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Original Article

Serological and Molecular Characterization of *Streptococcus iniae* in Cultured Nile tilapia *(Oreochromis niloticus)* in Thailand

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Abstract

Streptococcus iniae infections developed in on-growing tilapia farms located in the northeastern and central regions of Thailand during 2003-2011. In the present study, *S. iniae* isolates recovered from diseased tilapia were investigated using biochemical, serological and molecular techniques. Biochemical characterization showed different profiles. The arginine dihydrolase (ADH) activity was distinctive between *S. iniae*, isolates obtained from the outbreaks in central region were ADH-positive (ADH+ve) while northeastern isolates were ADH-negative (ADH-ve). The serological assay demonstrated corresponding results to the ADH activity. Serotype I and serotype II were ascribed to the ADH+ve and ADH-ve isolates, respectively. Despite the serotypic differences, phylogenetic analysis of 16S rRNA and superoxide dismutase A (*sod*A) genes demonstrated high genetic similarity with ≥96%identity amongst *S. iniae* isolates in Thailand, and isolates from aquatic animals elsewhere.

Keywords: Nile tilapia, serotype, *Streptococcus iniae*, Thailand

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Introduction

The commercial aquaculture of Nile tilapia *Oreochromis niloticus* has expanded dramatically in the last decades and has been responsible for a significant increase in global freshwater fish production, approximately 3.5 million metric tons in 2011. Thailand is now one of the leading tilapia producing countries that include China, Egypt, Indonesia, the Philippines and Brazil, and yields tilapia products exclusively for local consumption demand and international trade (Fitzsimmons et al., 2012). Along with the farming intensification, disease has become a significant problem due to poor water quality and the high stock densities used. Several diseases of bacterial pathogens have been occurred in intensive tilapia farms in many areas of Thailand, especially streptococcosis (Suanyuk et al., 2008; Yuasa et al., 2008). This disease is now considered to be the most threatening cause of mass mortality and economic loss in tilapia cultures throughout the country (Wongtavatchai and Maisak, 2008). The streptococcocal pathogen is an important bacterium that devastates fish cultures, mainly those cultivated in warm and freshwater, brackish or marine water (Dodson et al., 1999; Evans et al., 2002; Toranzo et al., 2005). Warm-water streptococcosis can cause acute to chronic, massive mortalities associated with septicemia and meningoencephalitis in fish species, especially in hybrid striped bass *Moronechrysops* x *M. Saxatilis* (Buchanan et al., 2005); hybrid tilapia *O. niloticus*x *O. aureus* (Shoemaker et al., 2001); rainbow trout *Oncorhynchus mykiss* (Perera et al., 1997; Eldar and Ghittino, 1999); Japanese flounder *Paralichthys olivaceus* (Nguyen et al., 2002) and barramundi *Lates calcarifer* (Bromage et al., 1999). Infected fish often shows similar clinical signs including anorexia, loss of orientation, unilateral or bilateral exophthalmia with corneal opacity, abdominal distention, hemorrhagic skin and, sometimes a protruding anal area with hyperemia can be found (Karsidani et al., 2010).

Although several members of *Streptococcus* sp. are the etiological agent of streptococcosis in many aquaculture species of the world, *S. agalactiae* and *S. iniae*are evidently the major pathogens of tilapia disease in Thailand. *S. agalactiae* infection has been commonly found to be a cause of streptococcosis occurring in farms located in all areas of the country, while *S. iniae* appeared in sporadic outbreaks (Wongtavatchai and Maisak, 2008; Suanyuk et al., 2010). The first isolation of *S. iniae* in Thailand was discovered in Nile tilapia reared on a commercial farm in Mukdahan province in the northeastern part of the country in December 2003. Three years later, issues of *S. iniae* infection in intensive tilapia culture were still occasionally observed in fish farms located in the northeastern region. In May 2011, a recurrence of *S. iniae* outbreaks occurred in on-growing Nile tilapia cultures in the northeastern and central region of Thailand, with typical signs of streptococcal infection. A streptococcal-like organism was isolated from the target organs of diseased fish and further confirmed to be *S. iniae* using polymerase chain reaction (PCR) by amplification of the 16S rRNA gene (Zlotkin et al., 1998). Although each outbreak of *S. iniae* infection caused considerable mortality and had a significant economic losses on the tilapia aquaculture of Thailand, limited documentation is available particularly on the genetic and serotypic characterization. This pathogen was also reported as a zoonotic agent causing disease in immunocompromised human in many countries, such as Canada (Weinstein et al., 1997), Hong Kong (Lau et al., 2003) and Taiwan (Sun et al., 2007).

