Determination of \textit{Gardnerella vaginalis} Genome Size by Pulsed-Field Gel Electrophoresis\textsuperscript{†}

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Abstract

The chromosomal DNA of four strains of \textit{Gardnerella vaginalis} were digested with rare cutting restriction enzymes and analyzed by pulsed-field gel electrophoresis (PFGE). The four strains studied were two clinical isolates (GVP 004 & GVP 007) and two American Type Culture Collection strains (ATCC 14018 & ATCC 14019). The restriction enzyme SfiI generated two DNA fragments of about 0.6 Mb and 1.1 Mb in all four strains giving a \textit{G. vaginalis} genome size of about 1.7 Mb. A similar genome size was calculated utilizing two more GC-rich sequence specific restriction endonucleases, NotI and Ascl. When digested with Ascl, the chromosomal DNA of all four strains gave rise to 11 to 12 DNA fragments ranging between 0.01 Mb to 0.43 Mb. DNA from the two clinical isolates were digested by NotI (yielding 7 to 9 fragments), while the DNA from the two ATCC strains were resistant to NotI digestion. In contrast to the clinical isolates, DNA from the two ATCC strains gave an identical profile for all restriction endonucleases tested. From double digestion experiments, the two SfiI sites could be localized on two Ascl fragments. From these PFGE studies, it is concluded that the \textit{G. vaginalis} genome is a circular DNA that ranges between 1.67 Mb and 1.72 Mb in size.

Key words: \textit{G. vaginalis}; PFGE; SfiI; NotI; Ascl; rare-cutting restriction endonucleases; genome size

1. Introduction

\textit{Gardnerella vaginalis} is a Gram-negative to Gram-variable bacteria that can be recovered from the normal vaginal flora of one out of every two women.\textsuperscript{1,2} \textit{G. vaginalis} predominates in the clinical situation known as bacterial vaginosis or BV.\textsuperscript{3,4} Additionally, \textit{G. vaginalis} has also been shown to be associated with \textit{G. vaginalis} bacteremia and with several other disorders including infections of the endometrium, fetal membrane and neonate.\textsuperscript{3,4} Clinically, BV is considered to be one of the most important vaginal infections due to the increased potential for upper genital tract infection\textsuperscript{5} and idiopathic premature delivery.\textsuperscript{6} Attempts have been made to determine whether \textit{G. vaginalis} present in normal flora differ from those present in BV.\textsuperscript{7,8}

Previous DNA fingerprint-based studies have shown that \textit{G. vaginalis} in BV represented a genetically mixed population.\textsuperscript{9} During our attempts to characterize \textit{G. vaginalis} at the DNA level,\textsuperscript{9–12} it became apparent that studies pertaining to the genomic organization of this organism may be extremely helpful. Now for the first time, the size of the \textit{G. vaginalis} is known. We have determined this genome size by utilizing rare-cutting restriction enzymes followed by an analysis of the resultant large DNA fragments in pulsed-field gel electrophoresis (PFGE) studies.

2. Materials and Methods

2.1. Bacterial strains and culture conditions

The four strains of \textit{G. vaginalis} employed are listed in Table 1. The two clinical isolates, GVP 004 and GVP 007, have been used in the previously reported DNA fingerprint-based restriction endonuclease analysis study.\textsuperscript{12} Cultures were grown on chocolate agar plates under anaerobic conditions, using a gas-pack system (BBL Microbiology System), at 37°C for about 40 h, as described previously.\textsuperscript{9,12}

2.2. DNA preparation

The protocol for embedding \textit{G. vaginalis} genomic DNA in low-melting point agarose plugs, was based on the instruction manual for the ImBed kit provided by New England Biolabs. However, the plugs were cast in a Bio-Rad
sample mold (Catalog # 170-3622).

