日本における正常なカキ稚貝からのカキヘルペスウイルス1型(OsHV-1)変異型の検出

誌名	魚病研究
ISSN	0388788X
著者名	嶋原,佳子 栗田,潤 桐生,郁也 西岡,豊弘 湯浅,啓 川名,守彦 釜石,隆 大迫,典久
発行元	日本魚病学会
巻/号	47巻4号
掲載ページ	p. 129-136
発行年月	2012年12月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

魚病研究 **Fish Pathology, 47 (4), 129–136, 2012. 12** © 2012 The Japanese Society of Fish Pathology

Surveillance of Type 1 Ostreid Herpesvirus (OsHV-1) Variants in Japan

Yoshiko Shimahara[1], Jun Kurita[1**], Ikunari Kiryu[1], Toyohiro Nishioka[1], Kei Yuasa[1], Morihiko Kawana[2], Takashi Kamaishi[1] and Norihisa Oseko[1*]

[1]*National Research Institute of Aquaculture, Fisheries Research Agency, Mie 519-0193, Japan*
[2]*Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Hokkaido 062-0992, Japan*

(Received July 5, 2012)

ABSTRACT—Ostreid herpesvirus 1 (OsHV-1) μVar is a variant of OsHV-1 and suspected of being the causative agent of acute mass mortality events of Pacific oysters during summers in Europe since 2008. In this study, the distribution of OsHV-1 was surveyed in the six main oyster-producing areas of Japan, using PCR targeting a C2/C6 fragment including ORF4. PCR products were amplified from 123 out of 1,714 oysters of three species of *Crassostrea* (*C. gigas, C. sikamea* and *C. ariakensis*), and 23 different nucleotide sequences, showing 96% to 99% similarity to the reference OsHV-1, were obtained. Although 18 sequences among the 23 obtained possessed a microsatellite deletion unique to OsHV-1 μVar, all PCR products contained two conserved nucleotides that were shared with the reference OsHV-1 and not with OsHV-1 μVar. Here, we found variable types of OsHV-1 in oysters in Japan, but their nucleotide sequences were not identical to those of OsHV-1 μVar.

Key words: Ostreid herpesvirus, OsHV-1, OsHV-1 μVar, *Crassostrea gigas*, *C. sikamea*, *C. ariakensis*, Japan, Pacific oyster

Mortalities of the larvae of the Pacific oyster *Crassostrea gigas*, associated with a herpes-like virus, were first noted in France (Nicolas *et al.*, 1992). The herpes-like virus was thereafter classified as the sole member of the genus *Ostreavirus* and named ostreid herpesvirus 1 (OsHV-1) (Davison *et al.*, 2005). The mortalities associated with OsHV-1 occurred during the summers of 1991 and 1993 (Nicolas *et al.*, 1992; Renault *et al.*, 1994). A morphologically similar virus was also found in New Zealand (Hine *et al.*, 1992). In the United States, OsHV-1, or a closely related virus, was detected during a mortality event of Pacific oysters (Friedman *et al.*, 2005; Burge *et al.*, 2006).

In 2008, the Pacific oyster aquaculture industry in France experienced acute events with high mortalities, reaching up to 100%, in spat and juveniles (Segarra *et al.*, 2010). These enormously damaging events were apparently different from the diseases caused by the OsHV-1 described above. The major feature of the mortalities was that OsHV-1 was detected at an abnormally high incidence (European Food Safety Authority, 2010; Segarra *et al.*, 2010; Renault, 2011; Renault *et al.*, 2012). By PCR amplification of OsHV-1 DNA obtained from the samples collected during the mortality event, and subsequent sequence analysis, the virus was demonstrated to be a newly reported variant of OsHV-1, referred to as ostreid herpesvirus 1 microvariant (OsHV-1 μVar) (Segarra *et al.*, 2010). OsHV-1 μVar differed from reference OsHV-1 (GenBank accession no. AY509253) (Davison *et al.*, 2005) by nucleotide mutations in the C2/C6 fragment including ORF4, and in the IA1/IA2 fragment including ORF42/43 (Segarra *et al.*, 2010). Notably the sequences in the C2/C6 area were more polymorphic than those in other regions (Renault *et al.*, 2012), and OsHV-1 μVar was characterized by a deletion of 12 consecutive nucleotides, non-synonymous substitutions, and other mutations in the C2/C6 fragment including ORF4 (HQ842610) (Segarra *et al.*, 2010). Since 2008, Pacific oysters have suffered from acute outbreaks of the disease, with higher mortalities in summer not only in France, but also in the UK, Ireland, the Netherlands, Spain, Australia and New Zealand, where either OsHV-1 μVar, or a very similar virus, was detected (Martenot *et al.*, 2012; Peeler *et al.*, 2012; Roque *et al.*, 2012; Renault *et al.*, 2012; Segarra *et al.*, 2010). The disease seems to occur most commonly in the Pacific oyster. The strong pathogenicity of OsHV-1 μVar was shown by injection of oysters with affected spat homogenates, and the infectivity of this

* Corresponding author
E-mail: ohseko@affrc.go.jp
** Present address: Headquarters, Fisheries Research Agency, Kanagawa 220–6115, Japan

virus was reported by Schikorski *et al.* (2011a, b). It is possible that this viral disease will spread worldwide in the near future.

