



Development of TaqMan real-time PCR assays for monitoring *Vibrio harveyi* infection and a plasmid harbored by virulent strains in European abalone *Haliotis tuberculata* aquaculture



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ABSTRACT

The Gram-negative bacterium *Vibrio harveyi* is known to be highly pathogenic for the European abalone *Haliotis tuberculata*, which is a gastronomically important marine gastropod with a high commercial value. Since 1998, some particular bacterial strains are described as implicated in recurrent mortality outbreaks in French farm and field stocks of abalone. Recently, a 9.6 kb plasmid named pVCR1, was shown to be harbored by one highly *V. harveyi* virulent ORM4 strain suggesting its involvement in virulence phenotype. Thus, we have developed in the present study two TaqMan real-time PCR assays allowing to (i) rapidly and specifically detect, by a duplex procedure and in less than 2 h, both *V. harveyi* and the presence of plasmid pVCR1 from unidentified bacterial colony and to (ii) quantify both *V. harveyi* and the plasmid pVCR1 in the hemolymph of abalone or its surrounding seawater. Quantification curves of *V. harveyi* or ORM4 strain seeded in hemolymph or artificial sea water samples were equivalent showing excellent qPCR efficacies and detection level as low as 18 *V. harveyi* cell-equivalent genomic DNA in a PCR reaction well. This qPCR allowed us to monitor *V. harveyi* ORM4 strain in experimentally infected *H. tuberculata*. These diagnosis assays could provide powerful and useful tools to better understand the epidemiology of vibriosis caused by *V. harveyi* in different cultured marine species including *H. tuberculata*.

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1. Introduction

The European abalone *Haliotis tuberculata* is a gastronomically important marine gastropod with a high commercial value but stocks of this mollusk are actually in decline in Europe (Huchette and Clavier, 2004). Numerous events of mortality have been reported in Europe in both farmed and wild populations of abalone in association with the detection of different pathogens, including the protozoan parasite *Haplosporidium montforti*, *Rickettsia*-like bacteria, or the Gram-negative bacterium *Vibrio harveyi* (Azevedo et al., 2006a,b; Balseiro et al., 2006; Huchette and Clavier, 2004). This last bacterium has been frequently involved in recurrent mortality outbreaks occurring seasonally, at the end of warm season, since 1998 in French farms and field stocks of *H. tuberculata* (Nicolas et al., 2002). *V. harveyi* has been widely recognized as a common pathogen of many commercially cultured fish and shellfish species worldwide (Aguirre-Guzman et al., 2001; Austin and Zhang, 2006; Lee et al., 2002; Liu et al., 1996; Nishimori et al., 1998; Pass et al., 1987;

Zhang and Austin, 2000) including abalone in Australia and Japan (Handlinger et al., 2005; Sawabe et al., 2007a).

Vibriosis outbreaks in *H. tuberculata* cultivated in France were shown to be driven by sea water temperature exceeding a 17 °C threshold (Huchette and Clavier, 2004; Travers et al., 2009a) and host physiology such as gametogenesis and reduced immune defense capacities (Travers et al., 2008). Although being non-specific, clinical signs of *V. harveyi* infection encompass a loss of muscular strength occurring concomitantly with the appearance of white pustules on the foot. Subsequently, diseased animals develop a fatal septicemia leading to up to 80% mortality within a few days to 3 weeks (Travers et al., 2009b). Furthermore, reproduction of the disease and mortality could be achieved by experimental infection using either intramuscular injection or bath contamination with ORM4 virulent *V. harveyi* strain. Recent studies focused on the identification of virulence factors displayed by this strain. ORM4 strain was found to inhibit phagocytosis and ROS production (Travers et al., 2009b) by a mechanism presumed by authors to involve the MAP kinase signal transduction pathway displayed by abalone hemocytes. Moreover, ORM4 strain harbored a 9.6 kb plasmid, named pVCR1, which was found to date only in pathogenic virulent strains of *V. harveyi* isolated during mortality outbreaks among *H. tuberculata* in France (Travers, 2008).

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