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Development of Fifteen Novel Microsatellite Markers from Rock Bream (*Oplegnathus fasciatus*)

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Abstract

Rock bream (*Oplegnathus fasciatus*) is one of the most economically valuable fish in Korea. In recent years, artificial breeding techniques with molecular and microsatellite markers have been developed to enhance rock bream resources. Microsatellite loci to define genetic diversity were screened in rock bream (n = 30) from Jeju areas of Korea and fifteen polymorphic microsatellite loci were newly identified and analyzed. The number of alleles per locus ranged from 9 to 34 while observed and expected heterozygosity ranged from 0.600 to 1.000 and from 0.772 to 0.977, respectively. These markers will serve as a foundation for future population genetic studies and the selective breeding technology of rock bream farming.

Keywords

***Oplegnathus fasciatus*, Rock Bream, Microsatellite Markers, Population Genetics**

1. Introduction

Rock bream (*Oplegnathus fasciatus*) is primarily an inhabitant of estuaries throughout Korea, Japan, Taiwan, and Hawaii [1] [2]. Recently, it is becoming one of the most valuable species in the fisheries industry in Korea. Therefore, the government has developed artificial breeding techniques to enhance rock bream resources [1]. Although it is an important commercial fish species, little is known about the genetic background of rock bream. Accordingly, screening for useful molecular markers is necessary for analyzing genetic information in rock bream [3].

Microsatellite DNA markers are powerful tools to measure genetic variation in wild and hatchery populations

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[4] [5]. The application of Microsatellite DNA markers has allowed rapid progress in a variety of aquaculture studies. Microsatellites have successfully been used to analyze genetic variation of several aquaculture fishes including Atlantic salmon [6] [7], barramundi [8], carp [9], red sea bream [10], Japanese flounder [5] [11], Korean black scraper [12] and tilapia [13]. In addition, several microsatellite-based studies have detected significant differences among geographically close salmonid populations [14].

In recent years, many rock bream farms in Korea have suffered substantial financial loss due to the occurrence of red seabream iridovirus disease (RSIVD) during summer months [15] [16]. To reduce this economic loss, we carry out experiments to isolate fish with iridovirus resistance. For this purpose, the high resolution of genetic map is necessary to facilitate searching for disease resistance traits and identifying candidate genes. Ozaki *et al*. (2010) reported linkage analysis of resistance to *Streptococcus iniae* infection in Japanese flounder based on 159 microsatellite markers [17]. Great links of microsatellite markers with resistant traits provide rise to quantitative trait loci (QTL) for resistance to a specific pathogen [18]. We isolated a polymorphic dinucleotide (CA) repeat sequence from CA repeat enriched genomic library. In this study, we reported on an additional 15 microsatellite markers for the rock bream.

2. Materials and Methods

Rock breams for constructing CA repeat enriched library and genotyping were purchased from culture farms in Jeju Province of Korea. CA repeat enriched library was constructed with an enrichment technique suggested by Hamilton *et al*. [19] Genomic DNA was extracted from 1 g of muscle tissue of cultured rock bream in Jeju Province of Korea by using standard phenol-chloroform extraction and ethanol precipitation. Purified genomic DNA was digested with *Rsa*I, *Hae*III, *Alu*I, and *Nhe*I restriction enzymes (New England Biolabs, Beverly, MA, USA) with proper conditions. The digested DNA fragments were ligated with specific linkers for amplification. The biotinylated repeat oligo (dCA_{16}) was hybridized with the digested DNA to purify DNA fragments containing a CA repeat motif. The microsatellite enriched elutant was amplified with an oligo adaptor primer. Amplified DNA was purified using Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany) and cloned with pBluescript II SK(-) vector (Stratagene, La Jolla, CA, USA). Cloned plasmids were extracted and sequenced with an ABI-3130xl sequencer. Mass sequences were analyzed with bioinformatical pipelines of Phred, Cross-match, RepeatMasker and SeqClean, and assembled with TGICL. Primer designable contigs were selected and primers were designed using Primer3Plus software.

