Polyphasic Approach to the Classification and Identification of *Gardnerella vaginalis* and Unidentified *Gardnerella vaginalis*-Like Coryneforms Present in Bacterial Vaginosis

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A taxonomic study of *Gardnerella vaginalis* and *G. vaginalis*-like coryneforms was performed in order to clarify the phylogenetic affiliation of these organisms and to improve future identification. We examined 50 strains by performing whole-cell protein and fatty acid analyses, a 16S rRNA sequence analysis, and an extensive phenotypic characterization analysis. The results of both chemotaxonomic techniques which we used divided the organisms into two main clusters, and the 16S rRNA sequence analysis revealed that the clusters represent different genera, which were easily distinguished by the results of classical phenotypic tests. The cluster I strains were identified as *G. vaginalis*, which was shown to be a close relative of the genus *Bifidobacterium*. An improved description of *G. vaginalis* is presented. The cluster II strains belong to or are closely related to *Actinomyces turicensis*.

Gardnerella vaginalis was first recognized by Leopold in 1953 and was said to resemble members of the genus *Haemophilus* (30). Both the pathogenic significance of *G. vaginalis* and the taxonomic status of this organism have been the subjects of debate. In 1955, Gardner and Dukes (16) named this organism *Haemophilus vaginalis* because it was a gram-negative, rod-shaped bacterium that was successfully isolated on blood agar media but not on other agar media and it was believed to be responsible for a characteristic vaginal discharge. For a long time this organism was thought to be the etiological agent of bacterial vaginosis, but its role in this disease has been controversial, in part because of a lack of appropriate detection and identification methods for this fastidious organism. Now, it is assumed that bacterial vaginosis is also associated with other microorganisms, including *Bacteroides* spp., *Mobiluncus* spp., *Mycoplasma hominis*, *Peptococcus* spp., and *Peptostreptococcus* spp. (3, 22, 26, 36, 43, 44). Unlike other *Haemophilus* strains, *H. vaginalis* strains do not require hemin (X factor), NAD (V factor), or coenzyme-like substances for growth (10, 28, 35). On the basis of a Gram stain reaction and cellular morphology similar to the Gram stain reaction and cellular morphology of members of the genus *Corynebacterium*, Zinneman and Turner (51) suggested that the organism should be removed from the genus *Haemophilus* and reclassified as *Corynebacterium vaginale*. Reyn et al. (39) studied the type strain of *H. vaginalis* by electron microscopy and concluded that the fine structure of its cell wall resembled that of a gram-positive bacterium and that it should probably be placed in the genus *Corynebacterium* or the genus *Butyrivacterium*. These authors also indicated that this bacterium may be closely related to the genus *Propionibacterium*. In contrast, on the basis of the results of their electron microscopy studies Criswell et al. (6, 7) concluded that the fine

structure of the cell wall was more consistent with the fine structure of a gram-negative organism. However, on the basis of the results of a volatile acid production study, Moss and Dunkelberg (31) showed that *H. vaginalis* should not be placed in the genus *Corynebacterium* or the genus *Butyrivacterium*. Also, it has been proposed that *Lactobacillus* is the proper genus for this organism (1, 15), but, as shown by Moss and Dunkelberg (31), acetic acid rather than lactic, butyric, or propionic acid is the principal fermentation end product, which makes placement in the genus *Lactobacillus* unsuitable.

In two more recent taxonomic papers published in 1980 (20, 37), the authors presented data obtained by a variety of biochemical methods and from DNA-DNA hybridization experiments and electron microscopy. The findings of these researchers revealed that *H. vaginalis* formed a good taxospecies that exhibited little or no similarity to established gram-positive or gram-negative genera. The need for a new genus designation led Greenwood and Pickett to propose the name *Gardnerella vaginalis* (20), a proposal supported by Piot et al. (37).

A reexamination of the cell wall ultrastructure of *G. vaginalis* ATCC 14018^T (T = type strain) and *G. vaginalis* clinical isolates led Sadhu et al. (40) to conclude that the cell walls of these organisms have a gram-positive type of organization and that the unusual thinness of the cell walls accounts for the gram-negative staining tendency of the organisms.

In this study, we used a polyphasic approach to clarify the phylogenetic affiliation of *G. vaginalis* and *G. vaginalis*-like coryneforms, which are found in the same niche.

