ANTAGONISTIC ACTION OF CELLS OF *LACTOBACILLUS DELBRUECKII* SSP. *LACTIS* TOWARD PATHOGENIC MICROORGANISMS IN FRESH MEAT SYSTEMS

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1995

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1997

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2000

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ACKNOWLEDGMENTS

I would like to express my sincere appreciation to all those who have contributed to my doctoral education at Oklahoma State University. I feel especially privileged to have had the opportunity to work with Dr. Stanley E. Gilliland as my major professor. I would like to thank Dr. Gilliland for his patience, encouragement, guidance, knowledgeable insight and friendship. Thank you for enabling me the opportunity to work and learn from you!

I would like to thank my committee members: Dr. Danielle Bellmer, Dr. William McGlynn and Dr. David Lalman. I thank you all for your valuable suggestions and assistance.

I would also like to extend my sincere appreciation to Jennifer Nangle for all of her friendship and enormous help throughout my research project. A special thanks to Erin, Amanda, Eric, J.R., Paloma, Trena and Kelly for all of their help and suggestions in the lab.

I am especially grateful to my wonderful husband Brandon and my wonderful family - Mom, Dad, Stan, Amy and Collin for all of their love, encouragement, patience and support. Thank you all for being there for me and believing in me!

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CHAPTER I

INTRODUCTION

Concerns over possible foodborne illness caused by consumption of contaminated improperly cooked meat has considerably heightened the public's awareness of foodborne illness after the 1992 foodborne illness outbreak on the West Coast. This outbreak was linked to *Escherichia coli* O157:H7 that was found in undercooked ground beef from a fast food chain. This organism has been identified as a rare serotype of an enterohemorrhagic *E. coli* which caused abdominal cramps, bloody diarrhea, and in some cases hemolytic uremic syndrome (Karmaili, 1989). The seriousness of the hemolytic uremic syndrome caused by *E. coli* O157:H7 (which was found to occur in young children and the elderly) caused fatalities in 3 to 5% of the cases (Weagant et al., 1994). Unlike other pathogens that can cause food borne illness, *E. coli* O157:H7 was found to be unique not only because it was potentially fatal but because the amount ingested to acquire the illness was less than 10 cells (Doyle and Schoeni, 1984).

Another pathogen of concern is *Salmonella*. *Salmonella typhimurium* is the most common found foodborne serovar found throughout the world (Hobbs, 1974). Although symptoms from illness due to *Salmonella* are not as severe or as potentially deadly as illness from *E. coli* O157:H7, *Salmonella* is considered a common cause for food borne illness (CDC,

1989). While most of the microflora transferred to carcasses during the slaughtering process are nonpathogenic, pathogens such as *Salmonella typhimurium* and *E. coli* O157:H7 can be present (Dickson and Anderson, 1992; Hardin et al., 1995).

Lactic Acid Bacteria (LAB) have been involved in food preservation dating back to biblical times. They belong to a large ubiquitous group of Gram positive rod or coccus shaped microorganisms, lack the ability to produce catalase, have the ability to ferment various carbohydrates, are facultative or microaerophilic, and have the ability to produce lactic acid from hexoses. Lactic acid bacteria also have the ability to produce various antimicrobial compounds such as lactic acid, acetic acid, diacetyl, carbon dioxide, reuterin, bacteriocins, and hydrogen peroxide (Daeschel, 1989). Of the mentioned inhibitory substances produced by lactic acid bacteria, hydrogen peroxide is the substance toward which we focused our research efforts. Because of their ability to produce hydrogen peroxide at refrigerated temperatures (Gilliland, 1980) lactic acid bacteria offer an alternative intervention for control of undesirable microorganisms in refrigerated food products. This is especially true if they can exert the preservative action without altering the desirable characteristics of the particular food product in which they are added to.

Lactic acid bacteria can exert antagonistic action toward the growth of spoilage and even pathogenic organisms in nonfermented foods at refrigerated temperatures (Daly et al., 1972; Gilliland and Speck, 1975;

Juffs and Babel, 1975; Gilliland, 1980; Martin and Gilliland, 1980; Gilliland and Ewell, 1983; Brashears et al., 1998). This antagonistic action toward spoilage and pathogenic organisms in both fermented and nonfermented foods has been attributed to the ability of the LAB to produce hydrogen peroxide at refrigerated temperatures.

The primary purpose of this study was to determine if a selected strain of *Lactobacillus delbrueckii* subsp. *lactis* could produce an antagonistic action toward food borne pathogens specifically *E. coli* O157:H7 and *S. typhimurium* in various fresh meat systems during refrigerated storage.

CHAPTER II

LITERATURE REVIEW

Preservation of Food by Lactic Acid Bacteria

For hundreds of years lactic acid bacteria have been used in the preservation of food. Dating back to early biblical times (Gilliland, 1985) foods were stored under conditions that resulted in changes to produce different foods with enhanced keeping qualities with a unique flavor and organoleptic characteristics. Over the years it was discovered that microorganisms (specifically lactic acid bacteria) were responsible for these observed changes. The changes imparted in food products by lactic acid bacteria were due to the fermentation of substrates available in the raw food products by the microorganisms.

The fermentation occurring in these food products is a metabolic process by which carbohydrates and related compounds are fermented with the release of energy in the absence of any external electron acceptors. The final electron acceptors are organic compounds produced directly from the breakdown of carbohydrates. As a result of this, only partial oxidation of the parent compound can occur and a small percentage of energy is released during this process (Gregory, 1974). Because lactic acid bacteria lack functional heme-linked electron transport systems or cytochromes, they have to obtain their energy needs

by substrate-level phosphorylation associated with the fermentation of carbohydrates.

This food fermentation or preservation by lactic acid fermentation is a process that changes the flavor and organoleptic characteristics of a product while considerably extending the shelf-life over that of the raw materials from which the product was originally made. In some instances the vitamin content of the fermented food is increased along with the resulting food being more digestible than the raw materials (Steinkraus, 1986; Jay, 1992). Kneifel et al. (1989) reported that dairy foods fermented with lactic acid bacteria increased concentrations of thiamin, pyridoxine, folic acid and biotin in the product by more than 20%. The primary preservation action that results from fermentation is a reduction in pH and in some cases removal of carbohydrates which would otherwise be used by other microorganisms (Kashet, 1987).

The lactic acid bacteria mentioned above belong to a large ubiquitous group of Gram positive rod or coccus shaped microorganisms. Lactic acid bacteria include the genera *Lactobacillus, Leuconostoc, Streptococus,* and *Pediococcus.* All members of these genera can be characterized by Gram reaction, the lack of ability to produce catalase, the lack of cytochromes, the ability to ferment various carbohydrates, facultative or microaerophilic metabolism, non-sporulating, inability to reduce nitrate, and the ability to produce lactic acid from hexoses. Some lactic acid bacteria are homofermentative while others are heterofermentative.

The metabolism of most sugars by homofermentative (homolactic) bacteria involves glycolysis resulting in almost exclusively lactic acid as an end product. When glucose is metabolized a homofermentative pattern may be observed, but not necessarily when pentoses are metabolized, because some homolactics produce both acetic and lactic acids when utilizing pentoses (Brown and Collins, 1977). London (1976) noted that the homofermentative character of homolactics may be shifted for some strains by altering conditions such as glucose concentration, pH, and nutrient limitation.

Heterofermentative lactic acid bacteria (heterolactics) are those that produce a mixture of end products from the fermentation of hexoses. The heterolactic metabolism involves the 6-phosphogluconate / phosphoketolase pathway and results in significant amounts of other end-products such as ethanol, acetate, and carbon dioxide in addition to lactic acid. Some heterolactics are known for producing acetylaldehyde and diacetyl, which are unique flavor and aroma components in some cultured foods. While the heterolactics are known for their flavor components, it has been noted that homolactics are able to extract about twice as much energy from a given amount of glucose that heterolactics (Forrest and Walker, 1971).

Other general characteristics of both homofermentative and heterofermentative lactic acid bacteria are their growth requirements. All lactic acid bacteria require preformed amino acids, B vitamins, and

purine and pyrimidine bases (Kandler, 1983). Most lactic acid bacteria are mesophilic with optimum growth around 37°C, however, some can grow below 5°C and others as high as 45°C. There is also quite a range when it comes to the influence of pH, some lactic acid bacteria can grow as low as pH 3.2, others as high as pH 9.6. Stamer (1976) noted that lactic acid bacteria exhibit only weak or no proteolytic and lipolytic activities. Thus from a taxonomic standpoint most of them are considered nonproteolytic and nonlipolytic.

Preservation Actions of Lactic Acid Bacteria

The lactic acid that is produced during lactic acid bacteria fermentation serves as an antagonist to other competing microflora by lowering the pH of the surrounding environment. This acidic environment allows the lactic acid bacteria to effectively compete and ultimately dominate fermenting ecosystems. Because of a higher acid tolerance lactic acid bacteria can out compete many pathogenic and spoilage microorganisms. Lactic acid bacteria can also produce some antimicrobial compounds other than lactic acid which can inhibit a variety of organisms. Some of these antimicrobial compounds are acetic acid, diacetyl, carbon dioxide, reuterin, bacteriocins, and hydrogen peroxide (Daeschel, 1989).

Acetic acid is produced primarily by heterofermentative lactic acid bacteria in equimolar amounts to lactic acid. Of the two acids (lactic and

acetic), acetic is the strongest inhibitor and has a wide range of inhibitory activity preventing the growth of yeasts, molds, and bacteria (Blom and Mortvedt, 1991). Acetic acid has a pK_a of 4.75, which is higher than that of lactic acid (pK_a of 3.08). Eklund, (1983) reported that at pH 4, only 11% of the lactic acid is undissociated and about 85% of acetic acid is undissociated, supporting the fact that acetic acid is a stronger inhibitor toward microorganisms than lactic acid at equal molar concentrations. Pinheiro et al (1968) reported that the primary inhibitory effect produced by leuconostocs was due to acetic acid. This finding implies that an extended shelf-life can be expected in products were acetic acid is a component or end product, due to the inhibition of psychrotrophs by the acetic acid. However, in a study conducted by Rubin (1978) it was observed that a mixture of lactic and acetic acid reduced the growth rate of *Salmonella typhimurium* more than either acid alone, suggesting a synergistic activity. Kandler (1983) found that under specific conditions of hexose limitation and availability of oxygen, homofermentative lactic acid bacteria may decompose lactic acid to acetic acid, formic acid, and carbon dioxide.

Diacetyl (2,3-butanedione) is a metabolic end product when citrate is metabolized via pyruvate into diacetyl (Lindgren and Dobrogosz, 1990). Diacetyl is noted as the aroma and flavor associated with butter and the "buttery" attributes in cultured dairy products. Jay (1982) reported that diacetyl was inhibitory to both pathogens and spoilage microorganisms.

Jay noted that diacetyl was more effective at pH <7 and that it's antimicrobial activity was antagonized by the presence of glucose, acetate, and Tween 80. Diacetyl was found to be more active against Gram negative bacteria, yeasts and molds than Gram positive bacteria and lactic acid bacteria were the least sensitive (Jay, 1982). Jay also notes that diacetyl is thought to react with the arginine binding protein of Gram negative bacteria and thereby interfering with the utilization of arginine.

Carbon dioxide is produced during the heterfermentative lactic acid fermentation of hexoses. The production of carbon dioxide contributes to a reduced Eh and is directly toxic to a number of putrefactive aerobic bacteria. Lindgren and Dobrogosz, (1990) reported that carbon dioxide formation helps create an anaerobic environment and the carbon dioxide in itself has an antimicrobial activity. The mechanism of this activity is unknown, however, King and Nagel (1975) suggest that it is because enzymatic decarboxylations are inhibited. Lindgren and Dobrogosz (1990) also suggested that the accumulation of carbon dioxide in the lipid bilayer causes dysfunction in permeability, and mention that at low concentrations carbon dioxide can stimulate the growth of some organisms, while at higher concentrations it can prevent growth.

Reuterin (3-hydroxypropionaldehyde) is produced by *Lactobacillus reuteri* (Axelsson et al., 1989). It is a low molecular weight, nonproteinaceous, highly soluble, pH neutral product produced by *L.*

reuteri from glycerin in the presence of coenzyme B_{12}. During the log phase, no reuterin is produced, however, when the cells enter the stationary phase, it begins to accumulate (Axelsson et al., 1989). Numerous researchers have found reuterin to have anti-bacterial, anti-fungal, anti-protozaoal and anti-viral activity (Axelsson et al, 1989; Chung et al., 1989; and Dobrogosz et al., 1989). Reuterin is thought to have such a broad anti-microbial activity because of its action against sulfhydryl enzymes and its inhibition of ribonucleotide reductase. Dobrogosz and coworkers (1989) reported reuterin to be an inhibitor of the substrate binding subunit of ribonucleotide reductase, which will directly interfere with DNA synthesis.

Bacteriocins have been defined by numerous researchers as antagonistic substances produced by lactic acid bacteria and are typically proteins that work against the same, or closely related species by adsorption to receptors on the target cells (Jack et al., 1994; de Vos et al., 1995; Veneman et al., 1995;Klaenhammer et al.,1992; Tag et al., 1976; and de Klerck and Smit, 1967). Bacteriocins are usually categorized into three classes, however, in 1993, Klaenhammer (1993) suggested a fourth class. The four classes of bacteriocins are as follows: Class I – lantibiotics; Class II – small (<10kDa), moderate (100°C) to high (121°C) heat stable, nonlanthionine containing membrane active peptides; Class III – large (>30 kDa) heat labile proteins; and Class IV –

complex bacteriocins which are proteins complexed lipid and/or carbohydrate.

The action of most bacteriocins is a two step process. The first step involves the adsorption of the bacteriocin to specific or nonspecific receptors on the target cell surface. During this first stage, the bacteriocin is still sensitive to the action of proteolytic enzymes. The second step involves the creation of pores in the membrane, and/or an efflux of cytoplasmic constituents. Because of this two step process bacteriocins of lactic acid bacteria have been the subject of intensive studies in recent years with regard to their acceptability and potential for biopreservation. Schillinger and Lucke, (1989) have investigated the antibacterial activity of *Lactobacillus sake* isolated from meat and found it to be due to a bacteriocin which is bactericidal. Other researchers have found the antimicrobial effect of bacteriocins to be bacteriostatic, specifically those of a glycoprotein nature (Lewus et al., 1992).

The most widely studied bacteriocins have been the colicins produced by some strains of *Escherichia coli*. The colicins have been well characterized as to their mode of action, host range, genetics, purification and ecological function (Daeschel, 1989). Even though the colicins have been thoroughly studied, the bacteriocin nisin is the best known and studied of the bacteriocins produced by lactic acid bacteria. Nisin, produced by some streptococci, is considered a class I bacteriocin and has a broad spectrum of activity against Gram-positive bacteria.

Research has shown that Gram- negative bacteria are only affected by nisin when their outer membranes are sublethally damaged (Ray, 1993; Sahl et al., 1995; and Venema et al.). Nisin is believed to target the cell membrane, and for its interaction to occur with the cell membrane it does not need any receptor, but it does need the presence of a membrane potential (Sahl et al., 1987; and Bruno and Montville, 1993).

Hydrogen Peroxide Production

Of the mentioned inhibitory substances produced by the lactic acid bacteria, hydrogen peroxide is another one that has been studied quite extensively. A number of lactic acid bacteria have the ability to produce hydrogen peroxide. Some strains produce more hydrogen peroxide than others (Gilliland and Speck, 1969). Of the lactobacilli, *Lactobacillus delbrueckeii* subspecies *lactis* tends to produce the highest amounts of hydrogen peroxide at refrigeration temperatures without growing or changing the pH of the medium (Gilliland, 1980).

Hydrogen peroxide can be formed by lactic acid bacteria in the presence of molecular oxygen during the production of lactate, pyruvate and NADH by flavin enzymes (Kandler, 1983). To be more specific, the hydrogen peroxide is produced by direct reduction of atmospheric oxygen catalyzed by a small number of flavoprotein oxidase enzymes, such as H_2O_2 forming NADH oxidase, hydrogen peroxide forming NADH oxidase, pyruvate oxidase, α-glycerolphosphate oxidase, lactate oxidase, and

NAD-independent lactic dehydrogenase (Condon, 1987; Esders et al, 1979; and Kandler, 1983). Condon (1987), reports that it is through the oxidase enzymes and NADH peroxidase, which are hydrogen peroxide scavengers, that O_2 and hydrogen peroxide can accept electrons from sugar metabolism, and as a result have a sparing effect on the use of metabolic intermediates, such as pyruvate or acetaldehyde as electron acceptors. Since lactic acid bacteria do not possess heme, they will not produce catalase and they do not utilize the cytochrome system, which reduces oxygen to water for terminal oxidation during their respiratory processes. For these reasons lactic acid bacteria can accumulate hydrogen peroxide in the growth medium (Condon, 1987). Whittenbury (1964) reported that there is no correlation between hydrogen peroxide production by lactic acid bacteria and a preference for anaerobic or aerobic growth conditions. Research findings conducted years later support Whittenbury's finding that regardless of their preference or requirement for aerobic or anaerobic conditions, some lactobacilli still formed detectable hydrogen peroxide (Kot et al., 1995; Kot et al., 1996; Collins and Aramaki, 1980; and Cogan et al., 1989). Whittenbury (1964) suggests these organisms contain flavoprotein oxidases which catalyze the production of hydrogen peroxide, but that certain organisms also possess enzymes which reduce the concentration of hydrogen peroxide to undetectable levels. Villegas and Gilliland (1998) reported the presence of a lactate oxidase in *Lactobacillus delbrueckeii* subspecies *lactis* which

used D-lactate in the presence of oxygen to produce hydrogen peroxide. This activity occurred at refrigeration temperatures.

Antagonist Action of Lactic Acid Bacteria Toward Pathogens and Spoilage Organisms

Research has shown that the antagonist action of lactic acid bacteria toward pathogens and spoilage organisms can be attributed to lactic acid, however, at refrigerated temperatures, where many lactic acid bacteria do not grow or produce lactic acid the antagonistic action may be attributed to the hydrogen peroxide produced by the lactic acid bacteria (Dahiya and Speck, 1968; Price and Lee, 1970; Gilliland and Speck, 1969, 1974, 1975, 1977; Martin and Gilliland, 1980; Haines and Harmon, 1973; Thomas et al., 1994; Daly et al., 1972; Juffs and Babel, 1975; Gilliland and Ewell, 1983).

Dahiya and Speck (1968) reported the toxic effects of hydrogen peroxide produced by lactobacilli. Their study identified hydrogen peroxide as the inhibitory agent in cultures of *L. lactis* and *L. bulgaricus* active against *Staphylococcus aureus*. Dahiya and Speck also reported that the concentration of hydrogen peroxide increased during storage of the lactobacilli at 5°C, and the maximum was attained after 5 days. Wheater and coworkers (1952) observed similar inhibition of *S. aureus* by a strain of *L. lactis*, they proposed that the inhibition was due to hydrogen peroxide, however, they were unable to detect any hydrogen

peroxide in the lactobacillus growth medium unlike Dahiya and Speck (1968). Other researchers during that time also found that lactobacilli could suppress the growth of *S. aureus*, however they only speculated that the effect was due to hydrogen peroxide (Iandolo, et al., 1965; Kao, 1966; Daly, et al., 1973; Raccach, et al., 1979).

Price and Lee (1970) reported hydrogen peroxide as the active substance produced by lactobacilli isolated from seafood in the inhibition of *Pseudomonas, Bacillus*, and *Proteus* species. Gilliland and Speck (1972) found that lactic streptococci produced autoinhibitory levels of hydrogen peroxide and it accumulated in the early stages of acid production then dissipated. However, since the lactic streptococci produced sufficient levels of peroxide to limit their acid production, they concluded that they might produce a sufficient amount to affect growth of food-borne pathogens such as staphylococci and salmonellae.

Reddy and coworkers (1970) conducted a study where a mixture of *Streptococcus lactis* and *Leuconostoc citrovorum* was inoculated in ground beef. Their results indicated that the lactic cultures inoculated in the ground beef retarded the growth of Gram-negative bacteria in the product during storage at 7°C. Daly and coworkers (1972) presented similar results for ground beef, milk and cottage cheese inoculated with *Streptococcus diacetilactis*. Gilliland and Speck (1975) examined the inhibition of psychrotrophic bacteria by lactobacilli in nonfermented refrigerated foods. Their results indicate the apparent inactivation of

Pseudomonas fragi and psychrotroph MC-60N in milk when *L. bulgaricus* was added. A rapid production of a bactericidal substance by the lactobacilli was noted, they concluded that this substance was hydrogen peroxide and that it appeared to be involved in causing the inhibition of the psychrotrophic bacteria at 5-7°C. Similar results were found by Juffs and Babel (1975) who examined commercial starter cultures containing mixed species of lactic cultures. The mixed species of lactic cultures were found to restrict the growth of psychrotrophs in raw milk and in autoclaved milk inoculated with a psychrotrophic culture stored at 3.5 and 7°C. They noted that the lactic cultures exhibiting the greatest inhibition of psychrotrophs at both temperatures produced more hydrogen peroxide and less acid than the other cultures.

Martin and Gilliland (1980) also examined the inhibition of psychrotrophic bacteria in refrigerated milk by lactobacilli isolated from yogurt. They found that higher populations of *L. bulgaricus* resulted in greater inhibition of the psychrotroph in autoclaved milk stored at 5.5°C. They also found that the intensity of inhibition of the psychrotrophic culture in autoclaved milk varied among the cultures of *L. bulgaricus* used, and the more inhibitory lactobacilli cultures produced more hydrogen peroxide in the refrigerated milk than did the less inhibitory ones. However, the same cell suspensions did not retard the growth of psychrotrophs in raw milk stored at 5.5°C. The researchers suggested that the lack of inhibition in raw milk was due to inadequate production

of hydrogen peroxide by the cultures of *L. bulgaricus* included in their study. However, in a study conducted later by Gilliland and Ewell (1983), it was found that *L. lactis* was able to significantly inhibit psychrotrophs in raw milk stored at 5 and 7°C. Results from their study also suggested that the measurement of hydrogen peroxide produced by the strains of *L. lactis* indicated that the strains most antagonistic toward psychrotrophs were the most active and produced the most hydrogen peroxide. These results support the findings of Martin and Gilliland (1980) that the more inhibitory the lactic culture at refrigeration temperature, the more hydrogen peroxide it produced.

Research has indicated that lactobacilli produce more hydrogen peroxide than lactic streptococci (Gilliland and Speck, 1969). It was also reported that of the lactobacilli, *L. lactis* produced more hydrogen peroxide than did *Lactobacillus helveticus*, *Lactobacillus jugurti* or *L. bulgaricus* (Premi and Bottazzi, 1972). The fact that *L. lactis* produces more hydrogen peroxide than *L. bulgaricus* may help explain why Martin and Gilliland (1980) were unable to find significant inhibition of psychrotrophs in raw milk using *L. bulgaricus*, while Gilliland and Ewell (1983) did find significant inhibition of psychrotrophs in raw milk using *L. lactis*.

Reinheimer and coworkers (1990) investigated the inhibition of coliform bacteria by lactic cultures. They performed a comparative study of the antibacterial activity of single and mixed strain cultures of species

of *Streptococcus*, *Leuconostoc*, and *Lactobacillus* against coliform bacteria such as *Escherichia coli* FDB, *Enterobacter aerogenes* B139 NRRL, and *Klebsiella* sp-strain T1 stored at 37, 25, and 10°C . The strains of *Lactobacillus* produced the most hydrogen peroxide. Antagonist action of the lactic cultures against the coliforms was reported at the various temperatures. The higher the temperature the more antagonistic the action. At the higher temperatures the antagonist action was primarily due to lactic acid, however, at the lower temperatures the antagonist action was attributed to the production of hydrogen peroxide.

Brashears and coworkers (1998) reported the antagonistic effect of *L. lactis* against *Escherichia coli* O157:H7 during refrigerated storage in trypticase soy broth (TSB) and on raw chicken meat . They examined the interaction of *L. lactis* and *E. coli* O157:H7 in TSB in the presence of catalase, they concluded from the results from this part of the experiment that the apparent killing action toward *Escherichia coli* O157:H7 was due to the production of hydrogen peroxide by the lactobacilli during storage at 5 and 7°C. They emphasized the fact that *L. lactis* does not grow at those temperatures but it can and does produce hydrogen peroxide, so it is unlikely that the antagonistic action was due to acid production of the culture. To further support this theory the authors reported that since the cells of lactobacilli were removed from the broth in which they had been grown prior to being inoculated into TSB or onto the raw chicken meat, it is not likely that substances

produced during their growth were involved. Not only were antagonist actions against *E. coli* O157:H7 reported, the lactobacilli exerted antagonist action toward coliforms in the natural flora of the raw chicken meat. They also noted that control samples were very slimy and foul smelling after a 5-day storage period, where as the samples that had the *L. lactis* added did not have the slimy appearance and foul odor. These results further suggest that the *L. lactis* not only acted antagonistically toward the *E. coli* O157:H7 added to the chicken pieces but also acted antagonistically on the growth of psychrotrophic spoilage organisms. Here again, it was demonstrated that the production of hydrogen peroxide at refrigerated temperatures by *L. lactis* was responsible for the antagonistic action toward pathogens and spoilage microorganisms.

Lactic Acid Bacteria in Meat Systems

For centuries most societies and cultures around the world have regarded meat as a nutritious, highly desirable food. Meat is the muscular tissue of red meat animals made up of contractile myofibrillar elements, soluble sarcoplasmic proteins, connective tissue and fat. Because meat is made up primarily of protein, fat, carbohydrates, amino acids and vitamins meat may serve as an excellent medium for the growth of microorganisms. Not only is meat comprised of nutritious ingredients for microbial growth but it also has two important properties that help facilitate microbial growth as well. The first of the two

properties is water content, which corresponds to adequate water activity and the second property is pH. Meat contains approximately 75% water, corresponding to a water activity level of around 0.99, which is suitable for most microbial growth. The pH value of meat, which may range from 7.0 to 5.0, fluctuates depending on species and time after slaughter.

Meat muscle in itself is essentially sterile. There have been reports of the presence of small numbers of bacteria in deep muscle (Gill et al., 1976). However, the ease in which sterile muscle tissue can be obtained by aseptic techniques shows that in carcasses from healthy animals bacteria are normally present only on the exposed surfaces of meat (Gardner and Carson, 1967; Gill et al., 1976). The largest source of microbial contamination occurring on the surface of meat is from the skin or hide of the particular animal being dressed and accidental contamination of gastrointestinal fluid during inviseration (Ayres, 1955).

Lactic acid bacteria are included in the normal microflora of fresh and processed meat. Of the lactic acid bacteria, lactobacilli are the predominant ones and are often accompanied by closely related lactic acid bacteria such as pediococci, leuconostocs, and enterococci (Sharpe, 1962). Lactic acid bacteria represent only a small fraction of the microorganisms which develop on fresh and cured meats stored under aerobic conditions, and are not believed to affect shelf life adversely (Haines, 1933; Ayres, 1960; Gardner et al., 1967; Kitchell and Ingram, 1967). Because most of the meat we deal with today involves some type

of packaging, especially vacuum packaging, lactic acid bacteria can rapidly achieve dominance in vacuum packages of both fresh and cured meats (Ingram, 1962). Research has indicated that lactic acid bacteria form 50 – 90% of the bacterial flora on vacuum-packed beef (Jaye et al., 1962; Pierson et al., 1970; Roth and Clark, 1972).

Reuter (1981) has indicated that despite chilling, psychrotrophic lactobacilli are favored for growth in packaged meat and meat products due to their tolerance of anaerobic conditions, of low pH values and of curing salts. Reuter also mentions that in many cases lactobacilli become the main component of the microflora suppressing other bacterial groups or species (such as psychrotrophs associated with spoilage) by antagonism. The antagonism exerted by lactobacilli towards spoilage organisms and pathogens has been addressed by numerous researchers (Reddy et al., 1970; Gilliland and Speck, 1975; Raccach et al., 1979; Gilliland, 1980; Reinheimer et al., 1990; Brashears et al., 1998). As mentioned previously, the antagonistic action exerted by lactobacilli toward spoilage organisms and pathogens has been, at least in part, attributed to the production of hydrogen peroxide.

Escherichia coli O157:H7

Escherichia coli O157:H7 has been linked epidemiologically to several outbreaks and cases of hemorrhagic colitis and hemolytic uremic syndrome dating back to 1982 (Martin, et al., 1986; Pai et al., 1984;

Riley, et al., 1983; Ryan, et al., 1986; Wells, et al., 1983; Karmali, 1989). It has been detected in numerous types of foods including raw milk, turkey sandwiches, cheese, apple cider, mayonnaise, ground beef, beef products, fermented sausage, potatoes, poultry, water and raw vegetables. However, in a majority of these cases the outbreak was linked to and even isolated from ground beef (Wells, et al, 1983; Lamothe, et al., 1983; Bryant et al., 1989; Doyle and Schoeni, 1987; Sekla, et al., 1990).