Thirty rock bream individuals from the Jeju Province of Korea were used for genotyping. The genomic DNA for PCR template was extracted through standard phenol-chloroform extraction and ethanol precipitation. Polymerase chain reaction (PCR) amplification was carried out in a ABI 2720 Thermal Cycler (Applied Biosystem, Foster City, CA, USA) in 25 µL of reaction mixture including approximately 50 ng of template DNA, 0.2 mMdNTP, 0.5 µM of each primer, ddH_2O, 1X PCR buffer (50 mM KCl, 2 mM $MgCl_2$, 10 mM Tris-HCl), and 1 unit *Taq* DNA Polymerase. Initial denaturation at 95˚C for 5 min, was followed by 35 cycles at 95˚C for 1 min, 50˚C for 1 min, 72˚C for 1 min and a final extension at 72˚C for 5 min. PCR products were electrophoresed on an ABI 3730xl Genetic Analyzer (Applied Biosystems, USA) using a POP7 gel matrix with GeneScan™–500 LIZ® Size Standard (Applied Biosystems, USA). GenSeScan Analysis (V.3.7, Applied Biosystems, USA) software was used to score microsatellite alleles, and allele size was manually verified.

We calculate the number of alleles per locus (k), observed and expected heterozygosities (Ho and He), polymorphic information content (PIC) and estimated null allele frequency (F) using the Curvus Program [20]. Linkage disequilibrium and the Hardy-Weinberg Equilibrium were determined using Genepop v4.0 [21].

3. Results and Discussion

We isolated 2039 unique repeat-containing sequences from enriched genomic DNA library and 621 primer pairs were designed from those sequences using Primer3Plus software. Fifteen of the primers were end-labeled at one primer of each pair with a fluorescent dye, 6-FAM, after the first screening step. The characteristics of the 15 microsatellite loci are shown in **Table 1**. A total of 312 different alleles were observed at the fifteen loci. The average number of alleles per locus was 21, ranging from 9 (RbJJCA07) to 34 (RbJJCA20). The size of alleles ranged from 116 (RbJJCA15) to 270 (RbJJCA25). Comparatively, a previous study by An *et al*. [3] reported rock bream to have a mean of 8 alleles in 9 loci with a total of 73 alleles observed.

Polymorphic information content (PIC) ranged from 0.726 (RbJJCA07) to 0.960 (RbJJCA20) with a mean of

Table 1. Characteristics of fifteen polymorphic microsatellite loci for rock bream *Oplegnathus fasciatus*.

