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 102 and 103 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. alginolyticusin preventing disease outbreak in marine aquaculture.

Keyword: -