Detection of Ostreid Herpesvirus 1 from adult Pacific Oysters *Crassostrea gigas* Cultured in Korea

Bo Young Jee¹, Su Jin Lee², Mi Young Cho¹, Soon Jeong Lee¹, Jin Woo Kim¹, Seung Hyuk Choi²,
Hyun Do Jeong² and Ki Hong Kim^{2*}

¹Aquatic Life Disease Control Division, National Fisheries Research and Development Institute, Busan 619-902, Korea

²Department of Aquatic Life Medicine, Pukyong National University, Busan 608-737, Korea

Abstract

The presence of ostreid herpesvirus 1 (OsHV-1) and the percentage of viral DNA detected in Pacific oyster *Crassostrea gigas* adults were investigated monthly between May and November 2012 at three locations along the southern coast of Korea. Among 210 oysters examined by polymerase chain reaction (PCR) analysis, OsHV-1 DNA was detected in only one oyster collected in August. The low detection rate of OsHV-1 DNA was consistent with the lack of reported OsHV-1-associated disease in *C. gigas* cultured in Korea. The sequence of the present PCR product amplified with the C2/C6 primer pair was identical to that of OsHV-1 μ Var except for one nucleotide, and the sequence amplified with Del36-37F2/Del36-37R showed a 605-bp deletion as in OsHV-1 μ Var. Although these sequence data are insufficient to determine genotype, the results suggest that the herpesvirus detected was similar to OsHV-1 μ Var. This is the first report on the presence of OsHV-1 in adult Pacific oysters cultured in Korea.

Key words: Ostreid herpesvirus 1, *Crassostrea gigas* adults, PCR analysis

Introduction

Since Farley et al. (1972) first described a herpes-type virus in the Eastern oyster *Crassostrea virginica*, herpes or herpes-like viruses have been reported worldwide from various bivalves including oysters, clams, and scallops (Renault and Novoa, 2004). Minson et al. (2000) named a virus isolated from infected Pacific oyster *Crassostrea gigas* larvae as ostreid herpesvirus 1 (OsHV-1), and Davison et al. (2005) reported the full genome sequence of OsHV-1 (GenBank accession no. AY509253). Initially, two OsHV-1 genotypes, OsHV-1 reference and OsHV-1 var, were reported in France (Arzul et al., 2001), but additional genotypes such as OsHV-1 microvariant (μ Var) (Segarra et al., 2010) and OsHV-1 μ Var Δ 9 (Martenot et al., 2011) have been newly added. OsHV-1 has been detected in *C. gigas* spat mortalities in France since 1993. However,

severe mortality outbreaks in Pacific oyster spat and juveniles have been reported in different member states of the European Union including France and Ireland since 2008, and OsHV-1 μ Var has been proposed as the possible culprit (Segarra et al., 2010; Schikorski et al., 2011a, 2011b).

Korea is among the top three countries in the world for production of Pacific oysters. However, as oyster farms are concentrated on the southern coastal area of Korea, an outbreak of highly infectious disease at a farm can bring highly disastrous results in the Korean oyster aquaculture industry. Although Moss et al. (2007) reported the presence of molluscan herpesvirus from *Crassostrea ariakensis* collected from Korea, no reports have been published on the presence of OsHV-1 in Korean Pacific oyster. The purpose of this study was to inves-

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***Corresponding Author**

E-mail: khkim@pknu.ac.kr

tigate the presence and prevalence of OsHV-1 in adult Pacific oysters in Korea.

Materials and Methods

Oysters

Crassostrea gigas adults (1-2 years old) were collected monthly between May 2012 and November 2012 from oyster farms located at Tongyeong, Goseong, and Geoje on the southern coast of Korea. Immediately after the oysters arrived in the laboratory each month, 30 oysters (10 oysters from each region) were sampled, and gill and mantle tissues were excised to analyze for the presence of OsHV-1 by polymerase chain reaction (PCR).

PCR analysis

The excised gill and mantle were lysed by adding 500 μ L of lysis buffer containing Proteinase K and incubated in a shaking water bath at 60°C for 3 h to extract total DNA. Then, 250 μ L of 6 M NaCl was added to each tube, followed by vigorous shaking of the tubes 20 times and a 10-min incubation on ice. The supernatants were recovered by centrifugation at 10,000 g for 10 min, mixed with 650 μ L isopropanol, and centrifuged at 10,000 g for 10 min. The resulted pellet was further air-dried for 5 min and eluted with RNase-free water.

