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Viruses infecting marine molluscs

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ABSTRACT

Although a wide range of viruses have been reported in marine molluscs, most of these reports rely on ultrastructural examination and few of these viruses have been fully characterized. The lack of marine mollusc cell lines restricts virus isolation capacities and subsequent characterization works. Our current knowledge is mostly restricted to viruses affecting farmed species such as oysters *Crassostrea gigas*, abalone *Haliotis diversicolor supertexta* or the scallop *Chlamys farreri*. Molecular approaches which are needed to identify virus affiliation have been carried out for a small number of viruses, most of them belonging to the *Herpesviridae* and *birnaviridae* families. These last years, the use of New Generation Sequencing approach has allowed increasing the number of sequenced viral genomes and has improved our capacity to investigate the diversity of viruses infecting marine molluscs. This new information has in turn allowed designing more efficient diagnostic tools. Moreover, the development of experimental infection protocols has answered some questions regarding the pathogenesis of these viruses and their interactions with their hosts. Control and management of viral diseases in molluscs mostly involve active surveillance, implementation of effective bio security measures and development of breeding programs. However factors triggering pathogen development and the life cycle and status of the viruses outside their mollusc hosts still need further investigations.

1. Introduction

Marine mollusc production is an increasingly important contributor to global food supply. Clams and cockles represent the first group of produced marine bivalves in the world with 5 millions of tons produced in 2012. It is closely followed by oysters while world mussels and pectinids production is less than 2 million tons (FAO, 2015). Although, in terms of production tonnage, abalone contributes a relatively small proportion of this aquaculture production, it is one of the most highly prized seafood delicacies in many parts of the world, particularly in Asia and, therefore, in terms of the value of production, is very important to many countries (Cook, 2014).

Despite this enjoyable situation, compared to other aquaculture activities the shellfish industry has shown rather slow growth notably because of limiting factors which primarily include infectious diseases including viral diseases (Bower, 2010). For example, in Europe, irido-like virus infections led to the almost total extermination of the Portuguese oyster, *C. angulata*, in French and European Atlantic waters in the early 1970's (Comps et al., 1976; Comps and Bonami, 1977). Since 1991, viruses belonging to the Herpesviridae family have been associated with high mortality rates of *Crassostrea gigas* hatchery-reared larvae and spat in France but also in New Zealand, USA and Mexico (Renault and Novoa, 2004). Wild and farmed abalones have experienced important mortality in Asia since the 90ies and subsequently in Australia since 2005. These mortalities have been attributed to a herpesvirus. In addition to these examples, many mortality events of marine molluscs remain unexplained and might be due to viruses.

These examples highlight the adverse effect of viruses on bivalve production. In addition considering that shellfish are filter feeders, they bioaccumulate in their tissues viruses present in the sea water and that might be pathogens for humans and higher vertebrates (Meyers, 1984). These viruses apparently do not affect bivalves but can indirectly impact the shellfish industry.

The fast growing development of New Generation Sequencing approach has started revealing the high diversity of viruses present in sea water (Brum et al., 2015; Martinez Martinez et al., 2014). A liter of seawater contains at least 100 billion viruses—the vast majority of which remain unidentified and uncharacterized (Weitz and Wilhelm, 2012). This diversity could be seen as a reserve of potential harmful pathogens for bivalves in the context of dysregulation of the ecosystem.

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In the present paper we will consider viruses which infect marine molluscs and replicate in their tissues. Although a wide range of viral families including *Herpesviridae, Papovaviridae, Togaviridae, Retroviridae, Reoviridae, Birnaviridae,* and *Picornaviridae* (Renault and Novoa, 2004; Meyers et al., 2009) have been reported in marine molluscs few of them have been fully characterized and most of these reports rely on ultrastructural examination. The lack of marine mollusc cell lines certainly restricts virus isolation capacities and subsequent characterization works. Molecular approaches which are needed to clearly identify virus affiliation have been carried out for a small number of viruses, most of them belonging to the *Herpesviridae* family.

The present paper aims to provide updated information on viruses pathogenic for marine molluscs in terms of diversity, pathogenesis, diagnosis, life cycle, interactions with their hosts and biosecurity. Most of information will concern members of the *Herpeviridae* family, however where and when possible, examples of members of other family will be included.

2. Genetic diversity of viruses infecting marine molluscs

There is currently a lack of molecular information concerning these viruses as the basic method for identification and examination of suspect samples remains histology. This technique enables the identification of cellular changes associated with viral infections without providing conclusive virus identification. Transmission electron microscopy examination was carried out providing information on viral ultrastructural features and putative assignment to particular viral families. However, only a few of them have been purified allowing an access to their genome and molecular characterization through sequencing. It is the case for birnairuses and herpesviruses infecting marine molluscs. As a consequence, both these virus groups have been studied more extensively than other mollusc viruses. They have been subjected to examination at molecular, epidemiological and ecological levels. Although birnaviruses have been detected in various marine molluscs, their infectivity for shellfish should still be regarded as weak or hypothetical. On the contrary, herpesviruses infecting marine molluscs are highly pathogenic.

