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Short Communication

Isolation and characterization of microsatellite markers for the Korean rockfish, *Sebastes schlegeli*

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ABSTRACT. The Korean rockfish (*Sebastes schlegeli*) is an important commercial fish that is widely used in aquaculture. We isolated and characterized 18 polymorphic microsatellite loci from the Korean rockfish using a $(GT)_{13}$-enriched genomic library. Polymorphism was assessed in 48 individuals from a single population collected from the northern coastal waters of the Yellow Sea. The observed and expected heterozygosities ranged from 0.0244 to 0.7660 (mean 0.4194) and 0.0244 to 0.8758 (mean 0.5002), respectively. Polymorphism at these loci indicated from two to 15 alleles (mean 5.7); 14 of 18 loci conformed to Hardy-Weinberg equilibrium. These markers should be useful for management and conservation studies of this species.

Key words: Korean rockfish; *Sebastes schlegeli*; Genetic structure; Microsatellite loci

INTRODUCTION

The Korean rockfish (*Sebastes schlegeli*) is the eurythermal, benthonic and semi-sedentary fish in the west of the North Pacific Ocean. It is also an important commercial fish in China, Korean and Japan (Hu et al., 2010). Overexploitation and environmental changes have caused a decline in Korean rockfish resources. Stock enhancement programs should be implemented to enrich fishery resources. It was reported that *S. schlegeli* has been listed as a mariculture and stock enhancement species in Korea at the beginning of 1990s (An and Park, 2009; Li, 2009).

It is important to estimate population genetic structure and genetic diversity to provide information for the conservation and management of *S. schlegeli* (Schwartz et al., 2007; Kitada et al., 2009; An and Park, 2009). Microsatellite markers are effective molecular markers that have been widely applied to management and conservation of this species because of the high level of polymorphism, co-dominant inheritance, and high abundance (Litt and Luty, 1989; Zhan et al., 2005, 2009). Six and fourteen microsatellite markers from *S. schlegeli* have already been isolated by Yoshida et al. (2005) and An and Park (2009), respectively. However, more microsatellite markers are needed for studying pedigree and building a genetic map. Here, we developed 18 polymorphic microsatellite markers for *S. schlegeli*.

MATERIAL AND METHODS

Forty-eight individuals of *S. schlegeli* were collected from the northern coastal waters of the Yellow Sea and preserved in alcohol until DNA extraction. Genomic DNA was extracted from muscle tissue using the phenol-chloroform procedure (Sambrook and Russell, 2001). Genomic DNA was simultaneously digested with *Mse*I (New England Biolabs, USA), and the digested DNA (10 µL) was ligated to *Mse*I adaptors (5'-TACTCAGGAACTCAT-3'/5'-GACGATGAGTCCTGAG-3'). Linker-ligated DNA was amplified in a 50-µL reaction mix using the adaptor-specific primer (5'-GATGAGTCCTGAGTAA-3'). Polymerase chain reaction (PCR) conditions were as follows: 20 cycles at 94°C for 30 s, 53°C for 1 min, 72°C for 1 min. The PCR products were purified using DNAmate (TaKaRa, Japan) and hybridized to a biotin-labeled $(GT)_{13}$ probe. The mixture was denatured at 94°C for 5 min, then at 53°C for 15 min. The hybrids were captured with streptavidin-coated magnetic beads (Promega, USA). Unhybridized DNA was washed away, and the remaining DNA was eluted from the magnetic beads and amplified using the adaptor-specific primer and the above program. Following purification, DNA fragments ranging from 500 to 1000 bp were selected by separation on 1.5% agarose gels. The fragments were ligated to pMD18-T vectors (TaKaRa), and transformed into *Escherichia coli* DH5α competent cells to construct an enriched microsatellite sequence library. After amplifying with $(GT)_{10}$ and M13 primers, 192 positive clones were obtained. The positive clones were sequenced on an ABI 3730 automated DNA sequencer (Applied Biosystems, USA).

Primers were designed for 80 microsatellite-containing clones using PRIMER PREMIER5 (Premier Biosoft International, USA) and tested for polymorphism from six *Sebastes schlegeli*. After preliminary screening, only 18 polymorphic microsatellite loci were tested on a sample of 48 individuals. PCR for all loci was performed separately in a 25-µL reaction volume containing 0.4 µM of each primer, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1X PCR buffer, 1 U Taq polymerase (Fermentas, Canada) and 50-100 ng DNA. Amplification was carried out with the following thermal profile: 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, optimal annealing temperature (Table 1) for 45 s, and 72°C for 45 s, and a final extension step at 72°C for 10 min. PCR products were separated on 6% denaturing polyacrylamide gels and vi-

sualized by silver-staining. Allele sizes were estimated according to the pBR322/*Msp*I marker.

The variability at each locus was measured in terms of number of alleles, expected heterozygosity and observed heterozygosity, and Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were tested using GENEPOP 4.0 (Raymond and Rousset, 1995). Null allele frequencies were calculated using Micro-Checker 2.2.3 (Van Oosterhout et al., 2004). The significant value for all diversity tests of significance was corrected by the sequential Bonferroni's procedure (Rice, 1989).

