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Evaluation of Typing of *Vibrio parahaemolyticus* by Three PCR Methods Using Specific Primers

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***Vibrio parahaemolyticus* is a halophilic bacterium frequently involved in human outbreaks of seafood-associated gastroenteritis. For epidemiological purposes, different molecular typing methods, such as pulsed-field gel electrophoresis (PFGE) or ribotyping, have been developed for this pathogen; however, these methods are mostly labor-intensive and time-consuming. In this work, we designed and evaluated three rapid PCR typing methods for this pathogen using primers designed on the basis of the following specific sequences: conserved ribosomal gene spacer sequence (RS), repetitive extragenic palindromic sequence (REP), and enterobacterial repetitive intergenic consensus sequence (ERIC). Typing patterns and clustering analysis indicated that these methods apparently differentiated *V. parahaemolyticus* strains from reference strains of interspecific *Escherichia coli*, *V. cholerae*, and *V. vulnificus* and were also valuable in subspecies typing of this pathogen. Forty domestic strains of *V. parahaemolyticus*, representing a wide range of PFGE patterns, were grouped into 15, 27, and 27 patterns, with discrimination indexes of 0.91, 0.97, and 0.98, by RS-, REP-, and ERIC-PCR, respectively. The discriminative abilities of these PCR methods closely approached or even exceeded those of PFGE and ribotyping. REP-PCR is preferable to ERIC-PCR because of the greater reproducibility of its fingerprints, while RS-PCR may be a practical method because it generates fewer amplification bands and patterns than the alternatives.**

Vibrio parahaemolyticus is a halophilic gram-negative bacterium that causes acute gastroenteritis in humans. Food poisoning caused by this pathogen is generally associated with the consumption of contaminated seafood; this organism is a crucial food-borne pathogen in Taiwan, Japan, and other coastal countries with high rates of seafood consumption (17). Clinical manifestations include diarrhea, abdominal cramps, nausea, vomiting, headache, fever, and chills, with incubation periods ranging from 4 to 96 h (4, 9).

Isolates of *V. parahaemolyticus* can be differentiated by serotyping, and 13 O groups and 71 K types have been identified (7). Serotyping is generally unable to differentiate all isolates originating from different regions or sources. Reliable molecular methods for strain typing would significantly aid epidemiological investigations. Recently, several molecular methods were developed for the subspecies typing of *V. parahaemolyticus*, namely, pulsed-field gel electrophoresis (PFGE) (33), ribotyping (29), and random amplified polymorphic DNA (RAPD) analysis (30). The PFGE method using *Sfi*I digestion is reliable, achieves high discrimination efficiency, and has been applied to typing of *V. parahaemolyticus* strains in many situations, such as the first pandemic O3:K6 strains (32), food poisoning outbreaks (28), environmental strains from seafood (31), and nosocomial outbreaks (12). However, the whole process takes several days to complete. Compared with PFGE, RAPD analysis has the merits of being less labor-intensive and faster to complete (30). Nevertheless, RAPD analysis, or the arbitrarily primed PCR method, which is based on short oligonucleotide primers, is impaired by lower discrimination efficiency (16, 30) and is complicated by variations in band intensity and the lack of reproducibility of certain minor bands (21).

By using a 22-mer primer specific for the enterobacterial repetitive intergenic consensus sequence (ERIC), Marshall et al. found that ERIC-PCR is useful for evaluating genetic and epidemiological relationships among *V. parahaemolyticus* strains (14). Besides ERIC-PCR, PCR methods based on the highly conserved ribosomal gene spacer sequence (RS) and the 38-bp repetitive extragenic palindromic sequence (REP) in *Enterobacteriaceae* and other bacteria have been used for the typing of pathogenic bacteria (25, 26). To develop a reliable rapid subspecies typing method for *V. parahaemolyticus*, the application of these three PCR methods (RS-, REP-, and ERIC-PCR) for typing 41 strains representing different PFGE patterns was evaluated.

MATERIALS AND METHODS

Bacterial strains. Forty strains of *V. parahaemolyticus* isolated from outbreaks in Taiwan during 1993 and 1994 and representing different PFGE patterns were analyzed here (28). Clinical strain ST550, O4:K13 and Kanagawa phenomenon positive and originating from Japan, was used as a reference strain (34). *Escherichia coli* JM109, *V. cholerae* 569B, and *V. vulnificus* CCRC12905 were used as interspecies reference strains. These bacterial cultures were stored at −80°C in tryptic soy broth (Difco Laboratories, Detroit, Mich.) containing 20% glycerol with no supplementary NaCl for *E. coli* and 3% NaCl for the *Vibrio* cultures. The *Vibrio* stock cultures were incubated in tryptic soy broth–3% NaCl at 37°C, agitated at 160 rpm for about 16 h, and streaked on tryptic soy agar–3% NaCl. The *E. coli* stock was cultured in Luria-Bertani broth medium (Difco) at 37°C, shaken at 160 rpm for about 16 h, and streaked on Luria-Bertani medium with 1.5% agar.

