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Molecular typing of *Vibrio parahaemolyticus* strains isolated from marine finfish/shellfish landed along south-west coast of India

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ABSTRACT

Vibrio parahaemolyticus is one of the most reported food-borne pathogen along the south-west coast of India, commonly occurring in marine fish. For epidemiological purposes, this pathogen is confirmed by various molecular typing methods, such as pulsed-field gel electrophoresis (PFGE) or ribotyping.These methods are labor intensive and time consuming. In the present study, rapid typing methods with specific sequences *viz.*, conserved ribosomal gene spacer sequence (RS), repetitive extragenic palindromic sequence (REP), and enterobacterial repetitive intergenic consensus sequence (ERIC) and RAPD (random amplified polymorphic DNA) were used. Marine fish / shellfish were collected from major landing centers located along the south-west coast of India and screened for *V. parahaemolyticus*. Following phenotypic characterisation, fingerprinting of bacterial strains was carried out by various typing methods *viz*., RAPD, ERIC, REP, and RS PCR. Cluster analyses revealed the conglomeration of bacterial isolates into three and four groups using RAPD and RS respectively while it revealed two major groups in each of the ERIC and REP PCR methods. ERIC-PCR method generated two major clusters, one with the finfish and cephalopod isolates and the other with the strains isolated from shellfish samples suggesting that the strains isolated from finfish samples showed higher genetic similarity with the strains from cephalopods than that of shellfish isolates. However, RS PCR generated fewer amplified bands (eight) as compared to other typing methods, thus giving scope for higher discrimination of various *V. parahaemolyticus* strains, suggesting it as a reliable practical method for routine use.

Keywords: Fingerprinting, Fish landing centers, PCR, Seafoods, *Vibrio parahaemolyticus*

Introduction

Global production of fish and fishery products has more than doubled since 1970, reflecting an increase in capture, and in particular, aquaculture production (Delgado *et al*., 2003). Cochin is the economic capital of Kerala and considered to be a major fishing hub of south-west coast of India, accounting for over 90% of statewide exports. *Vibrio parahaemolyticus* is a prevalent seafood borne pathogen in many Asian countries where marine foods are frequently consumed (Joseph *et al.,* 1983). The organism has been reported as the causative agent of gastroenteritis (Fujino *et al.,* 1953), wound infections and septicemia resulting from the consumption of contaminated seafoods. Recently with the emergence of pandemic O3:K6 strain of *V. parahaemolyticus* causing acute gastroenteritis, this pathogen has acquired greater significance (Matsumoto *et al.,* 2000).

Subspecies typing of *V. parahaemolyticus* isolates may be useful for studying the ecology of the bacterium as well as in tracking the organism causing disease. Recently, several molecular methods were developed for the subspecies typing of *V. parahaemolyticu*s, namely, random amplified polymorphic DNA (RAPD) analyses (Wong *et al*., 1999b). Analyses of the enterobacterial repititive intergenic consensus sequence (ERIC) PCR have been reported to be used for the sub-typing of *Vibrio cholerae* (Rivera *et al*., 1995). It was found to be a useful tool for evaluating genetic and epidemiological relationships among different *V. parahaemolyticus* strains (Marshall *et al*., 1999). Besides ERIC-PCR, methods based on the highly conserved ribosomal gene spacer sequence (RS) and the 38-bp repetitive extragenic palindromic (REP) sequence in *Enterobacteriaceae* and other bacteria have been used for the typing of pathogenic bacteria (Stubbs *et al*., 1999).

Though there are several reports available on the isolation and identification of *V. parahaemolyticus,* both by traditional and molecular methods, studies from this area on the genetic typing of this particular bacterial species are limited. Few authors have typed this microorganism by PFGE and ribotyping methods which are time consuming and labour intensive (Marshall *et al*., 1999). In the present study, we report the isolation, characterisation, and comparison of various rapid molecular typing methods like

RAPD, ERIC, REP and RS for evaluating genetic relationships of *V. parahaemolyticus* strains isolated from seafood samples collected from landing centers located in and around Cochin, along the south-west coast of India.

Materials and methods

Sample collection and processing

Samples were collected over a period of one year from landing centers located in and around Cochin to isolate *V. parahaemolyticus* strains from various marine fish/ shellfish samples. Samples were collected fortnightly and processed under aseptic conditions, within 2 h of collection. Finfish, shellfish and cephalopod samples (25 g) were homogenised with APW (225 ml) in a sterile polythene stomacher bag separately, and blended in a stomacher homogeniser (Stomacher 400 Seaward medicals, UK) at 230 rpm for 1 min. The samples were then incubated at 37 °C for a period of 18-24 h for enrichment (ICMSF, 1986).

Isolation and identification of V. parahaemolyticus

After 18-24 h of incubation, 0.5 ml of broth was aseptically pipetted into preset dried (56 ^{0}C, 45 min), thiosulphate citrate bile salt sucrose (TCBS) agar plates and incubated for 24-30 h. About 3 to 4 typical colonies (3-5 mm green or blue-green colonies with green or blue centre were picked from TCBS plates, and each one was inoculated into sterile sucrose medium supplemented with NaCl (3% w/v). Only sucrose non-fermenting colonies were streaked onto sterile tryptone soy agar (TSA) slants supplemented with NaCl (3% w/v) and maintained at room temperature for further identification. Halophilism tests were performed using NaCl-tryptone broth (T_1N_0, T_1N_3, T_1N_6, T_1N_8, and T_1N_{10}). Additional characterisation tests *viz*., Gram staining, catalase, cytochrome oxidase tests, triple sugar iron agar tests, lysine iron agar tests, arginine dehydrolase tests, lysine and ornithine decarboxylase tests, O/129 susceptibility, tests for glucose oxidation-fermentation in Hugh-Leifson broth, and arabinose, lactose, mannitol, mannose, cellobiose and inositol fermentation tests were performed following standard procedure (USFDA, 2001). The presumed positive cultures were further confirmed with a RAPID Hi-Vibrio ™ identification kit (KB007, Himedia, India).