Briefly, freshly grown and washed *G. vaginalis*, adjusted to A<sub>600</sub> of 0.38, was embedded in 1% InCert Agarose (FMC BioProducts) plugs. The plugs were then incubated under gentle agitation in the following sequence: i) 1 mg/ml lysozyme (in 10 mM Tris-HCl, pH 7.2; 50 mM NaCl; 10 mM EDTA-Na, pH 8.0; 0.2% Na deoxycholate; 0.5% N-laurylsarcosine) for 3 h at 37°C; ii) 1 mg/ml proteinase K (in 100 mM EDTA, pH 8.0; 0.2% Na deoxycholate; 1% N-laurylsarcosine) at 42°C for 20 h; iii) twice in a wash buffer (20 mM Tris-HCl; 50 mM Na deoxycholate; 0.5% ./V-laurylsarcosine) at 42°C for 20 h; and iv) twice in wash buffer (20 mM Tris-HCl; 50 mM EDTA-Na, pH 8.0) for 15 min each, all under gentle agitation. The plugs were finally washed in 0.1 x wash buffer and stored in the same buffer at 4°C.

### Table 1. Gardnerella vaginalis strains used in genomic size determination

<table>
<thead>
<tr>
<th>Culture Designation Clinical Background (Source)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC&lt;sup&gt;®&lt;/sup&gt; 14018 BV&lt;sup&gt;+&lt;/sup&gt; (C. D. Dukes 594; NCTC 10287)</td>
<td>ATCC&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td>ATCC 14019 BV (C. D. Dukes 317)</td>
<td>ATCC&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td>GVP* 004 Non-BV (Nath et al.&lt;sup&gt;*&lt;/sup&gt;)</td>
<td>ATCC&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td>GVP 007 Non-BV (Nath et al.)</td>
<td>ATCC&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*</sup>American Type Culture Collection, Rockville, MD., USA

* Bacterial Vaginosis

<sup>+</sup> *G. vaginalis* strains isolated at C.W. Post from vaginal exudate specimen supplied by Ann Marie Beddoe, MD, Assistant Professor of Obstetrics and Gynecology at the Mount Sinai Services of the City Hospital Center at Elmhurst, N.Y.


### 2.3. Restriction endonuclease digestion

Each plug was pre-equilibrated with 100 μl of the appropriate restriction buffer followed by 100 μl of the same fresh buffer supplemented with 5 to 20 units of restriction enzymes (New England Biolabs) and 0.1 mg/ml of acetylated bovine serum albumin (BSA) (New England Biolabs). Incubation was carried out at 37°C (50°C for *SfiI*) for 3 h under gentle agitation. This was followed by the addition of another 5 to 20 units of the enzyme and further incubation for 12 to 20 h. For double digestion, the plugs were incubated with each enzyme individually. First the lower salt specific enzyme was used in the lower salt restriction buffer. This was followed by a wash with the higher salt restriction buffer and then incubation with the second enzyme. The digestion was terminated by placing the plugs on ice and mixing with an equal volume of 0.25% bromophenol blue in 1.0 x TBE (90 mM Tris-borate; 2 mM EDTA-Na, pH 8.0). This resulted in a final concentration of 0.5 x TBE.

### 2.4. PFGE

Electrophoresis was carried out in a CHEF-DR II pulsed field system (Catalog # 170-3612, Bio-Rad) utilizing a 1% agarose (SeaKem, FMC BioProducts) gel in 0.5 x TBE buffer that was maintained at a constant temperature of 15°C. Depending on the size of the DNA fragments to be separated, different pulse time settings were employed at 200 V for 18 to 24 h. The pulse times employed were 60–120 sec, 5–65 sec, and 1–12 sec rampings for DNA fragment sizes of 0.5–1 Mb, 50–600 Kb and 7–250 Kb, respectively. A constant pulse time was used to resolve DNA fragments that migrated in close proximity. The gels were stained in 150 ml of 10 μg/ml ethidium bromide for 30 min and destained twice with 250 ml distilled water for 30 min each, all under gentle agitation, prior to photography under UV illumination.