Preparation of genomic DNA. Colonies on agar plates were picked, and their genomic DNA was isolated by the small-scale preparation method of Sambrook et al. (20), suspended in 10 mM Tris hydrochloride buffer–1 mM EDTA (pH 7.5), and stored at −20°C until required.

PCR primers. Three sets of amplification oligonucleotide primers were synthesized. For RS-PCR, a pair of 15-mer primers (L1, 5′-CAA GGC ATC CAC

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TABLE 1. Subspecies typing patterns determined for different strains of *V. parahaemolyticus* by different molecular methods[a]

Pattern determined by:						Strain	Location of isolation	Date of isolation (yr/mo/day)	Serotype
RS-PCR	ERIC-PCR	REP-PCR	PFGE[b]	Ribotyping[b]	RAPD analysis[b]				
A1	K	P	D3	A7	C5	677	Taipei	1994/6/30	K8
A1	K	P	D3	A7	C5	679	Peng-Hu	1994/6/30	K8
A1	K	P	D3	A7	C5	680	Peng-Hu	1994/6/30	K8
A2	F1	F2	B2	E5	B1	323	Miao-Li	Unknown	ND
A2	G1	E	A5	E2	D1	166	Taichung	1992/10/5	K29
A2	G2	D2	A5	G2	C3	168	Taipei	1992/9/28	ND
A2	H1	D1	A4	F1	D2	302	Miao-Li	1993/6/14	K29
A2	I	G	A6	F2	C3	197	Kaohsiung	1992/10/22	ND
A2	J	C1	E1	C3	C5	283	Kaohsiung	1993/7/4	K3
A3	B3	I2	ND	ND	ND	ST550	Japan	Unknown	K13
A3	G3	I1	G2	E1	B2	134	Kaohsiung	1992/9/20	ND
A3	L	P	D3	A7	C5	690	Peng-Hu	1994/6/30	ND
A3	N	Q	C4	B2	E1	757	Tai-nan	1994/12/13	K68
A3	N	Q	C4	B2	E1	758	Tai-nan	1994/12/13	K68
A3	N	Q	C4	B2	E1	759	Tai-nan	1994/12/13	K68
A3	P	P	B3	A2	C5	742	Ping-Tung	1994/10/15	K8
A4	Q	N	E2	F1	D2	554	Taichung	1993/10/2	K29
B	A1	L1	O1	C2	B1	402	Tai-nan	1993/7/28	K12
B	A1	L3	E3	A5	B1	355	Taipei	1993/6/27	ND
B	A2	L1	O1	C2	B1	403	Tai-nan	1993/7/28	K12
B	M	N	A6	F1	D2	415	Chia-Yi	1993/7/29	K29
B	O1	M	C3	A1	E1	418	Chia-Yi	1993/7/29	ND
B	O2	M	C5	G1	E1	436	Yun-lin	1993/8/3	K60
C	H1	D1	A4	F1	D2	304	Miao-Li	1993/6/14	K29
C	H1	N	A6	F1	D2	314	Miao-Li	1993/6/14	K29
D1	F2	A2	H1	E3	C5	325	Miao-Li	Unknown	ND
D1	P	R	H3	E3	E1	718	Tao-Yuan	1994/9/16	K41
D1	P	R	H1	E3	E1	720	Tao-Yuan	1994/9/16	K41
D2	C1	A1	H1	E3	E1	199	Taipei	1992/10/2	K41
E	A1	H	H1	I	B2	182	Tai-Tung	1992/10/16	ND
F	E	J	B2	H1	C5	272	Kaohsiung	1993/1/4	K6
G	D	F1	C2	A2	C3	145	Kaohsiung	1992/9/26	ND
H1	B1	L2	B1	A3	C3	364	Kaohsiung	1993/7/11	ND
H1	B2	K2	B1	A3	C3	626	Kaohsiung	1994/4/15	ND
H1	C2	K1	G3	D2	C3	473	Kaohsiung	1993/8/10	K15
H1	C2	K1	B1	A3	C3	474	Kaohsiung	1993/8/10	K15
H1	C2	K1	B1	A3	C3	487	Kaohsiung	1993/8/10	K15
H1	C3	O	J	G2	E1	434	Yun-lin	1993/8/3	K60
H2	G1	S	F3	E1	C3	135	Kaohsiung	1992/9/26	ND
I	ND	B	B1	A3	C4	702	Kaohsiung	1994/7/30	ND
J	H2	C2	K	C1	C3	308	Miao-Li	1993/6/14	ND

[a] ND, not determined.
[b] Data are from references 28 to 30.

CGT-3′, and G1, 5′-GAA GTC GTA ACA AGG-3′) was designed on the basis of the spacer sequences of 16S and 23S ribosomal DNAs (8). For REP-PCR, the primers contained multiple nucleotides at ambiguous positions in the consensus REP. The following pair of 18-mer primers was used for REP-PCR: REP-1D, 5′-NNN RCG YCG NCA TCM GGC-3′, and REP-2D, 5′-RCG YCT TAT CMG GCC TAC-3′, where M is A or C, R is A or G, Y is C or T, and N is any nucleotide (25). For ERIC-PCR, a pair of 22-mer primers (ERIC1R, 5′-ATG TAA GCT CCT GGG GAT TCA C-3′, and ERIC2, 5′-AAG TAA GTG ACT GGG GTG AGC G-3′) was designed on the basis of the core repeated sequence of ERIC (27).

