Impediometric Detection of *Campylobacter coli*

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**ABSTRACT**

The rapid automated bacterial impedance technique (RABIT) was examined as a method for the detection of two wild-type isolates of *Campylobacter coli* in broth media. Both isolates failed to produce a change in impedance that was sufficient for detection in any combination of six nonselective basal broth media, including Mueller-Hinton broth, nutrient broth no. 2, brain heart infusion broth supplemented with yeast extract (0.5% [wt/vol]), brucella broth, Campy broth supplemented with yeast extract (0.5% [wt/vol]), and Whitley impedance broth, at 37 and 42°C. Although the strains did proliferate in the media, changes in conductivity were very small (ranging from 0 to 1,000 μS) and were not significantly greater than the drift in conductance observed in the control broth medium. Additional work is therefore required to define a nonionic growth substrate that will produce charged ions upon metabolism that are detectable by RABIT.

Impedance techniques have become popular as rapid methods for the detection of bacterial pathogens (6, 27, 30) as well as a diverse variety of foodborne pathogens, including various serotypes of *Salmonella enterica* (7, 8) and *Listeria monocytogenes* (4). Since impedance techniques may have a role in the rapid screening of food products such as cooked chicken and associated foodstuffs, particularly in positive-release schemes (whereby food processors release product into the food chain only after confirmation of the absence of viable campylobacters in the product), it was the aim of this study to examine the suitability of this technique for the detection of *C. coli* with several basal broth media.

**MATERIALS AND METHODS**

**Campylobacter isolates.** Two wild-type *Campylobacter* strains, *C. coli* NI39 and *C. coli* NI43, were used in this study. These strains were isolated from freshly eviscerated porcine liver as previously described (16), and their phenotypic and genotypic characteristics are described in Table 1.

**Impedimetric detection with basal broth and with basal broth supplemented with growth supplements.** Six nonselective basal broth media, including Mueller-Hinton broth (Oxoid CM405, Oxoid Ltd., Basingstoke, UK), nutrient broth no. 2 (Oxoid CM67), brain heart infusion broth supplemented with yeast extract (0.5% [wt/vol]) (Oxoid L21), brucella broth (Gibco Life Technologies, Scotland), Whitely impedance broth (Don Whitley Scientific, UK), and Campy broth (10 g of tryptone soya broth [Oxoid CM129], 5 g of special peptone [Oxoid L72], 5 g of yeast extract [Oxoid L21], 5 g of sodium pyruvate, 0.75 g of Tris-HCl, and 1,000 ml of distilled water) (13). All media were reconstituted in accordance with the manufacturer’s instructions and were assessed for their ability to allow the growth of *C. coli* NI39 and

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TABLE 1. Characterization of porcine Campylobacter coli by biotyping, serotyping, and phage-typing methods employed in this studya

<table>
<thead>
<tr>
<th>C. coli strain</th>
<th>Characterization for biotyping scheme</th>
<th>Characterization for serotyping scheme</th>
<th>Characterization for phage-typing scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skirrow and Benjamin (24)</td>
<td>Penner and Hennessy (19)</td>
<td>Salama et al. (22)</td>
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<td></td>
<td>Roop et al. (21)</td>
<td>Lior (12)</td>
<td>Khakhria and Lior (10)</td>
</tr>
<tr>
<td>NI39</td>
<td>C. coli biovar 1</td>
<td>C. coli 2</td>
<td>UT</td>
</tr>
<tr>
<td>NI43</td>
<td>C. coli biovar 1</td>
<td>C. coli 2</td>
<td>UT</td>
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a UT, untypable; R, rough strain.

