「The Sea」 Journal of the Korean Society of Oceanography
Vol. 12, No. 2, pp. 102 — 111, May 2007

Genetic Polymorphism among Korean Salmonids Determined by RAPD (Randomly Amplified Polymorphic DNA) Analysis

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RAPD analyses using 60 OPERON primers and 13 URPs were performed in order to assess the genetic variation and frequency of polymorphisms in Korean salmonids. RAPDs were very reproducible and most useful at the sub-species level. In RAPD analysis, 138 polymorphic bands were detected between *Oncorhynchus masou* subspecies and 99 bands were generated in two types of rainbow trout. Estimated genetic distances between *O. masou* subspecies were 0.28794, and between wild rainbow trout and an albino mutant was 0.22786. Each species of salmonid was well characterized using URP 4R, the obtained bands could be useful as a species specific RAPD markers.

Key words: Genetic variation, Salmonid species, RAPD (Randomly Amplified Polymorphic DNA), OPERON primers, URPs

INTRODUCTION

Methods for distinguishing between individuals, populations, and species form the basis of many investigations in population biology, genetics and ecology. With the advent of new molecular biological techniques, there has been an increasing emphasis on the use of DNA characteristics as genetic markers. DNA polymorphisms have become available for discriminating between fish populations, species, and hybrids (Avise and Saunders, 1984; Perez, *et al*, 1999). Naturally occurring variations in DNA can be detected with several tests, each with its own advantages. RFLP-based DNA typing, is one common method, and has been used to determine genetic polymorphisms in the salmonids (Palva and Palva, 1987; Fields *et al.*, 1989; Bermingham *et al.*, 1991). VNTR-based DNA typing has also been applied in stock identification (Miller *et al.*, 1996, Babiak *et al*, 2002), population differentiation (O'Connell *et al.*, 1997), and in phylogenetic studies (Goodier and Dividson, 1998) in many salmonids.

Unfortunately, many of these techniques are expensive, time-consuming, protocols can be complicated and generally require large amounts of DNA for analysis (Elo *et al.*, 1997; Williams *et al.*, 1998). A simple method that requires little or no sequence data for the organism under study, is called RAPD (random amplified polymorphic DNA). RAPD bands are produced by amplification of genomic DNA with the single primers of arbitrary nucleotide sequence producing DNA bands of various lengths (Williams *et al.*, 1990). RAPD PCR has been efficiently used in fishes (Dinesh *et al.*, 1993; Bardakci and Skibinski, 1994; Foo *et al.*, 1995; Elo *et al.*, 1997) and fisheries (Garcia and Benzie, 1995; Harding *et al.*, 1997; Arias *et al.*, 1998; Grayson *et al.*, 1999; Hyytiä, *et al.*, 1999).

The method was originally developed using very short primers (10-12 bp) and low PCR annealing temperatures. The primers detect polymorphisms in nucleotide sequence that can be used as genetic markers (Williams *et al.*, 1990). The basis for RAPD analysis is that a primer is likely to find many sequences within the template DNA to which it can anneal at particular PCR annealing conditions (Welsh and McClelland, 1990; Williams *et al.*, 1990). Given the length and complexity of an organism's genome, PCR will amplify many random fragments that can vary in size when different species, subspecies, populations, or individuals are analyzed and can constitute the basis for identification.

However a drawback of the RAPD technique is that it is highly sensitive to reaction conditions. Small changes in reaction condition can cause variations in the amplification pattern. Hence, this method requires careful optimization to ensure reproducibility within laboratories, and suffer from a lack of portability between laboratories (Ellsworth, *et al.*, 1993; Bielawski *et al.*, 1995; Khandka *et al.*, 1997).

Further investigation has revealed that apparently any pair of long primers can be used to produce similarly complex DNA fingerprints. These fingerprints are, in essence, generated by the same mechanism as the RAPD method, the main difference being the use of longer "semi-random primers" (18 to 24 bases), hence the term long primer RAPD (LP-RAPD) (Weining and Langridge, 1991; Gilling and Holley, 1997).

In the present study, both decamer RAPD and LP-RAPD were examined in salmonids in order to determine the extent of DNA polymorphism and analyze the genetic differences among the salmonids by comparing the obtained RAPD profiles. Moreover,

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the discriminatory power of RAPD PCR at the intergeneric and interspecific level was evaluated.

MATERIALS AND METHODS

Genomic DNA Isolation

Preparation of genomic DNA was carried out from muscle, liver or brain according to two methodologies: 1) SDS/Proteinase K method and 2) guanidinium thiocyanate method.

Genomic DNA was extracted mainly by the SDS/Proteinase K method with some modications (McVeigh *et al.*, 1991; Taggart, *et al.*, 1992), and its procedure is detailed below. Approximately 0.3 g of tissue was placed in 750 μl of SDS/Proteinase K lysis buffer (10 mM Tris-HCl, pH 7.5; 100 mM EDTA; 1% SDS; 100 μl/ml Proteinase K) and incubated at 65 °C for 1-3 hrs. After digestion, proteins were precipitated by centrifugation at 12,000 *g* for 20 min. The supernatant was extracted once with phenol-chloroform (1:1) to further remove proteins. DNA was precipitated in 2.5 vol. of chilled 95% ethanol and incubated at -70 °C for a period ranging from 1 hr. to overnight. The visible DNA precipitate was removed from the ethanol with a glass rod and placed in Eppendorf tubes containing 70% ethanol. DNA was then pelleted by centrifugation at 10,000 *g* for 10 min. Pellets were air-dried at room temperature and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or distilled water.