Japan is one of the major producers of Pacific oysters, with a yearly production of 200,000 tons (Portal Site of Official Statistics of Japan, http://www.e-stat.go.jp/SG1/estat/List.do?lid=000001061498, accessed 9th February, 2012). OsHV-1 μVar is, at present, one of the major concerns for the aquaculture industry in Japan. OsHV-1 has been previously detected in wild and cultivated oysters collected in the Kyusyu and Mie Prefecture in 1999 and 2005, respectively (Moss *et al.*, 2007), but virus variation analyses of the nucleotide sequences of the C2/C6 fragment, which includes ORF4, were not performed. Recently, Renault *et al.* (2012) detected an OsHV-1 variant in samples collected in Japan. This OsHV-1 variant possessed a microsatellite deletion unique to OsHV-1 μVar in C2/C6, but the nucleotide sequence was not identical to OsHV-1 μVar (Renault *et al.*, 2012). In this study reported here, oyster samples were collected from major oyster-producing areas throughout Japan and extensively surveyed to determine whether OsHV-1 μVar was present in those areas.

Materials and Methods

Samples

Spat, or juveniles, of Pacific oysters were collected from Miyagi, Mie, Hiroshima, Okayama, Kumamoto and

Table 1. Origins of the oyster samples used in this study, and their C2/C6 sequence types

Location	Oyster species	Month of sampling	Water temperature (°C)	Number of samples	Shell length (mm)	Number of positives for OsHV-1	Types of sequence (number of animals)
Year 2007							
Miyagi	Pacific oyster*1	July	N.D.	23	21.0–39.7	7 (30.4%)	Type 1 (4), 2 (3)
	Pacific oyster	September	N.D.	24	34.1–55.1	2 (8.3%)	Type 1 (2)
	Pacific oyster	December	N.D.	24	13.6–37.9	3 (12.5%)	Type 1 (3)
Hiroshima	Pacific oyster	July	N.D.	24	65.0–86.4	0 (0%)	
	Pacific oyster	September	N.D.	24	39.1–64.8	7 (29.1%)	Type 1 (7)
	Pacific oyster	December	N.D.	24	50.9–78.6	0 (0%)	
Mie	Pacific oyster	July	N.D.	24	19.1–58.4	0 (0%)	
	Pacific oyster	September	N.D.	24	72.7–115.8	2 (8.3%)	Type 1 (21), 22 + 23** (1)
	Pacific oyster	December	N.D.	24	103.5–142.8	0 (0%)	
Ishikawa	Pacific oyster	July	N.D.	24	29.5–80.0	0 (0%)	
	Pacific oyster	September	N.D.	24	36.2–70.3	5 (20.8%)	Type 1 (1), 9 (3), 20 + 21** (1)
	Pacific oyster	December	N.D.	24	77.7–124.6	0 (0%)	
Kumamoto	Pacific oyster	July	N.D.	24	25.3–66.3	0 (0%)	
	Pacific oyster	September	N.D.	38	31.2–60.2	1 (2.6%)	Type 18 (1)
	Pacific oyster	December	N.D.	24	33.6–62.6	1 (4.2%)	Type 17 (1)
	Kumamoto oyster*2	July	N.D.	24	18.0–59.7	2 (8.3%)	Type 19 (2)
	Kumamoto oyster	September	N.D.	24	27.6–54.9	4 (16.7%)	Type 19 (4)
	Kumamoto oyster	December	N.D.	24	30.7–56.3	2 (8.3%)	Type 19 (2)
	Suminoe oyster*3	September	N.D.	24	19.3–101.7	1 (4.2%)	Type 18 (1)
Year 2011							
Miyagi	Pacific oyster	February	6.9	150	5–15.2	3 (2%)	Type 3 (1), 4 (1), 5 (1)
	Pacific oyster	August	28.7	45	N.D.	19 (42%)	Type 2 (13), 4 (1), 7 (1), 8 (2), 2 + 6** (1), 2 + 8** (1)
	Pacific oyster	October	19.0	150	11.0–31.3	30 (20%)	Type 2 (11), 4 (6), 6 (3), 7 (3), 11 (1), 12 (1), 13 (1), 14 (2), 15 (1), 16 (1)
	Pacific oyster	October	N.D.	150	18.3–38.0	16 (10.7%)	Type 4 (5), 6 (1), 9 (10)
Hiroshima	Pacific oyster	May	N.D.	150	13.5–33.6	4 (2.7%)	Type 1 (2), 2 (2)
	Pacific oyster	August	22.1	150	7.8–18.7	12 (8.0%)	Type 1 (12)
Mie	Pacific oyster	October	22–23	150	6.1–24.6	2 (1.3%)	Type 9 (1), 10 (1)
Okayama	Pacific oyster	November	17.4	150	9.7–14.2	0 (0%)	
	Pacific oyster	November	16.6	150	10.8–61.05	0 (0%)	

N.D.; No data
*1 Pacific oyster: *Crassostrea gigas*, *2 Kumamoto oyster: *C. sikamea*, *3 Suminoe oyster: *C. ariakensis*.
** Different types of OsHV-1 were simultaneously present in one animal.