Extraction and purification of chromosomal DNA

Genomic DNA from *V. parahaemolyticus* isolates was extracted following the method of Ausubel *et al.* (1987). Briefly, a colony with typical characteristics was picked from the TCBS plate and was grown overnight in LB broth containing NaCl (3% w/v) at 37 °C for 16-18 h. DNA was extracted from culture in exponential phase of growth of the organism after giving a wash with normal saline (NaCl 0.8% w/v), followed by resuspending in TE buffer (10 mM Tris–Cl, 1 mM Na_2EDTA, pH 8.0). Alkaline lysis was performed with sodium dodecyl sulfate (20% w/v) (SDS, SRL India), and proteinase K (20 mg ml^{-1}, Sigma, Aldrich , USA). After 1 h of incubation at 37 °C, NaCl (5 M) was added along with cetyl trimethyl ammonium bromide / NaCl solution to complex with the polysaccharides. DNA was purified from proteins and other cellular constituents using an equal volume of chloroform-isoamyl alcohol (24:1 v/v) followed by centrifugation (10,000 *g*) for 5 min. Further purification of the supernatant was achieved by adding an equal volume of phenol–chloroform–isoamyl alcohol (25:24:1 v/v) to the supernatant, followed by centrifugation (10,000 *g*). Sodium acetate (0.1 volume, 3M, pH 5.2) followed by chilled absolute alcohol was added to precipitate the DNA. The sample (0.2 ml) was centrifuged (12,000 *g*) for 10 min and the DNA pellet was washed once with cold ethanol (70% v/v) before being dried under vacuum. The purified DNA was resuspended in TE buffer and stored at –20 °C until further use.

RAPD, ERIC, RS and REP PCR assays

Vibrio parahaemolyticus isolates along with the positive (*V. parahaemolyticus*, NCMB 1902, MTCC 451) and negative controls (*V. alginolyticus*, MTCC 4439) were grown overnight at 37 °C on tryptic soy agar plate. Total DNA was purified from overnight culture broths following the standard procedure (Ausubel *et al*., 1987). The primers used for all the PCR's (RAPD, ERIC, RS, and REP) are listed in the Table 1. PCR amplification was optimised in a total reaction volume of 25 µl consisting of sterile Milli Q water (11.9 µl), 10X PCR buffer (2.5 µl), each primer (2.5 µl), dNTP mix (1.0 µl, 200 mM), template (1 µl), *Taq* DNA polymerase (0.6 µl, 3U μl^{-1}) and $MgCl_2$ (3.0 µl, 20 mM). All the amplifications were performed in a thermocycler (Eppendorf

Table 1. Oligonucleotide primers used in the typing of *V. parahaemolyticus*

Typing method	Primers	Nucleotide sequence	Reference
RAPD PCR	1	CAG GCG CAC A	
RS PCR	Forward	5'CAA GGC ATC CAC CGT3'	Wong and Lin, 2001
	Reverse	5'GAA GTC GTA ACA AGG3'	
ERIC PCR	Forward	5'ATG TAA GCT CCT GGG GAT TCA C3'	Wong and Lin, 2001
	Reverse	5'AAG TAA GTG ACT GGG GTG AGC G3'	
REP PCR	Forward	5'NNN RCG YCG NCA TCM GGC3'	Wong and Lin, 2001
	Reverse	5'RCG YCT TAT CMG GCC TAC3'	

Mastercycler, Germany) comprising of 30 cycles. Each cycle consisted of initial denaturation (95 °C, 7 min), annealing (52 °C, 1 min) and extension (72 °C, 5 min) for ERIC PCR; initial denaturation (95 °C, 7 min), annealing (45 °C, 1 min) and extension (65 °C, 5 min) for REP PCR; initial denaturation (95 °C, 7 min), annealing (55 °C, 1 min) followed by extension (70 °C, 5 min) for RS PCR, followed by a final extension (70 °C, 5 min) remaining same for all the three PCRs. Amplified products were resolved on agarose gels (2.0% w/v), stained with ethidium bromide (Sigma, 0.5 μg ml^{-1}). Electrophoresis was carried out at 7-10 V cm^{-1} for 90 min in tris acetate (TAE, pH 8.0) buffer. A 100 bp DNA ladder was used as molecular size marker (Gene RulerTM, Fermentas, Germany). The resolved bands were visualised on UV- transilluminator at a wavelength of 360 nm and photographed using an UV gel documentation system (Alpha Imager, Innotech Corporation, USA).

Cluster analyses

Similarity matrix was built for *V. parahaemolyticus* isolates using Dice's correlation co-efficient. Cluster analyses was performed and dendrogram was constructed using the data matrix of all the strains isolated from farm samples based on unweighted pair-group method with arithmetic means (UPGMA) (Sneath and Sokal, 1973) using the Gel Compar II software, version 4.0 (Applied-Maths, St-Martens-Latem, Belgium).

Results

Various PCR methods (RAPD, RS, REP, and ERIC-PCR) attempted in order to develop a reliable rapid subspecies typing method for *V. parahaemolyticus*, resulted in different banding patterns and the number of bands produced from each method are presented in Table 2.

