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Characterisation of anaerobic curved rods (*Mobiluncus* spp.) isolated from the urogenital tract

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Summary. Thirty-two strains of anaerobic curved rods isolated from vaginal secretions and one isolated from seminal fluid were examined. Growth of all strains on solid media was superior to growth in liquid media, and at 37°C they grew both anaerobically and in O_2 5% in N_2; they also grew anaerobically at 33°C but not at 42°C. No growth factors were identified, but strains grew more profusely at *p*H values above 5·0. The strains were screened in 80 biochemical tests, and for their susceptibility to 30 different antimicrobial agents. Most of the tests did not differentiate between the strains, but they were divided into four groups on the basis of cell morphology, metronidazole susceptibility, β-galactosidase activity and arginine and hippurate hydrolysis. Group 1 consisted of 19 strains conforming to the species *M. curtisi*; group 2 consisted of five strains conforming to the species *M. mulieris*; group 3 consisted of five strains that resembled *M. curtisi* morphologically, and group 4 consisted of four strains that resembled *M. mulieris* morphologically, but the strains in the latter two groups reacted differently in at least one of the three major differential biochemical tests. Of three strains of *M. curtisi* and three of *M. mulieris* chosen at random, one of *M. mulieris* had a SDS-PAGE and fast-protein liquid chromatography protein profile indistinguishable from that of *M. curtisi*. We conclude that further efforts are required to clarify the taxonomic status of the genus *Mobiluncus*.

Introduction

There has been much interest recently in anaerobic, curved, motile gram-negative bacteria present as components of the vaginal flora, especially as carriage appears to be associated with bacterial vaginosis (Durieux and Dublanchet, 1980; Skarin and Mårdh, 1982; Sprott *et al.*, 1982, 1983, 1984; Phillips and Taylor, 1982; Blackwell *et al.*, 1983; Spiegel *et al.*, 1980, 1983). These anaerobic curved rods have been given the genus name *Mobiluncus*, and two species, *M. curtisi* (*curtisii*) and *M. mulieris* have been proposed (Spiegel and Roberts, 1984). The two species can be differentiated by cell morphology and biochemical reactions, the most useful of these—β-galactosidase activity, arginine and hippurate hydrolysis and metronidazole susceptibility—having been proposed for differentiation (Nord *et al.*, 1984). *M. curtisi* organisms, referred to as "short forms" (SF) are considered to be 1–2 μm long, gram-variable, comma-shaped, resistant to metronidazole, and give positive results in all these differential biochemical tests. In contrast, *M. mulieris* organisms, referred to as "long forms" (LF), are considered to be 3–4 μm long, gram-negative, curved, sensitive to metronidazole, and give negative results in the three biochemical reactions. However, careful analysis of the literature shows that not all strains conform to these guidelines and we refer later to typical short forms (TSF) and typical long forms (TLF) for those that do, and atypical short forms (ASF) and atypical long forms (ALF) for those that do not. An additional problem is the fastidious nature of the organisms which complicates their isolation and subsequent cultivation. The purpose of this study was to investigate extensively the biochemical reactions of *Mobiluncus* species, to validate and better define any differential characteristics, to determine their antimicrobial susceptibilities with a view to identifying agents that might prove useful in the development of a selective medium, and to try to find cultural conditions or agents that would enhance growth and so facilitate their isolation.

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Materials and methods

Bacterial strains

Strains from various geographical sources (table I) were from women with bacterial vaginosis, except strain 32.T which was isolated from the seminal fluid of an infertile male. The strains are designated in the table as "short gram-variable" and "long gram-negative" according to the presumptive descriptions provided by the senders. They were subcultured every 5–7 days on Columbia blood agar (CBA) (Difco) and incubated anaerobically at 37°C.

Media

The growth of strains was tested in peptone-yeast-glucose broth and in the following broths and on their respective agar media: Brain Heart Infusion (BHI; Difco), Brucella (Oxoid) and Wilkins-Chalgren (Oxoid). The agar media were supplemented with horse blood 5%.

Agents used to enhance growth

Arginine-free base (Sigma) 2%, sodium hippurate (Sigma) 2%, sodium salts of formate and fumarate 0·3%, bovine serum 2·5% and campylobacter growth supplement—FBP (Oxoid) were added as single supplements to BHI broth to test for growth enhancement.

