A Numerical Taxonomic Study of Anaerobic Gram-negative Bacilli Classified as Bacteroides ureolyticus Isolated from Patients with Non-gonococcal Urethritis

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A numerical taxonomic study of 64 strains of anaerobic Gram-negative bacilli isolated from men with non-gonococcal urethritis, two unclassified laboratory strains of ‘corroding bacilli’, and 12 other strains of anaerobic Gram-negative bacilli, including nine received as anaerobic curved rods and three as ‘Bacteroides corrodens’ (B. ureolyticus), isolated from women with bacterial vaginosis, was undertaken. Seventeen reference anaerobic strains belonging to the genera Bacteroides, Fusobacterium, Mobiluncus, Mitsuokella and Wolinella were included. Morphological, biochemical and physiological characteristics were examined in 103 tests. The resemblance between the 95 strains was calculated using the $S_{SM}$, $S_f$ and $D_p$ coefficients for cluster analyses based on the UPGMA method. All three approaches gave similar groupings, and the estimated average probability of test error was 246%. The strains fell into 10 phenons. The unclassified strains from men and three from women with lower genital-tract infections, and the laboratory strains of ‘corroding bacilli’ clustered in one phenon with the reference strains of B. ureolyticus, indicating that they correspond to B. ureolyticus. The other unclassified strains of anaerobic curved rods clustered as a distinct phenon. They correspond to species of the newly described genus Mobiluncus. The taxonomic data and the compilation of diagnostic tables serve as a useful guide for the laboratory identification of clinical isolates regarded as B. ureolyticus.

INTRODUCTION

Non-gonococcal urethritis (NGU) and non-specific vaginitis (NSV) or bacterial vaginosis are two of the most common sexually transmitted diseases in men and women respectively. Various organisms are known, or have been thought, to be implicated in NGU infections, namely Chlamydia trachomatis (Darougar et al., 1971; Bowie et al., 1977 a, Richmond & Clarke, 1977; Taylor-Robinson & Thomas, 1980), Ureaplasma urealyticum (Taylor-Robinson, 1977, Taylor-Robinson et al., 1977, 1981; Taylor-Robinson & McCormack, 1979; Taylor-Robinson & Csonka, 1981), Clostridium difficile (Hafiz et al., 1975), Corynebacterium species (Furness et al., 1971, 1977), and Staphylococcus saprophyticus (Hovelius et al., 1979). Other organisms, namely Gardnerella vaginalis (Lapage, 1961; Jones et al., 1982; Kinghorn et al., 1982; Tabaqchali et al., 1983) and anaerobic curved rods (Holst et al., 1981; Sprott et al., 1983) are currently regarded as potential agents of NSV. Although various investigators have searched for anaerobes in lower genital-tract infections in men, evidence that they are associated with disease has not been

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Abbreviations: NGU, non-gonococcal urethritis; NSV, non-specific vaginitis.
substantiated (Bowie et al., 1977b; Hallen et al., 1977). However, using various approaches, strains of a Gram-negative, asaccharolytic, rod-shaped anaerobe were isolated from the urethra of men with NGU (Fontaine et al., 1982) and from vaginal specimens of women with NSV (Borriello et al., 1984). These organisms exhibited ‘twitching’ motility and many of their morphological and biochemical characteristics have been discussed fully in a previous paper (Fontaine et al., 1984a). In view of their recovery mainly from men with NGU these organisms were tentatively named ‘NGU strains’.

