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Identification of *Vibrio* Species isolated from Marine Fish using Polymerase Chain Reaction

Muhamad Faiz Bahari & [1]Sabri Mohd Yusoff
[1]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia

Abstract

Vibrio species are found in marine and estuarine environments. Vibriosis can cause more than 50% mortality in fish culture facilities once an outbreak is in progress. The objectives of this study were to subculture and identify *Vibrio* spp. that were isolated previously from marine fish; to develop a technique for simultaneous identification of several *Vibrio* spp. (*V. alginolyticus, V. parahaemolyticus, V. fluvialis* and *V. vulnificus*) using polymerase chain reaction (PCR); and to compare the rapid identification kit and PCRtechniques commonly used for the identification of the *Vibrio* spp. In this study, 20 isolates from four *Vibrio* species, which consisted of five eachof V. *alginolyticus, V. parahaemolyticus, V. fluvialis* and *V. vulnificus* isolates were provided by National Fish Health Research Centre (NaFisH). The species of *Vibrio* were identified using an identification kit, API 20E system. These organisms were isolated from various marine fish such as Asian Seabass (*Latescalcarifer*), Grouper (*Epinepheluscoioides*), Silver Pomfret (*Pampusargenteus*) and Red Snapper (*Lutjanuscampechanus*). The isolates were previously stored at -80˚C and subcultured onto TSA+. The pure cultures were then transferred to TSB+. These isolates were subjected to DNA extraction. Once the DNA is ready, PCR was used to optimise the products with the designated primers. All the PCR products were electrophoresed through 1% agarose gel for 1 h. The designated primers in this study were found suitable for the detection of *V. alginolyticus,V. parahaemolyticus,V. fluvialis* and *V. vulnificus*.Using the API 20E system, 15% (3/20) isolatesof *Vibrio* spp. were negative, indicating the the PCR technique is still required to confirm the result obtained by the use of the API 20E system.

Keywords: *Vibrio* species, Vibriosis, marine fish, PCR