Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Nanoconjugation of bicistronic DNA vaccine against *Edwardsiella tarda* using chitosan nanoparticles: Evaluation of its protective efficacy and immune modulatory effects in *Labeo rohita* vaccinated by different delivery routes

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ARTICLE INFO

Article history:
Received 9 January 2018
Received in revised form 10 February 2018
Accepted 22 February 2018
Available online xxxx

Keywords:
Nanoconjugation
DNA vaccine
GAPDH
Edwardsiella tarda
Labeo rohita
Chitosan nanoparticles

ABSTRACT

DNA-based immunization has proven to be an effective prophylactic measure to control aquatic animal diseases. In order to improve the efficiency of vaccine against fish pathogen, novel delivery mechanism needs to be adopted. In the present study we nanoconjugated the previously constructed DNA vaccine (pGPD + IFN) with chitosan nanoparticles (CNPs) by complex coacervation process. After construction of the vaccine, an *in vivo* vaccination trial was conducted in which 2 groups of rohu (*L. rohita*) fingerlings were vaccinated with CNPs-pGPD + IFN, one group by oral route (incorporated in feed for 14 days) and the other by immersion route (primary and booster immunised), whereas, a third group was intramuscularly (I/M) injected (initial and booster immunised) with naked pGPD + IFN and subsequently challenged with *E. tarda* (8.7×10^4 CFU/fish) at 35-day post initial vaccination. The protective immune responses were determined in terms of relative percentage survival (RPS), specific antibody production, non-specific immune response, expression kinetics of immune-related genes and pathological manifestation. Evaluation of RPS analysis revealed that CNPs-pGPD + IFN groups recorded highest RPS (81.82% and 72.73% in oral and immersion vaccinated fish group respectively) while the naked pGPD + IFN injected group showed 63.62% RPS when compared with 55% cumulative mortality of control group. In addition, NBT, myeloperoxidase activity, serum lysozyme activity and specific antibody titre in case of CNPs-pGPD + IFN groups showed higher activities during all the time points. Furthermore, CNPs-pGPD + IFN groups showed significant ($p < 0.05$) upregulation of different immune gene transcripts (IgHC, iNOS, TLR22, NOD1 and IL-1β) in three immunologically important tissues post immunization (both primary and booster dose) as well as after challenge. Thus, from this study, we can conclude that oral or immersion vaccination with CNPs-pGPD + IFN can orchestrate an effective immunisation strategy in organizing a coordinative immune response against *E. tarda* in *L. rohita* exhibiting minimum stress to the host with maximum efficacy.

1. Introduction

Indian major carps (IMCs) constitute the mainstay of Indian aquaculture with production over two million tonnes per year. However, this increase in production has led to occurrence of infectious diseases causing substantial losses to the carp farming. Thus the need for protection of the highly intensive culture system from infectious disease has becomes priority among fishers community. Among the various bacterial pathogens causing economic losses to the Indian aquaculture industry, *Edwardsiella tarda (E. tarda)*, a gram negative, facultative anaerobic bacterium belonging to the family Enterobacteriaceae, causes Edwardsiellosis/putrefactive systemic infection in both marine and freshwater fishes [1]. Pathological manifestation associated with the disease are distended

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https://doi.org/10.1016/j.vaccine.2018.02.099
0264-410X/

