Journal of General Microbiology (1980), **119**, 373–396. *Printed in Great Britain*

A Taxonomic Study of *Gardnerella vaginalis* (*Haemophilus vaginalis*) Gardner and Dukes 1955

By PETER PIOT,[1]* EDDY VAN DYCK,[1] MICHAEL GOODFELLOW[2] AND STANLEY FALKOW[3]

[1] *Laboratory of Bacteriology, Institute of Tropical Medicine, B-2000 Antwerp, Belgium*
[2] *Department of Microbiology, The Medical School, The University, Newcastle-upon-Tyne NE1 7RU*
[3] *Department of Microbiology, University of Washington, Seattle, Washington, U.S.A.*

(*Received* 5 *October* 1979)

Fifty-five strains received as *Haemophilus vaginalis* or as catalase-negative coryneform bacteria from the vagina together with 61 marker cultures were subjected to numerical phenetic analyses using 149 unit characters. The data were examined using the simple matching (S_{SM}), Jaccard (S_J) and pattern (D_P) coefficients and clustering was achieved using the average linkage algorithm. Cluster composition was not markedly affected by the coefficient used or by test error, estimated at 6·5 %. The *H. vaginalis* strains formed a tight cluster which was only distantly related to representatives of the genera *Arthrobacter*, *Cellulomonas*, *Corynebacterium sensu stricto*, *Erysipelothrix*, *Haemophilus*, *Kurthia*, *Lactobacillus*, *Listeria* and *Propionibacterium* but shared a high overall affinity to unclassified catalase-negative coryneforms which formed a discrete taxon, cluster 9. The *H. vaginalis* strains could be distinguished from the related strains in cluster 9 by several unrelated phenotypic characters.

Using the S1 endonuclease assay, DNA–DNA hybridizations were performed with representative strains from the numerical study as well as with reference strains of *Bifidobacterium* and *Actinomyces*. *Haemophilus vaginalis* was found to be a genotypically legitimate group and its DNA showed little homology with DNA from the marker strains tested. The DNA base composition of *H. vaginalis* was 42 to 44 mol % guanine plus cytosine. A new genus should be created to incorporate strains known as *H. vaginalis* or *Corynebacterium vaginale*. The name *Gardnerella vaginalis* proposed by Greenwood & Pickett (1979) is supported.

INTRODUCTION

The species *Haemophilus vaginalis* Gardner & Dukes 1955, proposed for isolates from the genito-urinary tract that are implicated in non-specific vaginitis (Leopold, 1953; Gardner & Dukes, 1954, 1955; Wurch & Lutz, 1955; Lutz *et al.*, 1956), has received little detailed study and is a source of confusion to clinicians and taxonomists alike (Dunkelberg, 1977). *Haemophilus vaginalis* does not require X- or V-factors or any definable coenzyme-like substances for growth (Lapage, 1961; Park *et al.*, 1968; Dunkelberg & McVeigh, 1969) and although it is now usually considered to belong to some other genus the name was retained as a temporary expedient in the current edition of *Bergey's Manual of Determinative Bacteriology* (Lapage, 1974) and is used for convenience here.

It has been proposed that *H. vaginalis* be renamed *Corynebacterium vaginale* (Zinnemann & Turner, 1963; Dunkelberg *et al.*, 1970*a*) but the type strain can readily be distinguished

0022-1287/80/0000-8997 $02.00 © 1980 SGM

from true corynebacteria. Thus, *H. vaginalis* NCTC 10287 contains 6-deoxytalose (Vickerstaff & Cole, 1969), lysine, glutamic acid and aspartic acid (Criswell *et al.*, 1971) in its cell wall, whereas true corynebacteria have arabinose, galactose and *meso*-diaminopimelic acid (Keddie & Cure, 1978; Keddie & Bousfield, 1980). *Lactobacillus* has also been proposed as a possible niche (Amies & Garabedian, 1963; Garabedian, 1969) but it probably does not belong to this genus or to *Eubacterium* or *Propionibacterium* as acetic acid is the principal fermentation end-product rather than lactic, butyric or propionic acids (Moss & Dunkelberg, 1969). The low GC content (42 mol %) of the DNA of the type strain (Lapage, 1974) clearly distinguishes it from both true corynebacteria and propionibacteria (Minnikin *et al.*, 1978).

Few reliable data are available on the properties of *H. vaginalis* strains although useful guidelines exist for their isolation and identification (Dunkelberg *et al.*, 1970*a*, *b*). The taxon is considered to be a good species (Lapage, 1974) but published characteristics vary, probably due to investigators using different media and inadequate samples of strains. There is even no consensus as to whether strains are Gram-positive or Gram-negative (Reyn *et al.*, 1966; Criswell *et al.*, 1971, 1972) although they do fall within Cure & Keddie's (1973) definition of 'coryneform' bacteria.

Numerical methods have helped to clarify the intra- and intergeneric relationships between coryneform bacteria in general (Jones, 1978) and between those from defined habitats such as the skin (Pitcher & Noble, 1978). In the present study, isolates from the vagina received as *C. vaginale*, *H. vaginalis* or as unclassified catalase-negative coryneform bacteria were compared, using numerical methods, with representatives of possibly related genera in an attempt to determine their taxonomic status.

In addition, DNA sequence homology between selected strains was examined and the DNA base composition of five strains was determined.

METHODS

Strains. The 124 test strains (Tables 1 and 2) were maintained on Diagnostic Sensitivity Test (DST) agar (Oxoid) supplemented with defibrinated horse blood (5 %, v/v) and subcultured every 48 h. Forty-seven of the strains were fresh cultures of catalase-negative coryneform bacteria isolated from the vagina on Casman agar base (BBL) supplemented with rabbit blood (5 %, v/v) (Casman, 1947); the remainder were derived from culture collections. All of the cultures were lyophilized.

Basal media. A basal medium was developed which supported the growth of all of the test strains but did not contain poorly standardized ingredients such as blood or serum. The medium finally adopted was a modification of the peptone yeast glucose agar of Holdeman *et al.* (1975) and contained: proteose peptone no. 3 (Difco), 20 g; yeast extract (Difco), 10 g; glucose, 2 g; Tween 80 (Koch–Light), 1 g; NaCl, 1 g; NAD (Sigma), 0·001 g; haemin (BDH), 0·01 g; Sörensen's phosphate buffer pH 7·2, 100 ml; Dulbecco's salt solution (Difco), 5 ml; distilled water, 1 l; pH 7·2. Granulated agar (1·35 %, w/v; BBL) was added for the solid basal medium, as other agars tested inhibited the growth of several nutritionally demanding strains. The two basal media, designated BM broth and BM agar, were used as a base for all tests unless otherwise stated.

Collection of data. Each strain was examined for 149 unit characters (Tables 2 and 3). Media were inoculated from 24 h BM agar slopes and incubated at 37 °C for 48 h in a 10 % (v/v) CO_2 incubator unless otherwise stated. Tests were generally carried out once but were repeated when inconclusive results were obtained.