In attempting to identify the NGU strains, difficulty was experienced in assigning them correctly to the classified species of the Bacteroidaceae. Although the strains had characteristics in common with Wolinella succinogenes (Tanner et al., 1981), and particularly with Bacteroides ureolyticus (Jackson & Goodman, 1978), phenotypic differences, and the paucity of information regarding species identification, called for a reappraisal of their differential characteristics. The problem was further complicated by inter-laboratory variation in test results using the same strains of B. ureolyticus (Fontaine et al., 1984b). These findings stimulated a re-examination of NCTC reference strains which has shown that some strains of B. ureolyticus were atypical and one (NCTC 10939) was incorrectly classified as B. ureolyticus (Taylor et al., 1986). Although Jackson & Goodman (1978) classified anaerobic Gram-negative corroding bacilli as B. ureolyticus, their proposals were not derived from numerical taxonomic studies. Because of these various problems, we fully characterized the NGU strains and strains of B. ureolyticus, examining their taxonomic status by numerical analysis. We discuss their taxonomic position in relation to other closely related strains, in particular W. succinogenes, as well as comparing several of their phenotypic features with reference strains of Bacteroides, Fusobacterium, Mobiluncus and the facultative anaerobes Eikenella corrodens and Campylobacter. Although not anaerobic, the latter two organisms had phenotypic or biochemical characteristics in common with the unknown clinical isolates. Finally, we attempted to delineate accurately the biochemical characteristics of B. ureolyticus and on the basis of this study to propose diagnostic tests which would facilitate the identification of this micro-organism.

**METHODS**

**Bacterial strains.** The field strains examined were 64 isolates from men with NGU, 3 similar strains from women with NSV, and 12 strains of Gram-negative and variable rods, also isolated from women with lower genital-tract infections. In addition, two laboratory strains of B. ureolyticus, one laboratory hamster strain, and two reference strains of B. ureolyticus (NCTC 10941 and NCTC 10948) were used (Table 1).

Reference strains, obtained from the National Collection of Type Cultures (NCTC) and the American Type Culture Collection (ATCC), included Bacteroides asaccharolyticus ((NCTC 19583), B. multiplicus (NCTC 10934), B. thetaiotaomicron (NCTC 19582), B. fragilis (NCTC 9343), B. praeacutus (NCTC 11158), B. melanimogenicus (NCTC 9336), B. vulgatus (NCTC 10583), B. distasonis (ATCC 8503), Eikenella corrodens (NCTC 10596), Campylobacter fetus (NCTC 5850), C. coli (NCTC 11353), C. jejuni biotype I (NCTC 11168), Fusobacterium varium (NCTC 10560), F. necrophorum (NCTC 10575) and Wolinella succinogenes (ATCC 29543).

**Cultivation and maintenance of strains.** The organisms were grown on a supplemented agar medium and in broth culture medium as described previously (Fontaine et al., 1984a). In addition, an agar medium of Brain Heart Infusion base (BHIA) was used. It comprised 3.7% (w/v) brain heart infusion (Oxoid), 1% special peptone (Oxoid), 0.5% yeast extract (Oxoid), 0.1% ornithine, 0.3% sodium formate, 0.3% sodium fumarate, 0.2% potassium nitrate, 0.05% dipotassium hydrogen phosphate, 0.05% sodium carbonate, 0.1% vitamin K1, (2-methyl-3-phytyl-1,4-naphthoquinone), 0.1% haemin and 1.2% agar. This medium (BHIA-FFN) was pre-reduced with 0.1% cysteine hydrochloride and sterilized by autoclaving at 121°C for 15 min. Strains were maintained by fortnightly subculture on either medium, supplemented with 5% (v/v) sheep blood and incubated at 37°C in an anaerobic cabinet. Otherwise, they were stored in laked horse blood at −70°C. Reference strains of Campylobacter spp. were grown and maintained by subculture on blood agar plates in a microaerophilic environment containing 75% nitrogen, 10% hydrogen, 10% carbon dioxide and 5% oxygen.

**Characterization of strains.** The morphological, cultural, physiological and biochemical characteristics of these strains, as well as their susceptibility to antimicrobial agents, have been described previously (Fontaine et al., 1984a). These and additional tests used in this study are described below; they were done in duplicate.