### 2.5. DNA size determination

The DNA fragment sizes were calculated from a plot of log molecular size and the distance migrated by composite DNA markers included in the same gel. For 0.1–1 Mb size DNA, the size markers used were Saccharomyces cerevisiae chromosomes (Catalog #s 170-3605 & 345 from Bio-Rad & New England Biolabs, respectively) and Lambda DNA ladder (Catalog # 170-3635, Bio-Rad). For 1–250 Kb fragments, Low range & Mid-size range II PFGE markers (Catalog #s 350 & 355–2 respectively from New England Biolabs) were used. The DNA fragment sizes reported here are the mean value with standard deviations at the same and different pulse conditions along with the number of independent determinations (n).

### 3. Results

The restriction endonucleases with the fewest restrictions on the *G. vaginalis* genome were enzymes which recognized GC-rich sequences (Table 2). The PFGE screenings were repeated at least twice under two different pulse conditions, 10–60 sec and 1–20 sec to separate mid-sized and small-sized DNA, respectively. Two octanucleotide endonucleases which recognize AT-rich sequences *PacI* and *Pmel*, generated more than 30 DNA fragments (results not shown). Three octanucleotides which recognize GC-rich sites, *SfiI*, *Ascl* and *NdeI*, generated the fewest DNA fragments were used in the subsequent analysis of the genomic size of the *G. vaginalis* strains listed in Table 1.

* SfiI generated two DNA fragments in all four *G. vaginalis* strains (Fig. 1). A comparison of these two fragments in all four strains revealed that the smaller of the two DNA fragments were of similar size while the larger DNA fragments showed slight variation. Accordingly, the genome sizes calculated varied between 1.70 to 1.75 Mb.
Table 2. A pulsed-field gel electrophoresis survey of *G. vaginalis* DNA fragments generated by selected GC-rich sequence-specific restriction enzymes

<table>
<thead>
<tr>
<th>Restriction Enzyme (Recognition Sequence)</th>
<th>ATCC 14018</th>
<th>ATCC 14019</th>
<th>GVP 004</th>
<th>GVP 007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascl (GG</td>
<td>CGCGCC)</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>AvoI (CPyCGPuG)</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>&gt;10</td>
<td>&gt;12</td>
</tr>
<tr>
<td>BglII (GCCCNNNN</td>
<td>NGGC)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;14</td>
</tr>
<tr>
<td>BstEII (G</td>
<td>GTNACC)</td>
<td>&gt;19</td>
<td>&gt;19</td>
<td>&gt;16</td>
</tr>
<tr>
<td>HaeII (PuGGCG</td>
<td>Py)</td>
<td>&gt;6</td>
<td>&gt;6</td>
<td>&gt;7</td>
</tr>
<tr>
<td>NdeI (GC</td>
<td>GGCCGC)</td>
<td>None</td>
<td>None</td>
<td>7</td>
</tr>
<tr>
<td>RsrII (CG</td>
<td>G A/TCCG)</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>&gt;9</td>
</tr>
<tr>
<td>SfiI (GGCCNNNN</td>
<td>NGGCC)</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>SgrAI (CPu</td>
<td>CCGGPyG)</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Smal (CCC</td>
<td>GGG)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;8</td>
</tr>
</tbody>
</table>

Table 3. *G. vaginalis* genome size calculated from *SfiI* generated DNA fragments resolved in pulsed-field gel electrophoresis

