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Review

Detection of ostreid herpesvirus 1 DNA by PCR in bivalve molluscs: A critical review

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Abstract

Herpes-like viral infections have been reported in different bivalve mollusc species throughout the world. High mortalities among hatchery-reared larvae and juveniles of different bivalve species have been associated often with such infections. The diagnosis of herpes-like viruses in bivalve molluscs has been performed traditionally by light and transmission electron microscopy. The genome sequencing of one of these viruses, oyster herpesvirus 1 (OsHV-1), allowed the development of DNA-based diagnostic techniques. The polymerase chain reaction (PCR) has been used for the detection of OsHV-1 DNA in bivalve molluscs at different development stages. In addition, the PCR used for detection of OsHV-1 has also allowed the amplification of DNA from an OsHV-1 variant. The literature on DNA extraction methods, primers, PCR strategies, and confirmatory procedures used for the detection and identification of herpesviruses that infect bivalve molluscs are reviewed.
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Keywords: Herpesvirus; OsHV-1; Detection; PCR; Bivalve molluscs; Oyster

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1. Introduction

Herpes-like viral infections have been identified in various marine bivalve mollusc species throughout the world, including the USA (Farley et al., 1972; Friedman et al., 2005), New Zealand (Hine et al., 1992), France (Nicolas et al., 1992; Arzul et al., 2001b; Renault and Arzul, 2001; Renault et al., 2000b, 2001b), Australia (Hine and Thorne, 1997), and Mexico (Vásquez-Yeomans et al., 2004). The first description of a virus morphologically similar to members of the *Herpesviridae* family in a bivalve mollusc was made by Farley et al. (1972) in the eastern oyster, *Crassostrea virginica*. Since then, a wide host range has been reported for herpes and herpes-like viruses infecting bivalve species, including the Pacific oyster *C. gigas* (Hine et al., 1992), the European oyster *Ostrea edulis* (Nicolas et al., 1992), the Antipodean flat oyster *O. angasi* (Hine and Thorne, 1997), the Chilean oyster *Tiostrea chilensis* (Hine et al., 1998), the Manila clam *Ruditapes philippinarum* (Renault et al., 2001a), the carpet shell clam *R. decussatus* (Renault and Arzul, 2001), the Portuguese oyster *C. angulata* (Arzul et al., 2001a), the Suminoe oyster *C. rivularis* (Arzul et al., 2001a) and the French scallop *Pecten maximus* (Arzul et al., 2001b). High mortalities among hatchery-reared larvae (Hine et al., 1992; Nicolas et al., 1992; Renault et al., 1994; Renault and Arzul, 2001) and juveniles (Comps and Cochennec, 1993; Renault et al., 1994, 2000b; Friedman et al., 2005) of different bivalve species have often been associated with herpes and herpes-like virus infections. Observations by transmission electron microscopy (TEM) revealed that larvae exhibit generalized infections, whereas focal infections were generally observed in spat (Renault et al., 1994). Viral infections were also observed in adult bivalves (Hine and Thorne, 1997; Arzul et al., 2001b, 2002; Vásquez-Yeomans et al., 2004), but adults are apparently less sensitive to such infections as compared to younger stages (Arzul et al., 2002; Renault and Novoa, 2004). The pathogenicity of herpes-like viruses was demonstrated by experimental transmission assays either for larval stages of *C. gigas* (Le Deuff et al., 1994) and other bivalve species (Arzul et al., 2001a,b). Similar experiments were conducted with juveniles and adults of *C. gigas*, but the results were inconclusive (Renault and Novoa, 2004). It is noteworthy that a highly pathogenic herpes-like virus was observed recently by TEM in the gastropod mollusc *Haliotis diversicolor supertexta* in Taiwan associated with high mortality rates (Chang et al., 2005).

