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Streptococcus iniae: An Unusual Important Pathogen Fish in Brazil

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Abstract

The current paper contains a report on the isolation of *Streptococcus iniae* in Nile tilapia (*Oreochromis niloticus*) in Brazil and South America. *S. iniae* is an important pathogen often associated with significant losses in fish production. It is also known for causing invasive infections in humans. An outbreak of infections characterized by exophthalmos, erratic swimming, ascites and melanosis occurred in intensive fish farming. Samples of kidney, brain and liver of the fish seeded into the culture medium yielded beta-hemolytic, gram-positive cocci isolate from the colonies. The partial sequencing of the 16S ribosomal gene was performed to identify the isolate. The sequence obtained showed 99% identity to 16S sequences of *S. iniae* present in the Genbank. A phylogenetic analysis was accomplished to confirm the species. Although *S. iniae* is frequently present in almost all continents, this work disclosed the second case of isolation of this pathogen in farmed fish both in Brazil and South America.

The tilapia culture is one of the fastest growing sectors in the food production industry worldwide. Intensive breeding is one of the most used systems for the species growth with high stocking rates. High feeding rates usually result in changes in the water quality and animal stress, enabling the incidence of infectious diseases [1-3]. The available literature reports that *Streptococcus iniae* and *Streptococcus agalactiae* constitute the primary cause of streptococcosis in intensive fish farming. *S. iniae* is an important pathogen in aquaculture, which may cause considerable economic losses and infect different species of marine and freshwater fish. Furthermore, it is an emerging zoonotic pathogen [4,5].

The first isolation of *S. iniae* occurred in the 1970s from skin lesions on dolphins (*Inia geoffrensis*). Then, it was subsequently identified in fish in North America, Middle East, Asia-Pacific region and Europe [4,6-8]. There are several strepococcosis reports in the Nile tilapia (*Oreochromis niloticus*) in Brazil, always associated with *S. agalactiae* [9-12]. Neverteless, only in 2012 Figueiredo et al. [13], described a meningoencephalitis outbreak of *S. iniae* in tilapia, the only case reported in South America.

The most common disease symptoms induced by *S. agalactiae* and *S.iniae* in fish are similar. They include exophthalmia, ascites, erratic swimming, lethargy, melanoseis, meningoencephalitis, septicemia and high mortality [4,10,12,14]. The objective of this study was to report the second case of isolation and molecular identification of *S. iniae* in the Brazilian Nile tilapia (*O. niloticus*).

An outbreak of the Nile tilapia (*O. niloticus*) mortality occurred in an intensive fish farming located in the basin of the Paranapanema River. Five fish with an average weight of 300 g exhibited clinical signs of exophthalmia, ascites, erratic swimming, lethargy and melanosis. Such five fish were sent alive in bags with water from the site and oxygen to the laboratory of Veterinary Microbiology and Infectious Diseases of the State University of Londrina. The fish were anesthetized with benzocaine solution, alcohol and water at a ratio of 1 g/10 ml/10 L [15], respectively, and submitted to autopsy.

Samples of kidney, brain and liver were collected and immediately inoculated onto Columbia agar (Difco Laboratories, Sparks, MD) supplemented with 5% defibrinated sheep blood, and incubated at 30°C for 48 hours under aeroçhilic conditions. The obtained colonies were submitted to Gram stain, catalase, esculin and serology tests using the Slidex Strepto kit (BioMérieux-France).

The isolated colonies underwent genomic DNA extraction as described by [16]. For molecular analyses, the strategy of partial amplification of the 16S rRNA region with the FD1 and RD1 primers was used, following the protocol described by the authors [17]. The amplification product was purified using the WizardSV Gel and PCR Clean-Up System (Promega) and sequenced by the Sanger method.

