Development of the first standardized biotic challenge model for Dover Sole (*Solea solea*) and its validation by assessing the protective potential of probiotic candidates

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Dover sole (*Solea solea*) is gastronomically highly appreciated and has a high market value. The species is therefore considered to be a very promising candidate for European aquaculture. Furthermore, ensuring a reliable supply of sole would reduce fishing pressure on wild Dover sole populations. However, as for many other fish species, sole production is hampered by amongst others high susceptibility to diseases and larval mortality, justifying the need for more research in this area. Infectious diseases are a major cause of larval mortality thereby decreasing survival and having a negative impact on the development of the fish embryo and larva.

Severe economic losses due to diseases in larviculture may be linked to vibriosis. Various *Vibrio* spp. have been cited as causative agents, with *Vibrio anguillarum* on top of the list. Efforts have been made to reduce infectious diseases, in the past mainly by applying antibiotics. Because of the emergence of acquired antimicrobial resistance, there is a great need for alternative measures to overcome diseases. In this respect, the amount of studies investigating the use and effect of probionts in aquaculture increased drastically. However, the mode of action of these probiotics is largely unknown. In addition, researchers become increasingly interested in studying bacteria-host interactions as understanding these may assist in combatting disease. Reliable experimental models not only enable experiments exploring the mode of action of probiotic candidates but also facilitate research aimed at unravelling the ways pathogens elicit disease and mortality. Nevertheless, only a handful of studies focused on the development of such models for fish larvae and for Dover sole, no such model is available yet.

Our research group developed a standardized biotic challenge model for Dover sole larvae adopting *Vibrio* spp. as potential pathogens. Five potentially pathogenic isolates were selected: the wild-type *V. anguillarum* HI610, *V. anguillarum* Fr and *V. harveyi* Fr that were both isolated from a disease outbreak in a French sea bass farm, *V. tapetis* isolated from skin blisters and liver of Dover sole and *V. tapetis* 2 originating from active skin ulcers of wild dab (*Limanda limanda*). Each isolate was added to the housing water of larvae at 10 days after hatching in a final concentration of 10^5, 10^6 or 10^7 colony forming units (CFU)/ml. No significant differences in survival were noticed between the larvae inoculated with *V. anguillarum* wild-type, *V. harveyi* Fr, *V. tapetis, V. tapetis 2* and the control group at any of the concentrations tested. *Vibrio anguillarum* Fr supplied at a final concentration of 10^6 CFU/ml well water resulted in a larval mortality of 36%, while the non-challenged control group displayed 5% mortality. When this isolate was administrated to the larvae at a concentration of 10^7 CFU/ml, 48% mortality was observed.

In addition to proving valuable in many other applications, this model is to be regarded as a powerful tool to evaluate the impact of (a)biotic components on larval health. Different probiotic candidates (administrated via the water and *Artemia* nauplii) were already assessed for their protective potential against *V. anguillarum* challenge and several prebiotic components will be evaluated in the future. In a next step, the impact of algal toxins on larval health will be considered amongst others by evaluating the susceptibility to disease agents.

Keywords: *Solea Solea*; Dover sole; larvae; *vibrio anguillarum*; probiotics