RESULTS AND DISCUSSION

The number of alleles per locus ranged from two to 15 (mean 5.7). The observed heterozygosity ranged from 0.0244 to 0.7660 (mean 0.4194), and expected heterozygosity ranged from 0.0244 to 0.8758 (mean 0.5002) (Table 1). Four loci departed significantly from HWE ($P < 0.05$) after sequential Bonferroni's correction (adjusted P value <0.00278). The observed departure from HWE is likely due to the evidence of "null" alleles (Py1-3, Py3-49, Py4-5, and Pyzj18). Three loci (Py3-8, Py3-9 and Pyzj8) showed significant pairwise linkage disequilibrium ($P < 0.05$), after Bonferroni's correction. Nevertheless, these newly developed loci in combination with existing loci isolated from this species will enrich the microsatellite marker resources to assess population genetic structure and examine patterns of gene flow in the *S. schlegeli*.

Table 1. Characteristics of microsatellite loci in *Sebastes schlegeli.*

Locus	Repeat motif	Primer sequence (5'-3')	Size range (bp)	Ta (°C)	A	N	H_O	H_E	H_O/H_E	P_{HW}
Py1-3†*	$(TG)_{14}$	F: ACTTTCACTGGTGCTTACT R: CATGATCTGTCCTTTTCTC	335-345	57	6	46	0.1518	0.5721	0.266	0.0004
Py2-12*	$(AC)_{17}$	F: TGACCAACAGGAAAATAC R: TTGAAAGATGACCCATTA	310-403	55	15	46	0.6957	0.8758	0.7944	0.0031
Py2-13	$(AC)_8$	F: CATTCCACCTGTCTGTCC R: AGATCGCCGATATTTGAT	180-252	55	6	48	0.625	0.6254	0.9994	0.0838
Py3-6*	$(TG)_{12}$	F: ACGTATGTTGGCTGAAAC R: TTTGGATAATGTGGCTTT	213-230	55	6	46	0.4130	0.5002	0.8257	0.0332
Py3-8	$(TG)_{10}$	F: CCCAGTAGGAGAAACTAAAC R: AATGAATACACCCTCAGAAA	260-275	55	3	45	0.1778	0.1847	0.7913	0.0441
Py3-9	$(TG)_6$	F: CCCAGTAGGAGAAACTAAA R: ATGAATACACCCTCAGAAA	260-270	55	3	48	0.2500	0.2432	1.028	0.6084
Py3-10	$(CA)_8$	F: TAAAGTGGTAGTTGGTGGTG R: TTACCGAAAGTGTAGAAAGC	259-270	55	4	47	0.7447	0.7433	1.0019	0.3982
Py3-27	$(CA)_{13}$	F: ACAGATAAGTTATGCACCAA R: TCGGGATAAGTAAGAGGA	307-350	55	8	47	0.7660	0.7463	1.0264	0.3737
Py3-29	$(TGG)_5$	F: TGCGGTGACTTATCCAGC R: TCAACAAGGGAGCAAAGG	315-346	55	4	48	0.5833	0.4939	1.1810	0.9661
Py3-41	$(TG)_8AGT(GA)_5$	F: CTGTTGGAGGGAGGTTAT R: GTGTCTGGTTGAGCGAGT	250-397	55	8	48	0.5625	0.6428	0.8751	0.0954
Py3-49†*	$(GT)_{16}$	F: ATCAATCACGCTCTTCTCA R: GTCACGTAGATGGCTGAAA	275-285	55	6	47	0.3404	0.6383	0.5333	0.0000
Py4-2	$(GT)_5$	F: TAACGCACTTACAGACAC R: ATTATAGGATGAAGCCAC	236-252	59	4	48	0.5	0.4143	1.2069	1.0000
Py4-4	$(CA)_8$	F: TCGGTGCAAGCAGGATGT R: TAGCAGGACTGGAGGGAGG	260-285	55	5	48	0.5417	0.4581	1.1825	0.0146
Py4-5†*	$(AC)_{21}$	F: ATGCACAGACAGAAATAC R: TCGGATGATAATCAATAC	215-245	54	11	48	0.6042	0.8743	0.6911	0.0000
Py4-8	$(CA)_5$	F: GTTCGCACAAACAGATAC R: ACTGATAGCACCAGAATAA	273-280	55	2	48	0.0417	0.0412	1.0121	1.0000
Pyzj8	$(TG)_{10}...(TG)_6$	F: CCCAGTAGGAGAAACTAAA R: CAAAACTAATGAATACACC	270-285	55	5	47	0.2553	0.2709	0.9424	0.3782
Pyzj16	$(AC)_5...(CT)_9...(CA)_5$	F: ATTTCACAGCCTCGTTTAG R: AGTAGGACAGGTGACTTCG	338-343	57.8	2	41	0.0244	0.0244	1.0000	1.0000
Pyzj18†*	$(GT)_6...(AC)_6...(TC)_6$	F: GTATTTGAGGGCATTTGT R: CAATTAGTGATGAGGAAGAG	290-302	55.3	5	48	0.2708	0.6550	0.4134	0.0001

Ta = optimized annealing temperature; A = number of alleles; N = number of individuals genotyped; H_O = observed heterozygosity; H_E = expected heterozygosity; P_{HW} = Hardy-Weinberg probability; †Locus deviated from Hardy-Weinberg equilibrium (adjusted P value <0.00278); *Locus may harbor null alleles (null allele frequency >5%).

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