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Differences by RAPD-PCR Analysis within and between Rockfish (*Sebastes schlegeli*) Populations from the Yellow Sea and the Southern Sea in Korea

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ABSTRACT

Polymerase chain reaction (PCR) amplification of DNA as 30 different arbitrary primers and random amplified polymorphic DNAs (RAPD) analysis were performed on genomic DNA extracted from the blood of the marine rockfish (*Sebastes schlegeli*) from the Yellow Sea and the Southern Sea. The unique properties of the genomic DNA were used to investigate the features of the population dynamics and origins of the species. Out of 30 primers, seven generated 207 highly reproducible RAPD polymorphic products, producing approximately 2.7 polymorphic bands per primer. About 67.4% of total amplified products (307) were either polymorphic (207) to rockfish. The degree of similarity varied from 0.22 to 0.63 as calculated by bandsharing analysis. Also, the average level of bandsharing was 0.39±0.02 within the rockfish strains. The electrophoretic analysis of RAPD-PCR products showed the relatively high levels of variation between different individuals in rockfish from the Yellow Sea. However, the RAPD outlines obtained with DNA of different rockfish strains from the Yellow Sea and the Southern Sea in Korea were very similar. Also, a small number of polymorphic bands were identified. Even if further analyses or more rockfish populations are required, this result implies RAPD analysis reflects genetic differences between the geographical strains of the rockfish.

(Key words: Bandsharing analysis, Genetic diversity, Polymorphism, Primer, RAPD-PCR, Rockfish)

Ⅰ. INTRODUCTION

The conventional method used for genetic selection is the biometrical approach combined with general genetic principles. Genetic markers have many potential applications in animal and plant genetics and breeding research. The polymorphic DNA markers that are shown to genetically link to a trait of interest can be used for individual identification, gene cloning, pathological diagnostics, and for trait improvement in fish breeding programs.

The DNA markers most commonly used are restriction fragment length polymorphisms (RFLP) (Lloyd et al., 1989; Beckenbach et al., 1990). Random primers based on the polymerase chain reac-

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tion produce a higher percentage of multiple-band outlines than RFLP probes. Genomic fingerprints generated by PCR amplification of DNA using arbitrary primers can be used for detecting DNA polymorphisms between organisms (Welsh and McClelland 1990; Welsh et al., 1991; Deragon and Landry 1992; Simpson et al., 1993; Orozco-Castillo et al., 1994; Chung et al., 1995; Koh et al., 1997; Lilley et al., 1997). Most fisheries applications of RAPD's have been at the fish species level (Smith et al., 1997; Liu et al., 1998; Yoon 1999).

Various primers detect polymorphisms in the presence or absence of specific nucleotide sequence information, and the polymorphisms function as genetic markers.

Polymorphisms are scored by the presence or absence of an amplification product at specific positions in the gel (Smith et al., 1997). Geographical populations have been distinguished by RFLP-PCR method in various fishes (Wilson et al., 1987).

In this study, DNA isolated from the blood was analysed by 30 random amplified polymorphic DNAs (RAPD) primers in order to identify genetic characteristics and genomic polymorphisms in rockfish (*Sebastes schlegeli*) from the Yellow Sea and the Southern Sea.

Ⅱ. MATERIALS AND METHODS

1. Blood Collection

Rockfish (*Sebastes schlegeli*) DNA samples were obtained from two aquaculture facilities in the periphery of the Yellow Sea (the western sea) and the Southern Sea in South Korea. The two facilities are approximately 350 km apart. These sites were chosen because they exceed the limit of larvae or broodstock transportation. RAPD analysis was performed on genetic DNA samples from a total of 100 rockfish using 30 random primers.

The DNA samples were collected from blood in two-year-old rockfish. The fish were anaesthetized with MS 222 (100 ppm). Blood samples were taken with sterile test tubes from the caudal vein into heparinized vials. The bloods obtained were refrigerated at 4℃ until use. All glassware, micropipette tips, centrifuge tubes, glass pipettes and solutions were autoclaved to avoid DNA recontamination.