Ishikawa Prefectures in 2007 and 2011. Kumamoto oysters, *C. sikamea*, and the Suminoe oyster, *C. ariakensis*, were collected from Kumamoto Prefecture in 2007 (Table 1, Fig. 1). Water temperatures at the time of sampling were between 6.9°C and 28.7°C. 1,714 oysters were dissected, and the entire tissue of the spat, excluding shells, were homogenized. Approximately 50 mg of the tissue from each sample were subjected to DNA extraction, using the Maxwell 16 Tissue DNA Purification Kit (Promega) following the manufacturer's instructions. For juvenile oysters larger than 20 mm in shell length, partial tissues including the mantle, muscle, gills and viscera were used for DNA extraction. All samples of oysters used in the present study were healthy, and no dramatic increases in mortality rate had been reported during culture of the oysters in each area.

PCR and sequence analysis

PCR, targeting the C2/C6 fragment including ORF4, was performed using the primer pairs C2: 5′-CTC TTT ACC ATG AAG ATA CCC ACC-3′, and C6: 5′-GTG CAC GGC TTA CCA TTT TT-3′ in a final reaction volume of 20 μL, as described by Renault and Arzul (2001) with modifications. The PCR reaction mixture contained 0.5 U of *TaKaRa Ex Taq* Hot Start Version (TaKaRa), 2 μL of 10× reaction buffer, 0.5 pmol of dNTP's and 20 pmol of each primer. One microliter of template DNA was added to each mixture. The program consisted of one cycle of denaturation at 94°C for 10 min, followed by 40 amplification cycles of 94°C for 30s, 63°C for 30s, and 72°C for 30s, with a final extension step of 72°C for 7 min.

PCR products were purified using Agencourt AMpure (Beckman Coulter) following the manufacturer's instructions, and sequenced directly using the BigDye Terminator Kit v3.1 (Applied Biosystems). When more than two types of nucleotide sequences were found in one sample, purified DNA was cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen) and the resultant plasmids were transformed into *Escherichia coli* strain DH5α. Plasmids from at least five independently derived clones were extracted using the QIAprep Spin Miniprep Kit (QIAGEN) and subjected to sequencing reactions. Sequencing reactions were purified with Agencourt CleanSEQ (Beckman Coulter) following the manufactur-

Fig. 1. Map of Japan, showing the six prefectures where oyster samples were collected.

er's instructions and loaded into an ABI PRISM 3130 *xl* Genetic Analyzer (Applied Biosystems). Basic alignments of nucleotide sequences were made using GENETYX-MAC software (GENETYX). Phylogenetic analysis was performed by the maximum-likelihood method using the MEGA5 program (Tamura *et al.*, 2011). The sequences obtained were compared, both with those of variants recently reported by Renault *et al.* (2012), reference OsHV-1 (AY509253) (Davidson *et al.*, 2005), and OsHV-1 μVar (HQ842610) (Segarra *et al.*, 2010).

