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☐ **Brief Communication** ☐

Different RAPD patterns between *Metagonimus yokogawai* and *Metagonimus* Miyata type

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Abstract: Genomic DNA from *Metagonimus yokogawai* and *Metagonimus* Miyata type was amplified by polymerase chain reaction based on the random amplification of polymorphic DNA (RAPD) technique. Eight random 10-mer oligonucleotide primers (OPA-02, 5-TGCCGAGCTG-3; OPA-09, 5-GGGTAACGCC-3; OPA-10, 5-GTGATCGCAG-3; OPA-11, 5-CAATCGCCGT-3; OPA-13, 5-CAGCACCCAC-3; OPA-17, 5-GACCGCTTGT-3; OPA-19, 5-CAAACGTCGG-3; OPA-20, 5-GTTGCGATCC-3) with a G+C content of 60-70% (Kit A, Operon Technologies Inc., California, USA) could produce distinguishable banding patterns between the two *Metagonimus* species. From the results of this study, it was suggested that *Metagonimus* Miyata type has a different DNA sequence from *M. yokogawai*.

Key words: *Metagonimus yokogawai*, *Metagonimus* Miyata type, random amplification of polymorphic DNA (RAPD)

Genus *Metagonimus* is one of the most important human infectious flukes in Korea. Nationwide survey data showed 0.3% infection rate on this parasite from the whole examined population (Ministry of Health and Social Welfare and Korea Association of Health, 1992). But certain focal endemic areas show much higher prevalence (about 20%; Yu *et al.*, 1994) than mean value.

It has been debated for several decades how many species are present among the genus *Metagonimus* since morphologically the worms belonging to the genus *Metagonimus* have some delicate differential points in their bodies. The well known species are *M. yokogawai* and *M. takahashii*. But Saito (1984) suggested two more subtypes of *M. yokogawai* such as *Metagonimus* Miyata type and *M.* Koga type. In Korea, *M. yokogawai*, *M. takahashii* and *Metagonimus* Miyata type have been reported (Kim *et al.*, 1987; Chai *et al.*, 1991 & 1993).

Random amplification of polymorphic DNA (RAPD) analysis is recently reported as a useful method to differentiate strains and species of *Schistosoma* and *Echinostoma* (Petrie *et al.*, 1996). We tried to apply this method to distinguish *M. yokogawai* and *Metagonimus* Miyata type.

Metacercariae (Mc) of *M. yokogawai* were collected by artificial digestion of *Plecoglossus altivelis* caught at Oshipcheon (stream), Samchok-gun, Kangwon-do. Mc of *Metagonimus* Miyata type were collected from *Zacco platypus*, caught at Talchongang (River), Chungju, Chungchongbuk-do. Adult worms of two kinds were obtained through oral infection

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to experimental rats (Sprague Dawley). The rats were sacrificed 2 weeks after the infection and the worms were isolated from the small intestine. Collected worms were stored at -20°C until used for DNA extraction.

For DNA extraction, the method of Sambrook *et al.* (1989) was followed. Adult worms were placed into a 1.5 ml tube containing lysis buffer (10 mM Tris-Cl, pH 8.0, 100 mM EDTA, 0.5% SDS). After thorough mixing and incubation overnight at 37°C, samples were treated with proteinase K (100 μg/ml) and RNase (20 μg/ml). Subsequently extraction with an equal volume of phenol: chloroform:isoamyl alcohol was performed. Total genomic DNA was finally precipitated overnight with addition of 1/20 volume 5 M NaCl and 2 volume 99% ethanol at -70°C. Genomic DNA pellet was obtained by centrifugation at 10,000 *g* for 10 min and resuspended in 50 μl of TE (10 mM Tris-Cl, pH 8.0, 1 mM EDTA) buffer. RNA in the sample was digested by addition of 0.1 volume RNase A (10 mg/ml) and incubated for 1 hr at 37°C. Extraction using phenol:chloroform: isoamyl alcohol was done as before. Residual phenol was removed by chloroform extraction. Total genomic DNA was precipitated as before, resuspended in 25 μl of TE buffer and stored at 4°C.

PCR amplification was done as follows. Twenty random 10-mer oligonucleotide primers with a G+C content of 60-70% (Kit A, Operon Technologies Inc., California, USA) were used for the initial study. Among them, 8 primers were chosen for further study as they proved to be suitable for distinguishing the *Metagonimus* species. The numbers and sequences of these primers were as follows: OPA-02, 5-TGCCGAGCTG-3; OPA-09, 5-GGGTAACGCC-3; OPA-10, 5-GTGATCGCAG-3; OPA-11, 5-CAATCGCCGT-3; OPA-13, 5-CAGCACCCAC-3; OPA-17, 5-GACCGCTTGT-3; OPA-19, 5-CAAACGTCGG-3; OPA-20, 5-GTTGCGATCC-3.

A total of 100 μl volume mixture containing sample DNA (90 ng), 2.5 mM $MgCl_2$, 100 μM of each dNTP (Promega), 25 pmol of each of primers and 2.5 units of *Thermus aquaticus* DNA polymerase (Promega) was amplified by PCR thermocycler (Perkin Elmer 9600). The PCR reaction was done at 94°C for 1 min (denaturation), at 35°C for 1 min (annealing), and at 72°C for 2 min (extension). This reaction was repeated for 45 cycles. Amplified products were analysed by electrophoresis in 8% polyacrylamide gel and detected by silver staining.