2. Sources of Genomic DNA

DNA extraction should be performed and according to the following separation and extraction procedures (Vierling et al., 1994). Samples of whole blood were placed into 0.2 ml microcentrifuge tubes, to which an equal volume of lysis buffer Ⅰ was added, then mixed gently by inverting the tube several times. The samples were incubated on ice for 30 min, centrifuged at 3,000 rpm (2,608 $\times$ g) for 10 min. at 4℃ to form a pellet. The supernatants were decanted and the pellet were resuspended with lysis buffer Ⅱ. Samples were transferred with aqueous phase to 1.5 ml Eppendorf tubes and 500 μg/ml proteinase K solution was added solutions. The solutions were gently mixed by inverting the tube and incubated at 37℃ for 6 min. There was added 400 μl of phenol to mixture and centrifuged for 1 min. at 1,000 rpm. 200 μl of phenol and 200 μl of chloroform : isoamylalcohol were added to the mixture. Samples were spun down for 1 min. at 1,000 rpm and added 700 μl of chloroform : isoamylalcohol. The cleared lysates were extracted with 2 volumes of ice-cold 70% ethanol, then centrifuged for 10 min. at 14,000 rpm (26,953 $\times$ g), then precipitated. The DNA pellets were air-dried for 30 min, and then dissolved 200 μl of TE buffer. Purity and concentration of DNA was measured with a spectrophotometer (Beckman DU 600 series). Purity was estimated by calculating the ratio of the absorbance (O.D. value) measured at 260~280nm. The final concentration was estimated with agarose elec-

trophoresis and ethidium staining.

3. Primers, Markers and Amplification Conditions

The primers, designed for other purposes and chosen arbitrarily for these experiments, were obtained from Operon Technologies, USA (Table 1). All of these decamer random primers had G + C content in the range 60～80%. Six 10-base oligonucleotide primer sequences (5' to 3') (Table 1), ΦX 174 DNA /*Hae* Ⅲ marker and lambda DNA /*EcoR* Ⅰ and *Hind* Ⅲ marker (Promega Co., USA) were used.

Amplification reactions were undertaken in volumes of 20 μl contained 10 ng of template DNA, 1×reaction buffer, 200 μM dNTP (Advanced Biotechnologies, LTD), 0.25 unit of Taq DNA polymerase (Perkin Elmer Cetus, USA), 1.0 unit primer (Operon Technologies, USA). Amplification was performed in a DNA Thermal Cycler (Perkin Elmer Cetus, USA). This mixture was overlaid with mineral oil, and followed an initial denaturation at 94℃ for 3 min. The thermal cycler programmed for 45 cycles at 94℃ for 1 min for denaturation, at 38℃ for 1 min for annealing, at 72 ℃ for 1 min for extension, using the fastest available transition between each temperature. Amplification products were separated by electrophoresis in 1.5% agarose gels with TBE and detected by staining with ethidium bromide. The gels were illuminated with UV light and photographed by UV DNA photographic system (Seoulin, Korea).

4. Analytical Method

Bandsharing (BS) calculation was somewhat modified the formula of Jeffreys and Morton (1987) and Mohd-Azmi et al., (2000): BS = N (Ba.......n) /(Ba + Bb+ .... +Bn). For instance, as follows: BS = 2 (Bab)/(Ba+Bb). Where Bab is the number of bands shared by individuals a and b, Ba is the total number of bands for individual a, and Bb is the total number of bands for individual b. If the comparison between the three lanes, the formula would be: BS = 3 (Nabc)/(Na+Nb+Nc) and so on. Only bands that were surely visible were scored. BS values were scored by the presence or absence

Table 1. Primers and primer sequences used for the detection of polymorphism in rockfish (*S. schlegeli*)

