conditions were possibly achieved due to its mode of action. Lytic *caudovirales* bacteriophages attach and inject viral DNA into bacteria, hijacking the host replication and translation mechanisms to produce phage parts, which assembled into capsids with genetic material and released from the infected cell through the activity of endolysins, peptidoglycan hydrolases the cell wall at the end of the cycle (Salmond & Fineran, 2015). Although bacteriophage applications against STEC in vacuum conditions has yet to be discussed in scientific reports, results of this research suggest that bacteriophage activity seems to be more effective than organic acids, even when *E. coli* adopts a less efficient metabolism when low O_2 concentrations are available. When compared to

organic acids, bacteriophage also depends on bacterial metabolism to perform its lytic cycle. The bacteriophage's better efficiency in lysing host cells is possibly attributed to independent molecular mechanisms that initiate viral DNA replication. Weigel and Seitz (2005) suggest that *E. coli* bacteriophages are able to regenerate closed ends of bacterial DNA to generate the phage genome and consequently produce phage structures. Previous research has suggested that *E. coli* strains are still able to replicate during cold storage under vacuum atmosphere (Dykes, Moorhead & Roberts 2001). Therefore, even with low metabolic rates, the active and independent bacteriophage mode of action is still able to induce lysis of the host, differently than organic acids, which possibly depend on higher bacterial metabolic rates to induce bacteria cell death.

The efficacy of bacteriophage applications in lowering *Enterobacteriaceae* in meats has been demonstrated by several authors in different conditions (Duc, Son, Honjoh & Miyamoto, 2018; Hudson et al., 2013; Hungaro, Mendonça, Gouvêa, Vanetti & Pinto, 2013; Liu et al., 2015; Stratakos & Grant, 2018; Sukumaran, Nannapanenni, Kiess & Sharma, 2015; Thung et al., 2017; Tomat, Casabonne, Aquili, Balagué & Quiberoni, 2018; Yeh et al., 2017; Yeh, de Moura, Van Den Broek & de Mello, 2018; Zinno, Devirgillis, Ercolini, Ongeng & Mauriello 2014), but to our knowledge, no studies evaluating the effects of bacteriophage activity on STEC under vacuum conditions have been published. Results of this research suggest that bacteriophage applications targeting STEC contamination in beef is more efficient either under aerobic or when meat is vacuum packaged compared to commonly used organic acids.

Conclusion

Bacteriophage applications lead to an optimal reduction of *E. coli* O157:H7 *(including stx 1 and stx2 gene-positives)* in contaminated beef when compared to organic acid applications. Latic and peroxyacetic acids did not reduce *E. coli* counts under vacuum conditions and minimally decreased contamination under aerobic atmosphere. Bacteriophage applications are more effective than organic acids when decontaminating beef under aerobic conditions as it is performed during subprimal and trimming fabrication for example. When reworking subprimals associated to STEC-positive trimmings after a High Event Period, beef packing plants must consider using bacteriophage solutions to eliminate possible *E. coli* contamination in vacuum-packaged beef.

References

Agilent. (2019). *E. coli* Cell Culture Concentration from OD_{600} Calculator. Available at: https://www.chem.agilent.com/store/biocalculators/calcODBacterial.jsp

Anderson, M. E., & Marshall, R. T. (1989). Interaction of Concentration and Temperature of Acetic Acid Solution on Reduction of Various Species of Microorganisms on Beef Surfaces. *Journal of Food Protection*, *52*(5), 312–315. https://doi.org/10.4315/0362-028x-52.5.312

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., King, D. A., Shackelford, S. D., … Koohmaraie, M. (2008). Source tracking of Escherichia coli O157:H7 and Salmonella contamination in the lairage environment at commercial U.S. beef processing plants and identification of an effective intervention. *Journal of Food Protection*, *71*(9), 1752–1760. https://doi.org/10.4315/0362-028X-71.9.1752

ATCC. (2015). ATCC Bacterial Culture Guide. Retrieved from http://www.scribd.com/doc/118917227/ATCC-Bacterial-Culture-Guide

Baldry M.G.C. & Fraser J.A.L. (1988). Disinfection with peroxygens. In: Industrial Biocides (ed. by K.R. Payne), 91–116. John Wiley & Sons, Inc., New York.

Barkocy-Gallagher, G. A., Arthur, T. M., Rivera-Betancourt, M., Nou, X., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2003). Seasonal Prevalence of Shiga Toxin-Producing Escherichia coli, Including O157:H7 and Non-O157 Serotypes, and Salmonella in Commercial Beef Processing Plants. *Journal of Food Protection*, *66*(11), 1978–1986. https://doi.org/10.4315/0362-028X-66.11.1978

Bell, R. G. (1997). Distribution and sources of microbial contamination on beef carcasses. *Journal of Applied Microbiology*, *82*, 292–300. https://doi.org/10.1046/j.1365-2672.1997.00356.x

Bosilevac, J. M., Nou, X., Barkocy-Gallagher, G. A., Arthur, T. M., & Koohmaraie, M. (2006). Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and Enterobacteriaceae and reduce the prevalence of Escherichia coli O157:H7 on preevisceration beef carcasses. *Journal of Food Protection*, *69*(8), 1808–1813. https://doi.org/10.4315/0362-028X-69.8.1808

Brashears, M. M., & Chaves, B. D. (2017). The diversity of beef safety: A global reason to strengthen our current systems. *Meat Science*, *132*, 59–71. https://doi.org/10.1016/j.meatsci.2017.03.015

Brichta-Harhay, D. M., Guerini, M. N., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., … Koohmaraie, M. (2008). Salmonella and Escherichia coli O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: An evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Applied and Environmental Microbiology*, *74*(20), 6289–6297. https://doi.org/10.1128/AEM.00700-08

Byrne, C. M., Bolton, D. J., Sheridan, J. J., McDowell, D. A., & Blair, I. S. (2000). The effects of preslaughter washing on the reduction of Escherichia coli O157:H7 transfer from cattle hides to carcasses during slaughter. *Letters in Applied Microbiology*, *30*, 142–145. https://doi.org/10.1046/j.1472-765x.2000.00689.x

Castillo, A., Lucia, L. M., Roberson, D. B., Stevenson, T. H., Mercado, I., & Acuff, G. R. (2001). Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef. *Journal of Food Protection*, *64*(1), 58–62. https://doi.org/10.4315/0362-028X-64.1.58

Desriac, N., Broussolle, V., Postollec, F., Mathot, A. G., Sohier, D., Coroller, L., & Leguerinel, I. (2013). Bacillus cereus cell response upon exposure to acid environment: Toward the identification of potential biomarkers. *Frontiers in Microbiology*, *4*(OCT), 1–13. https://doi.org/10.3389/fmicb.2013.00284

Dickson, J. S., & Kunduru, M. R. (1995). Resistance of acid-adapted salmonellae to organic acid rinses on beef. *Journal of Food Protection*, *58*(9), 973–976. https://doi.org/10.4315/0362-028X-58.9.973

Duc, H. M., Son, H. M., Honjoh, K. ichi, & Miyamoto, T. (2018). Isolation and application of bacteriophages to reduce Salmonella contamination in raw chicken meat. *LWT - Food Science and Technology*, *91*, 353–360. https://doi.org/10.1016/j.lwt.2018.01.072

Dykes, G. A., Moorhead, S. M., & Roberts, S. L. (2001). Survival of Escherichia coli O157:H7 and Salmonella on chill-stored vacuum or carbon dioxide packaged primal beef cuts. *International Journal of Food Microbiology*, *64*, 401–405. https://doi.org/10.1016/S0168-1605(00)00482-7

Ellebracht, J. W., King, D. A., Castillo, A., Lucia, L. M., Acuff, G. R., Harris, K. B., & Savell, J. W. (2005). Evaluation of peroxyacetic acid as a potential pre-grinding treatment for control of Escherichia coli O157:H7 and Salmonella Typhimurium on beef trimmings. *Meat Science*, *70*, 197–203. https://doi.org/10.1016/j.meatsci.2005.01.003

Foster, J. (1995). Low ph adaptation and the acid tolerance response of Salmonella-typhimurium. *Critical Reviews in Microbiology*, *21*(4), 215-237.

Fouladkhah, A., Geornaras, I., Yang, H., & Sofos, J. N. (2013). Lactic acid resistance of Shiga toxin-producing Escherichia coli and multidrug-resistant and susceptible Salmonella Typhimurium and Salmonella Newport in meat homogenate. *Food Microbiology*, *36*, 260–266. https://doi.org/10.1016/j.fm.2013.06.005

USDA-FSIS. (2015). United States Depatment of Agriculture - Food Safety Inspection Service, Compliance Guideline for Establishments Sampling Beef Trimmings for Shiga Toxin-Producing Escherichia coli (STEC) Organisms or Virulence Markers, August 2014. Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/e0f06d97-9026-4e1e-a0c2-1ac60b836fa6/Compliance-Guide-Est-Sampling-STEC.pdf?MOD=AJPERES

Hudson, J. A., Billington, C., Cornelius, A. J., Wilson, T., On, S. L. W., Premaratne, A., & King, N. J. (2013). Use of a bacteriophage to inactivate Escherichia coli O157: H7 on beef. *Food Microbiology*, *36*, 14–21. https://doi.org/10.1016/j.fm.2013.03.006

Hungaro, H. M., Mendonça, R. C. S., Gouvêa, D. M., Vanetti, M. C. D., & Pinto, C. L. de O. (2013). Use of bacteriophages to reduce Salmonella in chicken skin in comparison with chemical agents. *Food Research International*, *52*, 75–81. https://doi.org/10.1016/j.foodres.2013.02.032

Kelly, C. A., Cruz-Romero, M., Kerry, J. P., & Papkovsky, D. P. (2018). Assessment of performance of the industrial process of bulk vacuum packaging of raw meat with nondestructive optical oxygen sensing systems. *Sensors (Switzerland)*, *18*(5). https://doi.org/10.3390/s18051395

King, D. A., Lucia, L. M., Castillo, A., Acuff, G. R., Harris, K. B., & Savell, J. W. (2005). Evaluation of peroxyacetic acid as a post-chilling intervention for control of Escherichia coli O157:H7 and Salmonella Typhimurium on beef carcass surfaces. *Meat Science*, *69*(3), 401–407. https://doi.org/10.1016/j.meatsci.2004.08.010

Lagunas, D. P. (1992). *Anestesia en el paciente séptico con distrés respiratorio*. 47–60.

Leaper, S. (1984). Influence of temperature on the synergistic sporicidal effect of peracetic acid plus hydrogen peroxide on Bacillus subtilis SA22 (NCA 72-52). *Food Microbiology*, *1*(3), 199–203. https://doi.org/10.1016/0740-0020(84)90034-0

Liberti, L., Notarnicola, M., & Lopez, A. (1999). Advanced treatment for municipal wastewater reuse in agriculture. *Water Science and Technology*, *40*(4–5), 235–245. https://doi.org/10.1080/01919510008547217

Liu, H., Niu, Y. D., Meng, R., Wang, J., Li, J., Johnson, R. P., … Stanford, K. (2015). Control of Escherichia coli O157 on beef at 37, 22 and 4°C by T5-, T1-, T4-and O1-like bacteriophages. *Food Microbiology*, *51*, 69–73. https://doi.org/10.1016/j.fm.2015.05.001

Maciejewska, B., Olszak, T., & Drulis-Kawa, Z. (2018). Applications of bacteriophages versus phage enzymes to combat and cure bacterial infections: an ambitious and also a realistic application? *Applied Microbiology and Biotechnology*, *102*(6), 2563–2581. https://doi.org/10.1007/s00253-018-8811-1

Mataragas, M., Skandamis, P. N., & Drosinos, E. H. (2008). Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations. *International Journal of Food Microbiology*, *126*, 1–12. https://doi.org/10.1016/j.ijfoodmicro.2008.05.014

McEvoy, J. M., Doherty, A. M., Finnerty, M., Sheridan, J. J., McGuire, L., Blair, I. S., … Harrington, D. (2000). The relationship between hide cleanliness and bacterial numbers on beef carcasses at a commercial abattoir. *Letters in Applied Microbiology*, *30*(5), 390–395. https://doi.org/10.1046/j.1472-765x.2000.00739.x

Mirzaei, M. K., Eriksson, H., Kasuga, K., Haggård-Ljungquist, E., & Nilsson, A. S. (2014). Genomic, proteomic, morphological, and phylogenetic analyses of vB-EcoP-SU10, a podoviridae phage with C3 morphology. *PLoS ONE*, *9*(12), 1–19. https://doi.org/10.1371/journal.pone.0116294

Nou, X., Rivera-Betancourt, M., Bosilevac, J. M., Wheeler, T. L., Shackelford, S. D., Gwartney, B. L., … Koohmaraie, M. (2003). Effect of Chemical Dehairing on the Prevalence of Escherichia coli O157:H7 and the Levels of Aerobic Bacteria and Enterobacteriaceae on Carcasses in a Commercial Beef Processing Plant. *Journal of Food Protection*, *66*(11), 2005–2009. https://doi.org/10.4315/0362-028X-66.11.2005

Penney, N., Bigwood, T., Barea, H., Pulford, D., LeRoux, G., Cook, R., … Brightwell, G. (2007). Efficacy of a peroxyacetic acid formulation as an antimicrobial intervention to reduce levels of inoculated Escherichia coli O157:H7 on external carcass surface of hot-boned beef and

veal. *Journal of Food Protection*, *70*(1), 200–203. https://doi.org/10.4315/0362-028X-70.1.200