Escherichia coli O157:H7 is a Gram-negative rod shaped bacteria that unlike other generic *E. coli* will survive but not grow at refrigeration temperatures (Abdul-Raouf, et al., 1993 Weagant, et al., 1994). Doyle and Schoeni (1984) examined the survival and growth characteristics of *E. coli* O157:H7 in ground beef and found that it was able to survive for 9 months at -20°C with little change in number, indicating that the organism survives well in ground beef during storage. There have been some reports that the storage temperature of ground beef patties influences the subsequent heat tolerance of *E. coli* O157:H7. Jackson et al. (1995) reported that cells in frozen patties had greater heat tolerance than did cells in ground beef stored at 15°C. Ansay et al., (1999) reported that tempering (15°C for 4 hr) of ground beef patties prior to low temperature storage accelerated the decline in the numbers of *E. coli* O157:H7. Cheng and Kaspar (1998) investigated the growth and processing conditions affecting acid tolerance in *E. coli* O157:H7. They

also found that storage conditions may influence the acid tolerance of *E. coli* O157:H7 in ground beef. Their results imply that incubation at 15°C for 4 hr prior to storage at 4 or -20°C sensitized the surviving cells to synthetic gastric fluid. The results from the studies mentioned above suggest that refrigerated rather than frozen storage of ground beef increases the susceptibility of the pathogen to unfavorable conditions.

Because *E. coli* O157:H7 can survive at refrigerated and frozen temperatures and perhaps even become sensitized (due to various storage conditions) enough to be able to have greater heat and acid tolerance it is important that intervention strategies be examined for ground beef, beef products, or other meats that may be contaminated with *E. coli* O157:H7 (Doyle and Schoeni, 1984; Ansay et al., 1999). In work mentioned earlier conducted by Brashears and others (1998), an intervention strategy involving the use of lactic acid bacteria on the surface of poultry was investigated. Again, their results suggest that in fact the use of lactic acid bacteria on the surface of raw chicken meat will act antagonistically against *E. coli* O157:H7. These researchers attribute the antagonistic action of the lactic acid bacteria (which does not grow at refrigerated temperatures) against *E. coli* O157:H7 to the production of hydrogen peroxide by lactic acid bacteria at the refrigerated temperature.

REFERENCES

Abdul-Raouf, U.M., Beuchat, L.R., and Ammar, M.S. 1993. Survival and growth of *Escherichia coli* O157:H7 in ground, roasted beef as affected by pH, acidulants and temperture. Appl. Environ. Microbiol. 59:2364-2368.

Ansay, S.E., Darling, K.A., and Kaspar, C.W. 1999. Survival of *Escherichia coli* O157:H7 in ground beef patties during storage at 2, -2, 15 and then –2, and -20°C. J. Food Prot. 62:1243-1247.

Axelsson, L.T., Chung, T.C., Dobrogosz, W. J., and Lindgren, S.E. 1989. Production of a broad spectrum antimcirobial substance by *Lactobacillus reuteri.* Microb. Ecol. Health Dis. 2:131-136.

Ayres, J.C. 1955. Microbiological implications in handling, slaughtering, and dressing meat animals. Adv. Food Res. 6:109-161.

Ayres, J.C. 1960. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. Food Res. 25:1-18.

Blom, H. and Mortvedt, C. 1991. Anti-microbial substances produced by food associated micro-organisms. Biochem. Soc. Trans. 19:694-698.

Brashears, M.M., Reilly, S.S., and Gilliland, S.E. 1998. Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* O157:H7 on refrigerated raw chicken meat. J. Food Protect. 61:166-170.

Brown, W.V., and Collins, E.B. 1977. End products and fermentation balances for lactic streptococci grown aerobically on low concentrations of glucose. Appl. Environ. Microbiol. 33:38-42.

Bruno, M.E.C. and Montville, T.J. 1993. Common mechanistic action of bacteriocins from lactic acid bacteria. Appl. Environ. Microbiol. 59:3003-3010.

Bryant, H.E., Athar, M.A., and Pai, C.H. 1989. Risk factors for *Escherichia coli* O157:H7 infection in an urban community. J. Infect. Dis. 160:858-864.

CDC, 1989. Center for Disease Control, Morbidity and Mortality Weekly Report 38:38.

Cheng, C.M. and Kaspar, C.W. 1998. Growth and processing conditions affecting acid tolerance in *Escherichia coli* O1157:H7. Food Microbiol. 15:157-166.

Chung,T.C., Axelsson, L.T., Lindgren, S.E., and Dobrogosz, W.J. 1989. In vitro studies on reuterin synthesis by *Lactobacillus reuteri.* Microb. Ecol. Health Dis. 2:137-144.

Cogan, J.F., Walsh, D., and Condon, S. 1989. Impact of aeration on the metabolic end-products formed from glucose and galactose by *Streptococcus lactis.* J. Appl. Bacteriol. 66:77-84.

Collins, E.B. and Aramaki, K. 1980. Production of hydrogen peroxide by *Lactobacillus acidophilus.* J. Dairy Sci. 63:353-357.

Condon, S. 1987. Response of lactic acid bacteria to oxygen. FEMS Microbiol. Rev. 46:269-280.

Daeschel, M..A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol. 43:164-166.

Dahiya, R.S. and Speck, M.L. 1968. Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus.* J. Dairy Sci. 51:1568-1572.

Daly, C., La Chance, M., Sandine, W.E., and Elliker, P.R. 1973. Control of *Staphylococcus aureus* in sausage by starter cultures and chemical acidulation. J. Food Sci. 38:426-430.

Daly, C.W., Sandine, E., and Elliker, P.R. 1972. Interaction of food starter cultures and food-borne pathogens: Streptococcus diacetilactis versus food pathogens. J. Milk Food Technol. 35:349-357.

de Klerck, H.C. and Smit, J.A. 1967. Properties of a *Lactobacillus fermenti* bacteriocin. J. Gen. Microbiol. 48:309-316.

de Vos, W.M., Kuipers, O.P., van der Meer, J.R., and Siezen, R.J. 1995. Maturation pathway of nisin and other lantibiotics: post-translationally modified antimicrobial peptides exported by Gram positive bacteria. Microbiol. 17:427-437.

Dickson, J.S. and Anderson, M.E. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: a review. J. Food Prot. 55:133-140.

Dobrogosz, W.J., Casas, I.A., Pagano, G.A., Talarico, T.L., Sjoberg, B., and Karlsson, M. 1989. *Lactobacillus reuteri* and the enteric microbiota. In: The Regulatory and Protective Role of the Normal Microflora. Stockton Press, New York, pp 283-292.

Doyle, M.P. and Schoeni, J.L. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Envir. Micro. 48:855-856.

Doyle, M.P. and Schoeni, J.L. 1987. Isolation of *Escherichia coli O157:H7* from retail fresh meat and poultry. Appl. Envir. Micro. 53:2394-2396.

Eklund, T. 1983. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. J Appl. Bacteriol. 48:423-432.

Esders, T.W., Goodhue, C.T., and Schubert, R.M. 1979. Analysis of lactic acid or lactate using lactate oxidase. U.S. patent 4, 166,763.

Forrest, W.W., and Walker, D.J. 1971. The generation and utilization of energy during growth. Adv. Microbiol. Physiol. 5:213-274.

Gardner, G.A. and Carson, A.W. 1967. Relationship between carbon dioxide production and growth of pure strains of bacteria on porcine muscle. J. Appl. Bacteriol. 30:500-510.

Gill, C.O., Penney, N., and Nottingham, P.M. 1976. Effect of delayed evisceration on the microbial quality of meat. Appl. Environ. Microbiol. 31:465-468.

Gilliland, S.E. 1985. Bacterial Starter Cultures for Foods. CRC Press Inc., Boca Raton, FL.

Gilliland, S.E. and Ewell, H.R. 1983. Influence of combinations of *Lactobacillus lactis* and potassium sorbate on growht of psychrotrophs in raw milk. J. Dairy Sci. 66:974-980.

Gilliland, S.E. and Speck, M.L. 1969. Biological response of lactic streptococci and lactobacilli to catalase. Appl. Microbiol. 17:797-800.

Gilliland, S.E. and Speck, M.L. 1972. Interactions of food starter cultures and food-borne pathogens: Lactic streptococci versus staphylococci and salmonellae. J. Milk Food Technol. 35:307-310.

Gilliland, S.E. and Speck, M.L. 1974. Antagonism of lactic streptococci toward *Staphylococcus aureus* in associative milk cultures. Appl. Microbiol. 28:1090-1093.

Gilliland, S.E. and Speck, M.L. 1975. Inhibition of psycrotrophic bacteria by lactobacilli and pediococci in nonfermented refrigerated foods. J. Food Sci. 40:903-905.

Gilliland, S.E. and Speck, M.L. 1977. Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative cultures. J. Food Prot. 40:820-823.

Gilliland, S.E. 1980. Use of lactobacilli to preserve fresh meat. Proc. Recip. Meat Conf. 33:54-58.

Gregory, E.M. and Fridovich, I. 1974. Oxygen metabolism in *Lactobacillus plantaurm.* J. Bacteriol. 117:166-169

Haines, R.B. 1933. The bacterial flora developing on stored lean meat, specially with regard to slimy meat. J. Hyg. Camb. 33:645-654.

Haines, W.C. and Harmon, L.G. 1973. Effect of selected lactic acid bacteria on growth of *Staphylococcus aureus* and production of enterotoxin. Appl. Microbiol. 25:436-441.

Hardin, M.D., Acuff, G.R., Lucia, L.M., Oman, J.S. and Savell, J.W. 1995. Comparison of methods for decontamination from beef carcass surfaces. J. Food Prot. 58:368-374.

Hobbs, B.C. 1974. Food Poisoning and Food Hygiene. Edward Arnold Publishing, London.

Iandolo, J.J., Clark, C.W., Bluhm, L., and Ordal, Z.J. 1965. Repression of *Staphylococcus aureus* in associative culture. Appl. Microbiol. 13:646-649.

Ingram, M. 1962. Microbiological principles in prepacking meats. J. Appl. Bact. 25:259-281.

Jack, R.W., Tagg, J.R., and Ray, B. 1994. Bacteriocins of Gram-positive bacteria. Microbiol. Rev. 59:171-200.

Jackson, T.C., Hardin, M.D., and Acuff, G.R. 1995. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. J. Food Prot. 59:230-237.

Jay, J.M. 1992. Modern Food Microbiology. 4th ed. Chapman and Hall, New York, New York.

Jay, J.M. 1982. Antimicrobial properties of diacetyl. Appl. Environ. Microbiol. 44:525-532.

Jaye, M., Kittaka, R.S., and Ordal, Z.J. 1962. The effect of temperature and packaging material on the storage life and bacterial flora of ground beef. Food Technol. Champaign 16:95-98.

Juffs, H.S. and Babel, F.J. 1975. Inhibition of psychrotrophic bacteria by lactic cultures in milk stored at low temperature. J. Dairy Sci. 58:1612-1619.

Kandler, O. 1983. Carbohydrate metabolism in lactic acid bacteria. Antonei van Leeuwenhoek 49:202-224.

Kao, C.T. and Frazier, W.C. 1966. Effect of lactic acid bacteria on growth of *Staphylococcus aureus*. Appl. Microbiol. 14:251-255.

Karmali, M.A. 1989. Infection by verocytoxin producing *Escherichia coli*. Clin. Microbiol. Rev. 2:15-38.

Kashet, E.R. 1987. Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. FEMS Microbiol. Rev. 46:233-244.

King, A.D.J. and Nagel, C.W. 1975. Influence of carbon dioxide upon the metabolism of *Pseudomonas aeruginosa*. J. Food Sci. 40:362-366.

Kitchell, A.G. and Ingram, G.C. 1967. A survey of a factory for the sources of lactobacilli characteristic of vacuum packed Wiltshire cured bacon. Proc. 13th Meeting European Meat Res. Workers, Rotterdam, Paper B2.

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

Klaenhammer, T.R., Ahn, C., Fremaux, C., and Milton, K. 1992. Molecular properites of Lactobacillus bacteriocins. In: Bacteriocins, Microcins and Lantibiotics, springer-verlag, Berlin, pp.37-58.

Kneifel, W., Holub, S., and Wirthmann, M. 1989. Monitoring of B-complex vitamins in yogurt during fermentation. J. of Dairy Res. 56:651-656.

Kot, E., Furmanov, S., and Bezkorovainy, A. 1995. Ferrous iron oxidation by *Lactobacillus acidophilus* and it's metabolic products. J. Agri. Food Chem. 41:1276-1282.

Kot, E., Furmanov, S., and Bezkorovainy, A. 1996. Hydrogen peroxide production and oxidation of ferrous iron by *Lactobacillus delbrueckii* ssp *bulgaricus*. J. Dairy Sci. 79:758-766.

Lamothe, F., Gaudreau, C., Bernard, D., and Gill, S. 1983. Hemorrhagic colitis following the consumption of hamburger in Quebec. Can. Weekly Rep. 9:50-51.

Lewus, C.B., Sun, S., and Montville, T.J. 1992. Production of an amylase-sensitive bacteriocin by an atypical *Leuconostoc paramesenteroides* strain. Appl Environ. Microbiol. 58:143-149.

Lindgren, S.E. and Dobrogosz, W.J. 1990. Antagonistic activities of lactic acid bacteria in food and feed fermenttions. FEMS Microbiol. Rev. 87:149-163.

London, J. 1976. The ecology and taxonomic status of the lactobacilli. Ann. Rev. Microbiol. 30:279-301.

Martin, D.R. and Gilliland, S.E. 1980. Inhibition of psychrotrophic bacteria in refrigerated milk by lactobacilli isolated from yogurt. J. Food Prot. 43:675-678.

Martin, M.L., Shipman, L.E., Wells, J.G., Potter, M.E., Hedberg, K., Wachmuth, I.K., Tauxe, R.V., Davis J.P., Amolai, J., and Tilleli, J. 1986. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of hemoytic uremic syndrom (letter). Lancett ii:1043

Pai, C.K., Gordon, R., Sims, H.V, and Bryan, L.E. 1984. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7. Ann. Intern. Med. 101:738-742.

Pierson, M.D., Collins-Thompson, D.L., and Ordal, Z.J. 1970. Microbiological, sensory and pigment changes of aerobically and anaerobically packaged beef. Food Technol. Champaign 24:1171-1175.

Pinheiro, A.J., Liska, B.J., and Parmelee, C.E. 1968. Properties of substances inhibitory to Pseudomonas fragi produced by Streptococcus citrovorus and S. diactilactis. J. Dairy Sci. 51:183-189.

Premi, L. and Bottazzi, V. 1972. Hydrogen peroxide formation and hydrogen peroxide splitting activity in lactic acid bacteria. Milchwissenschaft 27:762-765.

Price, R.J. and Lee, S. 1970. Inhibition of *Pseudomonas* species by hydrogen peroxide producing lactobacilli. J. Milk and Food Tech. 33:13-18.

Raccach, M., Baker, R.C., Regenstein, J.M., and Mulnix, E.J. 1979. Potential application of microbial antagonism to extended storage stability of a flesh type food. J. Food Sci.44:43-46.

Ray, B. 1993. Sublethal injury, bacteriocins, and food microbiology. ASM News 59:285-291.

Reddy, S.G. Henrickson, R.L., and Olson, H.C. 1970. The influence of lactic cultures on ground beef quality. J. Food Sci. 35:787-791.

Reinheimer, J.A., Demkow, M.R., and Candioti, M.C. 1990. Inhibition of coliform bacteria by lactic cultures. Austral. J. Dairy Technol. 45:5-9.

Reuter, G. 1981. Psychrotrophic lactobacilli im meat products, in: Psychrotrophic Microorganisms in Spoilage and Pathogenicity. Roberts, T.A., Hobbs, G., Christian, J.H.B., and Skovgaard, N., Eds., Academic Press, New York, chp.23.

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.C., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A., and Cohen, M.L. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. 308:681-685.

Roth, L.A. and Clark, D.S. 1972. Studies on the bacterial flora of vacuum packaged fresh beef. Can. J. Microbiol. 18:1761-1766.

Rubin, H. E. 1978. Toxicological model for a two-acid system. Appl. Environ. Microbiol. 36:623-624.

Ryan, C.A., Tauxe, R.V., Hosek, G.W., Wells, J.G., Stoesz, P.A., McFadden, H.W., Smith, G.F., Wright, G.F., and Blake, P.A. 1986. *Escherichia coli* O157:H7 diarrhea in nursing home: clinical, epidemiological, and pathological findings. J. Infect. Dis. 154:631-638.

Sahl, H. G., Jack, R.W., and Bierbaum, G. 1995. Biosynthesis and biological activities of lantibiotics with unique post translational modifications. Eur. J. Biochem. 230:827-853.

Sahl. H.G., Kordel, M., and Benz, R. 1987. Voltage dependent depolarization of bacterial membranes and artificial lipid bilayers by the peptide antibiotic nisin. Arch. Microbiol. 149:120-124.

Schillinger, U. and Lucke, F.K. 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. 55:1901-1906.

Sekla, L.D., Milley, D., Stackiw, W., Sisler, J., Drew, J., and Sargent, D. 1990. Verotoxin-productin *Escherichia coli* in ground beef – Manitoba. Can. Dis. Weekly Rep. 22:103-105.

Sharpe, M.E. 1962. Lactobacilli in meat products. Food Manuf. 37:582-589.

Stamer, J.R. 1976. Lactic acid bacteria. In Food Microbiology: Public Health and Spoilage Aspects, ed M.P. deFigueiredo and D.F. Splittstoesser, Westport, Conn. 404-426.

Steinkraus, K.H. 1986. Fermented foods, feeds, and beverages. Biotech. Adv. 4:219-243.

Tagg, J.R., Dajani, A.S., and Wannamaker, L.W. 1976. Bacteriocins of Gram positive bacteria. Bacter. Rev. 40:722-756.

Thomas, E.L., Milligan, T.W., Joyner, R.E., and Jefferson, M.M. 1994. Antibacterial activity of hydrogen peroxide and the lactoperoxidase-hydrogen peroxide-thiocyanate system against oral streptococci. Infect. Immun. 62:529-535.

Veneman, K., Veneman, G., and Kok, J. 1995. Lactococcal bacteriocins: mode of action and immunity. Trends Microbiol. 3:299-303.

Villegas, E. and Gilliland, S.E. 1998. Hydrogen peroxide production by *Lactobacillus delbrueckii* subsp. *Lactis* I at 5°C. J. Food Sci. 63:1070-1074.

Weagant, S.E., Bryant, J.L., and Bark, D.H. 1994. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. J. Food Prot. 57:629-631.

Wells, J.G., Davis, B.R., Wachsmuth, I.K., Riley, L.W., Remis, R.S., Sokolow, R., and Morris, G.K. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. J. Clin. Microbiol. 18:512-520.

Wheater, D.M, Hirsch, A., and Mattick, A.T.R. 1952. Possible identity of "lactobacillin" with hydrogen peroxide produced by lactobacilli. Nature 170:623-624.

Whittenbury, R. 1964. Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. J. Gen. Microbiol. 35:13-26.

Yap, P.S. and S.E. Gilliland. 2000. Comparison of newly isolated strains of *Lactobacillus delbrueckii* subsp. *lactis* for hydrogen peroxide production at 5°C. J. Dairy Sci. 83:628-632.

CHAPTER III

ANTAGONISTIC ACTION OF CELLS OF *LACTOBACILLUS DELBRUECKII* SUBSP *LACTIS* TOWARD PATHOGENIC MICROORGANISMS IN FRESH MEAT SYSTEMS

ABSTRACT

Cells of *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 were added to various meat model systems previously inoculated with *Escherichia coli* O157:H7 or *Salmonella typhimurium* to determine if the lactobacilli were antagonistic toward the pathogens during storage at 5°C. Experiments where *L. delbrueckii* subsp. *lactis* RM2-5 was added to the surface of beef steaks by dipping or direct application onto the meat surface resulted in significantly ($P < 0.05$) reduced growth of psychrotrophs and coliforms and a slight decrease in numbers of *E. coli* O157:H7 over time compared to control samples to which no lactobacilli were added. Experiments involving the direct application of *L. delbrueckii* subsp. *lactis* RM2-5 onto the surface of freshly slaughtered beef and pork carcass samples inoculated with either *E. coli* O157:H7 or *S. typhimurium* exhibited significant ($P < 0.05$) declines in the numbers of the pathogens as well as reduced growth of psychrotrophs during storage at 5°C for 6 days. The results from the experiments suggest that cultures of lactobacilli could be used as an intervention technology for control of food borne pathogens. Results also suggest that an extension of the shelf-life of the

meat can result due to the decreased growth of psychrotrophic spoilage organisms.

INTRODUCTION

Concerns over possible foodborne illness caused by consumption of contaminated improperly cooked meat has considerably heightened the public's awareness of foodborne illness after the 1992 foodborne illness outbreak on the West Coast. This outbreak was linked to *Escherichia coli* O157:H7 that was found in undercooked ground beef from a fast food chain. This organism has been identified as a rare serotype of an enterohemorrhagic *E. coli* which caused abdominal cramps, bloody diarrhea, and in some cases hemolytic uremic syndrome (Karmaili, 1989). The seriousness of the hemolytic uremic syndrome caused by *E. coli* O157:H7 (which was found to occur in young children and the elderly) caused fatalities in 3 to 5% of the cases (Weagant et al., 1994). Doyle and Schoeni (1984) reported that unlike other pathogens that can cause food borne illness, *E. coli* O157:H7 was unique not only because it was potentially fatal but because the amount ingested to acquire the illness was less than 10 cells.

Another pathogen of concern is *Salmonella*. *Salmonella typhimurium* is the most common found foodborne serovar found throughout the world (Hobbs, 1974). Although symptoms from illness due to *Salmonella* is not as severe or as potentially deadly as illness from *E. coli* O157:H7, *Salmonella* is considered a common cause for food borne illness (CDC, 1989). While most of the microflora transferred to carcasses during the slaughtering process are nonpathogenic, pathogens such as *Salmonella*

typhimurium and *E. coli* O157:H7 can be present (Dickson and Anderson, 1992; Hardin et al., 1995).

The emergence of *E. coli* O157:H7 as a potentially deadly food borne pathogen has resulted in much research to detect the pathogen quicker and on ways to control it in the food supply. Some intervention strategies studied for use in fresh meat and meat products include: steam pasteurization (Nutsch 1998), various carcass washes (acid, hot water, etc.)(Dickson,1988; Dickson and Anderson, 1992; Barkate, 1993), steam vacuuming (Phebus, 1997), irradiation (Ehioba, 1988), ultra violet light(Yousef and Marth, 1988; Arrage et al., 1993), and ozone treatments (Kim et al., 1999).

Lactic Acid Bacteria have been involved in food preservation dating back to biblical times. Today they are widely used in the manufacture of many cultured foods. Because of this and their role in preservation of these products they offer an alternative intervention for control of undesirable microorganisms in other foods. This is especially true if they can exert the preservative action without altering the desirable characteristics of these foods. Lactic acid bacteria can exert antagonistic action toward the growth of spoilage and even pathogenic organisms in nonfermented foods at refrigerated temperatures (Daly et al., 1972; Gilliland and Speck, 1975; Juffs and Babel, 1975; Gilliland, 1980; Martin and Gilliland, 1980; Gilliland and Ewell, 1983; Brashears et al., 1998). In some cases this antagonistic action toward spoilage and

pathogenic organisms has been attributed to the ability of some lactic acid bacteria to produce inhibitory levels of hydrogen peroxide at refrigerated temperatures.

The primary purpose of this study was to determine if a selected strain of *L.delbrueckii* subsp. *lactis* could produce an antagonistic action toward food borne pathogens specifically *E. coli* O157:H7 and S. *typhimurium*, in various fresh meat systems during refrigerated storage.

MATERIALS AND METHODS

Sources and maintenance of cultures

The five strains of *Lactobacillus delbrueckii* subsp. *lactis* (RM2-5, RM1-9, RM4-1, RM4-7 and I) used in this study were from the stock culture collection of the Food Microbiology Laboratory in the Food and Agricultural Products Center at Oklahoma State University. All cultures of lactobacilli were maintained by subculturing in MRS broth (Difco Laboratories, Detroit, MI) using 1% inocula and incubation at 37°C for 18 h. *Escherichia coli* O157:H7 strain 43894 and *Salmonella typhimurium* strain 29631 were obtained from the American Type Culture Collection (ATCC). They were maintained by subculturing in Trypticase soy broth (TSB; BBL Microbiology Systems, Cockeysville, MD) using 1% inocula and incubation at 37°C for 18 h. All cultures were subcultured at least three times just before they were used experimentally and stored at 7°C between transfers. Stock cultures were maintained in either MRS agar or Trypticase Soy agar stabs at 7°C.

Microbial analyses

For all experiments samples were diluted using sterile peptone (0.01%) dilution blanks and plated using the pour plate technique. Initial dilutions for samples were prepared by aseptically weighing the sample into a sterile stomacher bag and adding nine times its weight of

sterile diluent to prepare a 1:10 dilution. After adding the diluent each sample was pummelled in a masticator (RUL Instruments, Barcelona, Spain) for 1 min. Appropriate additional dilutions were prepared using 0.1% peptone dilution blanks in accordance with standard procedures outlined by Vanderzant and Splittstoesser (1992). Depending on the experiment, samples were plated on the appropriate media to enumerate the organisms of interest. Violet red bile agar (VRBA) (Difco Laboratories) was used to enumerate *E. coli* O157:H7 and background coliforms, plates were incubated at 37° C for 24 h (we were able to use it for enumeration of *E. coli* O157:H7 when the inoculum level for the meat exceeded the numbers of coliforms present on the uninoculated meat). Lactobacillus selection agar (LBS) agar was used to enumerate *Lactobacillus delbrueckii* subsp. *lactis* as well as background lactobacilli, it was prepared from individual ingredients using the manufacturer's formulation (Baltimore Biological Laboratories, Cockeysville, MD). Plates of LBS agar were placed in a plastic bag and flushed with CO_2 for 20 seconds, sealed and incubated at 37° C for 48 h. Plate Count Agar (PCA; Difco Laboratories) was used to enumerate total aerobic bacteria, plates for this were incubated 7 d at 15° C. The lactobacilli MRS broth (Difco Laboratories) supplemented with1.5% agar (MRS agar) was used to enumerate the numbers of lactobacilli in the cell suspensions. These plates were incubated at 37° C for 48 h. Brilliant Green Agar (BGA; Difco

Laboratories) was used to enumerate *S. typhimurium*, plates were incubated at 37° C for 24 h.

Preparation of cultures for treatments

Cells of the lactobacilli were either from frozen or freshly prepared cultures. Both the frozen and fresh cultures were prepared by inoculating (1%) 200 ml of MRS broth with a freshly prepared MRS broth culture of the desired strain of *L. delbrueckii* subsp. *lactis* and incubating at 37°C for 18 h. Cells were harvested from the broth by centrifugation 20 min at 4,000 x *g* at 1°C. The pellets were resuspended, with the aid of approximately 20 sterile 2-mm glass beads, in 20 ml of cold, sterile aqueous 10% nonfat milk solids. The resulting concentrated culture when used fresh was held in a mixture of ice-water and used within 1 hour. The fresh concentrated culture was plated on MRS agar to determine the numbers of lactobacilli immediately after being concentrated. The concentrated culture that was frozen was aseptically dispensed in 2-g aliquots into 2-ml cryogenic vials and frozen at -196°C in liquid nitrogen until needed. After 1 day of frozen storage, a sample was removed and plated on MRS agar to determine the numbers of lactobacilli in the concentrated culture. Each vial of frozen concentrated culture was thawed prior to use by submersion in 25 ml of 40° C tap water for 20 min. The exterior of the vials were sanitized with 70% ethanol prior to opening.

Freshly prepared cultures of *E. coli* O157:H7 and *S. typhimurium* were used for the various experiments. Cells of either *E. coli* O157:H7 or *S. typhimurium* were harvested from an 18 hr TSB culture by centrifugation at 4000 x g for 20 min at 1°C, resuspended in 10 ml of sterile water and diluted to reach a final concentration of 1.0×10^5 CFU/ml and held in an ice-water bath for no longer than 1 hour prior to use. Cells of *E. coli* O157:H7 and *S. typhimurium* were plated on VRBA or BGA respectively immediately after being prepared to verify the number of cells present in the suspension before use.

Effects of freezing on *Lactobacillus delbrueckii* subsp. *lactis*

Five cultures of *L. delbrueckii* subsp. *lactis* (RM2-5, RM1-9, RM4-1, RM4-7 and I) were grown separately in MRS broth, concentrated and frozen as indicated above. Cultures were plated on MRS agar to determine the numbers of lactobacilli after 18 h growth in MRS broth, immediately after being concentrated, and after 1, 7 and 14 days of frozen storage (-196°C). This experiment was replicated three times.

