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Genetic Relatedness of *Vibrio cholerae* O1 and non-O1 by ERIC Polymerase Chain Reaction and Pulsed-Field Gel Electrophoresis

Yuherman[1], Son Radu[2], Gulam Rusul[3], Lum Keang Yeang[4], Ooi Wai Ling[2] and Jamal Khair[5]
[1]Department of Animal Production, Faculty of Animal Science, Universitas Andalas, Kampus Limau Manis, Padang, Indonesia
[2]Department of Biotechnology, [3]Department of Food Science, Faculty of Food Science and Biotechnology, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[4]Center for Strategic, Environment and Natural Resource, Malaysia Agricultural Research and Development Institute, Malaysia
[5]Division of Food Quality Control, Ministry of Health Malaysia, Jalan Dungun, Kuala Lumpur, Malaysia

Abstract: DNA fingerprinting by PCR amplification of enterobacterial repetitive intergenic consensus (ERIC) and pulsed-field gel electrophoresis (PFGE) were used to compare environmental and clinical isolates *of Vibrio cholerae* O1 and non-O1. All the *V. cholerae* O1 and non-O1 isolates were typable using ERIC PCR. Though PFGE generated banding patterns to discriminate the isolates into twelve fingerprints, eight isolates were untypable by PFGE due to consistent degradation of the bacterial DNA. Based on the dendrogram generated from ERIC-PCR method, three of the clinical isolates (C1, C2 and C3) were closely related to environmental isolates (E6 or E10). The results indicate that ERIC-PCR is a very discriminative and efficient method for studying genetic diversity of *V. cholerae* isolates.

Key words: ERIC-PCR, PFGE, *Vibrio cholerae* O1 and non-O1

Introduction

Cholera is a serious epidemic disease and continues to be a major health problem worldwide. *Vibrio cholerae*, the bacterium that causes cholera usually persist in the environment because it can grow in saltwater or in freshwater. This disease is characterized by devastating watery diarrhea which leads to rapid dehydration, often accompanied by vomiting and resulting in hypovolemic shock and acidosis (Salyers and Whitt, 1994; Kaper *et al*., 1995; Faruque *et al*., 1998). Not all strains of *V. cholerae* causes cholera and only a small number of strains have been responsible for all major outbreaks of cholera. Thus, the characterization of *V. cholerae* isolates by typing systems, which allow determination of isolate relatedness is required in epidemiologic investigation. Here, we compared two bacterial DNA-based analysis, enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and pulsed-field gel electrophoresis (PFGE) to study *V. cholerae* O1 and non-O1 isolates from clinical and environmental sources.

Materials and Methods

Bacterial isolates: The *Vibrio cholerae* O1 and non-O1 isolates examined in this study (Fig. 1) has been described previously (Radu *et al*., 1999).

Genomic DNA extraction for ERIC-PCR: Genomic DNA extraction was performed as described. The DNA sequence of ERIC primers employed (ERIC1R, 5'-ATGTAAGCTCCTGGGGATTCAC-3' and ERIC2, 5'-AAGTAAGTGACTGGGGTGAGCG-3') were previously reported by Versalovic *et al*. (1991). Amplification was performed in 25 μl volume containing 2.5 μl 10x reaction buffer, 1 mM each of dNTP, 5 pmol each of the forward and reverse primers, 2.5 U of Taq polymerase, 2 mM $MgCl_2$ and 10 ng of genomic DNA. PCR amplification was done as follows: denaturation at 92°C for 45s, annealing at 52°C for 1 min and elongation at 70°C for 10 min. A final elongation step at 70°C for 20 min at the end of 35 cycles was added. The PCR amplification products were fractionated by electrophoresis through 1.2% agarose gel and detected by staining with ethidium bromide.

