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Isolation and characterization of eight novel microsatellite loci from the brown alga *Sargassum horneri*

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Abstract *Sargassum horneri* (Turner) C. Agardh is one principal member of the ecologically important brown seaweeds that form submarine forests in the northwest Pacific coasts. However, no microsatellite marker has been available for *S. horneri* to our knowledge. In this study, eight polymorphic microsatellite loci were isolated for the first time from *S. horneri* using the protocol of fast isolation by AFLP of sequences containing repeats (FIASCO). The characteristics of these microsatellites were determined in a sample of 34 individuals of *S. horneri*. The number of the alleles per locus ranged from 2 to 5 (average 3.5). Mean observed and expected heterozygosity were 0.441 and 0.416, respectively. These polymorphic markers will provide a useful tool for further studies of population and conservation genetics in this species.

Keywords *Sargassum horneri* · Brown alga · Microsatellite · Genetic diversity · Population genetics · Phaeophyceae

Introduction

Sargassum horneri (Turner) C. Agardh is a large conspicuous brown seaweed that usually forms submarine forests in the northwest Pacific coasts. This alga has become the principal species of choice for reconstruction of seaweed beds in East Asian countries. It plays important ecological roles in providing nursery sites for marine animals and contributing significantly to nutrient uptake from mainland effluents due to the presence of large amounts of biomass in the near-shore coastal waters (Pang et al. 2009). Along the coast of Zhejiang Province in the East China Sea, the sessile populations of *S. horneri* which used to be distributed widely in near-shore waters or in waters around the islands have been observed to be disappearing gradually due to unknown reasons since the end of 1990s (Sun et al. 2008). In order to conserve and restore the natural populations and genetic resources of this species, it is essential to assess its genetic diversity and population structure. Microsatellite markers are robust tools for detecting genetic diversity due to their high variability (Liu and Cordes 2004). However, no microsatellite marker has been available for *S. horneri* to our knowledge. The protocol of fast isolation by AFLP of sequences containing repeats (FIASCO) has been widely used for isolation of microsatellite loci in many organisms (e.g., Li et al. 2009; Yang et al. 2009). In this study, eight novel polymorphic microsatellite loci were isolated and characterized for the first time from *S. horneri* by using the FIASCO method.

Materials and methods

Genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen). Three microsatellite enrichment libraries were constructed with probes $(CA)_8$, $(GA)_8$, and $(ACT)_8$ according to the FIASCO protocol (Zane et al. 2002). Primers were designed from the sequences with sufficient flanking regions using the program PRIMER PREMIER 5.0. Each primer pair was tested on 34 individuals collected from the following five localities: Nanji Island, Zhejiang province (nine individuals); Rudong, Jiangsu Province (seven individuals); Qingdao (four individuals), Weihai

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Table 1 Characterization of eight microsatellite loci developed for *Sargassum horneri*

Primer name	Sequence 5′-3′	Repeat motif	T_a (°C)	Size range (bp)	*A*	H_o	H_e	Accession number
Shorn18	F:AGGGAGAAGGTGTATCCAGA R:TGTTGCAGGCTGAGAGGGGAGA	$(TG)_4$…$(GA)_3$	53	116–120	2	0.000	0.059	KJ806639
Shorn26	F:GACTCCGAGCATGGTGTTGA R:AGCCTGAATGTGCCAGTAGA	$(ACT)_4$…$(AC)_8$	54	299–307	3	0.971	0.619	KJ806640
Shorn27	F:GCTATGTCAACAACCACCTCT R:TTCTGATTCGAGGTATTGTGC	$(ATC)_4$…$(ATC)_5$	54	335–350	4	0.677	0.508	KJ806641
Shorn30	F:ATGAAACATTGGCTTAGATG R:CAACATCGTGGACCGTGAAT	$(GATT)_5$	50	416–425	5	0.588	0.540	KJ806642
Shorn31	F:CGACACGTGATCACAAGGAC R:ACTATGGTATGTGACGTGGG	$(CT)_3A(CT)_3$…$(CT)_3$	54	307–323	4	0.382	0.419	KJ806643
Shorn34	F:GTGCTGCCAGAGTAGTTGTA R:AGAGTTGAAAGGCATAAGGA	$(AACCCT)_3$	50	284–296	2	0.118	0.212	KJ806644
Shorn41	F:ATGTGGCTGTTATCCCGAAGTA R:TGCCGATCAGGTCCGTGTAT	$(AT)_3$…$(AG)_8$	54	270–278	4	0.441	0.490	KJ806645
Shorn43	F:AGTAAAACTCCCCCGAAATAG R:GCGGCAGTTTCTAATCTTCT	$(TA)_5$…$(AT)_4$	53	216–224	4	0.353	0.485*	KJ806646

T_a annealing temperature, *A* number of alleles, H_o observed heterozygosity, H_e expected heterozygosity, *asterisk* represents significant deviations from Hardy–Weinberg equilibrium

(two individuals), and Dalian (12 individuals). PCR amplification was carried out in 20 μL volume containing 1× PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.5 μM fluorescent-labeled (forward) and unlabeled (reverse) primers, 0.5 U *Taq* DNA polymerase (Takara), and 20 ng of genomic DNA. PCR thermal cycle comprised an initial denaturation at 94 °C for 4 min, followed by 30 cycles of 94 °C for 30 s, locus-specific annealing temperature (T_a, Table 1) for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 7 min. Genotyping analysis was performed on an ABI 3730XL automated sequencer (Applied Biosystems, Carlsbad, CA) and allele sizes were determined with GeneMapper version 4.0.

The number of alleles, observed (H_o) and expected (H_e) heterozygosities, and the probability for Hardy–Weinberg equilibrium (HWE) test for each locus were computed using GENEPOP v 4.2.1 (Raymond and Rousset 1995). The 34 individuals were treated as a single population in the initial analysis with the purpose to detect the variations in microsatellite loci across the range of this species in China.

Results and discussion

Two hundred positive clones were sequenced and 45 of them contained microsatellite repeats with the flanking regions long enough for primer design. Eight polymorphic microsatellite loci were finally determined (Table 1). The number of the alleles per locus ranged from 2 to 5 with an average of 3.5. H_o and H_e ranged from 0.000 to 0.971 and from 0.059 to 0.619, with the mean values being 0.441 and 0.416, respectively. Significant deviation from HWE was found in the locus Shorn 43 after sequential Bonferroni correction (Rice 1989), probably as a result of pooling all the 34 individuals as a single population (Ingram et al. 2014). No linkage disequilibrium was detected among loci.

Although relatively low numbers of alleles were detected at most loci, variations were found in individuals across the distribution range of this species in China. Thus, these microsatellite markers are sufficiently variable for detailed studies of the population structure of *S. horneri* and will help to effectively conserve and manage natural stocks of this species.

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