Capsular typing of *Streptococcus agalactiae* (Lancefield group B streptococci) from fish using multiplex PCR and serotyping

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Abstract

Streptococcus spp. including *Streptococcus agalactiae* (Lancefield group B streptococci) are considered emerging pathogens responsible for approximately $1 billion USD in annual losses to the global tilapia (*Oreochromis* sp.) aquaculture industry. This study evaluated a published multiplex PCR for capsular (CPS) typing and commercially available antiserum to assign capsular serotype to a total of 40 *S. agalactiae* isolates including American Type Culture Collection (ATCC) isolates as controls. The multiplex PCR was modified from the original description to include primers specific for CPS types Ia, Ib, II and III, that are the CPS types reported from aquatic animals. Phenotypic variation was noted among the *S. agalactiae* isolates in this study based on API® 20 Strep strip results. The modified PCR assay that includes a 688 base pair (bp) amplicon based on the *cps*L gene diagnostic for *S. agalactiae* effectively typed piscine *S. agalactiae* isolates. Most isolates (28 of 30) from North, Central and South America were assigned to CPS type Ib based on the presence of three amplicons of 688, 621 and 272 bp. A commercial serotyping system mostly confirmed the molecular CPS types. The modified multiplex PCR assay can be used to determine the CPS types of *S. agalactiae* present on a farm and/or in a region, and an understanding of this will assist research and disease management strategies (i.e. vaccination and selective breeding).

Introduction

Streptococcosis, caused by *Streptococcus agalactiae* (Lancefield group B streptococci, GBS), is an emerging disease in farmed and wild fish worldwide (Shoemaker et al., 2017a). Two biotypes of *S. agalactiae* infecting fish were described based on biochemical characteristics and hemolytic ability against sheep red blood cells (i.e., non-hemolytic vs beta-hemolytic) (Sheehan et al., 2009). *Streptococcus agalactiae* is particularly problematic in tilapia (*Oreochromis* sp.) aquaculture and was initially named *S. difficile* (Eldar et al., 1994) and subsequently identified as non-hemolytic group B *S. agalactiae* by Vandamme et al. (1997). Multiple capsular (CPS) serotypes representing different multilocus sequence types (ST) including CPS type Ia (ST-7; ST-500), CPS type Ib (ST-261-like) and CPS type III (ST-283; ST-491) are presently impacting the industry (Suanyuk et al., 2008; Delannoy et al., 2013, 2016; Li et al., 2013; Liu et al., 2013; Soto et al., 2015). Early work suggested that CPS type was not restricted to a specific ST at least among human GBS strains (Jones et al., 2003). Metcalf et al. (2017) used whole

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