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

Yannick Labreuche[1], Philippe Soudant[2], Joel Henry[3], Frederique Le Roux[4], Christophe Lambert[2], Viviane Boulo[5], Arnaud Huvet [1], Didier Mazel[4], Celine Zatylnyj-Gaudin[3] Matthieu Garnier[1], Jean-Louis Nicolas[1]

(1) Unité Mixte de Recherche Physiologie et Ecophysiologie des Mollusques Marins (UMR 100), IFREMER, B.P 70, 29280 Plouzané, France
(2) Laboratoire des Sciences de l'Environnement Marin, Institut Universitaire et Européen de la Mer, Université de Bretagne Occidentale, place Copernic, Technopole Brest-Iroise, 29280 Plouzané, France
(3) Unité Mixte de Recherche Physiologie et Ecophysiologie des Mollusques Marins (UMR 100), Laboratoire de Biologie et Biotechnologies Marines, Université de Caen, esplanade de la Paix, 14032 Caen cedex, France
(4) Unité postulante Plasticité du Génome Bactérien, CNRS URA 2171, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris, France
(5) Génome, Populations, Interactions, Adaptation, UMR 5171 (IFREMER, CNRS, UMII), Université de Montpellier II, place E. Bataillon, CC 80, 34095 Montpellier, France

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.

Acknowledgements: This work was supported by the MOREST national project funded by IFREMER, the Regions Basse-Normandie, Bretagne, Pays de la Loire, Poitou-Charentes and by the Conseil General du Calvados. Y. Labreuche was supported by a doctoral grant from IFREMER and Region Bretagne