INHIBITION OF *ESCHERICHIA COLI* O157:H7 BY BACKGROUND MICROFLORA OF FRESH-CUT LETTUCE AND BABY SPINACH

by

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(Under the Direction of Mark A. Harrison)

ABSTRACT

Escherichia coli O157:H7 contamination of fresh-cut lettuce and spinach can occur from pre-harvest to product packaging. Natural microflora present on fresh produce may help reduce the pathogen load more than realized. The objectives of this study were to determine if naturally occurring microflora present on iceberg lettuce and spinach under conditions that mimic actual practices between production to retail sale are inhibitory toward *E. coli* O157:H7, and if so, to investigate possible means for the inhibition. Evidence of naturally-occurring microorganisms on fresh lettuce (295 isolates) and spinach (200 isolates) with possible antagonistic activity toward *E. coli* O157:H7 was documented. Possible inhibitory activity by several isolates was due either to acid production or bacteriocins.

INDEX WORDS: Lettuce, spinach, fresh-cut, inhibition, *Escherichia coli O157:H7*, antagonistic

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CHAPTER 1

INTRODUCTION

Foodborne illnesses associated with fresh-cut produce are a widespread and national problem. The U.S. may have a safe food supply but even in industrialized countries the percentage of people that contract foodborne diseases is near 30 percent a year (*3*). In the U.S., 325,000 hospitalizations due to foodborne illness occur each year (*1*). According to the Produce Marketing Association, the retail and foodservice sales of fresh-cut and whole produce were estimated to be $12 billion in 2006 (*2*). American consumers striving to increase their consumption of fresh produce, it has driven the focus and concern on the fresh-cut industry. As Americans demand more variety, they also expect safety, quality, and cheaper prices. Consumers are slowly veering away from frozen and canned foods and towards pre-cut and pre-packaged produce because of time savings, storage space limits, and less waste removal.

Ready-to-eat produce is very susceptible to contamination from many sources. Fresh, minimally processed produce has no true intervention steps for pathogen control. Because most produce is not cooked before being eaten, the produce undergoes no type of guaranteed pathogen reducing process. As a result the industry relies on Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) to help eliminate and reduce contamination. Also, there are voluntary Hazard Analysis Critical Control Points (HACCP) programs that can be employed to further reduce growth and contamination by pathogens. To help gain an understanding of the interactions a pathogen has with the native microflora on fresh produce a study was conducted.

This study evaluated the competitive, inhibitory, or antagonistic activity of native microflora obtained from samples of fresh-cut iceberg lettuce and bagged baby spinach against *Escherichia coli* O157:H7. Fresh-cut iceberg lettuce and baby spinach from the Salinas Valley, California were handled through simulated packing, transportations, and distribution channels, and microbiologically sampled at various points. To identify microbial isolates selected from the samples that were inhibitory against *E. coli* O157:H7, an agar spot method was used. Whether inhibitory activity was due to acid or bacteriocin production or the presence of other extracellular substances was also evaluated. The objective of this study was to identify *E. coli* O157:H7 inhibitory organisms naturally found on fresh-cut iceberg lettuce and baby spinach and identify their possible mode of inhibition.

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CHAPTER 2

LITERATURE REVIEW

Fresh-Cut Produce Industry

Fresh-cut and minimally processed vegetables are similar terms both meaning anything from slightly trimmed, lettuce heads cored and outer leaves stripped, to sliced, washed, bagged, trimmed or peeled carrots (*21*). The official definition from the United Fresh Produce Association states "any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state" (*28*). Also according to Produce Marketing Association the retail and foodservice sales of fresh-cut and whole produce were at $12 billion in 2006 (*23*). Fresh-cut and fresh whole vegetables have shown noticeable increases in consumption and per capita availability since 2000, along with an increase in the global distribution of these produces (*23*). This along with the government and other authorities suggesting Americans to increase their consumption of fresh vegetables has driven the focus of the fresh-cut industry. The U.S. Department of Agriculture's position on the food pyramid recommends people consume from 3.5 to 5 cups of vegetables a day, most of which is to come from dark green leafy vegetables. More consumers are looking for fast, ready-to-eat meals that also have healthy aspects. Bagged pre-washed and pre-cut vegetables are among this trend and a wide range of produce is supplied to consumers year round (*4*). Global trade in fresh fruits and vegetables is also giving people the variety for which they seek. The industry's new methods are

also helping extend shelf life with modified atmosphere packaging yielding minimally processed foods that consumers are ready to eat.

As Americans demand more variety they also expect safety, quality and cheaper prices. They are slowly veering away from frozen and canned foods to pre-cut and pre-packaged produce because of saved time, saved storage space, and less waste removal (*19*).

The fresh-cut industry is not ignoring these trends. The industry is seeing steady growth as the markets of fresh-cut vegetables alone achieved 1 billion dollar sales in 2006 and fresh-cut fruit continues to grow from 242 million dollars in 2006 (*32*). With this larger demand it is also important to continue food safety practices. Therefore, the fresh-cut industry needs to work with all members of the industry to obtain safe, high quality produce, while maintaining safe sanitary conditions.

The three industry-implemented programs for safety of produce are Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs), and Hazard Analysis Critical Control Points (HACCP). GAP guidelines are universally applied to the production of many different products focusing on sufficient, safe, and nutritious foods while also taking into account economic, social and environmental aspects. They are defined as three step processes of assessment and evaluation, implementation, and food safety program verification. Beyond the guides that the U.S. Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) provide, each processor or company develops a specific GAP, GMP, or HACCP program to apply to their process that is in accordance with these guides.

GMP guidelines are set for each processor of food, no matter the product, to ensure safety and economic aspects as well. GMPs as required by the U.S. FDA for food processors are found in the U.S. Code of Federal Regulations 21(CFR) Part 110.1 -- 110.99. GMPs cover all aspects

of a processing environment from the design of a sanitary facility to rules forbidding jewelry on workers (*1*). Unlike GAPs, GMPs are rules that are clearly defined and must be applied because in comparison a processing environment has easily defined food safety boundaries and the processing activities can be contained and controlled. They are promulgated by the FDA under the authority of the Federal Food, Drug, and Cosmetic Act. The regulations, enforceable by law, require manufacturers, processors and packagers of food to take proactive steps in an attempt to ensure their product is safe and pure.

HACCP is not yet mandatory for produce but many processors are using it because of its usefulness in implementing food safety in the production environment. It is designed as an overview analysis of the complete process that a company or processor completes with a food item. Then, the practices, production environment, and worker practices will be reviewed for hazard analysis or points where hazards can possibly be introduced into the food. Next the contamination point is assessed for concern and control points are developed to control these possible contaminations. The non-mandatory implemented HACCP plans are working well for produce due to the fact that it is a tool that relies on GAP and GMP and other plans to monitor and match the multiple needs of the various processing facilities.

Foodborne Illnesses Associated with Fresh Produce

Fresh-cut produce and fresh produce become vehicles of foodborne pathogens through a number of sources. The advances needed to increase production of fresh produce and those used to decrease cost are thought to have contributed to the increase in the occurrence of foodborne pathogens all over the U.S. The improper use of manure instead of chemical fertilizers, using untreated sewage for irrigation water, changes in the produce industry, social demographics, and

food consumption patterns may also be contributing to the increase in produce-associate outbreaks (*4*). These pathogens which include bacterial, parasitic, and viral agents are causing numerous illnesses and even death (*22*).

In the U. S., an estimated 76 million cases of foodborne illness occur each year (*29*). It is also generally accepted that this is underreported and there are many more cases that simply do not get documented. One system of monitoring foodborne illness incidents in the U.S. is though the FoodNet system which is a network of public health agencies and the Center for Disease Control and Prevention's Emerging Infections Program (*6*). This network only accounts for a little over fifteen percent of the U.S. population. The number of outbreaks reported has increased in recent years and the factors for this increase have to be examined. Some is due to improved surveillance mainly because of FoodNet. Other reasons include increased consumption as stated earlier along with an increase of popularity for salad bars and buffets that serve greater volumes of produce to a larger geographic area (*29*).

In 2007, FoodNet reported a total of 17,883 bacterial pathogen infections with 6,790 of those cases being salmonellosis, 5,818 being campylobacteriosis, 2,848 being shigellosis, 1,216 being *Cryptosporidium* infections, 545 being *E. coli* O157:H7 infections, 260 being *E. coli* non-O157 strain infections, 163 of yersiniosis, 108 of *Vibrio* infections, 122 listeriosis, and 13 cyclosporiasis (*8*). Of the 590 *E. coli* O157:H7 cases in 2006, at least 88 were outbreak-associated. This was because of 3 large multistate outbreaks associated with produce. One outbreak associated with bagged fresh spinach accounted for 32 cases and 2 more outbreaks associated with lettuce in 2 fast-food restaurants made up 14 more cases. In 2003 and 2004, the rates of *E. coli* O157:H7 infections were declining but in 2005 and 2006 these infections increased. The decrease was believed to be from the efforts of the USDA-FSIS and the beef

industry to reduce contamination of ground beef, so the reasons for the increases are not know (*6*).

According to the Center for Science in the Public Interest database, between 1990 and 2005, there were 713 outbreaks linked to produce and that accounted for 13% of foodborne illness outbreaks. Fifty percent of these produce related outbreaks were caused from foodservice establishments while 13% were from local homes and the remaining from schools, jails, the workplace, and other events. According to this data the average number of 48 ill people per produce related outbreak is higher than the average for beef, poultry, and seafood outbreaks. *Salmonella* was responsible for 18% and *E. coli* O157:H7 for 8% (*8*).

Sources of contamination surround produce from the day it is planted. *Clostridium* species as well as *Bacillus* species are commonly found in the soil as spores so they have the ability to transfer to ground harvested produce. *Listeria monocytogenes* is probably the most prevalent disease causing microbe in the soil according to Beuchat (*3*). Other pathogenic bacteria, parasites and viruses in the soil are likely from untreated manure or sewage. These microorganisms contaminate the soil and are transferred to the plant through handling processes. It is also possible at the retail level to get contamination from microbes in the soil if proper sanitation is not followed. The irrigation or flood waters used to water produce can also harbor pathogens that can contaminate even non-ground lying plants from raw sewage or surface run-off. Viruses are also known to remain viable on the surface of vegetables for up to 30 days (*2*). Treatment of sewage does not always result in bacteria or pathogen-free treated waste. *Listeria*, *Endamoeba*, *Salmonella*, *Ascari* cysts and *Taenia* cysts have been recovered from treated wastewater in fields. Feces remaining on the field to supply soil with nutrients have been shown to contain *E. coli* O157:H7 and *Cryptosporidium* that can remain viable for up to 70 days (*3*).

Combine that with a study by Elder and others that showed the frequency of *E. coli* O157:H7 in feces of slaughterhouse cattle to be 72% in 29 lots (*11*). Water used for irrigation, pesticide mixtures, plant growth regulators, and fertility management is another concern for pathogen contamination. Processing also has its concerns with rinsing, cooling, washing and transporting (*29*). Studies have shown pathogens like *E. coli* O157:H7 can transfer from irrigation water to below the top soil for more than two months after application (*15*). Not only does the quality of the water need to be addressed, but also the irrigation method, climate, soil, crop type, and water source. Another pathogen source is wild bird feces. These birds feed on the manure, garbage, sewage, or fish that may contain numerous pathogens (*17*). More contamination sources include dust, wild and domestic animals in the fields, and insects which could cause an unknown amount of cross-contamination in just the pre-harvest stages of production.

Post-harvest contamination can be similar to pre-harvest in that workers can still contaminate produce. Most harvesting techniques leave the product open and unprotected from dust, insects, and animals. Other points of concern are harvesting equipment (often from poor sanitation of the equipment), transporting containers, further processing equipment (sorting, packing, slicing, bagging, etc.), wash and rinse waters, ice, worker hygiene, transport vehicles, backhauling, improper storage, improper packaging, and improper handling at the preparation site. Packers and processors only sanitize their equipment about 50% of the time and most produce are in about 7 different containers through its process (*29*). This is a concern for pathogen introduction, and proper sanitation is the major defense. Also, new equipment should be analyzed for potential contamination and sanitation difficulties. Field worker hygiene should not be overlooked as manual labor is a huge part of the industry. In a recent survey by the USDA only about 50% of produce workers required their employees to wear gloves (*29*). Also,

restaurant workers and food providers, including those in the home, should be educated on food safety and the importance of proper handling and storage (*4*). Ice and water are a concern in post-harvest contamination in the use of water for washing, cooling, moving product, disinfecting, or adding waxes. *E. Coli* O157:H7 has been shown to transfer from ice to lettuce in one study (*18*). Proper water sanitation is the key to reducing contamination by water. Lettuce for instance can have a greater shelf life by washing in cold chlorinated water prior to bagging (*10*).

Temperature control is important to produce as a microbial control and also a quality control point, though it cannot be relied on as a sole control of pathogen growth. Abuse at the consumer level is the most common form of temperature abuse as most home refrigerators are not kept at suggested (3-4°C) levels (*30*).

Outbreaks from foodborne pathogens tied to produce have increased in frequency in recent years (*6*). Some specific fresh produce items of concern are berries, seed sprouts, melons, unpasteurized juices, and lettuce (*12*). Raw raspberries that were imported from Guatemala have been associated with several large *Cyclospora* outbreaks (*29*). The source was thought to be contaminated water used for pesticide application or poor harvester hygiene. Also frozen strawberries and raspberries have been linked to 2 or 3 outbreaks of hepatitis A and one incident of calicivirius thought to spread through contamination with human feces (*29*). The berries that are headed to fresh market are harvested by hand and field packed into retail containers with no wash step. Berries that are frozen are destemmed, transported in ambient temperatures to a processing facility, washed with potable water or chlorinated water, sometimes sliced, and then mixed with up to 30% sucrose and deep frozen. This additional processing (destemming, slicing) may explain the higher outbreak rate, as well as, freezing as it might be preserving the viruses in the berries (*29*).

Seed sprouts have been associated with foodborne illnesses over the years. They are not subject to the same sanitation requirements as foods because they are considered agricultural commodities. It is hard to isolate pathogens from the seed as populations are low compared to other microflora making them hard to detect. In addition, many pathogens can survive for months under the dry conditions for seed storage. Then while the seed is sprouted the pathogen has a near optimal environment for growth for 3 to 10 days (*29*). Alfalfa is the most infamous source but clover, radishes, and mung bean sprouts have also been identified with outbreaks. This higher rate of infection with alfalfa sprouts is due to higher consumption and the fact that they are commonly eaten raw. Pathogens that have been associated with sprouts include: *Salmonella, B. cereus*, *E. coli* O157:H7, and *Y. enterocolitica* (*29*).

