Journal of Food Protection, Vol. 64, No. 8, 2001, Pages 1138–1144

Effect of Different Levels of Beef Bacterial Microflora on the Growth and Survival of *Escherichia coli* O157:H7 on Beef Carcass Tissue†

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MS 00-402: Received 10 November 2000/Accepted 16 February 2001

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ABSTRACT

The influence of various levels of endogenous beef bacterial microflora on the growth and survival of *Escherichia coli* O157:H7 on bovine carcass surface tissue was investigated. Bacterial beef microflora inoculum was prepared by enriching and harvesting bacteria from prerigor lean bovine carcass tissue (BCT) and was inoculated onto UV-irradiated prerigor BCT at initial levels of 10^5, 10^4, 10^3, and $<10^3$ CFU/cm^2. Additional control BCT was inoculated with sterile H_2O. *E. coli* O157:H7 was inoculated onto all tissues at an initial level of 10^2 CFU/cm^2. Following a 48-h incubation at 4°C, BCT was incubated up to 14 days at 4 or 12°C, either aerobically or vacuum packaged. Regardless of the microflora level, there was no substantial growth of *E. coli* O157:H7 on BCT during storage at 4°C under either aerobic or vacuum-packaged conditions. Instead, viable cell numbers at 4°C remained constant, with no reduction in numbers associated with the different beef microflora levels. *E. coli* O157:H7 grew on all BCT stored at 12°C, regardless of microflora inoculation treatment, reaching higher populations on aerobic samples than on vacuum-packaged samples in 10 days. However, the presence of the beef microflora did appear to delay the onset of growth or slow the growth of the pathogen, and *E. coli* O157:H7 counts on BCT without added microflora were generally higher following 7 to 10 days of 12°C storage than those counts on BCT inoculated with beef microflora. These data demonstrate the importance of temperature control during meat handling and storage to prevent the outgrowth of this pathogen and indicate that proper sanitation and processing practices that prevent and reduce contamination of carcasses with *E. coli* O157:H7 are essential, regardless of background microflora levels.

Factors in the emergence of new foodborne pathogens or the increases in incidence of recognized pathogens as agents in foodborne illness can include changes in demographics, human behavior, agricultural practices, globalization, and food processing and distribution, as well as adaptive changes of the microorganisms themselves (for recent reviews, see *1, 20, 24).* The potential for microbial safety and spoilage problems due to new food sanitation, processing, or preservation technologies must be evaluated, as their introduction may present new opportunities for emerging or acknowledged foodborne pathogens to assert themselves. For example, increased reliance on refrigeration and increased production of extended shelf life refrigerated foods may have played a role in the emergence of *Listeria monocytogenes* as a major foodborne pathogen *(20).* Near coincident with the introduction of modified and controlled atmosphere foods such as sous vide and cook-chill products was the recognition of the possible risks for outgrowth of facultatively anaerobic or anaerobic psychrotrophic pathogens such as nonproteolytic *Clostridium botulinum, L. monocytogenes,* and *Yersinia enterocolitica,* due to atmosphere changes, reduced aerobic spoilage microorganisms, or reduced microbial competition *(12, 20).* Recognition of these possible hazards has led to the development of control measures to reduce these risks *(18, 20).* As another example, modern advances in dairy sanitation and processing practices are thought to be responsible for the increase in prominence of psychrotrophic strains of *Bacillus cereus* as significant causes of milk spoilage. These improvements have eliminated much of the traditional load of such heat-sensitive, psychrotrophic, postpasteurization contaminants as *Pseudomonas* or *Alcaligenes* spp., thus selecting for the more heat-resistant sporeformers such as *B. cereus (15, 19).*

During the last two decades, *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli* have emerged as prominent foodborne disease agents. Cattle are a reservoir of *E. coli* O157:H7, and the consumption of raw or undercooked beef and milk has most often been associated with *E. coli* O157:H7 foodborne infections *(5, 10, 11, 27).* Improvements in sanitation and the introduction of pathogen reduction interventions for carcasses have been offered as possible explanations for the emergence of this pathogen in beef *(16, 17).* Carcass intervention measures such as steam vacuum sanitizers, steam pasteurization, hot water, and antimicrobial chemical spray washes are not specific, reducing harmless meat microflora from carcasses in addition to bacterial pathogens *(7, 8, 21, 23).* The hypothesis is that fresh meats with high numbers of harmless, natural bacterial mi-

