J. Fish. Sci. Tech. 6(1), 13−19, 2003

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Genetic Polymorphism of Marsh Clam (*Corbicula leana*) Identified by RAPD-PCR

Jong-Man Yoon*, Kwan-Ha Park[1] and Sun-Nam Choe[2]

**[1]Department of Marine Biomedical Science, College of Ocean Science and Technology, Kunsan National University, Kunsan 573-701, Korea*

[2]Department of Food Science and Technology, College of Ocean Science and Technology, Kunsan National University, Kunsan 573-701, Korea

(Received July 2002, Accepted February 2003)

Genomic DNA from the muscle of marsh clam (*Corbicula leana*) from Gochang, Muan and a Chinese site was extracted to identify genetic differences and similarity by randomly amplified polymorphic DNAs-polymerase chain reaction (RAPD-PCR). Out of 20 primers, seven primers produced amplified fragments which were consistently polymorphic. A total of 1,246 amplified products were produced of which 530 were polymorphic (42.5%). The number of polymorphic bands produced per primer varied from 40 to 122 with an average of 75.7 in marsh clam from Gochang. 3.28 of the 23.0 polymorphic bands per lane were found to be polymorphic. Also, about 4.34% of total polymorphic bands were specific to marsh clam from Gochang. The major common bands of 0.28 kb generated by primer OPB-15 (GGAGGGTGTT) were present in every individuals, which were polymorphic. This common bands in every individuals should be diagnostic of specific strains, species and/or their relatedness. Primer OPB-19 (ACCCCCGAAG) produced the highest number of 12 specific bands. The intra-population variation was revealed in the band patterns identified by this primer. The random primer OPB-12 (CCTTGACGCA) yielded the amplified fragments which were consistently polymorphic between the marsh clams from Gochang and from Muan. This primer produced a total of 77 polymorphic bands: 31 bands from Gochang, 14 from Muan and 32 from the Chinese populations. An average of polymorphic bands were 1.8 from Gochang and 2.5 from the Chinese populations. This value obtained from the Chinese population was higher than those from the two domestic populations. Generally, the RAPD polymorphism generated by these primers may be useful as a genetic marker for strain or population identification of marsh clam.

Key words: *Corbicula leana*, Bandsharing analysis, Genetic similarity, Marsh clam, RAPD-PCR

Introduction

Marsh clam (*Corbicula leana*) is a commercially important mollusk species, which is distributed all over the Yellow Sea. This clam has been used as food materials in various ways in Korea. Recently population density of the marsh clam has been decreased significantly owing mainly to imprudent tidal land reclamation and reckless development during the last four decades. In consequence of the rapid increase in seed production of the marsh clam, there is a need to understand the genetic composition of the marsh clam populations in order to evaluate exactly the latent genetic effects induced by seed production operations. In spite of its economic and scientific importance, little information is available on the genetic relationships among the marsh clam populations in Korea.

So far the various molecular biological methods were used such as randomly amplified polymorphic DNAs (RAPD) (Welsh et al., 1991; Partis and Wells, 1996; Kim et al., 1997; Kwon et al., 1997; Garcia-Mas et al., 2000) based on the polymerase chain reaction (PCR).

*Corresponding author: jmyoon@kunsan.ac.kr

Genomic polymorphic bands generated by PCR amplification of DNA using arbitrary primers had some advantages for detecting DNA polymorphisms between organisms (Garcia-Mas et al., 2000). Thus, the applications of RAPD to aquaculture had been useful to identify genetic similarity and diversity between a few of fish species and mollusks apart from geographic sites (Smith et al., 1997; Callejas and Ochando, 1998; Tassanakajon et al., 1998; Klinbunga et al., 2000; McCormack et al., 2000).

The present work estimated the genetic variation within the populations of the marsh clam (*C. leana*) from Gochang and also elucidated genetic differences among the three populations from Gochang, Muan and a Chinese site using random primer OPB-12 (CCTTGACGCA).

Materials and Methods

Muscle collection

Muscle samples of the marsh clam (*C. leana*) were obtained from a brackish water site in the vicinity of Gochang and Muan in Korea and a Chinese site. The muscles were refrigerated at −70℃ until use. RAPD analysis was performed with DNA samples from a total of 23 marsh clams using 7 different random primers. The same DNA samples were used for each primer in this study. RAPD analysis was also performed with DNA samples from a total of 34 marsh clam using random primers OPB-12 in order to elucidate genetic differences among the three populations.