The investigation of *S. iniae* was performed by phenotypic, serological and molecular techniques besides clinical signs and necropsy examination, because the similar typical signs and gross lesions were commonly found in case of either piscine *S. iniae* or *S. agalactiae* infection (Eldar et al., 1994; Bromage and Owens, 2002; Geng et al., 2012). In addition to comparable lesions between *S. iniae* and *S. agalactiae* infections, initial microbiological properties, such as colony on solid agar, Gram-positive cocci, catalase and oxidase negative were not sufficient to distinguish these two pathogen species, thus the molecular technique was useful for identification of *S. iniae*.

Sequence analysis of 16S rRNA gene has been used for molecular identification of various streptococcal species, including *S. iniae* spreading in aquaculture (Karsidani et al., 2010; Al-Harbi, 2011; Figueiredo et al., 2012). However, the evolution rate of the 16S rRNA gene is slow, therefore the alternative house-keeping gene, superoxide dismutase A (*sodA*) gene, was suggested to be useful to investigate the relationship among the genetically related organisms (Kawamura et al., 1999). Superoxide dismutase A encoding gene was proven to be effective for molecular identification and phylogenetic analysis of Gram-positive bacteria (Poyart et al., 1998). Classifications of fish streptococcal pathogens with *sodA* gene sequencing analysis were reported in *S. phocae* (Alber et al., 2004), *S. agalactiae* (Kawamura et al., 2005) and *S. dysgalactiae* subsp. dysgalactiae isolated from farmed fish (Nomoto et al., 2008).

The study of serotypic and molecular characteristics of *S. iniae* pathogens is necessary for the following disease prevention regimes, particularly the vaccination. Different vaccination procedures should be applied depending on the regional prevalence of specific diseases (Brudeseth et al., 2013). In addition, the findings would be useful to ethological analysis and establish counter measures to prevent outbreaks of the disease. For these reasons, it is important to confirm the genetic and serological diversity of *S. iniae* pathogens spreading in the country.

In the present study, we investigated the phenotypic, serotypic and molecular characterization of *S. iniae* isolated from cultured Nile tilapia in Thailand. Sequencing and phylogenetic analysis of the partial 16S rRNA and *sodA* genes was performed to compare *S. iniae* strains isolated from Thai tilapia with strains described in the National Center for Biotechnology Information (NCBI).

Materials and Methods

Sample collection: Diseased Nile tilapia *Oreochromis niloticus* with high mortality were observed in northeastern and central Thailand, where fish were cultured in net cages and earthen ponds, respectively (Table 1). The moribund fish showing clinical signs of streptococcal infection were collected and transferred to the laboratory for further examination. Clinical signs and the gross lesions of the diseased fish were also recorded. Water temperature and water quality parameters, such as pH, dissolved oxygen, unionized ammonia and nitrite, were monitored at the sampling sites.

Bacterial isolation: Streptococcal bacteria isolated from pronephros of the diseased fish by plating on Tryptic Soya Agar (TSA; Oxoid, Basingstoke, UK) supplemented with 5% (v/v) sheep blood at 30±2°C for 24 h. The pure isolates were examined for the following characteristics to ensure the identification of streptococcal bacteria.

