NOVEL INTERVENTIONS FOR REDUCING PATHOGEN ATTACHMENT AND GROWTH ON FRESH PRODUCE

A Dissertation

by

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ABSTRACT

The objectives of this research were to 1) identify the native microbiota on surfaces of fresh fruit and leafy greens; 2) identify microorganisms antagonistic towards *Salmonella enterica* Typhimurium LT2 and *Escherichia coli* O157:H7 ATCC 700728 both *in vitro* and on produce surfaces; and 3) evaluate the ability of antimicrobial-bearing nano-encapsulates to prevent pathogen attachment and growth on produce surfaces. Produce (cantaloupe, tomato, endive, and spinach) was sampled from two farms for each produce type (n=30). Aerobic bacteria, lactic acid bacteria (LAB), yeasts/molds, enterococci, and coliforms were enumerated using appropriate media. For each sample, 4-12 isolated colonies from each medium were submitted to biochemical identification. Antagonism of recovered isolates against pathogens was determined using the Agar Spot method. Produce was spot-inoculated with a suspension of bacteria showing *in-vitro* antagonistic activity against *S. enterica* Typhimurium LT2 and *E. coli* O157:H7 then stored at 25°C for 24 h. Each sample was spot-inoculated with a suspension including both pathogens and stored at 25°C. At 0, 6, 12, and 24 h of storage, loose and strong attachment of pathogens on the surface was determined. Geraniol-loaded NPs were prepared by flash nanoprecipitation. Inhibition of *Escherichia coli* O157:H7 and *S.* Typhimurium LT2 was tested *in vitro* and on produce at 5°C, 15°C, and 25°C for up to 10 days. The organisms isolated from the surface of the various produce commodities were diverse; 1,389 isolates were isolated from the surfaces of cantaloupes, tomatoes, spinach, and endive. Of these, 109 (7.8%) showed antagonism activity *in vitro* against *S.* Typhimurium LT2 and 91 (6.6%) against *E. coli* O157:H7. *Staphylococcus*

antagonistic isolates showed larger zones of inhibition against both pathogens than the other antagonistic isolates recovered from produce. On produce surfaces, the endive-recovered isolate *Escherichia coli* and the cantaloupe-recovered isolated *Escherichia hermannii* depressed the growth of both pathogens. Geraniol loaded NPs inhibited *S.* Typhimurium and *E. coli* O157:H7 growth at 0.4 and 0.2 wt.%, respectively. Pathogen reductions on treated produce ranged from 1.2 to 6.0 $\log_{10}$ CFU/cm^2. In summary, antimicrobial NPs and microorganisms naturally present on produce may be useful for the post-harvest decontamination of fresh produce, from cross-contaminating microbial pathogens.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

TABLE Page

CHAPTER I

INTRODUCTION

Foodborne Illness and Produce

In the United States alone, 31 microbial pathogens are estimated to cause approximately 9.4 million instances of foodborne illness, 55,961 hospitalizations, and 1,351 deaths annually *(122)*. According to data published by the United States Centers for Disease Control and Prevention (CDC), 1,034 outbreaks of foodborne disease were reported in 2008 *(32)*. These outbreaks involved 23,152 reported cases of illness, 1,276 hospitalizations, and 22 deaths *(32)*. Within the reported instances of foodborne illnesses, *Salmonella* was the most common cause of hospitalizations related to foodborne disease outbreaks (62%), followed by the Shiga toxin–producing *Escherichia coli* (STEC) causing 17% of the reported, outbreak-associated hospitalizations *(32)*. During 1998-2008, a total of 13, 352 foodborne disease outbreaks, causing 271, 974 illnesses, were reported in the United States *(106)*. Within these outbreaks, many were associated with fresh and minimally processed produce, and more illnesses were attributed to leafy greens (22%) than to any other single commodity *(106)*. Leafy greens were also the second most frequent cause of hospitalizations (14%) and the fifth most recent cause of foodborne illness-related deaths *(106)*. Microbial pathogens have been found to be associated with a variety of produce related outbreaks, and Table 1 shows a small glimpse of produce-borne outbreaks in recent years. The average annual number of foodborne disease outbreaks reported to the CDC during 1998-2008 was more than double the average annual number reported during 1973-1997 *(67)*. The high number of

reported foodborne illnesses due to produce over the past years could be attributed to many factors including increased consumption, change in consumers' habits, and complex distribution systems *(53)*. Increased consumption has brought about increased production and distribution of fresh produce*(121)*.

TABLE 1. ***Microbial pathogens associated with produce-borne disease outbreaks.***

Organism	Produce Item Associated with Outbreak	Confirmed Cases	Country affected by Outbreak	Year	Reference
E. coli O157:H7	Spinach	199	United States	2006	*(95)*
E. coli O157:H7	Romaine lettuce	58	United States	2011	*(127)*
Shigella sonnei	Imported baby corn	218	Denmark and Australia	2007	*(86)*
Salmonella enterica Serotype Newport	Cucumbers	275	United States	2014	*(9)*
Salmonella enterica Serotype Braenderup	Mangoes	127	United States	2012	*(33)*
Salmonella enterica Serotype Typhimurium	Cantaloupes	261	United States	2012	*(34)*
Salmonella Saintpaul	Jalapeño and serrano peppers	1,440	United States	2008	*(31)*
Listeria monocytogenes	Cantaloupes	83	United States	2011	*(37)*
Cyclospora cayetanensis	Salad mix and cilantro	631	United States	2013	*(1)*
Yersinia pseudotuberculosis	Carrots	400	Finland	2006	*(116)*

Produce Contamination and Pathogen Attachment

Produce is often consumed as a raw, fresh commodity with little microbial reduction through processing, thus increasing consumer risk of disease from contaminants *(52)*. Produce can become contaminated with pathogens at any point of production including harvest, processing, and even at retail outlets, in foodservice establishments, and in the home kitchen *(77, 79)*. Figure 1 further demonstrates that there are many mechanisms/routes by which fresh produce can become contaminated with pathogenic microorganisms. Even transportation by consumers can affect the microbial safety of the produce *(19)*. However, the major source of microbial contamination of fresh produce is associated with human or animal feces *(79)*.

The quality of the water used for washing after harvest is critical, and when water comes in contact with produce, the quality of the water dictates the potential for contamination *(79)*. Water used to apply pesticides to plants and for post-harvest cooling and processing can transfer microbes directly to the produce *(75)*. Water used for irrigation may also be a source of microbial pathogens if it is cross-contaminated surface water, and if during irrigation, it comes in contact with the edible portions of the plant *(75)*. In addition, many crops will receive supplemental irrigation and protective topical sprays mixed with the water. Many commodities are cooled, moved/conveyed, or washed with water prior to their sale *(99)*. Experiments have shown that *Salmonella enterica* serovars from water can be taken up internally through the stem scar if the water is colder than the produce item such as in tomatoes *(45, 88)*. Pathogenic bacteria such as *Salmonella* have been isolated from irrigation water and have been transmitted by direct contact to the water

to other areas of production *(61)*. In 2005, tomatoes grown and packed on the eastern shore of Virginia were contaminated with *Salmonella* Newport causing over 500 causes of foodborne illness over 26 states . The cause of the outbreak was traced to the pond water that was used to irrigate the tomato fields. Therefore, water quality plays an important role in pre- and post-harvest microbiological quality of fruits and vegetables.

In addition to cross-contaminated irrigation waters, there are many other mechanisms/routes by which fresh produce can become contaminated with pathogenic microorganisms. Other pre-harvest sources of pathogenic microorganisms on fresh produce include soil, green or inadequately composted manure, air (dust), and wild and domestic animals *(16)*. Côté and Quessy *(38)* studied the persistence of *Salmonella* and

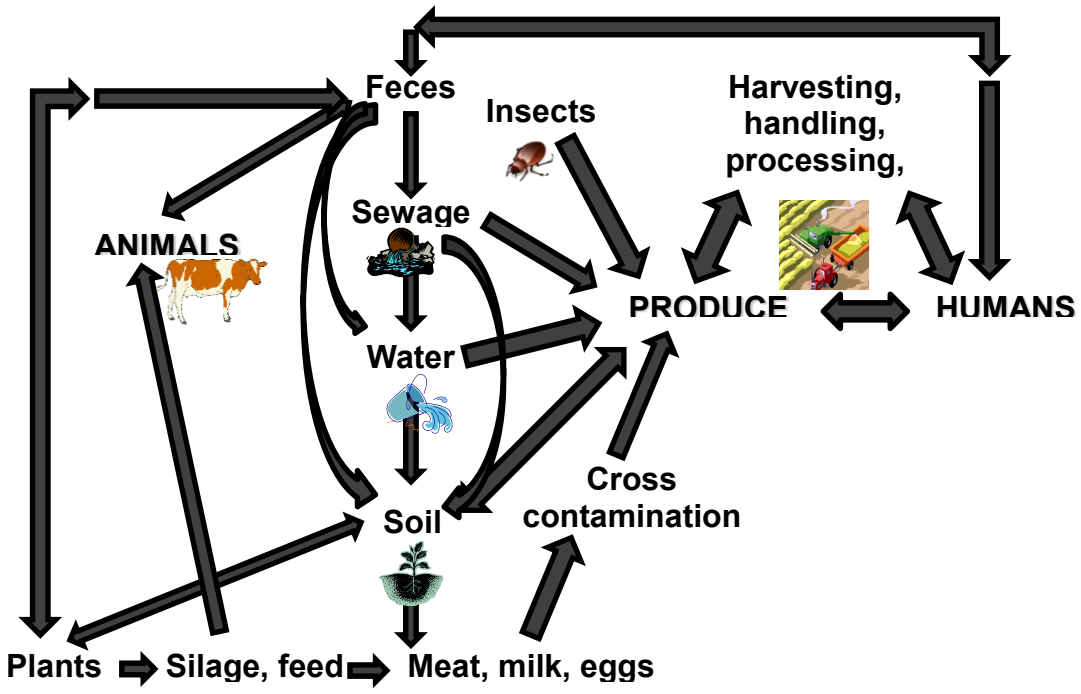

FIGURE 1. ***Produce contamination routes. Routes by which fresh produce can become contaminated with pathogens(16).***

E. coli in surface soil after application of liquid hog manure in fields of cucumbers and determined that both *Salmonella* and *E. coli* could survive for over 50 days. This study not only showed that manure and soil could play a role in the contamination of produce commodities, but also that early contamination with foodborne pathogens could lead to their persistence during harvest *(38)*. Harvesting may present the greatest opportunity for cross-contamination due to non-hygienic practices of employees, harvesting equipment, field containers, or minimal processing that occurs in the field such as is the case for romaine hearts and head lettuce *(75)*.

Worker hygiene and sanitation practices during production, harvesting, sorting, packing, and transport play a critical role in minimizing the potential for microbial contamination of fresh produce *(79)*. Important factors involved in worker hygiene and sanitation practices during production include workers' hands (the predominant vehicle to move produce from growing sites to packing and processing operations) and personal hygiene, which plays a role in physical contamination of produce with foreign material such as stones and glass fragments *(75)*. Furthermore, enteric pathogens such as *Salmonella enterica* and *E. coli* O157:H7 often originate from the intestinal tracts and/or fecal material of humans or animals, and the survival or growth of such pathogens on the produce item is influenced by the organism(s), produce item, and conditions of storage *(79)*. Certain conditions can inhibit the growth of bacteria on produce while other conditions will actually facilitate and favor the growth of bacteria such as storage temperature of the produce, relative humidity % during storage, produce pH and oxygen conditions during storage *(75)*. Therefore, microorganisms residing on fresh and fresh-cut

produce, throughout the journey from farm to fork, could will undergo cycles of subjection to unfavorable and hostile environments, periods of limited growth, along with periods of growth when conditions are favorable depending on storage conditions of the produce and the growth conditions of the microorganisms present *(75)*.

CHAPTER II

PRODUCE-BORNE *SALMONELLA ENTERICA* AND *ESCHERICHIA COLI* O157:H7

Salmonella Classification and Growth Requirements

Salmonella enterica has been isolated in the past from decaying fruits and vegetables and can contaminate fruits and vegetables upon harvesting due to cross-contamination with livestock feces *(53)*. *Salmonella enterica* is most prevalent in animal and human feces, raw meat, poultry, and eggs *(53)*. *Salmonella* serovars are Gram-negative, cytochrome oxidase negative, facultatively anaerobic enteric bacteria that are rod-shaped and motile with peritrichous flagella *(14)*. *Salmonella enterica* is unable to produce indole from tryptone and unable to convert acetoin from fermented acids; however, *Salmonella* is able to produce organic acids via fermentation without production of secondary metabolites, utilize citrate as a sole carbon source, and possesses the enzymes lysine and ornithine decarboxylase *(73)*.

Salmonella is divided into two different species, *Salmonella bongori* and *Salmonella enterica*, with over 2700 serotypes *(53)*. The species *S. enterica* is divided into six subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*) *(40)*. These subspecies are divided into various serovars or serotypes within the Kauffmann-White antigenic scheme, based on differences in reaction with antibodies of two major and/or other minor types of cell-surface antigens *(14, 39)*. For best growth, *Salmonella* require a pH between 6.6 and 8.2, and the minimum reported pH value for growth of *Salmonella* is 4.05 *(76)*. *Salmonella* are mesophilic and can grow within a temperature range of 2-54°C, while growth/replication temperatures below 7°C have been observed

only in bacteriological media and not in foods and growth at temperatures above 48°C are confined to mutants and tempered strains *(39)*. The optimum temperature range for growth is 35-37°C *(40)*. Under optimum conditions the minimum water activity needed for the growth of *Salmonella* is 0.94 and the maximum needed is >0.99, yet *Salmonella* can survive in food products with a low water activity *(14)*. Salmonellosis has also been associated with food products of low water activity such as some fermented meat products, hard cheese, peanut butter, chocolate, dried milk and cereal products and food ingredients such as black pepper and desiccated coconut *(14)*.

Salmonellosis

The species that affects humans by exerting pathogenesis is *Salmonella enterica (76)*, which can cause the illness salmonellosis by infection. The organism will grow and multiply in their host's body; *Salmonella* multiplies in the small intestine, colonizing and invading the intestinal tissues, producing an enterotoxin *(76)*. This will cause an inflammatory reaction and diarrhea *(14)*. *Salmonella* causes symptoms such as abdominal pain, diarrhea, chills, fever, nausea, and vomiting; the typical incubation period for *Salmonella* is 18 to 72 hours *(53)*, and the infectious dose of *Salmonella* has been reported to be as low as 10-100 cells *(14)*. Other *Salmonella* species such as *S.* Typhi, *S.* Paratyphi A, *S.* Paratyphi C are agents of typhoid and paratyphoid fevers, severe *Salmonella* caused diseases *(76)*. Illnesses caused by *Salmonella* can range from gastroenteritis to enteric (typhoid) fever and septicemia and chronic sequelae *(14)*. Septicemia is caused when

Salmonella are present in the blood stream and is characterized by high fever, malaise, pain in the thorax and abdomen, chills, and anorexia *(14)*.

Salmonella enterica Produce Outbreaks

The estimated incidence of foodborne illnesses linked to *Salmonella* is the highest among the major bacterial pathogens with more than 1 million illnesses estimated per year *(122)*. *Salmonella* has frequently been isolated from produce due to cross-contamination with livestock feces *(53)*. Recent produce-borne outbreaks associated with *Salmonella* include an outbreak in 2008 of *Salmonella* Saintpaul infections associated with jalapeño and serrano peppers *(31, 93)*, an outbreak in 2011 of *Salmonella* Panama associated with cantaloupe, and an outbreak of *Salmonella* Saintpaul in 2013 associated with cucumbers *(36)*. The outbreak of *Salmonella* associated with peppers in 2008 was caused by contamination in the irrigation water *(54)*. The outbreak of *Salmonella* on cantaloupe rind has been attributed to contamination while processing in the packing house *(54)*. These outbreaks show that enteric pathogens can contaminate produce through various routes as mentioned before and can also contamination a variety of produce items.

Escherichia coli O157:H7 Classification and Growth Requirements

Another pathogen frequently causing illness due to contamination in fresh produce is *Escherichia coli* O157:H7 *(53)*. The *E. coli* are Gram-negative, oxidase negative, rod-shaped, facultatively anaerobic rod-shaped organisms that cleave lactose and utilize glucose. Strains that possess flagella are motile with peritrichous flagella

(42). *E. coli* is able to produce indole from tryptone and forms organic acids via fermentation without production of secondary metabolites, but does not utilize citrate as its sole carbon source nor forms acetoin as a secondary metabolite from fermented acid *(73)*.

Enterohemorrhagic *E. coli* O157:H7

There are five recognized virulence groups for *E. coli*: enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC) *E. coli*; *E. coli* O157:H7 belongs to the EHEC virulence group *(76)*. *E. coli* O157:H7 is not only associated with meat and meat products; produce is also a prominent transmission vehicle of this organism *(79)*. The EHEC virulence group causes symptoms such as bloody diarrhea, abdominal pain, and can also lead to hemolytic uremic syndrome in which the red blood cells can be destroyed and the kidneys fail *(76)*. The incubation time for *E. coli* O157:H7 is two to five days *(53)* and the infectious dose has been reported to be as small as fewer than 50 bacterial cells *(130)*.

Escherichia coli O157:H7 Produce Outbreaks

The prevalence of foodborne illnesses per year of *E. coli* O157:H7 has been estimated to be over 60,000 per year *(122)*. One of the largest outbreaks in the United States occurred in 2006, a produce-borne outbreak linked to fresh spinach related to *E. coli* O157:H7 occurring across 26 states with an estimated 206 cases *(93)*. More recent

produce-borne outbreaks associated with *E. coli* O157:H7 include an outbreak in 2011 linked to Romaine lettuce, an outbreak in 2012 linked to organic spinach and spring mix blend, and an outbreak in 2012 linked to clover sprouts *(35)*.

CHAPTER III

INTERVENTIONS FOR FRESH PRODUCE

Antimicrobial Interventions for Fresh Produce

After harvesting, sanitizers are sometimes used in raw fruit and vegetable processing *(79)*. Sanitizers are sometimes used in raw fruit and vegetable processing and those approved for use and regulated by U.S. are listed in the U.S. Code of Federal Regulations (CFR), Title 21, Section 173 *(55)*. Sanitizers which can be used at a commercial level include chlorine (should not exceed 2000 ppm hypochlorite in wash water), chlorine dioxide (ClO_2) (not exceeding 3 ppm residual chlorine dioxide), hydrogen peroxide (H_2O_2) (up to 59 ppm in wash water), peroxyacetic acid (PAA) (up to 80 ppm in wash water), and ozone (O_3), yet commercial sanitizers are unlikely to eliminate all pathogens *(55, 75)*. Although chlorine is easy to apply and inexpensive, it is decomposed by organic matter and reaction products may be hazardous *(120)*. The effect of chlorine in a solution of chlorine bleach and water is due to available chlorine, present as hypochlorite and hypochlorous acid *(100)*. If chlorine is used in wash water, the produce must be rinsed with potable water following chlorine treatment according to 21CFR173.315 *(55)*. The regulations do not specify a permissible residual level of sodium hypochlorite and produce operations typically do not a use a sanitizer concentration greater than 200 ppm of total chlorine with a contact time of at least one minute *(55)*. Zhang *et al. (143)* obtained a 1.5 $\log_{10}$ CFU/cm^2 reduction after dipping tomatoes inoculated with *Salmonella enterica* Montevideo in a solution of 320 ppm chlorine at 25°C, a pathogen reduction which was not significantly different ($P<0.05$) than that obtained by dipping tomatoes in a 110 ppm

chlorine solution at 25°C. Weissinger *et al. (139)* showed less than a 1.0 $\log_{10}$ CFU/g reduction of *Salmonella enterica* Baildon for both inoculated lettuce and diced tomato after immersion for 40 s in a 120 or 200 ug/ml free chlorine solution. Chlorine dioxide is more potent than chlorine and is less corrosive than ozone. However, it must be generated on-site, is explosive at high concentrations (>10% in air) and is not permitted on cut fruits and vegetables *(120)*. Han *et al. (69)* inoculated uninjured and surface-injured green bell peppers with *E. coli* O157:H7. The peppers were then subjected to ClO_2 gas treatments of concentrations ranging from 0.15 to 1.2 mg/l for 30 min at 20°C *(69)*. The results indicated reductions of the injured surface ranging from 1.7 $\log_{10}$ at 0.15 mg/l ClO_2 and 6.5 $\log_{10}$ at 1.2 mg/l ClO_2 *(69)*. The results for the uninjured surface ranged from 2.9 $\log_{10}$-cycles reduction at a concentration of 0.15 mg/l ClO_2 and 8.0 $\log_{10}$-cycles reduction at a 1.2 mg/l concentration of ClO_2 *(69)*. This study showed that an increasing concentration of ClO_2 was more effective for 30 minutes for both injured and uninjured surfaces *(69)*. The study also showed that injured produce surfaces were more difficult to decontaminate than uninjured surfaces *(69)*.

Peroxyacetic acid has a broad spectrum of antimicrobial action and does not require on-site generation *(120)*. However, peroxyacetic acid is a strong oxidant *(120)*. Rodgers *et al. (117)* assessed peroxyacetic acid at 80 ppm for the reduction of *E. coli* O157:H7 and *Listeria monocytogenes* on inoculated produce. Produce (apples, lettuce, strawberries, and cantaloupe) were inoculated to bear 6.0 $\log_{10}$ CFU/g *E. coli* O157:H7 or *L. monocytogenes*, and then submerged in the sanitizer solution for up to 5 min, and examined for survivors. Peroxyacetic acid resulted in 4.4 $\log_{10}$ CFU/g reductions in both

pathogens *(117)*. These authors also assessed ozone bubbled through water to achieve 3 ppm for the reduction of *E. coli* O157:H7 and *L. monocytogenes* on produce inoculated to 6.0 $\log_{10}$ CFU/g. The results indicated a decrease in both pathogens of >5 $\log_{10}$-cycles following 2 to 5 minute exposure to ozone. Ozone is a more potent antimicrobial than chlorine and is not pH dependent; however, ozone requires on-site generation and is phytotoxic at high concentrations *(120)*. The threshold limit for long-term human exposure according to the U.S. Office for Safety and Health Administration (OSHA) is 0.1 ppm/day/work week but for short-term exposure it is 0.3 ppm for 15 minutes *(76)*. However, Ozone is GRAS for bottled water use and for use on a variety of fresh meats *(76)*.

Furthermore, pathogen attachment to surfaces of produce could play a role in the limiting efficacy of sanitizers *(138)*. Liao and Sapers *(87)* detected through examination via a scanning electron microscope that the attachment of *Salmonella* Chester was predominately on surfaces of injured tissue and stem and calyx regions (94%) but rarely on the unbroken skin (6%). Liao and Sapers *(87)* through the application of 6% hydrogen peroxide via immersion for 5 min reduced *Salmonella* Chester on apple skin by 3-4 logs; however, the population on the stem and calyx was only reduced by 1-2 logs. Laio and Sapers *(87)* suggested based on their results that a small portion of bacteria attached to stem and calyx was likely either resistant to or protected from the sanitizer treatment. The authors attributed the failure of the sanitizer to inactivate *Salmonella* to the firm attachment of bacteria on stem and calyx and to the partial resistance of attached bacteria to sanitizer *(87)*. Pathogens possess specific mechanisms

of attachment to produce such as pili and fimbriae, and the environment in which the pathogen has remained viable (water, manure, soil, etc.) can determine the surface molecules expressed and the metabolic state of the pathogen *(96)*. The site of attachment can also determine the strength of attachment of the pathogen, i.e. intact surface versus wounded surfaces *(96)*. Patel and Sharma *(107)* determined that the surface of intact produce that is covered by a hydrophobic waxy cuticle may allow hydrophobic *Salmonella* cells to attach, yet breaks in the cuticle can expose hydrophilic structures from within allowing intimate contact between bacterial cells and the produce surface. This would ultimately release previously unavailable nutrients to enteric bacteria, making them good sites for colonization. Patel and Sharma *(107)* also determined that the ability of *Salmonella* to attach to produce depends on the produce commodity itself as seen through differences in strength of attachment (S_R) between cabbage (0.05) and Romaine lettuce (0.25) by *Salmonella* Tennessee. However, more studies are needed to investigate the interactions between produce surfaces and *Salmonella (107)*. Thus, reduction in populations of microbiota on whole and fresh-cut produce is dependent upon the type of produce commodity, the background microbiota, and how microorganism(s) attach to the produce *(75)*.

Furthermore, the increasing demand of consumers for reduced-additive (including antimicrobial agents) and more "natural" foods have promoted the search for alternative antimicrobial agents that are naturally derived *(91)*. Naturally occurring antimicrobial compounds are abundant in the environment and essential oils derived from plants, herbs, and spices are known to inhibit foodborne pathogens *(91)*. The

antimicrobial compounds in plant materials are commonly contained in the essential oil fractions of leaves, flowers, bulbs, rhizomes, and other parts *(44)*. Clove (*Syzgium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) provide two frequently investigated antimicrobial phenolic compounds, eugenol [2-methyoxy-4-(2-propenyl)-phenol] and cinnamic aldehyde (3-phenyl-2-propenal), respectively *(43)*. Liu *et al. (90)* reported minimum inhibitory concentrations (MIC) of thymol, eugenol, berberine and cinnamaldehyde against *Salmonella* Typhimurium to be 256, 2048, 2048, and 1024 μg/ml, respectively. Catherine *et al. (30)* reported MICs of peppermint oil and eugenol ranging from 0.10% to 0.25% against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Yersinia enterocolitica*. Yun *et al. (141)* reported reductions of *Salmonella enterica* serovar Typhimurium on tomatoes by gaseous treatments for 18 h at 22°C of mustard essential oil (EO) (10 μl), isothiocynate (10 μl), cinnamon EO (250 μl), cinnamaldehyde (250 μl), oregano EO (250 μl), and carvacrol (250 μl) to be 6.18 ± 0.31, 4.56 ± 0.43, 3.79 ± 0.49 , 1.54 ± 0.32 , and 3.37 ± 0.85 $\log_{10}$ CFU/g, respectively.

The rose oil component geraniol (*trans*-3,7-Dimethyl-2,6-octadien-1-ol) also has the ability to inhibit the growth of foodborne bacterial such as *Salmonella enterica*, *Campylobacter jejuni*, *Listeria monocytogenes* and *E. coli* O157:H7 *(60, 80)*. Kim *et al. (80)* reported minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of geraniol in 1 % Tween 20 against *E. coli* O157:H7, *L. monocytogenes, Salmonella* Typhimurium, and *Vibrio vulnificus* ranging between 500 and 1,000 μg/ml. Friedman et al. (*18*) reported achieving 50 % lethality of inoculated *E. coli* O157:H7 and *Salmonella* cells in apples juice at levels of 0.089 and 0.031% geraniol,

respectively. A similar MIC for geraniol (MIC: 0.05 %) against *E. coli* O157:H7 grown in medium adjusted to pH 4.5 was reported; however, researchers observed that at pH 7.2 the MIC of geraniol against *E. coli* O157:H7 was >0.1 % (*19*). Raybaudi-Massilia *et al. (113)* reported a concentration of 2 μL/ml of lemongrass, cinnamon, and geraniol was needed to inactivate 3-4 $\log_{10}$ CFU/ml of *Salmonella* Enteritidis, *E. coli*, and *L.* innocua in apple and pear juices at 35°C after 24 h. The encapsulation of these naturally occurring essential oils may assist in: stabilizing the antimicrobial against deleterious reactions with food components and reducing the rate of the antimicrobial's release into the food *(129).*

Novel Interventions: Bio-controls

A biocontrol is the use of one or more organisms to inhibit or control other organisms *(76).* The manner in which an organism is controlled can be related to the presence of a live organism or could be due to indirect actions or agents such as the production of bacteriocins or via the competition for available nutrients *(76).* In food environments, the native microbiota of a food may have competitive advantages that could result in the suppression of undesirable or cross-contaminating microorganisms *(123).* It is possible that pathogens on foods may be inhibited and/or eliminated by the actions of competitors or antagonistic microbiota on foods *(123).*

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by some bacteria that are inhibitory to other bacteria, either within the same species or across genera *(115).* Lactic acid bacteria are a source of bacteriocins and have been extensively studied for the ability to inhibit/antagonize foodborne pathogens *(8, 21, 46,*

114, 115, 134). There are four classes of lactic acid bacteria bacteriocins. Class I bacteriocins are Lantibiotics *(81)*. These are small membrane-active peptides (<5 kDa) that contain the amino acids lanthionine, β-methyl lanthionine, and dehydrated residues *(81)*. Examples of lantibiotics are nisin and lactocin S *(81)*. Class II bacteriocins are small heat-stable, non-lanthionine containing membrane-active peptides (<10 kDa) characterized by Gly-Gly^{-1**+1}Xaa processing site in the bacteriocin precursor *(81)*. Examples of the second class include pediocin PA-l and lactococcin A *(81)*. Class III bacteriocins are large heat-labile proteins (>30 kDa) which include lactacins A and B *(81)*. Class IV bacteriocins are complex bacteriocins composed of protein plus one or more chemical moieties (lipid, carbohydrate) required for activity such as leuconocin S and plantaricin S *(81)*.

Other bacteria such as *Staphylococcus* spp. could potentially have antagonizing effects against foodborne pathogens. Their potential effects as antagonist to other bacteria were discovered over 100 years ago *(74)*. An early description of bacteriocin-like antagonism between Gram-positive bacteria occurred in 1885 by *Staphylococcus epidermis* inhibition of *Corynebacterium diptheriae* which led to the use of staphylococcal nasal and throat sprays for the treatment of diphtheria infection and carriage *(74)*. More recently, *Staphylococcus* ssp. antagonist effects against foodborne pathogens have been studied for their potential use in meat products *(83, 94, 125, 136, 137)*, dairy products *(22, 119)*, and spinach *(12)*.

Members of the genus *Bacillus* can also have potentially antagonizing effects against foodborne pathogens. Some *Bacillus* ssp. are known to produce a wide arsenal of

antimicrobial substances, including peptide and lipopeptide antibiotics, and bacteriocins *(2)*. These bacteriocins resemble bacteriocins produced by certain lactic acid bacteria. Many of the *Bacillus* bacteriocins belong to the lantibiotics (Class I) such as paenibacillin and lichenicidin while other members of the genus *Bacillus* produce nonmodified bacteriocins that resemble the pediocin-like bacteriocins (Class II) such as Coagulin and SRCAM 37 *(2)*. However, other bacteriocins produced by certain *Bacillus* ssp. are novel peptide sequences *(2)*.

Gram-negative organisms such as *Escherichia coli*, *Shigella*, and *Citrobacter* can produce colicins that have the ability to inhibit Gram-negative and Gram-positive bacteria *(28)*. *Aeromonas* and *Pseudomonas* spp. have also been shown to exhibit inhibitory properties against *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus (123)*. The use of Gram-negative organisms to inhibit pathogens in foods has not been fully explored. Novel methods incorporating naturally occurring microorganisms could aid in the inhibition of surface-contaminating food-borne pathogens.

Novel Interventions: Encapsulated Antimicrobials

Encapsulation can occur in various forms such as a membrane coating, a wall or membrane of spherical or irregular shaped, a multiwall structure with walls of the same or varying compositions or numerous cores within the same walled structure *(65)*. Micro- and nano-encapsulation of an antimicrobial within another food-grade material may assist in: (i) stabilizing the antimicrobial against deleterious reactions with food components;

(ii) stabilizing volatile antimicrobials against rapid evaporation; (iii) reducing the rate of the antimicrobial's release into the food, allowing lengthened exposure of microbes to antimicrobial pressure; and (iv) protection of the antimicrobial during processing *(129)*. A variety of nanoparticles (NPs) for the delivery of antimicrobials or drugs have been investigated including, but not limited to, liposomes, micelles, nanospheres, nanocapsules, solid lipid nanoparticles, microemulsions and carbon nanotubes *(84)*. An innovative type of NP delivery system are amphiphilic block copolymers which can form various types of nanoparticles such as micelles, polymersomes, nanospheres, and nanocapsules *(84)*. These polymers are obtained by the polymerization of more than one type of monomer, typically one hydrophobic and one hydrophilic, so that the resulting molecule is composed of regions that have opposite affinities for an aqueous solvent *(84)*.

Encapsulation methods have been used in order to improve the effectiveness of plant derived antimicrobials such as eugenol and cinnamic aldehyde *(4, 62, 63, 66, 108)*. Application of micelle-encapsulated eugenol to *L. monocytogenes* and *E. coli* O157:H7 at pH 5.0-7.0 completely inhibited both organisms' growth *in vitro* in tryptic soy broth after 24 h at 32°C *(62)*. Gaysinsky *et al. (64)* reported addition of eugenol-entrapping micelles to fluid milks inoculated with *L. monocytogenes* or *E. coli* O157:H7 inactivated pathogens in low fat milks (0 and 2% fat) and inhibited growth of pathogens in 4% fat milk. Cinnamon contains 0.5-10% volatile oil, of which 75% is cinnamic aldehyde and 8% is eugenol *(15)*. Clove contains 12-14% volatile oil, 95% of which is eugenol *(15)*. Cinnamon and cinnamic aldehyde have also demonstrated antimicrobial activity against

Gram-negative and Gram-positive foodborne bacteria such as *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella*. *(10, 59, 60, 62, 64, 78, 103).*

In addition to the encapsulation of differing food antimicrobials in differing structures, the incorporation of antimicrobials into food-grade polymers has allowed for the development of various antimicrobial-bearing edible films *(20, 24, 26, 97, 104, 112, 126, 133)*. These technologies might allow for preservation of antimicrobial activity prior to application to the food, increased opportunity for direct contact between antimicrobial and targeted microorganisms, and long-term suppression of microbial growth during storage as a result of diffusion of antimicrobial from the film to the surface of the produce commodity. Multiple polysaccharides and polypeptides have been explored for their utility to incorporate and deliver antimicrobials, though much research has been focused on chitosan, a polysaccharide obtained by de-acetylation of the naturally occurring polymer chitin *(3, 47, 111)*. Chitosan is polycationic in nature, and has been repeatedly reported to possess strong antimicrobial activity of its own, though observed antimicrobial efficacy has been shown to be increased when other antimicrobials are incorporated prior to casting of chitosan films *(47, 128)*. In addition to chitosan, alginates, whey-derived proteins, zein proteins, and other polymers have all been investigated for their utility in formulating antimicrobial-bearing edible films *(26)*. Spice essential oils (eugenol, cinnamic aldehyde) were incorporated into alginate films that were subsequently applied for the inhibition of spoilage microorganisms and *Salmonella enterica* serovar Enteritidis *(112)*. In addition to reductions observed in numbers of mesophilic and psychrotrophic bacteria, numbers of *S.* Enteritidis were significantly reduced (4.05-4.20 $\log_{10}$ cycle

reductions) by antimicrobial-bearing films over 21 days of refrigerated storage (5°C) on Piel de Sapo melons (*Cucumis melo* L.) surfaces *(112).* Nonetheless, these technologies are hindered by limitations similar to those facing other antimicrobial interventions such as the inability of the incorporated antimicrobial to consistently contact foodborne pathogens located between crevices of plant cells or instability of antimicrobials once released from polymer.

The objectives of this study were to 1) identify the native microbiota on surfaces of fresh fruits and leafy greens; 2) identify microorganisms antagonistic towards *Salmonella enterica* Typhimurium LT2 and *Escherichia coli* O157:H7 ATCC 700728 both *in* vitro and on produce; and 3) evaluate the ability of antimicrobial-bearing nano-encapsulates to prevent pathogen attachment and growth on produce surfaces. This study will generate data showing the impacts of produce physiology, processing, and intervention usage for the inhibition of pathogen attachment and growth on surfaces. Ultimately, this will help design and validate novel interventions for use in produce processing to control pathogen attachment and adherence.

CHAPTER IV

IDENTIFICATION OF NATURALLY OCCURING MICROORGANISMS ON PRODUCE COMMODITIES

Materials and Methods

Produce Sampling and Microorganism Recovery

Produce (endive, spinach, tomato, and cantaloupe) was sampled from farms located in South Texas. Each produce commodity was sampled from two different farms with n=15 from each farm during one season. From each lettuce and spinach sample, 25 g were taken and homogenized with 225 ml of 0.1% peptone water (Becton, Dickinson and Co., Sparks, MD). From each melon and tomato, three 10 cm^2 portions were aseptically excised from the surface. For cantaloupe, the excisions were macerated in 99 ml of 0.1% peptone water, and for tomato, the excisions were macerated in 99 ml of Phosphate-Buffered Saline (PBS; Thermo-Fisher Scientific, Waltham, MA). The use of PBS for tomato samples was decided after conducting a brief preliminary experiment. Although there was no significant difference ($p>0.05$) in bacterial counts (aerobic plate count) between using PBS and peptone water as diluents for tomato samples, there was a difference in the pH values of tomato samples diluted with peptone water versus tomato samples diluted with PBS, and PBS had the closest to neutral pH of the two diluents for tomato samples. All samples were plated on microorganisms-appropriate non-selective, selective, and selective/differential media according to standardized methods: aerobic bacteria on tryptic soy agar (TSA; Becton, Dickinson and Co.), Lactic Acid Bacteria (LAB) on de Man, Rogosa, and Sharpe (MRS; Becton, Dickinson and Co.) agar,

yeasts/molds on Dichloran Rose Bengal Chloramphenicol agar (DRBC; Becton, Dickinson and Co.), enterococci on Kenner Faecal streptococcal agar (KF; Becton, Dickinson and Co.), and coliforms on Violet Red Bile agar (VRBA; Becton, Dickinson and Co.) *(49)*. TSA was incubated aerobically at 35°C for 24 h prior to enumeration. MRS was incubated anaerobically at 35°C for 48 h prior to enumeration. For VRBA and KF, 1-mL aliquots of each dilution was added to separate petri dishes. Ten ml of tempered (48°C) agar was added to each plate, plates swirled to allow mixing of agar and sample aliquots, and allowed to sit until solidified. Once plates were solidified, plates were overlayed with 8-10 ml of tempered agar. KF agar was used to overlay KF plates and VRBA was used for VRBA plates. KF and VRBA were both incubated aerobically at 35°C for 48 h prior to enumeration. DRBC was incubated aerobically and upright for 5 days at 25°C prior to enumeration. From each sample, four colonies from each selective medium using the Harrison Disc method for random selection of colonies were isolated and from tryptic soy agar 4-12 colonies were isolated by choosing colonies displaying different colony diameters, colors, and morphologies *(70)*. Each isolated colony was subjected to a battery of tests for biochemical identification including Gram-stain, oxidase test, catalase test, oxidative/fermentative (OF) Basal Glucose test, and other biochemical tests *(73, 132)*. After biochemical tests, isolates with identical biochemical results, isolated from the same medium, and the same commodity were grouped together. One isolate from each group was randomly selected for biochemical identification resulting in approximately 100 isolates from each produce commodity selected and identified using Vitek 2 (bioMérieux N.A., Durham, NC) at the Texas A&M Center for Food Safety (CFS; College Station, TX)

with the assistance of CFS personnel. The Vitek 2 uses specific reagent cards: GN (Gram-negative fermenting and non-fermenting bacilli), GP (Gram-positive cocci and non-sporulating bacilli), BCL (Gram-positive spore-forming bacilli), CBC (Corynebacteria), and YST (yeast and yeast-like organisms) *(110)*. These reagent cards have 64 wells, each containing an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances *(110)*. Each card has a pre-inserted transfer tube used for inoculation *(110)*. Prior to inoculation, isolates were streaked on TSA and incubated for 24 h at 35°C *(110)*. Following incubation, a sterile swab was used to transfer a sufficient number of cells of a pure culture from the streaked TSA plate and suspended in 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube (bioMérieux) *(110)*. The turbidity was adjusted according to the appropriate McFarland turbidity range for the reagent card (0.50-0.63 for GN and GP) and measured using a turbidity meter called the DensiChek (bioMérieux) *(110)*. The test tube containing the microorganism suspension was then placed into a cassette and the identification card was placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube *(110)*. The filled cassette was placed manually into the vacuum chamber station *(110)*. After vacuum was applied and air is re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells *(110)*. Inoculated cards were then be passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator *(110)*. All card types were incubated on-line at 35.5 $\pm$ 1.0ºC *(110)*. Each card was removed from the

carousel incubator once every 15 min, transported to the optical system for reaction readings, and then returned to the incubator until the next read time *(110)*. Data was collected at 15 min intervals during the entire incubation period *(110)*. A transmittance optical system interprets the test reactions using different wavelengths in the visible spectrum *(110)*. During incubation, each test reaction was read every 15 min to measure either turbidity or colored products of substrate metabolism *(110)*. Calculations were performed on raw data and compared to thresholds to determine reactions for each test. The unknown bio-pattern was then compared to the database of reactions for each taxon, and a numerical probability calculation is performed. The Vitek 2 device then assigned identification to the unknown organism *(110)*.

Statistical Analyses

Microbiological data (plate counts) was logarithmically transformed (base 10) before statistical analysis. All quantitative analyses was conducted using JMP® Pro v11.0.0 (SAS Institute Inc., Cary, N.C.). Statistical differences between means were analyzed using a one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) ($p<0.05$).

Results

Cantaloupes

Across both farms (n=30) in one season, the populations of aerobic bacteria, fungi, enterococci, lactic acid bacteria (LAB) and coliforms were 6.1±0.4, 4.9±0.5, 2.6±1.0, 5.0±0.8 and 4.3±0.6 $\log_{10}$ CFU/cm^2, respectively. For Farm 1 (n=15) the mean populations of aerobic bacteria, fungi, enterococci, LAB and coliforms were 5.9±0.5, 4.6±0.6, 2.8±1.1, 4.6±0.9 and 4.1±0.7 $\log_{10}$ CFU/cm^2 respectively (Figure 2). For Farm 2 (n=15) the populations of aerobic bacteria, fungi, enterococci, LAB and coliforms were 6.2±0.4, 5.2±0.3, 2.4±0.9, 5.4±0.5, and 4.6±0.5 $\log_{10}$ CFU/cm^2 respectively (Figure 2). Aerobic bacteria, fungi, enterococci, and coliforms were not different across both farms, ($p \geq 0.05$). However, the LAB population on cantaloupes harvested from Farm 2 was higher than Farm 1, ($p < 0.05$). For cantaloupe isolates, tests revealed that of approximately 565 isolates, the following genera were present on melon surfaces: *Bacillus* (8%), *Enterococcus* (20%), *Enterobacter* (19%), *Leifsonia* (11%), *Pantoea* (5%), *Sphingomonas* (5%), and *Staphylococcus* (10%) (Table 2). Overall across both farms, 44% Gram-negatives and 56% Gram-positive organisms were isolated (Figure 3). For Farm 1 cantaloupes, 44% Gram-negatives and 56% Gram-positives were isolated from the rind and 42% Gram-negatives and 58% Gram-positive organisms were isolated from Farm 2 cantaloupes (Figure 3).

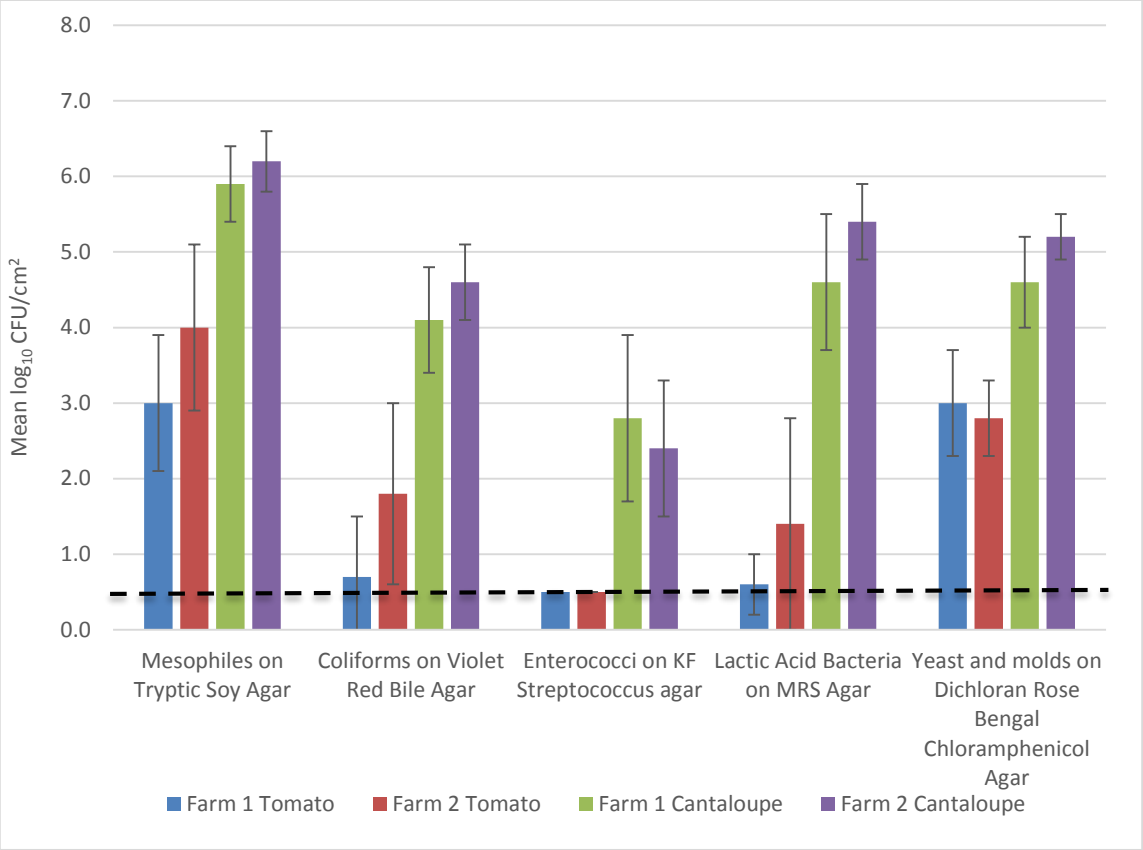

FIGURE 2. ***Native microbiota of tomatoes and cantaloupes sampled from two farms.*** Columns indicate mean $\log_{10}$ CFU/cm^2 of background microbiota and the error bars indicate standard deviation from sample means (n=15). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

TABLE 2. ***Cantaloupe-recovered bacterial isolates and numbers of isolates from sampled farms.***

Genus and species	Total Number of Isolates	Farm 1	Farm 2
Bacillus lentus	1	0	1
Bacillus vallismortis	43	5	38
Burkholderia cepacia	1	1	0
Buttiauxella agrestis	27	13	14
Corynebacterium minutissimum	1	1	0
Cronobacter sakazakii	27	17	10
Enterobacter cloacae	109	60	49
Enterococcus casseliflavus	4	3	1
Enterococcus faecalis	113	59	54
Enterococcus gallinarum	1	1	0
Escherichia hermannii	1	1	0
Gordonia spp.	2	0	2
Klebsiella pneumoniae ssp. *pneumoniae*	2	2	0
Kocuria kristinae	1	0	1
Leifsonia aquatica	62	36	26
Microbacterium spp.	15	11	4
Micrococcus lylae	1	1	0
Morganella morganii ssp. *sibonii*	3	0	3
Ochrobactrum anthropi	2	2	0
Pantoea spp.	30	21	9
Pseudomonas oryzihabitans	6	3	3
Rhizobium radiobacter	1	1	0
Serratia plymuthica	1	0	1
Sphingomonas paucimobilis	31	10	21
Staphylococcus gallinarum	5	4	1
Staphylococcus hominis ssp. *hominis*	1	0	1
Staphylococcus lentus	41	21	20
Staphylococcus sciuri	8	4	4
Staphylococcus xylosus	3	2	1
Streptococcus thoraltensis	7	6	1
Unable to be identified	19	12	7

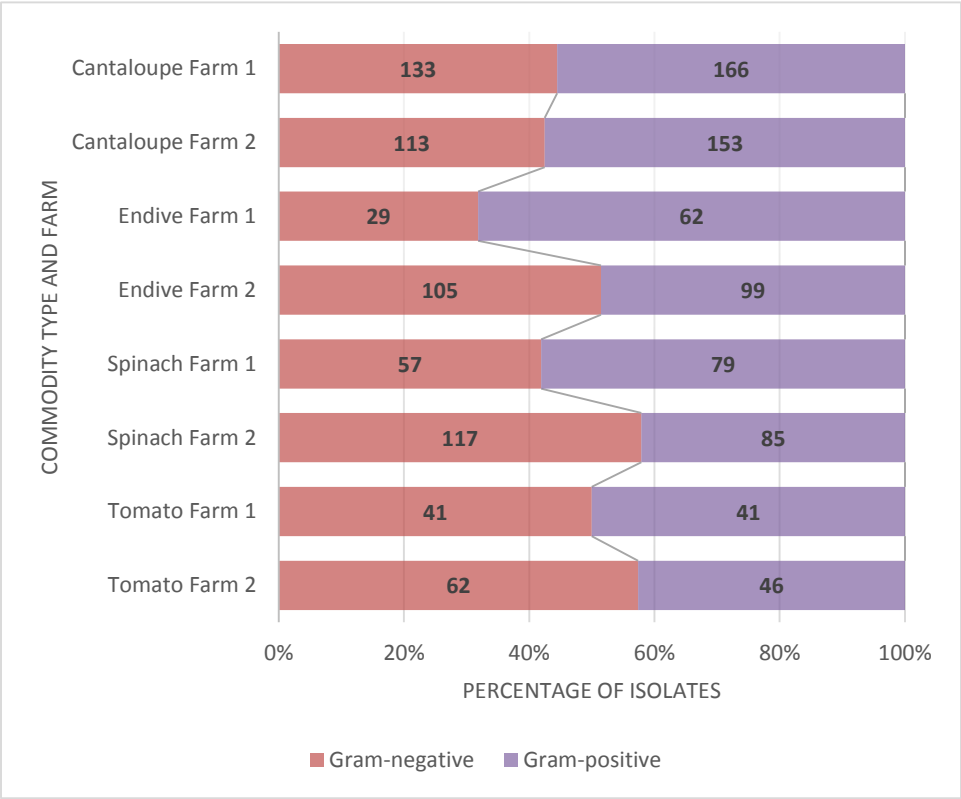

FIGURE 3. ***Percentage of Gram-positive and Gram-negative isolates from each farm source from each commodity type.*** Total number of isolates for each commodity, farm and Gram stain result are shown within the respective region.

Tomatoes

Across both farms (n=30) in one season, populations of aerobic bacteria, fungi, LAB and coliforms were 3.5±1.1, 2.9±0.6, 1.0±1.1 and 1.3±1.1 $\log_{10}$ CFU/cm^2, respectively. Numbers of enterococci for both farms remained below the detection limit (0.5 $\log_{10}$ CFU/cm^2). For Farm 1 (n=15) the mean populations of aerobic bacteria, fungi, LAB and coliforms were 3.0±0.9, 3.0±0.7, 0.6±0.4, and 0.8±0.8 $\log_{10}$ CFU/cm^2 respectively (Figure 2). For Farm 2 (n=15) the mean populations of aerobic bacteria, fungi, LAB and coliforms were 4.0±1.1, 2.8±0.5, 1.4±1.4, and 1.8±1.2 $\log_{10}$ CFU/cm^2 respectively (Figure 2). Coliforms, LAB, and fungi populations did not differ between tomato farms, ($p \geq 0.05$). However, aerobic bacteria numbers from Farm 2 were higher than Farm 1, ($p < 0.05$). For tomato recovered isolates, biochemical and Vitek tests revealed that from approximately 190 isolates, genera included: *Achromobacter* (6%), *Bacillus* (29%), *Enterobacter* (13%), *Micrococcus* (19%), *Sphingomonas* (21%), and *Staphylococcus* (5%) (Table 3). Overall across both farms, 54% Gram-negatives and 46% Gram-positive organisms were isolated (Figure 3). For Farm 1 tomatoes, 50% Gram-negatives and 50% Gram-positives were isolated from tomato surface and 54% Gram-negatives and 46% Gram-positive organisms were isolated from Farm 2 tomatoes (Figure 3).

TABLE 3. ***Tomato-recovered bacterial isolates and numbers of isolates from sampled farms.***

Genus and Species	Total Number of Isolates	Farm 1	Farm 2
Achromobacter denitrificans	11	0	11
Bacillus amyloliquefaciens	30	20	10
Bacillus licheniformis	25	10	15
Cronobacter sakazakii	2	2	0
Enterobacter cancerogenus	17	1	16
Enterobacter cloacae	7	5	2
Klebsiella pneumoniae ssp ozaenae	1	0	1
Leclercia adecarboxylata	8	0	8
Micrococcus leteus	19	9	10
Pantoea spp.	2	2	0
Pseudomonas oryzihabitans	1	0	1
Pseudomonas putida	1	0	1
Pseudomonas spp.	2	2	0
Pseudomonas stutzeri	4	0	4
Rhizobium radiobacter	2	1	1
Sphingomonas paucimobilis	40	25	15
Staphylococcus haemolyticus	1	1	0
Staphylococcus hominis ssp hominis	1	0	1
Staphylococcus sciuri	8	1	7
Unable to be identified	7	3	4

Spinach

Across both farms (n=30) in one season, mean populations of aerobic bacteria, fungi, enterococci, LAB and coliforms were 6.7±0.7, 5.3±0.6, 3.4±1.5, 4.7±1.3 and 5.5±0.9 $\log_{10}$ CFU/g, respectively. For Farm 1 (n=15) mean populations of aerobic bacteria, fungi, enterococci, LAB and coliforms were 6.2±0.6, 4.8±0.2, 2.2±0.9, 3.6±0.4 and 4.8±0.7 $\log_{10}$ CFU/g respectively (Figure 4). For Farm 2 (n=15) the mean populations of aerobic bacteria, fungi, enterococci, LAB and coliforms were 7.2±0.4, 5.9±0.3, 4.6±0.7, 5.8±0.8 and 6.1±0.5 $\log_{10}$ CFU/g respectively (Figure 4). Overall, populations from spinach-growing Farm 2 were significantly greater than Farm 1, ($p<0.05$), across all groups (aerobic bacteria, fungi, enterococci, LAB and coliforms). For spinach recovered isolates, biochemical and Vitek tests revealed that from approximately 339 isolates, genera recovered included: *Enterobacter* (8%), *Enterococcus* (18%), *Kocuria* (6%), *Pantoea* (13%), *Pseudomonas* (12%), *Sphingomonas* (12%), and *Staphylococcus* (8%) (Table 4). Overall across both farms, 51% Gram-negatives and 48% Gram-positive organisms were isolated (Figure 3). For Farm 1 spinach, 42% Gram-negatives and 58% Gram-positives were isolated from spinach leaf surface and 58% Gram-negatives and 42% Gram-positive organisms were isolated from Farm 2 spinach (Figure 3).

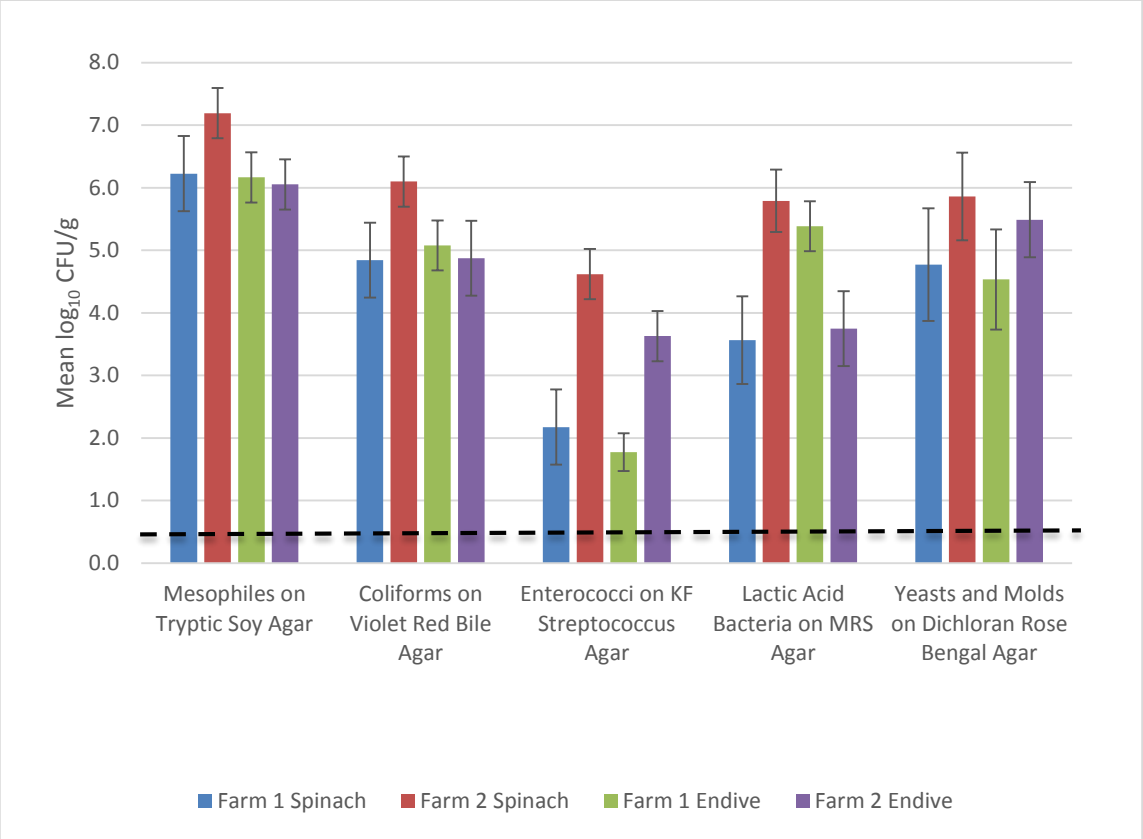

FIGURE 4. ***Native microbiota of spinach and endive sampled from two farms.*** Columns indicate mean $\log_{10}$ CFU/g of background microbiota and the error bars indicate standard deviations from sample means (n=15). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/g).

TABLE 4. ***Spinach-recovered bacterial isolates and numbers of isolates from sampled farms.***

Genus and species	Total Number of Isolates	Farm 1	Farm 2
Aerococcus viridans	3	3	0
Bacillus lentus	9	7	2
Enterobacter amnigenus 1	3	0	3
Enterobacter cancerogenus	3	0	3
Enterobacter cloacae complex	21	4	17
Enterococcus spp.	62	29	33
Erysipelothrix rhusiopathiae	17	5	12
Escherichia coli	2	1	0
Kocuria kristinae	21	4	17
Lactococcus garvieae	6	5	1
Listeria grayi	5	5	0
Pantoea agglomerans	8	8	0
Pantoea spp.	35	12	23
Pseudomonas aeruginosa	1	1	0
Pseudomonas fluorescens	12	2	10
Pseudomonas putida	19	10	9
Pseudomonas spp.	8	4	4
Rahnella aquatilis	6	0	6
Rhizobium radiobacter	5	3	2
Serratia marcescens	4	0	4
Serratia rubidaea	6	3	3
Sphingobacterium thalpophilum	1	0	1
Sphingomonas paucimobilis	40	17	23
Staphylococcus intermedius	23	10	13
Staphylococcus lentus	4	4	0
Staphylococcus warneri	1	0	1
Unable to be identified	8	1	7

Endive

Across both farms (n=30) in one season, the mean populations of aerobic bacteria, fungi, enterococci, LAB and coliforms were 6.1±0.4, 5.0±0.6, 2.7±1.2, 4.6±0.9 and 5.0±0.5 $\log_{10}$ CFU/g, respectively. For Farm 1 (n=15), the mean populations of aerobic bacteria, fungi, enterococci, LAB and coliforms were 6.2±0.3, 4.5±0.4, 1.8±0.8, 5.4±0.2 and 5.1±0.4 $\log_{10}$ CFU/g respectively (Figure 4). For Farm 2 (n=15), the mean populations of aerobic bacteria, fungi, enterococci, LAB and coliforms were 6.1±0.4, 5.5±0.4, 3.6±0.6, 3.7±0.6 and 5.5±0.4 $\log_{10}$ CFU/g respectively (Figure 4). Aerobic bacteria and coliforms did not significantly differ across both cantaloupe farms, ($p \geq 0.05$). However, the enterococci population from Farm 2 endive were significantly higher than Farm 1, ($p < 0.05$). The fungi and LAB populations from Farm 1 endive was significantly more than Farm 2, ($p < 0.05$). For endive recovered isolates, biochemical and Vitek tests revealed that from approximately 295 isolates, genera recovered included: *Aerococcus* (8%), *Bacillus* (7%), *Enterococcus* (12%), *Kocuria* (8%), *Lactococcus* (7%), *Pantoea* (17%), *Pseudomonas* (3%), and *Sphingomonas* (23%) (Table 5). Overall across both farms, 45% Gram-negatives and 55% Gram-positive organisms were isolated (Figure 3). For Farm 1 endive, 32% Gram-negatives and 68% Gram-positives were isolated from endive and 51% Gram-negatives and 49% Gram-positive organisms were isolated from Farm 2 endive (Figure 3).

