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Monitoring Beef Carcass Surface Microbial Contamination with a Luminescence-Based Bacterial Phosphatase Assay†

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ABSTRACT

A commercially available microbial phosphatase test kit (Fast Contamination Indicator; FCI) was evaluated as a rapid method for estimating microbial contamination levels on beef carcass tissues. A set of actual beef carcass surface sample swabs (n = 70) was tested using the assay as a means to rapidly (10 min) monitor carcass swab sample microbial contamination. A regression equation was developed in experiment 1 and tested on an independent population. There was agreement between this assay and the conventional plating method for total aerobic mesophilic bacteria (r = 0.93). The predicted total mesophilic aerobic bacteria count generated from the fitted regression line (predicted $\log_{10}$ CFU/cm^2 = 0.7505 × $\log_{10}$ FCI microbial phosphatase test values + 0.6726) showed a high correlation with actual aerobic mesophilic total counts (r = 0.88). The FCI test offers a simple and rapid method to estimate microbial contamination levels on beef carcasses.

Previously, data from this laboratory demonstrated a strong association between beef carcass hygiene, as measured using the total mesophilic aerobic plate count, and the likelihood of isolating biotype 1 *Escherichia coli* from that carcass *(7)*. Carcasses classified as having higher plate counts, i.e., poorer hygienic levels, were more likely to be *E. coli* biotype 1 positive. More recently, Wyss and Hockenjos *(9)* demonstrated a similar relationship between beef carcass hygiene and the incidence of verotoxin-positive *E. coli*. Taking full advantage of the potential implications of this knowledge and using this relationship as the basis for carcass monitoring require a means to rapidly assess carcass microbial loads.

Modified adenosine triphosphate (ATP) analysis methods were reported that estimate carcass microbial loads in 5 *(6)* to 15 min *(1)*. However, these methods, as reported, are not applicable to cold stored carcasses. Furthermore, their sensitivity is about 2.0 to 2.5 $\log_{10}$ CFU/cm^2. To increase sensitivity as well as to monitor microbial load from cold stored carcass samples, the *Limulus* Amoebocyte Lysate (LAL) method was applied *(3, 4, 8)*. Originally, the LAL test was used in the agglutination format, which requires several hours *(3, 5)*. Siragusa et al. *(8)* reported the modified colorimetric LAL method to estimate microbial loads on beef carcasses in 20 min.

The Fast Contamination Indicator (FCI) is a luminescence-based microbial phosphatase test which was developed for detecting fecal contamination on carcasses *(2)*. Based on the differences between heat stability of somatic and microbial phosphatase, the activity levels of each enzyme group can be distinguished with a heat inactivation step. The FCI test incorporates such a step to facilitate detection of microbial-derived phosphatase activity. Using a handheld luminometer, the FCI test requires a total of 10 min to perform after sampling. In this report, we present experimental data on the use of a microbial phosphatase test for estimating levels of mesophilic aerobic bacteria on both refrigerated and freshly slaughtered beef carcass tissues.

MATERIALS AND METHODS

Phosphatase assay. The commercial microbial phosphatase test kit (FCI; Charm Sciences Inc., Malden, Mass.) was used to determine microbial phosphatase activity. Carcass sample swabs (obtained as described below) were placed in a screw-capped sterile test tube (13 by 125 mm) containing 5 ml of sterilized distilled water and vortexed for 15 s at the highest setting. The swabbed content was expressed by squeezing against the side of the tube. One hundred-microliter aliquots of the samples were transferred to the FCI swab with a micropipetter. The swab was incubated at 75°C for 7 min in the incubator provided (Charm Sciences) to inactivate nonmicrobial phosphatase. After incubation, the swab was reinserted into the FCI test assembly and incubated at 45°C for 2 min. The FCI assay was then performed per manufacturer's instructions.

Bacterial enumeration. Carcass swab samples (obtained as described below) were diluted in buffered peptone water (Difco Laboratories, BBL Microbiology Systems, Sparks, Md.). One milliliter of each diluted sample was plated on duplicate Petrifilm Aerobic Count plates (3M, Minneapolis, Minn.) and incubated at 35°C for 24 h, then counted according to manufacturer's instructions. All counts were normalized to 1-cm^2 areas and transformed into the $\log_{10}$ value.

