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Molecular Typing of *Vibrio parahaemolyticus* Isolates, Obtained from Patients Involved in Food Poisoning Outbreaks in Taiwan, by Random Amplified Polymorphic DNA Analysis

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***Vibrio parahaemolyticus* is one of the most important food-borne pathogens in Taiwan, Japan, and other countries with long coastlines. This paper reports on the development of a new random amplified polymorphic DNA (RAPD) method for the molecular typing of this pathogen. The 10-mer primer 284 (5′-CAG GCG CAC A-3′) was selected to generate polymorphic amplification profiles of the genomic DNA at an annealing temperature of 38°C. A total of 308 clinical isolates of *V. parahaemolyticus* collected during food poisoning outbreaks in Taiwan, mostly occurring between 1993 and 1995, plus 11 environmental and clinical reference strains were analyzed by this RAPD method. A total of 41 polymorphic RAPD patterns were recognized, and these patterns were arbitrarily grouped into 16 types (A to P). Types A, B, C, D, and E were the major types, and subtypes C3, C5, E1, B1, D2, and A2 were the major patterns. The major types were phylogenetically more closely related to each other than to any of the minor types.**

Vibrio parahaemolyticus is a halophilic gram-negative bacterium that causes acute gastroenteritis in humans. It is one of the most important food-borne pathogens in Taiwan, Japan, and other countries with long coastlines (1). Isolates of *V. parahaemolyticus* can be differentiated by serotyping. Commercial serotyping antisera are available in Japan and other countries (e.g., from Denka Seiken, Tokyo, Japan). There are 13 O groups and 71 K types identified by these commercial antisera. Usually the serotyping method cannot differentiate all isolates which originate from different regions or sources. Dependable molecular methods for the typing of strains would greatly aid epidemiological investigations. However, molecular typing methods for the subspecies differentiation of *V. parahaemolyticus* have not been well developed. Recently, we described the pulsed-field gel electrophoresis (PFGE) method for the subspecies typing of this pathogen (10). This paper reports on the development of another molecular method, random amplified polymorphic DNA (RAPD), for the typing of *V. parahaemolyticus*. A total of 308 clinical isolates obtained during food poisoning outbreaks, mostly occurring from 1993 to 1995 in Taiwan, and several environmental and clinical reference strains were characterized by this procedure.

TABLE 1. *V. parahaemolyticus* cultures examined by RAPD in this study

Category	No.	Designation[a]	Origin	Remarks
Clinical isolate	308		Taiwan	295 collected 1993–1995
Reference environmental strain	4	Laboratory stock 109	Taiwan	
		Laboratory stock 226		
		CCRC12963		
		CCRC12958		
Reference clinical strain	7	CCRC10806	Japan	Type strain ATCC 17802
		CCRC12864	United States	
		CCRC12865	United States	
		CCRC13025	Taiwan	
		CCRC12863	Japan	
		CCRC13027	Taiwan	
		ST550	Japan	Reference 9

[a] CCRC, Culture Collection and Research Center, Hsin-chu, Taiwan, Republic of China; ST550 was furnished by T. Arai, Showa College of Pharmaceutical Sciences, Japan.

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MATERIALS AND METHODS

Bacterial strains. A total of 308 clinical isolates selected from the stool samples of patients involved in food poisoning outbreaks which occurred mostly from 1993 to 1995 in Taiwan were examined in this study. These isolates were identified by API (Montalieu-Vercieu, France) 20E identification strips and also by

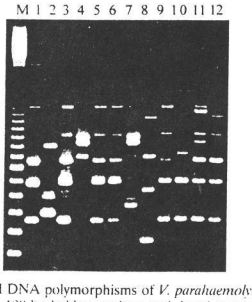

FIG. 1. Amplified DNA polymorphisms of *V. parahaemolyticus* isolates with primer 284. Lane M, 100-bp ladder marker; each band represents a 100-bp increment, with 300 bp at the low end. Lanes: 1, isolate DOH702 (pattern C4); 2, DOH714 (0); 3, DOH718 (E1); 4, DOH719 (I1); 5, DOH720 (E1); 6, DOH730 (C5); 7, DOH733 (I2); 8, DOH738 (N); 9, DOH740 (C5); 10, DOH741 (E4); 11, DOH747 (F1); 12, DOH755 (E1).

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