TABLE 5. ***Endive-recovered bacterial isolates and numbers of isolates from sampled farms.***

Genus and species	Total Number of Isolates	Farm 1	Farm 2
Aerococcus viridans	24	0	24
Alloiococcus otitis	3	3	0
Bacillus spp.	22	17	5
Brevundimonas spp.	1	0	1
Enterococcus casseliflavus	7	3	4
Enterococcus spp.	27	27	0
Escherichia coli	1	0	1
Kocuria kristinae	24	0	24
Lactococcus garvieae	22	3	19
Listeria grayi	1	0	1
Micrococcus spp.	1	1	0
Paenibacillus polymyxa	2	2	0
Pantoea agglomerans	4	4	0
Pantoea spp.	45	4	41
Providencia rettgeri	1	1	0
Pseudomonas aeruginosa	1	1	0
Pseudomonas oryzihabitans	7	4	3
Pseudomonas spp.	2	2	0
Rahnella aquatilis	7	0	7
Rhizobium radiobacter	2	1	1
Sphingobacterium thalpophilum	1	0	1
Sphingomonas paucimobilis	67	18	49
Staphylococcus aureus	1	0	1
Staphylococcus warneri	5	2	3
Vagococcus fluvialis	5	0	5
Unable to be identified	12	2	10

Discussion

The surfaces of produce can differ greatly in regards to tissue, structure, pH, and microorganisms native to the commodity/produce item *(92)*. The exact microbial composition of fruits and vegetables cannot be anticipated because it is possible for almost any organism to be present at some point in time; however, there are certain microorganisms that are commonly present in both fruits and vegetables *(17)*. The most numerous bacteria typically found on the surfaces of leafy greens are usually Gram-negative bacteria belonging to either the family Enterobacteriaceae or Pseudomonadaceae *(92)*. For the current study, the percentages of Gram-negatives and Gram-positives differed per farm. However, for spinach, overall, there were more Gram-negatives recovered (51%) versus Gram-positive bacteria. For both leafy green commodities the second highest population group identified were coliforms, Gram-negative oxidase-negative rods capable of fermenting lactic acid and gas within 48 h at 35°C. The highest population group was the aerobic bacteria, which can include both Gram-positive and Gram-negative organisms. Carlin *et al. (27)* also reported isolating *Enterobacter* ssp., *Pseudomonas* spp., and *Rahnella aquatilis* from the surface of endive, however, the frequency of isolation of these microorganisms were not described in the study. Fowler *et al. (58)* reported that mesophilic bacteria counts typically range from 5-7 $\log_{10}$ CFU/g for leafy greens. Ercolani *(50)* reported the populations of native microorganisms on leaf lettuce to be 7.8 $\log_{10}$ CFU/100g for aerobic bacteria, 4.8 $\log_{10}$ CFU/100 g for coliforms, and 3.4 $\log_{10}$ CFU/100g for fecal streptococci. Ailes *et al. (5)* reported the population of native microorganisms on spinach to be 5.8 ± 0.2 $\log_{10}$ CFU/g

for aerobic bacteria, 1.5 ± 0.2 $\log_{10}$ CFU/g for coliforms and 2.1 ± 0.2 $\log_{10}$ CFU/g for *Enterococcus*. Overall, in the current study, the results of endive and spinach were within this range.

The microflora of fruits can differ from that of leafy greens *(17).* Shi *et al. (124)* reported isolating similar microorganisms to those isolated in this study from tomatoes such as *Pantoea* spp., *Bacillus* spp., *Pseudomonas* spp., and *Enterobacter* spp; all were reported as commonly isolated, however, exact frequency was not mentioned. Bracket *(18)* also reported isolating *Bacillus* ssp.(1%), *Pseudomonas* spp. (13%), *Micrococcos luteus* (1%), and *Enterobacter* spp. (11%). Bracket also reported the population of native microorganisms on tomatoes to be 4-5 $\log_{10}$ CFU/g for aerobic bacteria, 3-4 $\log_{10}$ CFU/g for LAB, and 1-2 $\log_{10}$ CFU/g for yeasts and molds. In this current study, there were lower numbers of LAB, yet higher numbers of yeast and molds when compared to previous research. However, there were undetectable numbers of enterococci and lower numbers of all population groups for tomatoes when compared to results on the surfaces of cantaloupes. Melons in general have a pH close to neutral, 6.2-6.5, while tomatoes have a pH of 4.0-4.5*(57)*. The overall higher numbers on the surfaces of cantaloupes versus the surface of tomatoes could be attributed to the differences in pH. Nonetheless, differences in fertilizers used and environmental conditions at time of growth and harvest could also contribute to the differences observed *(17, 18)*. Materon *et al. (98)* reported average microbial populations for aerobic bacteria, fungi, and total coliforms on the surface of cantaloupes to be 5.9, 3.6, and 3.2 $\log_{10}$ CFU/cm^2 respectively. These levels were similar to the results of this study. Ukuku *et al.* reported the populations of

native microorganisms on cantaloupes to average 6.82 $\log_{10}$ CFU/cm^2 for total mesophilic aerobes, 2.90 $\log_{10}$ CFU/cm^2 for yeast and molds, 4.00 $\log_{10}$ CFU/cm^2 for Enterobacteriaceae, and 3.86 $\log_{10}$ CFU/cm^2 for LAB *(131)*. Overall, the results of this study are in line with previous published research. Furthermore, identifying these organisms and how these interact with foodborne pathogens could play a future role in increasing produce safety. Understanding how these organisms interact with pathogens, could allow for their utilization in pathogen interventions on fresh produce such as bio-control interventions.

CHAPTER V

IDENTIFYING ABILITY OF NATURALLY OCCURING MICROORGANISMS TO INHIBIT PATHOGENS ON PRODUCE SURFACES

Materials and Methods

Preparation of Pathogens for Assay of In Vitro *Inhibition by Microbial Pathogen Antagonists*

Rifampicin-resistant (Rif^R) isolates of *Salmonella enterica* serovar Typhimurium LT2 and *E. coli* O157:H7 American Type Culture Collection (ATCC) 700728 were obtained from the Department of Animal Science Food Microbiology Laboratory culture collection at Texas A&M University (College Station, TX). Cultures were maintained on tryptic soy agar (TSA; Becton, Dickinson and Co., Sparks, Md.) slants at 5°C. Working cultures were obtained by transferring a loopful of culture from TSA slants to 10 ml of tryptic soy broth (TSB; Becton, Dickinson and Co.) and incubating aerobically without agitation at 35°C for 24 h. *Salmonella* Typhimurium and *E. coli* O157:H7 strains were inoculated from TSA slants into 10 ml of TSB and incubated at 35°C for 24 h as described previously. After 24 h, a loop of each strain was transferred to fresh TSB for each and incubated at 35°C. After incubation, 10 ml of each culture were transferred to sterile 15 ml conical centrifuge tubes (Thermo-Fisher Scientific, Waltham, Mass.) for each culture. The suspension was then washed by centrifugation at 2191 x g in a Jouan B4i centrifuge (Thermo-Fisher Scientific) for 15 min at 22°C. Resulting bacterial pellets were suspended in 10 ml of Phosphate-buffered saline (PBS; EMD Millipore, Billerica, Mass.).

Centrifugation was repeated identically twice for 15 min at 22°C. The final re-suspension was in 10 ml of 0.1% peptone water (Becton, Dickinson and Co.) for each strain.

Pathogen Antagonism Assay In Vitro

Antagonism of produce surface-recovered isolates against pathogens was determined using the Agar Spot method *(56)*. Working cultures of the pathogen-antagonist candidate isolates were obtained by transferring a loopful of culture from -80°C cryostorage to either MRS broth (Becton, Dickinson and Co.) or TSB for non-MRS isolated colonies and incubated aerobically without agitation at 35°C for 24 h. Isolates were spotted (1 µL) onto MRS agar for isolates from MRS or TSA for non-MRS isolated colonies and incubated aerobically at 35°C for 24 h. *Salmonella* Typhimurium and *E. coli* O157:H7 strains were inoculated from TSA slants into 10 ml of TSB and incubated at 35°C for 24 h as described previously. After 24 h, a loop of each strain was transferred to fresh TSB for each and incubated at 35°C. Pathogens were prepared as described previously. After 24 h, 9 ml of molten TSA tempered to 48°C were seeded with prepared *S.* Typhimurium or *E. coli* O157:H7 to 6.0 $\log_{10}$ CFU/ml and then overlaid onto spotted plates. The plates were incubated at 35°C for 24 h. Inhibition halos produced by pathogen-antagonizing isolates were measured using a caliper. Horizontal and vertical planes were measured by caliper and averaged to generate a final total diameter that did not include the diameter of the pathogen-antagonizing isolate *(56)*. Therefore, isolates producing a mean inhibition halo $\geq$ 1.0 mm were designated antagonistic to pathogens *(56)*. The experiment was completed in duplicate with a total of two replications (n=4).

Pathogen Antagonism on Produce Surface Preliminary Experiments

Growth Curves

The objective of the following experiment was to determine growth rates of biosafety level 1 strains when compared to biosafety level 2 strains. Produce-recovered isolates of *Salmonella enterica* subsp. *enterica* serovars Montevideo and Poona, *Salmonella* Typhimurium American Type Culture Collection 13311 (ATCC, Manassas, VA, USA), *Salmonella* Typhimurium LT2 ATCC 700720 and strains of *E. coli* O157:H7 (designated P41, P8, and E34; beef cattle carcass isolates) and ATCC No. 700728, all resistant to 100 mg/L rifampicin, were obtained from the Food Microbiology Laboratory culture collection in the Department of Animal Science (Texas A&M University, College Station, TX, USA). Cultures were maintained on TSA slants at 5 °C. Working cultures were obtained as previously described. Biochemical identification of pathogens was conducted using Enterotube™ II (Becton, Dickinson and Co.) according to manufacturer instructions. *Salmonella* and *E. coli* O157:H7 strains were inoculated from TSA slants into 10 mL of TSB and incubated as previously described. Each strain was individually cultured in 10 ml TSB and incubated at 35°C for 24 h. Each culture was transferred to a conical centrifuge tube and cells harvested by centrifugation at 2191 x g in a Jouan B4i centrifuge (Thermo-Fisher Scientific, Inc.) for 15 min at 22°C. The supernatant was then discarded and the pellet resuspended in 10 ml of 0.1% peptone water. This procedure was repeated twice. Dilutions were made for each culture using 0.1% peptone water and 0.1 ml of the 1:10,000 dilution was transferred to tubes containing 9.9 ml of fresh TSB to achieve an initial concentration of 2.0 $\log_{10}$ CFU/ml.

Inoculated tubes were incubated aerobically at 35°C. At each time point a tube per each strain was removed and pour plated on tryptic soy agar (TSA) at the following hour points: 0, 1, 2, 4, 6, 8, 10, 12, 18, and 24. All plates were incubated aerobically without agitation at 35°C for 24 h. This experiment was completed with triplicate replications (n=3).

S. Typhimurium LT2 and *E. coli* O157:H7 Preliminary Growth Experiment on Produce Surface

S. Typhimurium LT2 and *E. coli* O157:H7 were prepared and washed via centrifugation as previously described. Produce (spinach, tomatoes, cantaloupes, and romaine lettuce) were purchased and washed in sterile running tap water and surface disinfected with 70% ethanol *(7)*. Due to availability, romaine lettuce was used in place of endive for this preliminary experiment. After drying for 1 h, 10 cm^2 pieces were excised using a flame-sterilized sterile cork borer and placed in sterile Petri dishes (VWR, Radnor, PA) in a Biological Safety Cabinet Class II A/B3 (NuAire, Plymouth, MN). Each sample was spot inoculated with ten 10 µl spots of one pathogen. A sample was spotted with 10 µl of 0.1% peptone water to serve as the negative control sample. Samples were left to dry at room temperature in the Biological Safety Cabinet Class II A/B3 for 1 h. At 0, 12, 24, and 48 h of storage, *S.* Typhimurium LT2 and *E. coli* O157:H7 were enumerated on TSA + Rifampicin (TSAR, 0.1 g/L; Sigma-Aldrich Co., St. Louis, MO). At each time point, three 10 cm^2 pieces were used for enumeration of the pathogens on the surface. The three 10 cm^2 were first placed into a polypropylene

wide-mouth bottle containing 99 ml of 0.1% peptone water and gently mixed by 15 inversion movements. Loosely attached cells in the peptone rinse were enumerated on TSAR *(25, 48)*. After this, for enumeration of strongly attached cells, the three 10 cm^2 samples were removed via flame-sterilized forceps from the bottle and transferred to stomacher bag with 99 ml of 0.1% peptone water and pummeled using a stomacher. After stomaching, the samples were enumerated on TSAR. Plates were incubated aerobically without agitation at 35°C for 24 h.

Preliminary Experiment for Pathogen Enumeration Using Selective/Differential Media Versus Selective Media and to Test Co-inoculation of Pathogens

S. Typhimurium LT2 and *E. coli* O157:H7 were prepared and washed via centrifugation as previously described. An isolate demonstrating antagonism towards these pathogens, *Pediococcus acidilacti*, from a previous study was used and two endive-recovered isolates (*Staphylococcus warneri* and *Lactococcus garvieae*) were also used along with a tomato-recovered isolate, *Leclercia adecarboxylata*. Working cultures of the antagonistic isolates were prepared by transferring a loopful of culture to 10 ml of MRS broth and incubating aerobically without agitation at 35°C for 24 h. After 24 h, a loop of each strain was transferred to fresh MRS for each and incubated at 35°C. After incubation of strains, 10 ml of each culture was transferred to sterile 15 ml conical centrifuge tubes for each culture. The suspension was then washed by centrifugation at 2191 x g in a Jouan B4i centrifuge for 15 min at 22°C. Bacterial pellets were re-suspended in 10 ml of PBS.

Centrifugation was repeated identically twice for 15 min at 22°C. The final re-suspension was in 10 ml of 0.1% peptone water.

Produce (Romaine Lettuce and Roma Tomato) was purchased at a local grocery store and washed in sterile running tap water and surface disinfected with 70% ethanol. After drying for 1 h, 10 cm^2 pieces were taken using a sterile cork borer and placed in sterile Petri dishes in a Biological Safety Cabinet Class II A/B3. Each sample was spotted with ten 10 µl spots of one produce antagonistic isolate, *L. adecarboxylata and P. acidilacti* for tomato samples and *S. warneri* and *L. garvieae* for lettuce samples. The samples were then stored at 22°C for 24 h. After 24 h, a set of samples was inoculated with ten 10 µl spots of one pathogen individually. Another set of samples was inoculated with a cocktail consisting of both pathogens. Samples were left at room temperature in the Biological Safety Cabinet Class II A/B3 for 1 h to allow the antagonistic isolates time to attach. At 0, 6 and 24 h. *S.* Typhimurium LT2 and *E. coli* O157:H7 were enumerated on selective TSA + Rifampicin (TSAR, 0.1 g/L; Sigma-Aldrich Co., St. Louis, MO) for samples containing one pathogen and enumerated on lactose-sulfite-phenol red-rifampicin agar (LSPR) prepared as outlined by Castillo *et al. (29)* for the selective and differential identification and enumeration of both pathogens. For each time point, loose and strong attachment of cells was determined by the procedure outlined by Cabrera-Diaz *et al. (25)*. At each time point, three 10 cm^2 pieces were used for enumeration of the total numbers of each pathogen. Three 10 cm^2 pieces were placed into a polypropylene wide-mouth bottle containing 99 ml of 0.1% peptone water and gently mixed by 15 inversion movements. Loosely attached cells in the peptone rinse were enumerated on either TSAR or LSPR

depending on inoculum. For enumeration of strongly attached cells, the three cm^2 samples were removed from the plastic bottle, and transferred to a stomacher bag with 99 ml of 0.1% PW. Then the sample was pummeled using a stomacher and enumerated on TSAR or LSPR depending on inoculum. Plates were incubated aerobically without agitation at 35°C for 24 h. The proportion of the total bacterial population which was physically attached to the surface was calculated by dividing the CFU/cm^2 of strongly attached cells by the total CFU/cm^2 of attached and loosely attached *(25).*

Preliminary Experiment for Determining the Antagonistic Isolate Inoculation Concentration and Pathogen Inoculation Concentration for Pathogen Antagonism on Produce Surfaces Experiments

Methods for this experiment were adapted from a previous study *(51).* Working cultures of the antagonistic isolates were obtained by transferring a loopful of culture from -80°C cryostorage to either MRS or TSA for non-MRS isolated colonies and incubated aerobically without agitation at 35°C for 24 h. *S.* Typhimurium LT2 and *E. coli* O157:H7 strains were inoculated from TSA slants into 10 ml of TSB and incubated at 35°C for 24 h as described previously. After 24 h, a loop of each strain was transferred to fresh TSB for each and incubated at 35°C. Pathogens were prepared as described previously. Isolates were also washed using the same previously described method as the pathogens. After 24 h, 9 ml molten MRS or TSA for non-MRS isolated colonies were tempered to 48°C and seeded with a prepared antagonistic isolate to a concentration of 6.0, 7.0 or 8.0 $\log_{10}$ CFU/ml. After solidification, 1 ml of 6.0 $\log_{10}$ CFU/ml of pathogen was spread on the

surface. Plates were created in duplicate. One set of plates was incubated at 22°C and another at 35°C aerobically for 24 h. MRS and TSA plates without antagonistic isolates were prepared as controls. After 24 h, plates were visually inspected for growth. The experiment was completed with duplicate replications (n=2).

Preliminary experiment to Determine Sampling Method for Cantaloupes and Tomatoes for Pathogen Antagonism on Produce Surfaces Experiments

S. Typhimurium LT2 and *E. coli* O157:H7 were prepared and washed via centrifugation as previously described. An isolate demonstrating antagonism towards these pathogens, *Pediococcus acidilacti*, from a previous study was used and two cantaloupe-recovered isolates (*Staphylococcus xylosus* and *Enterococcus casseliflavus*) were also used along with a tomato-recovered isolate, *Leclercia adecarboxylata*. Working cultures of the antagonistic isolates were prepared by transferring a loopful of culture to 10 ml MRS broth and incubating aerobically without agitation at 35°C for 24 h. After 24 h, a loop of each strain was transferred to fresh MRS for each and incubated at 35°C. After incubation of strains, 10 ml of each culture was transferred to sterile 15 ml conical centrifuge tubes for each culture. The suspension was then washed by centrifugation at 2191 x g in a Jouan B4i centrifuge for 15 min at 22°C. Bacterial pellets were re-suspended in 10 ml of PBS. Centrifugation was repeated identically twice for 15 min at 22°C. The final re-suspension was in 10 ml of 0.1% peptone.

Produce (Cantaloupes and Roma Tomato) was purchased at a local grocery store and washed in sterile running tap water and surface disinfected with 70% ethanol. After

drying for 1 h, 10 cm^2 pieces were taken using a sterile cork borer and placed in sterile Petri dishes in a Biological Safety Cabinet Class II A/B3 for the "SLICE" samples. Produce remained intact for the "WHOLE" samples. The SLICE samples were spotted with ten 10 µl spots of one produce antagonistic isolate, *L. adecarboxylata* or *P. acidilacti* for tomato samples, and *S. xylosus* or *E. casseliflavus* for cantaloupe samples. The SLICE samples were then stored at room temperature for 24 h. The whole intact fruits, cantaloupes and tomatoes, were spot inoculated with ten 10 µl of one produce antagonistic isolate in three separate areas on the intact fruit. After inoculation, the whole intact fruits were stored at room temperature for 24 h. After 24 h, the SLICE samples was inoculated with ten 10 µl spots of the pathogen cocktail. Also after 24 h, the intact fruits were inoculated with ten 10 µl spots of the pathogen cocktail. All fruit was left at room temperature for 1 h to allow time for the pathogens to attach. At 0 and 24 h *Salmonella* and *E. coli* O157:H7 were enumerated on selective/differential LSPR. For each time point, loose and strong attachment of cells was determined by the procedure outlined by Cabrera-Diaz *et al. (25).* At each time point, three 10 cm^2 pieces of the SLICE samples were used for enumeration of the total numbers of each pathogen. At each time point for the WHOLE samples, the three 10 cm^2 inoculated areas on the intact fruits were excised using the flame sterilized cork borer. The three 10 cm^2 for each sample were placed into polypropylene wide-mouth bottles containing 99 ml of 0.1% peptone water and gently mixed by 15 inversion movements. Loosely attached cells of pathogens in the peptone rinse were enumerated on LSPR. For enumeration of strongly attached cells, the three cm^2 samples were removed from the plastic bottle, and transferred to a stomacher bag with 99 ml of

0.1% peptone water and pummeled using a stomacher and enumerated on LSPR. Plates were incubated aerobically at 35°C for 24 h. The proportion of the total bacterial population physically attached to the surface was calculated by dividing the CFU/cm^2 of strongly attached cells by the total CFU/cm^2 of attached and loosely attached *(25)*.

Pathogen Antagonism on the Surface of Leafy Greens

This procedure was adapted from Alegre *et al. (4)* and Perez *et al. (55)*. *S.* Typhimurium LT2, *E. coli* O157:H7, and all epiphytic isolates demonstrating antagonism towards pathogens *in vitro* were washed as described previously. Unwashed and unwaxed hand harvested spinach and unwaxed and unwashed endive was purchased and washed in sterile water and surface disinfected with 70% ethanol. After drying for 1 h, 10 cm^2 pieces were taken using a sterile cork borer and placed in sterile Petri dishes in a Biological Safety Cabinet Class II A/B3. Each sample was spotted with ten 10 µl spots of one antagonistic epiphytic isolate at a concentration of 7.0 $\log_{10}$ CFU/ml. Previously determined antagonistic epiphytic isolates originally isolated from spinach were used on spinach samples and antagonistic epiphytic isolates originally isolated from endive were used for endive samples. After 24 h, ten 10 µl of a suspension containing *S.* Typhimurium LT2 and *E. coli* O157:H7 at a concentration of 5.0 $\log_{10}$ CFU/ml were spotted on the same location where the antagonistic isolate had been placed. Three 10 cm^2 pieces with only the pathogen suspension, three 10 cm^2 pieces with only the antagonistic epiphytic isolate, and three 10 cm^2 pieces with neither pathogen nor antagonistic epiphytic isolate served as controls (positive, negative controls,

respectively). Samples were stored at 25°C. At 0, 6, 12, and 24 h of storage, three 10 cm^2 pieces of the controls were placed in a stomacher bag with 99 ml of 0.1% peptone water, and the bag stomached (230 rpm) for 1 min. *Salmonella* and *E. coli* O157:H7 were enumerated on LSPR, and the antagonistic epiphytic isolate were enumerated on MRS or TSA for non-MRS isolated colonies. Three 10 cm^2 pieces without pathogens or antagonistic epiphytic isolates were plated on MRS agar, TSA, and LSPR to check for presence of other organisms. Loose and strong attachment of cells were determined by the procedure outlined by Cabrera-Diaz *et al. (25).* At each time point, three 10 cm^2 pieces were used for enumeration of the total numbers of each pathogen and isolate on the surface. Three 10 cm^2 were placed into polypropylene wide-mouth bottles containing 99 ml of 0.1% peptone water and gently mixed by 15 inversion movements. Loosely attached cells in the peptone rinse were enumerated on the previously described media corresponding to the isolate and pathogen being tested. For enumeration of strongly attached cells, the three 10 cm^2 samples were removed from the bottle, and transferred to stomacher bags with 99 ml of 0.1% peptone water and pummeled using a stomacher and enumerated on the previously described media corresponding to the isolate and the pathogen being tested. Plates were incubated aerobically without agitation at 35°C for 24 h. The experiment was repeated for a total of three replications. The proportion of the total bacterial population physically attached to the surface was calculated by dividing the CFU/cm^2 of strongly attached cells by the total CFU/cm^2 of attached and loosely attached *(25, 48)*.

Pathogen Antagonism on the Surface of Tomatoes and Cantaloupes

This procedure was adapted from Alegre *et al. (4)* and Perez *et al. (55). S.* Typhimurium LT2, *E. coli* O157:H7, and all epiphytic isolates demonstrating antagonism towards pathogens *in vitro* were washed as described previously. Unwaxed and unwashed tomatoes and cantaloupes were purchased and washed in sterile water and surface disinfected with 70% ethanol. After drying for 1 h, each intact fruit was spotted with ten 10 µl spots of one antagonistic epiphytic isolate in three separate 10 cm^2 areas for each isolate at a concentration of 7.0 $\log_{10}$ CFU/ml. Previously determined antagonistic epiphytic isolates originally isolated from tomatoes were used on tomato samples and antagonistic epiphytic isolates originally isolated from cantaloupes were used for cantaloupe samples. After 24 h, ten 10 µl of a suspension containing *S.* Typhimurium LT2 and *E. coli* O157:H7 at a concentration of 5.0 $\log_{10}$ CFU/ml was spot inoculated on the same location where the antagonistic isolate had been placed. Three 10 cm^2 areas with only the pathogen suspension, three 10 cm^2 areas with only the antagonistic epiphytic isolate, and three 10 cm^2 pieces with neither pathogen nor antagonistic epiphytic isolate served as controls (positive, negative controls, respectively) for each time point. Intact fruits were stored at 25°C. At 0, 6, 12, and 24 h of storage, three 10 cm^2 of each inoculated area containing both pathogens and antagonistic isolate, and three 10 cm^2 of each control were aseptically excised. At each time point three 10 cm^2 pieces of the controls were placed in a stomacher bag with 99 ml of 0.1% peptone water, and the bag stomached (230 rpm) for 1 min. *Salmonella* and *E. coli* O157:H7 were enumerated on LSPR, and the antagonistic epiphytic isolates were

enumerated on MRS or TSA for non-MRS isolated colonies. Three 10 cm^2 pieces without pathogens or antagonistic epiphytic isolates were plated on MRS agar, TSA, and LSPR to check for presence of other organisms. Loose and strong attachment of cells were as previously described *(25).* At each time point, three 10 cm^2 pieces were used for enumeration of the total numbers of each pathogen and isolate on the surface. Three 10 cm^2 were placed into polypropylene wide-mouth bottles containing 99 ml of 0.1% peptone water and gently mixed by 15 inversion movements. Loosely attached cells in the peptone rinse were enumerated on the previously described media corresponding to the isolate and pathogen being tested. For enumeration of strongly attached cells, the three 10 cm^2 samples were removed from the bottle, and transferred to stomacher bags with 99 ml of 0.1% peptone water and pummeled using a stomacher and enumerated on the previously described media corresponding to the isolate and the pathogen being tested. Plates were incubated aerobically without agitation at 35°C for 24 h. The experiment was repeated for a total of three replications. The proportion of the total bacterial population which was physically attached to the surface was calculated as previously described *(25, 48).*

Statistical Analyses

Microbiological data (plate counts) was logarithmically transformed (base 10) before statistical analysis. All quantitative analyses was conducted using JMP® Pro v11.0.0 (SAS Institute Inc., Cary, N.C.). Statistical differences between means were analyzed using a one-way analysis of variance (ANOVA) and Dunnet's t-test ($p < 0.05$)

for S_R and Tukey's Honestly Significant Difference (HSD) ($p < 0.05$) for total $\log_{10}$ CFU/cm^2 analyses.

Results

Antagonism Assay In Vitro

Isolates Recovered From Cantaloupes

Overall, 3.7% of the cantaloupe-recovered isolates demonstrated antagonistic activity against *S.* Typhimurium LT2, and 1.9% exhibited antagonistic activity against *E. coli* O157:H7 (Table 6). The majority of isolates that exhibited antagonistic activity against both pathogens were Gram-positive, non lactic acid bacteria (54.5%) (Table 7).

TABLE 6. ***Total produce isolates and the percentages of isolates from each commodity inhibitory towards*** **S.** ***Typhimurium LT2 and*** **E. coli** ***O157:H7***[a]

Produce	Total Number of Isolates	% Inhibitory to *S.* Typhimurium LT2	% Inhibitory to *E. coli* O157:H7
Cantaloupe	565	3.7	1.9
Tomato	190	6.8	4.0
Spinach	338	7.1	6.2
Endive	295	17.3	17.3

[a]Isolates producing a mean inhibition halo ≥ 1.0 mm in the *in vitro* antagonism assay were designated antagonistic to pathogens.

TABLE 7. *Produce isolates inhibitory towards* S. *Typhimurium LT2 and* E. coli *O157:H7 and the number of each corresponding to each classification*[a]

Produce	Coliform	Gram-negative, non coliform	Lactic acid bacteria	Gram-positive, non lactic acid bacteria
Cantaloupe	1	0	4	6
Tomato	4	0	0	3
Spinach	3	1	4	11
Endive	1	0	32	17

[a]Isolates producing a mean inhibition halo ≥ 1.0 mm in the *in vitro* antagonism assay were designated antagonistic to pathogens.

The majority of isolates exhibiting antagonistic activity against *S.* Typhimurium and *E. coli* O157:H7 were of the genus S*taphylococcus* (47.6 and 45.5%, respectively) (Table 8). Eighty-five point seven (85.7) and 81.8% of isolates exhibiting antagonistic activity against *S.* Typhimurium LT2 and *E. coli* O157:H7, respectively, were Gram-positive bacteria (Table 9). *Staphylococcus* cantaloupe-recovered isolates exhibited the greatest antagonistic activity against both pathogens (Table 9). *Staphylococcus xylosus* demonstrated the greatest antagonistic activity against *S.* Typhimurium LT2 (mean inhibition diameter 12.6 ± 2.6 mm); *Staphylococcus gallinarum* also exhibited similar antagonistic activity (mean inhibition zone diameter 12.3 ± 7.7 mm) against *S.* Typhimurium LT2 (Table 9). *Staphylococcus xylosus* also demonstrated the greatest antagonistic activity (mean inhibition zone diameter 6.0 ± 2.1 mm) along with *Staphylococcus gallinarum* (mean inhibition zone diameter 5.5 ± 1.8 mm) against *E. coli* O157:H7 (Table 9).

TABLE 8. ***Genera and species of cantaloupe-recovered isolates and the percentages of isolates inhibitory towards*** **S.** ***Typhimurium LT2 and*** **E. coli** ***O157:H7***

Microorganism	No. of isolates	% Inhibitory to *S.* Typhimurium	% *Inhibitory to E. coli* O157:H7
Bacillus lentus	*1*	*0.0*	*0.0*
Bacillus vallismortis	*43*	*0.0*	*0.0*
Burkholderia cepacia	*1*	*0.0*	*0.0*
Buttiauxella agrestis	*27*	*0.0*	*0.0*
Corynebacterium minutissimum	*1*	*0.0*	*0.0*
Cronobacter sakazakii	*27*	*0.0*	*0.0*
Enterobacter cloacae	*109*	*0.9*	*0.0*
Enterococcus casseliflavus	*4*	*25.0*	*25.0*
Enterococcus faecalis	*113*	*0.0*	*0.0*
Enterococcus gallinarum	*1*	*0.0*	*0.0*
Escherichia hermannii	*1*	*100.0*	*100.0*
Gordonia spp.	*2*	*0.0*	*0.0*
Klebsiella pneumoniae ssp. pneumoniae	*2*	*50.0*	*0.0*
Kocuria kristinae	*1*	*0.0*	*0.0*
Leifsonia aquatica	*62*	*0.0*	*0.0*
Microbacterium spp.	15	0.0	0.0
Micrococcus lylae	1	0.0	0.0
Morganella morganii ssp. *sibonii*	3	0.0	0.0
Ochobactrum anthopi	2	0.0	0.0
Pantoea spp.	30	0.0	0.0
Pseudomonas oryzihabitans	6	0.0	0.0
Rhizobium radiobacter	1	0.0	0.0
Serratia plymuthica	1	0.0	0.0
Sphingomonas paucimobilis	31	0.0	0.0
Staphylococcus gallinarum	5	80.0	60.0
Staphylococcus hominis ssp. *hominis*	1	100.0	100.0
Staphylococcus lentus	41	4.9	0.0
Staphylococcus sciuri	8	37.5	0.0
Staphylococcus xylosus	3	66.7	33.3
Streptococcus thoraltensis	7	85.7	57.1
Unable to be identified	19	5.3	5.3

TABLE 9. ***Isolates recovered from cantaloupes antagonistic to* S. *Typhimurium LT2 and* E. coli *O157:H7 and the measurement of the inhibition zone*[a]**

Microorganism	Lab ID	*S.* Typhimurium LT2 Zone of Inhibition (mm) Mean	Standard Deviation	*E. coli* O157:H7 Zone of Inhibition (mm) Mean	Standard Deviation
Enterobacter cloacae	98	2.0^{e}	0.2	NA	NA
Enterococcus casseliflavus	138	8.0^{abcd}	2.2	4.3^{a}	1.0
Escherichia hermannii	112	4.4^{bcde}	0.5	2.2^{a}	0.4
Klebsiella pneumoniae ssp *pneumoniae*	120	1.7^{e}	0.3	NA	NA
Staphylococcus gallinarum	110	12.3^{a}	7.7	5.5^{a}	1.8
Staphylococcus gallinarum	186	2.1^{e}	1.5	NA	NA
Staphylococcus gallinarum	187	2.9^{cde}	0.6	1.8^{a}	2.6
Staphylococcus gallinarum	122	5.6^{bcde}	2.1	2.9^{a}	3.4
Staphylococcus hominis ssp *hominis*	183	9.5^{ab}	1.4	3.7^{a}	1.2
Staphylococcus sciuri	147	2.3^{de}	0.2	NA	NA
Staphylococcus sciuri	179	3.9^{bcde}	0.8	NA	NA
Staphylococcus sciuri	185	3.4^{cde}	1.3	NA	NA
Staphylococcus xylosus	104	12.6^{a}	2.6	6.0^{a}	2.1
Staphylococcus xylosus	201	2.9^{cde}	0.6	NA	NA
Streptococcus thoraltensis	114	4.3^{bcde}	1.8	1.9^{a}	1.3
Streptococcus thoraltensis	141	3.2^{cde}	1.0	NA	NA
Streptococcus thoraltensis	145	8.2^{abc}	1.6	2.6^{a}	0.2
Streptococcus thoraltensis	149	3.8^{cde}	2.0	NA	NA
Streptococcus thoraltensis	158	1.6^{e}	1.9	2.7^{a}	3.4
Streptococcus thoraltensis	167	3.2^{cde}	0.5	NA	NA
Unable to be identified	151	5.8^{bcde}	0.4	2.6^{a}	0.7

[a]Mean values represent duplicate replications with duplicate samples (n=4); means with the same letter in the same column are not significantly different ($P \geq 0.5$). NA indicates not antagonistic.

Isolates Recovered From Tomatoes

Overall, the majority of isolates exhibiting antagonistic activity against *S.* Typhimurium LT2 were of the genus *Staphylococcus* (62%) (Table 10) and the majority of the isolates exhibiting antagonistic activity against *E. coli* O157:H7 were of the genus *Leclercia* (50%) (Table 10). The majority of isolates (69.2%) exhibiting antagonistic activity against *S.* Typhimurium LT2 were Gram-positive bacteria (Table 10). The majority of isolates (62.5%) exhibiting antagonistic activity against *E. coli* O157:H7 were Gram-negative bacteria (Table 10).

Staphylococcus isolates recovered from tomatoes exhibited the greatest antagonistic activity against both pathogens. *Staphylococcus hominis* ssp *hominis* demonstrated the greatest antagonistic activity (mean inhibition zone diameter 14.2 ± 5.2 mm) against *S.* Typhimurium LT2 (Table 11). *Staphylococcus haemolyticus* demonstrated the greatest antagonistic activity (mean inhibition zone diameter 7.1 ± 0.7 mm) against *E. coli* O157:H7 (Table 11). Overall, 6.8% of the tomato recovered isolates demonstrated antagonistic activity against *S.* Typhimurium LT2, and 4% exhibited antagonistic activity against *E. coli* O157:H7 (Table 6). The majority of isolates (57.1%) exhibiting antagonistic activity to both pathogens were coliforms (Table 7).

TABLE 10. ***Genera and species of tomato-recovered isolates and the percentages of isolates inhibitory towards*** **S.** ***Typhimurium LT2 and*** **E. coli** ***O157:H7***

Microorganism	No. of Isolates	% inhibitory to *S.* Typhimurium LT2	% inhibitory to *E. coli* O157:H7
Achomobacter denitrificans	11	0.0	0.0
Bacillus amyloliquefaciens	30	0.0	0.0
Bacillus licheniformis	25	0.0	0.0
Cronobacter sakazakii	2	0.0	0.0
Enterobacter cancerogenus	17	0.0	0.0
Enterobacter cloacae	7	0.0	0.0
Klebsiella pneumoniae ssp ozaenae	1	0.0	0.0
Leclercia adecarboxylata	8	50.0	50.0
Micrococcus leteus	19	0.0	0.0
Pantoea spp.	2	0.0	50.0
Pseudomonas oryzihabitans	1	0.0	0.0
Pseudomonas putida	1	0.0	0.0
Pseudomonas spp.	2	0.0	0.0
Pseudomonas stutzeri	4	0.0	0.0
Rhizobium radiobacter	2	0.0	0.0
Sphingomonas paucimobilis	40	0.0	0.0
Staphylococcus haemolyticus	1	100.0	100.0
Staphylococcus hominis ssp hominis	1	100.0	100.0
Staphylococcus sciuri	8	75.0	0.0
Unable to be identified	7	14.3	14.3

TABLE 11. ***Isolates recovered from tomatoes antagonistic to*** **S.** ***Typhimurium LT2 and*** **E. coli** ***O157:H7 and the measurement of the inhibition zone***[a]

		S. Typhimurium LT2 Zone of Inhibition (mm)		*E. coli* O157:H7 Zone of Inhibition (mm)	
Microorganism	Lab ID	Mean	Standard Deviation	Mean	Standard Deviation
Leclercia adecarboxylata	3013	5.9[ab]	2.0	3.1[c]	0.3
Leclercia adecarboxylata	3014	6.5[ab]	2.0	3.1[c]	0.8
Leclercia adecarboxylata	3162	8.9[ab]	1.8	4.8[abc]	1.2
Leclercia adecarboxylata	3231	2.1[b]	0.4	2.9[c]	0.6
Pantoea spp.	3256	NA	NA	4.1[bc]	1.3
Staphylococcus haemolyticus	3019	8.4[ab]	3.1	7.1[a]	0.7
Staphylococcus hominis ssp hominis	3002	14.2[a]	5.2	6.0[ab]	2.1
Staphylococcus sciuri	3010	5.5[b]	5.3	NA	NA
Staphylococcus sciuri	3011	9.5[ab]	5.5	NA	NA
Staphylococcus sciuri	3012	5.9[b]	2.0	NA	NA
Staphylococcus sciuri	3016	10.1[ab]	3.4	NA	NA
Staphylococcus sciuri	3017	4.9[b]	2.1	NA	NA
Staphylococcus sciuri	3018	9.8[ab]	4.5	NA	NA
Unable to be identified	3058	6.6[ab]	1.6	2.6[c]	0.2

[a]Mean values represent duplicate replications with duplicate samples (n=4); means with the same letter in the same column are not significantly different ($P \geq 0.5$). NA indicates not antagonistic.

Isolates Recovered From Spinach

Overall, 7.1% of the spinach recovered isolates demonstrated antagonistic activity against *S.* Typhimurium LT2, and 6.2% exhibited antagonistic activity against *E. coli* O157:H7 (Table 6). Overall, the majority of isolates (57.8%) showing antagonistic activity to both pathogens were Gram-positive, non lactic acid bacteria (Table 7). The majority of isolates exhibiting antagonistic activity against *S.* Typhimurium and *E. coli* O157:H7 were of the genus S*taphylococcus* (41.7 and 47.6%, respectively) (Table 12). Seventy-five (75%) and 90.5% of isolates exhibiting antagonistic activity against *S.* Typhimurium LT2 and *E. coli* O157:H7, respectively, were Gram-positive bacteria (Table 12).

Staphylococcus isolates recovered from spinach exhibited the greatest antagonistic activity against both pathogens. *Staphylococcus intermedius* demonstrated the greatest antagonistic activity (mean inhibition zone diameter 13.9 ± 3.8 mm) against *S.* Typhimurium LT2 (Table 13). *Staphylococcus intermedius* also demonstrated the greatest antagonistic activity (mean inhibition zone diameter 7.2 ± 0.4 mm) against *E. coli* O157:H7 (Table 13).

TABLE 12. ***Genera and species of spinach-recovered isolates and the percentages of isolates inhibitory towards*** **S.** ***Typhimurium LT2 and*** **E. coli** ***O157:H7***

Microorganism	No. of Isolates	% inhibitory to *S.* Typhimurium LT2	% inhibitory to *E. coli* O157:H7
Aerococcus viridans	3	0.0	0.0
Bacillus lentus	9	0.0	0.0
Enterobacter amnigenus 1	3	0.0	0.0
Enterobacter cancerogenus	3	0.0	0.0
Enterobacter cloacae complex	21	9.5	3.7
Enterococcus spp.	62	0.0	0.0
Erysipelothix rhusiopathiae	17	0.0	0.0
Escherichia coli	2	50.0	50.0
Kocuria kristinae	21	0.0	0.0
Lactococcus garvieae	6	0.0	0.0
Leuconostoc pseudomesenteroides	6	66.7	66.7
Listeria grayi	5	60.0	60.0
Pantoea agglomerans	8	0.0	0.0
Pantoea spp.	35	2.9	0.0
Pseudomonas aeruginosa	1	0.0	0.0
Pseudomonas fluorescens	12	0.0	0.0
Pseudomonas putida	19	0.0	0.0
Pseudomonas spp.	8	0.0	0.0
Rahnella aquatilis	6	0.0	0.0
Rhizobium radiobacter	5	0.0	0.0
Serratia marcescens	4	0.0	0.0
Serratia rubidaea	6	0.0	0.0
Sphingobacterium thalpophilum	1	0.0	0.0
Sphingomonas paucimobilis	40	5.0	2.5
Staphylococcus intermedius	23	39.1	39.1
Staphylococcus lentus	4	25.0	25.0
Staphylococcus warneri	1	0.0	0.0
Unable to be identified	8	12.5	12.5

TABLE 13. ***Isolates recovered from spinach antagonistic to* S. *Typhimurium LT2 and* E. *coli O157:H7 and the measurement of the inhibition zone*[a]

Microorganism	Lab ID	*S.* Typhimurium LT2 Zone of Inhibition (mm) Mean	*S.* Typhimurium LT2 Zone of Inhibition (mm) Standard Deviation	*E. coli* O157:H7 Zone of Inhibition (mm) Mean	*E. coli* O157:H7 Zone of Inhibition (mm) Standard Deviation
Enterobacter cloacae complex	2463	2.6^{bc}	3.0	NA	NA
Enterobacter cloacae complex	2617	3.9^{bc}	5.9	3.3^{a}	3.8
Escherichia coli	2568	4.8^{abc}	2.3	2.7^{a}	3.1
Leuconostoc pseudomesenteroides	2533	8.7^{abc}	4.4	3.5^{a}	1.2
Leuconostoc pseudomesenteroides	2536	6.9^{abc}	6.1	2.5^{a}	1.9
Leuconostoc pseudomesenteroides	2541	12.3^{ab}	4.8	5.1^{a}	1.8
Leuconostoc pseudomesenteroides	2544	7.8^{abc}	2.8	2.4^{a}	0.8
Listeria grayi	2528	6.3^{abc}	1.5	2.0^{a}	0.4
Listeria grayi	2530	10.8^{abc}	6.8	4.9^{a}	2
Listeria grayi	2554	10.5^{abc}	4.2	3.4^{a}	0.5
Pantoea spp.	2527	2.3^{c}	1.6	NA	NA
Sphingomonas paucimobilis	2537	4.0^{bc}	2.9	NA	NA
Sphingomonas paucimobilis	2562	8.8^{abc}	4.3	3.7^{a}	1.1
Staphylococcus intermedius	2553	13.9^{a}	3.8	3.2^{a}	0.3
Staphylococcus intermedius	2555	8.3^{abc}	1.8	3.7^{a}	0.6
Staphylococcus intermedius	2556	6.3^{abc}	5.1	3.1^{a}	1.3
Staphylococcus intermedius	2563	11.9^{abc}	4.7	6.2^{a}	1.5
Staphylococcus intermedius	2569	5.2^{abc}	1.3	3.2^{a}	0.7
Staphylococcus intermedius	2615	5.2^{abc}	1.6	4.3^{a}	2.3
Staphylococcus intermedius	2616	9.0^{abc}	1.4	3.5^{a}	1.8
Staphylococcus intermedius	2564	7.9^{abc}	2.3	7.2^{a}	0.4
Staphylococcus intermedius	2614	7.3^{abc}	3.4	6.5^{a}	4
Staphylococcus lentus	2565	4.2^{abc}	1.1	2.2^{a}	2.6
Unable to be identified	2603	7.2^{abc}	2.6	4.9^{a}	2.8

[a]Mean values represent duplicate replications with duplicate samples (n=4); means with the same letter in the same column are not significantly different ($P \geq 0.5$). NA indicates not antagonistic.

Isolates Recovered From Endive

Overall, 17.3% of the endive recovered isolates demonstrated antagonistic activity against *S.* Typhimurium and *E. coli* O157:H7 (Table 6). The majority of isolates exhibiting antagonistic activity against *S.* Typhimurium and *E. coli* O157:H7 were of the genus *Lactococcus* (41.2 and 41.2%, respectively) (Table 14). Ninety-eight (98.0%) and 96.1% of the isolates exhibiting antagonistic activity against *S.* Typhimurium LT2 and *E. coli* O157:H7 were Gram-positive bacteria (Table 14). Overall, the majority of isolates (64%) exhibiting antagonistic activity to both pathogens were lactic acid bacteria (Table 7). *Lactococcus* and *Bacillus* isolates exhibited the greatest antagonistic activity against both pathogens. *Lactococcus garvieae* and *Bacillus* spp. demonstrated the greatest antagonistic activity (mean inhibition diameter 17.8 ± 4.7 mm and 17.0 ± 1.7 mm, respectively) against *S.* Typhimurium LT2 (Table 15). *Lactococcus garvieae* and *Bacillus* spp. also demonstrated the greatest antagonistic activity (mean inhibition zone diameter 11.6 ± 1.7 mm and 8.9 ± 2.0 mm, respectively) against *E. coli* O157:H7 (Table 15).

TABLE 14. ***Genera and species of endive-recovered isolates and the percentages of isolates inhibitory towards*** **S.** ***Typhimurium LT2 and*** **E. coli** ***O157:H7.***

Microorganism	No. of Isolates	% inhibitory to *S.* Typhimurium LT2	% inhibitory to *E. coli* O157:H7
Aerococcus viridans	24	0.0	0.0
Alloiococcus otitis	3	0.0	0.0
Bacillus spp.	22	18.2	18.2
Brevundimonas diminuta	1	0.0	0.0
Enterococcus casseliflavus	7	100.0	100.0
Enterococcus spp.	27	0.0	0.0
Escherichia coli	1	100.0	100.0
Kocuria kristinae	24	0.0	0.0
Lactococcus garvieae	22	95.5	95.5
Listeria grayi	1	100.0	100.0
Micrococcus spp.	1	0.0	0.0
Paenibacillus polymyxa	2	0.0	0.0
Pantoea agglomerans	4	0.0	0.0
Pantoea spp.	45	0.0	0.0
Providencia rettgeri	1	0.0	0.0
Pseudomonas aeruginosa	1	0.0	0.0
Pseudomonas oryzihabitans	7	0.0	0.0
Pseudomonas spp.	2	0.0	0.0
Rahnella aquatilis	7	0.0	0.0
Rhizobium radiobacter	2	0.0	0.0
Sphingomonas paucimobilis	67	0.0	1.5
Sphingobacterium thalpophilum	1	0.0	0.0
Staphylococcus aureus	1	100.0	100.0
Staphylococcus warneri	5	80.0	80.0
Vagococcus fluvialis	5	100.0	80.0
Unable to be identified	12	58.3	58.3

TABLE 15. ***Isolates recovered from endive antagonistic to* S. *Typhimurium LT2 and* E. coli *O157:H7 and the measurement of the inhibition zone.*[a]**

Microorganism	Lab ID	*S.* Typhimurium LT2 Zone of Inhibition (mm) Mean	Standard Deviation	*E. coli* O157:H7 Zone of Inhibition (mm) Mean	Standard Deviation
Bacillus spp.	1475	13.5^{abcdefg}	0.9	8.9^{abcd}	2.0
Bacillus spp.	1481	17.0^{ab}	1.7	6.5^{abcdefgh}	7.5
Bacillus spp.	1485	6.8^{defghijk}	1.8	5.4^{abcdefgh}	0.5
Bacillus spp.	1505	3.2^{hijk}	6.5	2.5^{defgh}	2.9
Enterococcus casseliflavus	1402	11.3^{abcdefghi}	3.0	6.2^{abcdefgh}	2.0
Enterococcus casseliflavus	1446	5.9^{defghijk}	1.9	3.0^{defgh}	0.5
Enterococcus casseliflavus	1447	9.0^{abcdefghijk}	2.2	3.6^{cdefgh}	0.7
Enterococcus casseliflavus	1500	7.1^{cdefghijk}	3.6	3.9^{cdefgh}	1.5
Enterococcus casseliflavus	1502	6.3^{defghijk}	1.0	3.0^{defgh}	0.5
Enterococcus casseliflavus	1404	8.5^{bcdefghijk}	1.0	4.5^{bcdefgh}	0.9
Enterococcus casseliflavus	1448	9.6^{abcdefghijk}	2.8	5.2^{abcdefgh}	1.2
Escherichia coli	1472	6.9^{defghijk}	1.5	5.1^{abcdefgh}	1.5
Lactococcus garvieae	1388	16.3^{abc}	2.8	10.8^{ab}	1.6
Lactococcus garvieae	1437	12.2^{abcdefgh}	1.2	9.1^{abcd}	1.6
Lactococcus garvieae	1438	14.0^{abcde}	2.6	7.8^{abcdefg}	0.3
Lactococcus garvieae	1458	14.9^{abcd}	2.5	10.1^{abc}	2.8
Lactococcus garvieae	1460	8.7^{abcdefghijk}	3.5	4.1^{cdefgh}	0.6
Lactococcus garvieae	1466	9.9^{abcdefghijk}	6.7	11.6^{a}	1.7
Lactococcus garvieae	1467	17.8^{a}	4.7	9.0^{abcd}	2.7
Lactococcus garvieae	1469	12.6^{abcdefg}	1.6	6.1^{abcdefgh}	0.8
Lactococcus garvieae	1492	11.2^{abcddefghi}	4.0	6.3^{abcdefgh}	1.2
Lactococcus garvieae	1494	6.0^{defghijk}	0.9	3.3^{defgh}	0.6
Lactococcus garvieae	1495	4.8^{efghijk}	1.7	3.8^{cdefgh}	0.8
Lactococcus garvieae	1496	11.5^{abcdefghi}	1.4	8.0^{abcdefg}	1.1
Lactococcus garvieae	1501	9.7^{abcdefghijk}	0.9	7.3^{abcdefgh}	0.7
Lactococcus garvieae	1504	9.0^{abcdefghijk}	1.3	7.1^{abcdefgh}	1.1
Lactococcus garvieae	1395	3.3^{hijk}	6.6	5.6^{abcdefgh}	6.4
Lactococcus garvieae	1451	14.0^{abcde}	2.8	7.2^{abcdefgh}	0.3
Lactococcus garvieae	1459	16.8^{ab}	4.2	7.9^{abcdefg}	1.1
Lactococcus garvieae	1463	12.5^{abcdefgh}	2.8	7.4^{abcdefgh}	2.2
Lactococcus garvieae	1477	5.2^{efghijk}	0.7	1.1^{h}	2.1
Lactococcus garvieae	1478	5.4^{efghijk}	1.3	2.6^{defgh}	0.4
Lactococcus garvieae	1480	7.8^{bcdefghijk}	2.4	4.6^{bcdefgh}	1.7

Table 15 (continued)

Microorganism	Lab ID	*S.* Typhimurium LT2 Zone of Inhibition (mm) Mean	Standard Deviation	*E. coli* O157:H7 Zone of Inhibition (mm) Mean	Standard Deviation
Listeria grayi	1462	11.8^{abcdefgh}	4.1	4.2^{bcdefgh}	0.8
Sphingomonas paucimobilis	1273	NA	NA	1.9^{fgh}	2.3
Staphylococcus aureus	1486	11.9^{abcdefgh}	6.0	6.9^{abcdefgh}	4.8
Staphylococcus warneri	1417	14.8^{abcd}	1.5	8.8^{abcde}	1.4
Staphylococcus warneri	1418	10.9^{abcdefghij}	2.5	8.6^{abcdef}	1.5
Staphylococcus warneri	1450	13.2^{abcdefg}	3.3	8.3^{abcdefg}	1.5
Staphylococcus warneri	1482	13.6^{abcdef}	4.0	5.4^{abcdefgh}	6.3
Vagococcus fluvialis	1489	11.3^{abcdefghi}	3.4	3.4^{defgh}	0.4
Vagococcus fluvialis	1491	2.3^{ijk}	3.0	NA	NA
Vagococcus fluvialis	1479	4.9^{efghijk}	1.2	3.3^{defgh}	1.1
Vagococcus fluvialis	1490	1.9^{jk}	3.7	2.2^{efgh}	2.5
Vagococcus fluvialis	1506	1.2^{k}	2.3	1.7^{gh}	2.0
Unable to be identified	1414	6.2^{defghijk}	4.3	4.5^{bcdefgh}	1.6
Unable to be identified	1445	4.7^{fghijk}	2.2	1.8^{gh}	2.4
Unable to be identified	1468	6.0^{defghijk}	7.0	7.9^{abcdefg}	0.5
Unable to be identified	1488	8.2^{bcdefghijk}	3.2	4.4^{bcdefgh}	1.6
Unable to be identified	1442	10.8^{abcdefghij}	1.0	6.7^{abcdefgh}	1.1
Unable to be identified	1471	4.2^{ghijk}	1.7	1.7^{gh}	2.2
Unable to be identified	1484	1.7^{jk}	3.4	2.9^{defgh}	2.0

[a]Mean values represent duplicate replications with duplicate samples (n=4); means with the same letter in the same column are not significantly different ($P \geq 0.5$). NA indicates not antagonistic.

Pathogen Antagonism on Produce Surface Preliminary Experiments

Through preliminary experiments, it was determined that the *E. coli* O157:H7 Biosafety level 1 (BL1), ATCC 700728, strain behaved similarly to the Biosafety level 2 (BL2) strains in regards to mean generation time, ($p < 0.05$) (Table 16). It was also determined that *S.* Typhimurium LT2 (BL1) behaved similarly to the BL2 strains, ($p < 0.05$). The use of the BL1 strains would allow for the use of the same strains across several projects (Table 17).

TABLE 16. E. coli *O157:H7 strains, sources, biosafety level identification and mean generation times*.[a]

Strain	Original Source of Parent Strain	Biosafety Level	Mean Generation Time (min.)
R1	Beef carcass isolate	2	24.3 ± 4.0A
R18	Beef carcass isolate	2	23.5 ± 0.7A
R8	Beef carcass isolate	2	23.8 ± 1.5A
R41	Beef carcass isolate	2	22.4 ± 0.7A
R34	Beef carcass isolate	2	20.8 ± 1.9A
ATCC 700728	ATCC	1	22.1 ± 1.0A

[a]Values depict least square means of triplicate identical replications (n=3). Generation times were calculated from the linear portion of the exponential phase of growth for each strain *(118)*. Means within the same column across strains with different letters differ at $p < 0.05$, determined by one-way analysis of variance (ANOVA), with means separation by Tukey's Honestly Significant Differences (HSD) test.

TABLE 17. Salmonella enterica *strains, sources, biosafety level identification and mean generation times.*[a]

Strain	Original Source of Parent Strain	Biosafety Level	Mean Generation Time (min.)
Poona	Produce	2	25.5 ± 1.9A
Typhimurium	ATCC 13311	2	25.4 ± 1.3A
Montevideo	Produce	2	33.9 ± 6.5A
LT2	ATCC 700720	1	32.3 ± 3.3A

Values depict least square means of triplicate identical replications (n=3). Generation times were calculated from the linear portion of the exponential phase of growth for each strain *(118)*. Means within the same column across strains with different letters differ at $p < 0.05$, determined by one-way analysis of variance (ANOVA), with means separation by Tukey's Honestly Significant Differences (HSD) test.[a]

Preliminary experiments also determined there was no significant difference ($p \geq 0.05$) in inoculating the pathogens separately and plating on tryptic soy agar with rifampicin (0.1 g/L) versus inoculating the organisms together on the surface and plating on lactose-sulfite-phenol red-rifampicin agar (Tables 18-19). The results from this preliminary experiment would allow for co-inoculation of pathogens on the surface of the various produce commodities for the following study.

TABLE 18. ***Pathogen enumeration in the presence of antagonistic produce isolate on the surface of Romaine Lettuce using selective/differential media and selective media to test co-inoculation of pathogens.*** [a]

			S. Typhimurium LT2		*E. coli* O157:H7	
Antagonistic Produce Isolate	Agar used	Time Point (h)	Loosely Attached Mean log CFU/cm^2	Strongly Attached Mean Log CFU/cm^2	Loosely Attached Mean log CFU/cm^2	Strongly Attached Mean Log CFU/cm^2
Endive	LSPR	0	3.9 ± 0.5^{ab}	2.8 ± 0.2^{cd}	4.1 ± 0.5^{abcde}	2.5 ± 0.1^{cd}
Isolate 1417		6	3.9 ± 0.1^{ab}	3.2 ± 0.1^{abcd}	4.6 ± 0.6^{abcde}	3.6 ± 0.4^{abcd}
Staphylococcus		24	4.4 ± 0.1^{ab}	4.5 ± 0.2^{ab}	5.7 ± 0.3^{abc}	5.0 ± 0.7^{a}
warneri	TSAR	0	3.8 ± 0.3^{ab}	2.5 ± 0.0^{cd}	4.1 ± 0.2^{abcde}	2.3 ± 0.3^{cd}
		6	4.0 ± 0.2^{ab}	3.1 ± 0.0^{abcd}	4.7 ± 0.0^{abcde}	3.5 ± 0.1^{abcd}
		24	4.6 ± 0.1^{a}	4.0 ± 0.5^{abc}	5.4 ± 0.6^{abcd}	4.8 ± 1.0^{ab}
Endive	LSPR	0	3.5 ± 0.1^{ab}	2.1 ± 0.0^{d}	3.9 ± 0.3^{abcde}	2.3 ± 0.2^{cd}
Isolate 1467		6	3.4 ± 0.5^{ab}	2.1 ± 0.1^{d}	3.6 ± 0.5^{de}	2.2 ± 0.0^{cd}
Lactococcus		24	2.4 ± 0.3^{b}	1.7 ± 1.2^{d}	3.4 ± 0.2^{e}	1.5 ± 0.0^{d}
garvieae	TSAR	0	4.4 ± 0.2^{ab}	2.2 ± 0.3^{d}	3.9 ± 0.6^{bcde}	2.4 ± 0.1^{cd}
		6	3.7 ± 0.2^{ab}	2.9 ± 0.1^{cd}	3.8 ± 0.3^{cde}	3.0 ± 0.4^{cd}
		24	4.0 ± 1.1^{ab}	3.3 ± 0.8^{abcd}	3.8 ± 0.2^{cde}	4.0 ± 0.2^{abc}
Pathogen Only	LPSR	0	3.7 ± 0.0^{ab}	3.1 ± 0.8^{abcd}	4.1 ± 0.3^{abcde}	3.1 ± 0.8^{abcd}
		6	4.3 ± 0.3^{ab}	3.0 ± 0.6^{bcd}	4.8 ± 0.5^{abcde}	3.3 ± 1.1^{abcd}
		24	4.9 ± 1.1^{a}	3.9 ± 0.1^{abc}	5.8 ± 0.6^{ab}	4.8 ± 0.1^{ab}
	TSAR	0	3.7 ± 0.4^{ab}	3.1 ± 0.0^{abcd}	3.4 ± 1.2^{de}	2.9 ± 0.5^{bcd}
		6	4.5 ± 0.3^{ab}	3.2 ± 0.9^{abcd}	5.4 ± 0.3^{abcd}	4.0 ± 0.9^{abc}
		24	5.1 ± 0.0^{a}	4.7 ± 0.1^{a}	5.9 ± 0.2^{a}	5.1 ± 0.2^{a}

[a]LSPR indicates enumeration on selective/differential plating on lactose-sulfite-phenol red-rifampicin (LSPR) medium, and TSAR indicates selective plating on tryptic soy agar with the addition of 0.1 g/L of Rifampicin. The experiment was completed with two replications. Means with the same letter in the same column are not significantly different ($p \geq 0.05$).

