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Development of novel and polymorphic microsatellite DNA loci from *Haliotis ovina*

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ABSTRACT. Twelve microsatellite loci were developed from *Haliotis ovina* by the magnetic bead hybridization method. Genetic variability was assessed using 30 individuals from 3 wild populations. The number of alleles per locus ranged from 2 to 5, and the polymorphism information content ranged from 0.1228 to 0.6542. Observed and expected heterozygosities ranged from 0.0000 to 0.7778 and 0.1288 to 0.6310, respectively. These loci should provide useful information for genetic studies such as genetic diversity, pedigree analysis, construction of genetic linkage maps, and marker-assisted selection breeding in *H. ovina*.

Key words: Genetic markers; *Haliotis ovina*; Microsatellite markers

INTRODUCTION

The abalone is a marine gastropod and widely distributed along the coastal waters of tropical and temperate regions (Geiger, 1999). Among ~20 commercially important abalone (Jarayabhand and Paphavasit, 1996), *Haliotis ovina*, which is also primarily distributed in tropical areas, has high economic importance with regard to the fishing and farming industries. Although *H. ovina* is not a main target species for aquaculture in China, the genetic information of *H. ovina* is essential because of the rapid decline of China's natural resources due to overexploitation and pollution. Thus, further conservation strategies and recovery plans require basic genetic information on *H. ovina*.

Microsatellite analysis is an effective molecular tool and has a variety of applications. Until now, microsatellite markers in *H. rubra* (Huang and Hanna, 1998; Evans et al., 2000), *H. asinina* (Selvamani et al., 2000), *H. discus hannai* (Li et al., 2002), *H. kamtschatkana* (Miller et al., 2001), and *H. discus discus* (Sekino and Hara, 2001) have been reported. Also, microsatellite markers have been applied to analyze the genetic background of *H. rubra* (Evans et al., 2004; Li et al., 2006), *H. conicorpora* (Li et al., 2006), *H. discus hannai* (Li et al., 2003, 2004), *H. midae* (Evans et al., 2004), and *H. asinina* (Selvamani et al., 2001). To our knowledge, the genetic study of *H. ovina* has been limited, to date (Klinbunga et al., 2003; Li, 2006, 2009; Li et al., 2008).

MATERIAL AND METHODS

One wild individual from the Yingzhou population was used for simple sequence repeat (SSR) primer screening. Genomic DNA was extracted from the foot muscle using the CTAB method and examined by agarose gel electrophoresis (1%) and ultraviolet spectrophotometer. The sample was then digested with *Mbo*I (Fermentas, Canada) for 3 h (37°C). Subsequently, *Mbo*I adapter1 (5'-GATCGTCGACGGTACCGAATTCT-3') and *Mbo*I adapter2 (5'-GTCAAGAATTCGGTACCGTCGAC-3') were ligated to the digested products using T4 DNA ligase. The ligated product was hybridized with biotin-labeled SSR probes [$(GT)_{15}$ and $(CT)_{15}$], and the hybrid mixture was incubated with magnetic beads coated with streptavidin. The recovered DNA fragments were amplified using *Mbo*I primer (5'-GTCAAGAATTCGGTACCGTCGAC-3') and the polymerase chain reaction (PCR) products were purified by GenCleanPCR (GENEray, Shanghai, China) to remove the extra dNTPs and adaptors. The purified products (4.5 µL) were ligated to pMD19-T (0.5 µL) and then transformed into *Escherichia coli*. The transformants were selected on ampicillin plates.

A total of 576 colonies were chosen to mix with 60 µL LB liquid medium and then shake cultured for 3 h (37°C, 140 rpm). DNA fragments >500 bp were selected for sequencing by the Beijing Liuhehuada Company. A total of 40 pairs of primers were designed using Primer Premier 5.0 (Clarke and Gorley, 2001). Thirty individuals from Yingzhou (109°51'9"E; 18°22'37"N), Anyou (109°33'14"E; 18°12'11"N), and Yalong Wan (109°38'36"E; 18°11'13"N) were then used to analyze polymorphisms of the loci.

The PCR amplification conditions consisted of an initial denaturation at 94°C for 5 min; 30 cycles at 94°C for 45 s, annealing temperature (Table 1) for 45 s, and an extension at 72°C for 1 min; and a final extension at 72°C for 10 min. The PCR products were electrophoresed via Sequi-Gen Sequencing Cell (BIO-RAD), and the H_O, H_E, N_A, and PIC (polymorphism information content) were calculated using the GENEPOP 4.0 software (Rousset, 2010) and CERVUS 3.0 (Gao and Wu, 2005) (Table 1).