Locus/ GenBank No.	Primer sequences (5'-3')	Repeat motif	Ta	Size (bp)	N	K	Ho	He	PIC	P_{HW}	F (Null)
RbJJCA04/ JN251002	F: CCTTGCATCTTTGTGAGTGTG R: TTGTGCTGATGAAGGGACAA	$(TG)_{19}$	50	172 - 200	30	19	0.733	0.903	0.879	0.0000	0.1006
RbJJCA07/ JN251003	F: GCAGAGGAATACATCTGTGCAA R: GCAAGCGGAAATTTTAGTGG	$(CA)_{16}$	50	167 - 198	30	9	0.733	0.772	0.726	0.0452	0.0153
RbJJCA08/ JN251004	F: TGCAGAAATCAACACGTCTTTT R: GGGCTGGGCTGATTTACTT	$(CA)_{11}$	50	145 - 173	30	11	0.767	0.786	0.742	0.0459	0.0010
RbJJCA10/ JN251005	F: CGCAGTTTGCATGTGTTTTT R: CCAGGATCAGAGGAACCAGA	$(TC)_{40}$	50	186 - 228	30	25	0.900	0.953	0.934	0.0366	0.0200
RbJJCA12/ JN251006	F: TTCACCAAAGAGAGTTTGAAGAGA R: ACCCTTGACTTGGAGGGGTA	$(CA)_{24}$	50	122 - 180	30	24	1.000	0.947	0.927	0.0116	−0.0373
RbJJCA14/ JN251007	F: TGACACACATTTTTCCACGA R: GGCTTTGGAGATGAGTCCAG	$(CA)_{35}$	50	169 - 177	30	23	0.900	0.940	0.919	0.0316	0.0134
RbJJCA15/ JN251008	F: ACCGTCTTCCCTTTTTCTCC R: AGGCATGGGTTTGGTATTGA	$(AC)_{27}$	50	116 - 167	30	13	0.900	0.888	0.860	0.0563	−0.0136
RbJJCA17/ JN251009	F: AACACACACAACGCACCAAT R: TGAATCAGGTCCTGCTGTTG	$(TG)_{20}$	50	155 - 204	30	17	0.767	0.867	0.840	0.0024	0.0450
RbJJCA20/ JN251010	F: TTGACACCCTCACCTGGTTT R: CAACAGGACACCCACAGTGA	$(CA)_{24}$	50	163 - 235	30	34	0.900	0.977	0.960	0.0067	0.0322
RbJJCA23/ JN251011	F: ACAGTGAGGTATTTCAAGAATGAGA R: GAGGGCGTTGCTCTGAATAC	$(CA)_{57}$	50	184 - 239	29	27	0.966	0.957	0.938	0.0615	−0.0125
RbJJCA25/ JN251012	F: TCTGTGTGATCCAGGGAGAA R: CTGCACCATCAACAAAGTCG	$(TG)_{22}$	50	226 - 270	30	21	0.900	0.946	0.926	0.0254	0.0158
RbJJCA28/ JN251013	F: GACAGAGACAGACAGAGTGTCCA R: TGTCTCGATTTTGCAGACAGTT	$(CA)_{25}$	50	160 - 217	29	19	0.897	0.933	0.911	0.0025	0.0107
RbJJCA31/ JN251014	F: TCACCTTAGTGTCTCGCTTGG R: TTTGCAGCCCATTCTTTACA	$(AGGTG)_{19}$	50	124 - 208	30	27	0.933	0.940	0.920	0.0352	−0.0054
RbJJCA36/ JN251015	F: TCTCTGTATTGAAGTGCTGTGGA R: TGTGTTAAGTGGTTTTGTGTGTTG	$(CA)_{39}$	50	135 - 189	30	19	0.900	0.903	0.879	0.0621	−0.0089
RbJJCA39/ JN251016	F: GGGGATCACCTGTTTTTCAA R: GGAGAGGCTGTGTGTTAGGC	$(TG)_{25}$	50	145 - 275	30	24	0.600	0.955	0.936	0.0000	0.2223

0.887 for all 15 loci (**Table 1**). Boststein *et al*. classified markers as three grades according to PIC values which is highly informative (PIC > 0.5), reasonably informative (0.5 > PIC > 0.25), slightly informative (PIC < 0.25) [22]. Based on this classification, all loci from our study show highly informative values, indicating that all loci from our research could be useful markers for population genetics and quantitative trait locus (QTL) study of rock bream. In the case of rock bream individual RBJJCA20 the highest PIC value (0.960) was observed. This is a relatively high polymorphic value compared to other microsatellite markers from different fish species [23] [24].

Observed and expected heterozygosities ranged from 0.600 to 1.000 and from 0.772 to 0.977, respectively. After a Bonferroni correction (P = 0.0033) four microsatellite loci (RbJJCA04, RbJJCA17, RbJJCA28, RbJJCA39) exhibited significant departure from the Hardy-Weinberg equilibrium, possibly due to the presence of null alleles. It is thought that these null alleles were caused by genetic instability within this region.

4. Conclusion

In conclusion, the 15 microsatellite markers were developed from our CA repeat enriched library and these markers were shown highly informative values. The population structure of rock bream is under study. These polymorphic markers will be a useful tool for population genetic studies and QTL for disease resistance traits of rock bream. We also expect that these markers could provide information about the phylogeography of this species in Korea.

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