Pittman, C. I., Geornaras, I., Woerner, D. R., Nightingale, K. K., Sozfos, J. N., Goozdridge, L., & Belk, K. E. (2012). Evaluation of lactic acid as an initial and secondary subprimal intervention for Escherichia coli O157:H7, non-O157 Shiga toxin-producing E. coli, and a nonpathogenic E. coli surrogate for E. coli O157:H7. *Journal of Food Protection*, *75*(9), 1701–1708. https://doi.org/10.4315/0362-028X.JFP-11-520

Salmond, G. P. C., & Fineran, P. C. (2015). A century of the phage: Past, present and future. *Nature Reviews Microbiology*, *13*(12), 777–786. https://doi.org/10.1038/nrmicro3564

Smulders, F. J. M., & Greer, G. G. (1998). Integrating microbial decontamination with organic acids in HACCP programmes for muscle foods: Prospects and controversies. *International Journal of Food Microbiology*, *44*(3), 149–169. https://doi.org/10.1016/S0168-1605(98)00123-8

Stratakos, A. C., & Grant, I. R. (2018). Evaluation of the efficacy of multiple physical, biological and natural antimicrobial interventions for control of pathogenic Escherichia coli on beef. *Food Microbiology*, *76*(May), 209–218. https://doi.org/10.1016/j.fm.2018.05.011

Sukumaran, A. T., Nannapaneni, R., Kiess, A., & Sharma, C. S. (2015). Reduction of Salmonella on chicken meat and chicken skin by combined or sequential application of lytic bacteriophage with chemical antimicrobials. *International Journal of Food Microbiology*, *207*, 8–15. https://doi.org/10.1016/j.ijfoodmicro.2015.04.025

Thung, T. Y., Krishanthi Jayarukshi Kumari Premarathne, J. M., San Chang, W., Loo, Y. Y., Chin, Y. Z., Kuan, C. H., … Radu, S. (2017). Use of a lytic bacteriophage to control Salmonella Enteritidis in retail food. *LWT - Food Science and Technology*, *78*, 222–225. https://doi.org/10.1016/j.lwt.2016.12.044

Tomat, D., Casabonne, C., Aquili, V., Balagué, C., & Quiberoni, A. (2018). Evaluation of a novel cocktail of six lytic bacteriophages against Shiga toxin-producing Escherichia coli in broth, milk and meat. *Food Microbiology*, *76*(December 2017), 434–442. https://doi.org/10.1016/j.fm.2018.07.006

Tsai, Y. W., & Ingham, S. C. (1997). Survival of Escherichia coil O157:H7 and Salmonella spp. in Acidic condiments. *Journal of Food Protection*, *60*(7), 751–755. https://doi.org/10.4315/0362-028X-60.7.751

Unden, G., Becker, S., Bongaerts, J., Schirawski, J., & Six, S. (1994). Oxygen regulated gene expression in facultatively anaerobic bacteria. *Antonie van Leeuwenhoek*, *66*, 3–22. https://doi.org/10.1007/BF00871629

USDA-AMS. (2014). United States Depatment of Agriculture - Agricultural Marketing Service, Institutional Meat Purchase Specifications: Fresh Beef Series 100. Retrieved from https://www.ams.usda.gov/sites/default/files/media/IMPS_100_Fresh_Beef[1].pdf%5Cnhttp://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3003283

Uyttendaele, M., Jozwik, E., Tutenel, A., De Zutter, L., Uradzinski, J., Pierard, D., & Debevere, J. (2001). Effect of acid resistance of Escherichia coli O157:H7 on efficacy of buffered lactic

acid to decontaminate chilled beef tissue and effect of modified atmosphere packaging on survival of Escherichia Coli O157:H7 on red meat. *Journal of Food Protection*, *64*(11), 1661–1666. https://doi.org/10.4315/0362-028X-64.11.1661

van Mirlo, J., Hagens, S., Witte, S., Klamert, S., & Fieseler, L. (2019). Complete Genome Sequences of Escherichia coli Phages vB_EcoM-EP75 and vB_EcoP-EP335. *Microbiology Resource Announcements*, *8*(16), e00078-19. https://doi.org/https://doi.org/10.1128/MRA.00078-19

Von Wulffen, J., Sawodny, O., Takors, R., Löffler, M., Simen, J., Ederer, M., … Feuer, R. (2016). Transition of an anaerobic Escherichia coli culture to aerobiosis: Balancing mRNA and protein levels in a demand-directed dynamic flux balance analysis. *PLoS ONE*, *11*(7), 1–17. https://doi.org/10.1371/journal.pone.0158711

Weigel, C., & Seitz, H. (2006). Bacteriophage replication modules. *FEMS Microbiology Reviews*, *30*(3), 321–381. https://doi.org/10.1111/j.1574-6976.2006.00015.x

Wheeler, T. L., Kalchayanand, N., & Bosilevac, J. M. (2014). Pre- and post-harvest interventions to reduce pathogen contamination in the U.S. beef industry. *Meat Science*, *98*, 372–382. https://doi.org/10.1016/j.meatsci.2014.06.026

Wheeler, Tommy L., & Arthur, T. M. (2018). Novel Continuous and Manual Sampling Methods for Beef Trim Microbiological Testing. *Journal of Food Protection*, *81*(10), 1605–1613. https://doi.org/10.4315/0362-028x.jfp-18-197

Yang, X., He, A., Badoni, M., Tran, F., & Wang, H. (2017). Mapping sources of contamination of Escherichia coli on beef in the fabrication facility of a commercial beef packing plant. *Food Control*, *75*, 153–159. https://doi.org/10.1016/j.foodcont.2016.12.004

Yeh, Y., de Moura, F. H., Van Den Broek, K., & de Mello, A. S. (2018). Effect of ultraviolet light, organic acids, and bacteriophage on Salmonella populations in ground beef. *Meat Science*, *139*, 44–48. https://doi.org/10.1016/j.meatsci.2018.01.007

Yeh, Y., Purushothaman, P., Gupta, N., Ragnone, M., Verma, S. C., & de Mello, A. S. (2017). Bacteriophage application on red meats and poultry: Effects on Salmonella population in final ground products. *Meat Science*, *127*, 30–34. https://doi.org/10.1016/j.meatsci.2017.01.001

Zinno, P., Devirgiliis, C., Ercolini, D., Ongeng, D., & Mauriello, G. (2014). Bacteriophage P22 to challenge Salmonella in foods. *International Journal of Food Microbiology*, *191*, 69–74. https://doi.org/10.1016/j.ijfoodmicro.2014.08.037

Table 1. Killing efficacy of PhageGuard E™ (Bacteriophages EP75 and EP335) for four *E. coli* O157:H7 strains.

Strain	Bacteriophage application	Average CFU (4 plates)	Killing efficiency (%)
ATCC® 35150™	[a]Control	83.5	98.2
	2 × 1011 PFU/ml	1.5	
ATCC® 43895™	Control	181	97.2
	2 × 1011 PFU/ml	5	
ATCC® 43894™	Control	407	96.7
	2 × 1011 PFU/ml	13.5	
NCTC 13128	Control	103.5	98.3
	2 × 1011 PFU/ml	1.75	

[a] No bacteriophage applied

Table 2. Effects of the application of organic acids and bacteriophage solutions under vacuum (V) and aerobic conditions (non-vacuum, NV) on beef contaminated with 4 *E. coli* strains (ATCC® 35,150™, ATCC® 43,895™, ATCC® 43,894™, and NCTC 13128). Log CFU/cm^2.

		Antimicrobial treatment∗				
Nested model	Treatment time	CI	LA	PAA	PGE	Average∗∗∗
Vacuum (V)	30 min	2.07A	2.12Aa	1.80Ab	1.00B	1.75
	6 hr	2.37A	1.72Bb	2.30Aa	0.96C	1.84
	Average***	2.23A	1.92AB	2.06B	0.68C	SEM = 0.13
Non-vacuum (NV)	30 min	2.68	2.17	2.14	1.26	2.07
	6 hr	2.71	2.29	2.21	1.31	2.13
	Average***	2.70A	2.23B	2.17B	1.29C	SEM = 0.11

A,B,C Means in the same row having different superscripts are significant at $P = .0061$ for V and $P < .0001$ for NV. a,b Means in the same column within V having different superscripts are significant at $P < .0061$. SEM = Standard error of the mean.

∗ CI = Control Inoculated, LA = Lactic acid at 5%, PAA = Peroxyacetic acid at 400 ppm, and PGE = PhageGuard E™ at 1 × 108 PFU/ml.

∗∗ Average of means within treatment time ($P = .432$).

∗∗∗ Average of means within antimicrobial treatment ($P < .0001$).

Appendix V. Measuring Optical Density (OD_{600}) and diluting inoculum

1. Turn on the spectrophotometer and set the wavelength to measure 600 nm (OD_{600}).
2. Place a cuvette with LB broth in the placeholder, close the lid, and zero out the absorbance. This will be the baseline for the inoculum.
3. Insert a cuvette containing the overnight inoculum, shut the lid, and record the absorbance.
4. Convert the OD_{600} absorbance into CFU/mL (OD of 1.0 = 8 x 10^8 CFU/mL).

a. Chem.agilent *E. coli* cell calculator is available online

5. Calculate the dilution from the measured overnight culture to the desired concentration (CFU/mL) using $C_1V_1=C_2V_2$.
6. Using sterile BPW, dilute the bacterial inoculum.

CHAPTER THREE

Effects of MS Bacteriophages, Ultraviolet light, and Organic Acid Applications on Beef Surface Contaminated with STEC O157:H7 and the 'Big Six' Serotypes after a Simulated High Event Period Scenario

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Abstract

A high event period (HEP) occurs when beef processing facilities experience an elevated rate of Shiga Toxin-producing *E. coli* (STEC) positive trim samples. In order to avoid trim-associated vacuum-packaged primals and subprimals entering into commerce, beef that may be contaminated are treated with antimicrobials, re-packaged, and re-tested for STEC to ensure product wholesomeness. In this study, we evaluated the efficacy of Bacteriophages (P), Peroxiacetic Acid (PAA), Acidified Sodium Chlorite (ASC), and Ultraviolet light (UV) to reduce STEC O157:H7 and the Big Six populations in beef, after a simulated HEP scenario. In vacuum conditions, phage applications led to the greatest STEC reduction ($P<0.0001$). Under aerobic conditions, a combination of P + UV led to the lowest STEC loads ($P<0.0001$). Overall, treatments including bacteriophage provided best reductions when compared to non-phage treatments ($P<0.0001$ and $P<0.0001$, under vacuum and aerobic conditions, respectively). Bacteriophage solutions provided improved control of STEC O157:H7 and the Big Six serotypes when reworking product after a simulated HEP scenario.

Keywords: Acidified sodium chlorite, Bacteriophage, E. coli O157:H7, high event period, PAA ,STEC

Introduction

In the United States, the Food Safety and Inspection Service (FSIS) declared *E. coli* serotype O157:H7 adulterant in raw non-intact beef products in 1994. In 2012, six more serotypes (O26, O45, O103, O111, O121, O145) known as the "Big Six" were also added in the adulterant list (USDA-FSIS, 2012b). Regulatory guidelines mandate sampling of beef trim products and that no STEC positive raw beef products can be sold into commerce (USDA-FSIS, 2012a, 2014). The implementation of in-plant applications has demonstrated to be efficacious in decontaminating carcasses and reducing the presence of foodborne pathogens (Carlson et al., 2008; Castillo, Lucia, Goodson, Savell, & Acuff, 1998, 1999; Castillo, Lucia, Kemp, & Acuff, 1999; Castillo et al., 2001; Harris, Brashears, Garmyn, Brooks, & Miller, 2012; Harris, Miller, Loneragan, & Brashears, 2006; Marriott, Schilling, & Gravani, 2018a; Wheeler, Kalchayanand, & Bosilevac, 2014). However, studies have demonstrated that commonly used antimicrobial interventions are have decreased efficacy due to the development of acid resistant bacteria (Berry & Cutter, 2000; Dickson & Kunduru, 1995; Garren et al., 1998; Lin et al., 1996; Stopforth, Skandamis, Geornaras, & Sofos, 2007; Stopforth et al., 2004; Youssef, Yang, Badoni, & Gill, 2013). When a beef processing facility experiences a greater than 5% rate of STEC positive beef trim samples in a single time period, it is known as a high event period (HEP) (USDA-FSIS, 2014). The occurrence of a HEP indicates either a localized, out-of-control event or a systemic breakdown of preventative slaughter procedures that has led to contamination of beef products including primals, subprimals, and trimmings. The FSIS recommends to rework of

primals (Chuck, Brisket, Shank, Rib, Short Plate, Loin, Flank, Round) and subprimals (cuts derived from primals) by applying additional antimicrobial treatments to ensure no adulterated product is released into commerce (USDA-FSIS, 2014). Reworking procedures of subprimals occurs by first removing them from vacuum packaging and treating them with antimicrobials before repackaging. Subsequently, an aliquot of all repackaged products is tested for STEC, relying on antimicrobial effects under vacuum conditions. This process has been estimated to take approximately 30 minutes to 6 hours to complete depending on processing speed and production volume (Shebs, Lukov, Giotto, Torres, & de Mello, 2020).