ERIC-PCR analyses of V. parahaemolyticus isolates

The gel pattern of ERIC-PCR analyses of cultures isolated from landing centre samples, revealed 10 to 15 amplified bands with sizes ranging between 150 and 1,500 bp and were easily discernible in the *V. parahaemolyticus* isolates. Several bands with molecular sizes of 320, 400, 500, 550, 600, 700, 750, 800, and 1,200 bp were common in most of them while 500, 550, 600, 750, 800, and 1100 bp bands were clearly visible in all the tested strains (Fig. 1).

Table 2. Results of PCR Typing of *V. parahaemolyticus* from seafood samples by different methods, indicated by the size and number of bands

Method	Primers	Sequence	No. of bands	Size (Kbp)
RAPD PCR	1	CAG GCG CAC A	6-9	0.45-2.5
RS PCR	Forward	CAA GGC ATC CAC CGT	8	0.15-0.85
	Reverse	GAA GTC GTA ACA AGG		
ERIC PCR	Forward	ATG TAA GCT CCT GGG GAT TCA C	10-15	0.15-1.5
	Reverse	AAG TAA GTG ACT GGG GTG AGC G		
REP PCR	Forward	NNN RCG YCG NCA TCM GGC	10	0.1-2.0
	Reverse	RCG YCT TAT CMG GCC TAC		

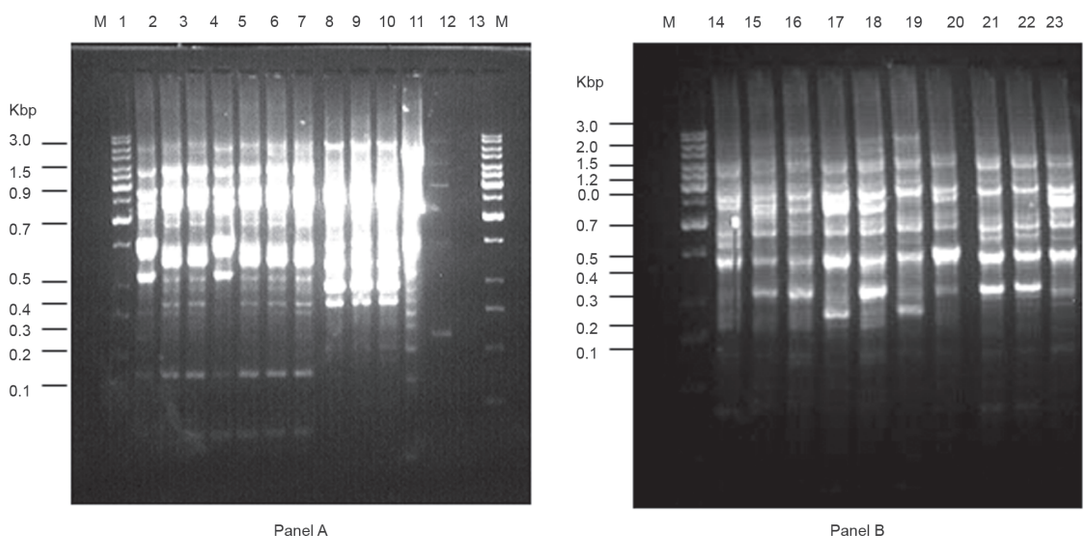


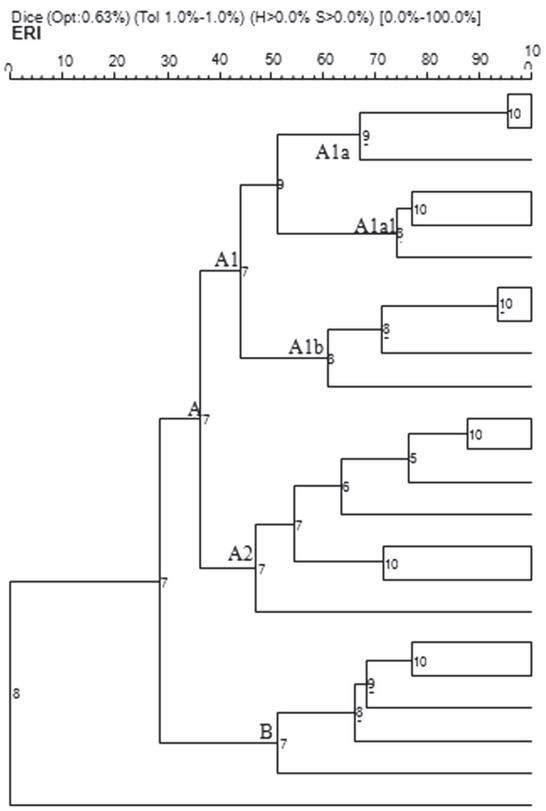
Fig. 1. ERIC-PCR profiles of *Vibrio parahaemolyticus* strains isolated from landing centre samples. Lane M: 100 bp DNA ladder, Lane 1-8: isolates from shellfish, Lane 9-11 & 14-16: isolates from finfish, Lane 17-23: isolates from cephalopods, Lane 12: *Vibrio alginolyticus* type strain (MTCC 4439), Lane 13: PCR negative control

The dendrogram of ERIC-PCR analyses of isolates revealed two major clusters *viz*., A and B based on the source of isolation. Group A included two sub-groups A1 and A2 consisting of isolates, specifically from shrimp samples. Subgroup A1a placed 14.3% of the strains, isolated from deepsea shrimps caught at a depth of and above 150 m clustered with A1a1 at S >50% which included cultures isolated from shallow water shrimps caught at a depth of 50 m. Subgroup A1a clustered with A1b subgroup at S >40%, placed cultures (14.3%) isolated from shallow water shrimps and *V. parahaemolyticus* type strain (NCMB 1902) which served as positive control was also included within the same cluster. Group A1 clustered with A2 at S >30% which placed 33.3% cultures isolated from fish and cephalopod samples. Group A clustered with group B at S < 25%, included the strains isolated from deepsea shrimp samples. Negative control formed a separate cluster distinctly away from the main cluster **(**Fig. 2**)**.

