Development of Thermal Surrogate Microorganisms in Ground Beef for In-Plant Critical Control Point Validation Studies

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ABSTRACT

In search of a suitable surrogate microorganism for in-plant critical control point validation, we compared the rates of thermal inactivation of three bacteria, Enterococcus faecium B2354, Pediococcus parvulus HP, and Pediococcus acidilactici LP, to those of Listeria monocytogenes and Salmonella. Ground beef samples containing 4 and 12% fat were inoculated with E. faecium, L. monocytogenes, and Salmonella Senftenberg 775W and heated at 58, 62, 65, or 68°C. The decimal reduction times (D-values) for E. faecium B2354 in 4 and 12% fat ground beef were 4.4 to 17.7 and 3.6 to 14.6 times greater, respectively, than those for L. monocytogenes or Salmonella Senftenberg 775W at all temperatures tested, with the greatest differences in D-values occurring at 58 and 62°C. Higher fat content protected bacteria from thermal inactivation in general, especially at temperatures lower than 68°C. The heat resistance in a broth medium at 62°C of two food-grade bacteria, P. parvulus HP and P. acidilactici LP, was compared with that of the three strains under study. The D-values of P. parvulus HP and P. acidilactici LP were lower than those of E. faecium B2354 but 4.1 and 2.5 times greater, respectively, than those of Salmonella Senftenberg 775W, the most resistant pathogen. These results indicate that thermal treatments of ground beef at 58 to 68°C that kill E. faecium B2354 will also kill Salmonella and L. monocytogenes, and the two Pediococcus isolates may serve as alternate surrogates for validation studies when a less heat-resistant surrogate is desired. However, additional studies in ground beef are needed with the Pediococcus strains in the desired temperature range intended for validation purposes.

Thermal processing of meat and poultry is among the most common of processing treatments used to ensure microbiological safety and is often a critical control point in hazard analysis critical control point systems for food processing. However, it is not feasible in food-processing facility settings to validate thermal processes with pathogenic bacteria. Hence, a suitable nonpathogenic (surrogate) microorganism is needed for process validation.

The possible use of indigenous microflora in raw meat for in-plant validation has been explored by Juneja (8). Comparative heat inactivation studies of beef have shown that the decimal reduction times (D-values) of indigenous microflora are about twice those of three groups of pathogens tested, namely Listeria monocytogenes, Salmonella, and Escherichia coli O157:H7. However, indigenous microflora in raw meat may vary among batches, depending on the source of contamination and the storage conditions. Accordingly, the heat resistance of the indigenous microflora would vary from one batch to another and would not likely be a consistent indicator for the thermal inactivation of pathogens. An alternative is to use as a surrogate a single nonpathogenic microorganism with known thermal inactivation characteristics, as is described in this study.

L. monocytogenes and Salmonella have been associated with many outbreaks of meatborne illness (3–6) and hence were selected for comparison studies. Another foodborne pathogen, E. coli O157:H7, has also been involved in outbreaks related to meat products (18). However, because E. coli O157:H7 has been determined consistently to be the most heat-sensitive pathogen among these three when tested in ground beef, turkey, or pork heated at 55 to 70°C (8, 14, 15), it was not included in our study. Furthermore, Salmonella Senftenberg 775W (ATCC 43845), the most heat-resistant Salmonella strain known, was selected for this study to simulate the worst-case scenario for a heat-resistant strain of Salmonella in meat (16).

The purpose of this study was to determine the relationship of the rates of thermal inactivation in ground beef of a potential surrogate microorganism, Enterococcus faecium B2354, to the most heat-tolerant non–spore-forming pathogens of concern in meat products, i.e., L. monocytogenes and Salmonella. The relationship of the thermal inactivation of two additional potential surrogate microorganisms, Pediococcus parvulus HP and Pediococcus acidilactici LP, to these two pathogens was also determined in a broth medium. The results obtained in this study will provide useful information in selecting surrogate microorganisms for in-plant thermal processing validation studies.