Comparison of cultures of *Lactobacillus delbrueckii* subsp. *lactis* for influence on the background microflora on fresh beef steaks

Fresh beef top round steaks approximately 0.63 cm thick were purchased from a local supermarket. Steaks were aseptically cut into 24 cubes (2.54 x 2.54 cm) using a sterile knife and cutting board. Six 1000

ml beakers containing 100 ml of a sterile 0.055M glucose solution (glucose dip) were prepared. Five ml of sterile aqueous 10% nonfat milk solids was added to the glucose dip in one beaker to serve as the control. Five g of thawed concentrated culture of each of the five cultures of *L. delbrueckii* subsp. *lactis* were added to each of the five remaining beakers (one culture per beaker). With the aid of sterile forceps, four cubes of meat were aseptically placed into each of the 6 beakers.

After the cubes of meat were placed into the beaker they were mixed by swirling the beaker continuously for 5 min. After 5 min cubes were removed with sterile forceps and placed into labeled 2 ounce Whirl Pak bags (Nasco) (1 cube per bag) and stored at 5°C. One bag from each treatment was removed for analyses on days 0, 3, 6 and 9. The experiment was replicated three times.

Influence of *Lactobacillus delbrueckii* subsp. *lactis* on *E. coli* O157:H7 in ground beef

A 454 g package of ground beef with 80% lean and 20% fat was purchased at a local supermarket. Sixty gram portions of ground beef were aseptically placed into each of four labeled sterile 1000 ml beakers (labeled A, B, C, and D). One ml of sterile water and 1 ml of sterile aqueous 10% nonfat milk solids were added to the meat in beaker A and mixed with a sterile spatula (treatment A = control). To the meat in beaker B, 1 g of thawed concentrated culture *L. delbrueckii* subsp. *lactis*

RM2-5 and 1 ml of sterile water were added and mixed as for A (treatment B). This treatment (B) was to determine the effects of the cells of *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 on the naturally occurring background microflora of the ground beef. The meat in beaker C was mixed in a like manner with 1 ml of a freshly prepared cell suspension of *E. coli* O157:H7 and 1 ml of sterile aqueous 10% nonfat milk solids (treatment C). Treatment C was to determine the fate of *E. coli* O157:H7 inoculated alone in the ground beef. The meat in beaker D was mixed with 1 ml of the resuspended *E. coli* O157:H7 and 1 ml of the concentrated *L. delbrueckii* subsp. *lactis* RM2-5 (treatment D). This treatment was to evaluate the antagonistic effects of the *Lactobacillus* toward *E. coli* O157:H7 in the meat.

After each treatment was prepared and the meat thoroughly mixed in each beaker, 13 – 15 g portions were packed into individual sterile 2 ounce Whirl Pak bags (Nasco) and stored at 5° C. One bag from each treatment was removed for microbial analysis on days 0, 3, 6 and 9 of refrigerated storage. The experiment was replicated three times.

Influence of *Lactobacillus delbrueckii* subsp. *lactis* on E. coli O157:H7 on steak surface

Two approaches were utilized to evaluate the influence of cells of *L. delbrueckii* subsp. *lactis* RM2-5 on *E. coli* O157:H7 on the surface of raw beef steaks. The first involved the application of the bacterial cells by

dipping the meat in suspensions of the bacteria. In these experiments an aqueous solution of glucose was used as suspending menstra for the cells of lactobacilli. The other approach was to directly inoculate the organisms onto a small area on the surface of the meat. In both the dip and the direct application experiments fresh beef top round steaks approximately 0.63 cm thick were purchased from a local supermarket. Steaks were aseptically cut into sixteen 2.54 x 2.54 cm cubes using a sterile knife and cutting board.

Dip experiments. Eight cubes of steak were dipped into 200 ml of sterile water at room temperature for 1 min, removed and four cubes were then dipped into 100 ml sterile 0.055M glucose for 3 min at room temperature, removed and placed into four individual 2 ounce Whirl Pak bags (Nasco) labeled Treatment A (control) and stored at 5° C. The other four cubes were dipped into 100 ml sterile 0.055M glucose containing 5 ml of concentrated cell suspension of the lactobacilli for 3 min at room temperature, removed, and placed into four individual 2 ounce Whirl Pak bags labeled Treatment B (lactobacilli alone) and stored at 5° C.

Eight additional cubes were dipped into 200 ml of sterile water containing 1 x 10^5 *E. coli* O157:H7 per ml at room temperature for 1 min, removed and four cubes were then dipped into 100 ml sterile 0.055M glucose for 3 min at room temperature, removed and placed in individual 2 ounce Whirl Pak bags labeled Treatment C (*E. coli* O157:H7)and stored

at 5° C. The remaining four cubes were dipped into 100 ml 0.055M glucose containing 5 ml of concentrated cell suspension of the lactobacilli for 3 min at room temperature, removed, placed into four individual 2 ounce Whirl Pak bags labeled Treatment D (*E. coli* O157:H7 and lactobacilli) and stored at 5° C.

One bag from each treatment was removed for microbial analyses on days 0, 3, 6 and 9. The experiment was replicated three times.

Inoculating cultures directly onto meat surface. Cells of *L. delbrueckii* subsp. *lactis* RM2-5 and *E. coli* O157:H7 and meat samples were prepared as described in the previous sections. For Treatment A (control) four cubes of beef steak were placed into four sterile plastic 100 x 15 mm petri dishes (Fisher Science Company), 0.1ml of sterile water and 0.1ml of sterile aqueous 10% nonfat milk solids were added to the geometric center of the top of each cube. For Treatment B (lactobacilli alone), 0.1ml of concentrated cell suspension of *L. delbrueckii* subsp. *lactis* RM2-5 and 0.1 ml of sterile water were added to the geometric center of the top of each of four cubes of steak in four individual petri dishes. Treatment C (*E. coli* O157:H7 alone) consisted of 0.1ml of cell suspension of *E. coli* O157:H7 and 0.1ml of sterile aqueous 10% nonfat milk solids placed on the geometric center of the top of each of four cubes of meat in four individual petri dishes. For Treatment D (*E. coli* O157:H7 and lactobacilli), 0.1ml of concentrated cell suspension of *L.*

delbrueckii subsp. *lactis* RM2-5 and 0.1ml of cell suspension of *E. coli* O157:H7 was added to the geometric center of the top of each of four additional cubes in four individual petri dishes.

After cubes were subjected to the assigned treatments all petri dishes containing the samples were stored at 5° C. One cube from each treatment was removed for microbial analysis on days 0, 3, 6 and 9. The experiment was replicated three times.

Direct Application on the Surface of Beef Carcasses

Samples of exposed surface areas of freshly slaughtered beef carcasses were obtained from a local slaughter house after evisceration and prior to final carcass washing. Samples were obtained by excising a 25 cm^2 core (approximately 0.635 cm thick) from the neck and shoulder areas. Four cores from each side of the carcass were taken, for a total of eight core samples taken from each animal. Each sample was aseptically placed, surface side up, into a labeled sterile plastic 100 x 15 mm petri dish (Fisher Science Company), ensuring carcass identification throughout the process. The samples were stored on ice and transported to the laboratory for treatment application and analyses.

Two separate experiments were conducted. One experiment involve the antagonistic action of *L. delbrueckii* subsp. *lactis* RM2-5 toward *E. coli* O157:H7 on the surface of beef carcasses and the other involved the antagonistic action of *L. delbrueckii* subsp. *lactis* RM2-5 toward S.

typhimurium . Each experiment was conducted identically with the exception of the pathogen used (*E. coli* O157:H7 or *S. typhimurium).*

After cores arrived at the laboratory they were subjected to one of four treatments A, B, C and D. For Treatment A (control) 0.1ml of sterile water and 0.1ml of sterile aqueous 10% nonfat milk solids were added to the geometric center of the top of each of the four core samples in four individual petri dishes. For Treatment B (lactobacilli alone), 0.1ml of concentrated cell suspension of *L. delbrueckii* subsp. *lactis* RM2-5 and 0.1 ml of sterile water were added to the geometric center of the top of each of four core samples in four individual petri dishes. Treatment C (pathogen alone) consisted of 0.1ml of cell suspension of the pathogen and 0.1ml of sterile aqueous 10% nonfat milk solids placed on the geometric center of the top of each of four core samples in four individual petri dishes. For Treatment D (pathogen and lactobacilli), 0.1ml of concentrated cell suspension of *L. delbrueckii* subsp. *lactis* RM2-5 and 0.1 ml of the pathogen were added to the geometric center of the top of each of four additional core samples in four individual petri dishes.

After carcass surface samples were subjected to the assigned treatments all samples were stored at 5° C. One sample from each treatment was removed for microbial analyses on days 0 and 6 or 8. For the experiment involving *E. coli* O157:H7 samples were plated on VRBA, PCA and LBS. For the experiment involving *S. typhimurium* samples

were plated on BGA, PCA and LBS. Both experiments were replicated five times.

Direct Application on the Surface of Pork Carcasses

The experiments involving the pork carcasses were carried out in a like manner as used for the beef carcass experiments. Due to the differences in the way that beef cattle and market hogs are slaughtered the only difference was the pork carcass samples were taken after the pigs had been scalded and eviscerated and samples taken were skin samples from the jowl and shoulder area rather than from skinned areas of the carcass. Two separate experiments were conducted. One experiment involved the antagonistic action of *L. delbrueckii* subsp. *lactis* RM2-5 toward *E. coli* O157:H7 and the other involved the antagonistic action of *L. delbrueckii* subsp. *lactis* RM2-5 toward *S. typhimurium* as was done for the beef carcass samples.

Statistical Analyses

Statistical analyses for all experiments were conducted as a split plot in a randomized complete block design with animals or meat samples as blocks in a 2 x 4 factorial arrangement of treatments in main units and 3 media in sub units. All experiments were repeated at least three times. Least significant difference analyses were used to compare means for significant differences at the 5% level of confidence. All data were

analyzed with the SAS program PROC MIXED and LSMEANS (SAS, 1985).

RESULTS

Effects of freezing on *Lactobacillus delbrueckii* subsp. *lactis*

Five strains of *L. delbrueckii* subsp. *lactis* (RM2-5, RM1-9, RM4-7, RM4-1, and I) were tested to confirm their ability to survive freezing and storage at -196° C in liquid nitrogen. Viable populations in the cultures as measured by plating on MRS agar initially (before freezing) and after 1, 7 and 14 d at -196° C revealed slight although not significant ($P > 0.05$) losses in counts for any of the five strains during the 14 days of storage (Figure B1, appendix, illustrates the survival of the cultures during frozen storage).

Influence of *Lactobacillus delbrueckii* subsp. *lactis* toward background microflora

The five strains of *L. delbrueckii* subsp. *lactis* were compared for their ability to influence the growth of the microflora on the surface of fresh cut beef steaks stored at 5°C. There were no significant differences ($P > 0.05$) in total plate counts among samples inoculated with any of the five cultures nor between any of the five cultures and the control sample on days 0 and 3. On day 6 there were no significant differences in total plate count ($P > 0.05$) among the five cultures, however, they were all significantly lower ($P < 0.05$) than the control sample (Table 1). Similar results were obtained on day 9 of storage with the exception of strain I which was not significantly different from the control or the other four

cultures. These results suggest that the *L. delbrueckii* subsp. *lactis* strains RM2-5, RM1-9, RM4-1 and RM4-7 all exerted inhibitory effects on the growth of psychrotrophic microorganisms on the surface of fresh cut beef stored at 5°C.

There were no significant ($P > 0.05$) differences in numbers of lactobacilli among the five strains of on any of the evaluation days. These results imply that similar populations of each culture adhered to the surface of the fresh beef steak. Each of the five strains had significantly higher ($P < 0.05$) numbers of lactobacilli than did the control on each evaluation day, this result was expected since the control was not inoculated with lactobacilli. The numbers of lactobacilli did increase in the control samples during storage, however, the numbers did not reach the numbers present in the inoculated samples.

On day 0 there were no significant ($P > 0.05$) differences in numbers of coliforms detected among the six samples including the control, however, there were significant differences ($P < 0.05$) in numbers between the control and the samples inoculated with the five cultures on days 3, 6 and 9 (Table 1). On each of those sample days the control had a significantly higher coliform count (an average of at least 2 log units greater) than did the samples which had been inoculated with each of the five strains of *L. delbrueckii* subsp. *lactis*. These results imply that all the strains of lactobacilli tested were effective in suppressing the growth of coliforms on the surface of fresh cut beef stored at 5°C. The magnitude

of the inhibitory action appeared much greater on the coliforms than on the psychrotrophs based on the counts on VRBA and on PCA incubated at 15°C.

Even though there was little or no difference among the strains of *L. delbrueckii* subsp. *lactis* in these experiments, strain RM2-5 was chosen for further study. In an earlier study (Yap and Gilliland, 1999) it was significantly more active in producing hydrogen peroxide at 5°C than were the other strains.

Ground Beef

Since an earlier study in our laboratory (Brashears et al., 1998) showed that *L. delbrueckii* subsp. *lactis* acted antagonistically against *E. coli* O157:H7 on raw chicken meat at refrigerated temperatures we conducted experiments to investigate the effects of *L. delbrueckii* subsp. *lactis* on *E. coli* O157:H7 in other meat models. The first meat model was ground beef stored at 5° C. A storage temperature of 5° C was chosen because this would mimic actual retail storage conditions of ground beef.

There were few significant differences in total plate counts among treatments on day 0, 3 and 6 of refrigerated storage. However, on day 9 treatment B (*L. delbrueckii* subsp. *lactis* RM2-5 alone) had a significantly lower ($P < 0.05$) total plate count (PCA at 15°C) than did treatment A (control) indicating that the lactobacilli was inhibitory to the background flora on the meat (Table 2).

There was no significant difference ($P > 0.05$) between counts on VRBA for samples inoculated with *E. coli* O157:H7(treatments C and D) on day 0 (Table 2). However, the VRBA counts on these two samples on day 0 were significantly higher ($P < 0.05$) than were those for treatments A and B which were not inoculated with *E. coli* O157:H7. These results illustrate that the inoculation level of *E. coli* O157:H7 was high enough so that counts on VRBA for the samples (Treatments C and D) provided a true representation of numbers of this pathogen present on day 0. Due to growth of coliforms in the control sample, on day 3 VRBA counts of treatment A (control) were not significantly different ($P > 0.05$) than treatments C and D that were inoculated with *E. coli* O157:H7. On day 6 treatment C had a significantly higher ($P < 0.05$) VRBA count than did treatments D and B. On day 9 treatments A and C did not have significantly different VRBA counts from each other, however, they both had significantly higher ($P < 0.05$) VRBA counts than B and D. These results show the inhibitory effect of the lactobacilli in treatment B on the naturally occurring coliforms on the meat. The increased numbers of coliforms observed on days 6 and 9 in treatment C are indicative of the growth of the coliforms present in the back ground flora of the meat since E. coli O157:H7 does not grow at 5° C (Doyle and Schoenil, 1987; Weagant et al., 1994; Jay, 1992). Thus the increase in numbers of background coliforms to numbers above the inoculation levels used makes it impossible to interpret any possible effect on this pathogen in

these experiments. It should be noted that populations of lactobacilli in treatments inoculated with *L. delbrueckii* subsp. *lactis* RM2-5 (treatments B and D) remained constant at approximately 1.0 x 10^7/g over 9 days of storage (data not shown).

Intact meat

A dip solution was used in preliminary experiments to inoculate the surfaces of intact pieces of meat with *E. coli* O157:H7, *L. delbrueckii* subsp. *lactis* RM2-5, or a combination of the two.

Glucose (0.055 M) was chosen as the dip with the idea that it would provide a readily available substrate for the lactobacilli to enhance their ability to produce hydrogen peroxide. Villegas and Gilliland (1998) reported that cells of *L. delbrueckii* subsp. *lactis* produced more hydrogen peroxide with glucose present than without it. As described in the materials and methods there were four treatments A (control), B (*L. delbrueckii* subsp. *lactis* alone), C (*E. coli* O157:H7 alone), and D (*E. coli* O157:H7 and *L. delbrueckii* subsp. *lactis*). The total plate counts (PCA at 15°C) increased on all samples during storage at 5° C, however there were no significant differences ($P > 0.05$) among the four treatments (A, B, C and D) on either days 0, 3 or 6. However, on day 9, treatment A (control) had significantly higher ($P < 0.05$) numbers than did treatment D. Although not statistically significant, treatment A had higher counts than did either treatment B or C (Table 3). These results may be

attributed to the antagonistic action of the *L. delbrueckii* subsp. *lactis* RM2-5 toward the growth of the naturally occurring bacteria on the surface of the fresh cut meat in treatments B and D.

There was no significant difference ($P > 0.05$) in VRBA counts between the two samples inoculated with *E. coli* O157:H7(treatments C and D) on day 0 (Table 5). The VRBA counts for these two samples were significantly higher ($P < 0.05$) than those for treatments A and B which were not inoculated with *E. coli* O157:H7. These results illustrate that the inoculation level of *E. coli* O157:H7 was high enough so that counts on VRBA for the samples inoculated with *E. coli* O157:H7 provided a true representation of numbers present on day 0 and were not influenced by the background flora on the meat. Throughout the entire 9 day storage at 5°C there were no significant differences ($P > 0.05$) between treatments A and B nor between B and D. Although not significant, there was a tendency for lower counts on VRBA for samples inoculated with *L. delbrueckii* subsp. *lactis* RM2-5 than in the ones not inoculated with the lactobacilli. The population of *E. coli* O157:H7 for treatments C and D both exhibited significant ($P < .05$) decreases during refrigerated storage. For treatment C there was a 1.2 Log reduction between day 0 and day 9 and for treatment D there was a 1.4 Log reduction over 9 days storage at 5°C. Although the total reduction between treatments C and D are not statistically different ($P < .05$) treatment D had larger reduction which

may be attributed to the antagonistic action of the lactobacilli toward the *E. coli* O157:H7.

It also should be noted that the use of the dip on the meat caused excess leaching of color during storage. A light pale pink to gray color also developed over storage time from the meat which had been submerged in the dip solutions.

Direct Surface Application

Since dipping meat samples might be an impractical method of inoculating meat surfaces, another method of inoculation was chosen for further evaluations of the potential antagonist action of the *Lactobacillus* against *E. coli* O157:H7 in meat models. In this method of application the inoculum (either the *E. coli* O157:H7, *L. delbrueckii* subsp. *lactis* RM2-5, or both) in a small volume was pipeted directly onto the surface of the meat. One series of experiments was done using cells of *L. delbrueckii* subsp. *lactis* RM2-5 from a concentrated culture that had been frozen and the second using a freshly prepared (i.e. nonfrozen) concentrated culture of *L. delbrueckii* subsp. *lactis* RM2-5. Again four treatments (A, B, C, and D) were used. Treatment A (uninoculated – control) permitted us to monitor the natural background microflora of the beef steaks over time. Treatment B (inoculated with only *L. delbrueckii* subsp. *lactis* RM2-5) permitted us to evaluate the influence of the lactobacilli on the natural background microflora of the beef steak

over time. Treatment C (inoculated with only *E. coli* O157:H7) was to enable us to monitor numbers of *E. coli* O157:H7 on the beef steak over time and to serve as the control for Treatment D. Treatment D (inoculated with both *E. coli* O157:H7 and *L. delbrueckii* subsp. *lactis* RM2-5) enabled us to determine how the lactobacilli influenced numbers of *E. coli* O157:H7 on the meat.

In the experiment using the fresh culture there were no significant differences ($P > 0.05$) among treatments for total plate counts on day 0 or 3 of refrigerated storage (Table 4). On day 6 the numbers on the meat inoculated with only the lactobacilli (Treatment B) were significantly lower ($P < 0.05$) than all other treatments. By day 9 there was not a significant difference ($P > 0.05$) between numbers on the samples not inoculated with lactobacilli (Treatments A and C), however, the counts for samples inoculated with lactobacilli (Treatments B and D) were significantly lower ($P < 0.05$) than treatments A and C. The counts for treatment B were still significantly lower ($P < 0.05$) than all other treatments. The lower total plate counts observed for treatments B and D can be attributed to the antagonistic action of the *L. delbrueckii* subsp. *lactis* RM2-5 against the naturally occurring microflora on the surface of the meat.

The counts on VRBA for day 0 revealed significantly higher numbers on the two samples inoculated with *E. coli* O157:H7 (Treatments C and D) than on the two not inoculated with the pathogen (Treatments A and

B). These results again illustrate that the inoculation level was high enough so that counts on VRBA for the samples inoculated with *E. coli* O157:H7 provided a true representation of numbers present on day 0. The numbers of *E. coli* O157:H7 in the samples inoculated with the pathogen alone exhibited very little change during storage. Those in the sample inoculated with both the *E. coli* O157:H7 and lactobacilli (Treatment D) exhibited some decline on days 3 and 6 but were not significantly ($P > 0.05$) different than those in Treatment C. On day 9 coliform counts were significantly lower ($P < 0.05$) in the samples inoculated only with the lactobacilli (Treatment B) than in the control (Treatment C) indicating that the lactobacilli were able to suppress the growth of naturally occurring coliforms on the surface of the meat (Table 4).

Results from the second experiment involving the direct application of cells of the lactobacilli from a frozen culture did not appear to provide as much control over total plate counts as we observed in the first experiment involving the fresh culture (Table 5). The meat inoculated only with lactobacilli (Treatment B) had significantly lower ($P < 0.05$) counts than the other treatments on day 0 and 3. On day 6 the control (Treatment A) had significantly higher ($P < 0.05$) counts than the other treatments (B, C and D) which were not significantly different from each other. By day 9 Treatment B had significantly lower ($P < 0.05$) counts than all of the treatments and the other treatments (A, C and D) were not

significantly different ($P > 0.05$) from each other. These results again indicate that it was the antagonist action of the *L. delbrueckii* subsp. *lactis* RM2-5 in treatments B and D that suppressed growth of the naturally occurring background microflora on the surface of the meat during storage.

The counts on VRBA for the experiment using frozen culture of the lactobacilli were similar to those observed in the previous experiments for days 0 and 3 of storage (Table 5). On day 6 there was not a significant difference ($P > 0.05$) in the treatments inoculated with *E. coli* O157:H7 (treatment C and D), however, there was a significant difference ($P < 0.05$) between the uninoculated treatments (treatments A and B). By day 9 there still was not a significant difference ($P > 0.05$) in the treatments inoculated with *E. coli* O157:H7 (treatment C and D), and the uninoculated treatments were not significantly different as well (Table 5).

Direct Application on the Surface of Beef Carcasses

***E. coli* O157:H7.** To determine the influence of *L. delbrueckii* subsp. *lactis* RM2-5 toward *E. coli* O157:H7 on the surface of freshly slaughtered beef carcasses samples of the carcass surface were excised aseptically from the neck and shoulder area of the carcass after evisceration and prior to the final carcass wash and aseptically placed into petri dishes with the surface side up. The samples were taken to the laboratory for

inoculation and evaluation before and after eight days of storage at 5° C. Treatment designations were similar to those in the direct application experiments in the previous section.

Inoculation of the samples of beef carcass surface with lactobacilli had no significant effect ($P > 0.05$) on the growth of psychrotrophic bacteria as indicated by increases in total plate counts during storage (Table 6). Populations of lactobacilli of treatments B and D that were inoculated with *L. delbrueckii* subsp. *lactis* Rm2-5, remained constant at approximately 1.0×10^7/g over the 8 day storage period (data not shown).

The counts on VRBA indicated that there were no significant differences ($P > 0.05$) in VRBA counts between samples inoculated with *E. coli* O157:H7 (Treatments C and D) nor between those not inoculated with *E. coli* O157:H7 (Treatments A and B)on day 0 (Table 9). However, the counts on VRBA were significantly higher ($P < 0.05$) in treatments C and D compared to treatments A and B which were not inoculated with *E. coli* O157:H7. This again enabled us to observe the behavior of *E. coli* O157:H7 on the samples based on counts on VRBA. On day 8 of refrigerated storage there was no significant difference ($P > 0.05$) in VRBA counts between treatments A and B, although the count for Treatment B VRBA was half a log unit lower than for Treatment A. Treatment D had a significantly lower ($P < 0.05$) VRBA count than Treatment C (approximately 1.3 Log units). This significant difference in VRBA counts

between treatment C and D indicates that the *L. delbrueckii* subsp. *lactis* RM2-5 was in fact able to act antagonistically against *E. coli* O157:H7 on the surface of freshly slaughtered beef carcasses.

S. typhimurium. Due to the promising results found in the experiment investigating the antagonist action of *L. delbrueckii* subsp. *lactis* RM2-5 toward *E. coli* O157:H7 on the samples of the surface of freshly slaughtered beef carcasses a similar experiment was conducted using *S. typhimurium.* The same protocol was used to investigate the influence of *L. delbrueckii* subsp. *lactis* RM2-5 toward *S. typhimurium* as was used in the previous experiments involving *E. coli* O157:H7.

Again, results revealed no significant effect ($P > 0.05$) of the lactobacilli on the growth of psychrotrophs on the samples based on monitoring counts on PCA (Table 7). As observed in the previous experiment (Table 6) it should be noted that, although not statistically significant, counts for treatment B (lactobacilli alone) were lower than for treatment A (control) on both day 0 and day 8. The number of lactobacilli of treatments B and D that were inoculated with *L. delbrueckii* subsp. *lactis* RM2-5, remained constant over the 8 day storage period (approximately 1.0×10^7/g; data not shown).

The numbers of salmonella were monitored by plating on BGA. Data indicated that there was not a significant difference ($P > 0.05$) in BGA counts between the samples inoculated with *S. typhimurium* (treatments

C and D) on day 0, (Table 7). However, the BGA counts were significantly higher ($P < 0.05$) for treatments C and D compared to treatments A and B that were not inoculated with *S. typhimurium*. These results illustrate that the inoculation level of *S. typhimurium* was high enough so that counts on BGA for the samples inoculated with *S. typhimurium* provided a true representation of numbers of this organism present on day 0 and were not influenced by background flora that is present on the samples. The background flora that formed colonies on the BGA (i.e. on the samples not inoculated with *S. typhimurium)* was not identified but likely were due to coliforms and not salmonella since coliforms would grow readily on BGA. The data from day 8 results indicates that the lactobacilli exerted inhibitory action toward this background flora in that the BGA counts for Treatment B were significantly lower ($P < 0.05$) than for treatment A. For the two treatments inoculated with *S. typhimurium* (treatment C and D) BGA counts for Treatment D which had been inoculated with both the salmonella and lactobacilli were significantly lower ($P < 0.05$) on day 8 than counts for Treatment C inoculated only with the salmonella (Table 7). The counts for Treatment D were approximately 1.5 log units lower than counts for Treatment C. This significant difference ($P < 0.05$) in BGA counts between treatment C and D indicate that the *L. delbrueckii* subsp. *lactis* RM2-5 was in fact able to act antagonistically against *S. typhimurium* on the surface of freshly slaughtered beef carcasses.

Neither of the pathogens increased in number for the samples inoculated only with the pathogen where as the number of pathogens in the samples additionally inoculated with the lactobacilli decreased during storage in both cases.

Direct Application on the Surface of Pork Carcasses

Because of the successful results obtained in experiments showing antagonistic effect that *L. delbrueckii* subsp. *lactis* RM2-5 had against the two pathogens on the surface of freshly slaughtered beef carcasses similar experiments were conducted on freshly slaughtered pork carcasses. The only exceptions were the samples were stored 6 days at 5°C for the *E. coli* O157:H7 experiment (with 10 replications) and 8 days for the S. *typhimurium* (with 5 replications). The experiments involving the pork carcasses were carried out in the same manner as for the beef carcass experiments. Treatment designations were similar to the treatments assigned in beef carcass application experiments discussed previously.

***E. coli* O157:H7.** The cells of *L. delbrueckii* subsp. *lactis* RM2-5 exhibited antagonistic action toward psychrotrophs and *E. coli* O157:H7 on the pork carcass skin samples (Table 8). Results from the plate counts on PCA revealed less than 1 per gram for all four treatments. On day 6 of refrigerated storage the PCA plate count on the uninoculated

skin (Treatment A) was significantly higher ($P < 0.05$) than all other treatments and the count on the sample inoculated only with *E. coli* O157:H7 was significantly higher ($P < 0.05$) than on either sample inoculated with lactobacilli (Treatments B and D). These results indicate that the lactobacilli was inhibitory to the growth of naturally occurring background microflora found on the surface of pork carcasses during refrigerated storage. Populations of lactobacilli for Treatments B and D that were inoculated with *L. delbrueckii* subsp. *lactis* RM2-5, remained constant over the 6 day storage period (approximately 1.0×10^7/g; data not shown).

Counts obtained on VRBA revealed less than one coliform per gram on samples that were not inoculated with *E. coli* O157:H7 (Treatments A and B). On days 0 and 6 the VRBA counts for Treatment D (inoculated with both *E. coli* O157:H7 and lactobacilli) were significantly lower ($P < 0.05$) on both days than for Treatment C (inoculated with only *E. coli* O157:H7). These results are interesting because both treatments were inoculated with the same level of *E. coli* O157:H7 and plated shortly after to obtain the day 0 data. These results indicate that the *Lactobacillus* was able to act antagonistically toward *E. coli* O157:H7 very rapidly on the pork skin. Results from day 6 were similar to results from day 0 (Table 8). Treatment D was still had significantly lower ($P < 0.05$) VRBA counts than treatment C.