In addition, the isolation of genomic DNA with guanidine salts was performed by using a commercial kit (DNAZOLTM Reagent, GIBCOBRL) according to the manufacturer instructions.

DNA Amplification

Two separate methods were employed for RAPD amplification. First, 60 decamer oligonucleotides from Operon Technologies (Alameda, CA, USA) were used. A standard procedure was arrived at based on the protocol of Elo *et al.* (1997) and Bielawski *et al.* (1995). PCR amplifications were done in a total volume of 25μl with 10 ng of template DNA, 4-6 pmol of a single primer, 1 units of *Taq* DNA polymerase (*Ex Taq*TM, TAKARA) and 0.2 mM of each dNTP. The reaction buffer contained 1.5 mM MgCl2, 10 mM Tris-HCl, pH 8.8, 50 mM KCl and 0.1% Triton X-100. The reaction mix was incubated in a thermal cycler (GeneAmp PCR system 2400, PE Applied Biosystems) programmed for 45 cycles of 30 sec at 94 °C, 30 sec at 40 °C, and 2 min at 72 °C, with a 5 min initial denaturation at 94 °C and a 7 min final extension at 72 °C.

Second, the long primer RAPD (LP-RAPD) analysis were performed with thirteen 20-mer URPs (universal rice primers),

Table 1. Decamer oligonucleotides for RAPD PCR

Primer		Sequence*	Primer		Sequence*	Primer		Sequence*
KIT A	OPA-01	CAGGCCCTTC	KIT B	OPB-01	GTTTCGCTCC	KIT C	OPC-01	TTCGAGCCAG
	OPA-02	TGCCGAGCTG		OPB-02	TGATCCCTGG		OPC-02	GTGAGGCGTC
	OPA-03	AGTCAGCCAC		OPB-03	CATCCCCCTG		OPC-03	GGGGGTCTTT
	OPA-04	AATCGGGCTG		OPB-04	GGACTGGAGT		OPC-04	CCGCATCTAC
	OPA-05	AGGGGTCTTG		OPB-05	TGCGCCCTTC		OPC-05	GATGACCGCC
	OPA-06	GGTCCCTGAC		OPB-06	TGCTCTGCCC		OPC-06	GAACGGACTC
	OPA-07	GAAACGGGTG		OPB-07	GGTGACGCAG		OPC-07	GTCCCGACGA
	OPA-08	GTGACGTAGG		OPB-08	GTCCACACGG		OPC-08	TGGACCGGTG
	OPA-09	GGGTAACGCC		OPB-09	TGGGGGACTC		OPC-09	CTCACCGTCC
	OPA-10	GTGATCGCAG		OPB-10	CTGCTGGGAC		OPC-10	TGTCTGGGTG
	OPA-11	CAATCGCCGT		OPB-11	GTAGACCCGT		OPC-11	AAAGCTGCGG
	OPA-12	TCGGCGATAG		OPB-12	CCTTGACGCA		OPC-12	TGTCATCCCC
	OPA-13	CAGCACCCAC		OPB-13	TTCCCCCGCT		OPC-13	AAGCCTCGTC
	OPA-14	TCGGCGATAG		OPB-14	TCCGCTCTGG		OPC-14	TGCGTGCTTG
	OPA-15	TTCCGAACCC		OPB-15	GGAGGGTGTT		OPC-15	GACGGATCAG
	OPA-16	AGCCAGCGAA		OPB-16	TTTGCCCGGA		OPC-16	CACACTCCAG
	OPA-17	GACCGCTTGT		OPB-17	AGGGAACGAG		OPC-17	TTCCCCCCAG
	OPA-18	AGGTGACCGT		OPB-18	CCACAGCAGT		OPC-18	TGAGTGGGTG
	OPA-19	CAAACGTCGG		OPB-19	ACCCCCGAAG		OPC-19	GTTGCCAGCC
	OPA-20	GTTGCGATCC		OPB-20	GGACCCTTAC		OPC-20	ACTTCGCCAC

*Primer sequences are given 5' to 3'.

which were developed by the Korea Rural Economic Institute. Amplification were performed with 50-200 ng of genomic DNA in reactions mixture of 20 μl, containing 10 mM Tris-HCl, pH 8.8, 1.5 mM MgCl2, 50 mM KCl, 0.2 mM of each dNTP, 0.2 μM primer, and 1 unit of *Taq* DNA polymerase (*Ex Taq*TM, TAKARA). The following program was used for LP-RAPD PCR : 35 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 2 min.

Banding Pattern Analysis

PCR amplification fragments were separated on 2% agarose gels in TAE buffer (40 mM Tris-acetate, 1 mM EDTA). The gels were run at 5 V/cm for 3.5 hr. The gels were then placed on a UV transilluminator and photographed. Images of each gel were captured with BIO-RAD Gel Doc 1000, and digitized images were converted, normalized, analyzed, and combined using Molecular Analyst Fingerprinting ver. 1.0 program (BIO-RAD).