Primer	Sequence(5' to 3')	GC content(%)	Primer	Sequence(5' to 3')	GC content (%)
FRP-01	CGCAGTACTC	60	FRP-16	GGCCACAGCG	80
FRP-02	GTCCTTAGCG	60	FRP-17	AACGTACGCG	60
FRP-03	CGCCCGATCC	80	FRP-18	GACCGACACG	70
FRP-04	GAGTGTCTCG	60	FRP-19	CATGTCCGCC	70
FRP-05	ATCTCCCGGG	70	FRP-20	CGCGAGGTGC	80
FRP-06	CATCCCGAAC	60	FRP-21	GACCGACACG	70
FRP-07	GCTCTGGCAG	70	FRP-22	CATGTCCGCC	70
FRP-08	GCTCTCCGTG	70	FRP-23	GGACCTACGG	70
FRP-09	ACCCCAGCCG	80	FRP-24	CGCAGTGGGC	80
FRP-10	AGGGCGCCAT	70	FRP-25	GGTGGTATAG	50
FRP-11	CTCTGGGATC	60	FRP-26	CCATATCCGG	60
FRP-12	GCACGGAGGG	80	FRP-27	GTGACTGGAG	60
FRP-13	GCACGGGTGA	70	FRP-28	TAGGGCCATC	60
FRP-14	CGCCCTGGTC	80	FRP-29	CGGCATTTGG	60
FRP-15	CGCGAACGGC	80	FRP-30	GTGACGGCTT	60

of an amplified product at specific positions in the same gel from the RAPD profiles. PCR amplification and bandsharing experiments on the same DNA sample were carried out to examine the efficiency and reproducibility and then the data obtained were used in this experiment and data analyses above-mentioned.

Ⅲ. RESULTS

1. Within-population Variations (from the Yellow Sea)

Of the 30 arbitrarily selected primers, seven random primers were used on the basis of the number and frequency of the polymorphisms produced. The bands in the molecular weight range from 1.6 to 5.1 kilobase pairs generated by random primer FRP-3 (CGCCCGATCC) were observed (Fig. 1). There were shown genomic DNA fingerprints generated using a primer (80% of GC contents) to amplify DNA isolated from the blood of 9 individuals. The similar band pattern was observed in 5.1

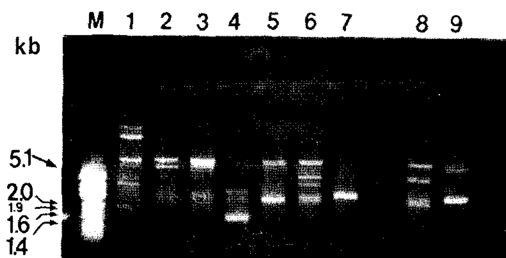

Fig. 1. Amplification products were electrophoresed on a 1.4% agarose gel with TBE (0.09 M Tris, pH 8.5; 0.09 M boric acid; 2.5 mM EDTA) and detected by staining with ethidium bromide. Individual specific RAPD patterns of rockfish amplified by arbitrary FRP-3 (CGCCC-GATCC). Each lane shows different individual DNA samples. M: Molecular size standard (Lambda DNA marker digested with *EcoR* Ⅰ and *Hind* Ⅲ).

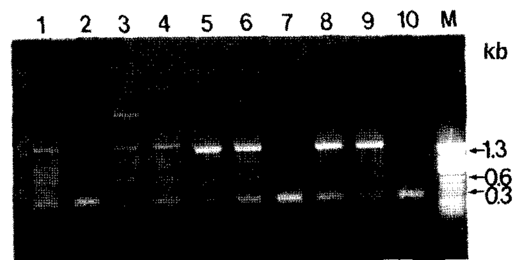

Fig. 2. Specific RAPD patterns of rockfish amplified by arbitrary primer FRP-9 (ACCCCAGCCG). Each lane (1~10) shows different individual DNA samples. The primer produced a single-band RAPD profile (lanes 2, 7 and 10). M: 194~1353 bp standard ladder (Φ X174 DNA marker digested with *Hae* Ⅲ).

kb. The primer yielded a single-band profile (lane 7). The Intra-specific variation was revealed in the band patterns ranged in 1.6 kb. The RAPD profiles obtained by FRP-9 (ACCCCAGCCG) with pooled DNA of individuals were some different (Fig. 2). The identical band pattern of RAPD-PCR products was observed in 0.3 kb. The Intra-specific variation was revealed in the band patterns ranged from 0.6 to greater than 1.3 kb. This primer produced a