S. typhimurium. Results from the enumeration of plate counts on PCA indicated that Treatment A (uninoculated control) had a higher ($P < 0.05$) count than Treatments C and D on day 0 (Table 9). On day 8 Treatments B and D (inoculated with *L. delbrueckii* subsp *lactis* RM2-5) were not significantly different from each other but had significantly lower ($P < 0.05$) counts than those not inoculated with lactobacilli (treatments A and C). These results may be attributed to the action of the lactobacilli in suppressing the growth of the naturally occurring microflora found on the surface of the pork carcass. The numbers of lactobacilli in treatments B and D that were inoculated with *L. delbrueckii* subsp. *lactis* RM2-5, remained constant over the 8 day storage period (approximately 1.0×10^7/g; data not shown).

The numbers of salmonella in samples inoculated with S. *typhimurium* were monitored by plating on BGA. This was possible because the BGA counts were significantly higher ($P < 0.05$) for treatments C and D compared to treatments A and B which were not inoculated with S. *typhimurium* (Table 9).

Results from BGA counts on day 8 indicated no significant differences ($P > 0.05$) between treatment A and B. The counts on BGA for treatments A and B likely were not *Salmonella* but coliforms. It should be noted that although not statistically significant treatment B had a notably lower count. On day 8 treatment D had a significantly lower ($P < 0.05$) BGA count than did treatment C which indicates the antagonistic

action of *L. delbrueckii* subsp. *lactis* RM2-5 toward *S. typhimurium* on the surface of pork carcass skin.

DISCUSSION

Of the five strains of lactobacilli that were initially used to investigate their influence toward the background microflora in fresh cut beef steaks, *L. delbrueckii* subsp. *lactis* RM2-5 exhibited somewhat better survival characteristics than the others, was antagonistic toward background microflora, and in a previous study (Yap and Gilliland, 2000) produced significantly more hydrogen peroxide than did the other strains tested. In previous studies (Gilliland and Speck, 1975; Brashears et al., 1998) the antagonistic effect of *L. delbrueckii* subsp. *lactis* toward the background microflora at refrigeration temperatures was attributed to the ability of this species to produce hydrogen peroxide at refrigerated temperatures. Because *L. delbrueckii* subsp. *lactis* RM2-5 was as effective or slightly more effective than the other four strains tested in our preliminary experiments and the fact that it has been reported to produce significantly greater amounts of hydrogen peroxide than the other strains we elected to use in subsequent experiments investigating the antagonist action against spoilage microflora and pathogens on meat.

Lactobacillus delbrueckii subsp. *lactis* does not grow at refrigerated temperatures, however, it does produce hydrogen peroxide (Yap and Gilliland, 2000; Dahiya and Speck, 1968; Gilliland and Speck, 1975, Brashears et al., 1998). Because the cells of *L. delbrueckii* subsp. *lactis* RM2-5 were removed from the broth in which they had been grown prior to being inoculated onto the meat, it is not likely that other substances

such as lactic acid or bacteriocins produced during their growth could have caused the killing action.

Results from the ground beef and meat dip experiments indicate that at refrigerated storage (5°C) *L. delbrueckii* subsp. *lactis* RM2-5 is effective in reducing the numbers of *E. coli* O157:H7. In these experiments the decrease in *E. coli* O157:H7 populations was not as great as differences seen in the experiments done in associative broth cultures (data not shown). However, it should be noted that in these experiments the *L. delbrueckii* subsp. *lactis* RM2-5 was effective in reducing the growth of psychrotrophs as well as background coliforms. The reductions in growth of psychrotrophs and coliforms generally became more pronounced the longer the samples were stored at 5°C. It is likely that because of the complex microbial environment in the ground beef as well as on the surface of the meat the lactobacilli were not able to be specifically antagonist to any one organism as observed in the broth experiments. However, granted the complex microbial environment that existed, the lactobacilli were still able to act antagonistically toward not only *E. coli* O157:H7 but other background spoilage microflora and coliforms as well.

The experiments where direct application of *L. delbrueckii* subsp. *lactis* RM2-5 onto the surface of the meat was investigated resulted in similar findings to the ground beef and dip experiments. In the experiments in which the meat was dipped into the bacterial suspension,

even though the antagonistic action of the lactobacilli was observed there were adverse quality and color characteristics that developed over time. The direct application of a suspension of *L. delbrueckii* subsp. *lactis* RM2-5 onto the surface on the meat eliminated the adverse quality and color effects observed with the use of the dips while still maintaining the effective antagonistic action of the lactobacilli toward *E. coli* O157:H7, background spoilage microflora and coliforms. Dipping the meat into an aqueous suspension of the lactobacilli probably would not be a very practical approach. The direct application could be achieved as a spray.

The counts on PCA for meat treated with direct application of the lactobacilli after 9 days of storage at 5°C which was 2.8 log cycles lower than for the control (Table 4) is a very desirable total count in regards to the shelf-life of that product. Recommended shelf-life of a fresh cut beef steak is approximately 3 to 5 days with an average total count in the range of 5 to 6 $Log_{10}CFU/g$ or cm^2 (Jay, 1992). These results reiterate how *L. delbrueckii* subsp. *lactis* RM2-5 was still able to act antagonistically toward not only *E. coli* O157:H7 but background spoilage microorganisms as well, perhaps having the potential to extend a product's shelf-life.

Preliminary experiments comparing the fresh and frozen cultures of lactobacilli indicated that there was little or no difference between the use of fresh or frozen cultures. Initially there appeared to be an advantage using the fresh culture in that more inhibition of the growth of

psychrotrophic microorganisms was obtained. However, since the use of a frozen culture provided the same initial populations of *L. delbrueckii* subsp. *lactis* RM2-5 and they were more convenient to use, they were used throughout the remainder of the study. Future research perhaps is needed to determine if freezing and frozen storage of the cultures of lactobacilli adversely influence their ability to inhibit undesirable organisms on meat during refrigerated storage.

Antagonistic effects of *L. delbrueckii* subsp. *lactis* RM2-5 toward both *E. coli* O157:H7 and *S. typhimurium* were observed when cells of the lactobacilli were directly applied on the surface of freshly slaughtered beef carcasses. Reductions in growth of background flora at 5°C on samples not inoculated with either of the pathogens also were caused by the lactobacilli. The antagonistic actions appeared to be greater than in the experiment involving the direct application on the surface of the steaks. This may be attributed to the fact that the surface of the beef carcass is primarily fat with little exposed lean tissue. The lean tissue may promote and sustain a more complex microbial environment where more complicated microbial competition exists. The surface of the freshly slaughtered beef carcass on the other hand, may not be inhabited by such a complex microbial environment due not only to its biochemical composition but to its relative age or freshness as compared to the meat surfaces used which were not as fresh as the newly slaughtered beef carcass.

When the direct application of *L. delbrueckii* subsp. *lactis* RM2-5 was tested against *E. coli* O157:H7 and *S. typhimurium* on the surface of freshly slaughtered pork carcasses the results were even better than on the beef carcass surface samples. The magnitude of these differences observed on the surface of pork carcasses as compared to the beef carcasses may be attributed to the biochemical make up of each carcass surface. The surface of a pork carcass has the skin intact with only the outer hair removed. Due to the nature of the way hogs are processed the carcass has been scalded, dehaired and singed. This process alone reduces the existing background microbial population. The beef carcass on the other hand, had the hide removed and a fat tissue surface exposed that most likely was subjected to cross contamination of microorganisms from both the outside and inside of the animal (Ayres, 1955). The fat tissue surface on the beef carcass is more capable of providing a source of more readily available nutrients for the microorganisms than would the surface of the pork carcass. Not only is the surface of the pork carcass initially cleaner but it is a surface that is not as porous and permeable as the fat tissue surface of the beef carcass.

One explanation for the better antagonistic action seen in the pork and beef carcass experiments than on the other meat systems may be due to a lack of or decreased amount of catalase present on the surface of the carcasses due to the skin barrier on the pork carcass and the fat

barrier as well as epimysium surrounding exposed muscle tissue on the beef carcass. The production of hydrogen peroxide that we believe is the primary compound responsible for the antagonistic action of *L. delbrueckii* subsp. *lactis* RM2-5 could be, at least partially, dissipated by the catalase present in meat (Jensen, 1954). Within the muscle fibers are myofibrils that are surrounded by the sarcoplasm which is a fluid phase. In this fluid phase of the sarcoplasma are various organelles one of which is the peroxisome. Contained inside the peroxisome are fatty acyl oxidases and catalase (DeDuve and Baudhuin, 1966). The biosynthesis of catalase is blocked under anaerobic conditions in the absence of heme, however under aerobic conditions in the presence of heme (such as in fresh cut meat) catalase can be synthesized (Hammes et al., 1995). It is possible that in the fresh steak experiments the catalase that may have been released from the peroxisomes during fabrication may have contributed to some of the dissipation of the hydrogen peroxide produced by the lactobacilli, therefore causing a decrease in the antagonistic action against the pathogens tested.

It should also be noted that in the experiments where beef steaks were used the meat was obtained from a local supermarket and as a result we could only approximate the actual age (time after slaughter) of the meat. In the beef and pork carcass experiments the actual age (time after slaughter) was known. It is possible that the age of the meat also could be a factor which caused the apparent difference in the intensity of

the antagonism on the beef steaks compared to the samples from the carcass surfaces and should be considered in future experiments. Because the steaks from the supermarket were approximately one week (after slaughter) older than the samples used in the carcass experiments the steaks may have had more time for the catalase present in the meat to express itself.

In recent years many decontamination processes or intervention technologies have been studied to help reduce the numbers of microorganisms, particularly pathogens such as *E. coli* O157:H7 and S. *typhimurium* on the surface of carcasses. These intervention technologies range from hot water rinses, organic acid rinses to steam pasteurization. It has been shown that the treatment of carcasses with hot water rinses and lactic or acetic acid can reduce the total numbers of bacteria by two or three orders of magnitude (Frederick, et al., 1994; Smulders et al., 1986). However, it also has been shown that species of different bacteria can vary in their susceptibility to organic acids and hot water rinses, in particular *E. coli* and *Salmonella* have been observed to be notably resistant (Brackett et al., 1994). Even though it has been shown that hotter rinses and stronger organic acids can achieve larger reductions in microbial populations and even start to effect *E. coli* and *Salmonella* populations, these hotter and stronger rinses will ultimately damage the quality and color characteristics of the meat (Bell et al., 1986; Woolthuis and Smulders, 1985).

The results from the experiments investigating the antagonistic action of *L. delbrueckii* subsp. *lactis* RM2-5 against *E. coli* O157:H7 and *S. typhimurium* in various meat models suggest that cultures of lactobacilli have a potential as an intervention technology. The results from the experiments conducted on the surfaces of beef and pork carcasses has indicated that the use of a culture of lactobacilli in the form of a rinse or spray application may help reduce the numbers of existing pathogens such as *E. coli* O157:H7 and *S. typhimurium* . Results also suggest that an extension of the shelf-life of the meat is possible due to the decreased growth of coliforms and psychrotrophic spoilage organisms. It should also be noted that there were no adverse quality or color effects apparent as a result of the application of the lactobacilli culture on the surface of beef and pork carcasses. Future research efforts should be focused on technologies to apply the cultures of lactobacilli to the carcass evenly, effectively and economically.

TABLE 1. Antagonistic action of five strains of *Lactobacillus delbrueckii* susbp.*lactis* toward naturally occurring background microflora found on fresh cut beef steaks during storage at 5° C.

Treatment	Type of organism enumerated	Count / g[a] Day 0	Day 3	Day 6	Day 9
Control		5.0 A	6.9 A	8.6 B	8.6 A
RM2-5		4.6 A	5.6 A	7.2 A	7.3 B
RM1-9	Total Plate Count[d]	4.6 A	5.9 A	7.7 A	7.4 B
I	at 15° C	4.9 A	6.3 A	7.7 A	7.9 A B
RM4-1		4.6 A	6.2 A	7.3 A	7.6 B
RM4-7		4.6 A	5.8 A	7.2 A	7.6 B
Control		1.0 B[b]	3.9 B	3.7 B	5.1 B
RM2-5		8.0 A	7.9 A	8.2 A	8.0 A
RM1-9	Lactobacilli[c]	7.7 A	7.8 A	7.7 A	7.5 A
I		7.9 A	7.6 A	6.8 C	7.1 A
RM4-1		8.0 A	8.3 A	8.3 A	8.0 A
RM4-7		8.1 A	8.3 A	8.0 A	7.9 A
Control		0.9 A	3.1 A	3.4 B	4.1 B
RM2-5		0.7 A	1.0 B	1.8 AC	1.8 A
RM1-9	Coliforms[e]	0.7 A	1.8 B C	2.0 A	2.1 A
I		0.8 A	2.4 C	2.3 A	2.6 A
RM4-1		0.7 A	1.4 B	1.5 C	1.8 A
RM4-7		0.7 A	0.9 B	1.3 C	1.4 A

[a]Microbial counts are expressed as $\log_{10}$ CFU/g; each value is the mean from four replicate trials.
[b]Means in the same column with the different letters are significantly different ($P < 0.05$).
[c]Numbers of lactobacilli were detected on LBS agar and are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials.
[d]Total Plate Counts were detected on PCA incubated 7 d at 15° C and are expressed as $\log_{10}$ CFU/g; each value is a mean from four trials.
[e]Coliforms were detected on VRBA and are expressed as $\log_{10}$ CFU/g; each value is a mean from four trials.

Table 2. Antagonistic action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 toward *Escherichia coli* O157:H7 in ground beef stored at 5° C.

Type of organism enumerated	Treatment	Count / g[a]			
		Day 0	Day 3	Day 6	Day 9
Total Plate Count[b] At 15° C	A (Control)	1.3 A[d]	3.2 A B	4.1 A	5.6 A
	B (Lactobacilli only)	1.9 A	2.4 B	3.8 A	4.3 B
	C (*E. coli* O157:H7 only)	2.2 A	3.5 A	3.9 A	5.4 A
	D (*E. coli* O157:H7 and lactobacilli)	2.0 A	3.5 A	4.4 A	5.0 A B
Coliforms[c]	A (Control)	1.4 A	2.8 A B	3.9 A B	5.0 A
	B (Lactobacilli only)	1.0 A	2.2 A	3.0 A	4.0 B
	C (*E. coli* O157:H7 only)	3.6 B	3.6 B	4.5 B	5.1 A
	D (*E. coli* O157:H7 and lactobacilli)	3.4 B	2.8 A B	3.2 A	3.9 B

[a] Microbial counts are expressed as $\log_{10}$ CFU/g; each value is the mean from three replicate trials.
[b]Total Plate Counts detected on PCA incubated 7 d at 15° C and are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials.
[c]Coliform counts detected on VRBA are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials.
[d]Means in the same column with the different letters are significantly different ($P < 0.05$).

Table 3. Antagonistic action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 toward *Escherichia coli* O157:H7 on the surface of fresh beef steaks after being treated in a glucose based dip solution and stored at 5° C.

Type of organism enumerated	Treatment	Count / g[a]			
		Day 0	Day 3	Day 6	Day 9
Total Plate Count[b] At 15° C	A (Control)	4.9$_{A}$[d]	6.3$_{A}$	7.1 $_{A}$	8.1 $_{A}$
	B (Lactobacilli only)	4.6 $_{A}$	5.7 $_{A}$	6.7 $_{A}$	7.4 $_{A\ B}$
	C (*E. coli* O157:H7 only)	5.1 $_{A}$	6.2 $_{A}$	6.9 $_{A}$	7.5 $_{A\ B}$
	D (*E. coli* O157:H7 and lactobacilli)	5.0 $_{A}$	5.6 $_{A}$	6.7 $_{A}$	7.1 $_{B}$
Coliforms[c]	A (Control)	1.3 $_{A}$	2.8 $_{A}$	3.2 $_{A}$	3.8 $_{A}$
	B (Lactobacilli only)	1.4 $_{A}$	2.0 $_{A}$	2.5 $_{A}$	3.1 $_{A}$
	C (*E. coli* O157:H7 only)	6.4 $_{B}$	5.7 $_{B}$	5.4 $_{B}$	5.2 $_{B}$
	D (*E. coli* O157:H7 and lactobacilli)	6.1 $_{B}$	5.2 $_{B}$	5.1 $_{B}$	4.7 $_{B}$

[a] Microbial counts are expressed as $\log_{10}$ CFU/g; each value is the mean from three replicate trials.
[b]Total Plate Counts detected on PCA incubated 7 d at 15° C and are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials.
[c]Coliform counts detected on VRBA are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials.
[d]Means in the same column with the different letters are significantly different ($P < 0.05$).

Table 4. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (fresh culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C.

Type of organism enumerated	Treatment	Count / g[a]			
		Day 0	Day 3	Day 6	Day 9
Total Plate Count[b] At 15° C	A (Control)	2.2 A[d]	2.9 A	4.1 A	5.8 A
	B (Lactobacilli only)	2.0 A	2.2 A	2.6 B	3.0 B
	C (*E. coli* O157:H7 only)	2.8 A	3.3 A	4.6 A	6.2 A
	D (*E. coli* O157:H7 and lactobacilli)	2.1 A	3.3 A	3.8 A	4.6 C
Coliforms[c]	A (Control)	1.0 A	1.0 A	1.8 A	2.8 A
	B (Lactobacilli only)	1.0 A	1.0 A	1.3 A	1.4 B
	C (*E. coli* O157:H7 only)	3.5 B	3.3 B	3.4 B	3.7 C
	D (*E. coli* O157:H7 and lactobacilli)	3.2 B	2.7 B	2.6 B	3.2 C

[a] Microbial counts are expressed as $\log_{10}$ CFU/g; each value is the mean from three replicate trials.
[b]Total Plate Counts detected on PCA incubated 7 d at 15° C and are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials.
[c]Coliform counts detected on VRBA are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials.
[d]Means in the same column with the different letters are significantly different ($P < 0.05$).

Table 5. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (frozen culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C.

Type of organism enumerated	Treatment	Count / g[a]			
		Day 0	Day 3	Day 6	Day 9
Total Plate Count[b] At 15° C	A (Control)	2.8 A [d]	4.1 A	5.8 A	6.6 A
	B (Lactobacilli only)	1.4 B	2.6 B	4.1 B	5.1 B
	C (*E. coli* O157:H7 only)	2.3 A B	3.9 A	4.4 B	6.2 A
	D (*E. coli* O157:H7 and lactobacilli)	2.5 A	3.8 A	4.8 B	5.2 B
Coliforms[c]	A (Control)	1.0 A	1.6 A	1.9 A	2.7 A B
	B (Lactobacilli only)	1.0 A	1.0 A	1.0 B	2.4 A
	C (*E. coli* O157:H7 only)	3.1 B	2.9 B	2.8 A	3.5 B
	D (*E. coli* O157:H7 and lactobacilli)	2.8 B	2.3 B	2.1 A	2.7 A B

[a] Microbial counts are expressed as $\log_{10}$ CFU/g; each value is the mean from three replicate trials.
[b]Total Plate Counts detected on PCA incubated 7 d at 15° C and are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials.
[c]Coliform counts detected on VRBA are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials.
[d]Means in the same column with the different letters are significantly different ($P < 0.05$).

Table 6. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 [a] against *Escherichia coli* O157:H7 on the surface of beef carcasses stored at 5 °C.

Enumeration Media	Day	Counts			
		A (Control)	B (Lactobacilli only)	C (*E. coli* O157:H7 only)	D (*E. coli* O157:H7 and lactobacilli)
PCA at 15°C [b]	0	3.0 A [c]	2.5 A B	3.2 A	2.0 B
	8	5.9 A	5.2 A	6.1 A	6.2 A
BGA [d]	0	1.3 A	1.0 A	5.4 B	4.7 B
	8	2.4 A	1.9 A	4.9 B	3.6 C

[a]Counts of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 remained constant over refrigerated storage time (approximately 1.0 x 10^7 CFU/cm^2).

[b]Total Counts on PCA incubated 7 d at 15 °C are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from five trials.

[c]Means in the same column with the different letters are significantly different ($P < 0.05$).

[d]Coliform counts detected on VRBA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from five trials.

Table 7. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 [a] against *Salmonella typhimurium* on the surface of beef carcasses stored at 5 °C.

Enumeration Media	Day	Counts			
		A (Control)	B (Lactobacilli only)	C (*E. coli* O157:H7 only)	D (*E. coli* O157:H7 and lactobacilli)
PCA at 15°C [b]	0	2.0$_{A}$ [c]	1.4 $_{A}$	2.2 $_{A}$	1.6 $_{A}$
	8	6.7 $_{A}$	5.8 $_{A}$	6.6 $_{A}$	6.4 $_{A}$
BGA [d]	0	1.0 $_{A}$	1.0 $_{A}$	4.8 $_{B}$	4.2 $_{B}$
	8	2.7 $_{A}$	1.3 $_{B}$	4.3 $_{C}$	2.8 $_{A}$

[a]Counts of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 remained constant over refrigerated storage time (approximately 1.0 x 10^7 CFU/cm^2).

[b]Total Counts on PCA incubated 7 d at 15 °C are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from five trials.

[c] Means in the same column with the different letters are significantly different ($P < 0.05$).

[d]*Salmonella typhimurium* detected on BGA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from five trials.

Table 8. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 [a] against *Escherichia coli* O157:H7 on the surface of pork carcasses stored at 5 °C.

Enumeration Media	Day	Counts			
		A (Control)	B (Lactobacilli only)	C (*E. coli* O157:H7 only)	D (*E. coli* O157:H7 and lactobacilli)
PCA at 15°C [b]	0	< 0.0 A [c]	< 0.0 A	< 0.0 A	< 0.0 A
	6	4.8 A	2.3 B	3.9 C	2.5 B
BGA [d]	0	< 0.0 A	< 0.0 A	4.0 B	2.9 C
	6	< 0.0 A	< 0.0 A	2.6 B	1.8 C

[a]Counts of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 remained constant over refrigerated storage time (approximately 1.0 x 10^7 CFU/cm^2).

[b]Total Counts on PCA incubated 7 d at 5 °C are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from five trials.

[c]Means in the same column with the different letters are significantly different ($P < 0.05$).

[d]Coliform counts detected on VRBA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from ten trials.

Table 9. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 [a] against *Salmonella typhimurium* on the surface of pork carcasses stored at 5 °C.

Enumeration Media	Day	Counts			
		A (Control)	B (Lactobacilli only)	C (*E. coli* O157:H7 only)	D (*E. coli* O157:H7 and lactobacilli)
PCA at 15°C [b]	0	4.2 A [c]	3.2 A B	3.1 B	2.9 B
	8	7.3 A	6.3 B	7.2 A	6.5 B
BGA [d]	0	1.0 A	1.0 A	4.3 B	3.9 B
	8	2.2 A	1.5 A	4.3 B	3.5 C

[a]Counts of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 remained constant over refrigerated storage time (approximately 1.0 x 10^7 CFU/cm^2).

[b]Total Counts on PCA incubated 7 d at 15 °C are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from five trials.

[c]Means in the same column with the different letters are significantly different ($P < 0.05$).

[d]*Salmonella typhimurium* detected on BGA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from five trials.

REFERENCES

Arrage, A.A., T.J. Phelps, R.E. Benoit, and D.C. White. 1993. Survival of subsurface microorganisms exposed to UV radiation and hydrogen peroxide. Appl. Environ. Microbiol. 59:3545-3549.

Ayres, J.C. 1955. Microbiological implications in handling, slaughtering, and dressing meat animals. Adv. Food Res. 6:109-161.

Barkate, M.L., G.R. Acuff, L.M. Lucia and D.S. Hale. 1993. Hot water decontamination of beef carcasses for reduction of initial bacterial numbers. Meat Sci. 35:397-401.

Bell, M.F., R.T. Marshall, and M.E. Anderson. 1986. Microbiological and sensory tests of beef treated with acetic and formic acids. J. Food Protect. 49:207-210.

Brackett, R.E., Y.Y. Hao, and M.P. Doyle. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. J. Food Protect. 57: 198-203.

Brashears, M.M., Reilly, S.S., and Gilliland, S.E. 1998. Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* O157:H7 on refrigerated raw chicken meat. J. Food Protect. 61:166-170.

CDC, 1989. Center for Disease Control, Morbidity and Mortality Weekly Report 38:38.

Dahiya, R.S. and Speck, M.L. 1968. Hydrogen peroxide formation by lactobacilli and its effect on *Staphylococcus aureus*. J. Dairy Sci. 51:1568-1572.

Daly, C.W., Sandine, E., and Elliker, P.R. 1972. Interaction of food starter cultures and food-borne pathogens: *Streptococcus diacetilactis* versus food pathogens. J. Milk Food Technol. 35:349-357.

DeDuve, C. and P. Baudhuin. 1966. Peroxisomes (microbodies and related particles). Physiol.Rev. 46:323

Dickson, J.S. 1988. Reduction of bacteria attached to meat surfaces by washing with selected compounds. J. Food Prot. 51:869-873.

Dickson, J.S. and M.E. Anderson. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. J. Food Prot. 55:133-140.

Doyle, M.P., and J.L. Schoenil. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394-2396.

Ehioba, R.M. 1988. Identification of microbial isolates from vacuum packaged ground pork irradiated at 1 kGy. J. Food Sci. 53:278-281.

Frederick, T.L., M.F. Miller, L.D. Thompson, and C.G. Ramsey. 1994, Microbiological properties of pork check meat as affected by acetic acid and temperature. J. Food Sci. 59:300-302.

Gilliland, S.E. and Speck, M.L. 1975. Inhibition of psycrotrophic bacteria by lactobacilli and pediococci in nonfermented refrigerated foods. J. Food Sci. 40:903-905.

Gilliland, S.E. 1980. Use of lactobacilli to preserve fresh meat. Proc. Recip. Meat Conf. 33:54-58.

Gilliland, S.E. and Ewell, H.R. 1983. Influence of combinations of *Lactobacillus lactis* and potassium sorbate on growht of psychrotrophs in raw milk. J. Dairy Sci. 66:974-980.

Hardin, M.D., Acuff, G.R., Lucia, L.M., Oman, J.S. and Savell, J.W. 1995. Comparison of methods for decontamination from beef carcass surfaces. J. Food Prot. 58:368-374.

Hobbs, B.C. 1974. Food Poisoning and Food Hygiene. Edward Arnold Publishing, London.

Hammes, W.P. and R.F. Vogel. 1995. The genus Lactobacillus, In: The Genera of Lactic Acid Bacteria. B. Wood and W. Holzapfel, editors. Blackie Academic and Professional, London.

Jay, J. 1992. Modern Food Microbiology. 4th ed. Chapman and Hall, New York, New York.

Jensen, L.B. 1954. Microbiology of Meats. 3rd ed. The Garrard Press, Publishers, Champaign, Illinois.

Juffs, H.S. and Babel, F.J. 1975. Inhibition of psychrotrophic bacteria by lactic cultures in milk stored at low temperature. J. Dairy Sci. 58:1612-1619.

Karmali, M.A. 1989. Infection by verocytoxin producing *Escherichia coli.* Clin. Microbiol. Rev. 2:15-38.

Kim, J., Yousef, A., and Dave, S. 1999. Application of ozone for enhancing the microbial safety and quality of foods: A review. J. Food Protect. 62:1071-1087.

Martin, D.R. and Gilliland, S.E. 1980. Inhibition of psychrotrophic bacteria in refrigerated milk by lactobacilli isolated from yogurt. J. Food Prot. 43:675-678.

Nutsch, A.L. 1998. Bacterial decontamination of meat surfaces through the application of steam pasteurization process. Ph.D. Dissertation, Kansas State University, Manhattan, KS.

Phebus, R.K. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. J. Food Protect. 60:476-484.

SAS Institute, Inc. 1985. SAS user's guide: statistics, ver. 5 ed. SAS Institute Inc., Cary, NC.

Smulders, F.J.M., P. Barendsen, J.G. Van Logtestijn, D.A. Mossel and G.M. van der Marel. 1986. Lactic acid: considerations in favour of its acceptance as a meat decontaminant. J. Food Technol. 21:419-436.

Vanderyant, C. and Splittstoesser, D.F. (eds). 1992. Compendium of methods for the microbiological examination of foods (3rd ed.) Amer. Washington, DC: Public Health Assoc.

Villegas, E. and Gilliland, S.E. 1998. Hydrogen peroxide production by *Lactobacillus delbrueckii* subsp. *Lactis* I at 5°C. J. Food Sci. 63:1070-1074.

Weagant, S.E., Bryant, J.L., and Bark, D.H. 1994. Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures. J. Food Prot. 57:629-631.

Woolthuis, C.H. and F.J.M. Smulders. 1985. Microbial decontamination of calf carcasses by lactic acid sprays. J. Food Protect. 48:832-837.

Yap, P.S. and S.E. Gilliland. 2000. Comparison of newly isolated strains of *Lactobacillus delbrueckii* subsp. *lactis* for hydrogen peroxide production at 5°C. J. Dairy Sci. 83:628-632.