Any type of cut melon is considered a hazardous food according to the FDA Food Code due to its low acid content and high water activity that is ideal for pathogen growth (*13*). Recent outbreaks involving mainly *Salmonella* have been assumed by investigators to have been contaminated in the field or during the washing step. It was then spread during the slicing of the melons. Other outbreaks have been shown to come from infected workers and utensils. The FDA Food Code recommendations are to wash melons before cutting, clean and sanitize utensils, refrigerate melons below 7°C, and they should not be exposed for more than four hours without refrigeration (*13*).

Unpasteurized juices also have the same risks as fresh and fresh-cut produce because they are minimally processed and are not thermally processed in any way. Only a small percentage of juices sold in the U.S. are unpasteurized as of August 1997. All unpasteurized juices require a warning label stating “WARNING: This product has not been pasteurized and therefore may contain harmful bacteria that can cause serious illness in children, the elderly, and persons with

weakened immune systems." (5). This requirement was due to three large scale outbreaks from commercial manufacturers of unpasteurized orange and apple juice. *Cryptosporidium, Salmonella*, and *E. coli* O157:H7 were all found in these unpasteurized juices and shows that multiple types of pathogens may be present.

Fresh lettuce heads and pre-cut lettuce salads have been in the headlines since the 1980's for foodborne illness outbreaks, but most have been small. From 1990 to 2005, the outbreaks became more common and greens-based salads along with plain lettuce and spinach have become more available to consumers. In the FDA's 2005 letter to California produce plants, they cite 18 lettuce outbreaks since 1995 that resulted in 409 cases of illness and 2 deaths linked to fresh or fresh-cut lettuce and an additional fresh-cut spinach outbreak. Of these 19 total outbreaks in California, 8 were able to be traced back to Salinas Valley, CA. The California produce companies were warned that since these commodities are eaten raw, they need to assess their GAP and GMP programs. The FDA further stated that river samples and data collected from local farms tested positive for the specific *E. coli* O157:H7 strain from the outbreak. The letter also stated the 2004 Produce Safety Action Plan and the goals contained within the plan (9).

Following the lettuce outbreaks in 2005, another outbreak was linked to spinach from one of the same companies that packaged the lettuce linked to the previous outbreaks. In September of 2006, the first *E. coli* O157:H7 illnesses were reported and more were reported in the following weeks. In the end, 205 confirmed illnesses and 3 deaths resulted. Researchers linked the illnesses back to bagged spinach and later to a processor in San Juan Baustia, CA. No *E. coli* O157:H7 was found at the packer but conditions were observed that would allow the spread of pathogens if they arrived on the incoming spinach. Using the package coding system from the

contaminated bags of spinach, researchers traced the contaminated lots back to 4 possible fields and tested all 4 fields for the specific *E. coli* O157:H7 strain that was the outbreak strain. The strain was identified in river water, cattle feces, and wild pig feces of a ranch, which was one mile from one of the fields, but was not found on any part of the fields or equipment. No definitive determination could be made, but irrigation water is thought to have been contaminated from surface water that contained cattle feces. Also, contamination could have occurred from wild pigs that were in the fields with the spinach (*7*).

Reduction of Pathogens on Produce

From the outbreaks discussed earlier we see there are many points where pathogens can contaminate fresh-cut produce and reside; therefore, the needs to eliminate these contamination points are crucial. In industry, there are a variety of sanitation methods used for different contact surfaces (wood, plastic, steel, iron) and items (troughs, crates, equipment) and while they each have a specific advantage their effectiveness may need reevaluation. Arguably the best way to reduce microbial contamination is through prevention. However, this is not always plausible as many produce items are ground harvested and there is no such thing as clean dirt. Also, typical scrubbing processes do not eliminate all microorganisms on a product (*29*). The goals with industry washing and sanitizing practices are to "adequately treat clean produce by a process that is effective in destroying or substantially reducing the number of microorganisms of public health concern as well as other undesirable microorganisms, without adversely affecting the quality of the product or its safety for the consumer" according to the FDA's Guide to Minimize Microbial Food Safety for Fresh Fruits and Vegetables (*12*). Each method is different depending on the produce type and the process step. Most use water for cleaning or transport as mentioned

earlier, and this water has to be cleaned and combined with sanitizers or detergents to help eliminate contaminants. Which sanitizer is used and the application of it is variable within the industry. Although there are alternatives the most effective treatment to reduce contamination levels is through washing fruits and vegetables in chlorinated water. Equipment can be sanitized with other chemicals such as chlorine dioxide, acidified sodium chlorite, bromine, iodine, quaternary ammonium compounds, acidic compounds, alkaline compounds, hydrogen peroxide, ozone, and even bacteriocins or plant derived antimicrobials (*12*). Other technologies researched for reducing pathogen levels on products and food contact surfaces include high pressure application, UV treatments, magnetic fields, irradiation, electronic pulsed fields, and ultrasound. There is very little research to the application of these techniques on fresh produce and the affect these treatments have on produce sensory aspects such as taste, appearance and texture, odor.

Biocontrol

Another treatment with some research behind it is biocontrol. Biocontrol is the application of beneficial microorganisms to prevent the growth and possible attachment, either through competition for physical space, nutrients, or production of antagonistic compounds, of unwanted microorganisms or pathogens on a surface or food. Researchers are looking towards the use of bacteria that are antagonistic to pathogens on fresh produce and salads. The most researched microorganisms are lactic acid bacteria (LAB). These are the most popular because they inhibit the growth of microorganisms through various mechanisms without causing unacceptable sensory changes. Inhibition by LAB is mediated by the acid environment produced as well as the antimicrobial compounds like bacteriocins, reuterins produced by *Lactobacillus reuteri* , hydrogen peroxide produced by some strains, and competition for space and nutrients

(*27*). LAB are also considered as food grade microorganisms and are generally recognized as safe (GRAS) by the FDA. They have historically been used to preserve meat and dairy products as well as fermented vegetables. Experiments have been conducted to look at LAB isolated from alfalfa sprouts to find antagonistic species against foodbourne pathogens such as *Salmonella, E. coli* O157:H7, and *Listeria.* Pathogens were found to have significantly reduced populations after 5 days (*33*).

A similar experiment performed by Liao and Fett (*20*) with natural microflora isolated from bell peppers, romaine lettuce, baby carrots, and alfalfa sprouts tested inhibition of growth of *Salmonella enterica* serovar Chester, *Listeria monocytogenes*, *E. coli* O157:H7, and *Erwinia carotovora* subsp. Carotovora on agar. They found 3 strains of *Bacillus* spp., 2 of *Pseudomonas*, and 1 of yeast that were inhibitory against the pathogens. While both *Pseudomonas* isolates are antagonistic on microbiological media, use on fresh produce is controversial because of their nature as spoilage organisms (*20*).

A study with fresh produce and fresh-cut produce looking for native microbiota from produce to inhibit 4 pathogens has also been conducted. *Staphylococcus aureus* ATCC 27664, *Escherichia coli* O157:H7 E009, *Listeria monocytogenes* LCDC 81-861, and *Salmonella* Montevideo were used as pathogens (*26*). The agar spot test was used along with combined inhibitory tests with all 4 pathogens. One isolate from cut lettuce was inhibitory to all 4 pathogens. Another project isolated native microflora from alfalfa sprouts and compared their inhibition of *Salmonella* against a *Pseudomonas fluorescens* strain (*21*). The single *Pseudomonas* strain showed maximum inhibition on the first day (4 log reduction) compared to the native microflora that showed maximum inhibition on the seventh day (5 log reduction). They

concluded that a multistrain culture might work better because some strains inhibited in the first days and some showed maximum inhibition later (*21*).

Fresh produce, specifically ready-to-eat salad mixes, were tested for *Lactobacillus casei*, *Lactobacillus plantarum*, and *Pediococcus* spp. inhibitory action against pathogens. These isolates were chosen due to their ability to produce antimicrobials. Strains isolated from salad were found to inhibit *Aeromonas hydrophila*, *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium, and *Staphylococcus aureus* (*31*). They found that *L. casei* was the most effective inhibitor of pathogen growth.

Another used *L. plantarum* isolated from salad because of its bacteriocin characteristics. It produces plantaricin D, a bacteriocin that has shown to have specific antagonistic effects against *L. monocytogenes*. Also, *P. syringiae* prevented growth of *E. coli* O157:H7 in cut apple wounds where there was a 2 log increase in untreated wounds (*16*). Recent research by Trias et al. (*27*) showed that 18 isolates from fresh vegetables and fruit identified by 16s rDNA sequencing and API 50CH varied from *Leuconostoc* spp. to *Weissella* spp. inhibited varying human pathogens. Treatments of cut lettuce and apples with the bacteria resulted in a 1 to 2 log reduction of *S.* Typhimurium and *E .coli* O157:H7, where the growth of *L. monocytogenes* was completely inhibited.

***Escherichia coli* O157:H7**

E. coli are part of the intestinal tract microflora of humans and other animals. The organism was originally called *Bacterium coli* and was isolated from infant's feces in 1885 by Theodor Escherich and was renamed in 1920 to *Escherichia*. Within the different strains there are pathogenic *E. coli* that can cause illness and death. Some strains can cause diarrhea while

others can cause urinary tract infections, respiratory illnesses, pneumonia, or HUS (hemolytic uremic syndrome). There are five types of *E. coli* that can be pathogenic. Enterotoxigenic *E. coli* (ETEC) is responsible for travelers diarrhea by LT and ST enterotoxins, enteropathogenic *E. coli* (EPEC) can cause severe diarrhea in infants, as it is mildly invasive and can elicit an inflammatory response, enteroinvasive *E. coli* (EIEC) is found only in humans and causes non-bloody diarrhea and dysentery, enteroaggregative *E. coli* (EAEC) is only found in humans and aggregate tissue cells of the intestinal mucosa and causes watery diarrhea without a fever, and finally enterohemorrhagic *E. coli* (EHEC) which causes hemorrhagic colitis (bloody diarrhea) which can progress to hemolytic uremic syndrome (HUS). EHEC strains, including *E. coli* O157:H7, are characterized by the production of vero toxin or shiga toxins. In 1982, *E. coli* O157:H7 was identified as a foodborne pathogen after an unusual outbreak of gastrointestinal illness with contaminated hamburgers (*25*). It was still not recognized as an important or threatening pathogen until 1993 after a large multistate outbreak linked to undercooked beef patties sold from fast-food restaurants. Since then, the U.S. averages an estimated 73,480 illnesses annually due to this pathogen resulting in 2,168 hospitalizations and 61 deaths (*24*). One-hundred-eighty three outbreaks and 5,245 illness cases from 1982 to 2002 were due to this pathogen. It occurs mostly in communities (53 outbreaks), then restaurants/food facilities (51 outbreaks), and then schools (16 outbreaks). Among these 183 outbreaks, the food vehicle was ground beef for 75 instances, in 42 it was unknown, in 38 it was produce-related, in 11 it was other beef products, in 10 it was other foods, and in 7 it was dairy products (*24*). More recently it has been recognized that *E. coli* O157:H7 could be on other foods like fresh produce. The recent outbreaks mentioned earlier with unpasteurized apple juice and fresh-cut produce have been a cause for concern in the fresh-cut industry. Because of these produce outbreaks, the

National Food Processors Association on behalf of the Food Irradiation Coalition sent a petition to FDA for a regulation to provide for the safe use of ionizing radiation for control of foodborne pathogens and extension of shelf-life in iceberg lettuce and spinach up to a maximum dose of 4.0 kGy (*14*).

Fresh Produce Related Outbreaks

Fresh produce related outbreaks due to foodborne pathogens were first reported in 1991 and have continued to increase as a major public health concern (*12*). The outbreaks tend to be seasonal, peaking in the summer and fall with about 75% occurring from July to October. Thirteen of these outbreaks were from lettuce, 7 from apple cider or juice, 6 from a mixed salad, 4 from coleslaw, 4 from melons, 3 from sprouts, and 1 from grapes(*8*). Of the 15 outbreaks that occurred in restaurants, 7 of these were from cross-contamination during food production. Of the remaining outbreaks, 20 were not due to kitchen-level cross contamination and none were due to imported produce. Six of the 24 multistate outbreaks were from produce.

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CHAPTER 3

INHIBITION OF *ESCHERICHIA COLI* O157:H7 BY BACKGROUND MICROFLORA OF FRESH-CUT LETTUCE AND BABY SPINACH[1]

[1] Johnston, M.A., and M.A. Harrison. To be submitted to *Journal of Food Protection*.

ABSTRACT

In recent years, there have been foodborne illness outbreaks associated with *Escherichia coli* O157:H7 from fresh-cut lettuce and spinach. Fresh-cut lettuce as well as spinach can become contaminated with pathogens from planting to post-harvest. Natural microflora already present on fresh produce may help reduce the pathogen load more than previous studies reveal. The objectives of this study were to examine and isolate natural microflora from fresh-cut iceberg lettuce and baby spinach to test for isolate inhibition against *E. coli* O157:H7, under conditions that mimic actual practices between production to retail sale. Evidence of naturally-occurring microorganisms on fresh lettuce (295 isolates) and spinach (200 isolates) with possible antagonistic activity toward *E. coli* O157:H7 was documented. Possible inhibitory activity by several isolates was due either to acid production or bacteriocins. No specific trend of inhibition zone size correlated with bacterial type was identified. Isolates with inhibitory activity were isolated from every step in the processing and handling of the fresh-cut iceberg lettuce and baby spinach. Higher number of isolates from lettuce was possibly due to greater sampling.

INTRODUCTION

In 2007, FoodNet reported a total of 17,883 bacterial pathogen infections, Five hundred and forty-five being *E. coli* O157:H7 infections (2). Of the 590 *E. coli* O157:H7 cases reported by FoodNet in 2006, at least 88 were outbreak-associated. Three large multistate outbreaks of *E. coli* O157:H7 associated with produce were the cause. One outbreak associated with bagged fresh spinach accounted for 32 cases, and two more outbreaks associated with lettuce in 2 fast-food restaurants made up 14 more cases each. In 2003 and 2004, the rates of *E. coli* O157:H7 infections were declining, but in 2005 and 2006 these infections increased. According to the Center for Science in the Public Interest database between 1990 and 2005 there were 713 outbreaks linked to produce that accounted for 13% of foodborne illness outbreaks. Fifty percent of these produce related outbreaks were caused from foodservice establishments while 13% were from local homes and the remaining from schools, jails, the workplace, and other events. Also according to this data the average of 48 ill people per outbreak is higher than the average for beef, poultry, and seafood outbreaks. *Salmonella* was responsible for 18% and *E. coli* O157:H7 8% (*4*).