Results and Discussion

In a recent study, Renault *et al.* (2012) analyzed three different genomic regions of OsHV-1 (ORF4; ORF35, -36, -37, and -38; and ORF42 and -43) from samples collected in France, Ireland, the United States, New Zealand, China and Japan. Of the three regions, ORF4, which was amplified with the C2/C6 primers,

```
OsHV-1 (AY509253)                    1 CTCTTTACCATGAAGATACCCACCAATGTGGTAAAGACGGAACAATCTTTTT-CTAGGATATGGAGCTGCGGCGCTATGGATTTAACGAGTGCCACCAAAAGTTGGGATAATGATTTTAGAATAGATGTGATGTGCGGCAAGATGAATGG 149
OsHV-1 uVar (HQ842610)                1 ....................................................-................................................................................................. 149
2010.002_France(JN800120).seq        1 -------.............................................-................................................................................................. 142
2010.012_France(JN800122).seq        1 -------.............................................-................................................................................................. 142
2009.Ireland_JN800129.seq            1 -------.............................................-................................................................................................. 142
2010.01_New_Zealand(JN800130).seq    1 -----------------------------------------------.....-................................................................................................. 102
2002.E50_China(JN800132).seq         1 -------.............................................-................................................................................................. 142
2010.158-144_Japan(JN800133).seq     1 -------.............................................-................................................................................................. 142
JPType1.seq                          1 ....................................................-................................................................................................. 149
JPType2.seq                          1 ....................................................-................................................................................................. 149
JPType3.seq                          1 ....................................................-................................................................................................. 149
JPType4.seq                          1 ....................................................-................................................................................................. 149
JPType5.seq                          1 ..................................G.................-................................................................................................. 149
JPType6.seq                          1 ....................................................-................................................................................................. 149
JPType7.seq                          1 ....................................................-................................................................................................. 149
JPType8.seq                          1 ....................................................-................................................................................................. 149
JPType9.seq                          1 ....................................................-................................................................................................. 149
JPType10.seq                         1 ....................................................-....A............................................................................................ 149
JPType11.seq                         1 ....................................................T................................................................................................. 150
JPType12.seq                         1 ....................................................-................................................................................................. 149
JPType13.seq                         1 ....................................................-................................................................................................. 149
JPType14.seq                         1 ....................................................-................................................................................................. 149
JPType15.seq                         1 ....................................................-................................................................................................. 149
JPType16.seq                         1 ....................................................-................................................................................................. 149
JPType17.seq                         1 ....................................................-................................................................................................. 149
JPType18.seq                         1 ....................................................-................................................................................................. 149
JPType19.seq                         1 ....................................................-................................................................................................. 149
JPType20.seq                         1 ....................................................-................................................................................................. 149
JPType21.seq                         1 ...................................................C-................................................................................................. 149
JPType22.seq                         1 ....................................................-................................................................................................. 149
JPType23.seq                         1 ....................................................-.....................................................................................T........... 149
                                       ...............................................****. ****.********************************************************************************.***********

OsHV-1 (AY509253)                  150 CAAGATACACAATGAGCTATTGCCCGACCACAAACCTAACGTTGTATTCGATTACGGATTAAGAAAATGGGTTCCACAATCTAAAATTAAAAAAACCACATGGGGG-CCAAGGAATTTAAA-CCCCGGGGAAAAA--GTATAAATAGGCG 295
OsHV-1 uVar (HQ842610)              150 .....................A.......................................................................C-...........-..............G.............A-............. 296
2010.002_France(JN800120).seq      143 .....................A.......................................................................C-...........G..............G.............A-............. 290
2010.012_France(JN800122).seq      143 .....................A.......................................................................C-...........G.A............G.............A-............. 290
2009.Ireland_JN800129.seq          143 .....................A.......................................................................C-...........-..............G.............A-............. 289
2010.01_New_Zealand(JN800130).seq  103 .....................A.......................................................................C-...........-..............G.............AA............. 250
2002.E50_China(JN800132).seq       143 .............................................................................................C-...........-..............-.............A-............. 288
2010.158-144_Japan(JN800133).seq   143 .....................A.......................................................................C-...........-..............G.............A-A............ 289
JPType1.seq                        150 .............................................................................................C-...........-..............G.............A-............. 296
JPType2.seq                        150 .............................................................................................C-...........-..............G.............A-A............ 296
JPType3.seq                        150 .............................................................................................C-...........-..............G.............A-A............ 296
JPType4.seq                        150 .............................................................................................C-..........C-..............G.............A-A............ 296
JPType5.seq                        150 .............................................................................................C-...........-..............G...G.........--............. 295
JPType6.seq                        150 .....................A.......................................................................C-...........-..............G.............A-............. 296
JPType7.seq                        150 .............................................................................................C-...........-..............G.............A-A............ 296
JPType8.seq                        150 .............................................................................................C-...........-..............G.............A-A............ 296