Phenotypic characterization: Conventional bacteriological techniques including Gram stain, catalase and oxidase production were applied to bacterial colonies for primary characterization. Biochemical differences among the isolates were determined by enzymatic tests with the API 20 STREP system (Biomerieux, Marcy L'Etoile, France). Lancefield antigen grouping of the streptococcal isolates were performed using a Slidex Strepto-kit (BioMerieux).

Serological determination: Cross-agglutination tests were conducted with antisera against *S. iniae* prepared from immunized tilapia according to Barnes et al. (2003). Streptococcal isolates those represented different outbreaks were selected for bacterin productions. Isolates were separately cultured in Tryptic Soya Broth (TSB; Oxoid). The cultures were incubated at 30±2°C for 12 h in a shaking incubator (Sheldon MFG., OR, USA). Bacterial pellets were harvested with the refrigerated centrifugation (SIGMA Laborzentrifugen GmbH, Germany) at 6500 g for 20 min and were inactivated by an addition of formalin at a final concentration of 3% (v/v). Formalin was removed by washing 3 times with sterile normal saline solution (NSS). The bacterial concentration was adjusted to $1x10^9$ cells/ml with a spectrophotometer (Spiral Biotech, Norwood, MA, USA) optical density of 1.1 at 540 nm wavelength.

Hyperimmune sera against 9 *S. iniae* isolates were obtained from juvenile tilapia (567±23 g body weight) immunized with bacterin (3×10^8 cells/fish) by intraperitoneal injection, 4 fish for each bacterial isolate. Each fish received a booster immunization 14 d after an initial dosage. Similar immunization protocol was carried using *S. agalactiae* and sterile NSS to acquire the control sera. Fourteen days after the booster immunization, the fish were anesthetized with MS-222 (Western Chemical, WA, USA) for blood sampling. Blood was allowed to clot overnight at 4°C, before the serum collected using centrifugation at 3500 g for 1 min. The serum was stored at 4°C until the examination of agglutinating antibody titers. The animal experiment was approved by the ethics committee of Chulalongkorn University Animal Care and Use Committee (CU-ACUC; Approval No. 11310078).

Serum antibody titer specific to *S. iniae* was measured by direct agglutination tests, in duplicate, in a microtiter plate (Klesius et al., 2000). One hundred µl of *S. iniae* suspension ($1x10^8$ cells/ml) in phosphate buffered saline (PBS) was added to each well, and serial 2-fold dilution of specific antiserum in PBS was then added to each well (100 µl). Plates were incubated overnight at room temperature (25°C). The antibody titers were read as reciprocal of the highest dilution of the sera showing bacterial agglutination. The agglutination test scheme was to evaluate serological response of tilapia against each *S. iniae* isolate and cross reactions against different isolates.

***Polymerase chain reaction (PCR) assay for Streptococcus iniae detection*:** Genomic DNA was extracted from a colony of the isolated bacterium using a Nucleospin® Extract I kit (Macherey-Nagel GmbH, Düren, Germany) following the manufacturer's instructions. PCR identification of *S. iniae* was conducted in a Tpersonal PCR cycler (Biometra, Goettingen, Germany) using 16S rRNA specific primers: Sin-1 and Sin-2 (Zlotkin et al., 1998). *S. iniae* ATCC29178 type strain was used as a positive control and distilled water was used as a negative control for the reaction. PCR amplicons were determined by 2% agarose gel electrophoresis in 1% Tris-Borate-EDTA buffer (National Diagnostics, GA, USA), stained with 0.5 µg/ml ethidium bromide (Sigma-Aldrich, Gillingham, Dorset, UK), and visualized under UV illumination (VilberLourmat Deutschland, Berlin, Germany). A 100 bp DNA ladder (GIBCO/BRL, MD, USA) was used as a molecular marker.

