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Resolution of Six Chromosomes of *Trichomonas vaginalis* and Conservation of Size and Number Among Isolates

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ABSTRACT: The electrophoretic karyotype of *Trichomonas vaginalis* isolates was determined by contour-clamped homogeneous electric field electrophoresis. Six chromosomal bands ranging between 50 kbp and 6 Mbp were reliably resolved by our separation method. Trichomonad chromosomes fell into 3 distinct size classes. The 3 maxichromosomes were approximately 5,700, 4,700, and 3,500 kbp. Two intermediate-sized chromosomes were ~1,200 kbp and 1,100 kbp. A minichromosome was ~75 kbp. The same size and number of chromosomes were present in 15 *T. vaginalis* isolates obtained from different geographic regions, reinforcing the idea of a highly conserved karyotype among trichomonal isolates worldwide.

Trichomonas vaginalis is a flagellated protozoan responsible for the world's most common nonviral sexually transmitted disease (World Health Organization, 1995). Diagnosis of trichomonosis (Kassai et al., 1988) is problematic, due to dramatic variation in host symptomatology, ranging from minor abdominal discomfort to severe inflammation, vaginal discharge, and vulvovaginal soreness or irritation (Honigberg, 1978; Kreiger et al., 1988; Wolner-Hanssen et al., 1989; Heine and McGregor, 1993; Petrin et al., 1998). Infection with this parasite has significant health consequences for women, including adverse pregnancy outcome (Cotch et al., 1997), increased susceptibility to human immunodeficiency virus (HIV) (Laga et al., 1991; Wasserheit, 1992), and possibly enhanced risk for cervical neoplasia (Zhang and Begg, 1994).

An extensive literature exists on the antigenic heterogeneity among *T. vaginalis* isolates (Kott and Adler, 1961; Honigberg, 1978; Torian et al., 1984; Alderete, Garza et al., 1986; Alderete, Kasmala et al., 1986; Alderete, Suprun-Brown et al., 1986; Garber et al., 1986; Kreiger et al., 1995). Type-specific antigens have been identified by classical immunologic techniques (Honigberg, 1978). More recently (Alderete, Garza et al., 1986), differences in the expression of high-molecular-weight immunogens among *T. vaginalis* strains were demonstrated. The use of monoclonal antibody reagents affirmed the existence of antigenic distinctions among isolates (Torian et al., 1984; Alderete, Kasmala et al., 1986; Alderete, Suprun-Brown et al., 1986; Kreiger et al., 1995). The discovery of the general property of phenotypic variation was originally defined on the basis of surface expression, or synthesis of repertoires of immunogens (Alderete, Kasmala et al., 1986, 1987), or both. It is now accepted that phenotypic variation by trichomonads is a response to specific parasite and environmental factors. This property that now includes iron-regulated proteins (Lehker and Alderete, 1992), viral-induced and repressed proteins (Provenzano et al., 1997), erythrocyte-binding proteins and hemolysins (Krieger et al., 1983; Dailey and Alderete, 1990; Lehker et al., 1990), proteinases (Dailey and Alderete, 1990; Provenzano and Alderete, 1995; Provenzano et al., 1997), and adhesins (Engbring and Alderete, 1998), among other environmentally regulated trichomonad proteins, contribute to this parasite's overall antigenic diversity and complexity. Antibody responses among patients with trichomonosis are toward proteins that are differentially expressed depending on the specific host microenvironment(s) (Lehker and Alderete, 1992). Therefore, populations of *T. vaginalis* organisms from fresh clinical isolates likely comprise a heterogeneous population, in which no 2 parasites are identical in the context of these numerous phenotypes. This heterogeneity among infecting parasites guarantees that some parasites will successfully infect a host. The ability to generate such a heterogeneous population will further assure survival in a constantly changing host vaginal environment. Apart from environmental control of particular phenotypes, the demonstrated isoenzyme variations among trichomonal isolates (Nadler and Honigberg, 1988) raises the possibility that genomic structural changes may also contribute to the reported antigenic heterogeneity among isolates.

More recently, different-sized chromosomes were identified for isolates of *T. vaginalis* (Yuh et al., 1997). Therefore, issues involving the antigenic, phenotypic, and genetic distinctions among these pathogenic human trichomonads make it important to establish reproducible experimental conditions for determining the size, number, and stability of chromosomes among isolates. We report on the optimization of conditions for pulsed-field gel electrophoresis (PFGE) in delineating the 6 chromosomes of *T. vaginalis* organisms.

