Isolation of Salmonellae from Food Samples

IV. Comparison of Methods of Enrichment

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ABSTRACT

TAYLOR, WELTON I. (Swift and Company, Chicago), AND JOHN H. SILLIKER. Isolation of salmonellae from food samples. IV. Comparison of methods of enrichment. Appl. Microbiol. 9:484–486. 1961.—A comparison of various methods of enhancing frequency of Salmonella isolations revealed that inoculation of a second enrichment broth, with culture from the first, was no improvement over the single direct enrichment method. It was inferior to centrifugation.

Selenite was observed to produce more positive isolations at 48 hr than at 24. No change occurred in tetra-thionate. Reconstitution of dried albumen with water produced a significant increase in isolations over direct inoculation of enrichment broth in the case of tetra-thionate but not selenite broth.

Pre-enrichment in lactose broth before inoculation of enrichment media was vastly superior to reconstitution in water for both enrichment broths. A comparison of results obtained using dulcitol, mannitol, lactose and carbohydrate-free purple broths in pre-enrichment indicated that the carbohydrate added was immaterial.

Potential solutions to the analytical problems posed by the needs for enumeration of small numbers of salmonellae from food samples have been presented in earlier publications of this series. Factors involved in the rationale governing the choice of media and both their experimental and empirical performances were discussed (Taylor, Silliker, Andrews, 1958), as were the merits of a methodology employing centrifugation (Silliker and Taylor, 1958). During the latter investigation, the inhibitory abilities of the enrichment broths were amply demonstrated. However, the possibility suggested itself that the debilitated condition of the salmonellae which have survived the rigors of the processing of eggs might make quantification impossible in inhibitory media. Several approaches for solution of the problem were considered. Would transfer of growth, from one enrichment broth to the other, aid in initiating growth for organisms static in the original broth? Would reconstitution in water add impetus to the initiation of growth? Will organisms which had not been demonstrable at 24 hr be isolated at 48 hr, perhaps, due to an extended lag phase? Would a noninhibitory medium be desirable for reconstitution of the product? In the current investigation, a variety of such tests, critically analyzed, will be presented.

MATERIALS AND METHODS

The two enrichment broths used in the experiments were cystine-selenite broth (North and Bartram, 1953) and brilliant green-tetra-thionate broth (McCullough and Byrne, 1952). Phosphate-buffered water was used as a diluent and for reconstitution (APHA, 1958). Carbohydrate pre-enrichment broths were 0.5% sugars in purple broth base. The control was purple broth base. Centrifugation was performed by the method and with the equipment described previously (Silliker and Taylor, 1958).

Inoculation of enrichment broths, pre-enrichment or water-reconstituted samples was at 37 C for 18 to 24 hr unless otherwise stated. The plating medium was brilliant green agar. Generic identification of Salmonella-suspect colonies was performed using dulcitol lactose iron agar slants (DLI) (Taylor and Silliker, 1958), and the ninhydrin test (Carlquist, 1956). Lactose-negative, dulcitol-positive, H2S-positive, ninhydrin-positive organisms were assumed to be salmonellae. Naturally not all Salmonella serotypes exhibit all of these characteristics, but many that do not, such as S. paratyphi A or S. typhosa, are not found in processed foods. The fact that pooled, blended product produces a multiplicity of serotypes in any given sample increased the odds immeasurably that biochemically atypical serotypes will not occur alone.

The following experiments were conducted with dried egg albumen known to be naturally contaminated with salmonellae; no stock cultures were used.

RESULTS AND DISCUSSION

The most probable number (MPN) method of Salmonella enumeration is based on qualitative positive isolations from quantitative inocula, the numerical value ascribed being computed from a statistical table of probabilities (Ayres, 1949). The modes of action of the two most widely used enrichment broths, selenite
and tetrathionate, are different. Neither supports growth of all salmonellae (Schneider, 1946; Banwart and Ayres, 1953). It would seem that if one sample aliquot were used, but that the first enrichment broth furnished the inoculum for the second broth, one might find a higher yield in Salmonella-positive isolations than before, because (i) coliforms or other non-salmonellae would undergo a second inhibition, thus further increasing the proportion of salmonellae in the enrichment aliquot, and (ii) the occasional Salmonella serotype inhibited by the first enrichment broth might grow well in the second. The results of experiments utilizing this principle were compared with concomitant centrifugation of the same Salmonella-contaminated egg material in Table 1. The net observable effect was that there was no statistically significant difference between dual transfer and direct inoculation and that centrifugation was far superior to both.