MATERIALS AND METHODS

Bacterial strains. *G. vaginalis* reference strains, including type strain, CCUG 3717, were obtained from the Instituut voor Tropische Geneeskunde (ITG), Antwerp, Belgium, and from the Culture Collection of the University of Göteborg (CCUG), Göteborg, Sweden. In addition, field isolates were obtained from the ITG and CCUG and our collection. A total of 50 isolates were included in the study (Table 1), and some of these isolates were also used in several previous studies (11–13, 20, 37). Bacteriological purity was checked by plating and exam-

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TABLE 1. Designations and sources of the strains used

Strain ^a	Other designation(s) ^a	Source
Cluster I strains		
<i>G. vaginalis</i> ITG 887 ^b	PN Edmunds T145	Human vagina, Edinburgh, United Kingdom
<i>G. vaginalis</i> ITG 894 ^b	PN Edmunds T64	Human vagina, Edinburgh, United Kingdom
<i>G. vaginalis</i> ITG 895 ^b	LMG 14324, PN Edmunds T66	Human vagina, Edinburgh, United Kingdom
<i>G. vaginalis</i> ITG 897 ^b	PN Edmunds T34	Human vagina, Edinburgh, United Kingdom
<i>G. vaginalis</i> ITG 899 ^b	PE Pease 646	
<i>G. vaginalis</i> ITG 1044 ^{b,c}	CDC 1412, LMG 14325	
<i>G. vaginalis</i> ITG 1045 ^{b,c}	CDC 1413, LMG 14326	
<i>G. vaginalis</i> CCUG 3717 ^{Tb,c}	NCTC 10287 ^T , ATCC 14018 ^T , Dukes 594 ^T	Human vagina, United States
<i>G. vaginalis</i> CCUG 4121 ^c	Dunkelberg V32	
<i>G. vaginalis</i> CCUG 4123 ^{b,c}	VDRL CV 208	Human vagina, United States
<i>G. vaginalis</i> CCUG 10059 ^{b,c}	Pickett 37369	Human vagina, United States
<i>G. vaginalis</i> ITG 132		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 145		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 146		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 286		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 290		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 293 ^b		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 300 ^b		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 802		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 805 ^b		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 823		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 828		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 831		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 833 ^c	LMG 14333, CCUG 32392	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 840		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 879		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> ITG 884		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> CCUG 3990 ^c		Human urine, Goteborg, Sweden
<i>G. vaginalis</i> CCUG 7154 ^c		Human ovarian abscess, Goteborg, Sweden
<i>G. vaginalis</i> CCUG 7712 ^c		Human amniotic fluid, Goteborg, Sweden
<i>G. vaginalis</i> CCUG 7751 ^c		Human urine, urosepsis, Goteborg, Sweden
<i>G. vaginalis</i> CCUG 7921 ^c		Human urine, Goteborg, Sweden
<i>G. vaginalis</i> CCUG 11278A ^c		Human urine, Goteborg, Sweden
<i>G. vaginalis</i> CCUG 26939 ^c		Human
<i>G. vaginalis</i> CCUG 26996 ^c		Human blood, Gavle, Sweden
<i>G. vaginalis</i> CCUG 28963 ^c		Human cervix, Boras, Sweden
<i>G. vaginalis</i> CCUG 30587 ^c		Human lung, Uppsala, Sweden
<i>G. vaginalis</i> CCUG 30841		Human urine, Umea, Sweden
<i>G. vaginalis</i> VDAB 2		Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> PVR 101		Human vagina, Antwerp, Belgium
Cluster II strains		
<i>G. vaginalis</i> -like strain ITG 75 ^{b,c}	LMG 14329, CCUG 32388	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> -like strain ITG 131 ^c	LMG 14330, CCUG 32389	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> -like strain LMG 14331 ^c	ITG 137, CCUG 32390	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> -like strain ITG 143 ^{b,c}	LMG 14736, CCUG 33040	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> -like strain ITG 147 ^{b,c}	LMG 14735, CCUG 33039	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> -like strain ITG 284 ^{b,c}	LMG 14737, CCUG 33041	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> -like strain ITG 296 ^c	LMG 14332, CCUG 32391	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> -like strain ITG 806 ^c	LMG 14738, CCUG 33042	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> -like strain ITG 886 ^c	LMG 14734, CCUG 33038	Human vagina, Antwerp, Belgium
<i>G. vaginalis</i> -like strain ITG 900 ^c	LMG 14733, CCUG 33037	Human penile ulcer, Antwerp, Belgium

^a ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; ITG, Instituut voor Tropische Geneeskunde, Antwerp, Belgium; LMG, Culture Collection of the Laboratorium Microbiologie Gent, University of Ghent, Ghent, Belgium; NCTC, National Collection of Type Cultures, London, England.

^b Analyzed by using experimental API galleries that included tests for 59 peptidases, 10 esterases, and 20 glycosidases and for the presence of fructose-6-phosphate phosphoketolase.

^c Examined by using the Rapid ID 32 Strep and API Coryne galleries.

ining living and Gram-stained cells. The organisms were grown on Columbia CNA agar (BBL, Becton Dickinson) supplemented with 6% fresh human blood, unless indicated otherwise, and were incubated aerobically at 37°C with 5% CO₂ enrichment.

Reference strains of all species of the genus *Bifidobacterium* were obtained from the Laboratorium Microbiologie Gent Culture Collection (LMG), Ghent, Belgium. In addition, phenotypic data for 192 strains obtained from humans and belonging to 10 groups or species of the genus *Bifidobacterium* (previously classified on the basis of numerical analysis data [2, 17, 18]) were available for comparison.

