Simultaneous detection of Photobacteriumdamselae, Vibrio alginolyticus, Vibrio harveyi and Vibrio parahaemolyticus using multiplex PCR amplification method

Abstract

The aim of this study was to develop a multiplex PCR amplification method that simultaneously detects the presence of four bacterial pathogens (Photobacteriumdamselae, V. alginolyticus, V. harveyi and V. parahaemolyticus), which are often synergistically caused disease to culture fish throughout the tropical waters, and occasionally cause food poisoning and wound infection to human. Specific multiplex PCR primers targeting conserve regions of virulence genes of the pathogens were designed and tested against different concentrations of MgCl and annealing temperatures. In addition to specificity against different bacterial species, the multiplex PCR was also tested against tissue and environmental samples known to harbor the pathogens. The result showed that the multiplex PCR was highly specific to the target pathogens. The optimum MgCl2 concentration and annealing temperature for successful multiplex PCR amplification of the pathogens were at5.0 mM and 56 °C, respectively. The detection limit of the multiplex PCR was at 10 pg of DNA template. Although the concentration of the pathogens in the environment is often lower, enrichment with tryptic soy broth supplemented with 2% NaCl (w/v) has shown to enhance the growth of the bacterial pathogens and hence improved detection. The rapidity, simplicity and cost-effectiveness of the multiplex PCR amplification method described in this paper provide a useful biosecurity tool for the determination of the pathogens in aquaculture farms and seafood processing industries throughout the tropical countries.