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SHORT COMMUNICATION

Isolation and characterization of 12 microsatellite loci in the noble scallop, *Chlamys nobilis*

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Introduction

Primarily distributed along the coastal waters of southern China and Japan, the noble scallop *Chlamys nobilis* has been a species of economic importance in the local mollusk industry for the tropical and subtropical zone of China. In recent years, the rising demand for scallop products and the decline of natural resource have led to increasing aquaculture practices of this species. Therefore, interest in and requirement for studies of genetic diversity and stock analysis of this species has increased in the scientific community. At the same time, mortality problems unfortunately began to affect this species in aquaculture farms since 2006 (Prof. Wang A, pers. comm.), and the demand for maintenance, management of high-quality stocks and genetic improvement have been stressed by both the industry sector and the scientific community. Consequently, the development and application of microsatellite markers are highly desirable for these purposes as in other species (Plieske & Struss 2001; Cruz, Ibarra, Fiore, Galingdo-Sánchez & Mendoza 2005; Cui, Shen, Yang, Gong & Gu 2005; Maremi, Kenji, Nadezhda, Aoi, Tomoki, Takafumi, Vladimir & Koji 2005; Zhan, Bao, Yao, Wang, Hui & Hu 2006; Wang, Ren & Yu 2008). In this note, we report the isolation and characterization of 12 polymorphic microsatellite loci from the noble scallop, *C. nobilis*.

Firstly, the total genomic DNA was extracted from the adductor muscle of a single individual, using the standard phenol–chloroform method (Sambrook & Russell 2000). Ten micrograms of clean genomic DNA was digested using Sau3AI. Then, fragments with an approximate size of 400–1000 base pair (bp) were extracted from agarose gel, and ligated to adaptors OligoA (5′-GCG GTA CCC GGG AAG CTT GG -3′) and OligoB (5′-GAT CCC AAG CTT CCC GGG TAC CGC-3′) in the same quantity. The ligation mixture was incubated overnight at 16 °C and ready for the next manipulation. Pre-hybridization polymerase chain reaction (PCR) amplification was then performed on a PTC-100 thermal cycler (MJ Research Ramsey MN, USA) using one of the linker oligos (OligoA). The reaction mixture (20 μL) contained 0.5 U of *Taq* polymerase (Tiangen, Beijing), 1 × PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer and approximately 20 ng of template DNA. Amplification was allowed to proceed for 25 cycles of 94 °C for 40 s, 60 °C for 1 min and 72 °C for 2 min.

Enrichment for microsatellites was performed primarily according to Fleisher and Loew (1996) with some modifications. The amplicons were denatured and hybridized to a combination of biotinylated probes $(CA)_{12}$ GCTTGA-Biotin, $(GA)_{12}$ GCTTGA-Biotin and $(ATG)_6$ GCTTGA-Biotin in 6 × SSC/0.1% SDS at 68 °C for 1 h. The DNA hybridized to the probes were then captured with streptavidin magnetic beads and washed (twice with 6 × SSC/0.1% SDS at room temperature, twice with 3 × SSC, 0.1% SDS at 68 °C and twice with 6 × SSC at room temperature). Following purification and elution from the beads, the DNA was denatured in 0.1 × TE at 95 °C and

Table 1 Characterization of eight microsatellite loci in the noble scallop, *Chlamys nobilis*

Locus/accession no.	Primer sequence (5′–3′)	T_a* (°C)	Repeat motif	Size rang (bp)	A†	Ho‡	He§	*HWE*¶ *P value*
Cn101/EF565375	F‖: ATCACCATTAGAACAACGGAACC R**: GGCCAAACCAAGCACAGATATTAT	55	$(CTCA)_{10}$	168–200	4	0.63	0.76	0.104
Cn102/EF565376	F: CTAAGGCCGATTGCAAGATAAC R: GTTGTGTGGAATTGTGAGCGGA	50	$(CA)_{22}$	148–180	3	0.28	0.28	0.622
Cn111/EF565377	F: GGAAGCTTGGGATCATCGGACAGT R: ACGGGAAAATCTAAAAAGGTTGAA	50	$(CA)_4C(CA)_6$	186–220	5	0.22	0.40	0.025
Cn113/EF565378	F: TAGGCCTAGGCATAACACA R: AATCATCAAGGTACCAGCAGACTA	50	$(CA)_5$ $CT(CA)_8$	190–218	7	0.40	0.68	<0.001
Cn119/EF565379	F: CCGTATGGCGAGGGGTTTG R: CGGTTTGTGTTTCTCG	50	$(GTTT)_6$	190–225	4	0.22	0.24	0.400
Cn121/EF565380	F: CCCTGTAAATGTGATGTAAAGAAT R: GAGAGCCCACTAATGTCAAAAC	50	$(ATC)_{21}$	210–258	12	0.33	0.88	0.005
Cn126/EF565381	F: TCGTTACATACATCTTCCTTGACA R: CGATGTTGTTATTGTTGTTGTTGA	52	$(CAT)_{26}CA(CCG)_8\ldots(TCG)_6(TCA)_{12}$	218–263	7	0.77	0.83	0.180
Cn128/EF565382	F: CATCCAGGCCCCGGTTGCTCA R: ACGACGACGACGACGACGACGAT	50	$(TCA)_6$ C $(CAT)_{13}$ $(TCG)_9$	120–350	8	0.77	0.78	0.370
Cn129/EU999746	F:CTACCTGTGTGTGTGGGACCAG R:GGTCCAACACACATGTAGTAC	50	$(TG)_{16}$	162–188	7	0.93	0.78	0.226
Cn130/EU999747	F:GGCCAAACCAAGCACAGATA R:CATTAGAACAACGGAACCAACAGG	51	$(GTGA)_{10}$	156–198	4	0.56	0.86	0.069
Cn131/EU999748	F: AAGCTTGGGGTGTGGTGGGTCTAT R: AAGCACTTACTCGCATTCTCACAA	50	$(TGC)_6\ldots(GT)_5$	140–160	5	0.69	0.83	0.114
Cn132/EU999749	F: ACCCATTCACGCTTGTTCC R:TATCCGTTAATCTACGTCAGTTC	52	$(GAT)_{24}$	202–216	7	0.50	0.85	<0.001