Incubation conditions

The strains were seeded on to CBA and the cultures incubated at 37°C in the following atmospheres: anaerobic (N_2 80%, $H_2$10%, CO_2 10%), aerobic, CO_2 5% in air, O_2 5% in N_2 and in a candle-jar. Cultures were also incubated in anaerobic conditions at 33°C and 42°C. All plate cultures were examined daily up to 10 days for bacterial growth.

Morphology

After various periods of anaerobic incubation on CBA, the strains were examined for colony morphology. Cell morphology was observed after staining by Gram's method.

Bacterial suspensions

A bacterial suspension equivalent to McFarland's tube no. 3 (*c.* 1×10^7 cfu/ml) was prepared by harvesting the growth on two CBA plates with cotton-wool swabs and expressing them in 3 ml of BHI broth. For a suspension equivalent to McFarland's tube no. 5 (*c.* 1×10^9 cfu/ml), the growth from ten plates was expressed in 2 ml of medium.

Effect of pH on growth

Suspensions of four TSF (*M. curtisi*) (A225, 69.10, A345, 2199) and four TLF (*M. mulieris*) (A201, A226, A229, 999.V) (McFarland's tube no. 5) were diluted in a series of eight ten-fold steps. A 30-μl sample of each dilution was inoculated on to CBA, unbuffered and buffered with mono- and di-basic sodium sulphate to *p*H 7·2, after which the media were incubated anaerobically at 37°C. The size and the numbers of colonies were estimated 48h and 72h later. Furthermore, the growth of strains in BHI broth, adjusted to *p*H values over a range from 4 to 8 (half unit intervals), was assessed.

Biochemical characterisation

Tests for catalase, oxidase and β-D-galactosidase (ONPG) activity, arginine, hippurate and starch hydrolysis and nitrate reduction were performed as described by Cowan (1974). For these, bacterial suspensions equivalent to McFarland's tube no. 3 were used. All tests were repeated on three separate occasions.Five different test kits (API Laboratory Ltd), namely API-AN1, AN2, AN3 (not commercially available), 20A and ZYM, containing a total of 73 different tests, were used. Fermentation reactions were evaluated with the API-AN3 test strips for L-arabinose, arbutine, cellobiose, fructose, galactose, glucose, glycerol, lactose, mannitol, melibiose, α-methyl-D-glucoside, α-methyl-D-mannoside, D-ribose, trehalose and D-xylose, and with the API 20A kit for maltose, mannose, melezitose, raffinose, rhamnose, salicin, sorbitol and sucrose. Aesculin hydrolysis, gelatin liquefaction, indole production and urease tests were performed with API 20A and tetrathionate reduction with API-AN3. Another 45 enzyme activities were assayed with the API ZYM and the API-AN1 and AN2 test strips.

Analysis of cell proteins

The cell proteins of three TSF (*M. curtisi*) (A225, 69.10, 2199) and TLF (*M. mulieris*) (A99, A201, A229) were analysed by SDS-polyacrylamide gel electrophoresis (PAGE), as described by Baron *et al.* (1984), and by fast protein liquid chromatography (FPLC), as described recently (Borriello *et al.*, 1985, 1986).

Antimicrobial susceptibility

Susceptibility to antimicrobials was determined by the disk-diffusion method. CBA was seeded with 100 μl of bacterial suspension (equivalent to McFarland's tube no. 3) and the plate culture was incubated anaerobically at 37°C for 5 days. Susceptibility to the antiseptic Irgasan (Ciba-Geigy) at a concentration of 100 μg/ml was assayed by the agar dilution method with the same medium.

Motility

This was determined by the hanging-drop technique.

Gas liquid chromatography (GLC)

For analysis by GLC, the strains were grown in chopped-meat-carbohydrate broth (Southern Group Laboratories) for one week anaerobically. The volatile and methylated fatty acids were extracted and detected as described elsewhere (Holdeman *et al.*, 1977).