TABLE 19. ***Pathogen enumeration in the presence of antagonistic isolates on the surface of tomatoes using selective/differential media and selective media to test co-inoculation of pathogens.***[a]

			S. Typhimurium LT2		*E. coli* O157:H7	
Antagonistic Isolate		Time Point (h)	Loosely Attached Mean log CFU/cm^2	Strongly Attached Mean Log CFU/cm^2	Loosely Attached Mean log CFU/cm^2	Strongly Attached Mean Log CFU/cm^2
		0	4.6 ± 1.1^{ab}	2.6 ± 0.1^{b}	3.9 ± 0.2^{bc}	2.6 ± 0.5^{d}
Tomato	LSPR	6	5.6 ± 0.0^{ab}	5.4 ± 0.0^{ab}	5.7 ± 0.0^{abc}	5.3 ± 0.0^{abc}
Isolate 3162:		24	5.6 ± 0.0^{ab}	5.4 ± 0.0^{a}	5.7 ± 0.0^{a}	5.4 ± 0.0^{a}
Leclercia		0	3.7 ± 0.2^{ab}	2.5 ± 0.7^{b}	3.8 ± 0.3^{bc}	3.4 ± 0.3^{abcd}
adecarboxylata	TSAR	6	3.8 ± 0.2^{ab}	3.7 ± 0.4^{ab}	4.1 ± 0.2^{abc}	2.8 ± 0.0^{cd}
		24	4.5 ± 1.2^{ab}	3.8 ± 1.0^{ab}	4.7 ± 0.5^{abc}	3.6 ± 0.5^{abcd}
		0	3.6 ± 0.3^{ab}	3.0 ± 0.4^{ab}	3.9 ± 0.3^{bc}	3.2 ± 0.1^{bcd}
	LSPR	6	3.4 ± 0.6^{b}	3.3 ± 0.7^{ab}	3.7 ± 0.7^{c}	3.2 ± 0.6^{bcd}
Pediococcus		24	4.9 ± 0.0^{ab}	4.8 ± 0.0^{ab}	5.7 ± 0.0^{a}	5.0 ± 0.0^{ab}
acidilacti		0	3.9 ± 0.5^{ab}	3.0 ± 0.4^{ab}	3.9 ± 0.3^{bc}	3.6 ± 0.0^{abcd}
	TSAR	6	3.9 ± 0.3^{ab}	3.0 ± 0.2^{ab}	3.8 ± 0.7^{bc}	3.2 ± 0.2^{bcd}
		24	5.2 ± 1.1^{ab}	4.5 ± 1.1^{ab}	5.5 ± 0.6^{ab}	5.0 ± 0.1^{ab}
		0	3.8 ± 0.1^{ab}	3.0 ± 0.4^{ab}	3.9 ± 0.6^{bc}	3.1 ± 0.5^{bcd}
	LSPR	6	3.8 ± 0.1^{ab}	3.2 ± 0.3^{ab}	3.8 ± 0.6^{bc}	3.2 ± 0.9^{bcd}
		24	3.9 ± 0.1^{ab}	3.4 ± 0.2^{ab}	5.0 ± 0.4^{abc}	5.2 ± 1.0^{ab}
Pathogen Only		0	4.1 ± 0.4^{ab}	2.7 ± 0.5^{b}	4.1 ± 0.2^{abc}	2.8 ± 0.1^{cd}
	TSAR	6	4.9 ± 0.9^{ab}	3.8 ± 0.8^{ab}	4.9 ± 0.6^{abc}	3.7 ± 0.7^{abcd}
		24	6.1 ± 1.2^{a}	4.7 ± 1.5^{ab}	5.4 ± 0.1^{ab}	4.5 ± 1.3^{abcd}

[a]LSPR indicates enumeration on selective/differential plating on lactose-sulfite-phenol red-rifampicin (LSPR) medium, and TSAR indicates selective plating on tryptic soy agar with the addition of 0.1 g/L of Rifampicin. The experiment was completed with duplicate replications. Means with the same letter in the same column are not significantly different ($p \geq 0.05$).

Preliminary experiments also determined there was no significant difference ($p \geq 0.05$) between excising samples then spot-inoculating versus spot-inoculating followed by excision (Tables 20-21).

TABLE 20. ***Cantaloupe-recovered isolates and their ability to antagonize two pathogens on the surface of cantaloupes using two different sampling methods.***

			S. Typhimurium LT2		*E. coli* O157:H7	
Produce Commodity And Isolate	Sampling Method	Time Point (h)	Loosely Attached Mean log CFU/cm^2	Strongly Attached Mean Log CFU/cm^2	Loosely Attached Mean log CFU/cm^2	Strongly Attached Mean Log CFU/cm^2
Cantaloupe Isolate 104 *Staphylococcus xylosus*	SLICE	0	4.1 ± 0.2^{a}	3.8 ± 0.3^{b}	4.2 ± 0.3^{d}	3.3 ± 0.3^{d}
		24	5.3 ± 0.7^{a}	5.3 ± 0.7^{ab}	6.2 ± 0.2^{ab}	5.7 ± 0.4^{ab}
	WHOLE	0	3.8 ± 0.0^{a}	3.5 ± 0.1^{b}	3.9 ± 0.0^{d}	3.2 ± 0.4^{d}
		24	4.7 ± 0.8^{a}	4.9 ± 0.4^{ab}	5.3 ± 0.1^{bcd}	5.1 ± 0.1^{bc}
Cantaloupe Isolate 138 *Enterococcus casseliflavus*	SLICE	0	4.0 ± 0.1^{a}	3.7 ± 0.2^{b}	4.2 ± 0.0^{d}	3.3 ± 0.3^{d}
		24	5.0 ± 1.2^{a}	5.3 ± 0.9^{ab}	6.0 ± 0.5^{ab}	5.8 ± 0.3^{ab}
	WHOLE	0	4.0 ± 0.1^{a}	3.5 ± 0.2^{b}	4.2 ± 0.4^{d}	3.1 ± 0.0^{d}
		24	5.1 ± 0.5^{a}	4.9 ± 0.8^{ab}	5.8 ± 0.8^{abc}	5.7 ± 0.7^{ab}
Cantaloupe Pathogen Only	SLICE	0	4.3 ± 0.0^{a}	4.0 ± 0.3^{ab}	4.3 ± 0.1^{cd}	3.7 ± 0.2^{cd}
		24	6.1 ± 1.3^{a}	6.2 ± 1.1^{a}	7.0 ± 0.6^{a}	6.8 ± 0.4^{a}
	WHOLE	0	4.2 ± 0.0^{a}	3.6 ± 0.2^{b}	4.1 ± 0.2^{d}	3.1 ± 0.1^{d}
		24	5.4 ± 0.4^{a}	5.4 ± 0.2^{ab}	5.9 ± 0.4^{ab}	5.6 ± 0.4^{ab}

[a]SLICE indicates 3-10cm^2 pieces were excised and then spot-inoculated with isolate. Then after 24 hours spot-inoculated with pathogen. WHOLE indicates the inoculation occurred prior to excision and excision took place < 30 minutes before plating. The experiment was completed with duplicate replications. Means with the same letter in the same column are not significantly different ($p \geq 0.05$).

TABLE 21. ***Tomato-recovered isolate 3162 and Pediococcus acidilacti and their ability to antagonize pathogens on the surface of tomatoes using two different sampling methods.***[a]

Produce Commodity And Isolate		Time Point (h)	*S.* Typhimurium LT2 Loosely Attached Mean log CFU/cm^2	*S.* Typhimurium LT2 Strongly Attached Mean Log CFU/cm^2	*E. coli* O157:H7 Loosely Attached Mean log CFU/cm^2	*E. coli* O157:H7 Strongly Attached Mean Log CFU/cm^2
Tomato Isolate 3162 *Leclercia adecarboxylata*	SLICE	0	3.8 ± 0.0^{a}	2.6 ± 0.0^{ab}	4.4 ± 0.0^{a}	3.4 ± 0.0^{abc}
		24	ND^{b}	ND^{b}	6.7 ± 0.0^{a}	5.8 ± 0.0^{ab}
	WHOLE	0	4.1 ± 0.0^{a}	2.9 ± 0.1^{b}	4.0 ± 0.1^{a}	2.6 ± 0.1^{bc}
		24	3.4 ± 1.9^{a}	2.3 ± 1.0^{b}	4.2 ± 1.8^{a}	2.6 ± 1.5^{bc}
Tomato *Pediococcus acidilacti*	SLICE	0	4.0 ± 0.1^{a}	3.1 ± 0.0^{ab}	4.8 ± 1.0^{a}	3.8 ± 1.9^{abc}
		24	5.3 ± 0.0^{a}	4.1 ± 0.0^{ab}	6.1 ± 0.0^{a}	4.8 ± 0.0^{abc}
	WHOLE	0	4.0 ± 0.0^{a}	2.7 ± 0.0^{b}	4.0 ± 0.0^{a}	2.2 ± 0.1^{bc}
		24	3.0 ± 2.0^{a}	2.6 ± 1.5^{b}	4.0 ± 2.6^{a}	2.9 ± 1.9^{abc}
Tomato Pathogen Only	SLICE	0	4.1 ± 0.2^{a}	2.7 ± 0.6^{b}	4.1 ± 0.0^{a}	4.0 ± 1.3^{abc}
		24	5.1 ± 0.0^{a}	5.4 ± 0.0^{a}	6.5 ± 0.0^{a}	6.5 ± 0.0^{a}
	WHOLE	0	3.7 ± 1.0^{a}	2.2 ± 0.6^{b}	4.4 ± 0.1^{a}	2.2 ± 0.3^{c}
		24	5.4 ± 1.0^{a}	4.3 ± 1.2^{ab}	5.5 ± 0.5^{a}	4.2 ± 1.3^{abc}

[a]SLICE indicates 3-10cm^2 pieces were excised and then spot-inoculated with isolate. Then after 24 hours spot-inoculated with pathogen. WHOLE indicates the inoculation occurred prior to excision and excision took place < 30 minutes before plating. ND indicates none detected. The experiment was completed with two replications. Means with the same letter in the same column are not significantly different ($p \geq 0.05$).

The final preliminary showed the ability of the pathogens to indeed grow and attach on the surface of each produce commodity over the period of 24 h (Tables 22-23). The findings of this preliminary experiment outlined the necessary time points for the final study on the surface of the produce commodities.

TABLE 22. E. coli *O157:H7 growth on the surface of various produce commodities.*[a]

Produce Commodity	Time Point (h)	Loosely Attached Mean log_{19} CFU/cm^2	Strongly Attached Mean log_{10} CFU/cm^2	Proportion physically attached
Cantaloupe	0	5.0 ± 0.0	4.8 ± 0.2	0.4 ± 0.1
	12	5.1 ± 0.7	5.4 ± 0.8	0.6 ± 0.0
	24	5.9 ± 1.6	6.2 ± 1.5	0.7 ± 0.0
	48	6.6 ± 1.6	6.6 ± 1.4	0.5 ± 0.1
Lettuce	0	5.0 ± 0.3	4.2 ± 0.1	0.2 ± 0.1
	12	5.3 ± 0.1	4.8 ± 0.6	0.3 ± 0.2
	24	5.9 ± 0.3	5.1 ± 0.8	0.2 ± 0.1
	48	5.0 ± 2.0	5.3 ± 1.6	0.7 ± 0.2
Spinach	0	5.3 ± 0.5	4.1 ± 0.5	0.1 ± 0.0
	12	5.4 ± 0.3	4.4 ± 0.5	0.1 ± 0.2
	24	5.6 ± 0.6	5.8 ± 1.2	0.6 ± 0.3
	48	5.8 ± 1.9	5.8 ± 1.7	0.5 ± 0.1
Tomato	0	5.0 ± 0.3	3.6 ± 0.3	0.0 ± 0.0
	12	5.1 ± 0.3	4.1 ± 0.1	0.1 ± 0.1
	24	6.1 ± 0.8	4.9 ± 1.1	0.1 ± 0.0
	48	6.8 ± 1.3	6.0 ± 1.8	0.2 ± 0.1

[a]*E. coli* O157:H7 at a concentration of 6.8 ± 0.1 mean log_{10} CFU/ml was spot inoculated (10 spots of 10µl) onto 10 cm^2 pieces of each produce commodity. At each time point, Three-10 cm^2 pieces were used to enumerate loosely attached cells and strongly attached cells on tryptic soy agar with rifampicin (TSAR, 0.1 g/L). Proportion of physically attached cells was calculated by dividing the CFU/cm^2 of strongly attached cells by the total CFU/cm^2 of attached and loosely attached.

TABLE 23. S. *Typhimurium LT2 growth on the surface of various produce commodities.*

Produce Commodity	Time Point (h)	Loosely Attached Mean $\log_{10}$ CFU/cm^2	Strongly Attached Mean $\log_{10}$ CFU/cm^2	Proportion physically attached
Cantaloupe	0	5.0 ± 0.2	4.8 ± 0.0	0.4 ± 0.1
	12	5.5 ± 0.0	5.4 ± 0.1	0.4 ± 0.1
	24	5.4 ± 0.1	5.6 ± 0.1	0.6 ± 0.0
	48	6.1 ± 0.1	6.4 ± 0.2	0.7 ± 0.1
Lettuce	0	4.9 ± 0.3	4.7 ± 0.4	0.4 ± 0.4
	12	5.5 ± 0.1	4.7 ± 0.4	0.1 ± 0.1
	24	5.8 ± 0.0	5.1 ± 0.3	0.2 ± 0.1
	48	5.8 ± 0.3	5.7 ± 0.5	0.4 ± 0.1
Spinach	0	4.7 ± 0.0	4.8 ± 0.1	0.6 ± 0.1
	12	5.3 ± 0.0	4.9 ± 0.3	0.3 ± 0.1
	24	5.7 ± 0.4	5.3 ± 0.5	0.3 ± 0.0
	48	5.2 ± 1.3	5.4 ± 0.9	0.6 ± 0.2
Tomato	0	4.9 ± 0.2	3.5 ± 0.3	0.0 ± 0.0
	12	5.5 ± 0.4	4.4 ± 0.0	0.1 ± 0.1
	24	6.3 ± 0.8	5.3 ± 1.2	0.1 ± 0.1
	48	7.0 ± 0.7	6.1 ± 0.9	0.1 ± 0.1

[a]*S.* Typhimurium LT2 at a concentration of 6.7 ± 0.2 Mean $\log_{10}$ CFU/ml was spot inoculated (10 spots of 10µl) onto 10 cm^2 pieces of each produce commodity. At each time point, Three-10 cm^2 pieces were used to enumerate loosely attached cells and strongly attached cells on tryptic soy agar with rifampicin (TSAR, 0.1 g/L). Proportion of physically attached cells was calculated by dividing the CFU/cm^2 of strongly attached cells by the total CFU/cm^2 of attached and loosely attached.

Pathogen Antagonism on the Surface of Leafy Greens

At 0 h, the populations of *E. coli* O157:H7 and *S.* Typhimurium LT2 on the spinach leaf surfaces in the presence of the spinach recovered antagonistic isolates were 3.7 ± 0.2 and 3.5 ± 0.2 $\log_{10}$ CFU/cm^2, respectively, and after 24 h, ranged from 4.9 ± 0.6 to 6.3 ± 0.4 and 3.8 ± 0.6 to 5.8 ± 0.3 $\log_{10}$ CFU/cm^2, respectively (Figures 5 and 6).

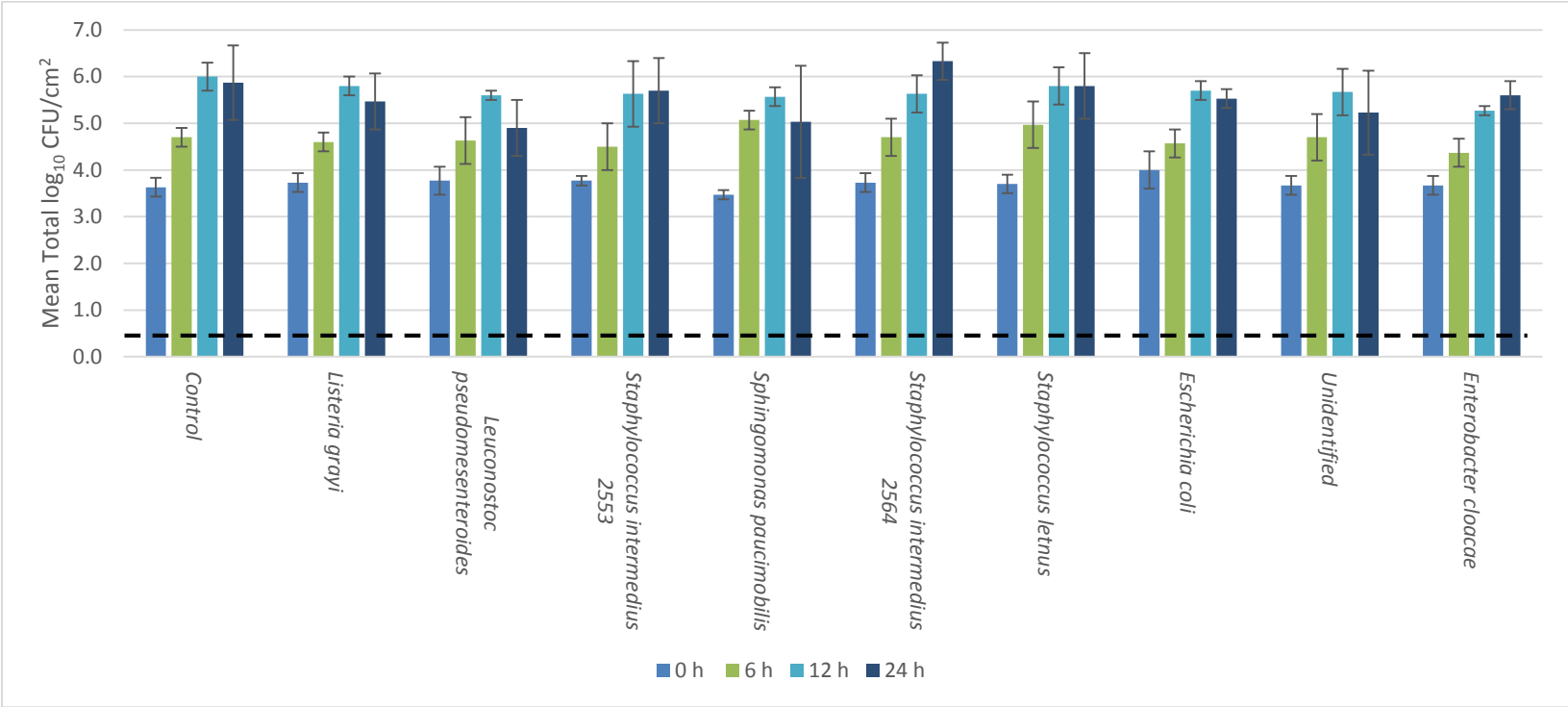

FIGURE 5. ***Surviving* E. coli *O157:H7 on spinach surfaces as influenced by antagonist addition.*** The isolates tested in this experiment previously showed antagonistic activity *in vitro*. Control indicates no antagonistic isolate was added; only pathogens were present. Bars represent the sum of loosely and strongly attached. Error bars indicate one standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2). There was no significant difference ($p \geq 0.05$) between adding antagonistic isolates and not at each time point for each pathogen.

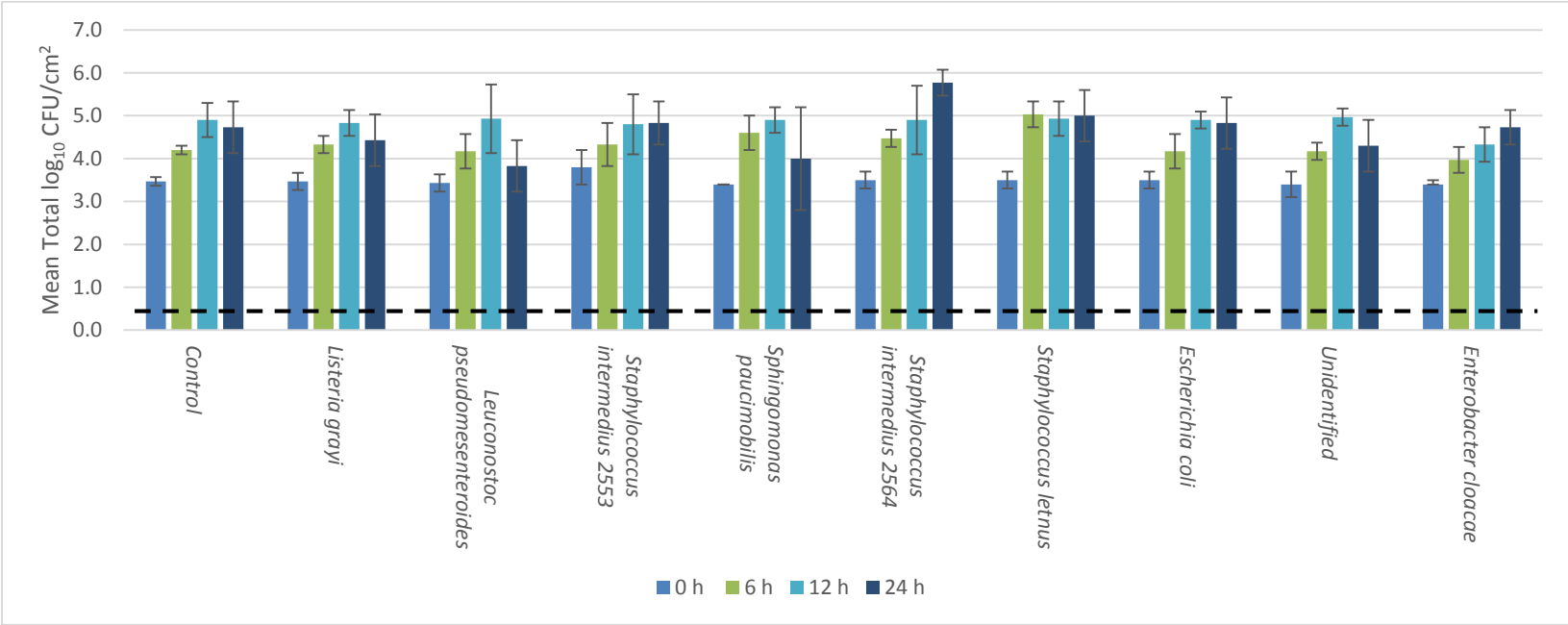

FIGURE 6. ***Surviving* S. *Typhimurium LT2 on spinach surfaces as influenced by antagonist addition.*** The isolates tested in this experiment previously showed antagonistic activity *in vitro*. Control indicates no antagonistic isolate was added; only pathogens were present. Bars represent the sum of loosely and strongly attached cells. Error bars indicate one standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2). There was no significant difference ($p \geq 0.05$) between adding antagonistic isolates and not at each time point for each pathogen.

Populations of the antagonistic isolates at 0 h were 5.7 ± 0.8 $\log_{10}$ CFU/cm^2 (Table 23). The population of the antagonistic isolate *Escherichia coli* 2568 was significantly higher over the 24 h period in the presence of the pathogens' when compared to the isolate on spinach without the pathogens presence (Table 24). However, the other antagonistic isolate populations did not differ significantly in the presence of the pathogens and without pathogens present. At the 12 h time point the antagonistic isolates overall were significantly higher with pathogens present than without out; however, at 0, 6, and 24 there was no difference in populations (Table 24).

The strength of attachment in the presence of the isolates after 24 h for *E. coli* O157:H7 and *S.* Typhimurium LT2 ranged from 0.02 ± 0.03 to 0.26 ± 0.28 and 0.09 ± 0.12 to 0.49 ± 0.10, respectively (Table 25). After 24 h, *Staphylococcus intermedius* 2553, *Leuconostoc pseudomesenteroides,* and an unidentified isolate 2603 produced a lower strength of attachment for *E. coli* O157:H7 (Table 24). However, an undidentified isolate 2603 produced the lowest strength of attachment after 24 h for *S.* Typhimurium (Table 25). There were no significant differences ($p \geq 0.05$) in the growth of both pathogens in the presence of the antagonistic isolates at each time point when compared to the control (no antagonistic isolate).

TABLE 24. ***Arithmetic means of pathogen-antagonizing inoculated bacteria on the surfaces of spinach.***

Isolate	Time Point (h)	With Pathogens Present Mean $\log_{10}$ CFU/cm^2	Isolate Only Mean $\log_{10}$ CFU/cm^2
Listeria grayi	0	6.3 ± 0.5	5.9 ± 0.5
	6	6.8 ± 0.7	5.6 ± 0.9
	12	7.2 ± 0.2	5.9 ± 0.1
	24	7.0 ± 0.8	6.9 ± 0.8
Leuconostoc pseudomesenteroides	0	5.8 ± 0.9	5.4 ± 0.2
	6	5.7 ± 0.3	5.3 ± 0.5
	12	6.2 ± 0.1	5.1 ± 0.2
	24	5.6 ± 0.6	5.2 ± 1.3
Staphylococcus intermedius 2553	0	5.6 ± 0.9	5.4 ± 1.0
	6	5.5 ± 0.4	5.3 ± 0.8
	12	6.4 ± 0.1	5.1 ± 0.3
	24	6.1 ± 0.7	5.2 ± 1.0
Sphingomonas paucimobilis	0	5.2 ± 1.1	6.7 ± 0.5
	6	5.9 ± 0.3	5.5 ± 1.4
	12	6.2 ± 0.3	5.2 ± 0.8
	24	6.7 ± 0.3	5.9 ± 1.1
Staphylococcus intermedius 2564	0	6.1 ± 0.5	6.6 ± 0.7
	6	6.1 ± 0.5	6.2 ± 0.1
	12	7.0 ± 0.6	6.2 ± 0.1
	24	6.5 ± 0.1	6.3 ± 0.1
Staphylococcus letnus	0	4.9 ± 0.9	4.6 ± 1.2
	6	6.0 ± 0.7	5.0 ± 1.3
	12	6.7 ± 0.6	5.3 ± 1.6
	24	5.8 ± 1.0	5.3 ± 2.2
Escherichia coli	0	5.4 ± 1.0	5.4 ± 0.7
	6	5.7 ± 0.8	4.6 ± 0.8
	12	6.7 ± 0.6	4.8 ± 0.4
	24	6.0 ± 0.4	4.3 ± 0.8
Enterobacter cloacae	0	6.5 ± 0.1	6.7 ± 0.7
	6	6.4 ± 0.1	6.4 ± 0.1
	12	6.7 ± 0.3	6.4 ± 0.3
	24	6.7 ± 0.7	6.7 ± 0.7
Unidentified 2603	0	5.9 ± 0.5	6.2 ± 0.1
	6	5.7 ± 0.4	6.2 ± 0.7
	12	6.3 ± 0.3	5.9 ± 0.5
	24	6.5 ± 1.1	5.6 ± 0.4

TABLE 25. *S_R values of* E. coli *O157:H7 and* S. *Typhimurium LT2 attached to spinach surfaces in the presence of naturally occurring antagonistic bacteria isolated from spinach surfaces.*[a]

Isolate	Time Point (h)	*E. coli* O157:H7 S_R Value	*S.* Typhimurium LT2 S_R Value
Control	0	0.02 ± 0.01	0.22 ± 0.09
	6	0.11 ± 0.10	0.26 ± 0.11
	12	0.03 ± 0.02	0.29 ± 0.24
	24	0.14 ± 0.11	0.49 ± 0.05
Listeria grayi	0	0.01 ± 0.01	0.12 ± 0.08
	6	0.03 ± 0.01	0.21 ± 0.15
	12	0.13 ± 0.12	0.37 ± 0.17
	24	0.11 ± 0.04	0.34 ± 0.14
Leuconostoc pseudomesenteroides	0	0.04 ± 0.04	0.23 ± 0.13
	6	0.03 ± 0.02	0.3 ± 0.08
	12	0.07 ± 0.05	0.5 ± 0.30
	24	0.1 ± 0.07	0.37 ± 0.09
Staphylococcus intermedius 2553	0	0.02 ± 0.02	0.16 ± 0.17
	6	0.07 ± 0.05	0.25 ± 0.18
	12	0.04 ± 0.03	0.42 ± 0.17
	24	0.08 ± 0.06	0.38 ± 0.25
Sphingomonas paucimobilis	0	0.04 ± 0.03	0.28 ± 0.16
	6	0.27 ± 0.29	0.3 ± 0.27
	12	0.06 ± 0.06	0.38 ± 0.28
	24	0.14 ± 0.09	0.4 ± 0.13
Staphylococcus intermedius 2564	0	0.02 ± 0.01	0.27 ± 0.24
	6	0.11 ± 0.04	0.49 ± 0.19
	12	0.15 ± 0.17	0.16 ± 0.20
	24	0.21 ± 0.17	0.44 ± 0.44
Staphylococcus letnus	0	0.01 ± 0.01	0.3 ± 0.24
	6	0.23 ± 0.34	0.33 ± 0.28
	12	0.14 ± 0.21	0.51 ± 0.25
	24	0.21 ± 0.28	0.32 ± 0.22
Escherichia coli	0	0.35 ± 0.46	0.29 ± 0.05
	6	0.05 ± 0.02	0.25 ± 0.03
	12	0.03 ± 0.02	0.28 ± 0.21
	24	0.26 ± 0.28	0.67 ± 0.30
Enterobacter cloacae	0	0.03 ± 0.02	0.32 ± 0.13
	6	0.02 ± 0.03	0.2 ± 0.15
	12	0.07 ± 0.06	0.5 ± 0.25
	24	0.21 ± 0.27	0.49 ± 0.10
Unidentified 2603	0	0.05 ± 0.02	0.23 ± 0.09

TABLE 25 (continued)

Isolate	Time Point (h)	*E. coli* O157:H7 S_R Value	*S.* Typhimurium LT2 S_R Value
Unidentified 2603	6	0.03 ± 0.01	0.21 ± 0.04
	12	0.11 ± 0.07	0.41 ± 0.12
	24	0.02 ± 0.03	0.09 ± 0.12

[a] Control indicates no antagonistic isolate was added; only pathogens were present. S_R = (strongly attached bacteria)/(loosely + strongly attached bacteria). Error bars indicate one standard deviation from sample means (n=3). There were no significant difference, ($p \geq 0.05$), between the Control S_R at each time point and the S_R of each pathogen in the presence of each antagonist isolate.

At 0 h, the populations of *E. coli* O157:H7 and *S.* Typhimurium LT2 on the endive surface in the presence of endive-recovered antagonistic isolates were 4.0 ± 0.2 and 3.9 ± 0.2 $\log_{10}$ CFU/cm^2, respectively. Populations of the antagonistic isolates at 0 h were 6.5 ± 0.6 $\log_{10}$ CFU/cm^2. After 24 h, populations of *E. coli* O157:H7 and *S.* Typhimurium LT2 ranged from 4.0 ± 0.3 to 5.8 ± 0.4 and 4.0 ± 0.5 to 4.9 ± 0.3 $\log_{10}$ CFU/cm^2, respectively (Figures 7 and 8).

Antagonistic isolate populations did not differ significantly in the presence of the pathogens when compared to without pathogens present for each isolate, ($p>0.05$) (Table 26). The endive recovered antagonistic isolate, *Escherichia coli* 1472, depressed the growth of both pathogens; overall pathogen load in the presence of this antagonistic isolate did not decrease from those at 0 h. The strength of attachment in the presence of the isolates after 24 h for *E. coli* O157:H7 and *S.* Typhimurium LT2 ranged from 0.02 ± 0.01 to 0.08 ± 0.07 and 0.28 ± 0.21 to 0.69 ± 0.14, respectively (Table 27).

After 24, *Enterococcus casseliflavus, Bacillus* spp., *Staphylococcus aureus,* and an unidentified isolate 1442 produced a lower strength of attachment for *E. coli* O157:H7 (Table 27). However, *Staphylococcus warneri, Bacillus* spp., and an unidentified isolate 1442 produced a lower strength of attachment for *S.* Typhimurium after 24 h (Table 27). Although there was no significant difference between the strength of attachment for the pathogens in the presence of antagonistic isolates versus pathogens only on the surface of endive, the S_R values did significantly increase ($p < 0.05$) over the period of 24 hours for both pathogens indicating an increase in attachment strength over the 24 hour.

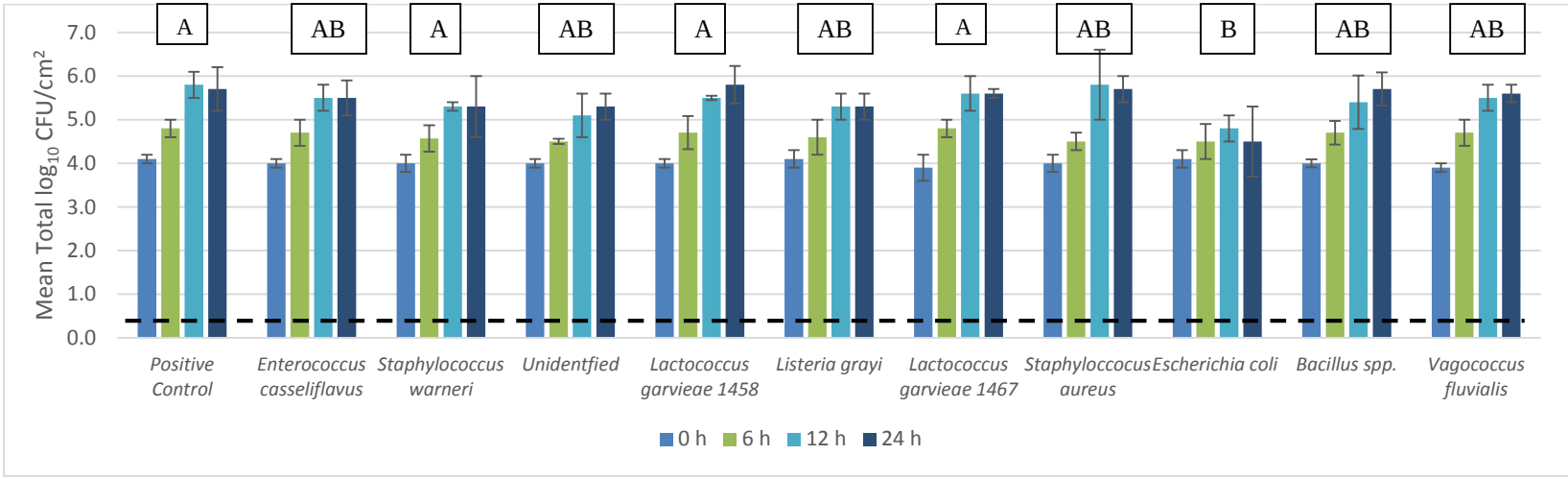

FIGURE 7. *Surviving* E. coli *O157:H7 on endive surfaces as influenced by antagonist addition.* The isolates tested in this experiment previously showed antagonistic activity *in vitro*. Positive control indicates no antagonistic isolate was added; only pathogens were present. Bars represent the sum of loosely and strongly attached. Error bars indicate one standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2). Isolates with the same letter did not differ from one another ($p \geq 0.05$).

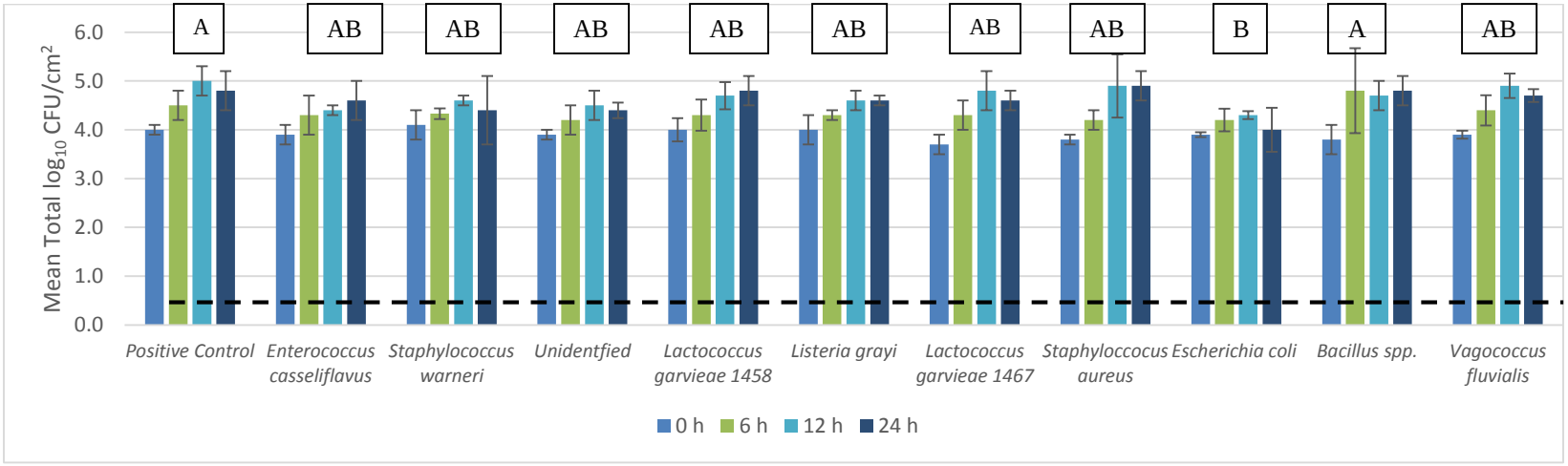

FIGURE 8. ***Surviving* S. *Typhimurium LT2 on endive surfaces as influenced by antagonist addition***. The isolates tested in this experiment previously showed antagonistic activity *in vitro*. Positive control indicates no antagonistic isolate was added; only pathogens were present. Bars represent the sum of loosely and strongly attached cells. Error bars indicate one standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2). Isolates with the same letter did not differ from one another ($p \geq 0.05$).

TABLE 26. ***Arithmetic means of pathogen-antagonizing inoculated bacteria on the surface of endive.***

Isolate	Time Point (h)	With Pathogens Present Mean $\log_{10}$ CFU/cm^2	Isolate Only Mean $\log_{10}$ CFU/cm^2
Enterococcus casseliflavus	0	6.4 ± 0.1	6.3 ± 0.1
	6	6.3 ± 0.1	6.2 ± 0.1
	12	6.3 ± 0.3	6.1 ± 0.1
	24	6.1 ± 0.1	6.1 ± 0.8
Staphylococcus warneri	0	5.9 ± 0.5	6.3 ± 0.9
	6	5.3 ± 0.2	5.8 ± 0.3
	12	5.7 ± 0.3	5.7 ± 0.7
	24	6.2 ± 0.8	5.6 ± 0.6
Unidentified 1442	0	6.1 ± 0.4	6.6 ± 0.3
	6	6.4 ± 0.1	6.5 ± 0.4
	12	6.5 ± 0.3	6.2 ± 0.1
	24	6.1 ± 0.6	6.2 ± 0.2
Lactococcus garvieae 1458	0	7.2 ± 0.4	7.2 ± 0.5
	6	7.0 ± 0.2	6.5 ± 0.5
	12	6.9 ± 0.2	6.8 ± 0.5
	24	6.6 ± 0.4	6.2 ± 0.0
Listeria grayi	0	6.4 ± 0.1	6.5 ± 0.2
	6	6.6 ± 0.4	6.5 ± 0.6
	12	6.4 ± 0.2	6.3 ± 0.2
	24	6.3 ± 0.1	5.6 ± 0.5
Lactococcus garieae 1467	0	6.8 ± 0.2	6.9 ± 0.2
	6	6.6 ± 0.0	6.8 ± 0.6
	12	6.8 ± 0.2	6.5 ± 0.4
	24	6.3 ± 0.2	6.2 ± 0.2
Staphylococcus warneri	0	7.1 ± 0.0	7.1 ± 0.1
	6	6.8 ± 0.2	6.7 ± 0.3
	12	6.7 ± 0.2	5.8 ± 1.4
	24	6.4 ± 0.2	6.4 ± 0.6
Escherichia coli	0	6.6 ± 0.2	6.9 ± 0.4
	6	6.7 ± 0.2	6.8 ± 0.4
	12	6.9 ± 0.4	6.7 ± 0.4
	24	6.7 ± 0.4	5.5 ± 1.3
Bacillus spp.	0	5.9 ± 0.3	6.0 ± 0.2
	6	6.1 ± 0.6	5.5 ± 0.6
	12	6.0 ± 0.2	5.2 ± 0.7
	24	6.0 ± 0.5	5.1 ± 0.8
Vagococcus fluvalis	0	5.6 ± 0.7	5.4 ± 0.4
	6	6.0 ± 0.6	5.3 ± 0.1
	12	6.0 ± 0.3	5.8 ± 0.5
	24	6.3 ± 0.1	4.8 ± 0.7

TABLE 27. *S_R values of* E. coli *O157:H7* and *S.* Typhimurium LT2 *attached to endive surfaces in the presence of naturally occurring antagonistic bacteria isolated from endive surfaces*.[a]

Isolate	Time Point (h)	*E. coli* O157:H7 S_R Value	*S.* Typhimurium LT2 S_R Value
Control	0	0.03 ± 0.02	0.11 ± 0.03
	6	0.10 ± 0.08	0.25 ± 0.08
	12	0.08 ± 0.05	0.30 ± 0.11
	24	0.16 ± 0.14	0.50 ± 0.1
Enterococcus casseliflavus	0	0.03 ± 0.02	0.18 ± 0.09
	6	0.02 ± 0.01	0.42 ± 0.15
	12	0.07 ± 0.05	0.31 ± 0.20
	24	0.09 ± 0.03	0.48 ± 0.32
Staphylococcus warneri	0	0.04 ± 0.03	0.45 ± 0.34
	6	0.10 ± 0.09	0.25 ± 0.30
	12	0.09 ± 0.02	0.45 ± 0.12
	24	0.18 ± 0.20	0.31 ± 0.24
Unidentified 1442	0	0.02 ± 0.01	0.15 ± 0.05
	6	0.10 ± 0.06	0.47 ± 0.12
	12	0.16 ± 0.23	0.23 ± 0.05
	24	0.09 ± 0.04	0.38 ± 0.08
Lactococcus garvieae 1458	0	0.06 ± 0.05	0.23 ± 0.06
	6	0.07 ± 0.03	0.37 ± 0.10
	12	0.04 ± 0.01	0.29 ± 0.09
	24	0.13 ± 0.10	0.50 ± 0.29
Listeria grayi	0	0.03 ± 0.01	0.16 ± 0.12
	6	0.06 ± 0.04	0.39 ± 0.16
	12	0.09 ± 0.05	0.40 ± 0.20
	24	0.16 ± 0.12	0.51 ± 0.11
Lactococcus garieae 1467	0	0.08 ± 0.07	0.36 ± 0.11
	6	0.08 ± 0.09	0.47 ± 0.22
	12	0.06 ± 0.03	0.37 ± 0.17
	24	0.15 ± 0.08	0.61 ± 0.07
Staphylococcus warneri	0	0.05 ± 0.03	0.30 ± 0.18
	6	0.06 ± 0.04	0.31 ± 0.21
	12	0.05 ± 0.02	0.30 ± 0.07
	24	0.12 ± 0.09	0.41 ± 0.13
Escherichia coli	0	0.08 ± 0.09	0.32 ± 0.21
	6	0.07 ± 0.03	0.18 ± 0.05
	12	0.17 ± 0.03	0.39 ± 0.22
	24	0.33 ± 0.25	0.69 ± 0.14
Bacillus spp.	0	0.03 ± 0.03	0.21 ± 0.11

TABLE 27 (continued)

Isolate	Time Point (h)	*E. coli* O157:H7 S_R Value	*S.* Typhimurium LT2 S_R Value
	6	0.13 ± 0.06	0.48 ± 0.45
	12	0.08 ± 0.04	0.35 ± 0.13
	24	0.12 ± 0.12	0.28 ± 0.21
Vagococcus fluvalis	0	0.08 ± 0.1	0.24 ± 0.1
	6	0.05 ± 0.02	0.32 ± 0.16
	12	0.11 ± 0.11	0.36 ± 0.12
	24	0.20 ± 0.23	0.45 ± 0.21

[a]Control indicates no antagonistic isolate was added; only pathogens were present. S_R = (strongly attached bacteria)/(loosely + strongly attached bacteria). Error bars indicate one standard deviation from sample means (n=3). There were no significant difference, ($p \geq 0.05$), between the Control S_R at each time point and the S_R of each pathogen in the presence of each antagonist isolate.

Pathogen Antagonism on the Surface of Tomatoes and Cantaloupes

Populations on tomato surfaces at 0 h of *E. coli* O157:H7 and *S.* Typhimurium LT2 in the presence of the antagonistic isolates were 3.2 ± 0.5 and 3.2 ± 0.2 $\log_{10}$ CFU/cm^2, respectively, and after 24 h, ranged from 4.7 ± 1.5 to 5.0 ± 1.4 and 3.9 ± 1.3 to 4.6 ± 1.3 $\log_{10}$ CFU/cm^2, respectively (Figures 9-10). Populations of the antagonistic isolates at 0 h were 6.0 ± 0.9 $\log_{10}$ CFU/cm^2. Antagonistic isolate populations did not differ significantly in the presence of the pathogens when compared to without pathogens present for each isolate, ($p \geq 0.05$) (Table 28).

The strength of attachment in the presence of the isolates after 24 h for *E. coli* O157:H7 and *S.* Typhimurium LT2 ranged from 0.02 ± 0.01 to 0.34 ± 0.50 and 0.06 ± 0.05 to 0.48 ± 0.44, respectively (Table 29). After 24 h, *Staphylococcus hominis* ssp. *hominis*, *Staphylococcus haemolyticus*, and *Leclercia adecarboxylata* produced a lower strength of attachment for *E. coli* O157:H7 (Table 29). *Staphylcooccus haemolytics* and *Leclercia adecarboxylata* also showed the lowest strength of attachment after 24 h for *S.* Typhimurium (Table 29). There were no significant differences ($p > 0.05$) in the growth of both pathogens in the presence of the antagonistic isolates at each time point when compared to the positive control (no antagonistic isolate).

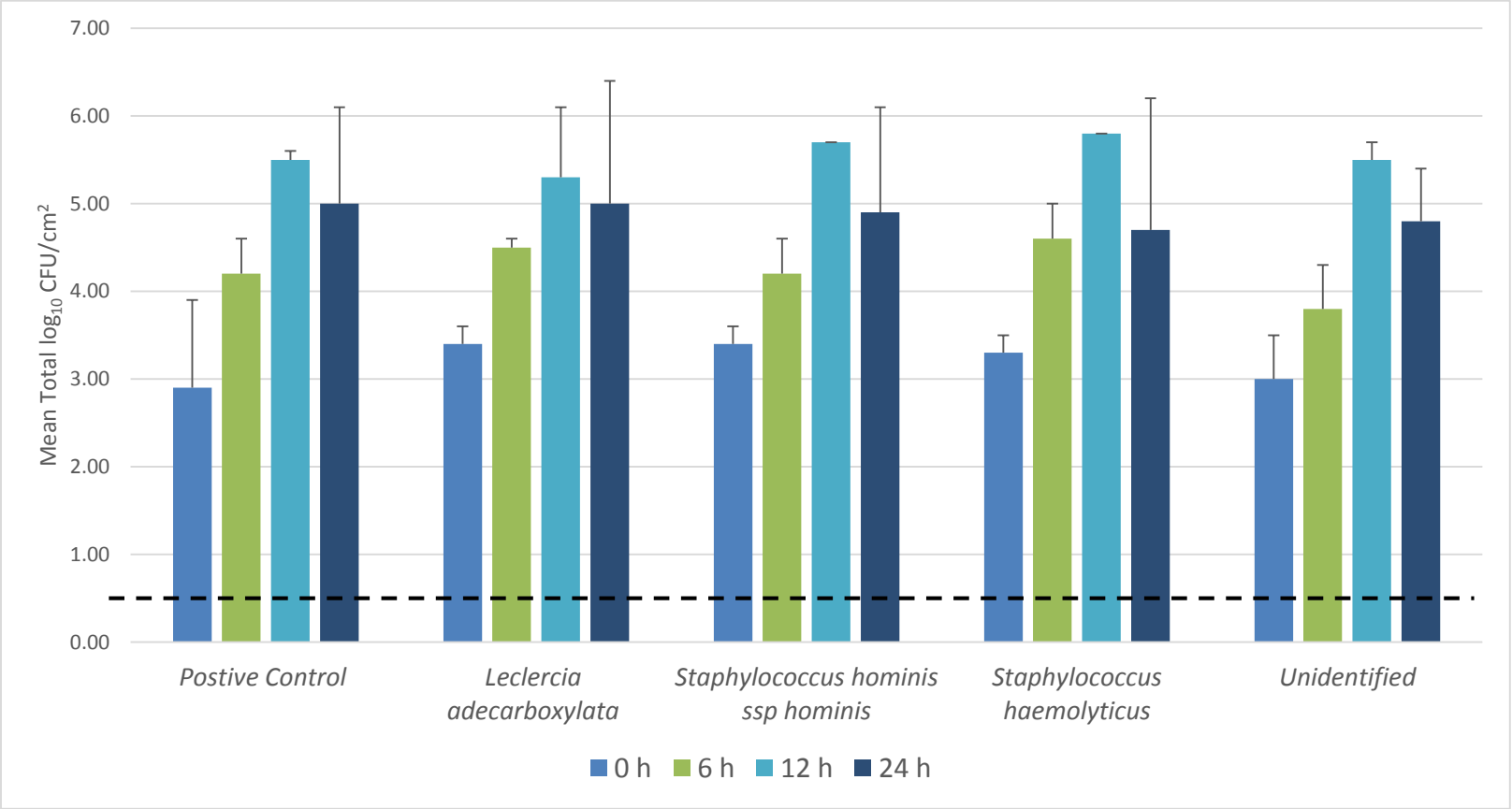

FIGURE 9. ***Surviving* E. coli *O157:H7* on tomato surfaces as influenced by antagonist addition.** The isolates tested in this experiment previously showed antagonistic activity in vitro. Positive control indicates no antagonistic isolate was added; only pathogens were present. Total $\log_{10}$ CFU/cm^2 is the total of Loosely Attached Cells ($\log_{10}$ CFU/cm^2) and Strongly Attached Cells ($\log_{10}$ CFU/cm^2). Error bars indicate one standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2). There was no significant difference ($p \geq 0.05$) between adding antagonistic isolates and not at each time point for each antagonist.

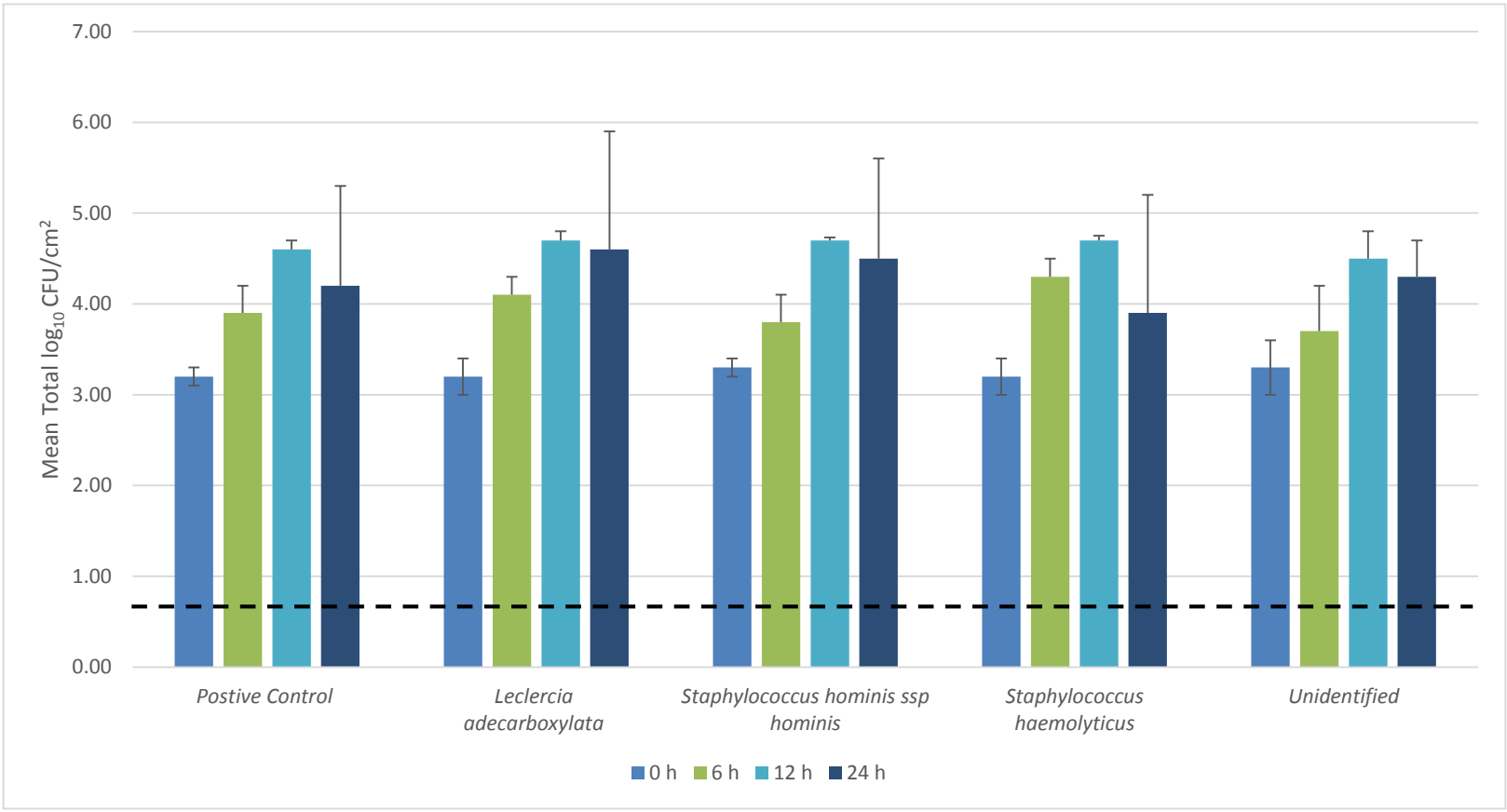

FIGURE 10. ***Surviving* S. *Typhimurium LT2 on tomato surfaces as influenced by antagonist activity.*** The isolates tested in this experiment previously showed antagonistic activity in vitro. Positive control indicates no antagonistic isolate was added; only pathogens were present. Total $\log_{10}$ CFU/cm^2 is the total of Loosely Attached Cells ($\log_{10}$ CFU/cm^2) and Strongly Attached Cells ($\log_{10}$ CFU/cm^2). Error bars indicate one standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2). There was no significant difference ($p \geq 0.05$) between adding antagonistic isolates and not at each time point for each antagonist.

TABLE 28. ***Arithmetic means of pathogen-antagonizing inoculated bacteria on the surface of tomatoes.***

Isolate	Time Point (h)	With Pathogens Present Mean $\log_{10}$ CFU/cm^2	Isolate Only Mean $\log_{10}$ CFU/cm^2
Staphylococcus hominis ssp. *hominis*	0	5.5 ± 0.2	5.5 ± 0.5
	6	5.2 ± 0.4	5.7 ± 0.4
	12	5.7 ± 0.8	5.9 ± 0.4
	24	6.3 ± 0.3	5.7 ± 0.1
Staphylococcus haemolyticus	0	5.4 ± 0.5	5.3 ± 0.2
	6	5.6 ± 0.4	5.5 ± 0.2
	12	5.9 ± 0.2	5.7 ± 0.5
	24	5.8 ± 0.6	5.6 ± 0.3
Unidentified 3058	0	6.1 ± 0.2	6.1 ± 0.2
	6	6.2 ± 0.1	5.9 ± 0.3
	12	6.2 ± 0.1	5.9 ± 0.4
	24	6.9 ± 0.3	6.0 ± 0.3
Leclercia adecarboxylata	0	4.9 ± 0.6	5.6 ± 0.8
	6	5.6 ± 0.5	5.1 ± 0.2
	12	5.9 ± 0.3	5.0 ± 0.3
	24	6.1 ± 0.7	5.3 ± 1.0

TABLE 29. ***S_R values of* E. coli *O157:H7* and *S.* Typhimurium LT2 *attached to tomato surfaces in the presence of naturally occurring antagonistic bacteria isolated from tomato surfaces*.**[a]

Isolate	Time Point (h)	*E. coli* O157:H7 S_R Value	*S.* Typhimurium LT2 S_R Value
Control	0	0.17 ± 0.23	0.10 ± 0.02
	6	0.04 ± 0.02	0.14 ± 0.07
	12	0.08 ± 0.05	0.11 ± 0.06
	24	0.16 ± 0.23	0.28 ± 0.18
Staphylococcus hominis ssp. *hominis*	0	0.04 ± 0.03	0.14 ± 0.06
	6	0.04 ± 0.03	0.06 ± 0.02
	12	0.04 ± 0.01	0.20 ± 0.03
	24	0.02 ± 0.01	0.48 ± 0.44
Staphylococcus haemolyticus	0	0.04 ± 0.02	0.11 ± 0.04
	6	0.05 ± 0.04	0.29 ± 0.33
	12	0.04 ± 0.04	0.12 ± 0.05
	24	0.03 ± 0.01	0.06 ± 0.05
Unidentified 3058	0	0.10 ± 0.10	0.05 ± 0.04
	6	0.06 ± 0.05	0.07 ± 0.05
	12	0.13 ± 0.16	0.22 ± 0.25
	24	0.34 ± 0.50	0.23 ± 0.03
Leclercia adecarboxylata	0	0.06 ± 0.04	0.10 ± 0.02
	6	0.06 ± 0.02	0.14 ± 0.03
	12	0.06 ± 0.02	0.21 ± 0.11
	24	0.06 ± 0.08	0.10 ± 0.01

[a]Control indicates no antagonistic isolate was added; only pathogens were present. S_R = (strongly attached bacteria)/(loosely + strongly attached bacteria). Error bars indicate one standard deviation from sample means (n=3). There were no significant difference, ($p \geq 0.05$), between the Control S_R at each time point and the S_R of each pathogen in the presence of each antagonist isolate.

At 0 h, the populations of *E. coli* O157:H7 and *S.* Typhimurium LT2 on the cantaloupe surface in the presence of cantaloupe recovered antagonistic isolates were 3.5 ± 0.3 and 3.5 ± 0.3 $\log_{10}$ CFU/cm^2, respectively (Figures 11 and 12). After 24 h, populations of *E. coli* O157:H7 and *S.* Typhimurium LT2 ranged from 2.8 ± 0.8 to 5.6 ± 0.1 and 2.5 ± 0.2 to 4.7 ± 0.8 $\log_{10}$ CFU/cm^2, respectively (Figures 11 and 12). The cantaloupe recovered antagonistic isolates *Escherichia hermannii* and an unidentified isolate 151 significantly ($p < 0.05$) depressed the growth of *E. coli* O157:H7. Overall, the *E. coli* O157:H7 concentration in the presence of the other antagonistic isolates did not decrease from the initial pathogen load at 0 h (Figure 11). The cantaloupe recovered antagonistic isolates *Escherichia hermannii*, *Enterococcus casseliflavus*, and an unidentified isolate 151 significantly ($p < 0.05$) depressed the growth of *S.* Typhimurium LT2. Overall, the *S.* Typhimurium LT2 concentration in the presence of the other antagonistic isolates did not decrease from those at 0 h (Figure 12).

