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Beyond Classical Approaches

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COMPARISON OF MOLECULAR TYPING OF *VIBRIO PARAHAEMOLYTICUS* ISOLATES USING RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) AND ENTEROBACTERIAL REPETITIVE INTERGENIC CONSENSUS – POLYMERASE CHAIN REACTION (ERIC-PCR) FINGERPRINTING.

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ABSTRACT

A total of 62 *Vibrio parahaemolyticus* isolated from local cockles (*Anadara granosa*) collected from Tanjong Karang, Kuala Selangor were characterized using Randomly Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD-PCR) and Enterobacterial Repetitive Intergenic Consensus- Polymerase Chain Reaction (ERIC-PCR) assays. The RAPD analysis in this study revealed heterogeneous isolates of *V. parahaemolyticus* in cockles by using two primers (GEN1-50-03 and GEN1-50-04). Additionally, highly reproducible fingerprints for the isolates unique for each of the isolates were observed using ERIC-PCR. Results from this study demonstrated that genotyping *V. parahaemolyticus* isolates by using RAPD and ERIC-PCR is feasible for differentiation of various strains. Both of ERIC-PCR and RAPD-PCR have shown to be rapid, sensitive and discriminative in typing the *V. parahaemolyticus* isolates from cockles.

Keywords: *V. parahaemolyticus*, cockles, RAPD-PCR, ERIC-PCR

INTRODUCTION

Vibrio parahaemolyticus is a Gram-negative marine bacterium that can cause seafood-borne gastroenteritis and traveler's diarrhea in humans after they consumed contaminated raw or partially cooked fish or shellfish, particularly oysters (Khan *et al.*, 2002). Outbreaks of *V. parahaemolyticus* food poisoning are most common in Japan and Southeast Asia, and they occurred occasionally in other parts of the world (Nishibuchi, 2004), and were associated with diverse serovars.

Reliable molecular methods have been developed for the subspecies typing of *V. parahaemolyticus* (Wong *et al.*, 1999; Wong and Lin, 2001; Khan *et al.*, 2002). This is where DNA-based procedures like Polymerase Chain Reaction (PCR) or DNA-sequencing approaches helped epidemiological investigations to be conducted more rapidly and thoroughly (Woodford and Johnson, 1998). RAPD analysis and ERIC-PCR are the two commonly used methods in PCR.

Nowadays, molecular typing methods are necessary for proving the similarity between the isolates. The aim of the present investigation was to use RAPD and ERIC-PCR assays to generate polymorphism in DNA patterns amenable to the differentiation of the *V. parahaemolyticus* strains isolated from cockles.

MATERIALS AND METHODS

Genomic DNA Isolation

Prior to amplification, genomic DNA of the sixty two *V. parahaemolyticus* strains were extracted by mini-preparation method of Ausubel *et al.*, (1987).

RAPD-PCR Primers and Amplification

Ten random primers of 10-mer were screened and three primers as shown in Table 1 were selected for further study as they provided reproducible and discriminatory patterns. Amplification reactions were performed in 25 µl volume containing 2.5 mM $MgCl_2$, 200 µM each dNTPs (Promega), 0.5 µM primer, 1.25 Units of *Taq* polymerase, 10-20 ng of genomic DNA. Amplifications were carried out in the thermal cycler (Perkin Elmer