Screening and characterization of novel microsatellite loci in *Lateolabrax japonicus*

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Genet. Mol. Res. 15 (4): gmr15048378
Received January 4, 2016
Accepted February 26, 2016
Published October 17, 2016
DOI http://dx.doi.org/10.4238/gmr15048378

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ABSTRACT. *Lateolabrax japonicus*, an economically important species, is widely consumed in the offshore coasts of China, Korea, and Japan. We identified 10 new *L. japonicus* microsatellite markers, using a modified protocol of fast isolation by AFLP of sequences containing repeats. Thirty *L. japonicus* individuals were collected from Xiamen, China, to evaluate the degree of polymorphism. The number of identified alleles ranged from three to five. The polymorphism information content varied from 0.267 to 0.711, whereas the observed and expected heterozygosities ranged from 0.249 to 0.706 and 0.294 to 0.751, respectively. One of the 10 loci (L10) deviated from the Hardy-Weinberg equilibrium. These new microsatellite markers will provide a useful tool for the determination of population genetic structure and genetic diversity in *L. japonicus*.

Key words: Genetic markers; *Lateolabrax japonicus*; FIASCO; Microsatellites

INTRODUCTION

Lateolabrax japonicus, also called sea perch, is one of the most promising cultured species in China. It is an economically valuable species, belonging to the order Perciformes, family Serranidae. This species is primarily distributed along the offshore coasts of China, Korea, and Japan. In China, *L. japonicus* is principally found in the southeastern coastal area, including Qingdao, Shidao, Qinhuangdao, and Zhoushan Island. The sea perch aquaculture originated in the 1970s and flourished in the late 1980s in China (Du et al., 2013). *L. japonicus* is a pelagic fish that has a high temperature and salinity tolerance compared to other fishes. As a carnivorous fish, its ingestion habits vary with age. Juveniles feed mostly on zooplankton, whereas adults mainly feed on shrimps, fish, and isopods (Sun et al., 1994). This species is becoming increasingly sought after, not only because of its good taste, low fat-content, rich nutrition, and medical value, but also because it is fast-growing and, thus, one of the most economically important marine fish species in China. In recent years, stocks of *L. japonicus* have declined sharply due to overfishing (Lou et al., 2000). In order to protect the species, improve stock quality, and keep the development of the fishery industry healthy and sustainable, it is necessary to gain a better understanding of the genetic structure of *L. japonicus*.

Microsatellites are short tandem repeated DNA sequences ranging in length from 1 to 6 bp (Weber and May, 1989). Microsatellite loci are flanked by conserved sequences, surrounding the variable core sequence. As powerful genetic markers, they are distributed throughout the eukaryote genome. Microsatellites possess many advantages, including high polymorphism, co-dominance, and ease of genotyping by polymerase chain reaction (PCR). For these reasons, they have been the genetic marker of choice for studies of population and evolutionary genetics; population differentiation (Li, 2006); and genome mapping and forensic studies (Goldstein and Schlötterer, 1999). The use of microsatellite markers has been reported in many fishes such as *Oncorhynchus mykiss* (Jackson, 1995), *Oreochromis niloticus* (Lee and Kocher, 1996), *Gadus morhua* (Herbinger et al., 1997), *Carassius gibelio* (Zhou et al., 2001), *Siganus fuscescens* (Ning et al., 2015), and *Coilia nasus* (Fang et al., 2015). There are also examples of studies of *L. japonicus* using microsatellite markers (Zhu and Chang, 1999; Jiang et al., 2007, 2008, 2009; Liu et al., 2009; Zhao et al., 2011).

In this study, we used a modified protocol for fast isolation by AFLP of sequences containing repeats to develop and characterize ten microsatellite loci for *L. japonicus*. These loci can be used for further studies of genetic diversity, population structure, and molecular identification of the cultivars. Furthermore, these markers may be used for the purpose of protecting this species.

MATERIAL AND METHODS

Total DNA was extracted from *L. japonicus* muscle from an individual captured in Xiamen, China, using the Genomic DNA Extraction kit (Tiangen, Beijing, China). The quantity and quality of the genomic DNA was tested using an ultraviolet spectrophotometer and by 1% agarose gel electrophoresis.