Colonial and cell morphology. Strains grown on BM agar were examined under a low power microscope for colonial characters after 2 d and for pigment production after 5 d incubation in the light at room temperature. Smears prepared from 1 and 2 d cultures grown on BM agar and Loeffler's serum (Cowan, 1974) slants were Gram-stained by means of Hucker's modification (Hucker & Conn, 1923) and examined at × 100 and × 1000 magnification. Motility was detected in stab cultures of semi-solid BM medium containing 0·2 % (w/v) granulated agar incubated for 5 d at 36 °C and room temperature and the type of flagellation was observed after Rhodes (1958).

Growth characteristics in liquid media were examined in BM broth. Haemolysis was detected on DST agar supplemented with 5 % (v/v) sterile horse blood and browning of blood was examined on Casman agar base slants supplemented with 5 % (v/v) sterile horse, sheep or rabbit blood, after 2 and 4 d incubation.

Growth requirements. The requirement for X- and V-factors was tested by observing satellitism around discs (BBL) containing the respective factors. X-factor requirement was also examined using the porphyrin test

Table 1. *Description and source of strains assigned to clusters based on pattern differences*

A. *Major clusters*

(*a*) Strains assigned to cluster 1

Subcluster 1a (*Listeria* species)

C4	*Listeria grayi*; D. Jones, The University, Leicester, C213; H. L. Seeliger L332/64
C1	*Listeria monocytogenes*; D. Jones, C52; NCTC 7973
C2	*Listeria monocytogenes*; D. Jones, C200; NCTC 5348

Subcluster 1b (*Streptococcus faecalis*)

C34	*Streptococcus faecalis*; D. Jones, C117; NCIB 6782
C35	*Streptococcus faecalis* var. *zymogenes*; ITG 36

(*b*) Strains assigned to cluster 2 (*Lactobacillus casei/plantarum*)

C27	*Lactobacillus casei* var. *rhamnosus*; D. Jones, C232; NCDO 243
C31	*L. casei* var. *rhamnosus*; M. E. Sharpe, National Institute for Research in Dairying, Reading, C16
C28	*Lactobacillus plantarum*; D. Jones, C233; NCDO 1752
C68	Coryneform sp.; ITG 148; vagina

(*c*) Strains assigned to cluster 4 (*Lactobacillus acidophilus/jensenii*)

C30	*Lactobacillus acidophilus*; M. E. Sharpe, A1
*C29, C29D	*Lactobacillus jensenii*; M. E. Sharpe; ATCC 25258; F. Gasser, 62G; human vaginal discharge
C101 to C104	*Lactobacillus* sp.; ITG 792, 793, 789, 800; vagina
C77, C78, C87, C94	Coryneform sp.; ITG 298, 299, 832, 877; vagina

(*d*) Strains assigned to cluster 6 (*Streptococcus* species)

C51	*Streptococcus agalactiae*; ITG 71
C38	*Streptococcus equisimilis*; NCTC 5371
C36	*Streptococcus pyogenes*; D. Jones, C9; C. S. Cummins, S6
C37	*Streptococcus* sp.; NCTC 8037
C106, C107	*Streptococcus* sp.; ITG 282, 283; vagina
C60	Coryneform sp.; ITG 135; vagina

(*e*) Strains assigned to cluster 8 (*Haemophilus vaginalis*)

*C43, C43D	*Haemophilus vaginalis*; NCTC 10287, ATCC 14018; K. Zinnemann; vagina
C46, C46D	*H. vaginalis*; P. N. Edmunds, Fife Area Health Authority, Kirkcaldy, T64 (group 2)
C47, C50	*H. vaginalis*; P. N. Edmunds, T66 (group 2), T34 (group 3)
C121, C122	*H. vaginalis*; R. E. Weaver, Center for Disease Control, Atlanta, Georgia, U.S.A., CDC 1412, 1413
C54 to C56, C69, C72 to C74, C79, C81 to C83, C85, C86, C89 to C93, C95, C97, C99, C105, C123	Coryneform sp.; ITG 77, 78, 130, 285, 290, 291, 293, 803, 822, 823, 826, 828, 831, 836, 838, 839, 840, 871, 879, 884, 898, 801, 805; vagina

(*f*) Strains assigned to cluster 9 (unclassified coryneforms)

C49	*Corynebacterium vaginale*; P. E. Pease, The University, Birmingham, 646 (group 2)
C45	*Haemophilus vaginalis*; P. N. Edmunds, T145 (group 6)
C108	*Streptococcus* sp.; ITG 284; vagina
C52, C57, C58, C64 to C67, C70, C76, C80, C88, C98, C118 to C120	Coryneform sp., ITG 75, 131, 132, 137, 143, 145, 147, 286, 296, 806, 833, 886, 300, 146, 802; vagina
C100	Coryneform sp.; ITG 900; penile ulcer

(*g*) Strains assigned to cluster 12 (*Haemophilus influenzae*)

C110	*Haemophilus influenzae*; NCTC 7279; Pittman type b; meningitis
C116	*Haemophilus parainfluenzae*; ITG 203
C111, C114	*Haemophilus paraphrophilus*; NCTC 10558, 10557

(*h*) Strains assigned to cluster 16 (*Corynebacterium sensu stricto*)

*C15	*Corynebacterium diphtheriae* var. *gravis*; D. Jones, C41; NCTC 3984
C16	*C. diphtheriae*; ITG 121
C20	*Corynebacterium pseudotuberculosis*; D. Jones, C42; NCTC 3450
C124	*Corynebacterium xerosis*; D. Pitcher, Institute of Dermatology, Homerton Grove, London; NCTC 9755
C24	*Corynebacterium* sp.; G. Delville, Université Catholique Louvain, Brussels, Belgium, 0122; skin of leprosy patient

B. *Minor clusters*

(*i*) Strains assigned to cluster 3 (*Lactobacillus cellobiosus/fermentum*)

C33	*Lactobacillus cellobiosus*; M. E. Sharpe, G1
C32	*Lactobacillus fermentum*; M. E. Sharpe, F1

(*j*) Strains assigned to cluster 5 (*Streptococcus sanguis*)

C39	*Streptococcus sanguis*; NCTC 7864
C40	*Streptococcus* M; NCTC 10711
C96	Coryneform sp.; ITG 883; vagina

(*k*) Strains assigned to cluster 7 (*Corynebacterium haemolyticum/pyogenes*)

C17, C17D	*Corynebacterium haemolyticum*; D. Jones, C45; NCTC 8452
C21	*Corynebacterium pyogenes*; D. Jones; NCTC 5225

(*l*) Strains assigned to cluster 10 (*Cellulomonas flavigena*)

C12	*Cellulomonas flavigena*; D. Jones, C96; NCIB 8078
C13	*C. flavigena*; D. Jones, C102; NCIB 8075