MATERIALS AND METHODS

Isolation and identification of food-grade microorganisms from commercial meat starter cultures. Two commercial meat starter cultures, HPS and LP (Chr. Hansen, Milwaukee, Wis.), were suspended in sterile lactobacilli deMan Rogosa Sharpe (MRS) broth (Difco, Becton Dickinson, Sparks, Md.) and streaked
onto lactobacilli MRS agar (Difco, Becton Dickinson) plates. After incubation at 37°C for 48 h, well-isolated colonies representing the dominant morphology on each plate were purified by streaking onto an MRS plate. Two isolates, one from HPS and one from LP, were identified by 16S rRNA gene sequencing (MIDI Labs, Newark, Del.) as *P. parvalus* and *P. acidilactici*, respectively, and were used for thermal inactivation studies.

**Identification of *Pediococcus* sp. NRRL B-2354, *Pediococcus* sp. NRRL B-2354 was identified by 16S rRNA gene sequencing (MIDI Labs) and the Vetek 2 automated system with the ID-GP card (card that identifies gram-positive bacteria) according to the manufacturer’s instructions (bioMérieux, Durham, N.C.).**

**Preparation of bacterial cultures.** Three bacterial strains, *Salmonella enterica* serovar Senftenberg 775W (ATCC 43845), *L. monocytogenes* 101M (serotype 4b, beef isolate), and *E. faecium* B2354 (formerly *Pediococcus* sp. NRRL B-2354 and *Micrococcus freundreichii*), were used for both ground beef and broth studies. *P. parvalus* HP and *P. acidilactici* LP were used only in broth studies. All strains were grown on Bile Escharotic agar (Becton, Dickinson) and stored at −80°C. To prepare test cultures, each strain was transferred to and incubated in 10 ml of tryptic soy broth with 0.6% yeast extract (TSBYE; Difco, Becton Dickinson) at 37°C for three consecutive 24-h intervals. After incubation, each culture was harvested by centrifugation at 5,000 × *g* for 10 min and then washed twice with sterile Bufferfield’s phosphate buffer (BBP, pH 7.2; Difco, Becton Dickinson) and resuspended in 0.25 ml of sterile BBP for ground beef studies and in 3 ml of sterile BBP for broth studies.

**Ground beef.** Ground beef with 4 and 12% fat content was purchased from a local retail store. Water activity, total moisture, and fat contents of the ground beef were determined on the basis of AOAC International methods, and pH was measured by a flat-surface pH electrode (Sensorex, Garden Grove, Calif.).

**Inoculation of ground beef.** Ground beef (75 g) was weighed on sterile foil and spread into a thin layer (ca. 1-cm thickness); then, 0.25 ml of each bacterial suspension (a total of 0.75 ml) was dripped in a circular pattern onto the meat to obtain a final concentration of 7 to 8 log CFU/g. The inoculated ground beef was kneaded aseptically by hand for 2 min to obtain even distributions of the inoculated bacteria. Ground beef (1 g) was placed into each of ca. 60 Whirl-Pak bags (Nasco, Modesto, Calif.). The bags were then compressed into a thin layer (ca. 1 mm thick) by rolling a bottle over the surface of each bag. The contents of two bags were initially sampled to enumerate inoculated bacteria. BBP (9 ml) was added to each bag, which was then hand massaged for 1 min. The beef suspension was serially diluted (1:10) in 0.1% peptone water, and 0.1-ml portions of appropriate dilutions were plated onto duplicate plates of modified Oxford agar (Difco, Becton Dickinson), xylose lysine thiosulfate agar (Difco, Becton Dickinson), and modified phenol red agar base (Difco, Becton Dickinson) for the enumeration of *L. monocytogenes* 101M, *Salmonella* Senftenberg 775W, and *E. faecium* B2354, respectively. The plates were incubated at 37°C for 24 h before enumeration. Thermocouples (copper constantan; 12-channel thermocouple scanner, Barnant, Barrington, Ill.) were placed into the center of each of three bags for temperature monitoring during heat treatment, and the remaining bags were vacuum sealed (model A352, Multivac, Kansas City, Mo.) and placed on ice until heat treatment.