* Annealing temperature.
† Allele number.
‡ Observed heterozygosity.
§ Expected heterozygosity.
¶ HWE, test of Hardy–Weinberg equilibrium.
‖ Forward primer.
** Reverse primer.

amplified using post-hybridization PCR using OligoA and the same cycling program as pre-hybridization PCR. Then, the DNA was spin-cleaned, quantified and TA cloned into the pMD18-T vector (Takara, Dalian). The ligation was used to transform DH10B competent cells and plated for cloning. White colonies were picked and checked with PCR, and those with insert length between 400 and 1000 bp were then sequenced with M13F or M13R primers in one direction using ABI 3730 XL (Applied Biosystems, Foster city CA, USA).

One hundred and five sequences were shown to contain microsatellites. However, many of them have insufficient or inappropriate flanking regions on one or both sides of simple sequence repeats, and some others possess only three to five repeats, holding less potential for polymorphism. Thus, primers were designed for each of 32 microsatellites with enough flanking sequences and more number of repeats (> 5) using PRIMERSELECT (DNAStar). The characterization of microsatellites was conducted with 50 unrelated individuals from a scallop cultivation farm in Shenzhen. Following DNA extraction, PCR was performed on a PTC-100 thermal cycler in a volume of 20 μL containing 0.25–0.5 U of *Taq* polymerase 1 × PCR buffer, 1.0–2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.2–1 μM of each primer and 20–100 ng of total DNA. The cycling conditions were as follows: 95 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, annealing temperature of each primer pair for 30 s and 72 °C for 40 s, with a final extension at 72 °C for 10 min. The PCR products were examined by electrophoresis on an 8% or a 10% non-denaturing polyacrylamide gel at 200 V for 5–6 h in 1 × TBE buffer, stained with ethidium bromide (0.5 mg mL^{-1}) and visualized with ultraviolet light. Allele size was determined against a 20 bp DNA size standard with software BIOCAPTMW (Vilber Lourmat). The number of alleles, heterozygosity, test of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were analysed using GENEPOP 3.4 (Raymond & Rousset 1995).

Of the 32 primer pairs tested, 10 did not generate expected PCR products, nine produced multibands or complicated patterns, one yielded a monomorphic band over individuals and 12 amplified clear and specific products, showing polymorphism in the sample population tested in the study. Out of these 12 loci, four, five and three were di-, tri- and tetra-nucleotide repeats respectively (Table 1). Although CA, GA and ATG probes were used for enrichment, some other repeat categories were also harvested, including (ATC)n, (TCG)n, (GAT)n, (GTTT)n, etc. The average allele number of these polymorphic markers was 6.08 per locus, with a range of 3–12. Locus Cn102 is the least polymorphic (with three alleles) and Cn021 the most polymorphic (with 12 alleles). The values of observed heterozygosity varied from 0.22 to 0.93 and the expected ranged varied from 0.24 to 0.88 respectively. After Bonferroni correction, exact tests for HWE revealed that eight (Cn101, Cn102, Cn119, Cn126, Cn128, Cn129, Cn130 and Cn131) loci conformed to HWE, and the other four (Cn111, Cn113, Cn121 and Cn132) showed significant departure from HWE (Table 1), indicating the possible existence of null alleles or genetic drift due to breeding manipulations. No significant linkage disequilibrium was detected for all the 12 polymorphic loci ($P > 0.05$ at eight loci and $P > 0.01$ at four loci). This is the second set of microsatellite markers developed for this species so far (Hui, Bao, Zhan, Hu, Lu, Chang & Hu 2006). These microsatellite loci would be useful for stock study and genetic analysis of *C. nobilis*. Nevertheless, one thing worth noting for these two sets of microsatellite loci is that the majority of loci are at a relatively low or a medium-low level of polymorphism in terms of number of alleles. Thus, more variable microsatellite loci are required in the future for studies such as pedigree analysis and stock management at a fine scale.

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