The primers used are listed in Table 1. Primary PCR reactions were conducted with primer pairs of C13/C5 (open reading frame [ORF]4), C13/C6 (ORF4), and Del-F/Del-R (ORF35, 36, 37, and 38), and amplification conditions were one cycle of 3 min at 95°C (initial denaturation), 30 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C, followed by 7 min at 72°C. Primer pairs used for nested PCR were C2/C4, C2/C6, and Del36-37F/Del36-37R, respectively, and 0.2 μ L of each primary PCR product was used as the template. The PCR conditions for the nested PCR were the same as those for the

Fig. 1. PCR analysis of ostreid herpesvirus 1 (OsHV-1) in the gill (G) and the mantle (M) of *Crassostrea gigas* collected from Tongyeong in August. Primary PCR was conducted with C13/C5 primer pair, and nested PCR was done with C2/C4 primer pair (right 352 bp). As a control, 18S ribosomal RNA gene was amplified with 18S-F/18S-R primer pair (left 537 bp). The figure is a part of the PCR results to show the positive band amplified with C2/C4 primer pair. MA, 1 kb ladder (Bioneer, Korea).

primary PCR. PCR products were analyzed on a 1% agarose gel containing Midori Green Advanced DNA stain (Nippon Genetics, Dürren, Germany) for visualization, purified using a gel purification kit (Nucleogen, Seoul, Korea), subcloned into pGEM-T Easy Vector (Promega, Madison, WI, USA), and sequenced. Multiple sequence alignments were generated using the CLUSTALW 1.8 program.

Results

In total, 210 Pacific oysters were PCR-analyzed to detect OsHV-1 DNA. The band corresponding to the partial 18S ribosomal RNA gene was detected from all analyzed specimens (Fig. 1). No amplified band was identified in any of the samples by primary PCR with the primer pairs for amplifying OsHV-1 DNA. However, nested PCR with the C2/C4 primers amplified a band of the expected size (352 bp) from only one oyster (Fig. 1), which was collected from Tongyeong in August; thus, the percentage of OsHV-1 DNA detection in *C. gigas* adults was less than 1% (1/210).

The sequence of the C2/C6 PCR product of the positive sample showed deletion of 13 consecutive nucleotides, and another 11 nucleotides were sporadically changed or deleted when compared to the OsHV-1 reference sequence (Fig. 2). Only one G (Fig. 2) was deleted in the present sample compared to that in the OsHV-1 μ Var sequence.

Nested PCR with the Del36-37F/Del36-37R primers amplified a band of expected size (384 bp) that represented a deletion of the middle region of the gene corresponding to 605 nucleotides in the OsHV-1 reference sequence (Fig. 3A). A comparison of the present sequence with those recorded in other countries was identical except for two nucleotides (Fig. 3B).

Table 1. Oligonucleotides used in this study

Primers	Sequence (5'→3')
18S-F	GTAACGGGTAACGGGGAATC
18S-R	CCCCGTGAGTCCCTCTTAAT
C13	CCTCGAGGTAGCTTTTGTC AAG
C5	CCGTGACTTCTATGGGTATGTCAG
C6	GTGCACGGCTTACCATTTTT
Del-F	GCTGCATATTGTCCTTCTAGC
Del-R	GATGAAAGTGCAGAAGAATACACTG
C2	CTCTTTACCATGAAGATACCCACC
C4	GCAGTTGTGGTATACTCGAGATTG
Del36-37F	ATACGATGCGTCGGTAGAGC
Del36-37R	CGAGAACCCCATTCCTGTAA

Fig. 3. Multiple alignment of nucleotides sequence of the PCR product (Del36-37F and Del36-37R primers) with ostreid herpesvirus 1 (OsHV-1) reference (A), and with OsHV-1 recorded from New Zealand (JN800252.1), Ireland (JN800250.1), USA (JN800249.1) and France (JN800248.1) (B). Identical nucleotides are indicated by black shading.vv

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