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☐ **Brief Communication** ☐

Genetic variance of *Trichomonas vaginalis* isolates by Southern hybridization

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Abstract: In the present study, genomic DNAs were purified from Korean isolates (KT8, KT6, KT-Kim and KT-Lee) and foreign strains (CDC85, IR78 and NYH 286) of *Trichomonas vaginalis*, and hybridized with a probe based on the repetitive sequence cloned from *T. vaginalis* to observe the genetic differences. By Southern hybridization, all isolates of *T. vaginalis* except the NYH286 strain had 11 bands. Therefore all isolates examined were distinguishable into 3 groups according to their banding patterns; i) KT8, KT6 and KT-Kim isolates had 11 identical bands such as 1 kb, 1.2 kb, 1.6 kb, 1.9 kb, 2.3 kb, 2.7 kb, 3.2 kb, 3.4 kb, 3.8 kb, 4.9 kb and 6.0 kb. ii) The metronidazole-resistant IR78 strain had the same bands as KT-Lee isolate at bands of 1 kb, 1.2 kb, 1.6 kb, 1.8 kb, 2.1 kb, 2.5 kb, 2.7 kb, 2.9 kb, 3.4 kb, 5.0 kb and 6.0 kb. Bands of CDC85, metronidazole-resistant strain, were similar to those of IR78 and KT-Lee, except that 3.2 kb replaced 2.9 kb. iii) NYH286 particularly had 12 bands and band patterns were similar to IR78 with a few exceptions as follows; i) 6.2 kb in place of 6.0 kb, ii) 2.0 kb and 2.2 kb instead of 2.1 kb. Through the results obtained, genetic variance of *T. vaginalis* isolates was demonstrated by Southern hybridization.

Key words: *Trichomonas vaginalis*, Southern hybridization, genetic diversity

Trichomonas vaginalis is one of the most widespread sexually transmitted diseases in the world with an incidence of 10 to 50% in patients of sexually transmitted disease clinics (Spence, 1986).

Among *T. vaginalis* isolates, phenotypic (Alderete *et al.*, 1986), isoenzymatic (Soliman *et al.*, 1982) and pathogenic differences (Mason and Forman, 1982; Min *et al.*, 1997) have been reported. Until now, a few reports were published on genetic variation among *T. vaginalis* isolates. Pace *et al.* (1992) reported that 3 strains of *T. vaginalis* showed different patterns by DNA hybridization with the probe Tv-E650-7.

The Southern blot analysis has been introduced for distinguishing species of *Giardia* isolate, differenciating the human infective and non-human infective isolates of *Trypanosoma brucei* and identification of *Leishmania* strains and *T. vaginalis* (Nash *et al.*, 1985; Rubino *et al.*, 1991; Guizani *et al.*, 1994; Hide *et al.*, 1994). In this study, the genetic variance of *T. vaginalis* were examined by Southern hybridization.

Seven isolates of *T. vaginalis* and one isolate of *Tritrichomonas foetus* were used in the study. *Trichomonas vaginalis* KT8, KT6, KT-

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Kim and KT-Lee isolates were obtained from Korean women with acute vaginitis (Ryu *et al.*, 1995). Metronidazole resistant *T. vaginalis* CDC85, IR78 and *T. foetus* KV strain were kindly provided by professor David Lloyd from the School of Pure and Applied Biology, Wales University, Cardiff. The NYH286 strain was purchased from ATCC (USA). Trophozoites were axenically cultured in TYM medium (Diamond, 1957).

For genetic analysis, it is necessary to get homogenous clone. The cloning of *T. vaginalis* was done according to the method applied to *Entamoeba histolytica* with a modification as 10% CO_2 incubator instead of anaerobic jar (Mueller and Petri, 1995). The agar (5%) and trophozoites (1×10^3) were mixed by inverting the tube 10 times and contents were then immediately poured into a Petri dish (100 by 15 mm). The colonies were visible to the naked eye after 4-5 days. Among seven isolates, KT8, KT-Lee and IR78 isolates had 2 colonies and CDC85 showed one colony (Fig. 1). We named the colony of each isolates as KT8C1 and KT8C2, KT-LeeC1 and KT-LeeC2, IR78C1 and IR78C2 and CDC85C, and cultivated each colony separately.

Trichomonad genomic DNA was isolated by a modified method of Rubino *et al.* (1991) and Ho *et al.* (1994). Briefly, cells were lysed with a NET buffer (containing 0.2% SDS) and DNA was extracted with phenol:chloroform, precipitated with sodium acetate, rinsed with ethanol, and dissolved in a TE buffer. RNase and proteinase K were treated subsequently, and then DNA was extracted with phenol: chloroform. The following procedures were the same as described above.

