Mobiluncus gen. nov., *Mobiluncus curtisii* subsp. *curtisii* sp. nov., *Mobiluncus curtisii* subsp. *holmesii* subsp. nov., and *Mobiluncus* *mulieris* sp. nov., Curved Rods from the Human Vagina

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A total of 22 strains of curved, anaerobic bacteria from the vaginae of women with bacterial vaginosis were found to be morphologically, biochemically, and genetically distinct from species in previously described genera. We placed these organisms in the genus *Mobiluncus* gen. nov., which includes curved, anaerobic, motile, rod-shaped bacteria that have multiple subpolar flagella and multilayered gram-variable cell walls, produce succinate and acetate, are stimulated by rabbit serum, and have guanine-plus-cytosine contents of 49 to 52 mol%. *Mobiluncus curtisii* is the type species (type strain, ATCC 35241). *Mobiluncus curtisii* strains are small (length, 1.7 µm) and gram variable, are stimulated by arginine, and produce ornithine, citrulline, and ammonia from arginine. Six of these strains migrate through soft (0.25%) agar and are nitrate negative, and these organisms are designated *Mobiluncus curtisii* subsp. *curtisii*. Six strains that do not migrate through soft agar and are nitrate positive are designated *Mobiluncus curtisii* subsp. *holmesii* (type strain, ATCC 35242). A total of 10 strains are designated *Mobiluncus mulieris* (type strain, ATCC 35243). These organisms are large (length, 2.9 µm) and gram negative, produce acid (pH <5.5) from glycogen, and are CAMP test positive. The levels of deoxyribonucleic acid homology were >75% within and ≤25% between strains of the two species.

Vibrio-like organisms were observed in vaginal fluids as early as 1895 (19) and were first isolated from the female genital tract by Curtis in 1913 (6). Prévot (26) described "*Vibrio mulieris*," the *Vibrio* of Curtis, in 1940. In 1954, Moore described five strains (22) which grew best in an anaerobic environment supplemented with CO₂. Durieux and Dublanche (7) characterized 18 serum-requiring strains of "vibrions succinoproduleurs" and separated these organisms into two groups, group VSP 1 containing cells which were 2 to 3 µm long, did not ferment glucose, and varied in nitrate reductase activity and group VSP 2 containing cells which were 5 to 6 µm long and fermented glucose but did not reduce nitrate. Peloux and Thomas (23) characterized four vaginal strains which were large (length, 5 to 6 µm) and saccharolytic. The guanine-plus-cytosine (G+C) content of one of these strains was 55 mol%. Recently Hjelm et al. (12) characterized 11 strains which fermented glucose, Holst et al. (14) studied 35 strains which were asaccharolytic, and Sprott et al. (35) examined 20 strains, including 8 short curved rods which utilized maltose, hydrolyzed hippurate, and hydrolyzed arginine and 12 long curved rods which were negative for all three of these characters.

Curved motile rod-shaped bacteria are of interest because they are associated with bacterial vaginosis (formerly known as nonspecific vaginitis) (9, 11, 24, 25, 29, 33, 34). However, previously published descriptions have been incomplete and inconsistent, and the taxonomic position of these organisms is uncertain. The purpose of this study was to characterize our strains and compare them with similar strains from other investigators, as well as with phenotypically similar species in previously described genera. The vaginal strains are distinct from previously described genera, and we propose that they be placed in two species in the genus *Mobiluncus* gen. nov.

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Meeting of the American Society for Microbiology, New Orleans, La., 1983.)

MATERIALS AND METHODS

Sources and maintenance of strains. We studied 22 strains of vaginal curved rod-shaped bacteria from 22 women who had clinical diagnoses of bacterial vaginosis, as determined by standard criteria (1). The curved motile rod-shaped organisms with strain designations beginning with BV (bacterial vaginosis) and SV (study of vaginosis) were isolated from vaginal fluids that were collected by methods described previously (32). The strains were isolated on brain heart infusion medium (Difco Laboratories, Detroit, Mich.) supplemented with 2% agar, 5% sheep erythrocytes, and 2% proteose peptone no. 3 or on brucella agar (Difco) supplemented with 5% sheep erythrocytes. The strains with designations beginning with BV were isolated by one of us (C.A.S.) from women in Seattle, Wash., and have been described briefly elsewhere (34). The strains with designations beginning with SV were provided by S. M. Finegold and Ellen Baron, Wadsworth Veterans Administration Hospital, Los Angeles, Calif. The remaining three strains were provided by Peter Piot, Institute for Tropical Medicine, Antwerp, Belgium. The sources of the other organisms studied were as follows: *Wolinella recta* FDC 285, *Wolinella* sp. strain FDC 286, *Bacteroides gracilis* FDC 401, and *Campylobacter concisus* FDC 484^T (T = type strain) were from Anne C. R. Tanner, Forsyth Dental Center, Boston, Mass.; strain VSMC, a *Wolinella succinogenes*-like organism of human origin, was from Marie B. Coyle, University of Washington, Seattle; *Succinivibrio dextrinosolvens* Bryant 24 was from M. P. Bryant, University of Illinois, Urbana; *Eubacterium lentum* A238-5 was isolated by one of us (C.A.S.) from a perforated appendix; and *Actinomyces odontolyticus* ATCC 17929^T, *Actinomyces bovis* ATCC 13683^T, *Anaerovibrio lipolytica* ATCC 33276^T, *Anaerobiospirillum succiniciproducens* ATCC 29305^T, *Campylobacter*

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fetus subsp. *venerealis* ATCC 19438^T, and *Selenomonas sputigena* ATCC 33150^T were obtained from the American Type Culture Collection, Rockville, Md.