Yousef, A.E. and E.H. Marth. 1988. Inactivation of Listeria monocytogenes by ultra volet energy. J. Food Sci. 53:571-574.

APPENDIX A

Interaction of *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 against *Escherichia coli* O157:H7 or *Salmonella typhimurium* in Trypticase Soy Broth

Broth Experiments

Broth experiments were conducted using freshly grown cultures prepared as described in the materials and methods section. An appropriate amount of each culture of either *Escherichia coli* O157:H7 or *Salmonella typhimurium* was inoculated into separate 100-ml portions of cold sterile TSB to yield and initial population of 1.0×10^5 CFU/ml. Each inoculated broth was mixed and aseptically dispensed in 25 ml aliquots into four sterile dilution bottles held in a mixture of ice and water. The freshly prepared concentrated cultures of *L. delbrueckii* subsp. *lactis* strains RM2-5 and I were added in the appropriate amounts to yield a population of approximately 1.0×10^8 CFU/ml to the 25 ml aliquots of either *E. coli* O157:H7 or S. *typhimurium* (Figure A1). Aliquots were labeled and stored at both 5 and 7°C. For the broth experiment involving *E. coli* O157:H7 microbial analyses were conducted on days 0, 3, 5 and 7 with 4 replicates. For the S. *typhimurium* broth experiment microbial analysis was conducted on days 0, 3, 5, and 7 with 3 replicates. The *E. coli* O157:H7 samples were plated on VRBA and MRS agar and the S. *typhimurium* samples were plated on BGA and MRS agar.

Broth Experiment Results

In the first broth experiment the antagonistic action of cells of *L. delbrueckii* subsp. *lactis* strains RM2-5 and I against *E. coli* O157:H7 at 5 and 7°C were investigated. Results from the storage at 5°C indicated

that there were no significant differences ($P > 0.05$) in populations of *E. coli* O157:H7 between the control and *L. delbrueckii* subsp. *lactis* RM2-5 or I treatments on day 0 and 3. On day 5 and 7, however, the control had a significantly ($P < 0.05$) higher population than did *L. delbrueckii* subsp. *lactis* RM2-5 and I (Table A1).

Results from the storage at 7°C were similar except that significant differences ($P < 0.05$) between the control sample and the *L.delbrueckii* subsp. *lactis* RM2-5 and I samples were seen starting at day 3. By day 7 there were significant differences ($P < 0.05$) among all three treatments. The numbers of *E. coli* were significantly lower in both samples inoculated with lactobacilli than in the control; those in the sample inoculated with strain RM2-5 were significantly lower than in the sample inoculated with strain I.

In the second broth experiment the antagonistic action of cells of *L. delbrueckii* subsp. *lactis* RM2-5 were investigated against *S. typhimurium* in trypticase soy broth stored at 5 and 7°C. Because the results from the first broth experiment indicated that the *L. delbrueckii* subsp. *lactis* RM2-5 culture was more effective than was strain I in its antagonistic action, only the *L. delbrueckii* subsp. *lactis* RM2-5 culture was used in the second broth experiment.

Results from both the 5 and 7°C storage time indicated that significant differences ($P < 0.05$) in populations of *S. typhimurium* did not start occurring until after day 3 of storage. After day 5 the control

sample contained significantly higher ($P < 0.05$) populations of *S. typhimurium* than the *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 sample (Table A2). These results indicate that *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 was effective in significantly reducing the numbers of *S. typhimurium* over extended storage time (greater than 3 d) at both 5 and 7°C.

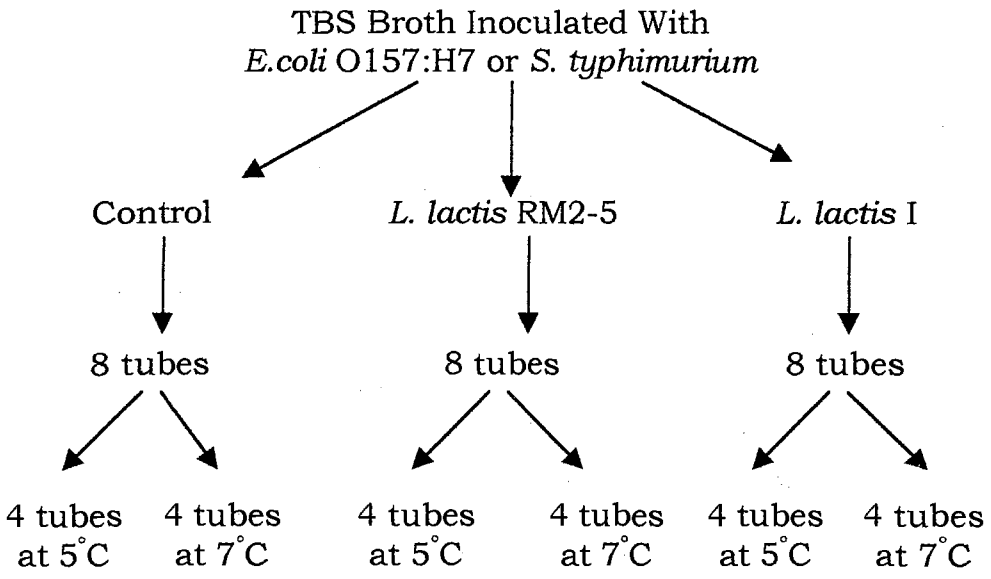

Figure A1. Schematic of broth sample preparation.

TABLE A1. Antagonistic action of *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and I toward *Escherichia coli* O157:H7 in Trypticase soy broth stored at 5 and 7° C.

	Number of *E. coli* O157:H7 (log_{10} CFU/ml)[a]		
	Storage at 5° C		
Day	Control	RM2-5	I
0	4.9^{Ab}	4.8^{A}	4.9 ^{A}
3	3.8^{A}	3.3 ^{A}	3.3 ^{A}
5	3.8^{A}	2.9 ^{A}	3.0 ^{B}
7	3.7^{A}	2.4 ^{B}	2.8 ^{B}
	Storage at 7° C		
Day	Control	RM2-5	I
0	4.9 ^{A}	4.8 ^{A}	4.9 ^{A}
3	4.7 ^{A}	3.6 ^{B}	3.9 ^{B}
5	4.7 ^{A}	2.7 ^{B}	3.3 ^{B}
7	4.4 ^{A}	2.2 ^{B}	3.3 ^{C}

[a]*E. coli* O157:H7 counts detected on VRBA are expressed as log_{10} CFU/ml; each value is a mean from three trials.
[b]Means in the same row having the same letter in common are not significantly different ($P > 0.05$).

TABLE A2. Antagonistic action of *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 toward *Salmonella typhimurium* in Trypticase soy broth stored at 5 and 7° C.

	Number of *S. typhimurium* (log_{10} CFU/ml)[a]	
	Storage at 5° C	
day	Control	RM2-5
0	6.0 A[b]	5.8 A
3	6.0 A	5.5 B
5	6.2 A	5.1 B
7	5.8 A	4.8 B
	Storage at 7° C	
day	Control	RM2-5
0	6.0 A	5.8 A
3	6.1 A	5.7 A
5	6.2 A	5.5 B
7	6.3 A	5.4 B

[a] *S. typhimurium* counts detected on BGA are expressed as log_{10} CFU/ml; each value is a mean from three trials.
[b]Means in the same row having the same letter in common are not significantly different ($P > 0.05$).

APPENDIX B
Graphical Presentations of Data

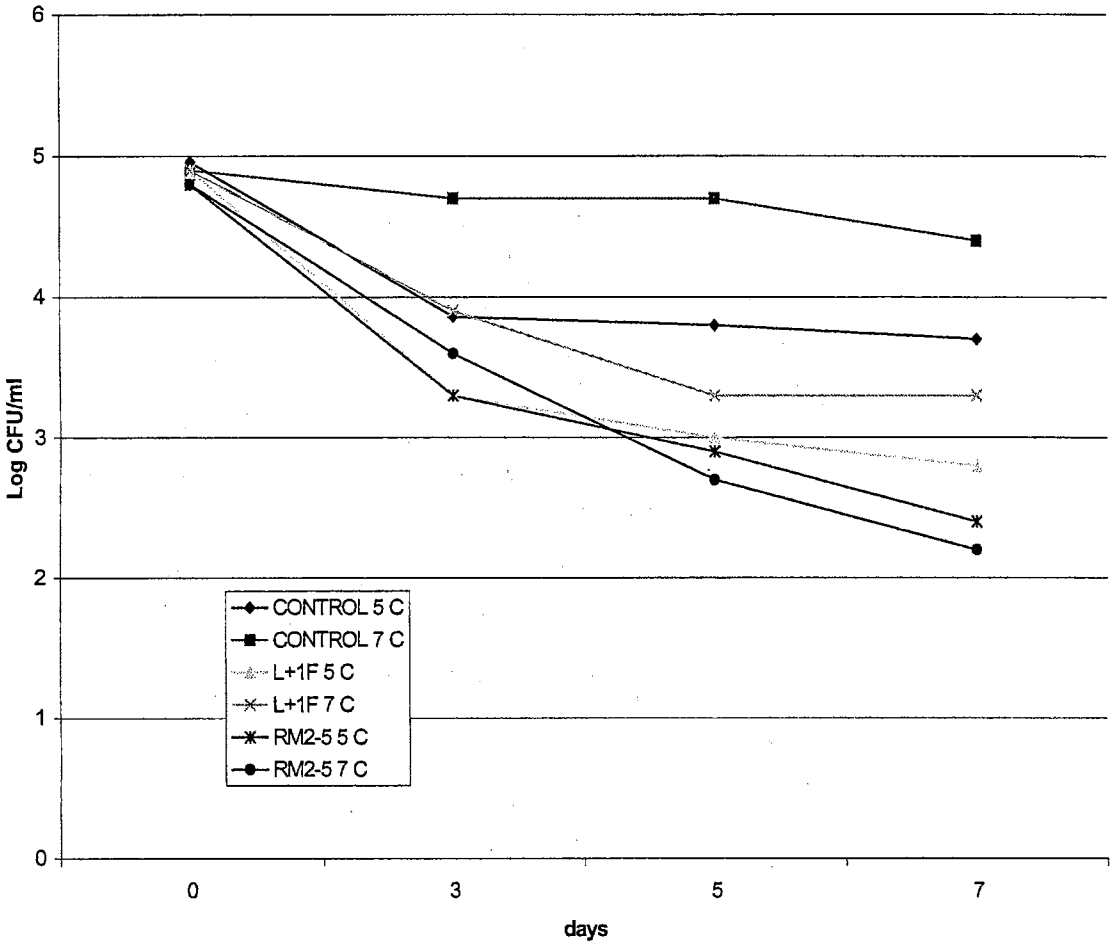

Figure B1. Antagonistic action of *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and I toward *Escherichia coli* O157:H7 in Trypticase soy broth stored at 5 and 7° C. *Escherichia coli* O157:H7 counts detected on VRBA are expressed as $\log_{10}$ CFU/ml; each value is a mean from three trials. This is a graphical presentation of the data shown in Table A1.

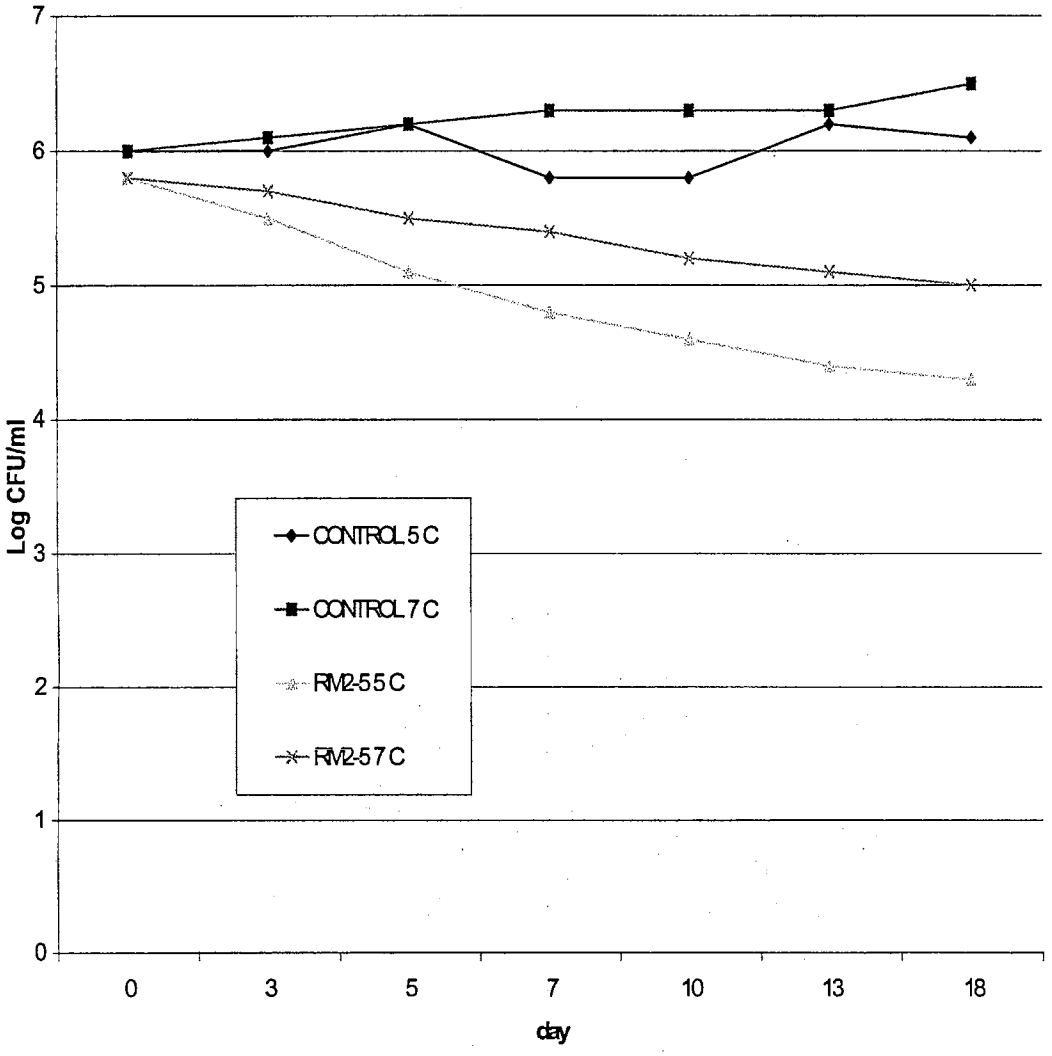

Figure B2. Antagonistic action of *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 toward *Salmonella typhimurium* in Trypticase soy broth stored at 5 and 7° C. *Salmonella typhimurium* detected on BGA are expressed as $\log_{10}$ CFU/ml; each value is a mean from three trials. This is a graphical presentation of the data shown in Table A2.

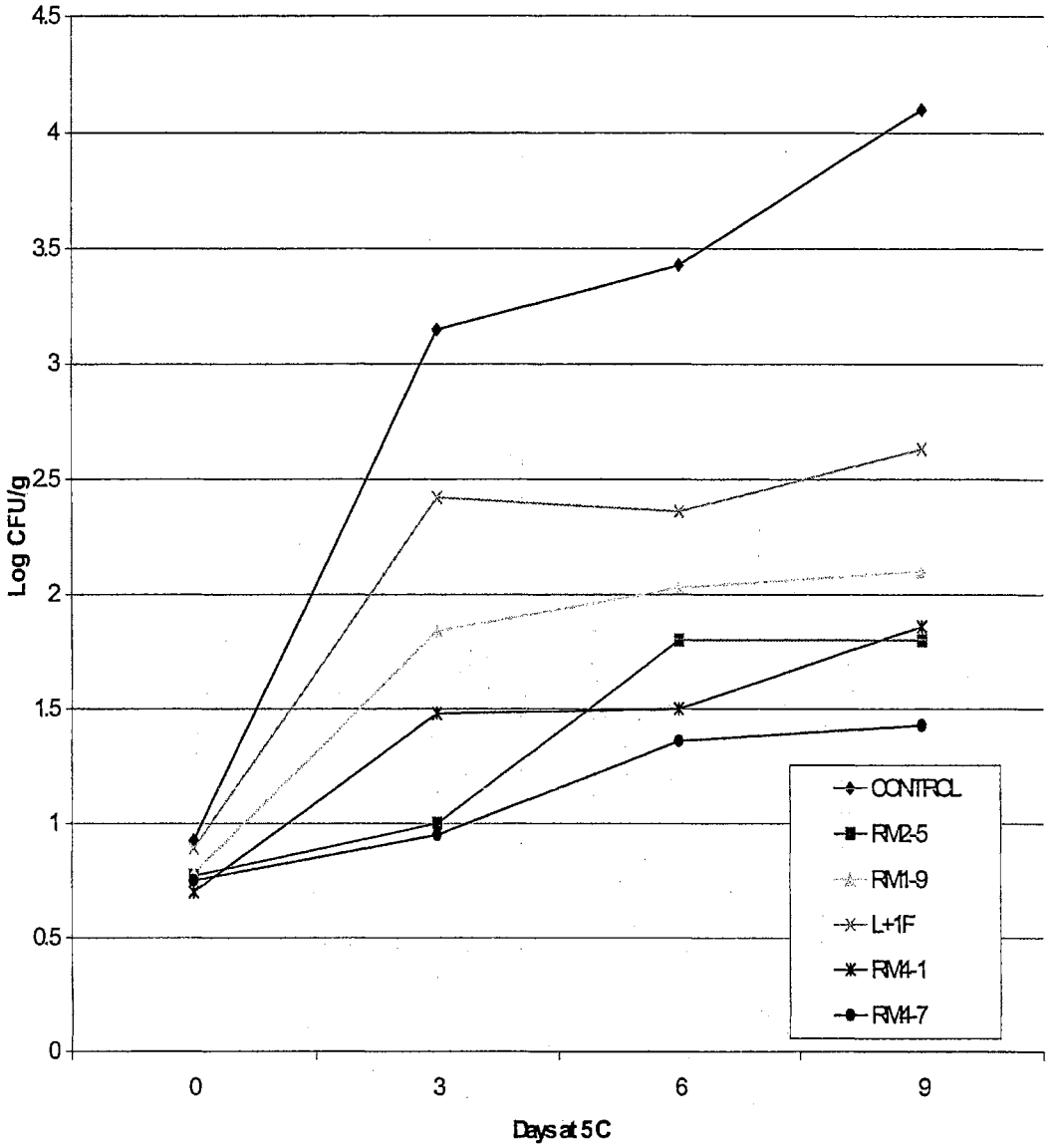

Figure B3. Antagonistic action of five freshly prepared strains of *Lactobacillus delbrueckii* susbp.*lactis* toward naturally occurring background microflora found on fresh cut beef steaks during refrigerated storage (5° C) on days 0, 3, 6 and 9. Coliform counts detected on VRBA are expressed as $\log_{10}$ CFU/g; each value is a mean from three trials. This is a graphical presentation of the data shown in Table 1.

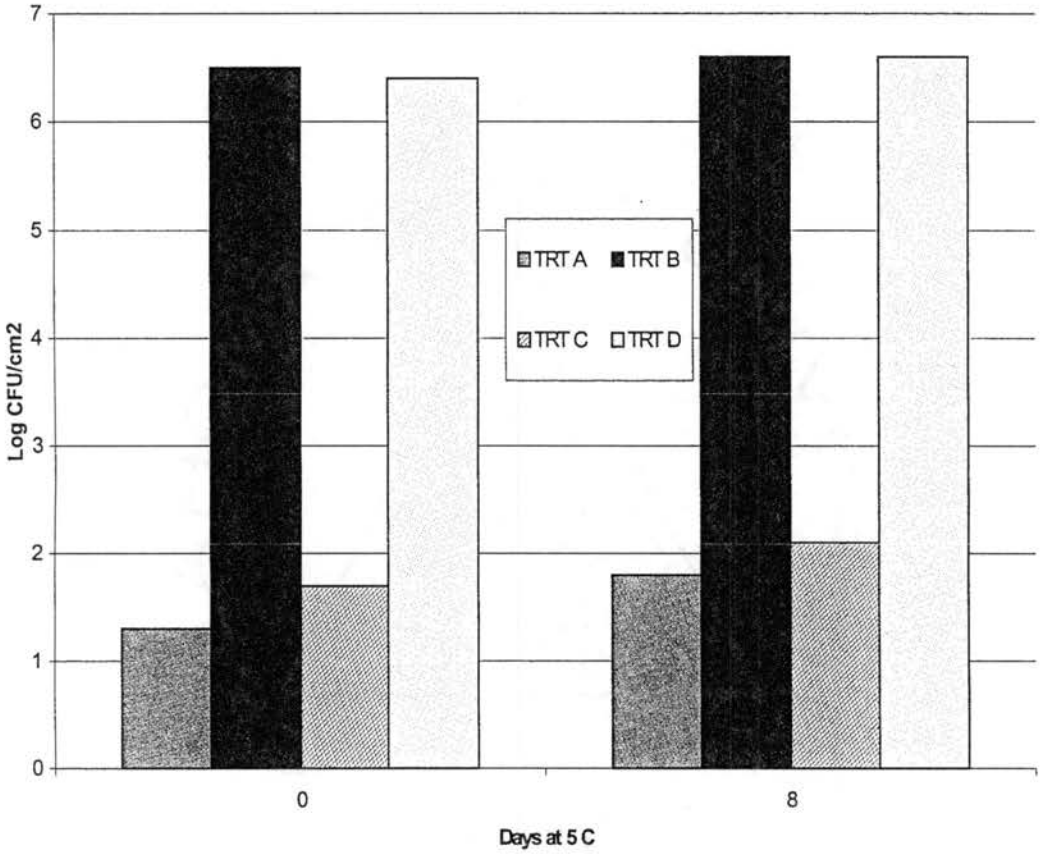

Figure B4. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Escherichia coli* O157:H7 on the surface of beef carcasses stored at 5 °C. Numbers of lactobacilli detected on LBS are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 6.

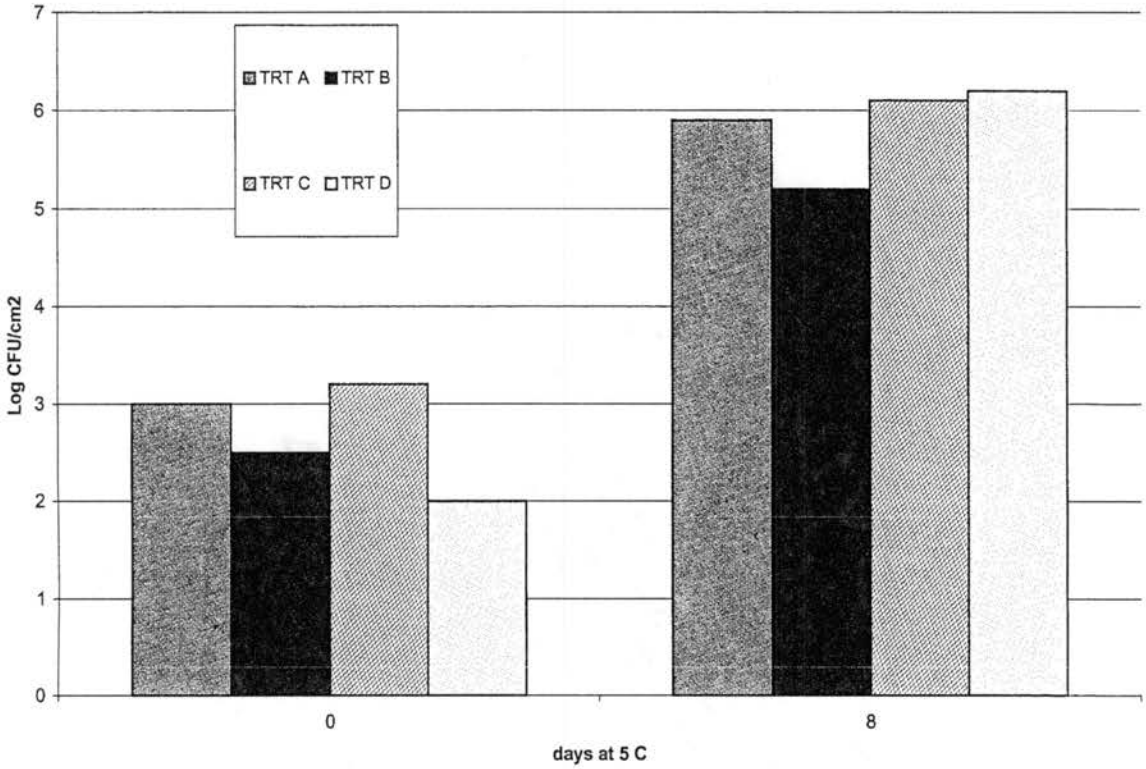

Figure B5. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Escherichia coli* O157:H7 on the surface of beef carcasses stored at 5 °C. Psychrotrophic counts detected on PCA (incubated at 15°C for 7 d) are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 6.

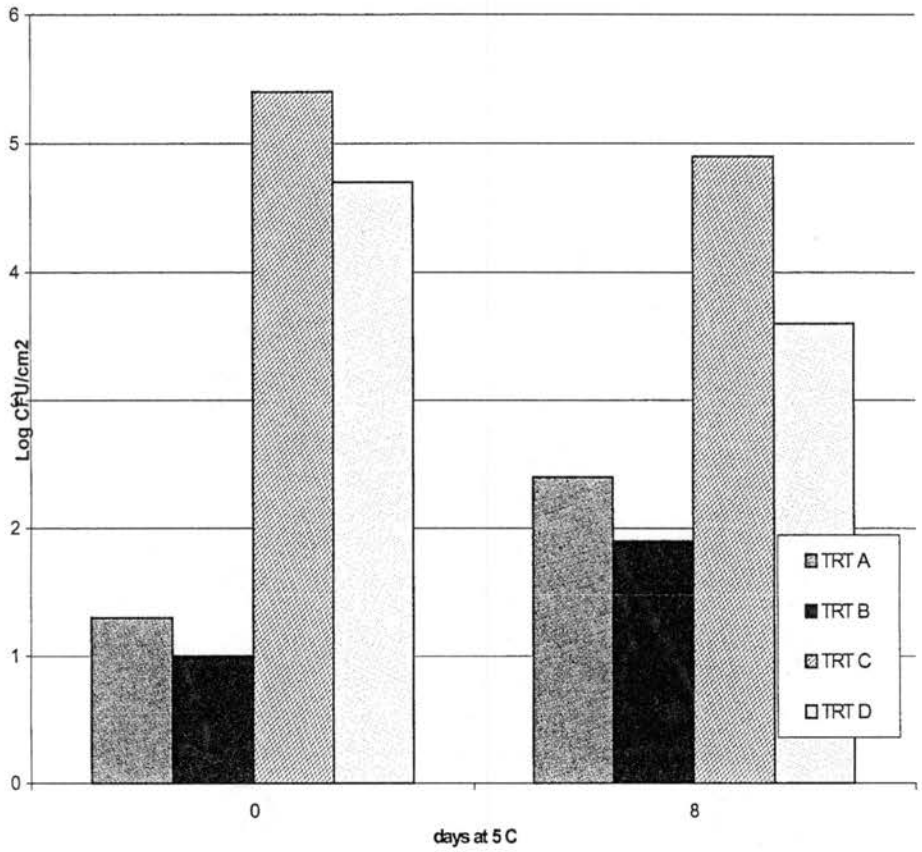

Figure B6. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Escherichia coli* O157:H7 on the surface of beef carcasses stored at 5 °C. *Escherichia coli* O157:H7 counts detected on VRBA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 6.