<table>
<thead>
<tr>
<th><em>SfiI</em> fragments</th>
<th>ATCC 14019</th>
<th>ATCC 14018</th>
<th>GVP 007</th>
<th>GVP 004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD Kb (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1,094</td>
<td>1,094</td>
<td>1,100</td>
<td>1,138</td>
</tr>
<tr>
<td>±21.0 (4)</td>
<td>±21.0 (4)</td>
<td>±12.2 (4)</td>
<td>±10.3 (5)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>608</td>
<td>608</td>
<td>610</td>
<td>615</td>
</tr>
<tr>
<td>±13.5 (4)</td>
<td>±13.5 (4)</td>
<td>±10.6 (7)</td>
<td>±9.6 (4)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,702</td>
<td>1,702</td>
<td>1,710</td>
<td>1,753</td>
</tr>
</tbody>
</table>

Figure 1. Pulsed-field gel electrophoresis profile of *SfiI*-digested *G. vaginalis* genomic DNA. The electrophoresis was performed with a pulsed time of 60 to 120 sec ramping at 200 V for 24 h. The *G. vaginalis* strains in four lanes were ATCC 14019, ATCC 14018, GVP 007, and GVP 004. The lanes at the two ends (Y) contained *S. cerevisiae* chromosomal DNA that was utilized as size markers.

for the four strains (Table 3). The higher value was for GVP 004, intermediate for GVP 007 and lower value for the two ATCC strains which incidentally, had identical *SfiI* profiles.

Ascl generated 12 fragments in the DNA from the ATCC strains. The two ATCC strains also had identical Ascl DNA profiles (lanes 5 & 6, Fig. 2 and lanes 3 & 4, Fig. 3). The total size of all the 12 Ascl DNA fragments in the ATCC strains was about 1.67 Mb (Table 4). Twelve Ascl DNA fragments, but of different sizes, were also obtained from GVP 004 (lane 8 in Fig. 2 and lane 6 in Fig. 3). Although the sizes varied, these 12 DNA fragments totaled about 1.72 Mb (Table 4). GVP 007 yielded Ascl DNA fragments (lane 7 in Fig. 2 and lane 5 in Fig. 3) with a total genome size of about 1.65 Mb (Table 4). The genome sizes based on Ascl DNA fragments in Table 4 were smaller than those determined from the *SfiI* fragments in Table 3. However, the same trend was seen: higher genomic value for GVP 004, intermediate for GVP 007 and lower for the two ATCC strains.

The *SfiI* profiles in Fig. 1 and the larger Ascl DNA fragments in Fig. 2 were of different sizes in all four strains (fragments A-E, Table 4). However, at least 5 smaller fragments in Fig. 3 (lanes 3-6) appeared very close to each other (DNA fragments H,I,J,K,L in the two ATCC strains were similar to fragments G,H,I,J,K in GVP 007 and G,H,I,J,L in GVP 004. Table 4). In the 2 ATCC culture Ascl DNA profiles, a pair of doublets (D,E & F,G in Table 4) that appeared as one wide intense band around 100 Kb in Fig. 2 (4th band from top in lanes 5 & 6) could be resolved into two doublets of 102 and 96 Kb, respectively, in Fig. 3 (lanes 3 & 4). These were considered as doublets from the band width and from the intensities when compared with similar bands in profiles from the
Table 4. Sizes of Ascl-cleaved G. vaginalis genomic DNA

