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*Short Communication*

# Microsatellite markers derived from Japanese scallop (*Mizuhopecten yessoensis*) expressed sequence tags

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**ABSTRACT.** Japanese scallop (*Mizuhopecten yessoensis*) is a cold-water shellfish, and a species of economic importance in China. In this study, we developed and evaluated simple sequence repeat (SSR) markers from the expressed sequence tags (ESTs) of *M. yessoensis*. The characteristics of 12 EST-SSR loci were investigated in 30 individual scallops, and the result revealed that the number of alleles per locus ranged from 2-4, with an observed heterozygosity ranging from 0.0333-0.7692, and an expected heterozygosity ranging from 0.0333-0.6312. Only two loci were found to depart significantly from the Hardy-Weinberg equilibrium ($P < 0.05$). The result of our study suggested that these markers could be considered as potential markers for studying the population structure of *M. yessoensis* and its intraspecific variation.

**Key words:** *Mizuhopecten yessoensis*; Expressed sequence tags; Japanese scallop; Molecular marker; Simple sequence repeats

 

The Japanese scallop *Mizuhopecten yessoensis* is a cold-water shellfish, and its original habitat is distributed across various regions of the world, including the northern part of Japan, the far eastern part of Russia, and the eastern part of the Korean Peninsula. Due to its large body mass, good taste, and nutritional value, *M. yessoensis* has become a high-value food. In 1983, *M. yessoensis* was introduced to China from Japan, and since then much research effort has focused on the development of better techniques for cultivating this species of scallop. However, despite increased cultivation, recent declines in quality and yield have prompted many studies on the genetic diversity of *M. yessoensis* (Wang et al., 2009; Li et al., 2010; Liu et al., 2010) to address concerns regarding its protection and genetic diversity in the hope of preventing a decline in its population.

Few studies have used DNA-based methods to study the population structure of *M. yessoensis* (Sato et al., 2005; Wang et al., 2009). Microsatellite DNAs, also known as simple sequence repeats (SSRs), are short (1-6 bp in length) tandem-repeat sequences that are widely dispersed in eukaryotic genomes. SSRs have been extensively used to study the genetic diversity and population structure of many species because of their high level of polymorphisms, co-dominant inheritance, and distribution throughout the genome (Xu et al., 2010; Yang et al., 2011; Zheng et al., 2012). Expressed sequence tags (ESTs) represent part of the transcribed genome of an organism and are an important resource for identifying microsatellites (Gao et al., 2010). In this study, we identified the microsatellites from *M. yessoensis* ESTs and analyzed the polymorphisms present in the EST-SSRs.

The SSRs of *M. yessoensis* genome were obtained by screening 3009 *M. yessoensis* ESTs obtained in our laboratory and 4711 *M. yessoensis* ESTs obtained from GenBank (at http://www.ncbi.nlm.nih.gov/Genbank/). The SSR sequences were identified using the software MISA (http://pgrc.ipk-gatersleben.de/misa/) and 58 of these EST-SSRs were randomly selected for polymerase chain reaction (PCR) using *M. yessoensis* genomic DNA as template. PCR primers were designed using the Primer 5.0 software with default parameters.

*M. yessoensis* genomic DNA was extracted from 30 different individuals obtained from a cultured population in Dalian, China. The DNA was extracted by a conventional phenol/chloroform extraction method. Each PCR reaction was conducted in a 25-μL volume containing 50 ng genomic DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 200 nM of each primer, and 1 U *Taq* polymerase (Takara). The PCR condition consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at one of the temperatures shown in Table 1 for 30 s, and extension at 72°C for 30 s, with a final extension step at 72°C for 10 min. The amplified-PCR products were separated in 10% non-denaturing polyacrylamide gel at 280 V for 1-2 h. After electrophoresis the gel was stained with ethidium bromide and visualized under ultraviolet light. The genetic diversity indices, including the observed and expected heterozygosities and tests for departure from the Hardy-Weinberg equilibrium (HWE) were performed using POPGENE32 version 1.32.