(*m*) Strains assigned to cluster 11 (*Haemophilus aphrophilus*)

C115	*Actinobacillus actinomycetemcomitans*; NCTC 9709, P. Holm; abscess
*C109, C113	*Haemophilus aphrophilus*; NCTC 5886, 5906

(*n*) Strains assigned to cluster 13 (*Erysipelothrix rhusiopathiae*)

*C5	*Erysipelothrix rhusiopathiae*; D. Jones, C50; NCTC 8163
C6	*E. rhusiopathiae*; D. Jones, C220; P. H. A. Sneath, 19

(*o*) Strains assigned to cluster 14 (*Arthrobacter globiformis*)

C7	*Arthrobacter globiformis*; D. Jones, C71; NCIB 8602
C8	*A. globiformis*; D. Jones, C75; NCIB 9334

(*p*) Strains assigned to cluster 15 (*Corynebacterium pseudodiphtheriticum*)

C126	*Brevibacterium* sp.; D. Pitcher; NCTC 11084; skin
C125	*Corynebacterium pseudodiphtheriticum*; D. Pitcher; NCTC 231
C127	Coryneform sp.; D. Pitcher, P87; skin

(*q*) Strains assigned to cluster 17 (*Corynebacterium bovis/flavidum*)

*C19	*Corynebacterium bovis*; D. Jones, C13; ATCC 7715
C23	*Corynebacterium flavidum*; NCTC 764

C. *Clusters containing single isolates*

C10	*Bacterium eurydice*; D. Jones, C208; L. Bailey, C208
*C11	*Brevibacterium incertum*; D. Jones, C24; ATCC 8363
C26	*Brochothrix thermosphacta*; D. Jones, C1D
C14	*Corynebacterium betae*; D. Jones, C35; NCPPB 363
C22	*Corynebacterium hoagii*; NCTC 10677
*C18	*Corynebacterium minutissimum*; D. Jones, C66; NCTC 10288
C59	*Eikenella corrodens*; ITG 134; vagina
C112	*Haemophilus* sp.; NCTC 10555; W. J. Ryan; peranal abscess
C25	*Kurthia zopfii*; D. Jones, C6; R. M. Keddie, S8
C3	*Listeria denitrificans*; D. Jones, C212; Pasteur Institute, Paris, L26
C117	*Propionibacterium jensenii*; D. Jones, C116; NCIB 5960
C63	Coryneform sp.; ITG 135; vagina

* Type strains; D, duplicate cultures; C, laboratory numbers.
ATCC, American Type Culture Collection, Rockville, Md, U.S.A.; ITG, Instituut voor Tropische Geneeskunde, Antwerp, Belgium; NCDO, National Collection of Dairy Organisms, Shinfield, Reading; NCIB, National Collection of Industrial Bacteria, Aberdeen; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden; NCTC, National Collection of Type Cultures, London.

Table 2. *Description and source of strains studied in DNA sequence homology experiments, but not in the numerical analysis*

Name as received	Strain designation	Source†
Actinomyces viscosus	46	B. L. Williams
**Bifidobacterium adolescentis*	ATCC 15703	—
**Bifidobacterium breve*	ATCC 15700	—
**Bifidobacterium infantis*	ATCC 15697	—
Branhamella catarrhalis	ITG 1642	—
Haemophilus vaginalis	Ellsw.	K. K. Holmes
Haemophilus vaginalis-like	52	R. F. Smith
Haemophilus vaginalis-like	88	R. F. Smith

Abbreviations as in Table 1.
† B. L. Williams, University of Washington, Seattle, U.S.A.; K. K. Holmes, University of Washington, Seattle, U.S.A.; R. F. Smith, Contra Costa Health Department, Martinez, California, U.S.A.

(Kilian, 1974). The requirement for increased CO_2 in the incubation atmosphere was determined by comparing growth on a lightly inoculated duplicate set of BM agar plates incubated in air and in an incubator with 10 % (v/v) CO_2, respectively. Growth on nutrient agar (Oxoid) and on peptone starch dextrose agar (Dunkelberg & McVeigh, 1969) was recorded after 2 d.

Biochemical tests. The benzidine test was carried out using the method of Deibel & Evans (1960), oxidase and urease activity were tested as described by Kovács (1956) and Stuart *et al.* (1945), respectively, catalase formation by adding a drop of H_2O_2 (3 %, v/v) to a loopful of bacteria on a glass slide, hydrogen sulphide production by suspending lead acetate paper strips above BM agar slopes supplemented with cysteine hydrochloride (0·25 %, w/v) and the formation of ammonia from peptone after 2 and 7 d as described by Jones (1975). Arginine, lysine and ornithine decarboxylases were detected using a micromethod (Shaw & Clarke, 1955), readings being taken at 4 and 24 h and compared with controls lacking the amino acid. Phenylalanine deaminase activity was detected using the micromethod of Bergquist & Searcy (1963), the production of a green colour on addition of 2 drops of ferric chloride solution (8 %, w/v) to 4 and 8 h cultures being recorded as a positive result. To examine hippurate hydrolysis, 5 ml of freshly prepared BM broth containing sodium hippurate (1 %, w/v) was inoculated and incubated for 48 h and then, after centrifuging the suspension, 0·2 ml of ferric chloride solution (12 %, w/v) was added to 0·8 ml of the clear supernatant. Nitrate reduction was observed in 10 ml BM broth containing potassium nitrate (0·1 %, w/v) (method 1; Cowan, 1974), the methyl red and Voges–Proskauer tests in BM broth supplemented with glucose (0·5 %, w/v) after 2 d (Cowan, 1974) and indole production in BM broth to which tryptone (0·5 %, w/v) had been added, the Kovács (1928) reagent being added after 2 d. The CAMP test (Christie *et al.*, 1944) was performed by cross-streaking test strains against a freshly inoculated line of *Staphylococcus aureus* ATCC 25923 on Tryptic Soy Agar (BBL) containing sheep blood (5 %, v/v) and examining plates after 2 d. Hydrogen peroxide inhibition was detected on BM agar plates swabbed with overnight suspensions in BM broth by adding a drop of H_2O_2 to the centre of dried plates which were then incubated for 2 d; zones of inhibition greater than 30 mm were recorded as positive. Tubes containing litmus milk (Oxoid) were heavily inoculated and examined daily for reduction and after 14 d for clotting, acid and alkaline production and digestion.

Degradation tests. Serum digestion was observed on heavily inoculated Loeffler serum slopes (Cowan, 1974) after 7, 14 and 21 d, and activity on BM agar plates containing egg yolk (10 %, v/v; Oxoid) was observed after 5 d when clearing, precipitation and the production of an oily iridescence around growth was noted. The basal medium for the remaining degradation tests was BM agar or BM broth minus Tween 80.