PAGE of whole-cell proteins. All strains were grown for 4 days at 37°C on Columbia agar (23 g of Special Peptone, 1 g of corn starch, 5 g of sodium chloride, 0.3 g of cysteine HCl, 5 g of glucose, 20 g of agar, 1 liter of H₂O) supplemented with 5% horse blood and were incubated in an anaerobic atmosphere containing 5% CO₂, 10% H₂, and 85% N₂. Polyacrylamide gel electrophoresis (PAGE) of whole-cell proteins was performed as described by Pot et al. (38).

Bifidobacterium reference strains were grown for 2 days under the same conditions.

A densitometric analysis, normalization and interpolation of the protein pro-

FIG. 1. Similarity dendrogram based on the protein patterns of *G. vaginalis* and *G. vaginalis*-like coryneform bacteria. Levels of correlation were expressed as percentages of similarity for convenience.

files, and a numerical analysis were performed by using the Gelcompar 3.1 software package (Applied Maths, Kortrijk, Belgium). Levels of correlation are presented below as percentages of similarity for convenience.

Cellular fatty acid analysis. After incubation for 48 h, a loopful of cells was

harvested, and the fatty acid methyl esters were extracted, separated, and identified as described previously (46).

Determination and analysis of 16S rRNA gene sequences. Genomic DNA was prepared by the standard procedures described by Giesendorf et al. (19). The 16S rRNA genes were amplified by PCR (32, 41), using a Perkin-Elmer Cetus GeneAmp PCR System 9600 apparatus. PCR was performed with forward primer 16F27 (annealing at positions 8 to 27) and reverse primer 16R1525 (annealing at the complement of positions 1525 to 1541; *Escherichia coli* 16S rRNA gene sequence numbering). The reaction conditions were the same as those described previously (25). PCR products were purified by using Centricon-100 microconcentrators (Amicon GmbH, Witten, Germany), and the sequence of the amplified 16S ribosomal DNA was determined directly by using an Applied Biosystems model 373A DNA sequencer and the protocols of the manufacturer (Perkin-Elmer, Applied Biosystems GmbH, Weiterstadt, Germany) for *Taq* cycle sequencing with fluorescent dye-labeled dideoxynucleotides. The sequencing primers which we used have been described previously (27).

The sequence data which we obtained were aligned with previously described 16S rRNA (and rRNA gene) sequences (9, 34) by using conserved regions and secondary-structure characteristics as references (21, 48). Sequence similarity values and evolutionary distances, which incorporated a correction factor (24) for reverse mutations, were calculated for masked (27) and unmasked sequence pair comparisons by using unambiguous nucleotide positions.

Phenotypic characterization. A total of 27 strains, including 17 cluster I strains (see below) which represented the diversity of this cluster, as determined by cellular protein and fatty acid analyses, and all 10 cluster II strains (see below), were examined by using Rapid ID 32 Strep and API Coryne galleries (bio-Mérieux, La Balme-les-Grottes, Montalieu-Vercieu, France), as described by the manufacturer. In addition, 13 cluster I strains and all of the cluster II strains were analyzed by using experimental API galleries that included tests for 59 peptidases, 10 esterases (tests performed with all strains except strains LMG 14331 and ITG 131), and 20 glycosidases (tests performed with all strains except strains LMG 14331, ITG 131, ITG 296, ITG 806, ITG 886, and ITG 900). The presence of fructose-6-phosphate phosphoketolase activity (42) in the same 13 cluster I strains and all of the cluster II strains was verified.

Nucleotide sequence accession number. The 16S ribosomal DNA sequence of strain LMG 14331, a representative of protein electrophoretic cluster II (see below), has been deposited in the GenBank database under accession number X87133.

RESULTS

SDS-PAGE of whole-cell proteins. All of the strains listed in Table 1 were examined by sodium dodecyl sulfate (SDS)-PAGE. Duplicate protein extracts of several strains were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The levels of correlation between duplicate protein patterns were more than 93% (data not shown).

The dendrogram obtained after we performed a numerical

FIG. 2. Electrophoretic protein profiles of representative strains belonging to both electrophoretic clusters and of two aberrant strains (*G. vaginalis* ITG 805 and ITG 884). The following molecular weight markers were used (track MWM, from left to right): trypsin inhibitor (molecular weight, 20,100), trypsinogen (24,000), carbonic anhydrase (29,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), egg albumin (45,000), and bovine albumin (66,000).