Genomic DNA samples (25 µL; 100 ng/µL) were digested using FastDigest *Tru*lI (Fermentas, Vilnius, Lithuania) at 65°C for 10 min. The digested fragments, ranging in size from 400 to 1200 bp, were ligated to *Mse*I adapter A (5'-ACGATGAGTCCTGAG-3')/*Mse*I adapter B (5'-TACTCAGGACTCAT-3') by T4 DNA ligase (Fermentas) at 37°C for 4 h. Subsequently, the samples were denatured at 95°C for 10 min and the denaturation products

were immediately hybridized (for 1 h at 61°C) with Bio-labeled probes consisting of $(CT)_{15}$ and $(GT)_{15}$. The linker-ligated fragments containing microsatellite repeats were captured using Streptavidin-coated Magnetic Sphere Particles (Promega, Madison, WI, USA). Target DNA fragments were amplified using *Mse*I adapter A, non-captured DNA fragments were washed away, and the PCR products were purified using GenCleanPCR (Generay Biotech Co. Ltd., Shanghai, China). The purified PCR products were then ligated into the PMD19-T vector (Takara, Shiga, Japan) at 16°C for 4 h, followed by transformation into OneShot chemically competent *Escherichia coli* DH5α (Tiangen) that were grown in a constant temperature incubator at 37°C for 14 h. Transformants were selected on lysogeny broth agar plates containing ampicillin (60 μg/mL). The positive clone fragments were amplified using M13 general primers and the PCR products were separated on 1% agarose gels. In this study, 150 positive clones containing DNA fragments ranging from 500 to 1000 bp were selected for subsequent sequencing by Invitrogen (Guangzhou, China).

We found that 149 fragments were successfully sequenced. Based on the search results from SSRhunter v. 1.3 (Nanjing Agricultural University, China), 111 clones contained microsatellites. A set of 49 primers were then designed using Primer Premier v. 5.0 (Premier, Canada). Finally, 28 primer pairs were selected for testing by amplifying the genomic DNA of 30 *L. japonicus* individuals captured in Xiamen, China. The assessment of the optimal annealing temperatures of the 28 primer pairs was performed on four individuals using gradient PCR. The annealing temperatures were found to range between 43.5° and 55.5°C. The PCR amplification was employed in a final volume of 10 μL consisting of 50 ng genomic DNA, 10X Taq buffer, 2 mM $MgCl_2$, 0.4 μM of each primer, 0.25 U Taq DNA polymerase (Fermentas), and 0.2 mM each dNTP. The cycling reaction conditions were as follows: pre-denaturation at 94°C for 5 min, followed by 32 cycles at 94°C for 30 s, 30 s at the optimized annealing temperature (Table 1), 72°C for 60 s, and a final extension step at 72°C for 10 min. The PCR products were resolved on 6% denaturing polyacrylamide gels using a Sequi-Gen Sequencing Cell machine (Bio-Rad, Hercules, CA, USA) and visualized by silver staining along with a 10-bp DNA ladder. Finally, the important genetic information and index of the 10 microsatellite loci identified as being polymorphic were analyzed using POPGENE 32 (v. 1.32) (Yeh et al., 2000) and CERVUS (v. 3.0), and those softwares were download from the China Science Software Network.

RESULTS AND DISCUSSION

In this study, 10 polymorphic microsatellite loci were successfully screened. The characterization of these loci are presented in Table 1. The number of polymorphic alleles per locus ranged from three to five with an average of four. The observed (H_O) and expected (H_E) heterozygosities ranged from 0.249 to 0.706 and 0.294 to 0.751, respectively. The polymorphism information content (PIC) varied from 0.267 to 0.711, with an average of 0.505. After applying a Bonferroni correction, nine of the 10 loci (all except for locus L10) were in Hardy-Weinberg equilibrium.