Growth in Tryptone-Yeast-Glucose (TYG) medium was examined. This medium contained 0.5% trypthone (Oxoid), 0.5% yeast extract (Oxoid) and 0.1% glucose (BDH) and was autoclaved at 121°C for 15 min. It was used alone or supplemented with (a) sterile 0.005% vitamin K1 (menadione), (b) sterile 0.005% haemin or (c) sterile heat-inactivated 1% bovine serum (Oxoid); growth was examined after incubation at 37°C for 4 d.
**Numerical taxonomy of strains of B. ureolyticus**

<table>
<thead>
<tr>
<th>Strain/species designation</th>
<th>Phenon (subphenon) assignment</th>
<th>Designation of phenon</th>
<th>Source*</th>
<th>Other information†</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1N1, P1N10, P4N5, P1N1, P2N14, P2N12, P3N9, P1N3, P1N7, P4N15, P1N8, P2N17, P1N18, P1N13, P1N14, P2N20, P1N20, P2N3, P2N8, P2N10, P1N21, P2N7, P3N12, P1N5, P1N9, P1N19, P2N13, P2N9, P1N12, P2N1</td>
<td>A (A1)</td>
<td><em>B. ureolyticus</em></td>
<td>PSCL</td>
<td>NGU</td>
</tr>
<tr>
<td>'B. corrodens' ST.98.12; CRC-HS1</td>
<td>A (A2)</td>
<td><em>B. ureolyticus</em></td>
<td>STMSL/CRCH</td>
<td>NSV; hamster strain</td>
</tr>
<tr>
<td>ST.105.7, ST.114.9</td>
<td>A (A2)</td>
<td><em>B. ureolyticus</em></td>
<td>STMSL</td>
<td>NSV</td>
</tr>
<tr>
<td>W1N2, W5N4, W2N13</td>
<td>A (A3)</td>
<td><em>B. ureolyticus</em></td>
<td>SSCW</td>
<td>NGU</td>
</tr>
<tr>
<td>W2N6, W5N6; 'B. corrodens'</td>
<td>A (A3)</td>
<td><em>B. ureolyticus</em></td>
<td>SSCW/CRCH</td>
<td>NGU x 2; Lab. strain</td>
</tr>
<tr>
<td>V50.7, V31.6, V22.1, V3.1</td>
<td>E</td>
<td><em>Mobiluncus</em> spp.</td>
<td>STMSL</td>
<td>NSV (ACR)</td>
</tr>
<tr>
<td>VF81, VF82, VSA25</td>
<td>E</td>
<td><em>Mobiluncus</em> spp.</td>
<td>PHLM/STMSL</td>
<td>NSV (ACR)</td>
</tr>
<tr>
<td>VMC37, ST.72.1</td>
<td>E</td>
<td><em>Mobiluncus</em> spp.</td>
<td>PHLM/STMSL</td>
<td>NSV (ACR)</td>
</tr>
</tbody>
</table>

* PSCL, Praed Street Clinic, London; SSCW, Shrodells Special Clinic, Watford; CRCH, Clinical Research Centre, Harrow; STMSL, St Thomas's Medical School, London; PHLM, Public Health Laboratory, Manchester; UOLS, University of Lund, Sweden.

† NGU, non-gonococcal urethritis; NSV, non-specific vaginitis; ACR, anaerobic curved rods.

Strains were tested on the agar media described for growth at different temperatures and in different atmospheres, namely 37 °C in air, in air plus 10% carbon dioxide, in a mixture of gases consisting of 5% oxygen, 10% carbon dioxide, 2% hydrogen and 83% nitrogen and for growth at 43 °C anaerobically. In addition, colony colour for all the strains growing under these conditions was noted.

Growth was examined on two selective media. (a) Bacteroides agar was prepared from 3-7% Brain Heart Infusion (BHI) base supplemented with 0.5% yeast extract, 0.001% haemin, 0.001% vitamin K₁, 0.05% cysteine hydrochloride, 0.03% sodium formaldehyde sulphoxylate, 0.1% Tween-80 and 1.8% agar (pH 7.2). It was sterilized by autoclaving at 121 °C for 15 min. Before use, 10% (v/v) horse blood, 100 µg kanamycin ml⁻¹ and 7.5 µg vancomycin ml⁻¹ were added. (b) Fusobacteria agar was prepared from BHI base to which 10% laked horse blood, 7.5 µg vancomycin ml⁻¹ and 10 µg paromomycin ml⁻¹ (Parke Davis) were added. An alternative medium for fusobacteria consisted of BHI supplemented with 10% horse blood, 0.1% Tween-80 and 10 µg nalidixic acid ml⁻¹.

