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- Note -

Development of Molecular Marker to Distinguish *Octopus minor* Sasaki Caught in Korea and that in China

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Octopus minor (*O. minor*) is widely distributed along the coastal regions of Korea, but most of them are caught in southern waters which are associated with one of the important fisheries stock. At present, *O. minor* from China has been introduced to the fishery markets in Korea. Here, we attempt to discriminate their origin for Korea or China using molecular techniques. Based on the *O. minor* mitochondrial DNA sequence, we developed a PCR-based origin discrimination system. The assay specificity was assessed by testing four individuals of *O. minor* from Sangdong, China, as well as 20 additional *O. minor* from Namhae, Muan, Yeosu and Jindo, Korea. Only four isolates of *O. minor* originated from China tested as positive in our distinction system. All PCR-positive products yielded identical sequences from Chinese *O. minor*, whereas Korean *O. minor* appeared to be PCR amplification. This result suggested that the primers used in this study are *O. minor* species specific, especially originated from China. The detection system appeared to be positive results in the use of 0.1 ng of Chinese *O. minor* DNA as template, however, the Korean *O. minor* even using 1 μg of DNA showed no amplification. Consequently, the assay provides a simple, rapid and accurate method for the detection of Chinese *O. minor*.

Key words : China, detection, DNA primer, Korea, PCR, octopus

Introduction

The octopus *Octopus minor* Sasaki is one of the most important fisheries resources in Korea. This species can be generally found around Korean coast. In particular, since southern waters are well developed by mud flats based on geographic characteristics, the region supports good habits for *O. minor* continuing a number of capture rate [4]. More recently, *O. minor* has been imported in great numbers from China to the Korean fisheries market. The new technique to identify *O. minor* imported from Chin is an impediment in terms of conserving native genetic stocks and rapid monitoring of those captured from Korean coast. The approach using DNA analysis methods has been useful regarding identification and detection against various organisms. Developed DNA techniques by specific probes and PCR primers allow targeted species to be distinguished from each other and from other species. However, little research has been undertaken to determine genetic studies between Korean and Chinese *O. minor*. The object of this study is to discriminate that of Chinese from Korean *O. minor* by PCR amplification with specific primer.

O. minor samples used in this study were collected from four localities in Korea and one locality in China. We used a total of 20 specimens obtained from Namhae (4 individuals), Muan (4 individuals), Yeosu (4 individuals), and Jindo (4 individuals) on the southern coast, as well as Sangdong (4 individuals) in China. The samples were captured from October to December, 2006. Total genomic DNA was extracted from 0.05 g of eyeball using a DNA Isolation kit (Roche Co.) after homogenizing. The extracted genomic DNA was frozen at -20°C until required. The partial region of the mtDNA COI gene was amplified and sequenced using HCO2918 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO1491 (5'-GGTCAACAAATCATAAAGATATTG G-3') primers [2]. PCR reactions were performed under the following conditions in 25 μl mixture: 1.25 unit *Taq* DNA polymerase (FastStar *Taq* DNA polymerase, Roche Co.); 1×PCR reaction buffer (Roche Co.); 0.1 mM dNTPs; 20 pmol of each primer; and 5-30 ng genomic DNA. Amplifications were performed with the MyCycler thermal cycler (Bio-Rad). The thermocycling profile included an initial denaturation step of 95°C for 4 min, followed by 35 cycles of 1 min at 94°C, primer annealing for 1 min at 50°C, and extension for 2 min at 72°C. The final extension step was in-

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creased up to 10 min. PCR product was separated by running at 50 V for 50 min in 2% agarose containing 0.5 μg ml^{-1} ethidium bromide and then checked for molecular size. The product was purified using a PCR Purification kit (Nucleospin® Extract) by following the manufacturer's instructions. The purified DNA was directly sequenced using an Applied Biosystem model ABI 3730XL automated sequencer and a Big Dye terminator cycle sequencing kit (Perkin-Elmer Applied Byosystems, UK). Sequence data were aligned using the multiple alignment program Clustal W [9]. Potential forward and reverse primers were selected manually using an on-line Primer design program (http://www-genome.wi.mit.edu/cgi-bin/primer), from aligned mtDNA COI gene sequences of *O. minor* individuals. The primer sequences are shown in Table 1. Specific PCR reactions were performed under the following conditions in 25 μl reaction volumes, with the same conditions as described above. The DNA was initially denatured at 95°C for 10 min followed by 35 cycles (denaturation at 94°C for 1 min, annealing at 48°C for 2 min, and extension at 72°C for 2 min). The final extension was established at 72°C for 5 min. The specificity of the PCR assay was assessed by amplifying DNA from 10 ng each of *O. minor* from China and Korea. For assessment of the sensitivity of the PCR assay, 0.001 to 1 ng of Chinese *O. minor* DNA was added into 1 μg of the genomic DNA of the Korean *O. minor*, and the mixture was subjected to the Chinese *O. minor* PCR detection assay. The PCR and gel electrophoresis were also carried out under the same conditions as described above.