In the United States, organic acid solutions and hot water are the most commonly used antimicrobial interventions in harvesting and processing plants. Acidic solutions such as Peroxyacetic acid disrupt chemiosmotic functions and interfere with the host cell membrane thereby affecting cell biochemical regulation and energy metabolism (Baldry & Fraser, 1988; Leaper, 1984; Liberti & Notarnicola, 1999). The efficacy of these chemical interventions has been demonstrated with high reductions (Harris et al., 2006; Laury et al., 2009; Zhao, Zhao, & Doyle, 2009), but also has been under scrutiny with decreased efficacy against foodborne pathogens (Bosilevac, Nou, Barkocy-Gallagher, Arthur, & Koohmaraie, 2006; King et al., 2005; Yeh, de Moura, Van Den Broek, & de Mello, 2018). These interventions are advantageous due to their cost, availability, and simple application (Mataragas et al., 2008). However, their potential decrease in

efficacy has been demonstrated with the development of acid resistant bacteria due to its exposure to sub-lethal applications (Bearson, Bearson, & Foster, 1997; Brown, Ross, McMeekin, & Nichols, 1997; Capita, Riesco-Peláez, Alonso-Hernando, & Alonso-Calleja, 2014; Cheng, Yu, & Chou, 2003; Foster & Hall, 1990; Goodson & Rowbury, 1989; Leyer, Wang, & Johnson, 1995; Wesche, Gurtler, Marks, & Ryser, 2009). Although organic acids are commonly applied during beef during processing, there are still occurrences of STEC outbreaks demonstrating a need for improved interventions. In this study, we evaluated individual and combined effects of two commonly used organic acids (Peroxyacetic acid and Acidified Sodium Chlorite), UV light, and a 7-phage novel bacteriophage cocktail on beef contaminated with STEC O157:H7 and the "Big Six" in both aerobic and vacuum conditions, simulating rework after a HEP.

Materials and methods

E. coli Strains and inoculum preparation

The seven pure STEC strains that were used in this study included the *E. coli* O157:H7 (ATCC® 35150™), O26 (ATCC® BAA-2196™), O45 (ATCC® BAA-2193™), O103 (ATCC® BAA-2215™), O111 (ATCC® BAA-2440™), O121 (ATCC® BAA-2219™), and O145 (ATCC® BAA-2192™). The frozen glycerol stock of each strain was streaked onto 1.6% agar LB agar plates in duplicate and incubated for 24 h at 37°C to ensure cultures are live and viable. A single isolated colony from each strain was transferred into independent sterile tubes containing 10 mL sterile LB broth and incubated at 37°C overnight with shaking (200 rpm). To prepare the inoculum, 1 mL of overnight pure

culture was transferred into 20 mL of LB broth and cultured overnight at 37°C with shaking. Subsequently, absorbance was measured at OD_{600} to calculate concentration (CFU/ml) by using the Agilent *E. coli* cell culture biocalculator (Agilent, 2019). Each culture was diluted to $1x10^8$ CFU/ml with sterile 0.1% Buffered Peptone Water (BPW) and then combined in equal amounts to create the final inoculum.

Phage preparation and stock titer

Stocks of bacteriophages Mello-Shebs 1 (MS1) 026, MS1 045, MS1 103, MS1 111, MS1 121, MS1 145, and MS1 157 were obtained directly from the University of Nevada, Reno - Food Safety Microbiology Laboratory Library.

Phage stock concentration was determined by the double-layer agar methodology described by Adams (1959) and modified by Yeh et al. (2017). In short, 100 µL of 10-fold diluted phage and 100 µL of Log-phase *E. coli* culture was added into 4 mL of molten LB agar (0.6% agar) tempered at 45°C. Solution was mixed by inverting twice then evenly distributed onto hard LB agar (1.6%) plates until solidified. Duplicated plates were incubated at room temperature overnight for plaque enumeration. Concentration of phage solutions were determined to be 10^8 PFU/mL.

Sample preparation and treatment design

Fresh beef rose meat (m. *Cutaneous trunci,* IMPS 194) (USDA-AMS, 2014) was obtained from a USDA inspected facility and transported under refrigeration (4°C) to the University of Nevada, Reno's Meat Quality Laboratory. The whole batch was screened for generic *E. coli* using 3M™ Petrifilm™ *E. coli*/Coliform count plates. Meat samples were fabricated into 10x10 cm^2 (100 cm^2) pieces (n=154) and randomly assigned to a Completely Randomized Design using the following model: $Y_{ij}=\mu + \alpha_i + \varepsilon_{ij}$. Y_{ij} was STEC count, μ was the grand mean across the treatments, and α_i was the effect of treatment from the grand mean specific to the 11 treatment levels. Model errors (ε_{ij}) were assumed to be random and independent. Treatments included: Control treated with BPW (C), Peroxyacetic acid (PAA, 400 ppm), Acidified Sodium Chlorite (ASC, 1200 ppm), Ultraviolet light - C (UV, 254 nm, 23°C for 30 s), a cocktail of all 7 MS1 Bacteriophages (P, 10^8 PFU/ml), PAA+ASC, PAA+UV, ASC+UV, P+PAA, P+ASC, and P+UV. The experiment was conducted separately treating samples under vacuum (n=77) and aerobic conditions (n=77).

Sample inoculation, antimicrobial applications, and bacterial enumeration

Beef samples were inoculated with a cocktail containing all seven STEC strains. The concentration of the inoculum was 1×10^8 CFU/ml, to yield approximately 3 Log CFU/cm^2. All samples were kept under refrigeration prior to inoculation and during the trial (5 ± 2°C). A volume of 500 µL of the inoculum was uniformly pipetted on meat and distributed throughout the whole surface area of the sample using a sterile plastic rod, samples were then wrapped with oxygen permeable film (aerobic conditions) or vacuum

packaged (model VMC10OP, VacPak-It, Lititz, PA, United States) and stored at 5°C for 30 min for bacterial attachment.

Subsequently, samples were removed from packages and treated with 500 µL of the antimicrobial solutions. Ultraviolet light (UV) was applied 2.5 cm above the sample surface by using a wand containing a single emitter. For combined treatments, UV was always applied first than PAA, ASC, and P. For treatments containing P, PAA and ASC were applied first. After treatments were applied, samples were re-wrapped or re-vacuumed. Re-vacuumed samples were used to simulate rework of contaminated subprimals after a HEP. Samples were then kept under refrigeration (5°C). After one hour, samples were removed from their package and swabbed (QS1000, Hygiena, Camarillo, CA, United States) for bacteria enumeration. Swabs were homogenized and the homogenate was vortexed, serially diluted, and spread-plated onto LB agar plates in duplicate. Plates were incubated inverted overnight at 37 °C, and *E. coli* colonies were enumerated (CFU/cm^2). In order to simulate an industry scenario, sample homogenates were not treated to neutralize possible residual effects of organic acids or phages. Antimicrobial neutralization procedures are not practiced by laboratories that provide service to meat processing facilities.

Color and lipid stability after UV light exposure

In order to evaluate the effects of UV treatment on color and lipid stability of beef samples, a separate trial using a different set of samples was

performed. A total of 10 samples were randomly assigned to a Control (n=5) and to the same UV treatment applied on contaminated samples (n=5). Instrumental color was recorded for L* (lightness; black = 0, white = 100), a* (redness; red = positive values; green = negative values), b* (yellowness; yellow = positive values; blue = negative values), hue angle (hue=arctan (b*/a*)), and chroma values [chroma=(a*2+b*2)1/2] using a Minolta chromameter (CM-700D; Minolta Inc., Osaka, Japan) with an 8 mm-diameter after 0, 1, 12, and 24 h after UV light exposure with sample measurements averaged from three locations on each sample. Lipid stability was measured 24 h after UV light exposure by performing the Thiobarbituric Acid assay described by Buege & Aust (1978) modified by Ahn et al. (1998). Briefly, 5 g of pulverized sample and 14 mL of deionized water and 1 mL of 10% Butylated hydroxyanisole (BHA, 10g of BHA diluted in 90 mL of Alcohol 90% and 5 mL of distilled water) were mixed, homogenized and centrifuged at 200 rpm for 5 min Subsequently, 1 mL of the homogenate was mixed with 2-Thiobarbituric Acid/Trichloroacetic Acid (TBA/TCA) and incubated at 70 °C for 30 min, prior to centrifugation at 2000 rpm for 15 min. Two-hundred (200) µL aliquots used to measure absorbance at 540 nm (Synergy HT, Biotek, USA). Lipid oxidation (TBA values) were expressed as malonaldehyde concentration (mg/Kg) and quantification was determined by comparing samples with solutions of known concentrations of TEP (1,1,3,3-tetraethoxypropane) in distilled water ranging from 0 to 50 nmol/mL.

Statistical analysis

The experiment was replicated 7 times with 1 repetition per treatment (n=154 total e.u., 77 e.u. for aerobic and 77 e.u. for vacuum; n=7 e.u. per fixed effect). Data obtained to evaluate the effects of UV application on color was analyzed as a repeated measures. The smallest Akaike and Bayesian information criteria indicated that Compound Symmetry was the best covariance matrix for color analysis. Data were analyzed using the GLIMMIX procedure of SAS® 9.3 package (SAS Institute, Inc., USA). When significance ($P \leq 0.05$) was detected for the fixed effect, means separations were performed by using the LSMEANS and DIFF functions. For contrasts, significance was detected by using the LSMESTIMATE function statements.

Results

Under vacuum conditions, UV only, PAA+ASC, PAA, and PAA+UV did not decrease STEC loads. Applications of the MS1 bacteriophage cocktail (P) and combined treatments of P+PAA, P+UV, P+ASC, and ASC+UV provided optimal bacteria reduction when compared to UV, C, PAA+ASC, PAA, PAA+UV, and ASC (P<0.0001, Figure 1). Samples treated with ASC+UV showed values statistically similar to treatments that provided the optimal reduction, but overall P only led to the highest numeric reduction (1.04 Log CFU/cm^2). Contrasts were evaluated for: all treatments containing P (P, P + PAA, P+UV, and P+ASC) versus all other treatments (UV, PAA, ASC, PAA+ASC, PAA+UV, and ASC+UV), all treatments containing P versus control (C) and all other treatments versus control (C) (Table

1). Under vacuum, all treatments containing P determined the best reduction when compared to other treatments (P<0.001). As observed in Figure 1, the contrast analysis confirmed that treatments containing P significantly reduced STEC loads when compared to control (P=0.0002), however, when evaluating the effects of all other treatments versus C, no difference was observed (P=0.3395).

Under aerobic conditions, combined treatments of P+PAA and P+UV were significantly more efficient than all other treatments (Figure 2). Overall, P+UV provided the greatest STEC reduction (1.33 Log CFU/cm^2) whereas UV, ASC, and PAA+ASC did not reduce bacteria populations. When looking at the contrasts analyses (Table 1), results for treatments applied in aerobic conditions were similar than results reported for vacuum. Treatments containing P provided improved reduction on STEC loads when compared to all other treatments and control. When comparing the effects of all other treatments against C, no statistical difference was observed (P=0.0781).

The effects of UV on color attributes and lipid stability are shown in Table 2. No detrimental effect of UV light was observed for lipid stability. Treated samples showed lower MDA values when compared to control. Regarding color, no interaction between both fixed effects of time and treatment were observed. However, samples were yellower at 12 h when compared to 0 and 24h (P = 0.0083) and less red (lower a* values) (P = 0.0003) and had lower Chroma values after 24 h (P = 0.0012). Values of Hue angle gradually increased from 0 to 24 h (P < 0.0001). Samples treated with UV were darker (P = 0.0330), redder (P = 0.0011), had a smaller hue angle (P = 0.0003) and higher Chroma values (P = 0.0059).

Discussion

Overall, the 7-phage cocktail and its treatment combinations demonstrated optimal reduction of STEC O157:H7 and the "Big Six" serotypes on beef surface, under both aerobic and vacuum conditions. A bacteriophage cocktail comprising seven phages targeting individual STEC serotypes, to our knowledge has not been reported for food application. We have demonstrated previously that each phage is able to lyse each serotype of STEC in vitro by at least 96.2% (Shebs, Yeh-Parker, Torres, & de Mello, 2019) which can be in part due to the ability of the phages to bind to LPS or OmpC surface receptors that are likely similar among the *E. coli* serotypes or other *Enterobacteriaceae*, enabling them to attach to more than one host species (Montag, Hashemolhosseini, & Henning, 1990; Singh, Singh, Williams, Jones, & Abdullah, 1995; Singh, Upshaw, Abdullah, Singh, & Klebba, 1992; Yu & Mizushima, 1982). Thus, moving between different hosts would allow acquisition of different genetic material through transduction that could widen the phages' host range to be effective against multiple foodborne pathogens and to discourage resistance against phage lysis (Fineran et al., 2009; Oechslin, 2018).

