NOTES

Application of the Rapid Lysine Decarboxylase Test for Early Isolation and Detection of Salmonellae in Sewage and Other Wastewaters

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A method for early isolation and detection of salmonellae in sewage and other wastewaters by using the rapid lysine decarboxylase test as a single biochemical reaction for screening the suspected Salmonella colonies is described. By this method, Salmonella isolation and identification can be completed within 2 to 3 days in contrast to the 5 to 7 days required for the conventional method.

In recent years emphasis has been placed on the presence or absence of salmonellae in sewage and its treated effluents in monitoring domestic waste treatment plants. In view of this, efforts are being made to develop simple and rapid methods for isolating, identifying, and enumerating these bacteria. In conventional methods, where enrichment procedures followed by streaking on selective differential media are involved, the time required to complete the whole analysis may range from 5 to 7 days (10, 15). The procedure adopted for confirming suspected Salmonella colonies that develop on selective media are quite cumbersome and time consuming. Each colony must be subjected to an array of various biochemical reactions before it is serologically confirmed, requiring 2 to 3 days. Development of an elevated-temperature technique (13–15) has, no doubt, improved the salmonella recovery rate from polluted waters; however, the time required to complete the test must be shortened.

Since, there is little chance of reducing the time necessary for enrichment and streaking on selective media, the solution would be to identify the Salmonella colonies more rapidly.

Several test schemes have been advocated for identifying members of the Enterobacteriaceae (17, 18), one of which is the lysine decarboxylase (LD) test (3, 5, 7, 11, 12). In the method of Edwards and Fife (4), LD activity and production of hydrogen sulfide by salmonellae are the only two criteria taken into consideration. Similarly, in Taylor’s dulcitol-lysine-lactose-iron agar method (16), the identity of salmonellae is established through dulcitol fermentation, hydrogen sulfide production, and lysine utilization. Huhtanen and Naghsik (9) also recommended the use of lysine-iron agar slants for identifying suspected Salmonella colonies developed on brilliant green agar (BGA) plates, a method that relies heavily on the LD reaction. They considered that all LD-negative cultures were also negative for Salmonella, and the remainder, either with or without an H2S reaction, were further confirmed with somatic antisera. Recently, Brooker et al. (2) have developed a very simple and rapid test for detecting LD activity in Enterobacteriaceae. With this test, results can be obtained within 1 to 4 h. The simplicity, specificity, and rapidity of this test have prompted me to use it for the early detection of Salmonella.

Thirty-nine wastewater samples collected on different days were used for Salmonella isolations. Samples of raw sewage, settled sewage, and its treated effluent were collected from the Institute’s wastewater stabilization pond. Wastewater was also collected from the local slaughterhouse and was fed into a laboratory-scale model of an anaerobic contact filter for treatment. Samples of both the raw slaughterhouse wastewater and the effluents from the contact filter were analyzed.

The samples were mixed well by vigorous shaking. Volumes of 1 ml were inoculated into tubes containing 10 ml of Kauffman tetraphionate broth, and volumes of 10 or more ml were inoculated into tubes or flasks containing equal volumes of double-strength medium. All of the inoculated tubes and flasks were kept in a water bath at 44°C for few minutes to raise the temperature of the contents and were then
transferred to a dry-air, water-jacketed incubator maintained at 41.5°C for 24 or 48 h. After 24 h of enrichment, a loopful of the culture from each tube/flask was streaked on BGA plates. The inoculated BGA plates were then incubated at 37°C for 18 h. A few suspicious colonies showing a pink or magenta hue on BGA plates were picked and inoculated into tubes containing 1 ml of lysine broth (2). While picking the colony, care was taken to avoid contamination. The whole colony was scooped to get sufficient inoculum to develop desired results within 1 to 4 h. With the same loop, nutrient agar (with 0.3% yeast extract) slope was inoculated. A 1-ml amount of sterile 0.5% plain agar, melted and cooled to 45 to 50°C, was added carefully to each tube to overlay the inoculated lysine broth. All of the tubes were then incubated in a dry-air, water-jacketed incubator at 37°C. The LD test was read at 1-, 2-, 3-, and 4-h intervals. Development of a green or blue color was considered to be positive indication of LD activity, whereas no change in the original straw-yellow color of the lysine broth was recorded as negative.