According to the Produce Marketing Association the retail and foodservice sales of fresh-cut and whole produce were at $12 billion in 2006 (*13*). This along with the government and other authorities suggesting Americans to increase their consumption of fresh produce has driven the focus and concern on the fresh-cut industry. As Americans demand more variety they also expect safety, quality, and cheaper prices. They are slowly veering away from frozen and canned foods to pre-cut and pre-packaged produce because of time savings, storage space limits, and less waste removal.

Ready-to-eat produce is very susceptible to contamination from many sources. Fresh minimally processed produce has no true intervention steps for pathogen contamination. Because most produce is not cooked before being eaten, the produce undergoes no type of guaranteed pathogen reducing process. As a result the industry relies on Good Agriculture Practices (GAPs) and Good Manufacturing Processes (GMPs) as programs to help eliminate and reduce contamination. Use of voluntary Hazardous Analysis Critical Control Points (HACCP) programs by some in the fresh-cut industry is another attempt to further reduce growth or contamination of pathogens on produce.

Previous studies have considered the possible presence of bacteria that are antagonistic to pathogens on fresh produce and salads. A study with fresh produce and fresh-cut produce looking for native microbiota from the produce to inhibit 4 pathogens has been conducted. *Staphylococcus aureus* ATCC 27664, *Escherichia coli* O157:H7 E009, *Listeria monocytogenes* LCDC 81-861, and *Salmonella* Montevideo were used as pathogens. One isolate was found from cut lettuce that inhibited all 4 pathogens (*14*).

The objective of this study was to identify isolate *E. coli* O157:H7 inhibitory organisms naturally found on fresh and packaged fresh-cut iceberg lettuce and baby spinach and to identify their possible modes of inhibition and identification. To isolate microbes from samples that were inhibitory against *E. coli* O157:H7, an agar spot method was used. Whether inhibitory activity was due to acid production, bacteriocin production, or the presence of other extracellular substances was also determined.

MATERIALS AND METHODS

Produce handling and sampling times

Fresh iceberg lettuce and spinach were harvested from fields in the Salinas Valley of California and shipped overnight to the Department of Food Science and Technology in Athens, GA. Variations on the way iceberg lettuce and spinach could be handled in commercial operations and after packaging were reflected in the handling and sampling plans. The greens were removed from the cooler and equilibrated to 25 or 32^{o}C to reflect two harvest conditions (one that is desirable and one that is abusive). The outer leaves of the iceberg lettuce heads were removed and the heads cored by hand. Investigators wore gloves to minimize introduction of microorganisms at this point. Samples were taken to enumerate the background microflora (aerobic mesophiles and psychrotrophic bacteria, coliforms, yeasts and molds, and lactic acid bacteria) on the lettuce and spinach.

Heads of lettuce were placed in plastic totes, held at 25 and 32^{o}C for up to 10 h and sampled for background microflora. Temperatures and times represented reasonable conditions and abusive conditions for transporting greens from the field to a cooling facility. After sampling, the lettuce was vacuum cooled to approximately 4^{o}C within 20-40 min. Totes were held at either 4 or 12^{o}C and the inoculated heads were sampled for background microflora at 0, 10, and 72 h. Sampling after 10 h represented transit to a fresh-cut operation in the general area where the lettuce was harvested, while 72 h represented the typical transit time from central California to eastern areas like Atlanta under a reasonable transit temperature and an abusive refrigeration temperature.

Lettuce was chop-cut (3.5-5.0 cm pieces) and held in chilled water (3-4^{o}C) with or without chlorine (pH adjusted) with agitation to simulate contact with water in fresh-cut

operations. After dewatering and bagging, lettuce samples were held at desired refrigeration and abusive storage temperatures and sampled over several days. Times and temperatures (4, 25, and 32°C) included abusive conditions that could occur during distribution of the retail product. Background microflora was sampled before and after exposure to chilled water, after bagging and on days 2, 5, 10, and 18.

Spinach was handled in a similar manner as the lettuce except the baby spinach was forced air cooled to approximately 4°C and the leaves were not chopped or shredded before packaging in macroperforated bags. Background microflora was sampled before and after exposure to chilled water, after bagging and on days 2, 5, and 10.

Sampling of Lettuce

Twenty-five g of lettuce were weighed out and 225 ml of D/E Neutralizing broth (Difco, Becton Dickinson, Sparks, Md.) was added in a sterile stomacher bag and stomached (Seward Stomacher 4000) at 230 rpm for 2 min. Serial dilutions were made in 0.1% peptone water and plated using a spiral plater (Spiral Biotech, Autoplater 4000, Bethesda, Md.) onto plate count agar (PCA, Difco) that was incubated at 37ºC for 24 h for mesophilies, PCA incubated at 4ºC for 8 to 10 d for psychrotrophic organisms, dichloran rose-bengal chloramphenicol agar (DRBC, Difco) incubated at 25ºC for 5 to 7 d for yeast and molds, violet red bile + 4-methylumbelliferyl-ß-D-gluconide agar (VRB+MUG, Difco) incubated at 32ºC for 24 h for coliform and *E. coli*. For lactic acid bacteria, 41 ml of 4x regular strength MRS (Difco) was added to the sample bags and shaken for 2 min. Dilutions were made with regular strength 9 ml MRS dilution tubes and plated on aerobic count Petrifilm (3M, St. Paul, MN) and placed in anaerobe jars at 37°C for 18-24 h. For each sample and media type, 8 random colonies were selected off of a plate using the

Harrison Disk Method (8). A cutout of the Harrison Disk gird was used under the original media plates to randomize selection. Figure 3.1 shows the sampling process for lettuce.

Sampling of Spinach

Twenty-five g portions of spinach were added to 225 ml of 0.1% peptone (Difco, Becton Dickinson, Sparks, Md.) and stomached at 230 rpm for 2 min. Samples were diluted, plated, and isolates selected as described above. Figure 3.2 flow chart shows the sampling process for spinach.

Preparation of *E. coli* O157:H7

E. coli O157:H7 isolates were used to determine the inhibitory activity of the spinach and lettuce isolates. Four strains of green fluorescent pigment-expressing (GFP) *E. coli* O157:H7, ATCC 4388, 123995, E0122, and 124546 (obtained from Dr. Li Ma and Dr. Larry Beuchat, UGA Center for Food Safety, Griffin,GA.) were used in this study. Each strain was transferred into 9 ml tryptic soy broth (Difco) with ampicillin (ampicillin sodium salt, Sigma, St. Louis, MO.,) (TSB+A) and incubated at 37°C in 24 h intervals. One ml of each strain were transferred into 9 ml TSB+A for three days prior to the experiment. *E. coli* O157:H7 strains were centrifuged (Beckman Coulter Fullerton, CA. Allegra ^{TM} X-22R Centrifuge) at 4,000 x g for 10 min. Each strain was diluted to approximately 10^4 cfu/ml and equal portions were combined to form an inoculum cocktail. Portions (20 µl) of the cocktail were evenly distributed onto PCA plates using the spiral plater and the lawn mode. Lawn mode on the spiral plater is a mode to apply an even amount of inoculum on the entire plate instead of dilutions. These plates will hereafter be called “lawn plates”.

Screening of Isolates for Inhibitory Action

Inhibitory activity produced by the spinach and lettuce isolates was determined using the agar spot test as previously described by Fleming et al (*6*). Each isolate selected was taken from the original media plate and placed onto a lawn *E. coli* O157:H7 plate made that day. These plates were then incubated at 37^{o}C for 24 h and examined for zones of inhibition surrounding each isolate colony. Zone diameters were measured with an electronic caliper (Digimax Slide, Scienceware, Pequannock, NJ).

Characterizing Isolates

Cellular morphology and Gram reaction were characterized for each isolate by microscopic examination of Gram stains of 20-24 h cultures. Identification of isolates was based on the biochemical reactions. All isolates were tested for catalase and oxidase (*8*). The API 20NE test system was used for non-enteric, oxidase positive, Gram negative rods, while the API 20E test system was used for non-fastidious, Gram negative, oxidase negative rods (bioMerieux, March-l'Etoile, France).

Supernatant Inhibition Assay

After isolates with inhibitory properties were selected, the isolates were screened to determine if the inhibitory property was an extracellular substance produced by each isolate. Cell-free broth preparations of the produce isolates were prepared by centrifuging the isolates from 24 h TSB for 15 min at 4,500 RPM (Allegra 22XR refrigerated centrifuge) at 4ºC prior to filtering the supernatant through a 0.2 µm sterile, Millipore filter (Fisher, Pittsburgh, PA). The agar spot test was then carried out using 2 µl of these sample preparations of the supernatant on each spot of the lawn plates.

Acid Production Assay:

Inhibition of the *E. coli* O157:H7 cocktail was also tested using the method of Lewus et al. (*11*). Isolates that exhibited inhibition to the *E. coli* O157:H7 cocktail lawn plates were cultured in 9 ml TSB at 37^{o}C for 24 h. Serial dilutions were prepared for each sample so that 30 to 300 colonies would grow on each plate of PCA and TSA without dextrose, with 0.5% yeast extract supplement (TSA+YE, Difco). These plates were incubated at 37^{o}C for 24 h and colony growth was checked for 30 to 300 colonies. After incubation, the colonies were overlaid with brain heart infusion agar (BHI, Difco) seeded with the same *E. coli* O157:H7 cocktail as used for the lawn plates at a population of 10^5 cfu/ml. PCA plates were used to allow acid production and TSA+YE plates were used to eliminate acid production due to dextrose presence. After solidification of the agar the plates were incubated at 37^{o}C for 24 h and examined for zones of inhibition.

Protease Sensitivity Assay

Sensitivity of the isolates to proteases was examined in order to determine if bacteriocins were produced. The method described by Lewus et al. (*11*) was used. Isolates were grown in TSB at 37^{o}C for 24 h. The proteases used in this experiment were protease type XXI (*Streptomyces griseus*;23 units/mg solid; Sigma), trypsin (bovine pancreas;1000 units/mg solid; Sigma), α-chymotrypsin (bovine pancreas; type II; 51 units/mg solid; Sigma), proteinase K (from *Tritirachium album;* 11.3units/mg solid; Sigma), and pepsin (porcine stomach mucosa; 3460 units/mg solid; Sigma-Aldrich). These proteases were prepared immediately before use by adding 10 mg/ml of sterile distilled water. Two µl of each protease was spotted on to TSA+YE plates and allowed to diffuse into the media. Isolates to be tested were spot inoculated adjacent to the spotted proteases. Positive controls without protease and negative controls with sterile

deionized water spotted in place of the proteases were also prepared. After 2 h to allow for diffusion and drying, plates were overlaid with 5 ml of BHI agar seeded with an *E. coli* O157:H7 cocktail to yield a final concentration of 10^3 on the plate. Plates were incubated for 24 h at 37^{o}C and examined for the absence of zones of inhibition. An absence of the zone compared to the control isolate spot and the negative sterile water spots showed sensitivity to that particular protease and confirmed that the isolate produces an inhibitory protein.

Replications

Three replications of each variation of the handling and packaging treatments were done.

RESULTS

Nine-hundred-forty-five lettuce samples were processed. From each of the different types of microbiological media used for each sample, colonies were isolated. This resulted in obtaining 36,970 isolates that were screened for antagonistic activity towards *E. coli* O157:H7. Of these, 295 isolates showed inhibitory activity toward E. coli O157:H7. Of the 295 inhibitory lettuce isolates, 287 (97%) were Gram negative bacilli, 4 were Gram positive bacilli, and 3 were Gram positive cocci. Two-hundred eleven were oxidase negative and 84 were oxidase positive. The Gram positive were not identified (Tables 3.1-3.15).

Four-hundred-sixty-two spinach samples were processed yielding 17,513 isolates. When these isolates were tested for antagonistic activity toward *E. coli* O157:H7 200 colonies showed some inhibition. Of the 200 inhibitory spinach isolates, 197(99%) were Gram negative bacilli, and 3(1%) were Gram-positive bacilli. One-hundred forty seven were oxidase negative and 49 were oxidase positive. Gram positive were not identified to the genus level (Tables 3.16-3.25).

Of the 514 isolates, 356(69%) were recovered from VRBA, 84 (16%) were from psychotropic plates, 60 (12%) were from mesophilic plates, 7 (1%) were from TSA, 5 (1%) from DRBC, and 2 (>1%) from anaerobic Petri film. Three hundred sixty-six (71%) were from the 25ºC field temperature samples while 145 (28%) were recovered from the 32ºC field temperature samples. Equal numbers of isolates were recovered from the 0, 10, and 72 h post-cool samples. Two-hundred-thirty-one(45%) were isolated from samples after chlorine washes, while 267 (52%) were isolated before chlorine washes.. *Burkholderia cepacia* and *Pseudomonas putida* were the most prevalent isolates exhibiting activity and were found on samples from each step in the processing and storage. Common inhibitory isolates were identified as *Pantoea*, *Klebsiella*, *Enterobacter*, and *Aeromonas*.

Nature of the Inhibitory Activity

Cells were centrifuged and filtered from the supernatant to determine if inhibition was due to supernatant particles smaller than 0.22 µm. No inhibition of the *E. coli* O157:H7 cocktail due to extracellular substances was shown by any of the isolates tested.

Protease tests results were combined into one column on Tables 3.1-3.25 labeled bacteriocin inhibition. Each test was concluded as possible bacteriocin inhibition if no zone of inhibition was seen on the plate for any of the 5 proteases. Therefore if one protease inhibited inhibition it was marked as yes to bacteriocin inhibition. Approximately 17% of the isolates produced a bacteriocin as part of their inhibitory activity.

Acid production results were also combined into one column in Tables 3.1-3.25 labeled acid inhibition. If the TSA+YE and the PCA plate both showed inhibition the result was recorded as no. If only the PCA plate showed inhibition the test was recorded as yes to acid inhibition. If both plates showed growth and no inhibition the test was repeated. If only the TSA

plate showed inhibition the test was inconclusive. Approximately 16% of the isolates produced acid as a part of their inhibitory activity.

DISCUSSION

Fresh-cut and ready-to-eat pre-bagged vegetables are supposed to be safe for consumption right out of the bag. This growing industry has been in stress lately due to the recent foodbourne illness outbreaks related to their product and the trouble in finding the source of this contamination (*13*). According to the investigation by the FDA and the California Department of Health and Services (CDHS) on the recent outbreak associated with pre-packaged spinach it was possibly due to water contamination from surface water runoff from nearby dairy lots or wild boars in the fields (*3*). While these problems need to be corrected, contamination was possible at multiple other points according to the investigation.

Due to these recent outbreaks of *Salmonella* and *E. coli* O157:H7 on fresh fruits and vegetables it is becoming necessary to look at other options and multiple hurdles to minimize them. Fresh fruits and vegetables may retain much of their naturally-occurring microflora even after handling and processing steps are complete. Pathogens may be introduced to this microflora and may or may not proliferate due to interactions with favorable, harmless microorganisms present on the product.