JPType9.seq                        150 .....................A.......................................................................C-...........-..............G.............A-............. 296
JPType10.seq                       150 .........................................................................................................-..............-.............--............. 295
JPType11.seq                       151 .....................A.......................................................................C-...........-..............G.............A-A............ 297
JPType12.seq                       150 .............A...............................................................................C-...........-..............G.............A-A............ 296
JPType13.seq                       150 .............................................................................................C-..........--..............G.............A-A............ 295
JPType14.seq                       150 .....................A.......................................................................C-...........-..............G.............A-A............ 296
JPType15.seq                       150 .....................A.......................................................................C-...........-..............G.............A-A............ 296
JPType16.seq                       150 .............................................................................................C-...........-..............G.............A-A............ 296
JPType17.seq                       150 .........................................................................................................-..............-.............--............. 295
JPType18.seq                       150 .............................................................................................C-...........-..............G.............A-............. 296
JPType19.seq                       150 .............................................................................................C-...........-..............G.............A-............. 296
JPType20.seq                       150 ...............................G.............................................................C-...........-..............G.............A-............. 296
JPType21.seq                       150 .............................................................................................C-...........-..............G.............A-............. 296
JPType22.seq                       150 .............................................................................................C-...........-..............G.............A-............. 296
JPType23.seq                       150 .............................................................................................C-...........-..............G.............A-............. 296
                                       *************.*******.*********.*************************************************************. **********. *.************.***.*********. .************

                                                                                                                            ↓ Microsatellite zone ↓    ↓ ORF 4
OsHV-1 (AY509253)                  296 CGATTTGTCAGTTTAGAATCATACCCACACACTCAATCTCGAGTATACCACAACTGCTAAATTAACAGCATCTACTACTACTACTACTACTACTGAAAAAATGCAGCCTTTCACAGAATTTTGCACCTTGACCAAAGCCATCACATC 445
OsHV-1 uVar (HQ842610)              297 ..............................--.....................................................------------.-................................................... 431
2010.002_France(JN800120).seq      291 ..............................--.....................................................------------.-................................................... 425
2010.012_France(JN800122).seq      291 ..............................--.....................................................------------.-................................................... 425
2009.Ireland_JN800129.seq          290 ..............................--.....................................................------------.-................................................... 425
2010.01_New_Zealand(JN800130).seq  251 ..............................--.....................................................------------.-................................................... 385
2002.E50_China(JN800132).seq       289 ..............................--.....................................................------------.-................................................... 423
2010.158-144_Japan(JN800133).seq   290 ..............................--.....................................................------------.-................................................... 424
JPType1.seq                        297 ..............................--.....................................................------------.-................................................... 431
JPType2.seq                        297 ..............................--.....................................................------------.-................................................... 431
JPType3.seq                        297 ..............................--.....................................................------------.-................................................... 431
JPType4.seq                        297 ..............................--.....................................................------------.-................................................... 431
JPType5.seq                        296 ..............................--.....................................................------------.-................................................... 430
JPType6.seq                        297 ..............................--.....................................................------------.-................................................... 431
JPType7.seq                        297 ..............................--...........................................................------.-................................................... 437
JPType8.seq                        297 ..............................--..................................................---------------.-................................................... 428
JPType9.seq                        297 ..............................--.....................................................------------.-................................................... 431
JPType10.seq                       296 .....................................................................................sts.........---.................................................... 442
JPType11.seq                       298 ..............................--.....................................................------------.-................................................... 432
JPType12.seq                       297 ..............................--.....................................................------------.-................................................... 431
JPType13.seq                       296 ..............................--.....................................................------------.-................................................... 430
JPType14.seq                       297 ..............................--.....................................................------------.-................................................... 431
JPType15.seq                       297 ..............................--.....................................................------------.-................................................... 431
JPType16.seq                       297 ..............................--.....................................................------------.-................................................... 431
JPType17.seq                       296 .....................................................................................---------..................................................... 436
JPType18.seq                       297 ..............................--.....................................................------------.-................................................... 434
JPType19.seq                       297 ..............................--.....................................................------------.-................................................... 431
JPType20.seq                       297 ..............................--.....................................................------------.-................................................... 431
JPType21.seq                       297 ..............................--.....................................................------------.-................................................... 431
JPType22.seq                       297 ..............................--C..............................................C.....------------.-................................................... 431
JPType23.seq                       297 ..............................--.....................................................------------.-................................................... 431
                                       ******************************  .**********************************************.***..            *.***************************************************
```

