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 Yaacob1, Bruno G. De Geest2, Jens Goethals3, Aline Bajeck4, Kristof Dierckens1, Peter Bossier1*, Daisy Vanrompay3+8

1Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Ghent, Belgium
2Department of Pharmaceutics, Ghent University, Ghent, Belgium
3Laboratory of Immunology and Animal Biotechnology, Ghent University, Ghent, Belgium
4Ecloserie Marine de Gravelines, France
5School 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 is performing a multitude of housekeeping and cytoprotective functions in prokaryotes. It 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

Heat shock proteins (HSPs) are abundant and ubiquitous in both eukaryotes and prokaryotes, performing a multitude of housekeeping and cytoprotective functions.