RS-PCR analyses of Vibrio parahaemolylticus isolates

The gel pattern of RS-PCR analyses of cultures isolated from landing centre samples revealed eight distinct bands with molecular sizes of 150, 220, 250, 300,320, 350, 650, 720, and 850 bp, and three amplified bands of molecular sizes 250, 300 and 650 bp were found to be common in all the tested *V. parahaemolyticus* isolates (Fig. 3).

The dendrogram of the same revealed four major clusters *viz.*, A, B, C, and D based on the source of samples rather than seasonality. A1 included 26.1% of the isolates of which A1a included isolates from deepsea shrimp samples caught at and above 150 m depth and clustered with A1b at S >53%. A1b placed strains isolated from inshore waters (<50 m depth). Subgroup A1 clustered with A2 at S >48% and included 13% of the isolates in A2a which clustered at S >65% and A2b which placed 17.4% of the isolates caught from deepsea region. Group A clustered with group B at S >30%, which included strains (13%) isolated from pelagic fish. Group B clustered with group C at S >28% included isolates from inshore region and *V. parahaemolyticus* positive control (NCMB 1902). Group C clustered with group D at S >20%, included strains (17.4%) isolated from fish and cephalopod samples. Negative control formed a separate cluster distinctly away from the main cluster (Fig. 4).



Vibrio	parahaemolyticus	H.woodmasoni	Dec
Vibrio	parahaemolyticus	H.woodmasoni	Jan
Vibrio	parahaemolyticus	A.alcocki	June
Vibrio	parahaemolyticus	M.dobsoni	March
Vibrio	parahaemolyticus	P.stylifera	Sep
Vibrio	parahaemolyticus	M.dobsoni	Jan
Vibrio	parahaemolyticus	P.stylifera	June
Vibrio	parahaemolyticus	NCMB, 1902	
Vibrio	parahaemolyticus	M.dobsoni	May
Vibrio	parahaemolyticus	M.dobsoni	Oct
Vibrio	parahaemolyticus	S.longipes	Feb
Vibrio	parahaemolyticus	S.indicus	Dec
Vibrio	parahaemolyticus	P.argentius	Jan
Vibrio	parahaemolyticus	S.longipes	Apri
Vibrio	parahaemolyticus	S.pharonis	March
Vibrio	parahaemolyticus	R.kanagurta	Oct
Vibrio	parahaemolyticus	L.duvacelli	Apri
Vibrio	parahaemolyticus	P.spinipes	Apri
Vibrio	parahaemolyticus	M.andamanensis	Nov
Vibrio	parahaemolyticus	H.gibbosus	Feb
Vibrio	parahaemolyticus	H.gibbosus	Feb
Vibrio	parahaemolyticus	H.gibbosus	Nov
Vibrio	alginolyticus	MTCC, 4439	

Fig. 2. Dendrogram illustrating the clustering of ERIC profile of *Vibrio parahaemolyticus* isolates from landing centre samples. Bars are shown at each node, corresponding to the standard deviation of values in that region of the similarity matrix. The average and the standard deviation of similarity values for the selected nodes are shown above the dendrogram. The similarity scale is shown above this dendrogram

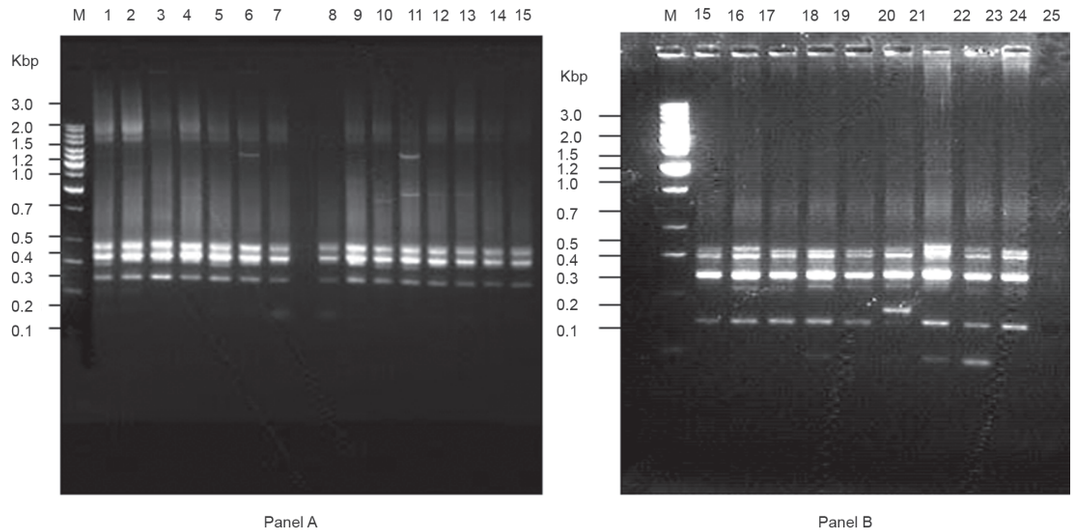


Fig. 3. RS-PCR profiles of *Vibrio parahaemolyticus* strains isolated from landing centre samples. Lane M: 100 bp DNA ladder, Lane 1-10: isolates from shellfish, Lane 11-14 & 16: isolates from finfish, Lane 17-24: isolates from cephalopod samples, Lane 15: *V. parhaemolyticus* type strain (NCMB 1902), Lane 25: *V. alginolyticus* type strian (MTCC 4439)