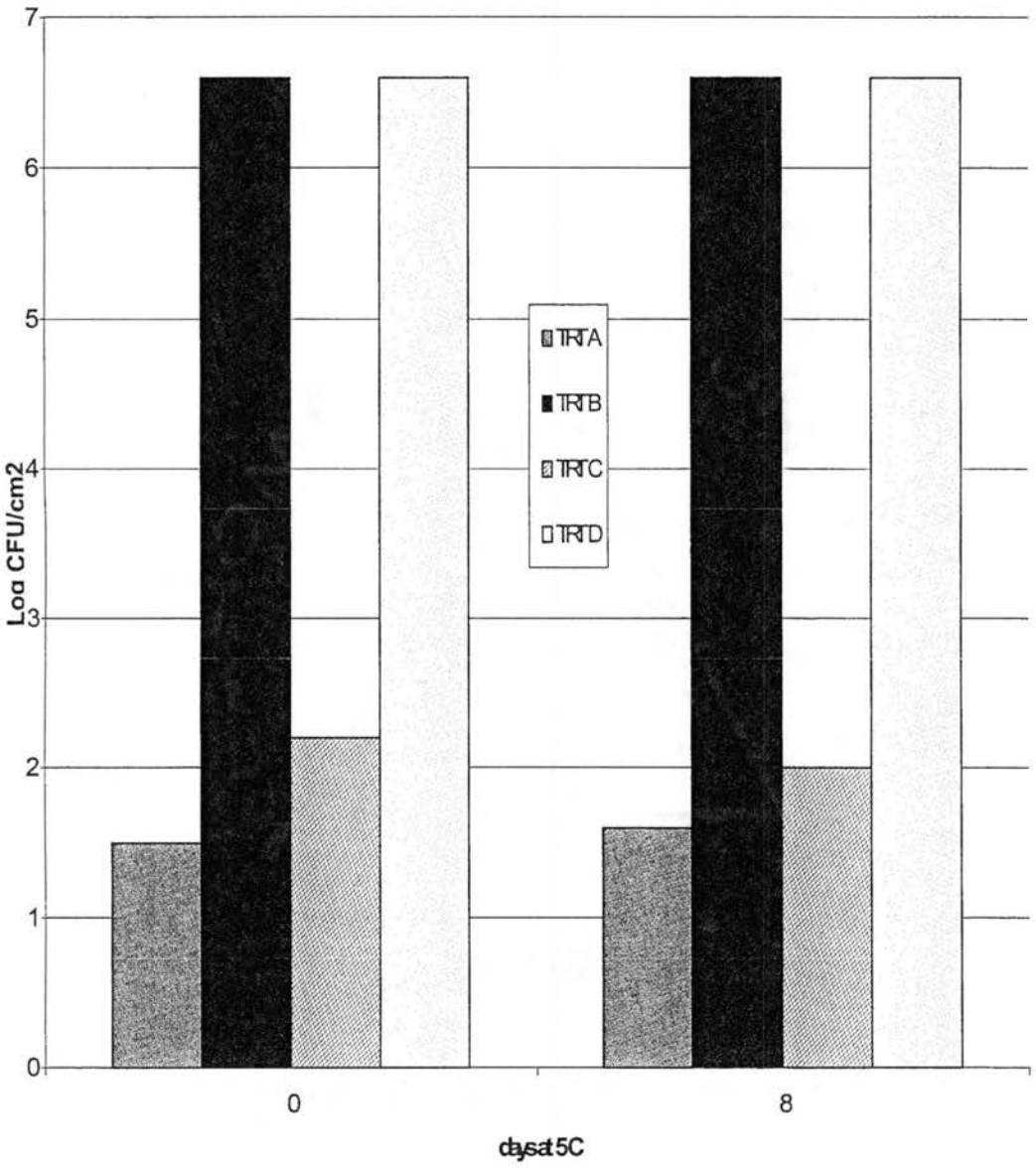

Figure B7. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Salmonella typhimurium* on the surface of beef carcasses stored at 5 °C. Numbers of lactobacilli detected on LBS are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Salmonella typhimurium*. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Salmonella typhimurium*. This is a graphical presentation of the data shown in Table 7.

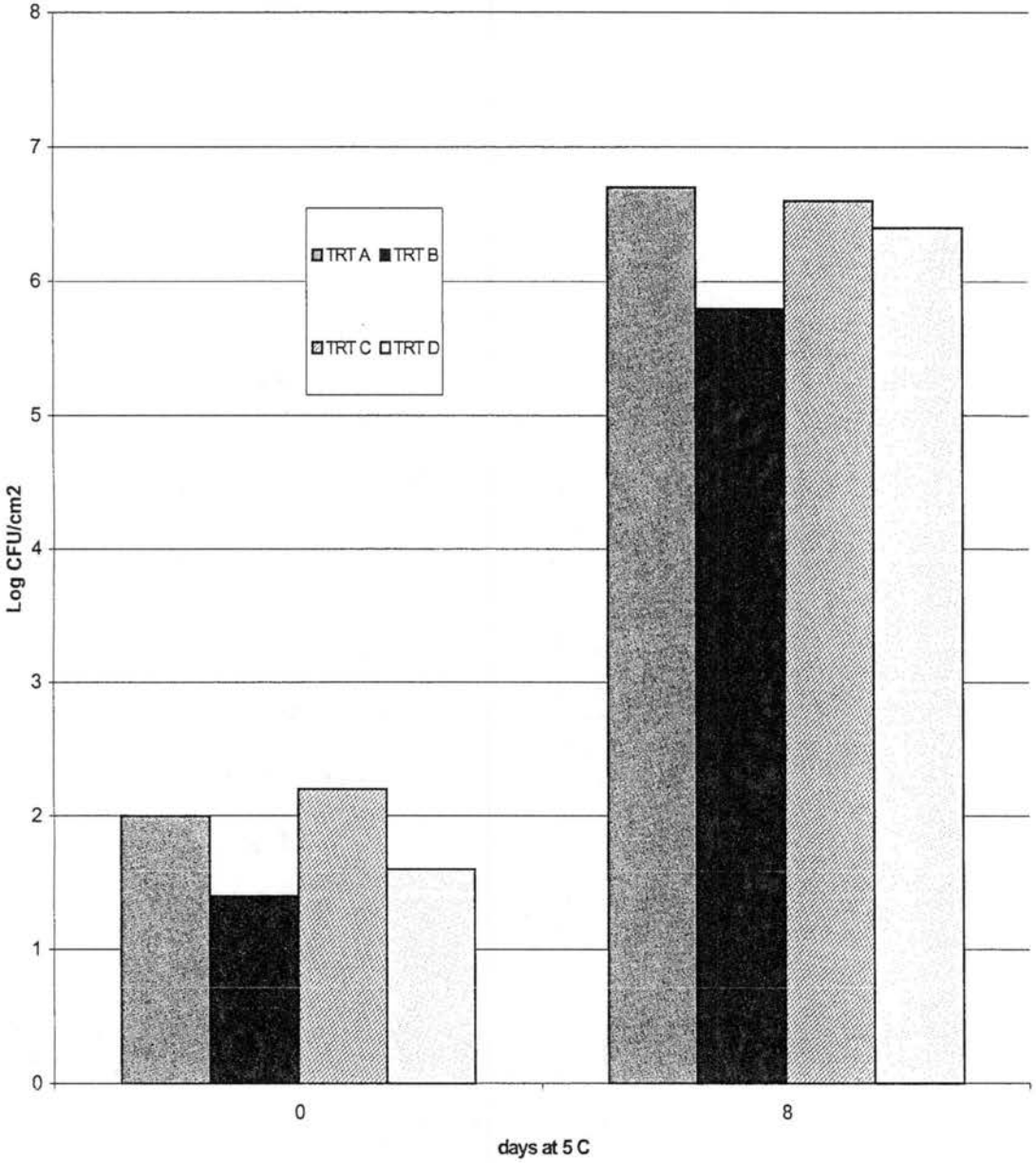

Figure B8. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Salmonella typhimurium* on the surface of beef carcasses stored at 5 °C. Psychrotrophic counts detected on PCA (incubate at 15°C for 7 d) are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Salmonella typhimurium*. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Salmonella typhimurium*. This is a graphical presentation of the data shown in Table 7.

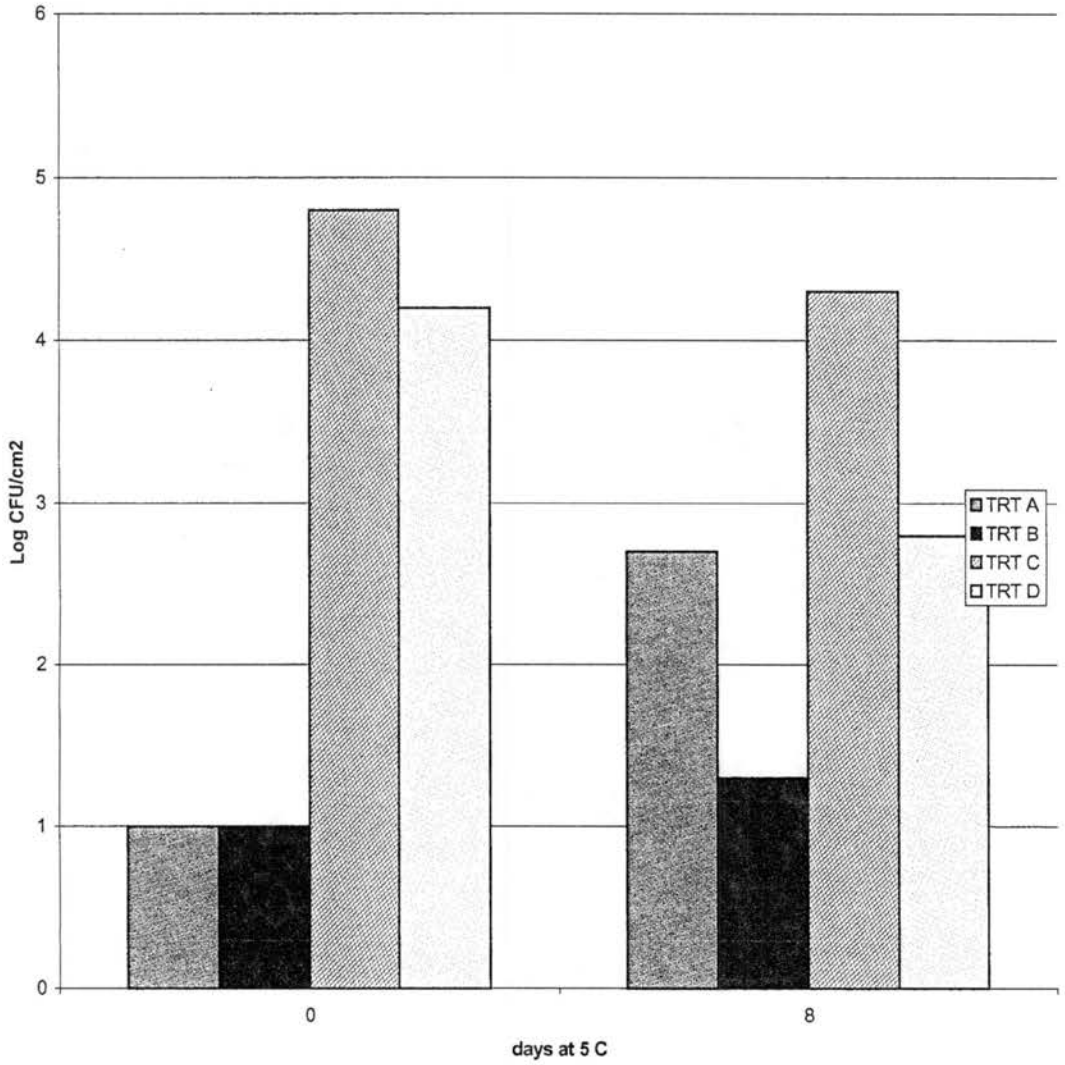

Figure B9. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Salmonella typhimurium* on the surface of beef carcasses stored at 5 °C. *Salmonella typhimurium* counts detected on BGA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Salmonella typhimurium*. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Salmonella typhimurium*. This is a graphical presentation of the data shown in Table 7.

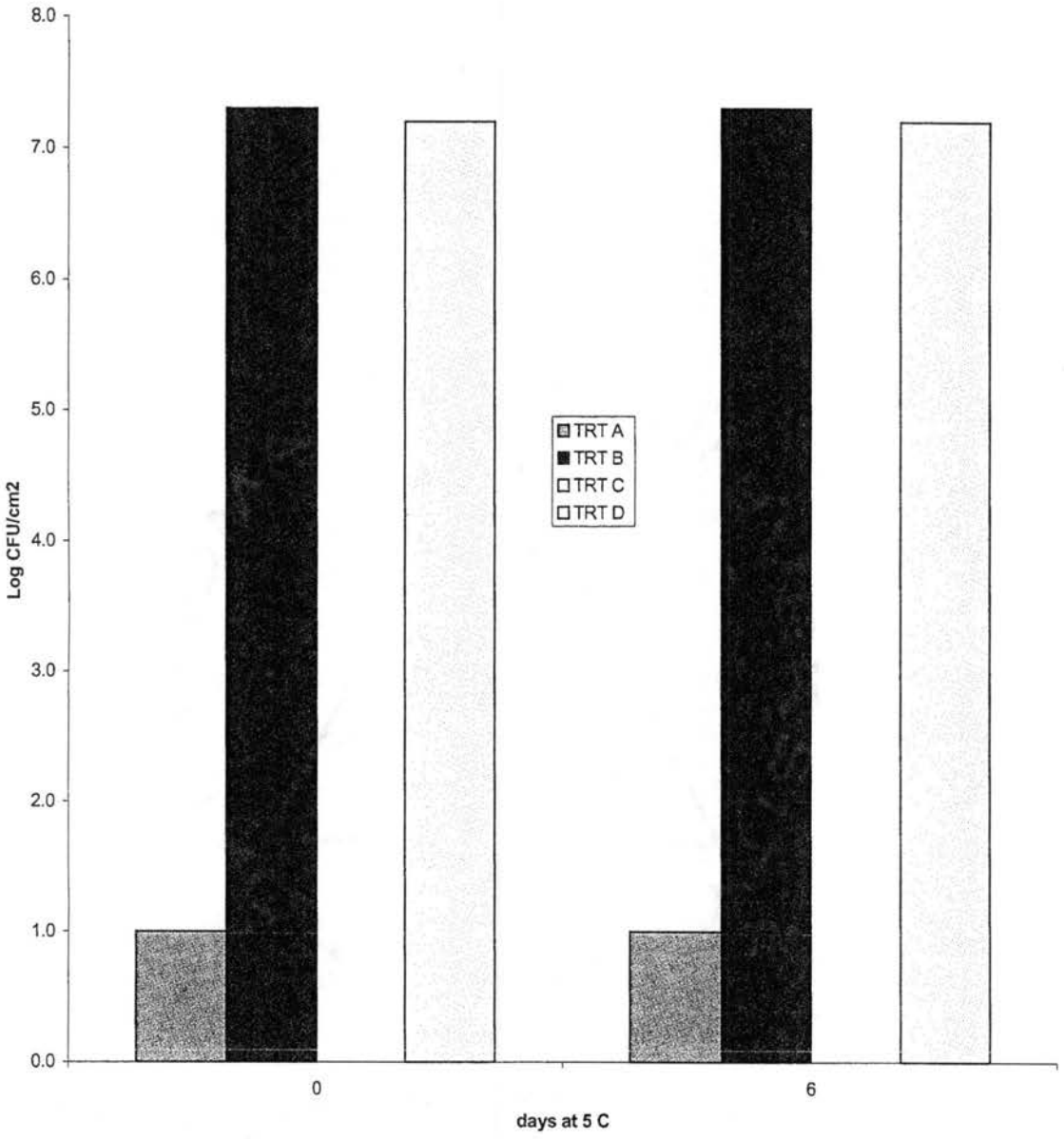

Figure B10. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Escherichia coli* O157:H7 on the surface of pork carcasses stored at 5 °C. Numbers of lactobacilli detected on LBS are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 8.

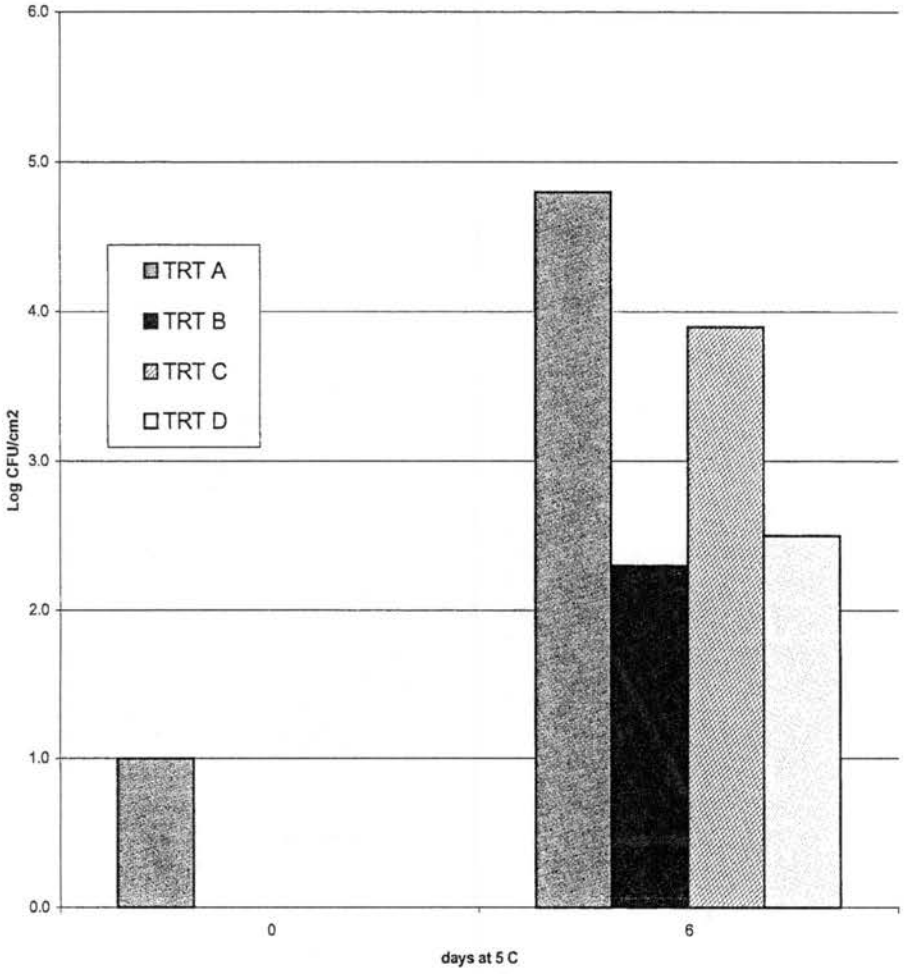

Figure B11. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Escherichia coli* O157:H7 on the surface of pork carcasses stored at 5 °C. Psychrotrophic counts detected on PCA (incubated at 15°C for 7 d) are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 8.

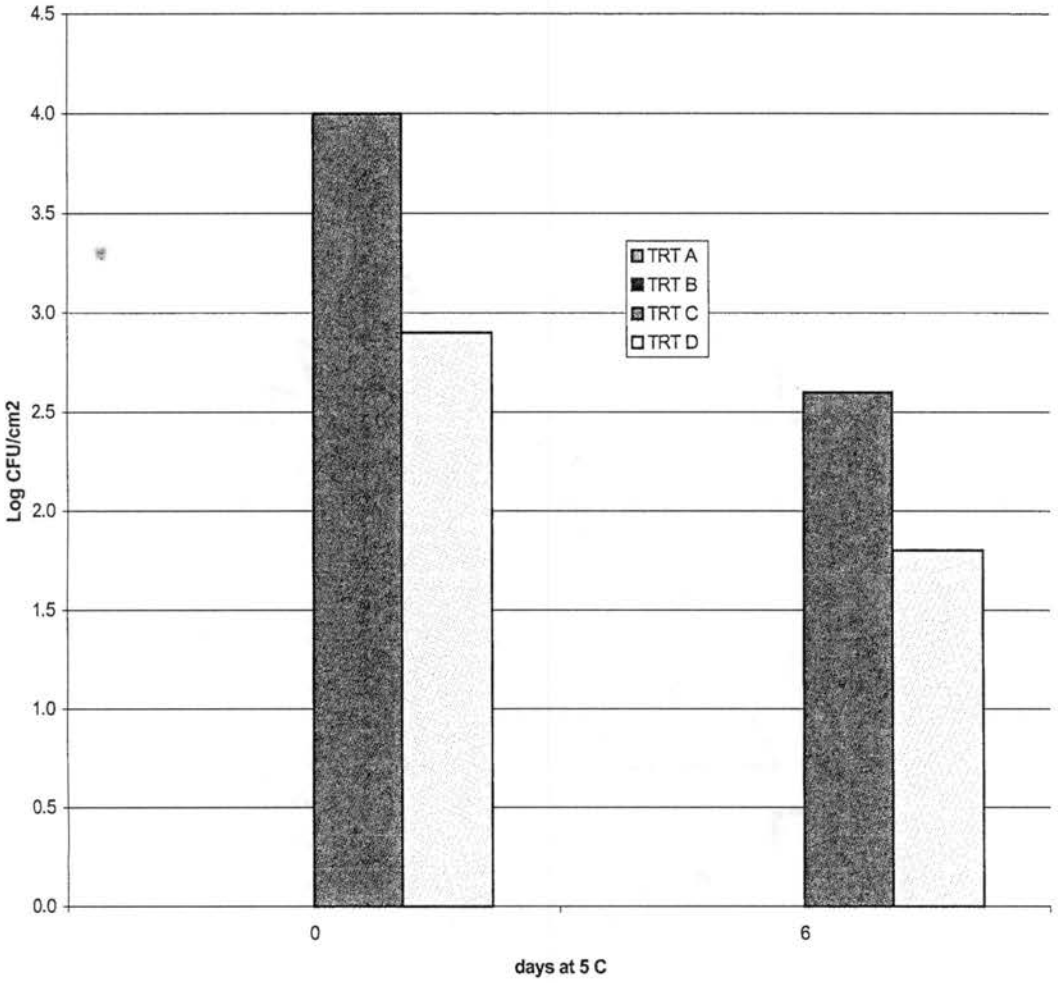

Figure B12. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Escherichia coli* O157:H7 on the surface of pork carcasses stored at 5 °C. *Escherichia coli* O157:H7 counts detected on VRBA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 8.

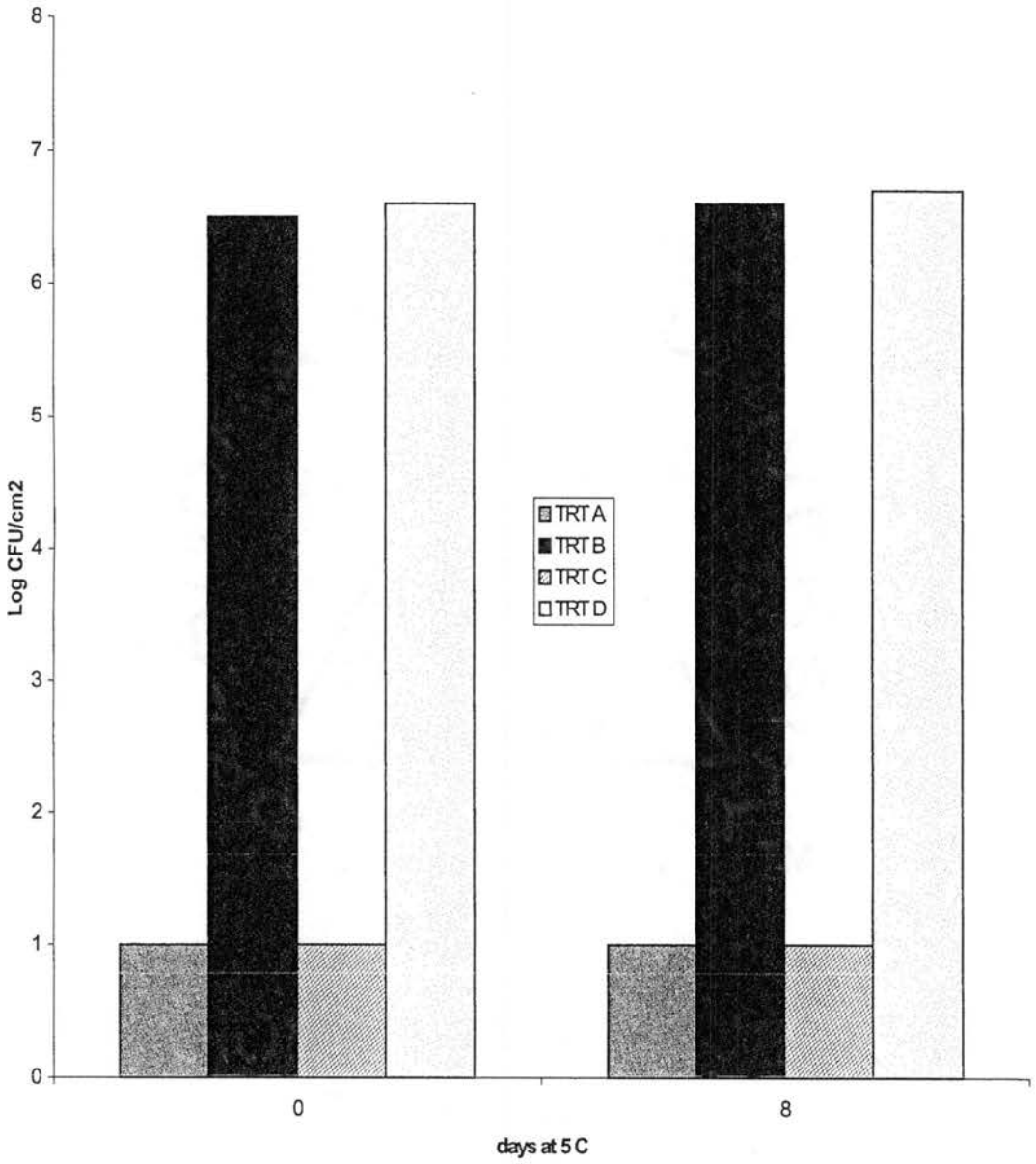

Figure B13. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Salmonella typhimurium* on the surface of pork carcasses stored at 5 °C. Numbers of lactobacilli detected on LBS are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Salmonella typhimurium*. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Salmonella typhimurium*. This is a graphical presentation of the data shown in Table 9.

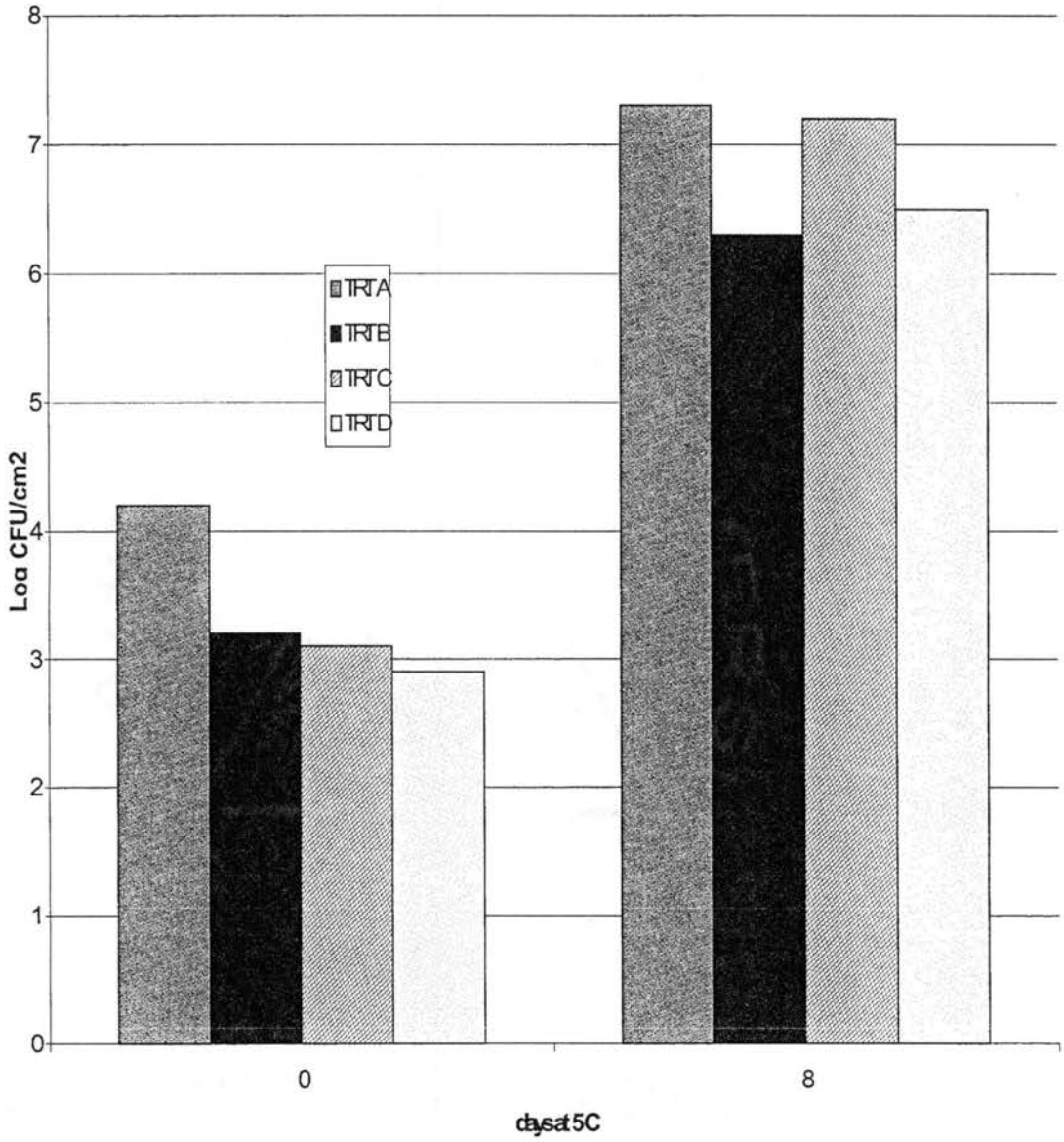

Figure B14. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Salmonella typhimurium* on the surface of pork carcasses stored at 5 °C. Psychrotrophic counts detected on PCA (incubate at 15°C for 7 d) are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Salmonella typhimurium*. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Salmonella typhimurium*. This is a graphical presentation of the data shown in Table 9.