To obtain intact chromosomal DNA (Riley and Krieger, 1992) for electrophoretic separation, parasites were embedded in agarose plugs. Briefly, 1×10^8 highly motile organisms at the logarithmic phase of growth in batch culture in complex trypticase–yeast extract–maltose medium supplemented with serum (Diamond, 1957) were washed in phosphate-buffered saline 3 times before suspension in 1 ml of buffer consisting of 10 mM Tris-HCl, pH 8.0, 20 mM NaCl, 100 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM aurintricarboxylic acid. Organisms were then placed in a 42 C water bath for 30 sec. Following addition of 1% diethylpyrocarbonate (DEPC), the parasites were resuspended in 1 ml of 1% warm low-melting point (LMP) agarose solution and carefully pipetted into a casting mold for incubation at 4 C for 30 min. The addition of aurintricarboxylic acid and DEPC was essential for neutralization of the numerous nucleases. This was important for visualization of DNA within the agarose gels after PFGE. The agarose plugs were then transferred to 25 ml digestion buffer containing 100 mM EDTA, 1% *N*-lauryl-sarcosine, 0.2% sodium deoxycholate, and 2 mg/ml proteinase K. Fresh proteinase K was added 2 additional times during a 24-hr period while incubating at 42 C. Finally, the agarose plugs were washed 10 times each for 1-hr periods in 50 ml wash buffer consisting of 20 mM Tris-HCl, pH 8.0 and 50 mM EDTA at 4 C. Agarose plugs containing trichomonad DNA were then placed in storage

buffer of 10 mM Tris-HCl, pH 8.0 and 0.05 mM EDTA and kept at 4 C until use.

To our knowledge, the separation of trichomonad chromosomes has not been described, necessitating our need to optimize the electrophoretic parameters for the separation of entire chromosomes in a CHEF-DR II PFGE system (Bio-Rad Laboratories, Richmond, California). This was accomplished by varying switch time, voltage, run time, and agarose concentrations. All samples were run on gels cast in a 14- × 12.7-cm mold with concentrations of LMP chromosomal-grade agarose ranging from 0.6% to 1.5% in 0.5× of a Tris–borate–EDTA (TBE) buffer (1× TBE is 44.5 mM Tris-HCl, 44.5 mM boric acid, and 1 mM EDTA). After the agarose solidified, the gel was loaded with the agarose plugs of embedded trichomonad DNA, and the samples were sealed into the wells with 1% LMP agarose prepared in 0.5× TBE. Next, the loaded gel was transferred to the electrophoretic chamber and left to equilibrate for 30 min before electrophoresis in 0.5× TBE buffer chilled to 14 C. The temperature in the electrophoretic chamber was maintained constant by recirculating the buffer at a flow rate of 1 L/hr through a cold waterbath set to 11 C. For electrophoresis extending beyond 24 hr, the buffer in the electrophoretic chamber was replaced daily with fresh, cold 0.5× TBE.

As shown in Figure 1A, we successfully achieved size separation of different-sized DNAs for the *T. vaginalis* isolate T068-II that has been studied extensively by us (Provenzano et al., 1997; Engbring and Alderete, 1998). The bands correspond to 5 μg DNA, and optimal separation of chromosomes depends on the run time, switch time, voltage, and agarose concentration. For example, lane 1 shows that the smallest chromosome (arrow) was isolated after 8 hr of electrophoresis at 200 V in 1.5% LMP agarose. Decreased concentrations of LMP agarose and voltage in concert with increased switch and run times were required to resolve larger chromosomal elements (lanes 2 through 4, arrows). The largest number of chromosomes (lane 5) was visualized only when electrophoresis was for 160 hr in a 0.6% LMP agarose gel at 50 V and a switch time of 3,600 sec. Six distinct DNA bands were resolved under these experimental conditions. Approximate sizes (in kbp) of the candidate chromosomes were 100, 1,100, 1,200, 3,500, 4,700, and 5,700, and DNA bands could be visualized based on comigrating digested λ DNA and commercially available yeast size markers (data not shown). It is noteworthy that additional alteration of conditions, including extended electrophoresis times, did not result in the detection of additional chromosomes. Furthermore, using conditions in lane 4, we examined increased amounts of DNA in the agar plugs that can influence the resolution of DNA bands. As shown in Figure 1B, no additional resolution apart from the 3 distinct sized bands were resolved.

