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# Molecular detection of *Aeromonas hydrophila* isolated from albino catfish, *Clarias* sp. reared in an indoor commercial aquarium

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**Abstract :** Moribund albino catfish, *Clarias* sp., displayed from an indoor private commercial aquarium were submitted in the laboratory for diagnostic examination. Dense culture of bacteria was recovered from the kidney and was characterized using Vitek System 2 and showed 98% probability to *Aeromonas* (*A.*) *hydrophila*. PCR result showed positive using *A. hydrophila* extracellular hemolysin gene ahh1 (130 bp) and aerolysin gene aerA (309 bp). The 16S rRNA gene was identical and exhibited 97% sequence similarity with the other known isolates of *A. hydrophila* available in the GenBank. In this paper, we reported the isolation and molecular detection of *A. hydrophila* from an albino catfish.

**Keywords :** aerolysin gene, *Aeromonas hydrophila*, catfish, extracellular hemolysin gene

Catfishes are an economically important group of fresh and brackiswater fish worldwide and several species have been introduced in aquaculture [10]. Recently, an albino catfish *Clarias* sp., appeared in the pet trade and displayed in commercial aquarium for exhibition. Many kinds of animals including tropical fish are usually kept in controlled condition like aquarium for exhibition but often suffer from stress or disease since their ecology and physiology are not always sufficiently understood [7].

An albino catfish from private commercial aquarium were submitted in our laboratory for diagnostic examination. In this paper, we reported the isolation and molecular detection of *Aeromonas* (*A.*) *hydrophila* in an albino catfish, *Clarias* sp., displayed in an indoor commercial aquarium in Seoul, South Korea.

Two moribund albino catfish (average body weight = 322.8 g, average total length = 33 cm) from commercial aquarium was submitted for diagnostic examination. Swabs from the gills, liver, spleen and kidney were streaked onto tryptic soy agar (Becton; Dickinson and Company, USA), and incubated at 25°C for two days. An isolated bacterium was re-streaked again onto fresh media to obtain the pure culture. Gram staining and motility test were performed. Vitek System 2 (bioMérieux, France) for Gram negative susceptibility card was used to further characterize the isolate.

Antibiotic susceptibility of bacterial isolate was determined by the disc diffusion method [3]. The sensitivity and resistance of isolated bacteria and zone diameter interpretative standards were determined according to the Clinical and Laboratory Standards Institute criteria for animal isolates [6].

Multiplex PCR for simultaneous detection of *A. hydrophila* extracellular hemolysin gene ahh1 (130 bp) and *A. hydrophila* aerolysin gene aerA (309 bp) was performed as previously described by Wang *et al*. [11]. Briefly, a colony of overnight culture was added into 100 μL of distilled water, the mixture was boiled for 10 min and centrifuged at 1,000 g for 10 min to sediment the cell debris. The DNA supernatants were transferred to new eppendorf tubes.

The hemolysin gene ahh1 forward primer, was 5′-GCCGAGCGCCCAGAAGGTGAGTT-3′ and the reverse

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primer, was 5′-GAGCGGCTGGATGCGGTTGT-3′. The aerolysin gene aerA forward primer was 5′-CAAGAA CAAGTTCAAGTGGCCA-3′ and the reverse primer was 5′-ACGAAGGTGTGGTTCCAGT-3′. The PCR mixture consisted of 20 μL reaction. The PCR amplification included an initial denaturation of at 95°C for 5 min followed by 50 cycles of denaturation at 95°C for 0.5 min, annealing of the primers at 59°C for 0.5 min and extension of at 72°C for 0.5 min. A final extension at 72°C for 7 min was used in the thermocycler (T-personal 48; Biometra, Germany). Volumes of each (10 μL) PCR product were subjected to electrophoresis in a 1.5% (w/v) agarose gel.

The representative PCR product sample was recovered from agarose gel and purified using the power gel extraction kit (Dyne Bio, Korea) as described in the manufacturer's instruction. Purified PCR amplification product was partially sequenced with 16S rRNA gene using universal sequencing primers (518F and 800R) at the Genomic Division, Macrogen, Korea, and by ABI PRISM Big Dye TM Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystem, USA). Electrophoresis of sequencing reaction was completed using the automated ABI PRISM 3730xl DNA Sequencer (Applied Biosystems, USA). The 16S rRNA sequence gene of the bacterial strain obtained in this study was analyzed and compared with entries in nucleotide sequence databases in the National Center for Biotechnology Information (NCBI website using Basic Local Alignment Search Tool program) and percentage sequence similarities were determined.