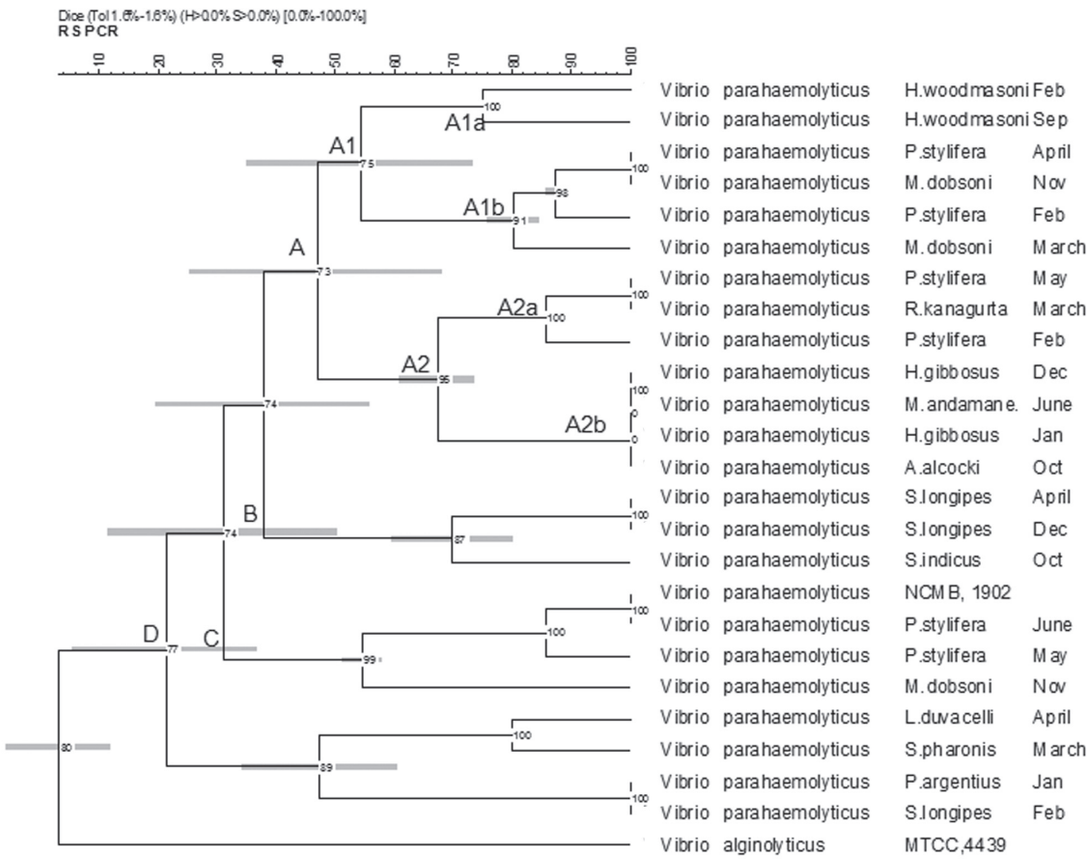


Fig. 4. Phylogenetic analyses of RS profile of *Vibrio parahaemolyticus* isolates from landing centre samples. Bars are shown at each node, corresponding to the standard deviation of values in that region of the similarity matrix. The average and the standard deviation of similarity values for the selected nodes are shown above the dendrogram. The similarity scale is shown above this dendrogram

REP PCR analyses of V. parahaemolyticus isolates

The gel pattern of REP-PCR analyses of landing centre samples revealed ten bands with molecular sizes of 100, 150, 320, 350, 520, 600, 720, 1000, 1500, and 2000 bp, while one amplified band with molecular size of 1000 bp was found in all *V. parahaemolyticus* isolates (Fig. 5).

The dendrogram depicted two major clusters, A and B mainly based on the source of sample rather than seasonality. A1 included 19.1% of the strains isolated from shrimp samples; a type strain of *V. parahaemolyticus* was also included within the same cluster. A1 clustered with A2 at S >63%. A2 placed cultures (42.9%) isolated from fish and cephalopod samples. Group A clustered with group B at S >60% which in turn formed two subgroups, B1 included cultures (28.6%) isolated from various shrimp samples and subgroup B1a included the cultures isolated from deepsea shrimps while B1b and B2 included strains from inshore shrimps (Fig. 6).

RAPD-PCR analyses of V. parahaemolyticus isolates

RAPD analyses of isolates from landing centre samples revealed three distinct clusters A, B and C depending upon the source of samples rather than seasonality. Group A is sub-grouped into A1 and A2. Sub-group A1 included group A1a1 with 17.4% of the cultures isolated from the deepsea shrimp samples during October-January. A1a1 clustered with sub-group A1a2 at S > 45% which placed isolates from shallow water shrimps caught at a depth of <50 m. Subgroup A1 clustered with A2 at S >20% which had 2 groups, A2a and A2b with 21.7% cultures isolated from deepsea shrimps caught at a depth of about 150-200 m and a type strain (NCMB 1902). Group A clustered with group B at S < 20% which placed 17.4% of the strains isolated from fish samples. Group B clustered with group C at S >10% which included 8.6% strains isolated from cephalopods and a *V. parahaemolyticus* type strain. Group C clustered with type strain *V. alginolyticus* at S >5% which served as a species negative control. However, the isolates from fish and cephalopods (reference strain, MTCC 451) formed distinctly separate group, with a minimum distance between them, from the shrimp isolates. Negative control (*V. alginolyticus* MTCC 4439) formed a single separate group distinctly separated from all the other isolates. The gel pattern revealed 6-9 bands ranging from 0.45 to 2.5 kb (Fig. 7). The characteristic fingerprint patterns obtained with primers are shown in dendrograms and gel pictures (Fig. 8).

