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Monitoring of fish pathogenic viruses from chum salmon (*Oncorhynchus keta*) in Korea

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ABSTRACT

A field survey was carried out to study the occurrence and distribution of salmonid pathogenic viruses. IHNV, IPNV and VHSV were tested from migrating chum salmon (*Oncorhynchus keta*) spawners and their offsprings from the Namdae River, the east coast of Korea, during 2006-2012. Detection rate of those viruses was compared by RT-PCR and RT-LAMP, and several samples were sequenced for the phylogenetic analysis. Of 901 samples tested, 218 samples (24.2%) were IHNV-positive by RT-LAMP, whereas 164 samples (18.2%) were IHNV-positive by nested RT-PCR. 296 out of 901 (32.9%) samples were IPNV-positive by RT-LAMP, whereas 195 samples (21.6%) were IPNV-positive by nested RT-PCR. all samples were VHSV-negative (0/901). Thus, the detection rates were increased when using RT-LAMP assay, compared with RT-PCR. The prevalence of those viruses was dramatically decreased since 2008, particularly in fry samples, maybe due to the use of disinfectants for eliminating active virus from fertilized eggs. In phylogenetic analysis, The IHNV isolates were clustered into the JRt genogroup including Japanese and other Korean isolates. The IPNV isolates were clustered into the genogroup Ⅰ or Ⅱ including asian isolates. In addition, all of the isolates were clustered with the rainbow trout isolates. Further studies are needed to clarify their origins and the potential pathogenicity.

Key words : Chum salmon, *Oncorhynchus keta*, Virus monitoring, RT-LAMP, RT-PCR

INTRODUCTION

Fish diseases of major importance in fish culture include infectious haematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV) and viral haemorrhagic septicemia virus (VHSV) (Knuesel et al., 2003). In Korea, chum salmon (*Oncorhynchus keta*) is an important salmonid fish species of which the

population management policies and artificial enhancement programs have been initiated since 1984. A recent decrease in the return rate of chum salmon in Korea is thought to be mainly due to global warming (Lee et al., 2007). However, other biotic factors, such as mortality at an early age and lack of nourishment, are also thought to be important, and infectious agents are thought to contribute at least partially to this decrease. In this study, we investigated infectious agents, especially major viruses in salmonids. The prevalence of IHNV, IPNV and VHSV were investigated from migrating chum salmon spawners and their offsprings by RT-PCR and RT-LAMP method. The isolated samples were sequenced and genotyping was conducted by direct sequencing and cloning methods for studying the phylogenetic relationships with other isolates from different geographical regions.

MATERIALS AND METHODS

Both spawners (adults) and their offsprings (fry) samples of chum salmon were randomly collected from the Namdae River, the east coast of Korea, during 2006-2012. For adult chum salmon, kidney from individual salmon were collected after artificial spawning and used for the RT-PCR and RT-LAMP analysis. For fry, 5 individual were pooled and considered as 1 fry sample for the RT-PCR and RT-LAMP analysis.

Each sample was homogenized, and total RNA was extracted using TRIzol reagent according to the manufacturer's instructions. The extracted RNAs were subjected to RT-PCR and RT-LAMP amplification, primer sets described in Table 1 and Table 2. RT-PCR and RT-LAMP were conducted as previously described (Suebsing et al., 2011). The resulting sequences were assembled with Genetyx Win Ver. 5.1. software, and multiple alignments were constructed using Clustal X (Thompson et al., 1997) to infer genetic relationships among the sequences using neighbor-joining criteria. The final phylogenetic tree was drawn with the MEGA 4.0 program (Tamura et al., 2007).

RESULTS AND DISCUSSION

Results of virus detection from migrating chum salmon spawners and artificially fertilized chum salmon fry are shown in Table 3. Of 901 samples tested, 218 samples (24.2%) were IHNV-positive by RT-LAMP, whereas 164 samples (18.2%) were IHNV-positive by nested RT-PCR. 296 out of 901 (32.9%) samples were IPNV-positive by RT-LAMP, whereas 195 samples (21.6%) were IPNV-positive by nested RT-PCR. all samples were VHSV-negative (0/901). Thus, the detection rates were increased when using RT-LAMP assay, compared with RT-PCR. The prevalence of those viruses was dramatically decreased since 2008, particularly in fry samples, maybe due to the use of disinfectants for eliminating active virus from fertilized eggs.

