FAS Fisheries and Aquatic Sciences

Original Article
Fish Aquat Sci 14(4), 347-354, 2011

Prevalence and Characterization of Typical *Aeromonas salmonicida* Chum Salmon Isolates in Korea

Yong-Seok Kim[1,a], Jang Won Yoon[2], Hyun-Ja Han[3,b], Rungkarn Suebsing[1] and Jeong-Ho Kim[1]*

[1]Department of Marine Bioscience and Technology, Gangneung-Wonju National University, Gangneung 210-702, Korea
[2]Department of Food Science and Engineering, Ewha Womans University, Seoul 120-750, Korea
[3] ECO Marine Bio Center, Chonnam National University, Yeosu 550-749, Korea
[a] Present address: Gangwondo Freshwater Resources Research Institute, Chuncheon 200-853, Korea
[b] Present address: Aquatic Life Disease Control Division, Fundamental Research Department, National Fisheries Research and Development Institute, Busan 619-705 Korea

Abstract

Aeromonas salmonicida is an important fish pathogen commonly associated with furunculosis in salmonids. Typical *A. salmonicida* strains have the surface virulence A-layer protein, a major virulence determinant encoded by the *vapA* gene. In this study, 880 chum salmon *Oncorhynchus keta* were collected from the east coast of Korea during 2006-2011, including 560 wild adults and 320 artificially hatched fry pools, and the presence of typical *A. salmonicida* was examined by PCR using the typical *A. salmonicida*-specific *vapA* gene primers. The results demonstrated that 34.5% of the samples (304/880 samples) were PCR positive, implying that a typical *A. salmonicida* infection is highly prevalent among chum salmon in Korea. Twenty typical *A. salmonicida* isolates were recovered based on their brown pigmentation on Trypticase Soy Agar (TSA) plates, which indicates the existence of the A-layer protein. Further biochemical analyses with the four randomly selected typical *A. salmonicida* isolates revealed some variations in their amino acid decarboxylation and carbohydrate fermentation activity. A phylogenetic analysis based on the entire *vapA* gene sequence suggested that the *A. salmonicida* isolates from chum salmon were clustered with those isolated from Atlantic salmon in Europe. Further study is needed to resolve such an interesting relationship in detail.

Key words: typical *Aeromonas salmonicida*, *Oncorhynchus keta,* furunculosis, chum salmon, *vapA* gene

Introduction

Aeromonas salmonicida is an important fish pathogen that has a geographically widespread distribution, with a broad host range and an economically destructive impact on cultivated fish, particularly salmonids (Trust et al., 1980; Fryer et al., 1988; Johnsen and Jensen, 1994; Mooney et al., 1995; Nomura et al., 2002). As a member of the family Aeromonadaceae, *A. salmonicida* has been classified into typical and atypical *A. salmonicida* pathogroups (Wiklund and Dalsgaard, 1998; Abbott et al., 2003). The "typical" group of *A. salmonicida* is frequently associated with furunculosis in salmonids. Such a group strain includes *A. salmonicida* subsp. *salmonicida*. In contrast, the "atypical" group of *A. salmonicida* is involved in various ulcerative diseases or atypical furunculosis in salmonids and non-salmonids (Wiklund and Dalsgaard, 1998). Other *A. salmonicida* subspecies such as *A. salmonicida* subsp. *achromogenes, masoucida, smithia*, and *pectinolytica* belong in this atypical *A. salmonicida* group (Wiklund and Dalsgaard, 1998). Although a classification has been created according to both genetic and biochemical characteristics of individual *A. salmonicida* strains, subspecies populations unable to be

Open Access http://dx.doi.org/10.5657/FAS.2011.0347

pISSN: 2234-1749 eISSN: 2234-1757

Received 1 July 2011; **Revised** 16 September 2011; **Accepted** 7 November 2011

***Corresponding Author**
E-mail: jhkim70@gwnu.ac.kr

classified under these criteria have been increasing; thus, more reliable classification methods are needed for atypical *A. salmonicida* strains (Lund et al., 2003).