To our knowledge, the use of a phage cocktail solution that targets all seven adulterant STEC serotypes has not been demonstrated beyond six phages. Also, such cocktails have not been compared to commonly used antimicrobial solutions such as PAA or ASC. Processors segregate primals and subprimals that

are associated with STEC positive trim lots. The products are subsequently tested for STEC and are often treated with an antimicrobial such as an organic acid before being repackaged and tested for STEC later to ensure no adulterated products are released into commerce. Products that are not decontaminated must be further processed by being sold to a cooker for a reduced price or recalled which reduces the profitability for the company and industry. In the United States, weak acids and oxidizing agents are commonly used to decontaminate carcasses and subprimals. Peroxyacetic acid (PAA) and acidified sodium chlorite (ASC) are both oxidizing agents that are approved for use on beef, poultry parts, and carcasses while UV-C light has yet to be approved for use on meat and poultry products (USDA-FSIS, 2019). These antimicrobials have been reported to be efficacious against bacterial pathogens (Harris et al., 2006; Laury et al., 2009; Penney et al., 2007; Ransom et al., 2003), while others have found little or inconsistent efficacy (Ellebracht et al., 2005; Gill & Badoni, 2004; King et al., 2005; Shebs, Lukov, Giotto, Torres, & de Mello, 2020; Stivarius, Pohlman, Mcelyea, & Waldroup, 2002; Stopforth et al., 2004; Uyttendaele et al., 2001; Yeh et al., 2018). Weak acids such as lactic acid (LA), acetic acid, or citric acid act by diffusing across the cell membrane and dissociating in the cytoplasm therefore lowering the pH and causing a disruption in the bacterial membrane and inhibition of mechanisms that control pH homeostasis. This disruption causes the bacteria to synthesize acid stress proteins to avoid acid-induced damage and to maintain optimal cytoplasmic pH levels (Bearson, Bearson, & Foster, 1997; Desriac et al., 2013; Foster, 1995; Salmond, Kroll, & Booth, 1984; Nair, Upadhyaya, Amalaradjou, & Venkitanarayanan, 2017). PAA is the combination of acetic

acid and hydrogen peroxide that creates peracetic acid or Peroxyacetic acid in equilibrium. PAA releases active oxygen in the form of superoxide radicals that oxidizes susceptible proteins that disrupt the chemiosmotic functions of membrane transport by disrupting the cell wall (Baldry & Fraser, 1988; Block, 2001; Fraser, Godfree, & Jones, 1984; Liberti & Notarnicola, 1999; Nair et al., 2017) ASC is the combination of sodium chlorite and an acid, most commonly citric acid. This combination produces an active chlorine dioxide or oxy-chlorous compound that can oxidize cell membrane surfaces (Gordon, Kieffer, & Rosenblatt, 1972; Kross, 1984; Thiessen, Usborne, & Orr, 1984; Villarreal, Baker, & Regenstein, 1990). Chlorine dioxide has been reported to have a much better antimicrobial effect compared to both chlorine water and hypochlorous acid (Benarde et al., 1965; Lillard, 1979).

The efficacy of PAA has been evaluated against many pathogens in different foods. PAA (0.01%) has been shown to be effective against *E. coli* after 10 minutes yielding over 5 Log CFU/ml reduction in solution (Briñez, Roig-Sagués, Hernández Herrero, López-Pedemonte, & Guamis, 2006), however it has not demonstrated to be as effective when applied to meat (Purnell, James, James, Howell, & Corry, 2014; Shebs et al., 2020; Stopforth et al., 2004; Yeh et al., 2018). On fresh beef carcass surface, 0.02% PAA resulted in a 3.32 Log CFU/cm^2 reduction of *E. coli* O157:H7 after 10 h of spray chilling, but there was no reduction after 24 or 48 h (Stopforth et al., 2004). On fresh beef surface, immersion in 0.02% ASC or 0.02% PAA reduced a 5-strain *E. coli* cocktail by 1.9

and 1.4 Log CFU/cm^2, respectively, although this was overshadowed by 0.5% cetylpyridinium chloride (CPC) which gave the optimal reduction of 4.8 Log CFU/cm^2 (Ransom et al., 2003). Bauermeister, Bowers, Townsend, & McKee (2008) demonstrated that a minimum PAA concentration of 0.0025% is an effective antimicrobial against *Salmonella typhimurium* on chicken, and higher concentrations of 0.015% and 0.02% showed an extended shelf life but had an off color and off odor. When Alonso-Hernando, Alonso-Calleja, & Capita (2013) compared the use of multiple antimicrobials against multiple pathogens on skinless chicken legs, they observed that ASC (1200 ppm) gave the best *E. coli* reduction (1.94 Log CFU/cm^2) followed by 2% citric acid (1.47 Log CFU/cm^2), while peroxyacids (220 ppm) did not result in any significant reduction. They also observed no significant decrease in *Salmonella enterica Enteritidis* with the use of peroxyacids. Similarly, King et al. (2005) did not observe any reduction in microbial counts when PAA was applied at 600 ppm or below at either 45°C or 55°C, but PAA of 1000 ppm reduced both *E. coli* O157:H7 and *Salmonella typhimurium* by up to 1.7 and 1.3 Log CFU/cm^2, respectively. However, these reductions were overshadowed by 4% lactic acid (2.7 and 3.4 Log_{10} CFU/ cm^2, respectively). When tested in ground beef, PAA (0.02%) yielded the lowest *E. coli* counts after seven days of retail display, while ASC (0.1%) resulted in the lowest APC counts (Pohlman et al., 2007). PAA (180 ppm) has also been demonstrated by Brightwell, Clemens, Adam, Urlich, & Boerema (2009) to be efficacious against vacuum packaged beef microflora, extending the shelf life. In our study, we did not observe a significant ($P > 0.05$) difference between samples treated with PAA and control samples under vacuum conditions, but we did observe a

significant ($P < 0.05$) reduction under aerobic conditions. Under vacuum conditions, the only significant reduction by PAA was in combination with bacteriophage (1.09 Log CFU/cm^2). Under aerobic conditions, PAA applied alone yielded a significant 0.48 Log CFU/cm^2 reduction and when combined with phage yielded a significant 1.07 Log CFU/cm^2 reduction.

Citric acid activated acidified sodium chlorite (ASC) has been well reported as an antimicrobial used against *E. coli* and *Salmonella* on meat with varying effectiveness. Ransom et al. (2003) immersed fresh beef contaminated with an *E. coli* O157:H7 cocktail into 0.02% ASC for 30 seconds and observed a significant 1.9 Log CFU/cm^2 reduction. Similarly, Kalchayanand et al. (2012) observed significant ($P < 0.05$) reductions of an STEC cocktail on fresh beef flanks when they sprayed 1000 ppm ASC (0.4-2.0 Log CFU/cm^2), but hot water (85°C) gave greater reductions (2.4-4.2 Log CFU/cm^2). Stopforth et al. (2004) applied ASC (0.12%) onto pre-chilled carcasses against acid habituated and non-acid habituated *E. coli* O157:H7 cells and observed a 2.1 and 2.4 Log CFU/cm^2 decrease after 48 hours of spray chilling, respectively, although CPC application yielded undetectable levels of *E. coli* cells. When Visvalingam & Holley (2018) evaluated multiple concentrations of ASC on contaminated beef surface prior to grinding, they observed no significant reduction of *E. coli* at 30 and 100 ppm, and only 200 and 400 ppm maintained a significant decrease over 4 days in vacuumed ground beef. Similarly, ASC (1000 ppm) applied onto beef trim prior to grinding and refrigerated storage yielded a 0.5 Log CFU/g reduction for *E. coli*

after 6 h with no change at 24 h (Harris et al., 2012). Pohlman et al. (2007) evaluated *E. coli* reduction in ground beef over 7 days and observed ASC (0.1%) yielded the best reduction of APC after 7 days storage (2.26 Log CFU/g), but PAA gave the highest reduction of *E. coli* (1.39 Log CFU/g). In our study, we did not observe a significant ($P > 0.05$) difference between control samples and ASC treated samples under both aerobic and vacuum conditions. When ASC was applied in combination with either UV-C light or with bacteriophage, then we observed a significant reduction ($P < 0.05$) compared to the control under both packaging conditions.

The application of UV light has been approved for use by the Food and Drug Administration (FDA) to decontaminate food, food contact surfaces, juices, and water because of its non-thermal and environmentally friendly nature (Bintsis et al., 2000; FDA, 2000; Sommers et al., 2010). UV light applied at 250-270 nm is considered germicidal UV-C with 254 nm the most common wavelength used for disinfection (Bintsis et al., 2000; Guerrero-Beltrán & Barbosa-Cánovas, 2004). The antimicrobial ability of UV-C light is based on the ability for the light to be absorbed by the DNA of microorganisms to cause thiamine and cytosine cross-linking which cannot be replicated, leading to cell death (Escalona et al., 2010; Guerrero-Beltrán & Barbosa-Cánovas, 2004; Hijnen et al., 2006; Sastry et al., 2000; Snowball & Hornsey, 1988). When applied onto alfalfa seeds, Sharma & Demirci (2003) reported a 4.89 Log CFU/g reduction of *E. coli* O157:H7 with pulsed UV light was applied for 1 min at 8 cm distance from the emitters. Similarly, Ozer & Demirci (2006) applied UV light for 1 min at 8 cm distance onto salmon fillets, but reported a maximum 1.09 Log CFU/g reduction of *E.*

coli O157:H7 and a maximum 0.86 Log CFU/g reduction of *Listeria monocytogenes* after 30 sec at 5 cm distance. Furthermore, the quality of the salmon fillets was visually altered by the UV light at close distances, indicating the ability of UV light to affect quality attributes of fresh meats and the importance of using a larger distance such as 8 cm to not affect quality attributes of products. When applied on goat meat, a combination of 1% lemongrass oil and UV-C light (10 cm distance for 2 min) reduced *E. coli* K12 to undetectable levels (6.66 Log CFU/mL reduction) compared to a maximum 1.18 Log CFU/ml reduction by UV-C light alone (Degala, Mahapatra, Demirci, & Kannan, 2018). When broad spectrum light was applied onto chicken breast against *Salmonella enterica* and *Listeria monocytogenes*, pulsed light (200-1000 nm) applied for 200 sec was shown to reduce these pathogens by 2 and 2.4 Log CFU/ml, respectively (Paskeviciute, Buchovec, & Luksiene, 2011). Similarly, chicken breast treated with a combination of UV-C light (40 min exposure time) and a bacteriophage solution (1 x 10^8 PFU/g) reduced a *Listeria monocytogenes* cocktail by 2.04 Log CFU/g within 1 h of treatment compared to a maximum 0.84 Log CFU/g and 1.58 CFU/g reductions with phage or UV-C light alone, respectively (Yang, Sadekuzzaman, & Ha, 2017). It has been demonstrated that a longer exposure time to UV-C light can result in a higher reduction of pathogens. As previously discussed, there is potential for UV-C light to affect quality attributes of meat products such as color and lipid oxidation, although careful application of UV-C light can circumvent such negative effects while offering efficient, non-thermal,

and non-chemical pathogen reduction. In our study, we observed a significant ($P < 0.05$) reduction when combining phage and UV-C light treatments which gave 0.83 and 1.46 Log CFU/cm^2 reductions for vacuum and aerobic conditions, respectively. Overall, control samples had better color stability. Meat color deterioration is typically caused by oxidation of myoglobin leads to higher L* and lower a*, b*, and Chroma values (King, Shackelford, Rodriguez, & Wheeler, 2011). When we evaluated the effects of UV light exposure on color and lipid oxidation, we observed no effects on lipid oxidation. Effect of time on color was expected since oxygenation of myoglobin is responsible for final color. Application of UV is known for causing photo-oxidation of myoglobin, however when applied in lower doses no detrimental effects on color or lipid stability are observed. In our study, samples treated with UV were darker, redder, had a smaller hue angle and higher Chroma values. Hue angle and Chroma values express color nuances and saturation whereas large angles and lower chroma values are associated to discoloration and browning. In this study, UV application improved color parameters. Although UV light exposure is associated to mechanisms that compromise color, results presented in this research showed no detrimental effects on color caused by UV light.

In this study, all antimicrobial combinations with phage gave significant ($P < 0.0001$) STEC reductions under both aerobic and vacuum conditions compared to all other antimicrobial treatments without phage. The efficacy of adding phage to other antimicrobial treatments has been previously reported (Leverentz et al., 2003; Sukumaran, Nannapaneni, Kiess, & Sharma, 2015; Yeh et al., 2018). Yeh et al. (2018) combined a bacteriophage preparation with lactic acid (LA), PAA, and tumbling UV light

and observed that the combination of bacteriophage and UV light yielded the greatest reduction (1.97 Log CFU/g) of *Salmonella* in ground beef. In addition, the combined application of phage with chemical antimicrobials (Chlorine, PAA, CPC) has shown to give significant ($P < 0.05$) reductions of *Salmonella* up to 2.5 Log CFU/cm^2 on chicken skin after 24 hours (Sukumaran et al., 2015). When comparing chemical antimicrobials to phage applications, Hungaro, Mendonça, Gouvêa, Vanetti, & Pinto (2013) demonstrated a 5-bacteriophage cocktail reduced *Salmonella enteritidis* on chicken skin with comparable efficacy to chemical antimicrobials, allowing them to be alternative interventions. Shebs et al. (2020) is the only study to our knowledge that evaluates the efficacy of phages under vacuum conditions compared to chemical antimicrobials, 5% LA and 400 ppm PAA. They observed significant reduction of *E. coli* O157:H7 under aerobic conditions and vacuum conditions with phage treatment, and no significant reduction with LA or PAA application under vacuum conditions.