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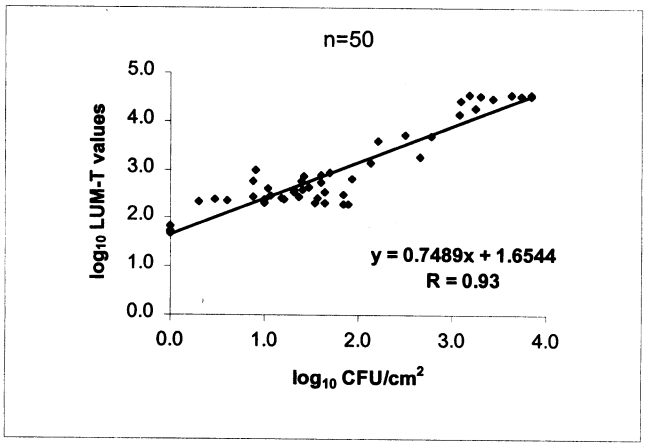

FIGURE 1. *Scatterplot of microbial phosphatase test values (log_{10} FCI microbial phosphatase test values) versus microbial counts (log_{10} mesophilic aerobic bacterial count per unit surface area) of inoculated beef carcass surface. Regression line is shown.*

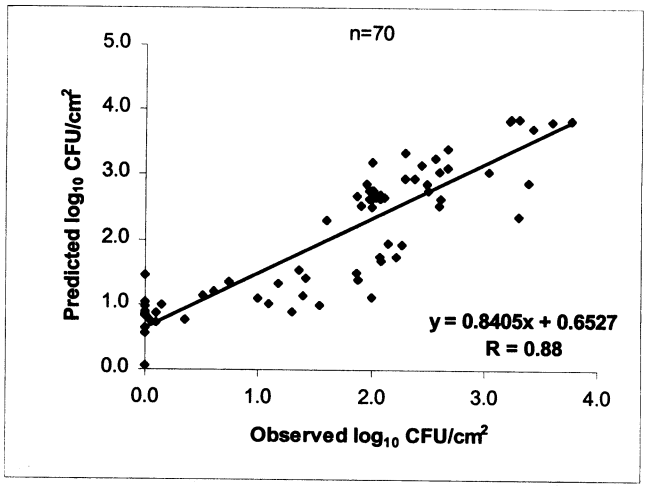

FIGURE 2. *Predicted beef carcass surface counts from the regression line equation calculated from data presented in Figure 1 and their relationship to the observed microbial counts (log_{10} mesophilic aerobic bacterial count per unit surface area) of those samples.*

Experiment 1: correlation of the microbial phosphatase test with microbial plate counts. This protocol was designed to obtain a range of microbial counts on actual beef carcass surface tissues. Beef carcass surface tissue sections (cutaneous trunci with intact superficial fascia, dimensions approximately 5 by 5 by 1 cm) were inoculated with bovine fecal suspensions. Fecal inocula were made as follows: on each day of an experiment, feces were obtained from three cows fed a corn–silage ration containing no antibiotics; 50 g of each feces sample (total of 150 g) was mixed together with 150 ml of sterile distilled water. This slurry was mixed using a metal spatula for 2 min, then passed through three layers of cotton cheesecloth (Kendall Co., Chicago, Ill.); the suspension was serially diluted in sterilized distilled water. One-milliliter aliquots of each dilution were spread over the surface of tissue sections and allowed to incubate at room temperature for 15 min prior to sampling. Samples were obtained by swabbing a 100-cm^2 area with a sterilized cotton-tipped swab (Puritan brand; Hardwood Products Co., Guilford, Maine), premoistened with sterilized distilled water, and returned to a screw-capped tube containing 5 ml of sterilized distilled water. Samples were vortexed vigorously for 15 s, then subjected to the microbial phosphatase test and bacterial plate count assays as described. After vortex mixing for 15 s at high speed, the swab samples were expressed by squeezing the swab against the side of the tube and discarded. Samples were then subjected to the assay as described above.

Experiment 2: estimation of microbial load in carcass surface samples with the microbial phosphatase test. Beef carcass surface samples were obtained from the abattoir of the Roman L. Hruska U.S. Meat Animal Research Center and from a commercial cow–bull beef processing plant. Samples were tested according to the method described above for the microbial phosphatase test. The log_{10} LUM-T values generated from the microbial phosphatase test were used to find predicted microbial counts. The predicted microbial counts were compared with actual microbial plate counts.

Statistical analysis. Bacterial counts were normalized to an area basis (CFU/cm^2) and transformed to the log_{10} values. Correlation coefficients and linear regression trendlines were calculated and plotted using the statistical tool package of Microsoft Excel 97 software.

RESULTS AND DISCUSSION

A highly significant ($P < 0.05$) linear relationship was observed between log_{10} total mesophilic bacteria numbers and the log_{10} LUM-T microbial phosphatase values ($r = 0.93$; Fig. 1) when analyzing all points in the scatterplot. From graphical analysis of the data, the microbial phosphatase test response values (log_{10} LUM-T) were linear to levels of approximately 1.5 log_{10} CFU/cm^2 total mesophilic counts. The previously reported R-mATP test *(6),* while indeed quicker, was less sensitive (response threshold greater than log_{10} 2.0 CFU/cm^2) and is dependent on the adenylate energy charge of the bacterial cells comprising the entire microbial population of the sample. Furthermore, the R-mATP test is currently not applicable to cold stored carcass samples *(6).* The sensitivity of the FCI assay was similar to that of the chromogenic LAL test *(8).* However, the FCI test requires a shorter assay time compared to the LAL test (ca. 10 min versus ca. 20 min, respectively). The FCI microbial phosphatase test offers two main advantages: (i) adaptability to testing chilled carcasses, and (ii) a low threshold of sensitivity (>1.5 log_{10} CFU/cm^2).