Each of eight primers made clearly distinguishable band patterns between *M. yokogawai* and *Metagonimus* Miyata type (Figs. 1 & 2). The banding patterns in Figure 1 and 2 strongly suggested that *M. yokogawai* and *Metagonimus* Miyata type have different genomic DNA sequences. Petrie *et al.* (1996) reported molecular characterization of *Echinostoma caproni* and *E. paraensei* by RAPD. They recommended the primers 3301 and 3303 which were originally designed for *Schistosoma* species as successfully distinguishable ones. Though we did not use those primers in this study, 8 primers among 20 commercially available primers were proved to be useful for discrimination of the two *Metagonimus* species. As reported separately, the restriction fragment length polymorphism (RFLP) patterns of internal transcribed spacer I of rDNA and mitochondrial COI gene of the 2 species were also proved different (Yu *et al.*,

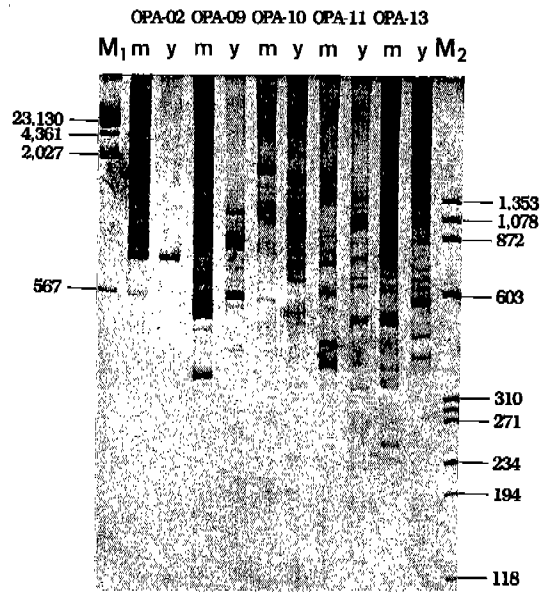

Fig. 1. Silver stained polyacrylamide gel showing RAPD profiles of *Metagonimus* species. OPA, primer number; m, *Metagonimus* Miyata type; y, *M. yokogawai*; M_1, M_2, marker.

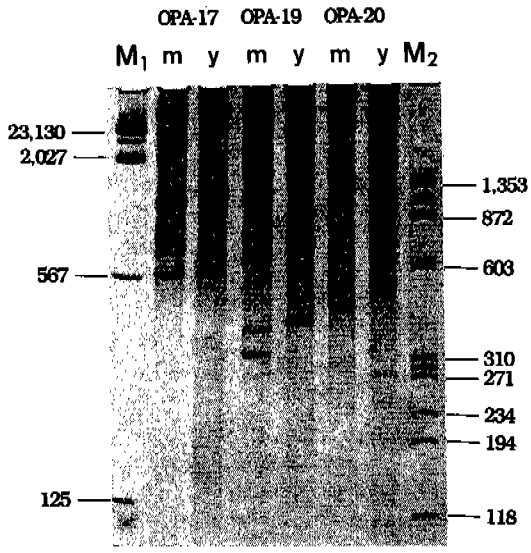

Fig. 2. Silver stained polyacrylamide gel showing RAPD profiles of *Metagonimus* species. OPA, primer number; m, *Metagonimus* miyata type; y, *M. yokgawai*; M_1, M_2, marker.

1997).

Biologically, it is well known that the second intermediate hosts of *M. yokogawai* and *Metagonimus* Miyata type are different. *M. yokogawai* is transmitted by *P. altivelis* and *Tribolodon taczanowskii*, and *Metagonimus* Miyata type by *Z. platypus* (Chai *et al.*, 1991 & 1993). Also morphologically, the location of testes, distribution of uterine tubules, egg sizes and ending point of vitelline follices of two species were also different (Chai *et al.*, 1991). From the results of this study, we could add strong evidences for *M. yokogawai* and *Metagonimus* Miyata type to be different species.

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=초록=

RAPD 분석을 이용한 요코가와 흡충과 미야타흡충의 분자생물학적 비교

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요코가와흡충과 미야타흡충의 genomic DNA를 RAPD 분석을 이용하여 비교하였다. 상업적으로 구입한 60-70%의 G+C 성분을 가진 무작위 10-mer oligonucleotide 표지자 (Kit A, Operon Technologies Inc., California, USA) 20개 중에서 다음의 8개를 이용하여 두 흡충간에 구별이 가능한 밴드양상을 관찰할 수 있었다: OPA-02, 5-TGCCGAGCTG-3; OPA-09, 5-GGGTAACGCC-3; OPA-10, 5-GTGATCGCAG-3; OPA-11, 5-CAATCGCCGT-3; OPA-13, 5-CAGCACCCAC-3; OPA-17, 5-GACCGCTTGT-3; OPA-19, 5-CAAACGTCGG-3; OPA-20, 5-GTTGCGATCC-3. 이 연구의 결과로 미야타흡충은 요코가와흡충과 서로 다른 유전자 염기서열을 가지고 있음이 암시되었다.

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