We next compared the chromosomes of 15 representative *T. vaginalis* isolates derived from different geographic regions. Importantly, the isolates have been studied in our laboratory for a variety of properties, including presence or absence of a double-stranded RNA virus (Wang and Wang, 1986; Khoshnan and Alderete, 1993), phenotypic variation (Alderete, Kasmala et al., 1986c; Alderete et al., 1987; Musatovova and Alderete, 1998), and cytoadherence and cytotoxicity (Engbring and Alderete, 1998). Patterns for 3 of the 15 isolates are presented in Figure 2. DNA was separated to visualize the 6 prominent chromosomes delineated in Figure 1. These included electrophoretic

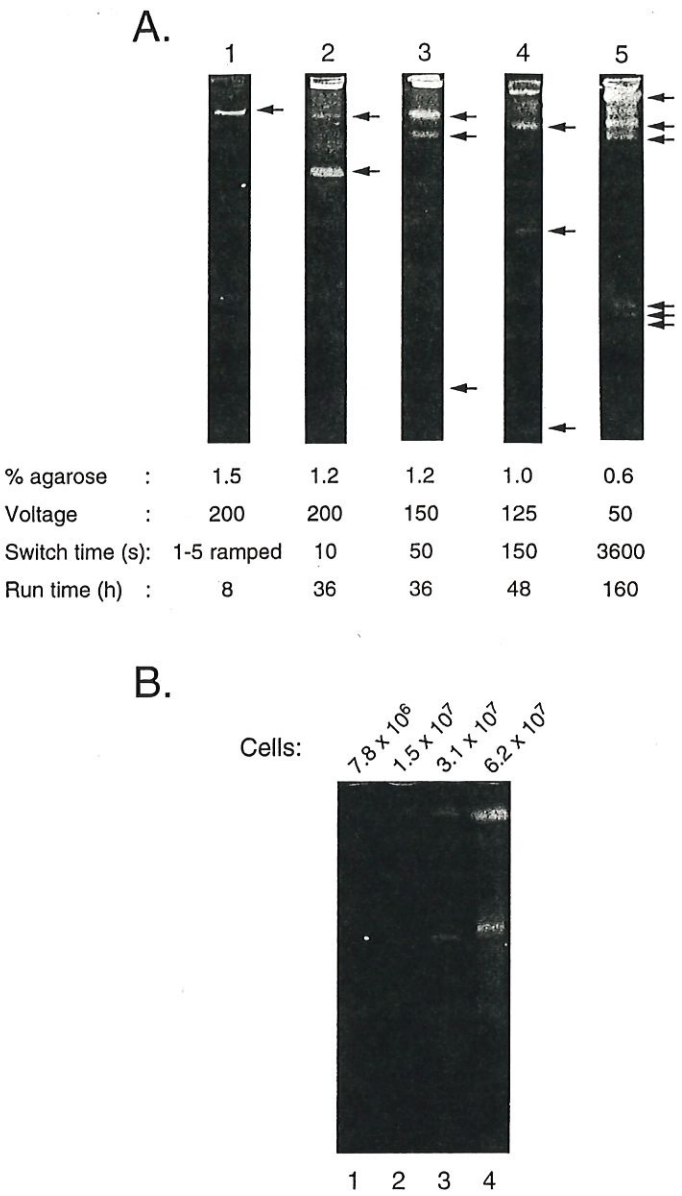

FIGURE 1. Establishment of electrophoretic conditions to separate the chromosomes of *Trichomonas vaginalis* isolate T068-II under varying PFGE conditions (**A**) versus electrophoresis of DNA from increasing numbers of trichomonads at 125 V for 48 hr with a 100-sec switch time in a 1.0% agarose gel (**B**).

conditions for the separation of the minichromosome <100 kbp (Fig. 2A), the intermediate-sized chromosomes of ~1,100 and 1,200 kbp (Fig. 2B), and the maxichromosomes of 3,500, 4,700, and 5,700 kbp (Fig. 2C). Except for the extra band with decreased intensity seen for isolate 347cz, all other 14 of 15 isolates were identical to those shown for the 3 isolates. Resolution of 6 DNA bands in all isolates suggests that at least 6 distinct chromosomal elements stably reside within *T. vaginalis* organisms. It remains to be determined whether the extra band for 347cz is a bona fide additional chromosome or, alternatively, an artifact generated during the preparation of this DNA.

In summary, we report on the conditions for the preparation of intact trichomonad chromosomes suitable for electrophoretic