TABLE 2. Mean fatty acid compositions of the strains studied^a

Cluster	No. of strains	% of:						
		14:0	16:1 ω 7c	16:0	18:2 ω 6,9c	18:1 ω 9c	Summed feature 7 ^b	18:0
I	40	6.9 $\pm$ 1.5	3.0 $\pm$ 0.9	36.6 $\pm$ 2.9	4.8 $\pm$ 1.3	35.2 $\pm$ 2.2	2.5 $\pm$ 2.0	9.2 $\pm$ 1.4
II	10	4.6 $\pm$ 0.4	6.2 $\pm$ 0.5	23.5 $\pm$ 2.2	8.0 $\pm$ 1.5	44.3 $\pm$ 3.3	4.4 $\pm$ 2.3	3.8 $\pm$ 0.5

^a Fatty acids that were present at concentrations of less than 1% in both groups are not shown, which explains why the values do not add up to 100%.

^b Summed feature 7 comprises 18:1 ω 7c, 18:1 ω 9t, or 18:1 ω 12t or any combination of these isomers.

analysis with all of the protein patterns is shown in Fig. 1. Figure 2 shows a representative selection of the patterns examined. In the numerical analysis, two major clusters were differentiated. In addition, two strains that had very similar protein patterns (ITG 884 and ITG 887) and strain ITG 805 occupied distinct positions on the dendrogram. Cluster I contained 37 strains, including the type and reference strains of *G. vaginalis*, several field isolates identified as *G. vaginalis* on the basis of the biochemical characteristics, and several strains received as unclassified *G. vaginalis*-like coryneforms, that grouped above a correlation level of 79%. The overall protein profiles of these strains were very similar, except in the 36,000- to 45,000-molecular-weight region (Fig. 2); in this region there were one to four dense protein bands whose positions were highly variable. The protein patterns of strains ITG 805, ITG

884 (Fig. 2), and ITG 887 differed in other ways, although the overall patterns were fairly similar. Cluster II contained 10 unclassified *G. vaginalis*-like coryneforms that grouped above a correlation level of 78%.

Our numerical analysis of these protein patterns and the patterns of *Bifidobacterium* reference strains revealed that cluster I and II strains and strains ITG 884, ITG 887, and ITG 805 differed from the taxa in the genus *Bifidobacterium* that have been described (data not shown).

Fatty acid methyl ester composition. A numerical analysis of the fatty acid components of the 50 strains which we examined revealed two subgroups (data not shown). The first subgroup contained all of the protein electrophoretic cluster I strains and aberrant isolates ITG 805, ITG 884, and ITG 887; the

TABLE 3. Levels of unmasked 16S rRNA gene sequence similarity between *G. vaginalis*-like strain LMG 14331 or *G. vaginalis* ATCC 14018^T and representative reference organisms

Taxon	Strain ^a	Nucleotide sequence accession no.	% Sequence similarity with <i>G. vaginalis</i> -like strain LMG 14331	% Sequence similarity with <i>G. vaginalis</i> ATCC 14018 ^T
<i>Actinomyces bovis</i>	DSM 43014 ^T	X53224	87.6	83.0
<i>Actinomyces radingae</i>	DSM 9169 ^T	X78719	90.9	83.1
<i>Actinomyces turicensis</i>	DSM 9168 ^T	X78720	98.4	82.5
<i>Actinomyces pyogenes</i>	ATCC 19411 ^T	M29522	86.9	83.2
<i>Actinomyces odontolyticus</i>	DSM 43331	X53227	94.1	83.5
<i>Actinomyces hyovaginalis</i>		X69616	94.7	82.6
<i>Aeromicrobium erythreum</i>	NRRL B-3381	— ^b	82.6	82.7
<i>Arcanobacterium haemolyticum</i>		X73952	87.4	82.7
<i>Arthrobacter globiformis</i>	DSM 20124 ^T	—	85.3	84.3
<i>Bifidobacterium bifidum</i>	ATCC 29521 ^T	—	81.5	93.1
<i>Bifidobacterium dentium</i>	ATCC 15423	—	80.9	92.4
<i>Bifidobacterium longum</i>	ATCC 15707 ^T	—	80.3	92.6
<i>Corynebacterium xerosis</i>	ATCC 373 ^T	—	84.1	82.8
<i>Dermatophilus congolensis</i>	ATCC 14637 ^T	—	86.6	84.1
<i>Gardnerella vaginalis</i>	ATCC 14018 ^T	—	84.9	—
<i>Gordona terrae</i>	DSM 43249 ^T	X79286	84.4	82.4
<i>Micrococcus luteus</i>	ATCC 381 ^T	—	85.5	84.1
<i>Mycobacterium tuberculosis</i>	NCTC 7416	X58890	83.0	81.5
<i>Nocardia asteroides</i>	DSM 43757 ^T	X80606	84.1	82.7
<i>Nocardioideus simplex</i>	NCIMB 8929 ^T	X53213	82.2	81.4
<i>Propionibacterium freudenreichii</i>	DSM 20271 ^T	—	82.0	82.5
<i>Pseudonocardia thermophilus</i>	ATCC 19285 ^T	X53195	85.1	83.2
<i>Rhodococcus globularis</i>	NCIMB 12315 ^T	X77779	83.2	81.6
<i>Rhodococcus rhodochrous</i>	DSM 43241 ^T	X79288	85.6	84.0
<i>Rothia dentocariosa</i>	ATCC 17931 ^T	—	85.3	83.9
<i>Saccharothrix australiensis</i>	DSM 43800 ^T	X53193	83.0	81.9
<i>Streptomyces albus</i>	DSM 40313	X53163	81.2	79.5
<i>Streptomyces phosalacineus</i>	DSM 43860 ^T	M55223	81.9	80.7
<i>Terrabacter tumescens</i>	NCIMB 8914 ^T	X53215	85.1	83.5
<i>Tsukamurella paurometabolum</i>	NCTC 10741 ^T	—	84.6	82.1
<i>Bacillus licheniformis</i>	DSM 13 ^T	X68416	74.4	76.3
<i>Fusobacterium nucleatum</i>	ATCC 25586 ^T	X55401	73.6	73.7

