Competitive exclusion or immune response? How does a *Vibrio* sp. prospective probiotic bacterium function in larvae of the oyster *Crassostrea* virginica?

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Developing "environmentally-friendly" methods for controlling microbial pathogenesis in aquaculture with use of probiotic bacteria is becoming increasingly preferred over use of chemical antimicrobials. Our recent research has shown that a naturally-occurring bacterium isolated from the digestive gland of an adult eastern oyster, *Crassostrea virginica*, could be used as a potential probiotic candidate in oyster larviculture. Challenge studies indicated that survival of 2-day old oyster larvae exposed to probiotic candidate OY15 (V*ibrio* sp.) was similar ($p<0.3883$) to that of control larvae with no added bacteria, indicating no harmful effects upon larval oysters. Further, addition of this probiotic candidate to oyster larvae challenged with a known, *Vibrio* sp. shellfish-larval pathogen (B183), significantly improved survival ($p<0.0141$) compared to the pathogen alone. Although the Kirby Bauer disc diffusion method suggested competitive exclusion as a possible mechanism for this probiotic effect, another possible mechanism was investigated. We conducted an *in vitro* study to determine the effects of probiotic candidate OY15, or the pathogenic strain B183, upon oyster hemocytes and their immune functions. This study indicated that probiotic candidate OY15 was stimulatory to oyster hemocyte immune functionality with no significant effects upon hemocyte mortality or percentages of granular and agranular hemocytes. In contrast, pathogen B183 caused immunosuppression of hemocyte immune functions, and significantly higher mortality of these cells.

Research utilizing molecular tools to determine effects of probiotic candidate OY15 upon the diversity of the microbial community associated with the culture of oyster larvae was also performed. Replicate larval cultures were treated with pathogen, probiotic, and both, as well as no added bacteria controls. Larvae and culture water were separated for bacterial-diversity analyses based upon 16S rRNA sequences detected. Results revealed diverse bacterial communities associated with oyster larvae and culture water, with significant differences between. Diversity of the oyster bacterial community did not change significantly with probiotic addition, suggesting no resulting, selective exclusion or retention of different bacteria within larvae. The pathogen B183 16S rRNA sequences could not be detected in larval or water preparations one day post infection, while OY15 16S rRNA sequences were evident one day post infection but could not be detected at day three. These results suggest that OY15 and B183 activities do not appear to influence larval or culture water bacterial communities but are likely directed at the oyster itself, consistent with the *in vitro* studies described above. Thus, although the probiotic bacterium was isolated based upon a competitive-exclusion assumption, the most-likely function appears to be immuno-activation.