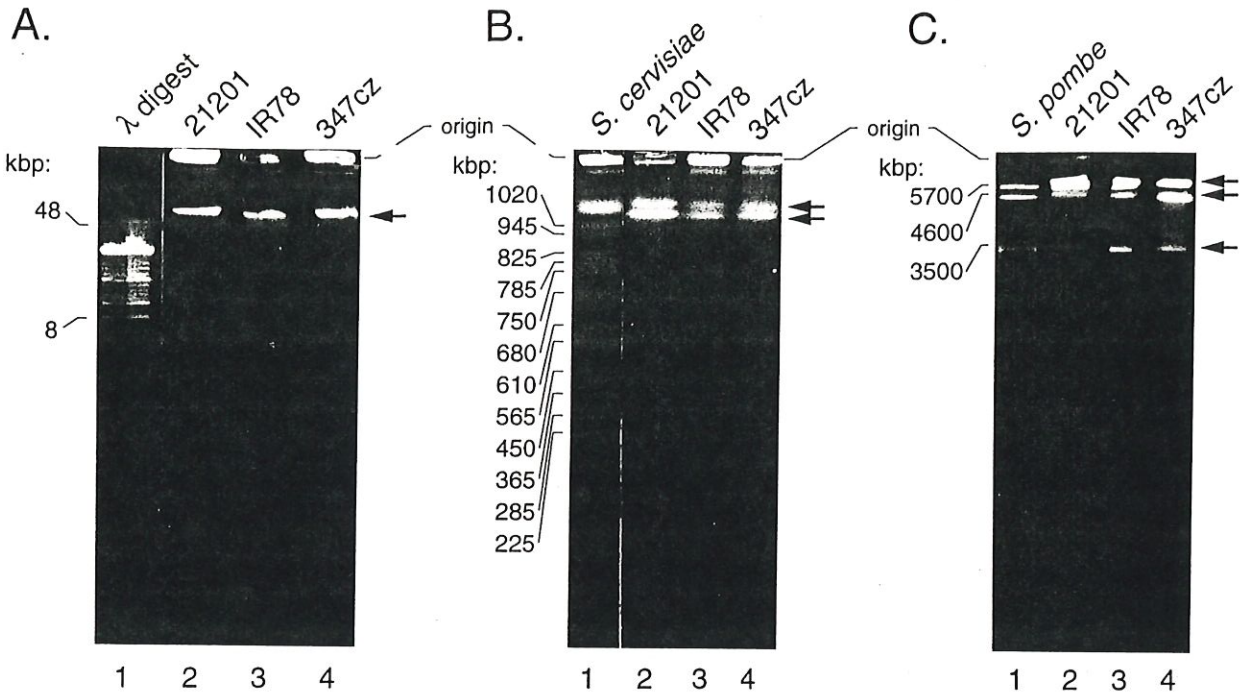

FIGURE 2. Comparisons of the number and size of chromosomes of representative *Trichomonas vaginalis* isolates 21201, IR78, and 347cz resolved under 3 PFGE conditions. (**a**) The minichromosomes resolved in electrophoretic conditions identical to those shown Figure 1A (lane 1). (**b**) The intermediate-sized chromosomal DNA electrophoresed in 1% agarose gel at 200 V for 24 hr at a ramped switch time of 60–200 sec. (**c**) The maxichromosomes resolved in 0.8% agarose at 45 V for 68 hr at a 2,000–3,000-sec ramped switch time. The origin label highlights the wells where the embedded trichomonad DNA plugs were loaded prior to PFGE. Size estimations of trichomonad chromosomes were obtained by comparing with digested λ DNA, *Saccharomyces cervisiae* strain YNN295 chromosomes, and *Schizosaccharomyces pombe* strain 972h chromosomes (Bio-Rad Laboratories, Hercules, California) and shown in lane 1.

separation by CHEF. Using optimized pulse and run times, we accomplished the separation of the trichomonad genome into 6 distinct chromosomes. Electrophoretic karyotyping revealed a remarkable conservation in the number and sizes of chromosomes among trichomonal isolates. Our results confirm the recent demonstration of 6 mitotic trichomonad chromosomes that also were segregated on the basis of 3 distinct sizes (Yuh et al., 1997).

In contrast to other protozoan parasites (van der Ploeg, 1984; Kemp et al., 1985; Corcoran et al., 1986; Giannini et al., 1986; Bishop and Miles, 1987; Upcroft et al., 1989; Korman et al., 1992), major chromosomal rearrangements are likely not a mechanism responsible for generating genetic heterogeneity in trichomonal populations. However, size variations among isolates involving small chromosomal segments cannot be discounted, because differences in chromosome sizes of <100 kbp were not detected by our gel system. The uniformity in chromosome size and number among isolates is consistent with experimental evidence that all isolates have exhibited identical properties despite phenotypic variations resulting from environmental regulation.

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Simplified Technique for Isolation, Excystation, and Culture of *Sarcocystis* Species from Opossums

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ABSTRACT: *Sarcocystis neurona* is a protozoan parasite that causes a neurological disease in horses called equine protozoal myeloencephalitis. The route of transmission is speculated to be by fecal–oral transfer of sporocysts shed from opossums. Controversy exists regarding both the natural life cycle for this parasite as well as the species identity of opossum *Sarcocystis.* To provide stage-specific material for species