Prevalence and Genetic Characterization of *Vibrio vulnificus* in Raw Seafood and Seawater in Malaysia

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**ABSTRACT**

*Vibrio vulnificus* is a highly invasive human pathogen that exists naturally in estuarine environment and coastal waters. In this study, we used different PCR assays to detect *V. vulnificus* in 260 seafood samples and 80 seawater samples. *V. vulnificus* was present in about 34 (13%) of the 260 seafood samples and 18 (23%) of the 80 seawater samples. Repetitive extragenic palindromic PCR (REP-PCR) and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) were applied to subtype the *V. vulnificus* isolates. Twenty-five REP profiles and 45 ERIC profiles were observed, and the isolates were categorized into 9 and 10 distinct clusters at the similarity of 80%, by REP-PCR and ERIC-PCR, respectively. ERIC-PCR is more discriminative than REP-PCR in subtyping *V. vulnificus*, demonstrating high genetic diversity among the isolates.

*Vibrio vulnificus*, a gram-negative halophilic bacterium of the family *Vibrionaceae*, is a worldwide inhabitant in the estuarine environment and coastal waters. *V. vulnificus* has been detected in oyster, shellfish, and the intestinal contents of fish (1, 4, 10, 13, 14, 19, 20). The infection and fatality rate caused by *V. vulnificus* is extremely high (40 to 60%) among people who have liver or gastric problems and immunodeficiencies (14). It can also cause severe wound infections in individuals who have skin lesions, preexisting cuts, and skin punctures. Like other vibrios, *V. vulnificus* is primarily transmitted to humans through consumption of raw or improperly cooked seafood harvested from waters harboring the organism, or by exposure of open wounds to warm seawater (9, 15).

Conventional culture methods for isolation and identification of *Vibrio* species include the use of selective media like CHROMagar *Vibrio*, followed by biochemical tests (21). However, these methods take several days to obtain definitive results at the species level. The culture method is also limited in identifying *V. vulnificus* when it is in a viable but nonculturable state. Hence, molecular methods such as PCR are broadly used because of their simplicity, rapidity, and ease of operation (2, 6, 16, 24). Brauns et al. (6) and Kumar et al. (16) applied monoplex PCR amplifications targeting the cytotoxin-hemolysin gene and gyrB gene, respectively, to detect *V. vulnificus* DNA. In 2010, Teh et al. (24) developed a multiplex PCR targeting gyrB gene for simultaneous identification of *Vibrio* spp. and *pmnA* genes for *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*.

Studies on molecular typing and genomic variation of *V. vulnificus* are helpful for epidemiological surveillance of this pathogen. Previous studies have used repetitive extragenic palindromic PCR (REP-PCR) and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) to investigate the relatedness among isolates and to track sources and the spread of *V. vulnificus* (7, 15).

The aims of this study were to determine the prevalence of *V. vulnificus* in different types of raw seafood marketed in retail stores and in seawater samples collected from different parts of Malaysia and to use REP-PCR and ERIC-PCR assays for analysis of variability among the *V. vulnificus* isolates.

**MATERIALS AND METHODS**

Isolation and identification of *V. vulnificus* on CHROMagar *Vibrio*. Two hundred sixty raw seafood samples, including fish, shrimps, prawns, cockles, oysters, clams, and squids, were purchased from different retail stores in Kuala Lumpur and Petaling Jaya, Malaysia, from August 2010 to May 2011. Eighty seawater samples were also collected from various locations in Port Dickson, Seri Kembangan, and Pahang, Malaysia, from February to April 2011. The samples were analyzed for the presence of *V. vulnificus*, using a previously described method (11).

In brief, seafood samples (25 g) or seawater samples (25 ml) were homogenized in 225 ml of alkaline peptone water and incubated for at least 6 h at 37°C. A loopful of enriched broth culture was streaked onto the surface of CHROMagar *Vibrio* (CHROMagar, Paris, France) and incubated overnight at 37°C. Presumptive *V. vulnificus* colonies (mauve) were transferred to Luria-Bertani broth containing 2.5% NaCl for further confirmation.

Identification of *V. vulnificus* by biochemical tests and API 20E. A series of conventional biochemical tests, including

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