Results

Colony morphology

All strains produced colonies that were small (1–2 mm in diameter), slightly convex, entire and transparent. The colony morphology of the SF and LF of *Mobiluncus*, whether typical or atypical forms, was indistinguishable, but β-haemolysis developed around colonies of LF more often than around those of SF.

Growth on different media

The rate of development or the size of colonies of the various strains of *Mobiluncus* species was not different on any of the solid media. Growth in liquid media was always poorer and slower than on solid media. None of the potential growth factors that were added had any significant effect on growth in either liquid or solid media.

Growth in different atmospheric conditions

All strains grew anaerobically at 33°C and 37°C, but not at 42°C. No growth occurred on CBA incubated in aerobic conditions, or in a candle-jar even after repeated subculture for 4 months that might have induced aerotolerance. All strains produced very small transparent colonies after incubation for 10 days in O_2 5% in N_2.

Influence of pH

The number or size of colonies produced by the various strains of SF and LF was not different on buffered or unbuffered CBA after incubation for either 48h or 72h. Only scanty growth occurred in broth media at *p*H 4, 4·5 and 5; more profuse growth occurred at all higher *p*H values tested.

Biochemical differentiation

On the basis of morphology in Gram's stains, metronidazole susceptibility, β-galactosidase activity and arginine and hippurate hydrolysis, the strains were divided into four groups:

"Typical" short forms (TSF) were gram-variable, comma-shaped rods, 1–2 μm long. They hydrolysed arginine and hippurate, exhibited β-galactosidase activity and were metronidazole resistant. These organisms are classed as *M. curtisi.*

"Typical" long forms (TLF) were gram-negative, curved rods with tapered ends, about 3–4 μm long. They did not hydrolyse arginine or hippurate or exhibit β-galactosidase activity and were metronidazole sensitive. These organisms are classed as *M. mulieris.*

"Atypical" short forms (ASF) were the same as the TSF morphologically, but gave different reactions in one or more of the three major differential biochemical tests.

"Atypical" long forms (ALF) were the same as the TLF morphologically, but gave different reactions in metronidazole susceptibility, or in the three major differential biochemical tests, or both.

According to the criteria above, of 20 strains received as short forms (table I), 16 were TSF and four (A275, A277, A345, MT72.10) were ASF; of 13 strains received as long forms (table I), five (A99, A200, A201, A226, A229) were TLF, four (A198, A221, A227, 999.V) were ALF, three (0.63, 99.12, 2313) were TSF and one (A199) was an ASF. This yielded a total of 19 TSF, five TLF, five ASF and four ALF (table II). Of the five ASF, two (A275, A345) were negative in tests for ONPG activity, two (A199, MT72.10) did not hydrolyse hippurate and one (A277) did not hydrolyse arginine (table II). Of the four ALF, one (999.V) hydrolysed hippurate, one (A198) was resistant to metronidazole but gave positive reactions in the other three major differential tests, one (A227) was resistant to metronidazole and gave negative reactions in the three major differential tests, and one (A221) was metronidazole resistant but gave positive reactions in tests for ONPG activity and arginine hydrolysis (table II).

Fermentation reactions. The results are shown in table III. All strains fermented ribose and xylose, but none fermented arbutine, lactose, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, salicin, sorbitol or sucrose. All the LF (typical and atypical) fermented arabinose, fructose, galactose and glucose, but only some of the short forms did so. The majority (80%) of the TLF and 50% and 25%, respectively, of the ALF gave positive results in tests for α-methyl-D-glucoside and trehalose, whereas all the short forms gave negative reactions. Moreover, none of the SF fermented *a*-methyl-D-mannoside or cellobiose, but some of the LF did.

Enzymic reactions. The results are shown in table

Table I. Source of strains of *Mobiluncus* spp.