In previous studies of this species, both more (Hu et al., 2007; Zhao et al., 2011) and fewer (Li et al., 2003, 2006; Liang et al., 2008; Fan et al., 2009) loci have been identified. The reason for this discrepancy may be related to differences in sampling sites and experiment skills. H_O and H_E values are important indicators used to evaluate the extent of population differentiation. Inbreeding natural selection, dumb alleles, the Wahlund effect, and other reasons may caused the H_O to be less than H_E.

Table 1. Characterization of 10 microsatellite loci isolated from *Lateolabrax japonicus*.

GenBank accession No.	Locus ID	Primer sequences (5'-3')	Repeat motif	Ta (°C)	N_A	Allele size (bp)	PIC	H_O	H_E
KT946737	B28	F: ACCACTCTTTCCCTCCATG R: CAGACTTTCCCGTTTACAGC	$(TC)_{11}$	55.5	4	132-141	0.491	0.4799	0.5201
KT946738	B60	F: GAGCCAGACACCATTTC R: GTCATTTATCCACCTTCC	$(ATGGA)_3$	51.0	5	260-296	0.526	0.4525	0.5475
KT946739	B97	F: ACAGGCCAGCATTTCAC R: ATTTCCTCCACCTCTTTCT	$(AG)_{24}$	51.0	3	166-180	0.403	0.5362	0.4638
KT946740	H40	F: TACCTACCTCACCCCAGAA R: GGACCAGAGCCCATTTAC	$(CA)_{20}$	46.8	3	219-240	0.267	0.7056	0.2944
KT946741	H69	F: TTCTTTGCCGTTTGGTTGTG R: GCAGCGGATCAGTGTCTTATTT	$(T)_{15}$	43.5	4	227-240	0.536	0.4519	0.5481
KT946742	H71	F: TTGCCCTCATAGTTCCC R: GCTGACTCGCTTTCTCC	$(ACACAA)_3$	53.2	5	138-156	0.711	0.2492	0.7508
KT946743	H72	F: CACATCCACTCCTACATC R: GTCACGACATTACTTTCTG	$(CA)_{10}$	51.0	4	151-169	0.532	0.3893	0.6107
KT946744	L10	F: CTTTACAAGTGTTGCTCC R: TTGAGTAGTCCTGAGTGG	$(GT)_{12}$	46.8	4	198-230	0.525	0.4494	0.5506*
KT946745	L30	F: CGAGGCTACAGTGGAAAT R: ACTGAACCAACTGACTCCC	$(TGGCT)_6$	55.5	5	245-280	0.572	0.3876	0.6124
KT946746	L36	F: TTTTGCGAACACCAGAGG R: AACGACGTGCCAATCCA	$(TG)_{32}$	48.8	3	308-318	0.492	0.4102	0.5898

Ta = annealing temperature; N_A = number of polymorphic alleles per locus; PIC = polymorphism information content; H_O = observed heterozygosity; H_E = expected heterozygosity; *indicates significant ($P < 0.05$) departure from expected Hardy-Weinberg equilibrium after Bonferroni correction for multiple tests ($k = 10$).

The PIC plays a significant role in measuring the polymorphism of marker genes or marker sequences (Lü, 1994). A PIC level below 0.25 indicates that the population has little polymorphism; whereas a PIC value above 0.5 indicates a high polymorphism. PIC values between 0.25 and 0.5 suggest a moderate population polymorphism (Botstein et al., 1980). In this study, we found four microsatellite loci with moderate polymorphism and six loci with high polymorphism. The overall PIC was 0.5054, which was higher than that reported for other sea perches (Liang et al., 2008; Fan et al., 2009), but lower than that previously reported for *L. japonicus* (Zhao et al., 2011).

The limitations of this study include that polyacrylamide gel electrophoresis (PAGE) experiments are affected by many factors, such as ambient temperature and silver staining conditions. Furthermore, PAGE results may be difficult to read. Hence, these are issues that need to be addressed in future studies. The 10 new microsatellite markers we developed in this study will provide important information for future studies of the population genetic structure and conservation genetics of *L. japonicus*.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the Key Program from Science and Technology Projects of Xiamen (#3502Z20143017) and the Science and Technology Plan Projects of Fujian Province (#2015N0010).

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