ADMINISTRATION OF BACTERIAL HEAT SHOCK PROTEIN 70 TO INDUCE A PROTECTIVE INNATE IMMUNE RESPONSE IN EUROPEAN SEA BASS LARVAE (*DICENTRARCHUS LABRAX*) AGAINST *VIBRIO ANGUILLARUM*

Eamy Nursaliza Yaacob[1,5], Bruno G. De Geest[2], Jens Goethals[3], Aline Bajeck[4], Kristof Dierckens[1], Peter Bossier[1*], Daisy Vanrompay[3*§]

[1]*Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Ghent, Belgium*

[2]*Department of Pharmaceutics, Ghent University, Ghent, Belgium*

[3]*Laboratory of Immunology and Animal Biotechnology, Ghent University, Ghent, Belgium*

[4]*Ecloserie Marine de Gravelines, France*

[5]*School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu, Terengganu, Malaysia*

ABSTRACT

We studied the use of recombinant *E. coli* heat-shock protein 70 (DnaK) encapsulated in alginate microparticles to protect European sea bass (*Dicentrarchus labrax*) larvae against *Vibrio anguillarum* infection. DnaK performs a multitude of housekeeping and cytoprotective functions in prokaryotes. It also stimulates the immune system of the model crustacean *Artemia*.

The experiment was performed by feeding alginate microparticles containing a low (0.5 mg) or high (1.0 mg) dose of the recombinant DnaK, to sea bass larvae at day 7 after hatching. Simultaneously, 2 groups (n = 120) of larvae were either fed with empty alginate microparticles or were receiving no microparticles (unfed) (negative controls). Larvae were infected with *V. anguillarum* after 18 h of feeding. Controls experienced an acute *V. anguillarum* infection resulting in high mortality. DnaK could not induce protection, as the mortality in the group receiving empty microparticles was statistically the same as in the groups fed with alginate microparticles containing the low or high dose of DnaK.

V. anguillarum significantly upregulated the expression of the *tlr3*, *tlr5*, *il1β, tnfα, cc1, cxcl8, cxcr4* and *ccr9* genes. Upregulation of pro-inflammatory cytokine genes, (*il1β, tnfα*), inflammatory chemokine genes (*cc1, cxcl8*) and chemokines receptor genes (*cxcr4, ccr9*) following bacterial infection is not uncommon in fish. However, to our knowledge, we are the first to demonstrate this in vibrio-infected axenic sea bass larvae.

Although there was a significant upregulation of *cas1, il1β, tnfα, cc1, cxcl8, cxcr4* and *ccr9* in the groups receiving DnaK, no protection against *V. anguillarum* was observed. We concluded that

axenic European sea bass larvae receiving recombinant DnaK prior to *V*. *anguillarum* challenge were not significantly protected from *V*. *anguillarum* infection.

KEYWORDS

Dicentrarchus labrax, sea bass, axenic, larvae, DnaK, innate immunity, *Vibrio anguillarum*

*These authors have contributed equally to this work.

§Corresponding author. Tel.: +32 2645972; Fax: +32 092648721

E-mail address: Daisy.Vanrompay@ugent.be