Sources of genomic DNA

Sliced muscles (approximately 5 g) were placed into 10 mℓ vials, to which an 4 volumes 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 30 min, centrifuged at 1,750×g for 10 min at 4℃. The pellets were transferred to 1.5 mℓ Eppendorf tubes with lysis buffer I, and then mixtures were centrifuged with microcentrifuge at 22,388×g for 1 min. The precipitates were dissolved with 0.8 mℓ of lysis buffer II (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 0.5% SDS). Samples were transferred to 1.5 mℓ Eppendorf tubes and added 15 μℓ of proteinase K solution (10 mg/mℓ). The mixtures were gently inverted and incubated at 65℃ for overnight. After incubation, 0.3 mℓ of 6 M NaCl was added and the mixture were gently pipetted for a few minutes. 0.6 mℓ of chloroform was 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 ice-cold 70% ethanol, centrifuged at 6,289×g for 5 min, and then precipitated. The DNA pellets were air-dried, and then dissolved in TE buffer. Purity was estimated by calculating the ratio of the absorbance (O.D. value) measured at 260−280 nm with a spectrophotometer (Shimadzu, Japan). The final concentration was estimated by agarose electrophoresis and ethidium bromide staining.

Primer and marker

The primers (G+C content, 60−70%), designed for other purpose and chosen arbitrarily for these experiments, were obtained from Operon Technologies, USA. Twenty decamer oligonucleotide primer sequences were used.

Amplification conditions

Amplification reactions were undertaken in volumes of 20 μℓ contained 10 ng of template DNA, 20 μℓ AccuPower premix (Bioneer Co., Korea) and 1.0 unit primer (Operon Technologies, USA). Amplification was performed in a DNA Thermal Cycler (Perkin Elmer, USA). This mixture was followed a predenaturation at 94℃ for 5 min. The thermal cycler programmed for 45 cycles at 94℃, for 1 min for denaturation, at 36℃, 1 min for annealing, at 72℃, 1 min for extension, at 72℃, 5 min for postextension. Amplified products were analysed by electrophoresis with φX174 DNA/*Hae* III marker (Promega Co., USA) in 1.4% agarose gels with TBE and detected by staining with ethidium bromide. The gels were viewed and photographed by photoman direct copy system (Seoulin Co., Korea).

Analytical method

Bandsharing (BS) calculation was somewhat modified by the formula of Jeffreys and Morton (1987) and Mohd-Azmi et al. (2000): 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 among the three lanes, the formula would be: BS=3 (Nabc)/(Na+Nb+Nc) and so on. Only bands that were readily visible were scored. BS values in RAPD outlines were scored by the presence or absence of an amplification product at specific positions in the same gel.

Results

Intra-population variations and bandsharing values

Of the 20 arbitrarily selected primers, seven random primers were used on the basis of the number and frequency of the polymorphisms produced. The genomic DNA polymorphic and/or specific bands were generated using a primer (60−70% of GC contents) to amplify DNA isolated from the muscle of 23 individuals (Figs. 1−4). Seven primers were used for generating a total of 1,246 scorable bands in Gochang population, ranging in size from less than 70 to larger than 1,350 base pairs (bp). These primers produced an average of 178 bands per primer (Table 1). Seven primers produced amplified fragments which were consistently polymorphic. A total of 1,246 amplified products were produced of which 530 were polymorphic (42.5%). The number of polymorphic bands produced per primer varied from 40 to 122 with an average of 75.7 in marsh clam from Gochang (Table 2). Also, about 4.34% of total polymorphic bands were either specific to marsh clam from Gochang as summarized in Table 2.