Alternatively, the banding patterns generated by RAPD were interpreted visually, amplification fragments were recorded as 1 (present) and 0 (absent). A data matrix was prepared from the 844 fragments scored (Table 2), and each fragments was treated as a unit character. For the analysis of characters, the RAPDistance Programs ver. 1.04 (Armstrong *et al.*, 1997) was used. The levels of similarity between pairs of traces were computed by using the Dice coefficient correlation, which was determined with the equation SD = [2nAB / nA + nB] × 100, where nAB is the number of matched fragments and nA + nB is the total number of fragments in profiles A and B (Armstrong, *et al.*, 1997). Data were clustered using the UPGMA method (Sneath and Sokal, 1973).

RESULTS

DNA Polymorphism Amplified By Decamer Arbitrary Primers

RAPD analyses using 60 different random sequence primers (OPERON kits A, B, and C) were conducted to assess the quality and frequency of polymorphisms in salmonids. DNA fingerprints were very reproducible; the only discrepancies were confined to the presence or absence of faint bands. However, because the amplification conditions were rigorously tested in optimization experiments and then the RAPD amplifications always repeated three times, the variable bands can be easily identified and removed from consideration.

Among the 60 primers tested, 18 were not able to reveal polymorphism, some primers gave few amplification products, or others amplified bands of similar size. A total 846 bands were generated by 42 RAPD primers. The number of fragments amplified varied from 6-36 for each primer, with an average of 20.1 bands per primer. For *Brachymystax lenok*, the total number of bands for each primer varied from 4-28, with an average of 15.9 bands per primer. Of the 846 bands, only 6 were monomorphic for all species tested.

Intergeneric band differences between *Brachymystax* and *Oncorhynchus* were observed with all the primers. Nine bands monomorphic in the genus *Oncorhynchus* were observed with six primers (OPC01, OPC05, OPB08, OPA09, OPC11, OPC15).

Interspecific polymorphisms of *Oncorhynchus* species were observed with almost all primers (especially, OPC01, OPC02, OPA15, OPC16, OPC18, and OPC19) (Fig. 1). 130 RAPD markers specific to *O. keta* were obtained with 39 primers; 107 markers specific to *O. mykiss* were obtained with 41 primers; and 98 bands specific to *O. masou* were obtained with 37 primers.

Polymorphisms within a species were also shown with 36 primers (Fig. 2). 50 RAPD markers were specific to *O. m. masou* and 47 markers were specific to *O. m. ishikawai*. In the rainbow trout, 28 RAPD markers were specific to the ainbow trout and its albino mutants included 45 specific bands. The average numbers of characteristic bands per primer estimated from the results presented in Table 3. The pairwise genetic distance is estimated from the data matrix by the Dice coefficience in the RAPDistance Programs ver. 1.04 (Armstrong *et al.*, 1997) (Table 4). The genetic distance between *B. lenok* and the other *Oncorhynchus*

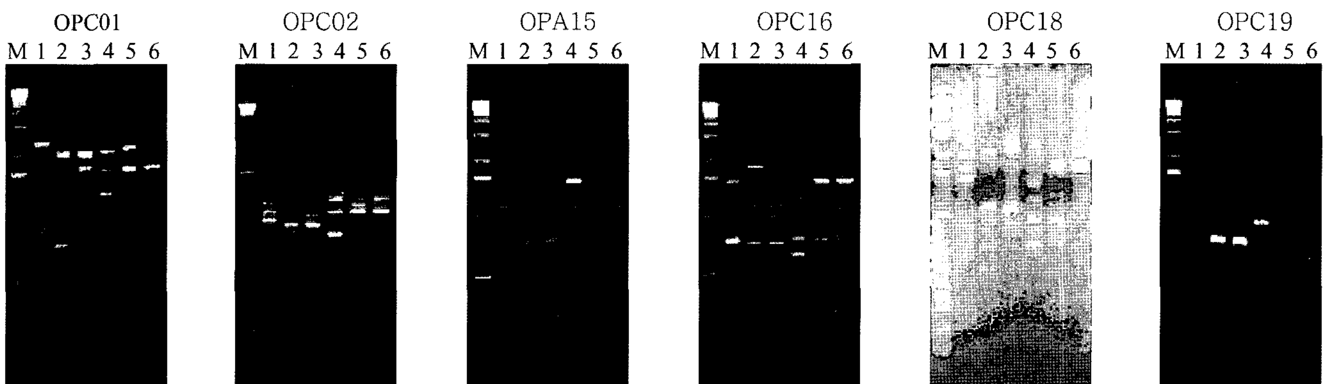

Fig. 1. RAPD profiles generated by six primers (OPC01, OPC02, OPA15, OPC16, OPC18, and OPC19), separated on a 2% agarose gel. Lane M, BRL/Gibco 1Kb molecular size marker; Lane 1, *B. lenok*; Lane 2, *O. masou ishikawai*; Lane 3, *O. masou masou*; Lane 4, *O. keta*; Lane 5, *O. mykiss*; Lane 6, Albino rainbow trout.

Table 2. The 846 characters amplified fragment data generated from 42 primers.