Bacterial strain*	Source
Short, gram variable	
A98, A223, A225, A228, A274, A275, A276, A277	National Collection of Type Cultures, London
69.10, 101.8, F81, F82, MT31.6, MT72.10	Department of Microbiology, St Thomas's Hospital Medical School, London
A25, A345, A408	Department of Bacteriology, University of Uppsala, Sweden
2199, 3405	Department of Medical Microbiology, University of Lund, Sweden
32.T†	St Bartholomew's Hospital, London
Long, gram negative	
A99, A198, A199‡, A200, A201, A221, A226, A227, A229	National Collection of Type Cultures, London
99.12‡	Department of Microbiology, St Thomas's Hospital Medical School, London
0.63‡	Department of Bacteriology, University of Uppsala, Sweden
999.V, 2313‡	Department of Medical Microbiology, University of Lund, Sweden

* Presumptive designation as provided by sender; subsequent classification of all strains is presented under "biochemical differentiation" in *Results* section.
† Isolated from seminal fluid.
‡ Further examination showed these to be short forms.

IV. All strains gave positive results for C_8 esterase lipase, α-glucosidase, leucine aminopeptidase and the following arylamidases: arginine, proline, alanine-phenylalanine-proline, phenylalanine-arginine and proline-arginine, whereas all strains gave negative reactions for cystine arylamidase, β-D-fucosidase, phospho-β-galactosidase, β-glucuronidase, C_{14} lipase, naphthol-AS-B1-phosphohydrolase and trypsin. For the other reactions, no difference between any of the groups was evident except for the test with α-galactosidase, in which almost all the short forms gave positive reactions and all but one of the long forms gave negative reactions.

Additional biochemical tests. All strains gave positive results in tests for nitrate and tetrathionate reduction and for starch hydrolysis, but negative results in tests for aesculin hydrolysis, catalase and

Table II. The grouping of *Mobiluncus* strains according to the biochemical tests recommended by the working group on diagnostic criteria for anaerobic curved rods (Nord *et al.*, 1984)

Group (number)	Strain no.	Gram staining reaction	Metronidazole susceptibility	β-Galactosidase (ONPG) activity	Arginine hydrolysis	Hippurate hydrolysis
Typical short forms (19)		Variable	Resistant	+	+	+
Atypical short forms (5)	A275, A345	Variable	Resistant	−	+	+
	A199, MT72.10	Variable	Resistant	+	+	−
	A277	Variable	Resistant	+	−	+
Atypical long forms (4)	999.V	Negative	Sensitive	−	−	+
	A198	Negative	Resistant	+	+	+
	A227	Negative	Resistant	−	−	−
	A221	Negative	Resistant	+	+	−
Typical long forms (5)		Negative	Sensitive	−	−	−

Table III. Fermentation reactions of strains of *Mobiluncus* spp.

Carbohydrate tested	Percentage of indicated strains* positive in fermentation test			
	TSF (19)	TLF (5)	ASF (5)	ALF (4)
D-Ribose, D-Xylose	100	100	100	100
L-Arabinose	95	100	100	100
Galactose	42	100	80	100
Fructose	53	100	40	100
Glucose	21	100	60	100
α-Methyl-D-glucoside	0	80	0	50
Trehalose	0	80	0	25
α-Methyl-D-mannoside	0	20	0	50
Cellobiose	0	20	0	25
Glycerol	0	20	0	25
Melezitose	5	20	0	0
Arbutine, lactose, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose	0	0	0	0

* Numbers in parentheses indicate numbers of strains tested.

oxidase activity, gelatin liquefaction, indole production and urease activity (table V). Furthermore, all strains showed a peculiar "corkscrew" motility.

Cell proteins

The three TSF (A225, 69.10, 2199) gave identical protein profiles by both SDS-PAGE analysis (fig. 1) and FPLC (fig. 2). Two of the three TLF strains (A201, A229) yielded profiles that were different from those of the SF strains by both SDS-PAGE and FPLC. The protein profiles of these two TLF were identical in FPLC and differed by only one major protein band on SDS-PAGE analysis. The remaining strain (A99), regarded as a TLF, had a protein profile indistinguishable from that of a TSF by both methods of analysis.

GLC profile

All strains had similar fatty-acid patterns representing production of large amounts of succinic acid and less of acetic and lactic acids.

Susceptibility to antimicrobials

The results of susceptibility tests are shown in table VI. However, details of susceptibility to metronidazole, which is an important differential characteristic, are shown in table II. It may be seen that several of the LF are atypical in being resistant to metronidazole. All strains were sensitive to the antiseptic Irgasan, bile salts and thallous acetate, but resistant to the three dyes tested (table VI).

