Virulence differences between GFP-tagged pathogens and their parental strains for blue mussel (*Mytilus edulis*) larvae

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Pathogens, especially vibrios, are largely responsible for larval diseases in shellfish aquaculture. Clarifying the mechanisms of *Vibrio* infections during the first hours is quite necessary for disease prevention. To make sure pathogens can be tracked *in vivo*, two strains of known pathogens of blue mussel (*Mytilus edulis*) larvae, *Vibrio hemicentroti* (ME09) and *V. anguillarum* (NB10) were labeled with green fluorescence protein (GFP), provided by *Escherichia coli* DH5α. Following a previously developed challenge model (Eggermont, et al., 2017), healthy two-day-old D-larvae were challenged with the GFP-tagged pathogens and their parental strains *in vivo,* at concentration of 10^4, 10^5 and 10^6 CFU·ml^{-1}, respectively. ME09-GFP and ME09 showed stronger toxicity to blue mussel larvae than NB10 and NB10-GFP. But all four strains showed less than 7% mortality at day 1. Specifically, ME09-GFP and ME09 caused a high mortality from 48 h onwards and a mortality of 85% at day 4 was observed for all concentrations. There was, however, a very low mortality among NB10 and NB10-GFP treatments at day 2. A significant larval mortality was only observed and was concentration-dependent from day 3 onwards. Besides, compared to their parental strains, the GFP-tagged vibrios were less virulent because they obviously delayed blue mussel larvae mortality. As more energy is required for this extra protein, incorporation of GFP can be a metabolic burden for the bacterial cells (Allision & Sattenstall, 2007). In the future, the invasion pathway of GFP-labeled pathogens is set up to be determined and the homochronous histological damage can be followed.

Keywords: Blue mussel; Larvae; *Vibrio spp.;* Green fluorescence protein (GFP)