For the identification of the bacterial species, the obtained sequences were compared to the sequences deposited in the GenBank [18] using the Blastn program. In addition to the direct comparison, we performed a phylogenetic study aligning the selected sequences using the ClustalW algorithm exporting the sequences in the Mega 6 program [19]. Accordingly, nine partial sequences of the 16 S rRNA gene isolates of *S. iniae* deposited in the GenBank until February 2015, which corresponded to the region delimited by the *primers*, were retrieved from the databases. Furthermore, we utilized two sequences of *S. agalactiae* besides another of *Streptococcus parauberis* that was used as a tree root. All sequences of *S. iniae* utilized were isolates from fish: three from Brazil [13], one from Taiwan (AY762259.1), one from Indonesia (KM209199.1), one from China (JQ990158.1) one from Israel (AF335573), one from Iran (FJ870987) and one from North America (NR025148.1) ATCC29178, besides the isolate obtained in this work. A phylogenetic tree was constructed using the *neighbor-joining* bootstrap method with 1,000 replicates.

Most of the isolated colonies were punctiform, gray, Gram-

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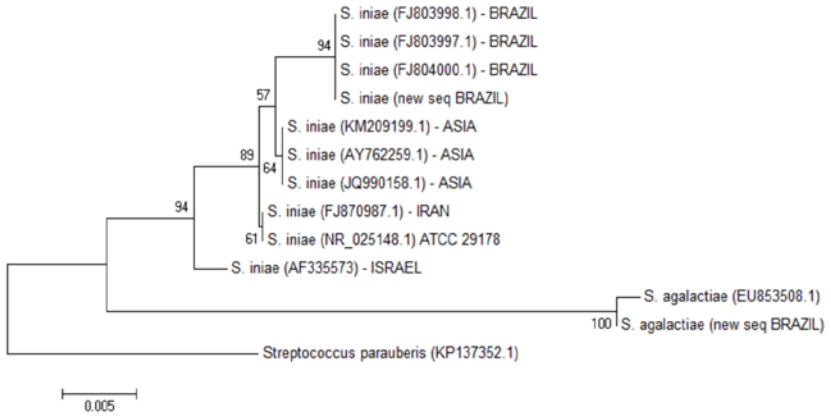

Figure 1: Phylogenetic tree constructed using the neighbor-joining, showing the distribution of different isolates of *S. iniae*and *S. agalactiae*, based on the partial sequence of the 16S gene. The tree was rooted by *S. parauberis*(outgroup). Bootstrap method with 1,000 replicates.

positive cocci, catalase-negative and esculin-negative. Serology allowed the classification under Lancefield group B, and identification as *S. agalactiae* (Data not shown). Nevertheless, one liver isolate of a fish presented β-hemolytic colonies and was not classified under any of the Lancifield groups. The partial 16S rRNA gene sequence showed 99% identity to the partial sequences of the 16S gene from the seven isolates of *S. iniae* with 100% coverage.

The analysis of the yielded phylogenetic tree indicated that the isolates identified as *S. iniae* were grouped into two clusters Figure 1. We can observe three groups corresponding to the tree root (*S. uberis*), a defined group composed of *S. agalactiae* and a group composed of the isolates of *S. iniae.* The isolate obtained in this work was grouped into a tree branch along with the three isolates from Brazil [13]. The remaining strains were distributed in the cluster. These results, together with the comparison data to other sequences clearly indicate that the isolate belongs to the *S. iniae* species.

The *S. iniae* strain isolated in this study showed greater genetic similarity to those isolated by Figueiredo et al. [13], when compared to the other strains. However, along with the others, it diverges from the isolates of *S. agalatiae.* The phylogenetic tree shows a clear geographical separation between the groups indicating genetic proximity and specialization of Brazilian isolates. This proximity may indicate that the occurrence of this important pathogen in Brazil is not occasional.

Streptococcosis is a disease that occurs in Brazil causing severe losses to the fish culture industry. Worldwide, *S. iniae* is described as one of the most important pathogens causing this disease. Currently, *S. agalactiae* is considered the main cause of streptococcosis in Brazilian intensive fish farming while the occurrence of *S. iniae* was reported only once in this country [13]. Although *S. iniae* is frequently reported in other continents, this work presented the second case of isolation of this pathogen in Brazilian and South American fish farming.

The overall results call for more extensive studies of *S. iniae* aiming to understand the actual distribution of this species in Brazil, its role in streptococcosis, and its diversity and epidemiological significance. These findings also urge the authorities to subsidize recommendations and encourage specific management practices for monitoring fish health and growth.

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