Gelatin degradation was detected in BM broths containing a sterile formalinized gelatin disc (Bio Mérieux); the broths were examined daily for up to 7 d for the disintegration of the discs. Aesculin hydrolysis was noted after 2 d in BM broth containing aesculin (0·1 %, w/v) when an immediate blackening of the medium was obtained on addition of 3 drops of an aqueous solution of ferric ammonium citrate (1 %, w/v). For the detection of casein degradation, an equal volume of skimmed milk (1 %, w/v; Oxoid), sterilized by autoclaving at 120 °C for 15 min, was added to the basal medium containing twice the normal concentration of agar; plates were examined for clearing around areas of growth for up to 14 d. Deoxyribonucleases were detected by flooding inoculated BM agar plates containing DNA (0·2 %, w/v; Sigma, calf thymus DNA) and $CaCl_2$ (0·08 %, w/v) after 4 d with 1 M-HCl and examining for zones of clearing around colonies. BM agar plates containing L-tyrosine (0·5 %, w/v) and starch (0·2 %, w/v), respectively, were examined after 14 d when zones of clearing around colonies were scored as positive. Tweens 40, 60 and 80 (Koch-Light) were added at 1 % (v/v) to BM agar containing $CaCl_2$ (0·01 %, w/v) and zones of clearing and precipitation of the calcium salts were recorded after 2 and 7 d (Jones, 1975). The casein, DNA and egg yolk plates were inoculated with a Steers inoculator (Steers *et al.*, 1959).

Enzymic tests. α-Glucosidase, β-glucosidase, β-glucuronidase, β-xylosidase and α-fucosidase were determined with chromogenic nitrophenylglycosides as described by Kilian & Bulow (1976) and β-galactosidase according to Le Minor & Ben Hamida (1962). Two d cultures were suspended in distilled water (pH 7·0) to a turbidity of a MacFarland standard no. 5 to no. 6 and 0·05 ml of this suspension was added to 0·05 ml of the enzyme substrate in a microtitre system (Sterilin). The resultant mixture was incubated at 37 °C and results were recorded after 4 and 24 h.

Using a commercial system (API-ZYM), α-galactosidase, β-glucosaminidase and α-mannosidase were determined with 6-bromo-2-naphthyl-α-D-galactopyranoside, 1-naphthyl-*N*-acetyl-β-D-glucosaminide, and 6-bromo-2-naphthyl-α-D-mannopyranoside as substrates. Tests were performed as instructed by the manufacturers and were read after 5 h incubation at 37 °C. The same system was used for the detection of alkaline and acid phosphatase, esterase (C_4), esterase (C_8), lipase (C_{14}), leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, chymotrypsin and phosphoamidase using the following substrates respectively: 2-naphthyl phosphate (pH 8·5 and pH 5·4), 2-naphthyl butyrate, 2-naphthyl caprylate, 2-naphthyl myristate, L-leucine-2-naphthylamide, L-valine-2-naphthylamide, L-cystine-di-2-naphthylamide, *N*-benzoyl-DL-arginine-2-naphthylamide, *N*-glutaryl-phenylalanine-2-naphthylamide, and naphthol-AS-BI-phosphodiamide (Buissière *et al.*, 1967).

Fermentation tests. Acid production from sugars (Tables 3 and 4) was determined using a rapid microtest adapted from Brown's (1974) procedure for *Neisseria gonorrhoeae.* The test medium consisted of 3 drops per ml of a freshly prepared buffered salt solution [BSS: K_2HPO_4, 0·04 g; KH_2PO_4, 0·01 g; KCl, 0·8 g; phenol red, 0·2 ml of a 1 % (w/v) aqueous solution; distilled water, 100 ml] containing one of the sugars, and dispensed in sterile microtitre plate wells (Sterilin). Using a 2 mm diameter loop, two loopfuls of bacteria from an overnight culture on blood agar were suspended in BSS (0·3 ml), and one drop of the resultant suspension was added to each of the wells containing a different substrate. The final concentration of the sugars (Tables 3 and 4) was 5·6 % (w/v), except for cellobiose (2·5 %, w/v), inositol (3 %, w/v), salicin (2·5 %, w/v) and starch (3 %, w/v). Whenever possible, 'AnalaR' grade sugars were used. Plates were incubated at 37 °C, and read at 4, 8 and 24 h. An inoculum control of basal medium without sugar was included for each strain in order to detect any change in pH of non-glycolytic origin.

Pilot experiments indicated that the method described above was preferable to traditional carbohydrate fermentation media, as acidification in the latter was found to depend on the rates of growth of the test strains.

Organic compounds as sole source of carbon. Strains were examined for their ability to grow on sodium acetate, citrate and malonate after 2 and 5 d using the medium and method of Gordon & Mihm (1957).

Antibiotic sensitivity studies. Sixteen antimicrobial agents (Tables 3 and 4) incorporated into BM plates were examined for their activity against test strains. With the exception of metronidazole, the data included in the computation consisted of the results obtained against only one concentration of the antimicrobial agent. In all cases, plates were inoculated with approximately 10^4 colony-forming units, using the Steers inoculator, and read after 2 d incubation. Sensitivity to optochin (ethyl hydrocuprein hydrochloride) was recorded by placing a disc (Rosco) containing 0·05 mg optochin on a BM plate inoculated with a swab and scoring zones of inhibition after 2 d as a positive result.

Tolerance to pH, temperature and chemical inhibitors. Strains were examined for their ability to grow on BM agar adjusted to pH 4·5, 5·5 and 8·0 with HCl or NaOH. Inoculated BM agar plates incubated in candle jars in incubators at 30, 37 and 45 ± 1 °C and at room temperature (20 ± 5 °C) were examined for growth after 2 and 7 d; plates incubated at 4 °C were read up to 21 d. To examine strains for their ability to survive heating, 18 h BM broth cultures were placed in a water bath at 60 ± 1 °C for 15 min and then single drops of immediately cooled suspension were added to BM agar plates which were examined for growth after 2 d.

For the inhibition tests, various concentrations of bile (Oxoid), bile salts (Oxoid), sodium chloride, sodium selenite, phenol, potassium tellurite, thallous acetate and tetrazolium (2,3,5-triphenyltetrazolium chloride) (Tables 3 and 4) were incorporated into BM agar and inoculated with a Steers replicator. The plates containing potassium tellurite were examined not only for growth but also for blackening after 2 and 5 d and were compared with negative controls. Similarly, the growth and reduction of tetrazolium was examined after 2 and 5 d.

Coding of data. Nearly all of the characters existed in one of two mutually exclusive states and were scored plus (+) or minus (−). Qualitative multistate characters, such as colony elevation, were scored plus (+) for the character state shown and minus (−) for the alternatives; quantitative multistate characters, for example, growth at various temperatures and in the presence of different concentrations of inorganic inhibitors, were scored by the additive method (Sneath & Sokal, 1974). Sensitivity to antimicrobial agents and resistance to inorganic inhibitors was scored plus (+). The $n \times t$ table contained data for 116 bacteria and 149 unit characters. The binary data were recorded on standard IBM punch cards.