Of the 58 primer pairs tested, 22 did not generate the expected PCR products, whereas 24 produced monomorphic bands, while 12 produced polymorphic bands. In these 12 polymorphic bands, the number of alleles per locus ranged from 2-4 (Table 1). Overall, the extent of polymorphism associated with the EST-SSRs in *M. yessoensis* genome appeared to be low, indicating *M. yessoensis* may have low genetic diversity. This may also reflect the presence of germplasm degeneration. The observed heterozygosity ranged from 0.0333-0.7692, whereas the expected heterozygosity ranged from 0.0333-0.6312. Departure from the Hardy-Weinberg equilibrium ($P < 0.05$) was only observed for the loci P1 and P10 (Table 1).

**Table 1.** Characterization of EST-SSRs from *Mizuhopecten yessoensis*.

| Locus (accession No.) | Repeat motif | Primer pair sequence (5'-3') | Expected size (bp) | T (°C) | $N_A$ | $H_O$ | $H_E$ | $P_{HW}$ |
|---|---|---|---|---|---|---|---|---|
| P1 (GH735872) | $(CT)_{13}$ | F: GAATGCATATCACTGTGAAGTAT<br>R: TACAAATCTGATAGTCACAGTAC | 315 | 54 | 3 | 0.2667 | 0.2435 | 0.89359 |
| P2 (GH735689) | $(TA)_7$ | F: GATGACGCAATAAATATGCAAGT<br>R: AGGTCAATAAATGAAAACATTGG | 298 | 54 | 3 | 0.3103 | 0.6152 | 0.00005 |
| P3 (GH735523) | $(TA)_4$ | F: TCGCTTCATCCATTCCTGAC<br>R: ATGTACCGACGTATGTTCCAAG | 269 | 58 | 2 | 0.0333 | 0.0333 | 1.00000 |
| P4 (JG968885) | $(AT)_5$ | F: CATTGCTAATACATGCTGGC<br>R: CTGTTAGTAGAATACAGTGAAGG | 266 | 56 | 2 | 0.5667 | 0.5034 | 0.483607 |
| P5 (GR867078) | $(TA)_5$ | F: GTCTTTAATGTGAACATACGCCC<br>R: CAGATGCTGAGTTGCCTACAAC | 250 | 59 | 2 | 0.5862 | 0.5064 | 0.38733 |
| P6 (GH735211) | $(TAACCC)_4$ | F: TAGTTACAGTGCCTCTTTG<br>R: GTTTATGTCCCATTATCCTG | 247 | 53 | 2 | 0.4167 | 0.5071 | 0.37203 |
| P7 (GT086446) | $(CAA)_6$ | F: CATATGCTTAGATACAACGAACA<br>R: GGTCTTACAAAATACGGGGAT | 149 | 56 | 4 | 0.5862 | 0.4967 | 0.78554 |
| P8 (GT565432) | $(GGTCAA)_3$ | F: GCAGGTCTCCAGTCTCCA<br>R: TACACCATGCACCATCACA | 180 | 56 | 2 | 0.2069 | 0.1887 | 0.57357 |
| P9 (GT565882) | $(CAC)_6$ | F: TCACAATGCCAGCTATCGA<br>R: CAAACAAGACCTCTGCCTCA | 192 | 56 | 2 | 0.0333 | 0.0333 | 1.00000 |
| P10 (GR867782) | $(GTG)_6$ | F: AGCCGTGGGAAGACAACAACAGC<br>R: ACAGGGTTTCGTTTACAGAGGTTAC | 148 | 60 | 4 | 0.4615 | 0.6312 | 0.00075 |
| P11 (GH735795) | $(ATG)_7$ | F: GATGCTTAGATACTTTACTC<br>R: TTACATCATAGAAGAGCTT | 149 | 50 | 3 | 0.7692 | 0.5799 | 0.13170 |
| P12 (GH735436) | $(TA)_4$ | F: AAGCAGGCACAGTGGTAAG<br>R: ATTTGCTACATTAGATAAGGATG | 234 | 55 | 2 | 0.0667 | 0.0655 | 0.894626 |

$T$ = annealing temperature; $N_A$ = number of alleles detected; $H_O$ = observed heterozygosity; $H_E$ = expected heterozygosity; $P_{HW} < 0.05$ indicates significant departure from Hardy-Weinberg equilibrium calculated for data from 30 individual scallops.

The polymorphic microsatellites derived from *M. yessoensis* identified in this study will be useful for analyzing the genetic structures of its population. It will also facilitate future studies to assess the genetic diversity within this commercially important scallop.

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