	OPA01	OPB01	OPC01	OPB02
B. lenok	010101001001001000010	00000000000100101000000	10010000101001001001	01100100000010010
O. m. masou	100010000110100011001	10100100100000000100100	00101010010001010110	10100010000001101
O. m. ishikawai	100010000100000010000	01010011010000000000101	00101010011000010110	10010010000001101
O. keta	101000110110000100000	00000000000001000010010	00100100100100000100	00001000001100000
O. mykiss	000000000000010100000	00001010001000010001100	01000101000010110100	00000000111000000
O. mykiss albino	000000000000010100100	00001010000010110001010	01000101000010110100	00000001011000000
Total number of fragments amplified	21	23	20	17

	OPC02	OPB04	OPC04	OPB05	OPC05
B. lenok	0000000100011000001	1100000110000101	0001100000010001000101	000010010001000000	011100010000100
O. m. masou	1100000001000110110	0000111000000110	1001001100001000100000	000100000000100000	100001101010000
O. m. ishikawai	1000000001000110110	0000011000000100	0001010001001100100000	000100001100110000	100001101110000
O. keta	0000001000100001000	0010000000010100	0100000010000100000010	000001000010001000	000011101011001
O. mykiss	0111111010100000000	0101000001101100	0010000000100110011000	110000100001000111	100101000010010
O. mykiss albino	0111111010100000000	0100000001101100	0010000000000100011000	101000000001000111	100101000010010
Total number of fragments amplified	19	16	22	18	15

	OPB06	OPC06	OPB07	OPC07
B. lenok	0000000101010010000	001000000101001000000100	110100110100010001000001001000000	00100001101000
O. m. masou	1101001000101100101	010101010000110110000010	110000000000100010000000100100011	01100000000000
O. m. ishikawai	1011000000100001101	010101010000010100000010	110000001000010010000000100101000	11100110000100
O. keta	0010100100000000000	010000100000100000010001	001010000100001000110000000000110	00100010010001
O. mykiss	0010110010000000010	100010000000000001100010	000000000011000101001110010010000	10101000100010
O. mykiss albino	0010110100000000010	100010001011000000101010	000001000001000100101010010010000	10111000100010
Total number of fragments amplified	19	24	33	14

	OPB08	OPC08	OPA09
B. lenok	0000000000100010010100010000	10100100101000101000110000	0000000100100001001000011
O. m. masou	0000000100010001000010101001	10000011000001000100010000	1111101000110100100100000
O. m. ishikawai	0000000100011000000010101001	10000010100101000100010000	1111001001100100101101000
O. keta	1010010100000000001010010100	11010010100000010010001000	0000000110000000000100000
O. mykiss	0101001011000000100011000010	00001000010010000000100111	0010010010001010010110100
O. mykiss albino	0101100011000100000011000010	00001000010010000001000111	0010010010001010010110010
Total number of fragments amplified	28	26	25

	OPC09	OPB10	OPC10	OPB11
B. lenok	000010000000000100011	01001000010000010100110010000	0000110000000110000000	0010001101100101010010
O. m. masou	011100011000001000000	10000110010000100010000000010	1000001010000000000011	0001011000001000100000
O. m. ishikawai	010100001011010000000	10000110011000101010000000010	0010000011000000000011	0001001000001010000000
O. keta	101101100100000000000	00010010100001000010001100101	0000000000010000000100	1000001010000000000101
O. mykiss	010000001000000011000	00110101100110000101000011000	0000000100000000110000	0010101000010000001000
O. mykiss albino	010000001000100010100	00000000100110000101000011000	0101000100101001011000	0100101000010000001000
Total number of fragments amplified	21	29	22	22

	OPC11	OPA12	OPB12	OPC12
B. lenok	100110001010010001000010001100100011	0001100101000	1010010000001110110	010001000000010000
O. m. masou	111101000100001000010001000000001100	0001000010100	0100101000110101000	101000010101001000
O. m. ishikawai	111101000100001000010001001000001100	0001001000010	0100100010000101000	000000000001001010
O. keta	110101000000100100101000110001011100	0110010000101	0000001000010111010	011101100100010000
O. mykiss	100101100000000010000100000010011100	1000000000001	0001000110000010010	000000001000000001
O. mykiss albino	100101010001000010000100000100011000	1000000000001	0000000101000110011	000011000010100101
Total number of fragments amplified	36	13	19	18

Table 2. (Continued)

	OPB13	OPC13	OPA14	OPB14
B. lenok	0001001100100001000	0011100010100100001	00000101000110010100100	0000000001000000100
O. m. masou	0100000010000100000	0111010000111010010	00000000001000000010100	0001000100000000010
O. m. ishikawai	0000000010010010100	0111010000111010110	00000000001001000010010	1001000000000110010
O. keta	1010110001000001001	0000001100010100000	01101000010100100000100	0011010110000101000
O. mykiss	0000000000000000010	0101010101000010000	10010010110000001000101	0100101000100000001
O. mykiss albino	0000000000001010010	1001010101000011000	10010010110000001001101	0100101000011000000
Total number of fragments amplified	19	19	23	19

	OPC14	OPA15	OPB15	OPC15	OPA16
B. lenok	100000000100001	010010	00101100000100111001	000000111110101010010	010000001101000001011101
O. m. masou	001010100111000	000001	01100010101010001010	001001001000010110001	100100000010000100100010
O. m. ishikawai	000000101001100	000001	01100010011000001000	001000001000010101001	101000000000100000100100
O. keta	010101000000010	000100	10010001101000001100	110100000000010000101	001010111000001000000000
O. mykiss	001001010000010	101000	01000001000101000000	001010001000110000001	000001000001000001000110
O. mykiss albino	001001010000010	101000	01000001000101000000	001010001001110000101	000000000001010010000110
Total number of fragments amplified	15	6	20	21	24