Previous studies have reported that both typical and atypical *A. salmonicida* infections occur worldwide, including in North America, Europe, and Japan (Trust et al., 1980; Fryer et al., 1988; Mooney et al., 1995; Nomura et al., 2002). Among the various *A. salmonicida* fish host species, salmonids are the most susceptible to furunculosis (Lund and Mikkelsen, 2004; Austin and Austin, 2007). In Korea, *A. salmonicida* subsp. *salmonicida* was isolated from cultured masu salmon (*Oncorhynchus masou*) in 1986 (Fryer et al., 1988) and more recently detected by the PCR from rearing water on a trout farm (Lee et al., 2002). However, *A. salmonicida* subsp. *salmonicida* has not been reported in other salmonids in Korea until now. In this study, the prevalence of typical *A. salmonicida* was examined in both migrating wild chum salmon and artificially hatched fry during 2006-2011 in Korea by PCR using typical *A. salmonicida*-specific *vapA* gene primers. Because the *vapA* gene encodes the surface virulence A-layer protein, the *vapA*-positive and A-layer-expressing typical *A. salmonicida* were isolated and further characterized using both biochemical and phylogenetic analyses.

Materials and Methods

Sample collection

Wild adults and artificially hatched chum salmon fry samples were randomly collected from the Namdae River basin and hatcheries at Yangyang City (located on the east coast of South Korea) during 2006-2011. The number of chum salmon samples in this study is listed in Table 1. Both kidney and spleen from individual wild adult chum salmon were collected after artificial spawning and used for the PCR analysis. Artificially hatched fry samples were randomly selected and euthanized with excess MS-222 (tricaine methane sulfonate; Sigma; St. Louis, MO, USA). Five individual fry were pooled and considered as 1 fry sample for the PCR analysis. Subsequent bacterial isolation was conducted for all PCR-positive adult samples as described below.

Isolation and identification of *A. salmonicida*

One hundred mg of adult chum salmon kidney were aseptically sampled and homogenized with 900 μL of phosphate-buffered saline. Each homogenate (100 μL) was spread directly on trypticase soy agar (TSA; Difco, Detroit, MI, USA) and furunculosis agar (FA) plates containing 1% tryptone (Difco), 0.5% yeast extract (Oxoid, Hampshire, UK), 0.1% L-tyrosine (Sigma), and 0.25% sodium chloride. If brown-pigmented colonies appeared on the plates after a 15ºC incubation for 2–7 days, they were selected and sub-cultured in trypticase soy broth (Difco) for a preliminary biochemical screening, including Gram staining and assessment of oxidase activity and catalase production, as previously described (Austin and Austin, 2007). Further biochemical characterization of the *A. salmonicida* isolates was performed using the API 20E kit (BioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Bacterial growth on Coomassie Brilliant Blue agar (CBBA) plates was examined as previously described (Cipriano and Bertolini, 1988) to confirm the presence of the surface A-layer.

Antimicrobial susceptibility

Antimicrobial susceptibilities were tested using a standard disk diffusion assay and Mueller-Hinton agar plates (Difco). The diameters of bacterial growth inhibitory zones were measured and interpreted according to NCCLS guidelines (National Committee for Clinical Laboratory Standard, 2001).

Genomic DNA preparation

Genomic DNA was extracted from 100 mg of salmon tissue sample or 5 mL of cultured bacterial cells using the AccuPrep Genomic DNA Extraction kit (Bioneer, Seoul, Korea) accord-

Table 1. Prevalence of the typical *A. salmonicida* infection among the chum salmon populations in Korea during 2006 and 2011

Type of sample	PCR analysis	2006	2007	2008	2009	2010	2011	Total
Adults	No. of the samples	60	140	120	120	120	ND	560
	A. salmonicida-positive	28	61	51	25	15	-	180
	A. salmonicida-positive (%)	46.7	43.6	42.5	20.8	12.5	-	32.1
Fry pools*	No. of the samples	ND	40	70	49	66	95	320
	A. salmonicida-positive	-	38	70	14	2	0	124
	A. salmonicida-positive (%)	-	95.0	100.0	28.6	3.0	0.0	38.8

ND, no data.
*One pool contains 5 random fries.

ing to the manufacturer's instructions. All genomic DNA was adjusted to a final concentration of 100 ng/μL for the PCR analysis.

PCR detection of typical *A. salmonicida* using the *vapA* gene-specific primers

The PCR amplification was performed to detect a partial *vapA* gene specific to typical *A. salmonicida* using the primers AP1 (5′-GGC TGA TCT CTT CAT CCT CAC CC-3′) and AP2 (5′-CAG AGT GAA ATC TAC CAG CGG TGC-3′), as previously described (Gustafson et al., 1992). The target gene was amplified with 25 μM of each primer and 2 μL of genomic DNA using the lyophilized AccuPower PCR Premix (Bioneer). Thermal conditions consisted of an initial incubation at 94ºC for 2 min, 30 cycles of 15 s at 94ºC, 30 s at 57ºC, and 90 s at 72ºC, with a final extension at 72ºC for 3 min (Byers et al., 2002). Genomic DNA from typical *A. salmonicida* (FPC 367 strain, Japan) was used as a positive control.