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croflora are less likely to be vehicles for foodborne disease than are cleaner meats with fewer bacteria, because the higher levels of normal beef microflora are likely to outcompete any pathogenic bacteria that may be present *(16, 17)*. Recent works have shown that background microflora in ground beef can inhibit the growth of *E. coli* O157:H7 in this product *(22, 28)*. Growth of this pathogen in ground beef was suppressed by levels of microflora that were present initially at levels greater than that of the *E. coli* O157:H7; however, the existing populations of the pathogen did not decline *(22, 28)*. The objective of the present study was to examine the effects of different levels of beef microflora on the growth and survival of *E. coli* O157:H7 on bovine carcass surface tissue during refrigerated storage.

MATERIALS AND METHODS

Bacterial inocula preparation. Streptomycin-resistant *E. coli* O157:H7 MARCS-1 *(7)* and a streptomycin-resistant strain of *E. coli* O157:H7 ATCC 43895 were inoculated from frozen 25% glycerol stock cultures into separate 10-ml volumes of tryptic soy broth (Difco, Becton Dickinson Microbiology Systems, Sparks, Md.) containing 250 μg/ml streptomycin. The nonstreptomycin-resistant parental strains of *E. coli* O157:H7 MARCS-1 and ATCC 43895 were initially isolated from human feces and ground beef, respectively. These cultures were incubated statically for 16 h at 37°C. Following incubation, the optical density at 600 nm of each culture was measured to confirm equivalent cell densities. To prepare the *E. coli* O157:H7 inoculum, 2 ml of each culture was mixed together, then diluted as necessary in sterile distilled H_2O (sdH_2O) to obtain a 150-ml volume containing approximately 10^4 CFU/ml. This preparation was immediately inoculated as described below to give an initial level of 10^2 CFU of *E. coli* O157:H7 per cm^2 of bovine carcass tissue.

The bacterial beef microflora inoculum was prepared by enriching and harvesting bacteria from lean bovine carcass tissue (BCT). For each experimental replication, BCT was obtained from the cutaneous trunci of two different prerigor carcasses immediately following slaughter at a local cow–bull processing facility. The BCT was placed in plastic bags in an insulated container to minimize cooling. Following transport to the laboratory, both BCT sections were vacuum packaged then incubated separately for 24 h at 10 and 15°C. After this initial incubation, the BCT was removed from the bags, and two 5- by 5-cm sections were aseptically excised from each piece (four total). Each 5- by 5-cm section was placed in a sterile filtered stomacher bag (Spiral Biotech, Bethesda, Md.) with 25 ml of brain heart infusion broth (Difco) and pummeled for 1 min using a stomacher lab blender (model 400; Tekmar, Cincinnati, Ohio). The excised 5- by 5-cm sections from each original BCT section were further incubated aerobically, in the bags with the brain heart infusion broth, for 24 h at 10 and 15°C. Following this second incubation, the BCT were pummeled again for 30 s. The entire retrievable volume of brain heart infusion broth was removed from each stomacher bag and placed into sterile 50-ml conical tubes. To remove large meat particles, these volumes were clarified by centrifugation at 50 × *g* for 2 min at 4°C. The supernatants were removed to fresh tubes, and cells were collected by centrifugation at 4°C for 30 min at 2,060 × *g*. Each of the cell pellets was resuspended in 4 ml of sdH_2O, then the four suspensions were combined to provide approximately 16 ml of meat microflora preparation. This initial microflora preparation was serially diluted further in sdH_2O (1:10 and 1:100) to provide three separate inocula containing three different levels of beef microflora, which were used immediately as described below.

Treatments were assigned to different beef microflora level categories based upon the initial levels of aerobic bacteria in the microflora preparations, as inoculated onto the BCT. To determine the total aerobic bacteria inoculated, a subsample of the initial microflora preparation was diluted as necessary in buffered peptone water (Difco) and spiral plated in duplicate onto tryptic soy agar plates (Difco), using a model D spiral plater (Spiral Biotech). The plates were incubated at 35°C for 24 h prior to enumeration.