The most common infectious problem of an ornamental fishes is bacterial disease. The majority of bacterial infections are caused by Gram-negative organisms to include the pathogenic genera of Aeromonas, Citrobacter, Edwardsiella, Flavobacterium, Mycobacterium, Pseudomonas and Vibrio [8].

In this study, the isolates were Gram-negative and motile. Vitek System 2 revealed the pathogen as *A. hydrohila* with 98% probability (data not shown). Result of the multiplex PCR (Fig. 1) confirmed positive using *A. hydrophila* extracellular hemolysin gene ahh1 (130 bp) and *A. hydrophila* aerolysin gene aerA (309 bp). Hemolysin and aerolysin and several extracellular products or ECP of *A. hydrophila* have been suggested as possible contributory factors on its pathogenesis [4]. 16S rRNA gene sequencing showed 97% similarity with *A. hydrophila* strain available in

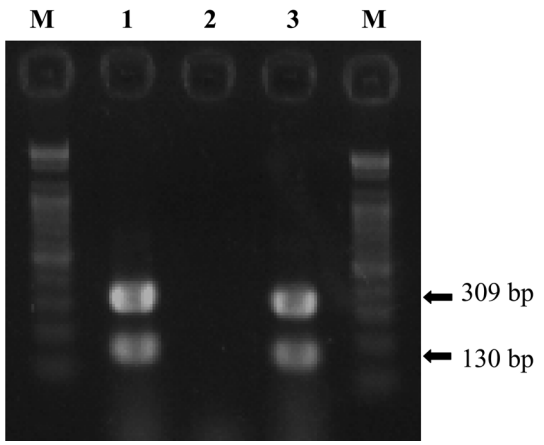


**Fig. 1.** Multiplex PCR amplification products profile of *Aeromonas* (*A.*) *hydrophila* extracellular hemolysin gene ahh1 (130 bp) and *A. hydrophila* aerolysin gene aerA (309 bp) isolated from the kidney of the albino catfish (Lane 3). Lane M: molecular weight marker using 100 bp ladder, Lane1: positive control, Lane 2: negative control.

Gene Bank (Accesion Numbers GQ184148, GQ141871, FJ494900 and FJ494895). The isolate was susceptible to chloramphenicol, ciprofloxacin, gentamicin, kanamycin, oxytetracycline, streptomycin, tetracycline, trimethoprim/sulfamethoxazole and resistant to erythromycin.

*A. hydrophila* is an autochthonal inhabitant of freshwater environments and is considered a primary pathogen of aquatic and terrestrial animals including humans [1]. This bacterial species is an important agent of disease in freshwater fish from warm environments in which it produces a hemorrhagic septicemia similar to those caused by other Gram-negative bacteria [4]. In these samples we observed severe hemorrhages surrounding the mouth, basement of the pectoral fins and the urogenital pores. Internal examination showed that the liver was pale and enlarged.

*A. hydrophila* had caused high mortality in catfish [12] and induced serious epidemics of ulcerative disease in fish in Southeast Asia and other regions of the world [2]. In addition, *A. hydrophila* is a secondary invader of injured tissues and causes diseases in stressed fish [9]. In this study, we speculated that stress is one of the contributory factors for the mortality of the catfish since this fish was held in an aquarium for display.

The origin and mode of infection of the *A. hydrophila* isolated from the albino catfish in the

present study is not known. Unfavorable environmental conditions like changes in temperature, deterioration of water quality, dissolved oxygen, pH and overcrowding could also affect the condition of the fish [5]. A drastic change in weather and organic material in the water led to the outbreaks of such disease [2]. Therefore, the maintenance of a biologically and chemically balanced environment and the appropriate regulation of physical conditions in the aquarium are required to avoid the disease.

In conclusion, this confirms the PCR detection using *A. hydrophila* extracellular hemolysin gene ahh1 (130 bp) and *A. hydrophila* aerolysin gene aerA (309 bp) in an albino catfish reared in commercial aquarium.

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