A Research Note

Procedures for Recovery of Stressed and Injured Cells of Yersinia Enterocolitica from Meat and Meat Products

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ABSTRACT

A new three-step procedure (TSP) for the recovery of Yersinia enterocolitica 0:3 from frozen meat, salted and dried meat products, raw dried meat products, and cooked perishable sausages, has been developed. The TSP is based mainly on enrichment in 0.15 M phosphate-buffered saline at 25°C. In the TSP, selected dilutions of samples are enriched for 1, 2, 3, 4, 24, and 48 h and then plated onto nonselective and selective agar media after or without alkali treatment. Additional enrichment was performed with half of the samples at 25°C for 24 h, and the rest at 4°C for up to 2 wk, followed by alkali treatment and plating on selective agar medium. Recovery of Y. enterocolitica was better using the TSP than the other method used for isolating the organism from meats.

Food microbiologists have long been concerned with the effectiveness of the procedures used for recovery of Yersinia enterocolitica from foods. In the last 20 years, many methods have been developed for isolating Yersinia species from different substances. Numerous preenrichment and enrichment media, solid selective media, and analytic procedures have been proposed (2,3,4,5,6,7,9,13,14,18,19,20,22).

The first fundamental contribution to the isolation of injured cells of Y. enterocolitica was reported by Restaino et al. (17). Later, Schiemann (20) published results regarding isolation of Yersinia species after cold and heat injury.

In view of the studies mentioned, and of results of our preliminary investigations (12), we have developed new analytical procedures for isolation of Y. enterocolitica from meat and meat products.

MATERIALS AND METHODS

Procedures for recovering Y. enterocolitica were carried out on beef and pork and meat products experimentally inoculated with Y. enterocolitica serotype 0:3, obtained from the collection of the Medical Academy, Sofia, Bulgaria. Samples of frozen pork that had been stored up to 6 months at -18°C (10); “veal pastrami,” which is a highly salted (4-5% NaCl), raw-dried uncultured product from veal meat; raw-dried uncultured products from pork and veal meat (delicacy “Preslav,” “Smiadovo” and “Manastirska”; sausages “Madara,” “Preslav,” and “Petrochan”), and cooked perishable sausage were investigated. The procedures were processed under industrial conditions according to the technical regulations of Bulgaria. Precautions were taken to prevent the spread of the test microorganisms. The recovery procedures were carried out at different intervals of time and at various steps of the technological process.

The investigations compared two methods. The first was the so-called routine method: 25 g of sample in 225 ml of 0.15 M phosphate-buffered saline (PBS), pH 7.6, was divided into two parts, one of which was enriched at 25°C for 48 h, and the other at 4°C for 3 wk. After alkali treatment (1), 0.1 ml of the enriched sample was plated on MacConkey agar with sterile bent glass rods.

The second method, the three-step procedure (TSP) which is the object of the present investigation, consists of three conditionally separated but mutually connected analytical procedures (Fig. 1). For enrichment procedures 0.15 M PBS, pH 7.6, was used, and nonselective (simple meat peptone agar) and selective (MacConkey agar) media were used for isolating Y. enterocolitica. A 10 g portion from each sample was added to a flask containing 90 ml of PBS warmed to 25°C. Enrichment was conducted at 25°C. In the second and third step procedures, further enrichments were conducted at 25°C for 24 h and at 4°C for up to 2 wk.

As shown in Fig. 1, the first procedures include removing 5 ml of enrichment culture at 1, 2, 3, and 4 h and adding this to 15 ml of PBS in tubes. Direct plating (0.1 ml) using bent glass rods on nonselective (simple meat peptone agar) agar is performed with incubation at 25°C for 48 h. Additional enrichment of the tubes at 25°C for 24 h was followed by alkali treatment (1) and plating (0.1 ml) on selective agar (MacConkey).

The second-step procedures involve adding 2 ml of 24-h enrichment culture to 18 ml of PBS in tubes, so as to have a 10-fold dilution. Subsequent tenfold dilutions to 10⁴ are made in PBS. Immediately after this, direct plating (0.1 ml) from every dilution was carried out on selective agar medium, without alkali treatment, followed by incubation at 25°C for 24-48 h. The contents of every tube were divided into two approximately equal parts, one of which was further enriched at 25°C for 24 h, and the other at 4°C for up to 2 wk. Alkali

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treatment of the additionally enriched samples was followed by plating on selective agar medium.

The third-step procedures were similar to the second, but adding 2 ml of 48-h enrichment culture to 18 ml of PBS in tubes and direct plating on selective agar medium was performed only after alkali treatment.

