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Short Communication

The isolation and characterization of twelve novel microsatellite loci from *Haliotis ovina*

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Twelve (12) microsatellite loci were developed from *Haliotis ovina* by magnetic bead hybridization method. Genetic variability was assessed using 30 individuals from three wild populations. The number of alleles per locus was from 2 to 5 and polymorphism information content was from 0.1228 to 0.6542. The 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*, microsatellites.

INTRODUCTION

Abalone belongs to marine gastropods and is widely distributed along the coastal waters of tropical and temperate areas (Geiger, 1999). Among about 20 commercially important abalone (Jarayabhand and Paphavasit, 1996), *Haliotis ovina*, which is also mainly distributed in tropical areas, has a high economic importance for it is both fished and farmed. Although, *H. ovina* is not a main target species for aquaculture in China, the genetic information of *H. ovina* is so essential because of the rapid decline of its natural resources due to overexploitation and the pollution of its living environment. Thus, further conservation strategies and recovery plans require the basic genetic information of *H. ovina.*

Microsatellite is an effective tool and has been applied to various genetic aspects. Up until now, microsatellite markers in *Haliotis rubra* (Huang and Hanna, 1998; Evans et al., 2000), *Haliotis asinina* (Selvamani et al., 2000), *Haliotis discus hannai* (Li et al., 2002), *Haliotis 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* (Li et al., 2006; Evans et al., 2004), *Haliotis conicorpora* (Li et al., 2006), *H. discus hannai* (Li et al., 2003, 2004), *Haliotis midae* (Evans et al., 2004) and *Haliotis asinine* (Selvamani et al., 2001). To our knowledge, the genetic study of *H. ovina* is very limited (Li, 2006, 2009; Li et al., 2008; Klinbunga et al., 2003).

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MATERIALS AND METHODS

One wild individual from Yingzhou population was used in SSR primer screening. Genomic DNA was extracted from foot muscle using CTAB method which was examined by agarose gel electrophoresis(1%) and ultraviolet spectrophotometer, and then was digested with *MboI* (Fermentas) for 3 h (37°C) and then, *MboI* adapter1 (5'- GATCGTCGACGGTACCGAATTCT-3') / *MboI* adapter2 (5'-GTCAAGAATTCGGTACCGTCGAC-3') were ligated to the digested products using T4 DNA ligase. The ligated product was hybridized with biotin-labeled sequence repeats (SSR) probes $(GT)_{15}$, $(CT)_{15}$, and the hybrid mixture was incubated with magnetic beads coated with streptavidin. The recovered DNA fragments were amplified using *MboI* primer (5'-GTCAAGAATTCGGTACCGTCGAC-3') and the polymerase chain reaction (PCR) products were purified by GenCleanPCR (Generay) to remove the extra dNTP and adaptors. The purified products (4.5 µl) was ligated to the 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 ul LB liquid medium, and then shake cultured for 3 h (37°C, 140 prm). DNA

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

Locus ID	Primer sequence (5'-3')	Na	Repeat motif	Allele size (bp)	PIC	Ho	He	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

Na, Number of alleles; Ta, annealing temperature; H_e, expected heterozygosity; H_o, observed heterozygosity; PIC, polymorphism information content.

fragments above 500 bp were selected for sequencing in Beijing Liuhehuada Company. 40 pairs of primers were designed using Primer Premier5.0 (Clarke and Gorley, 2001). 30 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 polymorphism of the loci.

The PCR amplification conditions consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 45 s, annealing temperature (Table 1) for 45 s, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The PCR products were electrophoresed on Sequi-Gen Sequencing Cell (BIO-RAD) and then the observed heterozygosity (H_o), expected heterozygosity (H_e), number of alleles (N_a) and the PIC (polymorphism information content) were calculated using the software GENEPOP 4.0 and CERVUS 3.0.

RESULTS AND DISCUSSION

Twelve (12) polymorphic microsatellite primers were developed. The number of alleles per locus was from two to five and PIC was from 0.1228 to

0.6542. The observed and expected heterozygosities ranged from 0.0000 to 0.7778 and 0.1288 to 0.6310, respectively. MICRO-CHECKER (Van Oosterhout et al., 2004) was applied to check microsatellite data. No genotyping error among the loci was detected.

Seven of the 12 newly developed microsatellite markers are considered to be high polymorphism (PIC>0.5) and could be useful in the further genetic studies in *H. ovina*, such as genetic diversity, pedigree analysis, construction of genetic linkage maps and marker-assisted selection breeding study. Besides, the genetic diversity index of *H. ovina* in our study was a little lower than that of *H. discus* (Zhan et al., 2008) and *Haliotis diversicolor* Reeve (Xin et al., 2008). 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. Also, this may relate to the samples we used to detect the polymorphism of the microsatellite loci and the microsatellite loci we chose to analyze the genetic diversity of *H. ovina*. So, in order to reveal the accurate genetic background of wild *H. ovina,* more sample areas and bigger samples size should be included in further study.

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