Two Rapid Methods to Identify Three Species of Pathogenic Vibrio in *Penaeus vannamei*

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**Abstract**

*Vibrio* species frequently infect *Penaeus vannamei* causing great economic losses to the Whiteleg shrimp industry. Rapid detection of pathogenic *Vibrio* infection would improve the fight against these diseases. In this study, single and multiple polymerase chain reaction (PCR) methods were developed to detect three species of pathogenic *Vibrio*: *Vibrio fluvialis*, *Vibrio anguillarum*, and *Vibrio alginolyticus*. Specific primers were designed for the toxR gene of *V. fluvialis*, the flaA gene of *V. anguillarum*, and the pyrH gene of *V. alginolyticus*. The bacteria were used as templates to establish a 25 µL reaction system for PCR amplification. The results showed that single and specific PCR amplification products of expected sizes were obtained (228bp, 1665bp, and 383bp, respectively). The lowest concentration detected for the three *Vibrio* species were $5.21 \times 10^2$, $2.70 \times 10^4$, and $2.48 \times 10^2$ colony forming units (cfu)/mL, respectively. We also developed a multiplex PCR method to identify the three *Vibrio* species accurately, and with improved identification efficiency. In addition, quantitative real-time PCR (qPCR) was used to identify the minimum detectable DNA concentration for the three *Vibrio* species ($1.0 \times 10^{-6}$ nmol/L for *V. fluvialis*, $1.0 \times 10^{-7}$ nmol/L for *V. anguillarum*, and $1.0 \times 10^{-8}$ nmol/L for *V. alginolyticus*). Technical requirements for ordinary PCR are low, therefore PCR is a feasible technique to detect and diagnose *Penaeus vannamei* bacterial disease.

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