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Genetic Variation and Polymorphism in Rainbow Trout, *Oncorhynchus mykiss* Analysed by Amplified Fragment Length Polymorphism

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The objective of the present study was to analyze genetic distances, variation and characteristics of individuals in rainbow trout, *Oncorhynchus mykis* using amplified fragment length polymorphism (AFLP) method as molecular genetic technique, to detect AFLP band patterns as genetic markers, and to compare the efficiency of agarose gel electrophoresis (AGE) and polyacrylamide gel electrophoresis (PAGE), respectively. Using 9 primer combinations, a total of 141 AFLP bands were produced, 108 bands (82.4%) of which were polymorphic in AGE. In PAGE, a total of 288 bands were detected, and 220 bands (76.4%) were polymorphic. The AFLP fingerprints of AGE were different from those of PAGE. Separation of the fragments with low molecular weight and genetic polymorphisms revealed a distinct pattern in the two gel systems. In the present study, the average bandsharing values of the individuals between two populations apart from the geographic sites in Kangwon-do ranged from 0.084 to 0.738 of AGE and PAGE. The bandsharing values between individuals No. 9 and No. 10 showed the highest level within population, whereas the bandsharing values between individuals No. 5 and No. 7 showed the lowest level. As calculated by bandsharing analysis, an average of genetic difference (mean±SD) of individuals was approximately 0.590±0.125 in this population. In AGE, the single linkage dendrogram resulted from two primers (M11+H11 and M13+H11), indicating six genetic groupings composed of group 1 (No. 9 and 10), group 2 (No. 1, 4, 5, 7, 10, 11, 16 and 17), group 3 (No. 2, 3, 6, 8, 12, 15 and 16), group 4 (No. 9, 14 and 17), group 5 (No. 13, 19, 20 and 21) and group 6 (No. 23). In AGE, the genetic distances among individuals of between-population ranged from 0.108 to 0.392. In AGE, the shortest genetic distance (0.108) displaying significant molecular differences was between individuals No. 9 and No. 10. Especially, the genetic distance between individuals No. 23 and the remnants among individuals within population was highest (0.392). Additionally, in the cluster analysis using the PAGE data, the single linkage dendrogram resulted from two primers (M12+H13 and M11+H13), indicating seven genetic groupings composed of group 1 (No. 15), group 2 (No. 14), group 3 (No. 11 and 12), group 4 (No. 5, 6, 7, 8, 10 and 13), group 5 (No. 1, 2, 3 and 4), group 6 (No. 9) and group 7 (No. 16). By comparison with the individuals in PAGE, genetic distance between No. 10 and No. 7 showed the shortest value (0.071), also between No. 16 and No. 14 showed the highest value (0.242). As with the PAGE analysis, genetic differences were certainly apparent with 13 of 16 individuals showing greater than 80% AFLP-based similarity to their closest neighbor. The three individuals (No. 14, No. 15 and No. 16) of rainbow trout between two populations apart from the geographic sites in Kangwon-do formed distinct genetic distances as compared with other individuals. These results indicated that AFLP markers of this fish could be used as genetic information such as species identification, genetic relationship or analysis of genome structure, and selection aids for genetic improvement of economically important traits in fish species.

Keywords: AFLP, Bandsharing value, Genetic distance, *Oncorhynchus mykiss*, Rainbow trout, Similarity matrix, Single linkage dendrogram

Introduction

Genetic polymorphisms are playing an increasingly important role as genetic markers in plant, animal and microorganism breeding programs. The polymorphic/specific markers proved useful for genetic improvement program of fish species providing invaluable data for marker-assisted selection (MAS) and genotype-assisted selection (GAS) including a variety of quantitative trait loci (QTL), target traits and economic trait loci (ETL) (Reiter et al., 1992; Hospital et al., 1997). Especially, identification of individual, or commercially-important fish species is necessary for efficient selective breeding and broodstock management, and for the measurement of various traits. DNA markers identified to be genetically linked to a

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trait of interest can be used for gene cloning, pathological diagnostics, transgenic techniques and for trait estimate in fish breeding programs. Also, the development of genetic markers in fish is needed to improve the efficiency of breeding by MAS and for the identification of economically important genes such as disease resistance genes, anti-freezing peptides genes and growth hormone genes.

The recent advance of genetic techniques with molecular biological methods showed a great potential to accelerate the biological perspectives. The polymerase chain reaction (PCR) was a rapid, simple, relatively cheap and sensitive procedure for in vitro amplification of specific DNA sequences using appropriate primers. There were so far used various conventional molecular biological methods including restriction fragment length polymorphism (RFLP) (Beckenbach et al., 1990; Garcia-Mas et al., 2000), randomly amplified polymorphic DNAs (RAPD) (Reiter et al., 1992; Liu et al., 1998; Yoon, 1999; Garcia-Mas et al., 2000; Mohd-Azmi et al., 2000; Song and Lee, 2000; Yoon and Kim, 2001), single-strand conformation polymorphism (SSCP) (Dekomien and Epplen, 2000) and microsatellite (MS) (Garcia de Leon et al., 1998; Waldbieser and Wolters, 1999; Li et al., 2000) based on PCR. Especially, the genetic similarity and polymorphisms were identified by not only RFLP markers but also RAPD (Garcia-Mas et al., 2000). The primer can detect polymorphisms in the presence or absence of specific nucleotide sequence information, and the polymorphisms can function as genetic markers. However, AFLP has advantages over other PCR-based techniques for DNA fingerprinting including RFLP and RAPD (Blears et al., 1998).