^a DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland. For other abbreviations see Table 1, footnote a.

^b —, sequence obtained from the Ribosomal RNA Database Project database (34).

second subgroup contained all of the protein electrophoretic cluster II strains (see above).

The fatty acid components of the two subgroups are shown in Table 2. The predominant fatty acids in all of the strains studied are 14:0, 16:1 ω 7c, 16:0, 18:1 ω 9c, 18:0, summed feature 6, and summed feature 7. Quantitative differences readily allow differentiation of the two subgroups.

16S rRNA sequence analysis. PCR amplification of the 16S rRNA genes between the nucleotides at positions 28 and 1525 allowed us to determine the nucleotides at 1,474 positions, which account for approximately 96% of the complete gene sequence (estimated by comparison with the 16S rRNA gene sequence of *E. coli*). Masked sequence comparisons (involving 1,349 nucleotide positions) of the 16S ribosomal DNAs revealed that strain LMG 14331 clustered with the gram-positive bacteria with high genomic DNA G+C contents (47) and most closely with species belonging to the genus *Actinomyces* (data not shown). Table 3 shows the results of unmasked comparisons between the sequence of strain LMG 14331 or *G. vaginalis* ATCC 14018^T and the sequences of representative reference strains belonging to the gram-positive high-G+C-content species. The highest level of sequence similarity (98.4%) was the level of similarity between LMG 14331 and *Actinomyces turicensis*. Analyses of partial sequences (analyses of about 30% of the complete gene, including the most variable regions of the 16S ribosomal DNA gene of *G. vaginalis* CCUG 3717^T) were performed to verify the sequence of *G. vaginalis* ATCC 14018^T rRNA in the Ribosomal RNA Database Project database (34). The sequences which we determined corresponded completely with the sequence present in the Ribosomal RNA Database Project database, and therefore the complete Ribosomal RNA Database Project sequence was used for phylogenetic analyses.

Phenotypic characterization. The following tests were negative for all of the strains examined: arginine dihydrolase, *N*-acetyl- β -glucosaminidase, urease, esculin hydrolysis, catalase, γ -glutamyl-transferase, *N*-carboxybenzoyl-glycyl-arginine arylamidase, pyroglutamic acid arylamidase, glycyl-tryptophan arylamidase, α -L-aspar-tyl-L-alanine arylamidase, α -L-aspartyl-L-arginine arylamidase, α -L-glutamyl- α -L-glutamine arylamidase, L-leucyl-L-leucyl-L-valyl-L-tyrosyl-L-serine arylamidase, *N*-benzoyl-L-alanine-4-methoxyarylamidase, *N*-carboxybenzoyl-glycyl-L-arginine arylamidase, *N*-acetyl-glycyl-L-lysine arylamidase, α -D-galactosidase, phospho- β -D-galactosidase, α -L-arabinosidase, β -D-glucosidase, β -D-galacturonohydrolase, β -D-glucuronidase, β -maltosidase, α -L-fucosidase, β -D-fucosidase, β -L-fucosidase, β -D-lactosidase, β -D-mannosidase, α -D-xylosidase, β -D-xylosidase, esterase C₆, C₁₆, and C₁₈, nitrate reduction, hydrolysis of gelatin, acetoin production, and acidification of mannitol, sorbitol, lactose, trehalose, raffinose, melibiose, melezitose, D-arabitol, methyl- β -D-glucopyranoside, tagatose, and cyclodextrin.

The following tests were positive for all of the strains examined: pyrazinamidase, alanyl-phenylalanyl-proline arylamidase, L-tyrosine arylamidase, L-phenylalanine arylamidase, L-lysine arylamidase, L-hydroxyproline arylamidase, L-histidine arylamidase, glycine arylamidase, L-arginine arylamidase, L-alanine arylamidase, glycyl-proline arylamidase, leucyl-glycine arylamidase, L-ornithine arylamidase, L-proline arylamidase, L-serine arylamidase, L-threonine arylamidase, β -alanine arylamidase, L-lysyl-L-alanine arylamidase, L-lysyl-L-lysine arylamidase, L-phenylalanyl-L-proline arylamidase, L-phenylalanyl-L-prolyl-L-alanine arylamidase, α -maltosidase, and acidification of maltose and ribose.