The PCR product of IHNV-positive and IPNV-positive samples taken from chum salmon fry in 2007 were sequenced. IHNV isolate was named ChYa07 and IPNV isolates were named ChYy07VR and ChYy07AB, respectively. The nucleotide sequences determined were registered in Genbank under accession number FJ230851, GQ866115, GQ866116. The deposited nucleotide sequences of 43 isolates of IHNV and 29 isolates of IPNV were used for Phylogenetic analysis (Kim et al., 2007; Nishizawa et al., 2005 and 2006).

Based on their G protein sequences, IHNV strains can be clustered into five genogroups. Four IHNV strains are reported in Korea at present time and are known to be grouped with the Japanese isolates (JRt genogroup). In this study, the isolate ChYa07 was found to be clustered in the JRt genogroup and was very closely related with other Korean isolates (Fig 1). Based on their VP2/NS junction regions, IPNV stains can be clustered into the genogroup Ⅰ(ChYy07VR) or Ⅱ (ChYy07AB) including Asian isolates (Fig 2). In addition, all of the isolates were clustered with the rainbow trout isolates. Currently, there is no information on how those strains were introduced into Korea. Further studies are necessary to clarify their origins and potential pathogenicity, for successful management in hatcheries and for protecting wild fish stocks.

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Table 1. Primers used for RT-PCR.

Virus	Primer	Orientation	Position	Primer sequence (5'→3')
IHNV	EXT1	Sense	563-583	GATCCCTACACCAGAGAC
	EXT2	Antisense	1235-1255	GGTGGTGTTGTTTCCGTGCAA
	INT1	Sense	623-643	TCACCCTGCCAGACTCATTGG
	INT2	Antisense	1085-1105	ATAGATGGAGCCTTTGTGCAT
IPNV	P1	Sense	1403-1425	AGAGATCACTGACTTCACAAGTGAC
	P2	Antisense	1738-1761	TGTGCACCACAGGAAAGATGACTC
	P3	Sense	74-90	CAACACTCTTCCCCATG
	P4	Antisense	225-241	AGAACCTCCCAGTGTCT
VHSV	VHSVF3	Sense	898-918	GATCAGGTCCCCCARRTCNGT
	VHSVR1	Antisense	450-471	TTCTTTGGAGGGCAAACNATH
	VHSVF4	Sense	539-588	GTACCCKTTCTTCCCCGAAC
	VHSVR2	Antisense	739-758	GTAGCRCCGRTCCAGTAGAC

Table 2. Primers used for RT-LAMP.

Virus	Primer	Position	Primer sequence (5'→3')
IHNV	F3[a]	110-127	CAGCCAAACCGTCCAACC
	B3[b]	299-316	TCGTTTCCGACCGACAGG
	FIP[c]	179-200/TTTT/128-145	CAGAGTGCATCCCTCGGGGTAGTTTTCGACACCGCAAGCGAATC
	BIP[d]	220-241/TTTT/269-288	ATGCCTCTCAACTGAGATGCCCTTTTATGTGGGATAGGCAATCAGC
	LF[e]	158-177	TGAAGAGCGGGTTTGACCAG
	LB[f]	243-267	TGAAGAGCGGGTTTGACCAG
IPNV	F3	1348-1367	AGAGGCATCAGAAAAGTGGC
	B3	1531-1548	ATAGCTTCCTGCCTCGGA
	FIP	1432-1453/TTTT/1374-1391	TGGTCTTGGTGAGGTCCCCAATTTTTCGTGCTGTCAACGCTCTT
	BIP	1465-1484/TTTT/1507-1524	GGACGCTACCTGTCACACGCTTTTGGCCCATGAGTCCATGAC
	LF	1438-1459	CTATAAGGGGAGCCGCCAT
	LB	1508-1539	CGGAGGCCGCTACCATGAT
VHSV	F3	579-596	AACATCACCCTGCCCAAC
	B3	776-795	CAGGTCGGTCTTGATCCATT
	FIP	642-663/TTTT/599-618	AAGCGTTTCTGAGGTAGGGCAATTTTACTGGCAAGGAGTCTACTGG
	BIP	694-715/TTTT/740-757	CACAGGGTGGTCAAGGCAATCGTTTTCCGTGCATGCCATTGTGA
	LF	625-641	TGGGCCTGAGGTGTAGCG
	LB	716-733	TTGCGGGTCACCACCCCT

[a] F3 : outer primer
[b] B3 : outer primer of complementary sequence
[c] FIP : forward inner primer
[d] BIP : backward inner primer
[e] LF : loop forward primer
[f] LB : loop backward primer

Table 3. The prevalence of salmonid viruses in chum salmon by RT-LAMP and nested RT-PCR assays.