	OPC16	OPB17	OPA18	OPB18	OPC18
B. lenok	0001100000010000	0000010001001000000101	00110000101000	000100010100010	11010110000100010
O. m. masou	0101001011001100	1001001010100011100100	00001000110110	001100001001100	00100000001000001
O. m. ishikawai	0100000011001100	1001001010100101000100	00001000101110	001100000000100	00100000001000101
O. keta	1010010100100010	0101000100010010011010	00100111000000	000010100001000	01000001010011100
O. mykiss	0001001010010001	0010100000100011000000	11100000000101	010010000010000	00001000100010000
O. mykiss albino	0001000000010001	0000000000100010000000	11100000000001	110011000010001	00001100100010000
Total number of fragments amplified	16	22	14	15	17

	OPA19	OPC19	OPB20	OPC20
B. lenok	010100000010100	1101101010	101010010001001000011010011	010001010000001011100000
O. m. masou	000000100000000	0100000100	001000010100000000100000001	011000010010100000001010
O. m. ishikawai	001000100000000	0000000100	000000010000000000100000101	011000010001100000001010
O. keta	100000001000010	0010010000	010000111010010010000000101	101010010100000110010101
O. mykiss	000010000001001	0000000101	001101000000000101000101101	100101101000010001100001
O. mykiss albino	000011010101001	0000000101	001001000000100101000101101	100101101000010001100001
Total number of fragments amplified	15	10	27	24

Note: 0, band absent; 1, band present. Albino represents albino rainbow trout

species ranged from 0.75958 to 0.89133, and distances at the interspecific level were 0.79566 to 0.84177. The distance between *O. masou masou* and *O. masou ishikawai* was 0.28794, and differences between rainbow trout and an albino mutant of rainbow trout was 0.22786. Based on the distance matrix, a generated dendrogram is shown in Fig. 3.

DNA Polymorphisms Amplified By Urps (Universal Rice Primers)

Six URPs (2F, 2R, 4R, 9F, 17R, and 41) were used for the LP-RAPD analysis (Fig. 4), which were selected by preliminary experiments using thirteen random URPs (1F, 2F, 2R, 4R, 6R, 8F, 9F, 17R, 25F, 30F, 32, 33F, and 41). This additional screening was performed in order to obtain bands more sensitive between subspecies.

No optimization of PCR conditions was attempted because the reproducibility of LP-RAPD PCR is greatly enhanced due to the high annealing temperatures used. The size of the amplified fragments ranged from 220 bp to 3 kb. Despite the occurrence of small sized bands, the reproducibility of the banding patterns between different DNA lots was high. However, in spite of that, the visual interpretation of RAPD banding patterns was difficult due to a large number of small fragments. Therefore, LP-RAPD fingerprints were not used in the cluster analysis.

Differences between wild rainbow trout and the albino mutant were observed in all the primers except 2F. Several DNA fragments specific to *O. m. masou* or *O. m. ishikawai*, were observed in the amplification with primers 2F, 4R, 17R, and 41.