This study simulates rework procedures to decontaminate STEC contaminated beef subprimals using a novel 7-bacteriophage cocktail, PAA, ASC, and UV-C light alone and in combinations. We observed the greatest reduction of STEC under vacuum conditions by P and P+PAA combined treatments, and under aerobic conditions by combined Phage and UV-C light treatments, while no significant reduction was observed in samples treated with ASC, UV, UV+PAA, PAA+ASC. Recently, commonly used chemical antimicrobials have been losing their efficacy that could be due to the development of acid resistance (Berry &

Cutter, 2000; Brackett, Hao, & Doyle, 1994; Diez-Gonzalez, Callaway, Kizoulis, & Russell, 1998; Hovde, Austin, Cloud, Williams, & Hunt, 1999). When bacteria are exposed to mild or sublethal levels of acidic solutions (5-6 pH), they are able to survive and synthesize acid resistance proteins that increases their survivability when re-exposed to lower pH solutions (2-4 pH) (Brudzinski & Harrison, 1998; Foster & Hall, 1990; Garren et al., 1998; Goodson & Rowbury, 1989; Leyer et al., 1995; Lin et al., 1996). The development of acid tolerant bacteria has become a food safety concern due to the most common antimicrobial interventions being acidic, requiring new and alternative antimicrobial solutions.

Bacteriophages have been studied for use as alternative food safety interventions because of their advantages including being non-thermal, non-oxidizing, and self-perpetuating (Goodridge & Abedon, 2003; Kazi & Annapure, 2016; Loc-Carrillo & Abedon, 2011; Oliveira et al., 2012). These characteristics are what make phages applicable for many applications, including HEP scenarios. Under such vacuum packaging conditions, the bacteria likely convert to anaerobic fermentation metabolism for energy production due to the reduction of O_2. Although vacuum packaging does not remove all traces of O_2 (Kelly, Cruz-Romero, Kerry, & Papkovsky, 2018), this shift to a less energy efficient metabolism could interfere with the phage lytic cycle, although the reductions of phage only treatments observed in this study were similar for vacuum (1.02 Log cfu/cm^2) and aerobic (0.95 Log cfu/cm^2) packaging. Under vacuum conditions, minimal oxygen is left within the package causing the *E. coli* to use fermentation as a way to adapt and survive, which greatly reduces the metabolic activity (James & Keevil,

1999; Stolper, Revsbech, & Canfield, 2010; Unden, Becker, Bongaerts, Schirawski, & Six, 1994) although it does not inhibit growth in all cases (Dykes, Moorhead, & Roberts, 2001) meaning that the metabolism of the bacteria is able to allow phage replication and cell lysis under vacuum conditions.

Optimal reduction of a 7-strain *E. coli* cocktail under both vacuum and aerobic packaging conditions was achieved by treatments containing our novel 7-bacteriophage cocktail. This could be due to the unique mechanism of the lytic cycle compared to mechanisms of chemical antimicrobials previously mentioned. The lytic cycle involves the hijacking of host replication mechanisms including ribosomes that begin to translate viral mRNA into protein virion structures. Lytic bacteriophages depend on the metabolism of the bacterial host to replicate and subsequently lyse the host cell via endolysin and hydrolase activity (Salmond & Fineran, 2015). At refrigeration temperatures, the bacterial metabolism slows and reduces the rate of protein synthesis and cellular growth (Berry & Foegeding, 1997; Broeze, Solomon, & Pope, 1978; Das & Goldstein, 1968; Farewell & Neidhardt, 1998). Although there have been studies that demonstrated better phage killing efficiency at higher temperatures (Liu et al., 2015; Tomat et al., 2018) there are reports of phage treatments that give efficacious foodborne pathogen reduction at low and refrigeration temperatures (Guenther et al., 2012; Hooton et al., 2011; Hungaro et al., 2013; Sukumaran et al., 2015; Tomat et al., 2018). Many studies have demonstrated bacteriophage applications on meats (Bigwood, Hudson, Billington, Carey-Smith, & Heinemann,

2008; Duc, Son, Honjoh, & Miyamoto, 2018; Hudson et al., 2013; Hudson, Billington, Wilson, & On, 2015; Hungaro et al., 2013; Liu et al., 2015; Shebs et al., 2020; Stratakos & Grant, 2018; Sukumaran et al., 2015; Thung et al., 2017; Tomat et al., 2018; Yeh et al., 2018, 2017; Zinno, Devirgiliis, Ercolini, Ongeng, & Mauriello, 2014), but few have demonstrated efficiency under vacuum conditions apart from this study or Shebs et al. (2020). Additionally, there are no studies to our knowledge that evaluate a phage cocktail that targets each USDA adulterant STEC serotype in combination with other antimicrobials. Results of this research suggest that bacteriophage cocktails that target the seven adulterant STEC serotypes in meats have higher pathogen killing efficacy than organic acids or UV-C light alone and should be further evaluated for their application in both aerobic and vacuum packaging conditions.

Conclusion

Bacteriophage application yielded the greatest reduction of all seven adulterant STEC on beef surface alone and in combinations with other antimicrobial treatments under simulated High Event Period protocols. Peroxyacetic acid and ASC treatments provided a limited decrease under both aerobic and vacuum conditions. The combination of PAA, ASC, and UV antimicrobial interventions with a 7-bacteriophage cocktail led to significant reductions of STEC under both vacuum and aerobic atmospheres compared to individual applications. During High Event Periods, reworking beef primals and subprimals to reduce STEC loads under vacuum packaged conditions

could become more effective with the addition of bacteriophages that target *E. coli* O157:H7 and the 'Big Six' to provide an additional barrier in robust food safety systems.

References

Adams, M. H. (1959). Methods of Study of Bacterial Viruses. In *Bacteriophages* (1st ed.). Retrieved from https://www.biodiversitylibrary.org/item/30293

Agilent. (2019). Agilent E. coli cell culture concentration from OD600 calculator. Available at: https://www.chem.agilent.com/store/biocalculators/calcODBacterial.jsp

Ahn, D. U., Olson, D. G., Jo, C., Chen, X., Wu, C., & Lee, J. I. (1998). Effect of Muscle Type, Packaging, and Irradiation on Lipid Oxidation, Volatile Production, and Color in Raw Pork Patties. *Meat Science*, *49*(1), 27–39. https://doi.org/10.1016/S0309-1740(97)00101-0

Alonso-Hernando, A., Alonso-Calleja, C., & Capita, R. (2013). Growth kinetic parameters of Gram-positive and Gram-negative bacteria on poultry treated with various chemical decontaminants. *Food Control*, *33*(2), 429–432. https://doi.org/10.1016/j.foodcont.2013.03.009

Baldry, M. G. C., & Fraser, J. A. L. (1988). Disinfection with peroxygens. In *Industrial biocides* (pp. 91–116). New York, NY, USA: John Wiley & Sons.

Bauermeister, L. J., Bowers, J. W. J., Townsend, J. C., & McKee, S. R. (2008). The microbial and quality properties of poultry carcasses treated with peracetic acid as an antimicrobial treatment. *Poultry Science*, *87*(11), 2390–2398. https://doi.org/10.3382/ps.2008-00087

Bearson, S., Bearson, B., & Foster, J. W. (1997). Acid stress responses in enterobacteria. *FEMS Microbiology Letters*, *147*(2), 173–180. https://doi.org/10.1016/S0378-1097(96)00503-4

Benarde, M. A., Israel, B. M., Olivieri, V. P., Granstrom, M. L., Benarde, A., Rutgers, M. A., & Granstrom M, M. L. (1965). Efficiency of Chlorine Dioxide as a Bactericide. *Applied Microbiology*, *13*(5), 776–780. Retrieved from http://aem.asm.org/

Berry, E. D., & Cutter, C. N. (2000). Effects of Acid Adaptation of Escherichia coli O157:H7 on Efficacy of Acetic Acid Spray Washes To Decontaminate Beef Carcass Tissue. *Applied and Environmental Microbiology*, *66*(4), 1493–1498. Retrieved from http://aem.asm.org/

Berry, E. D., & Foegeding, P. M. (1997). Cold Temperature Adaptation and Growth of Microorganisms. *Journal of Food Protection*, *60*(12), 1583–1594. Retrieved from http://meridian.allenpress.com/jfp/article-pdf/60/12/1583/2301527/0362-028x-60_12_1583.pdf

Bigwood, T., Hudson, J. A., Billington, C., Carey-Smith, G. V., & Heinemann, J. A. (2008). Phage inactivation of foodborne pathogens on cooked and raw meat. *Food Microbiology*, *25*(2), 400–406. https://doi.org/10.1016/j.fm.2007.11.003

Bintsis, T., Litopoulou-Tzanetaki, E., & Robinson, R. K. (2000). Existing and potential applications of ultraviolet light in the food industry - a critical review. *Journal of the Science of Food and Agriculture*, *80*(6), 637–645. https://doi.org/10.1002/(SICI)1097-0010(20000501)80:6<637::AID-JSFA603>3.0.CO;2-1

Block, S. S. (2001). *Disinfection, Sterilization, and Preservation* (Fifth). Retrieved from https://books.google.com/books?hl=en&lr=&id=3f-kPJ17_TYC&oi=fnd&pg=PR11&dq=Disinfection,+sterilization,+and+preservation.&ots=KnHoEy0LG2&sig=UdqJ2qUKyTvjUw_LQeNun12SjOk#v=snippet&q=paa cell&f=false

Bosilevac, J. M., Nou, X., Barkocy-Gallagher, G. A., Arthur, T. M., & Koohmaraie, M. (2006). Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and Enterobacteriaceae and reduce the prevalence of Escherichia coli O157:H7 on preevisceration beef carcasses. *Journal of Food Protection*, *69*(8), 1808–1813. https://doi.org/10.4315/0362-028X-69.8.1808

Brackett, R. E., Hao, Y. Y., & Doyle, M. P. (1994). Ineffectiveness of Hot Acid Sprays to

Decontaminate Escherichia coli 0157:H7 on Beef. *Journal of Food Protection*, *57*(3), 198–203. Retrieved from http://meridian.allenpress.com/jfp/article-pdf/57/3/198/1665411/0362-028x-57_3_198.pdf

Brightwell, G., Clemens, R., Adam, K., Urlich, S., & Boerema, J. (2009). Comparison of culture-dependent and independent techniques for characterisation of the microflora of peroxyacetic acid treated, vacuum-packaged beef. *Food Microbiology*, *26*(3), 283–288. https://doi.org/10.1016/j.fm.2008.12.010

Briñez, W. J., Roig-Sagués, A. X., Hernández Herrero, M. M., López-Pedemonte, T., & Guamis, B. (2006). Bactericidal efficacy of peracetic acid in combination with hydrogen peroxide against pathogenic and non-pathogenic strains of Staphylococcus spp., Listeria spp. and Escherichia coli. *Food Control*, *17*(7), 516–521. https://doi.org/10.1016/j.foodcont.2005.02.014

Broeze, R. J., Solomon, C. J., & Pope, D. H. (1978). Effects of low temperature on in vivo and in vitro protein synthesis in Escherichia coli and Pseudomonas fluorescens. *Journal of Bacteriology*, *134*(3), 861–874. https://doi.org/10.1128/jb.134.3.861-874.1978

Brown, J. L., Ross, T., McMeekin, T. A., & Nichols, P. D. (1997). Acid habituation of Escherichia coli and the potential role of cyclopropane fatty acids in low pH tolerance. *International Journal of Food Microbiology*, *37*(2–3), 163–173. https://doi.org/10.1016/S0168-1605(97)00068-8

Brudzinski, L., & Harrison, M. A. (1998). Influence of Incubation Conditions on Survival and Acid Tolerance Response of Escherichia coli 0157: H7 and Non-0157:H7 Isolates Exposed to Acetic Acid. *Journal of Food Protection*, *61*(5), 542–546. Retrieved from http://meridian.allenpress.com/jfp/article-pdf/61/5/542/1671173/0362-028x-61_5_542.pdf

Buege, J. A., & Aust, S. D. (1978). Microsomal Lipid Peroxidation. *Methods in Enzymology*, *52*(C), 302–310. https://doi.org/10.1016/S0076-6879(78)52032-6

Capita, R., Riesco-Peláez, F., Alonso-Hernando, A., & Alonso-Calleja, C. (2014). Exposure of Escherichia coli ATCC 12806 to Sublethal Concentrations of Food-Grade Biocides Influences Its Ability To Form Biofilm, Resistance to Antimicrobials, and Ultrastructure. *Applied and Environmental Microbiology*, *80*(4), 1268–1280. https://doi.org/10.1128/AEM.02283-13

Carlson, B. A., Ruby, J., Smith, G. C., Sofos, J. N., Bellinger, G. R., Warren-Serna, W., … Belk, K. E. (2008). Comparison of Antimicrobial Efficacy of Multiple Beef Hide Decontamination Strategies To Reduce Levels of Escherichia coli O157:H7 and Salmonella. *Journal of Food Protection*, *71*(11), 2223–2227. https://doi.org/10.4315/0362-028X-71.11.2223

Castillo, A., Lucia, L. M., Goodson, K. J., Savell, J. W., & Acuff, G. R. (1998). Use of hot water for beef carcass decontamination. *Journal of Food Protection*, *61*(1), 19–25. https://doi.org/10.4315/0362-028X-61.1.19

