

## Duplex PCR assay for the species-specific detection of the marine pathogen, *Vibrio alginolyticus*, using *dnaJ* and *ompK* genes

### ABSTRACT

*Vibrio alginolyticus*, is an important opportunistic pathogen for worldwide aquatic animals and marine environment. However, the microbiological methods like culture-based diagnosis and biochemical identification of *V. alginolyticus* is time consuming and unspecific. Thus, the aim of the study was to develop a duplex PCR assay for the species-specific identification of *V. alginolyticus*. To evaluate PCR specificity, this assay directed toward the *ompK*-virulence gene and *dnaJ* gene tested on six *Vibrio* species and three non- *Vibrio* species. Two specific bands with the expected sizes of 846 bp and 144 bp, respectively, were produced in isolates belong to *V. alginolyticus* and only one band were produced by others *Vibrio* species, 846 bp for *ompK* gene indicating a high specificity of duplex PCR assay. The sensitivity test of duplex PCR was detected by using different cells concentration of *V. alginolyticus*. The detecting capability of the duplex PCR from crude DNA was at 10<sup>2</sup> and 10<sup>3</sup> cells/mL. The sensitivity and efficacy of the assay were clarified using artificially infected *Artemia* and water culture which a clear PCR bands of 846 bp and 144 bp were generated from *Artemia* homogenates and water culture infected with *V. alginolyticus*. Our results showed that this newly developed duplex PCR would offer an accuracy and ideal tool for species-specific detection of *V. alginolyticus*in preventing disease outbreak in marine aquaculture.

**Keyword:** -