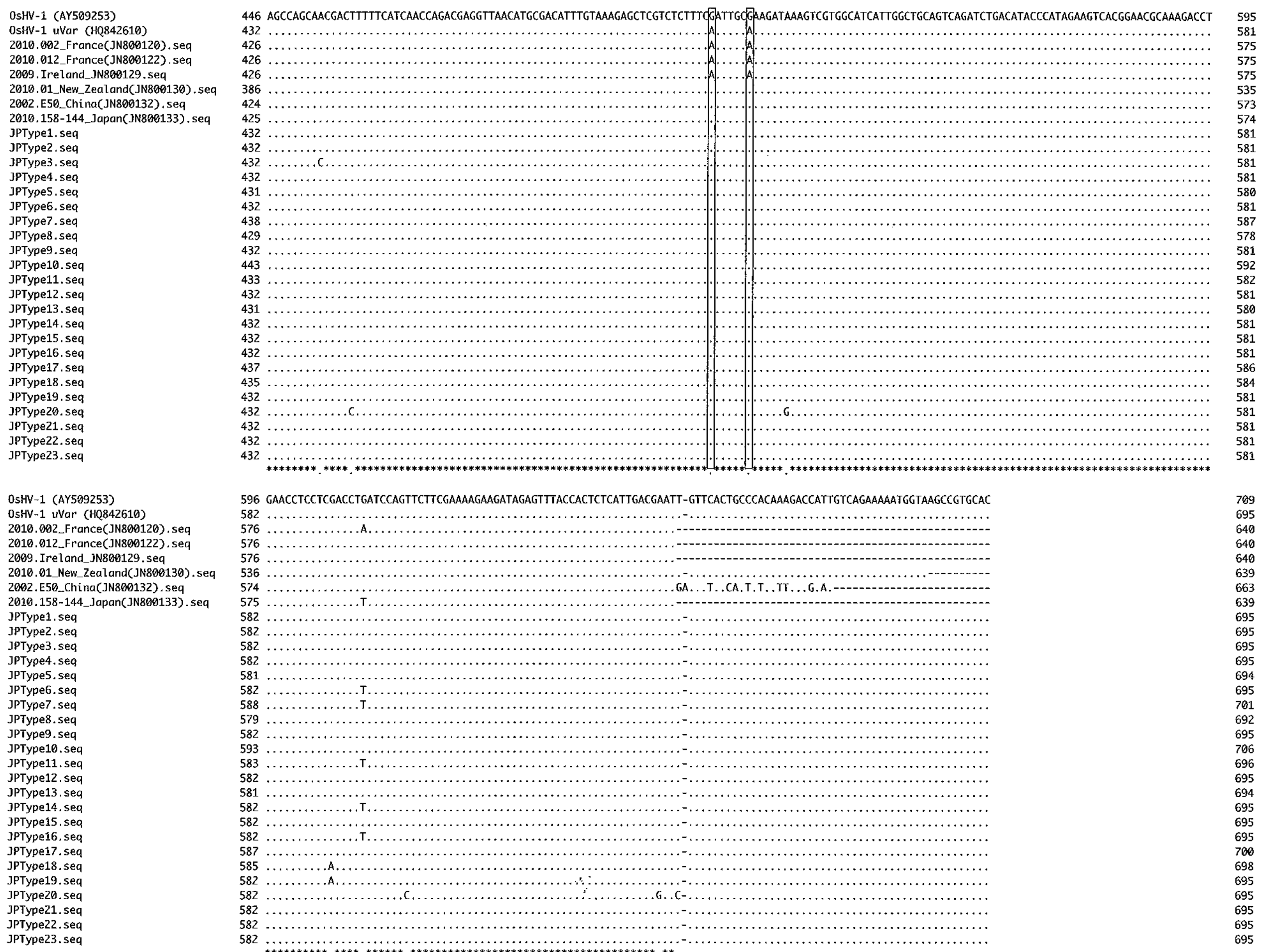

Fig. 2. C2/C6 sequence alignment of OsHV-1 variants obtained in this study (JPType 1–23). These variants are compared with those of reference OsHV-1 (AY509253), OsHV-1 μVar (HQ842610), and those described by Renault *et al.* (2012). The locations of the microsatellite zone and the initiation codon for ORF 4 are indicated. OsHV-1 μVar is characterized by a deletion of 12 consecutive nucleotides in the microsatellite zone, two non-synonymous substitutions, and the other mutations in this C2/C6 fragment. The major deletion in the microsatellite zone, and the substitutions of the two nucleotides in ORF 4, are framed. The substitutions of two nucleotides in ORF4 are conserved in all JPTypes found in the present study.

was the most polymorphic, and considered to be suitable for the subtyping of OsHV-1 (Renault *et al.*, 2012). In the present study, PCR targeting the C2/C6 area was carried out, and the nucleotide sequences of amplicons were analyzed. The C2/C6 product of approximately 700 bp was amplified from 37 out of 469 samples in 2007, and from 86 out of 1,245 samples in 2011 (Table 1). The C2/C6 product was detected in DNA extracted from spat taken from all sampling areas, except Okayama Prefecture, in 2011. The detection rate of the C2/C6 PCR product tended to be higher in the oysters collected in summer (Table 1). The C2/C6 PCR product was detected not only in samples from Pacific oysters, but also in those from both Kumamoto oysters and Suminoe oysters. The nucleotide sequences of the C2/C6 products were polymorphic and 23 different nucleotide sequences, JPType 1 to JPType 23 (Fig. 2), were obtained. The 23 sequences were deposited in the DDBJ/EMBL/GenBank Data Libraries under accession no. AB734057–AB734079. The 23 sequences showed 96% to 99% similarity to both reference OsHV-1 (AY509253) and OsHV-1 μVar (HQ842610).

A deletion of 12 consecutive nucleotides in the C2/C6 region is one of the characteristic features of OsHV-1 μVar. In our study, all of the C2/C6 products had a deletion, ranging from 3 to 15 bp, in the microsatellite zone. A deletion of 12 consecutive nucleotides in the microsatellite zone was found in a total of 18 types, JPType 1–6, 9, 11–16, 19–23. The position and the size of the deletion coincided with those of OsHV-1 μVar, whereas five sequence types, JPType 7, 8, 10, 17, and 18, possessed a deletion of 6, 15, 3, 9, and 9 consecutive nucleotides, respectively. All PCR products had two conserved nucleotides in ORF 4; these were also present in reference OsHV-1, but not in OsHV-1 μVar. Thus, OsHV-1 μVar, which is one of the major concerns for oyster producers in France at present, has not yet been found in Japan.

The polymorphisms of the C2/C6 fragment were recently investigated with samples collected in France, Ireland, the United States, New Zealand, China and Japan (Renault *et al.*, 2012). In addition, new variants of OsHV-1 μVar were found in France (Martenot *et al.*, 2011, 2012). Of the 23 variants obtained in our study, JPType 14 was identical to 2010/158–144 (JN800133) (Renault *et al.*, 2012), which originated in Japan. However, the other nucleotide sequences obtained in this study were not identical to those described recently by Renault *et al.* (2012) and Martenot *et al.* (2011, 2012). Therefore, none of the Japanese variants were identical to those reported previously in Europe, Oceania and America.

The phylogenetic tree of C2/C6 sequences generated by the maximum-likelihood method is shown in Fig. 3. Due to the high degree of sequence similarity between the variants, high bootstrap values were not obtained. However, our phylogenetic analysis produced a tree similar to that described by Renault *et al.* (2012), separating reference OsHV-1 and OsHV-1 μVar into distinct subgroups. The phylogenetic tree showed that the OsHV-1 variants in Japan were more variable than those detected in France. OsHV-1 μVar has been detected at high frequency during several outbreaks with high mortality in France since 2008