The remaining test results are shown in Table 4. Differences in test results between the Rapid ID 32 Strep and API Coryne galleries were found only for the presence of alkaline phosphatase in cluster I strains.

DISCUSSION

The phylogenetic positions of bacteria identified previously as *G. vaginalis* and other unidentified *G. vaginalis*-like coryneforms (37) isolated from bacterial vaginosis specimens have been controversial and unsettled for many years. In order to clarify the situation, we performed an integrated study in which we determined the genotypic and phenotypic characteristics of 50 *G. vaginalis* and *G. vaginalis*-like strains.

Strains studied. The strains which we studied included reference strains of *G. vaginalis* and field isolates which were identified by biochemical tests as *G. vaginalis* or unidentified *G. vaginalis*-like coryneforms. Among the latter strains were 26 isolates which belonged to two biochemical subgroups according to the data of Piot et al. (37). Our data (see below) allowed us to subdivide our collection of strains into two main subgroups, which were designated cluster I and cluster II.

There were some differences between our results and the results of Piot et al. (37). Piot et al. described strains ITG 132, ITG 145, ITG 146, ITG 286, ITG 300, ITG 802, ITG 833, ITG 887, and ITG 899 as members of the cluster containing the unclassified catalase-negative coryneforms, while our results showed that these strains are closely related to the *G. vaginalis* type strain (Fig. 1 and Table 2). These apparently contradictory results were confirmed after we repeated our analyses of these strains by starting with fresh cultures. Furthermore, the results of the fatty acid and protein electrophoresis analyses pointed toward the same conclusions. One of these strains, ITG 833, was included in the phenotypic analyses and produced a typical *G. vaginalis* pattern. The results of our various approaches confirmed each other, and it has been established with several taxa that high levels of similarity in whole-cell protein contents correlate with high levels of DNA-DNA homology (5). Our results therefore suggest that the nine isolates listed above are genuine *G. vaginalis* strains. Our results also revealed that the 10 cluster II strains from a homogeneous taxon, while the hybridization experiments of Piot et al. (37) revealed low levels of DNA homology for some of these strains. We again found that the results of all of our experiments correlated with each other and indicated that the 10 isolates examined form a homogeneous species. These findings indicate that the identities of some of the ITG isolates might be questioned as these isolates do not seem to correspond to the isolates studied by Piot et al. (37).

Taxonomic status of cluster I strains. Despite the considerable variability in a dense protein band region, 37 isolates produced very similar overall protein patterns. Ten *G. vaginalis* reference strains, including the type strain, belong to this cluster, and therefore, given the correlation between the similarity of whole-cell protein patterns and DNA homology values (5), we consider all of these isolates genuine *G. vaginalis* strains. Reference strain ITG 887 and two other isolates produced aberrant protein patterns. However, the overall protein patterns of these organisms shared many protein bands with the patterns of the *G. vaginalis* strains, and we found no differences when the same isolates were examined in the fatty acid analysis or by a battery of biochemical tests. Therefore, we consider all three of these strains protein electrophoretically aberrant *G. vaginalis* strains. A salient feature of all of the cluster I strains is the extreme variability in the dense protein bands in the 36,000- to 45,000-molecular-weight range (Fig. 2), a finding which has been reported by other workers (4).

The cellular fatty acid profiles of these strains contained a number of unidentified peaks for fatty acids which could not be differentiated under the gas chromatographic conditions which we used. Summed feature 6 comprises fatty acid 18:2 ω 6,9c or

TABLE 4. Phenotypic differences between *G. vaginalis* and *G. vaginalis*-like bacteria