All *vapA* genes from the *A. salmonicida* isolates were amplified using the complete *A. salmonicida vapA* gene-specific primers F-1 (5′-TCA ACG GAT AGG TTC AAC CC-3′) and R-1 (5′-CAG AGT GAA ATC TAC CAG CGG TGC-3′) as previously described to analyze the genetic diversity in typical *A. salmonicida*-specific *vapA* genes (Lund et al., 2003). After conducting 1% agarose gel electrophoresis, the PCR products were purified using the AccuPrep gel purification kit (Bioneer) according to the manufacturer's protocol, and the DNA nucleotide sequence was analyzed using the ABI PRISM 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) available at National Instrumentation Center for Environmental Management, Seoul National University (NICEM, Seoul, Korea).

Phylogenetic analysis

DNA nucleotide sequences of all *vapA* genes were analyzed by multiple-sequence alignment using ClustalW2 (Thompson et al., 1994). Phylogenetic trees were generated using the Molecular Evolutionary Genetic Analysis software (MEGA) version 4 and the neighbor-joining method with bootstrap values of 1000 replicates (Tamura et al., 2007).

Results

High prevalence of typical *A. salmonicida* isolates among chum salmon in Korea

To examine the prevalence of typical *A. salmonicida* infection among chum salmon in Korea, 880 chum salmon samples, including wild adults (n = 560) and artificially hatched fry pools (n = 320) (Table 1), were randomly collected and analyzed by the previously established PCR method using the *vapA* gene primers specific to typical *A. salmonicida*. The results demonstrated that approximately 34.5% of the samples (304/880 samples) produced the 421-bp amplicons specific to the *vapA* gene as expected (Fig. 1), implying that chum salmon infection by typical *A. salmonicida* is common in Korea (Table 1). Interestingly, the prevalence of typical *A. salmonicida* in the chum salmon fry pools was relatively higher in 2007 (95.0%) and 2008 (100%) than that in 2009 (28.6%), 2010 (3.0%), and 2011 (0.0%) (Table 1). A similar pattern was observed in the adult chum salmon samples. The prevalence of *A. salmonicida* in the wild adult chum salmon was approximately 46.7, 43.6, and 42.5% in 2006, 2007, and 2008, respectively, but decreased by 20.8% in 2009 and 12.5% in 2010 (Table 1).

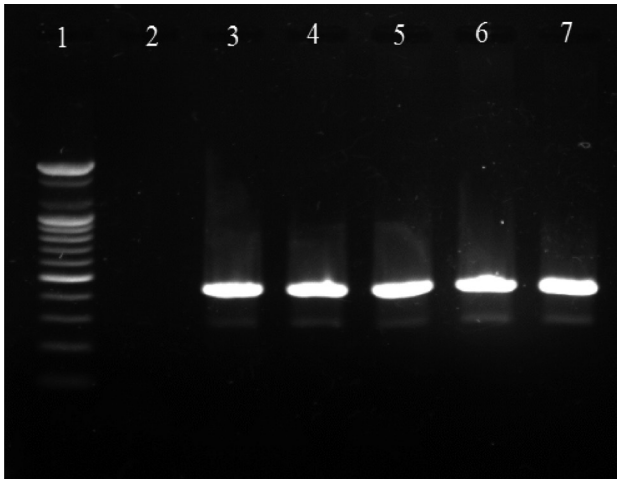

Fig. 1. Agarose gel electrophoresis of the PCR products using the typical *Aeromonas salmonicida*-specific *vapA* gene primers AP1 and AP2. Lanes 1, 100-bp DNA marker; 2, negative control (no template DNA); 3, positive control (FPC367); 4, sample 1; 5, sample 2; 6, sample 3; 7, sample 4.

Isolation of typical *A. salmonicida* from the chum salmon samples and biochemical characteristics of isolates

Twenty typical *A. salmonicida* isolates were recovered from the 304 wild adult chum salmon samples that were positive on the PCR using *vapA*-specific primers. As expected, all isolates were brown-pigmented when grown on TSA or FA plates, which is a characteristic of typical *A. salmonicida*. A preliminary screening confirmed that all 20 brown-pigmented isolates belonged to *A. salmonicida* because all were Gram-negative, oxidase- and catalase-positive, non-motile rods and possessed the ability to reduce nitrate and degrade gelatin (data not shown).