The development of a method for purifying herpes-like virus particles from infected *C. gigas* larvae facilitated the extraction of viral DNA and a partial genome characterization (Le Deuff and Renault, 1999). The genome was subsequently completely sequenced (GenBank accession no. AY509253) revealing a tenuous relationship with other herpesviruses. This oyster virus was classified as a member of the *Herpesviridae* family under the name oyster herpesvirus 1 (OsHV-1) (Minson et al., 2000) and was considered the only member of a new major class of herpesvirus (Davison, 2002; Davison et al., 2005). A variant of OsHV-1 (OsHV-1var) was also described in larvae of different bivalve species (Arzul et al., 2001b,c) and OsHV-1 and OsHV-1var are considered representatives of a single viral species. In contrast with vertebrate herpesviruses, which are generally confined to a single host, OsHV-1 has been identified in several bivalve species and interspecies viral transmission was demonstrated (Arzul et al., 2001a; Friedman et al., unpublished data).

Detection of viruses in mollusc bivalves cannot be done by classic serological methods because molluscs do not produce antibodies. In addition, viral replication in cell culture to facilitate the diagnosis is not possible due to the absence of mollusc cell lines. The diagnosis of herpes-like virus infections has traditionally been performed by light microscopy, as a first approach in order to detect cytological abnormalities, followed by transmission electron microscopy to complete the diagnosis (Hine and Thorne, 1997; Renault et al., 2000b). These procedures are time consuming, impractical for epidemiological surveys, and some viruses can be difficult to detect and identify when present in low amounts. As a result of these limitations, other diagnostic methods have been developed such as immunochemistry and nucleic acid-based techniques. A protocol using polyclonal antibodies produced in BalbC mice immunized with viral particles purified from infected *C. gigas* larvae has been used to detect OsHV-1 proteins (Le Deuff, 1995; Arzul et al., 2002). Another technique that has also been developed is *in situ* hybridization, which allows the detection of viral DNA (Renault and Lipart, 1998; Lipart and Renault, 2002; Barbosa-Solomieu et al., 2004). Both techniques have high sensitivity and specificity, and allow the visualization of viral proteins or DNA, respectively. Polymerase chain reaction (PCR) diagnostic methods have also been developed for detection of OsHV-1 DNA (Renault et al., 2000a; Renault and Arzul, 2001; Arzul et al., 2001a,b,c). PCR is considered a suitable tool for the diagnosis of OsHV-1 infections owing its specificity, high sensitivity relative to other methods (e.g., TEM), ease of sample processing, availability of reagents, and time and cost efficiency. However, as this test only detects viral nucleic acid, other tests such as histology, ISH or antigen-based tests must confirm PCR tests, particularly in a new species or new geographic location. In areas where the pathogen is endemic, PCR alone may be sufficient for diagnosis of its presence. A competitive PCR method was developed that can be used to demonstrate the presence of PCR inhibitors or to quantify OsHV-1 DNA (Renault et al., 2004). Different methods of DNA extraction (Renault et al., 2000a; Arzul et al., 2002; Batista et al., 2005; Friedman et al., 2005) as well as various primer pairs (Renault et al., 2000a; Arzul et al., 2001a,b,c; Renault and Arzul, 2001; Barbosa-Solomieu et al., 2004, 2005) have been designed and used to detect viral DNA using one-round or nested PCR. In addition, the PCR conditions used for detection of OsHV-1 also allow amplification of DNA from other closely related herpesviruses in different bivalve species, as was the case of OsHV-1var (Arzul et al., 2001b,c). In order to confirm the authenticity of PCR products, different techniques have been used, including digestion of PCR products with restriction enzymes (PCR-RFLP) (Arzul et al., 2001c; Renault et al., 2004; Barbosa-Solomieu et al., 2004, 2005) and DNA sequencing. The objectives of the present work are: (1) to provide a review of the different DNA extraction methods, PCR protocols, primers and confirmatory procedures that have been used in the detection of OsHV-1 DNA by PCR; (2) to discuss the adequacy of the

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