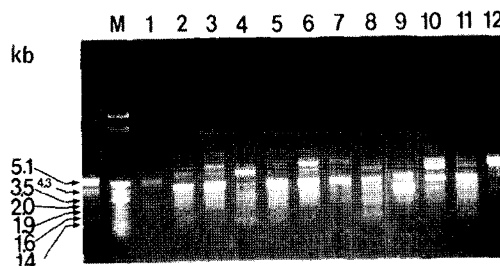

Fig. 3. Individual specific RAPD patterns in rockfish amplified by arbitrary FRP-12 (GCACGGAGGG). Each lane (1~10) shows different individual DNA samples. M: Molecular size marker (Lambda DNA marker digested with *EcoR* Ⅰ and *Hind* Ⅲ).

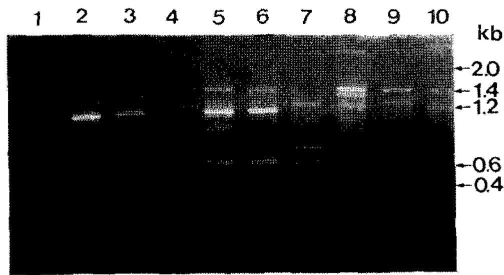

Fig. 4. RAPD-PCR products of rockfish amplified by arbitrary FRP-14 (CGCCCTGG-TC). Each lane (1～10) shows different individual DNA samples. M: Molecular size standard (Lambda DNA marker digested with *EcoR* I and *Hind* III).

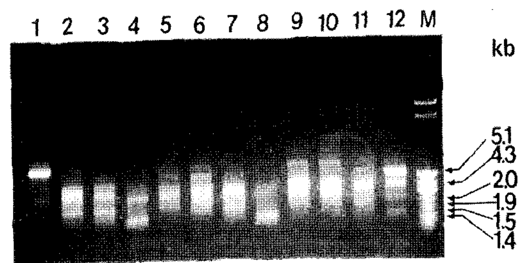

Fig. 5. Individual specific RAPD patterns in rockfish amplified by arbitrary FRP-16 (GGCCACAGCG). Each lane (1～10) shows different individual DNA samples. M: Molecular size marker (Lambda DNA marker digested with *EcoR* I and *Hind* III).

single-band RAPD profile (lanes 2, 7 and 10). The other primers FRP-12 (GCACGGAGGG) also generated RAPD band patterns showing DNA polymorphisms (Fig. 3). The similar band pattern was observed in 4.3 kb. The intra -specific variation was revealed in the band patterns ranged in 3.5 and greater than 5.1 kb. This primer produced a single -band RAPD profile (lane 1). The differences between strains should be diagnostic of specific strains and their relatedness. Another primer, FRP -14 (CGCCCTGGTC), detected a pair of RAPD major bands, approximately from 0.6 to 1.4 kb (Fig. 4). The DNA bands of from 0.6 to 1.4 kb were present in every individuals. Two corresponding bands of 1.0 and 1.2 kb were absent in 6 of 10 individuals, respectively, which were polymorphic and exhibited two patterns. The RAPD polymorphism generated by random primer FRP-16 (GGCCACAGCG) showed fingerprint bands (Fig. 5). The sizes of the bands recorded ranged from 1.4kb to approximately 5.1 kb. There were showed genomic DNA fingerprints generated using a primer (80% of GC contents) to amplify DNA isolated from the blood of 10 individuals. This primer yielded a single-band profile, which was polymorphic and exhibited two patterns (lanes 1 and 8).

