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Short Communication

Development and characterization of microsatellite loci for *Fenneropenaeus penicillatus* Alcock

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Eight novel microsatellite loci from the genome of *Fenneropenaeus penicillatus* Alcock were developed using the protocol of fast isolation by amplified fragment length polymorphism of sequences containing repeats (FIASCO). Thirty (30) wild individuals were used to analyze the polymorphism of these eight microsatellite markers. The results show that the number of alleles per locus and the polymorphism information content ranged from 2 to 7 and from 0.2076 to 0.7484, respectively. The observed and expected heterozygosity were 0.1724 to 0.9130 and 0.1639 to 0.7314, respectively. These microsatellite primers will be used for further population genetic studies, constructing genetic linkage maps or locating quantitative trait locus (QTL) of *F. penicillatus* Alcock.

Key words: Genetic markers, *Fenneropenaeus penicillatus* Alcock, microsatellites.

INTRODUCTION

Fenneropenaeus penicillatus Alcock is widely distributed in a broad geographic area from Pakistan to Indonesia in Indo-West Pacific. In China, it is native to Fujian and Guangdong Provinces. It is considered as a valuable source of animal protein with high protein and low fat. Also, its higher tolerance to water temperature and salinity comparing to other shrimps makes it a suitable species for aquaculture. So it was an important fishing and aquaculture target before 2000 in China. The aquaculture output of *F. penicillatus* Alcock reached its peak in the 1980s. However, the total aquaculture output decreased rapidly due to shrimp diseases happened in the 1980s. And the total catch of *F. penicillatus* Alcock has also fell in 1990s, which caught the government attention. Since then, Chinese government has kept on protecting its natural resources by putting some strict rules and recovering the natural resources by artificial releasing since the 2000s. Thus, in order to serve the purpose of better managing and efficient recovering the natural resources, as well as to find loci which relate to qualified characteristics in breeding, the genetic information of *F. penicillatus* Alcock is so essential. Up until now, researches concerning the genetic background of *F. penicillatus* Alcock is limited (Carolina et al., 2005). And ten microsatellite loci in *F. penicillatus* Alcock that were developed by Cao et al. (2011) were not sufficient, so eight more microsatellite loci are developed to serve the purpose of protecting the natural resources and aquaculture as shown in Table 1

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MATERIALS AND METHODS

Genomic DNA samples were extracted from a single wild *F. penicillatus* individual captured in Xiamen, China using gene DNA extraction kit SK 1252 according to the manufacturer's instructions. A total volume of 2000 µg (100 µg/µL) genomic DNA was digested with 10 U MseI in a 25 µL volume for three hours, and then 20 µL of the digested DNA was ligated with *Mse1I* adapter1 (5'- TACTCAGG-ACTCAT-3') /*Mse1I* adapter2(5'- GACGATGAGTCCTGAG-3') by T4 DNA ligase (5U) in 20 µL volume at 37°C for 3.5 h. The digestion-ligation mixture was subsequently denatured and then hybridized to $(CT)_{15}$ and $(GT)_{15}$ biotinylated oligonucleotide probes and fragments containing microsatellite repeats were captured with Streptavidin MagneSphere Paramagnetic Particles (Promega). Target DNA fragments were released from the beads after washings, and were amplified by using *Mse11*primer. After purification by GenCleanPCR (Generay), the purified products (4 µL) was ligated to the pMD19-T（Takara）at 16°C for 3 h and transformed into *Escherichia coli* for further selection on ampicillin plates. Positive colonies were transferred into 96-Well plates and further incubated at 37°C for 3 h.

Out of 330 clones, 76 positive clones with DNA fragments above

Table 1. Basic genetic information of eight microsatellite primers in *F. penicillatus* Alcock (sample size = 30 individuals).