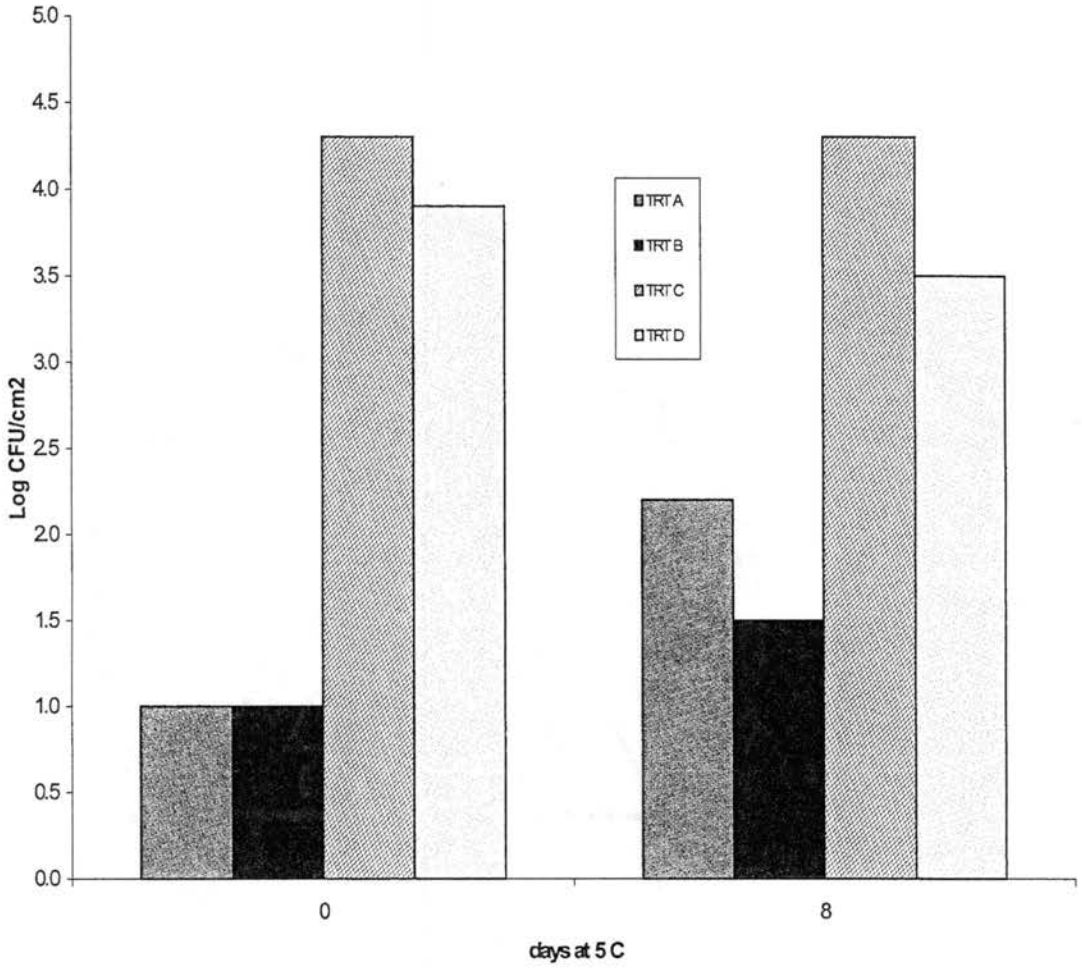

Figure B15. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Salmonella typhimurium* on the surface of pork carcasses stored at 5 °C. *Salmonella typhimurium* counts detected on BGA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Salmonella typhimurium*. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Salmonella typhimurium*. This is a graphical presentation of the data shown in Table 9.

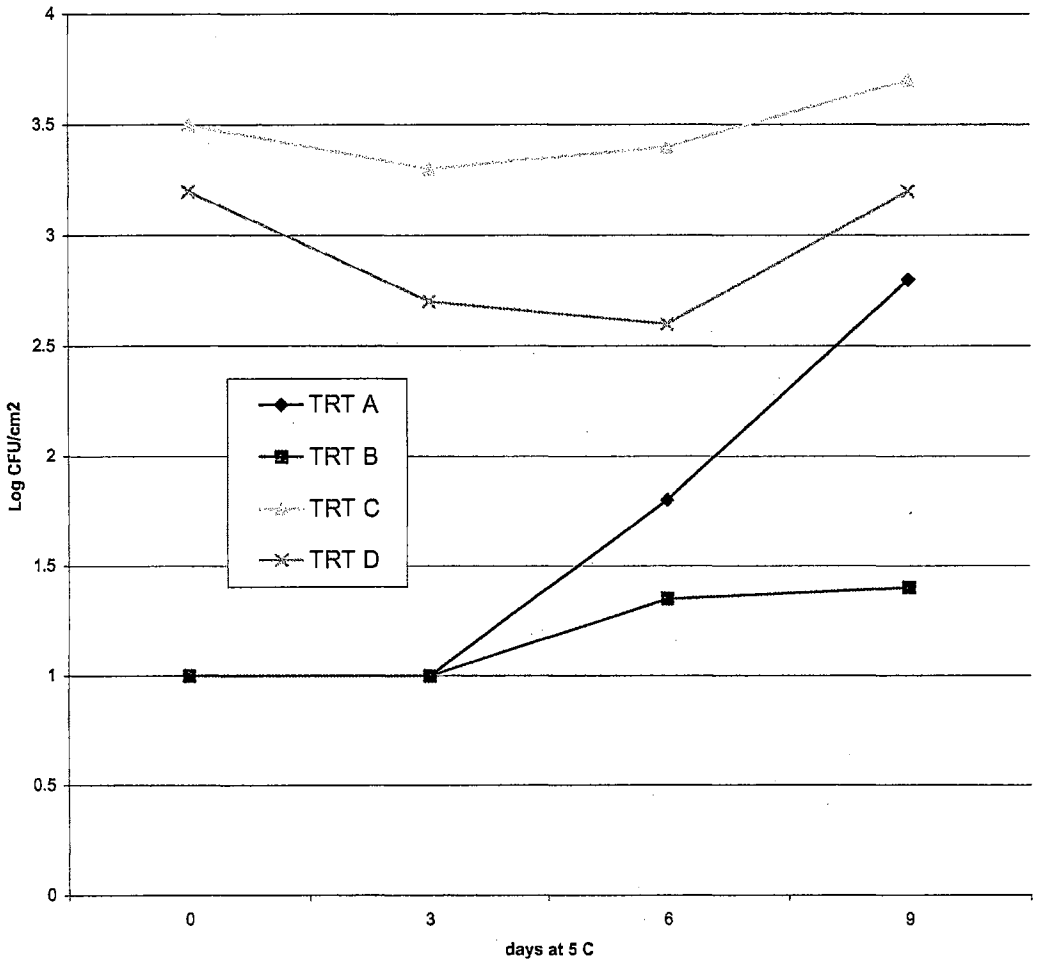

Figure B16. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (fresh culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C. *Escherichia coli* O157:H7 counts detected on VRBA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 4.

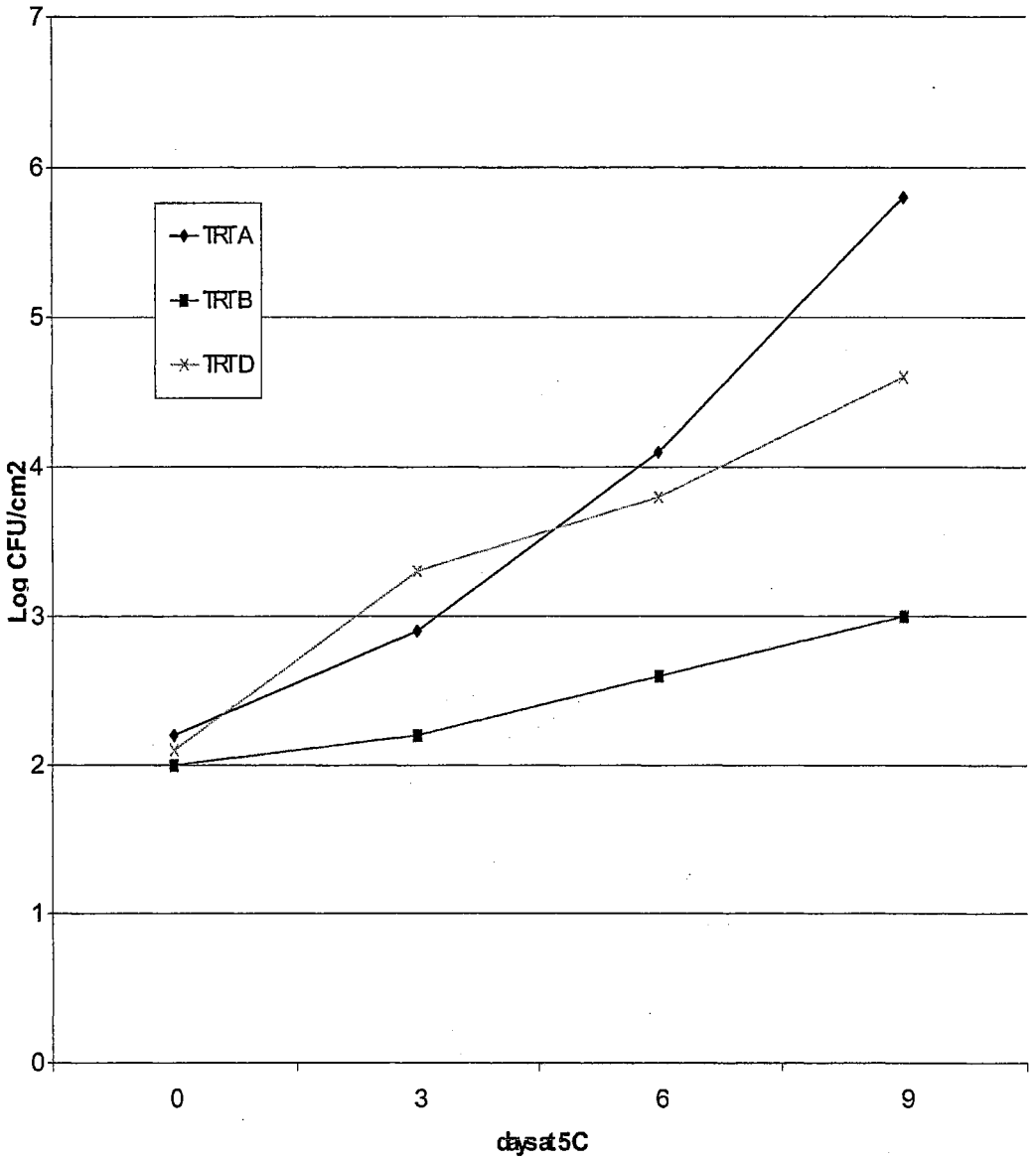

Figure B17. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (fresh culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C. Psychrotrophic counts detected on PCA (incubated at 15°C for 7 d) are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 4.

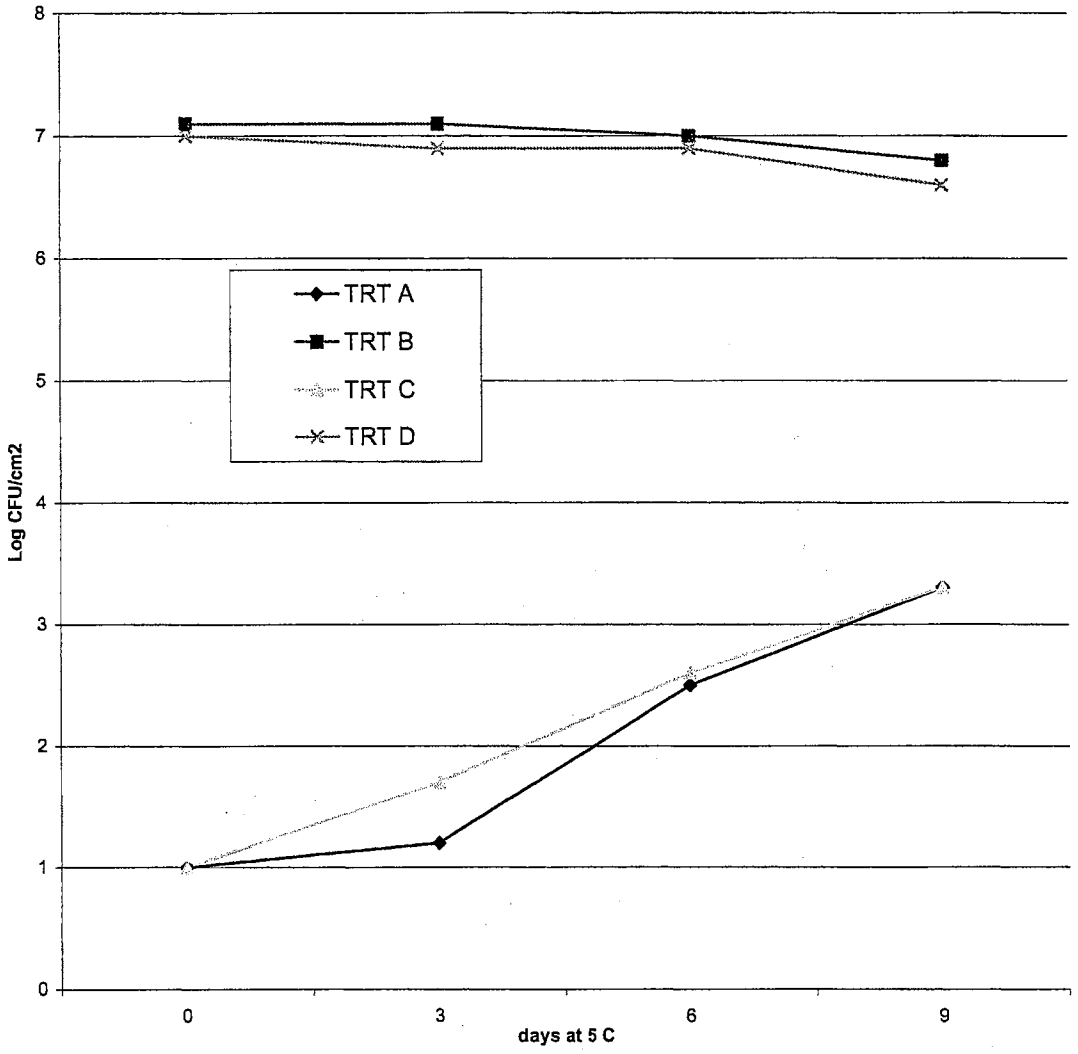

Figure B18. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (fresh culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C. Numbers of lactobacilli detected on LBS are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 4.

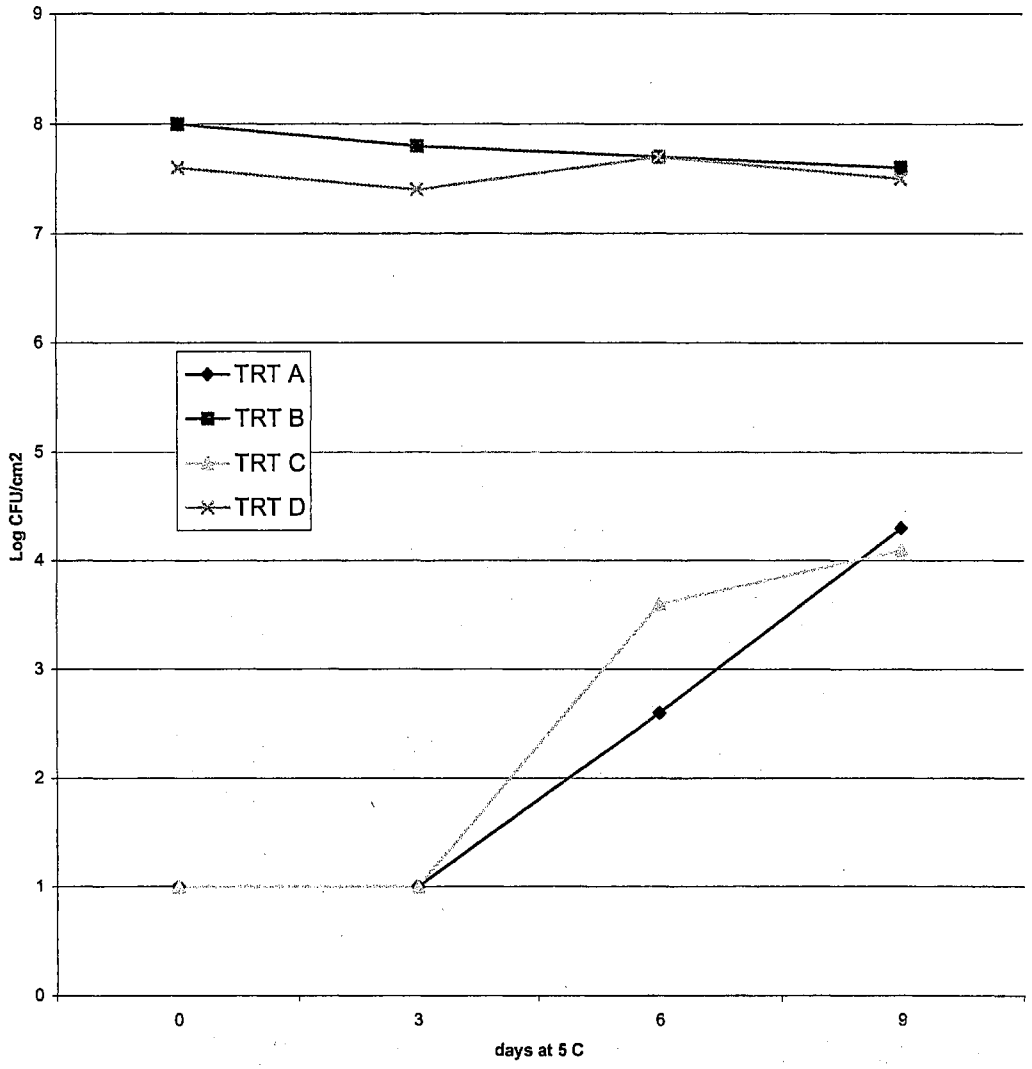

Figure B19. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (frozen culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C. Numbers of lactobacilli detected on LBS are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 5.

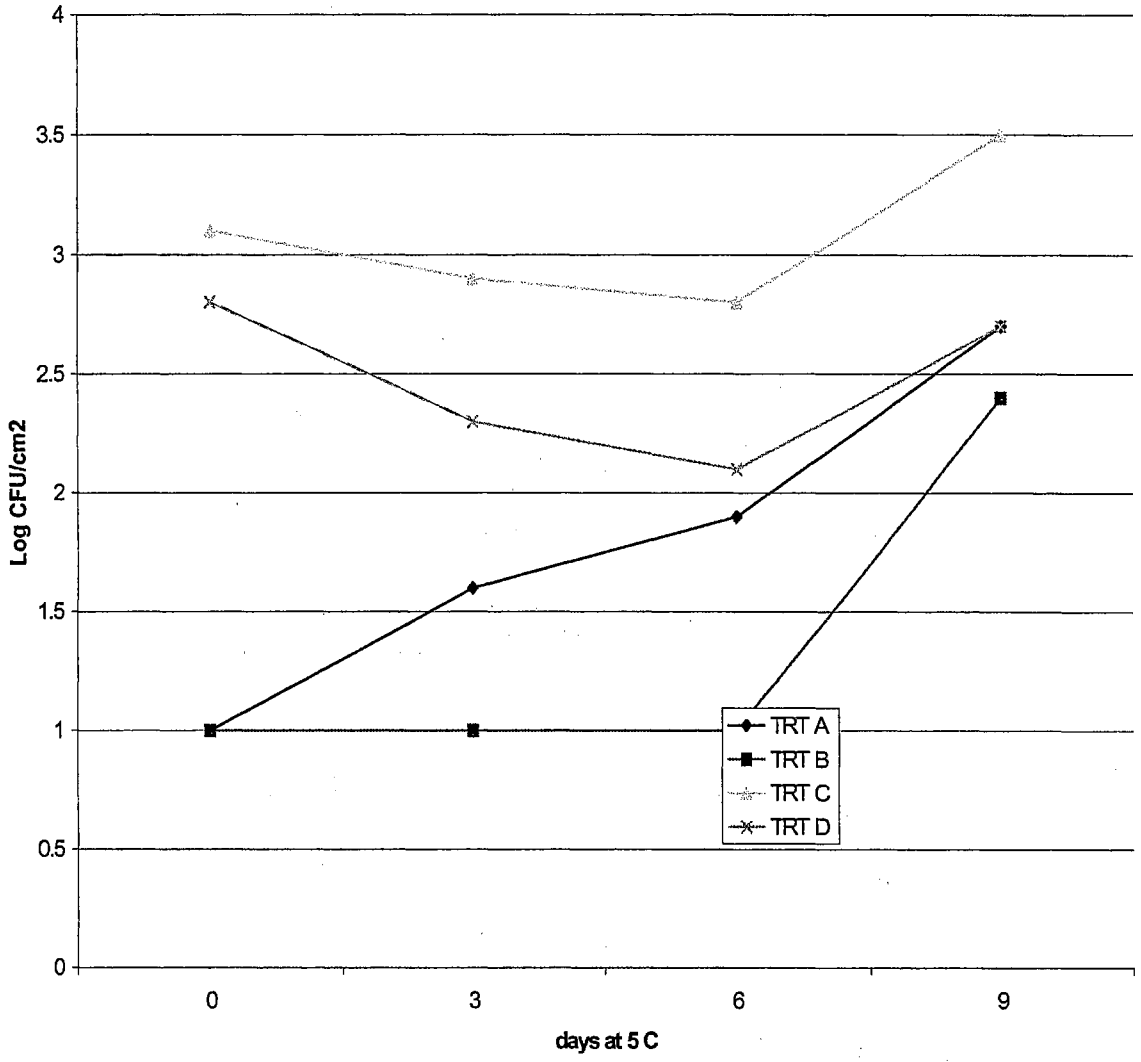

Figure B20. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (frozen culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C. *Escherichia coli* O157:H7 counts detected on VRBA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 5.

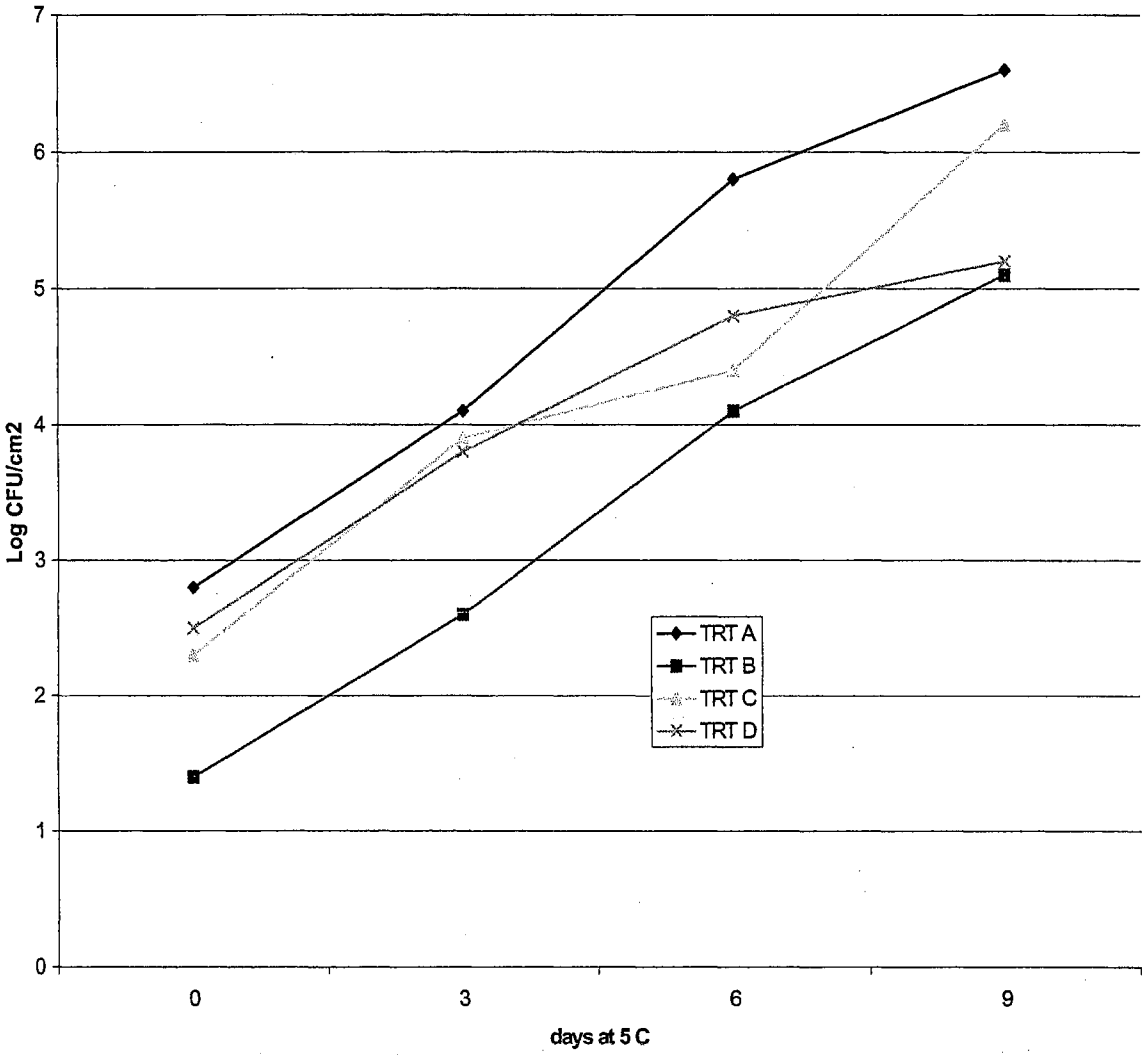

Figure B21. Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (frozen culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C. Psychrotrophic counts detected on PCA (incubated at 15°C for 7 d) are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 5.

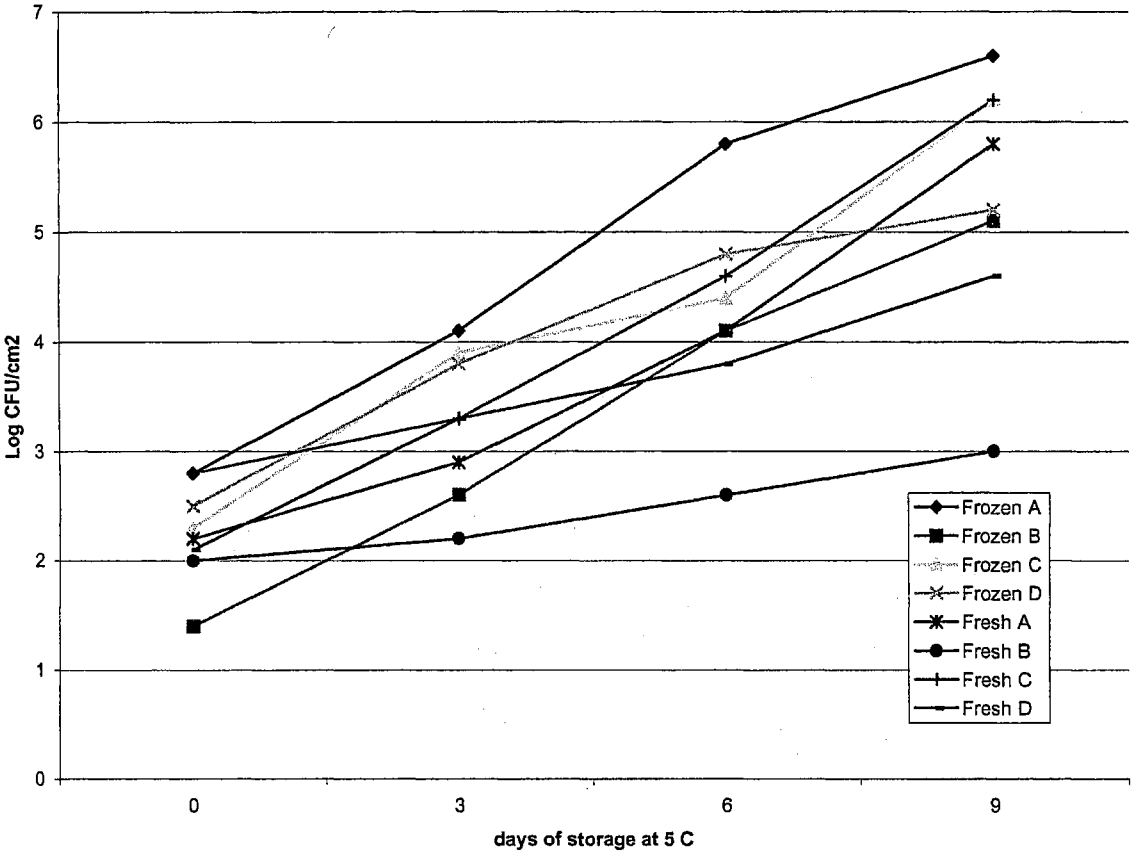

Figure B22. Comparison of psychrotrophic counts for fresh versus frozen culture directly applied onto the surface of fresh beef steaks. Psychrotrophic counts detected on PCA (incubated at 15°C for 7 d) are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 4 and 5.