On the nutrient agar slope, visible growth appeared within 4 to 6 h. This was used for slide agglutination test. LD-negative cultures were considered negative for *Salmonella* (9). The remainder were further confirmed by slide agglutination, using *Salmonella* poly O antiserum (Haffkine Biopharmaceutical Corporation Limited, Parel, Bombay, India) (1, 3, 6, 9).

The tetrothionate tubes that were negative for *Salmonella* colonies after 24 h of enrichment were incubated for another 24 h, and then cultures from these tubes were streaked onto BGA plates. Suspected colonies were picked and tested for *Salmonella* as described above.

In the initial stages, to develop confidence in the rapid LD test as a single biochemical reaction for screening *Salmonella* colonies developed on BGA plates, the modified Kohn method (8) was used in parallel. In all, 205 colonies isolated from six raw sewage and six settled-sewage samples were screened by both methods. The results revealed that, of 205 colonies tested, 129 showed typical biochemical reactions for *Salmonella* on Kohn medium. All 129 colonies were positive for LD activity within 2 to 4 h and gave a strong positive slide agglutination test with *Salmonella* poly O antiserum within 30 s, using growth from the slopes of composite medium no. 1 of the Kohn method, thereby confirming all cultures as *Salmonella*. The remaining 76 colonies showing negative biochemical reactions for *Salmonella* with the Kohn method were found to be negative for LD activity.

To further substantiate the reliability of this single test for screening salmonellae, more colonies were picked and analyzed. The results are given in Table 1, which includes data for colonies isolated after 24 and 48 h of enrichment in tetrahionate broth. Of 796 suspected *Salmonella* colonies isolated from BGA plates from 39 different wastewater samples, 645 (81%) were found to be positive for LD activity within 2 to 4 h. All 645 colonies were confirmed serologically as *Salmonella* by the slide agglutination test, using 6-h growth obtained on nutrient agar slopes. The remaining 151 colonies did not give any positive indication of LD activity within 4 h and, hence, were considered negative for *Salmonella*. These data prove beyond a doubt the reliability of this single biochemical test for screening suspected *Salmonella* colonies developed on BGA plates. Huhtanen and Naghsli (9) observed that 75% of the suspected *Salmonella* colonies on BGA plates had LD activity and that, of those, 94% proved to be *Salmonella*. The probability of a lactose-negative BGA colony being composed of *Salmonella* would, according to their findings, be approximately 0.70, this being the product of the probability that a colony would be LD positive (0.75) times the probability that the LD-positive cultures would be *Salmonella* (0.94). Our results confirm their findings.

It is evident from these studies that the rapid LD test of Brooker et al. (2) can be conveniently incorporated into existing *Salmonella* isolation techniques as a single biochemical reaction for

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. samples analyzed</th>
<th>No. of suspected Salmonella colonies tested</th>
<th>LD test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw sewage</td>
<td>6</td>
<td>175</td>
<td>118</td>
</tr>
<tr>
<td>Settled sewage</td>
<td>6</td>
<td>141</td>
<td>93</td>
</tr>
<tr>
<td>Effluent from sewage stabilization pond</td>
<td>4</td>
<td>58</td>
<td>54</td>
</tr>
<tr>
<td>Raw slaughterhouse wastewater</td>
<td>14</td>
<td>303</td>
<td>282</td>
</tr>
<tr>
<td>Treated slaughterhouse wastewater</td>
<td>9</td>
<td>119</td>
<td>98</td>
</tr>
</tbody>
</table>

* Confirmed as *Salmonella* by slide agglutination, using *Salmonella* poly O antiserum.
screening suspected *Salmonella* colonies developed on selective media, with an added advantage of early detection, thus reducing to a great extent the total time required to complete the analysis of the samples. This method will be of great help to persons working in wastewater treatment plants, particularly to those interested in studying the performance of their treatment processes in the reduction of salmonellae. Similarly, this technique may be of great value in clinical laboratories where large numbers of stool samples from gastroenteritis cases have to be examined for *Salmonella*. With this rapid and simplified method, a large number of samples can be handled at the same time, and results can be obtained within as short a period as 48 h for confirming the diagnosis.

It should be noted, however, that with this method only the presence or absence of *Salmonella* can be determined. Further identification of the isolates would require the use of biochemical and serological tests, including specific somatic and flagellar antisera.

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**LITERATURE CITED**