Computer analysis. Data were examined using the Clustan 1A program (Wishart, 1968) on an IBM 370/180 computer using both the simple matching coefficient (S_{SM}; Sokal & Michener, 1958), which includes both positive and negative matches, and the Jaccard coefficient (S_J; Sneath, 1957), which includes positive matches only. Clustering was achieved using the unweighted average linkage (UPGMA) algorithm (Sneath & Sokal, 1974). Vigour and pattern statistics were also calculated (Wishart, 1968) and a sorted shaded diagram was prepared from the product of the pattern coefficient (D_P)/UPGMA analysis.

Preparation of unlabelled DNA. Much difficulty was experienced in lysing most of the organisms. Although not optimal, the following procedure (partly suggested by J. Schiller) gave the highest yield of whole cell DNA. Strains were grown at 37 °C for 24 h in an anaerobic incubator in six flasks each containing 500 ml peptone starch dextrose broth (Dunkelberg & McVeigh, 1969). The cultures were incubated for a further 2 h in an orbital incubator (New Brunswick) in the presence of benzylpenicillin G (1 mg ml^{-1}). The cells were then harvested, washed twice with 0·01 M-Tris/HCl pH 8·2, resuspended in 0·02 M-Tris/HCl pH 7·0 plus 1 M-sucrose, and treated with lysozyme (final concentration 0·8 mg ml^{-1}) in 0·02 M-Tris/HCl pH 7·0 for 1 h at 37 °C. After harvesting, the cells were suspended in 0·01 M-Tris/HCl plus 0·01 M-EDTA plus 0·01 M-NaCl (TES), pH 8·0, and treated for 2 h on ice with sodium dodecyl sulphate (final concentration 20 mg ml^{-1}) and proteinase K (Sigma; final concentration 0·02 mg ml^{-1}). The crude DNA was deproteinized by two phenol extractions and two extractions with isoamyl alcohol/chloroform (1:24, v/v). Sodium acetate (final concentration 0·3 M) was added and DNA was precipitated by adding 2 vol. cold ethanol. DNA was recovered, resuspended in 10 ml distilled water and left overnight at 4 °C. The DNA solution was then brought to

0·1 M-NaCl, 0·05 M-Tris/HCl pH 8·1 and 0·05 M-EDTA pH 8·1, and treated with ribonuclease A (50 mg ml^{-1}; Sigma, bovine pancreas, type 1) for 1 h at 60 °C, followed by pronase (50 mg ml^{-1}) for 1 h at 37 °C. DNA was again deproteinized by one extraction with phenol and two with isoamyl alcohol/chloroform (1:24, v/v) and finally precipitated with 2 vol. cold ethanol. Ethanol-precipitated DNA was dissolved in distilled water and dialysed for 24 h at 4 °C against 2 l 0·42 M-NaCl. The DNA concentration and purity was determined spectrophotometrically (Gilford 250 spectrophotometer) at 258 and 280 nm.

Preparation of radiolabelled DNA. Radiolabelled DNA was prepared from unsheared DNA purified by the above procedure and dialysed against 0·003 M-Tris/HCl pH 8·0 for 24 h. A 1 μg sample of DNA was dried at 37 °C in a stream of compressed air and a mixture of 9×10^2 pmol GTP and $1{\cdot}8 \times 10^3$ pmol each of ATP, CTP and [^{3}H]TTP [40 to 60 mCi $mmol^{-1}$ (1·5 to 2·2 GBq $mmol^{-1}$); New England Nuclear Corp.] was dried under vacuum. The nucleotides were redissolved in 10 μl of a 10 × salt mixture consisting of 100 μl 1 M-Tris/HCl containing 0·1 M-$MgCl_2$ (pH 8), 100 μl bovine serum albumin (1 mg ml^{-1}) and 1·4 μl 2-mercaptoethanol and added to the dry DNA. Sterile distilled water was added to make 100 μl. After 10 min preincubation at 15 °C, 10 to 20 units of DNA polymerase I (Boehringer, grade 1) and 4 to 6 units of deoxyribonuclease (Sigma, beef pancreas) were added. The reaction was allowed to proceed at 15 °C for 90 min before it was terminated by adding 40 μl 0·5 M-EDTA. The reaction mixture was then extracted twice with chloroform/isoamyl alcohol (24:1, v/v) and loaded on a Sephadex G-75 column (0·6 × 7·5 cm). The column was eluted with 1 mM-Tris/HCl containing 0·25 M-EDTA (pH 8), and 25 fractions of 0·3 ml were collected. A 5 μl sample of each fraction was spotted on to a Whatman 3MM filter paper disc, precipitated with cold trichloroacetic acid (TCA) and counted in 5 ml of a toluene-based scintillation fluid in a liquid scintillation counter (Packard Instrument Co.). Fractions containing the highest specific activity (approximately 10^9 c.p.m. mg^{-1}) were pooled. The labelled DNA was then sheared as described previously and stored in 0·4 M-NaCl at −20 °C.

DNA reassociation and S1 endonuclease assay. Hybrid DNA duplexes were prepared and analysed with the single strand-specific endonuclease S1 by a modification of the method of Crosa *et al.* (1973). Reassociation mixtures (final volume 0·1 ml) contained 0·5 to 1·0 μg radiolabelled DNA, 15 mg unlabelled DNA and 0·42 M-NaCl. These mixtures were boiled for 11 min and incubated for 16 h at 60 or 67 °C. After incubation, 0·9 ml 0·42 M-NaCl was added to each mixture, and four 0·2 ml samples were removed and transferred to tubes containing 0·8 ml of reaction mixture such that the final mixture contained 0·1 mM-$ZnSO_4$, 0·15 M-NaCl, 0·03 M-sodium acetate buffer pH 4·5, and 20 μg sheared denatured DNA ml^{-1} (Sigma, type V, calf thymus). Two tubes from each reassociation were treated with 25 units of S1 (Sigma) for 20 min at 50 °C. The other two tubes were treated in a similar manner but without the addition of S1. The S1 reaction was terminated and the DNA duplexes were precipitated by chilling in an ice bath and adding 0·3 ml cold TCA and 40 μl 2 mM-DNA (Sigma, type III, herring sperm). The TCA-precipitated duplexes were collected on membrane filters (type HA2500; Millipore) and dried at 70 °C. The radioactivity associated with each filter was determined as previously described. The degree of polynucleotide sequence homology was calculated by determining the ratio between the average counts in the S1-treated and untreated samples after subtraction of background counts. Results were then normalized to the homologous reassociation values after correction for self-reassociation of the labelled DNA. Self-reassociation varied between 9 and 19 %. All experiments were carried out at least twice.

