Transfer Volume-Dependent Recovery of Salmonella from Minced Meat

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ABSTRACT

Six hundred 25-g samples of ground beef were divided into 3 groups of 200 each and 1 drop of a Salmonella broth culture was added to each sample. After storage at -27°C for 3-4 months, the samples were defrosted and blended with 225 ml of buffered peptone water. Ten ml of each suspension was preenriched at 37°C for 20 h and 10-fold dilutions of the material were made. One ml each of the preenriched culture and dilutions of \(10^{-2}\), \(10^{-4}\), \(10^{-6}\), and \(10^{-8}\) were transferred to selective enrichment media, and subsequently streaked onto selective agar plates. The mean percentage of Salmonella-positives obtained from all combinations of the selective media in relation to undiluted preenriched material and its \(10^{-2}\), \(10^{-4}\), \(10^{-6}\), and \(10^{-8}\) dilutions were for Salmonella typhimurium 70, 81, 84, 37, and 2, for Salmonella choleraesuis 64, 78, 66, 30, and 2, and for Salmonella anatum 60, 84, 75, 40, and 1, respectively. Colonies originating from diluted samples, particularly \(10^{-4}\) and further dilutions, usually represented pure cultures of salmonellae, but from undiluted material were frequently accompanied or outgrown by concomitant bacteria.

Salmonellae that have been subjected to an environmental shock, such as low or high temperature, reduced water activity, low pH or exposure to chemical preservatives, usually suffer cellular damage and may not grow in selective enrichment media. In general, this damage is reversible in a non-selective preenrichment medium, and following recovery the bacteria may show the same properties as before, including pathogenicity.

According to the International Standard Organization (ISO) method (15) for detection of salmonellae in meat and meat products, samples should be preenriched in buffered peptone water (BPW) at 37°C for 16-20 h before selective enrichment. Many other preenrichment media and incubation conditions have also been proposed (1-4, 6-8, 11, 13, 17, 18, 21). Little is known, however, about the amount of preenriched culture that should be transferred into selective enrichment medium for successful isolation of salmonellae.

D’Aoust (5) noted that transfer volume-dependent recovery was observed only with short (6 h) preenrichment incubation periods; at 24 h of incubation, 10-ml and 1-ml transfer volumes were equally sensitive. It appears also from results obtained by Price et al. (19) that transfer volumes greater than 1 ml do not increase method sensitivity.

Preliminary work in our laboratory suggested increased method sensitivity at reduced transfer volumes. The present study was undertaken to elucidate the interplay between inoculation of enrichment broths with diluted suspension of preenrichment culture and detection of Salmonella in frozen minced meat.

MATERIALS AND METHODS

Salmonella strains

Lyophilized strains of Salmonella typhimurium No. 1110, Salmonella choleraesuis No. 1236, and Salmonella anatum No. 680 were obtained from the Department of Microbiology, Veterinary Institute, Pulawy, Poland. Tubes of nutrient broth were inoculated with each strain and incubated at 37°C for 18 h.

Meat samples

Ground beef was purchased from a meat-producing plant in Olsztyn, Poland and divided into six hundred 25-g samples. One drop of a Salmonella broth culture was added to each sample, and the contents were mixed with a spatula. The samples were then stored in a freezer at -27°C for 3-4 months.

Salmonella assays

Each sample was thawed at room temperature for 2 h and blended with 225 ml of BPW (15). After 15 min, 10 ml of suspension was transferred into a tube which was subsequently incubated at 37°C for 20 h. From this preenriched culture, 10-fold dilutions were made with BPW and 1 ml each of the culture and its \(10^{-2}\), \(10^{-4}\), \(10^{-6}\), and \(10^{-8}\) dilutions were transferred to 10 ml of tetrahydroionate broth (TB) and selenite cystine broth (SC). After incubation for 24 h at 43°C (TB) or 37°C (SC),
the preenrichments were streaked onto brilliant green agar (BG) and Salmonella-Shigella agar (SS) plates. The plates were incubated at 37°C for 24 h. When the plates were examined, notations were made of the total number of well-isolated colonies, as well as the number of colonies that were "suspect" Salmonella. From the plates on which five or more suspect colonies were found, five colonies were subcultured into triple sugar iron (TSI) agar and incubated at 37°C for 24 h. Slide agglutination tests, using polyvalent H serum, group sera for somatic antigens, and sera for individual antigens were made on the material grown on TSI. All media except BPW were purchased from Difco. BPW was made of peptone produced by the Polish Factory of Sera, Vaccines and Media in Warsaw, and the sera were obtained from the Polish Salmonella Center in Gdynia.