Castillo, A., Lucia, L. M., Goodson, K. J., Savell, J. W., & Acuff, G. R. (1999). Decontamination of beef carcass surface tissue by steam vacuuming alone and combined with hot water and lactic acid sprays. *Journal of Food Protection*, *62*(2), 146–151. https://doi.org/10.4315/0362-028X-62.2.146

Castillo, A., Lucia, L. M., Kemp, G. K., & Acuff, G. R. (1999). Reduction of Escherichia coli O157:H7 and Salmonella Typhimurium on Beef Carcass Surfaces Using Acidified Sodium Chlorite. *Journal of Food Protection*, *62*(6), 580–584. https://doi.org/10.4315/0362-028X-62.6.580

Castillo, A., Lucia, L. M., Roberson, D. B., Stevenson, T. H., Mercado, I., & Acuff, G. R. (2001). Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in

subsequently produced ground beef. *Journal of Food Protection*, *64*(1), 58–62. https://doi.org/10.4315/0362-028X-64.1.58

Cheng, H. Y., Yu, R. C., & Chou, C. C. (2003). Increased acid tolerance of Escherichia coli O157:H7 as affected by acid adaptation time and conditions of acid challenge. *Food Research International*, *36*(1), 49–56. https://doi.org/10.1016/S0963-9969(02)00107-2

Das, H. K., & Goldstein, A. (1968). Limited capacity for protein synthesis at zero degrees centigrade in Escherichia coli. *Journal of Molecular Biology*, *31*(2), 209–226. https://doi.org/10.1016/0022-2836(68)90440-3

Degala, H. L., Mahapatra, A. K., Demirci, A., & Kannan, G. (2018). Evaluation of non-thermal hurdle technology for ultraviolet-light to inactivate Escherichia coli K12 on goat meat surfaces. *Food Control*, *90*, 113–120. https://doi.org/10.1016/j.foodcont.2018.02.042

Desriac, N., Broussolle, V., Postollec, F., Mathot, A. G., Sohier, D., Coroller, L., & Leguerinel, I. (2013). Bacillus cereus cell response upon exposure to acid environment: Toward the identification of potential biomarkers. *Frontiers in Microbiology*, *4*(284), 1–13. https://doi.org/10.3389/fmicb.2013.00284

Dickson, J. S., & Kunduru, M. R. (1995). Resistance of acid-adapted salmonellae to organic acid rinses on beef. *Journal of Food Protection*, *58*(9), 973–976. https://doi.org/10.4315/0362-028X-58.9.973

Diez-Gonzalez, F., Callaway, T. R. ;, Kizoulis, M. G., & Russell, J. B. (1998). Grain feeding and the dissemination of acid-resistant Escherichia coli from cattle. *Science*, *281*(5383), 1666–1668.

Duc, H. M., Son, H. M., Honjoh, K. ichi, & Miyamoto, T. (2018). Isolation and application of bacteriophages to reduce Salmonella contamination in raw chicken meat. *LWT - Food Science and Technology*, *91*, 353–360. https://doi.org/10.1016/j.lwt.2018.01.072

Dykes, G. A., Moorhead, S. M., & Roberts, S. L. (2001). Survival of Escherichia coli O157:H7 and Salmonella on chill-stored vacuum or carbon dioxide packaged primal beef cuts. *International Journal of Food Microbiology*, *64*, 401–405. https://doi.org/10.1016/S0168-1605(00)00482-7

Ellebracht, J. W., King, D. A., Castillo, A., Lucia, L. M., Acuff, G. R., Harris, K. B., & Savell, J. W. (2005). Evaluation of peroxyacetic acid as a potential pre-grinding treatment for control of Escherichia coli O157:H7 and Salmonella Typhimurium on beef trimmings. *Meat Science*, *70*, 197–203. https://doi.org/10.1016/j.meatsci.2005.01.003

Escalona, V. H., Aguayo, E., Martínez-Hernández, G. B., & Artés, F. (2010). UV-C doses to reduce pathogen and spoilage bacterial growth in vitro and in baby spinach. *Postharvest Biology and Technology*, *56*(3), 223–231. https://doi.org/10.1016/j.postharvbio.2010.01.008

Farewell, A., & Neidhardt, F. C. (1998). Effect of temperature on in vivo protein synthetic capacity in Escherichia coli. *Journal of Bacteriology*, *180*(17), 4704–4710. https://doi.org/10.1128/jb.180.17.4704-4710.1998

FDA. (2000). Irradiation in the production, processing and handling of food. , Pub. L. No. 21 CFR Part 179, Federal Register.

Fineran, P. C., Petty, N. K., & Salmond, G. P. C. (2009). Transduction: Host DNA Transfer by Bacteriophages. In *Encyclopedia of Microbiology* (pp. 666–679).

Foster, J. W., & Hall, H. K. (1990). Adaptive acidification tolerance response of Salmonella typhimurium. *Journal of Bacteriology*, *172*(2), 771–778. https://doi.org/10.1128/JB.172.2.771-778.1990

Foster, John W. (1995). Low ph adaptation and the acid tolerance response of salmonella typhimurium. *Critical Reviews in Microbiology*, *21*(4), 215–237.

https://doi.org/10.3109/10408419509113541
Fraser, J. A. L., Godfree, A. F., & Jones, F. (1984). Use of Peracetic acid in operational sewage sludge disposal to pasture. *Water Science and Technology*, *17*(4–5), 451–466. https://doi.org/10.2166/wst.1985.0151
Garren, D. M., Harrison, M. A., & Russelv, S. M. (1998). Acid Tolerance and Acid Shock Response of Escherichia coli 0157:H7 and Non-0157:H7 Isolates Provide Cross Protection to Sodium Lactate and Sodium Chloride. *Journal of Food Protection*, *61*(2), 158–161. https://doi.org/10.4315/0362-028X-61.2.158
Gill, C. O., & Badoni, M. (2004). Effects of peroxyacetic acid, acidified sodium chlorite or lactic acid solutions on the microflora of chilled beef carcasses. *International Journal of Food Microbiology*, *91*(1), 43–50. https://doi.org/10.1016/S0168-1605(03)00329-5
Goodridge, L., & Abedon, S. T. (2003). Bacteriophage biocontrol and bioprocessing: Application of phage therapy to industry. *SIM News*, *53*(6), 254–262.
Goodson, M., & Rowbury, R. J. (1989). Habituation to normally lethal acidity by prior growth of Escherichia coli at a sub-lethal acid pH value. *Letters in Applied Microbiology*, *8*(2), 77–79. https://doi.org/10.1111/j.1472-765X.1989.tb00227.x
Gordon, G., Kieffer, R. G., & Rosenblatt, D. H. (1972). The Chemistry of Chlorine Dioxide. *Progress in Inorganic Chemistry*, *15*, 201–286. Retrieved from https://onlinelibrary.wiley.com/doi/pdf/10.1002/9780470166161#page=206
Guenther, S., Herzig, O., Fieseler, L., Klumpp, J., & Loessner, M. J. (2012). Biocontrol of Salmonella Typhimurium in RTE foods with the virulent bacteriophage FO1-E2. *International Journal of Food Microbiology*, *154*, 66–72. https://doi.org/10.1016/j.ijfoodmicro.2011.12.023
Guerrero-Beltrán, J. A., & Barbosa-Cánovas, G. V. (2004). Review: Advantages and limitations on processing foods by UV light. *Food Science and Technology International*, *10*(3), 137–147. https://doi.org/10.1177/1082013204044359
Harris, D., Brashears, M. M., Garmyn, A. J., Brooks, J. C., & Miller, M. F. (2012). Microbiological and organoleptic characteristics of beef trim and ground beef treated with acetic acid, lactic acid, acidified sodium chlorite, or sterile water in a simulated commercial processing environment to reduce Escherichia coli O157:H7 and Salmon. *Meat Science*, *90*(3), 783–788. https://doi.org/10.1016/j.meatsci.2011.11.014
Harris, K., Miller, M. F., Loneragan, G. H., & Brashears, M. M. (2006). Validation of the use of organic acids and acidified sodium chlorite to reduce Escherichia coli O157 and Salmonella typhimurium in beef trim and ground beef in a simulated processing environment. *Journal of Food Protection*, *69*(8), 1802–1807. https://doi.org/10.4315/0362-028X-69.8.1802
Hijnen, W. A. M., Beerendonk, E. F., & Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review. *Water Research*, *40*, 3–22. https://doi.org/10.1016/j.watres.2005.10.030
Hooton, S. P. T., Atterbury, R. J., & Connerton, I. F. (2011). Application of a bacteriophage cocktail to reduce Salmonella Typhimurium U288 contamination on pig skin. *International Journal of Food Microbiology*, *151*(2), 157–163. https://doi.org/10.1016/j.ijfoodmicro.2011.08.015
Hovde, C. J., Austin, P. R., Cloud, K. A., Williams, C. J., & Hunt, C. W. (1999). Effect of Cattle Diet on Escherichia coli O157:H7 Acid Resistance. *Applied and Environmental Microbiology*, *65*(7), 3233–3235. https://doi.org/10.1128/AEM.65.7.3233-3235.1999
Hudson, J. A., Billington, C., Cornelius, A. J., Wilson, T., On, S. L. W., Premaratne, A., & King, N. J.

(2015). Use of a bacteriophage to inactivate Escherichia coli O157: H7 on beef. *Food Microbiology*, *36*(1), 14–21. https://doi.org/10.1016/j.fm.2013.03.006

Hudson, J. A., Billington, C., Wilson, T., & On, S. L. W. (2013). Effect of phage and host concentration on the inactivation of Escherichia coli O157:H7 on cooked and raw beef. *Food Science and Technology International*, *21*(2), 104–109. https://doi.org/10.1177/1082013213513031

Hungaro, H. M., Mendonça, R. C. S., Gouvêa, D. M., Vanetti, M. C. D., & Pinto, C. L. d. (2013). Use of bacteriophages to reduce Salmonella in chicken skin in comparison with chemical agents. *Food Research International*, *52*(1), 75–81. https://doi.org/10.1016/j.foodres.2013.02.032

James, B. W., & Keevil, C. W. (1999). Influence of oxygen availability on physiology, verocytotoxin expression and adherence of Escherichia coli O157. *Journal of Applied Microbiology*, *86*(1), 117–124. https://doi.org/10.1046/j.1365-2672.1999.00639.x

Kalchayanand, N., Arthur, T. M., Bosilevac, J. M., Schmidt, J. W., Wang, R., Shackelford, S. D., & Wheeler, T. L. (2012). Evaluation of commonly used antimicrobial interventions for fresh beef inoculated with shiga toxin-producing escherichia coli serotypes O26, O45, O103, O111, O121, O145, and O157:H73. *Journal of Food Protection*, *75*(7), 1207–1212. https://doi.org/10.4315/0362-028X.JFP-11-531

Kazi, M., & Annapure, U. S. (2016). Bacteriophage biocontrol of foodborne pathogens. *Journal of Food Science and Technology*, *53*(3), 1355–1362. https://doi.org/10.1007/s13197-015-1996-8

Kelly, C. A., Cruz-Romero, M., Kerry, J. P., & Papkovsky, D. P. (2018). Assessment of performance of the industrial process of bulk vacuum packaging of raw meat with nondestructive optical oxygen sensing systems. *Sensors (Switzerland)*, *18*(5). https://doi.org/10.3390/s18051395

King, D. A., Lucia, L. M., Castillo, A., Acuff, G. R., Harris, K. B., & Savell, J. W. (2005). Evaluation of peroxyacetic acid as a post-chilling intervention for control of Escherichia coli O157:H7 and Salmonella Typhimurium on beef carcass surfaces. *Meat Science*, *69*(3), 401–407. https://doi.org/10.1016/j.meatsci.2004.08.010

King, D., Shackelford, S., Rodriguez, A., & Wheeler, T. (2011). Effect of time of measurement on the relationship between metmyoglobin reducing activity and oxygen consumption to instrumental measures of beef longissimus color stability. *Meat Science*, *87*, 26–32. https://doi.org/10.1016/j.meatsci.2010.08.013

Kross, R. (1984). An innovative demand-release microbiocide. In *Basis for action and effects of dilution. Keynote lecture to the Second Biannual Conference on Progress in Chemical Disinfection*, *SUNY, Binghamton, New York*.