The various bands in the molecular weight ranged from 0.07 to 0.60 kilobase (kb) pairs were generated by random primer OPB-02 (TGATCCCTGG). It was observed in the marsh clam the population from Gochang (Fig. 1). The identical band patterns were observed in approximately 0.07 kb. The specific minor band patterns in the molecular weight of 0.60 kb were observed from lane 4. The RAPD profiles obtained by a primer with pooled DNAs of individuals were different. The identical bands of RAPD-PCR products by random primer OPB-03 (CATCCCCCTG) were observed in larger than 0.31 kb (Fig. 2). Also, the specific major band in the molecular weight of between 0.60 and 0.87 kb was observed in lane 13. The major common bands of 0.28 kb generated by primer OPB-15 (GGAGGGTGTT) were present in

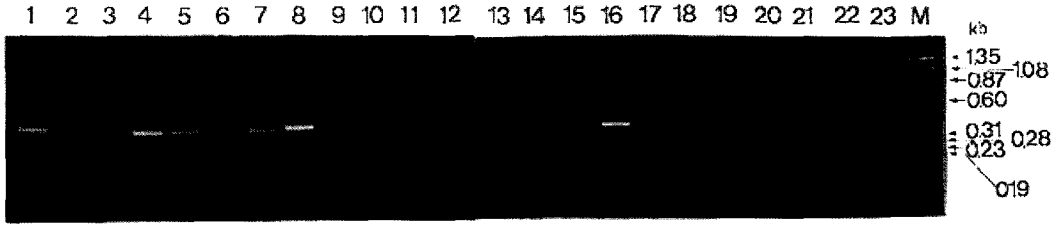

Fig. 1. Individual specific RAPD patterns of marsh clam (*C. leana*) from Gochang amplified by random primer OPB-02 (TGATCCCTGG). Amplification products were performed by a 1.4% agarose gel electrophoresis with TBE (0.09 M Tris, pH 8.5; 0.09 M boric acid; 2.5 mM EDTA) and detected by staining with ethidium bromide. Each lane shows different individual DNA samples. M: Molecular size marker (ϕX174 DNA marker digested with *Hae*III).

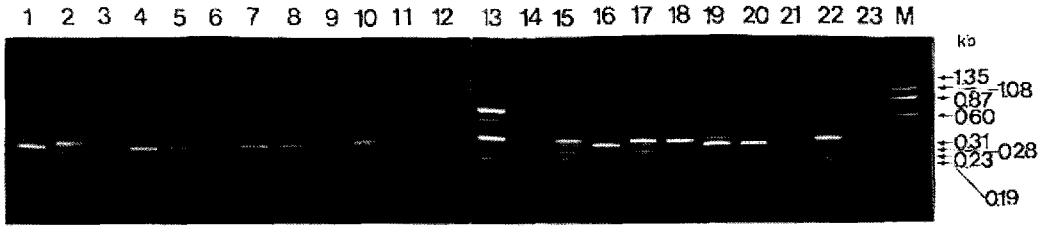

Fig. 2. RAPD-PCR products of marsh clam (*C. leana*) from Gochang amplified by arbitrary primer OPB-03 (CATCCCCCTG). Each lane (1−23) shows different individual DNA samples. M: Molecular size standard (ϕX174 DNA marker digested with *Hae*III).

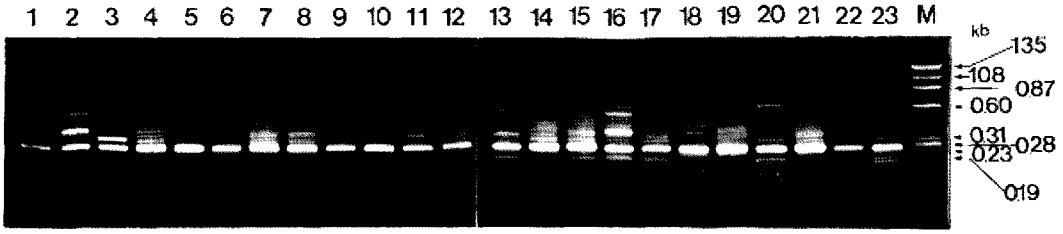

Fig. 3. RAPD profiles in marsh clam (*C. leana*) from Gochang amplified by primer OPB-15 (GGAGGGTGTT). Each lane (1−23) shows eleven individuals used. M: Molecular size marker (ϕX 174 DNA marker digested with *Hae*III).