C. coli NI43 with the rapid automated bacterial impedance technique (RABIT). All impedance measurements were performed on a RABIT system (Don Whitley Scientific). Late-log-phase (17-h) cultures of C. coli NI39 and C. coli NI43 were prepared by streaking the isolates onto blood agar no. 2 and incubating them microaerophilically for 24 h at 42°C. Plates were restreaked on five further occasions to avoid sublethally stressed cells. Late-log-phase (17-h) cells were harvested from two plates by scraping the surface with an inoculation loop (Sterilin, UK), and cells were resuspended in 0.1% (wt/vol) peptone saline solution (90 ml) that had recently been steamed and allowed to cool under reduced-oxygen conditions. Quadruple 0.5-ml aliquots of a 10⁻² serial dilution were inoculated into 5 ml of each medium to give a final inoculum of approximately 5.95 log CFU/ml. Growth tubes were placed in a temperature-controlled heating block and incubated aerobically at 37 ± 0.05°C. Impedance was monitored for 24 h at 6-min intervals. A value of 12 μS was chosen as the detection criterion in all cases. If three consecutive conductance changes are equal to or greater than the detection criterion value, then the time of the fourth value (6 min later) is recognized as the time to detection. The conductance of a replicate control tube without inoculum was also monitored for each treatment to estimate electrical drift that was not attributable to bacterial activity. Also, three additional treatments were examined: growth supplements, namely, FBP supplement (iron sulphate at 0.125 g/liter, sodium pyruvate at 0.125 g/liter, and sodium metabisulphite at 0.125 g/liter), diethiothreitol (0.15 g/liter), and FBP plus diethiothreitol, were investigated in combination with each of the six broth media described. Growth supplements were added to each basal broth to enhance the oxygen-quenching ability of the medium. All experiments were repeated at 42°C and replicated twice.

RESULTS AND DISCUSSION

C. coli NI39 and C. coli NI43 failed to produce a sufficient change in impedance in any combination of broth media. Although the strains did proliferate in the media, as described previously (15), changes in impedance were very small (ranging from 0 to 1,000 μS) and were not significantly greater than the electrical drift observed in the uninoculated control media. Since pure cultures and inocula containing high concentrations of campylobacters were used to optimize growth conditions, this limited change can be attributed to the complex physiological requirements of the campylobacters.

In impedance microbiology, the design of the growth medium is critical, because this medium must initially be able to support the growth and proliferation of the target organism. Additionally, it is important that noncharged components of the growth medium (e.g., glucose) be converted by the organisms into ionic by-products of metabolism (such as lactic acid) that allow the measurement of detectable changes in impedance (i.e., the resistance to the flow of an alternating current through the broth medium). Our previous study (15) showed that C. coli is capable of proliferation in Mueller-Hinton broth, brucella broth, nutrient broth no. 2, brain heart infusion broth supplemented with yeast extract, and Campy broth media, although this study did not examine the growth of C. coli in Whitley impedance broth. Furthermore, once a basal broth medium is defined, it does not always follow that this medium is an efficient signal producer. Previous studies have shown that Staphylococcus aureus will grow in nutrient broth but that growth in this medium does not produce a significant electrical signal (3). The design of a suitable broth for C. coli that fulfills both of these criteria is complicated. C. coli has been reported to be fastidious in nature (18), requiring amino acids and growth factors (28, 29). C. coli has limited capabilities with regard to metabolic activity and is not able to oxidize or ferment carbohydrates or glycolytic intermediates (11, 25). Instead, the organism obtains its energy from amino acids or intermediates of the tricarboxylic acid cycle (25). Of 11 short- and long-chain fatty acids tested, only acetate was used (25).

In conclusion, the detection of campylobacters with impedance has not been widely reported because of the complex physiological requirements of Campylobacter and because of the inability of antibiotics used in selective media to inhibit the growth of competing organisms. Although there is a growing demand in the food industry for rapid pathogen detection techniques, routine impedimetric detection of campylobacters may not be possible until a suitable detection medium is developed. Consequently, rapid nucleic acid–based detection techniques such as polymerase chain reaction and nucleic acid sequence–based amplification may offer practical alternatives to food-testing laboratories that presently wish to invest in non–culture-based technologies for the rapid detection of Campylobacter.

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REFERENCES


