GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Characterization of new microsatellite markers of *Siganus fuscescens* (Siganidae)

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Genet. Mol. Res. 12 (3): 2751-2754 (2013)
Received January 4, 2013
Accepted April 20, 2013
Published July 30, 2013
DOI http://dx.doi.org/10.4238/2013.July.30.12

ABSTRACT. *Siganus fuscescens*, which is a small commercially important marine fish, is wildly distributed in shallow waters throughout the tropical and subtropical Indo-Pacific and Eastern Mediterranean regions. It is part of a group known as rabbitfish. Fifteen new polymorphic microsatellite markers for *S. fuscescens* were identified, and 32 wild individuals were used to evaluate the degree of polymorphism of these markers. The number of alleles per locus ranged from 2 to 12, and the polymorphism information content ranged from 0.210 to 0.849. The observed and expected heterozygosities were 0.142-0.808 and 0.225-0.853, respectively. Although significant deviations from Hardy-Weinberg equilibrium were detected at 2 loci (Sf1-37-2 and Sf1-47), no significant deviations were detected at the other 13 loci. These microsatellite markers will provide a useful tool for studies on genetic diversity and differentiation of *S. fuscescens*.

Key words: Genetic markers; *Siganus fuscescens*; Microsatellite; Magnetic bead enrichment

INTRODUCTION

Siganus fuscescens, which is distributed from Japan to Eastern Australia in the Indo-Pacific, is a commercially important near-shore species. In China, *S. fuscescens* is widely scattered in the East and, South China Sea and the continental coast of Taiwan. A total volume of 15,700-25,800 tons of *S. fuscescens* was caught in the Indo-Pacific region between 1990 and 1995 according to the survey by the annual Statistical Manual of the FAO Fishery Resources (Carpenter and Nime, 2001). The wild stocks of *S. fuscescens* declined sharply due to overfishing and pollution of its habitat environment. Besides, international marine aquaculture experts recommended *S. fuscescens* as a new object for aquaculture because of its high protein value, pleasant taste, and easy recently developed farming technique (Lu, 1996; Ma and Liu, 2006). For the purpose of protecting and rationally exploiting its wild resources, studies on the germplasm resources of *S. fuscescens* are urgently required. Microsatellites are a useful tool to study population genetic diversity and differentiation due to its advantages such as co-dominance, high DNA polymorphism, reliability, etc. (Wang et al., 1994) and have been widely used in many species (Li, 2006; Hu et al., 2009). In this study, 15 new microsatellite markers were developed, which provide a useful tool in population genetic studies and the protection of species resources.

MATERIAL AND METHODS

Genomic DNA was extracted from the musculature of a single wild *S. fuscescens* captured in Xiamen, China, using gene DNA extraction kit DP304-03 (Tiangen) according to manufacturer instructions. The DNA was digested with restriction enzyme *Mse*I at 65°C for 10 min. The digested fragments ranging from 300 to 1200 bp were ligated to *Mse*I adapter 1 (5'-ACGATGAGTCCTGAG-3')/*Mse*I adapter 2 (5'-TACTCAGGACTCAT-3') by T4 DNA ligase at 37°C for 3.5 h. The ligation mixture was subsequently denatured and then hybridized to the biotinylated probes $(CT)_{15}$ and $(GT)_{15}$. Fragments containing microsatellite repeats were captured with Streptavidin-Coated Magnetic Sphere Particles (Promega,USA). The recovered DNA fragments were amplified by *Mse*I primer (5'-ACGATGAGTCCTGAG-3'). The DNA purified by GenCleanPCR (Generay) was ligated with the PMD19-T vector (Takara) at 16°C for 3 h and transformed into *Escherichia coli* competent cells for further selection on ampicillin plates. Recombinant clones were detected by PCR amplification using universal M13 primers, and the PCR products were visualized on 1% agarose gels. A total of 198 clones with DNA fragments over 500 bp were selected for sequencing by the BGI Company (Beijing). By analyzing the sequences, 148 of the clones were found containing microsatellites and 48 primer pairs were designed by the Primer Premier version 5.0 software.

A total of 32 wild *S. fuscescens* individuals collected from Xiamen, China, were used to analyze the polymorphism of these 15 microsatellite markers. PCR was performed in a 10-μL volume with 50 ng genomic DNA, 0.25 U Taq DNA polymerase (Fermentas), 10X Taq buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM forward primer, and 0.4 μM reverse primer. The PCR conditions were 94°C for 5 min, followed by 32 cycles at 94°C for 45 s, annealing temperature for 45 s (Table 1), and elongation at 72°C for 45 s, with a final extension at 72°C for 10 min. Amplified products were electrophoresed on Sequi-Gen Sequencing Cell and then the data matrix was analyzed to estimate the basic genetic information by POPGENE 32 (version 1.32) (Yeh et al., 2000) and CERVUS 3.0 (version 3.0) softwares.

Table 1. Characterization of 15 microsatellite loci isolated from *Siganus fuscescen*.