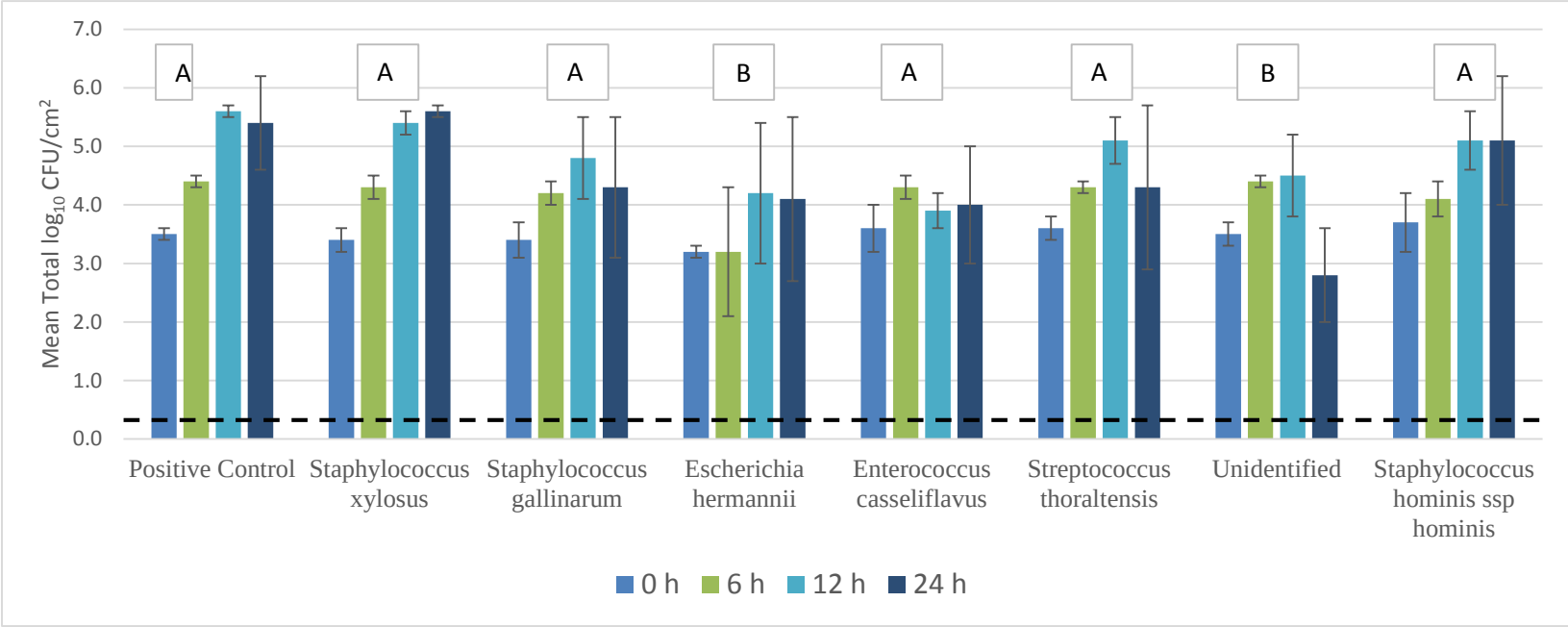

FIGURE 11. ***Surviving* E. coli *O157:H7 on cantaloupe surfaces as influenced by antagonist addition***. Positive control indicates no antagonistic isolate was added; only pathogens were present. Bars represent the sum of loosely and strongly attached. Error bars indicate one standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2). Isolates with the same letter did not differ from one another ($p \geq 0.05$).

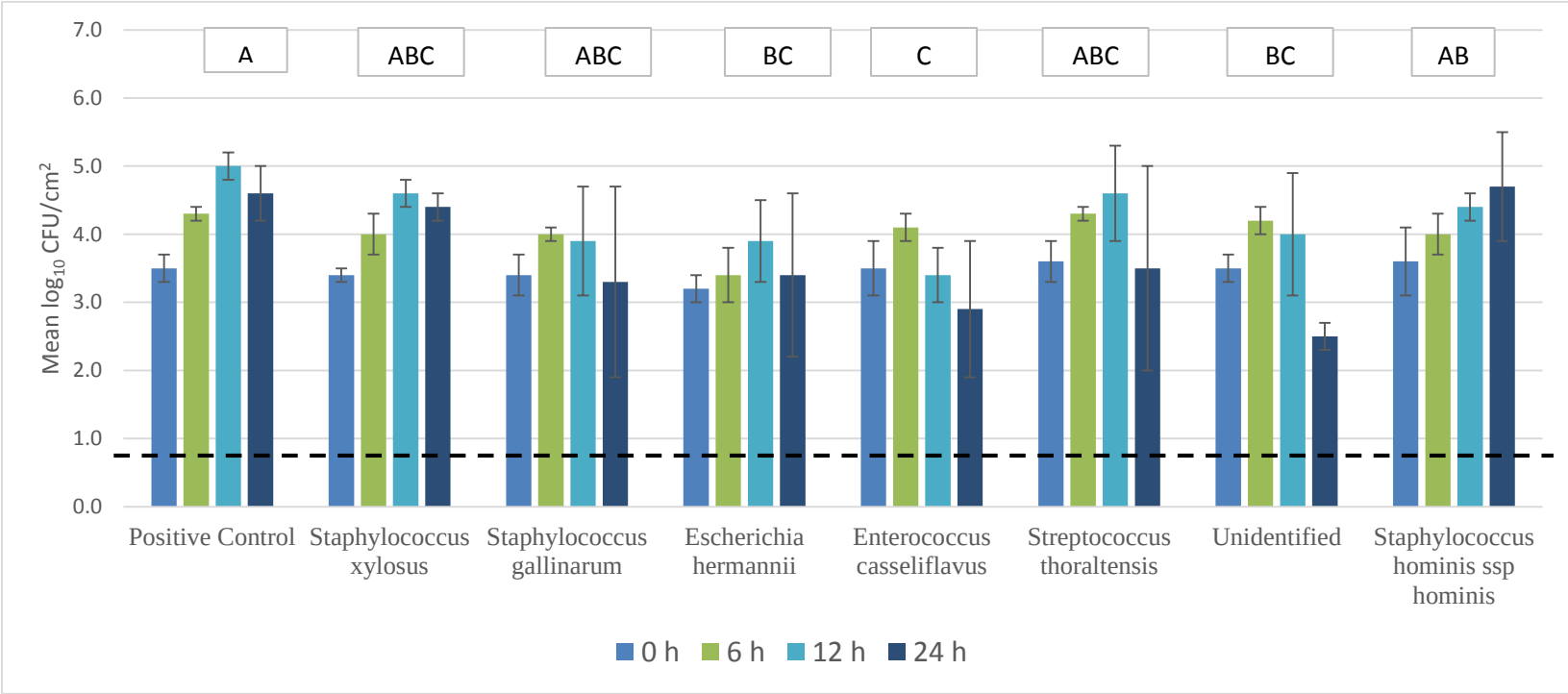

FIGURE 12. ***Surviving* S. *Typhimurium LT2 on cantaloupe surfaces as influenced by antagonist addition.*** The isolates tested in this experiment previously showed antagonistic activity in vitro. Positive control indicates no antagonistic isolate was added; only pathogens were present. Total $\log_{10}$ CFU/cm^2 is the total of Loosely Attached Cells ($\log_{10}$ CFU/cm^2) and Strongly Attached Cells ($\log_{10}$ CFU/cm^2). Error bars indicate one standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2). Isolates with the same letter did not differ from one another ($p \geq 0.05$).

Populations of the antagonistic isolates at 0 h were 6.1 ± 0.6 $\log_{10}$ CFU/cm^2. Antagonistic isolate populations did not differ significantly in the presence of the pathogens when compared to without pathogens present for each antagonistic isolate, ($p \geq 0.05$) (Table 30). The strength of attachment in the presence of the isolates after 24 h for *E. coli* O157:H7 and *S.* Typhimurium LT2 ranged from 0.35 ± 0.25 to 0.82 ± 0.1 and 0.38 ± 0.21 to 0.69 ± 0.25, respectively (Table 31). After 24 h, *Enterococcus casseliflavus* produced a lower strength of attachment compared to no antagonist application for both *E. coli* O157:H7 and *S.* Typhimurium LT2 (Table 31). Although there was no significant difference between the strength of attachment for the pathogens in the presence of antagonistic isolates versus pathogens only on the surface of the cantaloupe rind, the S_R values did significantly increase ($p < 0.05$) over the period of 24 hours for both *E. coli* O157:H7 and *S.* Typhimurium LT2 indicating an increase in attachment strength over the 24 hour.

TABLE 30. ***Arithmetic means of pathogen-antagonizing inoculated bacteria on the surface of cantaloupes.***

Isolate	Time Point (h)	With Pathogens Present Mean $\log_{10}$ CFU/cm^2	Isolate Only Mean $\log_{10}$ CFU/cm^2
Staphylococcus xylosus	0	6.3 ± 0.0	6.4 ± 0.1
	6	6.1 ± 0.3	6.0 ± 0.1
	12	6.5 ± 0.0	6.2 ± 0.1
	24	6.6 ± 0.3	6.4 ± 0.9
Staphylococcus gallinarum	0	6.0 ± 0.5	6.4 ± 0.1
	6	6.4 ± 0.3	6.4 ± 0.6
	12	6.4 ± 0.4	5.8 ± 1.7
	24	5.6 ± 0.6	6.5 ± 0.4
Escherichia hermannii	0	6.2 ± 0.6	5.6 ± 1.3
	6	6.0 ± 0.8	6.3 ± 1.1
	12	6.7 ± 0.2	6.5 ± 0.4
	24	6.4 ± 0.6	6.3 ± 0.7
Enterococcus casseliflavus	0	5.9 ± 0.2	6.1 ± 0.0
	6	6.1 ± 0.1	6.1 ± 0.1
	12	6.0 ± 0.4	6.1 ± 0.1
	24	5.7 ± 0.5	5.7 ± 0.4
Streptococcus thoraltensis	0	6.4 ± 0.1	6.8 ± 0.3
	6	6.4 ± 0.1	7.0 ± 0.0
	12	6.7 ± 0.2	7.0 ± 0.1
	24	6.5 ± 0.4	7.0 ± 0.3
Unidentified	0	5.4 ± 0.9	5.7 ± 0.7
	6	5.6 ± 0.5	5.5 ± 1.0
	12	5.7 ± 0.6	4.7 ± 0.4
	24	5.6 ± 0.6	5.1 ± 1.1
Staphylococcus hominis ssp. *hominis*	0	5.6 ± 0.5	5.8 ± 0.4
	6	5.4 ± 0.3	5.9 ± 0.6
	12	6.1 ± 0.7	5.8 ± 0.5
	24	6.4 ± 0.5	5.8 ± 0.5

TABLE 31. *S_R values of* E. coli *O157:H7* and *S.* Typhimurium LT2 *attached to cantaloupe surfaces in the presence of naturally occurring antagonistic bacteria isolated from cantaloupe surfaces*.[a]

Isolate	Time Point (h)	*E. coli* O157:H7 S_R Value	*S.* Typhimurium LT2 S_R Value
Control	0	0.25 ± 0.03	0.39 ± 0.06
	6	0.18 ± 0.07	0.44 ± 0.16
	12	0.37 ± 0.08	0.50 ± 0.05
	24	0.47 ± 0.09	0.48 ± 0.21
Staphylococcus xylosus	0	0.34 ± 0.07	0.4 ± 0.08
	6	0.50 ± 0.28	0.57 ± 0.22
	12	0.54 ± 0.27	0.26 ± 0.08
	24	0.50 ± 0.04	0.69 ± 0.25
Staphylococcus gallinarum	0	0.33 ± 0.06	0.49 ± 0.10
	6	0.28 ± 0.13	0.39 ± 0.13
	12	0.27 ± 0.10	0.47 ± 0.19
	24	0.45 ± 0.12	0.51 ± 0.17
Escherichia hermannii	0	0.24 ± 0.13	0.33 ± 0.18
	6	0.26 ± 0.09	0.54 ± 0.06
	12	0.29 ± 0.18	0.47 ± 0.27
	24	0.47 ± 0.09	0.63 ± 0.23
Enterococcus casseliflavus	0	0.41 ± 0.23	0.47 ± 0.13
	6	0.29 ± 0.06	0.54 ± 0.18
	12	0.69 ± 0.14	0.83 ± 0.11
	24	0.35 ± 0.25	0.38 ± 0.21
Streptococcus thoraltensis	0	0.29 ± 0.14	0.45 ± 0.03
	6	0.48 ± 0.22	0.49 ± 0.17
	12	0.38 ± 0.07	0.33 ± 0.09
	24	0.66 ± 0.10	0.57 ± 0.27
Unidentified	0	0.16 ± 0.12	0.28 ± 0.22
	6	0.38 ± 0.13	0.39 ± 0.14
	12	0.52 ± 0.07	0.75 ± 0.09
	24	0.82 ± 0.10	0.8 ± 0.08
Staphylococcus hominis ssp. *hominis*	0	0.45 ± 0.07	0.54 ± 0.11
	6	0.34 ± 0.15	0.37 ± 0.3
	12	0.48 ± 0.18	0.83 ± 0.08
	24	0.53 ± 0.34	0.53 ± 0.28

[a]Control indicates no antagonistic isolate was added; only pathogens were present. S_R = (strongly attached bacteria)/(loosely + strongly attached bacteria). Error bars indicate one standard deviation from sample means (n=3). There were no significant difference, ($p \geq 0.05$), between the Control S_R at each time point and the S_R of each pathogen in the presence of each antagonist isolate.

Discussion

Overall, 1,388 isolates were isolated from the surfaces of cantaloupes, tomatoes, spinach, and endive. Of these isolates, 109 (7.8%) showed antagonism activity *in vitro* against *S.* Typhimurium LT2 and 91 (6.6%) exhibited antagonism activity *in vitro* against *E. coli* O157:H7. Overall, *in vitro* the *Staphylococcus* isolates produced larger zones of inhibition against both pathogens than the other antagonistic isolates recovered from spinach, tomatoes, and cantaloupes indicating greater pathogen suppression. The tomato recovered isolate, *Staphylococcus hominis* ssp. *hominis*, produced the largest zone of inhibition (14.2 ± 5.2 mm) followed by the spinach recovered isolate, *Staphylococcus intermedius*, (13.9 ± 3.8 mm) and the cantaloupe recovered isolate, *Staphylococcus xylosus* (12.6 ± 2.6 mm) against *S.* Typhimurium LT2. The spinach recovered isolate, *Staphylococcus intermedius*, produced the largest zone of inhibition (7.2 ± 0.4 mm) followed by the tomato recovered isolate, *Staphylococcus haemolyticus* (7.1 ± 0.7 mm), and the cantaloupe recovered isolate, *Staphylococcus xylosus*, (6.0 ± 2.1 mm) against *E. coli* O157:H7. However, the endive recovered isolates *Lactococcus garvieae* produced larger zones of inhibition against both pathogens than the *Staphylococcus* isolates recovered from cantaloupes, tomatoes and spinach. *Lactococcus garvieae* produced a zone of inhibition of 17.8 ± 4.7 mm against *S.* Typhimurium LT2 and 11.6 ± 1.7 mm against *E. coli* O157:H7.

Staphylococcus spp. have been shown to have antagonizing effects against foodborne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus (12, 23, 82, 119, 136)*. Babic *et al. (12)* showed how the growth of *Listeria monocytogenes*

ATCC 19111 was affected by fresh-cut spinach containing *Pseudomonas fluorescens, Staphylococcus xylosus,* and an undefined culture of mesophilic aerobic microorganisms isolated from spinach. Babic *et al. (12)* showed that in TSB, a mix culture of *Listeria monocytogenes* and *Staphylococcus xylosus* achieved a concentration of 7.8 $\log_{10}$ CFU/ml compared to *Listeria monocytogenes* alone which achieved a final population of 9.0 $\log_{10}$ CFU/ml after 24 h with an initial *Listeria monocytogenes* population (0 h) of 2.44 $\log_{10}$ CFU/ml. Villani *et al. (136)* tested one hundred and twenty-five isolates of Micrococcaceae from Italian salami for antagonistic activities against *Listeria monocytogenes*. The researchers isolated colonies using the Harrison disc method for random selection and screened the colonies for Gram reaction, cell morphology and catalase test; only colonies that were catalase positive, Gram-positive, and shown to be irregular clusters were tested for antagonistic activity *(136).* The agar spot method was used to test for inhibitory activity, and only four isolates identified as *Staphylococcus xylosus* inhibited the growth of all five strains of *Listeria monocytogenes (*CAL, OH, V7, Scott A, ISS) tested *(136). Staphylococcus xylosus* 1E and 27E produced zones of inhibitions >10 mm (without the diameter of the spot) for all strains of *Listeria monocytogenes*; *Staphylococcus xylosus* 39A and 41A produced zones ranging from 3-5 mm (without the diameter of the spot) for all five strains *(136).* Although all strains were identified as *Staphylococcus xylosus,* not all strains exhibited the same antagonistic behavior nor did all the strains respond to the enzymatic tests identically *(136).* Although the foodborne pathogens used in this study were Gram-positive, the inhibition zones produced were similar to those from this study against *S.* Typhimurium LT2 and *E. coli*

O157:H7. The cantaloupe-recovered *Staphylococcus xylosus* 104 produced inhibition zones of 12.6 ± 2.6 mm and 6.0 ± 2.1 mm against *S.* Typhimurium LT2 and *E. coli* O157:H7, respectively (Table 9).

On produce surfaces the endive recovered isolate *Escherichia coli* 1472 and the cantaloupe recovered isolated *Escherichia hermannii* 112 depressed the growth of both pathogens and the cantaloupe recovered isolate *Enterococcus casseliflavus* 138 depressed the growth of *S.* Typhimurium LT2 on the surface of cantaloupes. Previous studies have shown that fresh vegetables can be sources of microorganisms with inhibitory properties against pathogenic bacteria *(27, 85, 123, 131, 134).* Leverentz *et al. (85)* inoculated the surfaces of Golden Delicious apples with either *Listeria monocytogenes* or *Salmonella* Poona and apple-recovered antagonistic isolates; overall counts of *Listeria monocytogenes* on the surfaces of Golden Delicious apples were reduced by 2.5-2.8 log units after 2-5 days of storage at 25°C with the addition of the apple-recovered antagonistic isolate *Gluconobacter asaii (85).* At 10°C the researchers saw reductions of 2.1 to 2.8 log units of *Listeria monocytogenes* after 5 days of storage at 10°C *(85).* However, the researchers did not see reductions of *Salmonella* Poona at either 10°C or 25°C *(85).* Ukuku *et al. (131)* investigated five classes (*Pseudomonas* spp., Enterobacteriaceae, yeast and mold, lactic acid bacteria and aerobic mesophilic bacteria) of native microflora inoculated on the surfaces of cantaloupe rind against *Listeria monocytogenes* on the surfaces of cantaloupe rind and found that populations of *L. monocytogenes* declined over the period of 15 days of storage at 5, 10 and 20°C. Schuenzel and Harrison *(123)* isolated various organisms from carrots, green peppers,

green and iceberg lettuce, green and purple cabbage, celery, and green and yellow onions *(123)*. From the organisms isolated, the isolates with inhibitory properties against *E. coli, Listeria monocytogenes*, *S. aureus*, and *Salmonella* Montevideo had originated from the surface of lettuce *(123)*. Of the bacterial isolates that demonstrated antagonism, 92% were Gram-negative rods and 8% were Gram-positive cocci *(123)*. Though the majority of the antagonism isolates in the present study were gram positive cocci, the isolates that were most effective on produce surface against *S.* Typhimurium LT2 and *E. coli* O157:H7 were Gram-negative rods. Competition for nutrients or colicin secretion could play a major role in the antagonistic activity on produce surface of these Gram-negative organisms *(28, 123)*. Though this study was not designed to determine the mode of action of the isolates, additional studies elucidating the inhibitory behavior of the isolates would provide a means to enhancing existing technologies or novel technologies that would ultimately reduce or eliminate pathogens from produce surfaces.

CHAPTER VI

EVALUATION OF GERANIOL-LOADED POLYMERIC NANOPARTICLES' ABILITY TO INHIBIT PATHOGEN GROWTH ON PRODUCE SURFACES*

Materials and Methods

Preparation of Plant Derived Compounds and Analysis of Formed Nanoparticles

Geraniol-loaded polymeric nanoparticles (NP) were prepared with a rapid nano-precipitation method previously reported *(6, 142).* Geraniol (>96.0%; CAS# 1-6-24-1) (TCI America, Portland, OR) and the amphiphilic triblock copolymer Pluronic® F-127 (PF127; CAS$ 9003-11-6) (Sigma-Aldrich Co.) were dissolved in tetrahydrofuran (THF; CAS# 109-99-9; Sigma-Aldrich Co.) to differing ratios to determine impact of blending ratios on resulting geraniol-containing NP size. Then, solution was rapidly impinged against milli-Q water to produce polymer-encapsulated geraniol-bearing NPs. The flow rate of water was 50.0 ml/min, and the flow rate of the THF solution was 5.0 ml/min. Following impingement processing, the NP-contained solution was placed under a fume hood for 7.0 h to remove THF.

Microorganisms and Inoculum Preparation for Nanoparticle Experiments

Salmonella Typhimurium LT2 (American Type Culture Collection [ATCC] No. 700720) (Manassas, VA) and *E. coli* O157:H7 (ATCC No. 700728) were obtained from the Food Microbiology Laboratory (Department of Animal Science, Texas A&M

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University) culture collection and revived according to previously published methods *(109)*. Working cultures were obtained by aseptically scraping a loopfull (10 µl) of culture from tryptic soy agar (TSA) slants into 9.0 ml sterile tryptic soy broth (TSB) followed by incubation at 35°C for 24 hr. A second passage was completed in identical fashion, with subsequent incubation at 35°C for 24 hr prior to antimicrobial assay completion.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Assays

Salmonella Typhimurium and *E. coli* O157:H7 cultures were diluted in 9.9 ml double strength TSB (2xTSB) to 5.0 log10 CFU/ml. To quantify the initial numbers of each pathogen inoculated into reaction plates, decimal dilutions were prepared in 0.1% (w/v) peptone water (Becton, Dickinson and Co.). Diluted cells were spread onto surfaces of TSA-containing Petri dishes; bacterial cells were enumerated following incubation of Petri dishes at 35°C for 24 hr. A micro-broth dilution assay was utilized to determine MIC and MBC of free and NP loaded geraniol against both bacterial organisms (Brandt *et al.*, 2010). Wells of a 96-well sterile microplate (Falcon®, Corning, Inc., Tewksbury, MA) were loaded with 200.0 µl free or NP171 encapsulated geraniol. Following loading, geraniol (free, NP-entrapped) was diluted via addition of 100.0 µl sterile phosphate-buffered saline (0.1%) (PBS), mixed, and then 100.0 µl of mixture was removed and loaded into adjacent wells. This process of dilution with PBS and transfer of 100 µl was repeated to produce two-fold dilutions of free or NP-loaded geraniol. Following loading of EOC in wells, prepared cultures were aseptically loaded

(100.0 µl) into reaction wells. Negative controls were prepared consisting of only sterile 2xTSB (100.0 µl) and sterile PBS (100.0 µl) to confirm no cross contamination of NPs. Positive controls consisted of 2x TSB (100.0 µl) and *S.* Typhimurium or *E. coli* O157:H7 in PBS (100.0 µl) to confirm pathogen ability to grow under experimental conditions. Additionally, geraniol-containing (free, encapsulated) non-inoculated controls were prepared to allow for baseline correction during determination of bacterial growth via observation of changes in optical density at 630 nm (OD630). A PF127 control was not included based on review of previously published research showing no inherent antimicrobial activity of the polymer against various Gram-positive and Gram-negative bacterial organisms (Chudasama *et al.*, 2010; Veyries *et al.*, 2000). Immediately following preparation (0 185 hr), and again at 24 hr incubation at 35°C, microplates were loaded into an Epoch UV/Visible scanning spectrophotometer (Bio-Tek® Instruments, Inc., Winooski, VT) and sample OD630 was read. Free or encapsulated geraniol producing a change in OD630 < 0.05 were deemed inhibitory to pathogen growth; the lowest concentration of free or NP-loaded geraniol producing pathogen inhibition was identified as the MIC (Branen and Davidson, 2004). Duplicate identical wells were constructed for all combinations of antimicrobial geraniol and microorganism, as well as required controls. The assay was replicated independently two times (n=4); MICs were identified as the lowest concentrations of free or entrapped geraniol inhibiting the pathogen. Bactericidal activity of free or encapsulated geraniol was assayed following completion of MIC determination. Numbers of surviving pathogens from wells containing inhibitory concentrations of free or encapsulated

geraniol were determined by spreading 0.1 ml of culture fluid directly from a sample well onto the surface of a TSA-containing Petri dish. Inoculated Petri dishes were then incubated for 24 hr at 35°C prior to enumeration of colonies. The lowest concentration of free or NP-loaded geraniol producing a >3.0 $\log_{10}$-cycle decline in numbers of pathogen ($\log_{10}$-transformed plate count of bacterial cells prior to microplate inoculation – $\log_{10}$- transformed plate count of bacterial cells following incubation of inoculated Petri dish from pathogen-inhibiting microplate well) was identified as the MBC for free or NP-encapsulated EOC for each pathogen.

Preliminary Experiment: Efficacy of Application Method

A small experiment was designed to determine optimal NP application method for produce decontamination. Working cultures of *E. coli* O157:H7 and *S.* Typhimurium were prepared as previously described. The cells were washed by centrifugation at 2191 x *g* in a Jouan B4i centrifuge for 15 min at 22°C. Resulting pellets were suspended in 10 ml PBS and again washed by centrifugation for 15 min at 22°C; centrifugation and washing procedures were repeated identically twice. After the final cycle, pellets were suspended in 10 ml PBS; both microbes were mixed and serially diluted in 0.1% peptone water to achieve an inoculum of 8.0 $\log_{10}$ CFU/ml. The concentration of the inoculum was confirmed via selective/differential plating on LSPR. Bunched, non-waxed spinach was locally purchased and immediately returned to the Food Microbiology Laboratory. Spinach leaves were washed in sterile water and surface disinfected with 70% ethanol *(7)*. After 1 h of drying at 25°C, 10 cm^2 samples were aseptically excised from the

spinach leaves with flame-sterilized implements. Samples were spotted with ten 10.0 µl spots of microbial cocktail on to the adaxial surfaces of the leaf. Samples were then stored at 25°C for 1 h to allow pathogen attachment to spinach surface. Nanoparticle treatments were applied to spinach samples to determine decontamination capacity of antimicrobial NPs as a function of application method. In the case of spraying, the impact of the number of spray applications was also tested, and in the case of immersion, the immersion period (2 and 5 min). The treatments were spray application of NPs via one, two or three sprays (~1.0 ml/spray), or spinach sample immersion in 20 ml of NP-containing buffer for 2 or 5 min. A negative control sample consisting of no added inoculum and no NPs was plated along with a positive control sample consisting of the inoculum without NP treatment. All samples were serially diluted in 0.1% peptone water and surviving microbial cells spread on LSPR; Petri dishes plates were incubated aerobically at 35°C for 24 h prior to pathogen enumeration. The procedure was replicated identically four times (n=4).

Nanoparticle Treatment on Surface of Produce (Spinach, Cantaloupe, and Tomato)

Working cultures of *E. coli* O157:H7 and *S.* Typhimurium were prepared as previously described. The cells were washed by centrifugation at 2191 x *g* in a Jouan B4i centrifuge (Thermo-Fisher Scientific, Inc., Waltham, MA) for 15 min at 22°C. Resulting pellets were suspended in 10 ml PBS and again washed by centrifugation for 15 min at 22°C; centrifugation and washing procedures were repeated identically twice. After the final cycle, pellets were suspended in 10 ml PBS; both microbes were mixed

and serially diluted in 0.1% peptone water to achieve an inoculum of 8.0 $\log_{10}$ CFU/ml. The concentration of the inoculum was confirmed via selective/differential plating on LSPR. Non-waxed spinach, non-waxed tomatoes, and non-waxed cantaloupes were purchased and returned to the Food Microbiology Laboratory. Three-10 cm^2 samples were aseptically excised from each produce commodity with flame-sterilized implements. Samples were spotted with ten 10.0 µl spots of microbial cocktail on the adaxial surfaces of the spinach leaf, and the outer surface of the fruits. Samples were then stored at 25°C for 1 h to allow pathogen attachment to surface. Treatments were applied via immersion for each treatment for 2 minutes. The treatments were as follows: nano-encapsulated geraniol, unencapsulated geraniol, and chlorine. Nano-encapsulated geraniol was prepared as previously described. Unencapsulated geraniol was prepared by adding geraniol to sterile milli-q water at a concentration equivalent to the amount loaded into the nanoparticles. For chlorine, 6.25% hypochlorite (Clorox Co., Oakland, CA) was mixed in sterile DI water to obtain a concentration of 200 mg/L (200 ppm). The pH of the chlorine treatment was adjusted to 7.0 prior to use. A negative control sample consisting of no added inoculum and no treatment was plated along with a positive control sample consisting of the inoculum without treatment. A set of samples for the enumeration of the background microbiota consisted of no added inoculum, but with treatment was also plated for each treatment. This set of samples were plated on E.coli/Coliform 3M Petrifilm™ Plates, APC 3M Petrifilm™ Plates, APC 3M Petrifilm™ Plates with serial dilutions in MRS broth to enumerate lactic acid bacteria, and LSPR for enumeration of *E. coli* O157:H7 and *S.* Typhimurium LT2. Following

plating on day zero, the prepared samples were aerobically incubated at 5°C and pulled for enumeration on days 3, 5, 7, and 10. A set of samples was re-contaminated on the third day of incubation in order to determine the ability of the antimicrobial interventions to disallow pathogen attachment to produce surface while also inhibiting pathogens that might contaminate produce prior to packing/processing. The inoculum for this set of samples was prepared as previously described. However, the inoculum for the re-contamination was serially diluted to achieve a finally log concentration one log lower than the initial day zero inoculum. On day five of incubation after plating, a set of samples was incubated at 15°C and another set at 25°C to simulate post processing temperature abuse conditions. These samples were pulled at day 7 and 10 for enumeration. All samples were serially diluted in 0.1% peptone water and all samples were spread on LSPR. Petri dishes plates were incubated aerobically at 35°C for 24 hour prior to pathogen enumeration. The procedure was replicated identically three times (n=3).

Statistical Analyses

Microbiological data (plate counts) will be logarithmically transformed (base 10) before statistical analysis. All quantitative analyses will be conducted using JMP v10.0.0 (SAS Institute Inc., Cary, N.C.). Statistical differences between means will be analyzed using a one-way analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) ($p<0.05$).

Results

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Assays

The MICs and MBCs of nano-encapsulated and unencapsulated geraniol applied to *S.* Typhimurium and *E. coli* O157:H7 are provided in Table 27. The MICs for nano-encapsulated geraniol were lower than those obtained for unencapsulated geraniol for both *S.* Typhimurium LT2 and *E. coli* O157:H7 ($p < 0.05$). The MIC of nano-encapsulated geraniol was slightly higher (0.25 wt.%) for *S.* Typhimurium than for *E. coli* O157:H7 (0.2 wt.%). Similar results were observed with respect to difference in pathogen-specific MICs for cells exposed to unencapsulated geraniol, where MICs of free essential oil components were 0.8 and 0.4 wt.% against *S.* Typhimurium LT2 and *E. coli* O157:H7, respectively. The MBC for unencapsulated geraniol did not differ between pathogens, though the MBC for the nano-encapsulated geraniol was lower for *E. coli* O157:H7 (0.4 wt.%) as compared to *S.* Typhimurium (0.8 wt.%) ($p < 0.05$) (Table 32).

TABLE 32. ***Least squares means of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of free and polymeric NP-encapsulated geraniol against*** **S.** ***Typhimurium LT2 and*** **E. coli** ***O157:H7(140).***[a]

	E. coli O157:H7		*S.* Typhimurium LT2	
	Encapsulated	Unencapsulated	Encapsulated	Unencapsulated
MIC[a] (wt. %)	0.20A	0.40B	0.25A	0.70B
MBC[b](wt. %)	0.40A	0.80B	0.80A	0.80A
Reduction[c] (log_{10} CFU/ml)	4.5 ± 0.1A	4.5 ± 0.1A	4.4 ± 0.1A	4.6 ± 0.2A

[a]MICs are defined as the lowest concentration of free or encapsulated geraniol producing <0.05 change in baseline-corrected optical density at 630 nm after 24 h incubation at 35°C from two independent replications with duplicate identical samples per replicate (n=4).

[b]MBCs the lowest concentration of free or encapsulated geraniol producing >3.0 log_{10}-cycle decrease in numbers of bacterial organisms (calculated as log_{10}-transformed plate count of bacterial cells prior to microplate inoculation – log_{10}-transformed plate count of bacterial cells following incubation of inoculated Petri dish from pathogen-inhibiting microplate well) across two independent replications with duplicate identical samples completed per replicate (n=4).

[c]Values depict mean log_{10} reductions in pathogen numbers from two independent replications, with duplicate identical samples per replicate (n=4), + one standard deviation. Log_{10} reductions were determined as the difference in pathogen-specific log_{10}-transformed plate counts of inoculated cells – surviving cells following completion of bactericidal activity assay.

[d]Values are means of duplicate identical replicates, with two identical samples per replicate (n=4). MIC and MBC means for encapsulated versus unencapsulated geraniol within a row not sharing common letters (A, B) after the mean MIC or MBC differ for each pathogen (*E. coli* O157:H7, *S.* Typhimurium LT2) at ($p < 0.05$).

Preliminary Experiment: Efficacy of Application Method

Nano-encapsulated geraniol reduced populations of both pathogens on the surfaces of spinach leaves, though reductions achieved were variable (Figure 13). The more effective method of NP delivery to pathogens inoculated onto spinach was through immersion. For *S.* Typhimurium, 2 and 5 min immersion produced reductions in pathogen numbers of 3.2 ± 1.7 and 4.2 ± 1.2 $\log_{10}$ CFU/cm^2, respectively. For *E. coli* O157:H7, immersion for 5 min in NP containing fluid resulted in a reduction in pathogen numbers of 4.2 ± 1.5 $\log_{10}$ CFU/cm^2 , while 2 min immersion produced only a 3.0 ± 2.0 $\log_{10}$ CFU/cm^2 reduction in the pathogen (Figure 13). Application of geraniol-containing NPs via spraying produced pathogen reductions not exceeding 1.5 $\log_{10}$ CFU/cm^2.

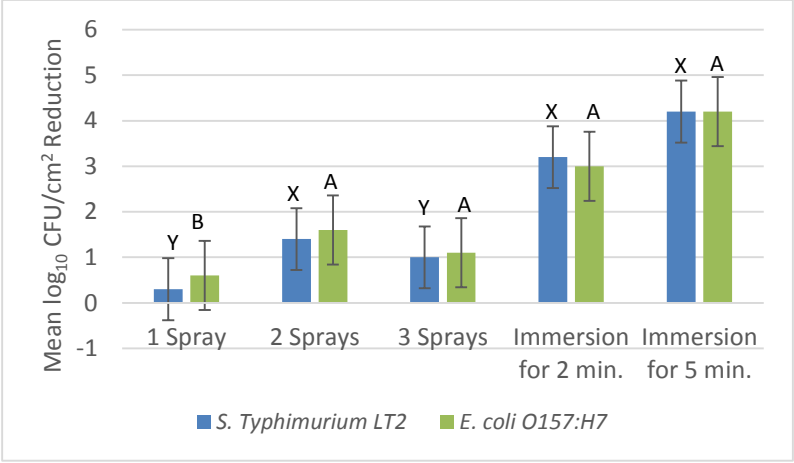

FIGURE 13. ***Least squares of mean reductions of* S. *Typhimurium and* E. coli *O157:H7 on spinach by application of nano-encapsulated geraniol using various application methods.*** Error bars indicate standard error about means (SEM). Mean populations of *E. coli* O157:H7 and *S.* Typhimurium LT2 on spinach prior to antimicrobial exposure were 6.2 ± 0.7 and 6.0 ± 0.5 $\log_{10}$ CFU/cm^2, respectively. Means for sharing same superscripted letters do not differ at p=0.05 (n 4). Limit of detection was 1.0 $\log_{10}$ CFU/cm^2*(140).*

Nanoparticle Treatment on Surface of Tomato

Inoculation of tomatoes with the bacterial pathogens resulted in 5.7 ± 0.3 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 and 5.9 ± 0.2 $\log_{10}$ CFU/cm^2 *S.* Typhimurium LT2 attaching to tomato surface on day 0 prior to incubation and treatments (Figure 14). After the initial application via immersion for 2 min. of the antimicrobials on day 0 *E. coli* O157:H7 concentrations on the surface were 3.8 ± 1.6, 4.7 ± 0.2, and 4.7 ± 0.3 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 14). Concentrations of *S.* Typhimurium LT2 on the surface were 3.4 ± 1.9, 4.6 ± 0.2, and 4.4 ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, and chlorine, respectively (Figure 14). Over the 10 day storage period at 5°C populations of both pathogens declined. At day 10 *E. coli* O157:H7 concentrations on the surface were 1.2 ± 1.1, 2.7 ± 1.4, 3.4 ± 0.7, and 3.3 ± 0.4 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 14). At day 10 *S.* Typhimurium LT2 concentrations on the surface were 1.3 ± 1.4, 3.3 ± 1.4, 4.3 ± 0.1, and 4.7 ± 0.4 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 14). Throughout the 10 day storage period 5°C treated tomatoes did not change visually and did not have visible mold growth (Figures 15-18).

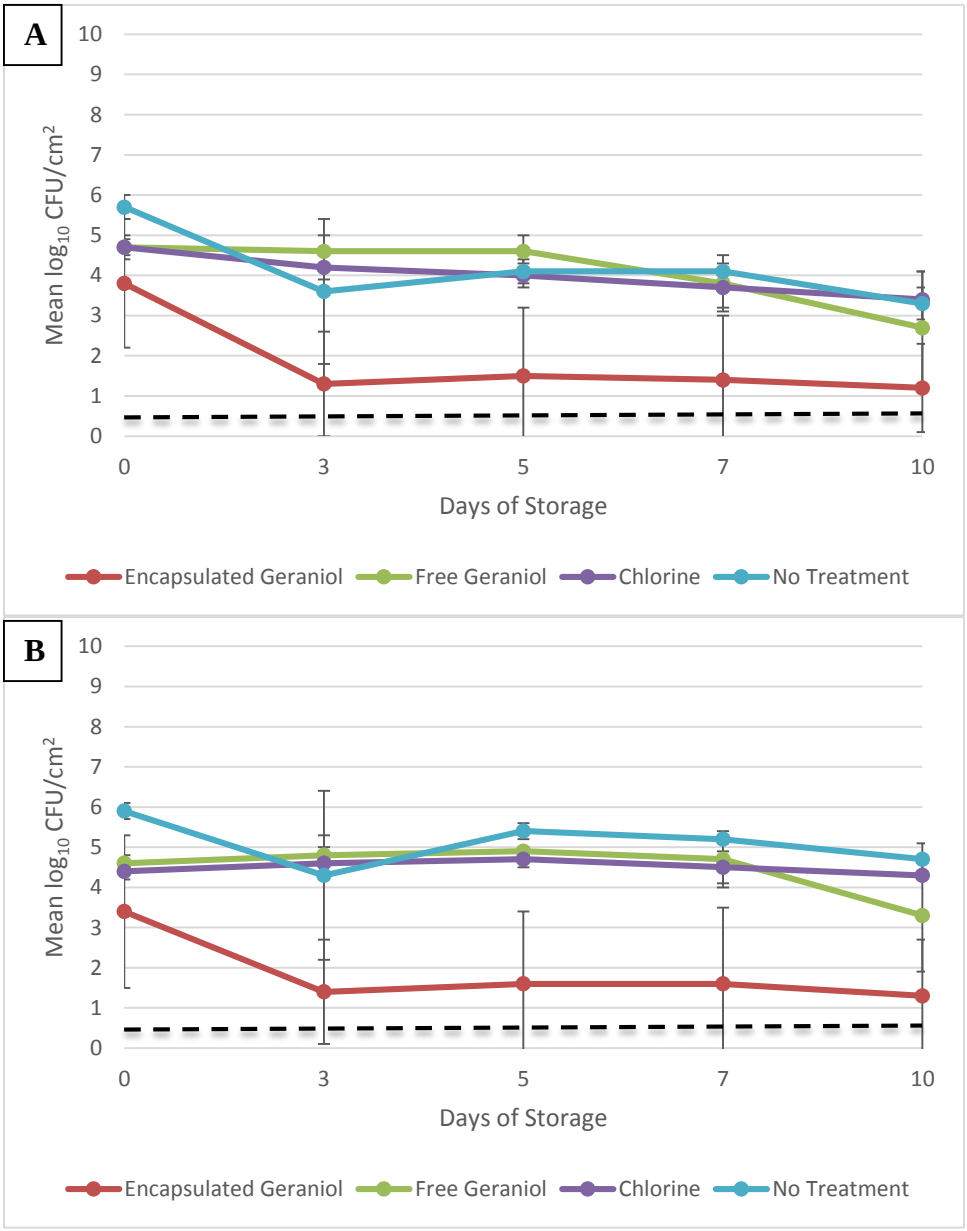

FIGURE 14. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of tomatoes stored at 5°C for 0, 3, 5, 7, and 10 days after the application of various treatments.*** Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

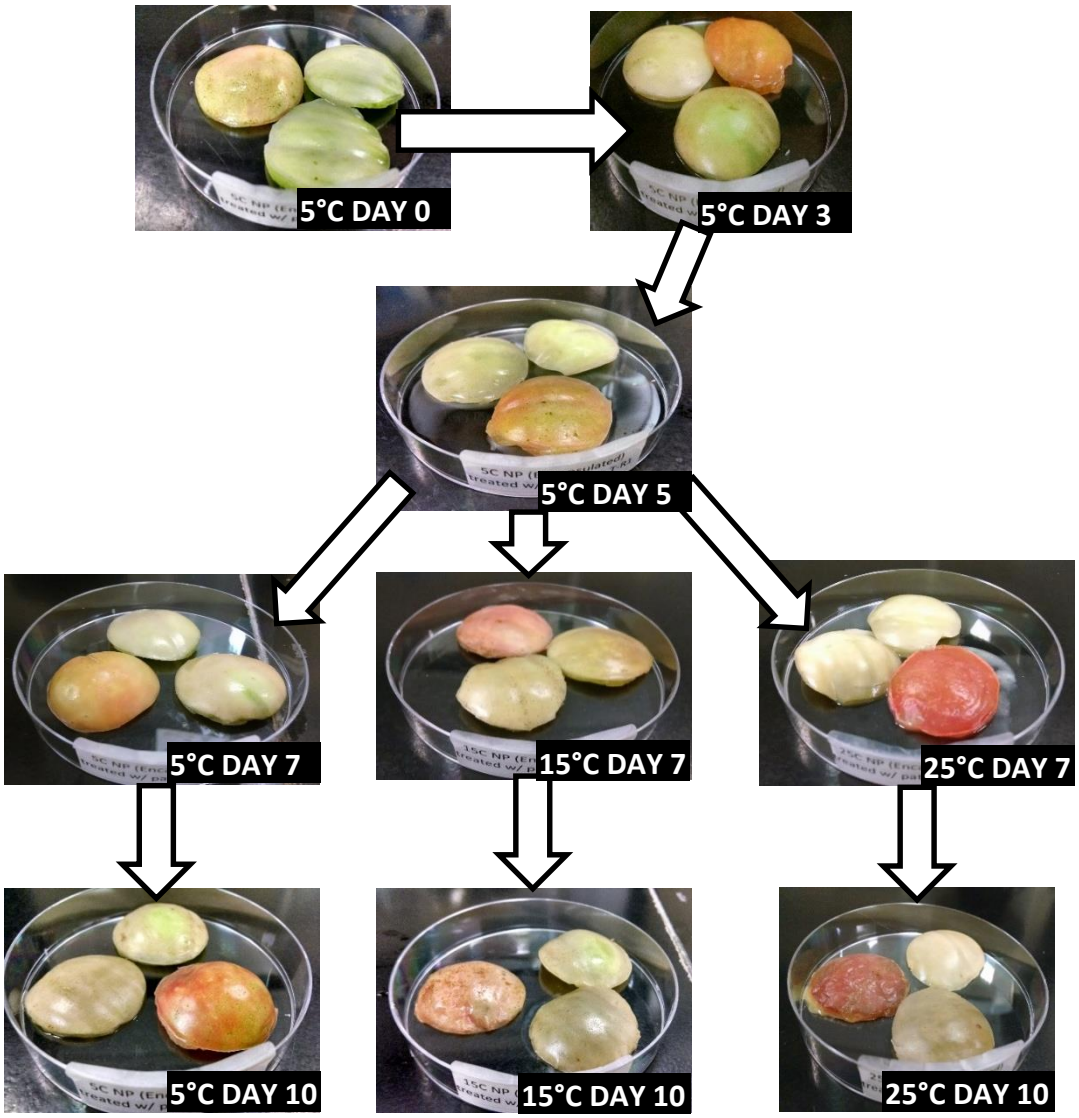

FIGURE 15. ***Nanoparticle-treated pathogen-inoculated tomato stored at various temperatures for 0, 3, 5, 7, and 10 days.***

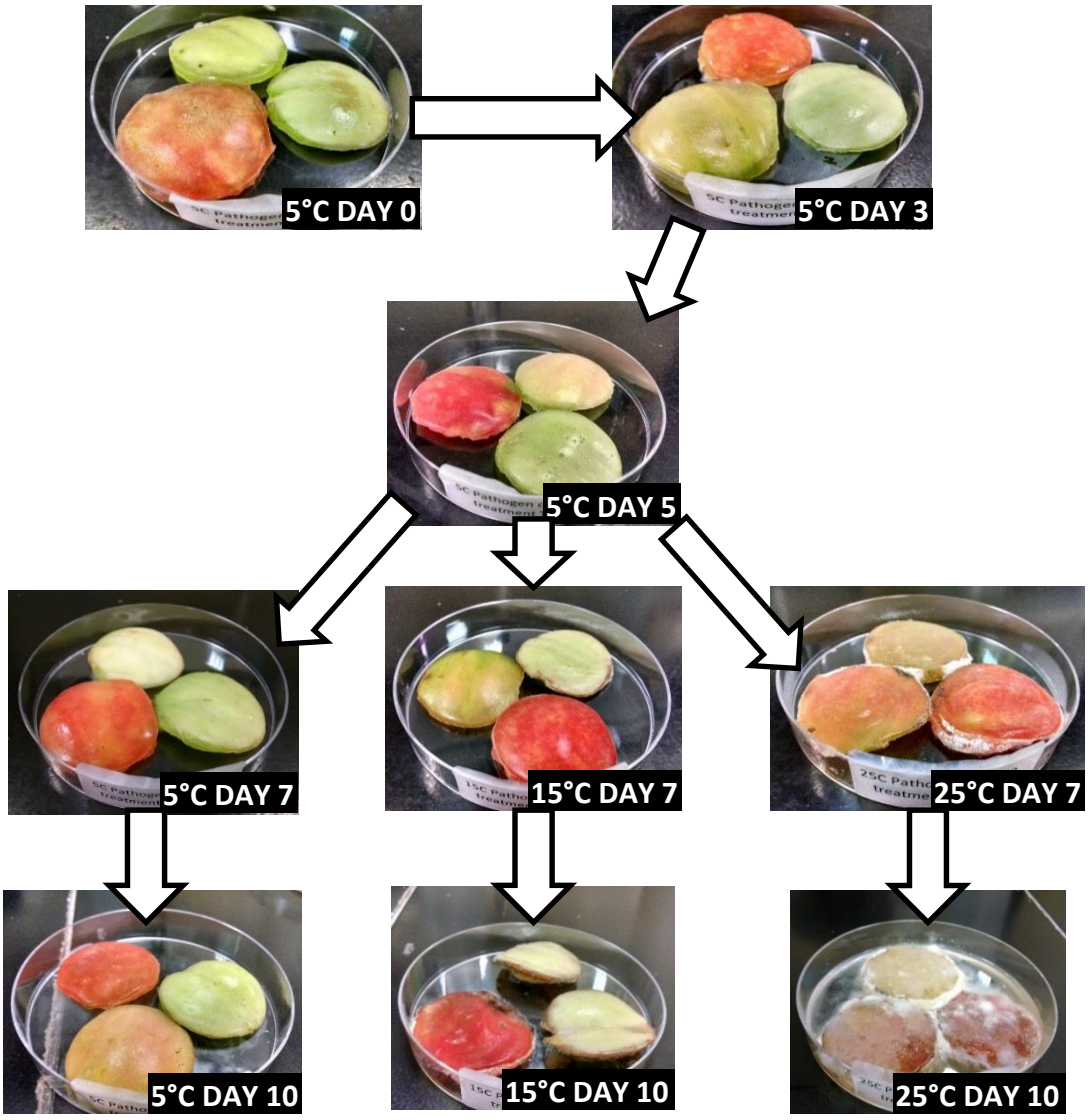

FIGURE 16. ***Untreated pathogen-inoculated tomato stored at various temperatures for 0, 3, 5, 7, and 10 days.***

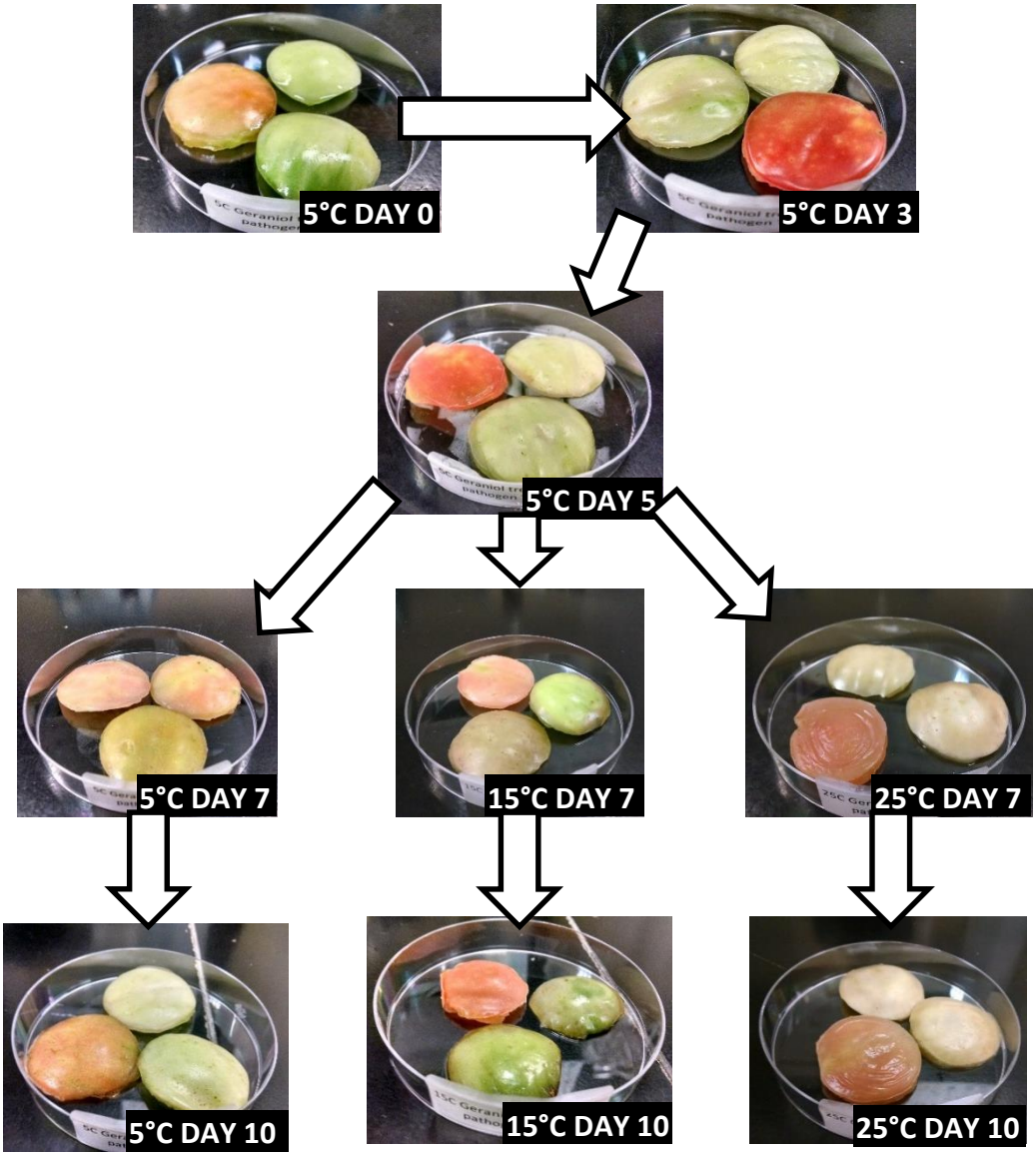

FIGURE 17. ***Unencapsulated geraniol-treated pathogen-inoculated tomato stored at various temperatures for 0, 3, 5, 7, and 10 days.***

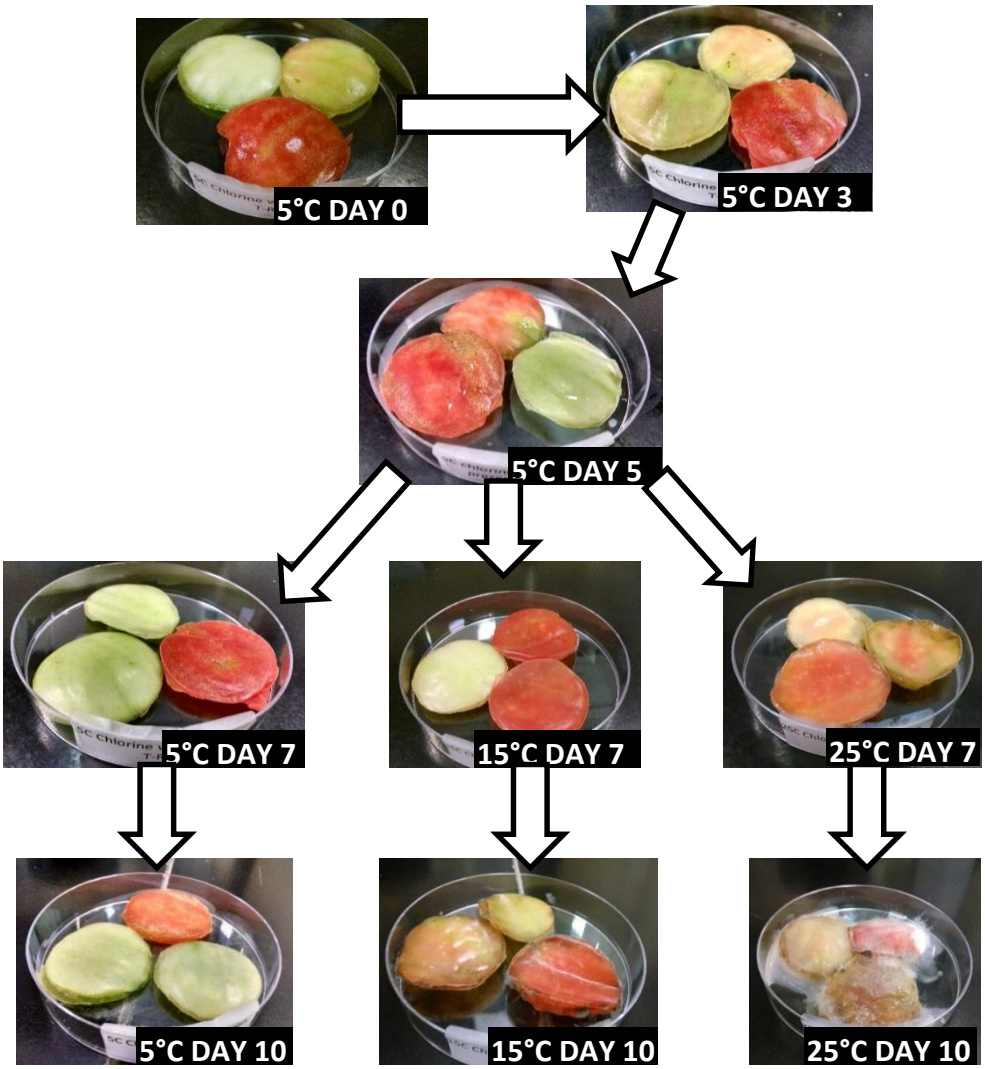

FIGURE 18. ***Chlorine-treated pathogen-inoculated tomato stored at various temperatures for 0, 3, 5, 7, and 10 days.***

On day 3 of storage, a set of tomatoes was re-contaminated with a bacterial suspension containing both pathogens with a concentration of 4.7 ± 0.3 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 and 4.7 ± 0.4 $\log_{10}$ CFU/cm^2 *S.* Typhimurium LT2 attaching to the surface. After the initial application of the pathogens on tomatoes previously pathogen-inoculated and treated with antimicrobial system, on day 3 *E. coli* O157:H7 concentrations on the surface were 4.4 ± 0.2, 4.9 ± 0.2, and 4.8 ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 19). After the initial application of the pathogens on already inoculated tomatoes on day 3, *S.* Typhimurium LT2 concentrations on the surface were 4.4 ± 0.3, 5.0 ± 0.2, and 4.8 ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 19).

At day 10, *E. coli* O157:H7 concentrations on the surface of the re-contaminated tomatoes were 1.5 ± 1.5, 3.8 ± 0.2, 3.8 ± 0.0, and 4.3 ± 0.6 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 19). At day 10 *S.* Typhimurium LT2 concentrations on the surface of the re-contaminated tomatoes were 1.6 ± 1.2, 4.0 ± 0.3, 4.2 ± 0.2, and 5.0 ± 0.5 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 19).

On day 3, non-inoculated treated samples were contaminated with pathogens to a concentration of 4.7 ± 0.3 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 and 4.7 ± 0.4 $\log_{10}$ CFU/cm^2 *S.* Typhimurium LT2 attaching to the surface to simulate post treatment contamination. On day 3, the *E. coli* O157:H7 concentration on the surface of the post treatment contaminated samples were 4.3 ± 0.1, 4.8 ± 0.0, and 4.8 ± 0.1 $\log_{10}$ CFU/cm^2 for

encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 20). On day 3, the *S.* Typhimurium LT2 concentration on the surface of the post treatment contaminated samples were 4.3 ± 0.1, 4.6 ± 0.2, and 4.7 ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 20). At day 10, *E. coli* O157:H7 concentrations on the surface of the post treatment contaminated tomatoes were 1.9 ± 1.2, 4.0 ± 0.0, 3.7 ± 0.2, and 3.9 ± 0.1 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment, respectively (Figure 20). At day 10 *S.* Typhimurium LT2 concentrations on the surface of the post treatment contaminated tomatoes were 1.8 ± 1.2, 3.9 ± 0.2, 4.0 ± 0.3, and 4.1 ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment, respectively (Figure 20).

