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Novel polymorphic microsatellite markers isolated from the pen shell *Atrina pectinata* (Mollusca: Bivalvia: Pinnidae)

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ABSTRACT. In this study, we isolated 21 novel polymorphic microsatellite DNA loci from the pen shell *Atrina pectinata* using magnetic-bead hybridization enrichment. The characteristics of these loci were tested using a population of 30 individuals collected from the Penglai coast, Shandong Province. The number of alleles ranged from 2 to 13, and polymorphism information content (PIC) varied from 0.1730 to 0.8954. Values for observed heterozygosity (H_O) and expected heterozygosity (H_E) ranged from 0.0714 to 0.9231 and from 0.1948 to 0.9237, respectively. Four loci deviated significantly from Hardy-Weinberg equilibrium. The newly developed microsatellite markers will be beneficial in assessing the genetic diversity, population structure and genetic conservation of *A. pectinata*, and in other relevant research.

Key words: *Atrina pectinata*; Microsatellite loci; Polymorphic

INTRODUCTION

The pen shell, *Atrina pectinata*, belonging to the family Pinnidae, is a kind of eurythermal, euryhaline and large wedge-shaped bivalve, which is widely distributed in the tropical and subtropical regions of the Indian and Western Pacific oceans (Wang et al., 1993). Its adductor muscle and mantle are very important sea foods in Korea, Japan, and China, which is attributed to its good taste and high nutritional value. In China, *A. pectinata* has high commercial and scientific research value because of the lack of large-scale culture. In the past decades, many problems such as overfishing, disease, deterioration of marine eco-environment have drastically reduced the amount of wild sources. However, to date, the research on the genetic characteristics of the *A. pectinata* population in China has been limited (Ren et al., 2005). Thus, to indentify the status of germplasm resources of Chinese *A. pectinata*, many further relevant studies on population structure and genetic variation are required.

Microsatellites or simple sequence repeats have been generally accepted as an effective tool to study genetic structure and diversity in animal and plant populations, and they have also been applied in many aquatic research fields. Nowadays, they are used successfully for paternity analysis and kinship studies (Gerloff et al., 1995), DNA fingerprinting and diversity studies (Katti et al., 2001), and genome mapping (Zane et al., 2002) in many organisms, because they have the advantage of abundant polymorphism, co-dominant inheritance, rapid and convenient detection, and so on. Therefore, the development of microsatellite markers can contribute significantly to obtaining more detailed insight into population genetics. Some microsatellite markers from *A. pectinata* have been isolated by Liu (2009) and Chen (2012). However, more effective microsatellite loci will be beneficial to the study of population genetics and construction of a genome map. Here, we present 21 new microsatellite markers in an important bivalve species from the family Pinnidae.

MATERIAL AND METHODS

Thirty individuals of *A. pectinata* were captured from the Penglai coast, Shandong Province, China. The live organisms were carried to the laboratory, and the adductor muscles of the samples collected were then preserved in alcohol until DNA extraction. Briefly, genomic DNA was extracted from adductor muscle after being digested by proteinase K/sodium dodecyl sulfate solution. DNA was purified using the standard phenol/chloroform method and precipitation with 3 M NaCl/ethanol (Aljanabi and Martinez, 1997). DNA was digested with restriction enzyme *Msp*I (Fermentas), and ligated to *Msp*I adaptors (AdapF5'-GATCATGAGT CCTGCT-3'/AdapR5'-CGAGCAGGACTCAGAA-3') using T4 DNA ligase (Fermentas). Subsequently, the fragments ranging from 300 bp to 1000 bp were selected by separation on 1% agarose gels. The purified fragments were then pre-amplified by polymerase chain reaction (PCR) using AdapF as primer to ensure successful and specific amplification. The PCR products were denatured at 94°C for 5 min and hybridized to biotin-labeled