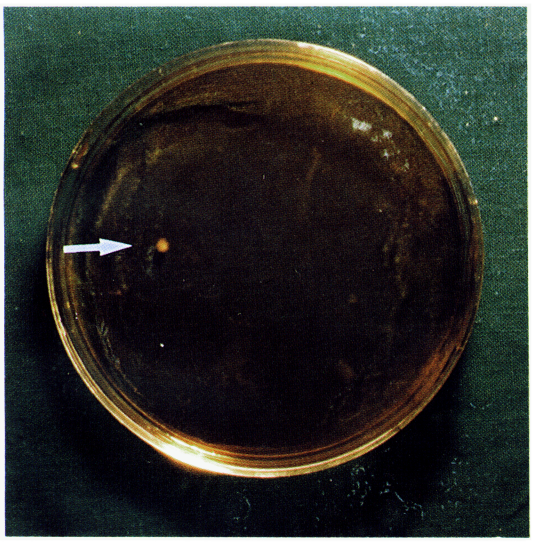

Fig. 1. Colony (arrow) of *T. vaginalis* in soft agar 5 days after seeding at a concentration of 1,000 trichomonad per plate

Field inversion gel electrophoresis (FIGE) was done using FIGE mapper (Bio-Rad). Five μg of *Eco*RI digested genomic DNA was loaded to each lane. Running condition was set at program 1 for 8 hr, and program 2 for 8 hr, and forward and reverse voltage was 180 V and 120 V, respectively. Restriction fragments separated on 0.8% agarose gels were denatured and transferred onto nylon membrane (Hybond-N^+, Amersham) by Southern blotting (Southern, 1975). For the probe preparation, PCR was done with *T. vaginalis* specific repetitive primers designed from Tv-E650-1, cloned by Pace *et al.* (1992). The forward and reverse primers were 5' GAGTTAGGGTCTAATGTTTGATGTG 3' and 5' AGAATGTGATAGCGAAATGGG 3' respectively. The amplification cycle comprised an initial 5 min denaturation at 95°C, then 1 min at 94°C, 1 min at 50°C, 1 min at 72°C for 35 cycle. About 337 bp sized product was identified and purified from agarose gels by using a Gene-clean kit (Qiagen, Germany). The purified 337 bp fragment was labeled with [α-^{32}P]dCTP by PCR as described previously (Konat *et al.*, 1994). Hybridization was carried out overnight in a 5× Denhardt's solution containing 6× SSPE, 50% formamide, salmon sperm DNA (100 μg/ml), and 0.5% SDS at 42°C. Membranes were washed twice with 2× SSPE and 0.5×SSPE. The autoradiographs were exposed to X-Omat film (Kodak, NY) with an intensifying screen for 3 weeks at –70°C.

By Southern hybridization, all six isolates of *T. vaginalis* except NYH286 strain had 11 bands in contrast to *T. foetus* showing no hybridization band (Fig. 2A). Bands shown by trophozoites of *T. vaginalis* before cloning were the same as those of colony-forming trophozoites after cloning. Namely, bands of the KT8 isolate were identical to KT8C1 and KT8C2; the same as in IR78 and KT-Lee (Fig. 2A).

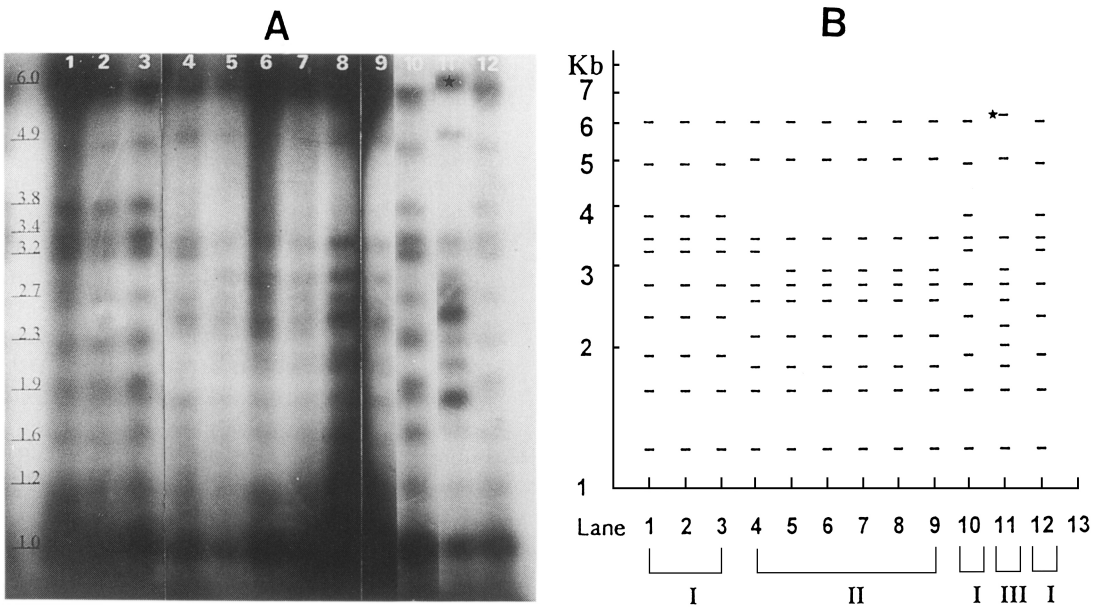

Fig. 2. Southern hybridization of 337 bp probe of *T. vaginalis* with *Eco*RI-digested trichomonad DNA. (A) Autoradiogram of Southern blot. Lane 1, KT8; 2, KT8C1; 3, KT8C2; 4, CDC85C; 5, IR78; 6, IR78C1; 7, IR78C2; 8, KT-Lee; 9, KT-LeeC2; 10, KT6; 11, NYH286; 12, KT-Kim; 13, *T. foetus* KV (B) Diagrammatic representation of banding patterns. Lanes 1-13 were identical to panel A. NYH286 strain (III group) had a peculiar 6.2 kb (★).