<table>
<thead>
<tr>
<th>Ascl fragments</th>
<th>ATCC 14019</th>
<th>ATCC 14018</th>
<th>GVP 007</th>
<th>GVP 004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean SD Kb (n)</td>
<td>418±6.2(3)</td>
<td>418±6.2(3)</td>
<td>423±4.3(4)</td>
<td>433±2.5(2)</td>
</tr>
<tr>
<td>A</td>
<td>380±8.2(3)</td>
<td>380±8.2(3)</td>
<td>363±9.0(4)</td>
<td>402±2.4(3)</td>
</tr>
<tr>
<td>B</td>
<td>292±6.2(3)</td>
<td>292±6.2(3)</td>
<td>235±7.1(5)</td>
<td>365±10.8(3)</td>
</tr>
<tr>
<td>C</td>
<td>102±6.9(5)</td>
<td>102±6.9(5)</td>
<td>206±5.2(6)</td>
<td>122±7.7(5)</td>
</tr>
<tr>
<td>D</td>
<td>102±6.9(5)</td>
<td>102±6.9(5)</td>
<td>185±8.1(6)</td>
<td>117±4.0(5)</td>
</tr>
<tr>
<td>E</td>
<td>96±0.7(4)</td>
<td>96±0.7(4)</td>
<td>96±0.7(4)</td>
<td>67±3.8(5)</td>
</tr>
<tr>
<td>F</td>
<td>96±0.7(4)</td>
<td>96±0.7(4)</td>
<td>66±3.0(4)</td>
<td>50±4.6(5)</td>
</tr>
<tr>
<td>G</td>
<td>49±5.2(4)</td>
<td>49±5.2(4)</td>
<td>46±4.8(6)</td>
<td>46±2.8(5)</td>
</tr>
<tr>
<td>H</td>
<td>46±3.5(4)</td>
<td>46±3.5(4)</td>
<td>13±1(1)</td>
<td>12±1.1(2)</td>
</tr>
<tr>
<td>I</td>
<td>13±1(1)</td>
<td>13±1(1)</td>
<td>8±1(1)</td>
<td>9±0.1(2)</td>
</tr>
<tr>
<td>J</td>
<td>8±1(1)</td>
<td>8±1(1)</td>
<td>46±2.8(5)</td>
<td>8±0.1(2)</td>
</tr>
<tr>
<td>Total</td>
<td>1,668</td>
<td>1,668</td>
<td>1,693</td>
<td>1,715</td>
</tr>
</tbody>
</table>

Table 5. Sizes of NolI-generated G. vaginalis genomic DNA

<table>
<thead>
<tr>
<th>NolI fragments</th>
<th>GVP 007</th>
<th>GVP 004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD Kb (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>439 ±7.2(4)</td>
<td>458 ±11.2(5)</td>
</tr>
<tr>
<td>B</td>
<td>331 ±7.4(4)</td>
<td>372 ±9.0(5)</td>
</tr>
<tr>
<td>C</td>
<td>231 ±6.6(5)</td>
<td>372 ±10.0(4)</td>
</tr>
<tr>
<td>D</td>
<td>227 ±6.5(5)</td>
<td>201 ±11.6(6)</td>
</tr>
<tr>
<td>E</td>
<td>140 ±7.6(7)</td>
<td>116 ±7.0(8)</td>
</tr>
<tr>
<td>F</td>
<td>118 ±6.6(7)</td>
<td>116 ±7.4(7)</td>
</tr>
<tr>
<td>G</td>
<td>115 ±6.1(7)</td>
<td>73 ±4.7(7)</td>
</tr>
<tr>
<td>H</td>
<td>74 ±4.3(7)</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>11 ±0.5(2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,686</td>
<td>1,708</td>
</tr>
</tbody>
</table>

clinical isolates. These Ascl DNA doublets in the ATCC culture could not be resolved further under the diverse pulse conditions utilized.

The restriction endonuclease NolI yielded no bands in DNA from either ATCC strain. The DNA from both ATCC strains appeared resistant to the action of NolI, as seen in lanes 1 & 2 of Fig. 2. DNA from GVP 007 gave rise to nine (lane 3, Fig. 2 & lane 1, Fig. 3) and DNA from GVP 004 gave rise to seven (lane 4, Fig. 2 & lane 2, Fig. 3) NolI DNA fragments, respectively. Based on the sum of all NolI DNA fragments, the genome sizes of these two clinical isolates were about 1.69 Mb for GVP 007 and 1.71 Mb for GVP 004 (Table 5). This is in close agreement with the results obtained from the Ascl DNA fragments (Table 4).