Beef tissue preparation. Lean BCT was obtained from prerigor carcasses, packed as described above, and transported to the laboratory for immediate use in experiments. The BCT was aseptically trimmed to 15- by 20- by 1-cm pieces and placed on sterile trays. To substantially eliminate any existing surface bacterial contamination, the excised BCT samples were sterilized by germicidal UV light (100 microwatts per cm^2) for 20 min on each side, as described by Cutter and Siragusa *(6)*. The internal surfaces of the BCT were UV treated first, then the BCT was transferred to a fresh sterile tray for the UV treatment of the external surface. BCT samples were inoculated immediately following this sterilization process.

Beef tissue inoculation and storage treatments. For each experimental replication, each of the three different microflora preparations was used to inoculate four 15- by 20- by 1-cm pieces of sterilized BCT. For each tissue piece, 3 ml of the appropriate inoculum was distributed over the external surface of the BCT using a pipette, then it was spread evenly over the entire external surface using the back of a sterile spoon. Four control BCT were inoculated in the same fashion using 3 ml of sterile sdH_2O. The BCT was allowed to stand at room temperature for 15 min to allow the inocula to adsorb prior to the application of the *E. coli* O157:H7 inoculum. Three-milliliter volumes of the *E. coli* O157:H7 inoculum were applied to the BCT as described for the microflora inocula. A 5- by 5-cm sample was aseptically excised from each tissue piece and placed in a sterile filtered stomacher bag (Spiral Biotech) for bacterial enumeration. Each tray was then covered with a plastic bag, which was loosely tented over the BCT so as not to touch the tissue surfaces, and stored at 4°C for 48 h. This initial aerobic incubation was done to simulate common practice in beef slaughter and fabrication operations, where beef carcasses may hang uncovered in a cooler for 24 to 72 h prior to fabrication. At 48 h, the BCT was again sampled for enumeration. At this time, at least three additional 5- by 5-cm samples were excised from each BCT, stored at either 4 or 12°C, and either aerobically or vacuum packaged. For aerobic storage, the excised samples were placed into separate sterile filtered stomacher bags, the tops of which were folded over once and secured with a binder clip. For vacuum packaging, samples were placed in vacuum-packaging bags (3.2-mil nylon-copolymer bags with an oxygen transmission rate at 23°C of 52 cm^3/m^2; Advantage Food Equipment Systems, Omaha, Neb.) and vacuum sealed (model LV10G; Hollymatic, Countryside, Ill.). Samples stored at 4°C were removed for bacterial enumeration at 7 and 14 days, and samples stored at 12°C were removed for bacterial enumeration at 4, 7, and 10 days. At each sampling time, the BCT surface pH was measured using a flat-surface combination probe (Corning, Inc., Corning, N.Y.).

Microbiological analyses. For bacterial enumeration, the excised 5- by 5-cm samples were pummeled for 2 min with 25 ml buffered peptone water containing 0.1% (vol/vol) Tween 20. Following pummeling, the filtered samples were serially diluted in

