

The road to an immune-priming strategy: a case of gnotobiotic European sea bass (*Dicentrarchus labrax*) larvae

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The search for alternative disease control in aquaculture practice is becoming more important to overcome the increasing occurrence of antibiotic resistance. Gnotobiotic animal models to study immune priming with prophylactic agents are an ideal platform to observe host-responses. Our study presents a 70 kDa recombinant bacterial heat shock protein, known as DnaK, to prime the innate immunity of gnotobiotic European sea bass larvae model that was developed at the Laboratory of Aquaculture and *Artemia* Reference Center (Dierckens *et al.*, 2009). Several studies have suggested that heat shock protein can mediate both innate and adaptive immune responses (Robert, 2003). Previously, DnaK significantly improved survival of *Artemia franciscana* against a *Vibrio campbellii* infection (Sung *et al.*, 2009). In the present study, two treatment groups of sea bass larvae were fed once with a high dose (1 mg) and a low dose (0.5 mg) of DnaK encapsulated in alginate microparticles at day 7 after hatching. After 18h, larvae were challenged with a pathogenic *Vibrio anguillarum* strain HI-610 at a density of 105 cfu ml⁻¹. The efficacy of DnaK to protect sea bass larvae against infection was monitored by counting the survival of the larvae after 18, 24 and 36h. A qPCR was conducted to observe the expression of nine innate immune-related genes. Our results showed that the survival of challenged larvae from both treatment groups (high and low dose) were not significantly different (p<0.05) compared to the alginate control group. However, gene expression analysis showed a significant up-regulation of the innate immune-related genes in the larvae fed with a high dose (1 mg) of DnaK-alginate microparticles compared to the control group after 18 and 24h of the *V. anguillarum* challenge. The significant up-regulation includes: pro-inflammatory genes (interleukin-1 β , interleukin-8 and tumor necrosis factor- α), the anti-inflammatory gene (interleukin-10) chemotactic cytokines receptor genes (CXCR4, CCR1 and CCR9) and the macrophage migration inhibition factor gene, MIF. Furthermore, the inflammatory cytokine-converting enzyme Caspase-1 was significantly increased for both dosages. Most of these genes were not significantly different 36h after challenge. Although no protection against *V. anguillarum* infection was observed in the survival, the recombinant DnaK protein did show a strong modulation on the innate immune responses on the gene expression level of the gnotobiotic European sea bass larvae. In conclusion, the use of DnaK as immunostimulants through non-diet feeding suggests to have a beneficial effect on disease resistance. Future studies focusing on the dose-response relationship are needed.

References

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