Susceptibility of *Vibrio parahaemolyticus* to Tris-Dependent DNA Degradation during Pulsed-Field Gel Electrophoresis

This study reports a characteristic of some *Vibrio parahaemolyticus* strains which are susceptible to electrophoresis-related DNA-degradation (ERDD) by pulsed-field gel electrophoresis (PFGE) in Tris-borate EDTA (TBE) buffer. *V. parahaemolyticus* is a halophilic bacterium naturally inhabiting coastal environments worldwide, with reported pandemic strains, warranting the need for surveillance and epidemiological research on clinical and food isolates for source tracking and risk assessment (10). PFGE has been widely used as the “gold standard” for molecular typing of bacterial isolates, to which other molecular typing techniques are compared (3–5).

The strains of *V. parahaemolyticus* used in this study are shown in Table 1. All of the characterized isolates were obtained from our culture collection (1). PFGE was performed according to the standard protocol for nontyphoidal *Salmonella* (Pulse-Net, Centers for Disease Control and Prevention, Atlanta, GA) (2, 6, 9) with modifications. Briefly, organisms were grown on tryptic soy agar with 2% NaCl (pH 8.5) at 35°C overnight and suspended in buffer (100 mM Tris, 100 mM EDTA [pH 8.5]) to a density of 0.6 ± 0.02 in a Dade Microscan turbidity meter (Dade Behring, Inc., West Sacramento, CA). Cell suspensions were treated with 4% formaldehyde to arrest the endogenous DNase activity or were untreated and immobilized into low-melting-point, SeaKem Gold (SKG) agarose plugs, lysed, washed, and digested with NotI or SfiI, as described elsewhere (6). Restriction fragments were resolved in 1% SKG agarose by PFGE using a CHEF DRIII apparatus (Bio-Rad Laboratories, Hercules, CA) containing 0.5× TBE buffer with or without 50 μM thioura. Restricted DNAs were electrophoresed at 6 V/cm for 22 h at 14°C, with pulse times ramping from 2 s to 40 s. Gels were stained, destained, and photographed using a Gel-Doc 1000 system (Bio-Rad) as described earlier (4).

The ERDD phenomenon was observed in 29 out of 164 (~18%) of the isolates from seafood using NotI digestion (figures not shown). Among the representative strains (Table 1), ERDD was observed irrespective of whether NotI or SfiI was used for digestion (figures not shown). Both clinical (tdh+/thh−) and environmental (tdh−/thh+) strains (Fig. 1) were susceptible. The effectiveness of thioura in preventing smearing of NotI-digested *V. parahaemolyticus* DNA can be seen in Fig. 1.

The use of formaldehyde before DNA isolation did not prevent ERDD in the selected strains, but addition of thioura (50 μM) to the running buffer resolved the problem without altering the macrorestriction profiles of the typeable strains (Fig. 1). We recommend doing electrophoresis using the regular buffer (TBE) to detect the ERDD types of *V. parahaemolyticus*, which can then be run separately by using thioura in the running buffer to obtain macrorestriction patterns. Using this protocol, the ERDD subtypes can be identified and their banding patterns revealed with minimum exposure, use, and disposal of the toxic compound thioura.

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### REFERENCES


### TABLE 1. Strains of *V. parahaemolyticus* used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Thermostable hemolysin (thl)</th>
<th>Thermostable hemolysin (tdh)</th>
<th>Source</th>
</tr>
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<tr>
<td>ATCC 17802</td>
<td>+</td>
<td>–</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>NY477</td>
<td>+</td>
<td>+</td>
<td>1, 8</td>
</tr>
<tr>
<td>S107-2</td>
<td>+</td>
<td>–</td>
<td>Ladysmith Harbor, British Columbia, Canada, May 2005</td>
</tr>
<tr>
<td>S107-5</td>
<td>+</td>
<td>–</td>
<td>Ladysmith Harbor, British Columbia, Canada, May 2005</td>
</tr>
<tr>
<td>S111-3</td>
<td>+</td>
<td>–</td>
<td>Ship’s Point, British Columbia, Canada, July 2005</td>
</tr>
<tr>
<td>S119-1</td>
<td>+</td>
<td>–</td>
<td>Ladysmith Harbor, British Columbia, Canada, August 2005</td>
</tr>
</tbody>
</table>
of *Vibrio parahaemolyticus* isolated from seafoods and cases of gastrointestinal disease in the UK. Int. J. Environ. Health Res. 18:283–293.

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