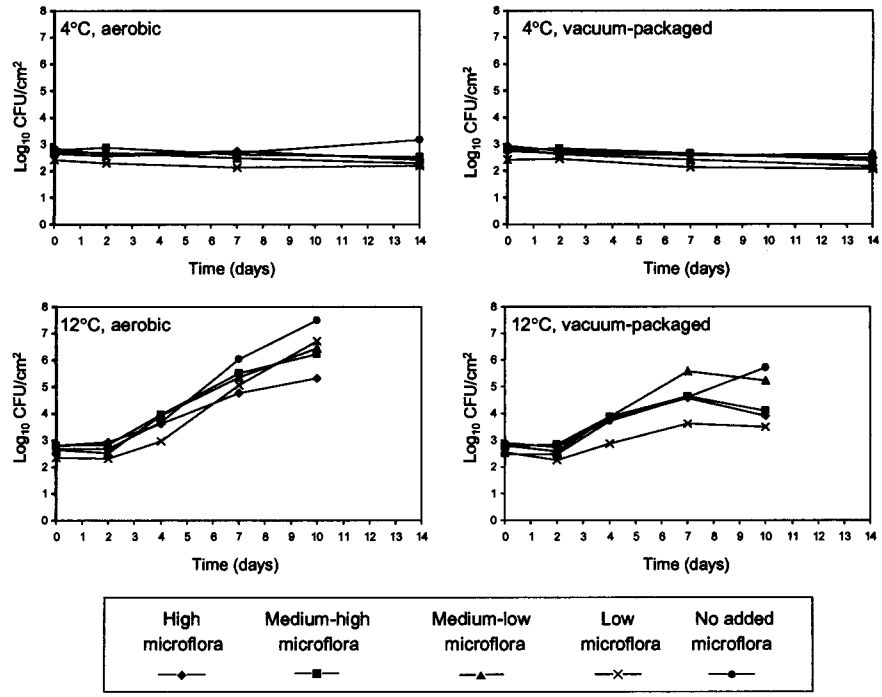

FIGURE 1. E. coli *O157:H7 populations over time on BCT inoculated with bacterial meat microflora at initial levels of* $\geq 10^5$ *and* $<10^6$ *CFU/cm*^{2} *(high microflora),* $\geq 10^4$ *and* $<10^5$ *CFU/cm*^{2} *(medium-high microflora),* $\geq 10^3$ *and* $<10^4$ *CFU/cm*^{2} *(medium-low microflora),* $\geq 10^2$ *and* $<10^3$ *CFU/cm*^{2} *(low microflora), and without added microflora, as determined by plating on SMAC. Inoculated beef tissues were incubated either aerobically or vacuum packaged at 4 and 12°C. The pooled standard error of the least squares means was 0.28, and the least significant difference was 0.76* $\log_{10}$ *CFU/cm*^{2}.

buffered peptone water if necessary and spiral plated or spread plated in duplicate on appropriate agar for the determination of populations of *E. coli* O157:H7, aerobic bacteria, and presumptive lactic acid bacteria (LAB). For enumeration of *E. coli* O157:H7, samples were plated on sorbitol MacConkey agar plates containing 250 μg/ml streptomycin (SMAC; Difco), which were incubated at 35°C for 24 h. For determination of populations of aerobic bacteria, samples were plated on tryptic soy agar plates that were incubated at 35°C for 24 h. For enumeration of LAB, samples were plated on Lactobacilli MRS (deMan Rogosa Sharpe; Difco) agar plates containing 0.02% (vol/vol) sodium azide, which were incubated in a GasPak jar (BBL, Becton Dickinson Microbiology Systems) at 30°C for 48 h, using AnaeroGen (Oxoid, Hampshire, UK) to generate an anaerobic atmosphere.

Statistical analyses. Seven independent replications of the experiment were done, with each replicate examining four different inoculum levels (three microflora levels and one uninoculated control), in each of two atmospheres (aerobic and vacuum packaged) and at each of two temperatures (4 and 12°C). Populations of bacteria on duplicate plates were averaged and converted to $\log_{10}$ CFU per cm^2. Least squares means of bacterial populations were analyzed as a completely randomized factorial design using the general linear models procedure of SAS (version 6.12; SAS Institute, Cary, N.C.). Statistical significance is defined as a $P \leq 0.05$ unless otherwise noted.

RESULTS

Beef bacterial microflora population categories. At various stages in the carcass dressing process, the majority of beef carcasses have aerobic bacteria counts of 2.00 to 3.00 $\log_{10}$ CFU/cm^2 *(2, 25)*. Therefore, to obtain adequate beef bacterial microflora for use in experiments, it was necessary to enrich prerigor BCT to increase bacterial numbers prior to their harvest. The prerigor BCT was enriched at two different low temperatures, first while vacuum packaged and then while immersed in aerobically incubated beef-based brain heart infusion broth. Two different temperatures and both anaerobic and aerobic incubations of the tissue were utilized during the enrichment process in an effort to prepare bacterial microflora preparations with both (i) adequate cell numbers for the targeted inoculation levels, and (ii) different bacterial populations in approximate proportions of those that may develop on beef early in refrigerated storage. Upon completion of experiments, the treatments were assigned to different beef microflora level categories based upon the initial number of aerobic bacteria inoculated per square centimeter of BCT, as determined by enumeration of aerobic bacteria in the microflora preparations prior to inoculation. The bacterial beef microflora treatment categories, range of initial CFU/cm^2 of aerobic beef microflora, and samples per treatment were as follows: (i) high microflora, ≥ 5.00 and <6.00 $\log_{10}$ CFU/cm^2, $n = 4$; (ii) medium-high microflora, ≥ 4.00 and <5.00 $\log_{10}$ CFU/cm^2, $n = 6$; (iii) medium-low microflora, ≥ 3.00 and <4.00 $\log_{10}$ CFU/cm^2, $n = 4$; (iv) low microflora, <3.00 $\log_{10}$ CFU/cm^2, $n = 6$; and (v) no added microflora, $n = 7$.