The four typical *A. salmonicida* isolates were randomly selected, designated AsCh06, AsCh07, AsCh08, and AsCh09, respectively, and further characterized by biochemical tests

(Table 2). The results showed that the AsCh08 and AsCh09 strains had the same biochemical phenotypes (API interpretation profile no. 0007124). However, they slightly differed from AsCh06 (API interpretation profile no. 0007024) and AsCh07 (API interpretation profile no. 2107124) in their biochemical activity, particularly for arginine dihydrolase, ornithine decarboxylase, mannitol fermentation, and sucrose fermentation (Table 2). The AsCh08 and AsCh09 strains were consistently able to produce all-blue colonies when cultured on CBBA plates due to surface A-layer protein expression. The antibiotic susceptibility test revealed that all typical *A. salmonicida* isolates were susceptible to various antibiotics including tetracyclines, aminoglycosides, β-lactam, and macrolides antibiotics but were resistant to nalidixic acid (data not shown).

Phylogenetic analysis of the typical *A. salmonicida* chum salmon isolates in Korea based on *vapA* gene diversity

All *vapA* genes were amplified from the genomic DNA of AsCh06-09 by PCR and analyzed by DNA nucleotide sequencing for the phylogenetic analysis. The DNA sequence data were deposited in the GenBank database with the accession number GU734698. The analysis showed that all isolates carried identical *vapA* genes (data not shown). Based on the *vapA* gene sequences, a phylogenetic tree was produced by comparing the AsCh08 isolate with the typical *A. salmonicida* isolates from other countries (Table 3). Interestingly, AsCh08 showed 99.9% similarity and co-clustered with typical *A. salmonicida* strains previously isolated from Atlantic salmon and salmonids in Scotland and Norway such as *A. salmonicida* subsp. *salmonicida* 4012 (AJ749882), 4017 (AJ749881), A449 (CP000644), and A450 (M64655) strains (Table 4, Fig. 2). Although further detailed study is needed, it should be noted that AsCh08 showed less similarity (~97% similarity) with other typical *A. salmonicida* isolates from Korea such as KCCM40239 (AB514572), RFAS1 (AB514573), and RFAS2 (AB514574) than with those from Atlantic salmon and salmonids in Scotland and Norway (Table 4, Fig. 2).

Table 2. Biochemical characteristics of the 4 *vapA*-positive and A-layer expressing typical *Aeromonas salmonicida* isolates from chum salmon in Korea

Biochemical characteristics	*A. salmonicida* isolates			
	AsCh06	AsCh 07	AsCh08	AsCh09
Gram stain	G(-)	G(-)	G(-)	G(-)
Morphology	Rod	Rod	Rod	Rod
Motility	-	-	-	-
Brown pigment production	+	+	+	+
Catalase	+	+	+	+
Oxidase	+	+	+	+
Oxidation-Fermentation	F	F	F	F
Nitrate reduction	+	+	+	+
b-galactosidase (ONPG)	-	-	-	-
Arginine dihydrolase (ADH)	-	+	-	-
Lysine decarboxylase (LDC)	-	-	-	-
Ornithine decarboxylase (ODC)	-	+	-	-
Urease (URE)	-	-	-	-
Tryptophane deaminase (TDA)	-	-	-	-
Indol production (IND)	-	-	-	-
Voges-Proskauer reaction (VP)	+	+	+	+
H_2S production	-	-	-	-
Gelatine degradation (GEL)	+	+	+	+
Citrate utilization (CIT)	-	-	-	-
Glucose fermentation/oxidation (GLU)	+	+	+	+
Mannitol fermentation/oxidation (MAN)	-	+	+	+
Inositol fermentation/oxidation (INO)	-	-	-	-
Sorbitol fermentation/oxidation (SOR)	-	-	-	-
Rhamnose fermentation/oxidation (RHA)	-	-	-	-
Sucrose fermentation/oxidation (SAC)	+	-	+	+
Melibiose fermentation/oxidation (MEL)	-	-	-	-
Amygdalin fermentation/oxidation (AMY)	-	-	-	-
Arabinose fermentation/oxidation (ARA)	-	-	-	-
Aesculin	-	-	-	-

Table 3. *Aeromonas salmonicida* strains used for phylogenetic analysis in this study