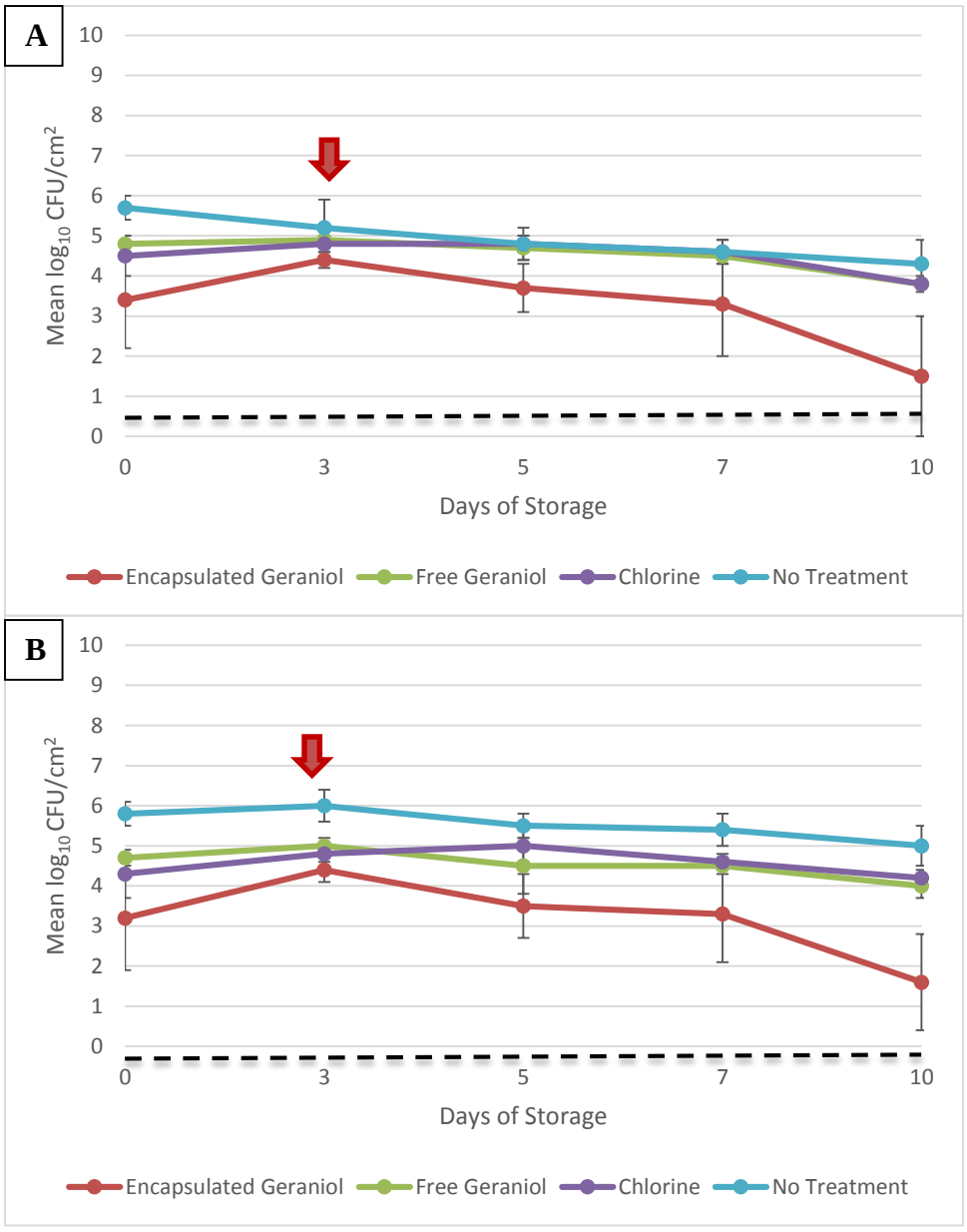

FIGURE 19. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of tomatoes stored at 5°C for 0, 3, 5, 7, and 10 days after the application of various treatments and with re-contamination of samples on day 3 of storage.*** Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2). Red arrow indicates when samples were re-contaminated.

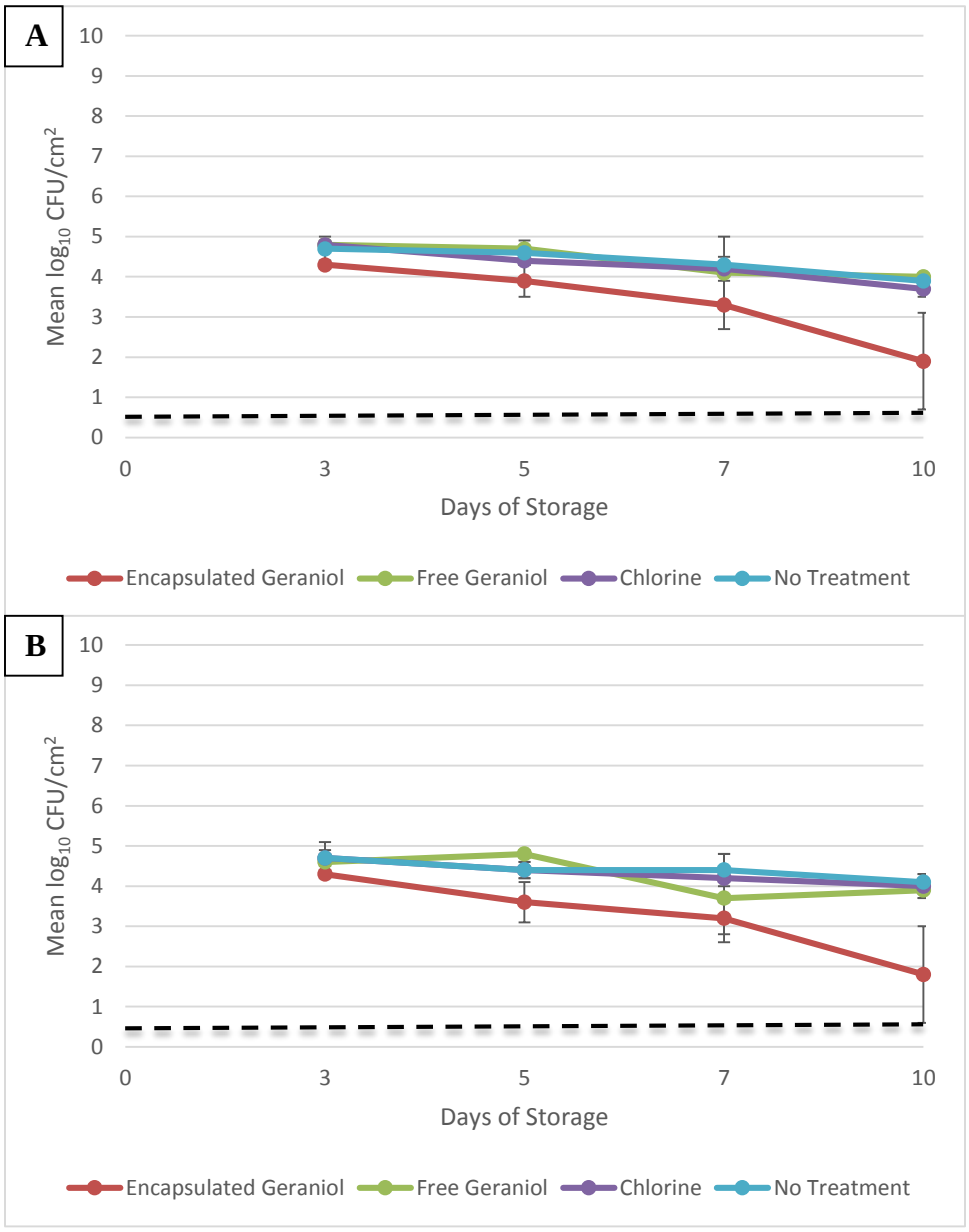

FIGURE 20. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of tomatoes stored at 5°C for 0, 3, 5, 7, and 10 days after the application of various treatments and with contamination of samples on day 3 of storage and no initial inoculation of pathogens on day 0.*** Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

Initial numbers of aerobic bacteria, LAB, and coliforms were 5.1 ± 1.2, 4.5 ± 1.3, and 5.9 ± 0.2 $\log_{10}$ CFU/cm^2 at day 0, respectively, prior to incubation (Figure 21). After the application of antimicrobial treatments and prior to storage, levels of aerobic bacteria, LAB, and coliforms were 3.3 ± 0.2, 3.2 ± 0.2, and 1.9 ± 1.3 $\log_{10}$ CFU/cm^2, respectively for encapsulated geraniol-treated, 4.2 ± 0.1, 3.9 ± 0.5, and 3.1 ± 1.0 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol-treated, and 4.5 ± 0.3, 4.0 ± 0.8, and 3.5 ± 0.5 $\log_{10}$ CFU/cm^2 respectively for chlorine-treated (Figure 21). After day 10 of storage at 5°C levels of aerobic bacteria, LAB, and coliforms on untreated samples were 6.8 ± 0.4, 6.8 ± 0.4, and 4.7 ± 0.4 $\log_{10}$ CFU/cm^2 respectively (Figure 21). After day 10 of storage at 5°C, levels of aerobic bacteria, LAB, and coliforms were 4.3 ± 0.1 $\log_{10}$ CFU/cm^2, 4.3 ± 0.1 $\log_{10}$ CFU/cm^2, and below detectable limits, respectively, for encapsulated geraniol-treated, 4.7 ± 1.7, 4.6 ± 1.6, and 1.4 ± 1.5 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol treated, and 6.0 ± 0.7, 5.8 ± 0.7, and 1.1 ± 0.7 $\log_{10}$ CFU/cm^2 respectively, for chlorine treated (Figure 21).

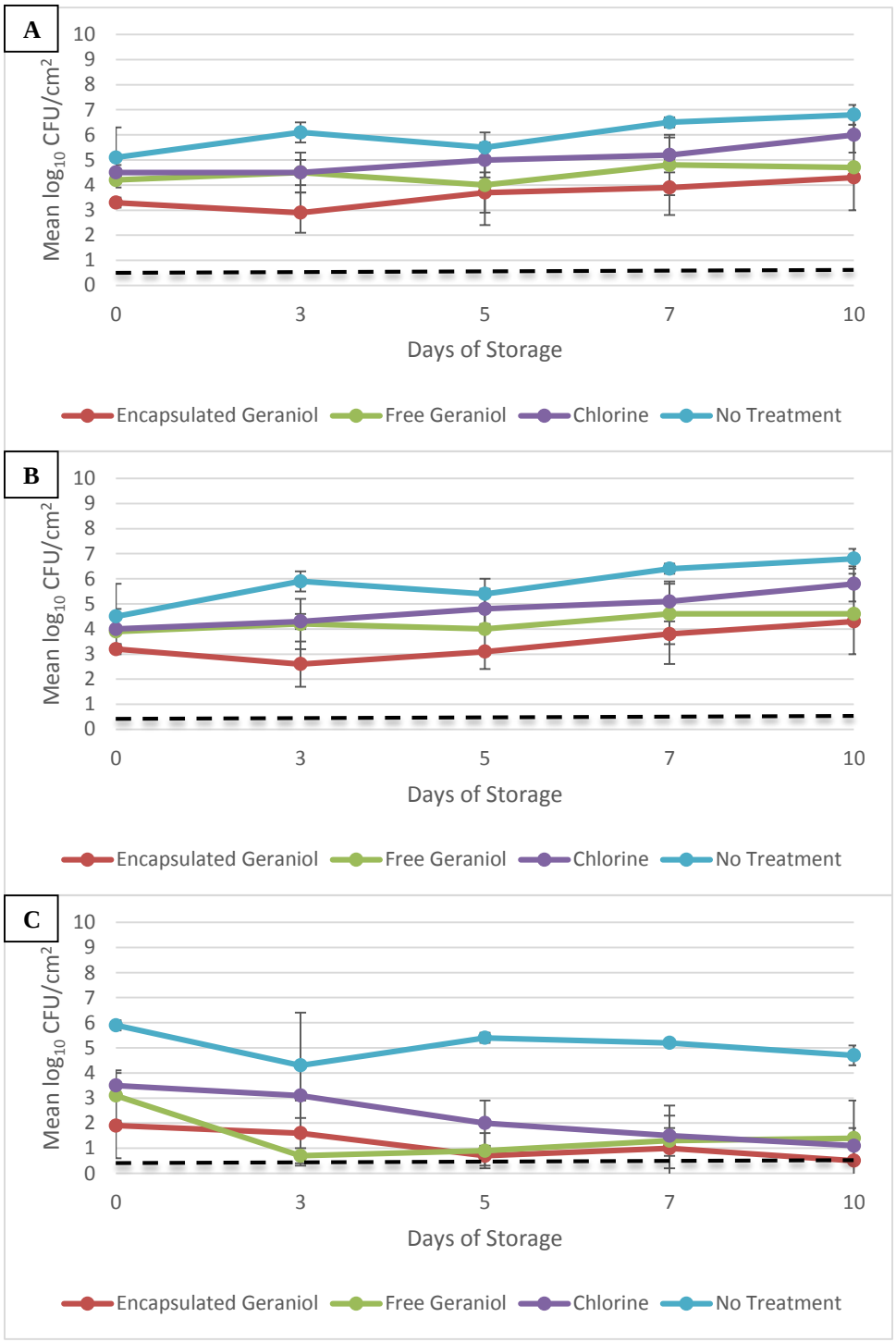

FIGURE 21. ***Means of A) aerobic bacteria, B) lactic acid bacteria, and C) coliforms on the surface of tomatoes stored at 5°C for 0, 3, 5, 7, and 10 days after the application of treatments.*** Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

On day 5 of storage at 5°C, samples of each inoculation treatment method (no recontamination, recontaminated, post treatment contaminated, and uninoculated with no contamination) and treatment type (encapsulated geraniol, unencapsulated geraniol, chlorine, and no treatment) were moved to 15°C or 25°C to simulate post processing temperature abuse. At day 10 at 15°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were not recontaminated were 2.4 ± 1.7, 5.9 ± 1.5, 6.4 ± 0.3, and 6.4 ± 1.1 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 22). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were not recontaminated were 2.5 ± 2.0, 5.4 ± 1.3, 6.1 ± 0.6, and 6.7 ± 0.5 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 22). At day 10 at 15°C encapsulated geraniol-treated tomatoes did not change visually and did not have visible mold growth (Figure 15). However, at day 10 at 15°C unencapsulated geraniol, chlorine, and untreated tomatoes appeared deteriorated, but did not have visible mold growth (Figures 16-18).

At day 10 at 15°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were recontaminated were 3.7 ± 0.6, 6.4 ± 0.7, 6.3 ± 0.2, and 6.2 ± 0.7 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 23). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were recontaminated were 3.0 ± 0.7, 6.4 ± 0.4, 6.4 ± 0.8, and 6.4 ± 0.6 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 23). At day 10 at 15°C, *E. coli* O157:H7 concentrations

on the surface for the post treatment contaminated samples were 3.2 ± 0.7, 2.1 ± 2.4, 6.1 ± 0.2, and 6.1 ± 0.7 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment, respectively (Figure 24). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the post treatment contaminated samples were 3.4 ± 1.6, 2.0 ± 2.5, 6.1 ± 0.3, and 6.3 ± 0.3 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment, respectively (Figure 24). At day 10 of storage at 15°C levels of aerobic bacteria, LAB, and coliforms on untreated samples were 8.3 ± 0.4, 7.5 ± 0.7, and 4.6 ± 3.6 $\log_{10}$ CFU/cm^2 respectively (Figure 25). At day 10 of storage at 15°C, levels of aerobic bacteria, LAB, and coliforms were 7.6 ± 0.2, 7.6 ± 0.2, and 1.7 ± 2.1 $\log_{10}$ CFU/cm^2, respectively for encapsulated geraniol treated, 7.7 ± 0.1, 7.2 ± 0.6, and 4.1 ± 3.2 $\log_{10}$ CFU/cm^2, respectively for free (unencapsulated) geraniol treated, and 7.8 ± 0.1, 7.0 ± 0.7, and 5.9 ± 0.3 $\log_{10}$ CFU/cm^2 respectively for chlorine treated (Figure 25).

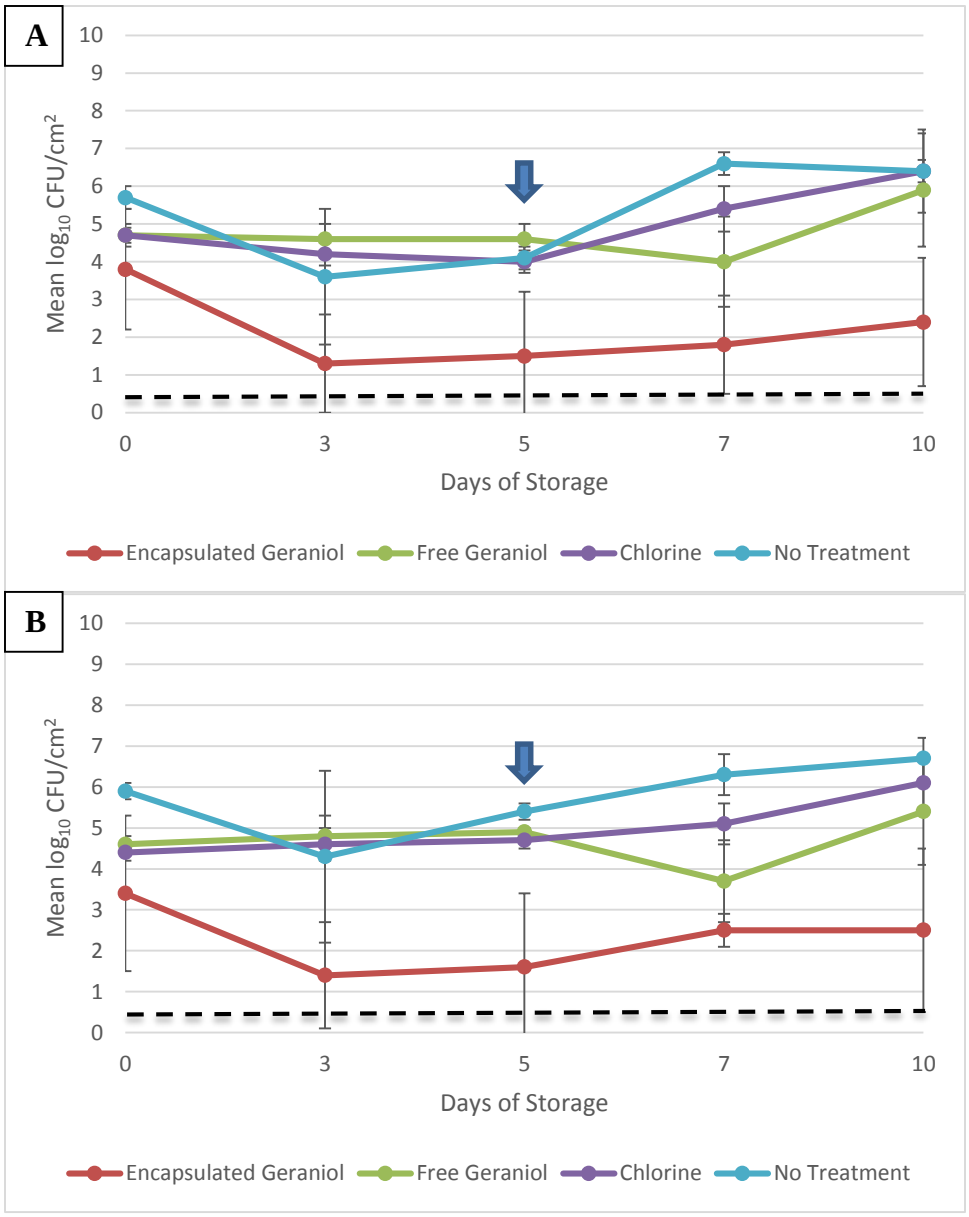

FIGURE 22. ***Means of A)*** **E. coli** ***O157:H7 and B)*** **S.** ***Typhimurium LT2 survivors on the surface of tomatoes stored at 15°C after the application of various treatments.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

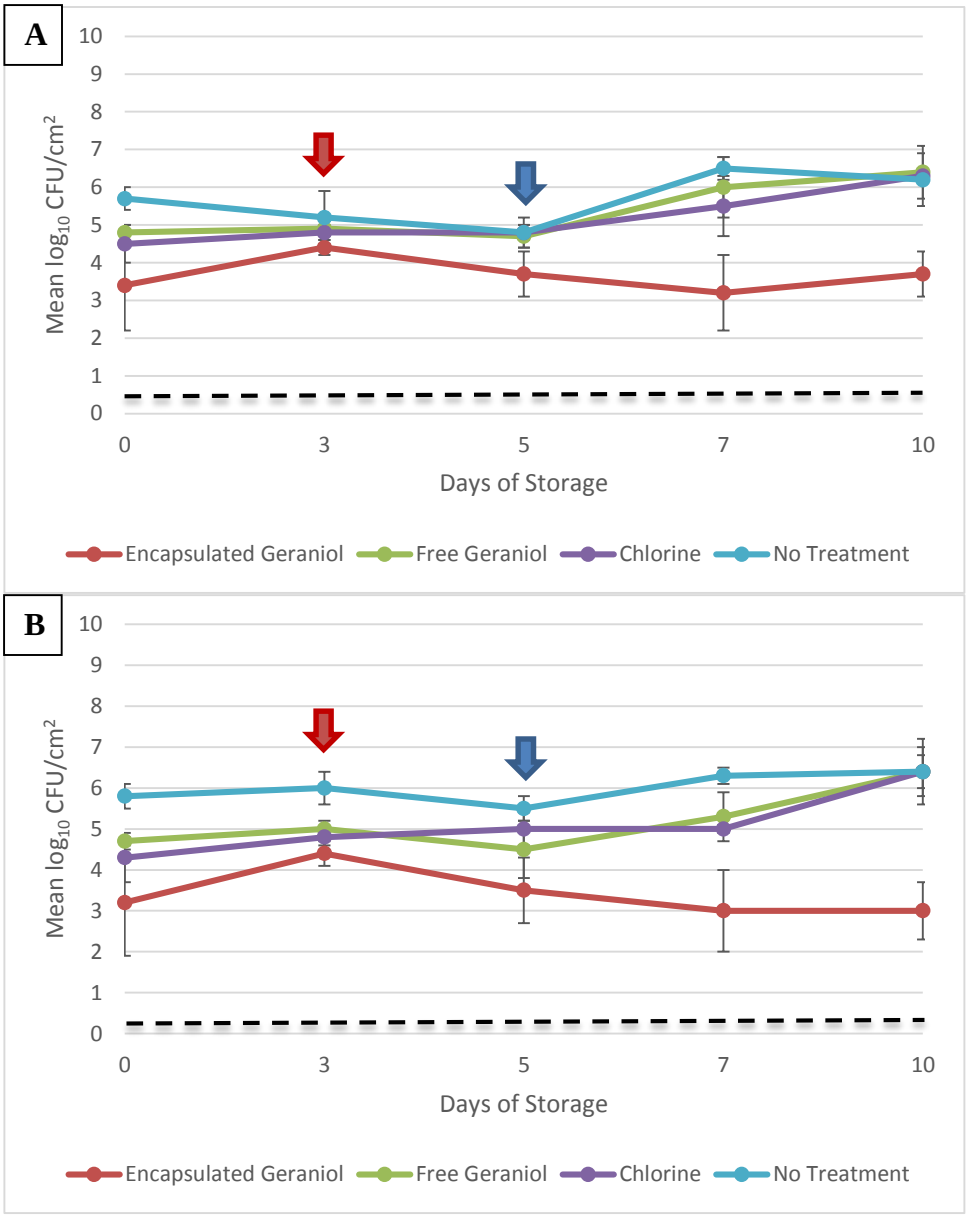

FIGURE 23. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of tomatoes stored at 15°C after the application of various treatments with re-contamination of samples on day 3 of storage.*** The blue arrow indicates when samples transitioned from 5°C to 15°C. The red arrow indicates when samples were re-contaminated. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

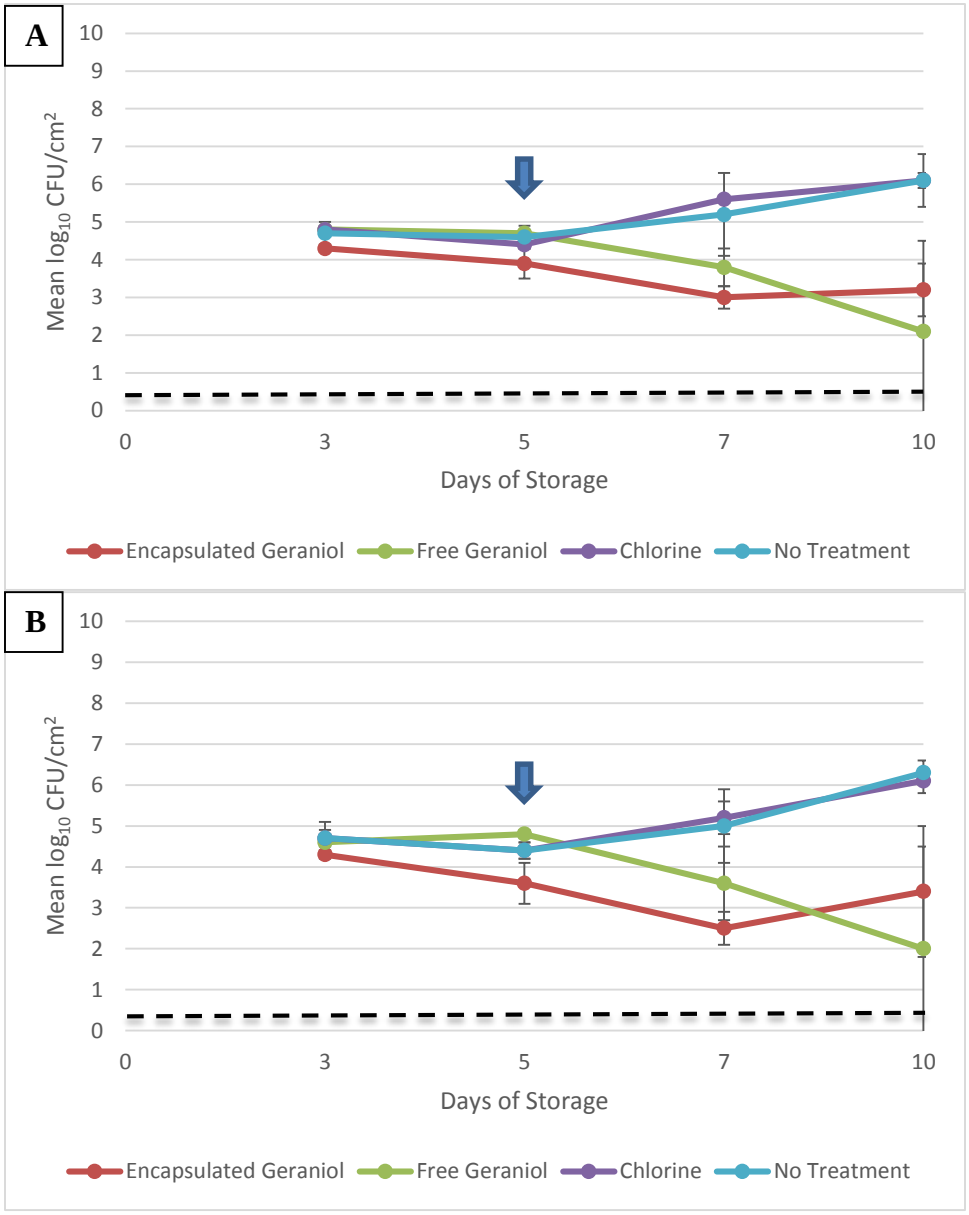

FIGURE 24. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of tomatoes stored at 15°C after the application of various treatments with contamination of samples on day 3 of storage and no initial inoculation of pathogens on day 0.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

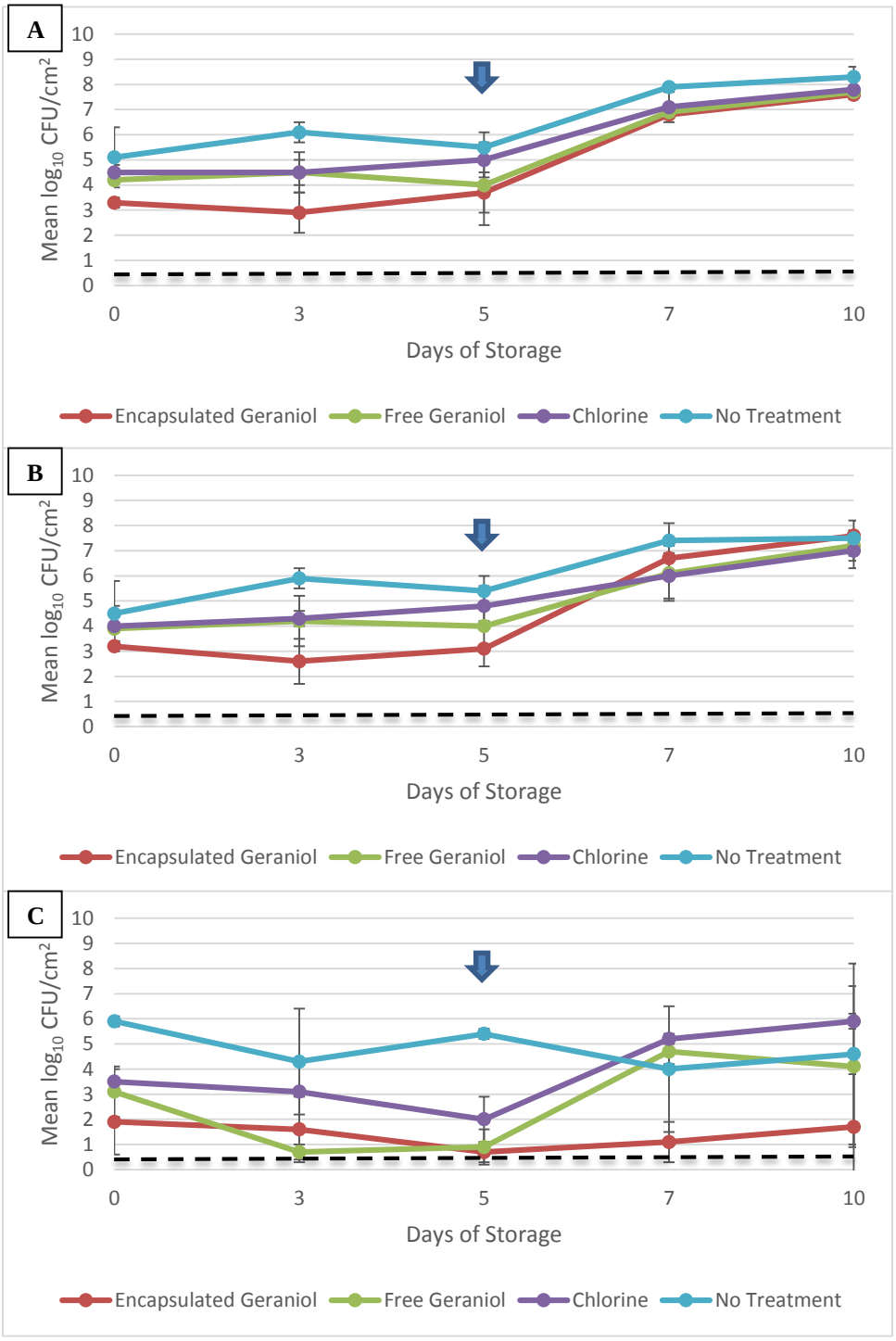

FIGURE 25. ***Means of A) aerobic bacteria, B) lactic acid bacteria, and C) coliforms on the surface of tomatoes stored at 15°C.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

At day 10 at 25°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were not recontaminated were 3.0 ± 4.2, 2.7 ± 3.8, 7.4 ± 0.5, and 7.6 ± 0.6 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 26). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were not recontaminated were 3.5 ± 4.6, 2.8 ± 3.9, 7.4 ± 0.5, and 7.8 ± 0.7 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 26). At day 10 at 25°C encapsulated geraniol treated tomatoes did deteriorate, but did not have visible mold growth (Figure 15). However, at day 10 at 25°C unencapsulated geraniol, chlorine, and untreated tomatoes appeared deteriorated, and the untreated and chlorine treated tomatoes had visible mold growth (Figures 16-18). At day 10 at 25°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were recontaminated were 4.1 ± 3.1, 6.9 ± 1.2, 7.4 ± 0.7, and 7.3 ± 0.4 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 27). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were recontaminated were 4.5 ± 3.5, 7.3 ± 0.6, 7.7 ± 0.6, and 7.4 ± 0.3 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 27). At day 10 at 25°C, *E. coli* O157:H7 concentrations on the surface for the post treatment contaminated samples were 3.6 ± 1.3, 4.4 ± 3.4, 7.3 ± 0.5, and 7.1 ± 0.7 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 28). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the post treatment contaminated samples were 5.1 ± 2.9, 4.3 ± 3.5, 7.2 ± 0.6, and 7.4 ± 0.5 $\log_{10}$ CFU/cm^2 for

encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 28). At day 10 of storage at 25°C levels of aerobic bacteria, LAB, and coliforms on untreated samples were 8.8 ± 0.4, 8.2 ± 0.1, and 7.5 ± 0.6 $\log_{10}$ CFU/cm^2 respectively (Figure 29). At day 10 of storage at 15°C, levels of aerobic bacteria, LAB, and coliforms were 7.8 ± 0.6, 7.7 ± 0.6, and 1.1 ± 1.0 $\log_{10}$ CFU/cm^2, respectively for encapsulated geraniol treated, 8..0 ± 0.6, 7.9 ± 0.7, and 5.0 ± 4.0 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol treated, and 7.9 ± 1.4, 7.6 ± 1.2, and 6.8 ± 0.8 $\log_{10}$ CFU/cm^2 respectively for chlorine treated (Figure 29).

Analysis of the pathogen concentrations on tomato surface as affected by the treatments indicated significantly lower numbers of both pathogens on tomato surface throughout the 5°C storage period and 15°C storage when treated with the nanoencapsulated geraniol than unencapsulated geraniol, chlorine and no treatment, ($p<0.05$), for all inoculation treatment methods (no recontamination, recontaminated, post treatment contaminated, and uninoculated with no contamination). However, treatments did not differ throughout the 25°C storage period for all inoculation treatment methods, ($p\geq0.05$).

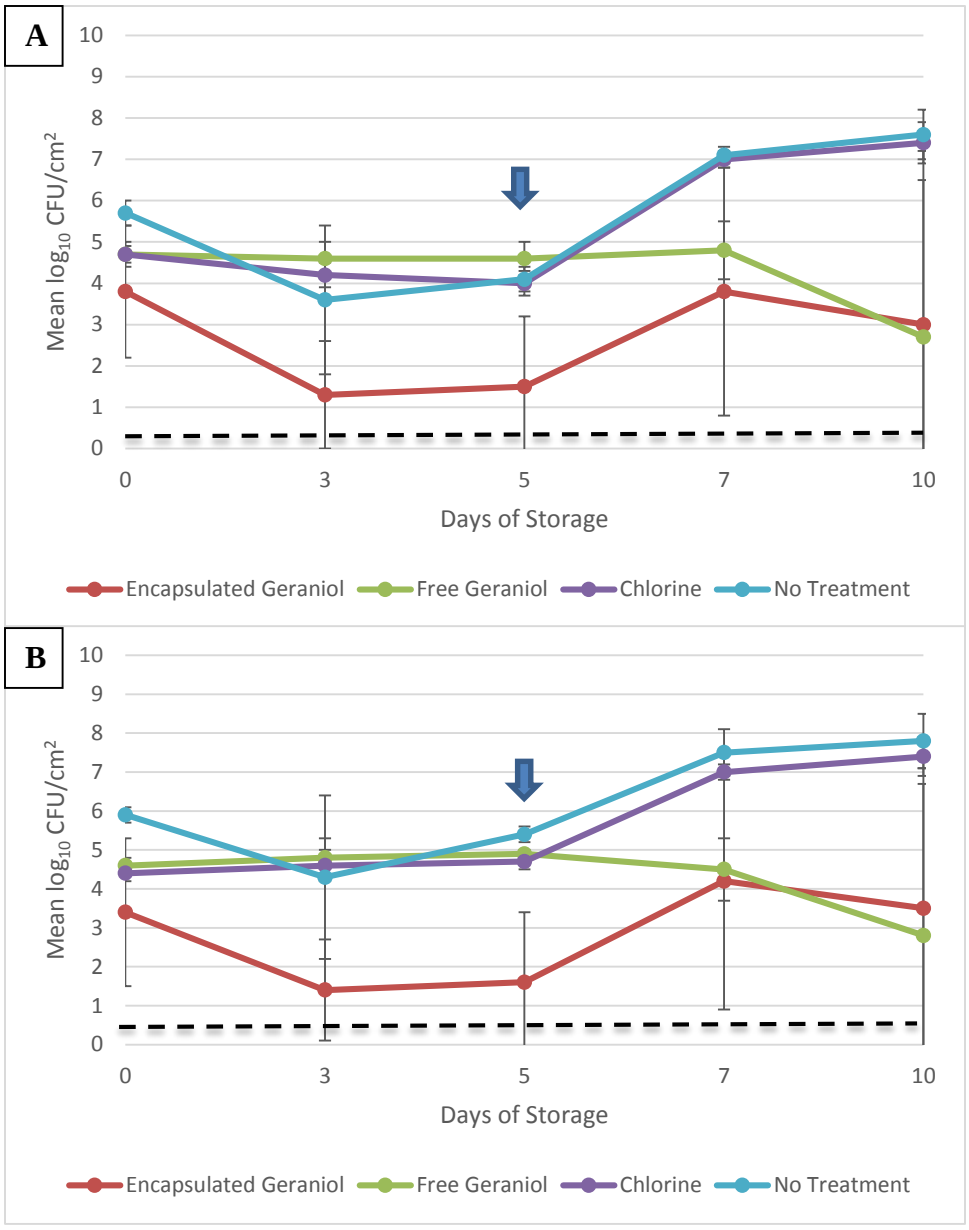

FIGURE 26. ***Means of A)*** **E. coli** ***O157:H7 and B)*** **S.** ***Typhimurium LT2 survivors on the surface of tomatoes stored at 25°C after the application of various treatments***. The arrow indicates when samples transitioned from 5°C to 25°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

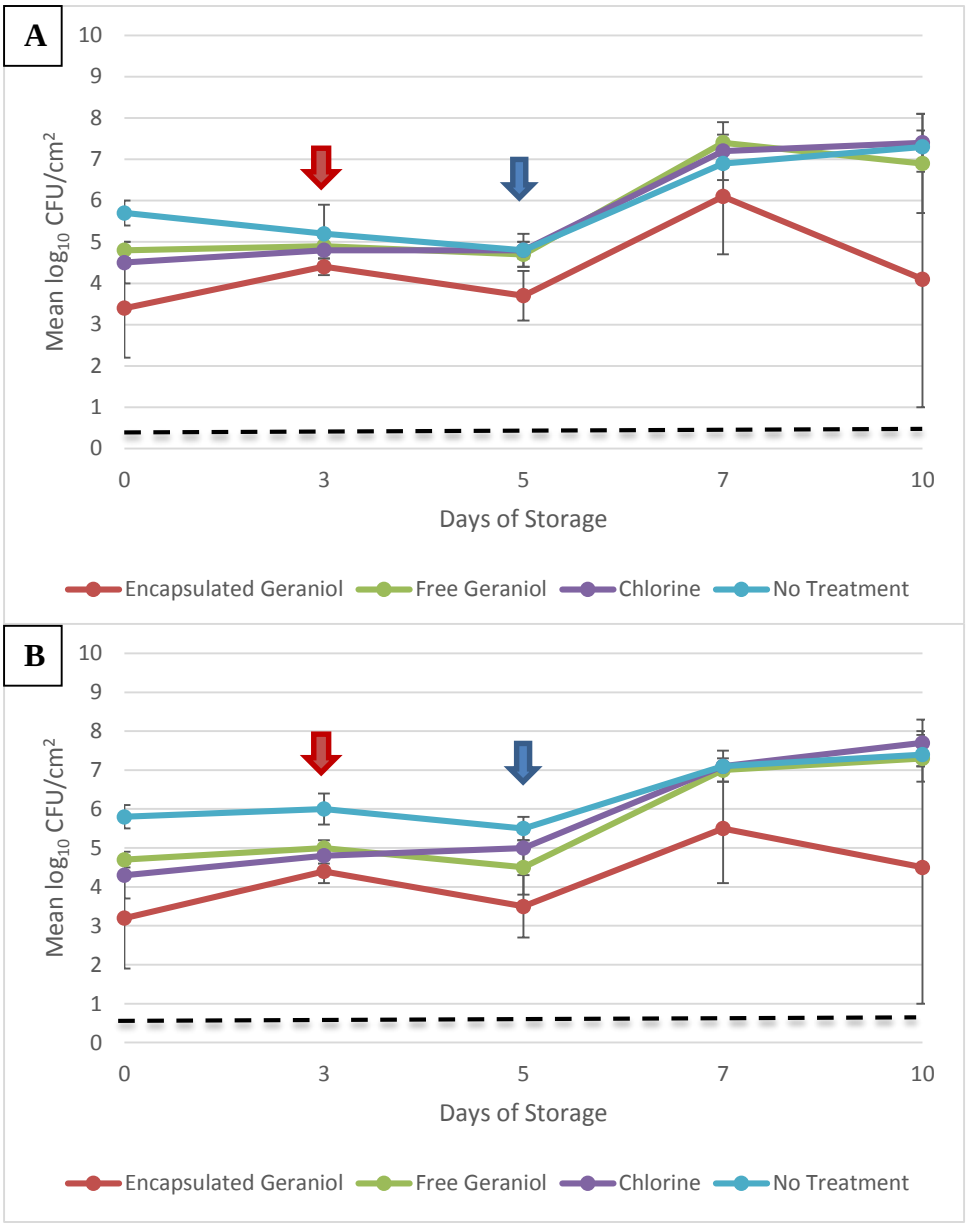

FIGURE 27. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of tomatoes stored at 25°C after the application of various treatments with re-contamination of samples on day 3 of storage.*** The blue arrow indicates when samples transitioned from 5°C to 25°C. The red arrow indicates when samples were re-contaminated. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (log_{10} CFU/cm^2).

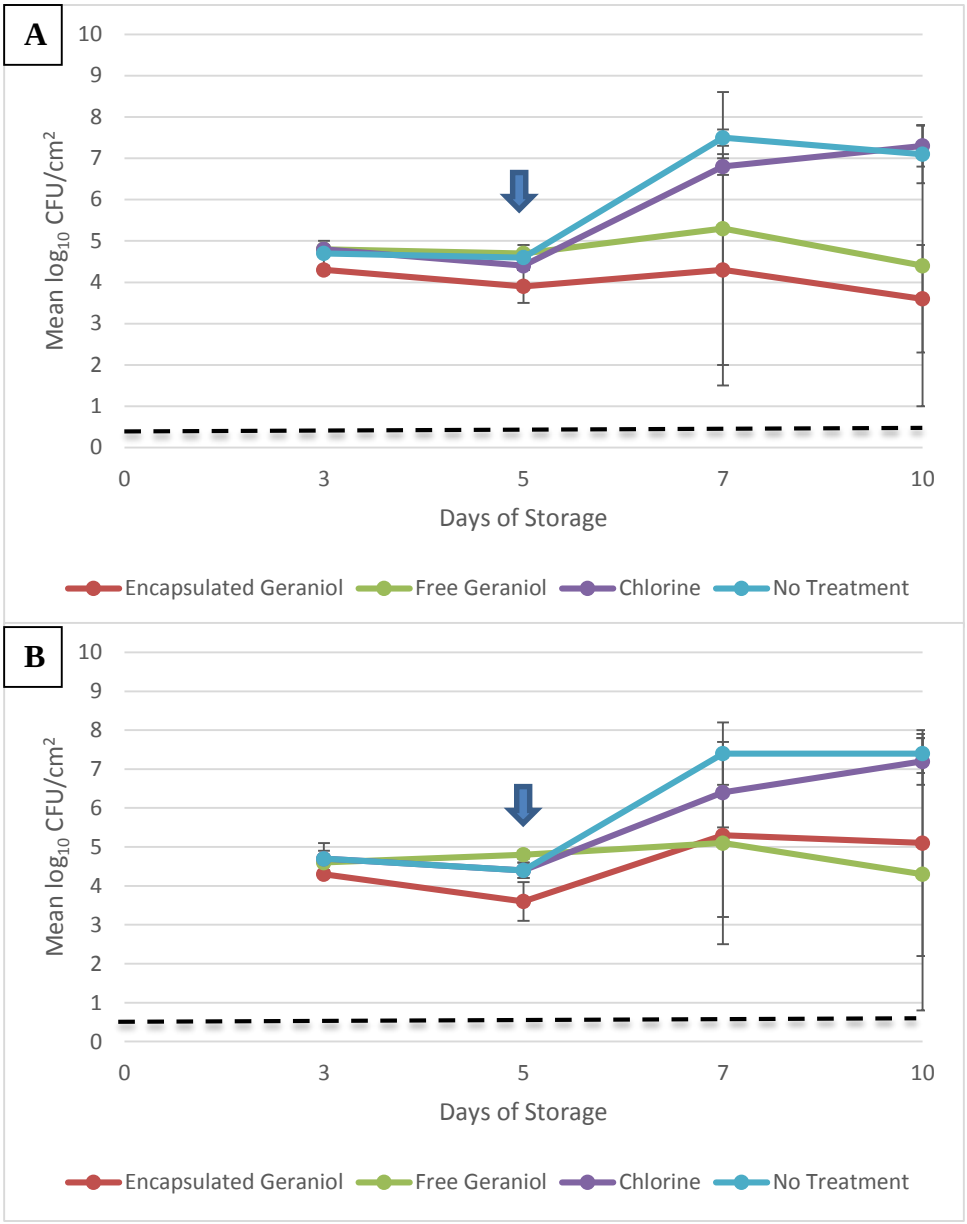

FIGURE 28. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of tomatoes stored at 25°C after the application of various treatments with contamination of samples on day 3 of storage and no initial inoculation of pathogens on day 0.*** The arrow indicates when samples transitioned from 5°C to 25°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

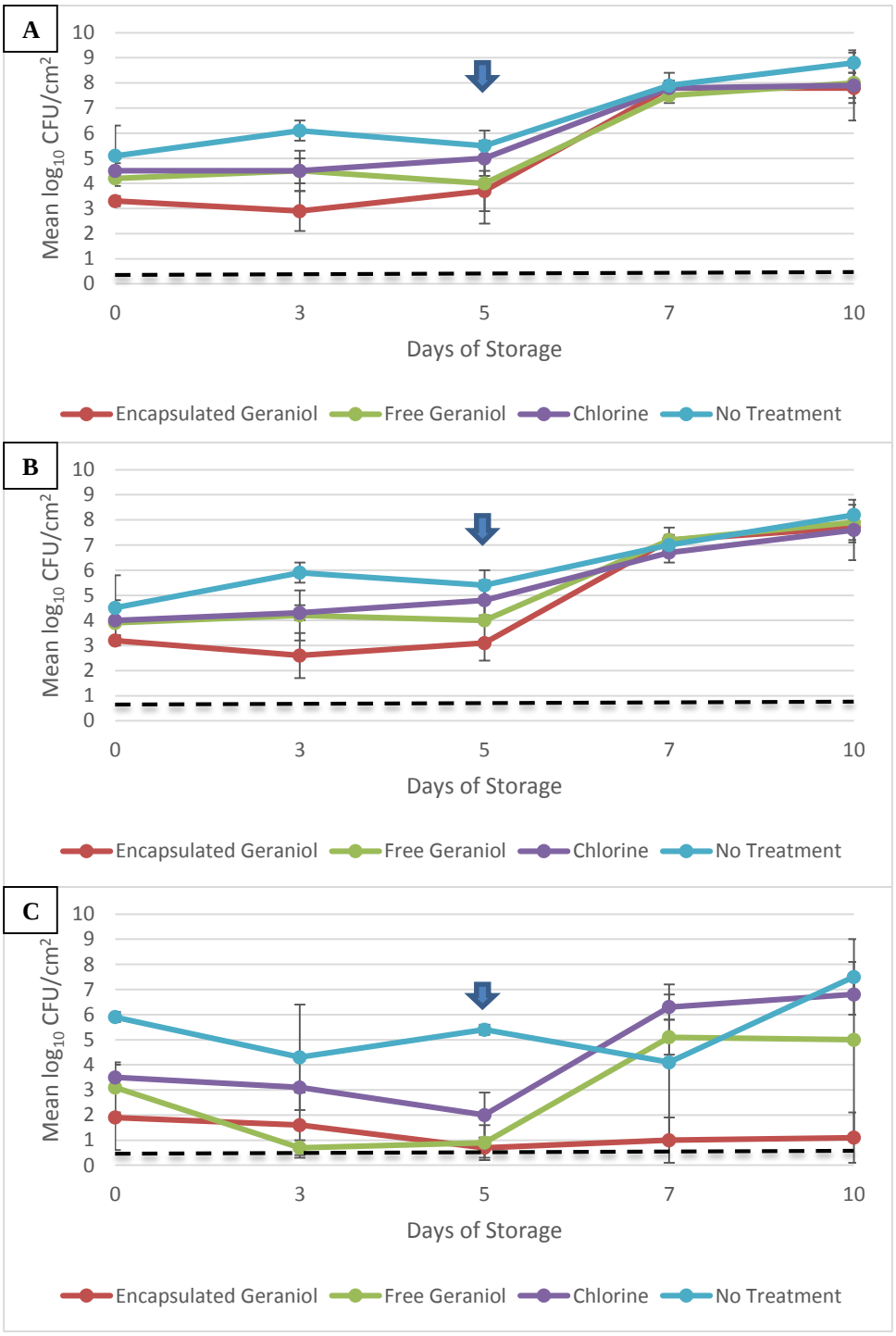

FIGURE 29. ***Means of A) aerobic bacteria, B) lactic acid bacteria, and C) coliforms on the surface of tomatoes stored at 15°C.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

Nanoparticle Treatment on Surface of Cantaloupe

Inoculation of cantaloupes with the bacterial pathogens resulted in 5.4 ± 0.4 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 and 5.4 ± 0.6 $\log_{10}$ CFU/cm^2 *S.* Typhimurium LT2 attaching to cantaloupe surface on day 0 prior to incubation and treatments (Figure 30). After the initial application via immersion for 2 min. of the antimicrobials on day 0, *E. coli* O157:H7 concentrations on the surface were 4.7 ± 0.3, 5.3 ± 0.1, and 4.9 ± 0.4 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 30). Concentrations of *S.* Typhimurium LT2 on the surface were 4.2 ± 0.5, 5.1 ± 0.3, and 4.5 ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, and chlorine, respectively (Figure 30). Over the 10 day storage period at 5°C populations of both pathogens declined. At day 10 *E. coli* O157:H7 concentrations on the surface were 1.0 ± 0.9, 4.2 ± 0.7, 2.2 ± 0.7, and 3.7 ± 0.4 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 30). At day 10 *S.* Typhimurium LT2 concentrations on the surface were 1.2 ± 1.2, 4.3 ± 0.8, 3.0 ± 0.3, and 4.4 ± 0.1 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 30).

On day 3 of storage, a set of cantaloupe samples were re-contaminated with a bacterial suspension containing both pathogens with a concentration of 4.7 ± 0.1 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 and 4.8 ± 0.1 $\log_{10}$ CFU/cm^2 *S.* Typhimurium LT2 attaching to the surface. After the initial application of the pathogens on already inoculated cantaloupes on day 3 *E. coli* O157:H7 concentrations on the surface were 4.6 ± 0.3, 5.1 ± 0.2, and 4.9 ± 0.3 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated)

geraniol, and chlorine, respectively (Figure 31). After the initial application of the pathogens on already inoculated cantaloupes on day 3, *S.* Typhimurium LT2 concentrations on the surface were 4.7 ± 0.3, 5.1 ± 0.2, and 4.8 ± 0.3 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 31). At day 10, *E. coli* O157:H7 concentrations on the surface of the re-contaminated cantaloupes were 2.2 ± 1.5, 4.2 ± 0.4, 3.5 ± 0.6, and 3.4 ± 0.7 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 31). At day 10 *S.* Typhimurium LT2 concentrations on the surface of the re-contaminated cantaloupes were 2.8 ± 1.0, 4.3 ± 0.4, 3.8 ± 0.5, and 4.3 ± 1.0 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 31).

On day 3, non-inoculated treated samples were contaminated with pathogens to a concentration of 4.7 ± 0.1 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 and 4.8 ± 0.1 $\log_{10}$ CFU/cm^2 *S.* Typhimurium LT2 attaching to the surface to simulate post treatment contamination. On day 3, the *E. coli* O157:H7 concentration on the surface of the post treatment contaminated samples were 4.4 ± 0.1, 4.6 ± 0.2, and 4.4 ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 32). On day 3, the *S.* Typhimurium LT2 concentration on the surface of the post treatment contaminated samples were 4.5 ± 0.1, 4.7 ± 0.2, and 4.9 ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 32). At day 10, *E. coli* O157:H7 concentrations on the surface of the post treatment contaminated cantaloupes were 1.6 ± 2.7, 3.8 ± 0.4, 3.5 ± 0.6, and 2.9 ± 0.5 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment,

respectively (Figure 32). At day 10 *S.* Typhimurium LT2 concentrations on the surface of the post treatment contaminated cantaloupes were 2.7 ± 1.2, 3.5 ± 0.4, 3.8 ± 0.3, and 3.5 ± 0.4 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 32).

Initial levels of aerobic bacteria, LAB, and coliforms were 4.8 ± 1.6, 4.3 ± 1.4, and 3.1 ± 2.4 $\log_{10}$ CFU/cm^2 at day 0, respectively, prior to incubation (Figure 33). After the application of treatments and prior to storage, levels of aerobic bacteria, LAB, and coliforms were 3.8 ± 0.9, 3.3 ± 0.6, and 0.9 ± 0.7 $\log_{10}$ CFU/cm^2, respectively for encapsulated geraniol-treated, 4.3 ± 0.9, 4.1 ± 1.1, and 1.6 ± 1.8 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol-treated, and 3.9 ± 0.6, 3.7 ± 0.8, and 1.9 ± 1.6 $\log_{10}$ CFU/cm^2 respectively for chlorine-treated (Figure 33). After day 10 of storage at 5°C levels of aerobic bacteria and lactic acid bacteria on untreated samples were 7.0 ± 0.3 and 5.8 ± 1.4, $\log_{10}$ CFU/cm^2 respectively (Figure 33). At day 10 of storage levels of coliforms for all treatments were below detection limits (Figure 33). After day 10 of storage at 5°C, levels of aerobic bacteria and LAB were 5.0 ± 1.4 and 3.5 ± 2.6 $\log_{10}$ CFU/cm^2 respectively for encapsulated geraniol-treated, 6.6 ± 0.4 and 6.4 ± 0.3 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol-treated, and 6.0 ± 0.5 and 6.2 ± 0.3 $\log_{10}$ CFU/cm^2 respectively for chlorine-treated (Figure 33).

