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## COMPARISON OF DIFFERENT *ONCORHYNCHUS MASOU* VIRUS (OMV) STRAINS BY DNA RESTRICTION ENDONUCLEASE CLEAVAGE ANALYSIS

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Seven strains of *Oncorhynchus masou* virus (OMV) genomes were analyzed with the restriction endonucleases *Bam*HI, *Eco*RI, *Hind*III and *Sma*I. The restriction patterns of OMV strain DNAs were divided into four groups. Restriction profiles of high passage strains (00-7812, 65th passage, and H-83, 60th passage) were different from those of low passage strains (00-7812, 8th passage, and H-83, 6th passage) when digested with *Bam*HI, *Hind*III and *Sma*I. However, no difference was observed between the restriction patterns of high and low passage viral DNA with *Eco*RI. There was no distinct difference observed between the restriction patterns of tumor tissue-derived and coelomic fluid-derived strains. By using <sup>32</sup>P-labelled DNA of standard OMV (strain 00-7812) as a probe, most of the fragments of other OMV strain DNAs were hybridized.

Key Words: OMV; restriction endonuclease patterns; salmonid herpesvirus

### INTRODUCTION

To date, at least two herpesviruses have been isolated from salmonid fish<sup>12,21</sup>. *Herpesvirus salmonis* was first isolated in 1975 from adult rainbow trout (*Oncorhynchus mykiss*) at the Winthrop National Fish Hatchery, Washington<sup>21</sup>. In 1978, *Oncorhynchus masou* virus (OMV) was isolated from adult landlocked masu salmon in Hokkaido, Japan<sup>12</sup>. *H. salmonis* was lethal to rainbow trout fry<sup>21,22</sup>. OMV was lethal to fry of chum salmon (*Oncorhynchus keta*), masu salmon, kokanee salmon (*Oncorhynchus nerka*), coho salmon (*Oncorhynchus kisutch*) and rainbow trout<sup>13,20</sup>. OMV, particularly has been demonstrated to induce epithelial tumors<sup>14</sup>.

Restriction endonuclease analysis of viral DNA has been used to differentiate

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serotypes of strains of herpes simplex virus (HSV)<sup>2)</sup>, Epstein-Barr virus<sup>17)</sup> and varicella zoster virus (VZV)<sup>16,23)</sup>. Differences in the distribution of restriction cleavage sites have been reported for strains of cytomegalovirus<sup>11)</sup> and Marek's disease virus (MDV)<sup>10)</sup> genomes. Recently, similar analyses have been carried out with the three salmonid herpesviruses<sup>7,8)</sup>. The DNA restriction patterns of *H. salmonis* were different from those of OMV strains. Furthermore it was noted that some DNA fragments of OMV strains disappeared or appeared anew during serial passage<sup>7)</sup>.

In this study, the restriction endonuclease cleavage patterns of seven OMV strains isolated from different tissues and different districts of Japan were analyzed. The restriction patterns of DNA from high passage strains *in vitro* were also analyzed, and compared with those from low passage strains. <sup>32</sup>P-labelled DNA of standard OMV (strain 00-7812) was hybridized with other OMV DNAs, but not *H. salmonis* DNA.

#### MATERIALS AND METHODS

**Cells:** Two cell lines, CHSE-214 and RTG-2 were used. They were derived from chinook salmon (*Oncorhynchus tshawytscha*) embryos and rainbow trout gonad, respectively. The cells were grown at 20°C as monolayers in Eagle's minimum essential medium (MEM, pH7.6; Nissui Seiyaku, Tokyo, Japan) supplemented with 7.5% fetal calf serum (FCS; Gibco). The same medium with 3% FCS was used for maintenance medium. All culture media contained 200 units of potassium penicillin G, 200 µg of streptomycin sulfate and 2.5 µg amphotericin B per ml.

**Viruses and viral DNAs:** Sources of *H. salmonis* and the seven strains of OMV, involving strains 00-7812 (as a standard strain), SO-8217, OIO-8201, TOT-8101, SHT-8201 and H-83 are listed in Table 1. The viruses were propagated in either CHSE-214 or RTG-2 cells at 10°C for *H. salmonis*, and at 15°C for OMV strains.