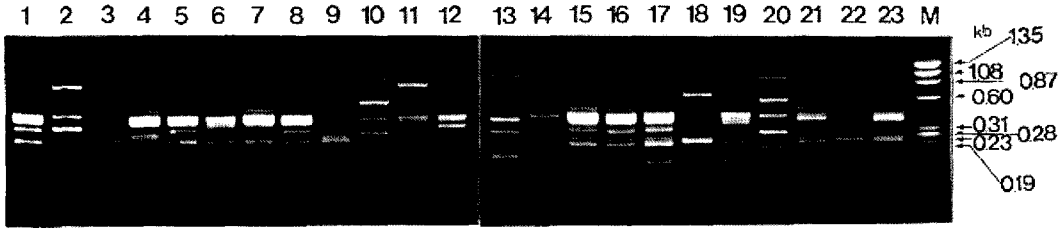

Fig. 4. Specific RAPD polymorphic bands generated in marsh clam (*C. leana*) populations by arbitrary primer OPB-19 (ACCCCCGAAG). Each lane (1−23) shows different individual DNA samples. M: Markers (ϕX174 DNA marker digested with *Hae*III).

Table 1. Total number of products and average number of products per lane generated for 7 primers in marsh clam (*C. leana*) from Gochang (23 bivalves/primer)

Primer No.	Total number of the products	Average number of the products per lane
OPB-02	85	3.7
OPB-03	187	8.1
OPB-07	228	9.9
OPB-11	192	8.3
OPB-12	195	8.5
OPB-15	198	8.6
OPB-19	161	7.0
Total	1,246	54.1
Average	178.0	7.73

Table 2. Number and bandsharing (BS) of polymorphic and specific products generated by 7 random primers of marsh clam (*C. leana*) from Gochang (23 bivalves/primer)

Primer No.	Total number of		Average number of polymorphic products	BS values
	polymorphic products	specific products		
OPB-02	40	3	1.7	0.54
OPB-03	88	2	3.8	0.52
OPB-07	99	3	4.3	0.56
OPB-11	122	0	5.3	0.40
OPB-12	71	2	3.1	0.64
OPB-15	48	1	2.1	0.76
OPB-19	62	12	2.7	0.61
Total	530	23	23.0	0.58 ± 0.04*
Average	75.7	3.29	3.28	

*Standard error

individual, which were poly-morphic (Fig. 3). This common bands which present in every individuals should be diagnostic for specific strains, species and/or their relatedness. Primer OPB-19 (ACCCCCGAAG) produced the highest number of specific bands, which was 12, as shown in Fig. 4. The specific minor band of 0.07 kb was present in lane 22, which was polymorphic. Also, the specific major band in the molecular weight of approximately 0.60 kb was observed in lane 2, 11 and 18 and also in lane 10, 13 and 20 (molecular weight, 1.08 kb). Especially, only a specific band (1.35 kb) was observed in lane 22. The intrapopulation variation was revealed in the band patterns identified by this primer.

The bandsharing values (BS) within the marsh clam population from Gochang altered from 0.40 to 0.76 as calculated by bandsharing analysis, showing an average level of 0.58 ± 0.04 (Table 2). Also, BS value generated by primer OPB-15 was higher than any other primers in the marsh clam population from Gochang, which was 0.76. On the contrary,

primer OPB-11 (GTAGACCCGT) generated the lowest level of BS value, which was 0.40.

Between-populations variations and bandsharing values

This random primer produced polymorphic DNA bands of which the sizes ranged from approximately 100 to 1,150 base pairs within the marsh clam from the three sites such as Gochang, Muan and a Chinese site (Fig. 5). The random primer OPB-12 (CCTTGACGCA) yielded the amplified fragments which were consistently polymorphic between the marsh clam from Gochang and that from Muan. This primer produced a total of 77 polymorphic bands, of which 31 bands in the marsh clam from Gochang, 14 from Muan and 32 in that from a Chinese site (Table 3). An average of polymorphic bands were 1.8 in the marsh clams from Gochang and also 2.5 in those from a Chinese site. This value obtained in the population from a Chinese site was higher than those of the two domestic populations. Also, this primer yielded a total of 18 specific bands, of which 10 bands in the marsh clam from Gochang, 2 from Muan and 6 from a Chinese site (Table 3). The specific major bands from less than 0.19 to 0.23 kb were present, which were polymorphic among popu-lations from three sites. In these ranges, the band patterns were highly reproducible and also very different among the three populations. As calculated by bandsharing analysis, the bandsharing values were 0.63, 0.89 and 0.74 in the marsh clams from Gochang, Muan and a Chinese site, respectively, as summarized in Table 3. BS value obtained from marsh clam population from Gochang was identified the lowest (0.63).