The strains were incubated on these media in an anaerobic cabinet at 37 °C for 4 d.

Stimulation of growth by nitrate was assessed by using medium prepared from nitrate broth (Difco) supplemented with 10 µg haemin ml⁻¹, 10 µg vitamin K₁, ml⁻¹, 0.1% Tween-80 and 0.1% cysteine hydrochloride. The strains were inoculated in 2 ml nitrate medium, and the cultures incubated anaerobically at 37 °C for 4 d. Uninoculated media were included as controls and treated similarly. The cultures were examined for the presence or absence of growth.

Deoxyribonuclease synthesis was examined using DNAase Agar (Oxoid) supplemented with 10 µg haemin ml⁻¹, 0.5% cysteine hydrochloride, 0.3% sodium formate and 0.3% sodium fumarate. The cultures were grown anaerobically at 37 °C for 4 d. Following incubation, DNA was precipitated with 1 M-HCl; the presence of DNAase activity was indicated by a clearing around the colonies.

Gelatin hydrolysis was tested using the method of Frazier (Cowan, 1974). The medium was supplemented with 0.1% cysteine hydrochloride, 0.3% sodium formate and 0.3% sodium fumarate. After inoculation the cultures were
incubated anaerobically at 37 °C for 4 d. Gelatin hydrolysis was indicated by a clear zone around individual colonies.

Crossley Milk Medium (Oxoid) was used to investigate acidity (pH change) and digestion. The medium was supplemented with 0.1% cystine hydrochloride, 0.3% sodium formate and 0.3% sodium fumarate. The cultures were incubated anaerobically at 37 °C for up to 14 d. Acidity was indicated by a change from purple (alkaline) to yellow (acid), and digestion by a clear, colourless or brown liquid.

Coding of characters. The data for each strain were coded as two-state characters: each feature was scored as 0 for a negative or 1 for a positive reaction. Five tests were constant for all strains examined. These were: dextran hydrolysis (negative); lecithinase production (negative); brown or black colony colour (negative); cystine arylamidase (negative); production of esterase C4 (positive). These and 11 other tests which gave poor reproducibility (see Results) were excluded from further analysis, so that each strain was analysed from one set of coded data based on 92 tests.

Computer analysis. Cluster analysis was performed using the CLUSTAN package, version 2 release 1 (Wishart, 1982) on the University of Surrey Prime 750 computer system. The programs GPROPS and GBEST (T. N. Bryant, unpublished programs) were used to determine the properties of each phenon and the most diagnostic characters for distinguishing phenons from each other. Resemblance matrices were calculated using the simple matching coefficient (SsM; Sokal & Michener, 1958), the Jaccard coefficient (Sj; Sneath, 1957) and the pattern difference coefficient (Dp; Sneath & Sokal, 1973). Each matrix was sorted by the unweighted pair group using arithmetic averages (UPGMA) method (Sneath & Sokal, 1973) and the cophenetic correlation coefficient (Sokal & Rohlf, 1962) was calculated for each analysis.

RESULTS

Reproducibility

The average probability (p) of an erroneous result was 2.46% when calculated on the basis of the results for 103 characters that varied for the 96 strains tested in duplicate. This is well within the range of acceptable errors of reproducibility of 10–15% (Sneath & Johnson, 1972). The average similarity between these duplicate strains was 97.6% as calculated by SsM. Eleven test characters had less than 85% reproducibility and were excluded from the taxonomic analysis. These were: growth of the organisms in TYG medium; growth in TYG medium plus 0.1% vitamin K1; growth on blood agar plus formate and fumarate at 43 °C; growth on blood agar plus formate and fumarate in an atmosphere containing 5% oxygen; production of hydrogen sulphide; tolerance of 2.5% sodium taurocholate, 0.5% sodium deoxycholate and 2.5% sodium taurocholate plus 2.5% sodium deoxycholate; reduction of 0.005% basic fuchsin and 0.005% methyl violet, and colony colour. Cluster analysis was based, therefore, on 92 characters.