This study evaluated natural microflora from multiple samples of fresh-cut lettuce and spinach under conditions that mimic those from the field to finished, packaged product. Four-hundred ninety-five inhibitors of *E. coli* O157:H7 were isolated from fresh-cut lettuce and baby spinach. Naturally-occurring microorganisms on foods have been recognized as having inhibitory activity toward foodborne pathogens. Bredholt et al. showed that lactic acid bacteria strains isolated from meat typically inhibit *E. coli* O157:H7 as well as *Listeria monocytogenes*

(*1*). Other studies have shown that fresh-cut produce are sources of competitive microorganisms (*5, 6, 7, 9, 10, 12, 14,* and *16).*

Liao and Fett (*12*) demonstrated inhibitory action against *Salmonella* Chester, *Listeria monocytogenes*, and *Escherichia coli* from *Pseudomonas* species on green pepper, romaine lettuce, baby carrots, alfalfa, and clover. The 6 isolates that inhibited at least one pathogen were *Bacillus* spp. (3 isolates), *Pseudomonas aeruginosa* (1 isolate), *Pseudomonas fluorescens* (1 isolate), and a yeast (1 isolate). On green pepper disks inoculated with the *P. fluorescens* and the yeast, growth of *S.* Chester and *L. monocytogenes* was reduced by 1 and 2 logs, respectively, over a period of 3 days.

Schuenzel and Harrison (*14*) screened isolates from fresh-cut produce for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Montevideo. Of 1,180 isolates screened, 37 (3.22%) were found to have various degrees of inhibitory activity against at least one pathogen. Many isolates were inhibitory against all 4 pathogens. Isolates that were most inhibitory were removed from finished lettuce shreds.

Jablasone et al., studied the interactions of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* on plants cultivated in a gnotobiotic system (*9*). Only *Enterobacter cloacae* reduced *E. coli* O157:H7 and *L. monocytogenes* levels by approximately 1 log cfu/g on lettuce.

Cooley et al., investigated *E. coli* O157:H7 survival and growth on lettuce in the presence of epiphytic bacteria (*5*). E. *coli* O157:H7 survival was enhanced 6-fold in the presence of the epiphyte *Wausteria paucula.* E. *coli* O157:H7 survival was decreased 20- to 30-fold in the presence of *Enterobacter asburiae.* They concluded *E. asburiae* may outcompete *E. coli*

O157:H7 by better utilization of some carbon and nitrogen substrates. They suggest using good agricultural practices to encourage the growth of competing bacteria.

This study was not designed to determine all the modes of inhibition of the isolates. The methods of Lewus et al. (*11*) helped to demonstrate whether the mode of inhibition was due to the presence of bacteriocins, acid production or some other undefined extracellular substance smaller than 0.22 μm.

In this study, the most effective inhibitors were most prevalent after the non-chlorine washings compared to the chlorine washings even after 18 d in storage. The reason could be due to less total bacteria present therefore the inhibiting microbes have more resources so they are easily recovered. So while the chlorine washings are beneficial for killing pathogenic microbes they also reduce the numbers of the inhibitory non-pathogenic microbes that might be present on the produce. *Pasteurella* spp was resilient and was recovered at every step with inhibition zones of 10 mm or greater for most samples. The natural microflora found on the fresh-cut lettuce and baby spinach inhibited the *E. coli* O157:H7 cocktail well, and half were above 10 mm inhibition zones showing the possibility of not only outcompeting but also inhibiting growth. None of the handling or processing steps appeared to decrease the amount of or strength of these natural inhibitors. Even after 18 d storage *Pantoea* spp. were isolated with similar inhibition to ones recovered from the start.

Aeromonas, an organism with potential public health significance was identified as 24 of the inhibitory isolates. These would probably not be used for inhibition or viewed as beneficial inhibitorory bacteria due to their nature to cause septicemia, meningitis, endocarditis, and is associated with traveler's diarrhea (*1*). However, evidence of a variety of naturally-occurring microorganisms on fresh lettuce and spinach with possible antagonistic activity toward *E. coli*

O157:H7 was documented. For those that pose no potential heath threat of their own, they may prove beneficial.

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CONCLUSION

Contamination of fresh-cut produce and minimally processed produce by pathogens is a growing problem. If the industry continues to grow to fit consumer demand, there is the likelihood that the industry will see an increase in the number and size of foodborne illness outbreaks. Native microflora with inhibitory activity toward foodborne pathogens may have shown that they can be useful in the fight against foodborne pathogens. Instead of focusing on eliminating all the organisms on produce, it should be established to eliminate the pathogenic organisms first and foremost. The isolates may be native to the California region but it is assumed that similar bacteria exist in other regions as well. The use of these bacteria as natural controls of foodborne pathogens may be applied to produce as well as other foods. Other research could be done to improve upon these results by applying these bacteria to inoculated produce and determine inhibition on produce items instead of media.

Table 3.1. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was cooled to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	% ID	T index	Comment
Cl	4	18	NO	NO	3.1	33-3	*Enterobacter cloacae*	92.2	0.51	GOOD IDENTIFICATION
Cl	25	5	NO	YES	3.7	21-2	*Aeromonas spp.*	87	0.24	LOW DISCRIMINATION
Cl	4	18	NO	N/A	3.4	33-4	*Rahnella spp*	78.4	1	LOW DISCRIMINATION
Cl	25	5	NO	N/A	4.4	37-3	*Aeromonas spp*	82	0.47	LOW DISCRIMINATION
N	4	-	NO	N/A	3.0	3-5	*Aeromonas spp*	86.6	0.56	LOW DISCRIMINATION
N	4	-	NO	N/A	3.0	4-1	*Unknown*	73.7	0.39	DOUBTFUL PROFILE
N	4	-	NO	N/A	3.1	4-2	*Vibrio spp*	99.7	0.59	VERY GOOD IDENTIFICATION
N	4	-	NO	N/A	3.0	4-3	*Unknown*	88.1	0.41	DOUBTFUL PROFILE
N	4	-	NO	N/A	3.0	4-4	*Aeromonas spp*	86.6	0.56	LOW DISCRIMINATION
N	4	-	NO	N/A	3.0	4-5	*Aeromonas spp*	91.5	0.76	LOW DISCRIMINATION
N	4	-	NO	N/A	3.0	5-1	*Aeromonas spp*	95.2	0.47	GOOD IDENTIFICATION
N	4	-	NO	N/A	2.0	5-2	*Vibrio spp*	98.9	0.75	GOOD IDENTIFICATION
no Cl	4	5	NO	NO	5.2	1-3	*Aeromonas spp*	82.1	0.18	LOW DISCRIMINATION
no Cl	4	18	NO	NO	1.1	31-5	*Unknown*	64.5	0.53	IDENTIFICATION NOT VALID
no Cl	25	2	YES	N/A	10.6	58-3	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
no Cl	25	2	NO	N/A	4.0	1-5	*Pseudomonas luteola*	75.8	0.48	GOOD IDENTIFICATION TO THE GENUS
no Cl	25	3	NO	YES	11.4	55-4	*Kluyvera spp*	99.3	0.89	VERY GOOD IDENTIFICATION
no Cl	25	3	NO	N/A	15.3	55-5	*Unknown*		-	UNACCEPTABLE PROFILE
no Cl	4	0	NO	N/A	4.0	5-3	*Vibrio spp*	98.9	0.75	GOOD IDENTIFICATION
no Cl	4	18	NO	N/A	2.9	32-1	*Raoultella ornithinolytica*	91	0.59	GOOD IDENTIFICATION
no Cl	4	18	NO	N/A	3.0	32-2	*Raoultella ornithinolytica*	91	0.59	GOOD IDENTIFICATION
no Cl	32	1	NO	N/A	12.1	53-5	*Kluyvera spp*	94.7	0.39	DOUBTFUL PROFILE
no Cl	25	3	NO	N/A	14.4	56-1	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	25	2	NO	N/A	12.2	56-4	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
no Cl	25	2	NO	YES	13.4	56-5	*Serratia plymuthica*	85.2	0.88	ACCEPTABLE IDENTIFICATION
no Cl	4	10	NO	N/A	5.0	60-2	*Escherichia coli*	89.6	0.8	LOW DISCRIMINATION
no Cl	4	10	NO	YES	10.1	60-3	*Kluyvera spp*	99.3	0.64	VERY GOOD IDENTIFICATION
no Cl	4	10	NO	N/A	7.1	60-4	*Kluyvera spp*	99.3	0.64	VERY GOOD IDENTIFICATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.2. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was cooled to 4°C and packaged for retail distribution in 12°C transport conditions.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Taxon	% ID	T index	Identification
Cl	25	2	NO	N/A	10.6	20-2	*Unknown*	-	-	UNACCEPTABLE PROFILE
Cl	25	5	NO	N/A	7.2	38-1	*Pantoea spp*	81	0.31	DOUBTFUL PROFILE
N	12	-	NO	NO	4.5	3-1	*Photobacterium damselae*	97.9	0.45	GOOD IDENTIFICATION
N	12	-	NO	N/A	7.9	21-1	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
no Cl	25	5	NO	NO	12.0	2-4	*Enterobacter amnigenus*	96.4	1	EXCELLENT IDENTIFICATION TO THE GENUS
no Cl	25	5	NO	NO	11.0	2-5	*Enterobacter amnigenus*	96.4	1	EXCELLENT IDENTIFICATION TO THE GENUS
no Cl	25	5	NO	NO	3.2	5-4	*Enterobacter spp*	92	0.5	DOUBTFUL PROFILE
no Cl	4	2	NO	N/A	8.1	20-3	*Pantoea spp*	67.4	0.35	LOW DISCRIMINATION
no Cl	4	10	NO	N/A	8.5	26-3	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	25	5	NO	N/A	7.1	2-3	*Vibrio spp*	98.9	0.75	GOOD IDENTIFICATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.3. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was cooled to 4°C for 10 hours and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Slide	Taxon	% ID	T index	Identification
Cl	4	5	NO	N/A	10.0	12-1	*Serratia liquefaciens*	69	1	VERY GOOD IDENTIFICATION TO THE GENUS
Cl	4	5	NO	N/A	10.5	11-5	*Pantoea spp*	94.9	0.87	GOOD IDENTIFICATION
Cl	4	10	YES	N/A	5.3	10-4	*Aeromonas spp*	99.5	0.87	VERY GOOD IDENTIFICATION
Cl	4	10	NO	N/A	11.5	54-1	*Pantoea spp*	99.4	0.83	VERY GOOD IDENTIFICATION
Cl	25	5	NO	NO	12.4	14-3	*Unknown*	-	-	UNACCEPTABLE PROFILE
Cl	25	5	NO	NO	8.7	14-4	*Unknown*	-	-	UNACCEPTABLE PROFILE
Cl	25	5	NO	N/A	11.7	23-5	*Pantoea spp*	76	0.88	LOW DISCRIMINATION
Cl	25	5	NO	N/A	10.5	27-4	*Unknown*	-	-	UNACCEPTABLE PROFILE
Cl	25	5	NO	N/A	13.3	14-5	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
no Cl	4	5	NO	N/A	13.7	20-5	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	4	5	NO	NO	11.9	16-4	*Enterobacter cloacae*	92.2	0.51	GOOD IDENTIFICATION
no Cl	4	5	NO	N/A	12.4	16-3	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	4	10	YES	N/A	7.0	38-2	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	4	10	NO	N/A	10.2	41-5	*Aeromonas sobria*	54.7	0.27	GOOD IDENTIFICATION TO THE GENUS
no Cl	4	18	NO	NO	8.2	37-5	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	4	-	NO	N/A	8.8	28-2	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	25	2	NO	NO	5.8	21-3	*Escherichia coli*	-	-	UNACCEPTABLE PROFILE
no Cl	25	2	NO	YES	9.5	11-3	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
no Cl	25	2	NO	N/A	10.0	11-4	*Serratia odorifera*	99.9	1	EXCELLENT IDENTIFICATION
no Cl	25	5	NO	NO	12.4	14-1	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	25	5	NO	NO	8.7	13-5	*Pantoea spp*	99.4	0.83	VERY GOOD IDENTIFICATION
no Cl	25	5	NO	N/A	7.8	13-4	*unknown*	81.3	0.28	LOW DISCRIMINATION
no Cl	25	5	NO	N/A	7.5	28-1	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	25	5	NO	N/A	6.8	40-1	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	25	5	NO	N/A	9.5	13-3	*Unknown*	98.7	0.4	DOUBTFUL PROFILE
no Cl	25	5	NO	N/A	9.4	14-2	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	32	2	NO	N/A	6.1	25-4	*Sphingomonas paucimobilis*	99.8	0.72	VERY GOOD IDENTIFICATION
no Cl	32	2	NO	N/A	11.6	3-2	*Serratia spp*	-	-	UNACCEPTABLE PROFILE

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.4. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was cooled to 4°C for 10 hours and packaged for retail distribution at 12°C.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Taxon	% ID	T index	Identification
Cl	4	18	NO	YES	1.8	7-1	*Aeromonas sobria*	73	0.73	VERY GOOD IDENTIFICATION TO THE GENUS
Cl	25	2	NO	NO	6.5	30-5	*Pantoea spp*	44	0.55	LOW DISCRIMINATION
Cl	25	2	NO	N/A	7.1	29-3	*Pseudomonas spp*	78	0.26	IDENTIFICATION NOT VALID
Cl	25	2	NO	N/A	8	18-1	*Pantoea spp*	-	-	UNACCEPTABLE PROFILE
no Cl	4	5	NO	N/A	8	12-4	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
no Cl	4	5	NO	N/A	11.7	12-3	*Serratia spp*	-	-	UNACCEPTABLE PROFILE
no Cl	4	5	NO	N/A	13.1	12-5	*Raoultella spp*	55.9	0.31	DOUBTFUL PROFILE
no Cl	4	18	NO	NO	12.3	8-1	*Rahnella spp*	78.4	1	LOW DISCRIMINATION
no Cl	4	18	NO	YES	12.4	7-4	*Pantoea spp*	51.1	0.25	DOUBTFUL PROFILE
no Cl	4	18	NO	YES	10.5	7-5	*Pantoea spp*	51.1	0.25	DOUBTFUL PROFILE
no Cl	12	-	NO	N/A	4.2	6-2	*Pseudomonas spp*	94.1	0.84	GOOD IDENTIFICATION
no Cl	25	5	NO	YES	9.7	2-1	*Enterobacter spp*	77.7	0.59	DOUBTFUL PROFILE

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.5. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was cooled to 4°C for 72 hours and packaged for retail distribution at 4°C

