

The poly-B-hydroxybutyrate (PHB) regulate response of mussel larvae challenged with *Vibrio coralliilyticus*

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Marine invertebrates, including blue mussel (*Mytilus edulis*) lack an acquired, memory type immunity based on T-lymphocytes subsets and clonally derived immunoglobulins. Blue mussels rely solely on the innate immune mechanisms that include both humoral and cellular responses. Antimicrobial peptides are an important component of the humoral defense system in marine invertebrates, providing an immediate and rapid response to invading microorganisms. Poly-B-hydroxybutyrate (PHB) is a natural polymer that can be depolymerized into water-soluble short chain fatty acid monomers. Short chain fatty acids have been described as being bacteriostatic and capable of downregulating the expression of virulence factors of bacterial pathogens. To date, knowledge of the immune reactions in bivalve larvae and of the mechanisms of gene expression regulation by PHB is still limited.

In this study, we investigated the immune response of mussel larvae “in vivo”, challenged with the pathogen *Vibrio coralliilyticus* (10^5 CFU mL⁻¹), in the presence or not of 1 mg L⁻¹ amorphous PHB. The expression of three genes that code for the antimicrobial peptides (AMPs) *mytimycin*, *mytilinB*, *defensin* and one gene that code for the hydrolytic enzyme *lysozyme* was followed-up in time. RNA were isolated from mussel larvae tissue at 3, 6, 12, 24, 48 hours post-exposure to the pathogen and AMPs were quantified by q-PCR using 18SrRNA gene as a housekeeping gene.

Our data demonstrated that AMPs genes had a tendency to be upregulated in infected mussel larvae, and the strongest expression was observed from 24h post-infection onwards. The *lysozyme* gene, on the other hand, was expressed evenly during the whole exposure time to the pathogen. Compared to the expression of the *lysozyme* gene, *mytimycin* was expressed 530 times stronger and *mytilinB* 6 times more. The expression of *defensin* gene was found to be expressed 350 times stronger than *lysozyme* 48h post-infection. Additionally, our results confirmed that both *mytilinB* and *defensin* genes were expressed in 2days-old blue mussel D-larvae in contrast to earlier findings. We further found that PHB positively governs the expression of AMPs genes in infected mussel larvae 6h post-infection but did not have a significant impact on the expression of the *lysozyme* gene under the same stressor conditions.

Keywords: mussel larvae; PHB; gene expression; antimicrobial peptides; lysozyme; phenoloxidase activity; mytimycin; mytilinB; defensin