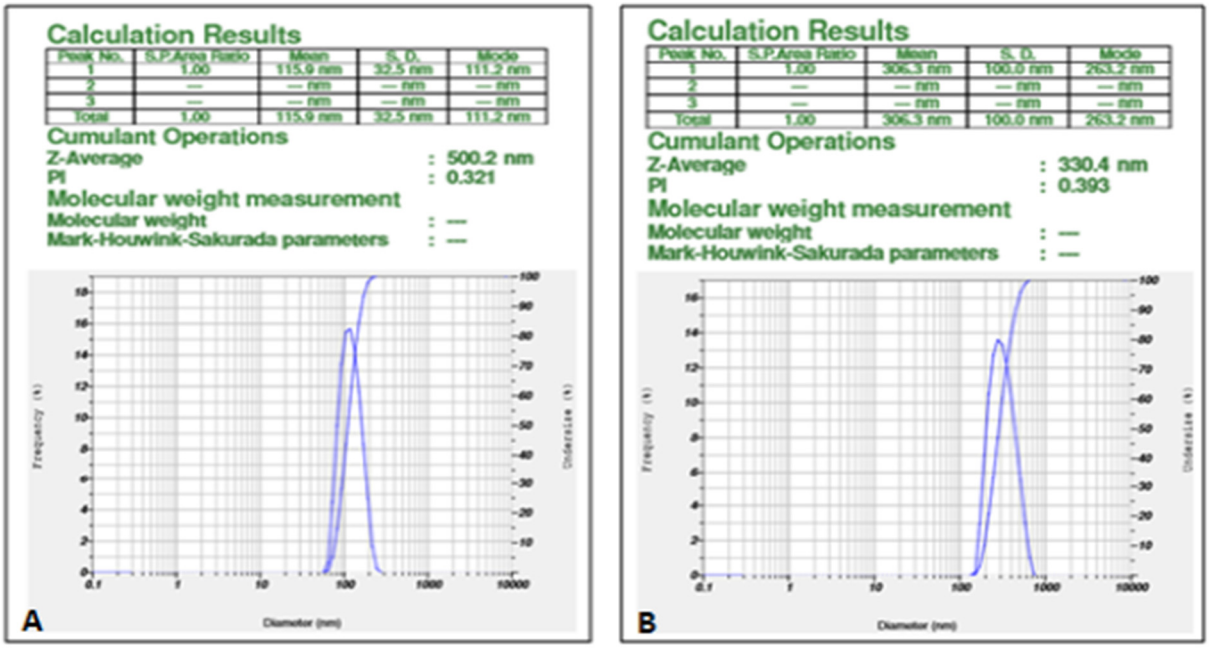

Fig. 1A. Particle size distribution of Chitosan Nanoparticles (CNPs) by dynamic light scattering (DLS) using HORIBA Scientific Nano particle analyzer SZ-100. (A) Blank CNPs; (B) CNPs-pGPD + IFN.

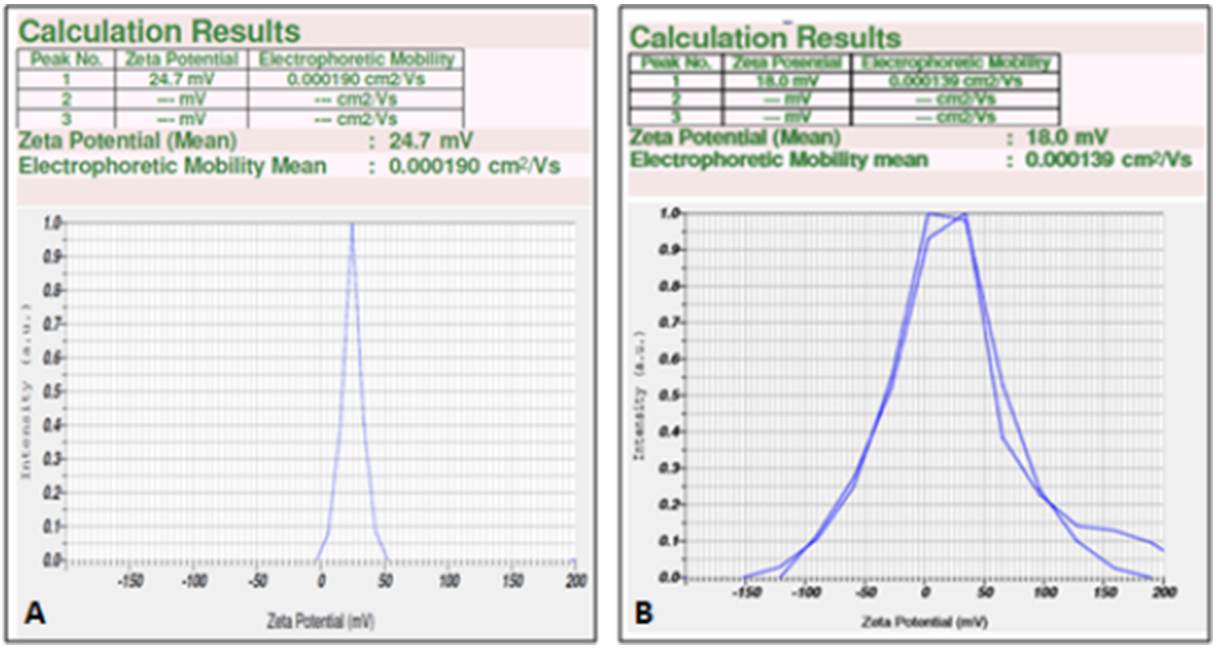

Fig. 1B. Zeta Potential of Chitosan Nanoparticles (CNPs) using HORIBA Scientific Nano particle analyzer SZ-100. (A) Blank CNPs; (B) CNPs-pGPD + IFN.

abdomen, prolapsed rectum, cutaneous lesions inside the musculature, fibrinous peritonitis along with necrosis of the hepatic and nephritic tissues [2].

In an attempt to control the spread of this disease, we previously developed a bicistronic DNA vaccine (designated as pGPD + IFN) containing a regular antigenic gene (glyceraldehyde-3-phosphate dehydrogenase gene of *Edwardsiella tarda*) along with an additional immune adjuvant gene (Interferon gamma gene of *Labeo rohita*) [3]. The vaccines construct was found to be successfully expressing the antigenic proteins both *in vitro* and *in vivo* and eliciting higher protective immunity in *L. rohita* against virulent *E. tarda* challenge but the mode of administration was intramuscular injection. Thus in lieu of the protective efficacy exhibited by the constructed vaccine, we presently focused on replacing the injection route of vaccine delivery by oral and immersion routes as the parental immunization method involves several limitation including stress on the fish, labour intensiveness, time requirements, unsuitability for administration in large number of small fish (<20 g) which are most susceptible to bacterial infection along with safety issues for fish as well as administrators [4].

Oral and bath immunization are attractive option to cater the disease outbreak, which offers convenience for mass vaccination and the advantage of zero handling stress to fish [5]. However, in contrast to the injection method, vaccine delivered through oral route,

Please cite this article in press as: Kole S et al. Nanoconjugation of bicistronic DNA vaccine against *Edwardsiella tarda* using chitosan nanoparticles: Evaluation of its protective efficacy and immune modulatory effects in *Labeo rohita* vaccinated by different delivery routes. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.02.099