Year	Sample type	no. positive/total					
		IHNV		IPNV		VHSV	
		Nested-PCR	RT-LAMP	Nested-PCR	RT-LAMP	Nested-PCR	RT-LAMP
2006	Adult	23/80	23/80	0/80	0/80	0/80	0/80
2007	Fry	21/32	32/32	3/32	7/32	0/32	0/32
	Adult	3/80	9/80	0/80	13/80	0/80	0/80
2008	Fry	23/32	32/32	0/32	11/32	0/32	0/32
	Adult	17/80	17/80	9/80	52/80	0/80	52/80
2009	Fry	9/49	12/49	47/49	47/49	0/49	0/49
	Adult	66/120	66/120	110/120	115/120	0/120	0/120
2010	Fry	0/66	ND	0/66	4/66	0/66	0/66
	Adult	0/120	3/120	13/120	17/120	0/120	0/120
2011	Fry	0/95	15/95	0/95	13/95	0/95	0/95
	Adult	2/107	9/107	13/107	17/107	0/107	0/107
2012	Fry	0/40	0/40	0/40	0/40	0/40	0/40
Grand total		164/901	218/901	296/901	195/901	0/901	0/901

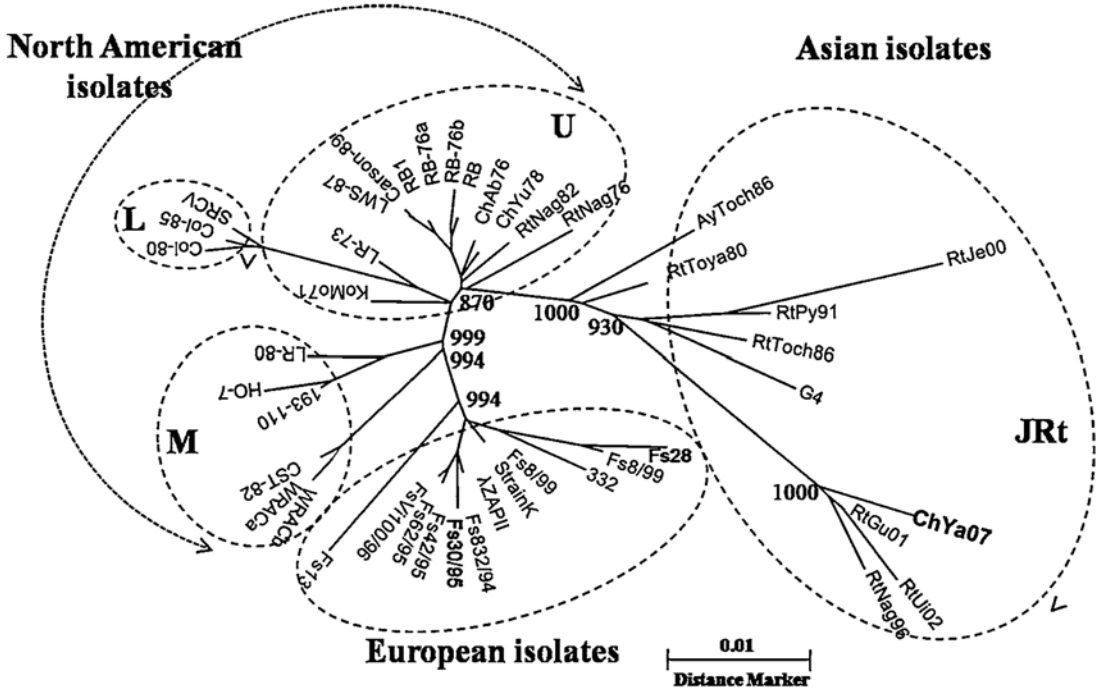

Fig 1. Molecular phylogenetic tree showing the genetic relationships among 43 isolates of IHNV based on the nucleotide sequence of the 1485-nt glycoprotein ORF, excluding the PCR primer sequences. Bootstrap values for 1000 replicates are shown at major nodes in the tree. The distance marker refers to the expected number of substitutions per site.