**RESULTS AND DISCUSSION**

The results obtained with the TSP method are shown in Table 1. The results obtained using the so-called routine method were the same as with the TSP method, when testing frozen meat, but the former took longer. Using the TSP method, we could recover *Y. enterocolitica* in the first-step procedure after the 2, 3, and 4 h enrichments, but the best recovery occurred after the additional enrichment. It was not difficult to distinguish *Y. enterocolitica* colonies on nonselective agar medium because few other *Enterobacteriaceae* microflora remain after a longer period of frozen storage (11). Besides, after 3 h enrichment, cold-injured cells start their multiplication (8,16). There was earlier good correlation of the results between the first- and second-step procedures: it appears that the third-step procedure is not necessary when testing frozen meat only.

**TABLE 1. Recovery, by the three-step procedure (TSP), of *Y. enterocolitica* from frozen, salted, raw dried, and heated meat products.**

<table>
<thead>
<tr>
<th>Step</th>
<th>Second enrichment</th>
<th>Frozen</th>
<th>Salted</th>
<th>Dried to 60°C (perishable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>None</td>
<td>6/12²</td>
<td>4/9</td>
<td>0/22</td>
</tr>
<tr>
<td></td>
<td>25°C</td>
<td>12/12</td>
<td>9/9</td>
<td>15/22</td>
</tr>
<tr>
<td></td>
<td>4°C</td>
<td>0/22</td>
<td>0/18</td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>None</td>
<td>7/12</td>
<td>6/9</td>
<td>18/22</td>
</tr>
<tr>
<td></td>
<td>25°C</td>
<td>12/12</td>
<td>9/9</td>
<td>18/22</td>
</tr>
<tr>
<td></td>
<td>4°C</td>
<td>18/22</td>
<td>10/18</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>None</td>
<td>12/12</td>
<td>4/9</td>
<td>5/22</td>
</tr>
<tr>
<td></td>
<td>25°C</td>
<td>12/12</td>
<td>5/9</td>
<td>6/22</td>
</tr>
<tr>
<td></td>
<td>4°C</td>
<td>9/9</td>
<td>8/22</td>
<td>9/18</td>
</tr>
</tbody>
</table>

²Number positive/number tested.

For isolation of the test microorganism from salted and dried meat products, the direct plating in the first- and second-step procedures did not give consistently positive results. Direct recovery is strongly dependent on the processing stage and above all on the variety and quantity of the microflora which normally develop during the ripening of these products. The moisture content of the meat products evaluated in this study was below 45%. According to our previous investigation (10), *Y. enterocolitica* O:3 multiples slowly up to 14 d in solutions of 2% and 4% NaCl, and remains viable more than 21 d in 6% NaCl. We surmise that the extent of drying (percent water content) is the main determinant of the injury and death of the test microorganism. Therefore, better recovery of the test microorganism in raw dried meat products was obtained with the second-step procedures (Table 1). Prolongation of the enrichment at 25°C and 4°C did not give the anticipated better results. We propose that the problem in recovering *Y. enterocolitica* from such meat products is the same as that in testing raw products of animal origin. Also, the influences of the normal microflora present in such products on *Yersinia* spp. are not clear (15,21); and some of their metabolic products, salts and other, may be acting as inhibitors.

When the TSP method was applied to cooked (heated to 60°C) perishable sausages the first step procedures did not recover the test microorganism. However, the organism was recovered by the second- and the third-step procedures. When we plated directly on selective agar medium without alkali treatment (second step), we did not isolate the organism, but we did after additional enrichment at 25°C and 4°C (1 wk). At the third step we did not have the same degree of recovery as at the second step. However, we did obtain 50% positive results (third step) after enrichment, alkali treatment, and plating on selective agar medium. Additional enrichment at 25°C for 24 h, and 4°C for up to 2 wk, was not necessary because the same results were obtained after these.

Our results suggest that the TSP is superior for the following reasons:
- The enrichment medium is 0.15 M PBS, pH 7.6, which favors recovery of injured cells of *Y. enterocolitica*.
- We incubated the first step enrichment at 25°C, which is
better than 4°C because it doesn’t act as an additional stress factor for injured cells.

- We made different dilutions and plated them directly on nonselective and selective agar media, excluding alkali treatment, which may be stressful to injured cells.

- Two step-enrichment procedures at the same temperatures at 4°C also, combined with dilutions, gave better results for recovery of injured cells.

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REFERENCES