Test	<i>G. vaginalis</i>		<i>G. vaginalis</i> -like bacteria	
	% of strains positive (<i>n</i> = 17)	Reaction of strain CCUG 3717 ^T	% of strains positive (<i>n</i> = 10)	Reaction of strain LMG 14331
Fructose-6-phosphate phosphoketolase	100	+	0	—
β-D-Galactosidase	71	+	0	—
α-D-Glucosidase	77	+	100	+
Alkaline phosphatase (Rapid ID 32 Strep)	76	+	0	—
Alkaline phosphatase (API Coryne)	0	—	0	—
Hydrolysis of hippurate	88	+	90	+
Acidification of glucose	82	ND	100	+
Acidification of glycogen	63	+	0	—
Acidification of pullulan	59	+	0	—
Acidification of saccharose	0	—	100	+
Acidification of L-arabinose	12	—	10	—
Acidification of xylose	0	—	100	+
L-Pyrrolidone arylamidase	92	+	40	—
L-Aspartate arylamidase	77	—	80	—
N-Benzoyl-leucine arylamidase	15	+	33	—
S-Benzyl-cysteine arylamidase	100	+	33	—
Methionine arylamidase	92	+	11	—
Glycyl-glycine arylamidase	23	+	33	—
Glycyl-phenylalanine arylamidase	69	+	33	—
L-Seryl-tyrosine arylamidase	92	+	80	—
N-Carboxybenzoxy-arginine-4-methoxyarylamidase	62	+	30	—
L-Glutamine arylamidase	85	+	20	—
α-L-Glutamate arylamidase	100	+	20	—
L-Isoleucine arylamidase	54	—	10	—
L-Tryptophan arylamidase	77	+	80	+
L-Alanyl-L-arginine arylamidase	69	+	60	—
L-Alanyl-L-phenylalanyl-L-proline arylamidase	62	+	100	+
L-Alanyl-L-phenylalanyl-L-prolyl-L-alanine arylamidase	85	+	50	—
L-Arginyl-L-arginine arylamidase	15	+	10	—
α-L-Glutamyl-L-histidine arylamidase	92	+	0	—
Glycyl-L-alanine arylamidase	23	+	80	+
Glycyl-L-arginine arylamidase	100	+	60	—
Glycyl-L-tryptophan arylamidase	23	+	0	—
L-Histidyl-L-leucyl-L-histidine arylamidase	100	+	20	—
L-Histidyl-L-serine arylamidase	100	+	20	—
L-Leucyl-L-alanine arylamidase	100	+	40	+
L-Phenylalanyl-L-arginine arylamidase	92	+	20	—
L-Prolyl-L-arginine arylamidase	100	+	70	+
L-Seryl-L-methionine arylamidase	69	+	10	—
L-Valyl-L-tyrosyl-L-serine arylamidase	85	—	0	—
N-Carboxybenzoxy-arginyl-4-methoxyarylamidase	38	—	20	—
L-Histidyl-L-phenylalanine arylamidase	38	—	0	—
L-Lysyl-L-serine-4-methoxyarylamidase	92	+	0	—
N-Acetyl-α-D-glucosaminidase	92	+	25	ND
α-D-Mannosidase	85	+	25	ND
Esterase C ₄	15	—	13	ND
Esterase C ₅	15	—	13	ND
Esterase C ₈	46	—	25	ND
Esterase C ₉	62	—	13	ND
Esterase C ₁₀	85	+	25	ND
Esterase C ₁₂	46	—	0	ND
Esterase C ₁₄	23	—	0	ND

^a +, positive; —, negative; ND, not determined.

18:0 ante or both. Because *G. vaginalis* lacks branched-chain fatty acids and the presence of 18:2 has been described previously (8, 23, 31), we assume that the peak corresponding to summed feature 6 contains only 18:2 ω6,9c. Summed feature 7 comprises one or more isomers of 18:1 (Table 2) and was detected in all of the strains examined.

The mean fatty acid composition of the cluster I strains (about 7% 14:0, about 3% 16:1 ω7c, about 37% 16:0, about 35% 18:1 ω9c, about 9% 18:0, about 5% 18:2 ω6,9c, and about

2% summed feature 7) corresponds well to the results of O'Donnell et al. (33) and Csángó et al. (8).

The results of our analysis of the variable regions of the 16S ribosomal DNA of *G. vaginalis* CCUG 3717^T confirmed the sequence of another subculture of the type strain in the Ribosomal RNA Database Project database. An extensive comparison of the latter sequence with the sequences of other gram-positive bacteria having high G+C contents revealed that *G. vaginalis* is closely related to the genus *Bifidobacterium* (level

of similarity with the type species, *Bifidobacterium bifidum*, 93.1%). The results of subsequent phenotypic analyses indicated that *G. vaginalis* exhibits fructose-6-phosphate phosphoketolase activity, a feature which was assumed to be specific to the genus *Bifidobacterium* (42). However, several investigators have reported that the DNA base ratio of *G. vaginalis* is approximately 42 mol% (23, 29, 37, 45), whereas the G+C content of *Bifidobacterium* DNAs vary from 55 to 67 mol% (2). This difference in DNA base ratio is far too large to consider *G. vaginalis* a *Bifidobacterium* species. The results of a comparison of the whole-cell protein patterns of *G. vaginalis* and *Bifidobacterium* reference strains revealed a number of similarities in the overall protein profiles but also allowed us to differentiate *G. vaginalis* from all *Bifidobacterium* species that have been described (data not shown). A comparison of the phenotypic characteristics of *G. vaginalis* and *Bifidobacterium* species (2, 17, 18) yielded several differential characteristics; however, these findings are beyond the scope of this paper. The various phenotypic analyses which we performed did not yield genus-specific characteristics which could be used to separate the two genera.