One of the most widely cultured salmonid for aquaculture is the rainbow trout native to the Pacific Coast drainages of North America from Alaska to California. Rainbow trout has been introduced as an aquaculture fish to the suitable waters in Kangwon-do in 1970s. The consumption of this fish has increased for a long time due to the rapid westernization of food habits and increased individual income from 1980s to the recent years. Accordingly, rainbow trout has become a popular fish in various types of restaurants (including a restaurant specializing in sliced raw fish, namely, hoejip) during the last two decades. In spite of its economic and scientific consequence, the genetic recessiveness of these trout populations has continuously made progress up to now in Korea. Thus, the applications of PCR-based molecular methods to rainbow trout aquaculture had been to identify the genetic variation and similarity of individuals and population in this fish species.

The most important of various merits are the capacity to investigate a variety of genomic DNA for polymorphism and AFLP is superior to any other systems in terms of the number of sequences amplified per PCR reaction and its reproducibility (Vos et al., 1995; Maughan et al., 1996; Young et al., 1998). The AFLP technique provides a reliable DNA fingerprinting technique for DNAs of complexity (Vos et al., 1995). Also, AFLP has numerous potential applications such as individual identification, the monitoring of animal and plant breeding, diagnostics of genetic diseases, pedigree analysis and the screening of DNA markers for MAS (Blears et al., 1998). AFLP can be applied to any DNA samples including plant (Mackill et al., 1996; Maughan et al., 1996; Bai et al., 1999; Yee et al., 1999; Purba et al., 2000; Huh and Huh, 2001), animal (Ajmone-Marsan et al., 1997; Young et al., 1998; Suazo and Hall, 1999; Knorr et al., 1999) and microbes (Lin et al., 1996), giving it the potential to become a overall DNA fingerprinting system. Despite the important roles of AFLP, applications of PCR-based AFLP to the teleost or shellfish species have so far been a little (Young et al., 1998; Barki et al., 2000; Felip et al., 2000). Therefore, in this study, genomic DNAs isolated from rainbow trout were (*O. mykiss*) digested by restriction enzymes, ligated by adapters and amplified by selective primers in order to identify the AFLP variations, to determine genetic distances and to detect the within-population and between-population genetic markers. Also, this study was to compare two different types of gels to establish which of them is more suitable to measure genetic diversity in this trout.

Material and Methods

Sample collection

Rainbow trout, *O. mykiss* were obtained from two aquaculture facilities apart from geographic sites in the Kangwon-do, Korea. DNA samples of fish anesthetized with MS 222 (100 ppm) were taken from liver tissues. Liver tissues were collected with sterile test tubes, immediately transported into liquid nitrogen and stored for further analysis. AFLP-PCR analysis was performed on genomic DNA samples from a total of 26 individuals.

Sources of genomic DNA

A piece of samples were used as DNA sources for PCR amplification. Thawed samples were placed into 1.5 ml Eppen-

dorf tubes, to which 2 volume of lysis buffer I (155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA) was added, and the mixture tube was gently inverted several times. The samples were incubated on ice for 5 min, centrifuged at 1,750 g for 10 min at 4°C to pellet. The supernatant was decanted with pellet, resuspended the nuclei in lysis buffer II (10 mM Tris-HCl; pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.5% SDS). Samples were transferred with aqueous phase to 1.5 ml Eppendorf tubes and added 15 μl proteinase K solution (10 mg/ml). The mixtures were gently inverted and incubated at 65°C for overnight. After incubation, there was added 300 μl of 6M NaCl and gently pipetted for a few of min. 600 μl of chloroform were added to the mixture and then inverted (no phenol). Samples were spun down at 22,388 g for 5 min. The cleared lysates were extracted with 2 volume of ice-cold ethanol, then centrifuged for 5 min. at 1,750 g, then precipitated. The DNA pellet was air-dried for 3 hrs, and then dissolved 200 μl of TE (10 mM Tris-HCl; pH 8.0, 1 mM EDTA) buffer. Purity and concentration of DNA were estimated by calculating the ratio of A_{260}/A_{280} measured with a spectrophotometer (Shimadzu, Australia).

Genomic DNA digestion and adapter ligation

The original AFLP protocol developed by Zabeau and Vos (1993) was followed with the minimum modifications. Approximately 500 ng of genomic DNA was first double-digested with two restriction enzymes (*Eco*R I and *Hind* III) at 37°C for three hrs. Double-stranded oligonucleotide adapters, homogeneous to one 5'- or 3'- end generated during restriction digestion, are ligated to the DNA fragments. The fragments were ligated with 5 pmol of *Eco*R I adapters and *Hind* III adapters at 37°C for three hrs, respectively. The ligated DNA fragments are amplified by PCR using primers complementary to the adapter and restriction site sequence with additional selective nucleotides at their 3'- end. The use of selective primers reduces the complexity of the mixtures. Selective primers under the stringent annealing conditions will amplify the fragments with complementary nucleotides extending beyond the restriction sites. Polymorphisms are revealed by analysis of amplified fragments on gels in AGE and PAGE, and comparison of the band patterns generated for each sample.

AFLP primers

Eight selective primers were synthesized to be complementary to the adapter/restriction-site sequences and to carry selective 3' nucleotides. Selective primer pairs included two *Eco*R I + 4 primers and two *Hind* III + 4 primers. There were shown a few of adapter + primer pair combinations (Table 1).

Amplification conditions

Amplification was performed in a DNA Thermal Cycler (Perkin Elmer Cetus, USA). Amplification reactions were performed in volumes of 20 μl contained 20 ng of genomic DNA, 10X buffer (50 mM KCl, 10 mM Tris-HCl, 2.0 mM $MgCl_2$), 200 μM dNTP (Perkin Elmer Cetus, USA), 1 unit of AmpliTaq polymerase (Perkin Elmer Cetus, USA) and 75 ng of each *Eco*R I + primer or *Hind* III + primer (Operon Technologies, USA). Each mixture was performed an initial predenaturation at 94°C for 3 min. Thermal Cycler programmed for 30 cycles at 94°C for 1 min for denaturation, at 56°C for 1 min for annealing, at 72°C for 1 min for extension and at last at 72°C for 5 min for extension again, using the fastest available transition between each temperature. Amplification products were analyzed by electrophoresis in 2% agarose gels with TBE (90 mM Tris; pH 8.5, 90 mM boric acid, 2.5 mM EDTA) and in 6% denaturing polyacrylamide gels with Tris-HCl (pH 8.8) for three hours. There were detected by staining with ethidium bromide and silver staining kit (Bioneer Co., Korea). The gels were illuminated with UV light and photographed by UV DNA photographic system (Seoulin Co. Korea) and photoman direct copy system (PECA products, USA), respectively.

