

Elucidating the efficacy of vaccination against Vibriosis in *Lates calcarifer* using two recombinant protein vaccines containing the Outer Membrane Protein K (r-OmpK) of *Vibrio alginolyticus* and the DNA Chaperone J (r-DnaJ) of *Vibrio harveyi*

ABSTRACT

Recombinant cell vaccines expressing the OmpK and DnaJ of *Vibrio* were developed and subsequently, a vaccination efficacy trial was carried out on juvenile seabass (~5 cm; ~20 g). The fish were divided into 5 groups of 50 fish per group, kept in triplicate. Groups 1 and 2 were injected with 10⁷ CFU/mL of the inactivated recombinant cells vaccines, the pET-32/LIC-OmpK and pET-32/LIC-DnaJ, respectively. Group 3 was similarly injected with 10⁷ CFU/mL of inactivated *E. coli* BL21 (DE3), Group 4 with 10⁷ CFU/mL of formalin killed whole cells *V. harveyi*, and Group 5 with PBS solution. Serum, mucus, and gut lavage were used to determine the antibody levels before all fish were challenged with *V. harveyi*, *V. alginolyticus*, and *V. parahaemolyticus*, respectively on day 15 post-vaccination. There was significant increase in the serum and gut lavage antibody titers in the juvenile seabass vaccinated with r-OmpK vaccine. In addition, there was an up-regulation for TLR2, MyD88, and MHCII genes in the kidney and intestinal tissues of r-OmpK vaccinated fish. At the same time, r-OmpK triggered higher expression level of interleukin IL-10, IL-8, IL-1 β in the spleen, intestine, and kidney compared to r-DnaJ. Overall, r-OmpK and r-DnaJ triggered protection by curbing inflammation and strengthening the adaptive immune response. Vaccinated fish also demonstrated strong cross protection against heterologous of *Vibrio* isolates, the *V. harveyi*, *V. alginolyticus*, and *V. parahaemolyticus*. The fish vaccinated with r-OmpK protein were completely protected with a relative per cent of survival (RPS) of 90 percent against *V. harveyi* and 100 percent against *V. alginolyticus* and *V. parahaemolyticus*. A semi-quantitative PCR detection of *Vibrio* spp. from the seawater containing the seabass also revealed that vaccination resulted in reduction of pathogen shedding. In conclusion, our results suggest r-OmpK as a candidate vaccine molecule against multiple *Vibrio* strain to prevent vibriosis in marine fish.

Keyword: Recombinant vaccine; Cross-protection; Immune response; Juvenile seabass