Laury, A. M., Alvarado, M. V., Nace, G., Alvarado, C. Z., Brooks, J. C., Echeverry, A., & Brashears, M. M. (2009). Validation of a lactic acid- and citric acid-based antimicrobial product for the reduction of Escherichia coli O157:H7 and Salmonella on beef tips and whole chicken carcasses. *Journal of Food Protection*, *72*(10), 2208–2211. https://doi.org/10.4315/0362-028X-72.10.2208

Leaper, S. (1984). Influence of temperature on the synergistic sporicidal effect of peracetic acid plus hydrogen peroxide on Bacillus subtilis SA22 (NCA 72-52). *Food Microbiology*, *1*(3), 199–203. https://doi.org/10.1016/0740-0020(84)90034-0

Leverentz, B., Conway, W. S., Camp, M. J., Janisiewicz, W. J., Abuladze, T., Yang, M., … Sulakvelidze, A. (2003). Biocontrol of Listeria monocytogenes on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. *Applied and Environmental*

Microbiology, *69*(8), 4519–4526. https://doi.org/10.1128/AEM.69.8.4519-4526.2003

Leyer, G. J., Wang, L. L., & Johnson, E. A. (1995). Acid adaptation of Escherichia coli O157:H7 increases survival in acidic foods. *Applied and Environmental Microbiology*, *61*(10), 3752–3755. https://doi.org/10.1128/AEM.61.10.3752-3755.1995

Liberti, L., & Notarnicola, M. (1999). Advanced treatment for municipal wastewater reuse in agriculture. *Water Science and Technology*, *40*(4–5), 235–245. https://doi.org/10.1080/01919510008547217

Lillard, H. S. (1979). Levels of chlorine and chlorine dioxide of equivalent bactericidal effect in poultry processing water. *Journal of Food Science*, *44*(6), 1594–1597. https://doi.org/10.1111/j.1365-2621.1979.tb09097.x

Lin, J., Smith, M. P., Chapin, K. C., Suk Baik, H., Bennett, G. N., & Foster, J. W. (1996). Mechanisms of Acid Resistance in Enterohemorrhagic Escherichia coli. *Applied and Environmental Microbiology*, *62*(9), 3094–3100. Retrieved from http://aem.asm.org/

Liu, H., Niu, Y. D., Meng, R., Wang, J., Li, J., Johnson, R. P., … Stanford, K. (2015). Control of Escherichia coli O157 on beef at 37, 22 and 4°C by T5-, T1-, T4-and O1-like bacteriophages. *Food Microbiology*, *51*, 69–73. https://doi.org/10.1016/j.fm.2015.05.001

Loc-Carrillo, C., & Abedon, S. T. (2011). Pros and cons of phage therapy. *Bacteriophage*, *1*(2), 111–114. https://doi.org/10.4161/bact.1.2.14590

Marriott, N. G., Schilling, M. W., & Gravani, R. B. (2018). Sanitizers. In *Principles of Food Sanitation* (pp. 175–198). https://doi.org/10.1007/978-3-319-67166-6_10

Mataragas, M., Skandamis, P. N., & Drosinos, E. H. (2008). Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations. *International Journal of Food Microbiology*, *126*, 1–12. https://doi.org/10.1016/j.ijfoodmicro.2008.05.014

Montag, D., Hashemolhosseini, S., & Henning, U. (1990). Receptor-recognizing proteins of T-even type bacteriophages. The receptor-recognizing area of proteins 37 of phages T4 TuIa and TuIb. *Journal of Molecular Biology*, *216*(2), 327–334. https://doi.org/10.1016/S0022-2836(05)80324-9

Nair, M. S., Upadhyaya, I., Amalaradjou, M. A. R., & Venkitanarayanan, K. (2017). Antimicrobial Food Additives and Disinfectants: Mode of Action and Microbial Resistance Mechanisms. In O. V. Singh (Ed.), *Foodborne pathogens and antibiotic resistance* (43rd ed., pp. 275–418).

Oechslin, F. (2018). Resistance development to bacteriophages occurring during bacteriophage therapy. *Viruses*, *10*(351), 1–23. https://doi.org/10.3390/v10070351

Oliveira, J., Castilho, F., Cunha, A., & Pereira, M. J. (2012). Bacteriophage therapy as a bacterial control strategy in aquaculture. *Aquaculture International*, *20*(5), 879–910. https://doi.org/10.1007/s10499-012-9515-7

Ozer, N. P., & Demirci, A. (2006). Inactivation of Escherichia coli O157:H7 and Listeria monocytogenes inoculated on raw salmon fillets by pulsed UV-light treatment. *International Journal of Food Science and Technology*, *41*(4), 354–360. https://doi.org/10.1111/j.1365-2621.2005.01071.x

Paskeviciute, E., Buchovec, I., & Luksiene, Z. (2011). High-Power Pulsed Light For Decontamination Of Chicken From Food Pathogens: A Study On Antimicrobial Efficiency And Organoleptic Properties. *Journal of Food Safety*, *31*(1), 61–68. https://doi.org/10.1111/j.1745-4565.2010.00267.x

Penney, N., Bigwood, T., Barea, H., Pulford, D., LeRoux, G., Cook, R., … Brightwell, G. (2007). Efficacy of a peroxyacetic acid formulation as an antimicrobial intervention to reduce levels of inoculated Escherichia coli O157:H7 on external carcass surface of hot-boned beef and

veal. *Journal of Food Protection*, *70*(1), 200–203. https://doi.org/10.4315/0362-028X-70.1.200

Pohlman, F. W., Quilo, S. a, Brown, a H., Crandall, P. G., Baublits, R. T., Story, R. P., … Rajaratnam, G. (2007). Microbial, instrumental color and sensory characteristics of ground beef processed from beef trimmings treated with potassium lactate, sodium metasilicate, peroxyacetic acid or acidified sodium chlorite as single antimicrobial interventions. *Journal of Muscle Foods*, *20*, 54–69. https://doi.org/10.1111/j.1745-4573.2008.00133.x

Purnell, G., James, C., James, S. J., Howell, M., & Corry, J. E. L. (2014). Comparison of Acidified Sodium Chlorite, Chlorine Dioxide, Peroxyacetic Acid and Tri-Sodium Phosphate Spray Washes for Decontamination of Chicken Carcasses. *Food and Bioprocess Technology*, *7*(7), 2093–2101. https://doi.org/10.1007/s11947-013-1211-8

Ransom, J. R., Belk, K. E., Sofos, J. N., Stopforth, J. D., Scanga, J. A., & Smith, G. C. (2003). Comparison of Intervention Technologies for Reducing Escherichia coli O157:H7 on Beef Cuts and Trimmings. *Food Production Trends*, *23*(1), 24–34.

Salmond, G., & Fineran, P. (2015). A century of the phage: Past, present and future. *Nature Reviews Microbiology*, *13*(12), 777–786. https://doi.org/10.1038/nrmicro3564

Salmond, C. V, Kroll, R. G., & Booth, I. R. (1984). The Effect of Food Preservatives on pH Homeostasis in Escherichia coli. *Journal of General Microbiology*, *130*, 2845–2850. https://doi.org/10.1099/00221287-130-11-2845

Sastry, S. K., Datta, A. K., & Worobo, R. W. (2000). Ultraviolet Light. *Journal of Food Science - Supplement*, *65*(8), 90–92. https://doi.org/10.1111/j.1750-3841.2000.tb00623.x

Sharma, R. R., & Demirci, A. (2003). Inactivation of Escherichia coli O157:H7 on inoculated alfalfa seeds with pulsed ultraviolet light and response surface modeling. *Journal of Food Science*, *68*(4), 1448–1453. https://doi.org/10.1111/j.1365-2621.2003.tb09665.x

Shebs, E. L., Lukov, M. J., Giotto, F. M., Torres, E. S., & de Mello, A. S. (2020). Efficacy of bacteriophage and organic acids in decreasing STEC O157:H7 populations in beef kept under vacuum and aerobic conditions: A simulated High Event Period scenario. *Meat Science*, *162*, 108023. https://doi.org/10.1016/j.meatsci.2019.108023

Singh, S. P., Singh, S. R., Williams, Y. U., Jones, L., & Abdullah, T. (1995). Antigenic Determinants of the OmpC Porin from Salmonella typhimurium. *Infection and Immunity*, *63*(12), 4600–4605. https://doi.org/10.1128/IAI.63.12.4600-4605.1995

Singh, S. P., Upshaw, Y., Abdullah, T., Singh, S. R., & Klebba, P. E. (1992). Structural Relatedness of Enteric Bacterial Porins Assessed with Monoclonal Antibodies to Salmonella typhimurium OmpD and OmpC. *Journal of Bacteriology*, *174*(6), 1965–1973. https://doi.org/10.1128/JB.174.6.1965-1973.1992

Snowball, M., & Hornsey, I. (1988). Purification of water supplies using ultraviolet light. In R. Robinson & U. Barking (Eds.), *Developments in food Moicrbiology* (pp. 171–191). Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201302076036

Sommers, C. H., Sites, J. E., & Musgrove, M. (2010). Ultraviolet light (254 nm) inactivation of pathogens on foods and stainless steel surfaces. *Journal of Food Safety*, *30*(2), 470–479. https://doi.org/10.1111/j.1745-4565.2010.00220.x

Stivarius, M. R., Pohlman, F. W., Mcelyea, K. S., & Waldroup, A. L. (2002). Effects of hot water and lactic acid treatment of beef trimmings prior to grinding on microbial, instrumental color and sensory properties of ground beef during display. *Meat Science*, *60*(4), 327–334. https://doi.org/10.1016/S0309-1740(01)00127-9

Stolper, D. A., Revsbech, N. P., & Canfield, D. E. (2010). Aerobic growth at nanomolar oxygen

concentrations. *Proceedings of the National Academy of Sciences*, *107*(44), 18755–18760. https://doi.org/10.1073/pnas.1013435107/-/DCSupplemental

Stopforth, J. D., Skandamis, P. N., Geornaras, I., & Sofos, J. N. (2007). Acid tolerance of acid-adapted and nonacid-adapted Escherichia coli O157:H7 strains in beef decontamination runoff fluids or on beef tissue. *Food Microbiology*, *24*(5), 530–538. https://doi.org/10.1016/j.fm.2006.05.007

Stopforth, J. D., Yoon, Y., Belk, K. E., Scanga, J. A., Kendall, P. A., Smith, G. C., & Sofos, J. N. (2004). Effect of Simulated Spray Chilling with Chemical Solutions on Acid-Habituated and Non-Acid-Habituated Escherichia coli O157:H7 Cells Attached to Beef Carcass Tissue. *Journal of Food Protection*, *67*(10), 2099–2106. https://doi.org/10.4315/0362-028X-67.10.2099

Stratakos, A. C., & Grant, I. R. (2018). Evaluation of the efficacy of multiple physical, biological and natural antimicrobial interventions for control of pathogenic Escherichia coli on beef. *Food Microbiology*, *76*, 209–218. https://doi.org/10.1016/j.fm.2018.05.011

Sukumaran, A. T., Nannapaneni, R., Kiess, A., & Sharma, C. S. (2015). Reduction of Salmonella on chicken meat and chicken skin by combined or sequential application of lytic bacteriophage with chemical antimicrobials. *International Journal of Food Microbiology*, *207*, 8–15. https://doi.org/10.1016/j.ijfoodmicro.2015.04.025

Thiessen, G. P., Usborne, W. R., & Orr, H. L. (1984). The efficacy of chlorine dioxide in controlling Salmonella contamination and its effect on product quality of chicken broiler carcasses. *Poultry Science*, *63*(4), 647–653. https://doi.org/10.3382/ps.0630647

Thung, T. Y., Premarathne, J. M. K. J. K., Chang, W. S., Loo, Y. Y., Chin, Y. Z., Kuan, C. H., … Radu, S. (2017). Use of a lytic bacteriophage to control Salmonella Enteritidis in retail food. *LWT - Food Science and Technology*, *78*, 222–225. https://doi.org/10.1016/j.lwt.2016.12.044

Tomat, D., Casabonne, C., Aquili, V., Balagué, C., & Quiberoni, A. (2018). Evaluation of a novel cocktail of six lytic bacteriophages against Shiga toxin-producing Escherichia coli in broth, milk and meat. *Food Microbiology*, *76*, 434–442. https://doi.org/10.1016/j.fm.2018.07.006

Unden, G., Becker, S., Bongaerts, J., Schirawski, J., & Six, S. (1994). Oxygen regulated gene expression in facultatively anaerobic bacteria. *Antonie van Leeuwenhoek*, *66*(1–3), 3–22. https://doi.org/10.1007/BF00871629

USDA-AMS. (2014). United States Department of Agriculture - Agricultural Research Service, Institutional Meat Purchase Specifications: Fresh Beef Series 100. Retrieved from https://www.ams.usda.gov/sites/default/files/media/IMPS_100_Fresh_Beef[1].pdf%5Cnhttp://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3003283

USDA-FSIS. (2012a). *United States Department of Agriculture - Food Safety Inspection Service, Shiga toxin-producing Escherichia coli in certain raw beef products*\. Retrieved from https://www.federalregister.gov/documents/2012/05/31/2012-13283/shiga-toxin-producing-escherichia-coli-in-certain-raw-beef-products

USDA-FSIS. (2012b). United States Department of Agriculture - Food Safety Inspection Service, USDA Targeting Six Additional Strains of E.coli in Raw Beef Trim Starting Monday | USDA. Retrieved March 23, 2020, from https://www.usda.gov/media/press-releases/2012/05/31/usda-targeting-six-additional-strains-ecoli-raw-beef-trim-starting

USDA-FSIS. (2014). *United States Department of Agriculture - Food Safety Inspection Service, Compliance Guideline for Establishments Sampling Beef Trimmings for Shiga Toxin-Producing Escherichia coli (STEC) Organisms or Virulence Markers*. Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/e0f06d97-9026-4e1e-a0c2-

1ac60b836fa6/Compliance_Guide_Est_Sampling_STEC_0512.pdf?MOD=AJPERES

USDA-FSIS. (2019). *United States Department of Agriculture - Food Safety Inspection Service, Safe and Suitable Ingredients used in the Production of Meat, Poultry, and Egg Products*. Retrieved from https://www.fsis.usda.gov/wps/portal/fsis/topics/regulations/directives/7000-series/safe-suitable-ingredients-related-document

Uyttendaele, M., Jozwik, E., Tutenel, A., De Zutter, L., Uradzinski, J., Pierard, D., & Debevere, J. (2001). Effect of acid resistance of Escherichia coli O157:H7 on efficacy of buffered lactic acid to decontaminate chilled beef tissue and effect of modified atmosphere packaging on survival of Escherichia Coli O157:H7 on red meat. *Journal of Food Protection*, *64*(11), 1661–1666. https://doi.org/10.4315/0362-028X-64.11.1661

Villarreal, M. E., Baker, R. C., & Regenstein, J. M. (1990). The Incidence of Salmonella on Poultry Carcasses Following the Use of Slow Release Chlorine Dioxide (Alcid). *Journal of Food Protection*, *53*(6), 465–467. Retrieved from http://meridian.allenpress.com/jfp/article-pdf/53/6/465/1660685/0362-028x-53_6_465.pdf

Visvalingam, J., & Holley, R. A. (2018). Evaluation of chlorine dioxide, acidified sodium chlorite and peroxyacetic acid for control of Escherichia coli O157:H7 in beef patties from treated beef trim. *Food Research International*, *103*, 295–300. https://doi.org/10.1016/j.foodres.2017.10.051

Wesche, A. M., Gurtler, J. B., Marks, B. P., & Ryser, E. T. (2009). Stress, Sublethal Injury, Resuscitation, and Virulence of Bacterial Foodborne Pathogens. *Journal of Food Protection*, *72*(5), 1121–1138.