Three groups of isolates were distinguished after hybridization (Fig. 2B). The KT8, KT6 and KT-Kim isolates of the first group had 11 identical bands: 1 kb, 1.2 kb, 1.6 kb, 1.9 kb, 2.3 kb, 2.7 kb, 3.2 kb, 3.4 kb, 3.8 kb, 4.9 kb and 6.0 kb. The second group comprised IR78, KT-Lee and CDC85. The metronidazole-resistant IR78 strain had the same bands as the KT-Lee isolate: 1 kb, 1.2 kb, 1.6 kb, 1.8 kb, 2.1 kb, 2.5 kb, 2.7 kb, 2.9 kb, 3.4 kb, 5.0 kb and 6.0 kb. CDC85 is known as metronidazole-resistant strain, IR78. The bands of CDC85 were similar to those of IR78 and KT-Lee, except that 3.2 kb replaced 2.9 kb. The NYH286 strain, the third one, had 12 bands, and its hybridization pattern had shared common bands with IR78 with a few exceptions as follows: i) 6.2 kb in place of 6.0 kb, ii) 2.0 kb and 2.2 kb instead of 2.1 kb (Fig. 2A). Both strains of IR78 and CDC85 were metronidazole-resistant although metronidazole-sensitive KT-Lee had bands identical to IR78. Thus, the present hybridization pattern showed no relation with metronidazole resistance.

In the present study we have used *Eco*RI as a restriction enzyme for digestion of genomic DNA. Another restriction enzyme treatment probably will show band patterns different from bands showed in this experiments. As the seven isolates of *T. vaginalis* were not enough to observe the genetic variance, we are planning a further research with more isolates of *T. vaginalis* and restriction enzymes for examining genetic differences of *T. vaginalis*.

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=초록=

Southern hybridization에 의한 질편모충의 유전학적 다양성

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질편모충 7개 분리주 (국내분리주 KT8, KT6, KT-Kim 및 KT-Lee, 외국 분리주 CDC85, IR78 및 NYH286주)의 유전학적 차이점을 관찰하고자 Southern hybridization을 하였다. 탐침 (probe)은 질편모충 DNA에 있는 반복적인 염기서열을 기초로 하여 337 bp의 탐침을 제작하였다. 질편모충 각 분리주를 클로닝하고 각각의 원충을 따로 배양하여 DNA를 분리하여 제한효소를 처리한 후 전기영동하고 Southern hybridization을 하였다. 질편모충 분리주에 관계없이 11개 내외의 분획이 관찰되었다. 콜로니가 2개 형성된 KT8, IR78 및 KT-Lee 분리주에서는 클로닝하기 전의 분리주와 클로닝하여 형성된 콜로니를 배양한 질편모충에서 같은 band pattern이 관찰되었다. 사용된 모든 질편모충을 band pattern에 따라 3군으로 나눌 수 있었는데, KT8 분리주는 국내 분리주인 KT6, KT-Kim 분리주와 같은 band pattern을 보여 1 kb, 1.2 kb, 1.6 kb, 1.9 kb, 2.3 kb, 2.7 kb, 3.2 kb, 3.4 kb, 3.8 kb, 4.9 kb 및 6.0 kb의 11개의 공통 분획을 보였다. 외국분리주로 metronidazole에 내성인 IR78 분리주는 국내 분리주인 KT-Lee 분리주와 같은 분획을 보여 1 kb, 1.2 kb, 1.6 kb, 1.8 kb, 2.1 kb, 2.5 kb, 2.7 kb, 2.9 kb, 3.4 kb, 5.0 kb 및 6.0 kb의 분획을 보였으며 IR78과 같이 약제 내성이 있다고 알려진 CDC85의 경우 IR78, KT-Lee 분리주와 비슷한 분획을 보였으나 2.9 kb가 없고 3.2 kb의 분획이 관찰되었다. 세번째 군에 해당되는 NYH286주는 12개의 분획을 보였는데 IR78, KT-Lee 분리주와 유사한 분획을 보였으나 그 차이점은 6.0 kb 대신 6.2 kb를, 2.1 kb 대신 2.0 kb와 2.2 kb를 나타냈다. 이상의 결과로 질편모충 여러 분리주의 유전학적 다양성이 관찰되었다.

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