A Research Note

Recovering Low Levels of Various Salmonella Serotypes from Deep-frozen Broiler Carcasses by Direct Enrichment

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ABSTRACT

Two hundred and forty broiler carcass halves were each inoculated with either 14 or 180 cells of Salmonella typhimurium. Each carcass half was then placed in a plastic bag, blast-frozen (-40 C) for 6 h, and stored at -23 C. After 1, 7 and 30 days of frozen storage, 80 of these samples were removed and allowed to thaw; then each carcass-half was shaken in its bag with 150 ml of added sterile water. Lactose broth was used to preenrich 40 of these rinse-fluid samples and selenite cystine broth was used for direct enrichment of the remaining 40 samples. S. typhimurium was successfully recovered from all 240 samples. Other serotypes successfully recovered by direct enrichment on similarly frozen carcass-halves stored for 30 days were Salmonella california, Salmonella derby, Salmonella heidelberg, Salmonella montevideo, Salmonella newport and Salmonella senftenberg. These data suggest that a preenrichment medium such as lactose broth may not be necessary for detection of salmonella on frozen broiler carcasses.

There have been several studies on the effect of freezing on bacterial cells. Ulrich and Halvorson (13) reported that the greatest destruction of viable cells occurred in the first 24 h of freezing. Van Eseltine et al. (15) observed that rapid freezing or freezing at temperatures below -24 C did not result in a reduction of bacterial numbers. According to Thomason et al. (12), preenrichment in lactose broth as suggested by North (8) has become the method for isolating salmonellae from dried and frozen foods in the United States. Silliker and Gabis (10) preenriched frozen raw meat samples in lactose broth for detection of salmonellae. The U.S. Department of Agriculture in the APHIS Microbiology Laboratory Guidebook (14) recommends that lactose broth be used for preenriching raw meat and products that have been subjected to freezing, drying or chemical treatment. Recently, it was suggested that buffered peptone water be used for preenrichment of sublethally injured salmonellae in frozen meat samples (3). We could find no evidence in the literature, however, to indicate that use of a preenrichment medium for detecting salmonellae in frozen poultry is actually necessary. A number of researchers working with unfrozen broiler carcasses have included the preenrichment step (2,4,5), perhaps in the belief that the bacteria on the carcass were subjected to considerable stress during commercial processing. In a recent study (1) with unfrozen broiler carcasses, however, we found that preenrichment with lactose broth did not increase the number of salmonella-positive samples when compared to direct enrichment with selenite cystine broth. This study was undertaken to determine if enrichment with selenite cystine, without a preenrichment step, could also be used for recovery of salmonella from broiler carcasses that had been blast frozen and stored at -23 C for up to 30 days.

MATERIALS AND METHODS

Experiment 1

Each of 120 freshly processed broiler carcasses was cut in half along the longitudinal axis. The carcass-halves in one group were each inoculated with a low level (about 14 cells) and those in the second group with a high level (about 180 cells) of a strain of Salmonella typhimurium resistant to 100 ppm of nalidixic acid. The inoculum (0.1 ml) was pipetted onto each carcass-half and thoroughly rubbed into the skin using a sterile bent glass rod. After inoculation, each carcass-half was placed in a plastic bag that was closed with a twist tie, then blast frozen (-40 C) for 6 h, and stored at -23 C. After 1, 7 and 30 days, 80 of the 240 halves were removed from storage and allowed to thaw completely; then each was vigorously shaken for 1 min in its bag with 150 ml of added sterile water. Concentrated (100) lactose broth (Difco) was added to 40 of these rinse fluid samples to yield a single-strength preenrichment medium. Concentrated (100) selenite cystine broth (Difco) was added to the remaining 40 samples to yield a single-strength direct enrichment medium. All 80 samples were then incubated for 24 h at 37 C. From the lactose samples, 10-ml portions were each aseptically transferred to 90 ml of selenite cystine broth and incubated for 24 h at 37 C. Each of the 80 samples incubated for 24 h at 37 C in selenite cystine were then streaked onto MacConkey agar (Difco) plates containing 100 ppm of nalidixic acid. The plates were also incubated for 24 h at 37 C. Colonies appearing on these plates were picked and serologically confirmed to be our marker organism.

Experiment 2

After observing the results of Experiment 1, we conducted a second experiment in which low levels (about 16-42 cells) of six additional strains of salmonellae resistant to nalidixic acid (Salmonella california, Salmonella derby, Salmonella heidelberg, Salmonella montevideo, Salmonella newport, and Salmonella senftenberg) were each inoculated onto 40 broiler carcass-halves. These 240 (6 strains x 40 carcasses) halves were each placed in a plastic bag, blast frozen (-40 C) for 6 h, and stored at -23 C for 30 days. Following this, they were removed, thawed, and sampled using the identical procedure described in Experiment 1.

RESULTS AND DISCUSSION

In Experiment 1, we recovered the inoculated S.
from all 240 broiler carcass halves regardless of inoculum level, length of frozen storage and/or medium used. The 24 h enrichment with selenite cystine was as effective as the 48-h preenrichment (lactose)-enrichment procedure in detecting the marker organism. Both methods resulted in 100% detection (20 positive recoveries from 20 samples).

For the second experiment we used only an inoculum level of approximately 30 cells because a number of published reports have indicated that levels of salmonellae on salmonella-positive carcasses are extremely low, e.g., 1 to 30/carcass (11), an average of 17/100 g of skin (7), and less than 100/100 g of skin (6). With S. california, S. derby, S. heidelberg, S. montevideo, S. newport and S. senftenberg, the single enrichment and the preenrichment followed by enrichment methods yielded the same number of positive detections (Table 1).

The results of this study strongly suggest that a preenrichment, or recovery, medium such as lactose may not be necessary for detecting the presence of low levels of salmonellae on frozen broiler carcasses. Under our experimental conditions, the stress of freezing and thawing, frozen storage, or a combination of these, was apparently not severe enough to warrant preenrichment. Ray and Speck (9) showed greater recovery of the nonlethally injured cells of frozen S. anatum NF3 with selenite cystine broth than with tetraphionate broth. Our use of selenite cystine broth in this study may partly explain our success in recovering salmonellae from nonlethally injured cells of frozen foods for many years. We feel that the results of this study warrant the further investigation of direct enrichment procedures for recovering salmonellae from broiler carcasses stored for long periods, as well as from other frozen foods. Since the extent to which a cell will be damaged by freezing can be affected by the physiological state of the cell as well as by the surrounding environment, direct enrichment for recovering naturally occurring salmonellae on frozen broiler carcasses should be investigated.

TABLE 1. Recovery of six Salmonella serotypes from frozen broiler carcasses after 30 days of storage (23°C) with lactose and selenite cystine broth.

<table>
<thead>
<tr>
<th>Salmonella serotype</th>
<th>Inoculum level (No. of cells)</th>
<th>Medium used†</th>
<th>Medium used†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lactose</td>
<td>Selenite cystine</td>
</tr>
<tr>
<td>S. california</td>
<td>16</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>S. derby</td>
<td>42</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>S. heidelberg</td>
<td>32</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>S. montevideo</td>
<td>24</td>
<td>20/20</td>
<td>19/20</td>
</tr>
<tr>
<td>S. newport</td>
<td>32</td>
<td>20/20</td>
<td>20/20</td>
</tr>
<tr>
<td>S. senftenberg</td>
<td>38</td>
<td>19/20</td>
<td>20/20</td>
</tr>
</tbody>
</table>

†Number of positive recoveries/number of samples.

REFERENCES