

Letters to the Editor

First Identification of *Streptococcus phocae* Isolated from Atlantic Salmon (*Salmo salar*)

Different genera and species of gram-positive, catalase-negative cocci are pathogenic to fish. The numbers of infections caused by these microorganisms have increased during the last decade, and such infections are responsible for significant economic losses in the fish farm industry (9). Identification of these bacteria can be difficult when using conventional microbiological criteria, and the organisms can be misidentified. Thus, *Enterococcus seriolocida*, described in 1991 as a new bacterial fish pathogen responsible for infection in eels and yellowtail, was assigned to the genus *Enterococcus* based on conventional phenotypic tests such as the ability to grow at 6.5% ClNa or pH 9.6 (12). Further biochemical and protein profiling, 16S rRNA sequencing, and DNA hybridization studies confirmed that *Lactococcus garvieae* and *E. seriolocida* were the same species (8, 16). Similarly, *Streptococcus shiloi* was proposed to accommodate clinical strains responsible for meningoencephalitis in tilapia and trout (6). Further DNA-DNA experiments revealed that this new streptococcal species should be considered a junior synonym of *Streptococcus iniae* (7). To avoid these identification problems, different molecular PCR assays have been developed to facilitate the identification of the gram-positive, catalase-negative cocci most frequently isolated from clinical specimens obtained from fish (2, 3, 20). During the development of a PCR assay for the specific detection of *S. iniae* (14), several strains of streptococci isolated from fish were requested from the bacterial collection of the FRS Marine Laboratory in Aberdeen, Scotland. Thirteen clinical isolates, eight identified as *S. iniae* (MT 2375, MT 2376, MT 2377, MT 2378, MT 2492, MT 2496, MT 2498, and MT 2500) and five identified as *Streptococcus* spp. (MT 2467, MT 2468, MT 2469, MT 2470, and MT 2471), were received. All streptococcal isolates, except MT2375 and MT2378, were recovered from clinical specimens of diseased Atlantic salmon (*Salmo salar*) farmed in Chile. However, only four (MT 2375, MT 2376, MT 2377, and MT 2378) of the eight isolates identified as *S. iniae* yielded the expected amplification product of 870 bp, which is specific for this bacterium (14). The biochemical characteristics of the four PCR-positive isolates were consistent with those previously described for this species (4). The remaining four isolates identified as *S. iniae* and five isolates identified as *Streptococcus* spp. could not be identified by PCR (13) as *S. iniae*, *Streptococcus difficilis*, *Streptococcus parauberis*, or *L. garvieae*, the gram-positive, catalase negative cocci most frequently associated with streptococcal infections in fish (5, 9). The present study was performed to identify and further characterize these streptococcal isolates obtained from clinical specimens of Atlantic salmon. The isolates were genotypically identified as *Streptococcus phocae*.

The nine streptococcal isolates were gram-positive, catalase-negative cocci that were beta-hemolytic on Columbia blood-agar plates. The type strain *S. phocae* CCUG 35103 was used for comparative purposes. All isolates were grown on Columbia blood-agar plates incubated aerobically at 30°C for 24 to 48 h. Biochemical characterization was achieved using the commercial system Rapid ID 32 Strep (version 2.0; bioMérieux España, S.A.) according to the manufacturer's instructions. All