For assessment of the species specificities of the OctoF3 and OctoR1 PCR assays, 20 individuals of *O. minor* were tested. Only four individuals from China tested were positive with the primer pair OctoF3-OctoR1 (1,012 bp), whereas there were no PCR products from Korea *O. minor* (Fig. 1). The sensitivity of the primers was tested by performing a PCR with serially diluted Korean and Chinese *O. minor* (Fig. 2). Based on the range of dilutions tested, the detection limit of the primers was 0.1 ng (Fig. 2a-b),

Table 1. Design of Octopus minor-specific oligonucleotide primers from mtDNA sequences

Primers	Sequence	Expected PCR product size (bp)
OctoF3 (forward)	5′-ATAATAATTTGCCTT-3′	1,012 bp
OctoR1 (reverse)	5′-AAAAAGCTTGAATAATT-3′	

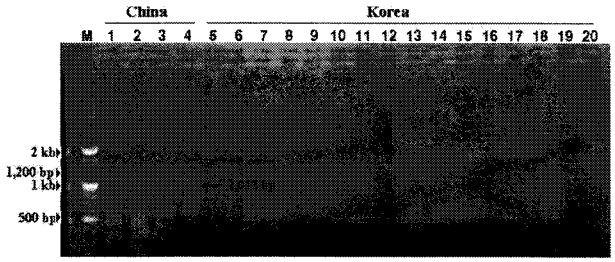

Fig. 1. Species specificity of the PCR-based assay for Chinese *Octopus minor* individuals were amplified with specific primers. PCR products for Chinese *O. minor* using OctoF3 (forward) and OctoR1 (reverse) targeted to a mtDNA COI gene were produced to 1,012 bp. Lanes 1-4, Sangdong, China; Lanes 5-8, Namhae, Korea; Lanes 9-12s, Muan, Korea; Lanes 13-16, Yeosu, Korea; Lanes 17-20, Jindo, Korea. M, molecular size marker (100 bp).

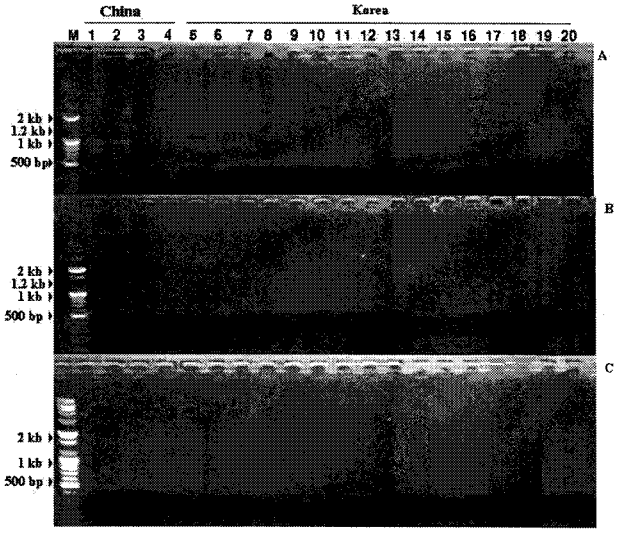

Fig. 2. Sensitivity of PCR-based assay for Chinese *Octopus minor*. Products are amplicons of 10-fold serial dilutions of Chinese *O. minor* starting from 1 ng with 1 μg of Korean *O. minor* from Namhae (A), 0.1 ng with 1 μg of Korean *O. minor* from Muan (B), and 0.01 ng with 1 μg of Korean *O. minor* from Yeosu (C). Lane 1-4, Sangdong, China; Lane 5-8, Namhae, Korea; Lange 9-12, Muan, Korea; Lange 13-16, Yeosu, Korea; Lange 17-20, Jindo, Korea. M, molecular size marker (100 bp).

but 0.01 ng DNA did affect the sensitivity of the assay (Fig. 2c). The effect of the mixture of 1 μg background DNA from Korean *O. minor* to the sensitivity of the assay was not observed.

In the analysis of sequences of the mtDNA COI gene, we have proven the relatively high similarity coefficients within the population of Korea, but slight genetic differ-

ences between Korean and Chinese *O. minor*. From the perspective of our results, it is assumed that a significant genetic divergence against *O. minor* will not be found, although Korea and China are separated geographically. Based on ecological characteristics, the boundary of the habitat (e.g. growth, feeding, reproduction, and so on) of an *Octopus* species is very small compared to other marine organisms [1,3,5-8]. This indicates that it is possible to estimate less genetic variability within the populations and genetic distance between the populations of *O. minor*.

In contrast, the PCR based method is expected to be used for the rapid differentiation of Chinese from Korean *O. minor*, although they have a high sequence homology. Consequently, designed sets of primers for variable regions will be required in the near future.

Acknowledment

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초록 : 한국산과 중국산 낙지구별을 위한 DNA 마커

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낙지는 우리나라 연안에 대부분 서식하는 종으로 특히 남해안 연안에서 많이 어획되고 있는 종이다. 현재, 중국산 낙지가 우리나라 수산시장에 많이 수입되고 있는 관계로 본 연구에서는 분자마커를 이용하여 한국산과 중국산 낙지를 구별하기 위하여 조사했다. 유전자 증폭을 이용하여 중국 산동지역에 서식하고 있는 낙지 4마리에 대하여 PCR 생성물이 보인 반면에, 남해, 무안, 여수, 진도에 서식하고 있는 낙지 미토콘드리아 DNA는 나타나지 않았다. 따라서 PCR 증폭으로 국내산 1 μg DNA 첨가 시 중국산 0.1 ng DNA까지 민감도를 보여, 앞으로 간편하고 신속한 중국산 낙지 구별을 위하여 좋은 도구로 이용될 것으로 보인다.