Of the above, the profile with URP 4R best described the six

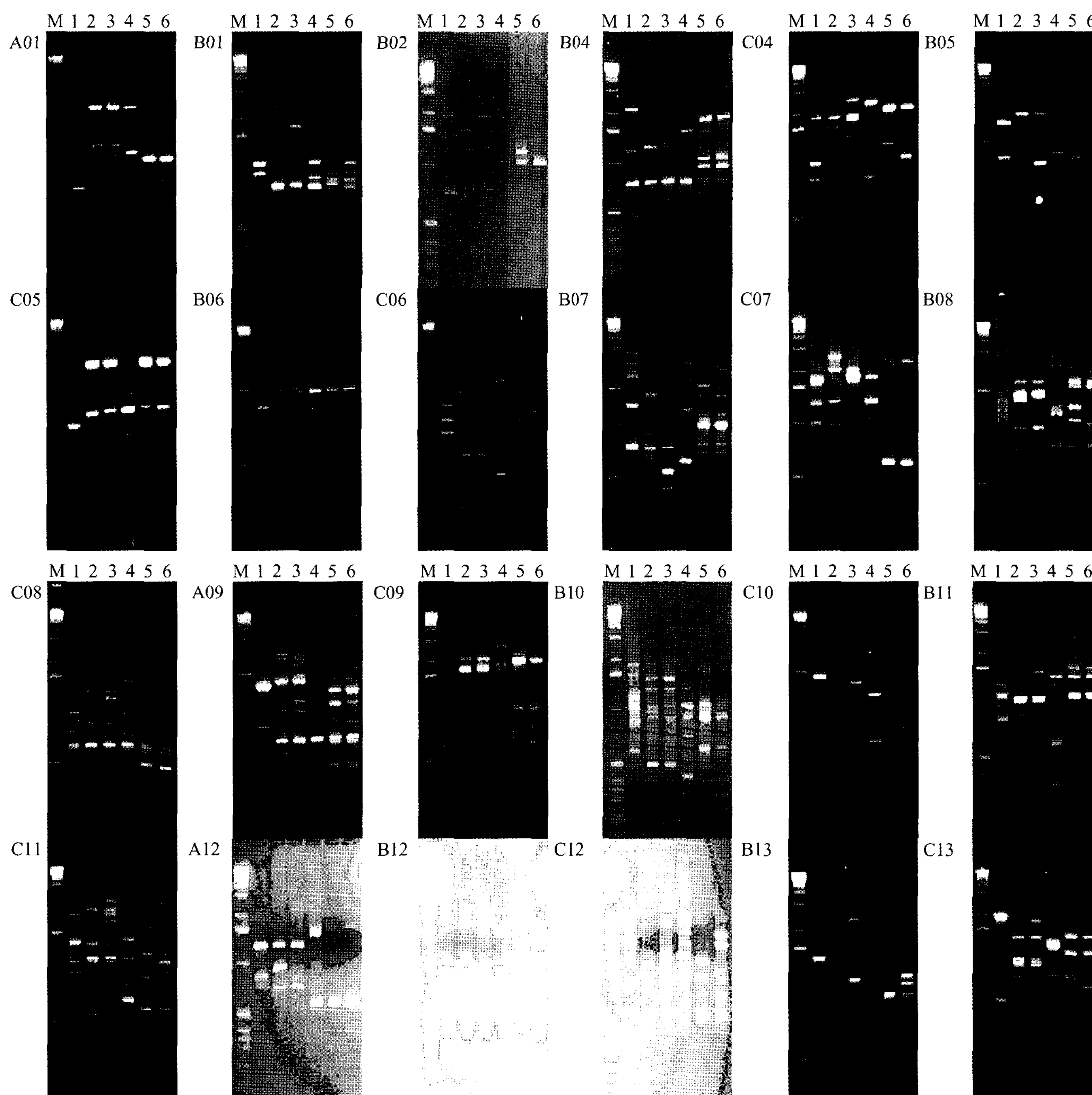

Fig. 2. RAPD profiles generated by 36 primers, separated on a 2% agarose gel. Lane M, BRL/Gibco 1Kb molecular size marker; Lane 1, *B. lenok*; Lane 2, *O. masou ishikawai*; Lane 3, *O. masou masou*; Lane 4, *O. keta*; Lane 5, *O. mykiss*; Lane 6, Albino rainbow trout. Primer set compared: A, OPA; B, OPB; C, OPC.

different salmonines. A 700 bp band was monomorphic in all the *Oncorhynchus* species. Bands at 1,050 bp and 1,630 bp fragments were specific for *B. lenok*, and a 340 bp band was specific for *O. keta*. A 500 bp band could distinguish between *O. m. masou* and *O. m. ishikawai*. While 700 bp and 2 kb fragments were common to both rainbow trout samples, other fragments were amplified from wild rainbow trout but not the albino mutants.

DISCUSSION

The results of RAPD analyses must always be carefully interpreted, especially when the nature of the amplified sequence is not known. RAPDs are sensitive to reaction conditions and often produce spurious and unrepeatable products if parameters are not carefully standardized (Ellsworth *et al.*, 1993; Khandka *et al.*, 1997). Yet, RAPD analysis have been used without apparent

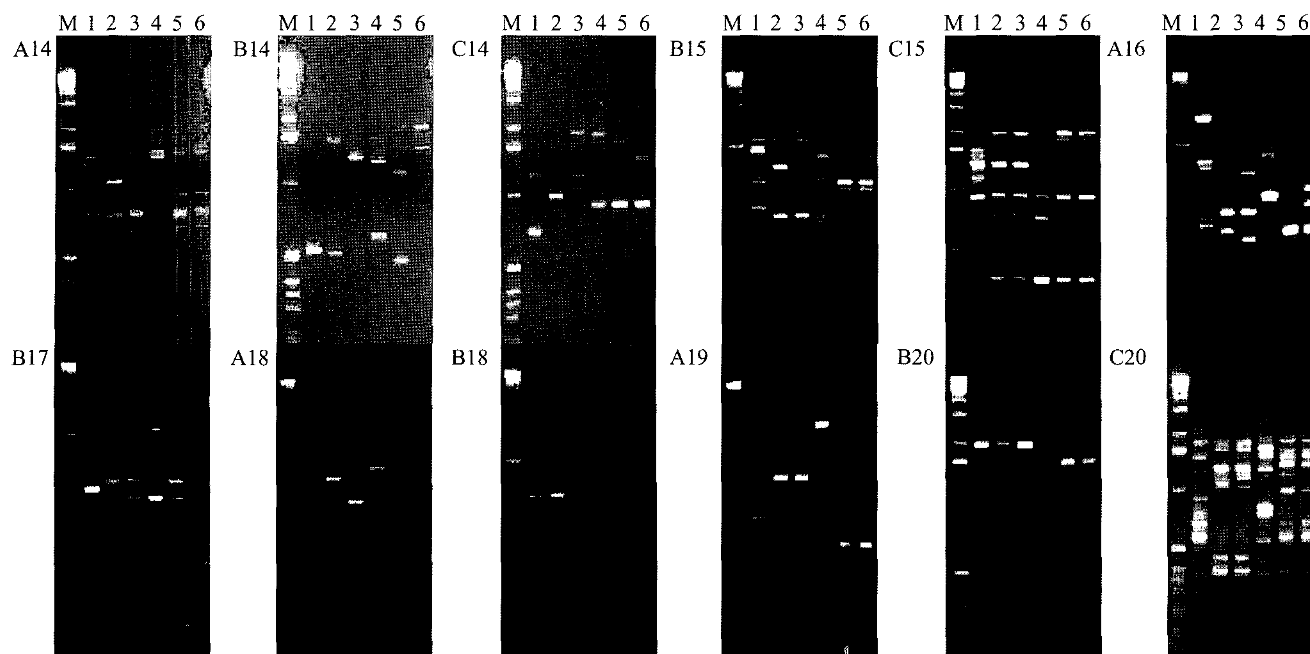

Fig. 2. (Continued).