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Slide	Taxon	% ID	T index	Identification
Cl	4	5	NO	YES	9.9	22-1	*Pantoea spp*	94.9	0.87	GOOD IDENTIFICATION
Cl	4	5	NO	YES	5.8	24-1	*Pantoea spp*	94.9	0.87	GOOD IDENTIFICATION
Cl	4	18	NO	YES	8.8	61-2	*Kluyvera spp*	99.3	0.64	VERY GOOD IDENTIFICATION
Cl	4	18	NO	N/A	7.9	25-1	*Pantoea spp*	44	0.55	LOW DISCRIMINATION
Cl	4	18	NO	N/A	7.0	25-2	*Pantoea spp*	-	-	UNACCEPTABLE PROFILE
Cl	4	18	NO	N/A	6.5	25-3	*Serratia spp*	70	0.52	LOW DISCRIMINATION
Cl	4	18	NO	N/A	8.1	24-3	*Aeromonas spp*	71.7	0.2	LOW DISCRIMINATION
Cl	4	18	NO	N/A	8.3	24-4	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
Cl	25	2	NO	NO	12.0	19-1	*Chryseobacterium indologenes*	98.8	0.45	GOOD IDENTIFICATION
Cl	25	2	NO	N/A	13.2	19-2	*Unknown*	-	-	UNACCEPTABLE PROFILE
Cl	32	2	NO	YES	12.3	18-3	*Pasteurella spp*	51.9	0.35	DOUBTFUL PROFILE
Cl	32	2	NO	N/A	13.0	18-4	*Aeromonas spp*	74.5	0.38	LOW DISCRIMINATION
no Cl	4	0	NO	YES	7.9	58-1	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
no Cl	4	2	NO	N/A	14.9	57-1	*Pantoea spp*	41.6	0.92	IDENTIFICATION NOT VALID
no Cl	4	10	NO	NO	7.4	60-5	*Kluyvera spp*	99.3	0.64	VERY GOOD IDENTIFICATION
no Cl	4	10	NO	N/A	6.0	61-1	*Kluyvera spp*	99.9	0.92	EXCELLENT IDENTIFICATION
no Cl	4	18	NO	NO	9.4	61-4	*Raoultella spp*	63.4	0.26	DOUBTFUL PROFILE
no Cl	4	18	YES	N/A	9.8	62-1	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
no Cl	4	18	NO	N/A	11.7	26-5	*Sphingomonas paucimobilis*	97.8	0.76	GOOD IDENTIFICATION
no Cl	4	18	NO	N/A	5.6	27-2	*Stenotrophomonas maltophilia*	92.4	0.84	GOOD IDENTIFICATION
no Cl	4	18	NO	N/A	7.7	62-2	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
no Cl	4	18	NO	YES	9.9	61-5	*Raoultella spp*	92	0.88	LOW DISCRIMINATION
no Cl	4	18	NO	N/A	10.4	62-3	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
no Cl	4	18	YES	N/A	10.4	62-4	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
no Cl	25	2	YES	NO	7.6	3-4	*Enterobacter spp*	60	0.67	DOUBTFUL PROFILE
no Cl	25	5	NO	YES	5.8	6-1	*Enterobacter cloacae*	96.6	0.67	GOOD IDENTIFICATION
no Cl	25	5	NO	NO	7.3	31-2	*Bordetella/Alcaligenes/Moraxella spp*	43.1	0.92	LOW DISCRIMINATION
no Cl	25	5	NO	NO	3.1	5-5	*Enterobacter cloacae*	95.1	1	GOOD IDENTIFICATION
no Cl	25	5	YES	N/A	13.0	31-4	*Psychrobacter spp*	45	0.67	DOUBTFUL PROFILE
no Cl	32	5	NO	NO	6.6	6-3	*Enterobacter amnigenus*	96.4	1	EXCELLENT IDENTIFICATION TO THE GENUS
no Cl	32	5	NO	N/A	13.7	41-3	*Unknown*	85.4	0.49	LOW DISCRIMINATION

- Indicates sample was not exposed to any further treatment or storage. N/A - Test resulted in inconclusive results.

Table 3.6. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was cooled to 4°C for 72 hours and packaged for retail distribution at 12°C.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Slide	Taxon	% ID	T index	Identification
Cl	4	0	NO	N/A	4.8	21-5	*Unknown*	-	-	UNACCEPTABLE PROFILE
Cl	4	2	NO	NO	10.1	16-5	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
Cl	4	2	NO	NO	12.4	9-3	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
Cl	4	2	YES	N/A	5.8	35-4	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
Cl	4	2	YES	N/A	5.0	35-5	*Klebsiella spp*	67.4	0.89	IDENTIFICATION NOT VALID
Cl	4	2	NO	N/A	6.5	36-1	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
Cl	4	5	NO	N/A	10.1	15-1	*Unknown*	81.3	0.28	LOW DISCRIMINATION
Cl	4	5	NO	N/A	12.5	15-2	*Cedecea spp*	89.1	0.31	DOUBTFUL PROFILE
Cl	4	5	NO	N/A	10.1	15-3	*Unknown*	-	-	UNACCEPTABLE PROFILE
Cl	4	18	NO	YES	9.8	30-3	*Escherichia coli*	89.2	0.3	DOUBTFUL PROFILE
Cl	4	18	NO	N/A	8	26-2	*Unknown*	-	-	UNACCEPTABLE PROFILE
Cl	25	2	NO	NO	2.2	7-2	*Escherichia coli*	89.2	0.3	DOUBTFUL PROFILE
Cl	25	2	NO	NO	12.3	9-1	*Serratia odorifera*	68.6	0.72	ACCEPTABLE IDENTIFICATION TO THE GENUS
Cl	25	2	NO	YES	11.1	8-4	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
Cl	25	2	NO	N/A	10.1	8-5	*Stenotrophomonas spp*	96.7	0.5	DOUBTFUL PROFILE
Cl	25	5	NO	N/A	5.0	28-3	*Pantoea spp*	44	0.55	LOW DISCRIMINATION
no Cl	4	0	NO	N/A	17.8	22-2	*Vibrio spp*	88.1	0.41	DOUBTFUL PROFILE
no Cl	4	2	YES	N/A	12.2	39-5	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	4	18	NO	N/A	7.2	26-1	*Unknown*	-	-	UNACCEPTABLE PROFILE
no Cl	25	2	NO	NO	10.3	47-5	*Pantoea spp*	95.4	0.69	VERY GOOD IDENTIFICATION TO THE GENUS
no Cl	25	2	NO	NO	3.8	9-4	*Aeromonas spp*	91.6	0.43	LOW DISCRIMINATION
no Cl	25	2	NO	N/A	12.5	23-1	*Brevundimonas vesicularis*	92.5	0.44	GOOD IDENTIFICATION
no Cl	25	2	NO	N/A	8.9	45-5	*Aeromonas spp*	-	-	UNACCEPTABLE PROFILE
no Cl	25	2	NO	N/A	10.0	47-3	*Pantoea spp*	99.9	0.5	VERY GOOD IDENTIFICATION
no Cl	25	2	NO	N/A	10.1	47-4	*Pantoea spp*	57.5	0.51	LOW DISCRIMINATION
no Cl	25	2	NO	N/A	8.2	48-1	*Pantoea spp*	57.5	0.51	LOW DISCRIMINATION
no Cl	25	5	NO	N/A	10.1	28-5	*Aeromonas spp*	-	-	UNACCEPTABLE PROFILE
no Cl	25	5	NO	N/A	8	22-3	*Vibrio spp*	98.9	0.75	GOOD IDENTIFICATION
no Cl	32	2	NO	NO	5.6	1-2	*Enterobacter spp*	-	-	UNACCEPTABLE PROFILE

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.7. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was precooled to 4°C for 10 hours and packaged for retail distribution

Transportation Temp.	Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Taxon	% ID	T index	Identification
4	no Cl	25	5	NO	NO	9.0	38-3	*Pasteurella spp*	49.7	0.72	LOW DISCRIMINATION
4	Cl	4	10	NO	NO	6.2	44-2	*Aeromonas spp*	99.5	0.87	VERY GOOD IDENTIFICATION
4	Cl	4	5	NO	N/A	7.9	38-4	*Photobacterium spp*			UNACCEPTABLE PROFILE
4	Cl	4	5	NO	N/A	5.8	38-5	*Pantoea spp*	99.9	0.63	VERY GOOD IDENTIFICATION
4	Cl	25	5	NO	N/A	8.9	39-4	*Pantoea spp*	99.9	0.63	VERY GOOD IDENTIFICATION
4	Cl	25	0	NO	YES	7.0	40-5	*Enterobacter spps*	49.8	0.3	DOUBTFUL PROFILE
4	no Cl	25	2	NO	N/A	13.0	41-2	*Unknown*			UNACCEPTABLE PROFILE
4	no Cl	4	10	NO	N/A	9.1	43-2	*Aeromonas sobria*	54.7	0.27	GOOD IDENTIFICATION TO THE GENUS
4	no Cl	4	10	NO	N/A	8.9	43-3	*Aeromonas sobria*	54.7	0.27	GOOD IDENTIFICATION TO THE GENUS
4	Cl	4	10	NO	N/A	8.2	44-3	*Rhizobium radiobacter*	99.5	0.67	VERY GOOD IDENTIFICATION
12	no Cl	25	5	NO	NO	5.4	37-2	*Enterobacter amnigenus*	96.4	1	EXCELLENT IDENTIFICATION TO THE GENUS
12	no Cl	4	10	NO	YES	15.7	57-5	*Enterobacter cloacae*	79.7	0.3	IDENTIFICATION NOT VALID
12	no Cl	25	5	NO	N/A	9.4	36-5	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
12	no Cl	25	5	NO	N/A	3.7	37-1	*Pantoea spp*			UNACCEPTABLE PROFILE
12	Cl	4	10	NO	N/A	7.5	44-5	*Rhizobium radiobacter*	99.5	0.67	VERY GOOD IDENTIFICATION
12	Cl	4	10	NO	N/A	7.2	45-1	*Ochrobactrum anthropi*	73.7	0.52	DOUBTFUL PROFILE
12	Cl	4	10	NO	N/A	13.9	45-2	*Pseudomonas luteola*	70.9	0.22	LOW DISCRIMINATION
12	Cl	4	10	NO	N/A	10.8	45-3	*Aeromonas spp*			UNACCEPTABLE PROFILE
12	no Cl	25	2	NO	N/A	13.6	51-1	*Escherichia coli*	89.6	0.8	LOW DISCRIMINATION
12	Cl	4	18	NO	N/A	11.9	56-2	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.8. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was precooled and postcooled to 4°C for 10 hours and packaged for retail distribution.

Transportation Temp.	Cl/no Cl	Store Temp.	Store Day	Acid Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	% ID	T index	Identification
4	no Cl	25	2	NO	N/A	3.8	37-4	*Aeromonas spp*	82.1	0.14	LOW DISCRIMINATION
4	no Cl	32	2	NO	N/A	2.5	32-4	*Enterobacter cloacae*	92.2	0.51	GOOD IDENTIFICATION
12	Cl	4	10	NO	N/A	13.7	56-3	*Pantoea spp*	96.3	0.28	GOOD IDENTIFICATION
12	Cl	25	2	YES	N/A	13.3	34-1	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
12	Cl	32	2	NO	N/A	2.0	32-5	*Escherichia coli*	89.2	0.3	DOUBTFUL PROFILE
12	no Cl	25	2	NO	N/A	7.4	33-5	*Rahnella aquatilis*	78.4	1	LOW DISCRIMINATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.9. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was precooled to 4°C for 10 hours and postcooled for 72 hours, then packaged for retail distribution.

Transportation Temp.	Cl/no Cl	Store Temp.	Store Day	Acid Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	% ID	T index	Identification
4	N	4	-	NO	NO	11.0	58-2	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
4	no Cl	4	5	YES	YES	13.8	55-1	*Serratia plymuthica*	50.9	0.79	LOW DISCRIMINATION
12	Cl	32	2	NO	YES	6.1	34-3	*Enterobacter sakazakii*	98.2	0.34	GOOD IDENTIFICATION
12	Cl	4	5	YES	N/A	11.4	29-4	*Pantoea spp*	93	0.72	GOOD IDENTIFICATION
12	Cl	32	2	YES	N/A	4.2	34-2	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
12	no Cl	4	2	YES	N/A	6.4	34-5	*Pseudomonas spp*	48.3	0.16	ACCEPTABLE IDENTIFICATION TO THE GENUS
12	Cl	32	2	YES	N/A	6.3	39-3	*Pasteurella spp*	91.5	0.52	GOOD IDENTIFICATION
12	N	12	-	NO	N/A	16.2	29-5	*Pantoea spp*	44	0.55	LOW DISCRIMINATION
12	no Cl	4	2	NO	N/A	6.5	35-1	*Pantoea spp*	48.5	0.46	LOW DISCRIMINATION
12	Cl	25	2	NO	N/A	9.8	46-1	*Pantoea spp*	98.3	0.63	GOOD IDENTIFICATION
12	no Cl	4	5	NO	N/A	10.6	50-2	*Rhizobium radiobacter*	99.8	0.96	VERY GOOD IDENTIFICATION
12	Cl	4	0	NO	N/A	8.9	30-4	*Vibrio spp*	65	0.22	LOW DISCRIMINATION
12	no Cl	4	2	NO	N/A	6.6	35-2	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
12	no Cl	32	2	NO	N/A	9.5	35-3	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
12	Cl	25	2	NO	N/A	5.7	36-3	*Serratia ficaria*	68.8	0.64	DOUBTFUL PROFILE
12	Cl	25	2	NO	N/A	3.7	36-4	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
12	N	12	-	NO	N/A	8.4	41-4	*Sphingomonas paucimobilis*	76.2	0.49	LOW DISCRIMINATION
12	no Cl	4	5	NO	N/A	7.2	50-3	*Vibrio spp*	98.9	0.75	GOOD IDENTIFICATION
12	Cl	4	5	NO	N/A	6.0	53-1	*Pantoea spp*	41.6	0.92	IDENTIFICATION NOT VALID

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.10. Microbial isolates selected from iceberg lettuce at 32°C 'field temperature' that was cooled to 4°C and packaged for retail distribution.