The strains were stored in double-strength skim milk at -20 or -70°C and were used within five passages of recovery from frozen stocks for each experiment.

Biochemical characterization. Media were inoculated and incubated in an anaerobic environment containing 80% nitrogen, 10% carbon dioxide, and 10% hydrogen. The cultures were incubated at 35°C for 5 days unless indicated otherwise. All media except starch broth, McClung-Toabe agar, and brucella blood agar were supplemented with 2% rabbit serum (GIBCO Laboratories, Grand Island, N.Y.) unless indicated otherwise. For some experiments broth media were supplemented with 1.8 mg of formate per ml and 1.8 mg of fumarate per ml (13) instead of rabbit serum.

Fermentation reactions, meat digestion, nitrate reduction, esculin and gelatin hydrolysis, and H₂S production were determined in prereduced anaerobically sterilized media (PRAS II media; Scott Laboratories, Fiskeville, R.I.). Fermentation reactions were tested in peptone-yeast extract (PY) broth supplemented with the appropriate carbon sources (13). Production of lipase and lecithinase was determined on McClung-Toabe agar (Regional Media Laboratories, Lenexa, Kans.). Production of ammonia, lipase, lecithinase, indole, H₂S, and catalase, hydrolysis of gelatin and esculin, and digestion of meat were determined by the methods described in the *Anaerobe Laboratory Manual* (13). H₂S production was determined in SIM medium (PRAS II medium) by using lead acetate strips (BBL Microbiology Systems, Cockeysville, Md.). Hippurate hydrolysis was determined by gas-liquid chromatography (17) after growth in PY broth supplemented with 5 mg of hippurate (Sigma Chemical Co., St. Louis, Mo.) per ml instead of rabbit serum.

Columbia broth (Difco) supplemented with 1% soluble starch (Difco), 10 µg of resazurin per/ml, and 2% rabbit serum was designated CSS broth. CSS broth was further supplemented with 1.5% agar (CSS agar) or 0.25% agar (CSS soft agar) for some experiments. Growth was evaluated in anaerobic, microaerophilic, capnophilic, and aerobic environments at 35°C and in an anaerobic environment at 25, 42, and 45°C on CSS agar. Plates to be incubated anaerobically were placed in GasPak jars (BBL) before removal from the chamber. A microaerophilic environment was obtained by evacuating a GasPak jar to a pressure of 20 lb/in² and refilling it twice with nitrogen and once with 80% nitrogen-10% hydrogen-10% carbon dioxide in the absence of catalyst. A capnophilic environment was created in sealed Mylar bags filled with 10% CO₂ in air. Aerobic plates were incubated in Mylar bags filled with air.

Synergistic hemolysis was determined by a modification of the CAMP test (8), using brucella agar supplemented with 5% sheep blood (Regional Media Labs) and *Staphylococcus aureus* ATCC 25923. Brucella agar (Difco) was supplemented with horse blood or rabbit blood (GIBCO).

Enzyme production was evaluated by using API ZYM test strips (Analytab Products, Plainview, N.Y.). Cultures were grown for 4 days at 35 or 42°C in CSS broth. The cells were harvested by centrifugation, washed twice with sterile saline, resuspended in saline, and adjusted to an optical density at 620 nm of 0.40 ± 0.01. Two drops of this suspension were added to each cupule, and the API ZYM strips were incubated for 4 h at 35°C. Reagents were added, and the reactions were interpreted according to the instructions of the manufacturer.

Motility. Broth cultures that were 3 h old were examined for motile cells by phase-contrast microscopy. Strains were also tested for migration through CSS soft agar which was dispensed into tubes (12 by 100 mm) and stored in the anaerobic chamber until the resazurin indicator became colorless. Several colonies of each strain were inoculated into the top 5 to 10 mm of medium. After 6 days of incubation, the distance from the meniscus of the agar to the advancing front of bacterial cells was measured. Migration through soft agar was interpreted as follows: negative, <15 mm; intermediate, 15 to 40 mm; positive, >40 mm.

Flagellum stains were prepared by the Ryu method (16).

Gas-liquid chromatography. After incubation for 5 days, culture samples were prepared for gas-liquid chromatography by methods adapted from methods described in the *Anaerobe Laboratory Manual* (13); these samples were analyzed on a chromatograph equipped with a flame ionization detector. The presence of less than 1 meq of acid per 100 ml was considered minor acid production, and the presence of more than 1 meq of acid per 100 ml was considered major acid production.

