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Genomic DNA Fingerprint Analysis of Biotype 1 *Gardnerella vaginalis* from Patients with and without Bacterial Vaginosis

SHIN-RU WU,[1]† SHARON L. HILLIER,[2] AND KAMALENDU NATH[1]*

Molecular Biology, Department of Biology, C. W. Post Campus of Long Island University, Brookville, New York 11548,[1] and Department of Obstetrics and Gynecology, RH-20, University of Washington, Seattle, Washington 98195[2]

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Of the 20 biotype 1 *Gardnerella vaginalis* isolates analyzed, 10 from patients with bacterial vaginosis and 10 from patients without bacterial vaginosis, none shared the same DNA fingerprint. However, a 1.18-kb *Hin*dIII fragment was common among 18 of the 20 biotype 1 isolates in a restriction fragment length polymorphism analysis with a 7.9-kb *G. vaginalis* DNA probe.

On the basis of the measurement of the activities of three enzymes, *Gardnerella vaginalis* isolates can be assigned to one of eight possible biotypes (7). When the distributions of different *G. vaginalis* biotypes from women with and without bacterial vaginosis (BV) in three countries were examined, biotypes 1, 2, and 5 were found to be the most predominant groups present at all locations (7). In a recent study, *G. vaginalis* strains of biotypes 1, 5, and 6 were found to be most common in all clinical situations (2). Of the 261 isolates examined in that study, biotype 7 was absent, while the lipase-positive group consisting of biotypes 1, 2, 3, and 4 was suggested to be more prevalent among women with BV. Since the association of a specific *G. vaginalis* biotype with BV is uncertain, we decided to use some of the DNA-based analysis methods to investigate the relationship of different *G. vaginalis* strains with BV, starting with biotype 1, which constitutes the present study; this will be followed by investigations of the subsequent biotypes.

(This study in part constitutes a master's thesis project of Shin-Ru Wu.)

The organisms positive for all three enzyme activities (β-galactosidase, lipase, and hippurate hydrolysis) belong to biotype 1. Twenty biotype 1 cultures, consisting of 10 isolates from individuals with BV and 10 isolates from individuals without BV (NBV) mentioned in the previous study (2), were used in a comparative genomic fingerprint analysis (Table 1). The original goal was to discover if any specific *G. vaginalis* DNA pattern was associated with BV.

Cultures were grown on chocolate agar or human bilayer Tween agar plates under anaerobic conditions by using a Gas Pak system (BBL Microbiology Systems) at 37°C for about 40 h (4, 5). RNA-free, high-molecular-size genomic DNA was isolated by a modification of an earlier procedure (3). Eighteen of the 20 DNA samples hybridized readily in a dot blot analysis with digoxigenin-labelled *G. vaginalis* ATCC 14018 DNA, which was carried out as described previously (4). The remaining two DNA preparations (those for isolates BV2 and NBV10) hybridized only at 10 times higher DNA concentrations (100 ng). All 20 DNA preparations were thus confirmed to be *G. vaginalis* DNA.

The DNA samples were digested with six restriction enzymes (*Bam*HI, *Eco*RI, *Cla*I, *Hae*II, *Hin*dIII, and *Msp*I) and were subjected to restriction endonuclease analysis (REA). For REA 750 ng of restriction enzyme-digested *G. vaginalis* DNA was separated in a 0.9% agarose (SeaKem, GTG) gel by electrophoresis at 23 V for 16.5 h by using 0.5× TBE buffer (45 mM Tris-borate, 1 mM EDTA [pH 8.0]).