A.salmonicida subspecies	Strain no.	GenBank Accession no.	Host	Country of origin
salmonicida	4128	AJ749890	Spotted wolfish *Anarhichas minor*	Norway
salmonicida	4099	AJ749891	Atlantic cod *Gadus morhua*	Norway
salmonicida	4137	AM937253	Spotted wolfish *Anarhichas minor*	Norway
salmonicida	aAs4088	AM937254	Spotted wolfish *Anarhichas minor*	Norway
salmonicida	4129	AJ749887	Spotted wolfish *Anarhichas minor*	Norway
salmonicida	4059	AM937252	Spotted wolfish *Anarhichas minor*	Norway
salmonicida	4067	AJ749885	Spotted wolfish *Anarhichas minor*	Norway
salmonicida	4065	AJ749884	Spotted wolfish *Anarhichas minor*	Norway
salmonicida	4050	AJ749892	Halibut *Hippoglossus hippoglossus*	Norway
salmonicida	RFAS1	AB514573	Korean rockfish *Sebastes schlegeli*	Korea
salmonicida	RFAS2	AB514572	Korean rockfish *Sebastes schlegeli*	Korea
salmonicida	KCCM402	AB514574	Korean rockfish *Sebastes schlegeli*	Korea
salmonicida	4012	AJ749882	Atlantic salmon *Salmo salar*	Scotland
salmonicida	A449	CP000644	Brown trout *Salmo trutta*	France
salmonicida	A450	M64655	Unknown	unknown
salmonicida	4017	AJ749881	Atlantic salmon *Salmo salar*	Norway
salmonicida	4122	AJ749893	European flounder *Platichthys flesus*	Finland
This study	AsCh08	GU734968	Chum salmon *Oncorhynchus keta*	Korea
achromogenes	4036(ATCC33659)	AJ749888	Brook trout *Salmo trutta*	UK
achromogenes	4111(NCBI1110)	AJ749889	Brook trout *Salmo trutta*	UK
achromogenes	aAs4101	AM937255	Atlantic cod *Gadus morhua*	Norway
achromogenes	4102	AJ749886	Atlantic cod *Gadus morhua*	Canada
achromogenes	117-92	AJ749879	Arctic Charr *Salvelinus alpinus*	Finland
masoucida	ATCC27013	AJ749883	Masou salmon *Oncorhynchus masou*	Japan
smithia	NCIMB 13210	AJ749880	Roach *Rutilus rutilus*	UK

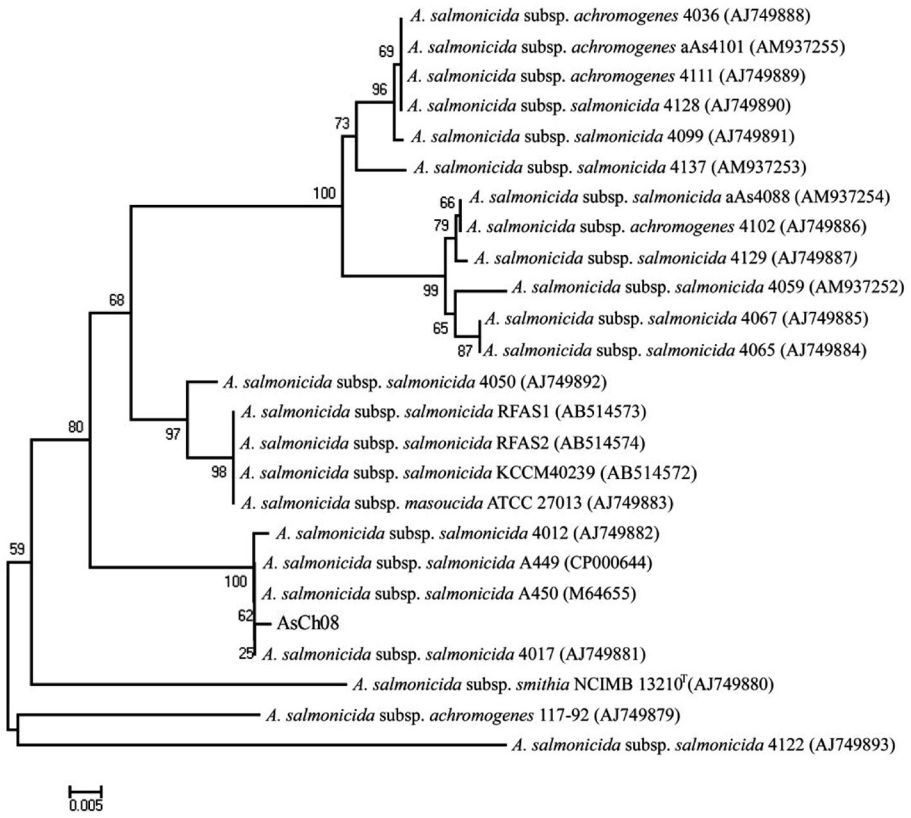

Fig. 2. Phylogenetic tree showing the genetic relationship of the chum salmon isolate (AsCh08) of typical *Aeromonas salmonicida* and the other *A. salmonicida* isolates based on the *vapA* gene sequences. The tree was constructed using neighbor-joining criteria with the bootstrap values at 1,000 replicates by MEGA4. Bar, 0.01 nucleotide substitution.