Effect of different beef bacterial microflora levels on *E. coli* O157:H7 on beef. *E. coli* O157:H7 populations during storage on BCT inoculated with the different levels of beef microflora are shown in Figure 1. Initial levels of *E. coli* O157:H7 on all BCT samples ranged from 2.37 to 2.92 $\log_{10}$ CFU/cm^2. Regardless of the microflora level, there was no substantial outgrowth of *E. coli* O157:H7 on BCT during storage at 4°C under either aerobic or vacuum-packaged conditions. Instead, viable cell numbers of *E. coli* O157:H7 at 4°C remained constant, with no reduction in numbers associated with the different beef microflora levels. At 4°C, slight growth of *E. coli* O157:H7 was seen only on aerobically stored BCT that had no added microflora. In this sample treatment, *E. coli* O157:H7 grew from a level of 2.67 $\log_{10}$ CFU/cm^2 on day 10 to 3.17 $\log_{10}$ CFU/cm^2 on day 14 ($P \leq 0.05$). While this indicates a possible

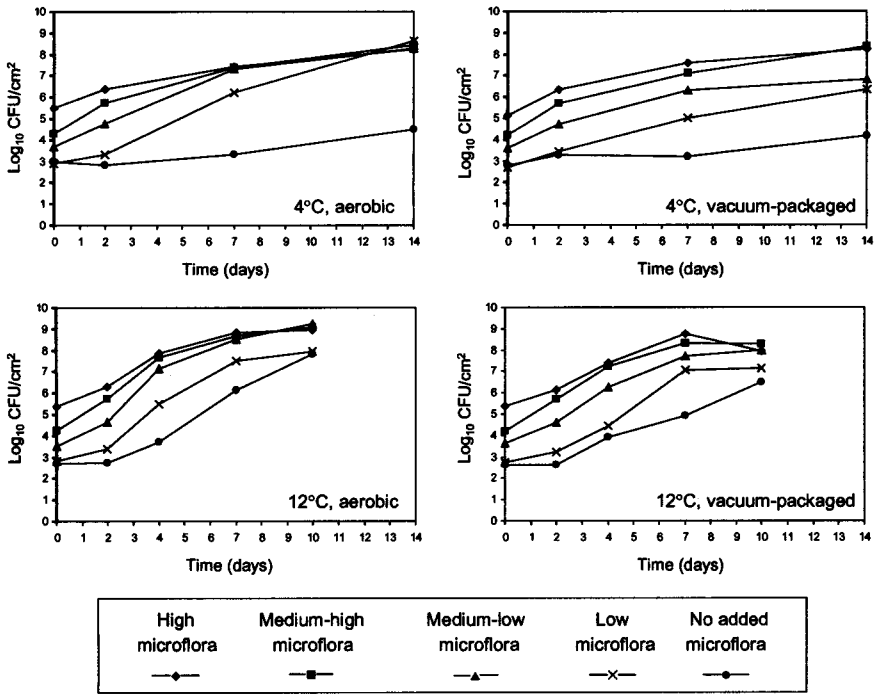

FIGURE 2. *Aerobic bacteria populations over time on BCT inoculated with bacterial meat microflora at initial levels of* $\geq 10^5$ *and* $<10^6$ *CFU/cm*^{2} *(high microflora),* $\geq 10^4$ *and* $<10^5$ *CFU/cm*^{2} *(medium-high microflora),* $\geq 10^3$ *and* $<10^4$ *CFU/cm*^{2} *(medium-low microflora),* $\geq 10^2$ *and* $<10^3$ *CFU/cm*^{2} *(low microflora), and without added microflora, as determined by plating on tryptic soy agar. Populations include inoculated* E. coli *O157:H7. Inoculated beef tissues were incubated either aerobically or vacuum packaged at 4 and 12°C. The pooled standard error of the least squares means was 0.36, and the least significant difference was 1.00* $\log_{10}$ *CFU/cm*^{2}.

effect of bacterial background microflora in preventing growth of this pathogen, this population was not significantly different from *E. coli* O157:H7 populations on high and medium-high microflora BCT on the same day, but it was different from populations on medium-low and low microflora BCT.

