

CHARACTERIZATION OF VIBRIO ISOLATED FROM *CRASSOSTREA GIGAS* SPAT SUFFERING SUMMER MORTALITY OUTBREAKS

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The French oyster production is mainly based on the pacific oyster, *Crassostrea gigas*. However, for several years, *C. gigas* spat has suffered severe mortality outbreaks. A multifactorial etiology implicating environment, oyster physiology, genetics and pathogens is heavily suspected. A herpesvirus and bacteria belonging to the *Vibrio splendidus* polyphyletic group have been isolated from summer mortality outbreaks. Moreover, several strains belonging to the genus *Vibrio* have been identified as pathogen for different mollusk species. However, few tools are available for the identification of these *Vibrios* and for the characterization of the virulence of the pathogenic strains.

The aims of this study are thus 1) to identify *Vibrios* isolated from oysters suffering summer mortality outbreaks using a polyphasic approach based on phenotyping and genotyping; 2) to select pathogenic strains by experimental infections; 3) to describe the pathology associated with these pathogens.

Cohabitation experiments were carried out to demonstrate the putative presence of a transmissible pathogen in oysters suffering summer mortality outbreaks. The *Vibrionaceae* flora was isolated from the haemolymph of moribund oysters. The isolated strains were phenotypically and genotypically characterized. The *Vibrionaceae* flora from diseased individuals is less diverse than in healthy individuals. It is mainly composed of strains belonging to the *V. splendidus* polyphyletic group.

The virulence of isolated strains was evaluated by bacterial injection in clams and oysters. Several pathogenic strains have thus been selected. Moreover, a cooperative effect between strains was reported: the virulence of two different bacterial strains was significantly enhanced when they were simultaneously injected in oysters. This phenomenon was observed for several species of *Vibrios* including *V. lentus* and *V. anguillarum*.

Histological studies on moribund or alive individuals experimentally infected (bacteria injection in the adductor muscle or in the pallal cavity) by two *V. lentus* strains showed that lesions and bacterial localization were similar. The bacteria were detected at the periphery of the adductor muscle. Infected animals presented important lesions of muscular fibers and abnormal nuclei in circulating hemocytes.

Thus this study allowed us 1) to observe a diminution of the diversity of the *Vibrionaceae* flora in the haemolymph of oysters suffering summer mortality outbreaks and 2) to set up a reproducible experimental model with the association of two strains. A new concept was demonstrated: the cooperation of two *Vibrio* strains resulting in the increase of the virulence and therefore of oyster mortality.