We concluded that the two organisms represent distinct genera belonging to a single phylogenetic lineage, and therefore our data supported the proposal of Greenwood and Pickett (20) and Piot et al. (37).

The following characteristics can be added to the description of *G. vaginalis* given by Greenwood and Pickett (20): positive for fructose-6-phosphate phosphoketolase, L-tyrosine arylamidase, L-pyrrolidone arylamidase, L-phenylalanine arylamidase, L-lysine arylamidase, L-hydroxyproline arylamidase, L-histidine arylamidase, glycine arylamidase, L-arginine arylamidase, L-alanine arylamidase, S-benzyl-cysteine arylamidase, methionine arylamidase, glycyl-proline arylamidase, leucyl-glycine arylamidase, L-seryl-tyrosine arylamidase, α -L-glutamate arylamidase, L-ornithine arylamidase, L-proline arylamidase, L-serine arylamidase, L-threonine arylamidase, β -alanine arylamidase, α -L-glutamyl-L-histidine arylamidase, glycyl-L-arginine arylamidase, L-histidyl-L-leucyl-L-histidine arylamidase, L-histidyl-L-serine arylamidase, L-leucyl-L-alanine arylamidase, L-lysyl-L-lysine arylamidase, L-phenylalanyl-L-arginine arylamidase, L-phenylalanyl-L-proline arylamidase, L-phenylalanyl-L-prolyl-L-alanine arylamidase, L-prolyl-L-arginine arylamidase, L-lysyl-L-serine-4-methoxyarylamidase, β -D-galactosidase, α -D-glucosidase, α -maltoosidase, N-acetyl- α -D-glucosaminidase, alanyl-phenylalanyl-proline arylamidase, and pyrazinamidase; and negative for arginine dihydrolase, N-acetyl- β -glucosaminidase, esculin hydrolysis, γ -glutamyltransferase, N-carboxybenzoxyl-glycyl-glycyl-arginine arylamidase, pyrrolutamic acid arylamidase, glycyl-tryptophan arylamidase, α -L-aspartyl-L-alanine arylamidase, α -L-aspartyl-L-arginine arylamidase, α -L-glutamyl- α -L-glutamine arylamidase, N-benzoyl-L-alanine-4-methoxyarylamidase, N-carboxybenzoxyl-glycyl-L-arginine arylamidase, N-acetyl-glycyl-L-lysine arylamidase, α -D-galactosidase, phospho- β -D-galactosidase, α -L-arabinosidase, β -D-glucosidase, β -D-galacturonohydrolase, β -D-glucuronidase, β -maltoosidase, β -D-fucosidase, β -D-fucosidase, β -L-fucosidase, β -D-lactosidase, β -D-mannosidase, α -D-xylosidase, β -D-xylosidase, esterase C₆, C₁₆, and C₁₈, nitrate reduction, hydrolysis of gelatin, acetoin production, and acidification of lactose, trehalose, raffinose, melibiose, melezitose, saccharose, D-arabitol, methyl- β -D-glucopyranoside, tagatose, and xylose.

Taxonomic status of cluster II strains. On the basis of the results of SDS-PAGE and fatty acid analyses, the 10 cluster II strains formed a homogeneous group. Sequence comparisons of the 16S ribosomal DNAs revealed a level of similarity of 98.4% between strain LMG 14331 and the type strain of *A.*

turicensis, which is within the range of values for a single species (14). The latter organism is a facultatively anaerobic, gram-positive, asporogenous, rod-shaped bacterium that has succinic and lactic acids as its main metabolic products and a DNA base composition of 57 mol% G+C. The two strains described as *A. turicensis* were isolated from mixed infections with anaerobes in a perianal abscess and pleural empyema (49, 50). When the phenotypic characteristics of cluster II and *A. turicensis* strains were compared, 16 common features were detected. We found differences only in α -L-fucosidase activity and in acidification of L-arabinose, lactose, mannitol, melezitose, melibiose, raffinose, and trehalose (in our study, 0 to 10% of the cluster II strains acidified these compounds, compared with one of the *A. turicensis* strains). Esterase C₄ was present in both of the *A. turicensis* strains, while only 13% of the cluster II strains were positive for the esterase C₄ test (49, 50). Both 16S rRNA sequence and phenotypic data showed that the cluster II strains are identical or closely related to *A. turicensis*. Additional chemotaxonomic and genotypic analyses will be required to confirm the relationship between the two taxa. Since *G. vaginalis* and the *A. turicensis*-like strains are found in the same ecological niche, features that distinguish these two species might be clinically important. *G. vaginalis* strains have fructose-6-phosphate phosphoketolase, α -L-glutamyl-L-histidine arylamidase, and L-lysyl-L-serine-4-methoxyarylamidase activities and do not acidify saccharose and xylose, while the *A. turicensis*-like bacteria have the opposite biochemical characteristics.

Strain LMG 14331, the cluster II reference strain, and all of the other cluster II strains have been deposited in the LMG culture collection.

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