E. coli O157:H7 grew on all BCT stored at 12°C, regardless of microflora inoculation treatment, reaching higher populations on aerobically stored samples in 10 days than on vacuum-packaged samples (Fig. 1). Vold et al. *(28)* previously reported that the availability of air can affect the growth of this microorganism in ground beef stored at 12°C, finding that growth inhibition by background microflora was more pronounced under anaerobic storage conditions. Although *E. coli* O157:H7 grew on all BCT treatments at this temperature, the presence of the beef microflora generally had a negative effect on the growth of *E. coli* O157:H7. Under aerobic storage, populations of the organism on BCT with no added microflora were higher at both 7 and 10 days, reaching a final population at day 10 of 7.50 $\log_{10}$ CFU/cm^2, which was significantly higher than populations on all microflora-inoculated BCT samples. The *E. coli* O157:H7 population at day 10 on the high microflora BCT was 5.33 $\log_{10}$ CFU/cm^2. The microflora effect was somewhat less clear-cut with vacuum-packaged BCT stored at 12°C. The *E. coli* O157:H7 population on BCT without added microflora at day 10 (5.70 $\log_{10}$ CFU/cm^2) was significantly higher than populations on high, medium-high, and low microflora BCT, but it was not higher than that on medium-low BCT. Although these data indicate that the presence of beef microflora on BCT can somewhat inhibit the outgrowth of this pathogen in cases of temperature abuse, the pathogen grew to high numbers on BCT at all microflora inoculation levels examined at 12°C.

Aerobic and LAB populations on beef. Populations of aerobic bacteria during storage on BCT inoculated with the different levels of beef microflora are shown in Figure 2. Because these populations were enumerated on tryptic soy agar, *E. coli* O157:H7 initially represented most of the aerobic microflora on BCT that were not inoculated with beef microflora (an average of 75.4% of aerobic bacteria at day 0), as well as much of the microflora on BCT inoculated with low microflora (an average of 46.2% of aerobic bacteria at day 0). At both temperatures and under both storage regimens, because of growth of the inoculated beef bacteria, these proportions dropped substantially in low microflora samples, to an average of less than 10% at day 2 and less than 1% thereafter. However, on BCT without added microflora, stored at 4°C, at 14 days *E. coli* O157:H7 counts represented 5.0% of aerobic bacteria on aerobic samples and 2.8% of aerobic bacteria on vacuum-packaged samples. At 10 days on BCT without added microflora, stored at 12°C, *E. coli* O157:H7 counts represented 47.8% of aerobic bacteria on aerobically stored samples and 15.5% of aerobic bacteria on vacuum-packaged samples.

Under aerobic storage at 4°C, the numbers of total aerobic bacteria on all inoculated BCT increased fairly rapidly (Fig. 2). By 14 days at 4°C, numbers of aerobic bacteria on all BCT inoculated with beef microflora ranged from 8.24 to 8.64 $\log_{10}$ CFU/cm^2 and were not significantly different. On vacuum-packaged samples stored at 4°C, the growth rates of aerobic bacteria appeared to be somewhat slower at the medium-low and low microflora inoculation levels when compared to the same aerobic samples at 4°C. While aerobic populations on BCT inoculated with high and medium-high microflora reached levels greater than 8.0 $\log_{10}$ CFU/cm^2 by 14 days, populations on BCT with medium-low and low microflora had grown to 6.81 and 6.32, respectively. Aerobic populations on BCT without added