Viral DNA was directly extracted from virus-infected cells as described previously<sup>7)</sup>.

**DNA restriction analysis:** Restriction digestion of viral DNA was carried out with 2-5 µg DNA as recommended by the supplier of the enzymes (Takara Shuzo Co., Kyoto, Japan). Digested DNAs were fractionated by electrophoresis in both 0.8% and 0.4% agarose gels at room temperature at 100 V for 4-6h. The gels were prepared in 50mM Tris-HCl, 1mM EDTA, and 0.5 µg ethidium bromide per ml. DNA fragments in the gel were photographed using a UV transilluminator (TL-15, Ultra Violet Products, Calif.).

**Southern blotting hybridization:** For analysis of DNA homology among the eight strains, viral DNA of a standard strain (00-7812, 6th passage) was purified as a probe by the method described by Hayashi *et al*<sup>7)</sup>. The purified DNA was <sup>32</sup>P-labelled by the nicktranslation method. The viral DNA fragments in the agarose gel were transferred onto a nitrocellulose filter according to the Southern method<sup>18)</sup>. The filter

Table 1. Source and pathogenicity of virus strains tested.

Strain	Isolation			passage	pathogenicity in salmonid fry <sup>a)</sup>	tumorigenicity <sup>b)</sup>
	place	year	tissue			
H. salmonis	Winthrop national fish hatchery	1975	coelomic fluids	6 8	+	—
OMV						
00-7812	Otobe, Hokkaido	1978	coelomic fluids	8 65	+	+
SO-8217	Shiribetsu, Hokkaido	1982	coelomic fluids	10	+	+
OIO-8201	Oippe, Aomori	1982	coelomic fluids	7	ND	ND
TOT-8101	Towada, Aomori	1981	tumor tissue	13	+	+
TYT-8101	Toya, Hokkaido	1981	tumor tissue	11	+	+
SHT-8201	Shiribetsu, Hokkaido	1982	tumor tissue	10	+	+
H-83	Chitose, Hokkaido	1983	coelomic fluids	6 60	+	ND ND

a) Experimental infection to chum salmon, masu salmon and rainbow trout.

b) Tumor formation in experimental inoculation of either chum salmon or masu salmon.

ND : not done.

was prehybridized at 42°C for 8h with a mixture containing 50% formamide, 5 X Denhardt's reagent (0.1% ficoll, 0.1% bovine serum albumin and 0.1% polyvinylpyrrolidone), 5 X SSC (1 X SSC consisted of 0.15M NaCl, 0.015M sodium citrate, pH 7.0), and 500  $\mu$ g calf thymus DNA per ml. It was then hybridized with the probe in a buffer containing 50% formamide, 5 X SSC, 1 X Denhardt's reagent, 10% dextran sulphate, and 100  $\mu$ g calf thymus DNA per ml at 42°C overnight. Following hybridization, the filter was washed three times with 2 X SSC-0.1% sodium laurylsulfate (SDS) at room temperature, and then shaken in 0.1XSSC-0.1% SDS at 55°C in a water bath. The filter was dried and autoradiographed on Kodak XR film with an intensifying screen for two or seven days at -80°C.