Discussion

The major problem encountered in attempting to elucidate the pathogenic role of the *Mobiluncus* species in bacterial vaginosis is the difficulty of isolating, culturing and recognising the organisms. They are fastidious and the development on primary isolation of only small colonies makes them difficult to recognise in cultures of vaginal specimens. This is in keeping with the observations of others that the isolation of these organisms is tedious (Durieux and Dublanchet, 1980), several weeks sometimes being required to obtain a pure culture (Thomason *et al.*, 1984). We tried to determine whether there were particular cultural conditions or agents that would enhance growth but no differences were observed in the growth of the strains on various solid and liquid media, although it was clear that they preferred growth on the former and only under anaerobic conditions or in O_2 5% in N_2. These findings are in agreement with those of other workers (Sprott *et al.* 1984) but differ in part from those reported by Holst *et al.* (1982) who found that only the metronidazole-resistant SF grew in microaerophilic conditions. We did not find any correlation between metronidazole-resistant strains and their tolerance of oxygen, metronidazole-sensitive strains growing equally as well as the resistant ones in O_2 5%. We found no improvement in growth of

Table IV. Enzyme profiles of strains of *Mobiluncus* spp.

Enzyme tested	Percentage of indicated strains* reactive in test			
	TSF (19)	TLF (5)	ASF (5)	ALF (4)
C_8 esterase lipase α-glucosidase Arginine arylamidase Leucine aminopeptidase Proline arylamidase Alanine-phenylalanine-proline arylamidase Phenylalanine-arginine arylamidase Proline-arginine arylamidase	100	100	100	100
Hydroxyproline arylamidase	95	100	100	100
Esterase C_4, leucine arylamidase	89	100	100	100
Lysylalanine arylamidase	89	100	100	100
Aspartic arylamidase	95	80	100	100
Ornithine arylamidase	95	80	100	100
Phenylalanine arylamidase	100	100	100	75
Serine arylamidase	95	80	100	100
Histidyl-phenylalanine arylamidase	84	100	100	75
Leucyl-glycine-arylamidase	100	80	100	75
Glutamine arylamidase	68	100	80	100
Histidine arylamidase	89	80	100	75
β-galactosidase (ONPG)	100	0	60	50
α-galactosidase	89	0	80	25
Acid phosphatase	37	60	40	50
Glutamyl-histidine arylamidase	26	20	40	50
Lipase C_{10}	16	20	40	25
Valine arylamidase	14	33	0	0
α-fucosidase	0	20	0	25
α-mannosidase	16	0	20	0
α-glutamyl-transpeptidase	10	0	20	0
Alkaline phosphatase	0	0	20	0
β-glucuronidase	0	0	20	0
Glutamyl-glutamic arylamidase	16	0	0	0
N-Acetyl-β-glucosaminidase	10	0	0	0
β-glucosidase, pyrrolidonic arylamidase	10	0	0	0
L-arabinosidase, β-mannosidase	5	0	0	0
Cystine arylamidase β-D-fucosidase Phospho-β-galactosidase β-glucuronidase C_{14} lipase Naphthol-AS-Bl-phosphohydrolase Trypsin, chymotrypsin	0	0	0	0

* Numbers in parentheses indicate numbers of strains tested.

the organisms at a particular *p*H value or on buffered agar, but noticed that they were tolerant of *p*H 8, the maximum value tested, and that their growth was sparse at *p*H values < 6. These findings might explain the reported low isolation rate of *Mobiluncus* species from the vaginas of healthy women (Spiegel *et al.*, 1980, 1983; Skarin and Mårdh, 1982; Blackwell *et al.*, 1983) because the normal vaginal environment has a *p*H value of 4·5 or less. The increased vaginal *p*H in bacterial vaginosis, probably due to diminished numbers of lactobacilli, may facilitate the growth of organisms of the *Mobiluncus* species.

Organisms within the genus *Mobiluncus* are divided into two species on the basis of their cell morphology, reaction in Gram's stain, metronidazole susceptibility, arginine and hippurate hydrolysis, and β-galactosidase reactions (Nord *et al.*, 1984). SDS-PAGE, DNA-DNA hybridisation and serological studies (Baron *et al.*, 1984; Moi and

Table V. Biochemical reactions of strains of *Mobiluncus* spp.