Gas-liquid chromatography was performed on samples of sham-inoculated media to determine the background levels of acetic, lactic, and succinic acids. If the concentration of any of these acids exceeded the background level by 20%, this was regarded as acid production.

Electron microscopy. The organisms were grown in CSS broth for 72 h. To prepare negative stains, 1 ml of a cell suspension was mixed with an equal volume of cold 1% glutaraldehyde in cacodylate buffer. Grids were prepared and stained with uranyl acetate. The remainder of the cells were harvested by centrifugation. Each cell pellet was fixed as described above, postfixed in 1% osmium tetroxide, and embedded in a mixture of Epon and Araldyte. Sections were stained with lead citrate. The grids were examined with a Philips model EM 400 electron microscope.

DNA purification. To prepare deoxyribonucleic acid (DNA), each strain was grown in 500 to 1,000 ml of broth for 5 days at 35°C in an anaerobic environment. The curved vaginal rod-shaped bacteria were grown in CSS broth supplemented with 0.04% cysteine hydrochloride. Starch and rabbit serum were replaced by 0.2% sodium formate and 0.3% fumaric acid for growth of *Campylobacter*, *Wolinella*, and *B. gracilis*, by 0.3% arginine hydrochloride for growth of *Eubacterium lentum*, and by 1% glucose for growth of all other organisms. The pH of each medium was adjusted to 7.0 ± 0.2. The bacterial cells were harvested by centrifugation at 4,810 × g for 10 min at 4°C. The resulting cell pellets were frozen at either -70 or -20°C until they were needed.

The DNA extraction methods of So et al. (31) and P. A. Totten (personal communication) were modified for our use. Approximately one-quarter of a cell pellet was suspended in 40 ml of TE [0.15 M tris(hydroxymethyl)aminomethane, 0.1 M ethylenediaminetetraacetic acid, pH 8.0], 40 mg of lysozyme was added, and the mixture was incubated at 37°C for 1 h. Then 10 mg of pronase (Sigma) was added, and the mixture was incubated at 55°C for 30 min. Sodium dodecyl sulfate was added to a final concentration of 0.4%, and the mixture was heated at 55°C until the cells were lysed (1 to 2 h). The solution was diluted 1:1 with TE, 2 mg of proteinase K (Sigma) was added, and the mixture was incubated overnight at 37°C. The suspension was mixed with an equal volume of tris(hydroxymethyl)aminomethane-saturated phenol (pH 8.0) and centrifuged at 2,400 × g for 5 min at 4°C. The aqueous phase was mixed with an equal volume of chloroform and centrifuged at 2,400 × g for 5 min at 4°C. The

aqueous phase was reextracted with chloroform. The DNA was precipitated with 0.3 M sodium acetate and cold ethanol and collected by centrifugation at $14,000 \times g$ for 20 min at 4°C. The pellet was suspended in $0.1 \times$ SSC (0.015 M NaCl plus 0.0015 M sodium citrate), and NaCl, ethylenediamine-tetraacetic acid, and tris(hydroxymethyl)aminomethane were added to final concentrations of 0.1, 0.05, and 0.025 mM, respectively. Boiled ribonuclease (500 mg; Sigma) was added, and the mixture was incubated at 60°C for 1 h. The DNA was extracted twice with chloroform-isoamyl alcohol (24:1) and precipitated with sodium acetate and cold ethanol. The DNA was suspended in $0.1 \times$ SSC and dialyzed against 0.42 M NaCl or other buffers, as described below. For hybridization, the DNA was sheared by sonication (4).

DNA-DNA hybridization. DNA was isolated from each of the 35 strains. The DNAs from four strains were labeled by nick translation, using 0.5 μ g of DNA and [3 H]thymine 5'-triphosphate, as described by Maniatis et al. (20); these DNAs were used as probes. The labeled DNAs were diluted to concentrations of 5,000 cpm/10 μ l. Test DNA (15 μ g) and 10 μ l of radioactive probe DNA were mixed, denatured in a total volume of 300 μ l, and then allowed to hybridize overnight at 65°C. The amount of reassociation between the probe DNA and the test DNA was assayed with S1 endonuclease as previously described (4). The levels of reassociation of unlabeled homologous DNA and unlabeled heterologous DNA (calf thymus DNA) with each labeled probe DNA served as controls in each experiment. The heterologous control measured the amount of self-annealing of the probe ($\leq 11\%$), and this value was subtracted from all other values. The homologous control always had $\geq 77\%$ of the total probe activity. Test results were normalized to 100% for the homologous DNAs.

Determination of thermal melting profiles. DNAs from the four vaginal curved rod-shaped organisms used as probes and an *Escherichia coli* control (G+C content, 51 mol%) were dialyzed against 4 liters of $0.5 \times$ SSC (0.075 M NaCl plus 0.0075 M sodium citrate, pH 7.0). The melting profiles were determined as described by Johnson (15), using a Gilford model 250 automatic recording spectrophotometer equipped with a heated cuvette holder. *Escherichia coli* was used as an internal standard for each experiment. Analyses were done in triplicate over a 1-week period.