**Heat treatment in ground beef.** The heat treatments were conducted in a circulating water bath (ThermoNESLAB, Newington, N.H.), which was preset and heated at the appropriate temperature at least 2 h before each study. The vacuum-sealed Whirl-Pak bags were placed in a stainless steel basket and separated from each other by wires. The three bags with thermocouples were placed in the middle and at the two ends of the basket to monitor temperatures of the bags’ contents during heat treatment. The basket was then placed into the water bath, with all the bags being fully submerged in the water. When the interior temperature of the three bags reached the desired temperature, three other bags were removed from the water bath and immediately placed in an ice water bath, and inoculated bacteria were enumerated for zero-time determination. Three bags were removed subsequently at each sampling time. At 58°C, the sampling times were at 2-min intervals for up to 14 min for the enumeration of *L. monocytogenes* 101M and *Salmonella* Senftenberg 775W and at 15-min intervals for up to 150 min for the enumeration of *E. faecium* B2354. At 62°C, sampling was every 30 s for up to 5 min for *L. monocytogenes* 101M and *Salmonella* Senftenberg 775W and at 5, 10, 15, 25, 35, 45, and 55 min for *E. faecium* B2354. Sampling at 65°C was every 10 s for up to 60 s for *L. monocytogenes* 101M and *Salmonella* Senftenberg 775W and every minute for up to 6 min for *E. faecium* B2354. Sampling at 68°C was every 5 s for up to 30 s for *L. monocytogenes* 101M and *Salmonella* Senftenberg 775W and every 10 s for up to 70 s for *E. faecium* B2354.

**Enumeration procedures of bacteria inoculated in ground beef.** Three selective media—modified Oxford agar, xylose lysine thiosulfate agar, and modified phenol red agar base—were used to enumerate *L. monocytogenes* 101M, *Salmonella* Senftenberg 775W, and *E. faecium* B2354, respectively. Tryptic soy agar (TSA) (12 ml) was overlayed onto each selective medium plate on the day of the experiment to enable the recovery of heat-injured cells (22). BBP (9 ml) was added to each Whirl-Pak bag, and then the bag was hand massaged for 1 min. The beef suspension was serially diluted (1:10) with 0.1% peptone water, and 0.1-ml portions of appropriate dilutions were plated onto duplicate overlaid plates and then incubated at 37°C for 24 h. In addition, the original cell suspension was enumerated by plating 1 ml (undiluted) onto four TSA overlaid selective plates (0.25 ml each) when low cell numbers were expected. All treatments were repeated at least three times.

**Inoculation, heat treatment, and enumeration of bacteria in broth.** Only one temperature, 62°C, was tested for all five strains in broth. For each strain, 1 ml of resuspended cells was transferred into 49 ml of prewarmed (ca. 64°C) TSBYE, and the tube was briefly vortexed before putting it into a circulating water bath at 62°C. When the temperature of the broth reached 62°C, as monitored by thermocouples, which occurred in less than 5 s, 1 ml was removed from the test tube and placed into a sterile tube in ice water for zero-time bacterial counts. Thereafter, 1 ml of bacterial suspension was removed at each sampling time. The cell suspension was serially diluted (1:10) with 0.1% peptone water, and 0.1-ml portions of appropriate dilutions were plated onto duplicate TSA with 6% yeast extract (TSAYE) plates. At later sampling times, the entire 1-ml sample was plated (four 0.25-ml portions) onto four TSAYE plates to enable the enumeration of small numbers of cells. The treatment was repeated three times.

**Calculation of D- and z-values.** The D-values (time to inactivate 90% of the cells), expressed in seconds, were determined from the linear portion of the survival curves by linear regression analysis by means of Microsoft Excel 2000 software (Microsoft
Corp., Redmond, Wash.). Only survival curves with more than five values in the linear portion and descending through more than 3 log cycles were used for D-value calculations. The z-values (changes in temperature required for the thermal destruction curve to traverse 1 log cycle) were determined by computing the linear regression of the mean log D-values versus their corresponding heating temperatures by means of Microsoft Excel 2000 software. The z-values were the absolute value of the inverse slope.

**Statistical analysis.** Each experiment was conducted at least three times, and three internal replications were employed in each replicate. The averaged data from each trial were analyzed by an analysis of variance by means of SAS software (SAS Institute, Cary, N.C.) to determine significant differences at a 95% confidence level ($P < 0.05$) among the treatments. The Student’s $t$ test was used to determine significant differences ($P < 0.05$) among the D-values of test strains in 4 and 12% fat content ground beef.

**RESULTS**

**Chemical characteristics of ground beef.** Moisture content, fat content, pH, and water activity of the ground beef were 72.6%, 3.7%, 5.5, and 0.990, respectively, for 4% fat content ground beef and 73.7%, 12.4%, 5.5, and 0.989, respectively, for 12% fat content ground beef. Variations of different batches of ground beef were within 1% for moisture and fat contents and 0.1 for pH value.