Activity tested	Percentage of indicated strains* positive in test			
	TSF (19)	TLF (5)	ASF (5)	ALF (4)
Nitrate and tetrathionate reduction	100	100	100	100
Starch hydrolysis	100	100	100	100
Arginine hydrolysis	100	0	80	75
Hippurate hydrolysis	100	0	60	50
β-galactosidase (ONPG)	100	0	60	50
Aesculin hydrolysis, catalase, oxidase, gelatin liquefaction, indole production, urease	0	0	0	0

* Numbers in parentheses indicate numbers of strains tested.

Danielsson, 1984; Taylor and Owen, 1984) also differentiate these organisms into two definite species. However, we found that four of the 13 strains received as LF were SF and, furthermore, not all the strains conformed to the proposed criteria for *M. curtisi* (our TSF) or *M. mulieris* (our TLF). Our findings were repeatable in tests undertaken on at least three different occasions and are in agreement with those of Påhlson *et al.* (1984) who found four of 15 SF to be atypical in that they did not hydrolyse hippurate or have ONPG activity and did not react with antisera to *M. curtisi* or *M.*

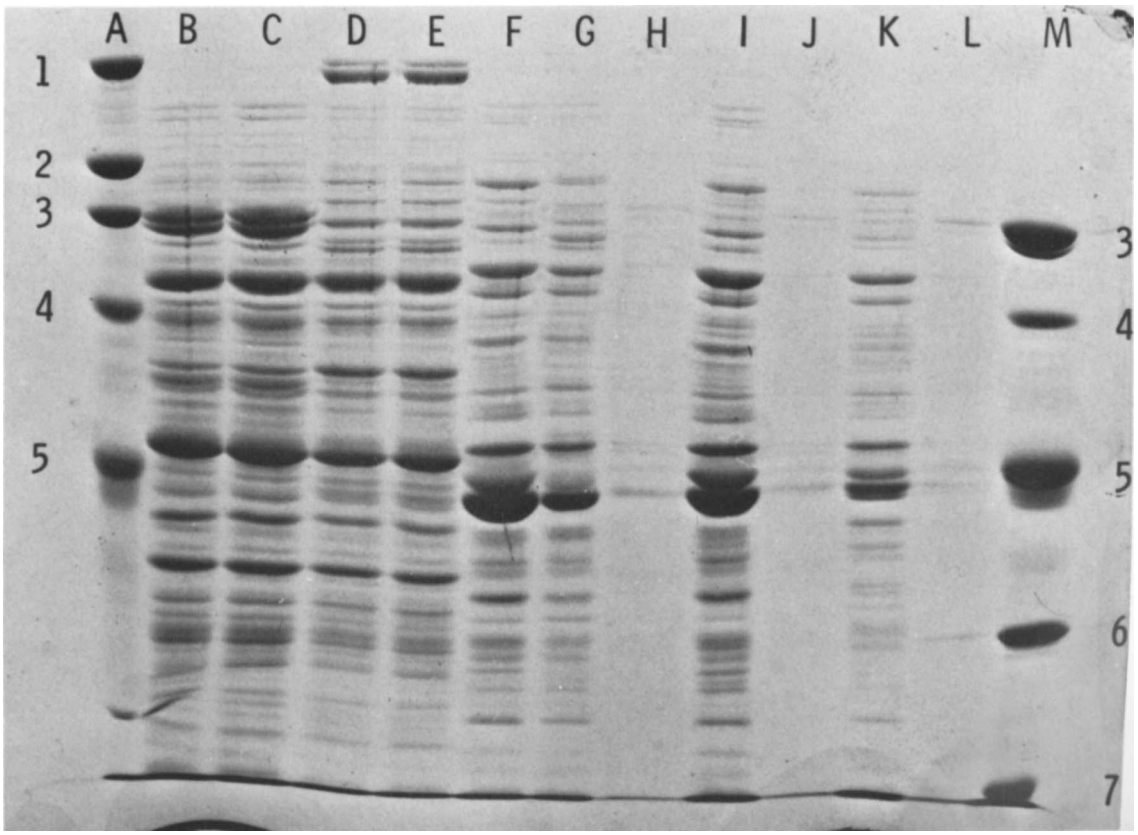

Fig. 1. SDS-PAGE patterns of major cell proteins of *Mobiluncus* spp. Tracks A and M contain protein standards of known mol. wts (10^3): 1—200, 2—116·25, 3—92·5, 4—66·2, 5—45, 6—31, 7—21. Tracks B and C refer to strain A201 (TLF), tracks D and E to strain A229 (TFL), track F to strain 69.10 (TSF), track G to strain A99 (TLF showing a TSF profile); tracks H, J and L are blank, track I refers to strain A225 (TSF) and track K to strain 2199 (TSF).