## RESULTS

### *DNA Restriction patterns of eight virus strains*

Restriction patterns of eight viral DNAs digested with four restriction endonucleases were analyzed by electrophoresis in 0.4% and 0.8% agarose gels. The DNA restriction profiles of four strains of OMV and *H. salmonis* were established when viral DNAs were digested with four different restriction endonucleases; *Bam*HI, *Hind*III, *Sma*I and *Eco*RI in 0.4% agarose gels (Fig. 1, A, B, C and D, respectively). No difference in the appearance of smaller fragments was observed when electrophoresed in 0.8% gels (data not shown). The restriction patterns of *H. salmonis* DNA (lane g of Fig. 1, A, B and C, lane e of Fig. 1, D) differed from those of other OMV strains (lanes a to f of Fig. 1, A, B, C and lane a to d of Fig. 1, D). From the restriction patterns of seven OMV strains, they could be divided into four groups (Table 2). The first was of strains TYT-8101 and standard strain (00-7812). The second consisted of strains H-83 and SO-8217. The third contained strains TOT-8101 and OIO-8201. The remaining subtype was strain SHT-8201. Compared with DNA fragments of standard OMV (Fig. 1, lane a), several DNA fragments of other OMV strains appeared to be new ones (indicated as ◀) while other fragments disappeared (indicated as ◁). Details of changes in DNA fragments migration patterns are summarized in Table 2. Changes in restriction fragments of OMV strain DNAs were located around map units 0 to 13, 32 to 44, 50 to 54, 75 to 77 and 80 to 82 in physical maps of genome of the standard strain (Fig. 2) reported in the previous study<sup>5</sup>).

Cleavage profiles of OMV strains 00-7812 (65th passage) and H-83 (60th passage) were compared with those of parent viruses. The results showed that the restriction patterns of these viruses changed after *in vitro* serial passages, when analyzed using *Bam*HI, *Hind*III and *Sma*I (Fig. 1A to D, lanes b and d). However no changes were observed in restriction patterns when digested with *Eco*RI (data not shown).