nine isolates exhibited nearly identical biochemical profiles (numerical code 04102001000), which correspond to a good identification (97.4%) of *Gardnerella vaginalis*. Sequencing of the 16S rRNA gene is extremely useful tool for the identification of unusual clinical isolates (11). Molecular genetic identification of the clinical isolates was attempted by sequencing the 16S rRNA gene of each streptococcal isolate (17). The determined sequences consisted of about 1,400 nucleotides each and the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>) was used to compare the sequences with those of other gram-positive, catalase-negative species available in the GenBank database. The 16S rRNA gene analysis revealed that all nine isolates were genotypically identical, displaying the highest sequence similarity (98.0%) with *S. phocae* (GenBank/EMBL accession number AF235052). Due to the relatively high number of undetermined bases of this sequence, *S. phocae* CCUG 35103^T was further sequenced as indicated above, and the sequence obtained was submitted to GenBank (see below). The 16S rRNA sequences of the clinical isolates and that of *S. phocae* CCUG 35103^T exhibited 100% similarity when they were compared. Biochemical comparison of the type strain and clinical isolates using the Rapid ID 32 Strep system revealed identical biochemical profiles. The genotypic identification of clinical isolates as *S. phocae* was consistent with the overall results of the phenotypic characteristics described for this species (15). These results corroborate the utility of 16S rRNA sequencing for the identification of unusual microorganisms and demonstrate that biochemical methods can fail to identify some of the species of streptococci isolated from clinical specimens of diseased fish. Commercial identification systems cannot identify these bacteria because they are not incorporated into the commercial system databases. However, the bacteria can easily be differentiated by several biochemical tests included in some identification systems (Table 1).

Until now, *S. phocae* has been isolated exclusively from different species of seals (10, 15, 19). To our knowledge, this is the first description of the identification of *S. phocae* isolated from fish. Pulsed-field gel electrophoresis (PFGE) is a highly discriminatory technique that has been applied to the epidemiological investigation of this bacterium (19). Clinical isolates of *S. phocae* analyzed in this study were molecularly characterized by PFGE (18) using the restriction enzymes ApaI (Promega Co.) and SmaI (MBI Fermentans). All *S. phocae* isolates displayed undistinguishable PFGE patterns with both restriction enzymes (data not shown), indicating that all the clinical isolates represent a single strain. *S. phocae* has usually been isolated from clinical specimens together with other bacteria or viruses (10, 19). Thus, although the PFGE results suggest a common source of infection and clinical significance, it was not possible to establish a link between disease in Atlantic salmon and *S. phocae*, due to the absence of additional pathological or epidemiological data.

Additional studies will be necessary to determine the clinical significance of this bacterial species for the salmon industry. In this regard, the design of species-specific primers for the iden-

TABLE 1. Biochemical characteristics included in the commercial identification strip Rapid ID 32 Strep that are useful for differentiating *S. phocae* from other streptococcal species responsible for streptococcal fish infections^a

Characteristic	Result for ^d :			
	<i>S. phocae</i> ^{b,c}	<i>S. iniae</i> ^c	<i>S. difficilis</i>	<i>S. parauberis</i>
Acid production from:				
Mannitol	–	+	–	+
Sorbitol	–	–	–	+
Lactose	–	–	–	+
Trehalose	–	+	–	+
Raffinose	–	–	–	+
Saccharose	–	+	+	+
Glycogen	–	+	–	–
Melezitose	–	+	–	–
Tagatose	–	–	–	+
Hydrolysis of arginine	–	V	+	+
Acetoin production	–	–	+	+
Production of:				
β-Glucuronidase	–	+	–	–
α-Galactosidase	–	–	–	+
Pyroglutamic acid arylamidase	–	+	–	+
N-Acetyl-β-glucosaminidase	–	+	+	+

^a Data obtained from references 4 to 6 and 15.

^b *S. phocae* can also be differentiated from *S. iniae* because the latter species is β-glucosidase positive.

^c *S. difficilis* and *S. iniae* are CAMP test positive, while *S. parauberis* and *S. phocae* are CAMP test negative.

^d V, variable.

tification as *S. phocae* (1) and the availability of tests to facilitate its identification will aid aquaculture laboratories to recognize this bacterium in the future and improve knowledge of its distribution and possible association with streptococcal infections in both wild and farmed fish.

Nucleotide sequence accession number. The nucleotide sequence corresponding to the 16S rRNA gene of *S. phocae* CCUG 35103^T has been assigned the accession number AJ621053 in the GenBank/EMBL database.

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