A Research Note

Effects of Enrichment Medium and Incubation Temperature on Recovery of Yersinia enterocolitica from Cooked Sausage

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ABSTRACT

Enrichment media and incubation temperatures were studied for the recovery of Yersinia enterocolitica from cooked sausage. The best enrichment condition for this purpose was phosphate-buffered saline, pH 7.6, incubated at 25°C for 24-48 h. Incubation at 4°C appeared to present an additional stress factor and more nutritive media interfered with the isolation of Y. enterocolitica from cooked sausage.

It is well-known that temperatures can affect microorganisms in different ways, and in some instances completely destroy bacteria (8,11,15). Studies have been done to characterize thermal damage to bacterial cells (2). Recovery of thermally damaged bacterial cells, such as of Y. enterocolitica from heat-processed foods of animal origin, is a crucial problem. The temperatures most often used for isolating Y. enterocolitica in enrichment media have been 4°C and 25°C, neither of which was clearly preferable in dealing with thermal injury (4,7,9,13). The purpose of this study was to compare enrichment media and temperatures for recovery of Y. enterocolitica from cooked sausage.

MATERIALS AND METHODS

Sausages (type "Kamchia" - produced from comminuted pork with 2.2% NaCl and 0.3% black pepper) were inoculated with Y. enterocolitica, serotype 0:3, obtained from the Institute of Infectious and Parasitic Diseases-Medical Academy, Sofia, Bulgaria. Ten ml of bacterial suspension was mixed with 100 g of sausage meat to yield a final concentration of 1.2 to 5.3 x 10^3 cfu/g. Samples of 12-15 g were wrapped in sterile gauze bags, yielding 6-8 samples, one of which was placed in the middle of each 5 cm diameter sausage. Heat treatments (drying the surface of the sausage, smoking at high temperature, and steam temperature) were carried out under production conditions. Temperatures inside the sausages were measured by thermocouples. When temperatures of 40, 45, 50, 55 and 60°C were reached inside the bags, the sausages were removed and cooled. Three enrichment media were evaluated in this study: (a) phosphate-buffered saline (PBS) at pH 7.6, (b) meat peptone broth (MPB), and (c) 1% peptone (PB). The contents of each bag were removed and ground separately. The resulting homogenate was divided into six 2 g portions and placed in duplicate test tubes containing 18 ml of either PBS, MPB or PB. Serial dilutions from the initial suspension were made in PBS, and 0.1 ml was plate in duplicated onto MacConkey agar for direct plate counts. In addition to this, the serial dilutions were incubated at 25°C for 2 d, and have been tested for presence of the test microorganism after enrichment. One tube of each set was incubated at 4°C for 21 d, and the second tube was incubated at 25°C for 2 d. At appropriate times each enrichment culture was sampled and streaked onto MacConkey agar plates, which were incubated at 25°C for 72-96 h. Colonies typical Y. enterocolitica were identified by using procedures described previously (12). Eighteen trials were performed.

RESULTS

At sausage temperatures up to 40°C, Y. enterocolitica recoveries were obtained after 1 wk of incubation at 4°C (Table 1). After only 24 h of incubation at 25°C, there was considerable growth of competitive microorganisms in MPB, and PB showed extensive changes due to microbial action, which hampered the recovery of Y. enterocolitica (data not shown). The Y. enterocolitica was not injured when heated to 40°C (Fig. 1). Y. enterocolitica was recovered from up to 10^3 dilutions of the sample (Fig. 1), indicating that the cells were not injured by heating at 40°C.

The Y. enterocolitica was not seriously injured at 45°C either (Fig. 1). Results obtained when the enrichment media were incubated at 25°C for 24 h (data not shown) and 48 h (Table 1) were the same. Why MPB and PB yielded better apparent recoveries of Y. enterocolitica at 25°C than at 4°C is not known. The test microorganism was recovered at dilutions up to 10^3 (Fig. 1). Hence, it appears that Y. enterocolitica is not seriously injured at 45°C.
TABLE 1. Number of heated sausage samples positive for *Y. enterocolitica* after enrichment at 4 and 25°C in PBS, MPB and PB.

<table>
<thead>
<tr>
<th>Internal temp. of sausage (°C)</th>
<th>Enrichment at (temp.) in (medium)</th>
<th>4°C</th>
<th>25°C</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PBS</td>
<td>MPB</td>
<td>PB</td>
</tr>
<tr>
<td>40</td>
<td>18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>45</td>
<td>18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>50</td>
<td>2</td>
<td>0</td>
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<td>55</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

PBS - phosphate buffered saline, pH 7.6.
MPB - meat-peptone broth.
PB - peptone broth.

<sup>a</sup>Sampled after 21 d of enrichment, except where indicated.
<sup>b</sup>Sampled after 2 d of enrichment.
<sup>c</sup>Number positive of 18 samples enriched and tested.
<sup>d</sup>Sampled after 7 d of enrichment.

PB - peptone broth.

DISCUSSION

Incubation at 4°C, the temperature most often recommended for enrichment of *Y. enterocolitica* from foods, was less effective than 25°C for recovery of the organism from cooked sausage. Our results confirm those of Doyle and Hugdahl (4), who obtained best recovery of non-injured *yersinia* cells from artificially contaminated ground beef and naturally contaminated porcine tongues by using small samples (1 g) and enrichment in PBS at 25°C. Incubation at 4°C probably stressed the injured cells additionally; metabolism of *yersinia* at this temperature is suppressed, making recovery difficult. The greatest metabolic effect is attained at 25°C, even though the temperature range for the metabolism of *Y. enterocolitica* is normally quite broad.

The selection of recovery media for this study was based on other workers' results with other species of thermally injured microorganisms (6,10,15). Andrews and Martin (1) and Carlsson et al. (3) observed almost no biochemical activity by heat-injured bacterial cells. Catalase activity was destroyed, which led to accumulation of H<sub>2</sub>O<sub>2</sub> in media rich nutritive substances (16). This accumulation was a result of the metabolic activity of the bacterial cells in the highly nutritious enrichment media and the concurrent lack of catalase activity from the injured bacterial cells.

The most suitable medium in our study for the recovery of *Y. enterocolitica* was PBS, probably for the following reasons: (a) PBS is poor in nutritive compounds and therefore didn't support accumulation of H<sub>2</sub>O<sub>2</sub> in the medium and (b) the incubation temperature (25°C) was inappropriate for the growth of other kinds of intrinsic microorganisms, especially those that were also heat injured. Additional investigations are needed to evaluate the usefulness of catalase as a component of the nutritive media, as suggested by Martin et al. (14) and Flowers et al. (5).

It was demonstrated in this study that the most appropriate enrichment medium for recovery of *Y. enterocolitica* from cooked meat products was PBS, pH 7.6, incubated at 25°C for 24-48 h. Incubation at 4°C appeared to be an additional stress factor, and more nutritive media were shown to interfere with isolation.

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REFERENCES

KOUNEV