

Administration of live-attenuated vaccine of *Vibrio harveyi* to improve survival of gnotobiotic brine shrimp (*Artemia salina*) model against multiple *Vibrio* infection

ABSTRACT

Newly developed live-attenuated protease derivative from pathogenic *Vibrio harveyi* strain Vh1 as a live vaccine to against Vibriosis of aquatic animals. In the current study, we used the gnotobiotic *A. salina* as model to evaluate the safety and efficacy of the live-attenuated. This study was conducted by bacterial safety experiment and bacterial efficacy experiment. During the bacterial safety, the wild type and live-attenuated of *V. harveyi* (MVh-vhs) were tested for 48 hours on the *Artemia* larvae (instar II). During the efficacy experiment, the *A. salina* larvae were incubated with different concentration of live-attenuated *V. harveyi* (MVh-vhs), then challenged with *V. harveyi*, *V. alginolyticus* and *V. parahaemolyticus*. The result of safety experiment showed that the high concentration of live-attenuated mutant *V. harveyi* (MVh-vhs) at concentration of 10⁹ CFU/mL is safe and had improved the *A. salina* larvae survival compared to other groups. On the other hand, pathogenic wildtype *V. harveyi* caused lethal effect on *A. salina* larvae by decreasing their survival. The surprising result of efficacy experiment showed that 10⁷ CFU/mL of live attenuated MVh-vhs with 6 hours post incubation with *A. salina* larvae contributed higher survival while 10⁹ CFU/mL of live attenuated MVh-vhs with 24 hours incubated *A. salina* larvae contributed higher survival against multiple *Vibrio* challenge. In this study, we concluded that the incubation time had affect bacterial concentration uptake by *A. salina* larvae and affect the effectiveness of *Artemia* bioencapsulation for targeted hosts.

Keyword: Vibriosis; *Artemia salina*; Live-attenuated vaccine; *Vibrio*; Serine protease