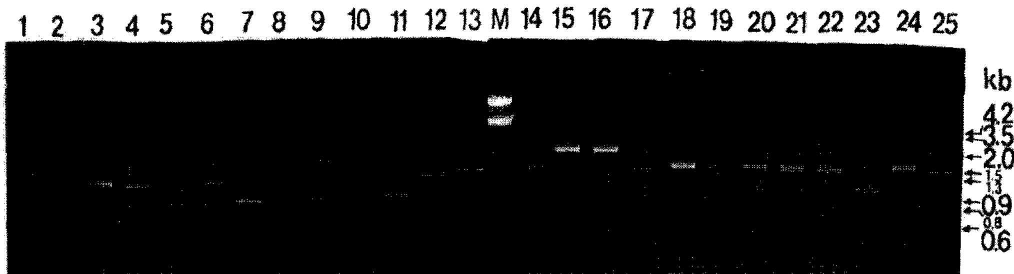

Fig. 6. Specific RAPD fingerprints generated in rockfish by arbitrary primer FRP-20 (CGCCGAGG-TGC). Each lane (1～25) shows different individual DNA samples (lanes 1～13 from the Yellow Sea and lanes 14～25 from the Southern Sea). M: Lambda DNA/*EcoR*I + *Hind* III.

2. Between-populations Variations and Band-sharing Values

The RAPD polymorphism generated by random primer FRP-20 (CGCGAGGTGC) showed fingerprint bands (Fig. 6). This showed genomic DNA fingerprints generated using a primer to amplify DNA isolated from the blood of 25 individuals from the Yellow Sea (lanes 1～13) and the Southern Sea (lanes 14～25). In this range from approximately 0.9 to 2.0 kb the patterns were highly reproducible and also very similar between two strains. However, this primer produced the sizes of polymorphic DNA bands ranged in 0.8 and 3.5 kb, respectively. This primer also yielded a single-band profile ranged in 2.0 kb, which were polymorphic. The bands exhibited the inter-strain -specific characteristics and were present in 2 of 12 individuals.

In this study, 23% of the random primers screened appeared amplified polymorphic products. In seven primers of the 30 RAPD primers used, the number of bands produced per primer varied from 2 to 6 with an average of 3.9. Especially, primer FRP-20 generated the highest number of fragments among the primers used with the average of 4.8 (Table 2). Seven primers produced amplified fragments that were consistently polymorphic between the individuals. A total of 207 amplification products were produced of which 18.7 were polymorphic. 2.7 of the 29.6 amplified bands were found to be polymorphic. About 67.4% of total amplified products (307) were either polymorphic (207) to rockfish. The degree of similarity varied from 0.22 to 0.63 as calculated by bandsharing analysis. Also, the average level of bandsharing was 0.39±0.02 within the rockfish strains (Table 3). A small number of polymorphic bands were identified, as shown in Fig. 6. In addition, the band patterns of population from the Yellow Sea (BS=0.62) were similar in comparison with those from the Southern Sea (BS=0.65) for reference to bandsharing value (Table 4).

Table 2. Number of RAPD products for single primer using agarose gel in rockfish from the Yellow Sea

Primers no	Range of products per lane	Average no. of products (Total)
FRP-3	1～5	4.0 (36)
FRP-9	1～5	2.4 (24)
FRP-12	3～6	3.9 (47)
FRP-14	3～7	4.5 (45)
FRP-16	2～6	3.3 (40)
FRP-20	2～6	4.8 (63)
FRP-24	2～6	4.3 (52)
Total		27.2(307)
Average	2～6	3.9(43.9)

Parentheses are the total number of products generated in rockfish.

Ⅳ. DISCUSSION

Polymorphic bands generated by PCR amplification of DNA using arbitrary primers had good merits for detecting DNA diversity and similarity between organisms (Welsh and McClelland 1990; Welsh et al., 1991). The amplification products can be generated as DNA fingerprint bands by gel electrophoresis, the patterns being characteristic of both the primers and the template DNA. Polymorphisms were scored by the presence or absence of amplification products at specific positions expressed by various primers (Smith et al., 1997). Intra -specific or inter-strain 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 (Welsh and McClelland 1990; Welsh et al., 1991; Orozco-Castillo et al., 1994; Vierling et al., 1994). It was used DNA extracted from rockfish blood which have the