Discussion

The PCR typing methods using specific primers designed on the basis of the repeated and conserved sequences in bacteria, and more stringent annealing conditions display more promising fingerprints than RAPD analyses. Marshall *et al*. (1999) found that ERIC-PCR using a 22-mer primer specific for the enterobacterial repetitive intergenic consensus sequence (ERIC) is useful for evaluating genetic and epidemiological relationships among *V. parahaemolyticus* strains. Besides ERIC-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 typing of pathogenic bacteria (Stubbs *et al*., 1999). To develop a reliable rapid

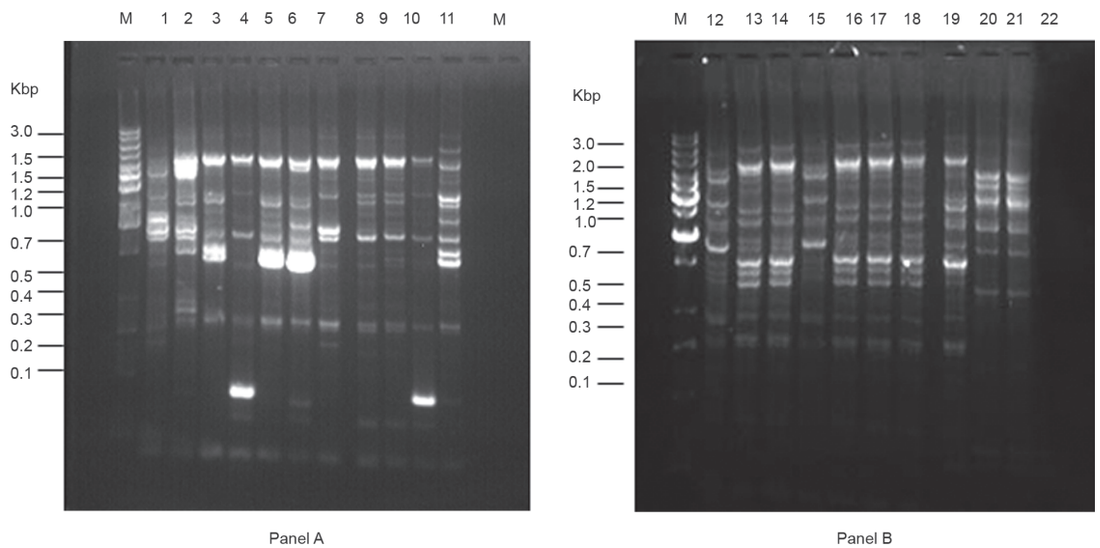


Fig. 5. REP-PCR profiles of *Vibrio parahaemolyticus* strains isolated from landing centre samples. Lane M: 100 bp DNA ladder, Lane 1: *V. parahaemolyticus* type strian (MTCC 451), Lane 2-9 isolates from shellfish, Lane 10-16 isolates from finfish, Lane 17-22 isolates from cephalopod samples

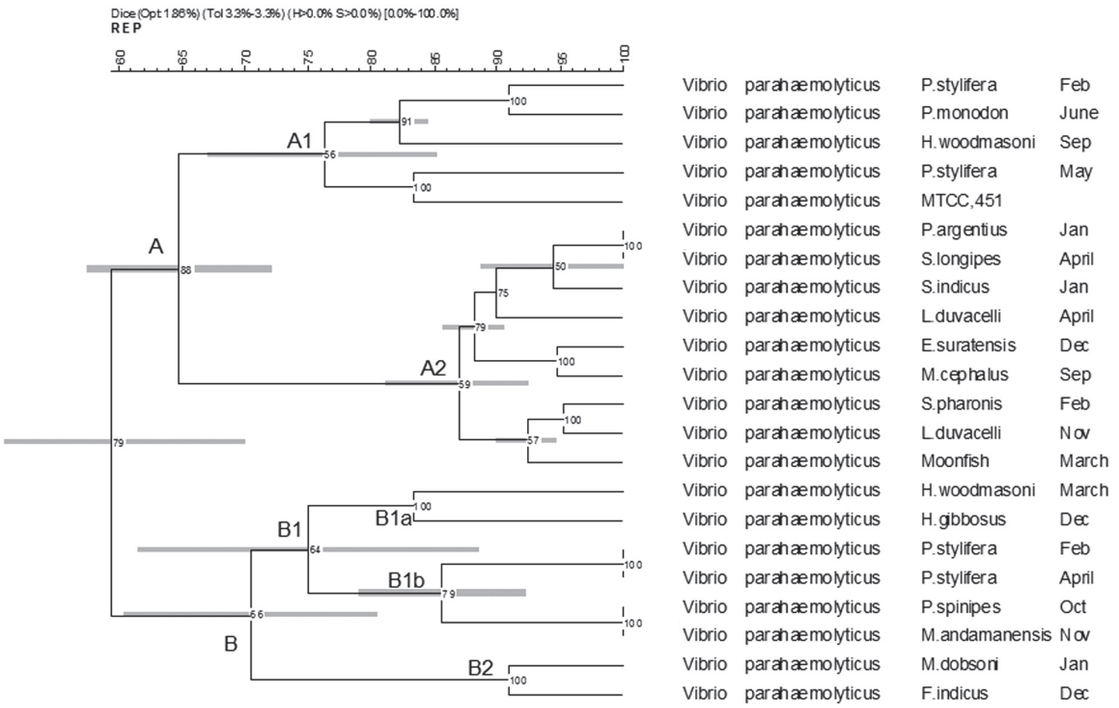


Fig. 6. Phylogenetic analyses of REP profile of *Vibrio parahaemolyticus* isolates from landing centre samples. Bars are shown at each node, corresponding to the standard deviation of values in that region of the similarity matrix. The average and the standard deviation of similarity values for the selected nodes are shown above the dendrogram. The similarity scale is shown above this dendrogram

subspecies typing method for *V. parahaemolyticu*s, the application of these PCR methods (RS, REP, ERIC and RAPD -PCR) for typing of isolates representing different banding patterns was evaluated and the number of bands produced from each source are presented in Table 2.