Table VI. Antimicrobial susceptibility of strains of *Mobiluncus* spp.

Antimicrobial to which strains were sensitive or resistant at indicated quantity (disk)			
Sensitive			
Bacitracin	10 I.U.	Streptomycin	5 μg
Cefoxitin	30 μg	Teicoplanin	30 μg
Clindamycin	2 μg	Tetracycline	30 μg
Ciprofloxacin	5 μg	Vancomycin	5 μg
Erythromycin	5 μg	Nitrofurantoin	100 μg
Gentamicin	10 μg	Thallous acetate	500 μg
Kanamycin	30 μg	Sodium taurocholate	2·5 μg
Minocycline	10 μg	Sodium deoxycholate	0·5 μg
Penicillin	1·5 μg	Irgasan	100 μg/ml*
Resistant			
Aztreonam	50 μg	Fusidic acid	10 μg
Colistin sulphate	10 μg	Neomicin	10μg
Nalidixic acid	10 μg	Brilliant green	0·001 μg
Polymixin B	300 μg	Methyl violet	0·001 μg
Sulphafurazole	100 μg	Victoria blue	0·001 μg
Sulphamethoxazole	2·5 μg		
Trimethoprim	1100 μg		

* Concentration in agar.

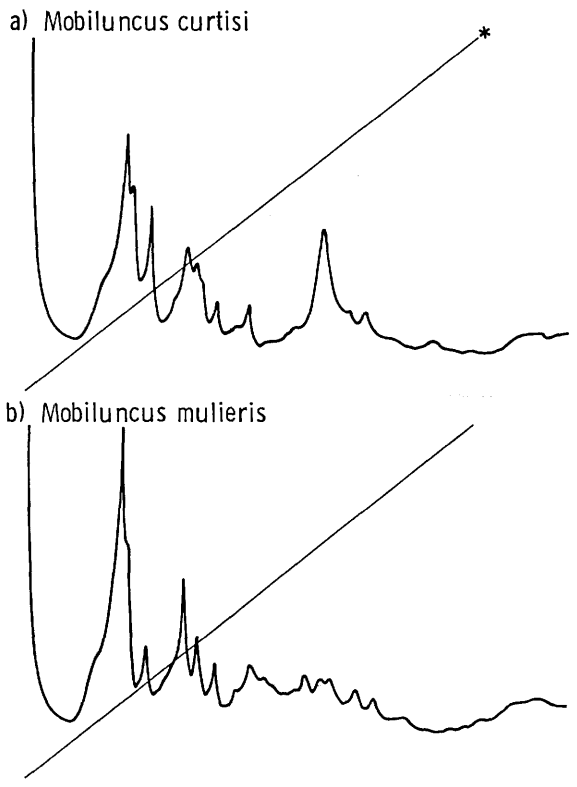

Fig. 2. Fast-protein liquid chromatography profile of cell proteins of *Mobiluncus* spp. a. Profile of strains 69.10 (TSF), A225 (TSF), 2199 (TSF) and A99 (TLF showing a TSF profile). b. Profile of strains A201 (TLF) and A229 (TLF). *Sodium chloride gradient 0–100% M NaCl.

mulieris. These authors did not find any ALF, but Fox and Phillips (1984) reported that two of the 14 LF they studied hydrolysed hippurate and one hydrolysed arginine.