2.1. Birnaviruses

Birnaviruses have been isolated from different bivalve species worldwide using different fish cell lines (Hill, 1976; Lo et al., 1988). Tellina virus 1 (TV-1) was isolated on the BF-2 (bluegill fry) fish cell line from *Tellina tenuis* in Great Britain (Hill, 1976) and assigned to the *Birnaviridae* family (Dobos et al., 1979). A virus assigned to the *Birnaviridae* has been also identified from cultured hard clams, *Meretix lusoria*, in Taiwan (Lo et al., 1988). Suzuki et al. (1998a) isolated a virus from Japanese pearl oysters (*Pinctada fucata)* presenting mass mortality named "Marine birnavirus" (MABV) (Suzuki et al., 1998a). A birnavirus was also isolated from Agemaki (Jack Knife Clam) *Sinovacura consticta* in Japan (Suzuki et al., 1998c). More recently, viruses interpreted as aquabirnaviruses were reported from Geoduck clams, *Panope abrupta,* and litteneck clams, *Protothaca staminea,* collected in Alaska (Meyers et al., 2009). Both viruses were not associated with abnormal mortality nor lesions detected by histology in the collected animals (Meyers et al., 2009).

MABV have been defined as a group belonging to the genus *Aquabirnavirus* and forming an independent genogroup to the infectious pancreatic necrosis virus (IPVN) infecting salmonids. Although MABV are relevant fish pathogens, they have also been isolated from a variety of marine shellfish (Inaba et al., 2009). Viruses isolated from shellfish and fish seem similar based on serological and genomic properties (Suzuki et al., 1997b, 1998b). High homologies were reported in the VP2/NS junction region of the virus genome between fish and shellfish isolates (Suzuki et al., 1998a,c; Zhang and Suzuki, 2003; Zhang and Suzuki, 2004; Inaba et al., 2009). All MABV are grouped in the same genogroup in the Aquabirnaviruses (Zhang and Suzuki, 2004). Although MABV and IPNV resemble each other, genogrouping based on the nucleotide sequence of the VP2/NS junction region separates them (Hosono et al., 1996). More recently, Nobiron et al. (2008) established that TV-1 is phylogenetically distant from all already known birnaviruses and defines a new genetic cluster among the birnaviruses.

Although the pathogenicity of certain MABV strains appears to be weak in shellfish, stressors such as changes in temperature, spawning and exposure to heavy metals can result in mortality events by increasing host susceptibility in some mollusc species (*Meretrix lusoria*, *Sinovacura constricta* and *P. fucata*). MABV may be considered as opportunistic pathogens able to induce a disease in marine molluscs under stressful conditions (Chou et al., 1994, 1998). Although MABV isolated from shellfish appear to be pathogenic to fish, assays to reproduce experimentally the infection in molluscs using birnavirus-like particles isolated on fish cell lines have shown inconsistent results.

2.2. Herpesviruses

Herpesviruses have been associated to mortality outbreaks resulting in high losses in several marine mollusc species, including the Pacific oyster, *Crassostrea gigas*, worldwide (Hine et al., 1992; Renault et al., 1994a,b; Burge et al., 2007; Vásquez-Yeomans et al., 2010).

A herpesvirus has been purified from naturally infected larval Pacific oysters collected in 1995 in a French commercial hatchery (Le Deuff and Renault, 1999) and its genome entirely sequenced (Davison et al., 2005) (GenBank accession number AY509253). This virus has been classified as ostreid herpesvirus type 1 (OsHV-1) within the *Malacoherpesviridae* family from the *Herpesvirales* order (Davison et al., 2009). The OsHV-1 genome is a double-stranded DNA of about 207 kbp (Davison et al., 2005). The overall genome organization is TR_L - U_L - IR_L - X - IR_S - U_S - TR_S in which TR_L and IR_L are inverted repeats flanking a unique region, U_L. However, a certain diversity of the viral genome was reported by Davison et al. (2005) as a small proportion of OsHV-1 genomes either lack the X sequence or contain an additional X sequence at the left terminus. Moreover, a 4.8 kbp region of U_L in inverse orientation was reported in approximately 20–25% of genomes (Davison et al., 2005). U_L and U_S were reported presenting two orientations in approximately equimolar amounts in viral DNA (Davison et al., 2005). The IR_L - IR_S junction is also not unique (Davison et al., 2005).

OsHV-1 genomic variants have been reported in different bivalve species in various geographical locations (Arzul et al., 2001a,b; Renault et al., 2001a,b; Friedman et al., 2005; Moss et al., 2007). A variant called "Var" was reported in 1997 during one episode of mortality affecting both larval Pacific oysters, *C. gigas*, and larval Manila clams, *Ruditapes philippinarum* (Arzul et al., 2001b; Renault et al., 2001a,b). Renault et al. (2012) reported that two *C. gigas* larval samples collected in a single commercial hatchery in 1993 presented high homologies with the variant Var. This variant was quite rarely detected in France and could be a virus infecting first clams. Interspecies transmission may be promoted through intensive farming conditions under which different bivalve species (oysters, clams) are kept at the same time in unnaturally close proximity.

Since 2008, massive mortality outbreaks among Pacific oysters, *C. gigas*, are reported in Europe (EFSA, 2010) in relation to the