The gel was stained with ethidium bromide (5 μg/ml) for 4 min, destained under running tap water for 15 to 30 min, and photographed under UV illumination. Pictures were taken with a Polaroid MP-4 land camera by using Polaroid type 52 and 55 films along with an orange filter (no. 23A; Kodak). The

TABLE 1. Certain common restriction fragments shared by *G. vaginalis* strains of biotype 1 from 10 patients with BV and 10 patients without BV

Culture[a]		*Hae*II fragments[b]			RFLP with pGVBH4[c]				
					*Hae*II		*Hin*dIII		
Hillier	Nath	7.5 kb	4 kb	3 kb	2.28 kb	1.18 kb	3.70 kb	1.48 kb	1.22 kb
PPL34.5	BV1	+	+	+	+	−	−	−	+
ASX41.9	BV2	−	−	−	−	−	−	−	−
AN265.4	BV3	+	+	+	+	−	+	+	+
a154.2f	BV4	−	+	−	+	−	−	+	+
ASn81.1	BV5	+	+	+	+	+	+	−	+
ASX42.1	BV6	+	−	−	+	+	+	+	+
ASn64.9	BV7	+	+	+	+	+	+	+	+
ASn11.1	BV8	+	−	+	−	+	−	−	+
ASx22.C	BV9	+	−	+	+	+	+	+	+
ASx23.a	BV10	+	−	+	+	+	+	+	+
PPL164.2r	NBV1	+	+	+	−	−	−	+	+
PPL127.2	NBV2	+	+	−	+	+	+	+	+
ASx142.K	NBV3	+	−	−	+	+	+	−	+
WAX2653J	NBV4	+	+	+	+	+	+	+	+
NAx261.44	NBV5	+	+	+	+	+	+	+	+
PPL128.2	NBV6	+	+	−	+	+	+	+	+
PPL18.4	NBV7	+	+	+	+	+	+	+	+
WAx265.31	NBV8	+	+	+	+	+	+	+	+
PPL19.32	NBV9	+	+	+	−	+	−	−	+
PPL169.2B	NBV10	−	−	−	−	−	−	−	−

[a] *G. vaginalis* strains isolated at the University of Washington (Hillier) and redesignated at the C. W. Post Campus (Nath) as BV1 to BV10 and NBV1 to NBV10 for isolates from individuals with BV and without BV, respectively.
[b] From the restriction profile in Fig. 1.
[c] From the Southern blot hybridization profiles in Fig. 2 and 3.

* Corresponding author. Mailing address: Molecular Biology, Department of Biology, C. W. Post Campus of Long Island University, Brookville, NY 11548. Phone: (516) 299-2240. Fax: (516) 299-4140.
† Present address: No. 21, Lane 1460, Chiu-Ju 4th Road, Kaohsiung, 80409, Taiwan, Republic of China.