RESULTS AND DISCUSSION

It is evident that recovery of salmonellae under all analytical conditions increased upon inoculation of enrichment media with diluted preenrichment culture (Table 1). The 10^-2 dilutions proved particularly effective with S. typhimurium whereas 10^-2 dilutions of preenrichment cultures were equally productive with the remaining strains. Dilution of 10^-3, unfortunately, was not included in the study but it is probable that a thousand-fold dilution of preenriched material would be the most satisfactory for recovery of all Salmonella types examined. Our more recent investigations based on a limited number of samples seem to confirm this hypothesis.

It should be stressed that in the course of these investigations, colonies that appeared on BG and SS plates originating from diluted material, particularly the 10^-2 and further dilutions, usually represented pure cultures of Salmonella. On the contrary, those from undiluted samples were very frequently accompanied by or outgrown by colonies of concomitant bacteria.

According to Vassiliadis (21), when dealing with meat products, the Rappaport-Vassiliadis medium (R-V) in 10-ml volume inoculated with 0.1 ml of the preenrichment medium, proved to be as effective as the R-V medium in 100-ml volume, inoculated with 1 ml of preenrichment medium, and clearly superior to the Muller-Kauffmann tetraphionate broth in 100-ml quantity.

D'Aoust (5) pointed out that, at 24 h of preenrichment, 10-ml and 1-ml volumes were equally sensitive, each giving 153 positive results. With 0.1-ml transfer volumes, 151 Salmonella isolations were obtained from the same number of samples examined. BPW, however, was not included in the above investigations.

Dilution of enrichment cultures before streaking on selective plates has also improved method sensitivity. Unpublished data from our laboratory showed that, frequently, better isolation of salmonellae may be obtained from heavily contaminated food samples containing a small number of salmonellae if, after incubation, the selective enrichment broth is diluted 1:100 or 1:1000 and these dilutions are streaked on plating media. Some authors also reported that subculture of the incubated enrichment broth into a second tube of the same medium, a secondary enrichment, may increase the yield of salmonellae from heavily contaminated samples (9,10,14).

In their examination of egg products, Montford and Thatcher (16) found that 10^-3 dilutions of SC broth cultures facilitated detection of salmonellae. They further

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**TABLE 1. Influence of diluting preenriched material on isolating salmonellae from frozen ground meat.**

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Liquid media</th>
<th>Selective mediaa</th>
<th>S. typhimurium</th>
<th>S. choleraeuis</th>
<th>S. anatum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB BG</td>
<td>No. of isolations</td>
<td>% Positive</td>
<td>Mean % Positive</td>
<td>No. of isolations</td>
</tr>
<tr>
<td>Material</td>
<td>TB SC</td>
<td>139 69</td>
<td>70</td>
<td>129 64 64</td>
<td>144 57 60</td>
</tr>
<tr>
<td>undiluted</td>
<td>TB SC</td>
<td>133 66</td>
<td>124 62</td>
<td>121 60</td>
<td></td>
</tr>
<tr>
<td>10^-2</td>
<td>TB SC</td>
<td>143 71</td>
<td>133 66</td>
<td>120 60</td>
<td></td>
</tr>
<tr>
<td>10^-4</td>
<td>TB SC</td>
<td>163 81</td>
<td>154 77 78</td>
<td>160 80 84</td>
<td></td>
</tr>
<tr>
<td>10^-6</td>
<td>TB SC</td>
<td>155 77</td>
<td>155 77</td>
<td>167 83</td>
<td></td>
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<tr>
<td>10^-8</td>
<td>TB SC</td>
<td>166 83</td>
<td>152 76</td>
<td>167 83</td>
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<tr>
<td></td>
<td>SC TB</td>
<td>166 83</td>
<td>160 80</td>
<td>175 87</td>
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<td>SC SS</td>
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<td>SC SS</td>
<td>165 82</td>
<td>131 65</td>
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<td>68 34 30</td>
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<td>SC SS</td>
<td>169 34</td>
<td>56 28</td>
<td>76 38</td>
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<td>57 28 81</td>
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<td>SC SS</td>
<td>171 39</td>
<td>57 28 81</td>
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*a TB, Tetrathionate broth; SC, selenite cystine broth; BG, brilliant green agar; SS, Salmonella-Shigella agar.*

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suggested that $10^{-2}$ to $10^{-3}$ dilutions of foods with high background flora should be used routinely.

The investigations presented here and results obtained by some other authors indicate that diluting preenriched or selectively enriched material may increase the number of Salmonella isolations from certain food samples. Our current experiments suggest that better results may also be obtained when 1 loopful instead of 1 ml of preenriched frozen meat in BPW is transferred into 10 ml of selective enrichment media. It should be stressed, however, that meat samples tested in these experiments were artificially contaminated and the diversity of Salmonella serotypes was limited. Therefore, more research is needed on the transfer volume-dependent recovery of Salmonella from various foods.

REFERENCES


