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Short Communication

Isolation and molecular detection of *Plesiomonas shigelloides* containing *tet*A gene from Asian arowana (*Scleropages formosus*) in a Korean aquarium

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An Asian arowana (dragonfish; *Scleropages formosus*) that had been reared for public exhibition in a private commercial aquarium in Seoul Korea was recently found dead. A bacterial pathogen was cultured from the fish's kidney, identified, and confirmed as *Plesiomonas shigelloides*, using a Vitek System®2, PCR and 16S rRNA gene sequencing. The isolated strain proved resistant to commercial antibiotics and the presence of the tetracycline resistance gene (*tet*A) was detected via genetic analysis. In this paper, we have described the isolation and identification of *P. shigelloides* from an Asian arowana reared in a private aquarium in Korea.

Key words: Asian arowana (dragonfish; *Scleropages formosus*), *Plesiomonas shigelloides*, tetracycline resistance gene (*tet*A).

INTRODUCTION

The Asian arowana (dragonfish; *Scleropages formosus*) is an ancient fish species of the *Osteoglossidae* family and one of the most primitive teleostean forms (Greenwood et al., 1966). It is distributed widely in Southeast Asia, including Cambodia, Indonesia, Laos, Malaysia, Myanmar, Vietnam, Thailand and Philippines (Kottelat et al., 2000).

The Asian arowana is one of most valuable species in the Asian ornamental trade (Kottelat et al., 2000). Due to high demand and overexploitation of natural populations, the Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES) has classified the arowana as a highly endangered species under its Appendix Ⅰ list (Joseph et al., 1986). However, the illegal harvest and trade of highly valued strains from wild populations continue to pose major problems threatening efforts to conserve this species (Ng and Tan, 1997).

Plesiomonas shigelloides are gram-negative, motile, non-spore-forming bacilli, which are facultatively anaerobic and oxidase-positive (González-Rey et al., 2000). *P. shigelloides* have been recognized as potential fish pathogens (González et al., 1999). Additionally, this bacterium has also been isolated from humans as well as domestic animals, including dogs, cats, goats, sheep and cows (Arai et al., 1980; Clark and Janda, 1991). In this study, we have described the isolation and molecular detection of *P. shigelloides* containing the *tet*A gene from an Asian arowana raised for exhibition in a commercial aquarium in Korea.

MATERIALS AND METHODS

Fish sample

In September 2009, the Asian arowana (total length 43.5 Cm, body weight 820 g) which was reared for public exhibition in a private commercial aquarium in Seoul, Korea, was found dead. The fish

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had evidenced symptoms including anorexia, lethargy, and depression, which had persisted for two weeks. Shortly after the fish died, it was submitted for postmortem examination.

Isolation and identification of causative agent

At necropsy, protrusion of the intestine, the presence of yellowish fluid in the abdominal cavity, and a pale and enlarged liver were noted. For bacterial isolation, sterile swabs from the kidney were streaked onto tryptic soy agar (TSA), and the inoculated plates were incubated for 24 h at 25°C. The suspected common colony was re-streaked on TSA to acquire a pure culture, which was then simply identified on the basis of microscopic analysis results, with the aid of a Vitek System®2 (bioMérieux®, France) for biochemical analysis.

Molecular analysis of *P. shigelloides*

Genomic DNA extraction was conducted as described previously (Jun et al., 2010). Genomic DNA was extracted by boiling the cells, followed by centrifugation. In an effort to accurately identify the isolated bacteria, *P. shigelloides,* from pure culture, PCR amplification was carried out as previously described by González-Rey et al. (2000). Negative (double-distilled water) and positive (*P. shigelloides*, ATCC 51903) controls were included in the PCR procedure. The purified PCR product was submitted to the Macrogen Genomic Division (Korea) and nucleotide sequencing was carried out using an ABI PRISM Big Dye TM Terminator Cycle Sequencing Kit (Applied BioSystems, USA). Electrophoresis of the sequencing reactions was completed using an automated ABI PRISM 3730XL DNA Sequencing System (Applied BioSystems, USA). The rRNA sequence gene of the bacterial strain acquired in this study was aligned with other bacteria of the same species (HQ663900.1) according to the sequence contained in the GenBank database; the multiple alignment algorithm in the MegAlign package (Windows Version 3.12e; DNASTAR Software Package, USA) was employed, and the percentage of sequence similarity was determined.

Antibiotic susceptibility test and molecular detection of the *tet*A gene

The antibiotic susceptibility of bacterial isolates was determined via the disc diffusion method (Bauer et al., 1966). The sensitivity and resistance of isolated bacteria and the zone diameter interpretive standards were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) criteria for animal isolates (CLSI, 2006). Multiplex PCR was conducted to amplify the tetracycline resistance genes (*tet*A, 211 bp; *tet*B, 391 bp; *tet*C, 897 bp; *tet*D, 844 bp; *tet*E, 744 bp) using isolated bacteria as described previously (Nawaz et al., 2006). Sequencing of the purified PCR product was carried out to confirm the presence of the tetracycline resistance genes at the Macrogen Genomic Division, in Korea. The sequence of the tetracycline resistance gene obtained in this study was aligned with those of the *Aeromonas* sp., *Klebsiella* sp. and *Pseudomonas* sp. plasmid tetracycline resistance genes (HM453327.1, HM371195.1 and FJ950710.1) available in the GenBank database, and the percentage sequence similarities were determined.