Table 3. Average number of specific bands per primer

Comparative item		Average number of specific bands per primer
Intergenic	*B. lenok*	3.90
Interspecific	*O. keta*	3.33
	O. masou	2.65
	O. mykiss	2.61
Intraspecific	*O. masou masou*	1.79
	O. masou ishikawai	1.57
	O. mykiss	1.40
	O. mykiss albino	1.88

difficulty (McCoy and Echt, 1993; Bardakci and Skibinski, 1994; Shigyo *et al.*, 1997; Tassanakajon *et al.*, 1997; Williams *et al.*, 1998). Problems with repeatability was addressed in this study by suing a higher annealing temperature (40 °C). In our conditions, optimized for salmonids, RAPD analysis is shown to be highly repeatable.

RAPD analysis is able to detect numerous polymorphisms between even closely related organisms. Many previous studies have shown variation at the subspecies level. Bardakci and Skibinski (1994) recently reported on the use of RAPD for species and subspecies identification in the cichlid fish subfamily, Tilapiinae; Yi *et al.* (1995) applied AP-PCR technique to the analysis of the relationships among six japonica and indica cultivars and 4 wild species of rice; Elo *et al.* (1997) studied genetic variation and inheritance of RAPD markers in 20 intra- and interspecific crosses between *Salmo salar* and *S. trutta*; Tassanakajon *et al.* (1997) used RAPD analysis to amplify the genome of black tiger prawns (*Penaues monodon*) to detect DNA markers and assess the utility of RAPD method for investigating genetic variation in wild *P. monodon* in Thailand.

In this study, 840 polymorphic bands generated by 42 primers can distinguish each species tested; 138 polymorphic bands from 41 primers detected differences between the *O. masou* subspecies; and 99 polymorphic bands were generated with 9 primers between wild rainbow trout and albino one. All polymorphic bands detected have potentialities as genetic marker at different taxonomical levels and prove the utility of the RAPD technique for discrimination between and within salmonid species.

Table 4. Matrix of genetic distance estimated from amplified fragment data

	B. lenok	*O. masou masou*	*O. masou ishikawai*	*O. keta*	*O. mykiss*	*O. mykiss* albino
B. lenok	-					
O. masou masou	.75958	-				
O. masou ishikawai	.86888	.28794	-			
O. keta	.89133	.79629	.79566	-		
O. mykiss	.87572	.80201	.80827	.81720	-	
O. mykiss albino	.85983	.83723	.84177	.80509	.22786	-

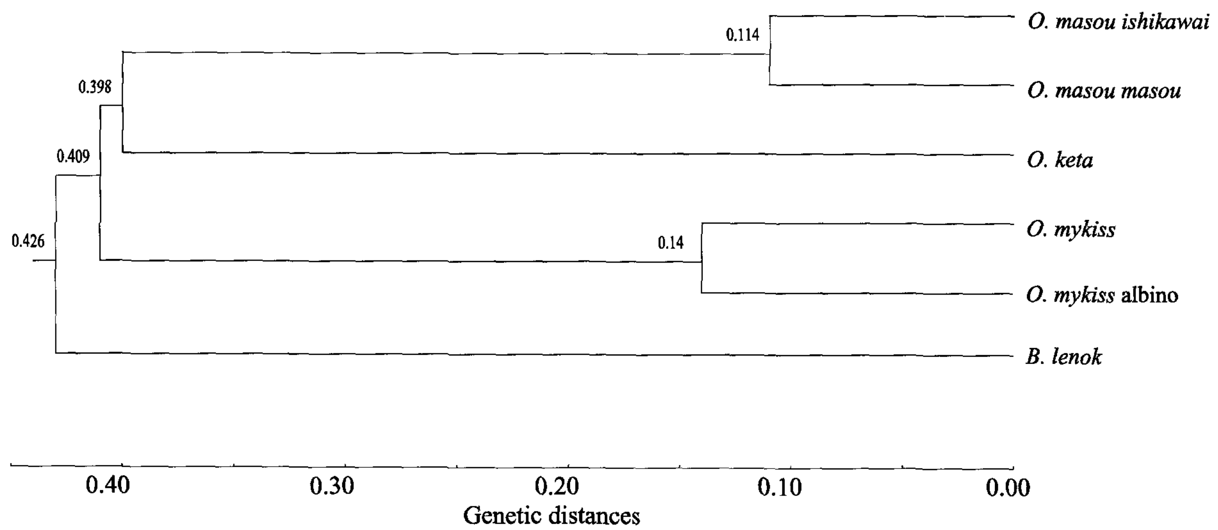

Fig. 3. Dendrogram demonstrating the relationships among the salmonines based on a compiled data set of genetic distance from the RAPD analysis.

