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Monitoring of *Aeromonas salmonicida* in Chum salmon (*Oncorhynchus keta*) from Namdae River, Korea

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ABSTRACT

In this study, 665 chum salmon (*Oncorhynkus keta*) were caught from Namdae river during 2006 and 2010, and monitored for typical *Aeromonas salmonicida*, a causative bacterium of furunculosis. 440 adults and 225 artificially hatched fry pools were examined by PCR using the typical *A. salmonicida*-specific *vapA* gene primers. The results demonstrated that 43.2% of the samples (287/665 samples) were PCR-positive, implying that typical *A. salmonicida* infection is prevalent among chum salmon in Korea. From the PCR-positive samples, 20 typical *A. salmonicida* isolates were recovered and their biochemical characteristics corresponded with those of known typical *A. salmonicida*. Moreover, phylogenetic analysis with entire *vap*A gene sequences suggested that Korean isolates were closely related with European isolates of Atlantic salmon. More studies are necessary to resolve this relationship in details.

Key words : *Aeromonas salmonicida*, *Oncorhynchus keta*, chum salmon

INTRODUCTION

Aeromonas salmonicida is an important fish pathogen having the geographically-widespread distribution with a broad host range. In particular, it has economically destructive impact on salmonid fish (Fryer et al., 1988; Mooney et al., 1995). Typical *A. salmonicida* is known to cause furunculosis in salmonid fish, whereas atypical *A. salmonicida* comprise many subspecies causing diseases both in salmonid and non-salmonid fish (Wiklund and Dalsgaard, 1998). Previous studies have reported that both typical and atypical *A. salmonicida* infections occur worldwide including North America, Europe, and Japan (Fryer et al., 1988; Mooney et al., 1995; Nomura et al., 2002), but there has been no report in Korea. In this study, the prevalence of typical *A. salmonicida* was examined with both migrating adult chum salmon and artificially hatched fry in Korea during 2006 and 2010 by PCR, using the typical *A. salmonicida*-specific *vapA* gene primers.

MATERIALS AND METHODS

Both wild adults and artificially hatched fry samples of chum salmon were randomly collected from the Namdae River basin and hatcheries at Yangyang City (located on the east coast of South Korea) during 2006 and 2010. For adult chum salmon, kidney from individual salmon were collected after artificial spawning and used for the PCR analysis. For fry, 5 individual were pooled and considered as 1 fry sample for the PCR analysis.

PCR was conducted as previously described with the known primers (Byers et al., 2002; Gustafson et al., 1992; Lund et al., 2003). For bacterial isolation, adult chum salmon individuals showing PCR positive signal were chosen and their kidney homogenates were spread on TSA agar. After incubation at 15ºC for 2~7 days, brown pigment producing colonies were selected, subcultured and their biochemical and genetic characterization were conducted. Phylogenetic tree was drawn with the entire vapA gene sequences, by MEGA (Tamura et al., 2007).

RESULTS AND DISCUSSION

The results demonstrated that 43.2% of the samples (287/665 samples) produced the 421-bp sized amplicons specific to the *vap*A gene as expected (data not shown), implying that chum salmon is commonly infected with typical *A. salmonicida* in Korea (Table 1). From those PCR-positive chum salmon samples, 20 typical *A. salmonicida* isolates were recovered based on their brown pigmentation on TSA plate, indicating the existence of A-layer protein. Further biochemical analyses with the 4 randomly-selected typical *A. salmonicida* isolates revealed some variations in their activities for amino acid decarboxylations and carbohydrate fermentations, but all other biochemical characteristics corresponded with those of typical *A. salmionicida* (data not shown). The phylogenetic tree was constructed with the *vap*A sequences of our isolate (AsCh08) with the typical *A. salmonicida* isolates from other countries. Interestingly, AsCh08 showed 99.9% similarities and closely clustered with typical *A. salmonicida* strains in Scotland and Norway such as *A. salmonicida* subsp. *salmonicida* 4012 (AJ749882), 4017 (AJ749881), A449 (CP000644) and A450 (M64655) strains (Fig. 1). Moreover, our isolate showed less similarity with other typical *A. salmonicida* isolates from Korea such as KCCM40239 (AB514572), RFAS1 (AB514573) and RFAS2 (AB514574) (Fig. 1). Further studies are necessary to solve this interesting phylogenetic relationship, as well as the pathogenicity of our typical *A. salmonicida* isolates to chum salmon.

REFERENCES

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.

Fryer JL, Hedrick RP, Park JW and Hah YC. 1988. Isolation of *Aeromonas salmonicida* from masu salmon in the Republic of Korea. J Wildlife 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

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.

Mooney JE, Powell C, 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.

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.

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.

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.

Table 1. Prevalence of the typical *A. salmonicida* infection among the chum salmon populations in Korea during 2006 and 2010.

