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*Short Communication*

# Development and characterization of new microsatellite markers of *Fenneropenaeus penicillatus*

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**ABSTRACT.** Thirteen new polymorphic microsatellite markers in *Fenneropenaeus penicillatus* were isolated and characterized. The polymorphism of the thirteen microsatellite markers was tested by 30 individuals from Lianjiang, China. It showed that the number of alleles per locus ranged from 3 to 6 and the Polymorphism Information Content (PIC) was from 0.324 to 0.706. The observed and expected heterozygosities were 0.3217-0.8023 and 0.1977-0.6783, respectively. Only one loci (LJ-19) deviated significantly from Hardy-Weinberg equilibrium (HWE) ($P < 0.00385$) after Bonferroni correction, while the other twelve markers were in HWE after Bonferroni correction ($P > 0.00385$). The thirteen polymorphic microsatellite markers could provide more genetic data for further research on cultivation and recovery of *F. penicillatus*.

**Key words:** *Fenneropenaeus penicillatus*; Polymorphic microsatellite loci; FIASCO

 

## INTRODUCTION

Red tail shrimp (*Fenneropenaeus penicillatus*) range extends from Pakistan to Indonesia in the Indo-West Pacific and was common in the 1990s in the east and south sea of China (Zhang et al., 2010). However *F. penicillatus* abundance has diminished mainly due to environmental issues. Fishing pressure was also thought to have contributed to a reduction in fish numbers and truncated age structures in some regions. In 2005, *F. penicillatus* was included in the Red List by the Chinese government as an endangered species (Wang and Xie, 2009). To slow down this tendency, efforts have been made. Microsatellite marker has show its predominance in the study of population genetic structure (Paul, 2000). Ten polymorphic microsatellite loci of *F. penicillatus* were developed for genetic conservation (Cao et al., 2012). This is not sufficient, so thirteen more polymorphic microsatellite loci of *F. penicillatus* are developed.

## MATERIAL AND METHODS

The microsatellite loci was developed according to the FIASCO protocol (Zane et al., 2002). By a modified cetyltrimethylammonium bromide extraction, genomic DNA was extracted from the muscle of a single wild *F. penicillatus* population captured in Lianjiang, China. After DNA concentration test, the genomic DNA (100 ng/μL) was digested with restriction enzyme *Mse*I (Fermentas, Vilnius, Lithuania) at 65°C for 180 min in a 25 μL volume. The digested fragments, ranging from 400 to 1200 bp, were ligated to *Mse*I adapter1 (5'-ACGATG AGTCCTGAG-3')/*Mse*I adapter2 (5'-TACTCAGGACTCAT-3') by T4 DNA ligase (Fermentas) at 22°C for 10 h. Denature the digestion-ligation fragments at 95°C for 10 min, then hybridize the fragments to the biotinylated oligonucleotide probes $(CT)_{15}$ and $(GT)_{15}$ at 61°C for 1 h. Next, the streptavidin-coated magnetic sphere particles (Promega, Madison, WI, USA) were used to capture and gather the microsatellite repeats in the fragments, while the noncaptured and loose DNA fragments were washed away. The recovered DNA fragments were amplified using *Mse*I adapter1. After purification by GenCleanPCR (Generay, Shanghai, China) to remove the extra adapters and dNTPs. Purified products (4 μL) were ligated to 1 μL PMD19-T vector (Takara, Shiga, Japan) at 16°C for 10 h. Afterwards, the product (5 μL) were transformed into 100 μL *Escherichia coli* (Invitrogen, Carlsbad, CA, USA) for further selection on ampicillin plates (60 ug/ml). Positive clones were cultured into 96-well plates at 37°C for 4.5 h with shaking. With universal M13 primer, the positive clones were detected by PCR amplification. After the visualization of the PCR products on 1% agarose gels, one hundred and eighty-one clones ranging from 400-1200 bp were sequencing by Life Technologies (Guangzhou, China).

One hundred and sixty-three fragments were successfully sequenced. After hunted by SSR hunter1.3, one hundred and forty-six microsatellite sequences met the requirements. Eighty-seven pairs of microsatellite amplification primers were designed by Primer Premier 5.0.32. After optimizing the amplification conditions for each primer pair in an Eppendorf Mastercycler Gradient System (Eppendorf, Hamburg, Germany), forty-seven primer pairs were successfully selected for testing by amplifying 30 wild individuals genomic DNA captured in Lianjiang, China. The PCR amplification was in a volume of 10 μL and performed as followings: denature for 5 min at 95°C; proceeded with 35 cycles of 30 s at 95°C, 30 s at annealing temperature (Table 1), and 40 s at 72°C; then extend at 72°C for 10 min; stored at

4°C. The amplified products were electrophoresed on polyacrylamide gels in a Sequi-Gen Sequencing Cell (Bio-Rad, Hercules, CA, USA) and visualized by silver staining. Finally, the basic genetic information was analyzed by the POPGENE 32 (version 1.32) (Yeh et al., 2000) and CERVUS 3.0 software (version 3.0).

## RESULTS AND DISCUSSION

Thirteen polymorphic microsatellite markers were screened, and the characterization of the thirteen markers were presented in Table 1.