Amplification conditions. Optimized PCR conditions were developed to produce reproducible fingerprints for *V. parahaemolyticus* strains. *V. parahaemolyticus* strain ST550 was used as a reference strain in every PCR experiment and was resolved in every electrophoresis gel, while the PCR assays were repeated three times with other *V. parahaemolyticus* strains to ensure reproducibility. PCR amplifications were conducted with a buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris HCl [pH 8.8], 1% Triton X-100) containing 200 μM each dATP, dCTP, dGTP, and dTTP, 50 pmol of primers, and 100 ng of template DNA in a final volume of 50 μl. Amplification was performed with a thermal cycler, Personal Cycler 20 (Biometra Biomedizinische Analytik Gmbh, Gottingen, Germany). All manipulations were conducted using dedicated DNA-free pipettes in a sterile field to minimize contamination risk. The reaction mixture was overlaid with a drop of sterile mineral oil and incubated in the thermal cycler at 95°C for 7 min. Then, 1.0 U of DyNAZyme II thermostable DNA polymerase (Finnzymes Oy, Espoo, Finland) was added, and the mixture was amplified at different temperature settings. RS-PCR was performed via denaturation at 90°C for 30 s, annealing at 55°C for 1 min, and extension at 70°C for 5 min; REP-PCR was performed via denaturation at 90°C for 30 s, annealing at 45°C for 1 min, and

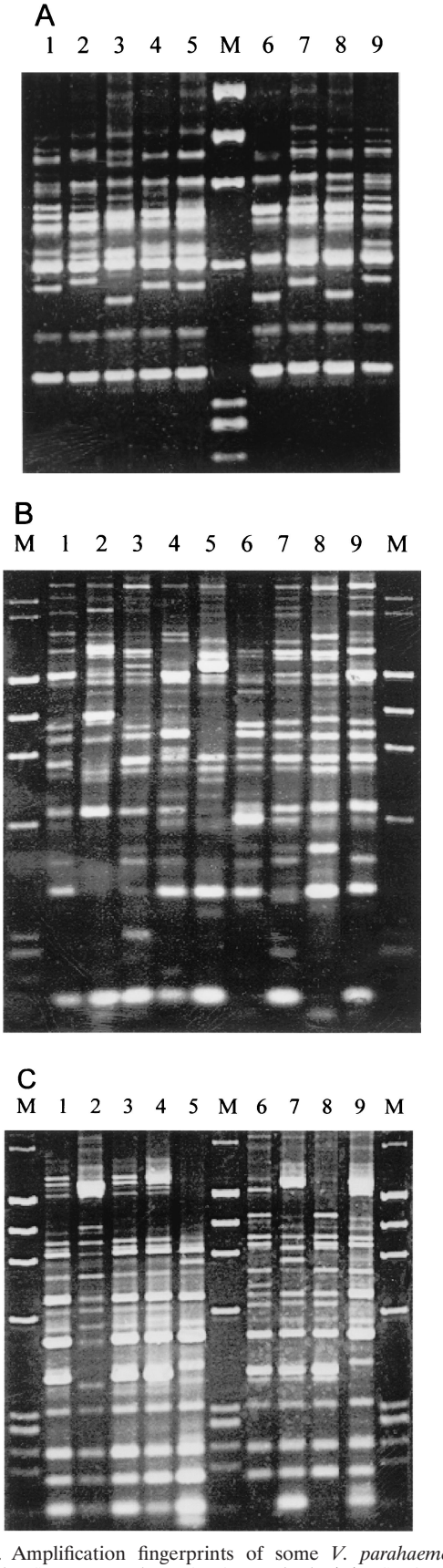

FIG. 1. Amplification fingerprints of some *V. parahaemolyticus* strains with RS-, REP-, and ERIC-PCR methods. (A) RS-PCR. Lanes: 1, strain 134 (pattern A3); 2, strain 135 (pattern H2); 3, strain 145 (pattern G); 4, strain 166 (pattern A2); 5, strain 168 (pattern A2); 6, strain 182 (pattern E); 7, strain 197 (pattern A2); 8, strain 199 (pattern D2); 9, strain ST550 (pattern A3); M, molecular size markers (from top to bottom, 1,353, 1,078, 872, 603, 310, and 281 bp). (B) REP-PCR. Lanes: 1, strain 134 (pattern I1); 2, strain 135 (pattern S); 3, strain 145 (pattern F1); 4, strain 166 (pattern E); 5, strain 168 (pattern D2); 6, strain 182 (pattern H); 7, strain 197 (pattern G); 8, strain 199 (pattern A1); 9, strain ST550 (pattern I2); M, molecular size markers (from top to bottom, 2,322, 2,027, 1,353, 1,078, 872, 603, 310, 281, and 271 bp). (C) ERIC-PCR. Lanes: 1, strain 355 (pattern A1); 2, strain 364 (pattern B1); 3, strain 402 (pattern A1); 4, strain 403 (pattern A2); 5, strain 415 (pattern M); 6, strain 418 (pattern O1); 7, strain 434 (pattern C3); 8, strain 436 (pattern O2); 9, strain ST550 (pattern B3); M, molecular size markers (from top to bottom, 2,027, 1,353, 1,078, 872, 603, 310, 281, and 271 bp).

extension at 65°C for 5 min; and ERIC-PCR was performed via denaturation at 90°C for 30 s, annealing at 52°C for 1 min, and extension at 70°C for 5 min. Following 30 reaction cycles, all the reaction mixtures were further incubated at 70°C for an additional 10 min.