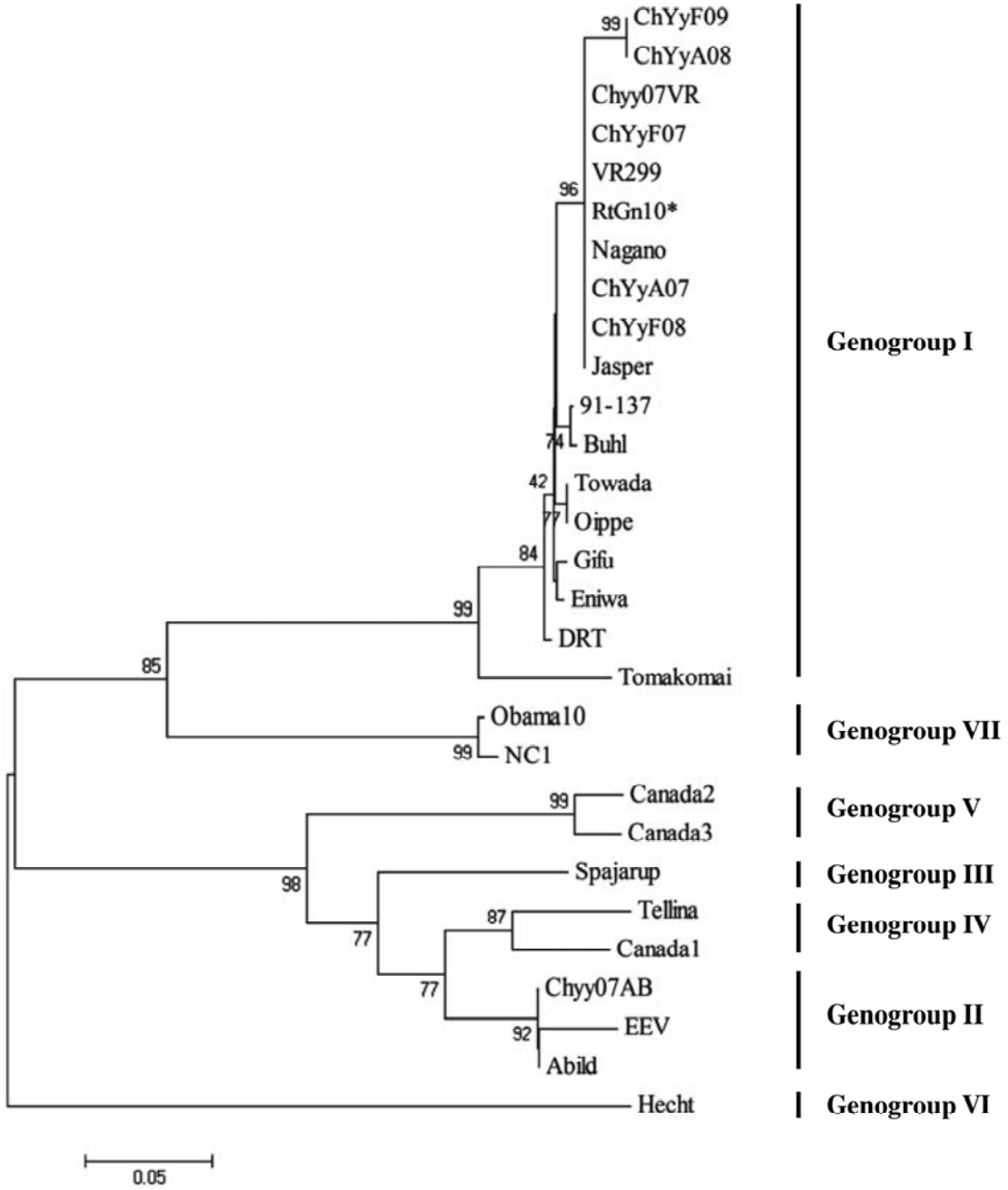

Fig 2. Molecular phylogenetic tree based on nucleotide sequence of the VP/NS junction region among 29 worldwide isolates of IPNV. Bootstrap values from 1,000 replicates are shown at major nodes. Bar, 0.05 replacement nucleotides per site.