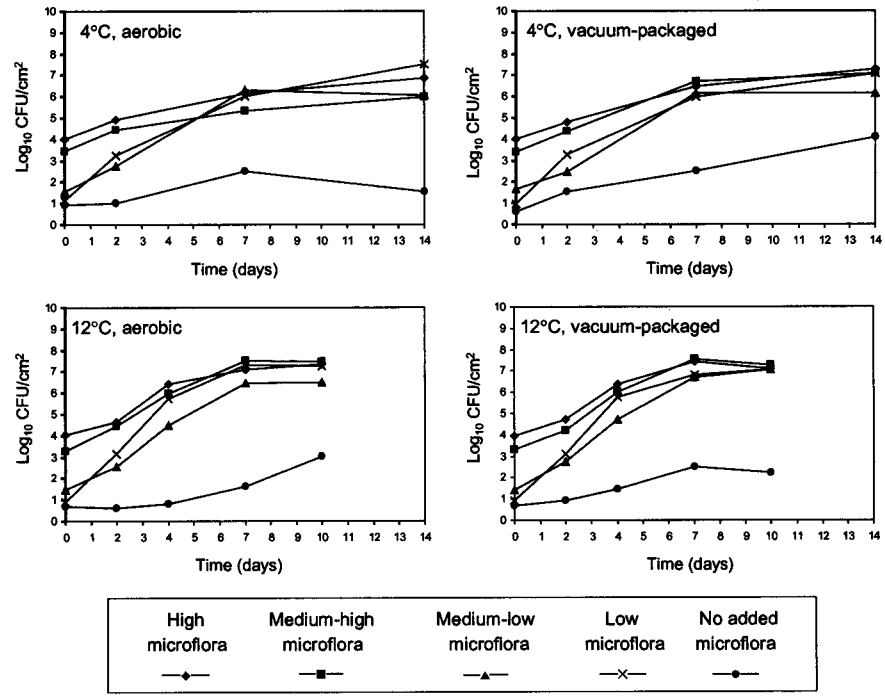

FIGURE 3. *Presumptive LAB populations over time on BCT inoculated with bacterial meat microflora at initial levels of ≥10^5 and <10^6 CFU/cm^2 (high microflora), ≥10^4 and <10^5 CFU/cm^2 (medium-high microflora), ≥10^3 and <10^4 CFU/cm^2 (medium-low microflora), ≥10^2 and <10^3 CFU/cm^2 (low microflora), and without added microflora, as determined by plating on MRS agar containing 0.02% sodium azide. Inoculated beef tissues were incubated either aerobically or vacuum packaged at 4 and 12°C. The pooled standard error of the least squares means was 0.67, and the least significant difference was 1.48 $\log_{10}$ CFU/cm^2.*

microflora attained levels of approximately 4 $\log_{10}$ CFU/cm^2 by day 14 on both aerobically stored and vacuum-packaged BCT at 4°C. This growth was represented by bacteria initially present on the BCT that were not inactivated by the UV treatment, as can be seen by comparison to the 4°C graphs in Figure 1 showing the populations of *E. coli* O157:H7 at the same time points. At 12°C, numbers of aerobic bacteria increased more rapidly, and similar to results seen at 4°C, the rates of growth under vacuum packaging compared to aerobic storage appeared to be somewhat slower. At days 4, 7, and 10 of aerobic 12°C storage, populations of aerobic bacteria in high, medium-high, and medium-low microflora treatments were not significantly different. Aerobic counts on low and no microflora BCT were different at days 4 and 7, but at day 10, they were not different at 7.94 and 7.82 $\log_{10}$ CFU/cm^2. Similar results for aerobic bacterial growth were seen on vacuum-packaged BCT stored at 12°C, except that populations within the same microflora inoculation treatments were about 1 log cycle lower by day 10 than those stored aerobically at the same temperature.

LAB populations on BCT during storage are shown in Figure 3. The UV irradiation treatment of the BCT substantially inactivated this group of bacteria; LAB counts on all BCT that were not inoculated with beef microflora remained low throughout storage. On all BCT inoculated with beef microflora, populations of LAB grew rapidly. In addition, within either 4 or 12°C treatments, there was little impact of package atmosphere on LAB growth rate. By 7 days at 4°C, LAB populations on BCT inoculated with the different levels of beef microflora were no longer different, either for aerobically stored or vacuum-packaged BCT. Similarly, by day 7 at 12°C, LAB counts had attained similar levels for each of the storage regimens.

Beef surface pH. On any given sampling day, the pH values of BCT surfaces were not significantly different between either the microflora level treatments or atmospheric storage regimens, indicating that pH was not responsible for differences in *E. coli* O157:H7 growth responses seen at 12°C (data not shown; $P \leq 0.05$). The initial pH values of BCT ranged from pH 6.85 to 7.27. At 10 and 14 days, the final sampling days for 12 and 4°C samples, respectively, the pH values ranged from 5.75 to 6.17.

