Short Paper

Species Identification and Polymorphisms Using RAPD-PCR in Penaeid Prawns *Penaeus japonicus* and *Metapenaeus ensis*

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Penaeid prawns are cultured and stocked in many of the coastal waters of Japan. It is thus necessary to investigate the genetic variability of the wild population and the hatchery stock in relation to the genetic management of the natural populations. However, until now genetic analysis has not been performed systematically in Penaeids. Since allozyme analysis showed a low level of sensitivity in Penaeid prawns,^{1,2} a new DNA analysis (RAPD) developed with PCR was used to examine and detect polymorphisms.^{3–5} RAPD, by adopting a short arbitrary sequence, may be a convenient method because prior knowledge of DNA sequences is not needed. RAPD has been applied in breeding programs of the penaeid prawn, *Penaeus monodon*.⁶

The objective of this study is to establish RAPD methods in penaeid prawns, and to assess their potential for the identification of species and degree of polymorphisms within each species.

Prawns samples were collected from two different places and identified morphologically: Wild adults of *Penaeus japonicus* and *Metapenaeus ensis* were collected in the coastal water of Kochi Prefecture. Thirty-three-day-old cultured postlarvae of *M. ensis* (mean body weight 0.017 g) were obtained from Kochi Prefectural Sea Farming Center.

Genome DNA were extracted by the SDS-phenol chloroform method⁶ from 100% alcohol preserved samples using similar volumes for all morphological structures. In the adult *P. japonicus* and *M. ensis*, the total genomic DNA was isolated from the pleopods,⁶ while for the postlarvae of *M. ensis*, the total genomic DNA was isolated from each whole homogenized individual specimen. In our analysis, we used three arbitrary sequenced primers of 10-mer oligonucleotides, Operon 10-mer kits, OPA and OPB (Operon Technologies Inc.).

PCR reactions were performed by modifying the protocol of Takagi and Taniguchi.⁵ Amplifications were done in 100 µl of reaction mix containing 1 µg of template DNA, 2.5 unit of Taq polymerase (Perkin Elmer Inc.), 0.2 mM dNTPs, 2 µM of primer, and 1 × reaction buffer. This buffer was the same as that supplied by the enzyme manufacturer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5

mM MgCl₂, 0.001% gelatin). Reactions were performed by a Mini-Cycler (MJ Research Inc.) programmed for one cycle at 94°C for 4 min, followed by 35 cycles at 94°C for 1 min, at 36°C for 1 min, at 72°C for 2 min, and another cycle at 72°C for 5 min. Then 15 µl of the reaction products were separated on a 5% polyacrylamide gel at 150 V/cm for 90 min in 1 × TBE buffer. Gels were stained with ethidium bromide and photographed on a UV trans-illuminator.

Forty primers were tested in the preliminary screening for the specimens of both species. Two primers, OPA-02 (5'TGCCGAGCTG 3'), and OPA-03 (5'AGTCACGCAC 3'), were found to generate individual prominent bands and they were subsequently used for further analysis.

Figure 1 shows the RAPD pattern obtained, using the primer OPA-03 from *P. japonicus* and *M. ensis*. A monomorphic pattern (Genotype I) was found in *P. japonicus*, while *M. ensis* had two genotypes (Genotype II, III). Figure 2 shows the band patterns of wild adults and cultured postlarvae of *M. ensis* using the same primer (OPA-

Fig. 1. Banding pattern of amplified DNA in two penaeus species using OPA-03 primer.

Counting from the right; lane 1, molecular size marker; lane 2–6, *P. japonicus*; lane 7–11, *M. ensis*.

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Fig. 2. Banding pattern of amplified DNA in wild adults and cultured postlarvae of *M. ensis* using OPA-03 primer. Counting from the right; lane 1, molecular size marker; lane 2-6, wild adults; lane 7-10, cultured postlarvae.

Fig. 3. Banding pattern of amplified DNA in *M. ensis* using OPA-02 primer.

Counting from the right; lane 1, molecular size marker; lane 2-11, *M. ensis*.

03). The genotype of postlarvae was monomorphic (Genotype III) and the Genotype II did not appear. Figure 3 shows the RAPD pattern of adults of *M. ensis* obtained using OPA-02. This primer showed a high degree of polymorphism. The numbers of genotypes observed in each species with OPA-02, 03 are listed in Fig. 4 and Table 1. There is no banding pattern sharing between the two species, in primers OPA-02 and OPA-03, which suggests that these patterns may be useful markers for species identification. Because of its monomorphic banding pattern, OPA-03 primer may be specially suitable for identification of *P. japonicus* and *M. ensis*. On the other hand, in OPA-02, many genotypes were detected, which suggests that OPA-02 may be suitable for population analysis in penaeid

Fig. 4. Schematic figure of genotype amplified DNA in two penaeus species using OPA-02 primer.

Counting from the left; lane 1, molecular size marker; lane 2-7, *P. japonicus*; lane 8-16, *M. ensis*. Numbers in parentheses show the observed number of each genotype.

Table 1. Number of polymorphic individuals estimated from RAPD-PCR fragments patterns detected with primer OPA-03

Species		Genotype		
		I	II	III
<i>M. ensis</i>	Adult	0	8	7
	Postlarvae	0	0	15
<i>P. japonicus</i>	Adult	11	0	0

prawns. The scarcity of polymorphism in *M. ensis* postlarvae suggests a decrease of genetic variability, suggesting that a bottle-neck effect had occurred during artificial propagation in cultured stocks. RAPD may also be useful in smaller specimens where morphological identification is difficult. However, Garcia and Benzie (1995) suggested that bacterial and algal contamination may happen in amplified products of smaller specimens. In addition, the band patterns detected in some primers slightly change during the PCR and electrophoretic processes. Therefore, the experimental conditions must be set up strictly.

We consider that the proper use of primer provides excellent sensitive markers for species identification, and for estimating genetic variability and inbreeding levels in penaeid prawns.

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