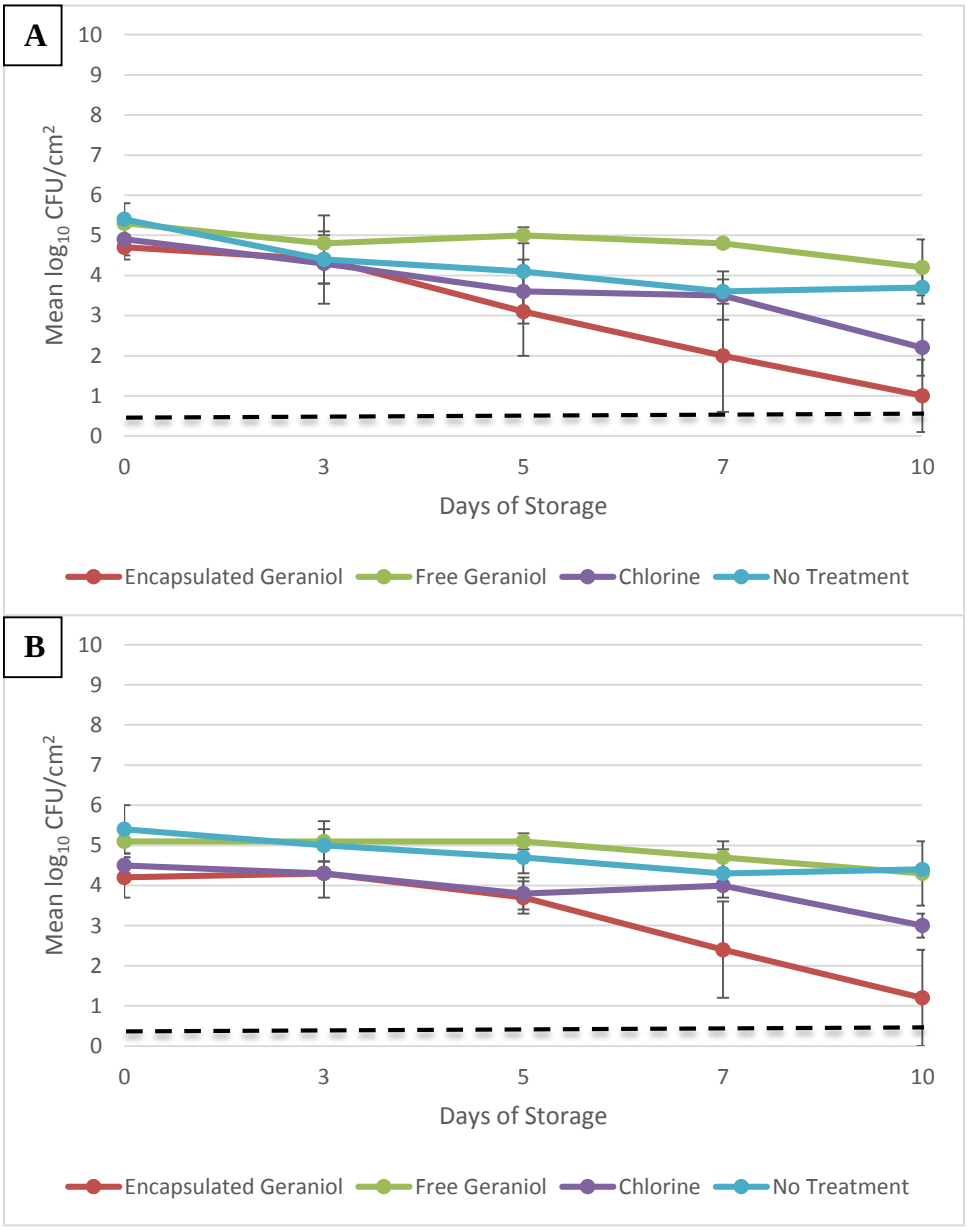

FIGURE 30. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of cantaloupes stored at 5°C for 0, 3, 5, 7, and 10 days after the application of various treatments***. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

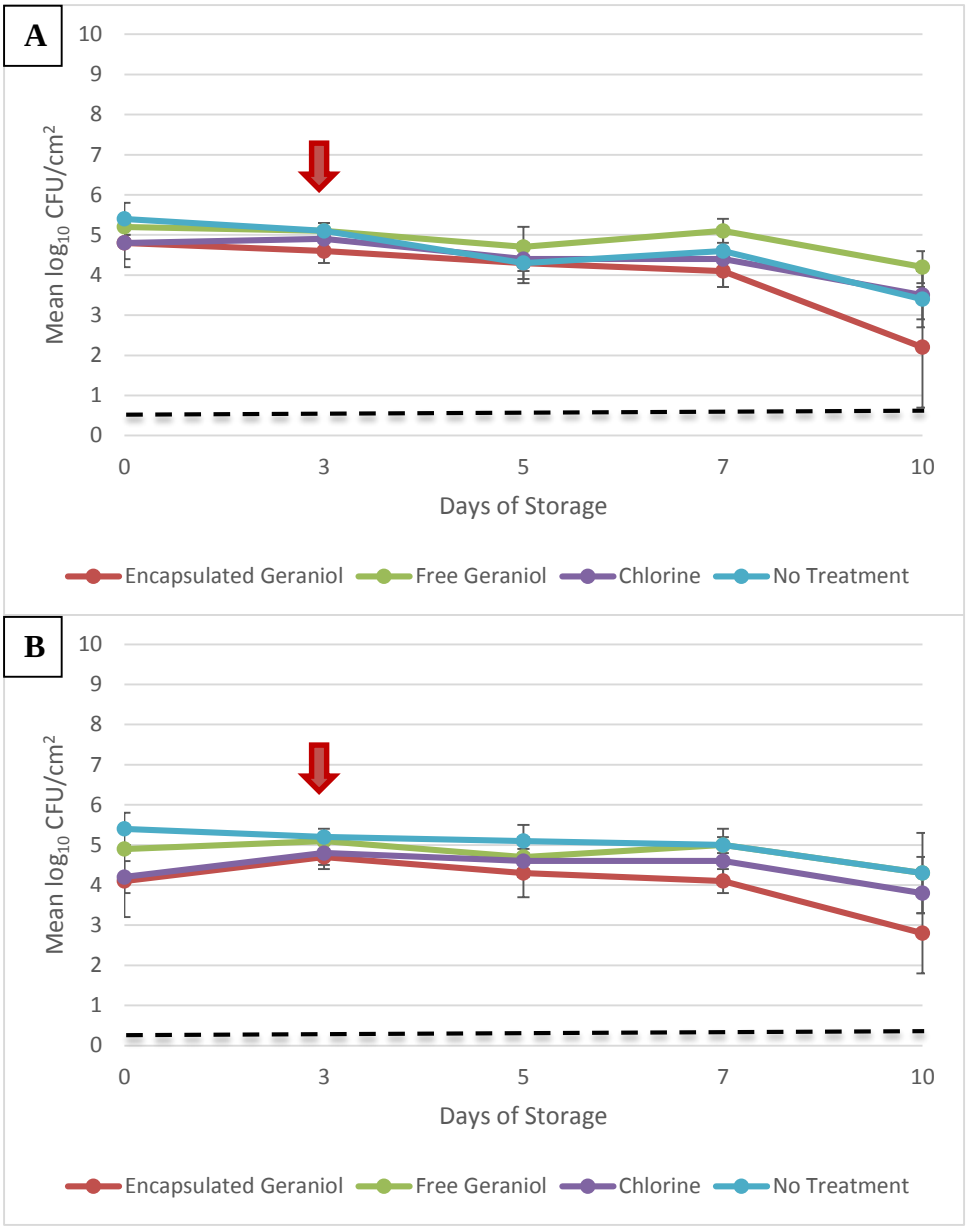

FIGURE 31. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of cantaloupes stored at 5°C for 0, 3, 5, 7, and 10 days after the application of various treatments and with re-contamination of samples on day 3 of storage.*** Error bars indicate standard deviation from sample means (n=3). The red arrow indicates when samples were re-contaminated. Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

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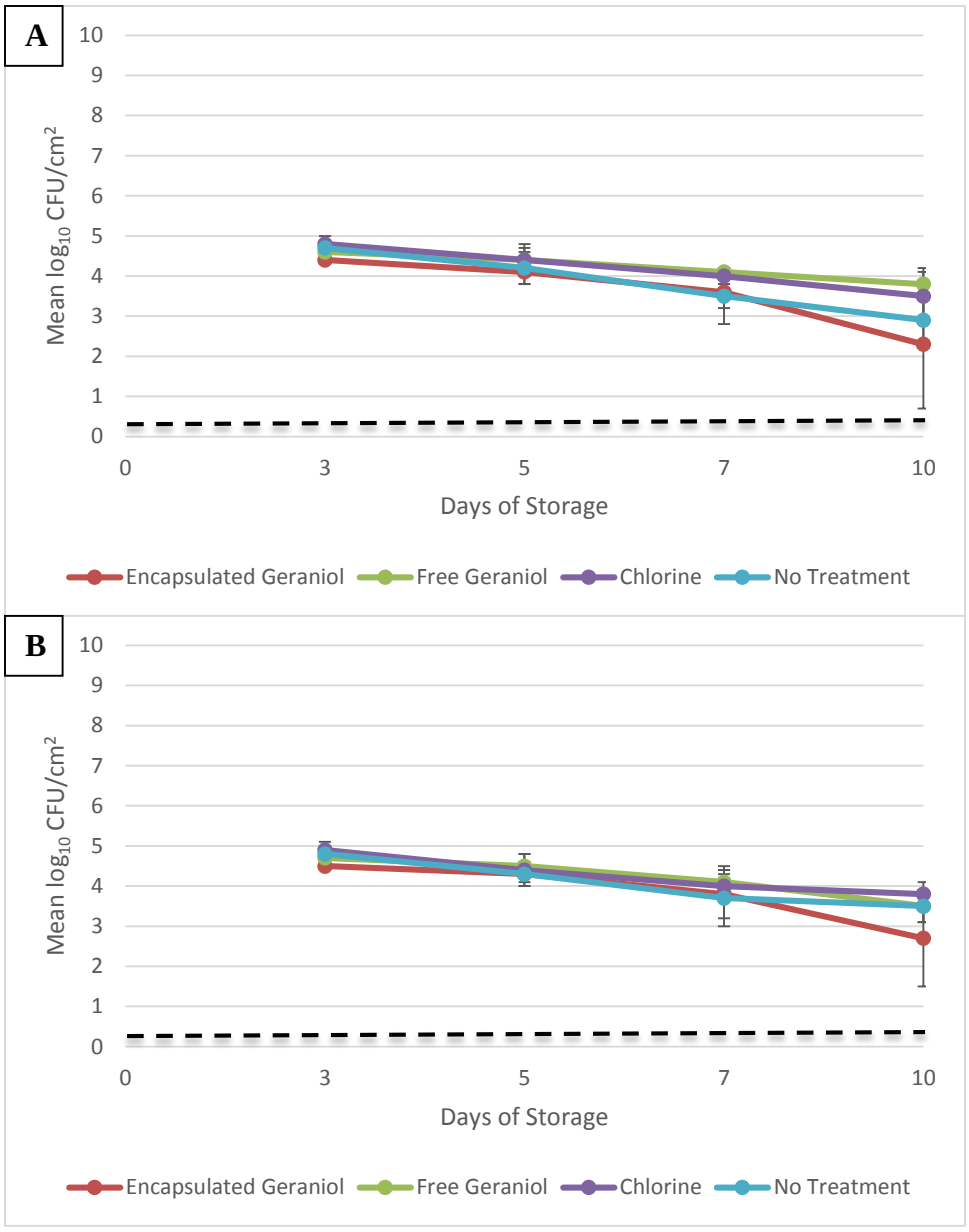

FIGURE 32. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of cantaloupes stored at 5°C for 0, 3, 5, 7, and 10 days after the application of various treatments and with contamination of samples on day 3 of storage and no initial inoculation of pathogens on day 0.*** Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

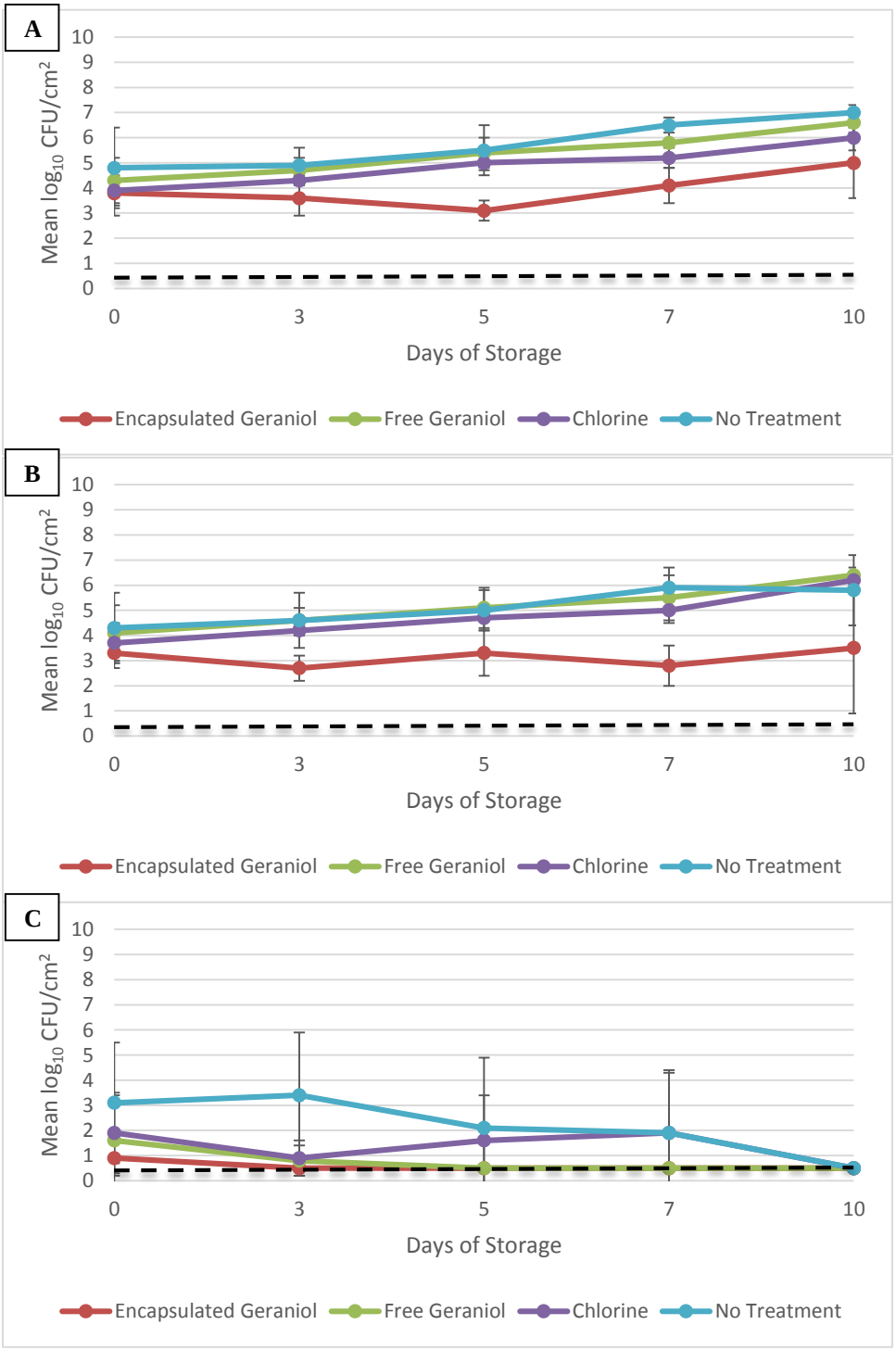

FIGURE 33. ***Means of A) aerobic bacteria, B) lactic acid bacteria, and C) coliforms on the surface of cantaloupes stored at 5°C for 0, 3, 5, 7, and 10 days after the application of treatments***. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

On day 5 of storage at 5°C, samples of each inoculation treatment method (no recontamination, recontaminated, post treatment contaminated, and uninoculated with no contamination) and treatment type (encapsulated geraniol, unencapsulated geraniol, chlorine, and no treatment) were moved to 15°C and 25°C to simulate post processing temperature abuse. At day 10 at 15°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were not recontaminated were 5.0 ± 1.3, 6.8 ± 0.9, 5.0 ± 2.0, and 5.2 ± 1.8 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment, respectively (Figure 34). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were not recontaminated were 4.5 ± 0.6, 6.2 ± 0.2, 4.4 ± 1.5, and 5.0 ± 1.9 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment, respectively (Figure 34).

At day 10 at 15°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were recontaminated were 5.2 ± 2.1, 6.6 ± 0.8, 5.3 ± 1.2, and 5.8 ± 1.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment, respectively (Figure 34). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were recontaminated were 4.2 ± 2.0, 6.3 ± 1.3, 4.7 ± 1.0, and 5.2 ± 0.9 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment, respectively (Figure 35). At day 10 at 15°C, *E. coli* O157:H7 concentrations on the surface for the post treatment contaminated samples were 3.9 ± 0.9, 5.6 ± 1.4, 5.3 ± 1.6, and 4.2 ± 1.1 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment,

respectively (Figure 36). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the post treatment contaminated samples were 3.3 ± 1.9, 5.3 ± 0.3, 4.6 ± 1.6, and 3.6 ± 1.4 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, chlorine, and no treatment, respectively (Figure 36). At day 10 of storage at 15°C levels of aerobic bacteria, lactic acid bacteria, and coliforms on untreated samples were 7.3 ± 1.1, 7.6 ± 0.9, and 2.0 ± 2.3 $\log_{10}$ CFU/cm^2 respectively (Figure 37). At day 10 of storage at 15°C, levels of aerobic bacteria and LAB were 7.2 ± 1.1 and 5.6 ± 0.4 $\log_{10}$ CFU/cm^2, respectively for encapsulated geraniol-treated, 7.8 ± 0.1 and 4.9 ± 3.9 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol-treated, and 7.3 ± 0.9 and 5.7 ± 2.0 $\log_{10}$ CFU/cm^2 respectively for chlorine treated (Figure 37). At day 10 of storage at 15°C, levels of coliforms were below detection limits for samples treated with unencapsulated and encapsulated geraniol (Figure 37). Levels of coliforms were 0.8 ± 0.6 $\log_{10}$ CFU/cm^2 for samples treated with chlorine (Figure 37).

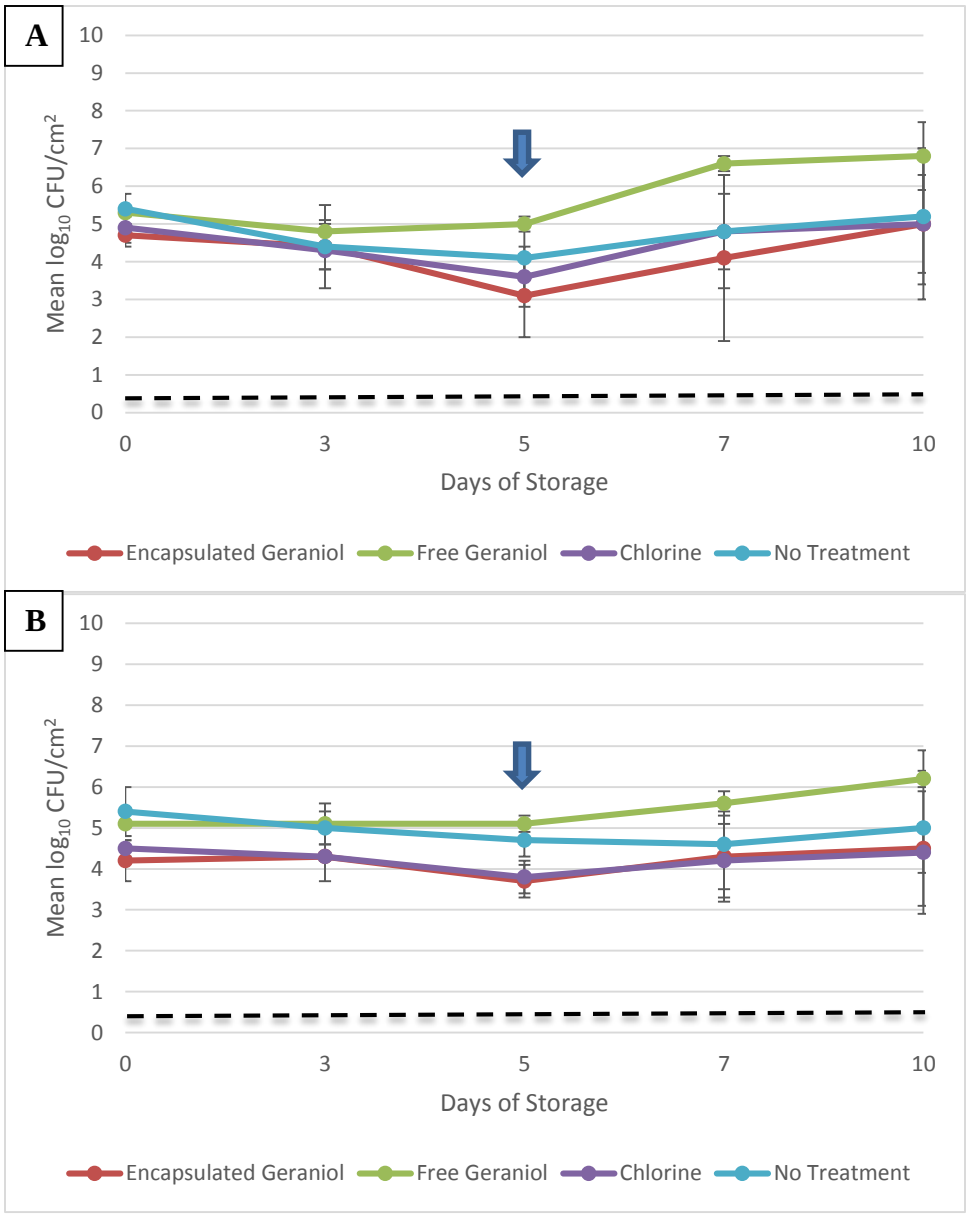

FIGURE 34. ***Means of A*) E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of cantaloupes stored at 15°C after the application of various treatments.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

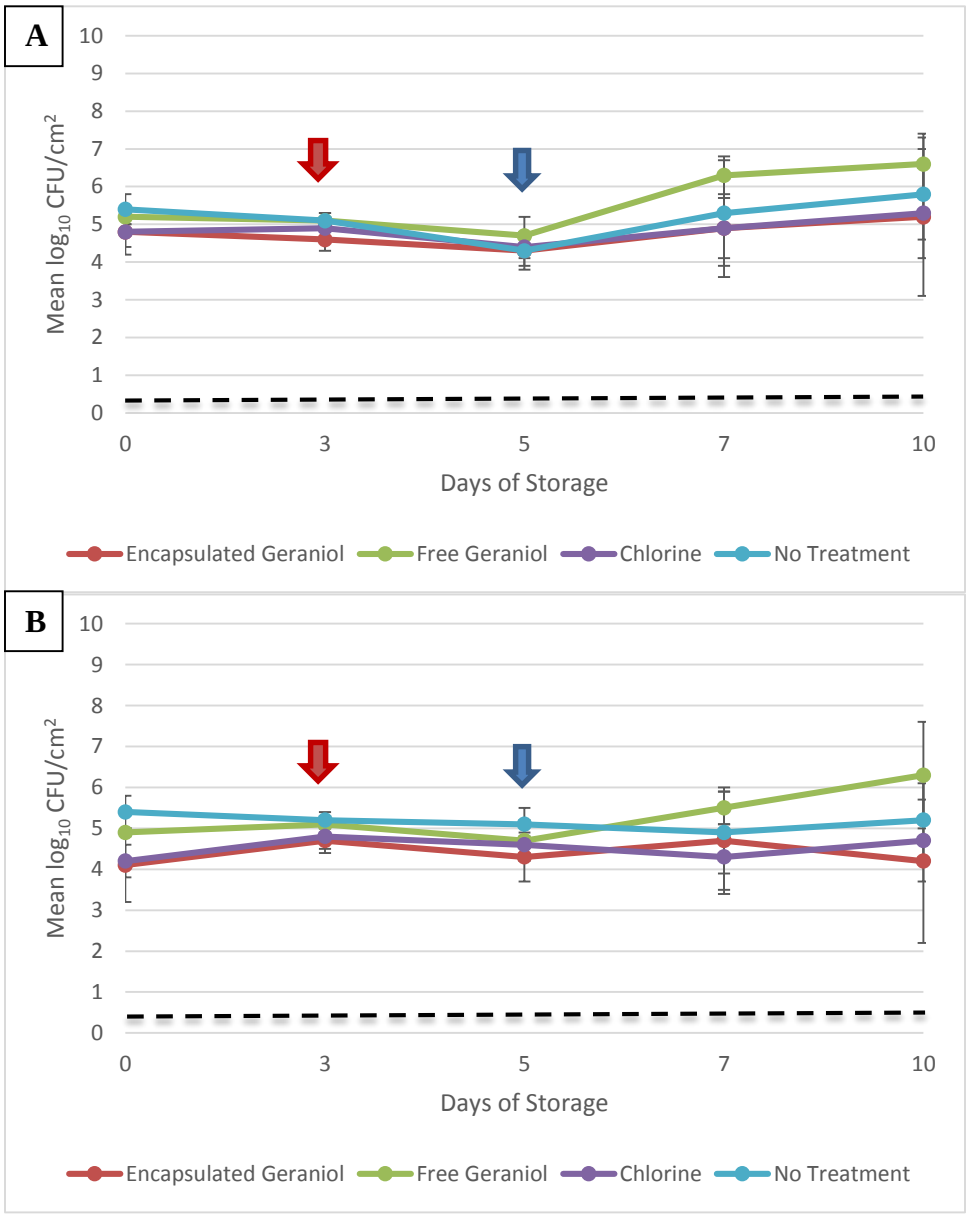

FIGURE 35. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of cantaloupes stored at 15°C after the application of various treatments with re-contamination of samples on day 3 of storage.*** The blue arrow indicates when samples transitioned from 5°C to 15°C. The red arrow indicates when samples were re-contaminated. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

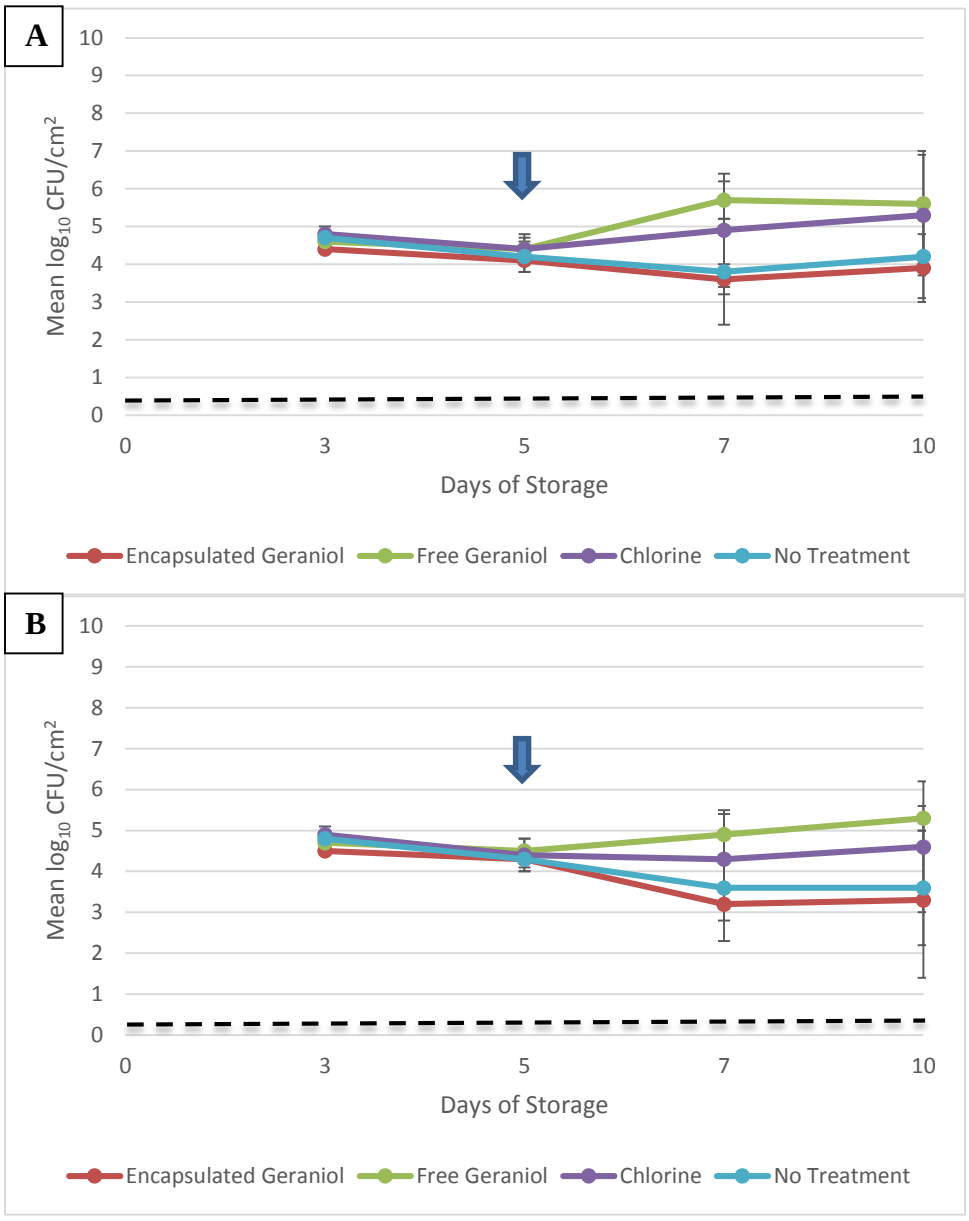

FIGURE 36. ***Means of A*) E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of cantaloupes stored at 15°C after the application of various treatments with contamination of samples on day 3 of storage and no initial inoculation of pathogens on day 0.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

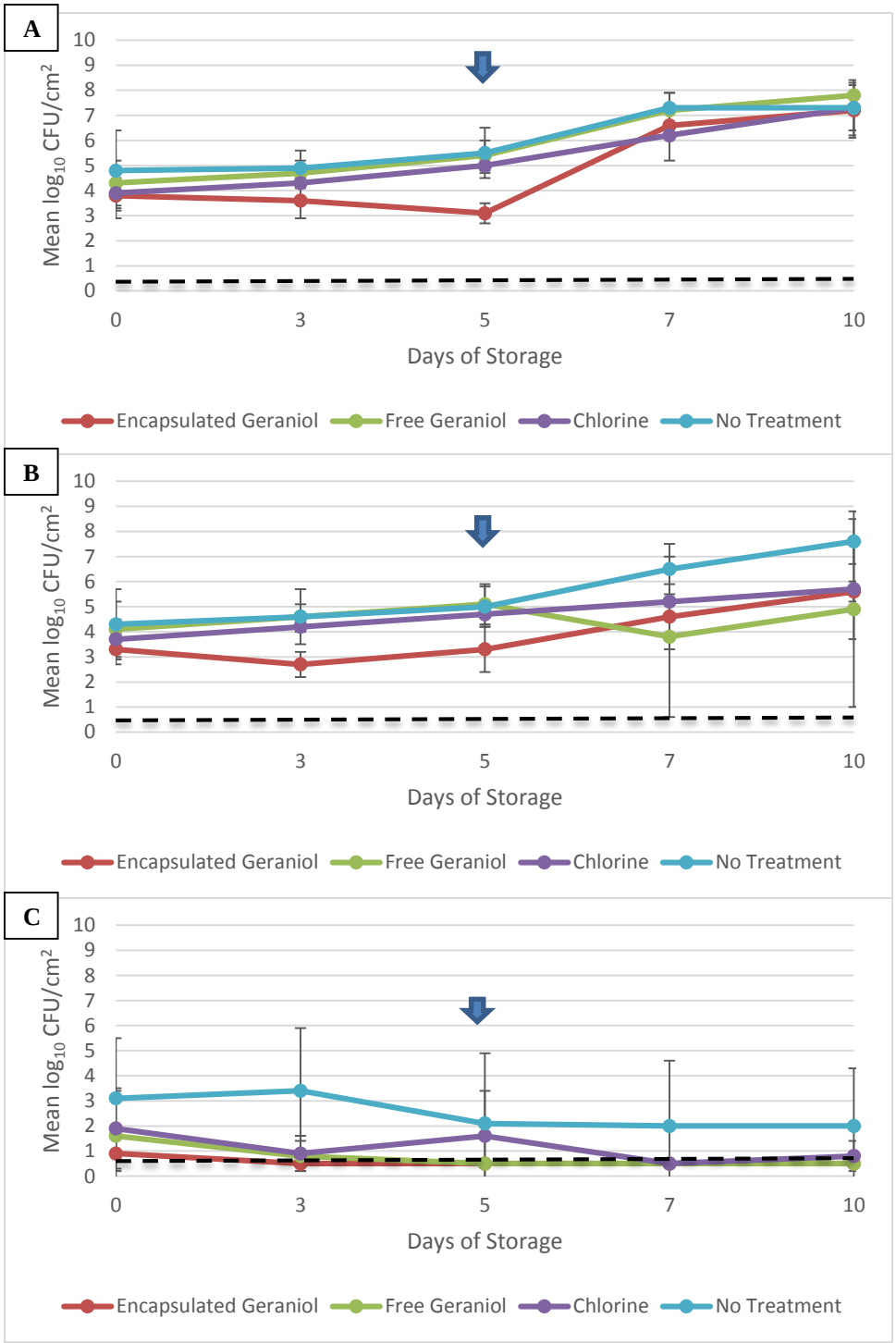

FIGURE 37. ***Means of A) aerobic bacteria, B) LAB, and C) coliforms on the surface of cantaloupes stored at 15°C.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

At day 10 at 25°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were not recontaminated were 7.3 ± 1.9, 7.9 ± 0.7, 6.7 ± 1.5, and 6.7 ± 1.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 38). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were not recontaminated were 6.8 ± 1.1, 7.5 ± 0.2, 6.6 ± 1.3, and 6.7 ± 0.9 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 38). At day 10 at 25°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were recontaminated were 7.0 ± 1.4, 8.0 ± 0.4, 7.4 ± 0.8, and 7.2 ± 1.0 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 39). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were recontaminated were 6.6 ± 0.7, 7.3 ± 0.3, 7.2 ± 0.6, and 7.1 ± 1.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 39). At day 10 at 25°C, *E. coli* O157:H7 concentrations on the surface for the post treatment contaminated samples were 8.1 ± 0.2, 8.2 ± 0.4, 6.9 ± 1.3, and 6.0 ± 2.0 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 40). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the post treatment contaminated samples were 7.9 ± 0.7, 7.9 ± 0.1, 6.8 ± 1.0, and 5.9 ± 1.4 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 40). At day 10 of storage at 25°C levels of aerobic bacteria, LAB, and coliforms on untreated samples were 8.0 ± 0.4, 7.8 ± 0.6, and 3.3 ± 2.4 $\log_{10}$ CFU/cm^2 respectively (Figure 41). At day 10 of storage at 25°C, levels of aerobic bacteria and LAB were 8.3 ±

0.5 and 7.2 ± 0.6 $\log_{10}$ CFU/cm^2, respectively for encapsulated geraniol-treated, 8.2 ± 0.6 and 7.7 ± 0.5 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol-treated, and 8.0 ± 0.5 and 7.2 ± 1.2 $\log_{10}$ CFU/cm^2 respectively for chlorine-treated (Figure 41). At day 10 of storage at 25°C, levels of coliforms were below detection limits for samples treated with encapsulated geraniol (Figure 41). Levels of coliforms were 2.0 ± 2.7 and 2.5 ± 1.3 $\log_{10}$ CFU/cm^2 for samples treated with unencapsulated geraniol and chlorine, respectively (Figure 41).

Throughout the 10 day storage period 5°C treated cantaloupes did not change visually and did not have visible mold growth (Figures 42-45). At day 10 at 15°C encapsulated geraniol-treated cantaloupes did not change visually and did not have visible mold growth (Figure 42). However, at day 10 at 15°C unencapsulated geraniol and chlorine appeared slightly deteriorated, but did not have visible mold growth (Figures 44-45). Untreated cantaloupe samples did have visible mold growth at day 10 at 15°C (Figure 43). At day 10 at 25°C all treatments except for samples treated with nanoencapsulated geraniol were deteriorated with visible mold growth (Figure 42-45).

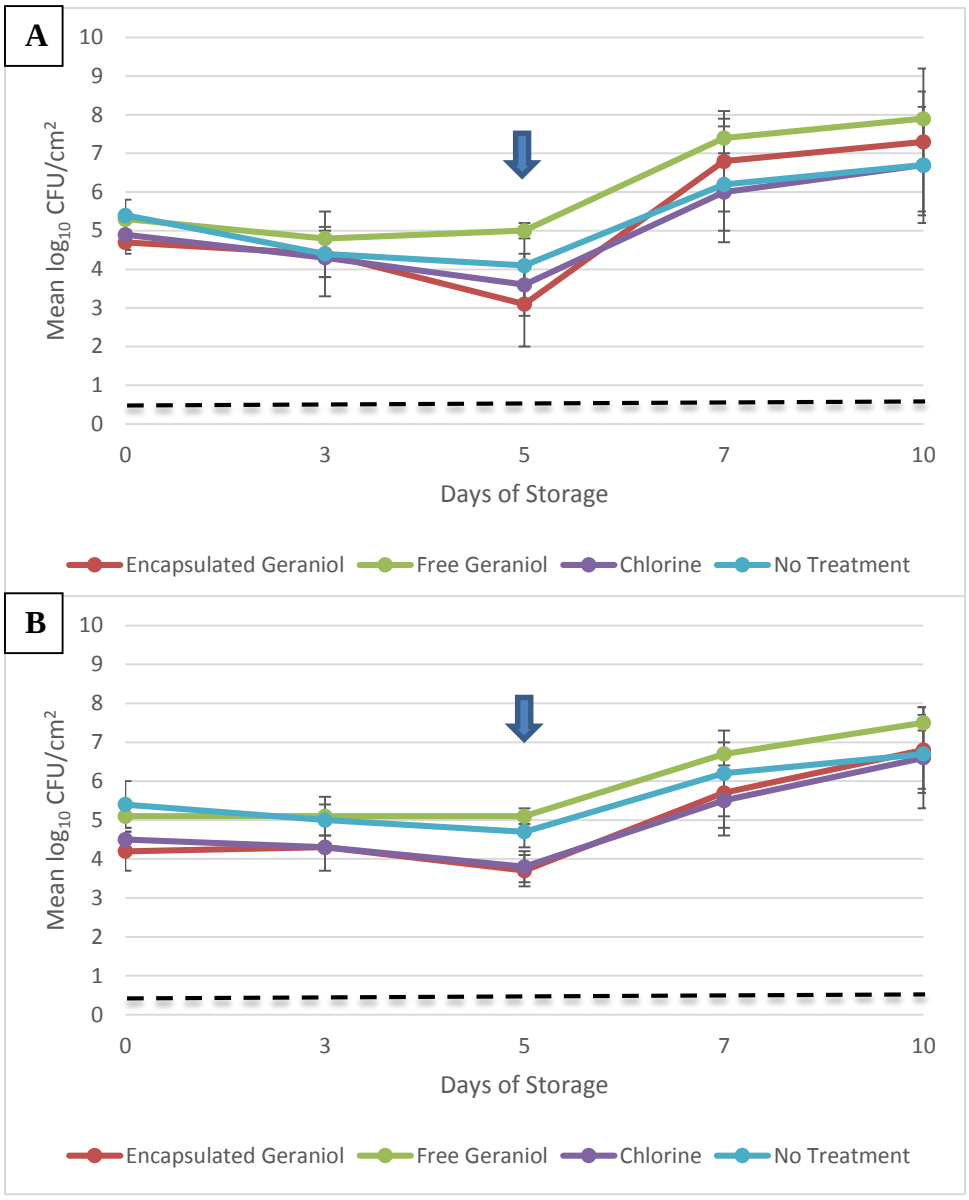

FIGURE 38. ***Means of A)*** **E. coli** ***O157:H7 and B)*** **S.** ***Typhimurium LT2 survivors on the surface of cantaloupes stored at 25°C after the application of various treatments.*** The arrow indicates when samples transitioned from 5°C to 25°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

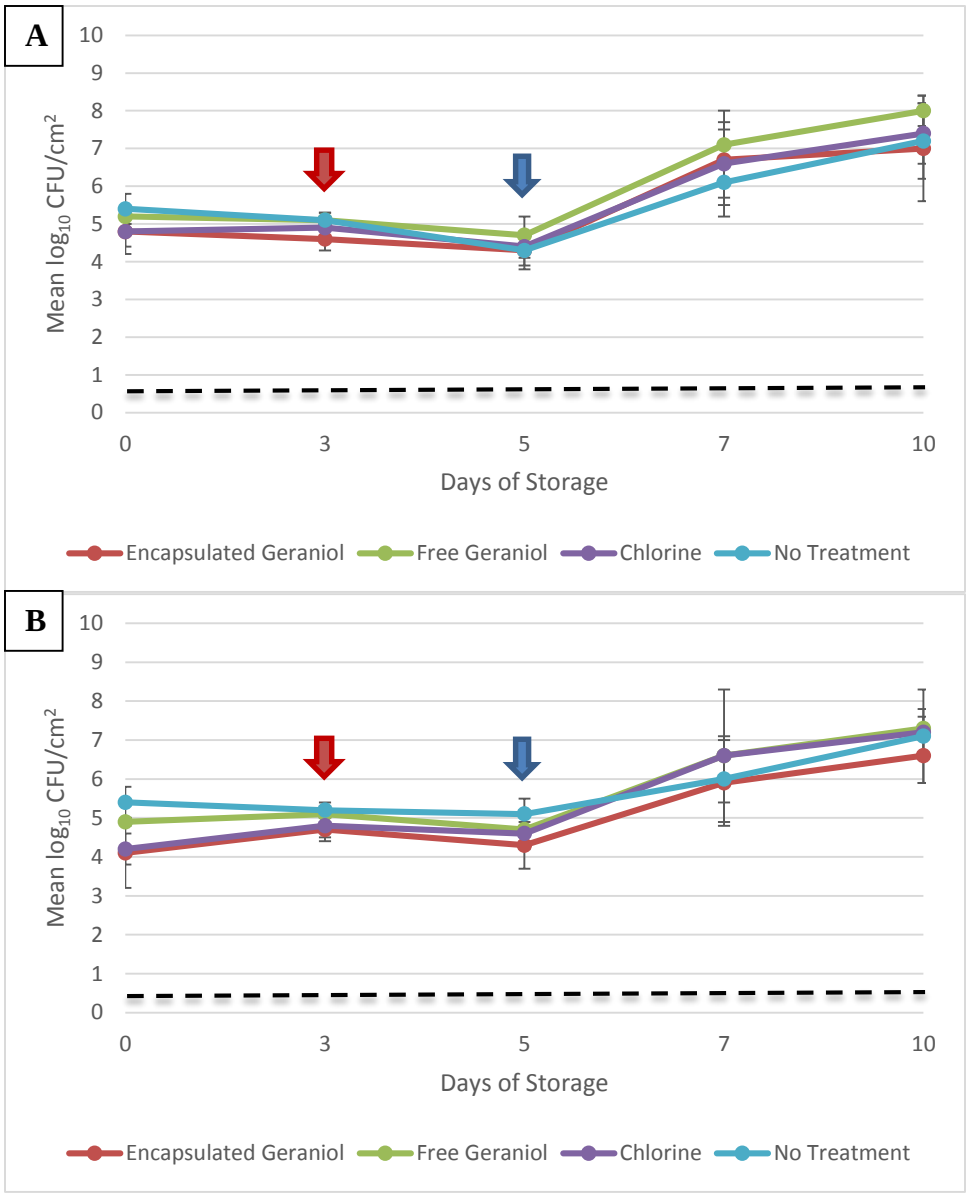

FIGURE 39. ***Means of A)*** **E. coli** ***O157:H7 and B)*** **S.** ***Typhimurium LT2 survivors on the surface of cantaloupes stored at 25°C after the application of various treatments with re-contamination of samples on day 3 of storage.*** The blue arrow indicates when samples transitioned from 5°C to 25°C. The red arrow indicates when samples were re-contaminated. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

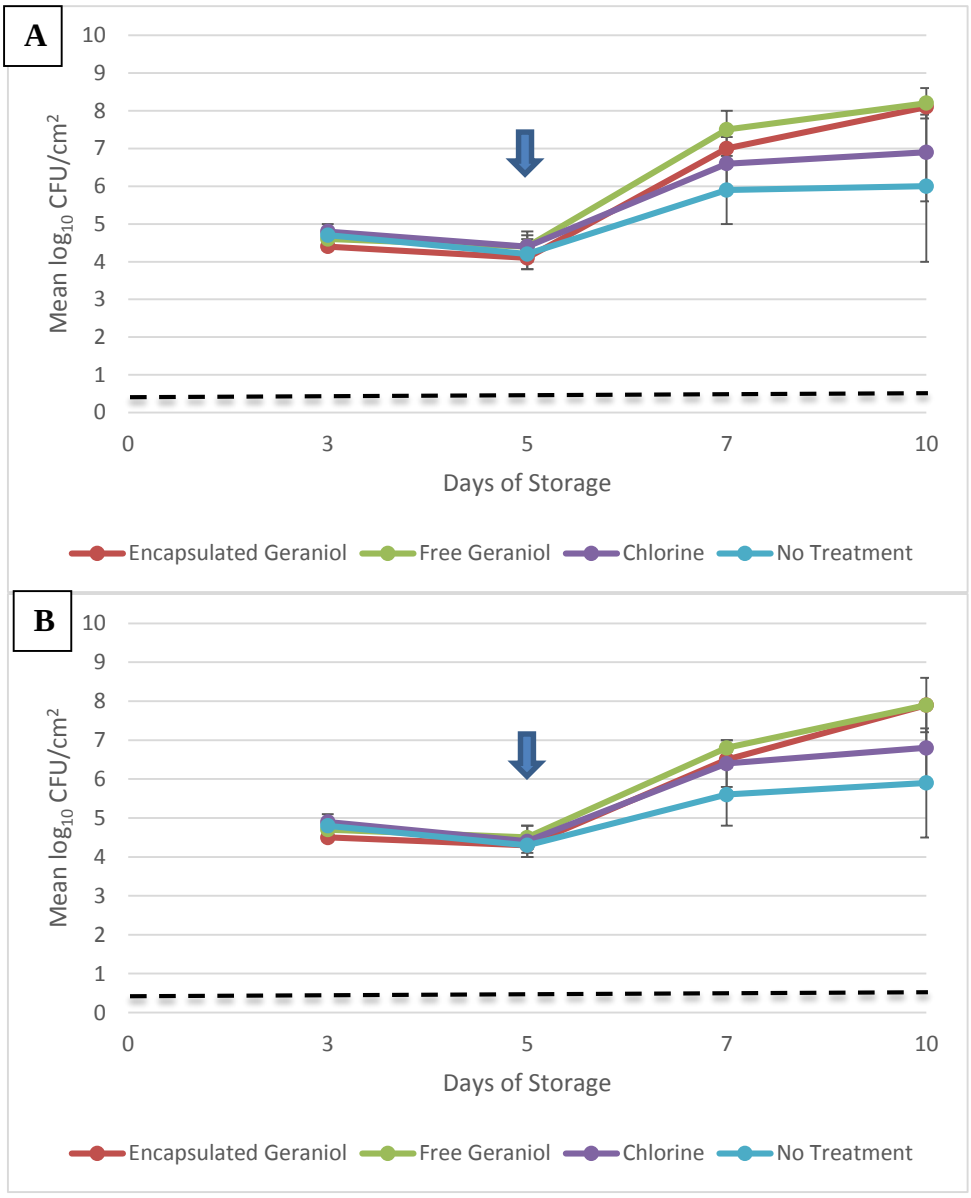

FIGURE 40. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of cantaloupes stored at 25°C after the application of various treatments with contamination of samples on day 3 of storage and no initial inoculation of pathogens on day 0.*** The arrow indicates when samples transitioned from 5°C to 25°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

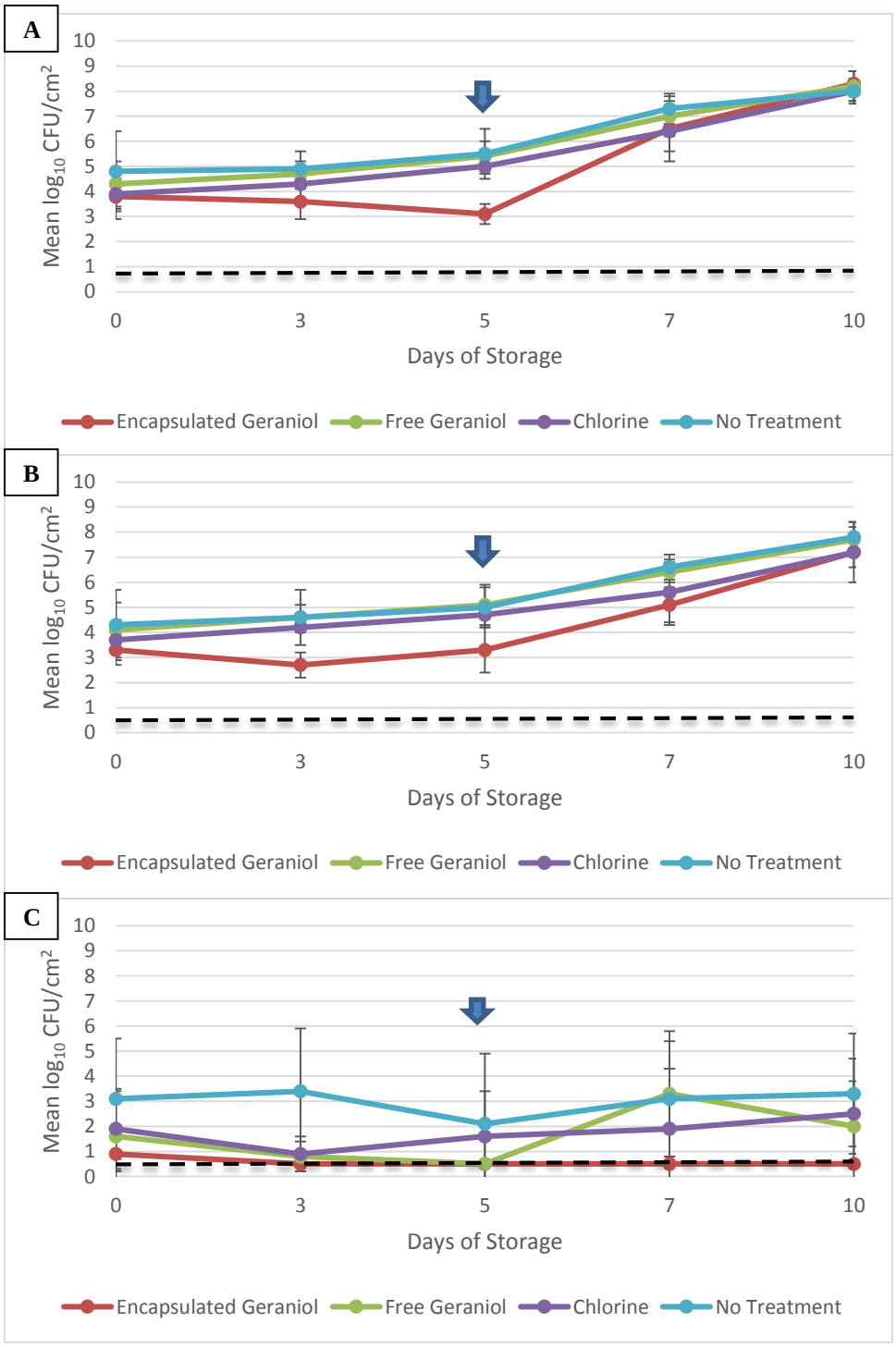

FIGURE 41. ***Means of A) aerobic bacteria, B) lactic acid bacteria, and C) coliforms on the surface of cantaloupes stored at 25°C.*** The arrow indicates when samples transitioned from 5°C to 25°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

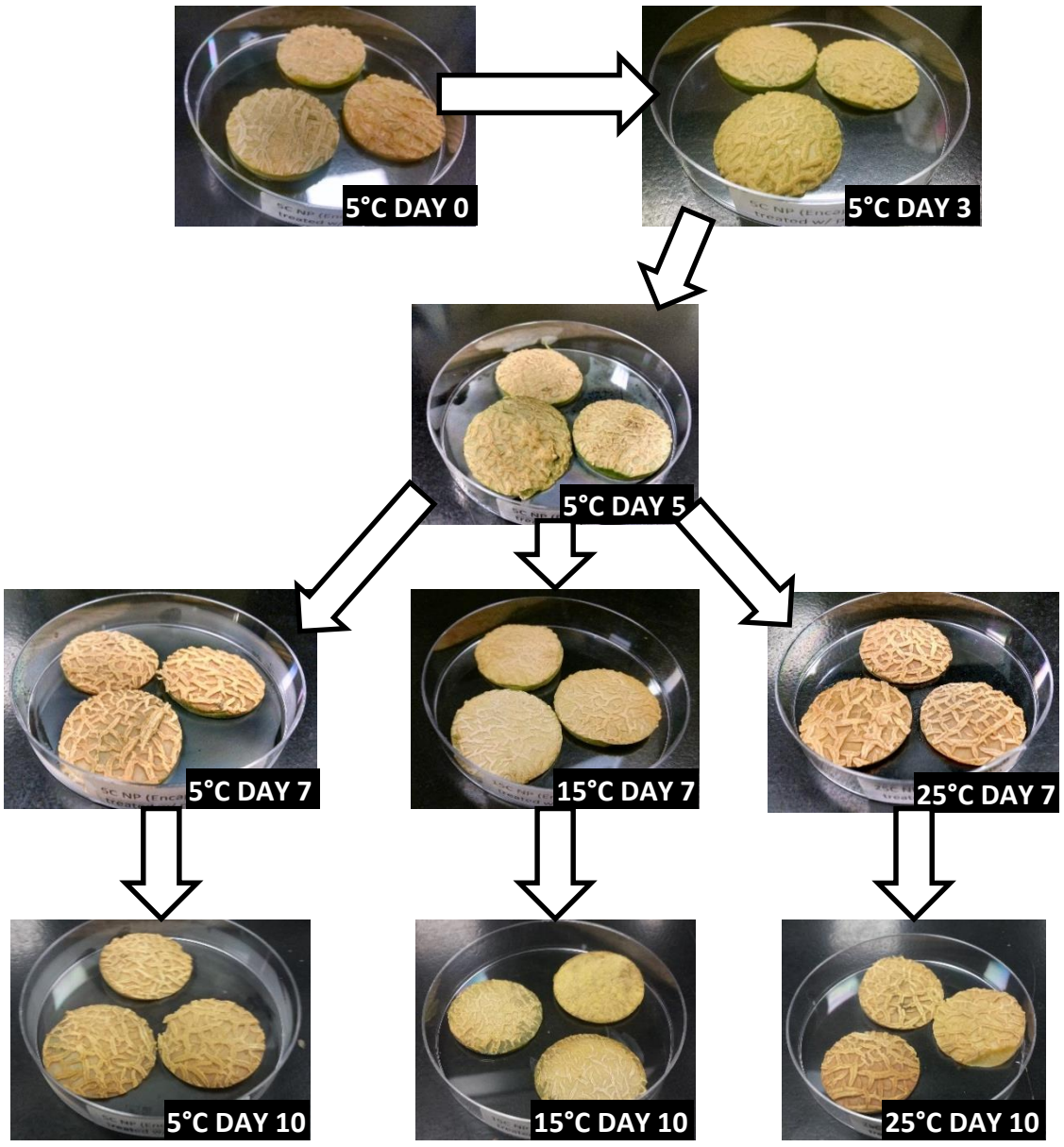

FIGURE 42. ***Nanoparticle-treated pathogen-inoculated cantaloupe stored at various temperatures for 0, 3, 5, 7, 10 days.***

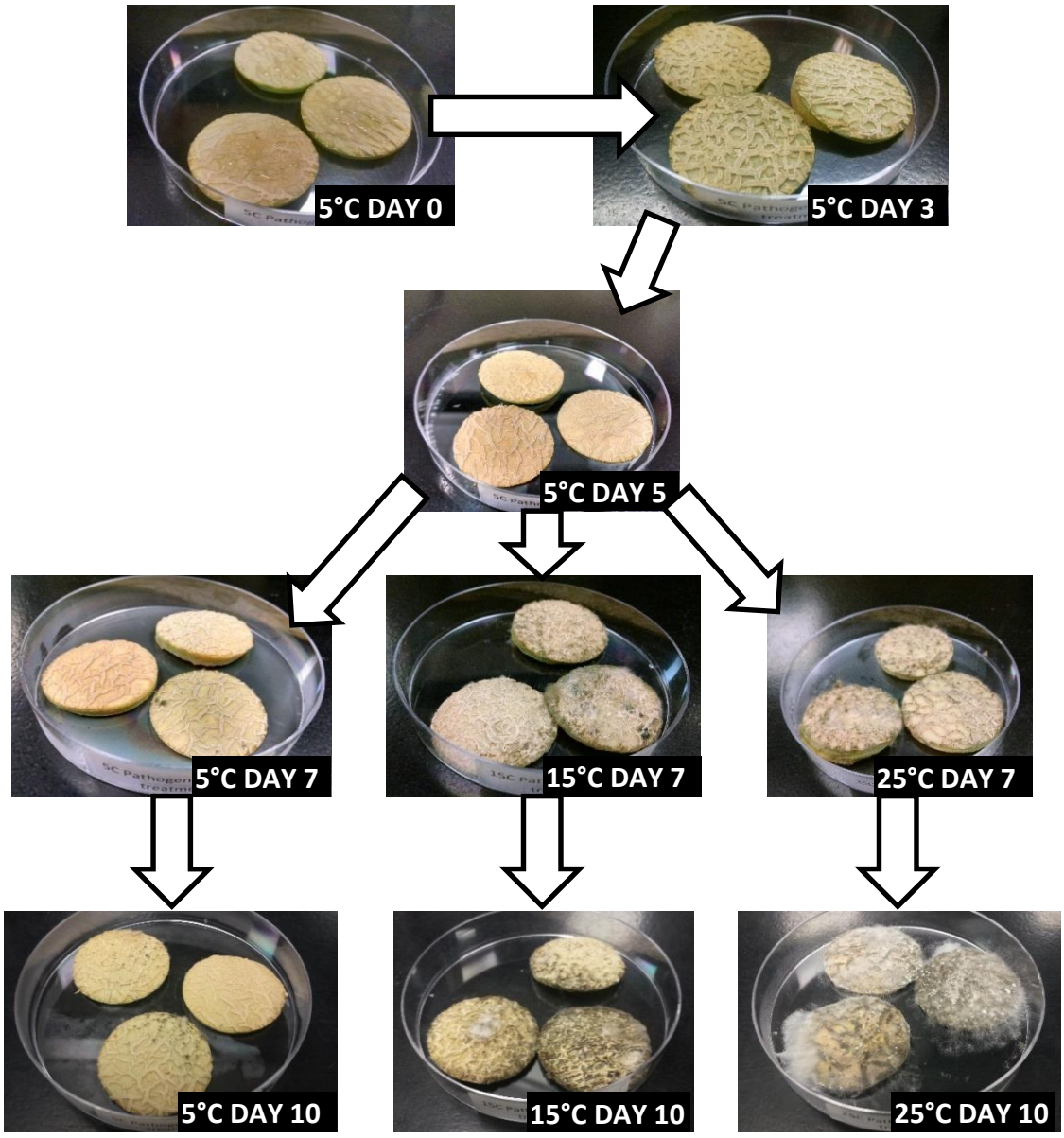

FIGURE 43. ***Untreated pathogen-inoculated cantaloupe stored at various temperatures for 0, 3, 5, 7, 10 days.***

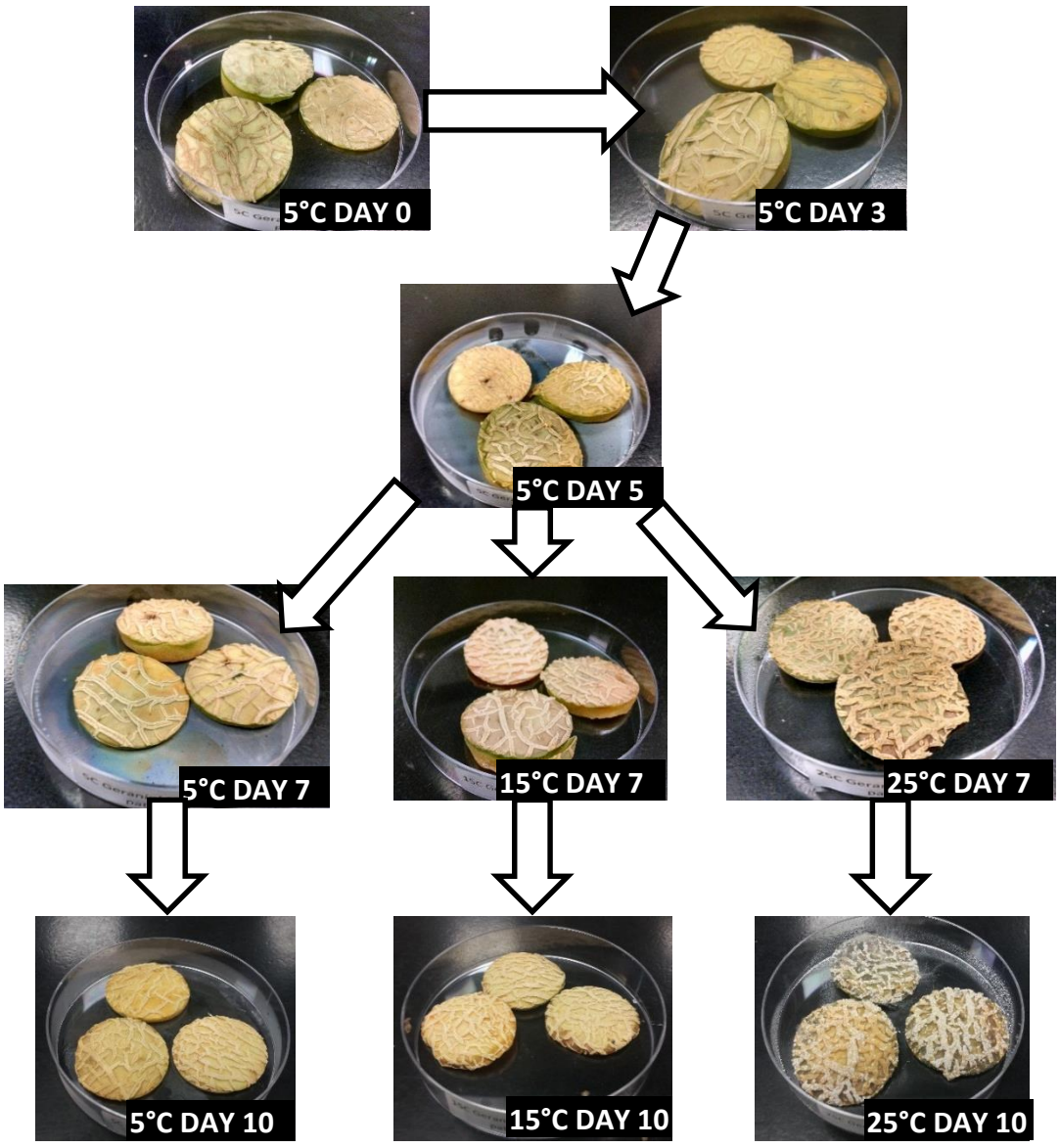

FIGURE 44. ***Unencapsulated geraniol-treated pathogen-inoculated cantaloupe stored at various temperatures for 0, 3, 5, 7, 10 days.***

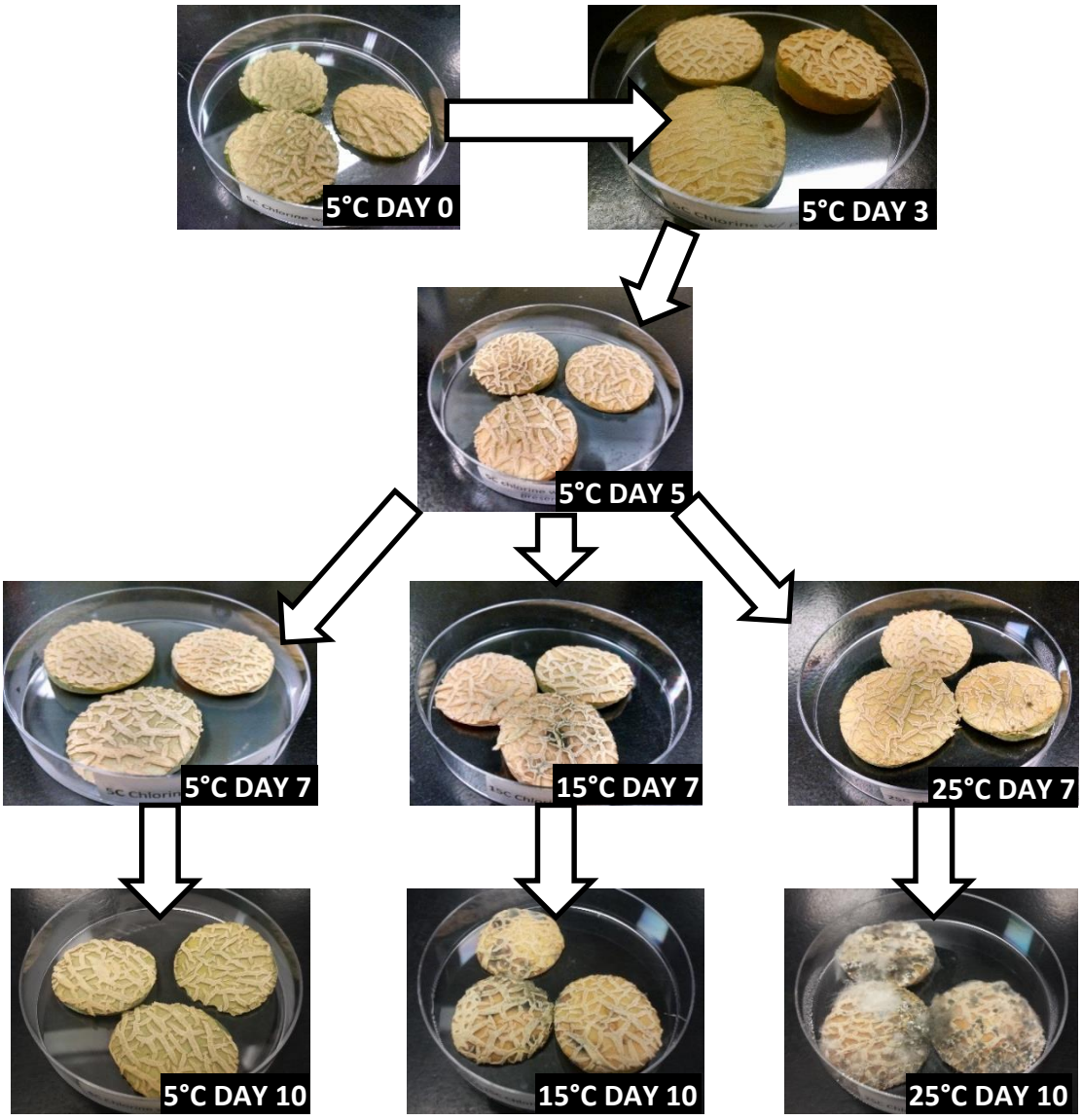

FIGURE 45. ***Chlorine-treated pathogen-inoculated cantaloupe stored at various temperatures for 0, 3, 5, 7, 10 days.***

Analysis of the pathogen concentrations on cantaloupe surface as affected by the treatments indicated significantly lower concentrations of both pathogens on cantaloupe surface throughout the 5°C storage period when treated with the nanoencapsulated geraniol than unencapsulated geraniol and no treatment, ($p < 0.05$), for the samples without recontamination and those that were recontaminated. Samples treated with nanoencapsulated geraniol and chlorine were not statistically different, ($p \geq 0.05$). However, for samples that were contaminated post treatment without the initial pathogen inoculation, there was no statistical difference between treatments, ($p \geq 0.05$). Treatments did not significantly reduce populations of both pathogens at 15°C and 25°C when compared to samples receiving no treatment for all inoculation treatment methods, ($p \geq 0.05$).