**Bacterial strain identification and name change of *Pediococcus* sp. NRRL B-2354.** Two strains of *Pediococcus* were obtained from commercial meat starter cultures for evaluation as additional surrogates. The 16S rRNA gene alignment profiles and phylogenetic analyses that were performed confirmed the identification of one isolate as *P. parvulus* and the other as *P. acidilactici*. *Pediococcus* sp. NRRL B-2354 was identified by 16S rRNA gene sequencing to be an *Enterococcus* sp. and was determined to be *E. faecium*, *E. hirae*, or *E. durans*, but the species could not be confirmed by sequence data only. Further analysis by the Vitek 2 microbial identification system identified the strain as *E. faecium*.

**Thermal inactivation of bacteria in ground beef.** In this study, no obvious shoulders or tails were observed in the thermal inactivation plots for any of the test strains at heating temperatures of 58 to 68°C. Examples of thermal inactivation plots for three bacteria in ground beef with 12% fat content at 62°C are shown in Figure 1. The D-values of *Salmonella* Senftenberg 775W, *L. monocytogenes* 101M, and *E. faecium* B2354 in 4 and 12% fat ground beef at 58, 62, 65, and 68°C are shown in Table 1. In 4% fat ground beef, there was no statistically significant difference in sensitivity to thermal inactivation between *L. monocytogenes* and *Salmonella* Senftenberg at any of the temperatures tested, except at 68°C, at which *L. monocytogenes* exhibited more thermal resistance than *Salmonella* Senftenberg ($P < 0.05$). This pattern was not observed in 12% fat ground beef, as there was no significant difference in the rates of thermal inactivation between the two pathogens at any of the four temperatures tested. The D-values for *E. faecium* B2354 in 4 and 12% fat ground beef were 4.4 to 17.7 and 3.6 to 14.6 times greater, respectively, than those for the most resistant pathogenic strain (*L. monocytogenes* or *Salmonella* Senftenberg 775W) at all temperatures tested.

Plots for the z-values of the three strains in 4 and 12% fat ground beef at 58 to 68°C are shown in Figures 2 and 3, respectively, and the z-values are summarized in Table 1. It is apparent from Figures 2 and 3 that *L. monocytogenes* and *Salmonella* Senftenberg 775W have similar thermal inactivation kinetics, especially in 12% fat ground beef. However, the z-values of *E. faecium* B2354 in both matrices were less than those of the two pathogens; hence, as heat temperature is increased above 68°C, the D-values of *E. faecium* would eventually become equivalent to or possibly less than those of the two pathogens, assuming the trends for rates of inactivation are consistent at higher temperatures.

**Influence of heating menstruum on rates of inactivation of test bacteria.** The fat content of ground beef influenced the rates of the thermal inactivation of all three test bacteria, with reduced rates of inactivation (higher D-values) in beef with higher fat content (Table 1). Significantly greater thermal protection by fat was observed in *E. faecium* B2354; however, this protective effect was greatly diminished at 68°C ($P < 0.05$). Interestingly, higher fat content significantly reduced the rates of the thermal inactivation of *Salmonella* Senftenberg 775W and *L. monocytogenes* 101M only at 58 and 65°C—not at the other two temperatures tested (62 and 68°C). The z-values of all three strains tested were less in 12% fat content ground beef than those obtained in 4% fat content ground beef.

**Thermal inactivation of bacteria in broth.** In search of a less heat-resistant surrogate than *E. faecium* B2354, the heat resistance values of two additional bacteria, *P. parvalus* HP and *P. acidilactici* LP, isolated from commercial meat starter cultures, were determined in broth at 62°C and compared to those of *L. monocytogenes* 101M, *Salmonella* Senftenberg 775W, and *E. faecium* B2354. The D-values for each microorganism under these conditions are shown in Table 2. The D-values of *P. parvalus* HP and *P. acidilactici* LP were substantially less than that of *E. faecium* B2354, but they were 4.1 and 2.5 times greater, respectively, than those of the most resistant pathogenic strain (*Salmonella* Senftenberg 775W).