In the current study, the PCR assays were repeated three times for each *V. parahaemolyticus* strain, and the reproducibility of the banding patterns was verified. Clustering revealed the grouping of isolates based on source of the sample rather than the seasonality of collection.

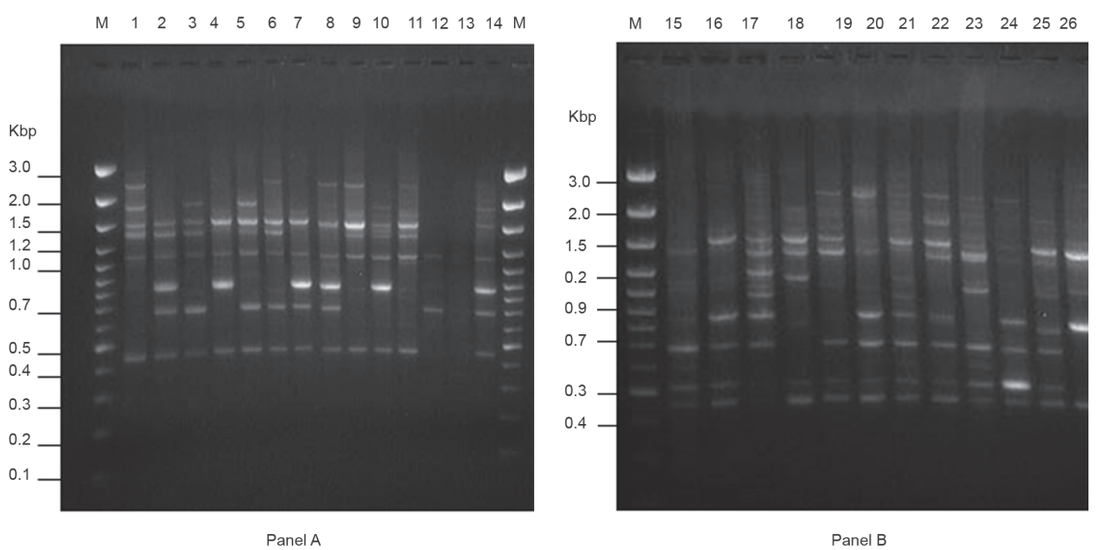


Fig. 7. RAPD-PCR profiles of *Vibrio parahaemolyticus* strains isolated from landing centre samples. Lane M: 100 bp DNA ladder, Lane 1-12 in Panel A and Lane 15-25 in Panel B: strains from different seafood isolates, Lane 13: PCR negative control, Lane 14: *V. parhaemolyticus* type strain (NCMB 1902), Lane 26: *V. parahaemolyticus* type strian (MTCC 451)

