

## **Oral vaccination of DNA encapsulated chitosan nanoparticles enhances the transcriptional responses of IFN and IFN-stimulatory genes in zebrafish**

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The use of oral vaccination in aquaculture has lagged behind injectable vaccines for a long time in protective immunity. In this study, we constructed the DNA vaccine using plasmid vector (pEGFPN2) and ORF012R gene of rock bream iridovirus (pEGFPN2-ORF012R). Then it was encapsulated to chitosan nanoparticles (CNPs) according to a complex coacervation method and denoted as pEGFPN2-ORF012R-CNPs. The pEGFPN2-ORF012R-CNPs had diameter of 189.5 nm. Encapsulation efficiency and loading capacity were determined as  $92.57\% \pm 0.87\%$  and  $9.32\% \pm 0.19\%$ , respectively. Final encapsulated product (pEGFPN2-ORF012R-CNPs) had +12.11 mV zeta potential. *In vitro* vaccine release assay showed that the plasmid DNA was sustainably released from the pEGFPN2-ORF012R-CNPs, up to  $84.26\% \pm 3.16\%$  of the total amount. By *in vitro* cell culture experiment we confirmed that the cloned pEGFPN2-ORF012R-CNP was expressed in HEK-293 cells. Oral vaccination was carried out by feeding of pEGFPN2-ORF012R-CNPs (250 ng/zebrafish/day) for 14 days. Quantitative real time PCR results clearly showed the transcriptional upregulation of IFN and IFN-stimulatory genes (Mx) in kidney and gut of zebrafish upon oral vaccination of pEGFPN2-ORF012R-CNPs compared to control fed diet, suggesting that CNPs is potential DNA vaccine delivery agent.

**Keywords:** Chitosan nanoparticles; Oral DNA vaccination; Zebra fish

**Acknowledgements** This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2017010990).

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