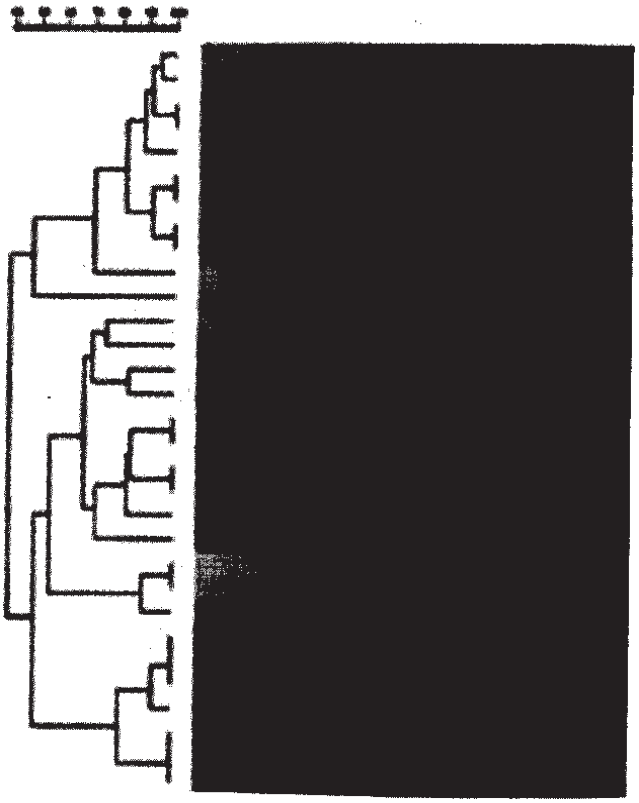

Fig. 1: ERIC PCR dendrogram and fingerprints profiles showing both clinical and environmental *Vibrio cholerae* O1 and non-O1 isolates. Similarity analysis was performed by using the Jeffrey's coefficient and clustering was done by UPGMA. Number in parenthesis indicates PFGE patterns. C, clinical isolates; E, environmental isolates and UT, untypable

Pulsed-field gel electrophoresis: Pelleted washed cells were harvested as described by Thong *et al*. (1996). *Spe*I macrorestriction fragments embedded in agarose were separated on 1% agarose gel by electrophoresis using the CHEF DR III apparatus (Bio-Rad). Electrophoresis was performed at 6 v/cm for 20 h at 14°C. Bacteriophage lambda DNA ladder PFGE marker (New England Biolabs) was used as size marker. Following electrophoresis, the gel was stained with ethidium bromide and DNA bands were visualized with UV transilluminator and photographed.

Results and Discussion

All isolates were typable using the ERIC primers. ERIC-PCR of the genomic DNA from all the *V. cholerae* O1 and non-O1 isolates resulted in amplification of fragments of DNA ranging in sizes between 0.3 to 3.2 kilobase pairs. Eight *V. cholerae* non-O1 isolates from environmental sources (E6, E7, E8, E9, E10, E11, E12 and E13) were not typable by the PFGE method since their DNA content was consistently degraded during digestion steps, even though the samples were run a second or a third time. For the other 23 *V. cholerae* isolates that were typable, eight PFGE patterns were differentiated after restriction by *Spe*I (Fig. 1, see number in parenthesis).

The dendrogram based on ERIC-PCR profiles is shown in Fig. 1. The 31 *V. cholerae* isolates were separated into 4 major clusters containing between one and 13 isolates each. ERIC-PCR was able to differentiate the clinical and environmental isolates as majority of the clinical and environmental clustered together (Fig. 1). However, three of the clinical isolates (C1, C2 and C3) clustered with the environmental isolates. In addition, the ERIC-PCR profiles generally grouped the *V. cholerae* O1 and non-O1 in different clusters. Since 8 of the *V. cholerae* non-O1 isolates were untypable by PFGE method, we could not generate a complete dendrogram for comparative purposes with that of the ERIC-PCR method. Hence, we could not make a proper comparison of the two fingerprinting methods to determine the location or clonal lineages of all isolates with the same ERIC-PCR profiles in the PFGE dendrogram. Therefore, there was no correlation that we can deduced accurately between the two typing methods.

It is interesting to note that apparently identical isolates of *V. cholerae* non-O1 were isolated in very different settings: *V. cholerae* non-O1 isolates no. C1, C2 and C3 are clinical isolates sent to us from the Institute for Medical Research and were recovered from symptomatic patients associated with the outbreaks of diarrhea in Kuala Lumpur; E6 and E10 environmental isolates were from surface water samples from another town, Malacca which is more than 100 km away from Kuala Lumpur. An explanation for these results might include the possibilities that these three clinical isolates could have occurred merely by chance or alternatively the patients might have been infected by the bacteria isolated and typed and originating from the same clonal lineages as the environmental isolates. The ability to use ERIC-PCR to determine isolates relatedness with some degree of accuracy could be used to monitor the epidemiological transmission of a particular isolate.

We are not claiming that PFGE is unreliable or not effective as it is possible that the eight isolates that were untypable when using the restriction enzyme *Spe*I, could be typed by using different restriction enzymes. Elsewhere, Hielm *et al*. (1998) reported that a large number of their *C. botulinum* isolates were undigestable by *Sma*I and suspected that GC methylation as a cause of non-digestion. In addition, Samore *et al*. (1996) suggested that DNA degradation by endonucleases was the cause for non digestion of their *C. difficile* isolates with *Sma*I. In conclusion, our study showed that ERIC-PCR can be used to differentiate environmental and clinical isolates of *V. cholerae* and that molecular types of isolates from the similar sources (clinical or environment) seem to be more closely related, suggesting a lesser degree of genetic diversity related to source of isolation.

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