

## Pacific oyster (*Crassostrea gigas*) microbiome in the pathogenesis of *Ostreid herpesvirus-1* (OsHV-1) infections

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Mass mortality disease outbreaks in Pacific oysters (*Crassostrea gigas*) have been reported globally, leading to drastic economic losses. Although *Ostreid herpesvirus-1* (OsHV-1) have been identified as a major pathogen associated with this heavy mortality, the oyster microbiome is also thought to play a role resulting in a polymicrobial pathogenesis. Hence, this study focused on evaluating the changes in the oyster microbiome, with particular reference to *Vibrio* spp., during progression of an experimental OsHV-1 infection. Pacific oysters (n=348) that were grown in three estuaries and free from OsHV-1, were recruited to the laboratory. Following an acclimation period of three days, the oysters were challenged with OsHV-1 ( $6.5 \times 10^5$  OsHV-1 DNA copies  $\mu\text{l}^{-1}$ ) by intra-muscular injection and were sampled on days 0, 1, 2, 3, 5 and 7 post-injection. OsHV-1 and *Vibrio* genomic DNA in nucleic acid extracts were quantified using real-time PCR assays while that from oysters before (A) and soon after OsHV-1 challenge (B); moribund oysters (C); survivors of OsHV-1 (D); and oysters that were not challenged with the virus (E), were used for microbiome analysis. The microbiome was identified by sequencing and analyzing the bacterial 16S rRNA gene (V1-V3). The bacterial diversity in oyster tissues changed after OsHV-1 injection ( $p < 0.05$ ). Oysters with the highest mortality also had the highest quantity of OsHV-1 and *Vibrio* ( $p < 0.05$ ). This group also had a higher initial bacterial diversity which decreased in the moribund oysters ( $p < 0.05$ ). The diversity of the microbiome of surviving and control oysters from two estuaries did not change compared to their pre-challenged counterpart. A strong correlation was observed between OsHV-1 and *Vibrio* quantity in OsHV-1 infected oysters ( $r = 0.6$ ;  $p < 0.001$ ). In conclusion, the higher *Vibrio* load with higher OsHV-1 DNA content and higher mortality suggests a role of *Vibrio* in mortality associated with OsHV-1 infections. Further studies are needed to evaluate the direct and indirect influences of *Vibrio* and other members of the resident microbial community of oysters during OsHV-1 infection.

Keywords: *Crassostrea gigas*, microbiome, *Ostreid herpesvirus-1*, Pacific oyster

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