Table 1. Adaptors and selective primer sequences (5' to 3') used in this study.

Adaptor	Selective primer sequences
*Eco*RI-adaptor For strand 5'-CTCGTAGACTGCGTACC-3' Rev strand 3'-AATTGGTACGCAGTCTAC-5'	E01: 5'-GAC TGC GTA CCA ATT CA-3' E11: 5'-GAC TGC GTA CCA ATT CAA C-3' E12: 5'-GAC TGC GTA CCA ATT CAC A-3' E13: 5'-GAC TGC GTA CCA ATT CAC T-3'
*Hind*III-adaptor For strand 5'-GACGATGAGTCCTGAC-3' Rev strand 3'-CTGACGCATGGTCGA-5'	H01: 5'-GAC TGC GTA CCA GCT TT-3' H11: 5'-GAC TGC GTA CCA GCT TTA C-3' H12: 5'-GAC TGC GTA CCA GCT TTA G-3' H13: 5'-GAC TGC GTA CCA GCT TTC T-3'

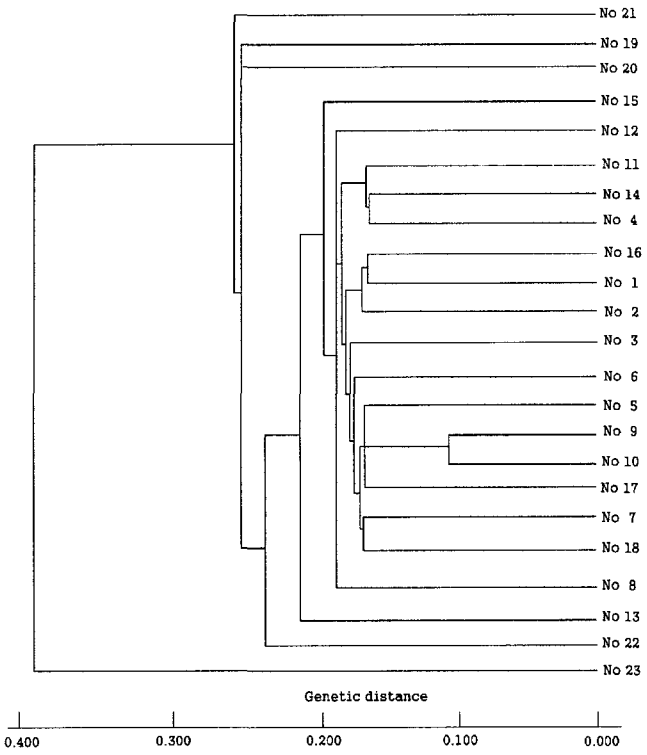

Fig. 1. Hierarchial dendrogram of genetic distances showing the relatedness among different individuals of two rainbow trout populations (No. 1~No. 23) generated according to the similarity matrix in Table 3 using AGE data.

Data analysis

To evaluate the AFLP variation and polymorphisms of individuals of within-population and between two populations, only bands that were readily visible were scored. AFLP outlines were compared only on samples lane in the same gel. Bandsharing calculation was somewhat modified the formula of Nei and Li (1979), Jeffreys and Morton (1987) and Mohd-Azmi *et al.* (2000): BS=N (Ba.....n)/(Ba+Bb+..... +Bn). Where Nabc is the number of fragments shared by individuals a, b and c for a single primer, Na is the total number of fragments for individual a screened within a population, Nb is the total number of fragments for individual b and Nc is the total number of fragments for individual c. If the comparison between the two lanes, the formula would be: BS=2 (Nab)/(Na+Nb) and so forth. An average of genetic similarity is calculated across all pairwise comparisons between individuals within two populations. Single linkage cluster analysis was performed on the similarity matrices in order to generate a dendrogram using pc-package program Systat version 10 (SPSS Inc., USA). Genetic differences and distances of individuals within population and between populations were calculated with dendrograms performed by Systat version 10. BS values were scored by the presence or absence of an amplified product at specific positions in the same gel from the AFLP profiles. PCR amplification and bandsharing experiments on the same DNA sample were carried out to examine the efficiency and then the data obtained were used in this experiment and data analyses above-mentioned.

Results

AFLP variation

Genomic DNA from rainbow trout was isolated, digested, ligated and preamplified at various times with PCR machines. The high degree of reproducibility of AFLP markers between experiments has been shown in this study and among different laboratories. The amplified products were separated by AGE with nine AFLP primer combinations and stained with ethidium bromide (Fig. 2A and B). Substantial amounts of polymorphism were seen for all the primers used. Each sample had unique banding patterns ranged from 0.4 to 1.5 kb showing individual identification in AGE. Individuals could be distinguished by the presence of unique bands. The characteristics and polymorphisms of AFLP fragments were analyzed by each primer combination by between AGE and PAGE (Table 2). A total of 131 AFLP fragments amplified by each AFLP primer pair, ranging in size from 0.5 to 1.5 kb.