Table 4. Pairwise comparison of the *vapA* gene sequences among the typical *Aeromonas salmonicida* isolates worldwide and the chum salmon isolate of *A. salmonicida* (AsCh08) in this study

No.	Accession No.	Nucleotide sequences identities (%)																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	AsCh08																								
2	CP000644	99.9																							
3	AJ749881	99.9	0.0																						
4	AJ749882	99.8	99.9	99.9																					
5	AJ749892	97.4	97.4	97.4	97.3																				
6	M64655	99.7	99.9	99.9	99.8	97.2																			
7	AB514573	97.2	97.2	97.2	97.2	98.3	97.1																		
8	AB514574	97.4	97.4	97.4	97.3	98.5	97.2	99.9																	
9	AB514572	97.2	97.2	97.2	97.2	98.5	97.1	99.7	99.9																
10	AJ749883	97.2	97.2	97.2	97.2	98.5	97.1	99.7	99.9	0.0															
11	AJ749891	96.3	96.3	96.3	96.3	96.9	96.2	96.6	96.8	96.8	96.8														
12	AJ749890	96.3	96.3	96.3	96.2	96.9	96.1	96.7	96.9	96.9	96.9	99.9													
13	AJ749889	96.3	96.3	96.3	96.2	96.9	96.1	96.7	96.9	96.9	96.9	99.9	0.0												
14	AM937254	95.5	95.5	95.5	95.4	96.4	95.3	96.1	96.3	96.3	96.3	98.5	98.5	98.5											
15	AJ749886	95.5	95.5	95.5	95.4	96.4	95.3	96.1	96.3	96.3	96.3	98.5	98.5	98.5	0.0										
16	AJ749887	95.4	95.4	95.4	95.3	96.4	95.3	96.1	96.2	96.2	96.2	98.3	98.4	98.4	99.9	99.9									
17	AJ749885	95.3	95.3	95.3	95.3	96.3	95.2	96.0	96.1	96.1	96.1	98.1	98.2	98.2	99.6	99.6	99.5								
18	AJ749884	95.3	95.3	95.3	95.3	96.3	95.2	96.0	96.1	96.1	96.1	98.3	98.3	98.3	99.8	99.8	99.6	99.9							
19	AJ749879	95.3	95.3	95.3	95.4	95.0	95.2	94.9	95.0	94.9	94.9	94.4	94.3	94.3	94.3	94.3	94.2	94.1	94.1						
20	AJ749888	93.4	93.4	93.4	93.4	94.1	93.3	93.9	94.0	94.0	94.0	97.1	97.1	97.1	95.7	95.7	95.6	95.3	95.5	91.5					
21	AJ749880	94.7	94.8	94.8	94.7	94.8	94.6	94.3	94.4	94.3	94.3	94.1	94.0	94.0	93.8	93.8	93.7	93.7	93.7	95.3	91.2				
22	AJ749893	92.4	92.5	92.5	92.5	92.6	92.4	92.3	92.4	92.4	92.4	92.4	92.4	92.4	92.0	92.0	91.9	91.9	91.9	93.1	89.5	92.3			
23	AM937255	31.3	31.3	31.3	31.3	32.0	31.3	31.8	31.8	31.8	31.8	34.0	34.0	34.0	33.2	33.2	33.1	32.9	32.9	30.1	34.9	30.4	29.5		
24	AM937253	31.1	31.1	31.1	31.0	32.1	31.1	31.8	31.8	31.9	31.9	33.3	33.4	33.4	33.0	33.0	32.9	32.8	32.8	30.1	34.3	30.1	29.5	98.1	
25	AM937252	29.0	29.0	29.0	28.9	29.6	29.0	29.4	29.4	29.5	29.5	30.8	30.7	30.7	31.8	31.8	31.7	32.0	32.0	28.2	31.5	28.4	27.5	89.4	89.4

