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Extracellular Enzymes Produced by *Vibrio alginolyticus* Isolated from Environments and Diseased Aquatic Animals

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Abstract

A total of 17 *Vibrio alginolyticus* isolates were obtained from environments, diseased fish and shrimp using CHROMagar Vibrio and confirmed by biochemical tests and PCR targeted to the *gyrB* gene. They were investigated for production of exoenzymes. All of the isolates from diseased fish and shrimp (n = 8) showed gelatinase, lecithinase, and caseinase activities, while 75% (6/8) of them possessed amylase and lipase activities. In environmental isolates (n = 9), the gelatinase and lecithinase activities were detected in all isolates. Four out of nine isolates (44%) possessed lipase, while 67% (6/9) of environmental isolates were positive for both caseinase and amylase activities. Interestingly, α-hemolysin activity was detected in all *V. alginolyticus* isolates from diseased fish and shrimp but it was detected in only 44% (4/9) of the environmental isolates, suggesting that they might be involved in bacterial pathogenesis. The arbitrarily primed polymerase chain reaction (AP-PCR) technique showed distinct DNA profiles of all isolates consisting of 7 to 11 bands ranging from 0.3 – 6.0 Kb (with a common band of 1.2 Kb). Fourteen DNA profiles were obtained from a dendrogram analysis with 20% maximum similarity. These results indicate genetically heterogenicity among *V. alginolyticus.*

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Keywords: Vibrio alginolyticus; exoenzymes; AP-PCR

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Nomenclature

AP-PCR	Arbitrarily primed polymerase chain reaction
ECPs	Extracellular products
PCR	Polymerase chain reaction
ng	nanogram
µl	microliter
rpm	revolutions per minute

1. Introduction

Vibrio alginolyticus is a member of the family Vibrionaceae, which are Gram negative bacteria with a curved rod shape, that are motile, non-spore-forming and that grow in 10% NaCl. It can be isolated from diseased marine-cultured animals with clinical symptoms of bacterial septicaemia and skin ulcer[1]. This bacterium is also an important opportunistic bacterial pathogen associated with seafood-borne infections in humans and is a normal inhabitant of estuarine and marine environments[2]. The pathogenesis for marine animals and humans following infection has been associated with only the virulent strains of *V. alginolyticus*. However, the avirulent strains do not infect and do not cause disease. Some genes may determine strain-specific characteristics such as virulence factors[3]. Extracellular products (EPSs) such as chitinases, hemolysins, alkaline proteases, cysteine proteases, alkaline metal-chelator-sensitive proteases, serine proteases and metalloproteases have been isolated from cell-free culture supernatants (CFS) of *V. harveyi, V. anguillarum, V. alginolyticus* and other species. These ECP have been proposed as virulence factors for fish and other marine organisms[4]. However, the structural and functional characteristics of the other genes of *V. alginolyticus* that encode exotoxins associated with fish and shrimp diseases are poorly known and represented in the DNA and Amino Acid International databases[5]. Characterization of pathogenic *V. alginolyticus* strains and their toxic ECP is a prerequisite for better understanding of pathogenesis, mechanisms of infection and control.

Arbitrarily primed polymerase chain reaction (AP-PCR) also referred to as the random amplified polymorphic DNA is a PCR-based technique for typing bacterial genomic DNA. This technique was first developed by John Welsh in 1990[6]. The technique is used for molecular epidemiological analysis because it is easy and fast, and it is also used for identification of strain-specific variations in DNA or specific fingerprints of bacteria[7]. AP-PCR is a PCR-based method that uses a short single primer (usually 10 bp) to amplify anonymous stretches of DNA. With this technique, there is no specific target DNA, so each particular primer will randomly anneal to the template DNA[8]. The DNA fragments generated are then separated and detected by gel electrophoresis.

In this study, 17 *V. alginolyticus* strains associated with diseased fish and shrimp in aquaculture farms and environments were isolated using CHROMagar Vibrio and confirmed by biochemical tests and PCR targeted to the *gyrB* gene. These strains were investigated for exoenzyme production and their genetic diversity was evaluated by AP-PCR.

2. Methods

2.1 Isolation of V. alginolyticus

For environmental isolates, six samples of water and three samples of sediments were collected from a fish aquaculture area at Khong-Jilhad, Krabi province, Thailand. The samples were enriched in alkaline peptone water (APW) and incubated at 37°C for 6-8 h. A loopful of culture broth was spread on CHROMagar Vibrio and incubated at 37°C for 18-24 h. After incubation, the presumptive milky white colonies on CV agar were randomly selected and then subcultured on thiosulphate-citrate-bile salt-sucrose agar (TCBS) and incubated overnight at 30°C. To isolate the bacteria from diseased fish, a group of seven cultured juvenile tiger groupers *Epinephelus fuscoguttatus* (with clinical signs of vibriosis including darkened body colour, white nodular skin lesions, haemorrhagic visceral organs, and usually pale gills and sudden death) were collected from a fish aquaculture area at