Cluster analysis

The results of the cluster analyses from three different computations, SsM, Sj and Dp, gave similar groupings. The cophenetic correlation coefficients for these analyses were 0.977, 0.947 and 0.941, respectively. The SsM/UPGMA classification will be described in detail since it had the highest cophenetic correlation and the inclusion of both positive and negative matches; it is preferable in this study because of the relatively large number of negative reactions recorded. However, some reference will be made to the analysis by Dp, which relates to the metabolic activity of strains and allows for differences in their growth rates (Sneath, 1968).

In the SsM/UPGMA dendrogram (Fig. 1), all the NGU strains clustered at 86% similarity (S). This phenon also contained the unclassified strains of B. ureolyticus isolated from women with bacterial vaginosis, a laboratory hamster strain and the reference strains of B. ureolyticus, including the type strain (NCTC 10941). Four other phenons (D, E, G, H) were delineated by other reference strains that grouped according to their taxa.

In the Sj/UPGMA analysis, the same pattern was observed, but the NGU strains clustered at the lower similarity level of 65%. In the Dp/UPGMA analysis, all the NGU strains except W5N4 and W2N13 clustered in one group at a similarity level of 89-94%. These two strains alone remained in subcluster A3, which was shifted slightly. Other changes due to variations in metabolic activity, resulted in B. praecucutus being grouped with the inactive fusobacteria, the hamster strain with W. succinogenes and B. asaccharolyticus with the other pigmented strain of B. melaninogenicus. Overall, clusters derived from the three computations did not affect the relationships between the reference strains or within the field strains.
Phenon A (86% S) contains all 64 clinical isolates labelled as NGU strains, two reference strains of *B. ureolyticus* including the type strain (NCTC 10941), five unclassified strains received as *B. ureolyticus*, three of them isolated from women with NSV and one unknown strain from a female laboratory hamster (Table 1). Although three subphenons (A1–A3) were evident within the cluster (Fig. 1), they were closely related and the strains were indistinguishable morphologically and biochemically. They were quite distinct from the other strains used in the study, with the exception of *W. succinogenes*. The tight clustering of the reference and laboratory strains of *B. ureolyticus* with the clinical NGU strains in the main cluster (phenon A) indicates that the latter are closely related and belong to the same species as *B. ureolyticus*. The characteristics distinguishing phenon A from other phenons in the study are shown in Table 2.
Table 2. Characteristics distinguishing the various phenons from each other by any combination of four tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Phenon...</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>J</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Propionic acid</td>
<td></td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Oxidase</td>
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<td>+</td>
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<td>α-Glucosidase</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>N-Acetyl-β-glucosaminidase</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Resistance to brilliant green</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<td>H2S from thiosulphate</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Arginine decarboxylase</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Lactic acid</td>
<td></td>
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<td>-</td>
<td>+</td>
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<td>-</td>
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<td>Growth on fusobacteria selective agar</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Growth on BA + formate and fumarate</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>in 5% O2</td>
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<td>+</td>
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<td>Growth in air + 10% CO2</td>
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<td>-</td>
<td>-</td>
<td>+</td>
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<td>-</td>
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<tr>
<td>Gelatin liquefaction</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Cell shape (mainly straight)</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
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</tbody>
</table>

Key: +, ≥90% strains gave positive reactions; + −, 50-89% gave positive reactions; − +, 13-49% gave positive reactions; −, ≤12% gave positive reactions; t, twitching motility; c, classical motility; BA, blood agar.