Gel electrophoresis. Following PCR, 10 μl of the reaction mixture was mixed with 2 μl of loading buffer (20). The mixture was electrophoresed in a horizontal 2% agarose gel (10 by 15 cm) in Tris-borate buffer at 100 V for 30 min. The process was continued at 75 V until the bromophenol blue tracking dye approached the front of the running gel. The amplified DNA bands were visualized following ethidium bromide staining and photographed under UV light. A mixture of lambda DNA digested with *Hin*dIII and ϕX174 DNA digested with *Hae*III (Finnzymes) was used to mark molecular masses.

Similarities among patterns. The size of each band was determined via Stratascan 7000 densitometry with one-dimensional analysis software (Stratagene, La Jolla, Calif.). Data were coded as 0 (negative) or 1 (positive). Following the method described by Martin-Kearley et al. (15), hierarchical cluster analysis was performed using the average linkage method with the squared Euclidean distance measure. The dendrogram was produced using the program SPSS for Windows, Release 6.0 (SPSS Inc., Chicago, Ill.) (15, 30). Finally, the discriminative abilities of different typing methods were calculated using the method of Hunter and Gaston (6).

RESULTS

The 40 domestic strains of *V. parahaemolyticus* used here have been previously examined and grouped into 22, 20, or 8 patterns through PFGE, ribotyping, or the RAPD method, respectively (Table 1). Strains with differences of one or more amplification bands were differentiated into different patterns here.

In RS-PCR, 8 to 15 amplified bands with sizes of between 330 and 1,000 bp were found in the *V. parahaemolyticus* strains. Bands ranging from 350 to 720 bp could be easily observed on the electrophoresis gel. Specifically, six amplified bands with molecular sizes of 350, 420, 610, 720, 750, and 870 bp were common in all strains (Fig. 1A), and two amplified bands (350 and 720 bp) occurred in all *V. parahaemolyticus* strains but not in *E. coli*, *V. cholerae*, and *V. vulnificus*. All 41 *V. parahaemolyticus* strains were grouped into 15 patterns, with A3 (17.1% of the total number of strains) being the predominant pattern (Fig. 2 and Table 2).

In REP-PCR, between 13 and 26 amplified bands ranging in size from 160 to 3,000 bp were discernible in the *V. parahaemolyticus* strains. Several amplified bands with molecular sizes of 200, 470, 500, 600, 640, 805, and 1,355 bp were common in most strains, while only the 805-bp band was present in all *V. parahaemolyticus* strains (Fig. 1B). The *V. parahaemolyticus* strains were grouped into 27 patterns, with P (12.2% of the

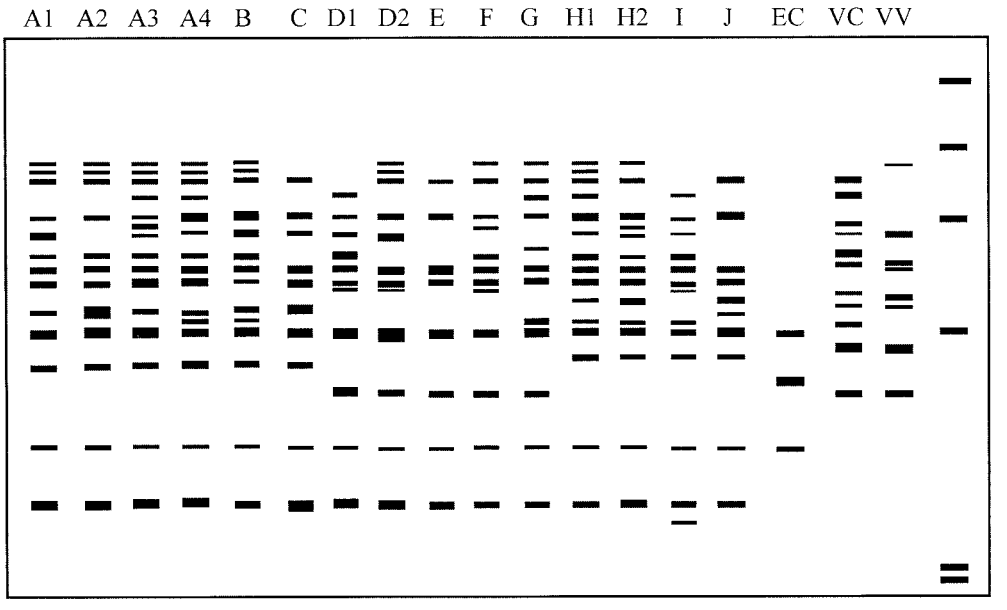

FIG. 2. Diagram of amplification patterns of *V. parahaemolyticus* with RS-PCR. EC, *E. coli*; VC, *V. cholerae*; VV, *V. vulnificus*. The rightmost lane contains the molecular size markers described in the legend to Fig. 1A.

total number of strains) being the predominant one (Fig. 3 and Table 2).