It has been observed by some investigators that dry egg samples inoculated directly into enrichment media seem to produce fewer salmonella isolations than reconstituted egg samples. Byrne, Rayman and Schneider (1955) reported that 2 hr of soaking and shaking of the egg as a 10% suspension in sterile distilled water facilitated salmonella recovery.

North (1961) used lactose broth for pre-enrichment and ascribed an increased frequency of salmonella isolation to its use. These investigations prompted the experiments comparing direct and pre-enrichment methods of analysis.

To reduce sampling error, which is often a factor of great magnitude when dried, naturally contaminated albumen is used, a homogeneous inoculum was prepared by making a 10% solution which was then mechanically shaken for 15 min. Replicate aliquots analyzed in four separate trials produced results as shown in Table 2. Streaking of 24-hr and 48-hr selenite aliquots revealed a significant increase in positive isolations. Tetrathionate did not. Incubation of the sample in buffered water was no improvement over direct inoculation into selenite.

**Table 1. Positive Salmonella isolations from 6 albumen samples**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dual enrichment</th>
<th>Centrifugation (Cent.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenite (S) 1/54*</td>
<td>S-S</td>
<td>2/54</td>
<td>8/54</td>
</tr>
<tr>
<td></td>
<td>S-T</td>
<td>0/54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T-S</td>
<td>12/54</td>
<td>31/54</td>
</tr>
<tr>
<td></td>
<td>T-T</td>
<td>17/54</td>
<td></td>
</tr>
<tr>
<td>Tetrathionate 13/54 (T)</td>
<td>S-S</td>
<td>2/54</td>
<td>8/54</td>
</tr>
<tr>
<td></td>
<td>S-T</td>
<td>0/54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T-S</td>
<td>12/54</td>
<td>31/54</td>
</tr>
<tr>
<td></td>
<td>T-T</td>
<td>17/54</td>
<td></td>
</tr>
</tbody>
</table>

Analysis: Chi-square, 4-fold table, Yates correction

<table>
<thead>
<tr>
<th></th>
<th>x²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S vs. S-S</td>
<td>1/54-2/54</td>
<td>0.000</td>
</tr>
<tr>
<td>S vs. Cent. S</td>
<td>1/54-8/54</td>
<td>4.34</td>
</tr>
<tr>
<td>T vs. T-T</td>
<td>13/54-17/54</td>
<td>0.41</td>
</tr>
<tr>
<td>T vs. Cent. T.</td>
<td>13/54-31/54</td>
<td>11.10</td>
</tr>
</tbody>
</table>

* Number positive/total number.

but was significantly better in tetrathionate. Incubation for 24 hr in lactose broth before tetrathionate and selenite enrichment produced the greatest number of positive salmonella recoveries, however.

Since both prolonged incubation in enrichment broths and pre-enrichment in lactose broth had shown promise, experiments were devised to determine whether greater incubation times and a wider selection of carbohydrates might improve these techniques.

Replicate samples of the same albumen produced the maximal number of salmonella-positive cultures in 72 hr although they were streaked daily for more than 1 week (Table 3). Purple sugar broth produced best results by far, and no preference for a carbohydrate was demonstrated in these trials. Tetrathionate, which has

**Table 2. Direct and pre-enrichment methods of analysis**

<table>
<thead>
<tr>
<th></th>
<th>Direct inoculation</th>
<th>Pre-enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Selenite (S)</td>
<td>Tetrathionate (T)</td>
</tr>
<tr>
<td>24 hr</td>
<td>48 hr</td>
<td>24 hr</td>
</tr>
<tr>
<td>25/50</td>
<td>36/50</td>
<td>2/50</td>
</tr>
<tr>
<td>17/40</td>
<td>13/40</td>
<td>30/40</td>
</tr>
</tbody>
</table>