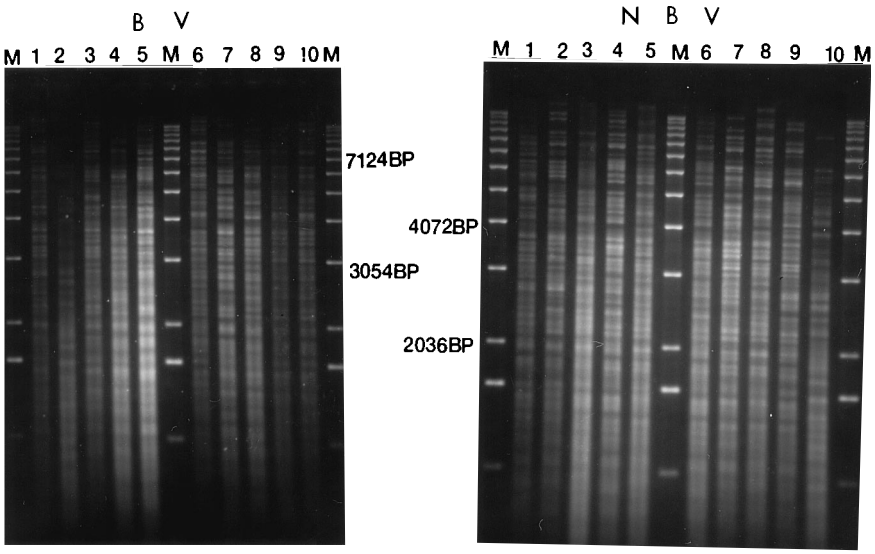

FIG. 1. REA of *Hae*II-digested DNAs from 20 biotype 1 *G. vaginalis* isolates. About 800 ng DNA was digested with 10 U of *Hae*II (Boehringer Mannheim) for 1.5 h; this was followed by digestion with another 10 U of *Hae*II for 1 h at 37°C. Electrophoresis was performed in a 0.9% agarose gel (12.5 by 15 cm) at 23 V for 14 h. After staining with ethidium bromide (5 μg/ml) and destaining, the gel was photographed under UV illumination by using an orange filter (Wratten 23; Kodak). The samples on the left (BV) represent the 10 samples from patients with BV and the samples on the right (NBV) represent the 10 samples from patients without BV described in Table 1. Lanes M, the 1-kb molecular size marker ladder (Gibco-BRL).

restriction patterns were evaluated visually by using both the prints and the negatives from type 55 film. The DNA fragment sizes were calculated from a plot of log molecular size and the distance that the DNA markers included in the same gel migrated.

DNAs from three isolates (BV8, NBV5, and NBV8) were resistant to *Bam*HI, similar to previously reported occasional *G. vaginalis* isolates (4, 5). Such observations have been reported for other organisms; for example, in one study (9), 8 of 93 *Staphylococcus aureus* isolates were resistant to *Bam*HI digestion but not to digestion with other restriction endonucleases. Whether the *Bam*HI resistance of the DNA in these isolates is due to GATC sequence-specific methylation or some other mechanism has not been resolved.

In REA, with all restriction enzymes tested, the restriction patterns were different in all isolates, as is evident in an *Hae*II profile (Fig. 1). A few bands, however, were shared by several isolates (Table 1), such as a sharp band at about 7.5 kb in 17 isolates, a two-band ladder above the 4-kb size marker in 13 isolates, a three-band ladder above the 3-kb size marker in 13 isolates, and so on. No distinction could be made between isolates from patients with BV and those from patients without BV on the basis of any REA. By individual REA the 20 isolates could be grouped into five (such as *Hae*II) or six (such as *Hin*dIII) subtypes on the basis of selected shared fragments.

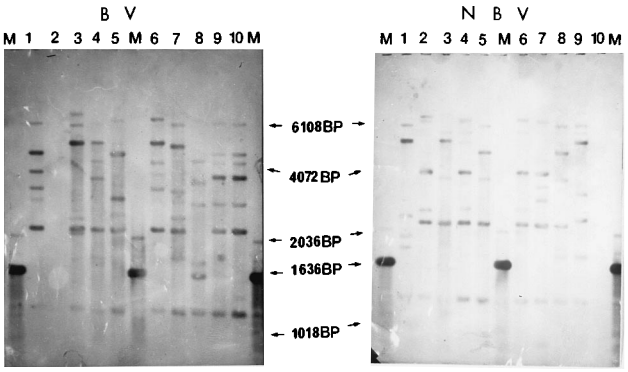

FIG. 2. RFLP analysis of Southern blots of *Hae*II digests. The Southern blots of *Hae*II restriction profiles in Fig. 1 were hybridized with pGVBH4 (along with a 1-kb ladder)-labelled probe by using a Genius nonradioactive DNA labelling and detection kit (Boehringer Mannheim). The hybridization was carried out at 65°C, and the color development was carried out at room temperature in the dark; both hybridization and color development were carried out overnight.

Since the restriction profiles were too difficult to analyze in detail, a modified subtyping method of selecting some well-resolved bands and comparing their presence in the REA (6) was used to subgroup the 20 strains on the basis of five of the six restriction profiles (Table 2). The *Msp*I DNA restriction profiles were not analyzed because nine of the isolates gave a smear unsuitable for comparative analysis. For the other five restriction enzymes two sets of bands were used (6), and five subtypes from each restriction profile were considered (Table 2). Except for BV9 and BV10, no two strains had exactly the same restriction-subtype distribution. A few strains had four of the five restriction-subtype distributions in common (BV2 and NBV10, BV3 and BV5, and BV1 and BV9 or BV10). When combined with the results of the restriction fragment length polymorphism (RFLP) analysis described below, although they were genomically close, none of the 20 strains was considered to have the same genomic fingerprint.