Decarboxylase and dihydrolase activity. Cultures were grown for 4 days in peptone-starch-dextrose broth supplemented with 0.1% arginine hydrochloride and in peptone-starch-dextrose broth supplemented with 0.1% lysine hydrochloride and 0.1% ornithine hydrochloride (3). The media were adjusted to pH 6.0 before autoclaving and were supplemented with 2% rabbit serum after autoclaving. The spent-culture media were filter-sterilized, and the cell-free supernatants were tested for putrescine, cadaverine, ornithine, and citrulline by using the thin-layer chromatographic methods of Chen et al. (3).

RESULTS AND DISCUSSION

All of the vaginal strains were curved, motile, anaerobic, rod-shaped organisms. These strains were either small (mean cell length, 1.7 μ m) or large (mean cell length, 2.9 μ m). No spores were detected, as determined by ethanol resistance of cultures (18) or by staining of cultures after 14 to 16 days of growth on chopped meat agar slants incubated anaerobically at 30°C. The strains were weakly (pH 5.5 to 6.5) or strongly (pH < 5.5) saccharolytic. The fermentation products included succinic and acetic acids, with or without lactic acid. All strains grew at 35 and 42°C but not at 45°C.

Some strains survived incubation at 25°C. These organisms grew at pH 6.0 to 7.2 and varied in their ability to grow at pH 5.0 and 7.9. They grew in the presence of 1% NaCl, but were partially or totally inhibited by 2% NaCl. When stained by the Kopeloff modification of the Gram stain (13), the strains of the small cell type were gram variable. Young cultures were more likely to appear to be gram positive. The strains of the large cell type were gram negative. Multiple lateral flagella were observed after flagellum staining of all 22 strains. Clusters of two to six flagella which were longer than the bacterial cells were observed in electron micrographs (Fig. 1A). A subterminal attachment site was sometimes observed (Fig. 1B).

Although the Gram reactions of the curved rod-shaped organisms were variable, electron micrographs of both groups revealed multilayered gram-positive cell walls lacking an outer membrane (Fig. 1C and D). Similar multilayered walls have been described for both gram-negative (21) and gram-positive (30) organisms. *Gardnerella vaginalis*, another gram-variable organism from human vaginae, also has a multilayered wall (10). The thinness of the peptidoglycan layer may explain the tendency of the curved rod-shaped organisms to stain gram negative. Cytoplasmic, spherical, electron-dense inclusions which may represent storage granules (27) or accumulations of phage heads were observed in all three strains examined (data not shown).

The apparent absence of an outer membrane suggests that the curved rod-shaped organisms more closely resemble gram-positive organisms than gram-negative organisms. This conclusion is supported by reports that these organisms are resistant to colistin and nalidixic acid (7) and are susceptible to penicillin (35) and vancomycin (unpublished data). Hydroxy fatty acids, which are commonly found in gram-negative cell walls, are absent from these organisms (28).

Although we originally described the curved rod-shaped bacteria of vaginal origin as asaccharolytic, we now have shown that these strains can reduce the pH values of media supplemented with rabbit serum. Growth in PY broth was improved by adding glycogen or maltose, carbohydrates which are found in human vaginae. More acetic acid and succinic acid were produced in PY broth supplemented with rabbit serum and glycogen than in PY broth supplemented with rabbit serum and glucose or in PY broth supplemented with rabbit serum alone. None of the strains was stimulated by formate-fumarate. When they were grown in the presence of formate-fumarate without rabbit serum, all strains produced a nonvolatile peak corresponding to methylmalic acid, which eluted at about 4.5 times the R_f of methylsuccinate. The identity of this compound was verified by mass spectrometry, and its production was not enhanced by rabbit serum.

The vaginal curved rod-shaped bacteria are different from members of the family *Propionibacteriaceae*. Neither *Propionibacterium* spp. nor *Eubacterium* spp. produce succinic acid or have polar flagella, and *Propionibacterium* spp. have G+C contents of 59 to 66 mol%. Previously described genera that produce succinate can also be differentiated from the vaginal curved rod-shaped organisms, as follows: *Actinomyces* spp. are nonmotile and have G+C contents of 60 to 63 mol%; *Anaerobiospirillum succiniciproducens* is a large (length 20- μ m) spiral organism with a G+C content of 44 mol%; *Bacteroides* spp. are either nonmotile or have peritrichous flagella; *Campylobacter* spp. are asaccharolytic, oxidase positive, have single polar flagella, and have G+C contents of 30 to 35 mol%; *Succinimonas amyolytica* cells are not curved and have single polar flagella; *Succinivibrio*