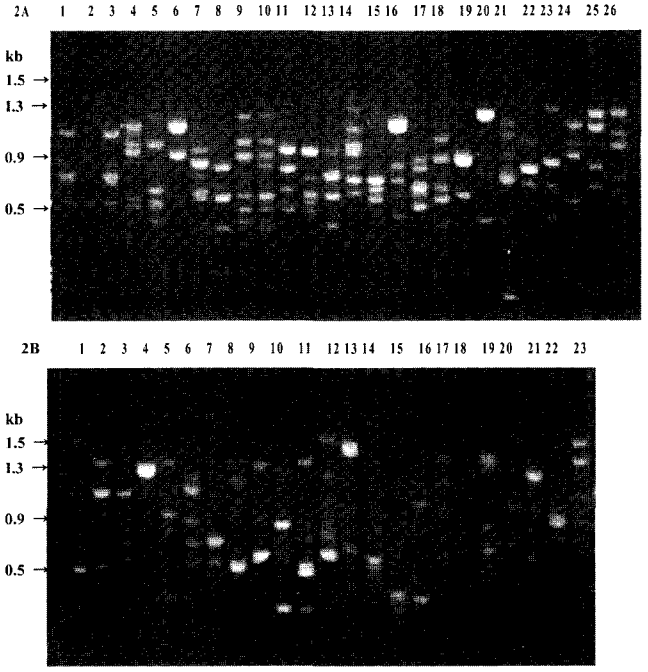

Fig. 2. Polymorphic AFLP profiles of rainbow trout individuals amplified with primer combinations (M11+H11) (A) and (M13+H11) (B) in agarose gel.

Table 2. AFLP analysis obtained with 9 primer combinations used to assess genetic variation of rainbow trout.

	Total number of bands amplified (A)	Size range of amplified products	Number of polymorphic bands identified (B)	Percentage of polymorphic bands (%)*
AGE	131	0.5-1.5	108	82.4
PAGE	288	0.1-1.5	220	76.4
Total	419		328	
Mean	23.2		18.2	79.4

*: The ratio of number of polymorphic bands identified(B)/total number of bands amplified (A)

The number of polymorphic loci detected per AFLP primer pair showed an average of 15 polymorphic fragments. Among 131 polymorphic fragments, the 108 fragments account for 82.4% of the total amplified fragments in rainbow trout.

The amplified products were also separated by PAGE (Fig. 4). Selective amplifications were performed with nine combinations of (E+4)-and (H+4)-primers. Of the 288 bands from the (M11)/(H13) amplification that were investigated, a total of 220 were polymorphic and accounted for 76.4% of the total amplified fragments. The number of polymorphic loci detected per AFLP primer pair in PAGE showed an average of 32 polymorphic fragments. There were shown mean bandsharing value and genetic variations for all 9 possible combinations of rainbow trout individuals calculated for each primer combination in AGE and PAGE (Figs. 2 and 4).

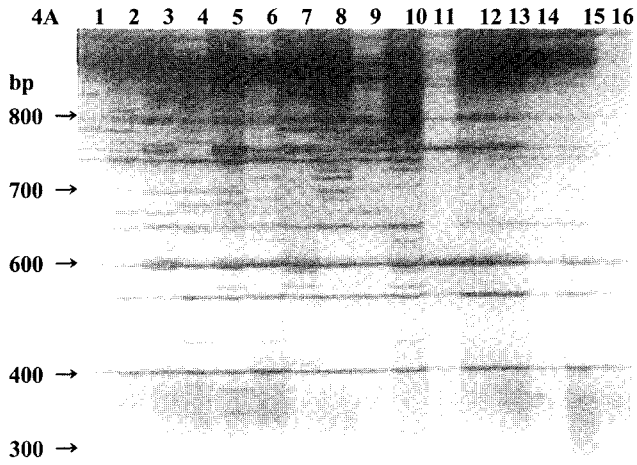

Fig. 4. Tightly linked polymorphic AFLP markers of rainbow trout individuals amplified with primer combinations (M12+H13) (A) in polyacrylamide sequencing gel.

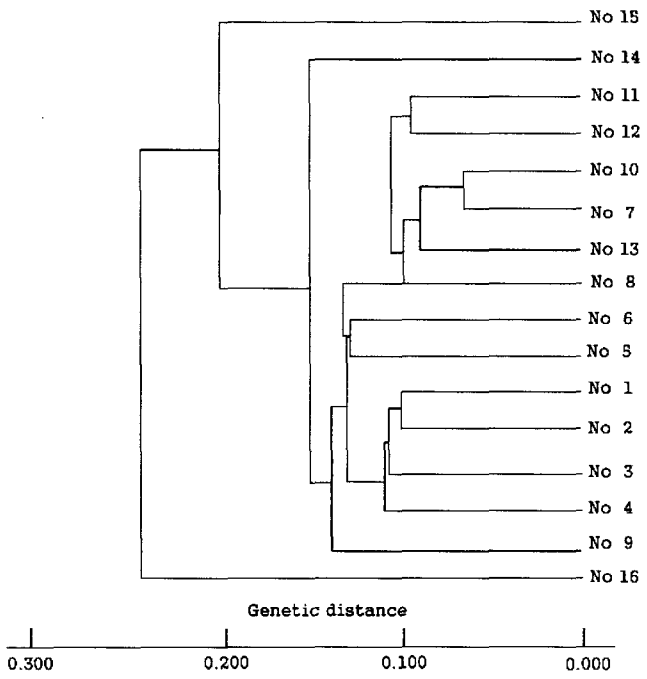

Fig. 3. Hierarchial dendrogram of genetic distances showing the relatedness among different individuals of rainbow trout population (No. 1~No. 16) generated according to the similarity matrix in Table 4 using PAGE data.