Nanoparticle Treatment on Surface of Spinach

Inoculation of spinach with the bacterial pathogens resulted in 6.1 ± 0.1 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 and 6.1 ± 0.1 $\log_{10}$ CFU/cm^2 *S.* Typhimurium LT2 attaching to spinach surface on day 0 prior to incubation and treatments (Figure 46). After the initial application via immersion for 2 min. of the antimicrobials on day 0 *E. coli* O157:H7 concentrations on the surface were 1.5 ± 1.6 and 3.7 ± 1.5 $\log_{10}$ CFU/cm^2 for free (unencapsulated) geraniol and chlorine, respectively (Figure 46). Concentrations of *S.* Typhimurium LT2 on the surface were 2.0 ± 1.4 and 3.5 ± 1.5 $\log_{10}$ CFU/cm^2 for free geraniol and chlorine, respectively (Figure 46). Concentrations of both pathogens were below the detection limit throughout the entire ten day storage period at 5°C for spinach

treated with nanoencapsulated geraniol (Figure 46). At day 10 populations of both pathogens on the surface of spinach treated with free geraniol were also below the detection limit (Figure 46). *E. coli* O157:H7 concentrations on the surface were 4.2 ± 0.7, 2.2 ± 0.7, and 3.7 ± 0.4 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free geraniol, chlorine, and no treatment, respectively (Figure 46). At day 10 *S.* Typhimurium LT2 concentrations on the surface were 1.0 ± 0.8 and 2.5 ± 0.3 $\log_{10}$ CFU/cm^2 for chlorine-treated and untreated spinach, respectively (Figure 46).

On day 3 of storage, a set of spinach samples were re-contaminated with a bacterial suspension containing both pathogens with a concentration of 4.9 ± 0.1 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 and 4.9 ± 0.0 $\log_{10}$ CFU/cm^2 *S.* Typhimurium LT2 attaching to the surface. After the initial application of the pathogens on already inoculated spinach on day 3 *E. coli* O157:H7 concentrations on the surface were 1.7 ± 1.0, 4.3 ± 0.3, and 4.4 ± 0.9 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 47). After the initial application of the pathogens on already inoculated spinach on day 3, *S.* Typhimurium LT2 concentrations on the surface were 1.5 ± 1.2, 4.2 ± 0.6, and ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 47). At day 10, *E. coli* O157:H7 concentrations on the surface of the re-contaminated spinach were below the detection limit for spinach samples treated with unencapsulated and nanoencapsulated geraniol, and 3.1 ± 0.1 and 3.4 ± 0.6 $\log_{10}$ CFU/cm^2 for samples treated with chlorine and untreated samples, respectively (Figure 47). At day 10, *S.* Typhimurium LT2 concentrations on the surface of the re-contaminated spinach were

below the detection limit for spinach samples treated with unencapsulated and nanoencapsulated geraniol, and 2.8 ± 0.3 and 3.5 ± 0.5 $\log_{10}$ CFU/cm^2 for samples treated with chlorine and untreated samples, respectively (Figure 47).

On day 3, non-inoculated treated samples were contaminated with pathogens to a concentration of 4.9 ± 0.1 $\log_{10}$ CFU/cm^2 *E. coli* O157:H7 and 4.9 ± 0.0 $\log_{10}$ CFU/cm^2 *S.* Typhimurium LT2 attaching to the surface to simulate post treatment contamination. On day 3, the *E. coli* O157:H7 concentration on the surface of the post treatment contaminated samples were 1.8 ± 1.2, 3.1 ± 1.8, and 4.8 ± 0.3 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 48). On day 3, the *S.* Typhimurium LT2 concentration on the surface of the post treatment contaminated samples were 1.7 ± 1.0, 3.2 ± 0.8, and 4.9 ± 0.2 $\log_{10}$ CFU/cm^2 for encapsulated geraniol, free (unencapsulated) geraniol, and chlorine, respectively (Figure 48). At day 10, *E. coli* O157:H7 concentrations on the surface of the post treatment contaminated spinach were below the detection limit for spinach samples treated with unencapsulated and nanoencapsulated geraniol, and 3.1 ± 0.6 and 1.9 ± 1.2 $\log_{10}$ CFU/cm^2 for samples treated with chlorine and untreated samples, respectively (Figure 48). At day 10, *S.* Typhimurium LT2 concentrations on the surface of the post treatment contaminated spinach were below the detection limit for spinach samples treated with unencapsulated and nanoencapsulated geraniol, and 3.1 ± 0.8 and 2.3 ± 0.5 $\log_{10}$ CFU/cm^2 for samples treated with chlorine and untreated samples, respectively (Figure 48).

Initial levels of aerobic bacteria, LAB, and coliforms were 4.7 ± 0.4, 4.3 ± 0.7, and 2.1 ± 1.4 $\log_{10}$ CFU/cm^2 at day 0, respectively, prior to storage (Figure 49). After the application of treatments and prior to storage, levels of aerobic bacteria and LAB 1.2 ± 0.6 and 0.7 ± 0.3 $\log_{10}$ CFU/cm^2, respectively for nanoencapsulated geraniol-treated. Levels of coliforms were undetectable throughout the storage period for samples treated with nanoencapsulated geraniol (Figure 49). Levels of aerobic bacteria, LAB, and coliforms were 2.8 ± 1.7, 1.3 ± 1.4., and 1.1 ± 1.0 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol-treated, and 4.0 ± 0.6, 3.7 ± 0.6, and 1.3 ± 0.7 $\log_{10}$ CFU/cm^2 respectively for chlorine-treated (Figure 49). After day 10 of storage at 5°C levels of aerobic bacteria, LAB, and coliforms on untreated samples were 4.2 ± 0.1, 3.7 ± 0.5, and 0.8 ± 0.5 $\log_{10}$ CFU/cm^2 respectively (Figure 49). At day 10 of storage levels of coliforms for unencapsulated geraniol and nanoencapsulated geraniol-treated spinach samples were below detection limits (Figure 49). Levels of LAB for spinach samples treated with nanoencapsulated geraniol were below the detection limits at day 10 of storage (Figure 49). Levels of aerobic bacteria at day 10 of storage at 5°C for nanoencapsulated geraniol-treated samples were 2.1 ± 2.2 $\log_{10}$ CFU/cm^2 (Figure 49). After day 10 of storage at 5°C, levels of aerobic bacteria and LAB were 2.4 ± 1.5 and 1.8 ± 2.0 $\log_{10}$ CFU/cm^2 respectively for unencapsulated geraniol-treated, 4.7 ± 0.8 and 3.7 ± 1.2 $\log_{10}$ CFU/cm^2 and respectively for chlorine-treated (Figure 49). Levels of coliforms for chlorine-treated samples were 0.7 ± 0.3 $\log_{10}$ CFU/cm^2 (Figure 49).

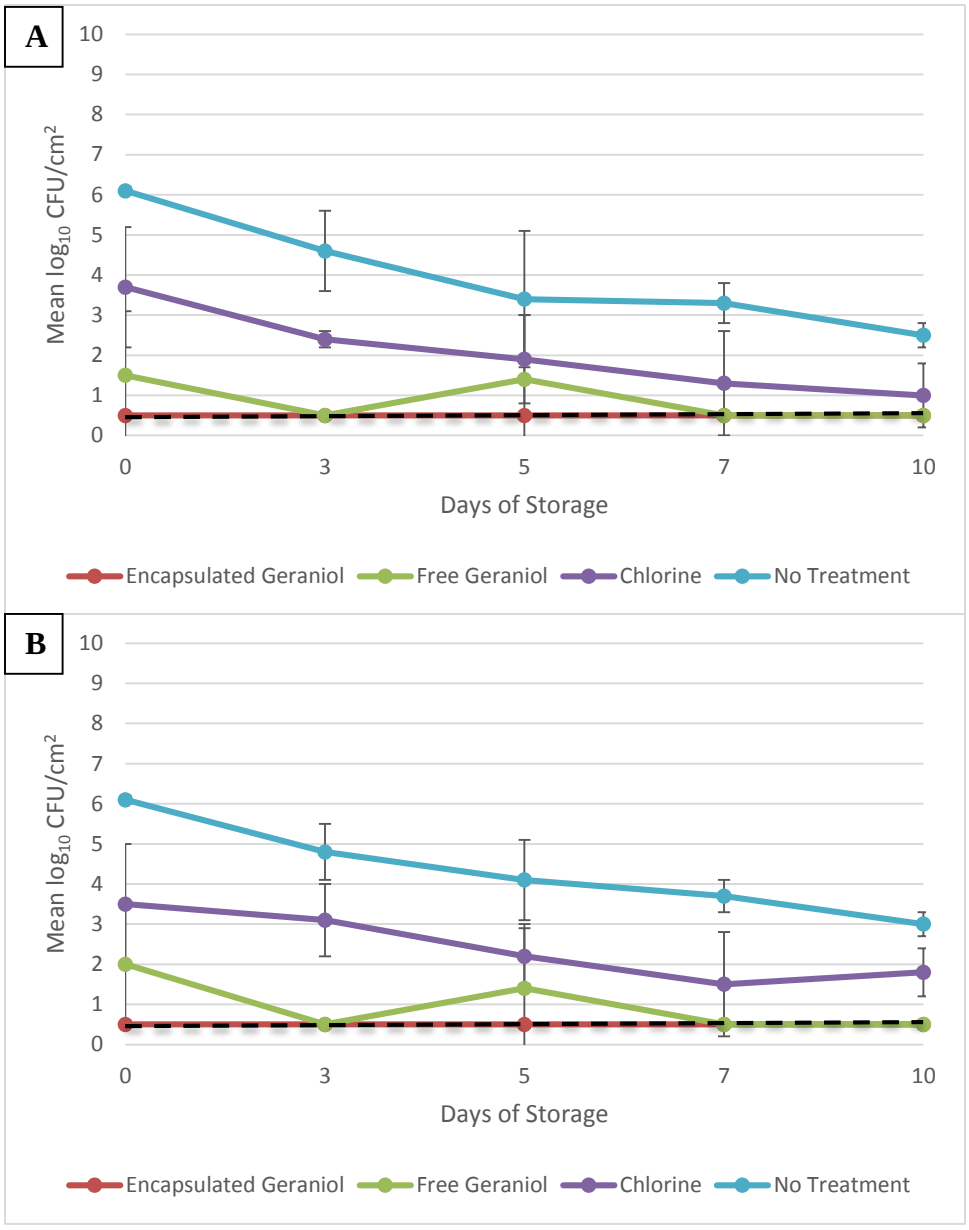

FIGURE 46. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of spinach stored at 5°C for 0, 3, 5, 7, and 10 days after the application of various treatments.*** Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

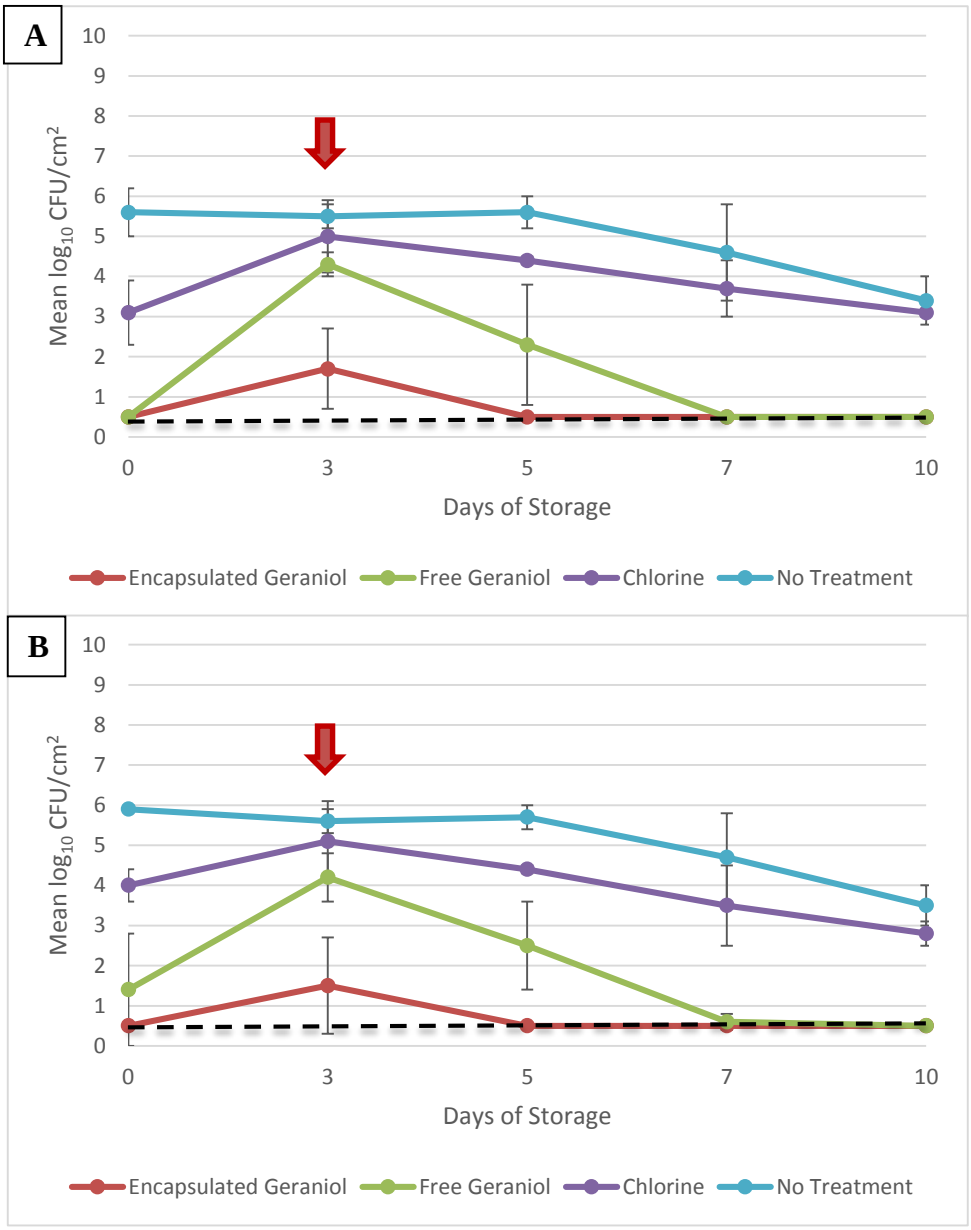

FIGURE 47. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of spinach stored at 5°C for 0, 3, 5, 7, and 10 days after the application of various treatments and with re-contamination of samples on day 3 of storage.*** Error bars indicate standard deviation from sample means (n=3). The red arrow indicates when samples were re-contaminated. Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

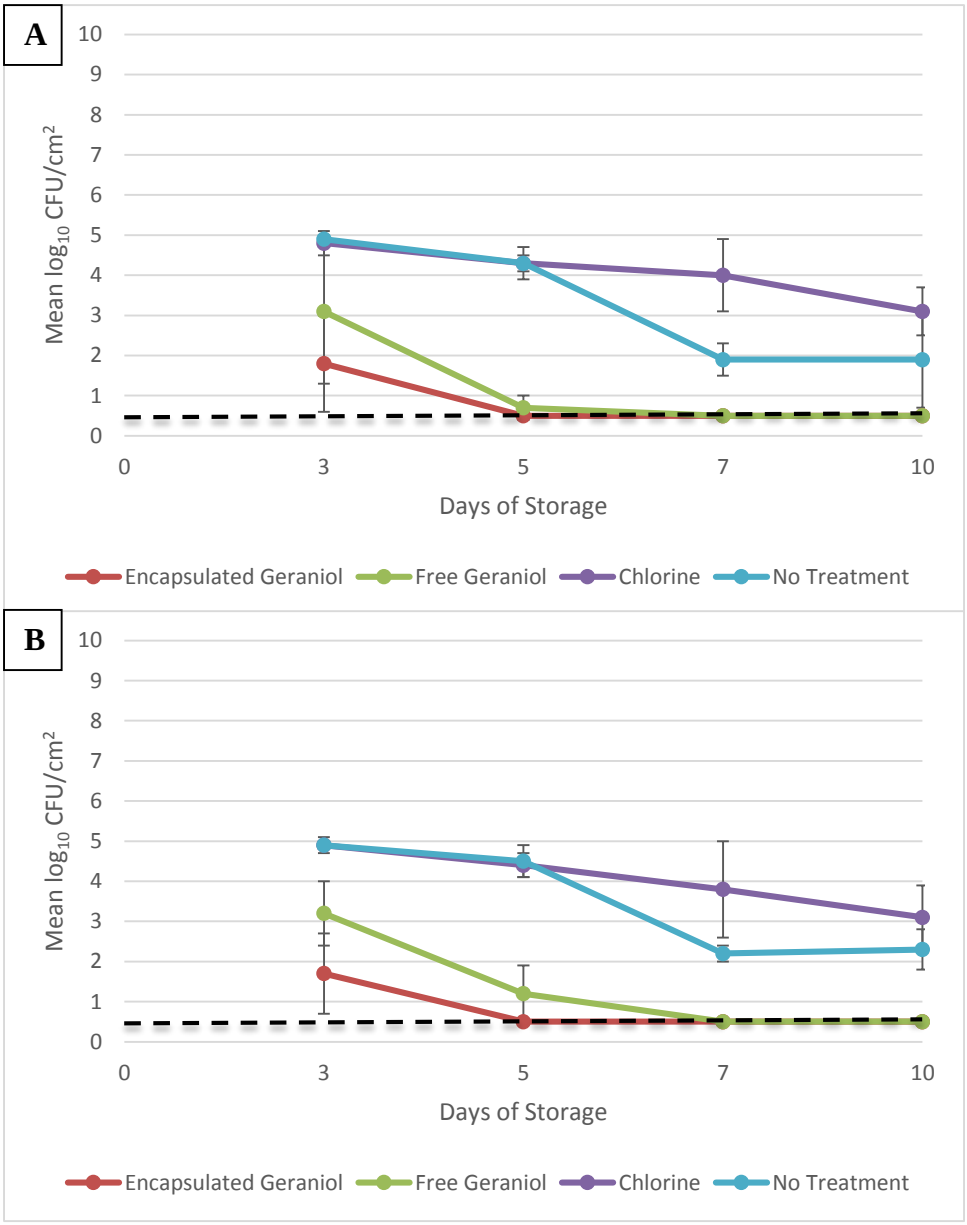

FIGURE 48. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of spinach stored at 5°C for 0, 3, 5, 7, and 10 days after the application of various treatments and with contamination of samples on day 3 of storage and no initial inoculation of pathogens on day 0.*** Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

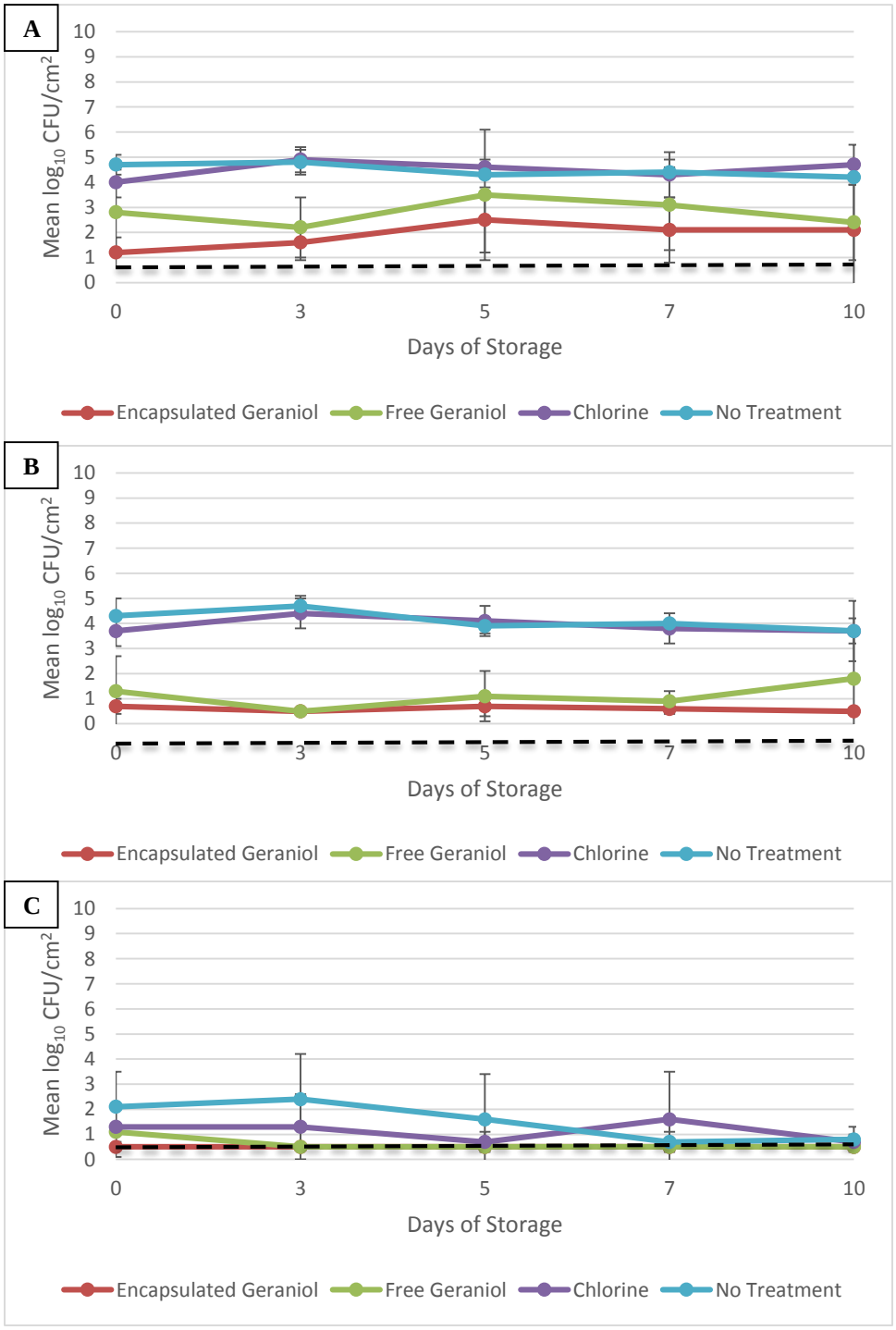

FIGURE 49. ***Means of A) aerobic bacteria, B) lactic acid bacteria, and C) coliforms on the surface of spinach stored at 5°C for 0, 3, 5, 7, and 10 days after the application of treatments.*** Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

On day 5 of storage at 5°C, samples of each inoculation treatment method (no recontamination, recontaminated, post treatment contaminated, and uninoculated with no contamination) and treatment type (encapsulated geraniol, unencapsulated geraniol, chlorine, and no treatment) were moved to 15°C and 25°C to simulate post processing temperature abuse. At day 10 at 15°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were not recontaminated were below detection limits for nanoencapsulated and unencapsulated geraniol and 1.0 ± 0.8 and 1.1 ± 0.8 $\log_{10}$ CFU/cm^2 for chlorine treated and untreatment spinach respectively (Figure 50). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were not recontaminated were below detection limits for nanoencapsulated and unencapsulated geraniol and 0.7 ± 0.3 and 1.6 ± 0.2 $\log_{10}$ CFU/cm^2 for chlorine and untreated spinach samples, respectively (Figure 50). At day 10 at 15°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were recontaminated were below detection limits for nanoencapsulated and unencapsulated geraniol and 2.7 ± 0.1 and 2.9 ± 0.3 $\log_{10}$ CFU/cm^2 for chlorine and untreated spinach samples, respectively (Figure 51). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were recontaminated were below detection limits for nanoencapsulated and unencapsulated geraniol and 2.8 ± 0.3 and 2.8 ± 0.1 $\log_{10}$ CFU/cm^2 for chlorine and untreated spinach samples, respectively (Figure 51). At day 10 at 15°C, *E. coli* O157:H7 concentrations on the surface for the post treatment contaminated samples were below detection limits for nanoencapsulated and unencapsulated geraniol and 2.2 ± 0.8 and 1.4 ± 0.7 $\log_{10}$ CFU/cm^2 for chlorine and

untreated spinach samples, respectively (Figure 60). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the post treatment contaminated samples were below detection limits for nanoencapsulated and unencapsulated geraniol and 2.3 ± 0.4 and 1.5 ± 0.6 $\log_{10}$ CFU/cm^2 for chlorine and untreated spinach samples, respectively (Figure 52). At day 10 of storage at 15°C levels of aerobic bacteria, LAB, and coliforms on untreated samples were 4.5 ± 0.5, 4.0 ± 0.6, and 1.7 ± 1.1 $\log_{10}$ CFU/cm^2 respectively (Figure 53). At day 10 of storage at 15°C, levels of aerobic bacteria were 0.8 ± 0.8 $\log_{10}$ CFU/cm^2 and below the detection limit for LAB and coliforms for samples treated with nanoencapsulated geraniol. At day 10 of storage at 15°C, levels of aerobic bacteria and LAB were 3.0 ± 2.4 and 2.8 ± 2.2 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol-treated, and 4.3 ± 0.1 and 4.2 ± 0.1 $\log_{10}$ CFU/cm^2 respectively for chlorine treated (Figure 53). At day 10 of storage at 15°C, levels of coliforms were below detection limits for samples treated with unencapsulated and encapsulated geraniol (Figure 53). Levels of coliforms were 1.6 ± 1.8 $\log_{10}$ CFU/cm^2 for samples treated with chlorine (Figure 53).

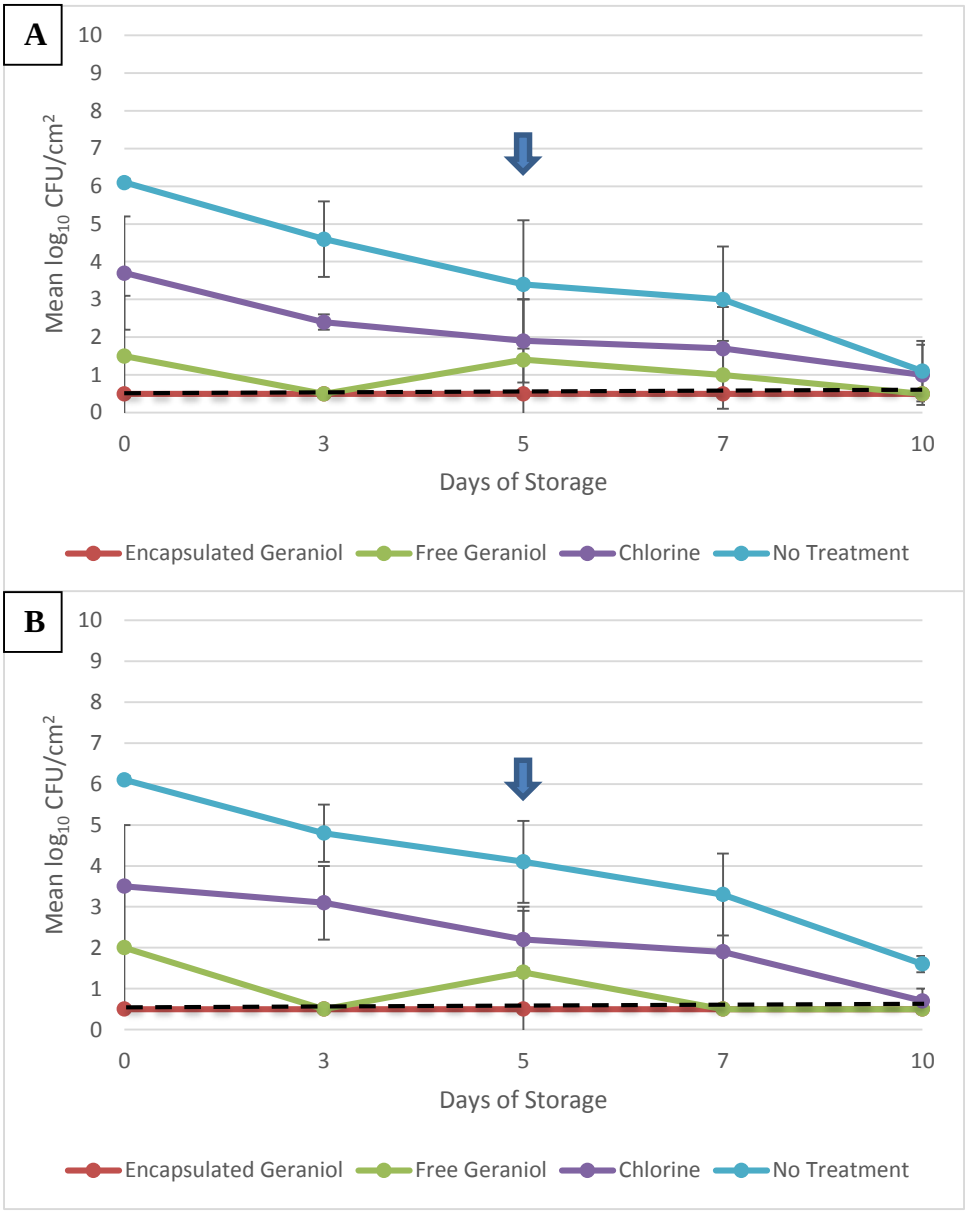

FIGURE 50. ***Means of A)*** **E. coli** ***O157:H7 and B)*** **S.** ***Typhimurium LT2 survivors on the surface of spinach stored at 15°C after the application of various treatments.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

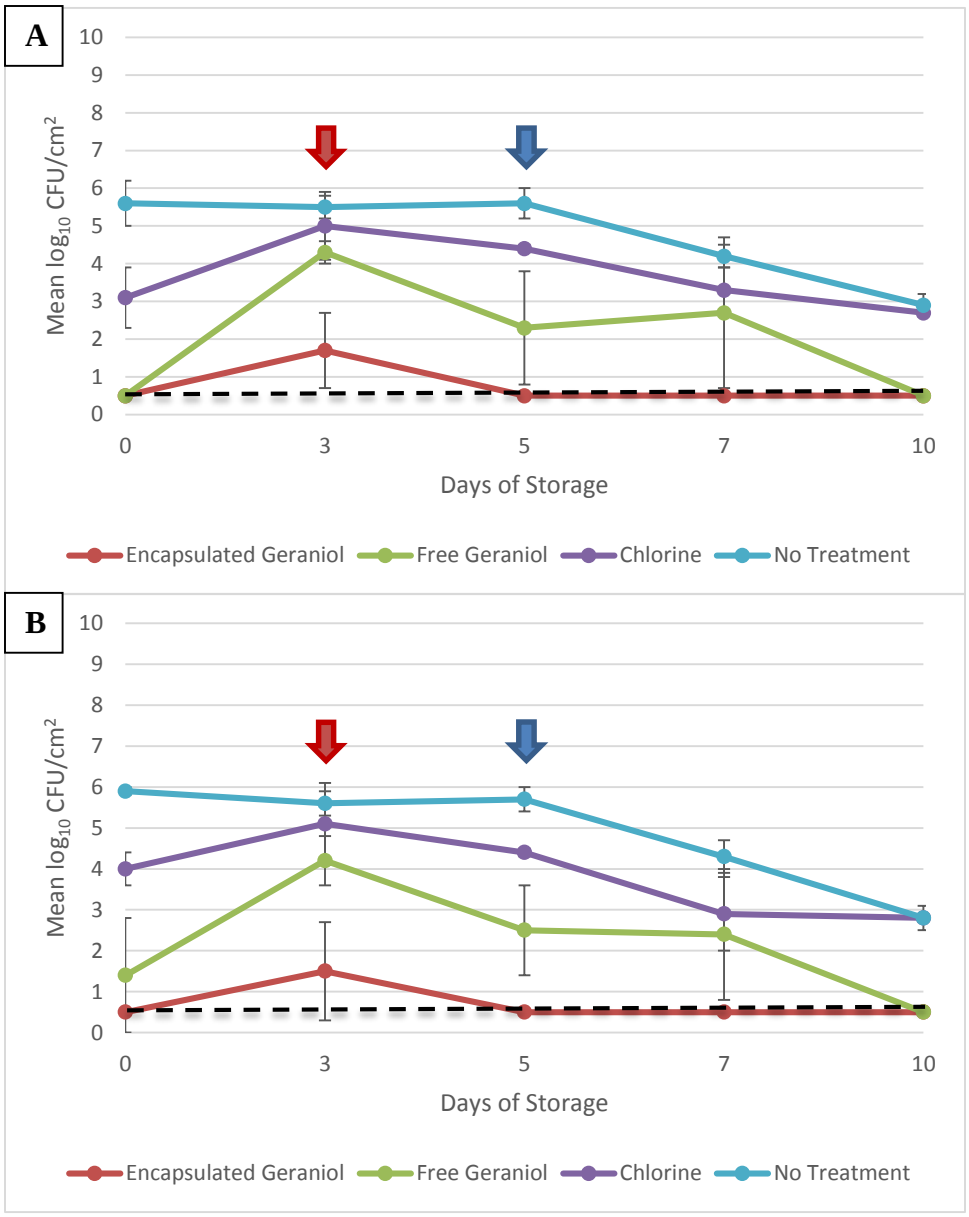

FIGURE 51. ***Means of A)* E. coli *O157:H7 and B)* S. *Typhimurium LT2 survivors on the surface of spinach stored at 15°C after the application of various treatments with re-contamination of samples on day 3 of storage.*** The blue arrow indicates when samples transitioned from 5°C to 15°C. The red arrow indicates when samples were re-contaminated. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

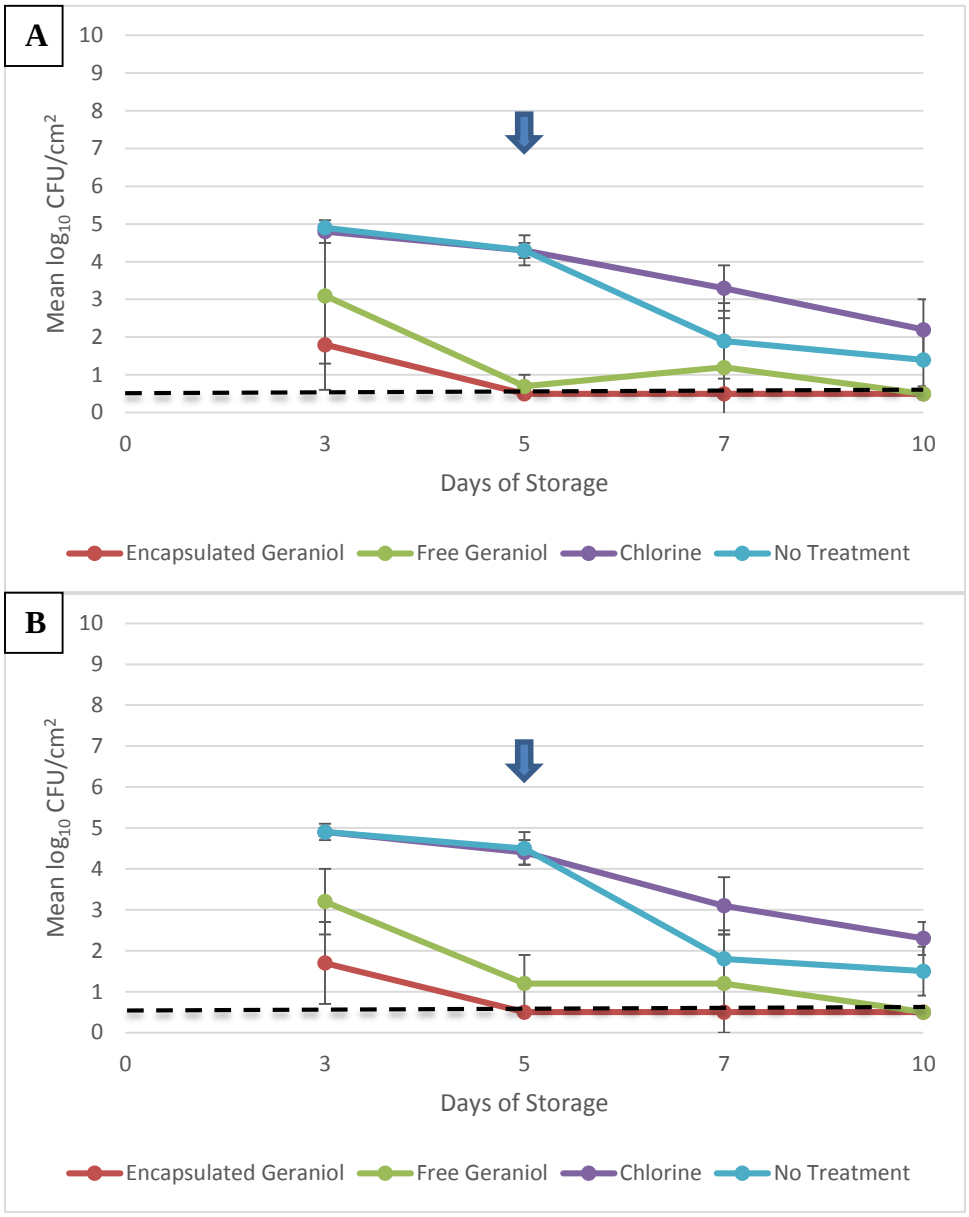

FIGURE 52. ***Means of A)*** **E. coli** ***O157:H7 and B)*** **S.** ***Typhimurium LT2 survivors on the surface of spinach stored at 15°C after the application of various treatments with contamination of samples on day 3 of storage and no initial inoculation of pathogens on day 0.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

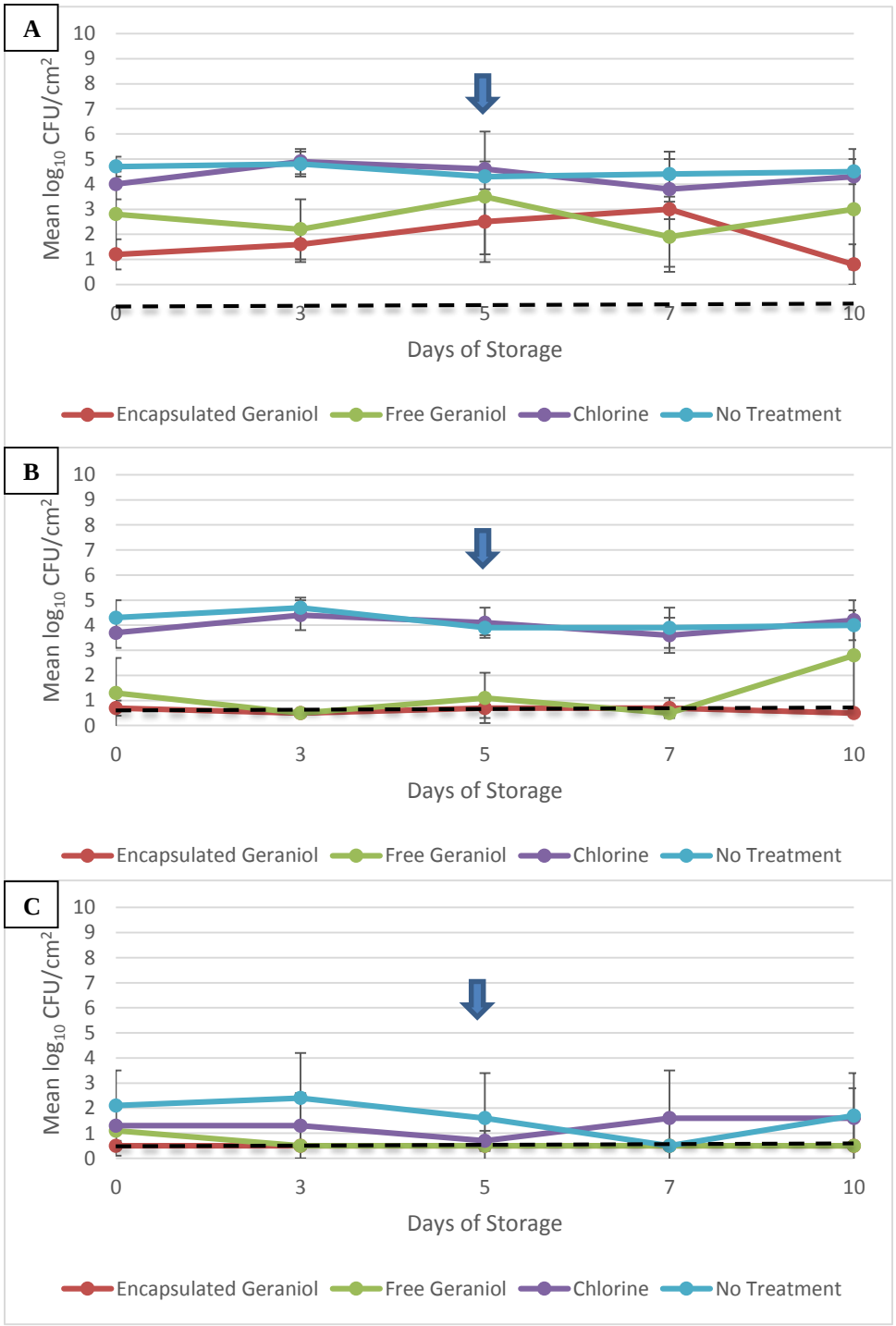

FIGURE 53. ***Means of A) aerobic bacteria, B) lactic acid bacteria, and C) coliforms on the surface of spinach stored at 15°C.*** The arrow indicates when samples transitioned from 5°C to 15°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

At day 10 at 25°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were not recontaminated were below detection limits for nanoencapsulated and unencapsulated geraniol and 4.5 ± 1.4 and 4.1 ± 2.0 $\log_{10}$ CFU/cm^2 for chlorine-treated and untreatment spinach respectively (Figure 54). At day 10 *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were not recontaminated were below detection limits for nanoencapsulated and unencapsulated geraniol and 4.4 ± 1.2 and 4.0 ± 1.9 $\log_{10}$ CFU/cm^2 for chlorine and untreated spinach samples, respectively (Figure 54). At day 10 at 25°C, *E. coli* O157:H7 concentrations on the surface for the inoculated samples that were recontaminated were below detection limits for nanoencapsulated geraniol and 3.8 ± 3.0, 5.9 ± 1.8 and 5.3 ± 0.5 $\log_{10}$ CFU/cm^2 for unencapsulated geraniol, chlorine and untreated spinach samples, respectively (Figure 55). At day 10, *S.* Typhimurium LT2 concentrations on the surface for the inoculated samples that were recontaminated were below detection limits for nanoencapsulated and 3.6 ± 2.1, 4.2 ± 1.2, and 5.0 ± 0.6 $\log_{10}$ CFU/cm^2 for unencapsulated geraniol, chlorine and untreated spinach samples, respectively (Figure 55). At day 10 at 25°C, *E. coli* O157:H7 concentrations on the surface for the post treatment contaminated samples were below detection limits for nanoencapsulated and unencapsulated geraniol and 6.4 ± 0.5 and 5.0 ± 0.6 $\log_{10}$ CFU/cm^2 for chlorine and untreated spinach samples, respectively (Figure 56). At day 10, *S.* Typhimurium LT2 concentrations on the surface for the post treatment contaminated samples were below detection limits for nanoencapsulated and unencapsulated geraniol and 6.0 ± 0.7 and 4.4 ± 0.8 $\log_{10}$ CFU/cm^2 for chlorine and untreated spinach samples, respectively (Figure

56). At day 10 of storage at 25°C, levels of aerobic bacteria, LAB, and coliforms on untreated samples were 6.7 ± 0.4, 6.6 ± 0.3, and 4.9 ± 0.1 $\log_{10}$ CFU/cm^2 respectively (Figure 57). At day 10 of storage at 25°C, levels of aerobic bacteria were 3.3 ± 2.0 $\log_{10}$ CFU/cm^2 and below the detection limit for LAB and coliforms for samples treated with nanoencapsulated geraniol. At day 10 of storage at 25°C, levels of aerobic bacteria and LAB were 4.7 ± 1.2 and 1.5 ± 1.8 $\log_{10}$ CFU/cm^2, respectively for unencapsulated geraniol-treated, and 7.4 ± 0.2 and 6.3 ± 0.1 $\log_{10}$ CFU/cm^2 respectively for chlorine-treated (Figure 57). At day 10 of storage at 15°C, levels of coliforms were below detection limits for samples treated with nanoencapsulated geraniol and 1.6 ± 1.9 and 5.1 ± 0.6 $\log_{10}$ CFU/cm^2 for samples treated with unencapsulated geraniol and chlorine, respectively (Figure 57).

Throughout the 10 day storage period 5°C treated spinach did not change visually and did not have visible mold growth (Figures 58-61). At day 10 at 15°C encapsulated geraniol treated spinach did not change visually and did not have visible mold growth (Figure 58). However, at day 10 at 15°C unencapsulated geraniol and chlorine appeared slightly wilted, but did not have visible mold growth (Figures 60-61). At day 10 at 25°C all treatments had deteriorated and appeared wilted (Figure 58-61).

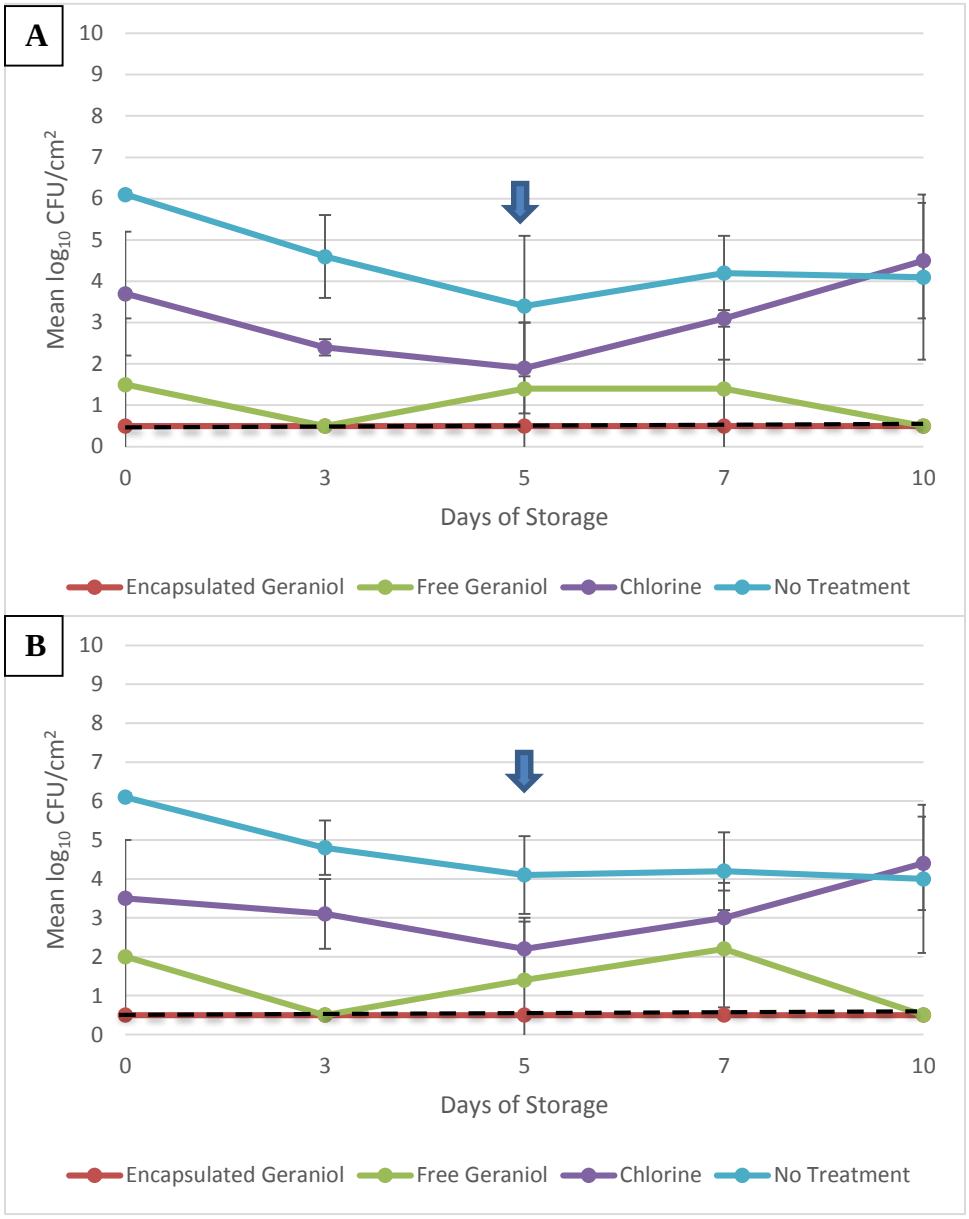

FIGURE 54. ***Means of A)*** **E. coli** ***O157:H7 and B)*** **S.** ***Typhimurium LT2 survivors on the surface of spinach stored at 25°C after the application of various treatments.*** The arrow indicates when samples transitioned from 5°C to 25°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

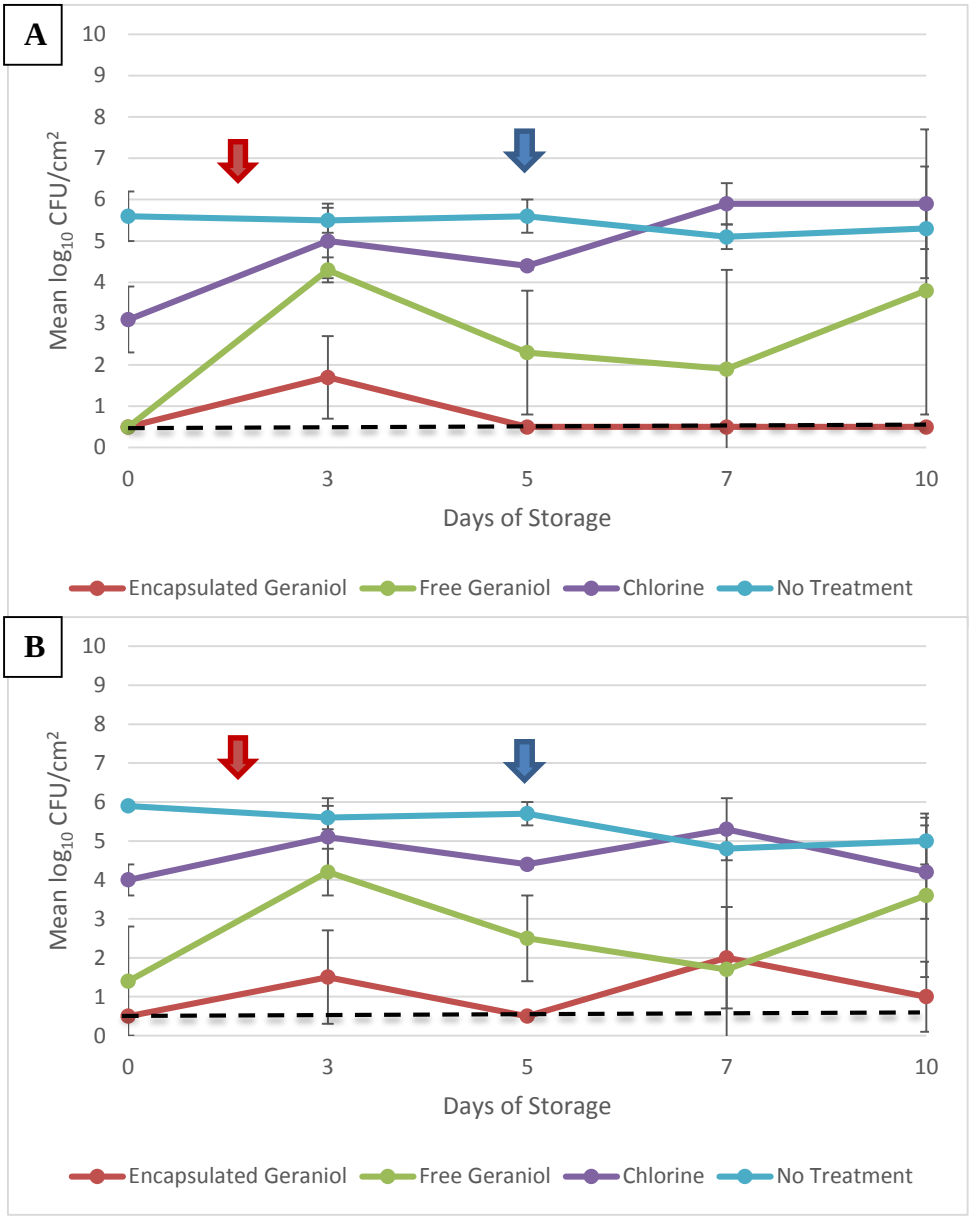

FIGURE 55. ***Means of A)*** **E. coli** ***O157:H7 and B)*** **S.** ***Typhimurium LT2 survivors on the surface of spinach stored at 25°C after the application of various treatments with re-contamination of samples on day 3 of storage.*** The blue arrow indicates when samples transitioned from 5°C to 25°C. The red arrow indicates when samples were re-contaminated. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

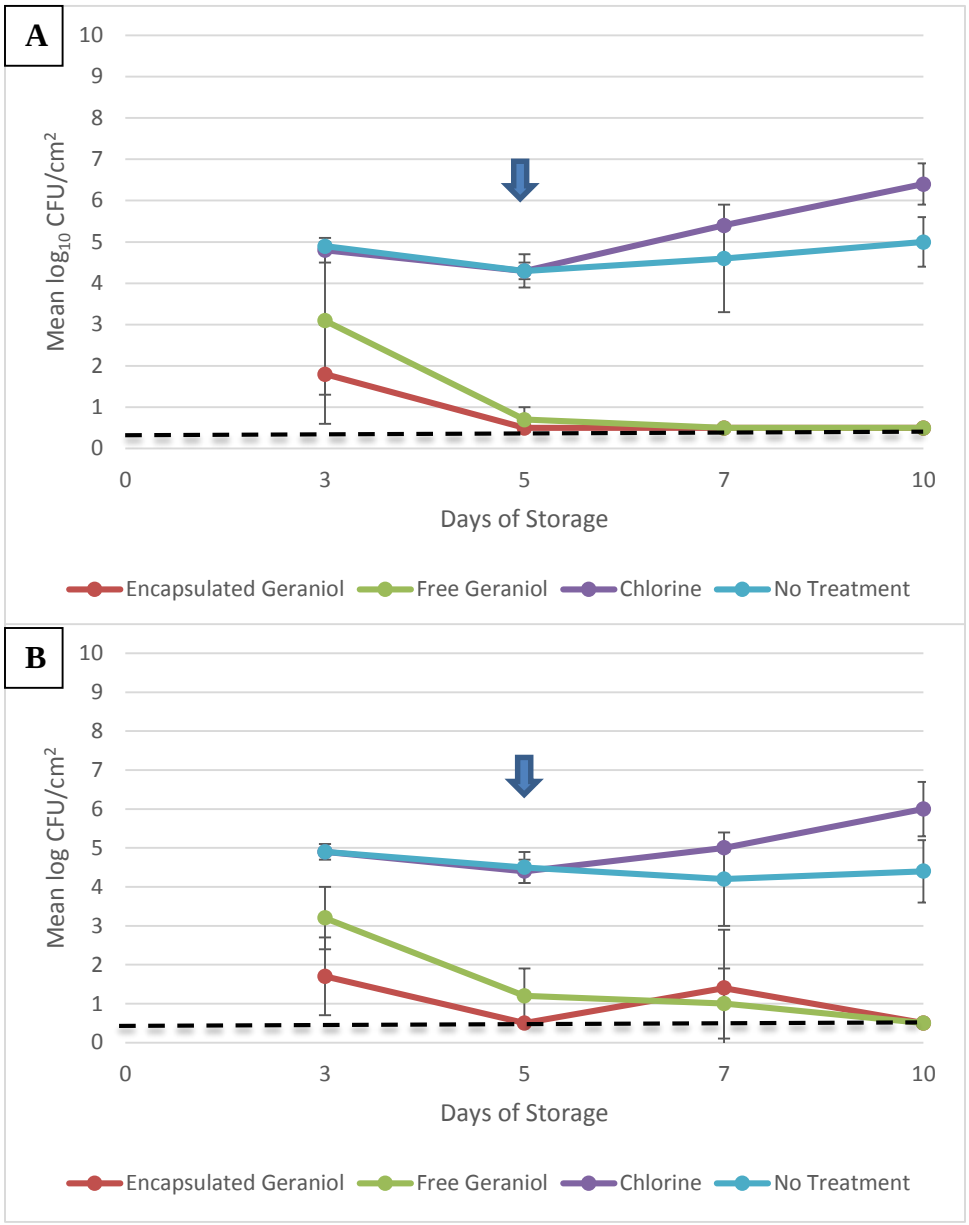

FIGURE 56. ***Means of A)*** **E. coli** ***O157:H7 and B)*** **S.** ***Typhimurium LT2 survivors on the surface of spinach stored at 25°C after the application of various treatments with contamination of samples on day 3 of storage and no initial inoculation of pathogens on day 0.*** The arrow indicates when samples transitioned from 5°C to 25°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

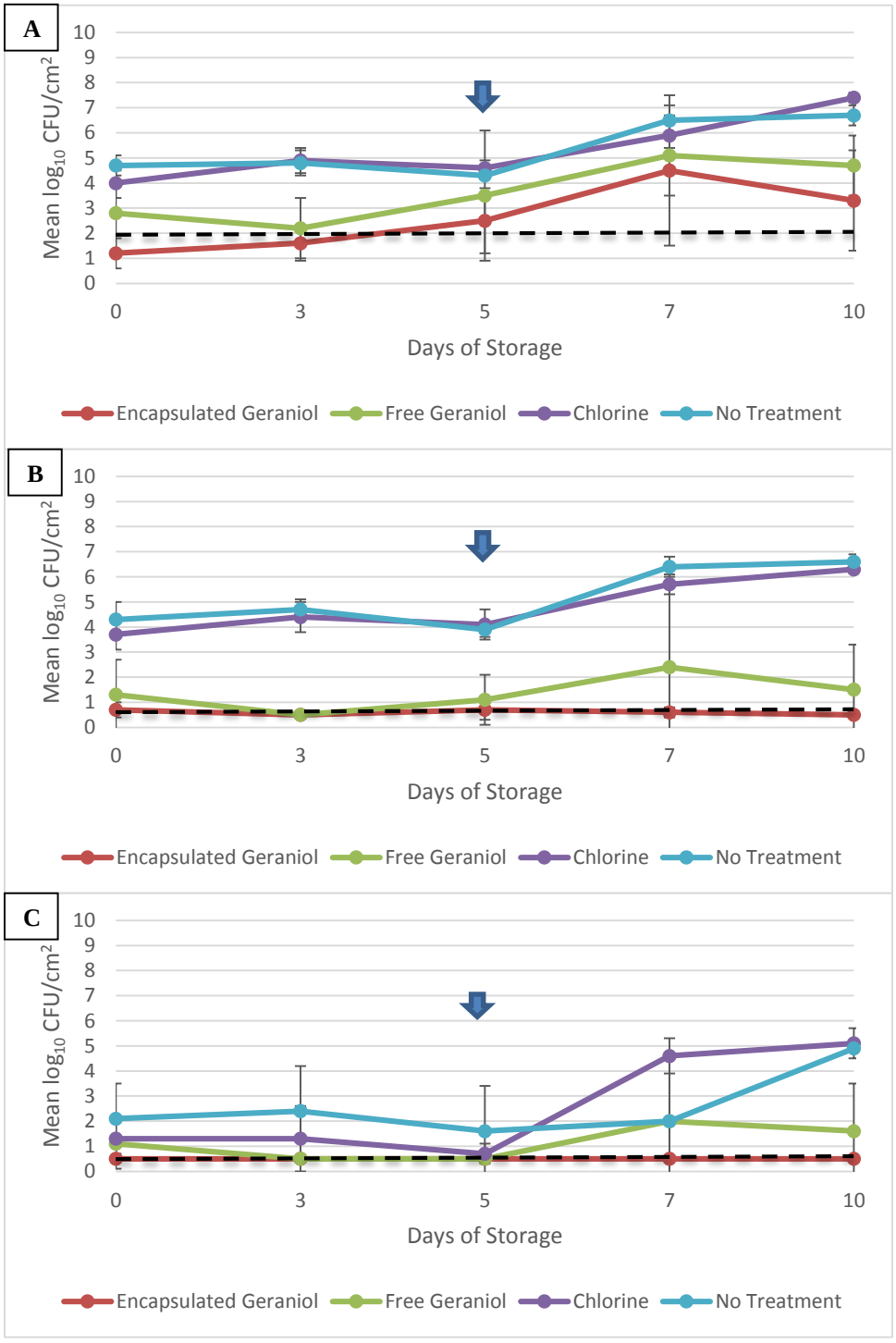

FIGURE 57. ***Means of A) aerobic bacteria, B) lactic acid bacteria, and C) coliforms on the surface of spinach stored at 25°C.*** The arrow indicates when samples transitioned from 5°C to 25°C. Error bars indicate standard deviation from sample means (n=3). Dashed line indicates minimum detection limit (0.5 $\log_{10}$ CFU/cm^2).

