CHARACTERIZATION OF THE VIRULENCE OF A *VIBRIO AESTUARIANUS* STRAIN PATHOGENIC TO THE PACIFIC OYSTER *CRASSOSTREA GIGAS*

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In France, annual mass mortalities of *Crassostrea gigas* oysters have been reported during summer since the 1980’s. Several studies on this subject have demonstrated that these mortality outbreaks resulted from complex interactions between the physiological and/or genetic status of the oysters, environmental factors and one or more infectious agents, among which are bacteria of the genus *Vibrio*. *Vibrio aestuarianus* was the most frequently encountered species isolated from the hemolymph of moribund and healthy oysters. Interestingly, these bacterial isolates exhibited variable virulence following experimental challenge of adult animals, this variation being apparently linked to the toxicity of bacterial extracellular products (also called ECPs).

As these data implicated some *V. aestuarianus* strains in mortality events, this work was aimed at investigating pathogenicity mechanisms of *V. aestuarianus* strain 01/32, which induced the highest oyster mortality after an experimental challenge. Studies of both *in vitro* and *in vivo* interactions between this strain and oyster immune cells established the central role of the ECPs in the pathogenesis. ECPs from *V. aestuarianus* 01/32 were indeed shown to be lethal when injected into oysters and to inhibit hemocyte adhesion and phagocytosis. Accordingly, biochemical and genetic approaches were further implemented to identify the major source of ECP toxicity. These two complementary approaches led to the characterization of a gene encoding a zinc-dependent metalloprotease and to the demonstration of its involvement in the lethal effect of ECPs. When expressed in a heterologous system, the metalloprotease conferred a toxic phenotype on the ECPs of the transconjugant and caused inhibition of hemocyte adhesive and phagocytic activities.
Taken together, these results demonstrate the critical role played by the metalloprotease in pathogenicity mechanisms of *V. aestuarianus* 01/32 during experimental infection of *C. gigas* oysters.

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