16S rRNA and sodA genes sequencing and phylogenetic analysis: Eleven bacterial isolates identified as *S. iniae* from previous steps were chosen to represent each regional outbreak. The target regions and oligonucleotide primers used for PCR amplification of *S. iniae* genes are listed in Table 2. Two primer pairs corresponding to conserved regions in the 16S rRNA gene (JW_16S_F and JW_16S_R) and the *sodA* gene (JW_sodAF1 and JW_sodAR1) were used (Maisak, 2010). PCR were performed for each gene amplification separately in a final volume of 40 µl containing 50 ng of genomic DNA, 0.5 µM of each primer, 250 µM of dNTP, and 1 unit of Taq DNA polymerase (iNtRON Biotechnology, USA) in a 1× PCR buffer (100 mMTrisHCl, 500 mMKCl, 20 mM$MgCl_2$). PCR mixture for 16S rRNA gene was amplified 30 cycles in the PCR Thermal Cycler with the following program: 94°C for 3 min (initial denaturation step), 30 cycles at 94°C for 30 sec (denaturation step), at 58°C for 30 sec (annealing step), and at 72°C for 60 sec (extension step), followed by a final extension at 72°C for 5 min. PCR condition for the *sodA* gene was conducted with the parameter: 94°C for 5 min, 30 cycles at 94°C for 45 sec, annealing step at 56°C for 45 sec, extension at 72°C for 45 sec, followed by a final extension at 72°C for 5 min.

Table 1 *Streptococcus iniae* isolates from Nile tilapia used in the study

Isolate no.	Isolation date	Host, culture system	Location
JW1	December 2003	80-90 g fish, cages in river	Mukdahan, northeastern
JW3	February 2004	260-300 g fish, cages in river	Nakornpanom, northeastern
JW4	March 2006	100 g fish, cages in river	Nongkhai, northeastern
JW6	March 2006	100-250 g fish, cages in river	Nongkhai, northeastern
JW7	April 2007	350 g fish, cages in river	Nakornpanom, northeastern
JW9	April 2007	450-600 g fish, cages in river	Mukdahan, northeastern
NS1-2011	May 2011	700 g fish, cages in river	Mukdahan, northeastern
NS8-2011	May 2011	50-70 g fish, cages in river	Mukdahan, northeastern
NS24-2011	May 2011	700-800 g fish, cages in river	Yasothon, northeastern
NS29-2011	May 2011	800 g fish, cages in river	Yasothon, northeastern
SI45-2011	May 2011	400-480 g fish, earthen pond	Nakornnayok, central
NS45-2011	May 2011	400-480 g fish, earthen pond	Nakornnayok, central
NSb45-2011	May 2011	360-450 g fish, earthen pond	Nakornnayok, central

Table 2 Oligonucleotide primers used in the study

Primer	Nucleotide sequence (5'-3')	Gene	Product
Sin-1	CTAGAGTACACATGTACTAAG	16S rRNA	300 bp
Sin-2	GGATTTTCCACTCCCATTAC		
JW_16S_F	AACGGGTGAGTAACGCGTAG	16S rRNA	1234 bp
JW_16S_R	TTCATGTAGGCGAGTTGCAG		
JW_ *sodA*F1	TGATGCTTTAGAGCCACAATTTGAT	*sod*A	512 bp
JW_ *sodA*R1	CATTGATGTAGTTTGGACGAACA		

The products from PCR assay were purified by NucleoSpin® Extract II kit (Macherey-Nagel GmbH) and sequenced using a BigDye® Terminator v3.1 cycle sequencing kit (1st BASE DNA Sequencing, Malaysia). The nucleotide sequences were analyzed using BioEdit V.7.2.0. Multiple sequences were aligned with sequences of reference strains available in the NCBI database by CLUSTALW. The phylogenetic relationship of *S. iniae* was created by the neighbour-joining method based on 1000 bootstrap replications with MEGA 5. Thereafter, the nucleotide sequences were submitted to the GenBank sequence database.

