Differentiation of Thermophilic Campylobacter by Colony Characteristics on MacConkey Agar Base (Difco)

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Thermophilic Campylobacter, Campylobacter jejuni (C. jejuni), Campylobacter coli (C. coli), and Campylobacter laridis (C. laridis), are recognized as major causes of bacterial diarrhea in humans and other animals [2, 8]. There are very few biochemical tests to differentiate the 3 species [1, 3]. C. jejuni can usually be distinguished from C. coli and C. laridis by the ability to hydrolyze hippurate. C. laridis is conspicuously different from C. coli in nalidixic acid resistance [1, 3]. Recently, however, hippurate-negative C. jejuni and nalidixic acid-resistant C. jejuni and C. coli have occasionally been found [7, 9], suggesting that these properties are not always reliable for identification purposes.

The aim of this study was to investigate simple differential characteristics among the thermophilic Campylobacter. The results indicate that C. laridis is clearly distinguished from C. jejuni and C. coli by colony characteristics in color and transparency on MacConkey Agar Base (Difco) plates.

A total of 143 strains were examined in the present study. They consisted of 65 C. jejuni, 50 C. coli, and 28 C. laridis strains isolated from cats [6], chickens, dogs, seagulls [5], and pigs, including 3 respective type strains obtained from Japan Collection of Microorganisms (JCM), Wako, Saitama, Japan. Among the seagull strains, 3 of C. jejuni, 2 of C. coli, and 5 of C. laridis were previously identified by deoxyribonucleic acid (DNA)-DNA hybridization test [5]. The fresh isolates from chickens, dogs, and pigs were identified by the methods of Benjamin \textit{et al.} [1].

MacConkey Agar (Difco and Eiken), MacConkey Agar Base (Difco), SS Agar (Difco and Nissui), and DHL Agar (Nissui) media were commercially obtained. In addition, MacConkey agar base was also self-prepared by using Bacto Peptone, Proteose Peptone (Difco), Bacto Bile Salts No. 3, sodium chloride, Bacto Agar, neutral red (Sigma), and crystal violet (Merck) to examine the effects of components and pH on

\textbf{Fig. 1.} Colonies of C. jejuni, C. coli, and C. laridis on MacConkey Agar Base (Difco) plates: left, with black background; right, with light background. Colonies of C. jejuni, C. laridis, and C. coli were arranged in upper two, middle two, and lower two rows, respectively. The respective type strains, C. jejuni JCM 2013\textsuperscript{3}, C. laridis JCM 2530\textsuperscript{3}, and C. coli JCM 2529\textsuperscript{3}, were situated at the left end of the first row of each species.
Table 1. Effects of concentrations of 4 components and pH of MacConkey Agar Base (Difco) on growth and colony characteristics of the thermophilic Campylobacter

<table>
<thead>
<tr>
<th>Bile Salts No. 3</th>
<th>Sodium chloride (g/l)</th>
<th>Neutral red (mg/l)</th>
<th>Crystal violet (mg/l)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0**&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>0.5&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;*&lt;/sup&gt;</td>
<td>6.0</td>
<td>0.2&lt;sup&gt;*&lt;/sup&gt;</td>
<td>6.2&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>5.0&lt;sup&gt;h&lt;/sup&gt;</td>
<td>12.0</td>
<td>0.4&lt;sup&gt;*&lt;/sup&gt;</td>
<td>6.5</td>
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<tr>
<td>1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.5</td>
<td>18.0</td>
<td>0.8&lt;sup&gt;*&lt;/sup&gt;</td>
<td>6.7</td>
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<tr>
<td>2.0</td>
<td>10.0**</td>
<td>24.0</td>
<td>1.0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5</td>
<td>12.5**</td>
<td>30.0&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.2</td>
<td>7.2</td>
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<tr>
<td>3.0</td>
<td>15.0**</td>
<td>36.0</td>
<td>1.4</td>
<td>7.5&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
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<td>42.0</td>
<td>1.6</td>
<td>7.7&lt;sup&gt;*&lt;/sup&gt;</td>
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<td>48.0</td>
<td>1.8</td>
<td>8.0&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>4.5&lt;sup&gt;**&lt;/sup&gt;</td>
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<td>2.0</td>
<td>8.2</td>
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</table>

a) Concentrations of each component or pH were changed individually with the remainders unchanged to the originals.