Wheeler, T. L., Kalchayanand, N., & Bosilevac, J. M. (2014). Pre- and post-harvest interventions to reduce pathogen contamination in the U.S. beef industry. *Meat Science*, *98*(3), 372–382. https://doi.org/10.1016/j.meatsci.2014.06.026

Yang, S., Sadekuzzaman, M., & Ha, S. Do. (2017). Reduction of Listeria monocytogenes on chicken breasts by combined treatment with UV-C light and bacteriophage ListShield. *LWT - Food Science and Technology*, *86*, 193–200. https://doi.org/10.1016/j.lwt.2017.07.060

Yeh, Y., de Moura, F. H., Van Den Broek, K., & de Mello, A. S. (2018). Effect of ultraviolet light, organic acids, and bacteriophage on Salmonella populations in ground beef. *Meat Science*, *139*, 44–48. https://doi.org/10.1016/j.meatsci.2018.01.007

Yeh, Y., Purushothaman, P., Gupta, N., Ragnone, M., Verma, S. C., & de Mello, A. S. (2017). Bacteriophage application on red meats and poultry: Effects on Salmonella population in final ground products. *Meat Science*, *127*, 30–34. https://doi.org/10.1016/j.meatsci.2017.01.001

Youssef, M. K., Yang, X., Badoni, M., & Gill, C. O. (2013). Survival of acid-adapted Escherichia coli O157: H7 and not-adapted E.coli on beef treated with 2% or 5% lactic acid. *Food Control*, *34*(1), 13–18. https://doi.org/10.1016/j.foodcont.2013.03.037

Yu, F., & Mizushima, S. (1982). Roles of lipopolysaccharide and outer membrane protein ompc of Escherichia coli K-12 in the receptor function for bacteriophage T4. *Journal of Bacteriology*, *151*(2), 718–722.

Zhao, T., Zhao, P., & Doyle, M. P. (2009). Inactivation of Salmonella and Escherichia coli O157:H7 on Lettuce and Poultry Skin by Combinations of Levulinic Acid and Sodium Dodecyl Sulfate. *Journal of Food Protection*, *72*(5), 928–936. https://doi.org/10.4315/0362-028X-72.5.928

Zinno, P., Devirgiliis, C., Ercolini, D., Ongeng, D., & Mauriello, G. (2014). Bacteriophage P22 to challenge Salmonella in foods. *International Journal of Food Microbiology*, *191*, 69–74.

https://doi.org/10.1016/j.ijfoodmicro.2014.08.037

Table 1. Significance of contrasts analysis for treatments applied under vacuum and aerobic conditions

	***P* - value**	
Contrasts	**Vacuum**	**Aerobic**
All treatments containing P[1] versus all other treatments[2]	<0.0001	0.0001
All treatments containing P[1] versus C[3]	0.0002	<0.0001
All other treatments[2] versus C[3]	0.3395	0.0781

[1]Bacteriophage (P), P + Peroxyacetic acid (PAA), P + Ultraviolet light (UV), and P + Acidified Sodium Clorite (ASC).

[2]UV, PAA, ASC, PAA+ASC, PAA+UV, and ASC+UV.

[3]Control (C) treated with Buffered Petpone water.

Table 2. Color and lipid stability of beef samples treated with UV light

Lipid stability

	Treatment[1]			
	Control	**UV**	**SEM[2]**	**P-value**
Mg MDA/kg	1.42[A]	0.99[B]		0.037

Color Parameters

	Time (h)				Treatment[1]			*P* - value		
	0	**1**	**12**	**24**	**Control**	**UV**	**SEM[2]**	**Time**	**Treat**	**Time*Treat**
L*	49.51	47.17	46.97	47.91	49.04[A]	46.74[B]	0.7265	0.3048	0.0330	0.9775
a*	14.44[a]	15.06[a]	14.39[a]	11.55[b]	12.89[B]	14.83[A]	0.3784	0.0003	0.0011	0.5641
b*	6.95[b]	7.73[ab]	8.37[a]	7.42[b]	7.65	7.59	0.3124	0.0083	0.8267	0.1423
Hue	25.72[c]	27.06[c]	30.29[b]	33.54[a]	31.12[A]	27.19[B]	0.9624	<0.0001	0.0003	0.2065
Chroma	16.05[a]	16.94[a]	16.68[a]	13.77[b]	15.04[B]	16.68[A]	0.4213	0.0012	0.0059	0.4494

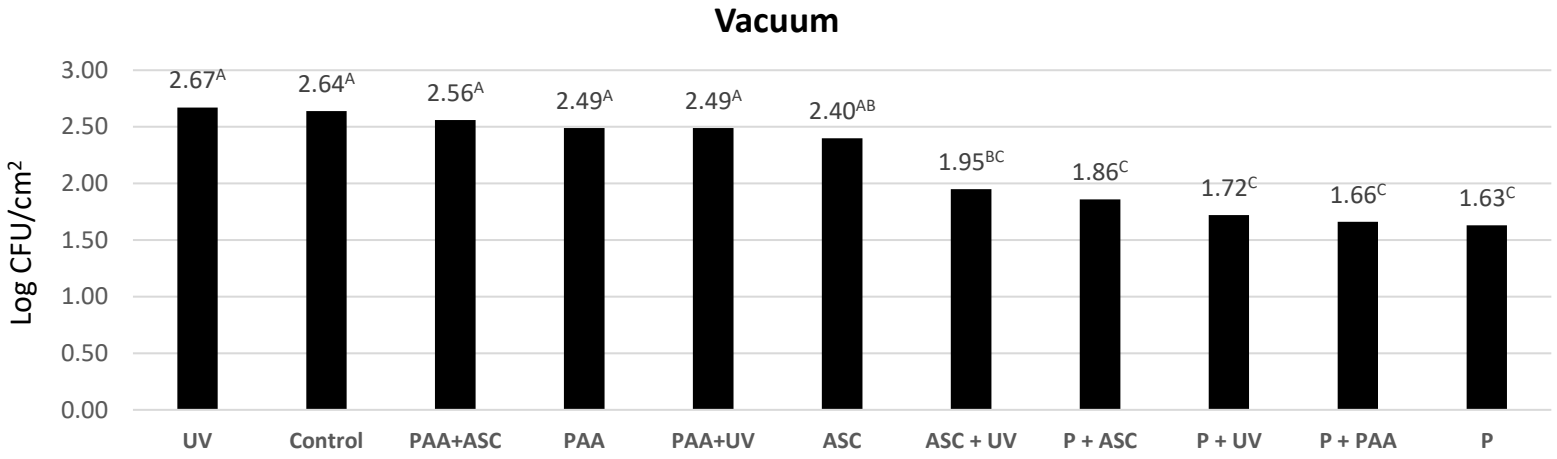
[1]Control=Not treated with UV light, UV= 254 nm, 23°C for 30 sec.

[2]Standard error for the mean for treatments.

[A,B]Different superscripts within treatment are significantly different.

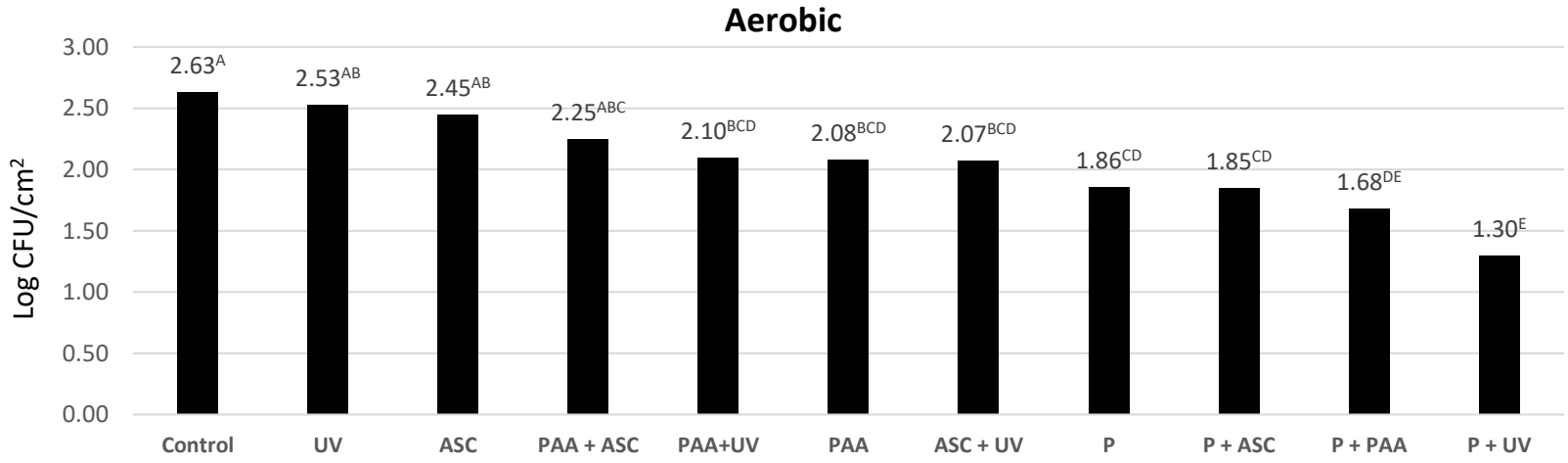
[a,b,c]Different superscripts within time are significantly different.

Figure 1. Least square means of STEC populations in beef samples (*m*. *Cutaneous* trunci) kept under vacuum and treated with BPW (C), Peroxyacetic acid (PAA, 400 ppm), Acidified Sodium Chlorite (ASC, 1200 ppm), Ultraviolet light - C (UV, 254 nm, 23°C for 30 s), MS1 Bacteriophages (P, 10^8 PFU/ml), PAA+ASC, PAA+UV, ASC+UV, P+PAA, P+ASC, and P+UV. Standard error of the mean = 0.2108.



A,B,C Means with different superscripts are significantly different at P<0.001.

Figure 2. Least square means of STEC populations in beef samples (*m*. *Cutaneous* trunci) kept under aerobic conditions and treated with BPW (C), Peroxyacetic acid (PAA, 400 ppm), Acidified Sodium Chlorite (ASC, 1200 ppm), Ultraviolet light - C (UV, 254 nm, 23°C for 30 s), MS1 Bacteriophages (P, 10^8 PFU/ml), PAA+ASC, PAA+UV, ASC+UV, P+PAA, P+ASC, and P+UV. Standard error of the mean = 0.1919.



A,B,C,D,E Means with different superscripts are significantly different at P<0.001.

Appendix VI. Phage amplification and concentration protocol

1. Inoculate 5x LB broth with fresh overnight culture at the ratio 1 mL culture to 5 mL 5x LB broth
2. Incubate at 37°C with shaking (230 RPM) for 1-3 hours
3. Inoculate 100 µL of high titer (≥10^9 PFU/mL) phage or 500 µL of low titer phage (≤10^8 PFU/mL)
4. Incubate at 37°C with shaking (230 RPM) overnight (18 ± 6 hours)
5. Centrifuge for 20 minutes at 5,000 x g
6. Filter liquid lysate with 0.2 µM syringe filter into a new labeled conical tube
    a. If propagating a large volume (>15 mL), filter supernatant with 0.45 µM syringe filter followed by 0.2 µM syringe filter
7. Transfer 12 mL into the upper reservoir of the 100 kDa Amicon filter (MilliporeSigma™ UFC910008)
8. Centrifuge at 4,000 × g for 9 min.
    a. Do not spin the filter dry, centrifugation times will vary based on concentration
    b. Do not exceed 12 mL if using a fixed angle rotor centrifuge
9. Remove the filtrate and add more phage lysate to upper reservoir and continue filtering through centrifugation until the phage lysate in the upper reservoir is less than 5 mL
10. Add 10 mL of SM Buffer into the upper reservoir to wash at 4,000 × g for 9 min. or until less than 10 mL of phage lysate
11. Perform the double agar assay as described by Adams (1959) and modified by Yeh et al. (2017) (Appendix IV)