Results

Clinical observation: The affected fish, with a wide range of body weight (50-500 g), showed variable clinical signs of streptococcal infection, including exophthalmia with opacity of the eye, abdominal distention and petechial hemorrhage on the body surface, obviously in operculum and pectoral area. Some fish exhibited subcutaneous abscesses at the base of the fin or tail. The internal lesions showed generalized hemorrhage at the brain and the visceral organs, especially in the intestines. In the abdominal cavity of clinical fish, serosanguineous fluid accumulation, enlarged pale liver and swollen spleen were found. During the disease outbreaks, water temperature ranged between 26-31°C, dissolved oxygen of 4.03-5.85 mg/l, pH was 7.12-7.74, unionized ammonia and nitrite were 0 mg/l.

Phenotypic characterization: *S. iniae* isolated from the diseased fish showed pinpointed (≤1 mm in diameter) colonies on blood agar, whitish round and exhibited β-haemolysis. The bacteria were Gram-positive cocci (0.9-1 μm in diameter) and yielded negative result on catalase and oxidase enzyme production. Some biochemical characteristics were found inconsistent amongst *S. iniae* isolates; the Voges-Proskauer, hippurate, esculin, mannitol utilization and arginine dihydrolase (ADH) (Table 3). Distinguishable difference within Thai isolates was the activity of ADH. The bacteria recovered from diseased tilapia from the central region were positive for ADH (ADH+ve), while other isolates from the northeastern region were ADH negative (ADH-ve). The Lancefield serogroup using Slidex Strepto-kit showed negative results to all isolates.

Serological determination: Serological analyses indicated the antigenic divergence between ADH+ve and ADH-ve*S. iniae*. The antiserum against the ADH+ve isolate (isolate no. SI45-2011, NS45-2011 and NSb45-2011) showed significant titer against its homologous isolate but did not agglutinate ADH-ve isolates. Similarly, tilapia immunized with ADH-ve isolates (isolate no. JW4, JW7, NS1-2011, NS8-2011, NS24-2011 and NS29-2011) exhibited high agglutinating titer against their homologous isolates, and negligible agglutination was found across ADH+ve isolate. Anti-*S.agalactiae* (isolate no. SA1) sera and sham-immunized sera presented titers<2 to any of *S. iniae* isolates (Table 4).

PCR amplification: Amplification of 300 bp band with primers specific for *S. iniae* 16S rRNA gene was detected in all bacterial samples. The specificity of the primers was tested using the pure genomic DNA

template extracted from *S. iniae* ATCC 29178 type strain.

Phylogenetic analysis of S. iniae 16S rRNA and sodA genes: The partial 16S rRNA gene sequences of *S. iniae* isolates in this study were submitted to the GenBank with the accession numbers of GQ169769-GQ169771, GQ338313-GQ338315 and KF555592- KF555596, and GenBank accession numbers for the *sodA* gene sequences of these isolates wereHM004083-HM004088 and KF555597- KF555601. The sequence analysis of partial 16S rRNA and *sodA* genes of *S. iniae*isolated from Thai tilapia revealed 96-100% similarity with the reference strains (*S. iniae* ATCC29178 type strain and *S.iniae* accession no. EU661272). The phylogenetic trees indicated that *S. iniae* from Thailand clustered into a group of other aquatic animals *S. iniae* strains from fish from China (accession no. JQ990158, HM053435 and EU622515), frog from Taiwan (accession no. AY762259), rainbow trout from Iran (accession no.FJ870987), barramundi from Australia (accession no. EU661272) and the Amazon freshwater dolphin ATCC29178 type strain (accession no. NR125048) with bootstrap percentage of 99-100. The genetic relationship among *S. iniae* isolates obtained from tilapia in different geographical areas of Thailand was shown in Fig 1. *S. iniae* isolates were clearly separated from the other Streptococcus species and distinguished from *S. agalactiae* (JW22 and JW25) recovered from diseased Nile tilapia in the same culture areas.