The possibility that the genus *Mobiluncus* could be divided into more than two species has been raised by several workers. Holst *et al.* (1982) grouped these organisms into three types, short, medium and long rods (considering the medium form as a subspecies of the long form) on the basis of cell morphology. DNA-DNA hybridisation studies (Christiansen *et al.*, 1984) supported a subspecies of the SF, the LF yielding a more homogeneous group. Spiegel and Roberts (1984) also proposed the division of *M. curtisi* into two subspecies (*M. curtisi* ssp. *curtisi* and *M. curtisi* ssp. *holmesi*), but discrepancies between laboratories on several major criteria for distinguishing the species and subspecies cast doubt on the value of these proposals (Owen *et al.*, 1984). Nevertheless, our results suggest that a categorical division of the organisms into just two species is unsatisfactory. The situation is complicated further by our finding that one strain that we identified as a TLF on the basis of biochemical tests and cell morphology had a cell protein profile by both SDS-PAGE and FPLC that was identical with that of a TSF. Baron *et al.* (1984) also described a LF possessing a major protein component of about 45,000 mol. wt which is typical of a SF. It is clear that further studies are necessary to elucidate the problem of speciation and sub-speciation of these bacteria.

The results reported in the literature for carbohydrate fermentations vary widely. Some workers have reported that all the strains were asaccharolytic (Holst *et al.*, 1982), whereas others have reported different patterns of fermentation (Fox and Phillips, 1984; Påhlson *et al.*, 1984; Taylor and Owen, 1984); our results are similar to those of Fox and Phillips (1984). Although the discrepancies may be due to different techniques, it is clear that the fermentation pattern is not a reliable criterion for differentiation of *Mobiluncus* spp.

The enzyme profiles also vary widely (Fox and Phillips, 1984; Hjelm *et al.*, 1984; Taylor and Owen, 1984). The majority of the enzymic reactions were shared by all strains examined, in agreement with Christiansen *et al.* (1984) and Taylor and Owen (1984). However, we found the test for α-galactosidase actively useful for distinguishing between TLF, which all gave negative results, and S.F., which generally gave positive results. The nitrate reduction test was suggested as a means of differentiating between the two groups (Baron *et al.*, 1984; Taylor and Owen, 1984), but in our hands all strains gave positive results, irrespective of rod length, a result in agreement with that of Christiansen *et al.* (1984).

The antibiotic susceptibility patterns recorded by us are in agreement with those reported by other workers (Påhlson *et al.*, 1984; Sprott *et al.*, 1984) apart from metronidazole susceptibility. We did not find that metronidazole differentiated clearly between the SF and LF because some of the latter were resistant to it, a finding also of Jones *et al.* (1985). We also tested a new antibiotic, aztreonam (Squibb), to which all strains of *Mobiluncus* species were resistant at high concentrations (up to 50 μg/ml). This antibiotic is a new cephalosporin active mainly against gram-negative aerobes, and may, therefore, be useful in the development of a selective medium. The antibiogram of *Mobiluncus* strains is unusual for "gram-negative" micro-organisms, in that they are sensitive to vancomycin and bacitracin and resistant to colistin, like the majority of gram-positive anaerobes. Of the gram-negative anaerobes only a few *Bacteroides* species, including members of the melaninogenicus-group, have been reported to have similar antibiotic susceptibilities (Sutter *et al.*, 1980). It is noteworthy that Spiegel and Roberts (1984) had suggested that *Mobiluncus* organisms might belong within the family *Bacteroidaceae*. However, electronmicroscope studies of their cell wall structure (Fox and Phillips, 1984; Skarin *et al.*, 1984) show that they do not have any outer membrane, as do gram-negative bacteria. Nevertheless, their cell wall structure does differ from typical gram-positive organisms and it is possible that *Mobiluncus* organisms are gram-positive bacteria that are easily de-stained, just as are eubacteria and some clostridia (Holdeman *et al.*, 1977).

In conclusion, the taxonomy of the genus *Mobiluncus* is still unclear in that not all strains fulfil the criteria proposed for the two species and further studies will be needed to resolve this problem. Furthermore, as these organisms are fastidious, the development of an effective selective medium is necessary to clarify their pathogenic role in bacterial vaginosis and to establish the possibility of their sexual transmission.

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