Phenon B (89.5% S) contained one strain of *B. praeacutus* (NCTC 11158) and the type strain of *W. succinogenes* (ATCC 29543). Like *B. ureolyticus*, *B. praeacutus* is one of the asaccharolytic species of *Bacteroides* possessing a low DNA G + C content, 28 mol% (Shah & Collins, 1983). Moreover, it has phenotypic features in common with *W. succinogenes* and *B. ureolyticus*. The grouping of *B. praeacutus* and *W. succinogenes* overlapped with that of the NGU strains of *B. ureolyticus* just outside the main cluster at the 85% level. However, *W. succinogenes* differed from *B. ureolyticus* in a number of characteristics (Table 3), as did *B. praeacutus* which, unlike *B. ureolyticus*, had no requirement for formate and fumarate and produced a wide range of volatile fatty acids, i.e. acetic, propionic, isobutyric, butyric and isovaleric. The taxonomic position of these strains is difficult to ascertain from their clustering. However, in the computation using $D_p$, *B. praeacutus* clustered with the metabolically inactive fusobacteria as expected and *W. succinogenes* grouped with the single hamster strain of *B. ureolyticus*.

Phenon C (83% S) contained a single reference strain of *E. corrodens* (NCTC 10596) and phenon D (95.7%) contained strains of three reference *Campylobacter* species, *C. coli*, *C. jejuni* and *C. fetus*, which clustered together and showed a considerable degree of similarity (99.2%) among themselves.

Phenon E (87% S) contained nine strains received as anaerobic curved rods isolated from women with NSV. This unclassified group of anaerobes was recently placed in the new genus *Mobiluncus*, comprising *M. curtisi* subsp. *curtisi* (type strain), *M. curtisi* subsp. *holmesii* and *M. mulieris* (Spiegel & Roberts, 1984). Only the last two taxa were tested. They formed a distinct cluster (85-5% S) and were quite different from the NGU strains in their morphology, growth requirements and sensitivity to antibiotics. Unlike the NGU strains they did not corrode agar medium. *Mobiluncus* strains were generally negative for oxidase, but they reduced nitrate to nitrite and produced alkaline phosphatase and esterase. About half of the strains decarboxylated lysine and ornithine, only one was resistant to vancomycin, and the majority were inactive fermentatively, like the NGU strains and reference strains of *B. ureolyticus*.

Phenon F (80-5% S) contained a single reference strain of *B. asaccharolyticus* which appeared different from the reference strain of *B. melaninogenicus* in phenon J (77% S), in keeping with other work (Holdeman et al., 1984).
Table 3. Diagnostic table for distinguishing B. ureolyticus from the indicated anaerobes

<table>
<thead>
<tr>
<th>Differential character</th>
<th>B. ureolyticus</th>
<th>W. succinogenes</th>
<th>M. mivini, M. curtisi subsp. hometri</th>
<th>B. asaccharolyticus</th>
<th>F. necrophorum/F. varium</th>
<th>‘B. fragilis group’</th>
<th>B. melanomogenes</th>
<th>B. (Mitsuokella) multiacidus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease production</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidase production</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine decarboxylase</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistance to vancomycin</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formate + fumarate requirement</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: +, > 90% strains gave positive reactions; +, 50-89% gave positive reactions; −, 13-49% gave positive reactions; −, < 12% gave positive reactions.

Phenon G (92.6% S) contained two reference strains of Fusobacterium: F. necrophorum and F. varium.

Phenon H (88.2% S) contained four reference strains of the ‘B. fragilis group’ of organisms, namely B. fragilis, B. vulgatus, B. distasonis and B. thetaiotaomicron. They formed a small cluster distinct from the other Bacteroides strains used in the study.

Phenon K (74.2% S) contained the single strain of B. multiacidus which was distinct from the other Bacteroides strains included in the study. This species has since been re-classified as Mitsuokella multiacidus (Shah & Collins, 1983). Besides the strains in phenons A and E, the other anaerobic strains used in the study are reference strains of the Bacteroidaceae. Four of these are represented as single strains (Fig. 1). Each defined phenon was examined for distinguishing features that could be weighted for identification. By comparing the frequencies of occurrence for the total number of characters within each defined phenon, a diagnostic table (Table 3) was constructed for the differentiation and identification of the anaerobes (see also Table 2).