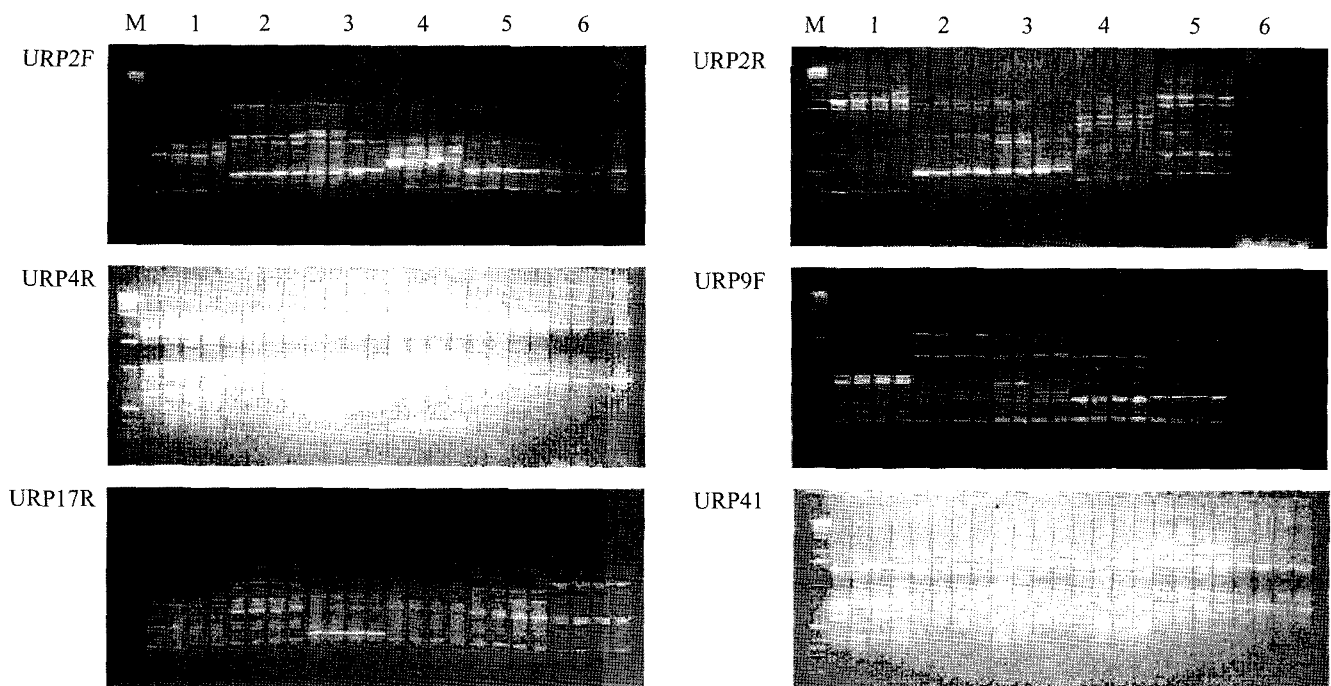

Fig. 4. LP-RAPD profiles generated by URPs, separated on a 2% agarose gel. Lane M, BRL/Gibco 1Kb molecular size marker; Lane 1, *B. lenok*; Lane 2, *O. masou ishikawa*; Lane 3, *O. masou masou*; Lane 4, *O. keta*; Lane 5, *O. mykiss*; Lane 6, *O. mykiss* albino. Each lanes has 4 samples.

These results are consistent with other work that has found high levels of RAPD band polymorphisn within species (Welsh and McClelland, 1990; Yi *et al.* 1995; Tassanakajon *et al.* 1997). The genetic distance between *O. masou* subspecies was estimated at 0.28794 and the value between wild rainbow trout and its albino mutant was 0.22786. These are similar to values reported for other species: Rice, 0-0.077 between varieties within subspecies; 0.269-0.318 between subspecies (Yi *et al.* 1995); and algae, 6 isolates of two species of *Porphyra* (0.364 to 0.714) (Kim *et al.* 1997).

mtDNA polymorphism are more useful in the discrimination of species than RAPDs. Nuclear DNA is approximately 180,000 times larger than the mitochondrial genome in salmonids, although mtDNA has a faster nucleotide substitution rate compared to nDNA and shows no recombination (Li and Grauer, 1991). In the case of *O. keta* and *B. lenok*, even though the two species

differed from other species, their variabilities varied in the same ranges between different comparative items (e.g. intergeneric comparision, interspecific comparision). On this respects, phylogenetic relationships of *Oncorhynchus* species can not be discussed, but their topology is identical with dendrogram from mtDNA sequence data. (Park *et al*., 2000).

LP-RAPD does not appear to be more sensitive to intraspecific and interspecific genetic variation that decamer RAPD analysis. Kim *et al*. (1997) reported that amplification using 21-mer primer pairs generates 5 to 30 bands that can be resolved on standard agarose gels.

RAPD analysis has proven to be a robust, dependable, and easy tool to use for identification of *O. masou* subspecies and in discrimination between rainbow trout and an albino mutant of rainbow trout. It has also proven more sensitive than sequence analysis for identification of sub species.

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2007년 3월 29일 원고접수
2007년 4월 18일 수정본 채택
담당편집위원: 강수경