Detection of *Streptococcus iniae* and *Streptococcus agalactiae* in tilapia (*Oreochromis* spp.) in Thailand

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**Introduction**

Streptococcosis is one of the most important bacterial diseases of farm-raised tilapia in Thailand and worldwide (2,4). This bacteria causes significant economic losses with chronic morbidity and mortality in affected farms. In Thailand, both *Streptococcus iniae* and *Streptococcus agalactiae* have been reported to cause Streptococcosis in Nile and red tilapia (2). According to the clinical signs of infected fish and bacterial morphology on culture media, it is difficult to differentiate *S. iniae* from *S. agalactiae*. Therefore, more sensitive and specific methods are critical for the detection and differentiation of these pathogens for proper control. The objective of this study is to study the prevalence of Streptococcosis in Nile and red tilapia through the application of colony PCR (4) to identify *Streptococcus iniae* and *Streptococcus agalactiae* in Thailand.

**Materials and Methods**

Infected fish were collected from red tilapia farms in central and west of Thailand (Figure 1). A total of 21 samples showing the clinical signs of Streptococcus infection were collected and isolated for bacterial infection. Bacteria were collected from the anterior kidney by aseptic technique. Bacteria were isolated on tryptic soy agar (TSA) and incubated at 30°C for 48 hr. Pure bacterial isolates were identified by Gram staining, and polymerase chain reaction (PCR). Two pairs of primer (Table 1) were designed to detect Lactate oxidase (lctO) and 16S rRNA/IMOD gene (5) (Table 1). PCR amplification of Lactate oxidase (lctO) and 16S rRNA/IMOD gene were modified according to the method of colony PCR (2). The PCR reaction of 20 µl containing 1.6 mM MgCl₂, 2 µl 10x buffer, 0.4 mM dNTP, 0.25 µl Taq polymerase, and 1.0 µl primers. Purified bacterial colonies were taken from TSA plates by a sterile pipette tip and then inoculated into a PCR reaction tube. The PCR conditions consist of preheating at 95°C for 5 min, followed of 40 cycles at 95°C for 30 s, 52°C for 30 s, 72°C for 30 s, and a final extension at 72 °C 7 min. PCR products were analyzed using gel electrophoresis in 2% agarose gel. The gel was stained with ethidium bromide. DNA bands were visualized under UV light.

**Figure 1.** Sampling locations: Both *S. iniae* and *S. agalactiae* were isolated from Kanchanaburi province, West of Thailand (red area). Other sampling locations with only *S. agalactiae* were indicated (*).  

**Table 1.** Primers used in this study

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Target gene</th>
<th>Primer name</th>
<th>Sequence (5’ to 3’)</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. iniae</em></td>
<td>Lactate</td>
<td>LOX-1</td>
<td>AGGGAAAAATCG CAAGTOCC-3’ 5’</td>
<td>870 bp</td>
</tr>
<tr>
<td></td>
<td>oxidase</td>
<td>LOX-2</td>
<td>ATATCTGATGG GCCGTCAAA-3’ 5’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(lctO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>16S rRNA/IMOD</td>
<td>F1</td>
<td>GAGTTTGGATCAT GGCTCAG-3’ 5’</td>
<td>220 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IMOD</td>
<td>ACCAACATGTGT TAATTACTC-3’</td>
<td></td>
</tr>
</tbody>
</table>

**Results and Discussion**

In Thailand, chronic loss of farm-raised tilapias were frequently reported throughout Thailand. The clinical signs of infected fish include erratic swimming, exophthalmia, opaque cornea, and chronic mortality rate with daily mortality ranging from 0.1 to 0.3 percent. From routine bacterial isolation, round shaped,
gray pinpoint colonies were isolated on TSA. All isolates were β-haemolytic (Figure 2), gram positive cocci. In addition, the colony multiplex PCR revealed a specific band product (220 bp) for S. agalactiae, while a 870 bp PCR product were observed in S. iniae (Figure 3). Among 21 Streptococcus isolates, 85.71% are S. agalactiae (18 out of 21). Notably, S. iniae has been detected in 3 out of 21 samples (14.28%). All of them were isolated from Kanchanaburi province where fresh water is used for tilapia culture. A previous report indicated that S. iniae could be isolated from diseased tilapia in fresh water environment (3). It is noteworthy that the species of streptococcus should be carefully interpreted. S. iniae can cause chronic low mortality while S. agalactiae can a cause more rapid and severe loss.(6)

In summary, we conclude that colony multiplex PCR is a sensitive and specific method for the detection and differentiation of Streptococcus infection in tilapia in Thailand. It is important that S. iniae could be isolated from red tilapia cultured in fresh water in Kanchanaburi province. Lastly, our findings highlight the application of diagnostic procedures for the implementation of correct control strategies e.g. vaccine design and antibiotic therapy.

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References