

Random amplified polymorphic DNA-PCR and ERIC PCR analysis on *Vibrio parahaemolyticus* isolated from cockles in Padang, Indonesia

¹Zulkifli, Y., ^{1*}Alitheen, N.B., ²Son, R., Raha, A.R., ⁴Samuel, L.,
¹Yeap, S.K. and ³Nishibuchi, M.

¹Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia,

²Center of Excellence for Food Safety Research, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia,

³Center for Southeast Asian Studies, Kyoto University, 46 Shimoadachi, Yoshida, Sakyo-ku, Kyoto 606-8501, Japan.

⁴Infectious Diseases Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Federal Territory, Malaysia

Abstract: In this study, RAPD-PCR and ERIC-PCR were used to study the epidemiology of *V. parahaemolyticus* isolated from cockles in Padang, Indonesia. The Gold Oligo OPAR3 primer produced bands ranged from 1-8 with sizes from 0.2 – 5.0 kb and the Gold Oligo OPAR8 primer produced 1-7 bands with sizes 0.7 – 1.5 kb. Both primers produced twenty five RAPD patterns with a few isolates failed to produce any products. Based on phylogenetic dendrogram, all the isolates can be divided into 6 major clusters with similarity between 0 to 52%. For the ERIC primer, it produced bands ranged from 3-15 with sizes from 0.1 – 5.0 kb and twenty seven different ERIC patterns. Construction of the phylogenetic dendrogram showed the isolates can be divided into 4 major clusters with similarity between 56 to 86%. The high diversity of both processes may be due to the multiple contamination sources of *V. parahaemolyticus*.

Keywords: *V. parahaemolyticus*, seafood, RAPD-PCR, ERIC-PCR, genomic fingerprinting

Introduction

Vibrio parahaemolyticus is one of the most widely recognized pathogenic *Vibrio* species due to numerous outbreaks and its wide occurrence in marine environment (Joseph *et al.*, 1983; Chiou *et al.* 1991; Mead *et al.*, 1999). This microorganism can be found in high number during summer in the United States and Europe, but all year round in Southeast Asian. The genus *Vibrio* consists of 28 species and twelve of them are recognized as human pathogens. The major species that contributed to the pathogenesis are *V. parahaemolyticus*, *V. cholerae*, *V. vulnificus*, and *V. alginolyticus*. In recent years, an increased awareness of the infections of some other *Vibrio* spp., including *V. mimicus*, *V. fluvialis*, *V. hollisae*, and *V. damsela* have been witnessed (Baffone *et al.*, 2000). Totally, *V. parahaemolyticus* has been implicated as a cause of at least a quarter of total food borne diseases caused by vibrios (Feldhusen, 2000).

DNA fingerprinting started when Jeffreys *et al.* (1985) developed minisatellite DNA analysis in humans that detected segments of DNA that are highly variable in human populations. The term

DNA fingerprint was coined to describe these unique patterns because they can be used in a manner similar to that of a true fingerprint to identify an individual. DNA fingerprinting is the identification of individual based on DNA markers. The patterns detected in DNA fingerprinting are unique to each individual with the exception of identical twins who share the same DNA fingerprints (Fairbanks and Andersen, 1999).

Development of Random Amplified Polymorphic DNA (RAPD) or arbitrarily primed PCR fingerprinting gave an advantage in which molecular preliminary information of the species studied is not necessary (Welsh and McClelland, 1990) and polymorphism pattern obtained usually varies among the species. In the previous studies, RAPD-PCR has been successfully used for genetic fingerprinting and molecular typing for many species, including fingerprinting of man (Jeffreys *et al.*, 1985), animals (Kostia *et al.*, 1996; Saez *et al.*, 2004), plant (Welsh and McClelland, 1990), microorganism such as *Lactobacillus*, *Salmonella*, *E. coli*, yeast, *Bacillus* (Miyata *et al.*, 1995; Giraffa *et al.*, 2004; Elegado *et al.*, 2004; Rengua-Mangia *et al.*, 2004; Foschino *et al.*, 2004; Svensson *et al.*, 2004)

*Corresponding author.

Email: noorjahan@biotech.upm.edu.my

Tel: +603-8946 7471 ; Fax: +603-8946 7593