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Streptococcus shiloi, the Name for an Agent Causing Septicemic Infection in Fish, Is a Junior Synonym of *Streptococcus iniae*

AVI ELDAR,[1,2] PAUL F. FRELIER,[3] LILIANA ASSENTA,[2] PATRICIA W. VARNER,[3] SARAH LAWHON,[3] AND HERVE BERCOVIER[1]*

Department of Clinical Microbiology, The Hebrew University-Hadassah Medical School, Jerusalem 91010,[1] and Department of Poultry and Fish Diseases, The Kimron Veterinary Institute, Bet Dagan 50250,[2] Israel, and Department of Veterinary Pathology, Texas A&M University, College Station, Texas 77843-4467[3]

***Streptococcus shiloi* strains, including the type strain, which were isolated in Israel and the United States, and *Streptococcus iniae* ATCC 29178^T (T = type strain) are phenotypically identical (as determined with API 20 STREP and API 50CH kits; beta-hemolytic on sheep blood agar). DNA-DNA hybridization experiments revealed levels of homology of 77 to 100%. Thus, *S. shiloi* should be considered a junior synonym of *S. iniae*. This bacterium is a major fish pathogen that is distributed worldwide.**

Streptococcus iniae was described in 1976 by Pier and Madin (7), who isolated this bacterium from skin lesions of a captive Amazon freshwater dolphin (*Inia geoffrensis*). In 1994, we described a new streptococcal species that was isolated from diseased rainbow trout (*Onchorynchus mykiss*) in Israel and was named *Streptococcus shiloi* (2). This name was validated in 1995 (4). *S. shiloi* differed from *S. iniae* in G+C content (37 mol% [2]; the G+C content of *S. iniae* is 32.9 mol% [7]), pathogenicity and host range. *S. shiloi* was shown to be the etiologic agent of an acute meningoencephalitis that affects trout (3), and it was also shown to be the causative agent of a similar disease in tilapia (*Oreochromis aurea* × *Oreochromis nilotica* hybrids) along with *Streptococcus difficile* (2). *S. shiloi* and *S. difficile* were shown to have broad geographical distributions and broad host ranges (2). *S. shiloi* was also isolated from diseased tilapia in Taiwan and the United States, and *S. difficile* was isolated in Japan from sea-cultured yellowtails (*Seriola quinqueradiata*) (2).

The purposes of this work were to elucidate the taxonomic position of *S. shiloi* and to compare *S. shiloi* Israeli field isolates collected over the last 5 years with isolates collected in the United States during the last 2 years.

A total of 15 American isolates were compared with 19 Israeli isolates, including the type strain of *S. shiloi* (strain ND 2-16 [= CIP 103769]), 1 strain isolated in Taiwan, and *S. iniae* ATCC 29178^T (T = type strain) (Table 1). All of the strains were isolated from diseased fish. The type of hemolysis was determined on Columbia agar base (Difco) supplemented with 5% (vol/vol) defibrinated sheep blood; the type of hemolysis produced on this medium was then compared with the type of hemolysis produced on the same medium supplemented with human or bovine blood. Biochemical reactions were determined with API 20 STREP and API 50CH systems (API, La Balmes Les Grottes, France). Most of the instructions of the manufacturer were followed; the only exception was the temperature of incubation, which was adjusted to 30°C. Results were read after 24 h of incubation. DNA was extracted by a previously described method (2). DNA-DNA hybridization experiments were performed by using the hydroxyapatite method described by Johnson and Ault (5) and Brenner et al. (1), except that the volumes used were modified (2). After denaturation by boiling, reassociation was allowed to proceed for 18 h at 60°C. Duplicate reactions were performed, and each run was performed twice. The levels of DNA relatedness (relative binding ratios) and the differences in melting points between the homologous reactions and the heterologous reactions for the labeled reference strains and other strains were calculated as described previously (1, 2). The rate of self-reassociation of the labeled DNA was routinely 2.5%, and this value was subtracted from the absolute hybridization ratios.

All 36 strains studied (Table 1) produced complete beta-hemolysis on the sheep blood-supplemented medium, whereas partial hemolysis was observed when the medium was supplemented with human or bovine blood. The reactions of the Israeli and American isolates, including *S. shiloi* ND 2-16^T and *S. iniae* ATCC 29178^T, were identical when we used the API 20 STREP kit. The Voges-Proskauer, hippurate, and α- and β-galactosidase activity tests were negative, whereas the esculin hydrolysis and pyrrolidonylarylamidase, alkaline phosphatase, leucine arylamidase, and arginine dehydrolase activity tests were positive. Ribose, mannitol, trehalose, starch, and glycogen were acidified by all of the isolates, whereas none of the isolates acidified arabinose, sorbitol, lactose, inuline, or raffinose. *S. iniae* ATCC 29178^T and six randomly selected Israeli and American strains were also tested by using API 50CH kits. Tests for acidification of ribose, galactose, D-glucose, D-fructose, D-mannose, mannitol, methyl-D-mannoside, *N*-acetylglucosamine, arbutin, esculin, salicin, cellobiose, maltose, sucrose, trehalose, melezitose, starch, glycogen, and β-gentobiose were positive for all of the isolates, including *S. iniae* ATCC 29178^T. Tests for acidification of D-arabinose, L-arabinose, galactose, methyl-D-glucoside, L-sorbose, D-xylose, L-xylose, methylxyloside, rhamnose, lactose, D-raffinose, D-fucose, L-fucose, glycerol, sorbitol, inositol, dulcitol, erythritol, adonitol, xylitol, D-arabitol, L-arabitol, amygdalin, inulin, lyxose, gluconate, 2-keto-gluconate, 5-keto-gluconate, turanose, and taganose were negative for all of the strains, including *S. iniae* ATCC 29178^T.