Transportation Temp.	Cl/no Cl	Store Temp.	Store Day	Acid Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Slide	Resulting ID	% ID	T index	Identification
4	Cl	4	10	NO	NO	12.5	23-3	*Vibrio spp*	88.1	0.41	DOUBTFUL PROFILE
4	Cl	4	10	NO	NO	10.4	23-4	*Escherichia coli*	89.6	0.8	LOW DISCRIMINATION
4	Cl	4	2	NO	NO	6.3	33-1	*Escherichia coli*	95.6	0.72	GOOD IDENTIFICATION
4	Cl	4	5	NO	N/A	14.8	52-4	*Unknown*	91.2	0.67	GOOD IDENTIFICATION
4	Cl	4	5	NO	N/A	11.1	52-3	*Escherichia coli*	89.2	0.3	DOUBTFUL PROFILE
4	Cl	32	2	YES	N/A	8.3	22-5	*Unknown*		--	UNACCEPTABLE PROFILE
4	no Cl	4	2	NO	NO	5.1	33-2	*Escherichia coli*	89.6	0.8	LOW DISCRIMINATION
4	no Cl	25	0	NO	N/A	14.1	36-2	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
12	Cl	25	5	NO	N/A	12.0	11-1	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
12	no Cl	4	5	NO	NO	3.3	8-3	*Escherichia coli*	89.2	0.3	DOUBTFUL PROFILE
12	no Cl	4	5	YES	N/A	5.5	23-2	*Cedecea lapagei*	89.1	0.31	DOUBTFUL PROFILE
12	no Cl	25	5	NO	NO	11.1	2-2	*Pantoea spp*	-	-	UNACCEPTABLE PROFILE
12	no Cl	25	5	YES	N/A	9.5	1-4	*Pasteurella spp*	63.9	0.55	IDENTIFICATION NOT VALID
12	no Cl	25	5	NO	N/A	5.3	10-3	*Aeromonas spp*	99.9	0.55	VERY GOOD IDENTIFICATION
12	no Cl	25	5	NO	N/A	4.3	10-2	*Sphingomonas paucimobilis*	74.2	0.54	LOW DISCRIMINATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.11. Microbial isolates selected from iceberg lettuce at 32°C 'field temperature' that was postcooled to 4°C for 10 hours and packaged for retail distribution.

Transportation Temp.	Cl/no Cl	Store Temp.	Store Day	Acid Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Slide	Resulting ID	% ID	T index	Identification
4	Cl	4	5	NO	N/A	10.7	3-3	*Serratia odorifera*	-	-	UNACCEPTABLE PROFILE
4	Cl	25	2	YES	N/A	7.7	20-4	*Unknown*	-	-	UNACCEPTABLE PROFILE
4	Cl	32	5	NO	N/A	2.9	21-4	*Unknown*	-	-	UNACCEPTABLE PROFILE
4	no Cl	25	5	NO	N/A	7.5	19-4	*Klebsiella pneumoniae ssp pneumoniae*	48.1	0.72	LOW DISCRIMINATION
4	no Cl	32	2	NO	No	11.0	17-1	*Unknown*	-	-	UNACCEPTABLE PROFILE
4	no Cl	32	2	NO	N/A	13.9	17-2	*Unknown*	-	-	UNACCEPTABLE PROFILE
4	no Cl	32	2	NO	N/A	8.5	22-4	*Pantoea spp*	94.9	0.87	GOOD IDENTIFICATION
12	Cl	25	2	NO	N/A	11.9	18-5	*Rhizobium radiobacter*	83.7	0.46	DOUBTFUL PROFILE
12	Cl	25	2	NO	YES	9.6	42-4	*Escherichia coli*	89.6	0.8	LOW DISCRIMINATION
12	no Cl	4	18	NO	YES	7.1	10-1	*Aeromonas spp*	99.9	0.55	VERY GOOD IDENTIFICATION
12	no Cl	4	18	NO	N/A	9.4	9-5	*Raoultella ornithinolytica*	55.9	0.31	DOUBTFUL PROFILE
12	no Cl	25	2	NO	N/A	9.2	19-5	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
12	no Cl	32	5	NO	N/A	3.2	28-4	*Unknown*	-	-	UNACCEPTABLE PROFILE

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.12. Microbial isolates selected from iceberg lettuce at 32°C 'field temperature' that was cooled to 4°C for 72 hours and packaged for retail distribution.

Transportation Temp.	Cl/noCl	Store Temp.	Store Day	Acid Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	% ID	T index	Identification
4	no Cl	4	18	NO	N/A	13.5	27-3	*Unknown*		-	UNACCEPTABLE PROFILE
4	no Cl	25	2	NO	NO	6.0	11-2	*Aeromonas spp*	82	0.47	LOW DISCRIMINATION
4	no Cl	25	5	NO	N/A	12.3	15-4	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
12	Cl	4	10	NO	NO	7.0	13-1	*Unknown*		-	UNACCEPTABLE PROFILE
12	Cl	4	10	NO	NO	8.3	13-2	*Chryseobacterium indologenes*	98.8	0.45	GOOD IDENTIFICATION
12	Cl	25	2	NO	NO	9.5	1-1	*Enterobacter amnigenus*	92	0.5	DOUBTFUL PROFILE
12	Cl	25	5	NO	NO	8	6-4	*Unknown*		-	UNACCEPTABLE PROFILE
12	Cl	25	5	YES	N/A	5.8	29-2	*Sphingomonas paucimobilis*	99.8	0.72	VERY GOOD IDENTIFICATION
12	Cl	25	5	NO	N/A	6.4	29-1	*Pantoea spp*	44	0.55	LOW DISCRIMINATION
12	no Cl	4	5	NO	NO	8	6-5	*Aeromonas sobria*	82.2	0.86	EXCELLENT IDENTIFICATION TO THE GENUS
12	no Cl	4	18	NO	N/A	5.0	27-5	*Aeromonas spp*	74.5	0.38	LOW DISCRIMINATION
12	no Cl	4	10	NO	N/A	6.9	39-1	*Pantoea spp*	99.9	0.63	VERY GOOD IDENTIFICATION
12	no Cl	4	10	NO	N/A	5.8	39-2	*Pantoea spp*	99.9	0.5	VERY GOOD IDENTIFICATION
12	no Cl	25	5	NO	NO	7.7	12-2	*Serratia odorifera*	99.9	1	EXCELLENT IDENTIFICATION
12	no Cl	25	2	NO	NO	4.9	17-5	*Enterobacter cloacae*		-	UNACCEPTABLE PROFILE
12	no Cl	25	2	NO	N/A	11.7	17-3	*Enterobacter cloacae*	92.2	0.51	GOOD IDENTIFICATION
12	no Cl	25	2	NO	N/A	6.9	17-4	*Enterobacter cloacae*	91.5	0.83	GOOD IDENTIFICATION
12	no Cl	25	2	NO	N/A	11.3	40-3	*Unknown*		-	UNACCEPTABLE PROFILE
12	no Cl	32	2	YES	N/A	12.0	7-3	*Unknown*		-	UNACCEPTABLE PROFILE

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.13. Microbial isolates selected from iceberg lettuce at 32°C 'field temperature' that was precooled to 4°C for 10 hours and packaged for retail distribution.

Transportation Temp.	Cl/noCl	Store Temp.	Store Day	Acid Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	% ID	T index	Identification
4	Cl	25	2	NO	N/A	14.8	52-5	*Vibrio spp*	97.3	0.38	GOOD IDENTIFICATION
4	Cl	32	0	NO	N/A	11.8	54-5	*Serratia marcescens*	-		UNACCEPTABLE PROFILE
4	no Cl	4	10	NO	N/A	8.2	45-4	*Rhizobium radiobacter*	99.5	0.67	VERY GOOD IDENTIFICATION
4	no Cl	4	10	NO	N/A	14.2	46-2	*Pseudomonas luteola*	-		UNACCEPTABLE PROFILE
4	no Cl	4	18	NO	YES	18.2	57-2	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
4	no Cl	4	18	NO	N/A	11.4	57-3	*Pantoea spp*	-		UNACCEPTABLE PROFILE
4	no Cl	4	18	YES	YES	19.0	57-4	*Chromobacterium violaceum*	-		UNACCEPTABLE PROFILE
4	no Cl	25	5	YES	N/A	6.0	40-2	*Chryseobacterium indologenes*	-		UNACCEPTABLE PROFILE
4	no Cl	32	2	YES	N/A	11.5	43-4	*Yersinia spp*	85.4	0.49	LOW DISCRIMINATION
4	no Cl	32	2	YES	N/A	9.2	44-1	*Pasteurella spp*	87	0.47	DOUBTFUL PROFILE
4	no Cl	32	2	NO	N/A	8.1	43-5	*Aeromonas spp*	-		UNACCEPTABLE PROFILE
4	no Cl	32	2	NO	N/A	7.6	49-4	*Escherichia coli*	89.6	0.8	LOW DISCRIMINATION
12	no Cl	12	10	NO	N/A	9.3	42-5	*Psychrobacter phenylpyruvicus*	91.3	1	GOOD IDENTIFICATION
12	no Cl	4	10	NO	N/A	5.0	43-1	*Aeromonas sobria*	54.7	0.27	GOOD IDENTIFICATION TO THE GENUS
12	no Cl	25	2	NO	N/A	15.5	53-2	*Enterobacter gergoviae*	69.8	0.3	DOUBTFUL PROFILE

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.14. Microbial isolates selected from iceberg lettuce at 32°C 'field temperature' that was precooled and postcooled to 4°C for 10 hours and packaged for retail distribution.

Transportation Temp.	Cl/no Cl	Store Temp.	Store Day	ACID inhibition	Bacteriocins inhibition	Inhibition (mm)	Sample no.	Resulting ID	% ID	T index	Identification
4	Cl	25	5	NO	NO	13.7	48-4	*Pantoea spp*	-		UNACCEPTABLE PROFILE
4	Cl	25	5	NO	N/A	10.3	48-5	*Pantoea spp*	57.5	0.51	LOW DISCRIMINATION
4	No cl	4	-	NO	NO	10.0	47-2	*Escherichia coli*	-		UNACCEPTABLE PROFILE
4	no Cl	4	5	NO	YES	11.2	49-2	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
4	no Cl	4	5	NO	N/A	13.2	49-3	*Serratia odorifera*	99.2	0.23	ACCEPTABLE IDENTIFICATION
4	no Cl	25	2	NO	NO	7.2	42-1	*Pseudomonas luteola*	95.8	0.35	DOUBTFUL PROFILE
4	no Cl	25	2	NO	NO	9.1	55-3	*Serratia ficaria*	68.8	0.64	DOUBTFUL PROFILE
4	no Cl	25	2	NO	N/A	11.7	42-2	*Proteus mirabilis*	-		UNACCEPTABLE PROFILE
4	no Cl	25	5	NO	YES	13.5	49-1	*Klebsiella spp*	48.1	0.72	LOW DISCRIMINATION
12	no Cl	4	2	NO	N/A	15.8	41-1	*Raoultella ornithinolytica*	92	0.88	LOW DISCRIMINATION
12	no Cl	25	5	NO	N/A	9.4	48-3	*Serratia spp*	-		UNACCEPTABLE PROFILE
12	no Cl	32	2	NO	N/A	3.9	32-3	*Rahnella aquatilis*	78.4	1	LOW DISCRIMINATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.15. Microbial isolates selected from iceberg lettuce at 25°C 'field temperature' that was precooled for 10 hours and postcooled for 72 hours to 4°C and packaged for retail distribution.

Transportation Temp.	Cl/no Cl	Store Temp.	Store Day	ACID inhibition	Bacteriocins inhibition	Inhibition (mm)	Sample No.	Resulting ID	% ID	T index	Identification
4	Cl	25	2	NO	N/A	1.6	49-5	*Escherichia coli*	89.2	0.3	DOUBTFUL PROFILE
4	Cl	25	2	NO	N/A	8.2	51-5	*Aeromonas hydrophila/caviae*	88.9	0.51	ACCEPTABLE IDENTIFICATION
4	no Cl	4	2	NO	NO	14.6	54-4	*Escherichia coli*	89.2	0.3	DOUBTFUL PROFILE
4	no Cl	25	2	NO	NO	11.7	50-5	*Vibrio spp*	98.9	0.75	GOOD IDENTIFICATION
4	no Cl	25	0	NO	N/A	12.5	44-4	*Pantoea spp*	-		UNACCEPTABLE PROFILE
4	no Cl	25	0	NO	N/A	6.0	51-2	*Serratia plymuthica*	56.7	0.62	GOOD IDENTIFICATION TO THE GENUS
4	no Cl	32	2	YES	N/A	15.1	54-3	*Pasteurella spp*	74	0.21	ACCEPTABLE IDENTIFICATION TO THE GENUS
4	no Cl	32	2	NO	N/A	11.0	53-3	*Serratia odorifera*	-		UNACCEPTABLE PROFILE
4	no Cl	32	2	NO	N/A	14.0	54-2	*Stenotrophomonas maltophilia*	80.4	0.86	ACCEPTABLE IDENTIFICATION
12	Cl	4	18	NO	NO	9.8	26-4	*Aeromonas hydrophila/caviae*	74.5	0.38	LOW DISCRIMINATION
12	Cl	25	5	NO	NO	12.0	47-1	*Unknown*	81.3	0.28	LOW DISCRIMINATION
12	Cl	25	5	NO	N/A	13.8	46-3	*Pantoea spp*	99.8	0.3	GOOD IDENTIFICATION
12	Cl	25	5	NO	N/A	11.7	46-4	*Escherichia coli*	44.6	0.38	DOUBTFUL PROFILE
12	Cl	25	5	NO	N/A	14.5	46-5	*Pantoea spp*	57.5	0.51	LOW DISCRIMINATION
12	Cl	25	2	NO	N/A	16.5	51-4	*Escherichia coli*	87.6	0.3	DOUBTFUL PROFILE
12	no Cl	25	0	NO	N/A	8.8	42-3	*Serratia plymuthica*	92	0.58	GOOD IDENTIFICATION
12	no Cl	25	5	NO	N/A	13.7	48-2	*Pantoea spp*	-		UNACCEPTABLE PROFILE
12	no Cl	25	2	NO	N/A	12.6	50-1	*Aeromonas hydrophila/caviae*	96	0.76	LOW DISCRIMINATION
12	no Cl	25	2	NO	N/A	14.3	53-4	*Aeromonas hydrophila/caviae*	93.5	0.52	LOW DISCRIMINATION
12	no Cl	32	2	NO	N/A	9.8	50-4	*Aeromonas hydrophila/caviae*	95.2	0.47	GOOD IDENTIFICATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.16. Microbial isolates selected from baby spinach at 25°C 'field temperature' that was cooled to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Slide	Resulting ID	ID %	T index	Comment
Cl	4	2	NO	N/A	7.2	72-1	*Unknown*	82.6	0.42	DOUBTFUL PROFILE
Cl	4	2	NO	NO	10.8	14-3	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
Cl	4	10	YES	N/A	13.1	89-5	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	4	10	N/A	NO	8.4	28-1	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	25	2	YES	NO	9	5-1	*Escherichia coli 1*	89.6	0.3	DOUBTFUL PROFILE
Cl	25	2	YES	NO	8.8	29-5	*Burkholderia cepacia*			UNACCEPTABLE PROFILE
Cl	-	-	YES	NO	7.7	76-5	*Unknown*	49.7	0.72	LOW DISCRIMINATION
Cl	-	-	NO	NO	5.6	52-1	*Raoultella ornithinolytica*	75	0.1	DOUBTFUL PROFILE
noCl	4	2	NO	YES	10.4	40-3	*Ochrobactrum anthropi*	73.9	0.4	DOUBTFUL PROFILE
noCl	4	5	NO	NO	3.5	75-1	*Aeromonas spp*			UNACCEPTABLE PROFILE
noCl	4	5	NO	N/A	9.4	14-2	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
noCl	4	5	NO	NO	9.9	57-4	*Pantoea spp*	67.6	0.38	LOW DISCRIMINATION
noCl	4	5	YES	YES	8.7	9-3	*Burkholderia cepacia*	98.6	0.54	GOOD IDENTIFICATION
noCl	4	5	NO	NO	11.4	7-2	*Achromobacter xylosoxidans*	74.9	0.81	LOW DISCRIMINATION
noCl	4	5	NO	NO	8.1	9-2	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
noCl	25	2	YES	NO	13.1	28-2	*Aeromonas hydrophila/caviae*			UNACCEPTABLE PROFILE
noCl	-	-	YES	NO	11.7	36-3	*Kluyvera spp*	78.4	0.34	IDENTIFICATION NOT VALID
noCl	-	-	YES	NO	6.6	51-1	*Escherichia coli*			UNACCEPTABLE PROFILE
noCl	-	-	NO	NO	11.1	51-2	*Pantoea spp*	40.7	0.45	IDENTIFICATION NOT VALID
noCl	-	-	YES	NO	9.7	89-1	*Pantoea spp*			UNACCEPTABLE PROFILE
noCl	-	-	N/A	NO	7.3	6-3	*Serratia marcescens*	97.5	0.45	DOUBTFUL PROFILE
noCl	-	-	N/A	N/A	11.9	94-1	*Aeromonas hydrophila/caviae*	87.8	0.26	LOW DISCRIMINATION
-	-	-	NO	NO	10.2	58-1	*Pantoea spp*			UNACCEPTABLE PROFILE
-	-	-	NO	NO	9.1	63-3	*Pantoea spp*	99.8	0.96	VERY GOOD IDENTIFICATION
-	-	-	NO	NO	9.2	65-1	*Unknown*	49.7	0.72	LOW DISCRIMINATION