FIG. 1. Electron micrographs of *Mobiluncus* spp. (A) Negatively stained *M. curtisii* subsp. *curtisii* BV345-16^T cell showing flagella. The flagella appear to originate subterminally (at the right). The blebs on the cell surface are artifacts of fixation but may have occurred because of the thin peptidoglycan layer (see below). Bar = 1 μ m. (B) Negatively stained *M. curtisii* subsp. *holmesii* BV376-6^T cell showing subterminal attachment of the flagella. Bar = 1 μ m. (C) Thin section of *M. curtisii* subsp. *curtisii* BV345-16^T cells showing the multilayered wall that is not clearly typical of gram-positive cell wall structure in both longitudinal section and cross-section (arrows). Bar = 1 μ m. (D) Thin section of *M. mulieris* BV27-6 cells showing the atypical multilayered gram-positive cell wall structure. Bar = 1 μ m.

dextrinosolvens cells have single polar flagella; and *Wolinella* spp. are asaccharolytic, exhibit growth that is stimulated by formate-fumarate, have single polar flagella, and have G+C contents of 42 to 49 mol%. To our knowledge, the G+C contents of *Succinimonas amylolytica* and *Succinivibrio dextrinosolvens* have not been determined. We found less than 1% DNA homology between the 4 probes and the other 13 strains examined, including the type strains of *Actinomyces bovis*, *Actinomyces odontolyticus*, *Anaerovibrio lipolytica*, *Anaerobiospirillum succiniciproducens*, *Campylobacter fetus* subsp. *venerealis*, *Campylobacter concisus*, and *Selenomonas sputigena* (see above).

Therefore, we propose a new genus, *Mobiluncus*, for the vaginal isolates. Although the characteristics of the vaginal isolates exclude them from the *Actinomycetaceae*, the *Bacteroidaceae*, and the *Propionibacteriaceae*, they are most similar to the *Bacteroidaceae*. We tentatively place two species of the new genus *Mobiluncus* in the family *Bacteroidaceae*, which includes other genera of curved, anaerobic,

rod-shaped organisms that are gram negative, have polar flagella, and produce succinic acid. The levels of DNA homology were >75% within and $\leq$ 25% between strains of the two species (Table 1).

***Mobiluncus* gen. nov.** *Mobiluncus* (mo.bi.lun'cus. L. adj. *mobilis* capable of movement, active; L. masc. n. *uncus* a hook; N.L. masc. n. *Mobiluncus* a motile curved rod) cells are anaerobic, gram variable or gram negative, curved, nonsporing, and rod shaped with tapered ends, occurring singly or in pairs with a gullwing appearance. They are motile by means of multiple subpolar flagella and possess a multilayered gram-positive type of cell wall.

Weakly (terminal pH, 5.5 to 6.5) or strongly (terminal pH, <5.5) saccharolytic. Fermentation products include succinic and acetic acids, with or without lactic acid. Growth is stimulated by rabbit serum. Growth is not stimulated by formate-fumarate. Oxidase and catalase are not produced.

The described species are found in human vaginae associated with bacterial vaginosis (nonspecific vaginitis).

TABLE 1. Relative levels of DNA sequence homology among *M. curtisii* and *M. mulieris* strains

Strain	% Homology with DNA from:			
	<i>M. curtisii</i> subsp. <i>curtisii</i> BV345-16 ^T	<i>M. curtisii</i> subsp. <i>holmesii</i> BV376-6 ^T	<i>M. mulieris</i> SV17J ^T	<i>M. mulieris</i> BV64-5
<i>M. curtisii</i> subsp. <i>curtisii</i>				
BV13-6	96	86	22	19
BV67-5	100	95	22	18
BV105-4	97	95	15	20
BV345-16 ^T	100	90	22	22
BV416-10	99	91	12	13
BV424-9	101	90	17	15
<i>M. curtisii</i> subsp. <i>holmesii</i>				
BV306-7	84	100	9	16
BV376-6 ^T	88	100	19	11
SV34N	85	102	18	21
SV40Q	82	95	14	14
SV51R	92	102	19	10
<i>M. mulieris</i>				
SV17J ^T	24	17	100	94
SV58S	22	17	89	79
2471	23	25	75	87
2472	22	9	83	100
2473	19	15	87	94
BV27-6	23	21	76	89
BV64-5	21	22	85	100
SV45E2	25	23	83	96
SV52T	25	13	83	88
BV26-9	12	12	88	81

The G+C content ranges from 49 to 52 mol%, as determined by the thermal melting method.

The type species is *Mobiluncus curtisii*.

***Mobiluncus curtisii* subsp. *curtisii* sp. nov.** *Mobiluncus curtisii* subsp. *curtisii* (cur.'ti'si.i. N.L. gen. n. *curtisii* named after A. H. Curtis, who isolated the first strain) cells are anaerobic, gram variable, curved, nonsporing, rod shaped, and motile by means of multiple subterminal flagella. The cells are <0.5 μ m wide and have a mean length of 1.7 μ m (Fig. 2A). Cells migrate through soft (0.25%) agar. Colonies are colorless, translucent, smooth, convex, and entire and reach a maximum diameter of 2 to 3 mm after 5 days of incubation.