Type of sample	PCR analysis	2006	2007	2008	2009	2010	Total
Adults	Number of the samples	60	140	120	120	ND[#]	440
	A. salmonicida-positive	28	61	51	25	-	165
	A. salmonicida-positive (%)	46.7	43.6	42.5	20.8	-	37.5
Fry pools*	Number of sample	ND[#]	40	70	49	66	225
	A. salmonicida-positive	-	38	70	14	2	124
	A. salmonicida-positive (%)	-	95.0	100.0	28.6	3.0	55.1

* One pool contains 5 random fries.

[#]ND: no data

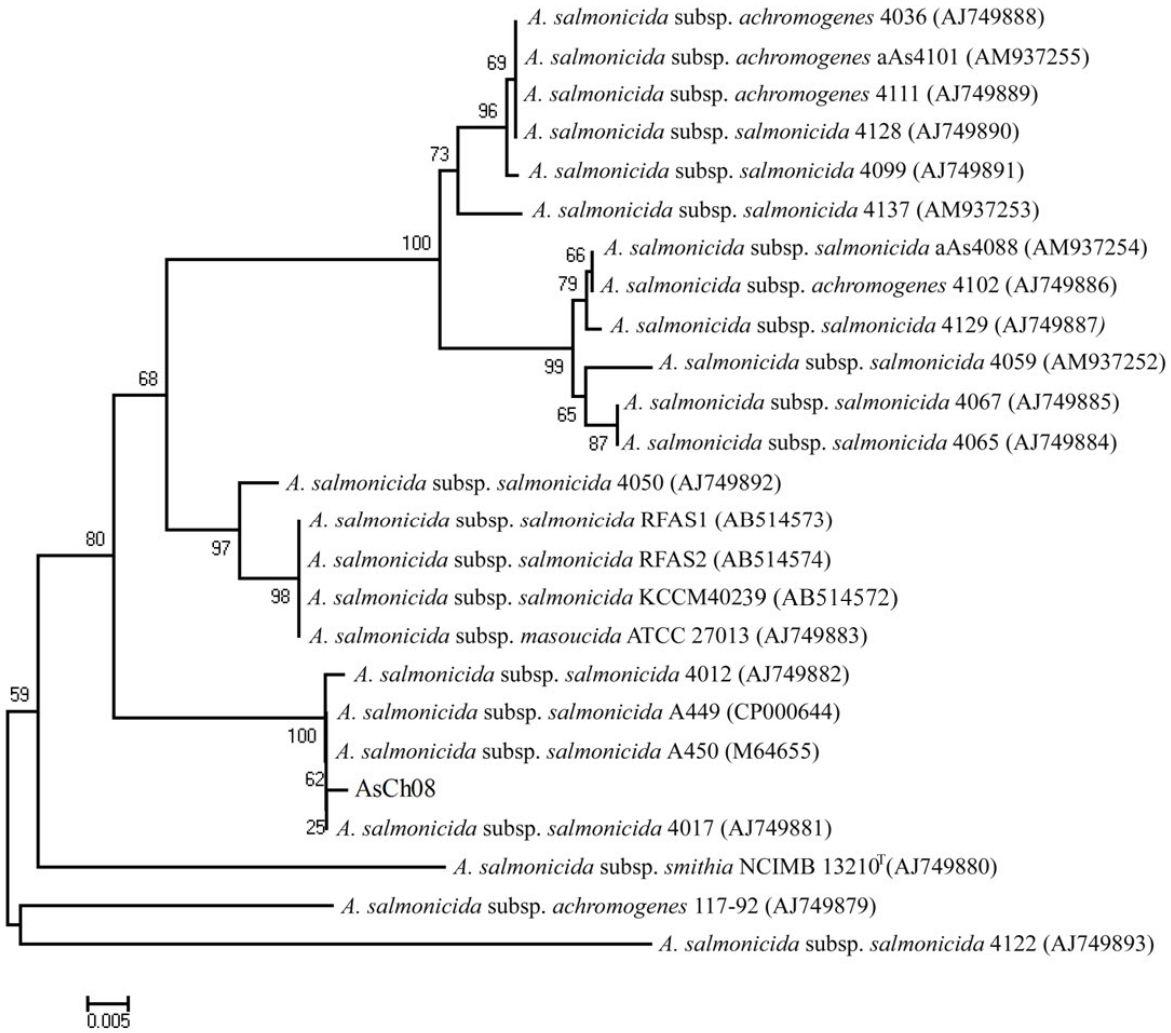

Fig. 1. Phylogenetic tree showing the genetic relationship of the chum salmon isolate (AsCh08) of typical *A. salmonicida* and the other *A. salmonicida* isolates based on the *vapA* gene sequences. The tree was constructed using neighbor-joining criteria with the bootstrap values at 1000 replicates by MEGA4. Bar, 0.01 nucleotide substitution.