**DISCUSSION**

One of the criteria in selecting surrogate microorganism(s) for thermal processing validation in food-processing facility settings is that it must be nonpathogenic to humans. *E. faecium* B2354, originally known as *M.freudenreichii* and then as *Pediococcus* sp. NRRL B-2354, was originally isolated from milk and dairy utensils (20). Three patents have been issued for its deliberate use in cheese manufacture for the purpose of enhancing the flavor as adjunct cultures (10, 13, 19). Its history of safety to humans through consumption in cheese and its heat resistance properties have made this strain an attractive surrogate microorganism in pasteurization studies as well as in studies of radiation inactivation of harmful bacteria by microwave energy (1,
FIGURE 1. D-value plots for L. monocytogenes 101M (A); Salmonella Senftenberg 775W (B); and E. faecium B2354 (C) in 12% fat content ground beef at 62°C.

TABLE 1. D-values and z-values of Salmonella Senftenberg 775W, L. monocytogenes 101M, and E. faecium B2354 in 4 and 12% fat content ground beef at 58, 62, 65, and 68°C.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Fat content (%)</th>
<th>D-values (in seconds) at:</th>
<th>z-values (°C)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>58°C</td>
<td>62°C</td>
<td>65°C</td>
</tr>
<tr>
<td>L. monocytogenes 101M</td>
<td>4</td>
<td>152 ± 39 A (A)</td>
<td>31.2 ± 2.2 A (A)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>264 ± 28 a (B)</td>
<td>36.2 ± 1.3 a (A)</td>
</tr>
<tr>
<td>Salmonella Senftenberg</td>
<td>4</td>
<td>154 ± 3 A (A)</td>
<td>40.2 ± 2.8 A (A)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>269 ± 11 a (B)</td>
<td>50.3 ± 2.3 a (A)</td>
</tr>
<tr>
<td>E. faecium B2354</td>
<td>4</td>
<td>2,724 ± 226 b (A)</td>
<td>476 ± 14.3 b (A)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3,902 ± 193 b (A)</td>
<td>736 ± 110 b (B)</td>
</tr>
<tr>
<td>Ratios</td>
<td>4</td>
<td>(17.7)</td>
<td>(11.8)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>(14.5)</td>
<td>(14.6)</td>
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</tbody>
</table>

*The D-values shown are the means of three independent trials, expressed as mean ± standard deviation. Means within each column with the same letter are not significantly different (P < 0.05). Capital letters are for 4% fat content ground beef, and lowercase letters are for 12% fat content ground beef. Means of the D-values of each microorganism at a specific temperature but a different fat content with the same letter in parentheses are not significantly different (P < 0.05).

b Ratios between D-values of E. faecium B 2354 and those of the most heat-resistant pathogen at the temperature tested.
2, 11, 12, 17, 20, 21). By more current bacterial identification procedures, this strain was identified as \textit{E. faecium}.

Another important criterion in selecting a surrogate microorganism for thermal processing validation is that its thermal inactivation rates (\(D\)- and \(z\)-values) be less than those of the most heat-resistant harmful microorganism that may occur in specific foods. Only microbes having greater thermal resistance than pathogens of concern are suitable surrogates. The \(D\)-values for \textit{E. faecium} B2354 in 4 and 12\% fat content ground beef were 4.4 to 17.7 and 3.6 to 14.6 times greater, respectively, than those for the most heat-resistant pathogenic strain (\textit{L. monocytogenes} or \textit{Salmonella} Senftenberg 775W) at all temperatures tested. Accordingly, heat treatments of ground beef at 58 to 68\°C sufficient to kill \textit{E. faecium} B2354 would also inactivate \textit{Salmonella} and \textit{L. monocytogenes}. Depending on the margin of safety desired, processors could use \textit{E. faecium} B2354 as a surrogate for validation studies of thermal processes in 4 and 12\% fat ground beef at 58 to 68\°C. There may be concern about the use of \textit{E. faecium} B2354 for thermal processing validation studies, because this strain is considerably higher (perhaps excessive) in its thermal resistance than is \textit{L. monocytogenes} or \textit{Salmonella} Senftenberg 775W, which would lead to substantial overprocessing of the product and increased energy costs for processing. However, this concern can be addressed by validation studies with a low cell population of \textit{E. faecium} B2354, depending on the ratio of the \(D\)-values between the surrogate and the target pathogen under the test conditions and on the margin of safety desired.