In ERIC-PCR, 12 to 25 amplified bands with sizes ranging between 160 and 1,690 bp were easily discernible in the *V. parahaemolyticus* strains. Several bands with molecular sizes of 270, 320, 520, 560, 660, 780, 900, 950, and 1,355 bp were common in most strains, while 270-, 520-, 660-, and 950-bp bands were present in all *V. parahaemolyticus* strains (Fig. 1C). The 39 domestic strains plus the reference strain from Japan were grouped into 27 patterns. Patterns A1, C2, H1, and P, each comprising three strains, were the most predominant patterns (7.5% of the total number of strains) (Fig. 4 and Table 2).

The clonal relationships among these *V. parahaemolyticus* strains were examined through cluster analysis of the PCR-generated patterns and are presented in dendrograms (Fig. 5, Fig. 6, and Fig. 7). Following cluster analysis, different patterns were arbitrarily classified into different types with strain dissimilarity values of 5 or more (33). Each type consisted of one to seven different patterns (Table 1). Compared with the interspecies reference strains, all the *V. parahaemolyticus* strains were closely related, according to analysis by the PCR methods; they differed significantly from the reference strains of *E. coli*, *V. cholerae*, and *V. vulnificus*, having dissimilarity values of 17 or more (Fig. 5 to 7). Strains of *V. parahaemolyticus* belonging to one or closely related PFGE patterns were generally grouped into closely related patterns by these PCR methods. Also, strains determined by one of these PCR methods to belong to strongly dissimilar patterns were generally noted as being dissimilar by the other two PCR methods (Fig. 5 to 7).

TABLE 2. Comparison of different molecular methods used in the subspecies typing of *V. parahaemolyticus*

Method	No. of:		% of strains with the predominant pattern	Discrimination index[a]
	Strains	Patterns		
PFGE	40	22	12.5	0.96
Ribotyping	40	20	12.5	0.95
RAPD	40	8	25.0	0.84
RS-PCR	41	15	17.1	0.91
REP-PCR	41	27	12.2	0.97
ERIC-PCR	40	27	7.5	0.98

[a] Simpson's index of diversity (14).

DISCUSSION

Several molecular methods have been developed and assessed for the typing of *V. parahaemolyticus*. PFGE is the method favored in our laboratory, owing to its highly reproducible fingerprints and strong discriminative ability (12, 31, 32). However, the ability of this method may be impaired by a high proportion of nontypeable isolates (23%), owing to DNA degradation during endonuclease digestion or other steps (14). A DNA degradation problem has been encountered with some strains in our laboratory, but these strains were successfully typed by repeating the experiment (28). The cause of DNA degradation is unknown; however, careful processing to avoid shearing interference and the use of a suboptimal enzyme reaction temperature of 37°C (optimum temperature of 50°C for *Sfi*I) may have reduced the level of DNA degradation in our study. Furthermore, difficulties were recently encountered with the typing of several strains collected from Japan and America in 2000, with the nontypeable rate reaching about 7% (unpublished data). Therefore, a combination of methods may be required to achieve the complete typing of different *V. parahaemolyticus* strains.

PCR typing methods using specific primers designed on the basis of the repeated and conserved sequences in bacteria and

