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Short Communication

Molecular detection of *Edwardsiella tarda* with *gyrB* gene isolated from pirarucu, *Arapaima gigas* which is exhibited in an indoor private commercial aquarium

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The pirarucu, *Arapaima gigas*, which was displayed in commercial aquarium was found dead and was submitted for diagnostic examination. Bacteria from different organs of the fish were characterized using Vitek System®2 and showed 98% probability to *Edwardsiella tarda.* Polymerase chain reaction (PCR) result showed positive for *E. tarda* gyr*B* gene. The 16S rRNA gene was identical and exhibited 99% sequence similarity with the other known isolates of *E. tarda* available in the GenBank. This paper reports the isolation and detection of *E. tarda* with the *gyrB* gene in pirarucu, *A. gigas*, which was exhibited in an indoor private commercial aquarium in Seoul, South Korea.

Key words: *Arapaima gigas*, commercial aquarium, *Edwardsiella tarda*, *gyrB* gene, pirarucu.

INTRODUCTION

The pirarrucu, *Arapaima gigas* (superorder Osteglossomorpha, order Osteoglossiformes, family Osteoglossidae and subfamily Heterotidinae) is an obligatory air-breathing osteoglossid which is endemic to the Amazonian basin and considered as the largest known freshwater scaled fish (Borella et al., 2009). This fish often grows up to 5 m in length and weighs as much as 200 kg (Kodama et al., 1987). They usually fed on small fishes and other items such as mollusks, crustaceans, insects and recently, on pelleted feeds (de Andrade et al., 2007).

Recently, pirarucu appeared in the pet trade and was displayed in commercial aquarium for exhibition and they required large tank and intensive care. Due to its enormous commercial importance, *Arapaima* became increasingly scarce and commercially extinct near larger Amazonian cities (Kodama et al., 1987). In 1975, pirarucu became one of a few fish species listed by the Convention on International Trade in Endangered Species (CITES) II convention (Farias et al., 2003). This fish is suitable for culture in captivity but the availability of fingerlings is still the critical point and there are not enough studies to support commercial production (Borella et al., 2009).

Edwardsiella tarda is the causative agent of Edwardsiellosis in many commercially important freshwater and marine fishes (Lan et al., 2008). This pathogen is also important from a public health point of view as it is known to cause disease in reptiles, birds, humans and other mammals (Mohanty and Sahoo, 2007). This study reports the molecular detection and isolation of *E. tarda* with the *gyrB* gene from pirarucu displayed in an indoor commercial aquarium in Seoul, South Korea.

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Abbreviations: TSA, Tryptic soy agar; **PCR,** polymerase chain reaction; **NCBI,** National Center For Biotechnology Information; **BLAST,** Basic Local Alignment Search Tool.

MATERIALS AND METHODS

Fish sample

Pirarucu (body weight = 20.1 kg, total length= 107 cm) that was found dead in one of the commercial aquaria was submitted for

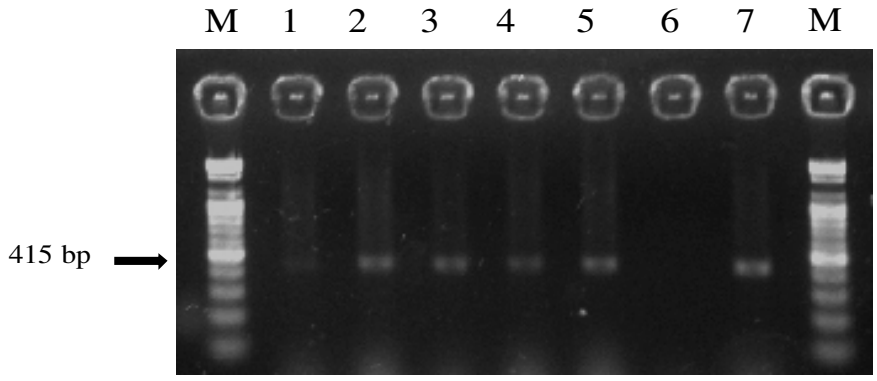

Figure 1. PCR amplification products profile of *E. tarda gyrB* gene (415 bp) isolated from different organs of pirarucu, *A. gigas*. Lane M, molecular weight marker using 100 bp ladder; Lane 1, gills; Lane 2, brain; Lane 3, liver; Lane 4, spleen; Lane 5, kidney; Lane 6, negative control; Lane 7, positive control.

diagnostic examination.

Isolation and identification of bacteria

Swabs from the gills, brain, liver, spleen and kidney were streaked onto tryptic soy agar (TSA) (Becton, Dickinson and Company, USA), and incubated at 25°C for two days. Isolated bacteria were restreaked again on fresh media to obtain the pure culture. Gram staining and motility test were performed. Vitek System®2 (bio-Mérieux®, France) was used to further characterize the isolate.