Discussion

Wild adult chum salmon samples positive for the *A. salmonicida vapA* gene were used for bacterial isolation, and the colonies producing a diffusible brown pigment, a characteristic of typical *A. salmonicida* isolates, were further examined. Biochemical analyses revealed that all brown-pigmented isolates were Gram-negative, oxidase-positive, catalase-positive, non-motile rods. They also produced dark blue colonies when cultured on CBBA plates, suggesting the presence of an A-layer (Cipriano and Bertolini, 1988). These results are consistent with the biochemical characteristics of typical *A. salmonicida* (Pavan et al., 2000). The PCR results showed that typical *A. salmonicida* was consistently detected at a high prevalence in both wild adult chum salmon and artificially hatched fry pools during 2006–2011. A similar long-term study reported a 1.7-50% prevalence of typical *A. salmonicida* infection in adult chum salmon from Japanese rivers (Nomura et al., 2002). Several other studies also showed that furunculosis is enzootic among wild salmon populations in rivers (Austin and Austin, 2007 and the references therein).

Typical *A. salmonicida* strains are thought to be extremely homogeneous. Therefore, epidemiological discrimination among the strains based on either phenotypic or biochemical properties has shown limited success in elucidating their geographical relatedness and/or host preference. Moreover, conventional genotypic methods such as plasmid profiling and ribotyping of *A. salmonicida* do not provide a clear epidemiological marker for furunculosis outbreaks (Nielsen et al., 1993, 1994). Sequencing of the 16rRNA gene is a robust tool for bacterial taxonomy, but genetic analysis of the 16S rRNA gene revealed that it is highly conserved in the genus *Aeromonas* (Martínez-Murcia et al., 2005). The *vapA* gene sequences of the five typical *A. salmonicida* isolates have been investigated, and results showed that they were identical and were grouped into the same cluster (Lund and Mikkelsen, 2004). Nonetheless, typical *A. salmonicida* strains were resolved in two clusters by geographical origin; one cluster contains mainly North American strains, and the other cluster contains mainly northern European strains (O'hlci et al., 2000). In the present study, the AsCh08 *vapA* gene sequence (GenBank accession no. GU734968) showed 99.9% similarity with that of *A. salmonicida* subsp. *salmonicida* strains A449 (GenBank accession number CP000644) and 4017 (GenBank accession no. AJ749881). In this study, AsCh08 was clustered with typical *A. salmonicida* type strain A450 (GenBank accession no. M64655) and type strains 4012 (GenBank accession no. AJ749882), 4017 (GenBank accession no. AJ749881), and A449 (GenBank accession no. CP000644). Unexpectedly, these strains were isolated from Atlantic salmon and salmonids in Scotland and Norway, all of which are geographically distant from Korea. Although this phenomenon cannot be explained, it would be interesting to investigate the possible relationship between these isolates and their geographical origin in detail.

A phylogenetic tree of 23 typical and atypical *A. salmonicida* strains has been reported based on their *vapA* gene sequences (Lund and Mikkelsen, 2004). The results demonstrated that most of the *vapA* gene sequence is highly conserved and that all typical *A. salmonicida* strains are localized in the same phylogenic cluster regardless of their geographic origin. Therefore, more discriminative genetic markers are required for epidemiological studies because it is still uncertain how this pathogen has been disseminated worldwide and how it affects wild salmonid populations.

In conclusion, typical *A. salmonicida* infection among chum salmon in Korea was monitored by PCR during 2006-2011, and the infection demonstrated a high prevalence. Although no information is currently available on the pathogenic potential of these pathogens and to what extent they have affected propagation of chum salmon in Korea, an appropriate control method should be considered to prevent possible clinical outbreaks in holding ponds and/or hatcheries. Affected wild adult chum salmon with typical *A. salmonicida* could act as carriers by transporting the pathogen to spawning areas (Nomura et al., 2002).

Acknowledgments

This research was partly supported by the Marine and Extreme Genome Research Center Program of the Ministry of Land, Transportation and Maritime Affairs, Republic of Korea. The authors thank Dr. Shotaro Izumi from Gunma Prefecture Fisheries Experimental Station, Japan, for providing typical *A. salmonicida* DNA as a positive control, and also thank all the staffs in Yangyang Salmon Station, Korea Fisheries Resources Agency for their help in collecting fish samples.

References

Abbott SL, Cheung WK and Janda JM. 2003. The genus *Aeromonas*: biochemical characteristics, atypical reactions, and phenotypic identification schemes. J Clin Microbiol 41, 2348-2357.