DNA base composition. Unlabelled DNA was prepared as described above and the mol % GC was determined as described by Mandel *et al.* (1968). Unsheared DNA (0·4 mg) was centrifuged in a Beckman model E ultracentrifuge at 44770 rev. min^{-1} for 18 h at 24 °C. Buoyant density and mol % GC were calculated as indicated by Mandel *et al.* (1968), using *Micrococcus luteus* (buoyant density 1·731 g cm^{-1}) as a reference.

RESULTS

Numerical study

Clustering of strains using the D_P coefficient with the UPGMA algorithm

The classification based on the pattern coefficient/UPGMA analysis is described in detail since it makes allowances for differences in growth rates, times of incubation and such factors which normally distort similarity values (Sneath, 1968).

All but 12 of the 116 strains were recovered in eight major (4 to 29 strains) and nine minor clusters (Fig. 1). The clusters, most of which were distinct and homogeneous, were named after the type or authentic named strains found within them (Table 1). Most of the isolates from the vagina were recovered in the related clusters 8 and 9, and the majority of the remainder were recovered in cluster 4.

The five strains in cluster 1 fell into two distinct subclusters. Subcluster 1a contained

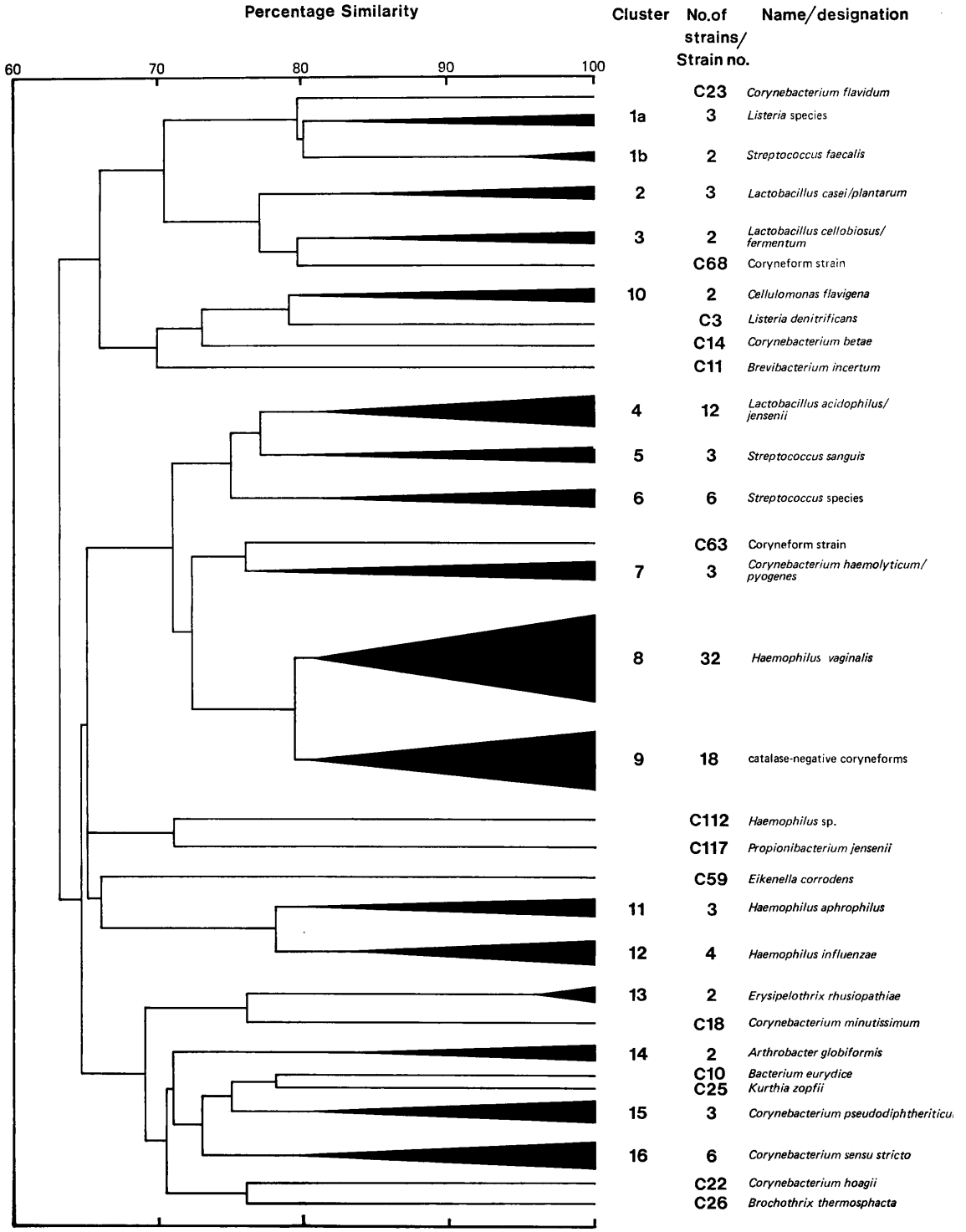

Fig. 2. A simplified dendrogram showing the relationships between clusters based on the S_{SM} coefficient and unweighted average linkage clustering (UPGMA).