**DISCUSSION**

This study was undertaken to determine the nature and taxonomic position of anaerobic Gram-negative bacilli that had been isolated from men with non-gonococcal urethritis (NGU strains) and which closely resembled B. ureolyticus. In view of their genotypic and phenotypic resemblances to B. ureolyticus our primary interest was to ascertain whether the NGU strains belonged to the same species as B. ureolyticus, which is taxonomically ill-defined. Moreover, although the NGU strains had appeared to be homogeneous in morphological and biochemical characteristics (Fontaine et al., 1984a, b), heterogeneity among strains had been reported in studies relating to their electrophoretic protein mobilities (Taylor & Owen, 1984).

The dendrograms produced using the three different coefficients, $S_{SM}$, $S_J$ and $D_p$, and UPGMA sorting were all similar. The high cophenetic correlation coefficients indicated that each dendrogram was a good representation of the resemblance matrix from which it was derived. In this study, a large number of matches were negative; however, the dendrograms based on the $S_{SM}$ and $S_J$ coefficients gave identical clustering of strains. In both analyses, there was tight clustering of the NGU strains and a previously unclassified laboratory strain with reference strains of B. ureolyticus, including the type strain (NCTC 10941), all of them being placed in phenon A.
The taxonomic status of *B. ureolyticus* had not previously been properly evaluated. More importantly, there was no consensus of opinion between different groups of workers on the biochemical reactions of *B. ureolyticus*, even when using the same strain (Fontaine et al., 1984b). Jackson et al. (1971) originally differentiated four strictly anaerobic strains from those that were facultatively anaerobic. Later, Jackson & Goodman (1978) characterized seven strains only and classified them as *B. ureolyticus*, of which NCTC 10941 and NCTC 10948 were used for comparison in this study. With so few strains, and a paucity of characterization tests and diagnostic tables, it is not surprising that strains of this anaerobe are sometimes difficult to identify. Placing reliance on the pitting of agar, as is often done, is unwise. It is not the best diagnostic feature, as other anaerobes, notably *W. succinogenes*, also corrode agar. Besides, pitting is a variable characteristic, depending on the type of medium and frequency of subculturing. This is true also of the interpretation of morphological features, which could vary among strains and within the same strain. Variation in the production of lactic acid and an unknown volatile fatty acid, β-haemolysis, proteolytic activity, production of H₂S in sulphide-indole motility medium, and growth of organisms in 5% oxygen, is not uncommon (Fontaine et al., 1984a, b). Such observations could only be made from the study of several strains. All these features are considered in this classification of strains identified as *B. ureolyticus*. Strains that appeared closely related to them, such as *W. succinogenes*, belonged to different taxa as demonstrated by this taxonomic analysis. The other *Bacteroides* species used in the study were markedly dissimilar from *B. ureolyticus*. Phenotypic characters that differentiate these organisms from all the *Bacteroides* spp. and *Mobiluncus* spp. are the production of oxidase and urease, and the requirement for formate and fumarate for growth.

In this investigation, all the reference strains clustered as expected in separate phenons with the exception of *W. succinogenes* and *B. praeacutus*, which grouped together in phenon B, despite being recognized as distinct species. However, some of the tests excluded because of poor reproducibility were those used to distinguish these two species. This study shows that a grouping of this nature can sometimes occur in a numerical taxonomic analysis which relates to phenotypic rather than genotypic relationships. Indeed, micro-organisms closely associated by virtue of phenotypic resemblances can be unrelated genetically (Stanier et al., 1977). The similarity between these two species was investigated further using clusterings based on the *D₁* coefficient and UPGMA sorting. Interestingly, *B. praeacutus* clustered with the inactive fusobacteria while *W. succinogenes* appeared with the hamster strain of *B. ureolyticus*, both of which are of animal origin. It is therefore considered that one of these strains is incorrectly classified in phenon B.

The present investigation is the first taxonomic approach towards the classification of clinical isolates of *B. ureolyticus*. The recognition of useful differential characteristics and the development of a relatively simple diagnostic table serve as useful guides to facilitate the identification of this micro-organism.

**REFERENCES**


Numerical taxonomy of strains of B. ureolyticus