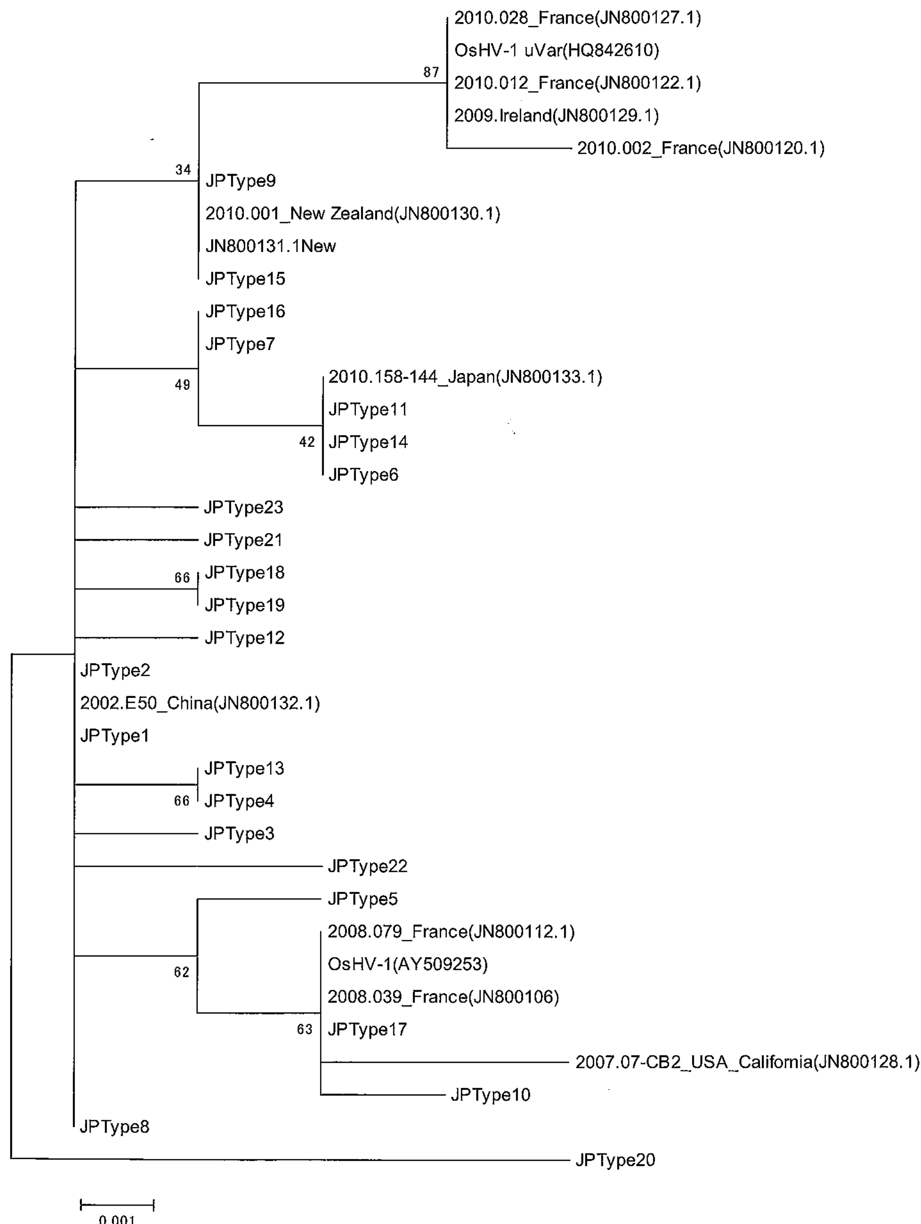

Fig. 3. Phylogenetic tree of the C2/C6 sequences generated by the maximum-likelihood method. Bootstrap values were obtained from 1,000 resampled data sets. OsHV-1 variants obtained in this study (JPType 1–23), reference OsHV-1, OsHV-1 μVar, and those described by Renault *et al.* (2012) were included in this analysis. The sequences of primers were excluded from the analysis.

(Segarra *et al.*, 2010 and Renault *et al.*, 2012). It seemed that the high prevalence of OsHV-1 μVar in France is due to its strong pathogenicity.

Although variable types of C2/C6 sequences were obtained in this survey, it remains unknown whether these OsHV-1 variants are as pathogenic to oysters as those found in Europe. Mortalities associated with ostreid herpesvirus are usually found in summer, concomitant with elevated water temperatures (Garcia *et al.*, 2011; Segarra *et al.*, 2010; Peeler *et al.*, 2012). It is well known among Japanese oyster farmers that aquaculture industry in Japan occasionally experiences relatively high mortality events of oysters in summer. However, large-scale mortality outbreaks, such as those seen recently in Europe, did not occur at least for the last few years. In order to determine the pathogenicity of OsHV-1 variants in Japan, experimental infections must be carried out; however, isolation and cultivation of OsHV-1 has not been successful due to the lack of suitable cell lines (Renault and Novoa, 2004). Further epidemiological studies must be undertaken in Japan to determine whether these variants affect oysters. In addition, the C2/C6 sequences will be analyzed when high mortality outbreaks accompany the detection of OsHV-1.

Acknowledgements

The authors are grateful to the Prefectures of Hiroshima, Ishikawa, Kumamoto, Mie, Miyagi, and Okayama for providing the oysters used in the present study. This study was supported by grants from the Food Safety and Consumer Affairs Bureau, Ministry of Agriculture, Forestry, and Fisheries of Japan.

Reference

Burge, A. B., F. J. Griffin and S. Friedman (2006): Mortality and herpesvirus infections of the Pacific oyster *Crassostrea gigas* in Tomales Bay, California, USA. *Dis. Aquat. Org.*, **72**, 31–43.

Davison, A. J., B. L. Trus, N. Cheng, A. C. Steven, M. S. Satson, C. Cunningham, R. M. Le Deuff and T. Renault (2005): A novel class of herpesvirus with bivalve hosts. *J. Gen. Virol.*, **86**, 41–53.

European Food Safety Authority (EFSA) (2010): Scientific Opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas*, **8**, 1894.

