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Efficacy of a *Streptococcus iniae* modified bacterin delivered using Oralject™ technology in Nile tilapia (*Oreochromis niloticus*)

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Abstract

This study evaluated the potential to orally deliver a previously developed *Streptococcus iniae* vaccine in tilapia using Oralject™ technology. This technology was developed to administer bioactive compounds to monogastric animals, and has been shown to be effective for delivery of a variety of antigens in numerous fish species. Two different formulations containing two doses of vaccine (four treatments) were fed to tilapia (4 tanks of 25 fish each) for 1 (Oralject-1 and Oralject-2 each containing 2×10^9 cells/g of feed) day (am and pm to satiation) or 5 (Oralject-1 and -2 each containing 2×10^8 cells/g of feed) days (once daily to satiation). The incorporated vaccine was a patented lyophilized modified bacterin (US Patent No. 6,379,677 B1). A positive control treatment [intraperitoneally (i.p.) injected *S. iniae* vaccine] and a negative control treatment (i.p. injection of tryptic soy broth, TSB) were included. Mean percent intake indicated that tilapia fed for 1 day (twice to satiation) the Oralject-1 consumed significantly ($P<0.05$) more feed than fish fed Oralject-2 (4.05% vs. 3.21%, respectively). Fish fed for 5 days either commercial feed or Oralject-1 or -2 also showed differences in feed intake; on most days, fish consumed significantly less ($P<0.05$) Oralject-2 ($\sim$1%) than the commercial diet or Oralject-1 ($\sim$2.5%). Tilapia were challenged 23 days post-vaccination by i.p. injection of 1×10^6 CFU *S. iniae*/fish. Mean percent mortality was 47.5 ($\pm$7.5) in the TSB-immunized challenged tilapia and was significantly higher ($P<0.001$) than in all immunized groups. No mortality occurred in the i.p.-vaccinated tilapia. Mortality ranged from 17.5 to 31.25 in the Oralject™ treatments. Relative percent survival was 100% in the i.p.-vaccinated tilapia and 63.1% in the most effective Oralject-vaccine-treated group. The results suggest that oral delivery of the lyophilized *S. iniae* vaccine using Oralject™ technology provided protection against streptococcal disease. These data validate an initial proof-of-principle for oral vaccination of tilapia using *S. iniae* in the Oralject™ system.
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Keywords: Oralject™; *Streptococcus iniae*; Oral vaccination; Nile tilapia

1. Introduction

Streptococcus iniae is one of the most significant Gram-positive pathogens in wild and cultured fish species worldwide. Estimates of losses in the US alone exceed $10 million annually (Shoemaker and Klesius,

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1997). Efficacious vaccines [bacterins or modified bacterins (i.e., containing extracellular products)] have been developed against *S. iniae* for delivery by injection (Eldar et al., 1997; Klesius et al., 1999; Klesius et al., 2000; Klesius et al., 2002). Attempts at immersion vaccination using these killed *S. iniae* vaccines have been unsuccessful. The injected vaccines while being effective are labor-intensive to deliver and induce stress (i.e., fish have to be handled). A potential mass delivery strategy is oral administration via feed (Vandenberg, 2004). Ease of delivery (i.e., feeding) would enable mass vaccination of large numbers of fish in hatcheries, ponds and even the environment.

Other bacterial vaccines for fish have been successfully delivered by oral administration; however, the efficacy of the vaccines has not been as good as by parenteral injection (Ellis, 1988; Newman, 1993). Recently, Romalde et al. (2004) reported the use of alginate microparticles containing formalin-killed *Lactococcus garviae* as an oral vaccine. The best protective rate based on relative percent survival (RPS) using this method was 50%. Romalde et al. (2004) were able to demonstrate that fish immunized with an aqueous vaccine by injection and boosted via oral delivery at 4 months were protected (RPS=87%). The aqueous vaccine alone failed to provide significant protection after the third month (RPS=40%) following intraperitoneal (i.p.) challenge (Romalde et al., 2004). A similar protective effect was suggested following oral re-immunization after initial immersion vaccination of European eels against *Vibrio vulnificus* (Esteve-Gassent et al., 2004). The oral vaccine was prepared by the addition of bacterial antigen to feed without a carrier or protective coating.

Vandenberg et al. (2003) proposed a novel delivery strategy (Oralject™) for oral vaccination of monogastric animals. The Oralject™ technology relies on the temporary reduction of the digestive processes by administration of anti-proteases and membrane permeability enhancers in combination with the vaccine. This approach permits the vaccine (i.e., antigen) to escape digestive hydrolysis and have enhanced vaccine component uptake (Vandenberg, 2004).

The objective of this study was to determine the efficacy of *S. iniae* modified bacterin incorporated in fish feed using Oralject™ technology[1] to provide protection against streptococcal disease in Nile tilapia (*Oreochromis niloticus*).

2. Materials and methods

2.1. Fish and feeding

A total of 600 Nile tilapia (*O. niloticus*) with a mean weight of 12.7 grams/fish was utilized in this study. Prior to the study 10 fish were found to be culture negative for *S. iniae* by standard microbiology (Shoemaker et al., 2001). Fish were weighed and divided into four replicate aquaria of 25 fish each for each treatment. Each aquarium was supplied with de-chlorinated city water (26±2 °C) at a rate of 0.5 l/min. Fish were acclimated for 1 week prior to treatment and fed at a rate of 2% body weight (BW)/day with Aquamax Grower 400 (Brentwood, MO). After the 7-day acclimation period, fish were fasted for 36 h. After feed withdrawal for 36 h, fish in group A were injected intraperitoneally (i.p.) with 100 μl of sterile tryptic soy broth (TSB) (Table 1). Fish from group B were injected i.p. with lyophilized *S. iniae* vaccine (Klesius et al., 2000) resuspended in TSB at a rate of 100 μl per fish (equivalent to 4×10^8 CFU/fish). The lyophilized *S. iniae* vaccine was prepared by culturing *S. iniae* isolate ARS-60 for 72 h in tryptic soy broth prior to killing with formalin. The *S. iniae* cells were then removed from the culture fluid via centrifugation. After removal, the culture fluid was concentrated (20-fold) via use of a 2-kDa hollow fiber filter. Following sterile filtration (0.2 μm pore size), formalin-killed cells were added back to a final concentration of 4×10^9 cells/ml. Two different Oralject containing vaccine formulations were fed to the other tilapia. Fish from groups C and D were fed for 1 day (am and pm) to satiation with Oralject formulations-1 and -2 containing 2×10^9 cells/g feed, respectively. Fish from groups E and F were fed for 5 days once daily to satiation with Oralject formulations-1 and -2 containing 2×10^8 cells/g feed, respectively. Amount of feed consumed for each group was recorded daily during the 1-day treatment and 5-day treatment. Feed consumed was expressed as a percentage of the initial total weight of the fish in the tank. Following the feeding of the vaccine formulations, all fish were fed Aquamax Grower 400 (Brentwood, MO) at a rate of 2% initial body weight once daily.

2.2. Experimental challenge and antibody determination

All groups of fish (20/tank) were challenged 23 days after final feeding of the orally delivered vaccine.

[1] Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendations or endorsement by the USDA.