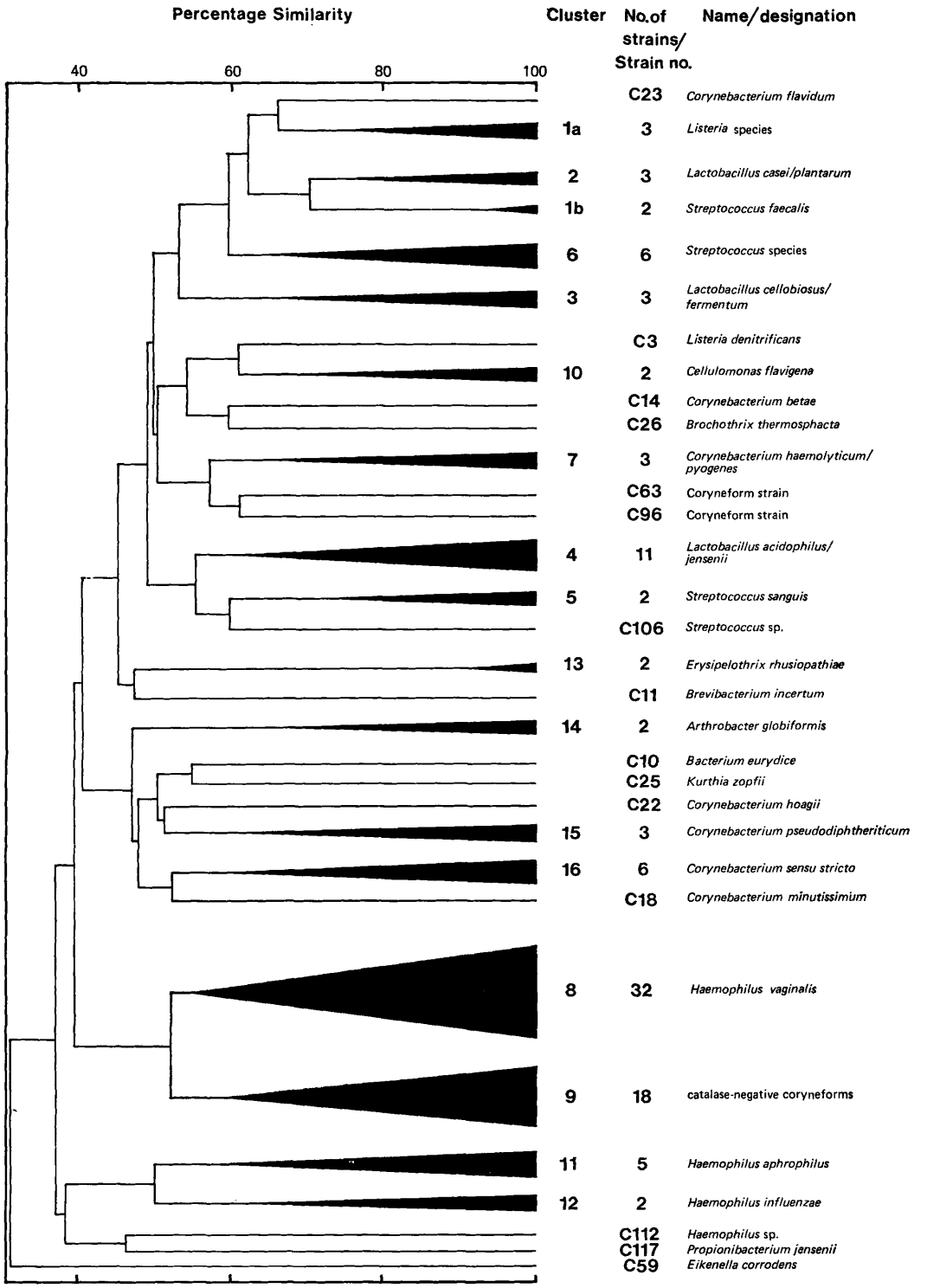

Fig. 3. A simplified dendrogram showing the relationships between clusters based on the S_J coefficient and unweighted average linkage clustering (UPGMA).

Listeria monocytogenes C1 and C2, which were closely related, and *Listeria grayi* C4. The two strains of *Streptococcus faecalis* (C34 and C35) formed subcluster 1b and they also shared quite a high affinity with *Lactobacillus casei* C27 and C31 in cluster 2.

Clusters 2, 3 and 4 contained reference strains of *Lactobacillus*. Cluster 2 consisted of the two *L. casei* strains, *Lactobacillus plantarum* C28 and the vaginal isolate C68 and shared a relatively high affinity with cluster 3 which accommodated *Lactobacillus cellobiosus* C33 and *Lactobacillus fermentum* C32. Cluster 4, the largest *Lactobacillus* taxon, contained eight vaginal isolates, the duplicate cultures of *Lactobacillus jensenii* (C29 and C29D) and, on the periphery of the cluster, *Lactobacillus acidophilus* C30. The strains in cluster 4 shared a higher intergroup similarity with some of the streptococci in clusters 5 and 6 than with the lactobacilli of clusters 2 and 3 (Fig. 1). Cluster 5 comprised *Streptococcus sanguis* C39, *Streptococcus* M (C40) and vaginal isolate C96, while seven streptococci, including *Streptococcus agalactiae* C51, *Streptococcus equisimilis* C38 and *Streptococcus pyogenes* C36, formed cluster 6.

The two largest clusters, 8 and 9, contained strains which shared a relatively high affinity with one another (Fig. 1). Cluster 8 consisted of 29 vaginal isolates including duplicate cultures of the type strain of *Haemophilus vaginalis* (C43 and C43D). Although these isolates formed a compact cluster there was some evidence that they fell into two related subclusters containing 16 and 11 strains, respectively (Fig. 1). Strains C50 and C86 did not fall into either of the subgroups. Cluster 9 was also homogeneous and contained 17 vaginal isolates, an isolate from a penile ulcer (C100) and one received as a *Streptococcus* strain (C108). Vaginal isolate C63, which formed a single member cluster, was loosely associated with clusters 8 and 9. Cluster 7, which contained *Corynebacterium haemolyticum* C17 and C17D and *Corynebacterium pyogenes* C21, showed its closest affinity with clusters 8 and 9.

Cluster 10 comprised *Cellulomonas flavigena* C12 and C13 and was sharply separated from the other clusters. *Listeria denitrificans* C3 shared a relatively high similarity with the two cellulomonads and, to a lesser extent, an affinity also existed between them and *Brevibacterium incertum* C11 and *Corynebacterium betae* C14 which formed single member clusters.

Most of the reference *Haemophilus* strains fell into the related clusters 11 and 12 (Fig. 1). Cluster 11 contained *Haemophilus aphrophilus* C109 and C113 and *Actinobacillus actinomycetemcomitans* C115, while cluster 12 comprised *Haemophilus influenzae* C110, *Haemophilus parainfluenzae* C116 and *Haemophilus paraphrophilus* C111 and C114. *Haemophilus* strain C112 formed a single member cluster.

Clusters 13 and 14 showed little similarity to one another or to any of the other clusters. The former consisted of *Erysipelothrix rhusiopathiae* C5 and C6; the latter contained *Arthrobacter globiformis* C7 and C8.

Clusters 15, 16 and 17 contained reference strains of *Corynebacterium* and formed a loose aggregate taxon (Fig. 1). The first contained *Corynebacterium pseudodiphtheriticum* C125 and skin isolates C126 and C127, while *Corynebacterium bovis* C19 and *Corynebacterium flavidum* C23 were loosely associated in cluster 17. Cluster 16 accommodated *Corynebacterium diphtheriae* C15 and C16, *Corynebacterium pseudotuberculosis* C20, *Corynebacterium xerosis* C124 and an isolate from the skin of a leprosy patient (C24).

The remaining reference strains, *Bacterium eurydice* C10, *Brochothrix thermosphacta* C26, *Corynebacterium hoagii* C22, *Corynebacterium minutissimum* C18, *Eikenella corrodens* C59, *Kurthia zopfii* C25 and *Propionibacterium jensenii* C117, showed little similarity with one another or with any of the clusters and formed single member clusters.

Clustering of strains using the S_{SM} and S_J coefficients with the UPGMA algorithm

Cluster composition was only marginally affected when the S_J and S_{SM} coefficients were used with the UPGMA algorithm, though some of the clusters were arranged differently (Figs 2 and 3). In each of these analyses clusters 3, 4, 7, 10, 13, 14 and 15 were recovered in their entirety.