DISCUSSION

Background microflora have been demonstrated to inhibit the growth of *E. coli* O157:H7 in ground beef *(22, 28)*. Palumbo et al. *(22)* monitored the growth of this organism in irradiated (no or low background flora) and fresh (naturally occurring background flora) ground beef. The *E. coli* O157:H7 strains grew in irradiated ground beef held at 8, 12, and 15°C, but their growth was inhibited in fresh ground beef. Although growth was inhibited, the populations of the pathogen in the fresh ground beef remained constant throughout the study *(22)*. Vold et al. *(28)* inoculated hygienically prepared ground beef with background microflora harvested from commercial ground beef that had been packaged either aerobically or anaerobically. The monitored background microflora were lactic acid bacteria, which were present initially at levels of 10^4 to 10^5 CFU/g; initial levels of *E. coli* O157:H7 were 10^3 CFU/g. The ground beef was stored at 12°C in order to study growth and survival of the pathogen under abusive temperature conditions. In the absence of added microflora, *E. coli* O157:H7 stored aerobically grew rapidly *(28)*. Growth of *E. coli* O157:H7 was inhibited by the high levels of microflora at this temperature, and this inhibition was greater when the ground beef was stored anaerobically *(28)*. Sim-

ilar *E. coli* O157:H7 growth inhibition on BCT was not observed in the current study. Although the presence of higher levels of beef microflora resulted in slower growth of *E. coli* O157:H7 on BCT at 12°C, the pathogen grew at all microflora levels examined. In addition, storage at 4°C was adequate to control the growth of *E. coli* O157:H7 on BCT regardless of beef microflora populations. The observed differences are most likely due to the differences in the "media" examined (ground beef versus beef carcass surface tissue) and the effects these differences may have on the growth of both *E. coli* O157:H7 and the different bacterial microflora species present on carcass tissue and ground beef. For example, grinding and comminution of meat ruptures tissue cells, releasing fluids and nutrients that provide a ready source of moisture and substrates for bacteria. As another example, exhaustion of preferred substrates upon bacterial growth on the meat surfaces may lead to changes in the composition of bacterial flora *(13)*.

The results of *E. coli* O157:H7 survival on BCT during storage at 4°C are consistent with those reported by Dorsa et al. *(9)*, who introduced low levels of bacterial pathogens as postprocessing contaminants onto BCT that had been washed with water (32°C), hot water (72°C), lactic acid, acetic acid, trisodium phosphate, or left untreated. In this work *(9)*, no substantial outgrowth of *E. coli* O157:H7 was seen, and any growth, inhibition, or inactivation of *E. coli* O157:H7 following inoculation and during 21 days of 4°C storage was not associated with differences in such general microflora populations as mesophilic aerobic bacteria, lactic acid bacteria, or pseudomonads. Instead, inhibition or inactivation of *E. coli* O157:H7 populations on the BCT treated with the organic acids and trisodium phosphate was attributed to the combination of refrigeration temperature and residual activity of the antimicrobials *(9)*. On untreated BCT and BCT treated with water or hot water, between days 0 and 7, there were initial increases of about 1.00 $\log_{10}$ CFU/cm^2 or less of *E. coli* O157:H7, then populations remained stable or decreased slightly throughout the remainder of the study *(9)*. Taken together, this study *(9)* and the present study indicate that carcass pathogen reduction interventions that also reduce the background microflora of beef carcasses will not result in unchecked growth of the remaining *E. coli* O157:H7 on carcasses when temperatures are kept appropriately low.

At the higher temperature of 12°C, the presence of background microflora on BCT did not stop the growth of *E. coli* O157:H7, but it did appear to slow the growth of the pathogen. This indicates a possible role for beef microflora on BCT in controlling ultimate populations of this pathogen in cases of higher abusive storage temperatures. The application of protective microflora has been both suggested and examined as a method to control pathogen growth during temperature abuse of refrigerated foods *(3, 4, 14, 26)*.

In summary, growth and survival of *E. coli* O157:H7 on BCT stored at 4°C were not significantly affected by the presence of different levels of endogenous beef bacterial microflora. *E. coli* O157:H7 populations remained steady throughout 14 days at 4°C, with no population reductions associated with the different beef microflora levels. At 12°C, *E. coli* O157:H7 grew on BCT at all microflora levels examined, although growth of the pathogen was generally slower on high microflora BCT compared to growth on BCT without added microflora. These data demonstrate the importance of temperature control during meat production and storage to prevent the outgrowth of this pathogen and indicate that proper sanitation and processing practices that prevent and reduce contamination on carcasses with *E. coli* O157:H7 are needed, regardless of background microflora levels.

ACKNOWLEDGMENTS

We thank Rebecca Hartford and Kim Kucera for technical assistance and Ken Ostdiek for beef tissue collection.

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