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Preparation, characterization and evaluation of the immune effect of alginate/chitosan composite microspheres encapsulating recombinant protein of *Streptococcus iniae* designed for fish oral vaccination

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Abstract

Streptococcus iniae has caused serious harm to the fish farming industry in recent years. Vaccination is a potential approach for preventing and controlling disease, being oral vaccination the most suitable vaccination route in fish. Alginate and chitosan microspheres have been widely used as controlled release systems for oral vaccination in fish. In this study, we prepared and characterized alginate/chitosan composite microspheres encapsulating the recombinant protein serine-rich repeat (rSrr) of S. iniae. We evaluated effect of these microspheres on the immune system of channel catfish. The microsphere preparation conditions were optimized by Response Surface Method and target microspheres were obtained under 1.68% alginate (w/v), the W/O ratio 3.6:7.4 (liquid paraffin with 4% Span 80, v/v) with stirring at 1,000 rpm, 9.64% $CaCl_2$ (w/v) and 0.95% chitosan (w/v) with an encapsulation efficiency of 92.38%. The stability and safety of rSrr-microspheres were evaluated in vitro and in vivo, respectively. Furthermore, compared with control group, oral vaccination with rSrr-microspheres induced higher serum antibody titers, higher lysozyme activity, higher total protein and higher expression of immune-related genes, and resulted in higher relative percent survival (RPS) with the value of 60% for channel catfish against *S.iniae* infection. Our results thus indicate that alginate/chitosan microspheres encapsulating rSrr can be used as oral vaccine for channel catfish, providing efficient immunoprotection against *S. iniae* infection.

Keywords: alginate/chitosan microspheres; oral vaccine; immune effect; immunoprotection; fish

1. Introduction

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