Antibiotic susceptibility test

Antibiotic susceptibility of bacterial isolate was determined by the disc diffusion method (Bauer et al., 1966). The sensitivity and resistance of isolated bacteria and zone diameter interpretive standards were determined according to the CLSI (Clinical and Laboratory Standards Institute) criteria for animal isolates (CLSI, 2006).

Extraction of bacterial DNA and polymerase chain reaction (PCR) amplification

The methods of Lan et al. (2008) that was previously published for bacterial DNA extraction, primers and PCR amplification were used. Briefly, a colony of overnight culture was added into 100 µl of distilled water, the mixture was boiled for 10 min and centrifuged at 1000 xg for 10 min to sediment the cell debris. The DNA supernatants were transferred to fresh Eppendorf tubes.

The forward primer, *gyr*BF1, was 5'- GCATGGAGACCTTCAGC AAT-3' and the reverse primer, *gyr*BR1, was 5'-GCGGAGATTTT GCTCTTCTT-3'. The expected length in polymerase chain reaction (PCR) is 415 bp. The PCR mixture consisted of 20 ul. The amplification conditions were 30 cycles at 94°C for 1 min, 51.5°C for 30 s and 72°C for 30 s, and then an extra extension step of 72°C for 10 min in the thermocycler (T-personal 48; Biometra, Germany) . Volumes of each (5 ul) PCR product were subjected to electrophoresis in a 1% (w/v) agarose gel.

16S rRNA sequencing

The representative PCR product sample was recovered from agarose gel and purified using the power gel extraction kit (DyneBioInc, 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 Inc., Korea, and by ABI PRISM Big Dye TM Terminator Cycle Sequencing Ready Reation 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 (BLAST) program (http://www.ncbi.nlm.nih.gov/BLAST), and percentage sequence similarities were determined.

RESULTS AND DISCUSSION

E. tarda is a common pathogen which has been isolated from eel (Egusa, 1976), channel catfish (Meyer and Bullock, 1973), tilapia (Kubota et al., 1981), olive flounder (Nakatsugawa, 1983), carp (Sae-Qui et al., 1984), seabream (Baxa et al., 1985) and stripebass (Herman and Bullock, 1986). It was isolated also from cetaceans (Buck et al., 1991), seals (Regalla, 1982) and human (Vandepitte et al., 1983). It causes septicemia with extensive skin lesions affecting internal organs such as liver, spleen and muscle.

In this study, the isolated bacterium was gram negative, motile and it showed 98% probability using the Vitek System®2 test (data not shown). PCR result (Figure 1) showed that the isolates from different organs (gills, brain, liver, spleen and kidney) were positive for *E. tarda gyrB* gene marker. The *gyrB* gene is a single-copy gene, present in all bacteria which encode the ATPase domain of DNA gyrase, an enzyme essential for DNA replication (Huang, 1996). Lan et al. (2008) previously developed this PCR method that is useful for the detection of *E. tarda* in diseased fish based on using the *gyrB* gene as a taxonomic marker. Result of the 16S rRNA gene of *E. tarda* was identical and exhibited 99% sequence simila-

rity with the other known isolates of *E tarda* available in the GenBank (Accession No. GQ180183, FJ405297 and FJ405292).

Earlier studies indicated that *E. tarda* is susceptible to various antibiotics (Mohanty and Sahoo, 2007). In this study, the isolated bacterium was susceptible to ampicillin, gentamicin, chloramphenicol, ciprofloxacin and trimethoprim/sulfamethoxazole and resistant to streptomycin and tetracycline.

Considering that pirarucu is a commercial fish and an endangered species of the Amazonian diversity, efforts and knowledge must be made on the association of the opportunistic pathogen that affects this fish. However, the mode of origin of the infection of *E. tarda* isolated from pirarucu is not known. It was reported that the pathogenesis of *E. tarda* is multifactorial, the mechanisms are poorly understood and the site of attachment and penetration are not known although the intestine and abraded skin are most likely sites for penetration of the bacteria (Mohanty and Sahoo, 2007). Stress factors such as overcrowding, sudden change of temperature, pH and dissolved oxygen fluctuations might contribute to the development of the disease. 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 (Kodama et al., 1987; Choresca et al., 2007).

The isolation of *E. tarda* from aquarium could be a reservoir, pose a risk to other fishes and could be transmitted to human. *E. tarda* is also known as pathogen of human and can lead to severe enteritis (Lewbart, 2001). Ornamental fish can be sub clinically infected or carrier of pathogen and it is believed that they can be a reservoir for the organisms (Humphrey et al., 1986). It was reported that a case of diarrhea in human infant has been traced to *E. tarda* in the home aquarium (Vandepitte et al., 1983). It was suggested that proper aquarium hygiene should be observed. In addition, appropriate regulations of physical conditions in the aquarium are required to avoid stress and occurrence of diseases.

In conclusion, this confirms the detection of *E. tarda* in pirarucu, *A. gigas*, exhibited in a commercial aquarium by PCR with the amplification product of 415 bp using *gyrB* gene as a taxonomic marker.

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