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Prevalence and characterisation of potentially virulent *Vibrio parahaemolyticus* in seafood in Malaysia using conventional methods, PCR and REP-PCR

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ABSTRACT

A previously developed multiplex PCR targeting *gyrB* of Vibrios at genus level and *pntA* genes for specific detection of *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* was evaluated. The sensitivity of the multiplex PCR on spiked seafood was 1.5×10^3 CFU g^{-1}. One hundred and fifty seafood samples were collected from retail stores and hypermarkets in different locations in Kuala Lumpur, Petaling Jaya and Seri Kembangan. The prevalence of *V. parahaemolyticus* was 29% (43/150). The *pntA* primers for *V. parahaemolyticus* detection were 100% specific and comparable to the *toxR* gene-based PCR. Six (12%) and 2 (4%) isolates contained *trh* and *tdh* genes, respectively. Repetitive Extragenic Palindromic PCR (REP-PCR) was used to genetically characterize the *V. parahaemolyticus* isolates in which 41 REP profiles were observed and all the isolates were categorized into 11 distinct clusters at the similarity of 80%. *tdh*-positive isolates shared a low level of similarity with *trh*-positive isolates. The prevalence of *V. parahaemolyticus* and particularly the presence of virulent gene such as *trh* and *tdh* among the isolates reiterate a high risk of contamination for seafood consumers in Malaysia. DNA fingerprinting of *V. parahaemolyticus* in this study indicates a high genetic diversity among the isolates and REP-PCR was able to distinguish the isolates with different virulotypes.

1. Introduction

Vibrio parahaemolyticus is an important human pathogen that is widely distributed in estuarine and marine environments. *V. parahaemolyticus* is associated with gastroenteritis when contaminated raw or insufficiently cooked seafood are consumed (Özer et al., 2008; Pruzzo, Huq, Colwell, & Donelli, 2005). Since this species is highly abundant in marine products, they have become a major concern in the production and trade of seafood (DePaola, Nordstrom, Bowersm, Wells, & Cook, 2003).

In Southeast Asian countries, including Malaysia, there is a high probability of *V. parahaemolyticus* outbreaks, due to the ambient optimum temperature and appropriate climate for the growth of this species (Elhadi, Radu, Chen, & Nishibuchi, 2004; Okuda et al., 1997). Virulent *V. parahaemolyticus* isolates in raw seafood have also been reported in Malaysia (Sujeewa, Norrakiah, & Laina, 2009). Thus, the prevalence of pathogenic Vibrios in seafood is of public health concern.

Conventional methods of isolation and identification of *Vibrio* spp. involve the presumptive identification based on selective media such as thiosulfate-citrate-bile salts-sucrose agar (TCBS) and CHROMagar™ *Vibrio* followed by biochemical tests, including oxidase, Triple Sugar Iron (TSI), Sulfur reduction-Indole-Motility (SIM), Methyl Red (MR) and Voges–Proskauer (VP), and salt tolerance tests (Barrow & Feltham, 1993; Kaysner & Depaola, 2004). As the conventional methods are time consuming and lack sensitivity, molecular approaches have been developed and utilized as an alternative approach due to their higher sensitivity and specificity. Teh, Chua, and Thong (2010) reported a simple multiplex PCR targeting *gyrB* and *pntA* gene to differentiate *V. parahaemolyticus*, *Vibrio cholerae*, *Vibrio vulnificus* and other *Vibrio* spp. This multiplex PCR demonstrated high specificity in *Vibrio* species identification but has yet to be evaluated for its sensitivity in seafood, the main source of Vibrios infection.

Although the function of *toxR* gene is to stimulate the expression of thermostable direct haemolysin gene (*tdh*), it is present in either pathogenic or non-pathogenic *V. parahaemolyticus* isolates (Sujeewa et al., 2009). Therefore, it has been used for identification of *V. parahaemolyticus* (Sechi, Dupre, Deriu, Fadda, & Zanetti, 2000). Conversely, the presence of *tdh* gene and/or the *tdh-related haemolysin* gene (*trh*) determines the virulence and pathogenicity of

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