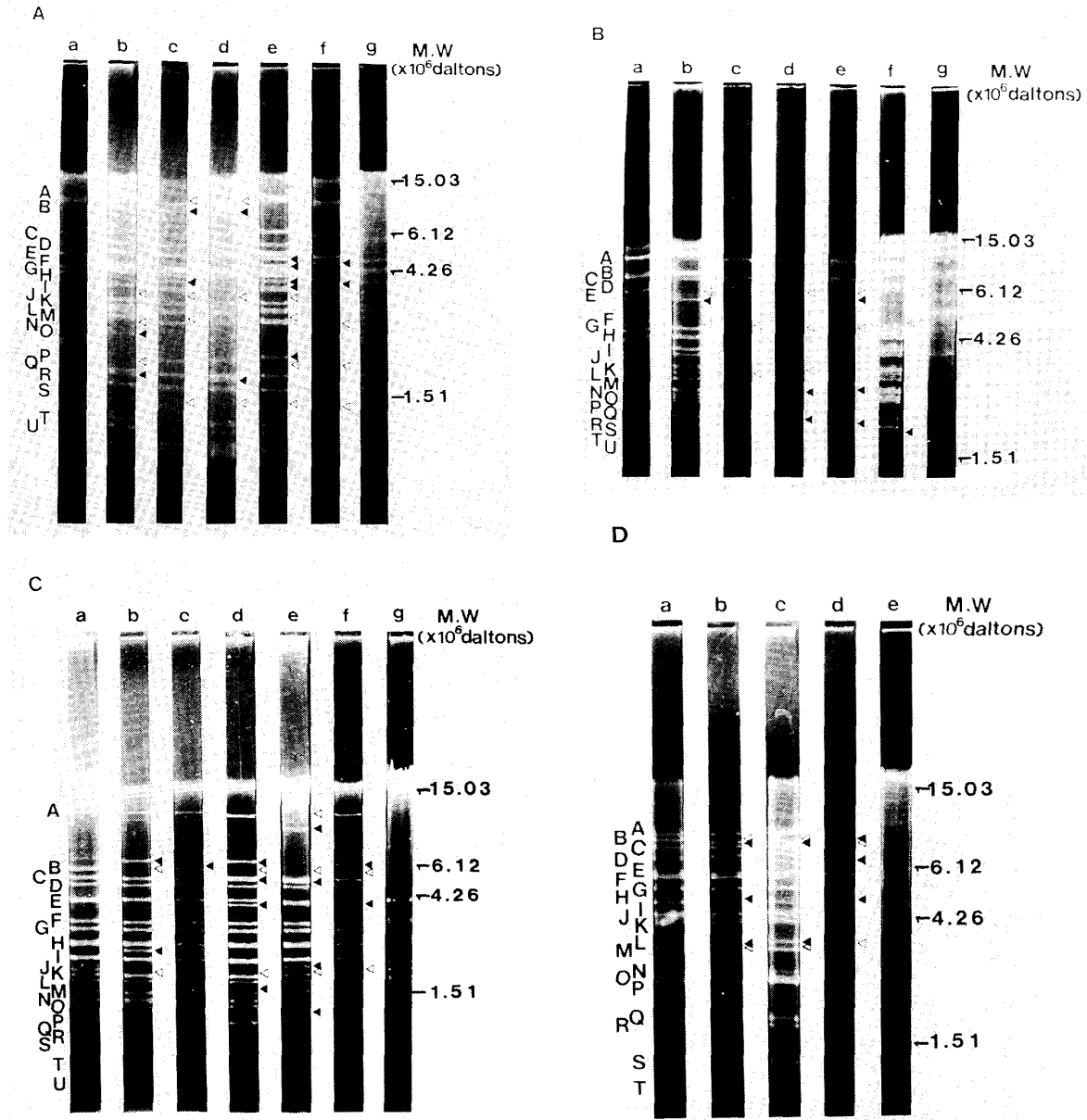


Fig. 1 DNA restriction patterns of OMV and *H. salmonis* with *Bam*HI (A), *Hind*III (B), *Sma*I (C) and *Eco*RI (D). The restriction fragments were electrophoresed in 0.4% agarose gel at 100V for 6h. In A, B and C, Lane a: standard OMV strain (00-7812, 8th passage), b: 00-7812 (65th passage), c: H-83 (6th passage), d: H-83 (60th passage), e: strain OIO-8201, f: strain SHT-8201 and g: *H. salmonis*. In D, Lane a: standard strain (00-7812, 8th passage), b: H-83 (6th passage), c: strain SHT-8201, d: strain OIO-8101 and e: *H. salmonis*.

Table 2. Comparison of genome variation in restriction fragments observed in OMV strains.

Strain	group	Changes in migration patterns of restriction fragments with							
		<i>Bam</i> HI		<i>Eco</i> RI		<i>Hind</i> III		<i>Sma</i> I	
		FrM <sup>a)</sup>	NFr <sup>b)</sup>	FrM <sup>a)</sup>	NFr <sup>b)</sup>	FrM <sup>a)</sup>	NFr <sup>b)</sup>	FrM <sup>a)</sup>	NFr <sup>b)</sup>
TYT-8101	1	none	none	none	none	none	none	none	none
H-83	2	A, J, N, S	B°, H°	B, L	B°, I'	F, K	none	none	B'
SO-8217	2								
OIO-8201	3	J, M, P, S	E°, F', H°, I', P'	B, L	B°	E, F	E°, N', R°	A, B, J	A°, C°, J', P'
TOT-8101	3								
SHT-8217	4	N, P, S	E°, H'	B, L	B', D°, I'	E	S°	B, J	B', E°

a) FrM : Fragment missing. Variation was recorded taking the DNA fragment migration pattern of the standard strain (00-7812, 8th passage).

b) NFr : Novel fragment. Novel fragments were formally designated in relation to migration of the standard strain DNA fragments, e. g., P' designates a fragment migrating faster than fragment O but slower than fragment Q of the standard strain DNA. Novel fragments labelled O° migrate slower than fragment O of the standard strain DNA.