Bandsharing values and genetic distances

In the present study, the average bandsharing values of the individuals between two populations apart from the geographic sites in Kangwon-do ranged from 0.084 to 0.841 in AGE and PAGE (Table 3). The bandsharing value between individuals No. 9 and No. 10 showed the highest level within population, whereas the bandsharing value between individuals No. 5 and No. 7 showed the lowest level. A similarity matrix based on Nei and Li's index of similarity was used to perform single linkage cluster analysis in order to obtain the Euclidean distances and dendrogram (Table 3 and 4). The average bandsharing values of all of the samples within rainbow trout population ranged from 0.084 to 0.738 in view of the result of AGE. As calculated by bandsharing analysis in AGE, the average level of genetic difference (mean±SD) was approximately 0.590±0.125 in the individuals of this population. Also, as calculated by bandsharing analysis in PAGE, the average level of genetic difference was approximately 0.654±0.081 in the individuals of this population. In view of PAGE, the bandsharing value between individual No. 7 and No. 10 showed the highest level within rainbow trout population, whereas the bandsharing value between individual No.

Table 3. Similarity matrix including bandsharing values and genetic differences calculated using Nei and Li's index of similarity for rainbow trout, *O. mykiss* individuals obtained in AGE data.

	Bandsharing values of individuals in rainbow trout																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	-	0.343	0.545	0.349	0.225	0.343	0.367	0.375	0.292	0.367	0.382	0.556	0.556	0.325	0.536	0.584	0.347	0.472	0.254	0.243	0.384	0.375	0.254
2	0.657	-	0.389	0.325	0.286	0.472	0.343	0.486	0.397	0.333	0.347	0.292	0.567	0.292	0.367	0.452	0.575	0.310	0.250	0.167	0.375	0.143	0.167
3	0.455	0.611	-	0.375	0.191	0.459	0.432	0.422	0.431	0.488	0.583	0.382	0.325	0.361	0.333	0.500	0.606	0.200	0.307	0.516	0.606	0.434	0.473
4	0.651	0.675	0.625	-	0.321	0.325	0.481	0.516	0.534	0.517	0.393	0.393	0.417	0.686	0.382	0.417	0.467	0.500	0.486	0.277	0.358	0.182	0.245
5	0.775	0.714	0.809	0.679	-	0.111	0.084	0.225	0.536	0.557	0.431	0.384	0.439	0.374	0.443	0.614	0.422	0.398	0.333	0.393	0.565	0.675	0.398
6	0.657	0.528	0.541	0.675	0.889	-	0.361	0.343	0.291	0.349	0.534	0.523	0.375	0.411	0.254	0.350	0.649	0.450	0.606	0.444	0.473	0.629	0.311
7	0.633	0.657	0.568	0.519	0.916	0.639	-	0.467	0.477	0.536	0.481	0.649	0.614	0.508	0.400	0.231	0.586	0.697	0.508	0.450	0.374	0.467	0.341
8	0.625	0.514	0.578	0.484	0.775	0.657	0.533	-	0.542	0.267	0.391	0.333	0.389	0.468	0.268	0.459	0.584	0.584	0.508	0.343	0.584	0.500	0.608
9	0.708	0.603	0.569	0.466	0.464	0.709	0.523	0.458	-	0.738	0.367	0.565	0.398	0.343	0.310	0.202	0.584	0.558	0.516	0.500	0.467	0.500	0.511
10	0.633	0.667	0.512	0.483	0.443	0.651	0.464	0.733	0.262	-	0.467	0.504	0.356	0.356	0.389	0.431	0.606	0.585	0.455	0.508	0.564	0.467	0.552
11	0.618	0.653	0.417	0.607	0.569	0.466	0.519	0.609	0.633	0.533	-	0.258	0.084	0.548	0.293	0.267	0.455	0.450	0.473	0.299	0.649	0.564	0.354
12	0.444	0.708	0.618	0.607	0.616	0.351	0.351	0.667	0.435	0.496	0.742	-	0.443	0.375	0.459	0.311	0.522	0.311	0.325	0.216	0.484	0.667	0.397
13	0.444	0.433	0.675	0.583	0.561	0.625	0.386	0.611	0.602	0.644	0.916	0.547	-	0.167	0.222	0.100	0.254	0.534	0.411	0.582	0.393	0.667	0.455
14	0.675	0.708	0.639	0.314	0.626	0.589	0.492	0.532	0.657	0.644	0.452	0.625	0.833	-	0.258	0.393	0.341	0.227	0.450	0.321	0.536	0.416	0.398
15	0.464	0.633	0.667	0.618	0.557	0.746	0.600	0.732	0.690	0.611	0.707	0.541	0.778	0.742	-	0.365	0.250	0.254	0.268	0.200	0.313	0.375	0.431
16	0.416	0.548	0.500	0.583	0.386	0.650	0.769	0.541	0.798	0.569	0.733	0.689	0.900	0.607	0.635	-	0.444	0.350	0.236	0.416	0.361	0.347	0.336
17	0.653	0.425	0.394	0.533	0.578	0.351	0.414	0.416	0.416	0.394	0.545	0.478	0.746	0.659	0.750	0.556	-	0.444	0.486	0.450	0.573	0.361	0.415
18	0.528	0.690	0.800	0.500	0.602	0.550	0.303	0.416	0.442	0.415	0.550	0.689	0.466	0.773	0.746	0.650	0.556	-	0.361	0.377	0.389	0.472	0.595
19	0.746	0.750	0.693	0.514	0.667	0.394	0.492	0.492	0.484	0.545	0.527	0.675	0.589	0.550	0.732	0.764	0.514	0.639	-	0.236	0.300	0.334	0.437
20	0.757	0.833	0.484	0.723	0.607	0.556	0.550	0.657	0.500	0.492	0.701	0.784	0.418	0.679	0.800	0.684	0.550	0.623	0.764	-	0.404	0.386	0.500
21	0.616	0.625	0.394	0.642	0.435	0.527	0.626	0.416	0.533	0.436	0.483	0.616	0.607	0.464	0.687	0.639	0.427	0.611	0.700	0.596	-	0.222	0.446
22	0.625	0.857	0.566	0.818	0.325	0.371	0.533	0.500	0.500	0.533	0.436	0.333	0.333	0.584	0.625	0.653	0.639	0.528	0.666	0.614	0.778	-	0.508
23	0.746	0.833	0.464	0.755	0.602	0.689	0.659	0.392	0.489	0.448	0.646	0.603	0.545	0.602	0.569	0.664	0.585	0.405	0.563	0.500	0.554	0.492	-

Genetic differences of individuals

Table 4. Similarity matrix including bandsharing values and genetic differences calculated using Nei and Li's index of similarity for rainbow trout, *O. mykiss* individuals obtained in PAGE data.