Analysis: Chi-square

<table>
<thead>
<tr>
<th></th>
<th>x²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁₄ vs. S₄₄</td>
<td>25/50-36/50</td>
<td>4.2</td>
</tr>
<tr>
<td>S₂₄ vs. S₄₄ water</td>
<td>25/50-17/40</td>
<td>0.2</td>
</tr>
<tr>
<td>S₂₄ vs. S₄₄ lactose</td>
<td>25/50-30/40</td>
<td>4.8</td>
</tr>
<tr>
<td>T₂₄ vs. T₄₄ water</td>
<td>2/50-13/40</td>
<td>11.0</td>
</tr>
<tr>
<td>T₂₄ vs. T₄₄ lactose</td>
<td>2/50-36/40</td>
<td>63.9</td>
</tr>
</tbody>
</table>

**Table 3. Comparison of direct and pre-enrichment options**

<table>
<thead>
<tr>
<th></th>
<th>Selenite (S)</th>
<th>Tetrathionate (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>72</td>
<td>24</td>
</tr>
<tr>
<td>4/40</td>
<td>5/40</td>
<td>4/40</td>
</tr>
</tbody>
</table>

Purple sugar broths

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lactose</th>
<th>Mannitol</th>
<th>Dulcitol (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>T</td>
<td>S</td>
<td>T</td>
<td>24</td>
</tr>
<tr>
<td>24/40</td>
<td>39/40</td>
<td>24/40</td>
<td>32/40</td>
<td>25/40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24/40</td>
</tr>
</tbody>
</table>

Analysis: Chi square

<table>
<thead>
<tr>
<th></th>
<th>x²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁₄ vs. S₂₄</td>
<td>4/40-5/40</td>
<td>0.0</td>
</tr>
<tr>
<td>S₁₄ vs. Scontrol</td>
<td>4/40-24/40</td>
<td>19.8</td>
</tr>
<tr>
<td>S₂₄ vs. Scontrol</td>
<td>4/40-16/20</td>
<td>29.4</td>
</tr>
<tr>
<td>T₁₄ vs. T₂₄</td>
<td>4/40-6/40</td>
<td>0.1</td>
</tr>
<tr>
<td>T₁₄ vs. Tcontrol</td>
<td>4/40-39/40</td>
<td>29.1</td>
</tr>
<tr>
<td>T₂₄ vs. D₂₄</td>
<td>4/40-16/20</td>
<td>29.4</td>
</tr>
<tr>
<td>Scontrol vs. Tcontrol</td>
<td>24/40-39/40</td>
<td>14.6</td>
</tr>
</tbody>
</table>
proven more inhibitory to both salmonellae and coliforms upon direct inoculation, emerged as the enrichment medium of choice after pre-enrichment in nonselective broths. It is noteworthy that dulcitol preenrichment broth, streaked directly after 24 hr, produced more salmonellae than did the direct inoculum enrichment broths (16/20 vs. 4/20).

Both pre-enrichment in nonselective media and centrifugation are significant improvements over the direct inoculation of salmonella-containing albumen samples into tetraethionate and selenite broths. Although tetraethionate is clearly marked as the medium of choice after pre-enrichment, no such clear-cut preference may be made in direct inoculations. In direct inoculations, usually one will be superior in one experiment and the other will prove superior in the next. This is borne out in the three tables shown: In Table 1, tetraethionate is clearly the better; in Table 2, selenite is superior; in Table 3, they are equal. In the years of experience we have had with these media, we have volatilized with the latest modification of each medium. For a given sample, where replicates are analyzed using both media, some conclusions may be drawn just from the appearance of the streaked plates; from selenite, the ratio of salmonellae to coliforms is much lower so that relatively few salmonellae colonies are found per plate, suggesting that an unknown number of positive samples could be missed simply because plates having but few colonies of noncoliform appearance may be overlooked by technic-ians (Taylor, 1958), or that the ratio is such that all colonies are coliforms, although there may be salmonellae in the broth aliquot. Tetraethionate, conversely, is characterized by positive plates almost totally comprised of salmonella colonies, but, since tetraethionate is obviously more inhibitory to all organisms, one feels that, when a sample has but few salmonellae in it, "no growth" plates may frequently occur. Our conclusion was that accuracy was best obtained with duplicate samples using both media; use of either alone must be considered a calculated risk.

LITERATURE CITED


Schneider, M. D. 1946. Investigation of Salmonella content of powdered whole egg with not more than two percent moisture content. II. General survey on occurrence of species of Salmonella in high-quality egg powder. Food Research 11:313-318.


