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Development of a SYBR green based real-time polymerase chain reaction assay for specific detection and quantification of *Vibrio parahaemolyticus* from food and environmental samples

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Abstract

Vibrio parahaemolyticus is a foodborne pathogen and their human infection is regularly associated with the consumption of raw or undercooked seafood and contaminated water supplies. Many conventional biochemical identification and confirmation procedures are performed to detect the presence of this pathogen, both from seafood or environmental samples. However, these procedures not only require two or more days to complete, they do not have the capabilities to determine the number of *V. parahaemolyticus* cells in any given samples. Thus, in this study we describe the development of a rapid SYBR green based real-time PCR assay, targeting the thermo labile (*tl*) gene of *V. parahaemolyticus* for the detection and enumeration of this bacterium from seafood and environmental samples. We report that the real-time PCR assay and the primers designed are highly specific, and only generated the desired amplicons with *V. parahaemolyticus* DNA samples against other bacteria and fungi species. Our assay is also highly sensitive, and, is able to detect *V. parahaemolyticus* with high coefficient values in concentrations as low as 1.0 pg/µl DNA for pure genomic DNA solutions and 10 cells/ml in serially diluted cell suspension and spiked samples. This assay can be completed in less than 3 hours and may be used as a tool for rapid determination of *V. parahaemolyticus* densities in the food industries, environmental risk assessment and for clinical diagnostics purposes.

Introduction

Vibrio spp. have been recognized as one of the leading cause of foodborne outbreaks in the Asian region countries such as Malaysia, Japan, China, India, Taiwan and Korea (Lesley *et al.*, 2011; Nelapati *et al.*, 2012; Theethakaew *et al.*, 2013). Among the genus *Vibrio*, *Vibrio parahaemolyticus* is one of the most prominent species associated with human illness (Lesley *et al*., 2011). Formerly known as *Pasteurella parahaemolytica*, this bacterium was first isolated in Japan in 1950 and has since been detected all around the world (Lesley *et al.*, 2011; Bechlars *et al.*, 2013; Stephens *et al.*, 2013).

V. parahaemolyticus is an enteric pathogen that can cause gastroenteritis in human, usually after the ingestion of undercooked or mishandled seafood (Stephens *et al.*, 2013). It is a halophilic (salt-requiring), non-sporing, Gram-negative facultatively anaerobic bacterium that lives naturally in brackish saltwater worldwide (Johnson *et al.*, 2010; Broberg *et al*., 2011; Quiroz-Guzmán *et al*., 2013). This bacterium is an invasive organism affecting primarily the colon. *V. parahaemolyticus* grows optimally in 37°C in which it can reach generation time up to 8 or 9 times in water and 12 to 18 times in seafood (Nelapati *et al*., 2012). Symptoms of gastroenteritis caused by *V. parahaemolyticus* include watery diarrhea, abdominal cramps, vomiting, headache, chills, nausea and fever (Sakata *et al*., 2012). The pathogenesis of *V. parahaemolyticus* is based on the presence of several commonly documented virulence factors, namely thermo labile (*tl*), thermo stable direct hemolysin (*tdh*) and tdh-related hemolysin (*trh*) genes (Kim *et al*., 2008; Rizvi and Bej, 2010; Theethakaew *et al*., 2013).

To date, many biochemical identification and confirmation procedures are performed to detect and confirm the presence of this pathogen, especially from seafood samples. However, these procedures require 2 or more days to complete (Roman *et al.*, 2011; Sakata *et al*., 2012). Standard PCR is also a method of choice, in which rapid detection of pathogens in biological materials can be achieved. However, it does not allow quantitative analysis of template DNA or cell concentrations (Gao *et al*., 2004). More recently, real-time Polymerase Chain Reaction (real-time PCR) have been applied on the studies of *V. parahaemolyticus* (Cai *et al*., 2006; Rizvi and Bej, 2010). Real-time PCR is not only more sensitive,

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