Discussion

This study provided primary epidemiological data of *S. iniae* outbreaks in Nile tilapia farming in Thailand. Some biochemical profiles of *S. iniae* isolated from Nile tilapia in the present study were variable, but most of biochemical characters were similar to those of *S. iniae* recovered from other fish species: Asian sea bass or barramundi *Lates calcarifer* (Bromage et al., 1999; Suanyuk et al., 2010) and Amazon freshwater dolphin *Inia geoffrensis* (Pier and Madin, 1976).The biochemical inconsistency between *S. iniae* isolates obtained from fish was also reported in the other studies. It was suggested that biochemical variations of *S. iniae* may associate with the differences amongst isolates, host species, geographical locations, or environmental factors such as water temperature and salinity (Kanai et al., 2006; Russo et al., 2006; Nho et al., 2009; Shoemaker et al., 2010). In this study, it was clear that *S. iniae* isolates from the central region were ADH+ve, and the northeastern region were ADH-ve. Serotypic classifications of *S. iniae* were found distinctive and related to their ADH biotypes. The typing of serotype I for ADH+ve *S. iniae* and the serotype II for ADH-ve *S. iniae* were studied in rainbow trout infections (Bachrach et al., 2001; Barnes et al., 2003).

Nile tilapia antiserum against the ADH+ve *S. iniae* did not agglutinate ADH-ve isolates, but had high antibody titers to its homologous strain. Likewise, the antisera to ADH-ve isolates showed significant agglutinating titers across ADH-ve isolates, however titers against their heterologous isolates were low. Our results were similar to those of serological tests reported in rainbow trout. Rainbow trout serotype I-antisera (against ADH+ve *S. iniae*) demonstrated no cross-reactivity to serotype II strains (against ADH-ve *S. iniae*). The present study

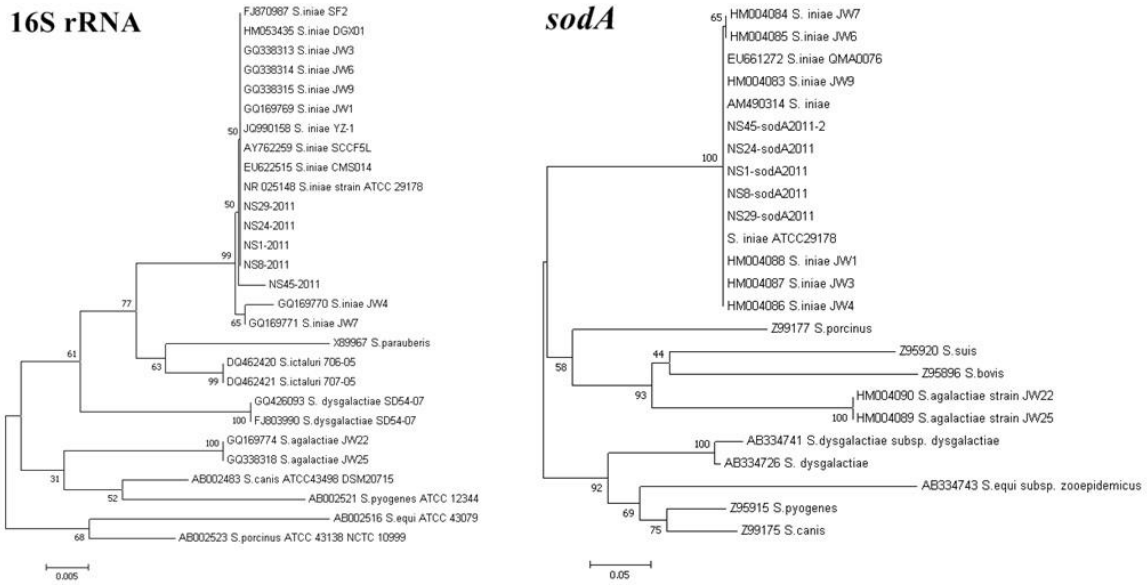

Figure 1 Neighbour-joining phylogenetic tree based on 16S rRNA and *sodA* gene sequences of *S. iniae* isolates from tilapia in Thailand, *S. iniae* isolates from other sources of aquatic animal species and other members of streptococci. Bootstrap percentages calculated from 1000 replications are displayed at branch points. Scale bars represent distance values.