(AC)15 and (AG)15 probes at 65°C for 30 min in a PCR amplifier. Fragments containing microsatellite repeats were enriched by Streptavidin Magnesphere (Promega) and amplified by AdapF to make it double-stranded. The amplification products were ligated into PMD-18 vector (Takara) at 16°C for 30 min, and the ligated fragments were electroporated into Top10 competent *Escherichia coli* cells (Transgene) and cultured on a LB substrate with ampicillin, IPTG and x-Gal overnight (12-14 h). The recombinant colonies were transferred into LB culture medium with ampicillin, and shaken for 8 h at 37°C. A 1-μL aliquot of bacterial suspension was used as template to determine the length of plasmid insert sequence, and the positive clones were then selected for sequencing using the ABIPRISM 3730 Genetic Analyzer (Applied Biosystems). Finally, 109 clones were sequenced, where 93 (85.32%) clones provided available sequences and 66 contained microsatellite loci with at least five uninterrupted repeats. Primer pairs were designed with the highest possible annealing temperature using Primer Premier 5.0.

The primer pairs were tested on 30 individuals collected from Penglai. PCR amplifications were performed in a volume of 25.0 μL, including 50 ng genomic DNA, 1X PCR buffer (Transgene), 0.2 mM dNTPs, 0.5 μM each primer, and 1.0 U Taq DNA polymerase (Transgene). The thermal profile included an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing temperature (Table 1) for 30 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min.

The PCR products were resolved on an 8% denaturing polyacrylamide gel in 1X TBE buffer containing 8 M urea. After electrophoresis at 450 V for 3 h and silver nitrate staining, molecular sizes of the amplified fragments were estimated using a pBR322DNA/*Bsu*RI Marker. Alleles were counted artificially, and allele size was identified using the ladder as a length reference. Observed (H_O) and expected (H_E) heterozygosities and tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were calculated using the POPGENE software (Version1.31), and PIC was determined with the program PIC-CALC (Version 0.6). Results were corrected for multiple simultaneous comparisons using Bonferroni correction (Rice, 1989).

RESULTS AND DISCUSSION

The characteristics of 21 loci are shown in Table 1 and their sequences have been submitted to GenBank. In total, 21 of 66 loci were polymorphic in the population of 30 individuals tested, while the remaining loci were monomorphic or showed poor amplification. Among the 21 detectable loci, the number of alleles observed ranged from 2 to 13 with an average of 6.35. H_O and H_E ranged from 0.0714 to 0.9231 and from 0.1948 to 0.9237, with an average of 0.46616 and 0.609375, respectively. Four loci deviated significantly from HWE after Bonferroni correction (adjusted P = 0.00238), while 17 loci showed no deviation from HWE. No significant genotypic linkage disequilibrium was found between any pair of loci. In this study, we isolated 21 new polymorphic microsatellite markers from *A. pectinata*, which provide a useful, powerful tool for future research on the assessment of germplasm resources and population conservation of *A. pectinata*.

Table 1. Characteristics of 21 microsatellite loci isolated from *Atrina pectinata* tested in 30 individuals collected from Penglai coast, Shandong Province, China.