Genetic differences of individuals	Bandsharing values of individuals															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	-	0.770	0.602	0.585	0.654	0.596	0.613	0.615	0.676	0.590	0.727	0.642	0.687	0.720	0.686	0.635
2	0.230	-	0.630	0.625	0.634	0.591	0.648	0.676	0.654	0.608	0.636	0.556	0.569	0.613	0.608	0.417
3	0.398	0.370	-	0.633	0.664	0.572	0.683	0.628	0.664	0.612	0.528	0.592	0.712	0.589	0.665	0.473
4	0.415	0.375	0.367	-	0.581	0.561	0.625	0.595	0.599	0.590	0.545	0.608	0.650	0.597	0.578	0.591
5	0.346	0.366	0.336	0.419	-	0.728	0.774	0.737	0.651	0.585	0.652	0.611	0.679	0.607	0.659	0.620
6	0.404	0.409	0.428	0.439	0.272	-	0.616	0.732	0.564	0.731	0.488	0.723	0.660	0.664	0.676	0.561
7	0.387	0.352	0.317	0.375	0.226	0.384	-	0.720	0.685	0.853	0.694	0.706	0.828	0.743	0.664	0.592
8	0.385	0.324	0.372	0.405	0.263	0.368	0.280	-	0.717	0.741	0.689	0.771	0.821	0.681	0.703	0.606
9	0.324	0.346	0.336	0.401	0.349	0.436	0.315	0.283	-	0.701	0.591	0.631	0.689	0.574	0.541	0.609
10	0.410	0.392	0.388	0.410	0.415	0.269	0.147	0.259	0.299	-	0.687	0.816	0.833	0.734	0.658	0.560
11	0.273	0.364	0.472	0.455	0.348	0.512	0.306	0.311	0.409	0.313	-	0.841	0.792	0.718	0.661	0.645
12	0.358	0.444	0.408	0.392	0.389	0.273	0.294	0.229	0.369	0.184	0.159	-	0.825	0.789	0.700	0.758
13	0.313	0.431	0.288	0.350	0.321	0.340	0.172	0.179	0.311	0.167	0.208	0.175	-	0.762	0.680	0.634
14	0.280	0.387	0.411	0.403	0.393	0.336	0.257	0.319	0.426	0.266	0.282	0.211	0.238	-	0.709	0.526
15	0.314	0.392	0.335	0.422	0.341	0.324	0.336	0.297	0.459	0.342	0.339	0.300	0.320	0.291	-	0.504
16	0.365	0.583	0.527	0.409	0.380	0.439	0.408	0.394	0.391	0.440	0.355	0.242	0.366	0.474	0.496	-

2 and No. 16 showed the lowest level.

In the cluster analysis using the AGE data, the single linkage dendrogram resulted from two primers (M11+H11 and M13+H11), indicating six genetic groupings composed of group 1 (No. 9 and 10), group 2 (No. 1, 4, 5, 7, 10, 11, 16 and 17), group 3 (No. 2, 3, 6, 8, 12, 15 and 16), group 4 (No. 9, 14 and 17), group 5 (13, 19, 20 and 21) and group 6 (No. 23) (Fig. 1). The genetic distances among individuals of between-population apart from the geographic sites in Kangwon-do, ranged from 0.108 to 0.392. The shortest genetic distance (0.108) displaying significant molecular differences was between individuals No. 9 and No. 10. Especially, the genetic distance between individuals No. 23 and the remnants among individuals within population was highest (0.392). Additionally, in the cluster analysis using the PAGE data, the single linkage dendrogram resulted from two primers (M12+H13 and M11+H13), indicating seven genetic groupings composed of group 1 (No. 15), group 2 (No. 14), group 3 (No. 11 and 12), group 4 (No. 5, 6, 7, 8, 10 and 13), group 5 (1, 2, 3 and 4), group 6 (No. 9) and group 7 (No. 16) (Fig. 3). By comparison with the individuals in PAGE, genetic distance between No. 10 and No. 7 showed the shortest value (0.071), also between No. 16 and No. 14 showed the highest value (0.242). As with the PAGE analysis, genetic heterogeneity was certainly apparent with 13 of 16 individuals showing greater than 80% AFLP-based similarity to their closest neighbor. The three individuals (No. 14, No. 15 and No. 16) of *O. mykiss* in Kangwon-do formed distinct genetic distances as compared with other individuals.

Discussion

AFLP analysis is a good PCR method, developed by Keygene, Inc., which selectively amplifies DNA fragments (Zabeau and Vos, 1993). AFLP method detected high numbers of polymorphic genetic markers with minimal cost and time requirements is a major objective of molecular genetics (Maughan et al., 1996). The most important advantages of AFLP are the capacity to inspect a genome for polymorphism and its reproducibility (Blears et al., 1998; Hansen et al., 1999; Suazo and Hall, 1999). Especially, AFLP markers are extremely useful, being abundant, highly variable and relatively simple to analyze linkage groups in rainbow trout. These markers provide fundamental information about the size and structure of the salmonid genome (Young et al., 1998). AFLP markers provided useful information for identification of genotypes and construction of a molecular linkage map in tef, *Eragrostis tef* (Bai et al., 1999). AFLP markers were evaluated for their usefulness in the genetic analysis of sugar beet and wild *Beta* species (Hansen et al., 1999). AFLP is the selective amplification of restriction fragments from a digest of genomic DNA using the PCR (Blears et al., 1998). Molecular genetic polymorphisms are identified by the presence or absence of DNA fragments following restriction and amplification of genomic DNA. However, low sensitivity to the amplification conditions were seen (Suazo and Hall, 1999), as compared with other method such as RAPD-PCR (Song and Lee, 2000; Yoon and Kim, 2003).