Table 3 Biochemical characteristics of *Streptococcus iniae* from diseased Nile tilapia in Thailand and *S. iniae* from previous studies, tested with API 20 STREP system (BioMerieux)

Characteristic	*Streptococcus iniae*				
	This study (*n* = 13)	**Red tilapia[a]**	**Asian sea bass[a]**	**Barramundi[b]**	**ATCC 29178[c]**
Haemolysis	β	β	β	β	β
Catalase	-	-	-	-	-
Oxidase	-	-	-	-	-
Voges-Proskauer	- (6)	-	-	-	-
Hippurate	- (7)	-	-	-	-
Esculin	+ (6)	+	+	+	+
Pyrrolidonylarylamidase	+	+	+	+	+
α-Galactosidase	-	+	-	-	-
β-Glucuronidase	+	+	+	+	+
β-Galactosidase	-	+	-	-	-
Alkaline phosphatase	+	+	+	+	+
L-leucinearylamidase	+	+	+	+	+
Arginine dihydrolase	- (3)	+	+	+	+
Acid production from:					
Ribose	+	+	+	+	+
Arabinose	-	-	-	-	-
Mannitol	+ (3)	+	+	+	+
Sorbitol	-	-	-	-	-
Lactose	-	-	-	-	-
Trehalose	+	+	+	+	+
Inulin	-	-	-	-	-
Raffinose	-	-	-	-	-
Starch	+	+	+	+	+
Glycogen	+	+	+	+	+

+: positive reaction,- : negative reaction,[a](Suanyuk et al., 2010), [b](Bromage et al., 1999), [c](Pier and Madin, 1976)
Numbers in parenthesis denote isolates tested that gave the opposite result.

Table 4 Agglutinating titers of Nile tilapia antisera against *Streptococcus iniae* homologous and heterologous isolates

Isolate immunized	ADH	Agglutinating titer against:									
		JW4	JW7	NS1-2011	NS8-2011	NS24-2011	NS29-2011	SI45-2011	NS45-2011	NSb45-2011	SA1
JW4	-	64	32	32	32	32	32	4	4	4	2
JW7	-	64	128	64	64	64	64	8	8	4	2
NS1-2011	-	64	64	128	64	64	64	4	4	4	2
NS8-2011	-	64	64	64	128	64	64	8	8	4	2
NS24-2011	-	64	64	64	64	128	64	8	8	4	2
NS29-2011	-	64	64	64	64	64	128	8	8	4	2
SI45-2011	+	<2	2	<2	2	2	2	512	512	512	2
NS45-2011	+	<2	2	<2	2	2	2	512	512	512	2
NSb45-2011	+	<2	2	<2	2	2	2	512	512	512	2
Control											
S. agalactiae (SA1)	+	<2	<2	<2	<2	<2	<2	<2	<2	<2	1,024
Sham-immunized sera[a]	NA	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2

[a]Sham-immunized sera were obtained from the fish injected with sterile normal saline solution, ADH: arginine dihydrolase, NA: not applicable

suggested serological diversity among *S. iniae* observed in our study may relate to the different geographical distribution of *S. iniae* pathogens occurring in tilapia farms.

Despite variable biochemical properties and geographical origins, the high level of genetic similarity of *S. iniae* isolates from different locations of Thailand assessed by sequencing and phylogenetic analysis was demonstrated. The 16S rRNA and *sodA* gene sequences of the representative tilapia *S. iniae* isolates are closed to *S. iniae* strains from other countries. From the interview of case owners, it was possible that the initial outbreak of *S. iniae* in Thailand was associated with the non-warranted movement of tilapia fry from China. Streptococcosis in fish species in China is commonly caused by *S. iniae* (Zhou et al., 2008), but the pathogenic streptococcal bacteria usually found in Thai tilapia farms is *S. agalactiae*.

No genetic relatedness between *S. iniae* and *S. agalactiae* recovered from the diseased Nile tilapia in Thailand despite their coincident outbreaks.