b) *, Concentrations or pH which made all strains grow well but did not allow clear distinction between colonies of C. larisidis and those of the other thermophilic campylobacters.

c) **, Concentrations at which degrees of growth varied from strain to strain, that is, well growth to no growth.

d) Underlines indicate the concentrations or pH at which the colonies of C. larisidis were clearly distinguishable from those of the other thermophilic campylobacters.

e) Original concentration or pH of MacConkey Agar Base (Difco).

O2-CO2-N2 mixture (5:10:85) for 2 days. Growth degrees and colony characteristics were examined for all strains.

All strains grew well on MacConkey Agar (Difco and Eiken) and commercial and self-prepared MacConkey agar base, but none on SS Agar (Difco), 3 strains (2 of C. coli and 1 of C. larisidis) on SS Agar (Nissui), and 8 strains (1 of C. jejuni, 5 of C. coli, and 2 of C. larisidis) on DHL Agar. On the other hand, Itoh et al. [4] reported that 10 to 30% of C. jejuni and C. coli strains, 18.8% of C. coli strains, and 72% of C. coli strains grew on MacConkey Agar (Eiken), SS Agar (Nissui), and DHL Agar (Nissui), respectively. It is not clear why these differences were observed between their results and ours, but the cell suspensions used as inocula in the present study may cause such differences.

Almost transparent and slightly reddish violet colonies of all C. larisidis strains on MacConkey agar base were clearly distinct from those of all C. jejuni and C. coli strains which were opaque and reddish white (Fig. 1). When the concentration of components or pH of self-prepared MacConkey agar base were altered individually, all strains could grow well and colonies of all C. larisidis strains were also distinct from C. jejuni and C. coli strains under conditions with Bile Salts No. 3 of 1.5 (original) to 3.0 g/l, sodium chloride of 5.0 (original) to 7.5 g/l, crystal violet of 1.0 to 2.2 (original, 1.0) mg/l, and pH 6.5 to 7.2 (original, 7.1), respectively (Table 1). At the other range of concentrations or pH, growth degrees varied with the concentrations, pH, and strains, and colonies of C. larisidis were not clearly distinguishable. Neutral red was not influential in the test range of 0 to 48 mg/l, although it gave a tint of red to the colony, which was somewhat helpful in the differentiation. When Bile Salts No. 3, sodium chloride, or crystal violet was completely omitted, no differential effect was observed. Therefore, synergistic effects of the 3 components seemed to be required.

All strains grew well on MacConkey Agar (Difco and Eiken), but the colonies of C. larisidis were not always distinguishable from those of C. jejuni and C. coli.

Contrast between the colonies was conspicuous with blackish background and appropriate illuminating angle. The contrast was not so conspicuous when the cell concentration of inocula was more than 3.5 or less than 0.1
MacFarland turbidity standard and when strains were streaked directly from colonies.

According to the results of the present study, MacConkey Agar Base (Difco) is considered to be useful as a differential medium of C. laridis from C. jejuni and C. coli by the colony characteristics in color and transparency.

REFERENCES


要約

コロニー性状による thermophilic Campylobacter の鑑別（短報）：金内長司・伊藤和夫・原沢亮11、尾形学
（麻布大学農政公衆衛生学第2講座、11宮崎大学農学部畜産微生物学講座）——Campylobacter laridis は Mac-
Conkey Agar Base (Difco) 上でほぼ透明な淡赤紫色のコロニーを形成し、不透明な淡白桃色のコロニーを形成する C. jejuni および C. coli と明瞭に区別された。