The number of alleles per locus ranged from 3 to 6, and the polymorphism information content (PIC) varied from 0.324 to 0.706 by CERVUS3.0. The observed and expected heterozygosities were 0.3217-0.8023 and 0.1977-0.6783, respectively. Deviations from Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium (LD) were tested by POPGEN32. Only one loci (LJ-19) deviated significantly from Hardy-Weinberg equilibrium (HWE) ($P < 0.00385$) after Bonferroni correction. The main reason for this was heterozygosity deficiency caused by Wahlund effects, inbreeding or null alleles.

The thirteen polymorphic microsatellite markers could provide more genetic data for further research on cultivation and recovery of *F. penicillatus*.

**Table 1.** Characterization of 13 microsatellite primers in *Fenneropenaeus penicillatus* (sample size = 30 individuals).

| GenBank accession No. | Locus ID | Primer sequence (5'-3') | Ta (°C) | Repeat motif | Allele size (bp) | $N_A$ | PIC | $H_O$ | $H_E$ | P-HWE |
|---|---|---|---|---|---|---|---|---|---|---|
| KM095655 | LJ-7 | F: TGGGTCCGATTCCT<br>R: GGTGGATGCGAAAA | 49 | $(ACC)_5$ | 165-178 | 4 | 0.551 | 0.5857 | 0.4143 | 0.6273 |
| KM095656 | LJ-15 | F: TAAGGCAGTTGAAACGATGTCC<br>R: CCTTTCTTTCTTCTCACGCACC | 42 | $(TCGC)_3$ | 125-130 | 3 | 0.434 | 0.5435 | 0.4565 | 0.3476 |
| KM095657 | LJ-19 | F: AGCGTCTCGTCTCCTCCTCT<br>R: GAACTTTCGCTTGGACTTGG | 50 | $(TG)_{34}$ | 155-177 | 4 | 0.514 | 0.5311 | 0.4689 | 0.0000* |
| KM095658 | LJ-36 | F: AAGAGGATGAGAAGGC<br>R: CACAGAGCAGAGAGCG | 53 | $(AC)_{20}GC(AC)_9GC(AC)_8N(CT)_{18}$ | 220-227 | 3 | 0.478 | 0.7415 | 0.2585 | 0.5302 |
| KM095659 | LJ-40 | F: TTATGCTGAGACGGAGGGAATG<br>R: CAGACTGACACACAGGCAAACA | 58 | $(TGTC)_4$ | 240-265 | 6 | 0.706 | 0.3217 | 0.6783 | 0.0082 |
| KM095660 | LJ-43 | F: CAGTAGAAAGCAAACGAATGGG<br>R: ACGACGTATGCAAATCAAAACC | 41 | $(ATAG)_4ATAA(ATAG)_3$ | 281-302 | 4 | 0.336 | 0.6594 | 0.3406 | 0.6181 |
| KM095661 | LJ-47 | F: AAAGGTCGGGAAGA<br>R: CCACAAACGCACAT | 53 | $(AG)_{27}$ | 265-300 | 5 | 0.387 | 0.8023 | 0.1977 | 0.9997 |
| KM095662 | LJ-49 | F: ATGGCGTGATAAGGATTG<br>R: GAGGGAAAGGAAGATACAGA | 59 | $(TC)_5$ | 192-200 | 4 | 0.431 | 0.5838 | 0.4162 | 0.3538 |
| KM095663 | LJ-54 | F: GCAGGGACAGACAGAG<br>R: ACGAACGAGCAAGAGT | 47 | $(CT)_{15}$ | 102-108 | 3 | 0.390 | 0.5517 | 0.4483 | 0.0901 |
| KM095664 | LJ-61 | F: GCAAACAGGAGAAC<br>R: TGTGGACTGAGGCT | 44 | $(AG)_{20}GG(AG)_8(TG)_{29}(AG)_9$ | 178-192 | 5 | 0.347 | 0.7624 | 0.2376 | 0.9978 |
| KM095665 | LJ-63 | F: TGTAAACCGCCATATCCTCT<br>R: TGACTTTGTCCGTCCTTCTG | 58 | $(TGGG)_3$ | 153-182 | 4 | 0.324 | 0.7429 | 0.2571 | 0.8801 |
| KM095666 | LJ-71 | F: GACGAGGATGGAAAGCAA<br>R: GAAAGGGGTAGGGGAAAA | 43 | $(AC)_8$ | 210-225 | 5 | 0.583 | 0.4123 | 0.5877 | 0.0269 |
| KM095667 | LJ-78 | F: TGAGTAATGAAAATCCGT<br>R: GCAGCATAGAGTATAGACAG | 45 | $(TG)_9$ | 200-225 | 5 | 0.326 | 0.7422 | 0.2578 | 0.9951 |

Ta, annealing temperature; $N_A$, number of polymorphic alleles per locus; PIC, polymorphic information content; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; and P-HWE, P values for the Hardy-Weinberg expectation test (adjusted P = 0.00385). *Denotes significant departure from the Hardy-Weinberg equilibrium.

 

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