N/A - Test resulted in inconclusive results.
- Indicates sample was not exposed to any further treatment or storage.

Table 3.17. Microbial isolates selected from baby spinach at 25°C 'field temperature' that was postcooled for 10 hours to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Slide	Resulting ID	ID %	T index	Comment
Cl	4	2	N/A	NO	11.1	3-4	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
Cl	4	2	NO	YES	12.7	82-5	*Pantoea spp 1*	85.1	0.33	ACCEPTABLE IDENTIFICATION
Cl	4	2	YES	YES	12.4	2-4	*Pantoea spp 2*	40.7	0.45	IDENTIFICATION NOT VALID
Cl	4	2	YES	NO	12	7-4	*Pantoea spp 2*	42.2	0.95	LOW DISCRIMINATION
Cl	4	2	NO	NO	9.5	25-3	*Pantoea spp 2*	48.7	0.29	GOOD IDENTIFICATION TO THE GENUS
Cl	4	2	NO	NO	7.4	59-1	*Pseudomonas luteola*			UNACCEPTABLE PROFILE
Cl	4	2	NO	YES	6.4	52-4	*Pseudomonas luteola*	55.2	0.42	LOW DISCRIMINATION
Cl	4	2	YES	YES	10.5	2-2	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	4	5	YES	YES	6	80-3, 19-5	*Unkown*	56.9	0.9	LOW DISCRIMINATION
Cl	4	5	NO	NO	7.1	7-3	*Ralstonia pickettii*	45	0.85	LOW DISCRIMINATION
Cl	4	10	NO	NO	6	54-1	*Pantoea spp 2*	40.7	0.45	IDENTIFICATION NOT VALID
Cl	-	-	NO	NO	7.3	32-1	*Citrobacter freundii*	59.6	0.23	IDENTIFICATION NOT VALID
Cl	-	-	N/A	YES	7.9	55-5	*Unknown*			UNACCEPTABLE PROFILE
Cl	-	-	No	NO	7.6	32-2	*Pseudomonas luteola*			UNACCEPTABLE PROFILE
Cl	-	-	NO	NO	7	33-5	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION
Cl	-	-	NO	NO	11.9	33-3	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION
Cl	-	-	NO	YES	8.6	12-4	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION
noCl	4	2	NO	YES	8	69-2	*Unkown*	56.9	0.9	LOW DISCRIMINATION
noCl	4	2	NO	NO	8.5	57-1	*Pseudomonas oryzihabitans*	40	0.77	LOW DISCRIMINATION
noCl	4	5	YES	YES	10.6	24-4	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
noCl	-	-	N/A	YES	9.2	33-2	*Ochrobactrum anthropi*	73.9	0.4	DOUBTFUL PROFILE
noCl	-	-	No	NO	4.9	13-2	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
noCl	-	-	YES	NO	7.9	37-1	*Pseudomonas oryzihabitans*			UNACCEPTABLE PROFILE
noCl	-	-	No	NO	8.6	33-1	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION
noCl	-	-	YES	NO	9.4	12-2	*Burkholderia cepacia*	98.6	0.54	GOOD IDENTIFICATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.18. Microbial isolates selected from baby spinach at 25°C 'field temperature' that was postcooled for 72 hours to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	ID %	T index	Comment
Cl	4	2	NO	N/A	9.7	3-1	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	25	2	NO	NO	10.1	13-3	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
noCl	25	2	YES	NO	5.6	19-2	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
noCl	4	2	YES	NO	7.8	22-4	*Escherichia coli*	89.6	0.3	DOUBTFUL PROFILE
noCl	4	5	YES	YES	12	23-3	*Escherichia coli*	89.6	0.3	DOUBTFUL PROFILE
noCl	25	2	NO	YES	9.1	30-2	*Pantoea spp*	38.2	0.58	IDENTIFICATION NOT VALID
Cl	4	2	NO	YES	6.2	32-4	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION
noCl	4	5	NO	YES	11.7	34-5	*Enterobacter cloacae*			UNACCEPTABLE PROFILE
Cl	-	-	NO	NO	7.8	55-1	*Serratia rubidaea*	48.4	0.61	GOOD IDENTIFICATION TO THE GENUS
Cl	-	-	NO	NO	9.1	55-2	*Alcaligenes faecalis*	71.1	0.67	LOW DISCRIMINATION
noCl	25	2	NO	YES	8.9	65-2	*unknown*	85.7	0.39	DOUBTFUL PROFILE
Cl	-	-	YES	N/A	6.8	65-3	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	-	-	N/A	NO	5.8	65-5	*Escherichia coli*	89.2	0.3	DOUBTFUL PROFILE
noCl	4	5	NO	NO	6.4	67-3	*Pantoea spp*			UNACCEPTABLE PROFILE
Cl	4	10	NO	NO	7.2	67-5	*Pantoea spp*	40.7	0.45	IDENTIFICATION NOT VALID
Cl	4	10	NO	YES	7.4	73-1	*Unknown*	49.7	0.72	LOW DISCRIMINATION
noCl	4	5	YES	NO	8	76-1	*Escherichia coli*	89.6	0.8	LOW DISCRIMINATION
Cl	4	5	YES	YES	7.9	79-1	*Unknown*	56.9	0.9	LOW DISCRIMINATION
Cl	4	5	YES	NO	10.2	92-1	*Klebsiella pneumoniae ssp pneumoniae*	48.1	0.72	LOW DISCRIMINATION
-	-	-	YES	NO	11.8	90-3	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.19. Microbial isolates selected from baby spinach at 25°C 'field temperature' that was precooled for 10 hours to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	ID %	T index	Comment
Cl	4	2	YES	NO	5.7	21-5	*Stenotrophomonas maltophilia*	51.3	0.1	DOUBTFUL PROFILE
Cl	4	5	NO	NO	8.9	8-2	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	4	10	NO	NO	5.7	36-1	*Serratia plymuthica*	66.4	0.27	DOUBTFUL PROFILE
Cl	4	10	NO	NO	12.8	66-4	*Escherichia coli*	90.5	0.24	ACCEPTABLE IDENTIFICATION
Cl	4	10	N/A	NO	5.1	4-2	*Escherichia coli*	89.6	0.3	DOUBTFUL PROFILE
Cl	4	10	NO	NO	8.2	54-4	*Klebsiella pneumoniae ssp ozaenae*			UNACCEPTABLE PROFILE
Cl	4	10	NO	NO	9.3	54-3	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
Cl	4	10	NO	NO	8.8	91-3	*Pantoea spp*	70.3	0.81	VERY GOOD IDENTIFICATION TO THE GENUS
Cl	4	10	NO	NO	7.9	38-4	*Pseudomonas oryzihabitans*	47.4	0.42	DOUBTFUL PROFILE
Cl	4	10	NO	YES	4.9	57-3	*Yersinia enterocolitica*	80.3	0.48	LOW DISCRIMINATION
Cl	-	-	N/A	YES	9.7	38-1	*Ochrobactrum anthropi*	88.6	0.27	ACCEPTABLE IDENTIFICATION
Cl	-	-	YES	NO	10.6	34-3	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION
noCl	4	2	N/A	YES	11.1	38-5	*Pantoea spp*	44.3	0.17	DOUBTFUL PROFILE
noCl	4	5	NO	NO	5	53-2	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
noCl	4	5	NO	NO	13.5	2-3	*Pantoea spp*	40.7	0.45	IDENTIFICATION NOT VALID
noCl	4	10	NO	NO	6.3	80-2	*Ochrobactrum anthropi*	73.9	0.4	DOUBTFUL PROFILE
noCl	4	10	NO	N/A	5.8	68-2	*Pantoea spp*	50.9	0.34	IDENTIFICATION NOT VALID
noCl	4	10	NO	YES	10	68-1	*Unknown*	56.9	0.9	LOW DISCRIMINATION
-	-	-	NO	YES	8.5	55-3	*Pseudomonas luteola*	54.3	0.6	LOW DISCRIMINATION
-	-	-	NO	YES	6.7	79-2	*Pseudomonas oryzihabitans*	40	0.77	LOW DISCRIMINATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.20. Microbial isolates selected from baby spinach at 25°C 'field temperature' that was pre- and postcooled for 10 hours to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	ID %	T index	Comment
Cl	4	2	NO	YES	11.3	70-5	*Ochrobactrum anthropi*	73.9	0.4	DOUBTFUL PROFILE
Cl	4	5	NO	NO	7.3	24-2	*Escherichia coli*	98.9	0.47	GOOD IDENTIFICATION
Cl	4	5	YES	NO	6.6	24-3	*Escherichia coli*	89.6	0.3	DOUBTFUL PROFILE
Cl	4	5	NO	YES	6	26-1	*Klebsiella oxytoca*	74.8	0.33	DOUBTFUL PROFILE
Cl	4	5	YES	YES	10.9	73-4	*Unknown*	56.9	0.9	LOW DISCRIMINATION
Cl	4	10	N/A	N/A	12.6	81-3	*Escherichia coli*	89.6	0.8	LOW DISCRIMINATION
Cl	4	10	NO	YES	10.3	81-4	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
Cl	4	10	NO	YES	9.4	58-3	*Pantoea spp*	99.4	0.3	GOOD IDENTIFICATION
Cl	4	10	NO	NO	10.9	76-4	*Unknown*	56.9	0.9	LOW DISCRIMINATION
Cl	4	10	NO	YES	8.6	20-4	*Burkholderia cepacia*	99.9	0.72	VERY GOOD IDENTIFICATION
Cl	4	10	NO	NO	8.5	19-1	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION
Cl	4	10	NO	NO	6.7	20-3	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION
Cl	4	10	NO	NO	12.6	6-2	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	4	10	NO	NO	9	20-5	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	25	2	NO	NO	11.4	49-3	*Klebsiella spp*	38.3	0.22	DOUBTFUL PROFILE
Cl	-	-	NO	YES	10.3	31-3	*Pantoea spp*	-	-	UNACCEPTABLE PROFILE
Cl	-	-	NO	NO	13	66-1	*Escherichia coli*	95.6	0.72	GOOD IDENTIFICATION
Cl	-	-	NO	YES	9.3	37-3	*Ochrobactrum anthropi*	49.9	0.52	IDENTIFICATION NOT VALID
Cl	-	-	NO	NO	10.3	31-4	*Burkholderia cepacia*	95	0.46	GOOD IDENTIFICATION
Cl	-	-	NO	YES	7.9	56-4	*Alcaligenes faecalis*	71.1	0.67	LOW DISCRIMINATION
Cl	-	-	NO	YES	9.5	31-1	*Delftia acidovorans*	99.9	0.5	DOUBTFUL PROFILE
Cl	-	-	NO	NO	13.1	27-3	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
noCl	4	2	NO	NO	4.3	29-1	*Burkholderia cepacia*	97.5	0.47	GOOD IDENTIFICATION
noCl	4	10	YES	NO	10.2	81-5	*Unknown*	99	0.22	DOUBTFUL PROFILE
noCl	4	10	NO	NO	9.2	53-1	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
noCl	4	10	NO	NO	9.1	91-5	*Pantoea spp*	94.9	0.87	GOOD IDENTIFICATION
noCl	25	2	NO	N/A	7.3	39-4	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
noCl	-	-	NO	NO	7.4	56-5	*Pantoea spp*	95.5	0.35	GOOD IDENTIFICATION
noCl	-	-	NO	YES	8.9	55-4	*Ewingella americana*	80.4	0.47	LOW DISCRIMINATION
noCl	-	-	NO	YES	9.5	37-2,55-5	*Burkholderia cepacia*	99.4	0.6	VERY GOOD IDENTIFICATION
noCl	-	-	NO	NO	11.7	28-4	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION

- Indicates sample was not exposed to any further treatment or storage. N/A - Test resulted in inconclusive results.

