

PCR DETECTION OF PUTATIVE AEROLYSIN AND HEMOLYSIN GENES IN AN *AEROMONAS HYDROPHILA* ISOLATE FROM INFECTED KOI CARP (*CYPRINUS CARPIO*)

A.Uma¹, G.Rebecca², S.Meena¹ and K.Saravanabava¹

¹ Shrimp Disease Diagnosis Laboratory, Vaccine Research Centre- Viral Vaccines, Centre for Animal Health Studies, Tamilnadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai-51

ABSTRACT

Aeromonas hydrophila is an opportunistic and primary bacterial pathogen of a variety of aquatic, terrestrial animals and humans. *A. hydrophila* causes infectious dropsy in ornamental and food fishes and its pathogenicity has been associated with the virulence factors such as aerolysin and hemolysin. The detection of the presence of such virulence factors is a better indicator of the potential health risk. In our study, hemolysin and aerolysin gene was detected by PCR in an *A. hydrophila* isolated from infected Koi carp. Infection due to bacterial pathogen with such virulent factors through contact with infected fish while handling them, water or other constituents of fish life environment poses health hazards to humans.

Key words: *Aeromonas hydrophila*, PCR, virulence factors

INTRODUCTION

A. hydrophila is a gram-negative bacteria (Nordmann and Poirel, 2002), that causes infections in food and ornamental fishes, there by posing a threat to the development of the aquaculture enterprise. Koi carp, *Cyprinus carpio* is an important ornamental fish species and it also serves as a food fish. Aerolysin and hemolysin genes are reported to be the putative virulence genes of *A. hydrophila* (Shome et al., 2005). Aerolysin, produced by some strains of *A. hydrophila*, is an extracellular, soluble, hydrophilic protein exhibiting both hemolytic and cytolytic properties. Aerolysin binds to specific glycoprotein receptors on the surface of eukaryotic

cells before inserting into the lipid bilayer and forming holes. Hemolysins are exotoxin protein produced by bacteria and the lytic activities of hemolysins on red blood cells are reported to be important for nutrient acquisition or for causing certain conditions such as anemia (Griffiths et al., 1988). The present study was carried out with an objective to screen the presence of putative virulent genes like aerolysin and hemolysin in an *A. hydrophila* isolate from infected Koi carp.

² Student, SASTRA University, Thanjavur. Corresponding author: Dr. A.Uma, Associate professor, Shrimp disease diagnosis laboratory, Vaccine research centre – viral vaccines, Tamilnadu veterinary and animal sciences university, Madhavaram milk colony, Chennai- 51. umaarumugam@yahoo.com

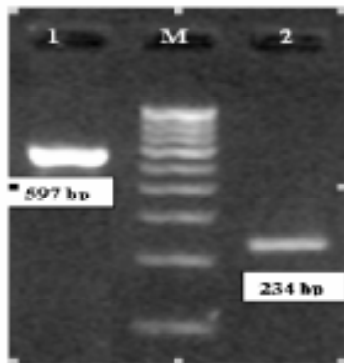
MATERIALS AND METHODS

Koi carp with clinical symptoms of bacterial infection was used for isolation of bacteria. Isolation of *A. hydrophila* was done using a specific media (Aeromonas isolation HiveTM medium base) and the isolate was coded as SDDL 05/09. Genomic DNA was extracted from 24h old cultures in nutrient broth. About 1ml of culture was taken, pelleted at 3000 rpm and used for extraction of DNA using a commercial DNA extraction kit (Bangalore Genei, Bangalore) following the manufacturer's protocol. PCR primers and protocol of Kingombe et al. (1999) and Sen and Rodgers, (2004) were used for partial amplification of aerolysin and hemolysin genes of SDDL05/09 respectively.

PCR reaction was performed in a total volume of 25 μ l with 22 μ l of 1 X master mix (Bangalore Genei, Bangalore) 20 pmoles each of

Fig.1

PCR amplification of hemolysin (lane 1) and aerolysin (lane 2) genes of *Aeromonas hydrophila* isolate SDDL05/09 (lane M-100 bp molecular weight marker)



forward and reverse primer (1 μ l) and 1 μ l of the extracted DNA. PCR cyclic conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation for 15 sec at 95 °C, annealing for 30sec at 66 °C, extension for 30 sec at 72 °C and final extension for 7 min at 72 °C. The PCR amplified products were detected by resolving 8 μ l of products in an agarose gel by gel electrophoresis with ethidium bromide at 100V for 30 min. The PCR products were purified and sequenced using commercial automated sequencing services (M/s MWG, Bangalore).

RESULTS AND DISCUSSION

PCR amplification of DNA from *A. hydrophila* isolate SDDL 05/09 using specific primers for aerolysin and hemolysin genes resulted in the expected PCR products of 234bp and 597bp sizes respectively (Fig-1). The sequence information of these genes was confirmed with the Genbank database of the National Center for Biotechnology Information (NCBI) by using the BLASTN program (<http://www.ncbi.nlm.nih.gov/>).

Screening of specific cytotoxin and hemolysin genes has been reported to be the most effective way of detecting and characterizing *Aeromonas* virulence factors (Yousr et al., 2007). Many researchers have documented various diseases caused by *Aeromonas* sp., such as gastroenteritis (Chopra et al., 1999), endocarditis (Brouqui and Raoult, 2001), wound infection (Ouderkirk et al., 2004), acute suppurative cholangitis (Chan et al., 2001). Some strains are reported to be invasive to epithelial cells and one of the major virulence factors in gastroenteritis is aerolysin (Chu and Lu, 2005). Chopra et al. (1993) have demonstrated the cytotoxic activity of aerolysin. The presence of aerolysin and hemolysin genes in our isolate from infected koi carp shows that it is a virulent strain

and transmission of such virulent strains through contacts during handling of fish results in infection to human. The source of the organism may be ambient environment, secondary contamination due to catching, transporting, handling and etc. may also contribute for its distribution. Suitable management measures like maintaining good water quality in the rearing systems, proper feed management and use of approved antibiotics in proper levels for control would help to prevent and control the *A. hydrophila* infection in fishes but also its spread to humans.

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