

Protection and Immune Responses of DNA Vaccines Targeting Virulence Factors of *Streptococcus iniae* in Nile Tilapia (*Oreochromis niloticus*)

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Abstract : *Streptococcus iniae* (SI) is a devastating pathogenic bacteria causing heavy mortality in farmed fish. The application of commercialized bacterin vaccine has been reported failures as the outbreaks of the new serotype of SI were emerged in farms after vaccination and subsequently caused severe losses. In the present study, we attempted to develop effective DNA vaccines against SI infection using Nile tilapia (*Oreochromis niloticus*) as an animal model. Two monovalent DNA vaccines were constructed by the insertion of coding sequences of cell wall-associated virulence factors-encoding genes, comprised of *eno* (α -enolase) and *mtsB* (hydrophobic membrane protein), into cytomegalovirus expression vector (pCI-neo). In the animal trial, 30-g Nile tilapia were injected intramuscularly with 15 μ g of each vaccine (mock vaccine group was injected by naked pCI-neo) and maintained for 35 days prior challenging with pathogenic SI at the dosage of 107 CFU/fish. At 13 days post-challenge, the relative percent survival of pEno, pMtsB and mock vaccine were 57%, 45% and 27%, respectively. The expression levels of immune responses-associated genes, namely, IL1 β , TNF- α , TGF- β , COX2, IL-6, IL-12 and IL-13, were investigated from the spleen of experimental animal at 7 days post-vaccination (PV) and 7 days post-challenge (PC) using quantitative RT-PCR technique. Generally, at 7 days PV, the pEno vaccinated group exhibited highest level of up-regulation (1.7 to 2.9 folds) of every gene, but TGF- β , comparing to pMtsB and mock vaccine groups. However, at 7 days PC, pEno group showed significant up-regulation (1.4 to 8.5 folds) of immune-related genes as similar as mock vaccine group, while pMtsB group had lowest level of up-regulation (0.7 to 3.3 folds). Summarily, this study indicated that the pEno and pMtsB vaccines could elicit the immune responses of the fish and the magnitude of gene expression at 7 days PV was also consistent with the protection level conferred by the vaccine.

Keywords : gene expression, DNA vaccine, Nile tilapia, *Streptococcus iniae*

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