Table 3.21. Microbial isolates selected from baby spinach at 25°C 'field temperature' that was precooled for 10 hours and postcooled for 72 hours to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	Acid Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	ID %	T index	Comment
Cl	4	2	NO	NO	10.3	25-2	*Escherichia coli*	-	-	UNACCEPTABLE PROFILE
Cl	4	2	N/A	YES	12	25-1, 10-1	*Unknown*	-	-	UNACCEPTABLE PROFILE
Cl	4	5	NO	YES	8.4	15-3	*Pantoea spp*	40.7	0.45	IDENTIFICATION NOT VALID
Cl	4	5	NO	YES	8.3	84-5	*Pantoea spp*	99.2	1	VERY GOOD IDENTIFICATION
Cl	4	10	YES	YES	9.3	56-1, 89-4	*Pantoea spp*	58.3	0.4	GOOD IDENTIFICATION TO THE GENUS
Cl	4	10	NO	NO	8.3	66-3	*Ochrobactrum anthropi*	88.6	0.27	ACCEPTABLE IDENTIFICATION
Cl	4	10	NO	NO	9.6	56-2, 58-5	*Pantoea spp*	67.6	0.38	LOW DISCRIMINATION
Cl	4	10	NO	YES	8	39-1,89-3	*Pantoea spp*	56.8	0.63	IDENTIFICATION NOT VALID
Cl	4	10	NO	YES	10.9	39-2, 58-4	*Serratia ficaria*	48	0.4	DOUBTFUL PROFILE
Cl	25	2	N/A	NO	10.5	75-4	*Kluyvera spp*	77.7	0.14	IDENTIFICATION NOT VALID
Cl	-	-	NO	YES	11.2	43-5	*Pantoea spp*	94.4	0.2	DOUBTFUL PROFILE
noCl	4	2	NO	NO	8	80-4	*Unknown*	56.9	0.9	LOW DISCRIMINATION
noCl	4	2	NO	NO	11.8	27-1	*Escherichia coli*	89.6	0.3	DOUBTFUL PROFILE
noCl	4	2	NO	NO	9.7	22-1	*Escherichia coli*	94	0.64	LOW DISCRIMINATION
noCl	4	2	NO	NO	8.2	24-1	*Escherichia coli*	89.6	0.3	DOUBTFUL PROFILE
noCl	4	2	NO	NO	11.3	22-2	*Escherichia coli*	89.6	0.3	DOUBTFUL PROFILE
noCl	4	2	N/A	NO	8.6	27-2	*Pantoea spp*	40.7	0.45	IDENTIFICATION NOT VALID
noCl	4	2	NO	NO	9.7	80-5	*Unknown*	49.7	0.72	LOW DISCRIMINATION
noCl	4	2	NO	NO	8.8	6-4	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
noCl	4	5	NO	NO	4.1	21-1	*Burkholderia cepacia*	97.5	0.47	GOOD IDENTIFICATION
noCl	4	10	NO	NO	11	23-4	*Escherichia coli*	98.8	0.47	GOOD IDENTIFICATION
noCl	4	10	NO	NO	13.6	43-2	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
noCl	4	10	NO	YES	6.6	19-3	*Achromobacter xylosoxidans*	-	-	UNACCEPTABLE PROFILE
noCl	25	2	NO	NO	9.1	35-4	*Escherichia coli*	94	0.64	LOW DISCRIMINATION
noCl	25	2	NO	NO	11.6	11-3	*Pantoea spp*	94.3	0.37	DOUBTFUL PROFILE
noCl	25	2	NO	NO	8.8	4-4	*Pseudomonas putida*	99.6	0.46	DOUBTFUL PROFILE
-	-	-	NO	YES	12	42-1	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
-	-	-	NO	NO	10.2	42-5	*Klebsiella pneumoniae ssp pneumoniae*	38.3	0.22	DOUBTFUL PROFILE
-	-	-	NO	NO	11.6	42-4	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
-	-	-	YES	YES	10.5	24-5	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION

- Indicates sample was not exposed to any further treatment or storage. ` N/A - Test resulted in inconclusive results.

Table 3.22. Microbial isolates selected from baby spinach at 32°C 'field temperature' that was cooled to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Sample No.	Resulting ID	ID %	T index	Comment
Cl	25	2	YES	NO	7.8	49-2	*Escherichia coli*	-	-	UNACCEPTABLE PROFILE
Cl	25	2	NO	NO	6.3	85-2	*Escherichia coli*	89.6	0.8	LOW DISCRIMINATION
Cl	4	2	NO	NO	6.4	85-1	*Klebsiella pneumoniae ssp ozaenae*	48.8	0.9	IDENTIFICATION NOT VALID
Cl	4	10	YES	NO	5.8	60-2	*Kluyvera spp*	-	-	UNACCEPTABLE PROFILE
Cl	4	5	YES	YES	8	84-3	*Pantoea spp*	56.8	0.63	IDENTIFICATION NOT VALID
Cl	25	2	NO	N/A	7.7	84-1	*Serratia plymuthica*	50.9	0.79	LOW DISCRIMINATION
Cl	4	10	YES	YES	6.8	62-1	*Serratia plymuthica*	-	-	UNACCEPTABLE PROFILE
Cl	25	2	NO	N/A	12.6	85-3	*Burkholderia cepacia*	99.7	0.22	DOUBTFUL PROFILE
noCl	4	5	YES	NO	6.1	61-2	*Escherichia coli*	97.7	0.84	GOOD IDENTIFICATION
noCl	4	2	NO	YES	10.7	93-5	*Klebsiella pneumoniae ssp pneumoniae*	48.1	0.72	LOW DISCRIMINATION
noCl	-	-	NO	YES	9	82-3	*Unknown*	98.6	0.62	GOOD IDENTIFICATION
noCl	-	-	NO	YES	11.1	82-2	*Providencia stuartii*	96.8	0.42	GOOD IDENTIFICATION
noCl	4	5	YES	YES	6.4	62-2	*Pseudomonas luteola*	98.1	0.49	GOOD IDENTIFICATION
noCl	25	2	NO	NO	6	47-1	*Serratia plymuthica*	40.3	0.32	DOUBTFUL PROFILE
noCl	4	5	YES	YES	11.1	41-1	*Serratia liquefaciens*	-	-	UNACCEPTABLE PROFILE
-	-	-	N/A	NO	9.6	60-3	*Enterobacter sakazakii*		-	UNACCEPTABLE PROFILE
-	-	-	NO	YES	7.8	8-1	*Ralstonia pickettii*	45	0.85	LOW DISCRIMINATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.23. Microbial isolates selected from baby spinach at 32°C 'field temperature' that was postcooled for 10 hours to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	ACID Inhibition	Bacteriocins Inhibition	Inhibiton (mm)	Sample No.	Resulting ID	ID %	T index	Comment
Cl	4	2	NO	NO	7.5	78-4	*Pantoea spp*	50.6	0.44	GOOD IDENTIFICATION TO THE GENUS
Cl	4	2	YES	YES	9	78-3	*Unknown*	49.7	0.72	LOW DISCRIMINATION
Cl	4	5	NO	NO	7.7	16-3	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	25	2	YES	NO	10.2	16-1	*Aeromonas hydrophila/caviae*		-	UNACCEPTABLE PROFILE
Cl	25	2	YES	NO	11.2	63-4	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
Cl	-	-	NO	YES	10.3	81-2	*Klebsiella pneumoniae ssp pneumoniae*	48.1	0.72	LOW DISCRIMINATION
noCl	4	2	NO	YES	10.9	78-2	*Unknown*	49.7	0.72	LOW DISCRIMINATION
noCl	4	2	NO	NO	10.1	78-1	*Pantoea spp*	42.2	0.95	LOW DISCRIMINATION
noCl	4	5	NO	YES	7.4	8-4	*Pantoea spp*	89.9	0.18	DOUBTFUL PROFILE
noCl	4	5	YES	NO	10.7	18-3	*Burkholderia cepacia*	-	-	UNACCEPTABLE PROFILE
noCl	25	2	YES	NO	6.7	50-2	*Burkholderia cepacia*	-	-	UNACCEPTABLE PROFILE

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.24. Microbial isolates selected from baby spinach at 32°C 'field temperature' that was postcooled for 72 hours to 4°C and packaged for retail distribution.

Cl/noCl	Store Temp.	Store Day	Acid Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Slide	Resulting ID	ID %	T index	Comment
Cl	4	2	NO	NO	5.2	47-4	*Klebsiella pneumoniae ssp rhinoscleromatis*	98.9	0.84	GOOD IDENTIFICATION
Cl	4	2	YES	YES	8.1	47-2	*Klebsiella pneumoniae ssp pneumoniae*	44.8	0.52	DOUBTFUL PROFILE
Cl	4	2	NO	YES	7.5	47-3	*Klebsiella pneumoniae ssp pneumoniae*	98.7	0.3	DOUBTFUL PROFILE
Cl	4	5	NO	NO	6.7	90-5	*Klebsiella oxytoca*	75.8	0.17	DOUBTFUL PROFILE
Cl	4	10	YES	NO	4.9	12-3	*Burkholderia cepacia*	98.6	0.54	GOOD IDENTIFICATION
Cl	25	2	YES	NO	10.1	86-1	*Kluyvera spp*	99.3	0.14	DOUBTFUL PROFILE
Cl	-	-	YES	NO	6.3	51-3	*Escherichia coli*	89.6	0.3	DOUBTFUL PROFILE
noCl	4	5	YES	YES	7.1	43-1	*Aeromonas hydrophila/caviae*	87.8	0.26	LOW DISCRIMINATION

- Indicates sample was not exposed to any further treatment or storage.
N/A - Test resulted in inconclusive results.

Table 3.25 Microbial isolates selected from baby spinach at 32°C 'field temperature' that was precooled for 10 hours to 4°C and packaged for retail distribution.

Post-cool (h)	Cl/noCl	Store Temp.(°C)	Store Day	Acid Inhibition	Bacteriocins Inhibition	Inhibition (mm)	Slide	Resulting ID	ID %	T index	Comment
0	Cl	4	2	NO	NO	9.5	63-5	*Pseudomonas putida*	95.1	0.69	GOOD IDENTIFICATION
0	Cl	-	-	NO	NO	7.2	41-5	*Escherichia coli*	-	-	UNACCEPTABLE PROFILE
0	Cl	-	-	YES	NO	7.5	58-2	*Pseudomonas luteola*		-	UNACCEPTABLE PROFILE
10	Cl	4	10	YES	NO	10.5	44-1	*Pantoea spp*	59.5	0.33	DOUBTFUL PROFILE
10	Cl	4	2	NO	YES	9.8	70-3	*Pantoea spp*	40.7	0.45	IDENTIFICATION NOT VALID
10	Cl	4	10	NO	YES	10	43-4, 44-5	*Pseudomonas luteola*		-	UNACCEPTABLE PROFILE
10	-	-	-	NO	NO	10.1	85-5	*Pseudomonas putida*	99.6	0.96	VERY GOOD IDENTIFICATION
72	Cl	-	-	NO	NO	8.6	46-5	*Aeromonas spp*		-	UNACCEPTABLE PROFILE
72	noCl	-	-	NO	YES	9.5	59-2	*Unknown*	82.6	0.42	DOUBTFUL PROFILE
-	-	-	-	NO	YES	10.4	60-5	*Unknown*	56.9	0.9	LOW DISCRIMINATION

- Indicates sample was not exposed to any further treatment or storage. Also indicates ID%
N/A - Test resulted in inconclusive results.

Figure 3.1 Lettuce Diagram for sampling per replication.

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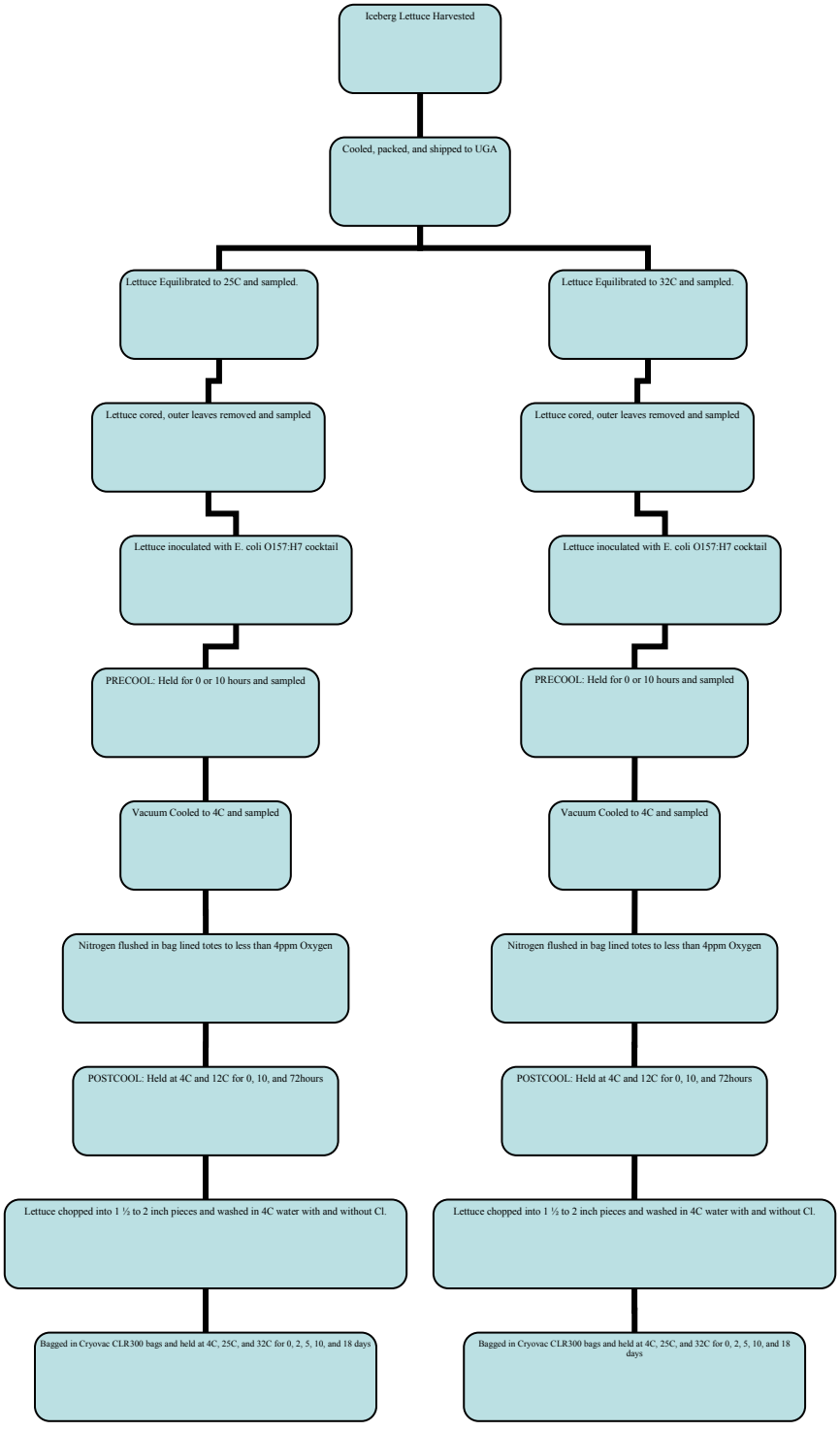

Figure 3.2 Spinach Diagram for Sampling per replication