The restriction fragment analysis was easier when the Southern blots were hybridized with *G. vaginalis* DNA fragments as probes. For example, by using a 7.9-kb *G. vaginalis* ATCC 14018 *Bam*HI insert in pBR322 (pGVBH4) in an RFLP analysis of *Hae*II digests, 15 isolates were found to share a 2.28-kb fragment and 14 isolates were found to share a 1.18-kb fragment (Fig. 2 and Table 1). Of these, 12 isolates contained both *Hae*II fragments.

In a similar RFLP analysis of *Hin*dIII digests, numerous fragments were found in common between 18 of the 20 DNAs hybridizing with the pGVBH4 probe (Fig. 3). Most remarkable was a 1.22-kb fragment that was present in all hybridizing *Hin*dIII DNAs of biotype 1 isolates (Table 1). A 1.48-kb frag-

TABLE 2. Composite of REA subtypes of 20 biotype 1 *G. vaginalis* isolates

G. vaginalis strain	REA[a]				
	*Bam*HI	*Cla*I	*Eco*RI	*Hae*II	*Hin*dIII
BV1	B3	C4	E4	HA1	HN1
BV2	B5	C5	E5	HA5	HN5
BV3	B1	C3	E3	HA1	HN2
BV4	B1	C3	E3	HA4	HN4
BV5	B1	C3	E3	HA1	HN1
BV6	B4	C2	E4	HA4	HN1
BV7	B1	C1	E4	HA1	HN1
BV8	B4	C1	E4	HA3	HN2
BV9	B1	C1	E4	HA3	HN1
BV10	B1	C1	E4	HA3	HN1
NBV1	B2	C1	E2	HA1	HN4
NBV2	B3	C1	E2	HA2	HN1
NBV3	B3	C3	E1	HA4	HN4
NBV4	B1	C1	E3	HA1	HN2
NBV5	B4	C3	E3	HA1	HN3
NBV6	B1	C1	E3	HA2	HN4
NBV7	B1	C1	E2	HA1	HN1
NBV8	B4	C3	E1	HA1	HN1
NBV9	B1	C1	E1	HA1	HN1
NBV10	B5	C5	E5	HA5	HN6[b]

[a] Subtypes were based on two sets of bands selected from each REA and indicated as 1 (both sets of bands), 2 (only the larger set), 3 (only the smaller set), 4 (none of the two), and 5 (BV2 and NBV10 with similar profiles but unlike any of the profiles for the remaining 18 strains). Selected band sets included B (*Bam*HI), a 4.5-kb single band and a doublet just below 4 kb; C (*Cla*I), a 5.0-kb single band and a doublet around 3.5 kb; E (*Eco*RI), a triplet near 4 kb and a doublet above 3 kb; HA (*Hae*II), a doublet above 4 kb and a triplet above 3 kb; HN (*Hin*dIII), a 5.5-kb single band and a band below 3.0 kb.

[b] NBV10 was resistant to *Hin*dIII digestion, and hence, it was assigned to subtype 6 in the *Hin*dIII REA grouping.

ment and a 3.4-kb fragment were present in 13 different isolates, 9 of which contained both fragments (Table 1). Additionally, 11 isolates shared a 2.68-kb and 9 isolates shared a 1.95-kb fragment.

The restriction patterns of BV2 and NBV10 DNAs were consistently different from those of the other 18 isolates but were closer to each other, as evidenced in the *Hae*II profile (Fig. 1). Their failure to hybridize in a Southern blot is similar to the case for DNA from a previous culture (GVP 001) that hybridized very poorly with ATCC 14018 DNA (4). The strains in these two biotype 1 cultures thus seem to belong to one subtype. Although no morphological or biochemical analysis was carried out to explain the failure of these two strains to hybridize, the difference in the hybridization efficiency could indicate the presence of more than one species of *Gardnerella*. For example, the *Bacteroides* species have been distinguished on the basis of high-level (>75%) and low-level (<24%) DNA homologies (1).