Discussion

Lately, the RAPD-PCR has been applied to detect DNA polymorphisms in various animals/plants/microbes using a large number of random primers (Callejas and Ochando, 1998; Tassanakajon

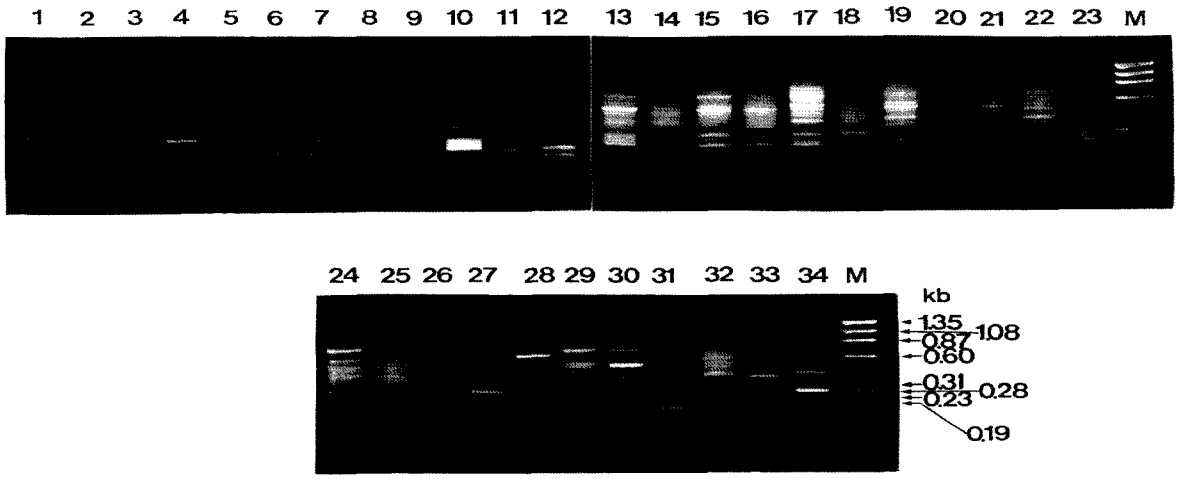

Fig. 5. Individual specific RAPD patterns amplified with arbitrary decamer OPB-12 (CCTTGACGCA) in marsh clam (*C. leana*) from three sites. Each lane (1−34) shows different individual DNA samples (lanes 1−12 from Gochang, lanes 13−23 from Muan and lanes 24−34 from a Chinese site). M indicates φX174 DNA molecular size marker digested with *Hae*III.

Table 3. Total and average number of polymorphic and specific products generated for OPB-12 (CCTTGACGCA) among marsh clam (*C. leana*) populations from three sites (11 or 12 bivalves)

Primer	Number of polymorphic products			Number of specific products			BS values		
	Gochang	Muan	Chinese	Gochang	Muan	Chinese	Gochang	Muan	Chinese
OPB-12	31 (2.6)	14 (1.3)	32 (2.9)	10 (0.83)	2 (0.18)	6 (0.55)	0.63	0.89	0.74

Numbers in the parentheses are the average numbers of polymorphic and specific products generated by random primer OPB-12 in marsh clam.

et al., 1998; Das et al., 1999; Huang et al., 2000). In this study, a number of polymorphic and specific bands were identified by RAPD-PCR method and bandsharing analysis using random primer (OPB-02, -03, -07, -11, -12, -15 and -19), as shown in Figs. 1−4 and summarized in Tables 1−2. Polymorphisms were scored by the presence or absence of the band pattern of amplified products at specific positions expressed by various primers (Smith et al., 1997; Tassanakajon et al., 1998). The sizes of amplified fragments obtained in this study were from less than 70 to larger than 1,350 bp. This indicated that the genome size of the marsh clam was similar to that of the blue catfish analyzed by Liu et al. (1998). Generally, the size and number of the bands were strictly dependent on the nucleotide sequence of the primer and the source of the template DNA, resulting in a genomespecific DNA band.