Considering the resolution of two gel electrophoresis such as AGE and PAGE in this study, it is possible to construct a high resolution with the large number of primers available in PAGE in rainbow trout. While minor band, less than 150 bp, unidentified in AGE, AFLP fragments ranged from approximately 100 bp were detected in PAGE. A total of fragments, an average of fragments per primer and polymorphic DNA bands identified in PAGE were much more than those in AGE. This result was in accordance with that of Bai et al. (1999). However, PCR products were resolved in agarose-Synergel instead of polyacrylamide and were visualized by ethidium bromide staining (Suazo and Hall, 1999). Generally, the number of fragments that can be analyzed simultaneously, is dependent on the recognition site digested by restriction enzymes, primers and G+C contents. Typically, 50~100 restriction fragments are amplified and detected on denaturing polyacrylamide gels (Vos et al., 1995). However, the genetic frequency of polymorphic fragments was generally very low in polyacrylamide gel electrophoresis. The AFLP markers have so far been detected in various animals, plants and microbes. The 332 AFLP markers used in the segregation analysis were produced using 28 primer pairs for an average of 12.1 markers/primer pair in rainbow trout (Young et al., 1998). The total of AFLP markers identified by 9 primer combinations ranged from 70 to 200 in channel catfish, *Ictalurus punctatus*, blue catfish, *I. furcatus* and their F_1, F_2 and backcross hybrids, *I. furcatus* (Liu et al., 1998). The polymorphic levels were diversified from 38 to 75% according to primer combinations. The average percent polymorphism between *indica* and *japonica* accessions in rice was 65.35% for AFLP (Mackill et al., 1996). The number of polymorphic loci detected per AFLP primer pair in a sample of 23 accessions in soybean ranged from 9 to 27 (Maughan et al., 1996). The polymorphic fragments in wild and cultivated soybean ranged from about 17 to 36% of the total observed (Maughan

et al., 1996). On average, most of the diversity was detected within populations, with 79% of the variation being within and 21% being between populations of Indian and Kenyan tea (Paul et al., 1997). 96.4% were polymorphic among the accessions in sugar beet (*Beta* spp.) (Hansen et al., 1999). The level of polymorphism within tef, *Eragrostis tef* accessions was low (18%) (Bai et al., 1999). 18% of the AFLP amplification products were polymorphic among accessions in azuki, *Vigna angularis* (Yee et al., 1999). The total number of bands screened was 783, of which 181 (23.1%) found a polymorphic pattern among the melon lines (Garcia-Mas et al., 2000). One hundred and fifty eight scorable band levels were generated of which 96 (61%) were polymorphic (Purba et al., 2000). Also, 31% out of fragments identified by a few of primer combinations were polymorphic in Italian Holstein (Ajmone-Marsan et al., 1997). AFLP markers were approximately 60% in a cross within White Leghorns (Knorr et al., 1999). DNA was analyzed by using three different primer combinations producing from 61 to 63 polymorphic markers (55.7 to 88.9%) (Barki et al., 2000). As expected, the polymorphic ratio of AFLP products considerably varied according to life species.

When comparisons of the performance of two types of molecular markers in measuring genetic diversity have been carried out in rainbow trout species, the level of polymorphism detected with AFLP (the average of 76.4%) in AGE was substantially higher than that identified with RAPD (the average of 69.5%) (Yoon, 1999). A few of polymorphic RFLP, RAPD and AFLP markers were scored on plant species and the genetic similarity measured comparatively (Garcia-Mas et al., 2000). Also, a higher percentage (83%) of AFLP primer pairs generated polymorphic bands within azuki, *Vigna angularis* compared with RAPD (26%) (Yee et al., 1999). In addition, bandsharing scores were calculated as an expression of similarity of RAPD fingerprints of animals from either the same or different breeds (Jeffreys and Morton, 1987; Gwakisa et al., 1994; Liu et al., 1998; Mohd-Azmi et al., 2000). The intra-specific divergence estimates based on sequence were less than the inter-specific divergence estimated from restriction fragment analysis (Beckenbach et al., 1990). This can be used as a potential genetic marker for linkage analysis with economically important traits in fish (Liu et al., 1998) and in livestock (Mohd-Azmi et al., 2000). Intra-specific variations in the pattern were observed for each primer and such data should be of value not only in the discrimination of the correlation with the economic traits but also in the construction of phylogenetic trees or dendrograms (Yee et al., 1999; Garcia-Mas et al., 2000; Yoon and Kim, 2003). Recently, this result implies the genetic variation or diversity due to introduction of foreign genes or improved breeds within the rainbow trout in Korea as compared with the results obtained in this study. The AFLP polymorphism generated by the primer may be used as a genetic marker for species or strain identification in important aquacultural fish species, rainbow trout.