Table 3. Bandsharing values (BS) within the rockfish strain from the Yellow Sea

Primers no	Total number of polymorphic products	Average number of polymorphic products	BS values
FRP-3	30	3.3	0.30
FRP-9	19	1.9	0.42
FRP-12	31	2.6	0.39
FRP-14	35	3.5	0.22
FRP-16	30	2.5	0.33
FRP-20	32	2.4	0.63
FRP-24	30	2.5	0.47
Total	207	18.7	2.76
Average	29.6	2.7	0.39±0.02 *

* mean ± standard error

Table 4. Comparison of the RAPD profile of different rockfish strains from the Yellow Sea (lanes 1~13) and the Southern Sea (14~25) obtained with arbitrary primer FRP-20 (CGCGAGG-TGC)

Lane no	Range of products	Total no. of products	Average no. of polymorphic products	BS
1~13	3-6	76	2.2 (29)	0.62
14~25	1-6	62	1.8 (22)	0.65
Total		138	2.0 (51)	1.27
Average		69	(25.5)	0.64

BS: bandsharing value

Parentheses are the total number of polymorphic products generated in rockfish from the Yellow Sea and the Southern Sea, respectively.

genome size of from 300 bp to greater than 5.1 kb. The random primer, FRP-20 generated the highest number of fragments among the primers used with the average of 4.8, as summarized in Table 2. The random primers, FRP-12 generated RAPD band patterns showing DNA polymorphisms, as shown in Fig. 3.

In this study, the intra-specific variation was revealed in the band patterns ranged in 3.5 and greater than 5.1 kb, as shown in Fig. 3. This primer produced a single-band RAPD profile (lane 1). The differences between strains would be diagnostic of specific strains and their relatedness. The other random primer (FRP-16) also yielded a single-band profile, which was polymorphic and exhibited two patterns (lanes 1 and 8).

The number of intra-strain polymorphisms was greater than that of inter-strain polymorphisms (Welsh et al., 1991). Liu et al., (1998) reported that some intra-specific RAPD variations were observed for different strains or for individuals. They screened a collection of RAPD markers in catfish and identified 22 primers that revealed 171 strain-specific genetic markers. Also, Johnson et al., (1994)

screened a collection of RAPD markers in zebrafish and identified 116 primers that revealed 721 strain -specific genetic markers. These workers have found more variation within a strain than between strains considered as a whole.

However, Beckenbach et al., (1990) observed that the intra-specific divergence estimates based on sequence were less than the inter-specific divergence estimated from restriction fragment analysis. This can be used as an potential genetic marker for linkage analysis with economically important traits in fish (Smith et al., 1997; Liu et al., 1998). In this study, the random primer (FRP-20) produced the sizes of polymorphic DNA bands ranged in 0.8 and 3.5 kb, respectively. This primer also yielded a single-band profile ranged in 2.0 kb, which were polymorphic. The bands exhibited the inter-strain -specific characteristics and were present in 2 of 12 individuals. RAPD marker would be particularly useful in marker-assisted selection (MAS) programs in these important aquaculture species. Therefore, these bands are potential polymorphic markers.

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). In this study, random primer, FRP-20 generated the highest number of fragments among the primers (the average of 4.8). This specific primer was found to be useful in the individual identification, resulting from the different DNA polymorphism among individuals (Koh et al., 1997; Liu et al., 1998). RAPD markers produced by primers were effective in determining polymorphism between sorghum lines (Vierling et al., 1994). In this study, approximately 9.1 % of total amplified bands were either specific to rockfish, as shown in Figs. 1～6. 2.7 of the 29.6 amplified bands were found to be polymorphic, as summarized in Table 3. The degree of similarity varied from 0.22 to 0.63 as calculated by bandsharing analysis. Also, the average level of bandsharing was 0.39 ± 0.02 within the rockfish strains (Table 3). For reference, the bandsharing values showed 0.39 in rainbow trout (Yoon 1999) and about 0.73 in zebu cattle (Gwakisa et al., 1994). The RAPD outlines obtained with DNA of different rockfish strains from the Yellow Sea and the Southern Sea in Korea were very similar. There was no significant difference between the two populations far apart from each other. However, this result that a small number of polymorphic bands were identified, implies the genetic variation or diversity due to introduction within the rockfish strains from different geographical regions compared with rainbow trout (Yoon 1999). This genetic diversity in rockfish may have been caused by a transitory increase in broodstock numbers in the hatchery from which the Yellow Sea or the Southern Sea population was derived. Rockfish may have high levels of genome DNA diversity due to the introduction of the wild strains from the Yellow Sea or the Southern Sea. This demonstrates the utility of the technique in strain or breed identification (Welsh et al., 1991; Bishop et al., 1993; Simpson et al., 1993; Gwakisa et al., 1994; Vierling et al., 1994; Lilley et al., 1997; Liu et al., 1998; Oidtmann et al., 1999).

