Temperature Range for Growth of *Escherichia coli* Serotype O157:H7 and Selected Coliforms in *E. coli* Medium

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*Escherichia coli* serotype O157:H7 and five other fecal and nonfecal coliforms were tested for minimum and maximum temperatures for growth in *E. coli* medium by using a temperature gradient incubator with a mean temperature increment of 1.67°C (±0.392). The temperature range for growth of *E. coli* O157:H7 is inconsistent with that of other fecal coliforms, suggesting that this pathogen is excluded with standard enumeration procedures used for foods and water.

*Escherichia coli* is recommended as an indicator of fecal contamination in water and foods (15). The role of enteropathogenic strains of this organism in causing infectious diarrheal diseases in infants and children is well established (4, 5, 8, 10, 11, 18). The enterohemorrhagic *E. coli* serotype O157:H7 has been given a considerable amount of attention recently because of its implication in sporadic cases and outbreaks of hemorrhagic colitis (9, 12, 13, 14) and hemolytic uremic syndrome (2, 7). Various meat products have been implicated with outbreaks of these diseases (3, 12, 16), and indications are that the incidence of this organism in meats is not a rare occurrence (3). This serotype of *E. coli* exhibits characteristics typatypical of the *E. coli* group of organisms such as the inability to ferment sorbitol (17), the absence of β-D-glucoronidase activity (9), and the inability to grow at temperatures normally used in most probable number (MPN) procedures for enumerating fecal coliforms.

This study was initiated to measure the temperature range for growth of O157:H7 in conjunction with other fecal and nonfecal coliforms. The normal procedure for the enumeration of fecal coliforms in water and foods requires incubation at 44.5°C in *E. coli* medium (EC medium) (Difco Laboratories) after screening for lactose fermentation and gas production in lauryl sulfate tryptose broth (Difco) at 35°C. Growth with gas production within 48 h in EC medium at 44.5°C is presumptive for the presence of fecal coliforms. Confirmation for the presence of *E. coli* is done with eosin methylene blue agar and the IMViC tests (1). This study describes the temperature range for growth of selected members of the coliform group as indicated by gas production and turbidity in EC medium.

The bacteria used in this study were *E. coli* serotype O157:H7 isolated from human stool, *E. coli* ATCC 11775 (kindly provided by Russell Herwig, University of Washington), *E. coli* biotype I (IMViC reaction, + + + +) isolated in our laboratory from a clam, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and another nonfecal coliform isolated in our laboratory from a scallop. Each culture was grown to log phase in lauryl sulfate tryptose medium and 0.1 ml of a dilution (10⁶ cells per ml) was used to inoculate 10 ml of EC medium with each tube containing an inverted gas vial. The tubes were incubated in a temperature gradient block incubator capable of incubating 12 cultures, each at 32 different temperatures (11). The incubator was set at a temperature range of 0 to 50°C, and the temperatures were read at zero time and after 24 and 48 h of incubation. The mean increment between tubes was 1.67°C ± 0.392. A constant gradient was obtained over the set temperature range as shown in the regression line plot (Fig. 1). The minimum and maximum temperatures for turbidity with gas production were recorded at 24, 36, and 48 h of incubation (Table 1).

For *E. coli* O157:H7, after 24 h of incubation, the temperature range for turbidity with gas was 24.3 to 41.0°C. After 36 h, the minimum temperature changed to 19.3°C with no change in the maximum. At the end of 48 h, there was no further change; however, turbidity only (no gas) was evident at a low temperature of 16.4°C and at a high of 42.5°C. For *E. coli* ATCC 11775, the minimum temperature for turbidity with gas after 24 h was 29.2°C. With extended incubation, this temperature decreased to 26 and 22.7°C at 36 and 48 h, respectively. The maximum temperature of 44.5°C was obtained after 24 h for this typical member of the fecal coliforms.

For *E. coli* biotype I, which has the IMViC reaction + + + +, typical of type I *E. coli*, showed a minimum temperature of 32.8°C for turbidity and gas production at 24 h. At 36 and 48 h, the temperature decreased to 31.0°C and 29.2°C, respectively. The maximum growth temperatures obtained were also typical of fecal coliforms, with 44.5°C after 24 and 36 h and an increase to 46°C after 48 h. This organism would be detected by MPN procedures used for fecal coliforms in water and foods (44.5°C) and shellfish (45.5°C) as used by the U.S. Food and Drug Administration (6).

**TABLE 1. Temperature ranges for growth of coliform bacteria in EC medium at three incubation times**

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Temp (°C) range for growtha after incubation in EC medium for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
</tr>
<tr>
<td>O157:H7</td>
<td>24.3-41.0</td>
</tr>
<tr>
<td>ATCC 11775</td>
<td>29.2-44.5</td>
</tr>
<tr>
<td>Biotype I (from clam)</td>
<td>32.8-44.3</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>26.0-41.0</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>27.6-41.0</td>
</tr>
<tr>
<td>Nonfecal coliform (from scallop)</td>
<td>32.8-42.5</td>
</tr>
</tbody>
</table>

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a Growth is indicated by turbidity with gas production.
For *Klebsiella pneumoniae*, after 24 h of incubation, the range for growth was 26 to 41°C. At 36 and 48 h, the minimum temperature decreased to 22.7°C whereas the maximum remained unchanged. Turbidity without gas was present at a low temperature of 17.5°C at 48 h.

The minimum temperatures for turbidity with gas for *Enterobacter aerogenes* ATCC 13048, a member of the coliform group, were 27.6, 26, and 24.3°C after 24, 36, and 48 hours of incubation, respectively. A maximum temperature of 41°C was obtained in 24 h and remained unchanged with further incubation.

For the organism isolated from scallop and classified as a nonfecal coliform after screening by Food and Drug Administration procedures (6), the minimum temperatures for turbidity and gas were 32.8, 31, and 27.7°C after 24, 36, and 48 hours of incubation, respectively. A maximum temperature of 42.5°C was reached in 24 h and remained unchanged with further incubation.

These data show that *E. coli* O157:H7 does not grow at temperatures typically used in MPN procedures for the enumeration of *E. coli* and other fecal coliforms. Instead, the organism grows well in the temperature range characteristic of nonfecal coliforms such as *K. pneumoniae* and *E. aerogenes*. Thus, it is possible that this pathogenic *E. coli* is omitted in the normal screening for fecal coliforms by standard procedures with incubation at 44.5°C. Therefore, the classical definition of fecal coliforms with growth at 44.5°C does not fit *E. coli* serotype O157:H7.

**LITERATURE CITED**