The bandsharing values approach based on the presence or absence of amplified DNA bands was used to estimate similarity indices, as summarized in Table 3 and 4. In the present study, the average similarity index between individuals No. 9 and No. 10 showed the highest level within rainbow trout population in AGE (0.738), whereas the similarity index between individuals No. 5 and No. 7 showed the lowest level (0.084). Also, in the PAGE, the similarity index between individual No. 7 and No. 10 showed the highest level within rainbow tout population (0.853), whereas the similarity index between individual No. 2 and No. 16 showed the lowest level (0.417). As calculated by bandsharing analysis in AGE, the average level of genetic difference was approximately 0.590± 0.125 in the individuals of this population. Also, in PAGE, the average level of genetic difference was approximately 0.654±0.081 in the individuals of this population. On the whole, the similarity index using PAGE data was higher than that obtained from AGE. All individuals also revealed close genetic affinities, as compared with rainbow trout individuals, as summarized in Fig. 1. It appears from the AFLP-PCR data that this large genetic difference in rainbow trout population may be genetic polymorphism. Callejas and Ochando (1998) indicated that Spanish barbel species (*Barbus bocagei* and *B. graellsii)* were more related to each other than *B. sclateri* by means of the cluster analysis of the genetic similarity values obtained from RAPD data. The average level of bandsharing obtained by the five random primers used was 0.40±0.05 in the wild crucian carp population, contrast with 0.69±0.08 observed in the cultured crucian carp population (Yoon and Park, 2001). The degree of similarity in two carp species varied from 0.46 to 0.67 as calculated by bandsharing analysis (Yoon, 2001). The average level of bandsharing was approximately 0.57±0.03 between the species common carp and Israeli carp generated using various random primers. The genetic relationships of 3 goat populations in China were studied by genetic differentiation coefficient and genetic similarity coefficient (Geng et al., 2002). The result showed that genetic relationship between LCG and CCG was the

closest (genetic distance=0.0106), then CG and CCG (genetic distance=0.0109). They made mention that RAPD marker was more effective in analyzing the genetic relationship of populations. RAPD data analysis, including distance and parsimony methods, family clustering and the analysis of molecular variance, were applicable for the study of genetic relationships among species of the genus *Digitalis* (Nebauer et al., 2000). Namely, they stated that the species relationships revealed by RAPD-PCR approach were fully consistent with those previously obtained using morphological affinities. This result showed a similar tendency to that of Orozco-Castillo et al. (1994) that RAPD analysis reflected morphological differences between the sub-groups and morphological origin of the coffee material.

In this study, the single linkage dendrogram obtained by the cluster analysis using the AGE data, resulted in six genetic groupings composed of group 1 (No. 9 and 10), group 2 (No. 1, 4, 5, 7, 10, 11, 16 and 17), group 3 (No. 2, 3, 6, 8, 12, 15 and 16), group 4 (No. 9, 14 and 17), group 5 (13, 19, 20 and 21) and group 6 (No. 23). The genetic distances among individuals of between-population apart from the geographic sites in Kangwon-do, ranged from 0.108 to 0.392. The shortest genetic distance (0.108) displaying significant molecular differences was between individuals No. 9 and No. 10. Especially, the genetic distance between individuals No. 23 and the remnants among individuals within population was highest (0.392). Additionally, in the cluster analysis using the PAGE data, the single linkage dendrogram resulted from two primers (M12+H13 and M11+H13), indicating seven genetic groupings composed of group 1 (No. 15), group 2 (No. 14), group 3 (No. 11 and 12), group 4 (No. 5, 6, 7, 8, 10 and 13), group 5 (No. 1, 2, 3 and 4), group 6 (No. 9) and group 7 (No. 16). By comparison with the individuals in PAGE, genetic distance between No. 10 and No. 7 showed the shortest value (0.071), also between No. 16 and No. 14 showed the highest value (0.242). As with the PAGE analysis, genetic heterogeneity was certainly apparent with 13 of 16 individuals showing greater than 80% AFLP-based similarity to their closest neighbor. The three individuals (No. 14, 15 and 16) of *O. mykiss* in Kangwon-do formed distinct genetic distances as compared with other individuals. Thus, the crossing utilization of three individuals (No. 14, No. 15 and No. 16) of rainbow trout used in this study seems to be fit undoubtedly to raise the productivity of this species and also for avoiding close inbreeding from other kinds of matings.

The phenogram using RAPD data obtained across all primers revealed close relationships between accessions identities in native American maize accessions (Moeller and Schaal, 1999). The similarity index for Tamaroa white and Tamaroa/Tuscarora white was quite high (similarity=0.8). Also, they stated that Mandan red and Arikara bronze showed close genetic affinity, which confirmed historical records. Generally speaking, the potential of RAPDs to identify diagnostic markers for strain, breed, species and population identification in plants (Orozco-Castillo et al., 1994; Moeller and Schaal, 1999), in livestock (Jeffreys and Morton, 1987; Mohd-Azmi et al., 2000; Geng et al., 2002), in parasites (Dias Neto et al., 1993) and in fish (Partis and Wells, 1996; Callejas and Ochando, 1998; Klinbunga et al., 2000; Yoon and Park, 2001) has also been demonstrated. In this study, AFLP-PCR analysis has revealed significant genetic distances among rainbow trout individuals. High levels of genetic polymorphisms and the existence of population differentiation among rainbow trout individuals showed AFLP-PCR approach is one of the most suitable tools for individuals and population biological DNA studies.

Consequently, AFLP markers of these rainbow trout could be used as genetic information such as species identification, genetic relationship or analysis of genome structure, and selection aids for genetic improvement of economically important traits in fish species. Although AFLP is complicated, requires special equipment and is more expensive, it is reproducible, and the capacity to reveal several bands and small amounts of DNA are needed when compared with those of RFLP and RAPD (Garcia-Mas et al., 2000). Development of an intra-specific genetic map of animal, plant and microbe would be valuable for quantitative trait loci, trait mapping and marker-assisted selection, and further genetic analyses may be useful for enhancing the efficiency of molecular breeding programs (Hospital et al., 1997). Especially, molecular genetic markers, such as microsatellite loci, quantitative trait loci and genomic mapping, will be useful for selection of broodstock for multiple reproductive traits or health- and production-related traits in fisheries science (Waldbieser and Wolters, 1999).

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