RESULTS AND DISCUSSION

Twelve polymorphic microsatellite primers were developed. The number of alleles per locus ranged from 2 to 5, and the PIC ranged from 0.1228 to 0.6542. The H_O and H_E ranged from 0.0000 to 0.7778 and 0.1288 to 0.6310, respectively (Table 1). MICRO-CHECKER (Van Oosterhout et al., 2004) was applied to check microsatellite data. Genotyping error was not detected among the loci.

Table 1. Basic genetic information for 12 microsatellite markers in *Haliotis ovina*.

Locus ID	Primer sequence (5'-3')	N_A	Repeat motif	Allele size (bp)	PIC	H_O	H_E	Ta (°C)	GenBank accession No.
YB4	F: ACACGAACCAAGATTAGAGG R: TGAGAGAGGAGAACAAGGAA	3	$(TC)_5(CA)_3N_2(CA)_{20}$	190-225	0.2763	0.0741	0.1426	49	JN561131
YB14	F: TGGTCGCTGGAGAATCGT R: TGCCGTGACACTGGAAAG	5	$(CTCA)_3(CA)_2(CTCA)$	180-250	0.5395	0.5517	0.5638	45	JN561132
YB15	F: GACGACACCGATAGGAGA R: AAGAGGGACAGAGGCTTG	5	$(CA)_{26}CG(CT)_{20}$	180-210	0.4013	0.1852	0.3026	40	JN561133
YB22	F: GGAACTTCAACATCCCCT R: TTCAAACTTAGAACCCGC	2	$(GA)_{18}$	275-285	0.5169	0.0769	0.5077	46	JN561134
YB23	F: ATTTCCCCGAGTACACCATACG R: TAGGACTTCAGATTGACGAGCG	3	$(GA)_9N_2(GA)_{19}$	235-285	0.3361	0.1154	0.1802	55	JN561135
YB48	F: ACTGTGTCTGAGTGGGGTATT R: AAGTTTTTTTGTGAGTGAGCA	4	$(CA)_{22}$	160-175	0.6184	0.7778	0.6101	44	JN561136
YB55	F: TTGCCTATGTCAGCACAGTTC R: AAGCAATCAACCAATCACCTG	5	$(GT)_{17}$	185-210	0.5360	0.5357	0.5143	46	JN561137
YB68	F: TGTGCTGTGCTATAAATGTCAC R: TTGTCTTTGTATCGGAGGTTG	3	$(CA)_{18}$	200-200	0.5936	0.4706	0.5419	46	JN561138
YB70	F: TCCATTTTGTGATGACTCC R: GACGACACTTTGTTGCTCT	3	$(CA)_4N_2(CA)_{21}$	160-200	0.1228	0.0667	0.1288	45	JN561139
YB77	F: GATGTAGCAAAATGTAACCCC R: ATCCCCTCGCAAACCCAG	3	$(GA)_{47}$	250-270	0.6542	0.6000	0.6310	46.5	JN561140
YB87	F: CTGATCTCTGTGCCAGGTACTA R: GACCAAACTAACATTCTCACGC	2	$(GA)_{52}$	135-150	0.5231	0.2500	0.3577	47	JN561141
YB88	F: CAAAGTTCAGTTGATTACTGGC R: TAACATTCCTGGTATTGCGAC	2	$(CA)_3N_2(CA)_8N_2(CA)_{21}$	125-140	0.1754	0.0000	0.1317	50.5	JN561142

Ta = annealing temperature; PIC = polymorphism information content.

Seven of the 12 newly developed microsatellite markers were highly polymorphic (PIC > 0.5) and could be useful in further genetic studies on *H. ovina*, such as genetic diversity, pedigree analysis, construction of genetic linkage maps, and marker-assisted selection breeding studies. In addition, the genetic diversity index of *H. ovina* in our study was a slightly lower than those of *H. discus* (Zhan et al., 2008) and *H. diversicolor*. This may be due to the overfishing of its wild resources, which could result in small population size and the overexploitation of its natural environment. Furthermore, this result could be related to the samples used to detect the polymorphism of the microsatellite loci and the microsatellite loci used to analyze the genetic diversity of *H. ovina*. Thus, in order to reveal the accurate genetic background of wild *H. ovina*, more sample areas and larger sample sizes should be utilized in future studies.

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