Growth is stimulated by arginine. Starch and hippurate are hydrolyzed in the absence of serum. Citrulline, ornithine, and ammonia are produced from arginine. Weakly (pH 5.5 to 6.5) to strongly (pH <5.5) saccharolytic (Table 2). Cultures are more turbid in PY broth supplemented with rabbit serum and glycogen or maltose than in PY broth supplemented with glucose and rabbit serum or rabbit serum alone. Other biochemical characteristics are shown in Table 2. The fermentation products from glycogen include succinate (>1 meq/100 ml), trace amounts of fumarate (<1 meq/100 ml), and moderate amounts of acetate (<1 meq/100 ml).

Alpha-glucosidase (10 to 30 nmol of product), phosphoamidase ($\leq$ 5 nmol), and leucine aminopeptidase (5 to 10 nmol) are produced. Esterase lipase (<5 nmol) is produced by five of six strains, acid phosphatase is produced by one strain, α -galactosidase is produced by two strains, and alkaline phosphatase is produced by one strain. Butyrate esterase, lipase, valine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin, β -glucuronidase, β -glucosi-

dase, *N*-acetyl- β -glucosamidase, α -mannosidase, and α -fucosidase are not produced at 35 or 42°C.

Nitrate is not reduced to nitrite. A weak CAMP reaction (Fig. 3) is produced on brucella agar supplemented with 5% sheep blood when *Staphylococcus aureus* ATCC 25923 is used.

The G+C content of the DNA is 51 to 52 mol%, as determined by the thermal melting method.

Isolated from human vaginae; associated with bacterial vaginosis.

The type strain is strain BV345-16 (= ATCC 35241).

***Mobiluncus curtisii* subsp. *holmesii* subsp. nov.** The characteristics and sources of *Mobiluncus curtisii* subsp. *holmesii* (holme'si.i. N.L. gen. n. *holmesii* named after K. K. Holmes, a researcher of sexually transmitted diseases) are similar to those of *M. curtisii* subsp. *curtisii* except as follows. Cells do not migrate through soft (0.25%) agar. Weakly saccharolytic (pH 5.5 to 6.5) (Table 2). The fermentation products from glycogen in the presence of serum include trace amounts of pyruvate (<1 meq/100 ml). Trace amounts of oxaloacetate (<1 meq/100 ml) are produced by five of six strains.

Esterase lipase ($\leq$ 5 nmol) is produced by all strains. β -Galactosidase and α -galactosidase are produced by two of six strains, and alkaline phosphatase is produced by one of six strains.

Nitrate is reduced to nitrite.

The type strain is strain BV376-6 (= ATCC 35242).

***Mobiluncus mulieris* sp. nov.** *Mobiluncus mulieris* (mulie'ris. L. gen. n. *mulieris* of a woman; from "*Vibrio mulieris*," which was suggested by Prévot [26]) cells are anaerobic, gram negative, curved, nonsporing, rod shaped, and motile by means of multiple subterminal flagella. Migration through soft (0.25%) agar is variable. The cells are <0.5 μ m wide and have a mean length of 2.9 μ m (Fig. 2B). Colonies are colorless, translucent, smooth, convex, and entire and reach a maximum diameter of 2 to 3 mm after 5 days of incubation.

Growth is not stimulated by arginine. Hippurate is not hydrolyzed. Citrulline, ornithine, and ammonia are not produced from arginine. Starch is hydrolyzed in the absence of serum. Strongly saccharolytic (pH <5.5). Cultures are more turbid in PY broth supplemented with rabbit serum and glycogen or maltose than in PY broth supplemented with rabbit serum and glucose or in PY broth supplemented with rabbit serum alone. Other biochemical characteristics are shown in Table 2. The fermentation products from glycogen include succinate (>1 meq/100 ml), acetate (>1 meq/100 ml), and trace amounts of fumarate (<1 meq/100 ml); 8 of 10

FIG. 2. Gram stains of *Mobiluncus* spp. The cells were <0.5 μ m wide. (A) Gram-variable cells of *M. curtisii*. The mean cell length was 1.7 μ m. Bar = 20 μ m. (B) Gram-negative cells of *M. mulieris*. The mean cell length was 2.9 μ m.