Locus ID	GenBank accession No.	Primer sequence (5'-3')	Repeat motif	N_A	Ta (°C)	Allele size (bp)	*Ho*	H_E	PIC
Sf1-27-2	JX987789	F: CGCTCCACCCCCTCTACC R: CAAGTGTGTCCCCCCGCAA	$(GT)_8$	3	53	322-345	0.467	0.587	0.522
Sf1-27-3	JX997373	F: GGGGAGAGAGTGCAGAGTGT R: GTGAGTGGGTGTGTGGTGAC	$(CT)_{14}(CA)_5$	3	60	165-171	0.375	0.576	0.486
Sf1-27-3	JX997374	F: ACCCACTCACATACACTCCT R: CACCACTATTGAATTAGCCTG	$(CA)_4(CA)_{25}$	10	60	130-173	0.781	0.853	0.849
Sf1-35	JX997375	F: TAACTCTGAGGCGAGTGT R: AATCTGATGGATGTGCTT	$(CA)_{27}(GA)_3$	4	53	178-188	0.406	0.433	0.486
Sf1-37-2	JX997376	F: TGTTCAGGAAAGGAGGA R: GTGGGCAGTAGAGGTTA	$(CA)_6AA(CA)_{36}$	3	53	242-251	0.142	0.520	0.572*
Sf1-47	JX997377	F: GCTCTCTCTTTCCCCCCACAG R: TCACACAAACTCCCCCTCCCAC	A_{14}	3	60	194-201	0.406	0.576	0.504*
Sf3-12-1	JX997378	F: GACCCCCTTCTCACTCTC R: ATCACTGATCTGACCCCA	$(CTT)_{11}$	5	60	245-272	0.562	0.669	0.593
Sf3-14	JX997379	F: AGAATAAAAAGGAGAAGTGG R: GTGAGGGAGTAAGTGAATGA	$(CT)_{10}$	6	56	104-116	0.656	0.693	0.647
Sf3-15	JX997380	F: ATACGCATACACGCAAGC R: ATGTCGGTGGGTCTGATT	$(AC)_4(TC)_6(AC)_9AT(AC)_5$	6	56	158-180	0.438	0.649	0.585
Sf3-33	JX997381	F: TTTTGTCAAGAGGACCGGGGG R: AGATGTGAGGGGAGGGGTGG	$(GA)_{10}(GACAGA)_3$	3	56	298-318	0.219	0.225	0.210
Sf3-36	JX997382	F: TCACAAACTTTAAGACAG R: AAGACATCAATTCAACAC	$(CA)_{11}CG(CA)_4CG(CA)_3$	10	56	97-117	0.719	0.790	0.761
Sf3-39	JX997383	F: GATGTTGGCTCTGGTCCC R: ATCGCTTCACTGCTCTCC	$(TG)_{19}(GA)_8$	12	56	164-168	0.808	0.843	0.848
Sf3-44	JX997384	F: TAACGCTTTTTCTCAACCT R: CCATCTGATCTCCACTTCT	$(TG)_{24}$	4	50	318-330	0.563	0.615	0.615
Sf3-63	JX997385	F: ATGGAACAGGTTGTGATACG R: TTTGTGAGAAAGAAAAGGAA	$(CA)_{28}$	3	56	173-188	0.250	0.225	0.210
Sf3-66	JX997386	R: GAGAGCAGAGAGGTCTGAG F: TTATCCTTGTTGGTAGCAA	$(AG)_3G(GA)_3T(AG)_{26}$	2	50	315-330	0.406	0.324	0.271

N_A = number of polymorphic alleles; Ta = annealing temperature; H_O = observed heterozygosity; H_E = expected heterozygosity; PIC = polymorphism information content. *Significant departure ($P < 0.003$) from expected Hardy-Weinberg equilibrium conditions after correction for multiple tests (k = 15).

RESULTS AND DISCUSSION

The number of alleles, observed heterozygosity and expected heterozygosity were calculated by POPGENE 32 and the polymorphic information content was calculated by CERVUS 3.0. According to the results, the number of alleles per locus ranged from 2 to 12 and the polymorphism information content varied from 0.210 to 0.849. The observed and expected heterozygosities were 0.142-0.808 and 0.225-0.853, respectively. Deviations from Hardy-Weinberg equilibrium (HWE) and evaluated genotypic linkage disequilibrium were tested using POPGENE 32. Error analysis was carried out by MICRO-CHECKER (Van Oosterhout et al., 2004). Among the 15 loci examined, 13 loci were in HWE while 2 loci (Sf1-37-2 and Sf1-47) deviated significantly from HWE after correction ($P < 0.003$, Table 1), which might have been due to the presence of null alleles confirmed by MICRO-CHECKER. The characterization of the loci is presented in Table 1.

In conclusion, the new 15 loci isolated in this study could provide a useful tool for further population genetic studies on *S. fuscescens*.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (#31272668).

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