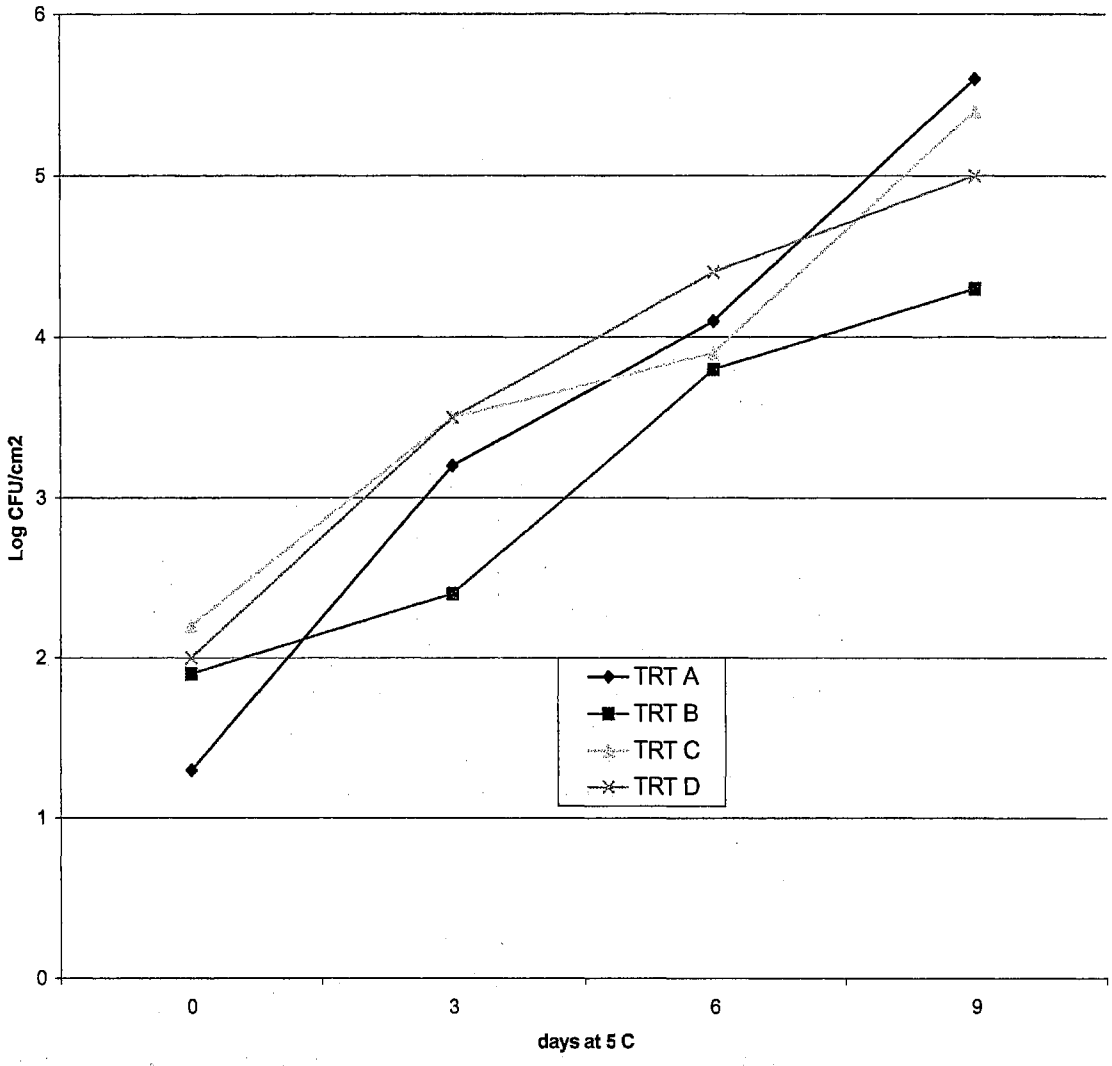

Figure B23. Antagonistic action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 toward *Escherichia coli* O157:H7 in ground beef stored at 5° C. Psychrotrophic counts detected on PCA (incubated at 15°C for 7 d) are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 2.

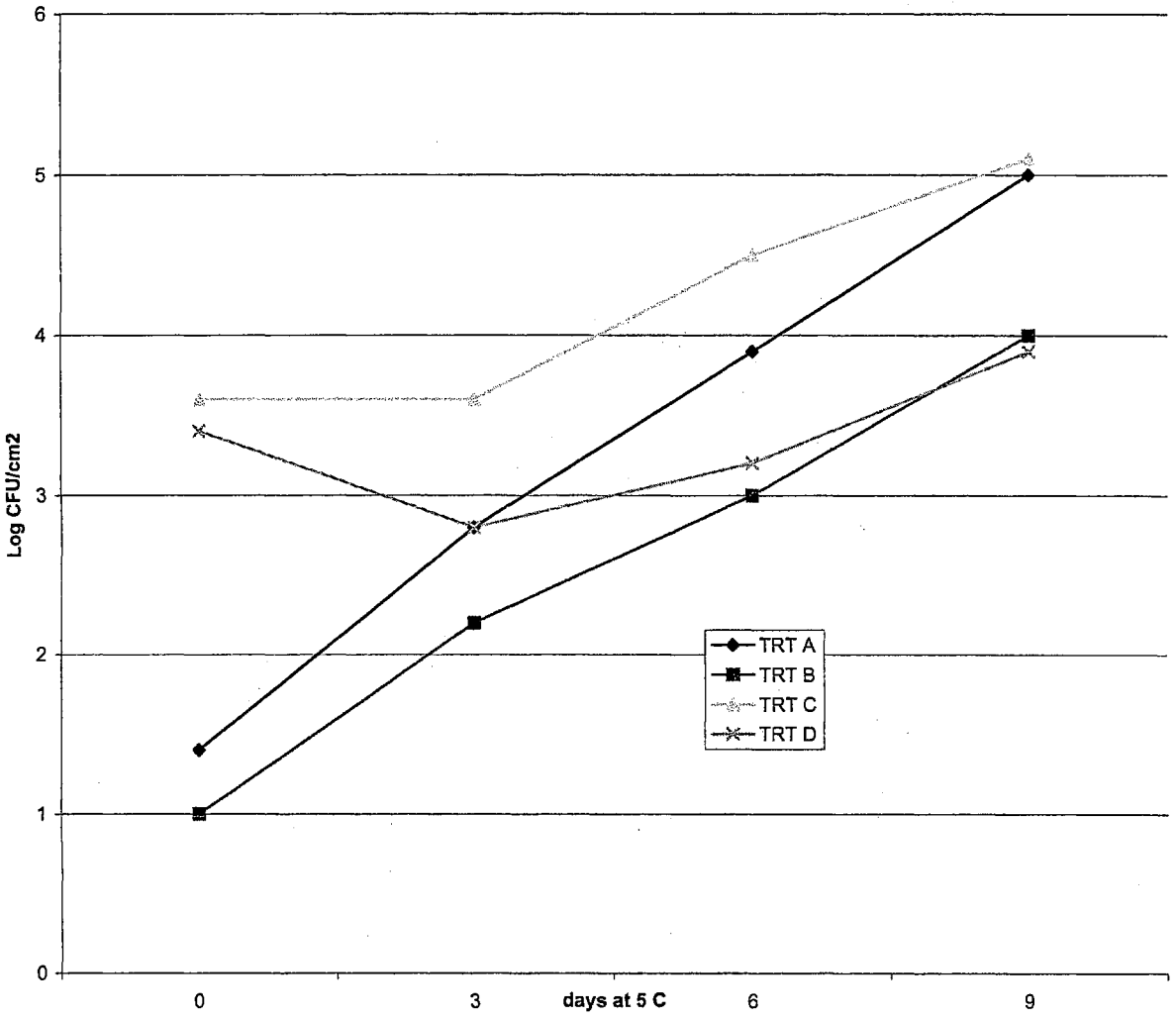

Figure B24. Antagonistic action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 toward *Escherichia coli* O157:H7 in ground beef stored at 5° C. *Escherichia coli* O157:H7 counts detected on VRBA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 2.

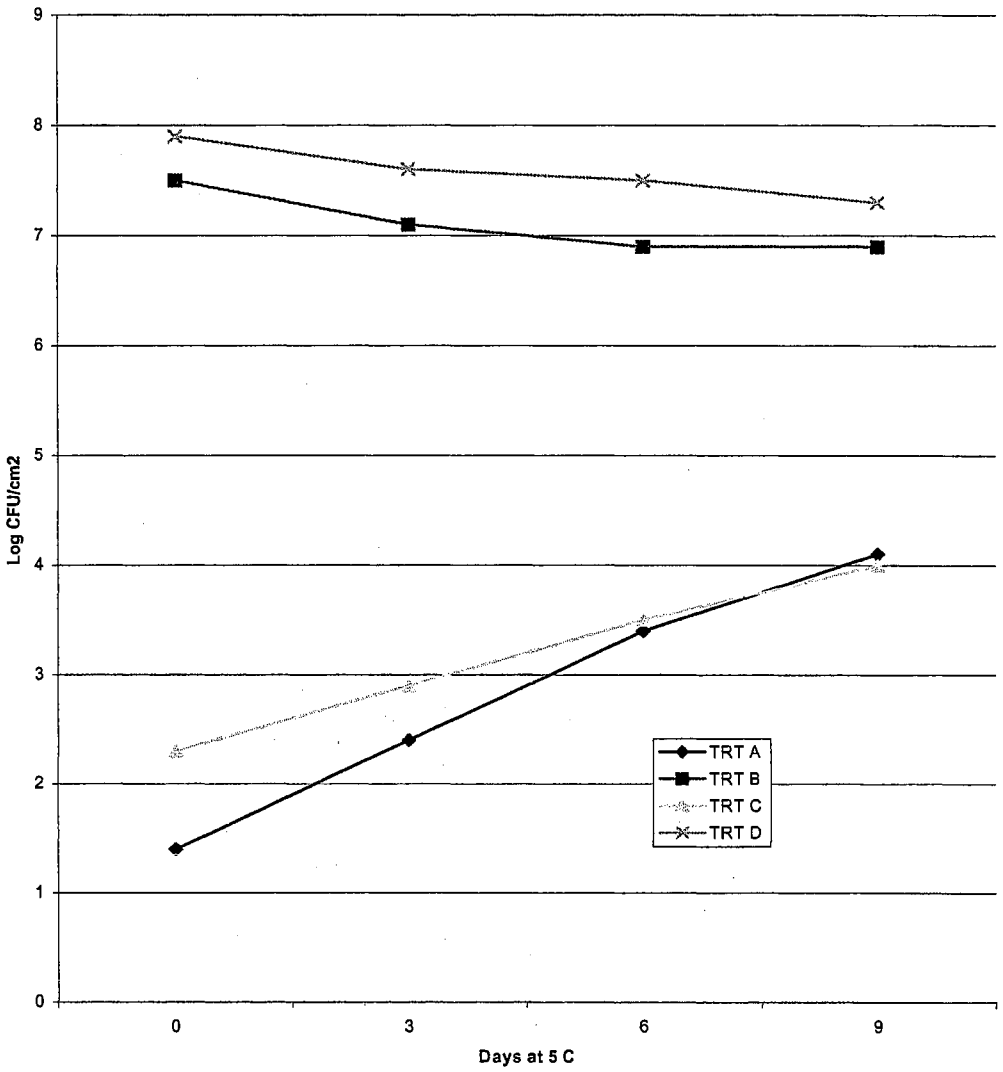

Figure B25. Antagonistic action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 toward *Escherichia coli* O157:H7 on the surface of fresh beef steaks after being treated in a glucose based dip solution and stored at 5° C. Numbers of lactobacilli detected on LBS are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 3.

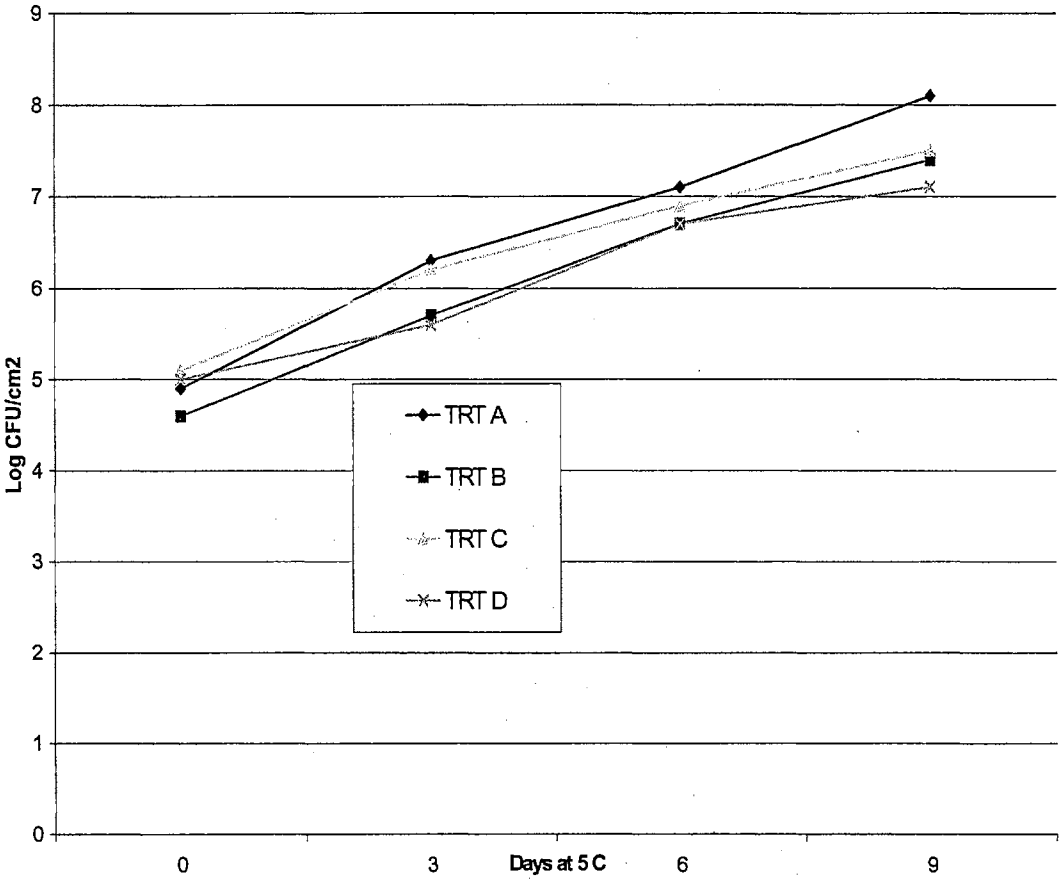

Figure B26. Antagonistic action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 toward *Escherichia coli* O157:H7 on the surface of fresh beef steaks after being treated in a glucose based dip solution and stored at 5° C. Psychrotrophic counts detected on PCA (incubated at 15°C for 7 d) are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 3.

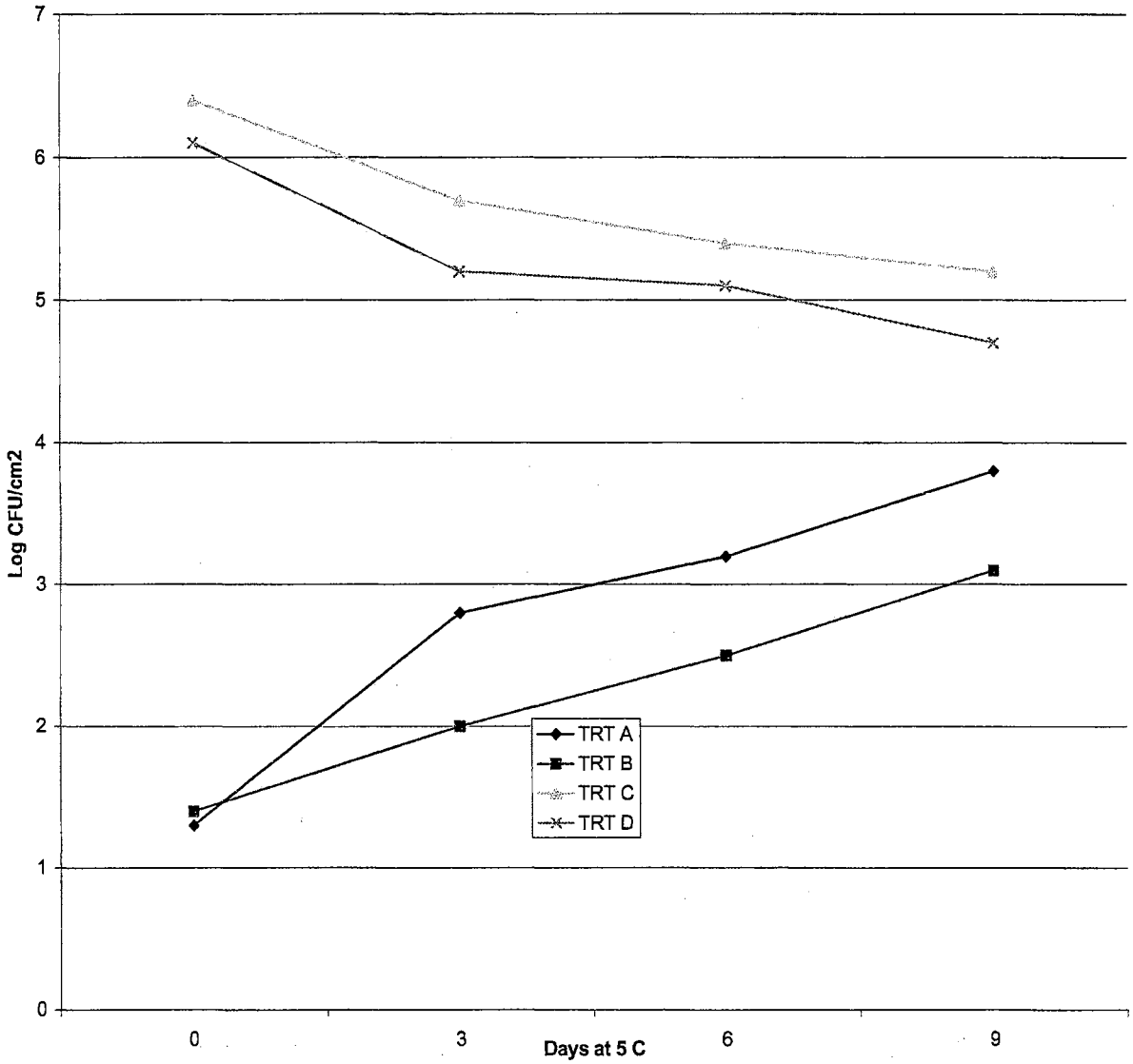

Figure B27. Antagonistic action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 toward *Escherichia coli* O157:H7 on the surface of fresh beef steaks after being treated in a glucose based dip solution and stored at 5° C. *Escherichia coli* O157:H7 counts detected on VRBA are expressed as $\log_{10}$ CFU/cm^2; each value is a mean from six replications. Treatment A = control. Treatment B = inoculated only with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5. Treatment C = inoculated only with *Escherichia coli* O157:H7. Treatment D = inoculated with *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and *Escherichia coli* O157:H7. This is a graphical presentation of the data shown in Table 3.

APPENDIX C

STATISTICAL ANALYSES

Table C1 – Antagonistic action of five freshly prepared strains of *Lactobacillus delbrueckii* susbp.*lactis* toward naturally occurring background microflora found on fresh cut beef steaks during refrigerated storage (5° C) on days 0, 3, 6 and 9..

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	2	4.432170	2.216085	7.23	0.0013
Treatment	5	20.596863	4.119373	13.45	< .0001
Time	3	43.213538	14.404513	47.02	< .0001
Time*Treatment	15	29.266772	1.951118	6.37	< .0001
Error (a)	46	50.389383	1.095421	3.58	< .0001
Media	2	1068.800306	534.400153	1744.25	< .0001
Treatment*Media	10	208.252528	20.825253	67.97	< .0001
Time*Media	6	45.042426	7.507071	24.50	< .0001
Error (b)	30	11.340574	0.378019	1.23	0.2254

Table C2 – Antagonistic action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 toward *Escherichia coli* O157:H7 in ground beef stored at 5° C.

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	2	21.3077083	10.6538542	13.64	< .0001
Treatment	3	102.1975000	34.0658333	43.60	< .0001
Time	3	39.5358333	13.1786111	16.87	< .0001
Time*Treatment	9	14.0566667	1.5618519	2.00	0.0726
Error (a)	30	28.6956250	0.9565208	1.22	0.2870
Media	2	101.6816667	101.6816667	130.14	< .0001
Treatment*Media	6	195.2858333	65.0952778	83.31	< .0001
Time*Media	6	4.4575000	1.4858333	1.90	0.1492
Error (b)	18	4.951667	0.5501852	0.70	0.7005

Table C3 – Analysis of variance of table 3 - Antagonistic action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 toward *Escherichia coli* O157:H7 on the surface of fresh beef steaks after being treated in a glucose based dip solution and stored at 5° C

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	2	21.5234722	10.7617361	20.33	< .0001
Treatment	3	91.6169444	30.5389815	57.69	< .0001
Time	3	33.4191667	11.1397222	21.04	< .0001
Time*Treatment	9	18.8480556	2.0942284	3.96	0.0005
Error (a)	30	11.8720833	0.3957361	0.75	0.8077
Media	2	129.7372222	64.8686111	122.55	< .0001
Treatment*Media	6	246.507222	41.0845370	77.61	< .0001
Time*Media	6	18.9716667	3.1619444	5.97	< .0001
Error (b)	18	16.0127778	0.8895988	1.68	0.0668

Table C4 – Analysis of variance of table 4 - Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (fresh culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C.

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	2	11.3646094	3.7882031	4.61	0.0065
Treatment	3	178.0571094	59.3523698	72.28	< .0001
Time	3	14.3564544	4.7854948	5.83	0.0018
Time*Treatment	9	17.2894531	1.9210503	2.34	0.0281
Error (a)	30	31.7091406	0.0746476	0.86	0.6966
Media	2	155.9819531	155.9819531	189.96	< .0001
Treatment*Media	6	252.1058594	84.0352865	102.34	< .0001
Time*Media	6	1.5877344	0.5292448	0.64	0.5902
Error (b)	18	6.1857031	0.6873003	0.84	0.5860

Table C5 – Analysis of variance of table 5 - Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 (frozen culture) against *Escherichia coli* O157:H7 on the surface of fresh beef steaks stored at 5 °C.

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	2	18.6089583	9.3044792	16.27	< .0001
Treatment	3	161.9536458	53.9845486	94.38	< .0001
Time	3	40.9003125	13.6334375	23.84	< .0001
Time*Treatment	9	30.8067708	3.4229745	5.98	< .0001
Error (a)	30	42.2677083	1.4089236	2.46	0.0069
Media	2	196.3676042	196.3676042	343.31	< .0001
Treatment*Media	6	274.5236458	91.5078819	159.98	< .0001
Time*Media	6	6.9853125	2.3284375	4.07	0.0148
Error (b)	18	27.1951042	3.0216782	5.28	0.0002

Table C6 – Analysis of variance of table 6 - Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Escherichia coli* O157:H7 on the surface of beef carcasses stored at 5 °C.

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	4	32.354500	8.0886250	13.22	< .0001
Treatment	3	80.5576667	26.8525556	43.88	< .0001
Time	1	42.9603333	42.9603333	70.20	< .0001
Time*Treatment	3	1.2736667	0.424556	0.69	0.5592
Error (a)	28	19.7108333	0.7039583	1.15	0.3155
Media	2	28.8781667	14.4390833	23.59	< .0001
Treatment*Media	6	249.863833	41.6439722	68.05	< .0001
Time*Media	2	58.2861667	29.1430833	47.62	< .0001
Error (b)	6	10.7518333	1.7919722	2.93	0.0138

Table C7 – Analysis of variance of table 7 - Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Salmonella typhimurium* on the surface of beef carcasses stored at 5 °C.

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	4	22.5730000	5.6432500	9.05	< .0001
Treatment	3	68.2329167	22.7443056	36.48	< .0001
Time	1	69.7687500	69.7687500	111.91	< .0001
Time*Treatment	3	5.1942500	1.7314167	2.78	0.0483
Error (a)	28	18.3736667	0.6562024	1.05	0.4202
Media	2	50.5715000	25.2857500	40.56	< .0001
Treatment*Media	6	237.9398333	39.6566389	63.61	< .0001
Time*Media	2	140.023500	70.0117500	112.30	< .0001
Error (b)	6	9.0505000	1.5084167	2.42	0.0360

Table C8 – Analysis of variance of table 8 – Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Escherichia coli* O157:H7 on the surface of pork carcasses stored at 5 °C.

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	9	7.9015000	0.8779444	3.08	0.0021
Treatment	3	272.2835000	90.7611667	318.05	< .0001
Time	1	50.2335000	50.2335000	176.03	< .0001
Time*Treatment	3	11.1645000	3.7225000	13.04	< .0001
Error (a)	63	21.2271667	0.3369392	1.18	0.2085
Media	2	234.4885833	117.2442917	410.85	< .0001
Treatment*Media	6	985.1537500	164.1922917	575.37	< .0001
Time*Media	2	190.6952500	95.3476250	334.12	< .0001
Error (b)	6	18.4957500	3.0826250	10.80	< .0001

Table C9 – Analysis of variance of table 9 - Antagonist action of *Lactobacillus delbrueckii* susbp.*lactis* RM2-5 against *Salmonella typhimurium* on the surface of pork carcasses stored at 5 °C.

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	4	3.2496667	0.8121667	2.15	0.0847
Treatment	3	84.0815833	28.0271944	74.22	< .0001
Time	1	49.7940833	49.7940833	131.86	< .0001
Time*Treatment	3	0.5169167	0.1723056	0.46	0.7138
Error (a)	28	8.3953333	0.2998333	0.79	0.7462
Media	2	114.1020000	57.0510000	151.08	< .0001
Treatment*Media	6	309.3006667	51.5501111	136.51	< .0001
Time*Media	2	72.2346667	36.1173333	95.64	< .0001
Error (b)	6	5.0813333	0.8468889	2.24	0.0501

Table C10 – Analysis of variance of table A1 - Antagonistic action of *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 and L+1 Farr toward *Escherichia coli* O157:H7 in Trypticase soy broth stored at 5 and 7° C.

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	4	1.8401667	0.9200833	7.21	0.0026
Treatment	5	34.9040833	6.9808167	54.71	< .0001
Time	3	21.2856667	7.0952222	55.60	< .0001
Time*Treatment	15	2.6289167	0.1752611	1.37	0.2191
Error (a)	46	4.8698333	0.1058659	0.83	0.7232
Media	1	441.6126042	441.6126042	3460.80	< .0001
Treatment*Media	3	3.2419792	1.0806597	8.47	0.0003
Time*Media	3	14.1578125	4.7192708	36.98	< .0001
Error (b)	9	0.4192708	0.0465856	0.37	0.9433

Table C11 – Analysis of variance of table A2 - Antagonistic action of *Lactobacillus delbrueckii* subsp. *lactis* RM2-5 toward *Salmonella typhimurium* in Trypticase soy broth stored at 5 and 7° C.

Source	DF	Sum of squares	Mean square	F Value	Pr > F
Replication	2	1.30254902	0.65127451	9.02	0.0041
Treatment	3	2.25305322	0.75101774	10.40	0.0012
Time	6	30.87892725	5.14648787	71.26	< .0001
Time*Treatment	18	28.28654894	1.57147494	21.76	< .0001
Error (a)	54	5.66411765	0.10489107	1.45	0.2450
Media	1	57.25444444	57.25444444	792.75	< .0001
Treatment*Media	3	0.16000000	0.16000000	2.22	0.1624
Time*Media	6	0.37722222	0.18861111	2.61	0.1144
Error (b)	18	0.20166667	0.10083333	1.40	0.2850

VITA

Michele Marie Otremba Senne

Candidate for the Degree of

Doctor of Philosophy

Thesis: ANTAGONISTIC ACTION OF CELLS OF *LACTOBACILLUS DELBRUECKII* SPP *LACTIS* TOWARD PATHOGENIC MICROORGANISMS IN FRESH MEAT SYSTEMS

Major Field: Food Science

Biographical:

Personal Data: Born in Santa Maria, California, August 24, 1973, the daughter of Stan and Anna Otremba. Married to Brandon Senne on December 11, 1999.

Education: Bachelor of Science degree in Animal Science from California Polytechnic State University, San Luis Obispo, in March, 1995; Master of Science degree in Animal Science with a concentration in meat science from Kansas State University, Manhattan, KS, in June, 1997; Completed requirements for the Doctor of Philosophy degree at Oklahoma State University on November 21, 2000.

Professional Experience: Director of Pork Safety for the National Pork Producers Council, February 2000 – current. Member of ASAS; AMSA; IFT and NMA.

Academic Honors: Recipient of the Glenn Rapp Foundation Distinguished Graduate Research Fellowship: 1998 – 2000.