TABLE 2. Phenotypic characteristics of 22 strains of *Mobiluncus* spp.^a

Characteristic	<i>M. curtisi</i> subsp. <i>curtisi</i> strains						<i>M. curtisi</i> subsp. <i>holmesii</i> strains						<i>M. multieris</i> strains										
	BV 13-6	BV 67-5	BV 105-4	BV 345-16 ^f	BV 416-10	BV 424-9	BV 306-7	BV 319-5	BV 376-6 ^f	SV34N	SV40Q	SV51R	SV1J ^T	SV58S	2471	2472	2473	BV 27-6	BV 64-5	SV45E2	SV52T	BV 26-9	
Fermentation of:																							
Arabinose	- ^b	-	-	-	-	-	-	-	-	-	-	-	vw	vw	-	-	-	-	-	-	vw	vw	-
Fructose	w	vw	vw	-	-	vw	-	vw	-	vw	vw	vw	ac	ac	vw	vw	vw	ac	-	vw	w	w	-
Galactose	w	ac	-	ac	-	vw	-	vw	-	vw	-	vw	vw	vw	-	-	vw	-	vw	vw	vw	vw	-
Glucose	ac	w	w	w	vw	w	-	vw	-	-	-	w	ac	ac	ac	w	ac	vw	w	-	-	-	w
Glycogen	vw	vw	vw	vw	vw	vw	vw	vw	-	vw	vw	w	ac	ac	ac	ac	ac	ac	ac	ac	ac	ac	w
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	ac	ac	ac	ac	ac	-	ac	ac	-	vw	
Lactose	vw	-	vw	-	-	-	-	-	-	-	vw	vw	ac	-	-	-	-	-	vw	-	-	-	-
Maltose	ac	w	w	w	w	w	-	vw	vw	vw	vw	vw	ac	ac	ac	ac	ac	ac	ac	ac	ac	ac	ac
Mannose	vw	-	-	-	-	-	-	-	-	-	-	-	vw	vw	vw	vw	vw	-	-	-	-	-	-
Melibiose	ac	ac	w	ac	-	ac	-	w	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	vw	vw	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	vw	vw	-	-	-	-	-	-	-	-	-
Ribose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	vw	w	vw	w	w	-	-	w	-
Starch	-	-	-	-	-	-	-	-	-	-	vw	vw	w	ac	ac	w	ac	-	-	w	w	-	-
Sucrose	-	-	-	-	-	-	-	-	-	-	-	-	ac	ac	ac	ac	ac	-	-	-	-	-	-
Trehalose	-	-	-	-	-	-	-	-	-	-	-	-	ac	ac	ac	ac	ac	-	-	-	-	-	-
Xylose	w	ac	ac	vw	vw	w	vw	w	-	vw	vw	vw	ac	w	ac	ac	ac	vw	ac	w	w	w	ac
pH in PY broth	7.4	7.4	7.3	7.4	7.4	7.4	7.4	7.4	7.4	7.5	7.4	7.4	7.2	7.1	6.9	6.9	7.1	7.0	7.2	7.1	7.1	7.1	6.9
Nitrate	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+
Arginine stimulation	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Hippurate hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
NH ₄ ⁺ from ammonia	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
CAMP test	m	m	m	m	m	m	m	m	m	m	m	m	st	st	st	st	st	st	st	st	st	st	st
Migration through soft agar	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	±
GLC ^c	a,S	a,S	a,S	a,S	a,S	a,S	a,S	a,S	a,S	a,S	a,S	a,S	A,S	A,S	A,J,S	A,J,S	A,J,S	A,J,S	A,J,S	A,S	A,S	A,S	A,S

^a All of the isolates are motile and hydrolyze starch. None produces acid from adonitol, amygdalin, arginine, cellobiose, erythritol, esculin, inulin, mannitol, melezitose, salicin, or sorbitol. None hydrolyzes esculin, digests meat, forms spores, or produces gelatinase after 30 days of incubation. Lecithinase, lipase, indole, catalase, oxidase, and H₂S are not produced.

^b +, Positive; -, negative; ±, intermediate; vw, very weak acid (pH 5.5 to 6.0); ac, strong acid (pH <5.5); m, mild; st, strong.

^c Results of gas-liquid chromatography (GLC) after growth in PY broth supplemented with glycogen and 2% rabbit serum: a, <1 meq of acetate per 100 ml; i, <1 meq of lactate per 100 ml; S, >1 meq of succinate per 100 ml.

FIG. 3. CAMP reactions showing weak enhancement of hemolysis by *M. curtisii* subsp. *curtisii* (Ia) and *M. curtisii* subsp. *holmesii* (Ib) and strong enhancement of hemolysis by *M. mulieris* (II). *Staphylococcus aureus* ATCC 25923 (sa) was included as a negative control.

strains produce trace amounts of oxaloacetate (<1 meq/100 ml), 6 of 10 strains produce trace amounts of pyruvate (<1 meq/100 ml), and 5 of 10 strains produce trace amounts of lactate (<1 meq/100 ml).

Alpha-glucosidase (15 to ≥ 40 nmol of product), phosphoamidase (≤ 5 nmol), leucine aminopeptidase (<5 to 20 nmol), and esterase lipase (<5 nmol) are produced. Acid phosphatase is produced by 1 of 10 strains. Butyrate esterase, lipase, valine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosamidase, α -mannosidase, and α -fucosidase are not produced at 35 or 42°C.

Nitrate is reduced to nitrite by 1 of 10 strains. A strong CAMP reaction (Fig. 3) is produced on brucella agar supplemented with 5% sheep blood when *Staphylococcus aureus* ATCC 25923 is used.