Figure 2 presents the carcass surface test data from a set of uninoculated and feces-inoculated carcass tissues. The log_{10} FCI microbial phosphatase activity values were used to predict log_{10} CFU/cm^2 microbial levels with the equation generated from Figure 1 (predicted log_{10} CFU/cm^2 = 0.7505 × log_{10} LUM-T values + 0.6726). The relationship between the predicted surface counts (log_{10} total CFU/cm^2) and the actual total mesophilic bacterial surface counts was significant ($P < 0.05$, $r = 0.88$; Fig. 2) at microbial levels >1.5 log_{10} CFU/cm_2. The response of the FCI microbial phosphatase test was highly correlated to actual microbial numbers on beef carcass tissues.

Siragusa et al. *(7)* reported a strong association between categorization or classification of mesophilic bacteria levels with occurrence of biotype 1 *E. coli* on beef carcass. Similarly, we used the actual mesophilic aerobic bacterial

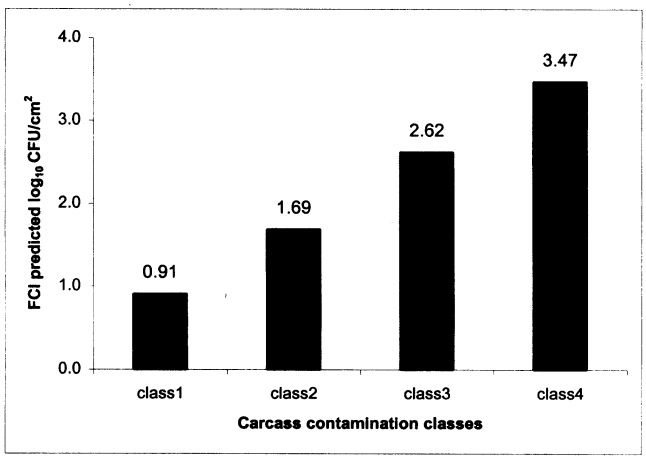

FIGURE 3. *The relationship between the Lum-T value and the microbial count-based classification of beef carcass samples. Classification criteria defined in text. Values above bars are the log_{10} Lum-T value means within each category.*

counts of the present data set to classify these swab samples into one of four different contamination classes (class 1 = 0 to 1 log_{10}; class 2 = ≥1 to 2 log_{10}; class 3 = ≥2 to 3 log_{10}; and class 4 = ≥3 to 4 log_{10}). For each class, the mean of that data set of FCI microbial phosphatase values (log_{10} LUM-T) was obtained. The relationship between the average of the FCI-measured microbial phosphatase values and the contamination classes showed strong association (Fig. 3). The agreement provides a useful means to gauge or monitor the categories of contamination levels on beef carcasses in a time frame useful for process monitoring. As with other such rapid carcass microbial level monitors, the FCI microbial phosphatase assay is not a substitute for accurate quantitative microbial counting methods, such as the standard plate count, which are highly useful for other tests requiring greater accuracy such as spoilage or shelf-life assays.

The FCI microbial phosphatase test can be applied by food animal processors to monitor microbial loads. One hypothetical example of its use is that processors could monitor the FCI microbial phosphatase test values of carcasses through several weeks to find trend curves that are related to actual microbial count data such as coliform levels or mesophilic aerobic plate counts. Using these data, processors could set acceptable limits for their process and monitor their process by the FCI microbial phosphatase assay. Selection criteria for the beef carcasses with higher microbial levels versus lower might be useful for prioritizing which beef stocks should be further processed sooner versus being shipped or held under refrigeration longer before further processing.

The aforementioned experimental design was carried out to assess a means of monitoring carcasses to achieve optimal hygiene before subsequent final interventions applied at the prechilled stage. The efficacy of this assay for gauging microbial levels on carcass samples taken from poststeam-pasteurized or similarly heat-treated samples remains to be determined. Although no attempt was made here to ascertain the effects of antimicrobial interventions on the microbial phosphatase–microbial level relationship, this question remains to be addressed if this monitor is to be applied to postintervention carcasses. The success of generic microbial tests to achieve a higher level of carcass hygiene requires obtaining carcass data in a structured, regular, and controlled monitoring program with subsequent evaluation of the information in order to rectify process deviations to prevent carcass contamination prior to the carcasses being further processed. In conclusion, we have reported laboratory-acquired data that show that a microbial phosphatase test is effective for monitoring beef carcass hygiene levels in artificially contaminated samples that are more representative of red meat animal processing before application of any final antimicrobial intervention.

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