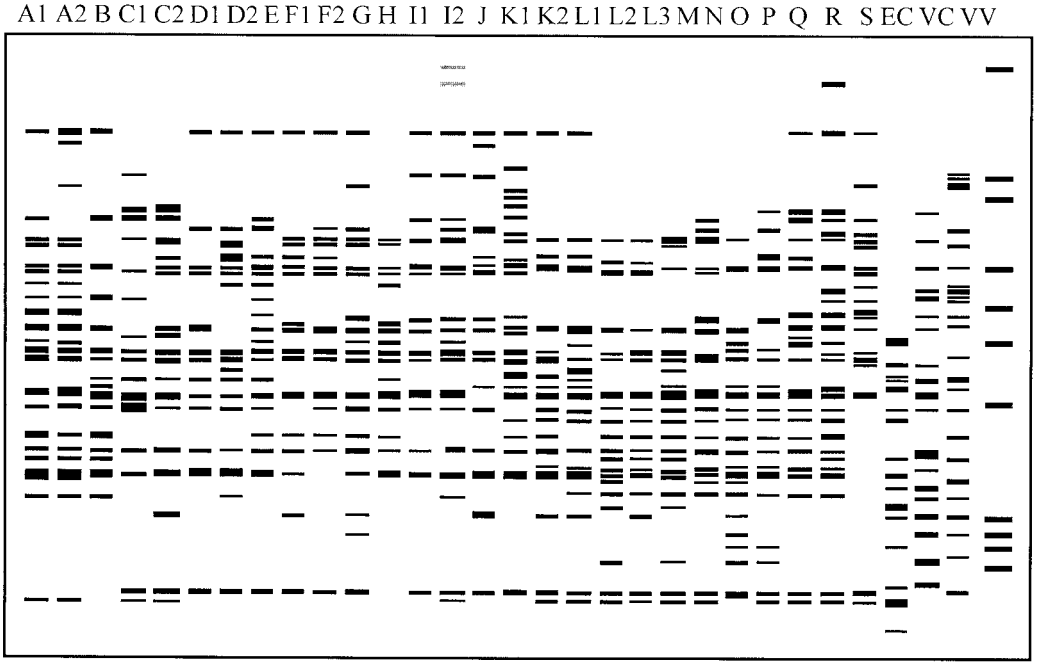

FIG. 3. Diagram of amplification patterns of *V. parahaemolyticus* with REP-PCR. EC, *E. coli*; VC, *V. cholerae*; VV, *V. vulnificus*. The rightmost lane contains the molecular size markers described in the legend to Fig. 1B.

more stringent annealing conditions display more promising fingerprints than RAPD analysis (11). Spacer regions within the 16S and 23S genes in prokaryotic rRNA genetic loci exhibit significant length and sequence polymorphisms in different species and are flanked by highly conserved sequences (8). Multiple copies of these loci occur in bacteria (24). Therefore, amplification using primers designed on the basis of these flanking sequences will generate polymorphic fingerprints which can be used to distinguish bacterial strains at the species and subspecies levels (1, 2, 8). RS-PCR has been applied to typing of species from many genera, including *Listeria*, *Staphylococcus*, and *Salmonella* (8, 10), but had not yet been applied

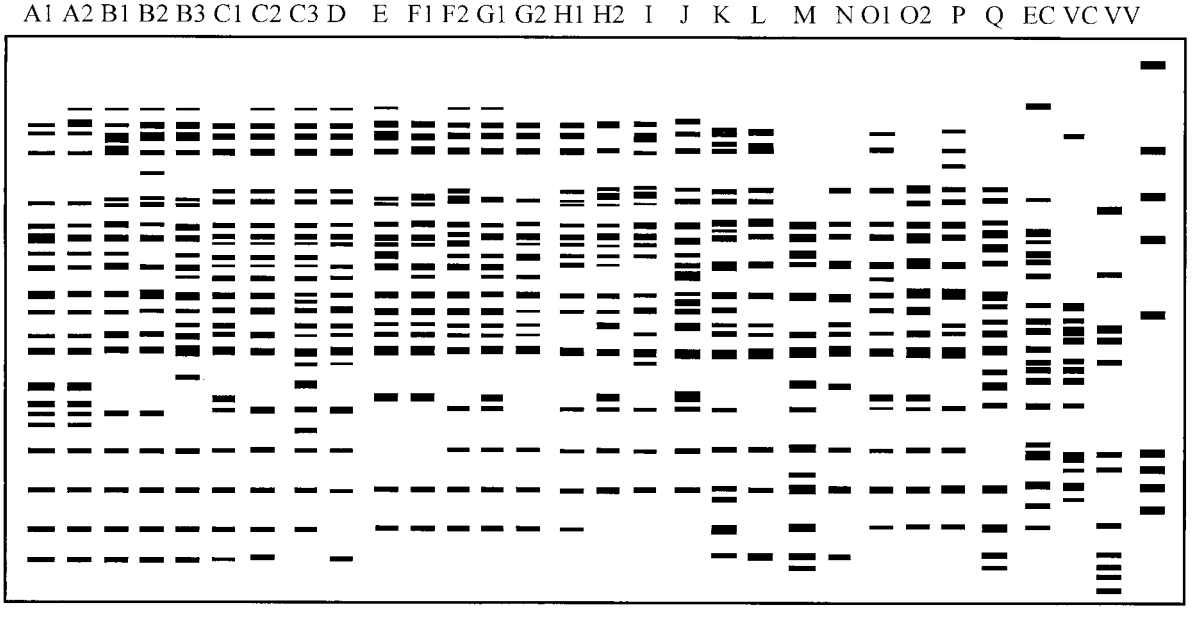

FIG. 4. Diagram of amplification patterns of *V. parahaemolyticus* with ERIC-PCR. EC, *E. coli*; VC, *V. cholerae*; VV, *V. vulnificus*. The rightmost lane contains the molecular size markers described in the legend to Fig. 1C.