Similar to the Ascl fragments, only few smaller NolI DNA fragments were common to both clinical isolates in lanes 1 & 2 of Fig. 3 (fragments F,G,H for GVP 007 corresponding to fragments E,F,G for GVP 004, respectively, in Table 5). The five NolI bands in GVP 004 DNA (lane 4 in Fig. 2 and lane 2 in Fig. 3) are comprised of two doublets of 372 and 116 Kb (B,C and E,F, respectively, in Table 5). None of these pairs could be resolved into individual bands by varying pulse conditions.

In contrast, the six NolI bands in GVP 007 DNA (lane 3 in Fig. 2 and lane 1 in Fig. 3) were comprised of two pairs of doublets (231 & 227 Kb and 118 & 115 Kb corresponding to fragments C & D and F & G, respectively, in Table 5) could be resolved by varying pulse conditions, accounting for the eight NolI fragments. Additionally, a ninth NolI fragment of 11 Kb (fragment I in Table 5) could be resolved under a pulse condition of 5-15 sec for 18 hr (not shown).

When the NolI- and Ascl-digested plugs were subjected to electrophoresis in a 1% agarose gel with no pulse (regular gel electrophoresis), no bands were observed. This suggested that DNA fragments smaller in size than those detected under PFGE were not present.

The appearance of a band in the two ATCC culture lanes treated with NolI (lanes 1 & 2 in Fig. 2 and lanes 2 & 4 in Fig. 4) raised the question of whether the G. vaginalis genome is linear or whether the two ATCC strains contain a single NolI site. A SbfI-NolI double digestion resulted in the formation of only the 2 SbfI fragments in the 2 ATCC cultures (lanes 1 & 3, Fig. 4), confirming the absence of a NolI site in the genomes of these two strains. In other experiments, no bands appeared in NolI-digested ATCC strains. This, however, did not rule out the possibility of a linear G. vaginalis genome.

If the genome of G. vaginalis is circular, two SbfI sites should reside within two fragments generated by other restriction endonucleases. In the cases of GVP 007 and GVP 004 DNA, the SbfI-NolI double digest profile hardly
Figure 2. Pulsed field gel electrophoresis profile of *NotI*- and *Ascl*-digested *G. vaginalis* genomic DNA. The electrophoresis was performed with a 15 to 65 sec pulse ramping for 22 h. The order of the four *G. vaginalis* strains in lanes 1-4 for *NotI* and in lanes 5-8 for *Ascl* is the same as in Fig. 1. The two end lanes (L) contained the lambda concatamer marker.

Figure 3. Resolution of the smaller *NotI* and *Ascl* generated *G. vaginalis* genomic fragments. The electrophoresis was performed at a 7-sec constant pulse for 18 h. Lanes 1 and 2 represent *NotI*-digested GVP 007 and GVP 004, respectively, while lanes 3-6 represent *Ascl*-digested ATCC 14019, ATCC 14018, GVP 007, and GVP 004. The markers used included the mid-size marker (M) and low-size marker (L).

differed from the *NotI* profile (lanes 5 & 6 for GVP 007 and lanes 7 & 8 for GVP 004 in Fig. 4). The possible exception is the largest *NotI* fragment that appeared slightly smaller in the *SfiI*-*NotI* double digest. However, the *SfiI* sites could be readily discerned within two *Ascl* fragments in all four strains.

Thus in Fig. 5, the second and third *Ascl* bands from top in lane 1 for GVP 007 corresponding to fragments B and C in Table 4 were cleaved by *SfiI* (Lane 2) into 242 + 112 Kb and 207 + 41 Kb fragment sizes, respectively.
Similarly, the second and third Ascl-bands from the top in lanes 3 & 5 for the two ATCC strains were cleaved by SfiI (lanes 4 & 6) into 241 + 120 Kb and 207 + 85 Kb fragment sizes, respectively. The SfiI sites on GVP 004 DNA reside on the second (207 + 207 Kb) and third (248 + 123 Kb) Ascl bands from the top (not shown).