In search for a less heat-resistant surrogate than \textit{E. faecium} B2354, the heat resistance of \textit{P. parvulus} HP and \textit{P. acidilactici} LP was substantially less than those of \textit{E. faecium} but were greater than those of \textit{Salmonella} Senftenberg 775W, the more resistant of the two pathogens. Hence, these two \textit{Pediococcus} strains show promise as alternate surrogates for thermal inactivation validation studies; however, additional studies of foods and an appropriate range of temperatures are needed.

\textit{L. monocytogenes} 101M and \textit{Salmonella} Senftenberg 775W were selected for study on the basis of their heat tolerance (16) (and preliminary studies). \textit{Salmonella} Senftenberg 775W is reportedly ca. 30 times more heat resistant than \textit{Salmonella} Typhimurium. The thermal inactivation data reported in the present study were largely consistent with those reported elsewhere, although it is difficult to compare the \(D\)-values obtained in this study with those in previous reports because of the differences in meat composition and product formulations. Goodfellow and Brown (7) reported \(D\)-values at 51.6, 57.2, and 62.7\°C of 61 to 62, 3.8 to 4.2, and 0.6 to 0.7 min, respectively, for a mixture of six \textit{Salmonella} strains of different serotypes in ground beef. Juneja et al. (9) determined the \(D\)-values of an eight-strain mixture of \textit{Salmonella} in ground beef (65.5\% mois-

\begin{table}[h]
\centering
\begin{tabular}{|l|c|}
\hline
Bacterial strains & \(D\)-values (min) \textsuperscript{a} \\
\hline
\textit{L. monocytogenes} 101M & 1.71 ± 0.02 \textsuperscript{A} \\
\textit{Salmonella} Senftenberg 775W & 1.86 ± 0.03 \textsuperscript{B} \\
\textit{E. faecium} B2354 & 11.7 ± 0.4 \textsuperscript{C} \\
\textit{P. parvulus} HP & 7.65 ± 0.30 \textsuperscript{D} \\
\textit{P. acidilactici} LP & 4.68 ± 0.04 \textsuperscript{E} \\
\hline
\end{tabular}
\caption{\textit{D}-values of \textit{Salmonella} Senftenberg 775W, \textit{L. monocytogenes} 101M, \textit{E. faecium} B2354, \textit{P. parvulus} HP, and \textit{P. acidilactici} LP in TSBYE broth at 62\°C}
\end{table}

\textsuperscript{a} The \(D\)-values shown are the means of three independent replicate trials, expressed as mean ± standard deviation. Means with the same letter are not significantly different (\(P < 0.05\)).
ture and 12.5% fat content) to be 8.65, 5.48, 1.50, and 0.67 min at 58, 60, 62.5, and 65°C, respectively. Murphy et al. (15) reported that the D-values of a six-strain Salmonella mixture, including Salmonella Senftenberg 775W in ground beef (49.7% ± 1.5% moisture and 34.4% ± 1.1% fat content), ranged from 36.91 min at 55°C to 0.063 min at 70°C. A possible explanation, at least in part, for the higher D-values in the latter two studies than those in our study is that there were differences in the composition of the ground beef. These differences underline the importance of validation under specific test conditions.

The composition of heating menstrua (food products) is known to have a major influence on the thermal resistance of bacteria. Results of our study showed reduced rates of thermal inactivation (higher D-values) in beef with higher fat content for all three strains tested. Such protection was most apparent at lower heating temperatures.

In conclusion, E. faecium B2354 was consistently and considerably more heat tolerant than L. monocytogenes 101M and Salmonella Senftenberg 775W under the conditions tested (4 and 12% fat content ground beef heated at 58 to 68°C). The heat resistance values of two Pediococcus strains, P. parvulus HP and P. acidilactici LP, when tested in broth at 62°C, were less than that of E. faecium B2354 but were 4.1 and 2.5 times greater, respectively, than that of Salmonella Senftenberg 775W, the most resistant of the two pathogens when evaluated in broth. These results indicate that thermal treatments of ground beef at 58 to 68°C that kill E. faecium B2354 will also kill Salmonella and L. monocytogenes. The two Pediococcus isolates may serve as alternate surrogates for validation studies when a less heat-resistant surrogate is desired; however, additional studies of ground beef are needed with these strains in the desired temperature range intended for validation purposes.

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