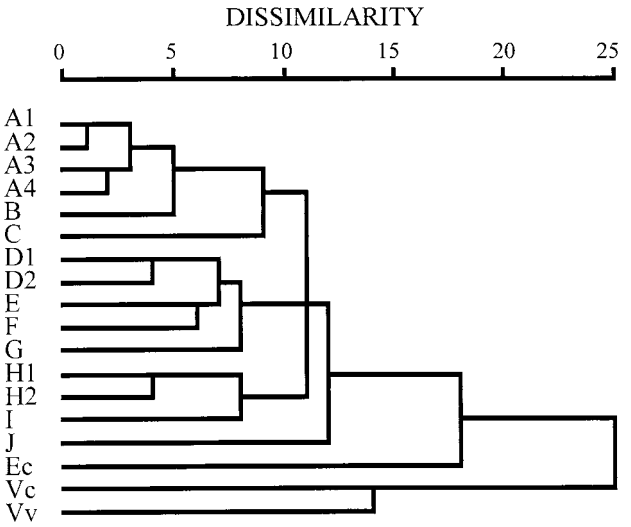

FIG. 5. Dendrogram illustrating the clustering of amplification patterns of *V. parahaemolyticus* with RS-PCR. The dendrogram was produced using the squared Euclidean distance measure and average linkage clustering method with the program SPSS for Windows, Release 6.0. The dissimilarity units are arbitrary, being based on the sum of the squared Euclidian distance measure. Strains were arbitrarily grouped into different types. Letters at left designate the patterns.

to *V. parahaemolyticus*. The 16S-23S rRNA intergenic spacer regions of *V. parahaemolyticus* contain different tRNA compositions, and similarities in the nucleotide sequences of the noncoding regions flanked by the tRNA genes have been noted (13).

REP-PCR and ERIC-PCR are both based on the presence of repetitive conserved sequences in bacteria. The REP-PCR method is based on the presence of 38-bp REPs in *Enterobacteriaceae* and other bacteria and has been applied to many species (14, 19, 25, 26). With REP-PCR, the fingerprinting profiles differentiate toxigenic *V. cholerae* O1 strains from nontoxigenic O1 and non-O1 strains, while ERIC-PCR further differentiates toxigenic O1 strains into El Tor and classical biotypes (22). This work is the first to apply the RS-PCR and REP-PCR methods to the typing of *V. parahaemolyticus*.

ERIC-PCR is the most widely adopted of the above three PCR typing methods and has been applied to the typing of many species, including *V. cholerae* (18, 23) and *V. parahaemolyticus*. Marshall et al. (14) examined 38 clinical strains of *V. parahaemolyticus* from outbreaks on Canada's Pacific coast and several environmental strains using ERIC-PCR, ribotyping, PFGE, and restriction fragment length polymorphism analysis of the genetic locus encoding the polar flagellum. Six ERIC-PCR patterns were identified by using a single primer for the amplification, and it was concluded that ERIC-PCR and ribotyping were useful for evaluating genetic and epidemiological relationships among *V. parahaemolyticus* strains (14).

All three PCR typing methods described here could differentiate *V. parahaemolyticus* from other species and effectively differentiate intraspecific strains. The *V. parahaemolyticus* strains examined here were deliberately selected to represent a variety of different patterns and have been typed using PFGE, ribotyping, and RAPD analysis. The discriminative ability of these PCR methods can thus be evaluated and compared with that of other published methods. PFGE, ribotyping, REP-PCR, and ERIC-PCR exhibited an excellent discrimination index of 0.95 or higher (Table 2). Based solely on the discrimination index (Simpson's index of diversity [14]), REP-PCR and ERIC-PCR will be selected as the two best rapid PCR typing methods for *V. parahaemolyticus*. However, REP-PCR could be the better of the two owing to its higher rate of reproducible fingerprints. In the current study, the PCR assays were repeated three times for each *V. parahaemolyticus* strain, and the reproducibility of the banding patterns was observed. In ERIC-PCR, some of the minor light amplification bands were inconsistent, thus complicating pattern differentiation. Among the three PCR methods, RS-PCR generated fewer amplification bands than REP-PCR and ERIC-PCR and thus fewer subspecies patterns and a slightly lower discrimination index (0.91) (Table 2). However, since the RS-PCR patterns were more easily discernible visually than the REP-PCR or ERIC-PCR patterns, they may be a practical method for routine use.

Although the discriminative ability of these PCR typing methods differed from 0.91 to 0.98, these methods are effective for typing strains from outbreaks. When the typing of strains in each outbreak is examined, the results obtained by these PCR methods mirrored those of the PFGE method for some outbreaks, although they differed slightly for other outbreaks. For