In this study, seven primers generated 530 highly polymorphic markers, producing approximately 3.28 polymorphic bands per primer in the marsh clam from Gochang. Especially, about 4.34% of total polymorphic bands were either specific to the marsh clam from Gochang. These results indicated that there were population-specific RAPD fragments in the marsh clam and there were differences in frequencies of the seven primer fragments, as reported in catfish (Liu et al., 1998), livestock (Jeffreys and Morton, 1987; Koh et al., 1998; Mohd-Azmi et al., 2000), seaweed (Kim et al., 1997; Hong et al., 1997) and melon (Garcia-Mas et al., 2000).

Especially the specific minor band patterns in the molecular weight of 0.60 kb generated by random primer OPB-02 were observed from lane 4. Also, primer OPB-19 produced the highest number of specific bands, which was 12, as shown in Fig. 4. In this study, we identified that about 1.85% of total amplified bands were specific to the marsh clam. This specific primer was also found to be useful for the individual identification of the marsh clam, resulting from the different DNA polymor-phism among individuals. It was same to the result of Liu et al. (1998) obtained in catfish of the species. Some intraspecific or intrastrain RAPD variations were also observed in different strains or individuals (Welsh et al., 1991; Liu et al., 1998). Additionally, McCormack et al. (2000) identified that a minimum of 93% of genotypic variance occurred among individuals within populations. They found more variations within a strain or a population than between strains and populations considered. Especially, in marine organisms, the percentages of polymorphic bands in the five geographic populations investigated with the black tiger shrimp (*Penaeus monodon*) varied from 51.5 to 57.7% (Tassanakajon et al., 1998). They reported that RAPD analysis yielded a total of 252 genotypes.

In this study, the bandsharing values within the marsh clam population from Gochang were altered from 0.40 to 0.76 showing an average level of 0.58 ± 0.04 (Table 2). Also, BS value generated by the primer OPB-15 was higher than those by any other primers in the marsh clam populations, which was 0.76. On the contrary, the primer OPB-11 generated the lowest level of BS value (0.40). As mentioned in Table 3, the average level of bandsharing value in the population from Gochang showed lower than those in the populations from the other two sites. The bandsharing value of Gochang population obtained by the random primer OPB-12 apparently showed the lowest value (0.63). On the contrary, the bandsharing value in the population from Muan showed a larger shift as compared with those of the populations from the other two sites (0.89). In general, bandsharing values were calculated as an expression of similarity of RAPD polymorphic bands of animals from either the same or the different breeds (Jeffreys and Morton, 1987; Mohd-Azmi et al., 2000). Besides, this analysis method could be used as an potential genetic marker for linkage analysis with economically important traits in fish (Smith et al., 1997; Liu et al., 1998; Tassa-nakajon et al., 1998). This result also demonstrated this technique is useful for identification of strains or breeds (Welsh et al., 1991; Dias Neto et al., 1993; Liu et al., 1998). The potential of RAPDs to identify diagnostic markers for strain or species identification in mice (Welsh et al., 1991), parasites or pathogens (Dias Neto et al., 1993; Kwon et al., 1997), livestock (Koh et al., 1998), plants (Deragon and Landry, 1992; Das et al., 1999) and fish (Partis and Wells, 1996; Liu et al., 1998) has also been demonstrated. RAPD-PCR system could be very useful for the rapid certification, quality control of clam seed production and all the projects based on

PCR amplification of specific plant DNA fragments (Deragon and Landry, 1992).

In the present study, we demonstrated the successful use of RAPD analysis to identify within-population variations of the marsh clam. In the future, studies dealing with many individuals, sampling sites and populations of the marsh clam will be necessary. Also, additional researches such as microsatellite, minisatellite, AFLP and sequence techniques are likely to maximize the efficiency of efforts in various fields of interest in aquaculture organisms.

Acknowledgments

The authors are grateful to the Fisheries Science Institute of Kunsan National University for equipments and funding in the program year of 2002.

References

Callejas, C. and M.D. Ochando. 1998. Identification of Spanish barbel species using the RAPD technique. J. Fish Biol., 53, 208−215.