4. Discussion

Based on PFGE analysis, the G. vaginalis genome appears to be about 1.7 Mb. The value obtained with SfiI was slightly higher. This could be attributed to errors in the size determination of the two large DNA fragments generated by this enzyme. The megabase size fragment could not be sized accurately due to limited size markers available in the S. cerevisiae chromosomes at this range and the inherent errors associated in the determination of this size range DNA.13

Based on two other enzymes that generated restriction fragments smaller than 500 Kb, the genome size of the four G. vaginalis strains was calculated as being between 1.67 and 1.72 Mb. This 1.7 Mb genomic size, which is about 40% of the E. Coli genome size, is similar to that reported for other small bacteria, such as the pathogens Campylobacter jejuni and C. coli.14,15 thermophile Streptococcus thermophilou16 and obligate intracellular bacteris Rickettsiella melolanthae.17

Although most prokaryotic genomes are circular, there are exceptions, such as the linear 950 Kb genome of Borrelia burgdorferi.18 The circular nature of the G. vaginalis genome was established from the observation that it contained two and not one restriction site for SfiI, accounting for the two fragments in Fig. 1. These two restriction sites were established from cleavage of 2 out 12 Ascl fragments by SfiI as shown in Fig. 5.

The lack of a clear cut SfiI site on NotI DNA fragments in the two clinical isolates in Fig. 4 suggests that these two sites either overlap (for example a composite NotI-SfiI sequence of the type GC|GGCCGCNN|NGGCC with the NotI cleavage site, GC|GGCCGC at the left bar and the SfiI cleavage site, GGCCNNNN|NGGCC at the right bar) or that these two sites are situated very close to each other. Of course, no such conclusion could be drawn from similar experiments performed with the two ATCC cultures since their genomes were resistant to NotI digestion.

Organisms such as C. coli and C. jejuni with a low G+C content (28-38 mol%) have been shown to be resistant to NotI or SfiI digestion.14,15 Other DNAs with low G+C content, such as Clostridium acetobutylicum DNA (30 mol%) is resistant to NotI.19 Haemophilus influenzae DNA (39 mol%) is resistant to both NotI and SfiI,20 while H. parainfluenza DNA (39 mol%) is resistant to SfiI, but is readily cleaved by NotI.21 Organisms such as the Lactococcus strains, with 35-40 mol% G+C content are digested by both NotI and SfiI.16

The G+C content of G. vaginalis DNA is 42-44 mol% (summarised in ref. 5). This is in accordance with a rare cleavage by GC-rich sequence-specific endonucleases such as SfiI and NotI (Table 2). SfiI generated only two DNA fragments in all four G. vaginalis strains tested. This is
Figure 5. Comparison of Ascl profiles with Sfil-Ascl double digestion profiles of G. vaginalis genomic DNA. The electrophoresis was carried out at a 5- to 50-sec pulse for 22 h. The odd number lanes represent Ascl profiles while the even numbers Sfil-Ascl double digestion profiles of GVP 007, ATCC 14018, and ATCC 14019. The marker used was Lambda concatamers (L).

the smallest number of genomic fragments generated by any restriction endonuclease reported for any organism. Additionally, as we have discussed before, DNA of some G. vaginalis, such as the ATCC strains, appear to be resistant to Notl digestion. A differential sensitivity of different strains of G. vaginalis DNA to selected restriction endonucleases was shown previously for BamHI in non-pulsed restriction endonucleases analysis studies.9,12

It is quite remarkable that the two ATCC cultures gave identical restriction profiles in pulsed-field gel electrophoresis. This is in agreement with our previous report of identical restriction profiles in non-pulsed restriction endonuclease analysis for ATCC 14018 and ATCC 14019.10 However, all clinical isolates that have been checked so far have never shown identical restriction profiles.9,12 Since repeated cultures obtained from the American Type Culture Collection Center gave the same results, it is evident that the two ATCC cultures represent the same strain.

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