Austin B and Austin D. 2007. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. 4th ed. Springer-Praxis, Chichester, GB, pp. 25-89.

Byers HK, Gudkovs N and Crane MS. 2002. PCR-based assays for the fish pathogen *Aeromonas salmonicida*. I. Evaluation of three PCR primer sets for detection and identification. Dis Aquat Org 49, 129-138.

Cipriano RC and Bertolini J. 1988. Selection for virulence in the fish pathogen *Aeromonas salmonicida*, using Coomassie Brilliant Blue agar. J Wildl Dis 24, 672-678.

Fryer JL, Hedrick RP, Park JW and Hah YC. 1988. Isolation of *Aeromonas salmonicida* from masu salmon in the Republic of Korea. J Wildl Dis 24, 364-365.

Gustafson CE, Thomas CJ and Trust TJ. 1992. Detection of *Aeromonas salmonicida* from fish by using polymerase chain reaction amplification of the virulence surface array protein gene. Appl Environ

Microbiol 58, 3816-3825.

Johnsen BO and Jensen AJ. 1994. The spread of furunculosis in salmonids in Norwegian rivers. J Fish Biol 45, 47-55.

Lee C, Cho JC, Lee SH, Lee DG and Kim SJ. 2002. Distribution of *Aeromonas* spp. as identified by 16S rDNA restriction fragment length polymorphism analysis in a trout farm. J Appl Microbiol 93, 976-985.

Lund V, Espelid S and Mikkelsen H. 2003. Vaccine efficacy in spotted wolffish *Anarhichas minor*: relationship to molecular variation in A-layer protein of atypical *Aeromonas salmonicida*. Dis Aquat Org 56, 31-42.

Lund V and Mikkelsen H. 2004. Genetic diversity among A-proteins of atypical strains of *Aeromonas salmonicida*. Dis Aquat Org 61, 257-262.

Martínez-Murcia AJ, Soler L, Saavedra MJ, Chacón MR, Guarro J, Stackebrandt E and Figueras MJ. 2005. Phenotypic, genotypic, and phylogenetic discrepancies to differentiate *Aeromonas salmonicida* from *Aeromonas bestiarum*. Int Microbiol 8, 259-269.

Mooney J, Powell E, Clabby C and Powell R. 1995. Detection of *Aeromonas salmonicida* in wild Atlantic salmon using a specific DNA probe test. Dis Aquat Org 21, 131-135.

National Committee for Clinical Laboratory Standard. 2001. Clinical Laboratory Standard of Antimicrobial Susceptibility (NCCLS) (2001) Testing: Eleventh Information Supplement. National Committee for Clinical Laboratory Standard of Antimicrobial Susceptibility, Wayne, PA, US.

Nielsen B, Olsen JE and Larsen JL. 1993. Plasmid profiling as an epidemiological marker within *Aeromonas salmonicida*. Dis Aquat Org 15, 129-135.

Nielsen B, Olsen JE and Larsen JL. 1994. Ribotyping of *Aeromonas salmonicida* subsp. *salmonicida*. Dis Aquat Org 18, 155-158.

Nomura T, Honma H, Kasai H and Yoshimizu M. 2002. Isolation of *Aeromonas salmonicida*, causative agent of furunculosis, from chum salmon caught in the river or coast. Bull Fish Sci Hokkaido Univ 53, 45-50.

O'hlci B, Olivier G and Powell R. 2000. Genetic diversity of the fish pathogen *Aeromonas salmonicida* demonstrated by random amplified polymorphic DNA and pulsed-field gel electrophoresis analyses. Dis Aquat Org 39, 109-119.

Pavan ME, Abbott SL, Zorzópulos J and Janda JM. 2000. *Aeromonas salmonicida* subsp. *pectinolytica* subsp. nov., a new pectinase-positive subspecies isolated from a heavily polluted river. Int J Syst Evol Miccrobiol 50(Pt 3), 1119-1124.

Tamura K, Dudley J, Nei M and Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 4, 1596-1599.

Thompson JD, Higgins DG and Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22, 4673-4680.

Trust TJ, Khouri AG, Austen RA and Ashburner LD. 1980. First isolation in Australia of atypical *Aeromonas salmonicida*. FEMS Microbiol Lett 9, 39-42.

Wiklund T and Dalsgaard I. 1998. Occurrence and significance of atypical *Aeromonas salmonicida* in non-salmonid and salmonid fish species: a review. Dis Aquat Org 32, 49-69.