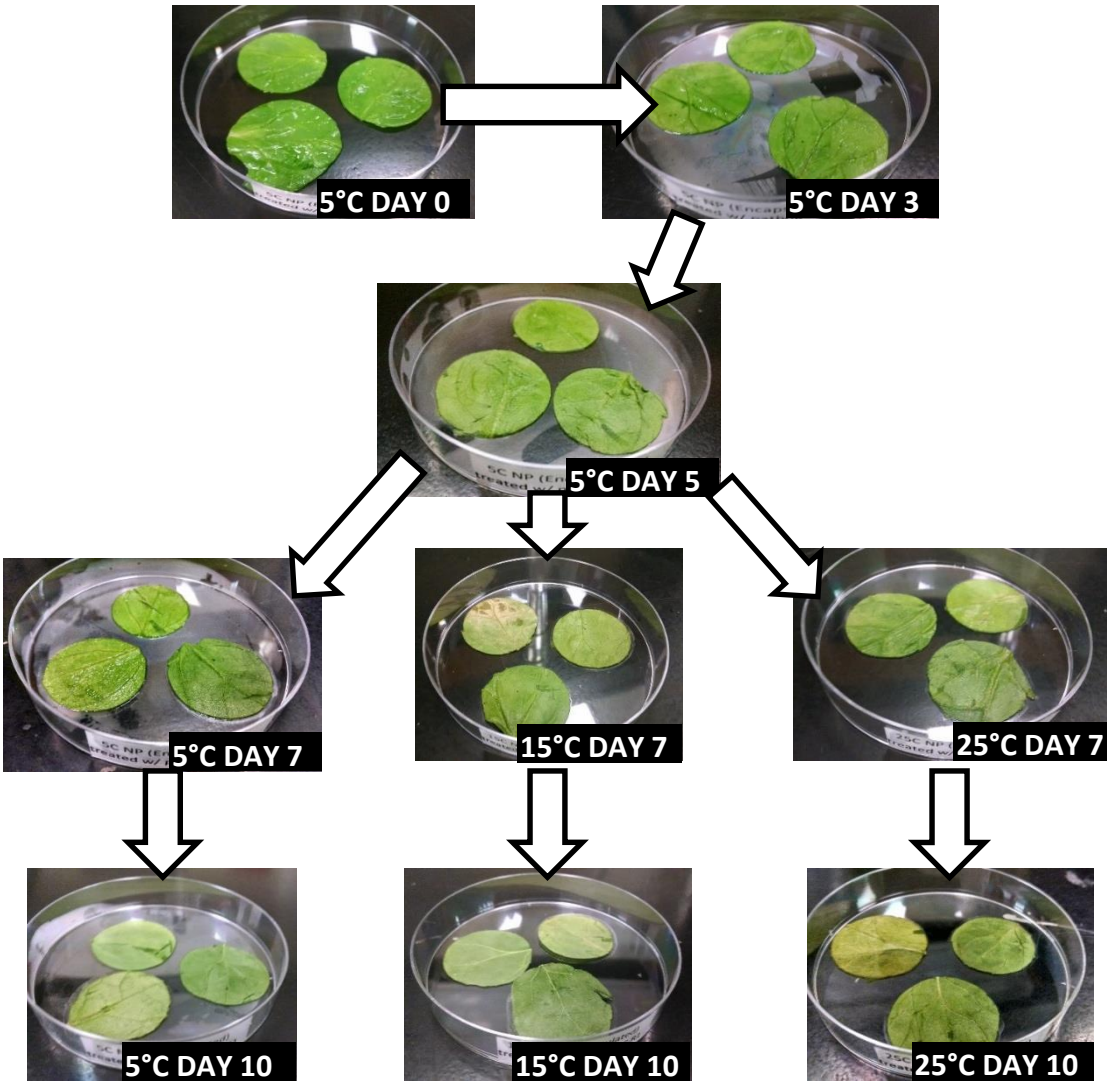

FIGURE 58. ***Nanoparticle-treated pathogen-inoculated spinach stored at various temperatures for 0, 3, 5, 7, 10 days.***

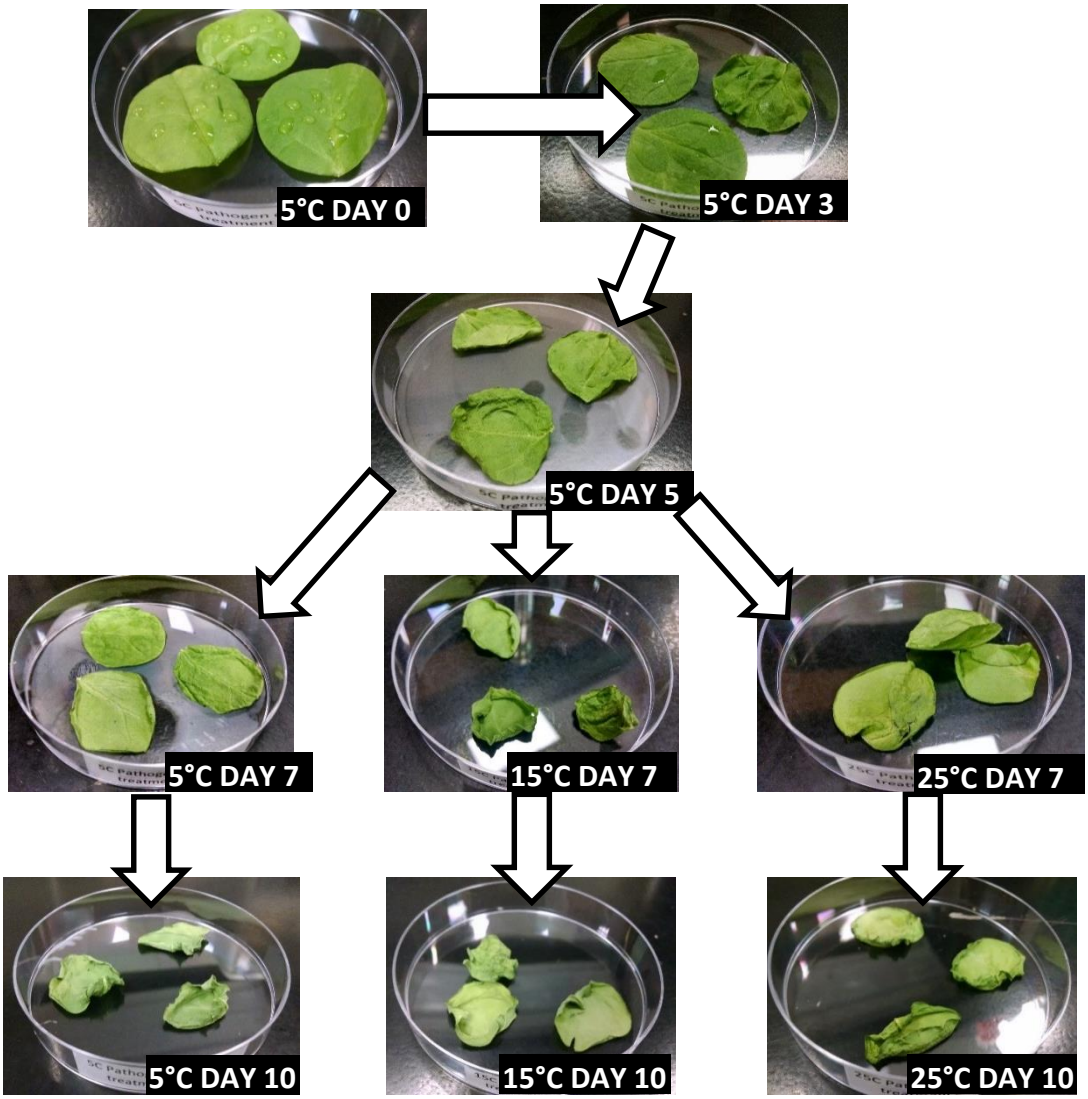

FIGURE 59. ***Untreated pathogen-inoculated spinach stored at various temperatures for 0, 3, 5, 7, 10 days.***

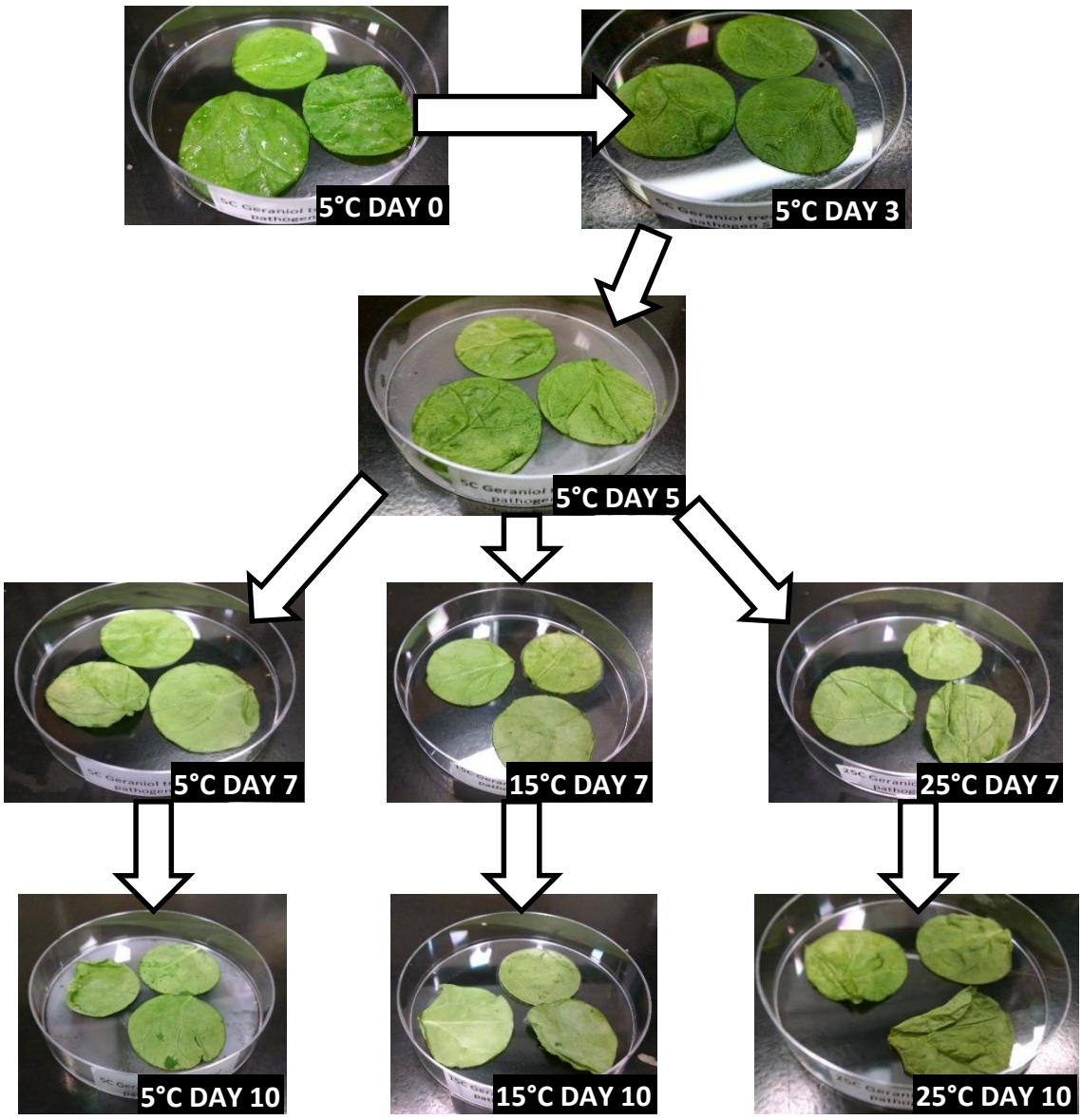

FIGURE 60. ***Unencapsulated geraniol-treated pathogen-inoculated cantaloupe stored at various temperatures for 0, 3, 5, 7, 10 days.***

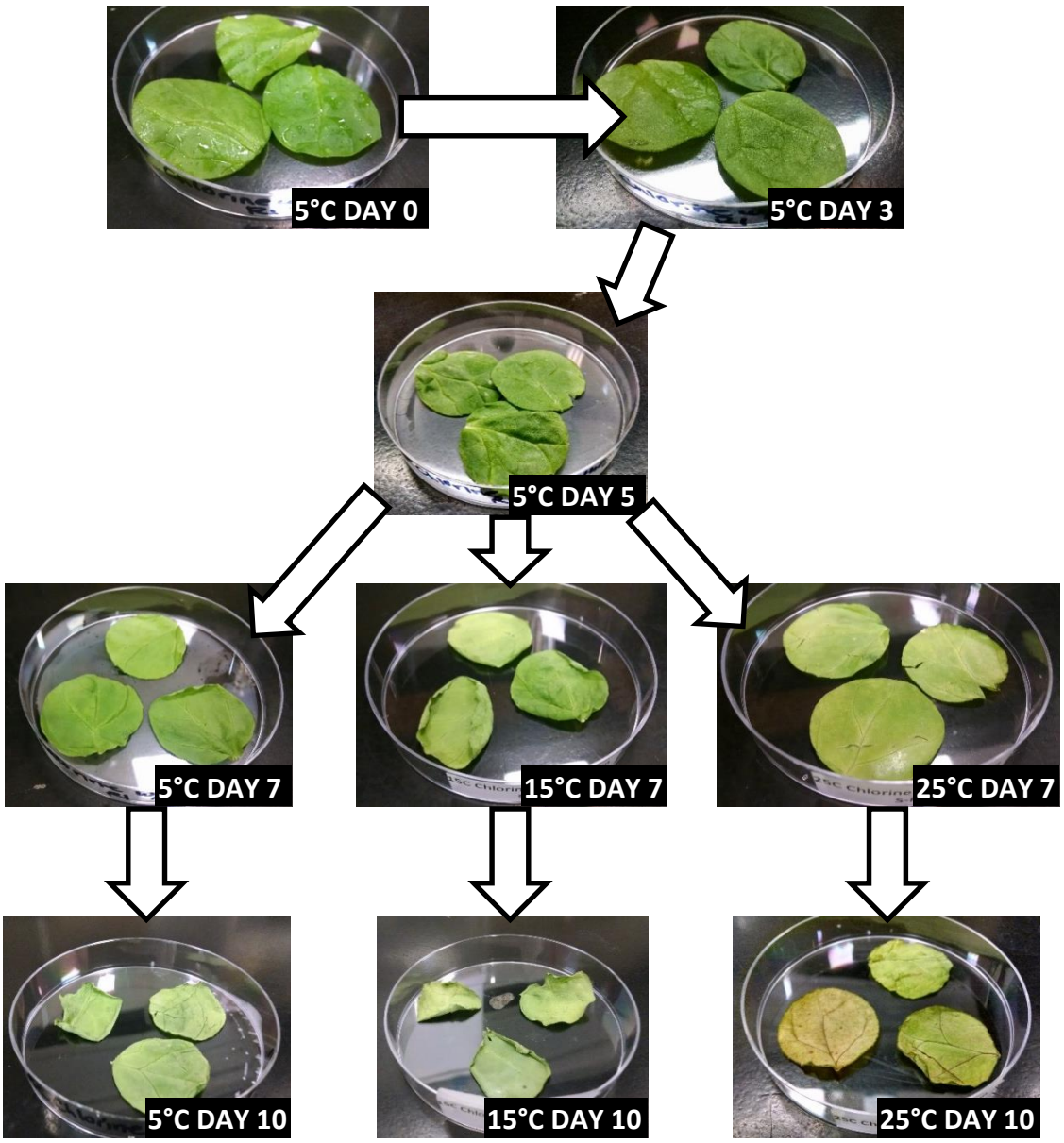

FIGURE 61. ***Chlorine-treated pathogen-inoculated spinach stored at various temperatures for 0, 3, 5, 7, 10 days.***

Analysis of the pathogen concentrations on spinach surface as affected by the treatments indicated significantly lower concentrations of *E. coli* O157:H7 and *S.* Typhimurium LT2 on spinach surface throughout the 5°C storage period when treated with the nanoencapsulated geraniol and free (unencapsulated) geraniol, ($p < 0.05$). However, there was no significant difference between unencapsulated and encapsulated geraniol on samples that were not recontaminated. Pathogen concentrations were significantly lower throughout the 5°C storage period when treated with nanoencapsulated geraniol for samples receiving additional contamination at day 3 and for samples that were post treatment contaminated, ($p < 0.05$). Overall, at 15°C and 25°C nanoencapsulated geraniol and free (unencapsulated) geraniol treated spinach did not differ significantly, ($p \geq 0.05$), in the populations of pathogens on the surface. However, both geraniol treatments were significantly lower than untreated samples, ($p < 0.05$).

Discussion

The antimicrobial essential oil components in NPs inhibited pathogen growth at lower geraniol concentrations as compared to unencapsulated geraniol. Similar decreases in MICs of plant-derived antimicrobials following nano-encapsulation were reported for cinnamon bark extract-containing poly-D,L lactide co-glycolide (PLGA) nanoparticles tested against *S.* Typhimurium and *L. monocytogenes*, as well as for cinnamon bark extract, clove bud extract, *trans*-cinnamaldehyde, and eugenol entrapped within β-cyclodextrin inclusion complexes *(71, 72)*. Our findings are also in accord with others reporting greater susceptibility of *E. coli* O157:H7 to free and nano-encapsulated plant phenolic acids as compared to *Salmonella (68)*. Other authors have previously theorized that nano-entrapment within polymeric nanocapsules of plant-derived antimicrobials (including geraniol) enhances their interaction with pathogenic cells through allowing greater suspension of active compound(s) in aqueous medium as well as limiting interactions with medium components that would degrade antimicrobial activity (e.g., partitioning within fat phases in emulsified foods, oxidation, etc.) *(89, 102).*

Raybaudi-Massilia *et al. (113)* through transmission electron microscopy micrographs of *Salmonella* Enteritidis in the presence of essential oil, lemongrass, and without essential oil in apple juice was able to show that in the presence of the essential oil the cell membrane can be damaged resulting in leakage of cell contents. These results were attributed to the hydrophobicity characteristic of essential oils; this characteristic enables the essential oil to spread through the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable *(113)*. The

permeability, therefore, causes leakage of ions and other cell contents and brings about an extensive loss of cell contents or the exit of critical molecules and ions, leading to death *(113)*. Plant derived compounds also have the potential to control mold growth *(41, 101)*. Mohammadi *et al. (101)* showed that cinnamomum essential oil loaded chitosan nanoparticles extended the shelf life of cucumbers up to 21 days at 10 ± 1°C while uncoated fruit were unmarketable in less than 15 days at 10 ± 1°C. Mohammadi *et al. (101)* also showed that the encapsulated oils decreased both disease severity and incidence of *Phytophthora*-inoculated cucumbers during 7 d storage at 4°C followed by 2-3 day storage at 20°C.

Data collected prior to the commencement of the produce experiments demonstrated that a rapid nanoprecipitation method for encapsulating the plant-derived terpene geraniol in the polymer Pluronic® F-127 was demonstrated to produce a unimodal population of NPs, with variable particle hydrodynamic size that differed according to PF127:geraniol mixing ratio *(140)*. Geraniol release against dialysis water followed an exponential release kinetic, with 50 % of drug being released within the first 7.25 h of storage at 25°C *(140)*. The release profile analysis did indicate that reduced temperature storage of EO-loaded NPs during distribution could slow the rates of NP degradation and EO loss *(140)*. Furthermore, as seen with both cantaloupe and tomato samples (though variable) the storage of nanoencapsulated geraniol-treated pathogen-inoculated produce samples at 5°C enabled the continuous release of geraniol from the NPs throughout the 10 day storage period.

Initial pathogen reductions on treated produce surfaces (cantaloupe, tomatoes, and spinach) ranged from 1.2 to 6.0 $\log_{10}$ CFU/cm^2 and were below the detection limit for spinach. Pathogen numbers continued to decline over the 10 day storage period at 5°C. Overall, numbers did not decline when transferred to temperatures of abuse (15°C and 25°C). If viable organisms were present on the surface, numbers increased when samples were transferred to 25°C. Aerobic bacteria, LAB, and coliforms followed trends similar to those of pathogens except untreated samples showed an increase in aerobic bacteria and LAB at all temperatures throughout the 10 day storage period. Reductions in pathogen numbers observed in the current study are similar to findings from previous research exploring the capacity of plant-derived antimicrobials to decontaminate inoculated produce. Viazis *et al. (135)* reported that application of 0.5% *trans*-cinnamaldehyde dispersed in TSB produced a 3.3 $\log_{10}$-cycle reduction in *E. coli* O157:H7 numbers on baby spinach leaves following 10 min exposure at 23°C. Application of 10% emulsified clove oil, as well as 5% and 10% zataria oil extract, produced reductions of 2.5-3.5 $\log_{10}$-cycles in *E. coli* O157:H7 on surfaces of baby-leaf salad vegetables after 5 days of storage at 7°C *(11)*. Orue *et al*. *(105)* reported reductions in numbers of *Salmonella*, *Shigella sonnei*, and *E. coli* on spinach following 20 min exposure to various essential oil mixtures ranged from 2.0 to 3.0 $\log_{10}$ CFU/g. These researchers also reported that *E. coli* O157:H7 reductions were greater than those of *Salmonella*, similar to findings presented in the current study. Baskaran *et al.* reported that application of 0.15% and 0.35% *trans*-cinnamaldehyde, 0.15% and 0.30% carvacrol, and 0.5% and 1% β-resorcyclic acid as wash treatments (applied separately) for apples

were all effective in reducing *E. coli* O157:H7 compared to a plain water wash treatment and reduced pathogens by 4-5 $\log_{10}$ CFU/apple in 5 min *(13).* In summary, nano-encapsulation of geraniol enhanced antimicrobial activity against the enteric pathogens *S.* Typhimurium LT2 and *E. coli* O157:H7. Nano-encapsulation increased bioavailability and transportation of geraniol as seen through both the decreased MIC and the lower numbers of surviving pathogens on the surfaces of fruits and vegetables as compared to free (unencapsulated) geraniol and chlorine-treated. Though a great deal of research has been completed detailing the efficacy of plant-derived essential oil components to inhibit the growth of foodborne bacterial pathogens, studies detailing their utility on fresh produce after encapsulation in food-grade encapsulating materials are novel. Further studies are needed to understand the release kinetics on the surface produce commodities in order to determine the cause of the variability seen on the surfaces of cantaloupes and tomatoes.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The surfaces of produce can differ greatly in regards to tissue, structure, pH, and microorganisms native to the produce *(92).* Overall, the organisms isolated from the surface of the various produce commodities (cantaloupe, tomato, spinach, and endive) were diverse. Overall, 1,389 isolates were isolated from the surfaces of cantaloupes, tomatoes, spinach, and endive. Of these isolates, 47.3% were Gram-negative bacteria and 52.7% were Gram-positive bacteria. Of these isolates, 109 (7.8%) showed antagonism activity *in vitro* against *S.* Typhimurium LT2 and 91 (6.6%) exhibited antagonism activity *in vitro* against *E. coli* O157:H7. Overall, *in vitro* the *Staphylococcus* antagonistic isolates showed larger zones of inhibition against both pathogens than the other antagonistic isolates recovered from spinach, tomatoes, and cantaloupes. The tomato-recovered isolate, *Staphylococcus hominis* ssp. *hominis*, showed the largest zone of inhibition (14.2 ± 5.2 mm) followed by the spinach-recovered isolate, *Staphylococcus intermedius*, (13.9 ± 3.8 mm) and the cantaloupe-recovered isolate, *Staphylococcus xylosus* (12.6 ± 2.6 mm) against *S.* Typhimurium LT2. The spinach-recovered isolate, *Staphylococcus intermedius*, showed the largest zone of inhibition (7.2 ± 0.4 mm) followed by the tomato-recovered isolate, *Staphylococcus haemolyticus* (7.1 ± 0.7 mm), and the cantaloupe-recovered isolate, *Staphylococcus xylosus*, (6.0 ± 2.1 mm) against *E. coli* O157:H7. However, the endive-recovered isolates *Lactococcus garvieae* produced larger zones of inhibition against both pathogens than the *Staphylococcus* isolates recovered from cantaloupes, tomatoes and spinach.

Lactococcus garvieae produced a zone of inhibition of 17.8 ± 4.7 mm against *S.* Typhimurium LT2 and 11.6 ± 1.7 mm against *E. coli* O157:H7. On produce surfaces the endive-recovered isolate *Escherichia coli* 1472 and the cantaloupe-recovered isolated *Escherichia hermannii* depressed the growth of both pathogens and the cantaloupe-recovered isolate *Enterococcus casseliflavus* depressed the growth of *S.* Typhimurium LT2 on the surfaces of cantaloupes.

In summary, this research also demonstrated the potential for using geraniol as an antimicrobial for decontamination of produce. Geraniol loaded NPs inhibited *S.* Typhimurium and *E. coli* O157:H7 growth at 0.4 and 0.2 wt.%, respectively. Initial pathogen reductions on treated produce surfaces (cantaloupe, tomatoes, and spinach) ranged from 1.2 to 6.0 $\log_{10}$ CFU/cm^2 and were even below the detection limit for spinach. Pathogen numbers continued to decline over the 10 day storage period at 5°C. Overall, numbers did not decline when transferred to temperatures of abuse (15°C and 25°C). If viable organisms were present on the surface, numbers increased when samples were transferred to 25°C. Aerobic bacteria, LAB, and total coliforms followed similar trends to pathogens except untreated samples showed an increase in aerobic bacteria and lactic acid at all temperatures throughout the 10 storage period. In summary, antimicrobial NPs and microorganisms naturally present on produce surfaces may be useful for the post-harvest decontamination of foods, such as fresh produce, from cross-contaminating microbial pathogens.

REFERENCES

1. Abanyie, F., J. R. Harris, R. E. Wiegand, R. L. Hall, B. Herwaldt, E. B. Gray, Y. Qvarnstrom, P. T. Cantey, A. J. Da Silva, H. Bishop, N. Wilson, A. E. Fiore, L. Slutsker, M. Parise, R. R. Harvey, I. Williams, M. E. Wise, S. Bosch, R. Tauxe, S. Lance, L. Gaul, V. Cantu, M. Desvignes-Kendrick, K. Irvin, A. Fields, A. Wellman, and J. Beal. 2015. 2013 multistate outbreaks of *Cyclospora cayetanensis* infections associated with fresh produce: focus on the Texas investigations. *Epidemiol. Infect.*:8p.

2. Abriouel, H., C. M. A. P. Franz, N. B. Omar, and A. Gálvez. 2011. Diversity and applications of *Bacillus* bacteriocins. *FEMS Microbiol. Rev.* 35:201-232.

3. Agnihotri, S. A., N. N. Mallikarjuna, and T. M. Aminabhavi. 2004. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J. Control. Release* 100:5-28.

4. Ahmed, K., Y. Li, D. McClements, and H. Xiao. 2012. Nanoemulsion and emulsion based delivery systems for curcumin: encapsulation and release properties *Food Chem.* 132:799-807.

5. Ailes, E. C., J. S. Leon, L.-A. Jaykus, L. M. Johnston, H. A. Clayton, S. Blanding, D. G. Kleinbaum, L. C. Backer, and C. L. Moe. 2008. Microbial concentrations on fresh produce are affected by postharvest processing, importation, and season. *J. Food Prot.* 71:2389-2397.

6. Akbulut, M., P. Ginart, M. E. Gindy, C. Theriault, K. H. Chin, W. Soboyejo, and R. K. Prud'homme. 2009. Generic method of preparing multifunctional fluorescent nanoparticles using flash nanopercipitation. *Adv. Funct. Mater.* 19:718-725.

7. Alegre, I., I. Viñas, J. Usall, N. Teixidó, M. J. Figge, and M. Abadias. 2013. Control of foodborne pathogens on fresh-cut fruit by a novel strain of *Pseudomonas graminis*. *Food Microbiol.* 34:390-399.

8. Amézquita, A., and M. M. Brashears. 2002. Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by Lactic Acid Bacteria. *J. Food Prot.* 65:316-325.

9. Angelo, K. M., A. Chu, M. Anand, T.-A. Nguyen, L. Bottichio, M. Wise, I. Williams, S. Seelman, R. Bell, M. Fatica, S. Lance, D. Baldwin, K. Shannon, H. Lee, E. Trees, E. Strain, and L. Gieraltowski. 2015. Outbreak of *Salmonella* Newport infections linked to cucumbers - United States, 2014. *MMWR Morb. Mortal. Wkly. Rep.* 64:144-147.

10. Aureli, P., A. Costantini, and S. Zolea. 1992. Antimicrobial activity of some plant essential oils against *Listeria monocytogenes. J. Food Prot.* 55:344-348.

11. Azizkhani, M., P. Elizaquível, G. Sánchez, M. V. Selma, and R. Aznar. 2013. Comparative efficacy of *Zataria multiflora* Boiss., *Origanum compactum* and *Eugenia caryophyllus* essential oils against *E. coli* O157:H7, feline calicivirus and endogenous microbiota in commercial baby-leaf salads. *International Journal of Food Microbiology* 166:249-255.

12. Babic, I., A. E. Watada, and J. G. Buta. 1997. Growth of *Listeria monocytogenes* restricted by native microorganisms and other properties of fresh-cut spinach. *J. Food Prot.* 60:912-917.

13. Baskaran, S. A., A. Upadhyay, A. Kollanoor-Johny, I. Upadhyaya, S. Mooyottu, M. A. Roshni Amalaradjou, D. Schreiber, and K. Venkitanarayanan. 2013. Efficacy of plant-derived antimicrobials as antimicrobial wash treatments for reducing enterohemorrhagic *Escherichia coli* O157:H7 on apples. *J. Food Sci.* 78:M1399-M1404.

14. Bell, C., and A. Kyriakides. 2002. *Salmonella* a practical approach to the organism and its control in foods. Blackwell Science, Ames, Iowa.

15. Beuchat, L. R. 1994. Antimicrobial properties of spices and their essential oils. p. 167-179. *In* V.M. Dillon, and R.G. Board (ed.), Natural antimicrobial systems and food preservation CAB Intl., Wallingford.

16. Beuchat, L. R. 1995. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59:204-216.

17. Brackett, R. E. 1987. Microbiological consequences of minimally processed fruits and vegetables. *J. Food Qual.* 10:195-206.

18. Brackett, R. E. 1988. Changes in the microflora of packaged fresh tomatoes. *J. Food Qual.* 11:89-105.

19. Brackett, R. E. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharvest Biol. Tech.* 15:305-311.

20. Brasil, I., C. Gomes, A. Puerta-Gomez, M. Castell-Perez, and R. Moreira. 2012. Polysaccharide-based multilayered antimicrobial edible coating enhances quality of fresh-cut papaya. *LWT-Food Sci. Technol.* 47:39-45.

21. Breidt, F., and H. Fleming. 1997. Using lactic acid bacteria to improve the safety of minimally processed fruits and vegetables. *Food Tech.* 51:44-51.

22. Brito, M. A. V. P., G. A. Somkuti, and J. A. Renye Jr. 2011. Isolation of bacteriocin-producing *Staphylococci* from Brazilian cheese. *J. Food Safety* 31:365-370.

23. Brito, M. A. V. P., G. A. Somkuti, and J. A. Renye Jr. 2011. Production of antilisterial bacteriocins by *Staphylococci* isolated from bovine milk. *J. Dairy Sci.* 94:1194-1200.

24. Brown, C. A., B. Wang, and J.-H. Oh. 2008. Antimicrobial activity of lactoferrin against foodborne pathogenic bacteria incorporated into edible chitosan film. *J. Food Prot.* 71:319-324.

25. Cabrera-Diaz, E. 2007. Characterization and evaluations of *Escherichia coli* Biotype I strains for use as surrogates for enteric pathogens in validation of beef carcass interventions. [Doctoral dissertation]. Doctoral dissertation, Texas A&M University.

26. Cagri, A., Z. Ustunol, and E. T. Ryser. 2004. Antimicrobial edible films and coatings. *J. Food Prot.* 67:833-848.

27. Carlin, F., C. N. the, and C. E. Morris. 1996. Influence of background microflora on *Listeria monocytogenes* on minimally processed fresh broad leaved endive (*Cichorium endivia* var. *latifolia*). *J. Food Prot.* 59:698-703.

28. Cascales, E., S. K. Buchanan, D. Duché, C. Kleanthous, R. Lloubès, K. Postle, M. Riley, S. Slatin, and D. Cavard. 2007. Colicin biology. *Microbiol. Molecul. Biol. Rev.* 71:158-229.

29. Castillo, A., L. M. Lucia, K. J. Goodson, J. W. Savell, and G. R. Acuff. 1998. Use of hot water for beef carcass decontamination. *J. Food Prot.* 61:19-25.

30. Catherine, A. A., H. Deepika, and P. S. Negi. 2012. Antibacterial activity of eugenol and peppermint oil in model food systems. *J. Essent. Oil Res.* 24:481-486.

31. CDC. 2008. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items- United States, 2008. *MMWR Morb. Mortal. Wkly. Rep.* 57:929-934.

32. CDC. 2011. Surveillance for foodborne disease outbreaks- United States, 2008. *MMWR Morb. Mortal. Wkly. Rep.* 60:1197-1202.

33. CDC. 2012. Multistate Outbreak of *Salmonella* Braenderup Infections Associated wtih Mangoes (Final Update) Available at:

http://www.cdc.gov/salmonella/braenderup-08-12/index.html. Accessed August 16 2015.

34. CDC. 2012. Multistate outbreak of *Salmonella* Typhimurium and *Salmonella* Newport infections linked to cantaloupe (Final Update). Available at: http://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/index.html. Accessed August 16 2015.

35. CDC. 2013. Reports of selected *E. coli* outbreak investigations. Available at: www.cdc.gov/ecoli/outbreaks.html. Accessed 11 August 2013.

36. CDC. 2013. Reports of selected *Salmonella* outbreak investigations. Available at: http://www.cdc.gov/salmonella/outbreaks.html. Accessed 11 August 2013.

37. Cosgrove, S., A. Cronquist, G. Wright, T. Ghosh, R. Vogt, P. Teitell, A. Gelfius, C. Spires, T. Duvernoy, S. merriweather, M. Freeman, P. Griffin, K. Jackson, L. Joseph, B. Mahon, K. Neil, B. Silk, C. Tarr, R. Tauxe, E. Trees, M. Ibraheem, M. Imanishi, N. Jain, J. mcCollum, and K. O'Connor. 2011. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe — United States, August–September 2011. p. 1357. *In* Centers for Disease Control and Prevention.

38. Côté, C., and S. Quessy. 2005. Persistence of *Escherichia coli* and *Salmonella* in surface soil following application of liquid hog manure for production of pickling cucumbers. *J. Food Prot.* 68:900-905.

39. Cox, J. 1999. *Salmonella.* p. 1928-1937. *In* R.K. Robinson, C.A. Batt, and P. Patel (ed.), Encyclopedia of Food Microbiology Academic Press, San Diego.

40. D'Aoust, J.-Y. 2000. *Salmonella.* p. 1233-1299. *In* B.M. Lund, T.C. Baird-Parker, and G.W. Gould (ed.), Microbiological Safety and Quality of Food, vol. 1-2. Springer-Verlag, New York.

41. da Cruz Cabral, L., V. Fernández Pinto, and A. Patriarca. 2013. Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. *Int. J Food Microbiol.* 166:1-14.

42. Darnton, N. C., L. Turner, S. Rojevsky, and H. c. Berg. 2007. On torque and tumbling in swimming *Escherichia coli. J. Bacteriol.* 189:1756-1764.

43. Davidson, P. M., and A. S. Naidu. 2000. Phyto-phenols. p. 266-294. *In* A.S. Naidu (ed.), Natural food antimicrobial systems CRC Press, Boca Raton, FL.

44. Davidson, P. M., J. N. Sofos, and A. L. Branen (ed.). 2005. Antimicrobials in food, 3rd Edt. CRC Press, Boca Raton.

45. de Roever, C. 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Control* 9:321-347.

46. de Vuyst, L., and F. Leroy. 2007. Bacteriocins from lactic acid bacteria: production, purification, and food applications. *J. Mol. Microbiol. Biotechnol.* 13:194-199.

47. Devlieghere, F., A. Vermeulen, and J. M. Debevere. 2004. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiol.* 21:703-714.

48. Dickson, J., and M. Koohmaraie. 1989. Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. *Appl. Environ. Microbiol.* 55:832-836.

49. Downes, F. P., and K. Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, fourth edition. American Public Health Association, Washington, DC.

50. Ercolani, G. L. 1976. Bacteriological quality assessment of fresh marketed lettuce and fennel *Appl. Environ. Microbiol.* 31:847-852.

51. Escartín, E. F., R. T. Vitela, and A. C. Ayala. 1984. Antagonismo de cepas de *Streptococcus*, *Lactobacillus* y *Leuconostoc* procedentes de quesos frescos no pasteurizados contra algunas bacterias enteropatógenas. *Rev. Latinoam. Microbiol.* 26:17-51.

52. Fatica, M. K., and K. R. Schneider. 2011. Salmonella and produce: Survival in the plant environment and implications in food safety. *Virulence* 2:573-579.

53. FDA. 2013. Chapter IV. Outbreaks associated with fresh and fresh-cut produce. Incidence, growth, and survival of pathogens in fresh and fresh-cut produce. Available at: http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm091265.htm. Accessed April 2013.

54. FDA. 2013. Outbreak investigations. Available at: http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm272351.htm. Accessed 11 August 2013.

55. FDA. 2015. Chemicals used in washing or to assist in the peeling of fruits and vegetables. *In*, 21CFR173.315, Silver Spring, MD.

56. Fleming, H. P., J. L. Etchells, and R. N. Costilow. 1975. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. *Appl. Microbiol.* 30:1040-1042.

57. Foods, I. C. o. M. S. f., and Icmsf. 2000. Micro-organisms in Foods 6: Microbial Ecology of Food Commodities. Aspen Publishers, Incorporated.

58. Fowler, J. L., and J. F. Foster. 1976. Microbiological survey of three fresh green salads--can guidelines be recommended for these foods? *J. Milk Food Tech.* 39:111-113.

59. Friedman, M., R. Buick, and C. T. Elliott. 2004. Antibacterial activities of naturally occurring compounds against antibiotic-resistant *Bacillus cereus* vegetative cells and spores, *Escherichia coli*, and *Staphylococcus aureus. J. Food Prot.* 67:1774-1778.

60. Friedman, M., P. R. Henika, and R. E. Mandrell. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes,* and *Salmonella enterica. J. Food Prot.* 65:1545-1560.

61. Gallegos-Robles, M. A., A. Morales-Loredo, G. Alvarez-Ojeda, A. Vega-P, Y. Chew-M, S. Velarde, and P. Fratamico. 2008. Identification of *Salmonella* serotypes isolated from cantaloupe and chile pepper production systems in Mexico by PCR-restriction fragment length polymorphism. *J. Food Prot.* 71:2217-2222.

62. Gaysinsky, S., P. M. Davidson, B. D. Bruce, and J. Weiss. 2005. Growth inhibition of *Escherichia coli* O157:H7 and *Listeria monocytogenes* by carvacrol and eugenol encapsulated in surfactant micelles. *J. Food Prot.* 68:2559-2566.

63. Gaysinsky, S., P. M. Davidson, B. D. Bruce, and J. Weiss. 2005. Stability and antimicrobial efficiency of eugenol encapsulated in surfactant micelles as affected by temperature and pH. *J. Food Prot.* 68:1359-1366.

64. Gaysinsky, S., T. M. Taylor, P. M. Davidson, B. D. Bruce, and J. Weiss. 2007. Antimicrobial efficacy of eugenol microemulsions in milk against *Listeria monocytogenes* and *Escherichia coli* O157:H7. *J. Food Prot.* 70:2631-2637.

65. Gibbs, B. F., S. Kermasha, I. Alli, and C. N. Mulligan. 1999. Encapsulation in the food industry: a review. *Int. J. Food Sci. Nutr.* 50:213-224.

66. Gomes, C., R. G. Moreira, and E. Castell-Perez. 2011. Poly (DL-lactide-co-glycolide) (PLGA) nanoparticles with entrapped *trans*-cinnamaldehyde and eugenol for antimicrobial delivery applications *J. Food Sci.* 76:N16-N24.

67. Gould, L. H., K. A. Walsh, A. R. Vieira, K. Herman, I. T. Williams, A. J. Hall, and D. Cole. 2013. Surveillance for Foodborne Disease Outbreaks -- United States, 1998-2008. *MMWR Surveill. Summ.* 62:1-34.

68. Gutierrez, J., G. Rodriguez, C. Barry-Ryan, and P. Bourke. 2008. Efficacy of plant essential oils against foodborne pathogens and spoilage bacteria associated with ready-to-eat vegetables: antimicrobial and sensory screening. *J. Food Prot.* 71:1846-1854.

69. Han, Y., D. M. Sherman, R. H. Linton, S. S. Nielsen, and P. E. Nelson. 2000. The effects of washing and chlorine dioxide gas on survival and attachment of *Escherichia coli* O157:H7 to green pepper surfaces *Food Microbiol.* 17:521-533.

70. Harrigan, W. F. 1998. Laboratory methods in food microbiology, 3rd edt. Academic Press San Diego, CA.

71. Hill, L. E., C. Gomes, and T. M. Taylor. 2013. Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. *LWT-Food Sci. Technol.* 51:86-93.

72. Hill, L. E., T. M. Taylor, and C. Gomes. 2013. Antimicrobial efficacy of Poly (DL-lactide-co-glycolide) (PLGA) nanoparticles with entrapped cinnamon bark extract against *Listeria monocytogenes* and *Salmonella* Typhimurium. *J. Food Sci.* 78:626-632.

73. Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams (ed.). 1994. Bergey's manual of determinative bacteriology, 9th edt. Williams & Wilkins, Baltimore, Maryland.

74. Jack, R. W., J. R. Tagg, and B. Ray. 1995. Bacteriocins of Gram-positive bacteria. *Microbiol. Rev.* 59:171-200.

75. James, J. (ed.). 2006. Microbial hazard identification in fresh fruits and vegetables. John Wiley & Sons, Inc., San Francisco, Calif.

76. Jay, J. M., M. J. Loessner, and D. A. Golden. 2005. Modern food microbiology. Springer Science+Business Media, LLC.

77. Johnston, L. M., L.-A. Jaykus, D. Moll, M. C. Martinez, J. Anciso, B. Mora, and C. L. Moe. 2005. A field study of the microbiological quality of fresh produce. *J. Food Prot.* 68:1840-1847.

78. Johny, A. K., M. J. Darre, A. M. Donoghue, D. J. Donoghue, and K. Venkitanarayana. 2010. Antibacterial effect of *trans*-cinnamaldehyde, eugenol, carvacrol, and thymol on *Salmonella* Enteriditis and *Campylobacter jejuni* in chicken cecal contents *in vitro*. *J. Appl. Poultry Res.* 19:237-244.

79. Kader, A. A. (ed.). 2002. Postharvest technology of horticultural crops, 3rd edt. University of California, Oakland, Calif.

80. Kim, J., M. R. Marshall, and C.-i. Wei. 1995. Antibacterial activity of some essential oil components against five foodborne pathogens. *J Agri. Food Chem.* 43:2839-2845.

81. Klaenhammer, T. R. 1993. Genetics of bacteriocins produced by lactic acid bacteria. *Microbiol. Rev.* 12:39-86.

82. Lauková, A., and M. Mareková. 1993. Antimicrobial spectrum of bacteriocin-like substances produced by rumen *Staphylococci Folia Microbiol.* 38:74-76.

83. Lauková, A., M. Simonová, and V. Strompfová. 2010. *Staphylococcus xylosus* S03/1M/1/2, bacteriocin-producing meat starter culture or additive. *Food Control* 21:970-973.

84. Letchford, K., and H. Burt. 2007. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *Eur. J. Pharm. Biopharm.* 65:259-269.

85. Leverentz, B., W. S. Conway, W. Janisiewicz, C. P. Abadias, and M. J. Camp. 2006. Biocontrol of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* serovar Poona on fresh-cut apples with naturally occuring bacterial and yeast antagonists. *Appl. Environ. Microbiol.* 72:1135-1140.

86. Lewis, H., M. Kirk, S. Ethelberg, R. Stafford, K. Olsen, E. Nielsen, M. Lisby, S. Madsen, and K. Molbak. 2007. Outbreaks of shigellosis in Denmark and Australia associated with imported baby corn, August 2007- final summary. . *Euro Surveill.* 12:E071004.

87. Liao, C.-H., and G. M. Sapers. 2000. Attachment and growth of *Salmonella* Chester on apple fruits and *in vivo* response of attached bacteria to sanitizer treatments. . *J. Food Prot.* 63:876-883.

88. Lin, C.-M., and C.-I. Wei. 1996. Transfer of *Salmonella montevideo* onto the interior surfaces of tomatoes by cutting. *J. Food Prot.* 60:858-863.

89. Liolios, C. C., O. Gortzi, S. Lalas, J. Tsaknis, and I. Chinou. 2009. Liposomal incorporation of carvacrol and thymol isolated from the essential oil of *Origanum dictamnus* L. and *in vitro* antimicrobial activity. . *Food Chem.* 112:77-83.

90. Liu, Q., H. Niu, W. Zhang, H. Mu, C. Sun, and J. Duan. 2015. Synergy among thymol, eugenol, berberine, cinnamaldehyde and streptomycin against planktonic and biofilm-associated food-borne pathogens. *Let. Appl. Microbiol.* 60:421-430.

91. López-Malo Vigil, A., E. Palou, and S. M. Alzamora. 2005. Naturally occurring compounds-plant sources. p. 429-452. *In* P.M. Davidson, J.N. Sofos, and A.L. Branen (ed.), Antimicrobials in foods, 3rd edt. Taylor and Francis, New York.

92. Lund, B. M. 1992. Ecosystems in vegetable foods. *J. App. Bacteriol. Symp. Supple.* 73:115S-126S.

93. Lynch, M. F., R. V. Tauxe, and C. W. Hedberg. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiol. Infect.* 137:307-315.

94. Mah, J.-H., and H.-J. Hwang. 2009. Inhibition of biogenic amine formation in a salted and fermented anchovy by *Staphylococcus xylosus* as a protective culture. *Food Control* 20:796-801.

95. Maki, D. G. 2006. Don't eat the spinach- controlling foodborne infectious disease. *New Engl. J. Med.* 355:1952-1955.

96. Mandrell, R. E., L. Gorski, and M. T. Brandl. 2006. Microbiology of fruits and vegetables. CRC Press Inc, Albany, CA.

97. Matan, N. 2012. Antimicrobial activity of edible film incorporated with essential oils to preserve dried fish (*Decapterus maruadsi*) *Int. Food Research J.* 19:1733-1738.

98. Materon, L. A. 2003. Survival of *Escherichia coli* O157:H7 applied to cantaloupes and the effectiveness of chlorinated water and lactic acid as disinfectants. *World J. Microbiol. Biotechnol.* 19:867-873.

99. Matthews, K. R. (ed.). 2006. Microbiology of fresh produce. ASM Press, Washington D.C. .

100. McGlynn, W. 2004. FAPC-116 Guidelines for the use of chlorine bleach as a sanitizer in food processing operations. *In* FAPC (ed.), Oklahoma Cooperative Extension Service.

101. Mohammadi, A., M. Hashemi, and S. M. Hosseini. 2015. Chitosan nanoparticles loaded with Cinnamomum zeylanicum essential oil enhance the shelf life of cucumber during cold storage. *Postharv. Biol. Tech.* 110:203-213.

102. Mourtzinos, I., N. Kalogeropoulos, S. E. Papadakis, K. Konstantinou, and V. T. Karathanos. 2008. Encapsulation of nutraceutical monoterpenes in β-cyclodextrin and modified starch. *J. Food Sci.* 73:S89-S94. .

103. Mytle, N., G. L. Anderson, M. P. Doyle, and M. A. Smith. 2006. Antimicrobial activity of clove (*Syzgium aromaticum*) oil in inhibiting *Listeria monocytogenes* on chicken frankfurters. *Food Control* 17:102-107.

104. Olivas, G. I., and G. V. Barbosa-Cánovas. 2005. Edible coatings for fresh-cut fruits. *Crit. Rev. Food Sci.* 45:657-670.

105. Orue, N., S. García, P. Feng, and N. Heredia. 2013. Decontamination of *Salmonella*, *Shigella*, and *Escherichia coli* O157:H7 from leafy green vegetables using edible plant extracts. *Journal of Food Science* 78:M290-M296.

106. Painter, J. A., R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo, and P. M. Griffin. 2013. Attribution of Foodborne Illnesses, Hospitalizations, and Deaths to Food Commodities by using Outbreak Data, United States, 1998-2008. *Emerg. Infect. Diseases* 19:407-415.

107. Patel, J., and M. Sharma. 2010. Differences in attachment of *Salmonella enterica* serovars to cabbage and lettuce leaves. *Int. J. Food Microbiol.* 139:41-47.

108. Pérez-Conesa, D., L. McLandsborough, and J. Weiss. 2006. Inhibition and inactivation of *Listeria monocytogenes* and *Escherichia coli* O157:H7 colony biofilms by micellar-encapsulated eugenol and carvacrol. *J. Food Prot.* 69:2947-2954.

109. Perez, K. L., L. M. Lucia, L. Cisneros-Zevallos, A. Castillo, and T. M. Taylor. 2012. Efficacy of antimicrobials for the disinfection of pathogen contaminated green bell pepper and of consumer cleaning methods for the decontamination of knives. *Int. J. Food Microbiol.* 156:76-82.

110. Pincus, D. H. 2013. Microbial identification using the Biomérieux Vitek 2 System. *In* M.J. Miller (ed.), Encyclopedia of Rapid Microbiological Methods PDA-DHI, Baltimore, MD.

111. Pranato, Y., S. K. Rakshit, and V. M. Salokhe. 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *Food Sci. Tech.* 38:859-865.

112. Raybaudi-Massilia, R. M., J. Mosqueda-Megar, and O. Martín-Belloso. 2008. Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. *Int. J. Food Microbiol.* 121:313-327.

113. Raybaudi-Massilia, R. M., J. Mosqueda-Melgar, and O. Martín-Belloso. 2006. Antimicrobial activity of essential oils on *Salmonella* Enteritidis, *Escherichia coli*, and *Listeria innocua* in fruit juices. *J. Food Prot.* 69:15779-1586.

114. Reina, L. D., F. Breidt, H. P. Fleming, and S. Kathariou. 2005. Isolation and selection of lactic acid bacteria as biocontrol agents for nonacidified, refrigerated pickles. *J. Food Sci.* 70:M7-M11.

115. Riley, M. A., and O. Gillor (ed.). 2007. Research and applications in bacteriocins. Horizon Bioscience.

116. Rimhanen-Finne, R., T. Niskanen, S. Hallanvuo, P. Makary, K. Haukka, S. Pajunen, A. siitonen, R. Ristolainen, H. Pöyry, J. Ollgren, and M. Kuusi. 2008. *Yersinia pseudotuberculosis* causing a large outbreak associated with carrots in Finland, 2006. *Epidemiol. Infect.* 137:342-347.

117. Rodgers, S. L., J. N. Cash, M. Siddiq, and E. T. Ryser. 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe *J. Food Prot.* 67:721-731.

118. Ross, T., and T. A. McMeekin. 1994. Predictive microbiology. *Int. J. Food Microbiol.* 23:241-264.

119. Ryser, E. T., S. Maisnier-Patin, J. J. Gratadoux, and J. Richard. 1994. Isolation and identification of cheese-smear bacteria inhibitory to *Listeria* spp. *Int. J. Food Microbiol.* 21:237-246.

120. Sapers, G. M., J. R. Gorny, and A. E. Yousef (ed.). 2006. Microbiology of fruits and vegetables. Taylor & Francis Group, LLC, Boca Raton, FL.

121. Sapers, G. M., E. B. Solomon, and K. R. Matthews (ed.). 2009. Produce contamination problem - causes and solutions. Elsevier.

122. Scallan, E., R. Hoekstra, F. Angulo, R. Tauxe, M.-A. Widdowson, S. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States-major pathogens. *Emerg. Infect. Dis.* 17:7-15.

123. Schuenzel, K. M., and M. A. Harrison. 2002. Microbial antagonists of foodborne pathogens on fresh, minimally processed vegetables. *J. Food Prot.* 65:1909-1915.

124. Shi, X., Z. Wu, A. Namvar, M. Kostrzynska, K. Dunfield, and K. Warriner. 2009. Microbial population profiles of the microflora associated with pre- and postharvest tomatoes contaminated with Salmonella typhimurium or Salmonella montevideo. *J Appl. Microbiol.* 107:329-338.

125. Simonová, M., V. Strompfová, M. Marciňáková, A. Lauková, S. Vesterlund, M. L. Moratalla, S. Bover-Cid, and C. Vidal-Carou. 2006. Characterization of

Staphylococcus xylosus and *Staphylococcus carnosus* isolated from Slovak meat products. *Meat Sci.* 73:559-564.

126. Sipahi, R., M. Castell-Perez, R. Moreira, C. Gomes, and A. Castillo. 2012. Improved multilayered antimicrobial alginate-based edible coating extends the shelf life of fresh-cut watermelon (*Citrullus lanatus*). *LWT-Food Sci. Technol.* 51:9-15.

127. Slayton, R. B., G. Turabelidze, S. D. Bennett, C. A. Schwensohn, A. Q. Yaffee, F. Khan, C. Butler, E. Trees, T. L. Ayers, M. L. Davis, A. S. Laufer, S. Gladbach, I. Williams, and L. B. Gieraltowski. 2013. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 associated with romaine lettuce consumption, 2011. *Plos One* 8:e55300-e55300.

128. Sudarshan, N. R., D. G. Hoover, and D. Knorr. 1992. Antibacterial action of chitosan. *Food Biotechnol.* 6:257-272.

129. Taylor, T. M., P. M. Davidson, B. D. Bruce, and J. Weiss. 2005. Liposomal nanocapsules in food science and agriculture *Crit. Rev. Food Sci.* 45:587-605.

130. Tilden, J., W. Young, A.-M. McNamara, C. Custer, B. Boesel, M. A. Lambert-Fair, J. Majkowski, D. Vugia, S. B. Werner, J. Holingsworth, and J. G. Morris. 1996. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *Am. J. Public Health* 86:1142-1145.

131. Ukuku, D. O., W. F. Fett, and G. M. Sapers. 2004. Inhibition of *Listeria monocytogenes* by native microflora of whole cantaloupe. *J. Food Safety* 24:129-146.

132. Vanderzant, C., and R. Nickelson. 1969. A microbiological examination of muscle tissue of beef, pork, and lamb carcasses. *J. Milk Food Tech.* 32:357-361.

133. Vargas, M., C. Pastor, A. Chiralt, D. J. McClements, and C. González-Martínez. 2008. Recent advances in edible coatings for fresh and minimally processed fruits. *Crit. Rev. Food Sci.* 48:496-511.

134. Vescovo, M., C. Orsi, G. Scolari, and S. Torriani. 1995. Inhibitory effect of selected lactic acid bacteria on microflora associated with ready-to-use vegetables. *Let. Appl. Microbiol.* 21:121-125.

135. Viazis, S., M. Akhtar, J. Feirtag, and F. Diez-Gonzalez. 2011. Reduction of *Escherichia coli* O157:H7 viability on leafy green vegetables by treatment with a bacteriophage mixture and *trans*-cinnamaldehyde. *Food Microbiology* 28:149-157.

136. Villani, F., O. Pepe, G. Mauriello, G. Salzano, G. Moschetti, and S. Coppola. 1994. Antimicrobial activity of *Staphylococcus xylosus* from Italian sausages against *Listeria monocytogenes. Let. Appl. Microbiol.* 18:159-161.

137. Villani, F., L. Sannino, G. Moschetti, G. Mauriello, O. Pepe, R. Amodio-Cocchieri, and S. Coppola. 1997. Partial characterization of an antagonistic substance produced by *Staphylococcus xylosus* 1E and determination of the effectiveness of the producer strain to inhibit *Listeria monocytogenes* in Italian sausages. *Food Microbiol.* 14:555-566.

138. Wang, H., H. Feng, W. Liang, Y. Luo, and V. Malyarchuk. 2009. Effect of surface roughness on retention and removal of *Escherichia coli* O157:H7 on surfaces of selected fruits. *J. Food Sci.* 74:E8-E15.

139. Weissinger, W. R., W.Chantarapanont, and L. R. Beuchat. 2000. Survival and growth of *Salmonella baildon* in shredded lettuce and diced tomatoes, and effectiveness of chlorinated water as a sanitizer *Int. J. Food Microbiol.* 62:123-131.

140. Yegin, Y., K. L. Perez-Lewis, M. Zhang, M. Akbulut, and T. M. Taylor. 2016. Development and characterization of geraniol-loaded polymeric nanoparticles with antimicrobial activity against foodborne bacterial pathogens. *J. Food Eng.* 170:64-71.

141. Yun, J., X. Fan, and X. Li. 2013. Inactivation of *Salmonella enterica* serovar Typhimurium and quality maintenance of cherry tomatoes treated with gaseous essential oils. *J. Food Sci.* 78:M458-M464.

142. Zhang, M., and M. Akbulut. 2011. Adsorption, desorption, and removal of polymeric nanomedicine on and from cellulose surfaces: efect of size. *Langmuir* 27:12550-12559.

143. Zhuang, R. Y., L. R. Beuchat, and F. J. Angulo. 1995. Fate of *Salmonella montevideo* on and in raw tomatoes as affected by temperature and treatment with chlorine *Appl. Environ. Microbiol.* 61:2127-2131.