The DNAs of *S. shiloi* ND 2-16^T and *S. iniae* ATCC 29178^T were labeled and hybridized with each other and with the DNAs of field strains (Table 1). The high levels of DNA-DNA homology (77 to 100%) and the low $\Delta T_{m(e)}$ values (1.4°C or less) for the various isolates collected in Israel and the United

* Corresponding author. Mailing address: Department of Clinical Microbiology, The Hebrew University-Hadassah Medical School, POB 1172, Jerusalem 91010, Israel. Phone: 972-2-758256. Fax: 972-2-784010.

TABLE 1. Levels of DNA-DNA homology for *S. iniae* and *S. shiloi* strains[a]

Strain	Origin			Relative binding ratio with[b]:	
	Date (mo/yr)	Host[c]	Location	*S. shiloi* ND 2-16^T	*S. iniae* ATCC 29178^T
4B10M321	9/93	Tilapia brain	Texas	96	90 (1.2)[d]
4B10M322	9/93	Tilapia brain	Texas	77	ND[e]
4B10M331	10/93	Tilapia brain	Texas	89	79 (1.4)
4B10M332	10/93	Tilapia brain	Texas	100	93 (0.9)
4B10M34	10/93	Tilapia brain	Texas	97	93 (1.2)
4B10M38	11/93	Tilapia brain	Texas	92	ND
4B10M411	12/93	Tilapia brain	Texas	100	ND
4B10M412	12/93	Tilapia brain	Texas	93	ND
4B10M43	1/94	Tilapia brain	Texas	100	ND
4B10M45	1/94	Tilapia brain	Texas	100	ND
4B10M45	1/94	Tilapia brain	Texas	90	ND
4B10M47	2/94	Tilapia brain	Texas	ND	ND
4B10M52	3/94	Tilapia brain	Texas	91	ND
4B10M60	4/94	Tilapia brain	Texas	93	ND
Idaho 1	1994	Tilapia kidney	Idaho	91	ND
S. iniae ATCC 29178^T	1976	Freshwater dolphin skin abscess	United States	90 (0.8)	100 (0.0)
S. shiloi ND 2-16^T	8/89	Tilapia brain	Afikim, Israel	100 (0.0)	95 (0.5)
ND 04	8/88	Trout kidney	Afikim, Israel	90 (0.7)	ND
ND 24	5/89	Trout brain	Ein Hamifraz, Israel	98 (0.5)	ND
ND 26	5/89	Tilapia brain	Afikim, Israel	93	ND
CH 1	6/86	Tilapia kidney	Taiwan	89	ND
Dan 1	4/89	Trout brain	Dan, Israel	95 (0.9)	78 (1.0)
Dan 2	4/89	Trout brain	Amir, Israel	92 (0.4)	ND
Dan 3	12/91	Trout brain	Dan, Israel	77	ND
Dan 4	12/91	Trout kidney	Dan, Israel	93	83 (1.3)
Dan 5	12/91	Trout kidney	Dan, Israel	86	ND
Dan 6	12/91	Trout brain	Dan, Israel	89	ND
Dan 7	12/91	Trout kidney	Dan, Israel	93	ND
Dan 10	1/92	Coho salmon brain	Dan, Israel	91	ND
Dan 12	2/92	Trout kidney	Dan, Israel	90	93 (1.1)
Dan 14	2/92	Coho salmon brain	Dan, Israel	95	ND
Dan 15	2/92	Trout brain	Dan, Israel	91	ND
Dan 16	2/92	Trout brain	Dan, Israel	92	ND
Dan 18	4/92	Trout brain	Dan, Israel	83	ND
Dan 35	11/94	Trout brain	Dan, Israel	78	ND
Dan 36	11/94	Trout brain	Dan, Israel	81	90 (1.1)

[a] Strains were isolated from diseased fish in Israel, the United States, and Taiwan.
[b] The relative binding ratios are the means of the values from four determinations, expressed as percentages of homologous binding.
[c] The scientific names of the fish are as follows: tilapia, *Oreochromis aurea* × *Oreochromis nilotica* hybrids; trout, *Onchorynchus mykiss*; Coho salmon, *Onchorynchus kisutch*; Amazon freshwater dolphin, *Inia geoffrensis*.
[d] The values in parentheses are $\Delta T_{m(e)}$ values (in degrees Celsius) [$\Delta T_{m(e)}$ is the difference in melting points between the homologous reaction and the heterologous reaction]. These values reflect the levels of divergence in related DNA sequences and were not determined for all reactions.
[e] ND, not done.

States (Table 1) support the biochemical findings, indicating that all of the isolates which we tested are similar and belong to the same species. These organisms should be referred to as *S. iniae* and not *S. shiloi*. These two names are synonyms but because *S. iniae* (7) was published before *S. shiloi* (2, 4), only the name *S. iniae* should be retained. The differences in G+C contents which have been observed may have been due to technical differences, and the data that we published previously (2) (which were determined by the high-performance liquid chromatography method) are probably more accurate than the data initially reported for *S. iniae*, which were determined by the denaturation method (7). Our conclusions have practical importance. *S. iniae*, which was described in 1976 on the basis of strains isolated in the United States (7), may have come to Israel in imported fish eggs in the early 1980s. The United States now imports tilapia from Israel. Disease due to *S. iniae* in tilapia in the United States may be caused by imported *S. iniae* strains or local *S. iniae* strain; only a molecular epidemiological study could answer this question. While only poorly defined group B streptococci were shown to infect fish in the United States or Japan (6, 8), our data show that *S. iniae* not only is a pathogen of captive freshwater dolphins, but also is a major fish pathogen responsible for fish mortality in trout in Israel and in tilapia in the United States, Israel, and Taiwan.

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