RESULTS AND DISCUSSION

The results of our microscopic examination demonstrated that the isolate was gram-negative, motile and oxidase-positive. Additionally, according to the results generated using the Vitek System®2 (bioMérieux®, France), *P. shigelloides* was isolated with a probability of 99%. The identification was further confirmed via a PCR assay based on the 23S rRNA gene. The isolated strain contained the amplification product: 284bp of the 23S rRNA gene for *P. shigelloides* (Figure 1-a). Moreover, as the result of 16S rRNA gene sequencing, *P. shigelloides* consisting of approximately 950 nucleotides was isolated, and evidenced a 100% sequence similarity with other *P. shigelloides* strains (HQ663900.1) contained in the GenBank database. As a consequence, the identification of *P. shigelloides* was confirmed.

The antibiotic resistance profile of the *P. shigelloides* isolate showed that it was resistant to amoxicillin/clavulanic acid, ampicillin, chloramphenicol, tetracycline and trimethoprim/sulfamethoxazole. However, it was found to be sensitive to amikacin, cefepime, cefotaxime, ciprofloxacin, and gentamicin. The assay detected the presence of the *tet*A gene in *P. shigelloides* by amplifying the 211 bp PCR product from the genomic DNA of the isolate (Figure 1-b). The results of *tet*A gene sequencing showed a 100% similarity with other reported *tet*A genes of *Aeromonas* sp. (HM453327.1), *Klebsiella* sp. (HM371195.1) and *Pseudomonas* sp. (FJ950710.1) available in the GenBank database.

Tetracycline evidenced excellent *in vitro* activity against *P. shigelloides* and was identified as an excellent drug for treatment in cases of *P. shigelloides* infection (Reinhardt and George, 1985). In our study, the *P. shigelloides* isolate proved resistant to tetracycline, in contrast to the results reported by Stock and Wiedemann (2001), who noted that 74 *P. shigelloides* isolates were susceptible to tetracycline. Additionally, Kain and Kelly (1989a) noted previously that among 72 *P. shigelloides* isolates, 26% of the strains were resistant to tetracycline; the administration of tetracycline was recommended for therapy only if the isolate was known to be susceptible.

Antimicrobial-resistant bacteria present in an aquatic environment may be transferred to fish, other animals, and humans who come in contact with this ecosystem via wound infections deriving from exposure to contaminated water or fish while handling fish (Petersen and Dalsgaard, 2003). Antimicrobial resistance determinants selected in aquatic environments may, in some cases, be transmitted to human pathogenic bacteria (Smith et al., 1994). It is alarming that a strain of *P. shigelloides* resistant to tetracycline and harboring the *tet*A gene was isolated from an aquarium fish exhibited in an indoor private commercial aquarium. Additionally, the symptoms of patients who were infected with tetracycline-resistant *P. shigelloides* strains required a significantly longer time to resolve than the case with patients infected with more-susceptible strains (Kain and Kelly, 1989b).

In this paper, we confirmed the isolation and molecular detection of *P. shigelloides* containing the *tet*A gene from an Asian arowana reared in a commercial aquarium in

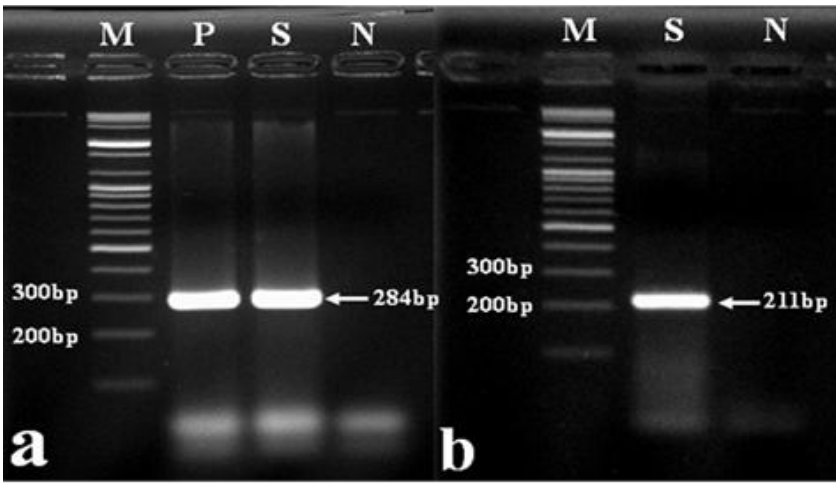

Figure 1. a: The amplification products obtained using the PCR assay for detection of *P. shigelloides* in Asian arowana. Lanes M, 100-bp DNA ladder; lane P, positive control (*P. shigelloides* ATCC 51903, 284bp); lane S, positive for *P. shigelloides* (284bp); lane N, negative control. b: A multiplex PCR assay was performed to amplify the tetracycline resistance genes (*tet*A to E) from the isolate. Lanes M, 100-bp DNA ladder; lane S, the presence of the *tet*A gene (211bp); lane N, negative control.

Korea.

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