Abstract

Research Title : Detection and Identification of *Vibrio harveyi*, *V. parahaemolyticus*and *V. vulnificus*in Marine Shellfish by Using Multiplex PCR

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Year : 2016

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A Multiplex PCR was developed for simultaneous identification of three major pathogenic *Vibrio* species, including *V. harveyi*, *V. parahaemolyticus* and *V. vulnificus*. Three pairs of specific primer were located in *vhhP2* gene (157 bp), *tlh* gene (450 bp) and *rpoS* gene (273 bp), which were corresponded to *V. harveyi*, *V. parahaemolyticus* and *V. vulnificus*, respectively. The ice-cold storage of life mussel (*Pernaviridis*), blood clam (*Tegillarcagranosa*) and baby clam (*Paphiaundulata*) were purchased from five local markets in Bangkok. One gram of flesh from each sample was dissected out, minced and homogenized with glass homogenizer in 9 ml of 2% NaCl Alkaline peptone water and then incubated at 37°C. 1 ml of homogenized samples on 0, 3, and 6 hours were diluted in ten-fold serial dilution and 100 µl of each dilution was spread on TCBS agar plate and incubated at 37°C overnight for colony counting. The colonies with different morphology from each homogenate sample were collected with sterile tooth pick, dissolved in phosphate buffer saline and immediately extracted DNA (NaOH/Tris-HCl) before storage at -20°C prior to testing by triplex PCR method. The detection limit of this method for *Vibrio* detection was estimated at 10^4CFU/g of shellfish sample. Accuracy of Multiplex PCR method was same as conventional method, however, more rapid and eases to interpretation. In conclusion, this developed triplex PCR should be suitable for simultaneous detection of *Vibrio* species for food safety assessment and monitor distribution of food-borne pathogen.