The DNA fingerprint heterogeneity among the 20 isolates of the same biotype extends our previous DNA-based findings of a genetically mixed population of *G. vaginalis* in patients with BV (5). No discrimination could be made between the isolates from patients with BV and the isolates from patients without BV on the basis of DNA fingerprint analysis. However, for the first time we have found a restriction fragment, the 1.22-kb *Hin*dIII fragment hybridizing with pGBH4, that is common to all 18 of the 20 hybridizing DNAs determined by Southern blot analysis. Whether this represents a unique marker for only biotype 1 cultures or all *G. vaginalis* isolates poses an intriguing question. If the latter was to hold true, this 1.22-kb *Hin*dIII fragment can serve as an indicator of *G. vaginalis* in various clinical situations. Use of cloned DNA fragments in RFLP analysis is becoming extremely useful in microbial epidemiology (8). In summary, a genomic fingerprint study of one of eight possible *G. vaginalis* biotypes shed no light into the fac-

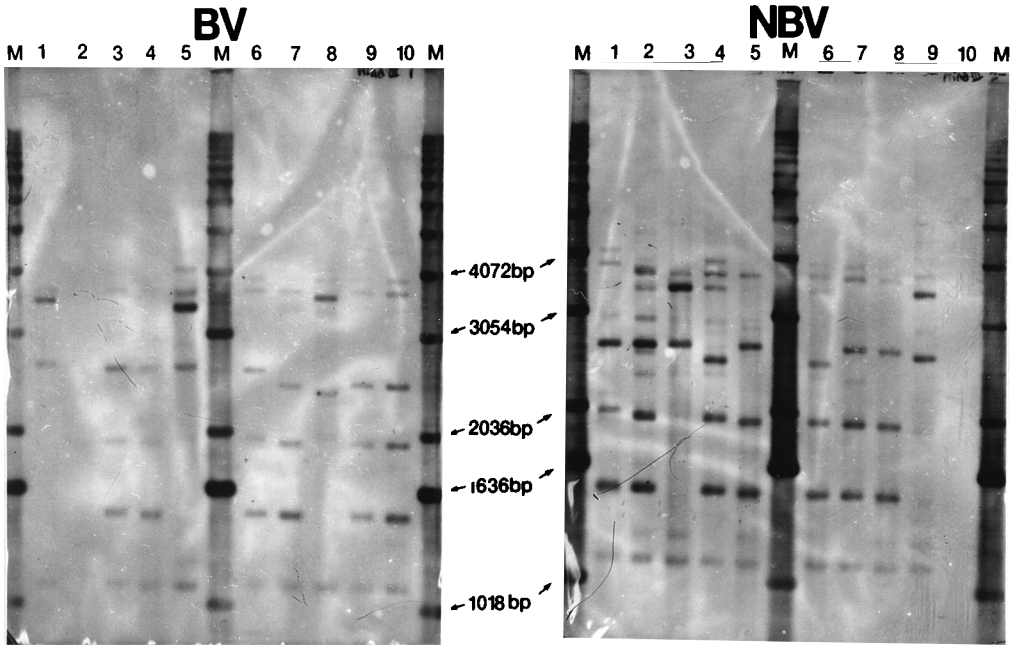

FIG. 3. RFLP analysis of Southern blots of *Hin*dIII digests. *Hin*dIII restriction profiles, similar to that described for *Hae*II in the legend to Fig. 1, were transferred onto a maximum-strength Nytran membrane (Schleicher & Schuell) and the Southern blot was hybridized with pGVBH4 (along with a 1-kb ladder) as described for the *Hae*II filter in the legend to Fig. 2.

tors that contribute to the development of BV when *G. vaginalis* is but one of several BV-associated bacteria.

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