Locus	Accession No.	Primer sequences(5'-3')	Repeat motif	Size/bp	TA/°C	N_A	H_O	H_E	PIC
Ap02	JQ411058	F: ACACTTTCGTTCATCCAC R: CTTTATACGGCCATGTTTCA	$(AC)_7$	111	58	8	0.5714	0.7875	0.7435
Ap03	JQ411059	F: CTCCAAGAAATAGCGGCAT R: CGGCGTCAAGTTACAATCAC	$(GC)_7(GT)_5$	207	60	2	0.4667	0.3638	0.2938
Ap06	JQ411060	F: CGGGCTCTGATAAAAGTC R: GCGCGAAGGATGTAAATAAT	$(CG)_6(TG)_3AG(TG)_{18}$	200	57	7	0.5385	0.8250	0.7820
Ap07	JQ411061	F: TCCGAGAACTTATTGCGACAC R: CGCTTTGCTGTCCGTGTT	$(AC)_8(AT)_6$	201	60	4	0.5333	0.6062	0.5171
Ap09	JQ411062	F: CGGCTCTCGCAATACGATAACG R: CACACCAAGAGACAGAACCACA	$(TG)_{10}$	337	62	9	0.3793	0.7604	0.7246
Ap10*	JQ411066	F: TTGCTTTCTACGCCACAG R: TCTGAAACCGATATTGAACACTG	$(TC)_{12}$	298	57	6	0.1852	0.6737	0.6130
Ap12	JQ411064	F: TCAACATTTCACCCAGTA R: TAATCACTACGATACGGA	$(GT)_7$	178	57	6	0.7241	0.8155	0.7723
Ap16	JQ411065	F: TCGTGATAGTTCCTTTCCA R: ATGTTTTACCTGGACGGA	$(CA)_8$	254	57	6	0.6897	0.8028	0.7592
Ap18	JQ411066	F: TTGTGTTATGCGTGGGAG R: CGTGCTTCAGTGGTTTAGT	$(CT)_6$……$T)GC_5$	228	62	2	0.0714	0.1948	0.1730
Ap23	JQ411067	F: GCAAAAATAAATAGACGGAGGGC R: GACAGACAGGCAGACAGA	$(AC)_9$……$C)AGAC_6$	124	62	5	0.3667	0.6497	0.5922
Ap25	JQ411068	F: AGTGTGTAGTGACGGGATGT R: GCCAATGGTATGAACGGAAAGT	$(AC)_9$	194	60	4	0.2667	0.4028	0.3494
Ap30*	JQ411069	F: ATTTGCTTTCTACGCCAC R: GTAAAACCTCTGAAACCGA	$(TC)_{12}$	308	60	6	0.1818	0.7791	0.7265
AP40	JQ411070	F: GCCAACTGAAAATACTCCA R: GGTTGTGTGTATATGTGC	(AG)16T(AG)8	195	57	10	0.4231	0.8801	0.8489
AP42	JQ411071	F: ACGAATTAGGTTAATGTTGAC R: GCCGTGCTTATATGTGTA	$(AT)_2(AC)_3AG(CA)_4(GA)_{20}$	205	57	12	0.7500	0.9034	0.8740
AP46	JQ411072	F: TACATTCTGCGTATCGTC R: CGGTCAAACTTGCTAACAC	$(TG)_8(AG)_4(TG)_8$	224	58	13	0.7586	0.9147	0.8903
AP48	JQ411073	F: GCCACCACCATATAGCTG R: GTCGTAATTCTCCAAATGCAG	$(AG)_{14}G(GA)_{15}$	314	61	7	0.4000	0.7861	0.7354
AP39	JQ411074	F: CGATCATAATGGAAGTGTC R: TCGTGAAATAGACATCTTGTTATG	$(CT)_{16}$	302	58	6	0.7931	0.8113	0.7663
AP49*	JQ411075	F: GTAGCATGACGCGCGAAG R: TACCCTTCTGATATCCCGTGC	$(CA)_{18}CT(CA)_3(CG)_6$	173	61	6	0.7233	0.3333	0.6638
APQ142	JQ411076	F: TATTCTCCGATTAGGTTGCCGTC R: GCATACCGCCCAATGTTACG	$(GT)_7$	110	60	2	0.2160	0.2414	0.1897
APR152	JQ411077	F: ACTCGCAGGCTTGAAATAG R: GTGAAAATGAATGAGATCGGGT	$(AC)_{13}$	226	57	12	0.9130	0.9237	0.8954
APU157*	JQ411078	F: AGAGTATGGAATGGACTATCAG R: AGACTACCTCCATAGAAACAC	$(AC)_9$	388	58	7	0.9231	0.8318	0.7929

*Significant deviation from HWE after Bonferroni's correction ($P < 0.00238$). F = forward primer/ R = reverse primer; TA = annealing temperature; N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphic information content.

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