Friedman, C. S., R. M. Estes, N. A. Stokes, C. A. Burge, J. S. Hargove, B. J. Barber, R. A. Elston, E. M. Burreson and K. S. Reece (2005): Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tamales Baty, California, coincides with summer mortality episodes. *Dis. Aquat. Org.*, **63**, 33–41.

Garcia, C., A. Thebault, L. Degremont, I. Arzul, L. Miossec, M. Robert, B. Chollet, C. Francois, J-P. Joly, S. Ferrand, N. Kerdudou and T. Renault (2011): Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006. *Vet. Res.*, **42**, 73.

Hine, P. M., B. Wesney and B. E. Hay (1992): Herpesviruses associated with mortalities among hatchery-reared larval Pacific oysters *Crassostrea gigas*. *Dis. Aquat. Org.*, **12**, 135–142.

Martenot, C., E. Oden, E. Travaille, J. P. Malas and M. Houssin (2011): Detection of different variants of Ostreid Herpesvirus 1 in the Pacific oyster, *Crassostrea gigas* between 2008 and 2010. *Virus Res.*, **160**, 25–31.

Martenot, C., S. Fourour, E. Oden, A. Jouraux, E. Travaille, J. P. Malas and M. Houssin (2012): Detection of the OsHV-1 μVar in the Pacific oyster *Crassostrea gigas* before 2008 in France and description of two new microvariants of the Ostreid Herpesvirus 1 (OsHV-1). *Aquaculture*, **338–341**, 293–296.

Moss, J. A., E. M. Burreson, J. F. Cordes, C. F. Dungan, G. D. Brown, A. Wang, X. Wu and K. S. Reece (2007): Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay. *Dis. Aquat. Org.*, **77**, 207–223.

Nicolas, J. L., M. Comps and N. Cochennec (1992): Herpes-like virus infecting Pacific-oyster larvare. *Bull. Eur. Ass. Fish Pathol.*, **12**, 11–13.

Peeler, E. J., R. A. Reese, D. L. Cheslett, F. Geoghegan, A. Power and M. A. Thrush (2012): Investigation of morality in Pacific oysters associated with *Ostreid herpesvirus*-1 μVar in the Republic of Ireland in 2009. *Prev. Vet. Med.*, **105**, 136–143.

Renault, T. (2011): A review of mortality outbreaks in the Pacific oyster, *Crassostrea gigas*, reported since 2008 in various European Union Member States and the related implementation of Council Directive 2006/88/EC. *OIE Bulletin*, **3**, 51–52.

Renault, T. and I. Arzul (2001): Herpes-like virus infections in hatchery-reared bialve larvae in Europe: specific viral DNA detection by PCR. *J. Fish Dis.*, **24**, 161–167.

Renault, T., N. Cochennec, R. M. Ledeuff and B. Chollet (1994): Herpes-like virus infecting Japanese oyster (*Crassostrea gigas*) spat. *Bull. Eur. Ass. Fish Pathol.*, **14**, 64–66.

Renault, T., P. Moreau, N. Faury, J-F. Pepin, A. Segarra and S. Webb (2012): Analysis of clinical Ostreid Herpesvirus 1 (*Malacoherpesviridae*) speciments by sequencing amplified fragments from three virus genome areas. *J. Virol.*, **86,** 5942–5947.

Renault, T. and B. Novoa (2004): Viruses infecting bivalve mullucs. *Aquat. Living Resour.*, **17**, 397–409.

Roque, A., N. Carrasco, K. B. Andree, B. Lacuesta, L. Elandaloussi, I. Gairin, C. J. Rodgers and M. D. Furounes (2012): First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain. *Aquaculture*, **324–325**, 303–306.

Schikorski, D., N. Faury, J. F. Pepin, D. Saulnier, D. Tourbiez and T. Renault (2011a): Experimental ostreid herpesvirus 1 infection of the Pacific oyster *Crassostrea gigas*: Kinetics of virus DNA detection by q-PCR in seawater and oyster samples. *Virus Res.*, **155**, 28–34.

Schikorski, D., T. Renault, D. Saulnier, N. Faury, P. Moreau and J. F. Pepin (2011b): Experimental infection of Pacific oyster *Crassostrea gigas* spat by ostreid herpesvirus 1: demonstration of oyster spat susceptibility. *Vet. Res.*, **42**, 27.

Segarra, A., J. F. Pepin, I. Azul, B. Morga, N. Faury and T. Renault (2010): Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. *Virus Res.*, **153**, 92–99.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar (2011): MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, **28**, 2731–2739.

日本における正常なカキ稚貝からのカキヘルペスウイルス１型（OsHV-1）変異型の検出

嶋原佳子・栗田　潤・桐生郁也・西岡豊弘
湯浅　啓・川名守彦・釜石　隆・大迫典久

わが国のカキ主産地６県におけるカキ稚貝を対象にカキヘルペスウイルス１型（OsHV-1）の検出をPCRにより試みた。供試した1,714検体のうち123検体からOsHV-1ゲノム上のC2/C6領域が検出され，塩基配列が一部異なる23種類が得られた。すなわち，日本には多様性に富んだOsHV-1変異型が広く存在しているが，いずれも高病原性を有するOsHV-1 μVarとは一致しなかった。

魚病研究，**47**（4），129–136（2012）