Abstract

Vibrio vulnificus and several other *Vibrio* spp. are autochthonous microorganisms in Gulf of Mexico water (gulf water). The current study describes the development, optimization, and application of state-of-the-art DNA and protein-based detection methodologies that would allow for rapid, specific, and sensitive identification of *V. vulnificus* and selected clinically important *Vibrio* spp. in shellfish. Also, the overexpression of the DEAD-box protein identified at 4°C provided an insight into the cold-adaptive response in *V. vulnificus* , which questioned the existence of a quiescent state in *V. vulnificus* . The SYBR Green I ® and TaqMan-based real-time PCR assays exhibited specific detection of 84 *V. vulnificus* isolates using oligonucleotide primers for *vvh* . The minimum level of detection by the real-time PCR methods were 1 pg of purified genomic DNA or 10^{2}-10^{3} *V. vulnificus* in 1 g of unenriched oyster tissue homogenate or 10 ml of gulf water. This detection level was improved to one *V. vulnificus* following 5 h of enrichment. The entire detection method, including sample processing, enrichment, and real-time PCR amplification, was completed within 8 h, making it a rapid single-day assay. The multiplex PCR assay, using *vvh* and *viuB* as targets, successfully distinguished the clinical and the environmental strains of *V. vulnificus* . Its application on enriched natural oysters detected the presence of pathogenic *V. vulnificus* in 15% of the *vvh* positive samples. Next, the gene-specific DNA microarray coupled with multiplexed PCR offered a specific and sensitive (1 CFU/gm) method for comprehensive detection of total and pathogenic *V. vulnificus, V. cholerae* , and *V. parahaemolyticus* in shellfish and gulf water. This method proved to be an improvement over the Covalink(TM) NH hybridization assay. Using phage-displayed random peptide library and FACS analysis, a novel peptide was identified that could be used in the development of an "intact-cell"-based detection assay for clinically important *Vibrio* spp. The rapid and sensitive detection of *V. vulnificus* and other pathogenic *Vibrio* spp., in compliance with the Interstate Shellfish Sanitation Conference (ISSC) guidelines, would help ensure a steady supply of "safe-to-eat" postharvest treated oysters to consumers and thereby reduce *Vibrio* -associated illnesses or outbreaks.