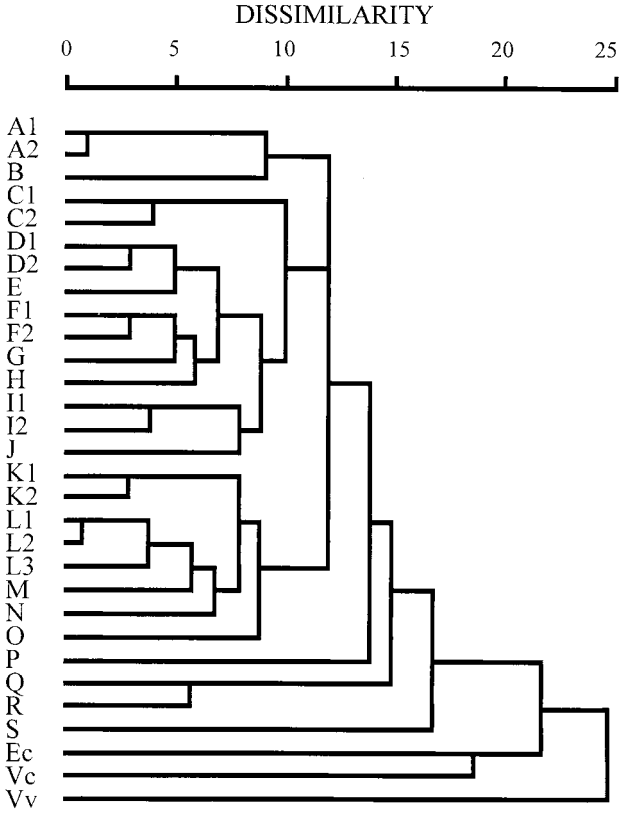

FIG. 6. Dendrogram illustrating the clustering of amplification patterns of *V. parahaemolyticus* with REP-PCR. See the legend to Fig. 5 for details.

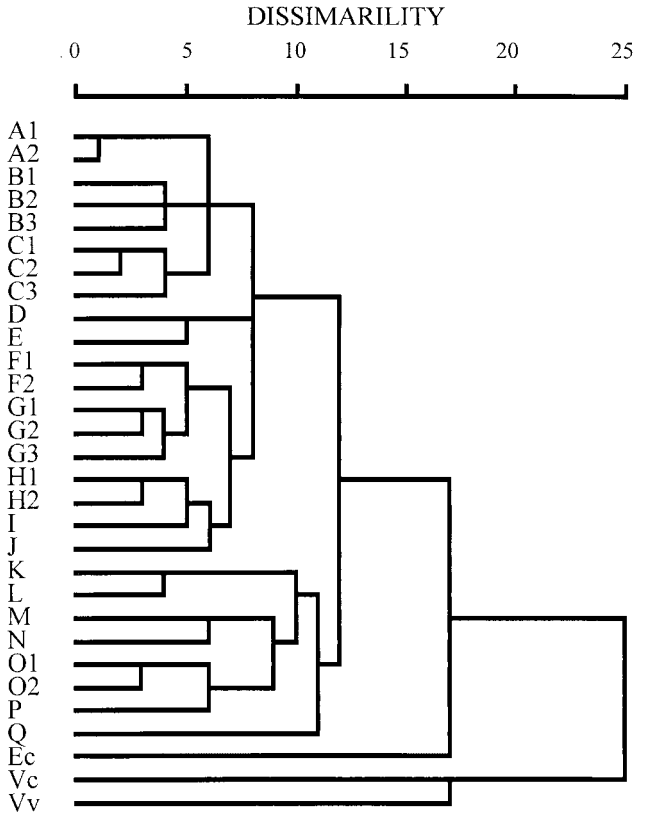

FIG. 7. Dendrogram illustrating the clustering of amplification patterns of *V. parahaemolyticus* with ERIC-PCR. See the legend to Fig. 5 for details.

example, the outbreak occurring in Miao-Li on 14 June 1993 was typed as A4, A4, K, and A6 by PFGE, A2, C, J, and C by RS-PCR, H1, H1, H2, and H1 by ERIC-PCR, and D1, D1, C2, and N by REP-PCR for strains 302, 304, 308, and 314, respectively (Table 1). In another example, the outbreak occurring in Peng-Hu on 30 June 1994 was typed as D3 by PFGE and P by REP-PCR but as A1 and A3 by RS-PCR and K and L by ERIC-PCR (Table 1). The use of a combination of these PCR methods could achieve even higher discriminative ability when fine and rapid typing is required.

The presence of the repeatable fingerprints in REP-PCR and ERIC-PCR suggested the presence of these repetitive consensus sequences (REP and ERIC) in *V. parahaemolyticus*. In another *Vibrio* species, *V. cholerae*, the presence of ERIC has been confirmed to be located near the hemolysin gene. Meanwhile, ERIC of *V. cholerae* is highly homologous with those found in *Enterobacteriaceae*. A previous study has speculated that a transpecific genetic exchange has affected a group of *E. coli* hemolysin genes and that ERIC has thus "hitchhiked" with the hemolysin gene (5). Besides the presence of ERIC, the possibility of fingerprints being formed by random amplification cannot be excluded, and Gillings and Holley (3) confirmed that ERIC-PCR fingerprints may be thus produced. Gillings and Holley performed PCR with ERIC primers using salmon and lambda DNA templates without ERIC, and the fingerprints were formed and changed according to different annealing conditions used in the PCR procedure (3).

In conclusion, RS-PCR, REP-PCR, and ERIC-PCR are suitable rapid typing methods for *V. parahaemolyticus*. All three methods have high discriminative ability, but REP-PCR is superior to ERIC-PCR owing to the better reproducibility of fingerprints produced with this method. Nevertheless, RS-PCR, with a slightly lower discriminative ability, may be a more practical method because fewer amplification bands and patterns are generated, simplifying review and interpretation of data.

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