Das, S., J. Rajagopal, S. Bhatia, P.S. Srivastava and M. Lakshmikumaran. 1999. Assessment of genetic variation within *Brassica campestris* cultivars using amplified fragment length polymorphism and random amplification of polymorphic DNA markers. J. Biosci., 24, 433−440.

Deragon, J.M. and B.S. Landry. 1992. RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. PCR Methods Appl., 1, 175−180.

Dias Neto, E., M. Steindel, L.K.F. Passos, C. Pereira de Souza, D. Rollinson, N. Katz, A.J. Romanha, S.D.J. Pena and A.J.G. Simpson. 1993. The use of RAPDs for the study of the genetic diversity of *Schistosoma mansoni* and *Trypanosoma cruzi*. DNA Fingerprinting, 69, 339−345.

Garcia-Mas, J., M. Oliver, H. Gomez-Paniagua and M.C. Vicente. 2000. Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theor. Appl. Genet., 101, 860−864.

Hong, Y.K., C.H. Sohn, K.W. Lee and H.G. Kim. 1997. Nucleic acid extraction from seaweed tissues for polymerase chain reaction. J. Mar. Biotechnol., 5, 95−99.

Huang, B.X., R. Peakall and P.J. Hanna. 2000. Analysis of genetic structure of blacklip abalone (*Haliotis rubra*) populations using RAPD, minisatellite and microsatellite markers. Mar. Biol., 136, 207−216.

Jeffreys, A.J. and D.B. Morton. 1987. DNA fingerprints of dogs and cats. Anim. Genet., 18, 1−15.

Johnson, S.L., C. Midson, E.W. Ballinger and J.H. Postlethwait. 1994. Identification of RAPD primers that reveal extensive polymorphisms between laboratory strains of zebrafish. Genomics, 19, 152−156.

Kim, L.G., H.J. Jin, Y.S. Kim, J.Y. Park, K.W. Nam and Y.K. Hong. 1997. Discrimination of two red algae *Acrosorium polyneurum* and *A. yendoi* using polymerase chain reaction technique. J. Kor. Fish. Soc., 30, 585−588.

Klinbunga, S., P. Ampayup, A. Tassanakajon, P. Jarayabhand and W. Yoosukh. 2000. Development of species-specific markers of the tropical oyster (*Crassostrea belcheri*) in Thailand. Mar. Biotechnol., 2, 476−484.

Koh, M.C., C.H. Lim, S.B. Chua, S.T. Chew and S.T. Phang. 1997. Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. Meat Sci., 48, 275−285.

Kwon, M.G., J.Y. Lee, S.I. Park, T. Iida, I. Hirono and T. Aoki. 1997. RAPD analysis of atypical *Aeromonas salmonicida* isolated in Japan. Fish Pathol., 32, 109−115.

Liu, Z., P. Li, B.J. Argue and R.A. Dunham. 1998. Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. Furcatus*) and their F_1, F_2 and backcross hybrids. Anim. Genet., 29, 58−62.

McCormack, G.C., R. Powell and B. Keegan. 2000. Comparative analysis of two populations of the brittle star *Amphiura filiformis* (Echinodermata: Ophiuroidae) with different life history strategies using RAPD markers. Mar. Biotechnol., 2, 100−106.

Mohd-Azmi, M., A.S. Ali and W.K. Kheng. 2000. DNA fingerprinting of red jungle fowl, village chicken and broilers. Asian-Aus. J. Anim. Sci., 13, 1040−1043.

Partis, L. and R.J. Wells. 1996. Identification of fish species using random amplified polymorphic DNA (RAPD). Mol. Cell. Probes, 10, 435−441.

Smith, P.J., P.G. Benson and S.M. McVeagh. 1997. A comparison of three genetic methods used for stock discrimination of orange roughy, *Hoplostethus atlanticus:* allozymes, mitochondrial DNA, and random amplified polymorphic DNA. Fish. Bull., 95, 800−811.

Tassanakajon, A., S. Pongsomboon, P. Jarayabhand, S. Klinbunga and V. Boonsaeng. 1998. Genetic structure in wild populations of black tiger shrimp (*Penaeus monodon*) using randomly amplified polymorphic DNA analysis. J. Mar. Biotechnol., 6, 249−254.

Welsh, J., C. Petersen and M. McClelland. 1991. Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. Nucleic Acids Res., 19, 303−306.