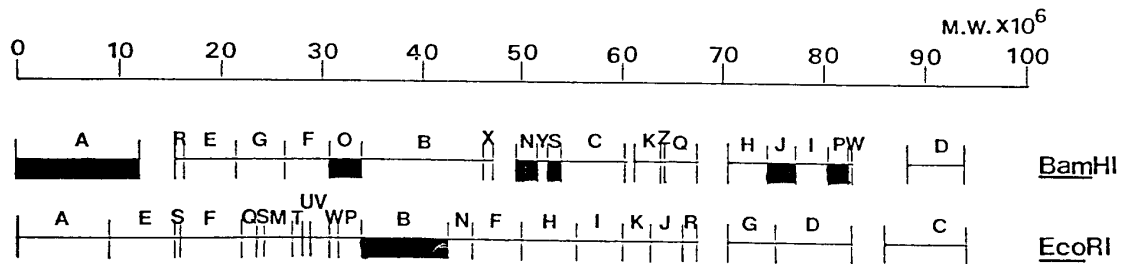


Fig. 2 Location of alternated restriction fragments found in OMV strain genomes on physical maps of standard 00-7812 strain DNA for *Bam*HI and *Eco*RI. Solid bars depict genome regions known to vary among strains.

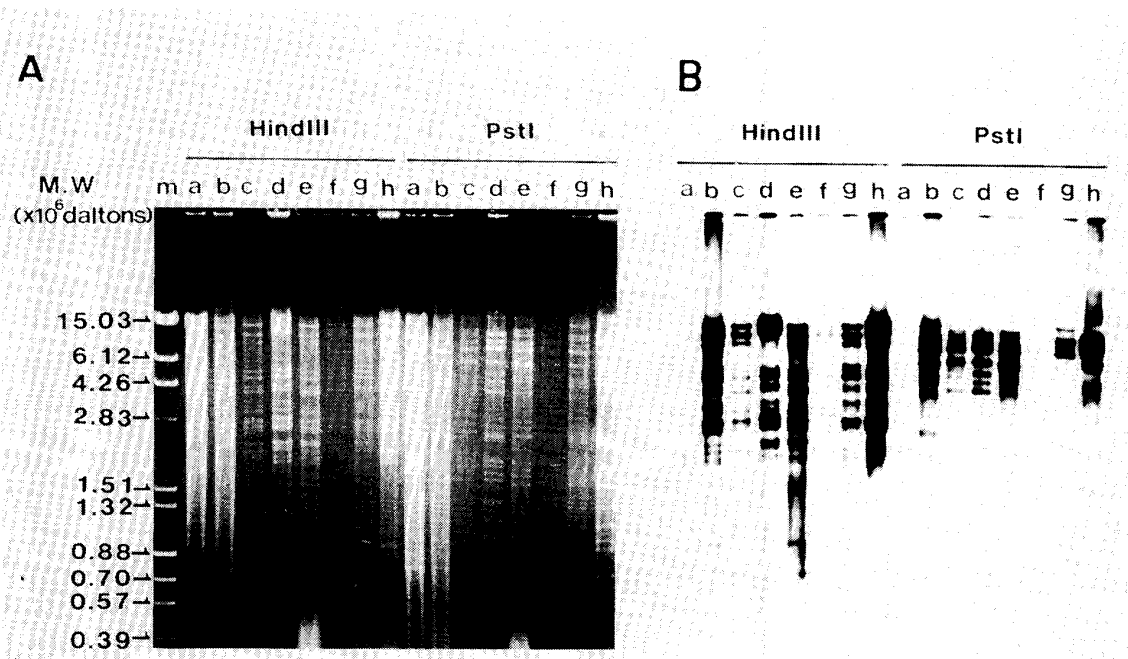


Fig. 3 Restriction fragment homology among seven OMV strains. The digestion products with *Hind*III and *Pst*I were electrophoresed in a 0.4% agarose gel, and stained with ethidium bromide (A). <sup>32</sup>P-labelled probe of viral DNA of standard strain (00-7812) was hybridized to Southern blots of restriction endonuclease digests with *Hind*III and *Pst*I (B). Lane a: *H. salmonis*, b: strain 00-7812, c: strain SO-8217, d: strain OIO-8201, e: strain H-83, f: strain TOT-8101, g: strain TYT-8101 and h: strain SHT-8201.



### *Southern blotting hybridization*

<sup>32</sup>P-labelled DNA of the standard strain 00-7812 digested with *Hind*III and *Pst*I, was hybridized with seven OMV strains and *H. salmonis* to identify DNA fragments which shared sequence homology with 00-7812 DNA. As shown in Fig. 3 (B), *H. salmonis* DNA fragments (lane a) did not hybridize, while DNA fragments of all remaining OMV strains (lanes b to h) hybridized with 00-7812 DNA.