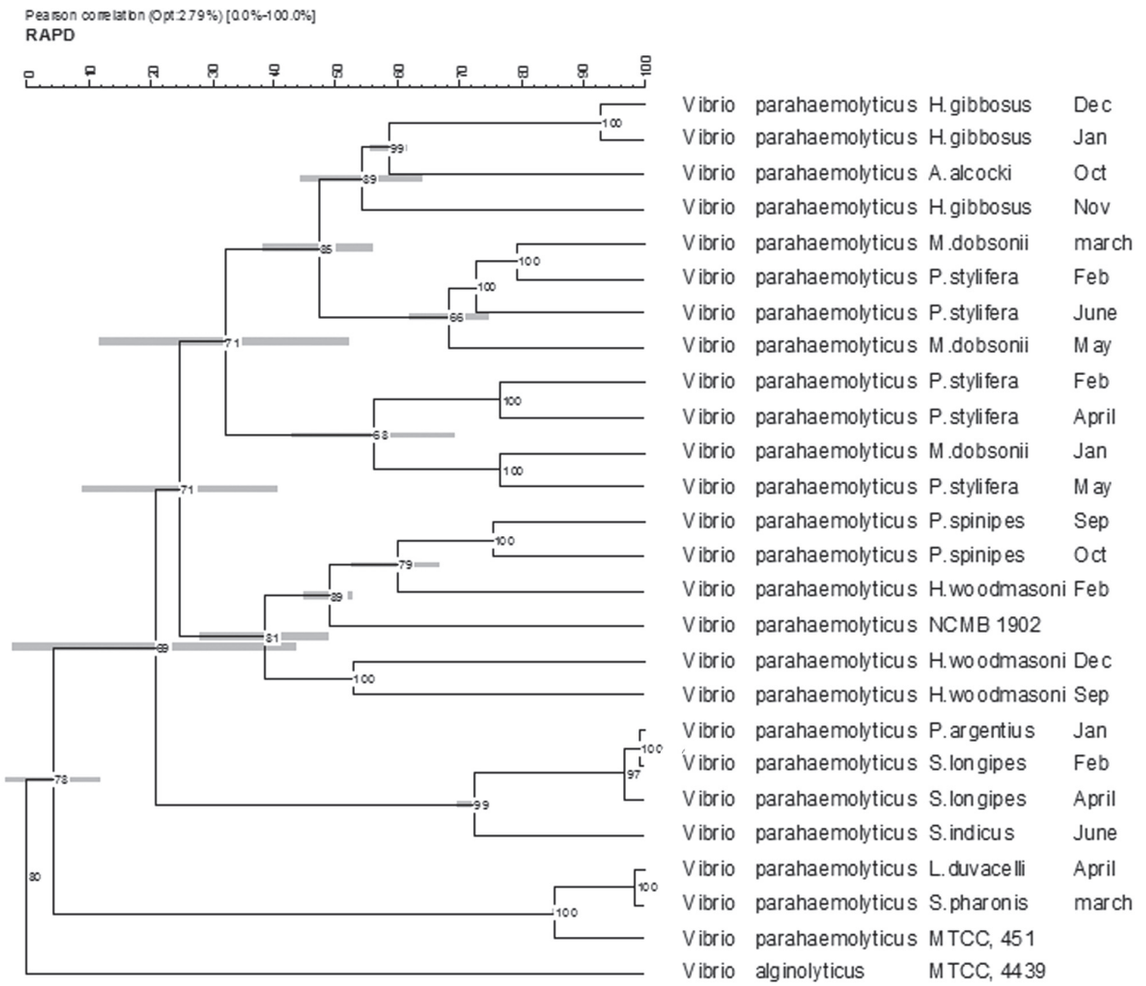


Fig. 8. Dendrogram illustrating the clustering of RAPD profile of *Vibrio parahaemolyticus* isolates from landing centre samples. Bars are shown at each node, corresponding to the standard deviation of values in that region of the similarity matrix. The average and the standard deviation of similarity values for the selected nodes are shown above the dendrogram. The similarity scale is shown above this dendrogram

ERIC-PCR is the most widely adopted method of the three PCR typing methods used, and has been applied for typing of many species, including *V. cholerae* (Shangkuan *et al*., 1997) and *V. parahaemolyticus* (Wong and Lin, 2001). In the present study, ERIC-PCR method generated two major clusters (A & B) of which A1 and A2 represented clusters formed from the finfish and cephalopod isolates. B1 and B2 formed clusters from the strains isolated from shellfish samples suggesting that the strains isolated from finfish samples showed higher genetic similarity with isolates from cephalopods than with shellfish isolates. ERIC PCR was used to specifically identify O3:K6 *V. parahaemolyticus* isolates in less than 6 h in United States (Khan *et al*., 2001). Marshall *et al.* (1999) compared 38 clinical strains of *V. parahaemolyticus* isolates from the 1997 Canadian outbreak using ERIC-PCR. Using a single primer for the amplification, ERIC-PCR was useful for evaluating genetic and epidemiological relationships among *V. parahaemolyticus* strains (Marshall *et al*., 1999). Similar observations were made using REP and RS PCRs but with little variations in the subgroups (Chakraborty and Surendran, 2009). However, their study revealed clustering of toxigenic strains into a single group by RAPD analysis. Both REP-PCR and ERIC-PCR are 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 for many species (Stubbs *et al*., 1999). In earlier works, REP-PCR was used to differentiate toxigenic *V. cholerae* O1 strains from non-toxigenic and non-O1 strains based on the fingerprint profiles.

All the three PCR typing methods described in this study could differentiate *V. parahaemolyticus* from other species (negative control), and effectively project the diversity within various groups of samples. In the present study ERIC-PCR was found to be rapid, relatively easy to perform compared to that of REP-PCR. However, in ERIC-PCR, some of the minor amplification bands were found to be inconsistent, thus complicating pattern differentiation. Similarly, few authors (Maluping *et al*., 2005) also confirmed the presence of ERIC and REP sequences in the genome of this bacterial species but their study indicated that REP-PCR is inferior to RAPD and ERIC-PCR owing to the fact that it is less reproducible. Among the three PCR methods, RS-PCR generated fewer amplification bands compared to REP-PCR and ERIC-PCR, and thus giving scope for higher discrimination (Table 2). However, since the RS-PCR patterns were more easily discernible visually than the REP-PCR or ERIC-PCR patterns, the former can be suggested as a practical method for routine use. Though the discriminative efficiency of these PCR typing methods differed from each other, these methods are effective for typing of *V. parahaemolyticus* strains. Wong and Lin (2001) reported that the results obtained by these PCR methods mirrored those of the PFGE method for some outbreaks, although they differed slightly in other outbreaks. Few earlier studies (Anderson and Ordall, 1972) reported on the similarities among some strains of *V. parahaemolyticus* at molecular genetic level. Recently, Zanetti *et al*. (1999) observed intraspecies variation in *V. alginolyticus* strains using DNA amplification.

DNA-based typing of isolates is of great importance for tracking epidemiological and causal relationships. The ERIC, REP, and RS PCR methds employed in this study to understand the genetic diversity among the isolates, can be used in molecular subspecies typing of *V. parahaemolyticus* independently or as a supplement to other typing methods when very sensitive typing is required. RS-PCR, REP-PCR, and ERIC-PCR are suitable rapid typing methods for *V. parahaemolyticu*s and all the three methods have high discriminative ability. However, ERIC-PCR was found to be superior to REP-PCR owing to its better reproducibility of fingerprints produced with this method. ERIC-PCR method generated two major clusters, one with the finfish and cephalopod isolates and the other with the strains isolated from shellfish samples suggesting that the strains isolated from finfish samples showed higher genetic similarity with the cephalopods than that of shellfish isolates. Nevertheless, RS-PCR seems to be more practical method with fewer amplification bands and patterns, simplifying the interpretation of data and exhibiting high discriminative ability.

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