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The Use of Colony Hybridization in the Isolation of Thermostable Direct Hemolysin-Producing *Vibrio parahaemolyticus* from Foods Implicated in an Incidence of Food Poisoning

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Humans are infected by *Vibrio parahaemolyticus* by ingesting uncooked contaminated marine fishes and shellfishes (1). Its major bacterial pathogenic factors are thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) (2).

In general, in optimum temperatures, *V. parahaemolyticus* grows more rapidly than other food poisoning-causing bacteria, and negligence in temperature control during food processing and storage is the major cause of poisoning by

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mantis shrimps in salt polymyxin broth
↓ masticated
↓ 35°C, overnight → PCR
spread on TCBS agar
↓ 35°C, overnight
the *V. parahaemolyticus*-like colonies are collected and suspended in PBS aliquots
↓
aliquots are titrated and *tdh*$^+$ is detected by PCR
↓
aliquots are placed on TCBS agar
↓ 35°C, overnight
colonies are transferred to nylon filters and hybridized with a *tdh*-probe
↓
colonies showing hybridization signals on X-ray films are selected and suspended in PBS
colonies are plated on TCBS agar
↓ 35°C, overnight
single colonies are selected
↓
the unique *tdh*$^+$ colonies are detected by PCR

PBS: phosphate-buffered saline

Fig. 1. Flow chart of the screening process of *tdh*$^+$ colonies using hybridization

this pathogen.

Isolation of TDH-positive bacteria from the stool specimens of patients is relatively easy. However, isolation of this bacteria from foods or sea water is extremely difficult, though isolation of TDH-positive bacteria from implicated foods by using the modified Wagatsuma medium was reported (3). We recently succeeded in an application of colony hybridization for isolation of *tdh*$^+$ (gene encoding TDH) bacteria from foods implicated in a food poisoning case. The information is detailed in this report.

In November 1999, food poisoning was caused by *Sushi* prepared by a restaurant in Sendai City. The major symptoms of the patients were diarrhea, abdominal pain, and vomiting. From stool specimens of two patients, TDH-positive *V. parahaemolyticus* O3:K6 was isolated (isolates VP1 and VP2). In order to identify foods implicated in this incident, we tried to detect TDH-producing bacteria from various foods stored in the restaurant. The *tdh* gene was detected by PCR from only a frozen stock of boiled mantis shrimps after culture in salt polymyxin broth. *V. parahaemolyticus* of various serotypes were isolated from thiosulfate-citrate-bilesalts-sucrose (TCBS) agar plates inoculated with the broth but none of the 264 isolates were *tdh*$^+$.

In order to screen thousands of colonies, colony hybridization by using a *tdh* probe was performed. The outline of the procedures is shown in Figure 1. The bacteria grown in salt polymyxin broth were plated on ten TCBS agar plates. A total of 1,369 transparent and sucrose non-fermenting *V. parahaemolyticus*-like colonies appeared. After culture overnight, the colonies were transferred to nylon filters (Hybond™-N+, Amersham LIFE SCIENCE, Buckinghamshire, England) and treated with alkaline solution for DNA denaturation and cross-link with the filter. After prehybridization, the filters were probed with the peroxidase-labelled *tdh* probe (2) by using ECL™ direct nucleic acid labeling and detection systems (Amersham LIFE SCIENCE). Three plates were positive for *tdh*$^+$ candidate colonies. Forty colonies were checked for the *tdh* gene by PCR. One colony (VP3) was positive. Therefore, the detection rate was 0.07% (1/1,369).

The isolate obtained by the colony hybridization method from the mantis shrimp (VP3) actually produced TDH. VP3's serotype, which was O3:K6, biochemical markers, and the pattern of pulsed-field gel electrophoresis (PFGE) of DNA digests were identical to the patient isolates' (Fig. 2, lanes 1-3). Though of the A type prevalent most recently, their PFGE pattern was different from any of the reported subtypes (4). Two *tdh*$^-$ O3:KUK isolates from the same mantis shrimps (VP4-5) were examined for PFGE pattern. One (VP4) had a pattern resembling that of VP1-3 but was not of the A type (Fig. 2, lane 4), and another (VP5) had a pattern entirely different (Fig. 2, lane 5).

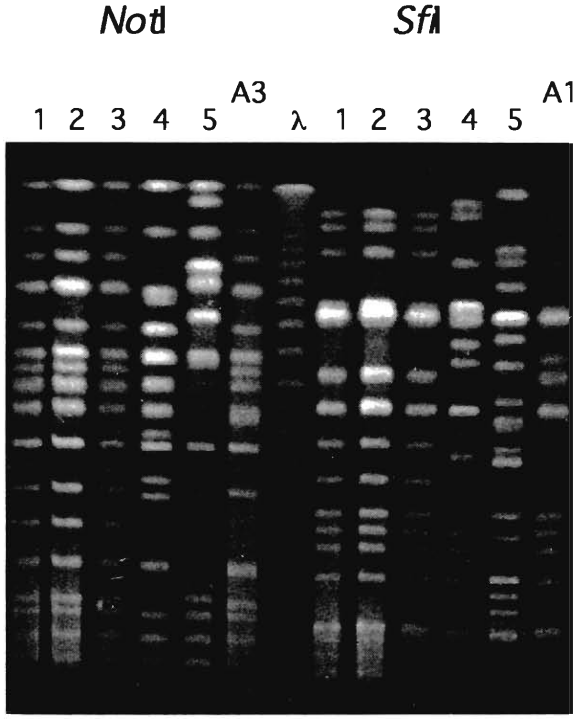

Fig. 2. Pulsed-field gel electrophoresis (PFGE) patterns showing *Not*I- or *Sfi*I- chromosomal DNA digests of *Vibrio parahaemolyticus* isolates. Lane A3: the standard strain of A3 subtype; Lane A1: the standard strain of A1 subtype; Lane λ : λ DNA ladder; Lane 1-2: VP1-2 (*tdh*$^+$ isolates from patients); Lane 3: VP3 (*tdh*$^+$ isolates from the mantis shrimps); Lane 4-5: VP4-5 (*tdh*$^-$ isolates from the mantis shrimps)

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