The G+C content is 49 to 50 mol%.

The type strain is strain SV17J (= ATCC 35243).

The salient characteristics which differentiate *M. curtisii* from *M. mulieris* are shown in Table 3.

Although our strains of *M. curtisii* and *M. mulieris* appear to differ from many of the European strains described previously, these differences may only reflect variations in methods. The three Belgian strains characterized in this study were *M. mulieris* strains. We tested seven additional strains sent to us by A. Skarin. One was a *M. curtisii* subsp. *curtisii* strain, three were *M. curtisii* subsp. *holmesii* strains, and three were *M. mulieris* strains. The levels of DNA relatedness of phenotypically similar strains reported by Mårdh et al. (P.-A. Mårdh, G. Christensen, E. Holst, L. Larsson, B. R. Møller, A. Skarin, and I. Thelin, Abstr. Int.

Soc. Sex. Transm. Dis. Res. V, Seattle, Wash., 1983, Abstr. 24, p. 56) differ only within the expected analytical error. The G+C contents reported here are 3 to 4 mol% lower than the G+C content reported for a strain phenotypically similar to *M. mulieris* that was examined by Peloux and Thomas (23), who used the buoyant density method.

Our observation of arginine utilization by *M. curtisii* but not by *M. mulieris* supports the independent observations of Sprott et al. (35). Because the Eh (oxidation-reduction potential) decreases 60 U when the pH increases 1 U, production of ammonia in vivo makes the environment more anaerobic. Other organisms present in the vaginal flora which also produce ammonia from arginine include *Streptococcus* spp. (5) and the mycoplasmata (2). Holst et al. (14) noted that pure cultures of the curved rod-shaped organisms smelled of cat urine. This may be attributable to the ammonia.

Because *M. curtisii* and *M. mulieris* do not produce putrescine or cadaverine in peptone-starch-dextrose broth supplemented with lysine and ornithine, these species apparently are not the source of the amine odor associated with bacterial vaginosis.

In studies of similar vaginal organisms, Hjelm et al. (12) detected production of butyrate esterase by 10 of 11 strains and production of acid phosphatase by 7 of 11 strains. Holst et al. detected leucine aminopeptidase only in long variant (*M. mulieris*) strains and butyrate esterase in all strains (14). We detected leucine aminopeptidase in six of seven strains sent to us by Skarin, four of which were the short variant (*M. curtisii*). Differences in our results may reflect differences in growth media or concentrations of inocula.

M. mulieris but not *M. curtisii* gave a strongly positive CAMP test on brucella agar supplemented with 5% sheep blood (Fig. 3). There was minimal stimulation of hemolysis by strains of either species when the CAMP test was performed on human blood bilayer-Tween agar (36) or on brucella agar supplemented with 5 or 10% horse or rabbit blood. Peloux and Thomas demonstrated a similar phenomenon by using *Clostridium perfringens* (23) and four strains that probably were *M. mulieris* strains.

Extracellular capsular material as detected by electron microscopy was associated only with *M. curtisii* subsp. *holmesii*.

The starch, maltose, and trehalose reactions are unreliable in the presence of serum because of the hydrolytic action of

TABLE 3. Differential characteristics of *Mobiluncus* spp.

Characteristic	<i>M. curtisii</i> subsp. <i>curtisii</i>	<i>M. curtisii</i> subsp. <i>holmesii</i>	<i>M. mulieris</i>
Length of cells (μ m)	1.7	1.7	2.9
Gram reaction	Variable	Variable	Negative
Stimulated by arginine	+	+	—
NH ₄ ⁺ from arginine	+	+	—
Hippurate hydrolyzed	+	+	—
CAMP reaction	Weak	Weak	Strong
Produces >1 meq of acetate per 100 ml	—	—	+
Acid (pH <5.5) from glycogen	—	—	+
Acid (pH ≤ 6.0) from melibiose	+(—)	—(+)	—
Nitrate reduced	—	+	—(+)
Migrates through soft agar	+	—	v
Acid (pH <5.5) from trehalose	—	—	v

+ , Positive; — , negative; +(—), usually positive; —(+), usually negative; v, variable.

serum. All of the strains hydrolyzed starch broth in the absence of serum within 10 days. Starch hydrolysis was also detected as clearing of starch agar (3.5% Columbia broth, 1% proteose peptone no. 3, 0.75% glucose, 1% corn starch, and 1.5% agar) after incubation for 4 days. Starch is hydrolyzed either to maltose by amylase or to D-glucose by amyloglucosidase. Therefore, starch-hydrolyzing organisms that produce acid from glucose or maltose would be expected to produce acid from starch. The reasons why this pattern was not demonstrated by all strains of *Mobiluncus* are unknown.

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ADDENDUM IN PROOF

M. curtisii subsp. *holmesii* strain BV319-5 was examined for percent homology with the four strains of *Mobiluncus* spp. used as probes, but the data have been deleted from Table 1 because we have reason to doubt the purity of the culture. We are currently reexamining this strain.

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