Locus ID	Primer sequences (5-3)	Anneal °C	Repeat motif	Size range	A	P-HWE	PIC	Ho	He	GenBank accession no.
FP-2	F: ATAATACAGCCTTTCCCACC R: ACACGGACAAACGCACAT	60	$(TG)_4C(GT)_{12}$	264-274	3	0.2549	0.6428	0.3448	0.6364	*JQ822199*
FP-6	F: CAGTTTCTGGTCAAGGGTT R: GTTTTGTTTTCAAGGTGGG	40	$(AC)_{12}AA(AC)_{21}TC(AC)_8(TC)_{23}N(TC)_7TA(TC)_{17}$	320-360	3	0.9178	0.3460	0.2593	0.2369	*JQ822200*
FP-11	F: ATGGACCAGAGCCAAGTAC R: ATTAGATTTTGGGGAAGAG	40	$(CT)_{16}N(TC)_9$	107-125	3	0.1804	0.5463	0.4667	0.4805	*JQ822201*
FP-22	F: CGCTCTTATGGAGGAACA R: GTTAGCTTACGGTTTGTGC	40	$(GA)_{26}$	200-240	5	0.0093	0.7129	0.9130	0.6879	*JQ822202*
FP-48	F: CAGCCCTGACGTACTCCT R: TTGGCAGAACGAAGCATA	60	$(TC)_5$	216-220	3	0.9771	0.2076	0.1724	0.1639	*JQ822203*
FP-49	F: GTCTTCCGTGGGCGTTAC R: TGGTCTTGGTCGGGTTTG	48	$(TC)_{24}N(CA)_{40}$	182-188	2	0.0559	0.5615	0.6316	0.4438	*JQ822204*
FP-62	F: ACAAGCACGCACGCAAAC R:CAGTCATAGGCAGGCAGA	60	$(AC)_6N(AC)_{12}N(AC)_{10}N(AC)_{24}$	230-278	7	0.1126	0.7484	0.7200	0.7314	*JQ822205*
FP-64	F: GTGTTGCGATAAATCACAGT R: GTCCACGAATAAAGCAGAAG	40	$(GA)_{24}C\ (GA)_7(GT)_{17}$	150-168	3	0.0078	0.4096	0.2069	0.4301	*JQ822206*

F= Forward primer, R= reverse primer, (A), number of alleles per locus, (Ho) observed heterozygosity and (He) expected heterozygosity. P-HWE P-values for the Hardy–Wienberg Expectation test (adjusted P = 0.00625), polymorphic information content (PIC).

500 bp were shake cultured for 3 h (37°C, 300 prm) and then sequenced. After analysis of the sequences, 61 clones were found containing microsatellites. Thirty-three (33) pairs of primers were successfully designed by using Primer Premier 5.0 (Clarke and Gorley, 2001). Thirty (30) wild individuals from 3 populations collected in Xiamen, Lianjiang and Zhanjiang were used to analyze the polymorphism of these eight microsatellite markers. Polymerase chain reactions (PCR) were performed in volume of 10 µL. The PCR protocols included: initial denaturation of 5 min at 94°C, followed by 32 cycles with 30 s at 94°C, 30 s at annealing temperature (Table 1), 30 s at 72°C, and a final extension at 72°C for 10 min. The PCR products were electrophoresed on Sequi-Gen Sequencing Cell. Number of alleles per locus (*A*), observed (*Ho*) and expected (*He*) heterozygosity and polymorphic information content (*PIC*) were calculated by the software POPGEN32 and CERVUS 3.0 (Table 1). Also Deviations from Hardy-Weinberg equilibrium (HWE) and evaluated genotypic linkage disequilibrium (LD) were tested using POPGEN32 (Raymond and Rousset, 1995).

RESULTS AND DISCUSSION

According to the results, the number of alleles per locus ranged from 2 to 7 and the polymorphism information content ranged from 0.2076 to 0.7484. The observed and expected heterozygosities

ranged from 0.1724 to 0.9130 and from 0.1639 to 0.7314, respectively (Table 1). No deviation from the Hardy–Weinberg equilibrium in the tested population after Bonferroni correction (adjusted $P = 0.00625$). And no loci showed significant linkage disequilibrium after Bonferroni correction. There was no evidence for allelic dropout or null alleles, as shown by MICRO-CHEKER.

Number of alleles per locus (*A*) of eight loci ranged from 2 to 7, which was similar to that of *Fenneropenaeus chinensis* (Zhang et al., 2004), but was much lower than that of *Penaeus monodon* by Pongsomboon et al. (2000) and by Xu et al. (1999). This may due to the loci applied as well as the samples. *F. penicillatus* Alcock populations underwent a severe decline in the late 1980s and early 1990s, which contributed to the small population size and the low polymorphism of wild populations. However, we needed more population genetic information to ensure the hypothesis.

The eight novel microsatellite markers described here will be useful for analyzing the genetic structure within/ between populations of *F. penicillatus* Alcock.

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