The number and size of the fragments generated strictly depend on the nucleotide sequence of the primer used and on the source of the template DNA, resulting in a genome-specific fingerprint of random DNA fragment. The potential of RAPDs to identify diagnostic markers for strain or species identification in mice (Welsh et al., 1991), in parasites or pathogens (Welsh and McClelland 1990; Bishop et al., 1993; Simpson et al., 1993; Lilley et al., 1997; Oidtmann et al., 1999), in livestock (Gwakisa et al., 1994; Chung et al., 1995; Koh et al., 1998), in plants (Deragon and Landry 1992; Orozco-Castillo et al., 1994) and in fishes (Lloyd et al., 1989; Johnson et al., 1994; Liu et al.,

1998; Yoon 1999) has also been demonstrated. There were strain-specific RAPD fragments in rockfish and there were differences in frequencies of seven primer fragments, as have been reported in catfish (Liu et al., 1998), livestock (Koh et al., 1997) and sorghum (Vierling et al., 1994). In addition to mapping and breeding applications, RAPD-PCR system could be very useful for the rapid certification and quality control of seed production and for all projects based on PCR amplification of specific plant DNA fragments (Deragon and Landry 1992).

Further analysis is required to identify primers that amplify sufficient bands shared by the species to permit a quantitative analysis. Additionally, it seemed to be essential to get better data on the genetic distances between a large number of rockfish populations. Also, in order to interpret more readily differences between two rockfish samples, other molecular genetic methods such as microsatellite, minisatellite, mtDNA and intron polymorphisms and so on should have been provided. Also, in future, additional many populations, a variety of sampling sites and genetic differences between wild and cultured rockfish and so forth will be necessary to get much better results.

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요 약

황해 및 남해산 조피볼락 (*Sebastes schlegeli*) 개체군 사이의 RAPD-PCR 분석에 의한 차이

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황해와 남해산이 각각 50마리씩 총100마리의 조피볼락 (*Sebastes schlegeli*)의 DNA를 혈액으로부터 추출하여 30개의 무작위 primer를 사용한 RAPD(random amplified polymorphic DNAs)-PCR (polymerase chain reaction) 방법으로 분석하였다. genomic DNA의 독특한 특징들이 그 어종군의 특징을 알아내기 위해서 사용되었다. 30개의 primer 중에서 7개의 primer로 부터 증폭된 전체 산물 (307) 중에서 약 67.4%인 207개의 다형성의 산물 (polymorphic products)이 나타났고, 1개의 primer당 약 2.7개의 다양성의 산물 (polymorphic bands)이 확인되었다. 황해산의 경우 bandsharing analysis 를 통해서 볼 때 0.22로부터 0.63까지의 bandsharing value가 확인되었고, 이러한 수치는 0.39±0.02의 평균값을 나타내었다. 황해산과 남해산 2개체군의 RAPD-PCR 산물의 전기영동적 분석을 통해서 볼 때 황해산 조피볼락의 개체들 사이에서 변이가 약간 높게 나타났지만 매우 유사한 특징을 나타내었다. 그러나 일부 개체에서 적은 수이지만 polymorphic bands가 확인되었다. 더 많은 개체군과 다른 연구방법을 통한 연구가 있어야 되겠지만, 이러한 결과는 RAPD 방법을 통하여 2 지역산 조피볼락의 유전적 차이를 어느 정도 확인할 수 있는 가능성을 제시해 주고 있다.

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