Generally, epidemics of streptococcosis in fish occur when high increase of water temperature in the summer season, over stocking density and adverse water quality (Hernández et al., 2009). *S. agalactiae* has been recognized as a pathogen suitable to tropical areas (water temperatures more than 27°C) compared with *S. iniae* which may prefer colder temperate regions (Mian et al., 2009). *S. iniae* outbreaks in tilapia farms in Thailand were dissimilar to above reports, infections were detected sporadically throughout the year, regardless the water temperature (26-31°C) and with regular water features. The origin of this bacterium in Thailand has not been established. Streptococcal infection is horizontally transmitted from dead or infected fish to fish, through water-born exposure, particularly through the wound infection (Bowater et al., 2012). *S. iniae* was discovered in high numbers from the water column because the infected tilapia shed this pathogen mainly through excrement during the first week post infection (Baums et al., 2013). Sharing of common sources of water in aquaculture, particularly net pen floating in the river, may be a notable factor behind the outbreaks in tilapia farms. *S. iniae* infection reported in this work was found only in the on-growing tilapia but not in larvae or fry stages. The findings of this research can be applied to the etiological study and the study will give the beneficial information in order to prevent disease outbreaks.

The present study revealed high genetic similarity amongst *S. iniae* isolates from tilapia aquaculture in Thailand, nevertheless, the existing of two distinctive serotypes were demonstrated in *S. iniae* isolates from different sources. The serological variousness among the isolates was shown to be the reason for the lack of vaccine efficacy (Agnew and Barnes, 2007). The verification of serological diversity with geographical distribution data of *S. iniae* outbreaks is requisite for vaccination strategy and effective vaccine development to be employed in the tilapia aquaculture of Thailand.

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บทคัดย่อ

การจำแนกทางซีรัมและอณูชีววิทยาของเชื้อสเตรปโตคอคคัส อินิเอ้ ที่ระบาดในปลานิล *(Oreochromis niloticus)* เพาะเลี้ยงในประเทศไทย

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การศึกษาเชื้อสเตรปโตคอคคัส อินิเอ้ ที่เป็นสาเหตุของโรคในฟาร์มเลี้ยงปลานิลขุนทางภาคตะวันออกเฉียงเหนือและภาคกลางของประเทศไทยในช่วงปีค.ศ. 2003-2011พบว่าผลการทดสอบคุณสมบัติทางชีวเคมีของเชื้อจากปลาป่วยแต่ละพื้นที่แสดงความแตกต่างของคุณสมบัติทางชีวเคมี โดยเฉพาะเอนไซม์อาร์จินีน ไดไฮโดรเลส (ADH) โดยพบว่าเชื้อสเตรปโตคอคคัส อินิเอ้ สายพันธุ์จากภาคกลางของประเทศไทยมีคุณสมบัติเป็นบวก (ADH+ve) แต่เชื้อจากภาคตะวันออกเฉียงเหนือมีคุณสมบัติเป็นลบ (ADH-ve) ผลการทดสอบปฏิกิริยาทางซีรัมสามารถจำแนกเชื้อที่มีคุณสมบัติ ADH+ve เป็นซีโรไทป์ 1และเชื้อที่มีคุณสมบัติ ADH-ve เป็นซีโรไทป์ 2อย่างไรก็ตามเมื่อวิเคราะห์ความสัมพันธ์เชิงวิวัฒนาการของไรโบโซม (16S rRNA) และยีน superoxide dismutase A (*sod*A) พบความเหมือนกันทางพันธุกรรม ≥96% ระหว่างเชื้อสเตรปโตคอคคัส อินิเอ้ จากภาคตะวันออกเฉียงเหนือและภาคกลางของประเทศไทยและเชื้อสเตรปโตคอคคัส อินิเอ้ ที่แยกได้จากสัตว์น้ำในประเทศอื่นๆ

คำสำคัญ: ปลานิล ซีโรไทป์ สเตรปโตคอคคัส อินิเอ้ ประเทศไทย

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