### DISCUSSION

Since the isolation of strain 00-7812 in 1978, at least 163 strains serologically identical to OMV have been isolated from northern Japan<sup>6,12-14,20</sup>. In this study, the genomes of seven typical OMV strains were analyzed with restriction endonucleases. It was found that OMV strains, although serologically identical, showed different viral DNA restriction patterns. The alteration in restriction fragments of HSV strain DNA was due to the gain or loss of cleavage sites in their genomes<sup>9</sup>. Another mechanism for the alteration in restriction patterns was seen in the VZV strains of DNA, and was due to small DNA base sequence changes in their genomes<sup>19</sup>. The VZV DNA restriction fragments, which differed between the various strains, appeared on the physical map within four regions of the genome<sup>19</sup>. In the present study, the alteration in restriction patterns might be due to the changes in small DNA base sequences in their genomes, since the migration of fragments from *Bam*HI-J and -S; *Eco*RI-B and -L; *Hind*III-E and -F and *Sma*I-B and -J frequently vary among OMV strains examined. Six altered regions could appear in the physical map of the standard OMV strain.

The results of Southern blot hybridization indicated that *H. salmonis* was different from all OMV strains tested. The results also supported those previously obtained by serological and genetic analyses in this laboratory<sup>7,8</sup>. The optimal temperature for growth of the strain *H. salmonis* (10°C) was lower than that of strains 00-7812 and H-83 (15°C)<sup>6</sup>. The sizes of viral particles and nucleocapsids of strains H-83 and 00-7812 were larger than those of *H. salmonis*<sup>6,12,21</sup>. Strains 00-7812 and H-83 were not closely related to *H. salmonis* serologically, but were similar to each other according to cross-neutralization test and cross-ELISA<sup>8</sup>. The results of the present study and previous reports show that salmonid herpesviruses are divided into at least two groups based upon physical, serological and genomic characteristics.

The synthesis of defective herpesvirus DNA after serial passages of the virus has been reported in several herpesviruses<sup>1,3,4</sup>. Discrete DNA regions of low passage virus-derived DNA can be tandemly repeated in DNA molecules of high passage virus. It is reported that restriction endonuclease cleavage patterns of HSV DNA changed during *in vitro* passages<sup>9,15</sup>. Two strains of OMV (00-7812 and H-83) also showed changes in the cleavage patterns during passage<sup>7</sup>. The changes occurred during the 28th to 36th passage of both OMV strains. Since all OMV strains examined in the

present study were within 13 passages (Table 1), the alterations in restriction cleavage profiles between OMV strains were generally of a subtle nature. It was known that the change of restriction profile occurred before 36 passages<sup>7)</sup>. However, no further changes could be observed following more than 60 passages in the present experiment. This result indicates that OMV DNAs are relatively stable. The *EcoRI* restriction patterns of 00-7812 and H-83 DNAs were stable through more than 60 *in vitro* passages, indicating that *EcoRI* was the most suitable for the identification of salmonid herpesvirus strains.

The seven strains of OMV examined in the present study were isolated from different places in Hokkaido and Honshu. They were isolated from either coelomic fluids of apparently normal fish or tumor tissues. When the restriction patterns of these strains were compared, no obvious genetic relationship was observed between strains isolated from apparently normal or tumor-bearing fish. Virulent strains of MDV contained some specific-fragments, such as *BamHI*-D, H, *SalI*-E, F, and *SmaI*-G, which were not found in the avirulent strains of MDV<sup>10)</sup>. Since strains of OMV tested here showed pathogenicity to the fry of salmonid fish, it is important that a comparison of virulent and avirulent, or oncogenic and non-oncogenic strains of OMV be made by analysis of their genomes with restriction endonucleases.

Investigation of the genomes of other fish herpesviruses such as carp herpesvirus or channel catfish virus should provide more information about chronological and geographical epidemics.

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