The classification based on the S_{SM}/UPGMA analysis differed from the D_P/UPGMA analysis only in the clustering behaviour of a few individual strains (cf. Fig. 1 and 2). Thus, the vaginal isolate C68 was no longer included in cluster 2 but formed a single member cluster, *Streptococcus* strain C106 was on the periphery of cluster 4 as opposed to cluster 6, while *Corynebacterium bovis* C19 joined cluster 16 leaving *Corynebacterium flavidum* C23 as a single member cluster. In both the S_{SM} and S_J analyses *Haemophilus vaginalis* C47 was found in cluster 9 as opposed to cluster 8, while the reverse was true for vaginal isolates C67 and C70.

In the S_J/UPGMA analysis the strains in cluster 1 were more closely related to those in cluster 2 than was apparent in the other analyses (Fig. 3) and *Corynebacterium flavidum* C23 showed a loose relationship with the listeriae in subcluster 1a. In this analysis, cluster 7 showed a closer affinity to the *Lactobacillus* and *Streptococcus* clusters than to the *Haemophilus vaginalis* strains of clusters 8 and 9. Vaginal isolate C68 showed its highest affinity with the lactobacilli in cluster 3, while coryneform strain C96 and *Streptococcus* strain C106, originally in clusters 5 and 6, respectively, formed single member clusters. *Haemophilus parainfluenzae* C116 and *Haemophilus paraphrophilus* C114 were transferred to cluster 11 from cluster 12.

Reproducibility of results

Inclusion of duplicate strains (Table 1) in the analysis enabled experimental test error to be estimated. The probability (p) of an erroneous result averaged 6·5 %, equal to an observed S_{SM} value of about 87 % between duplicate cultures. A small number of tests was responsible for most of the test error. These tests were those for colony morphology, haemolysis, growth in the presence of phenol (0·1 %, w/v), tetracycline (0·5 mg l^{-1}), thallous acetate (0·1 %, w/v) and metronidazole (32 mg l^{-1}), acid production from mannose and reaction in litmus milk. Some tests were found to be insufficiently reproducible and the results of them were not included in the data matrix. These tests were acid production from glycogen, sensitivity to sulphamethoxazole (128 mg l^{-1}) and the Gram reaction, which depended on the medium used and on the age of the culture. However, with the exception of the *Haemophilus* strains, *Actinobacillus actinomycetemcomitans* and *Eikenella corrodens*, all of the test strains were uniformly or partially Gram-positive in their early-exponential phase on Loeffler's inspissated serum.

Characterization of the major and minor clusters

The properties of the major and minor clusters are given in Tables 3 and 4, respectively.

The strains in cluster 8 (*Haemophilus vaginalis*) were facultatively anaerobic, non-motile, Gram-negative to Gram-variable rods measuring 0·3 to 2 μm and were negative for the benzidine, catalase, indole, nitrate, ornithine decarboxylase, oxidase, phenylalanine deaminase and Voges–Proskauer tests. They grew optimally at 37 °C and at pH 6·5 to 7·0, but usually not below pH 6·0, and formed colourless, translucent, smooth colonies with a diameter of 0·5 mm after 24 h on blood agar and showed diffuse β-haemolysis on rabbit blood and, infrequently, on horse blood. Acid but not gas was formed from glucose and maltose, acid phosphatase, α-glucosidase and leucine aminopeptidase were produced, hippurate was degraded, starch was uniformly hydrolysed but variable reactions were noted on egg yolk medium, litmus milk and with the formation of α- and β-galactosidase. Most strains were sensitive to the antibiotics tested, to hydrogen peroxide and to the inorganic inhibitors (Table 3).

The isolates in cluster 9 (unclassified coryneforms) could be distinguished from those in cluster 8 by a number of tests of presumptive diagnostic importance (Table 3). Thus, they formed smaller colonies, grew on nutrient agar, were α-haemolytic and grew in BM agar containing bile (0·5 %, v/v), sodium chloride (2 %, w/v) or trimethoprim (5 mg l^{-1}).

(*continued on p.* 386)

Table 3. *Percentage frequencies of positive characters found in the major clusters*

	Listeria species/ *Strep. faecalis*	*Lactobacillus casei/plantarum*	*Lactobacillus acidophilus/ jensenii*	*Streptococcus* species	*Haemophilus vaginalis*	Unclassified coryneforms	*Haemophilus influenzae*	*Corynebacterium sensu stricto*
Cluster no.	1	2	4	6	8	9	12	16
No. of strains	5	4	10	7	29	19	4	5
Colonial characters:								
Margin entire	100	25	10	100	100	100	75	80
Elevation convex	100	100	80	100	90	95	75	100
Elevation 'fried egg'	0	25	60	0	0	5	0	0
Colonies translucent	0	50	100	14	86	84	100	20
Colonies leave impression in agar	20	75	100	57	52	26	0	20
Browning of blood	100	100	100	100	93	84	100	20
α-Haemolysis	0	75	40	43	7	84	0	40
β-Haemolysis	60	25	60	43	90	5	0	20
Homogeneous growth in broth	100	100	50	57	7	58	25	80
Micromorphological and staining characters:								
Rods formed	60	100	100	14	100	100	100	100
Motility	80	0	0	0	0	0	0	0
Flagella	60	0	0	0	0	0	0	0
Growth requirements:								
CO_2 (10 %) enhances growth	0	0	0	0	34	26	0	0
X-factor	0	0	0	0	0	0	25	0
V-factor	0	0	0	0	0	0	100	0
Growth on:								
Nutrient agar	100	100	100	100	10	79	0	100
Peptone starch dextrose agar	100	100	100	100	100	100	0	100
Biochemical tests:								
Arginine decarboxylase	40	25	70	100	3	0	0	0
Benzidine	60	0	0	0	0	0	75	100
CAMP	0	0	0	14	0	0	0	0
Catalase	60	0	0	0	0	0	100	100
Hippurate hydrolysis	100	75	10	14	86	44	0	20
H_2O_2 inhibition	0	25	0	0	100	95	100	20
H_2S	20	0	0	0	0	5	25	20
Indole	0	0	0	0	0	0	25	0
Litmus milk acid	100	100	30	43	52	26	0	0
Litmus milk alkaline	0	0	60	14	45	74	100	100
Litmus milk coagulation	60	100	50	71	41	26	0	20
Litmus milk digestion	20	25	10	29	0	5	0	20
Litmus milk peptonization	0	0	10	29	10	11	0	20
Litmus milk reduction	100	100	50	14	7	5	0	0
Methyl red	100	100	100	100	100	100	75	100
NH_3 from peptone	0	50	30	0	7	5	100	80
$NO_3 \rightarrow NO_2$	0	0	0	0	0	0	100	60
$NO_3 \rightarrow N_2$	0	0	0	0	0	0	0	20
Ornithine decarboxylase	0	0	0	0	0	0	25	0
Oxidase	0	0	0	14	0	0	100	0
Urease	0	0	0	0	3	0	75	20
Voges–Proskauer	100	50	10	57	0	0	0	20
Degradation tests:								
Aesculin	100	75	100	0	3	0	0	0
Casein	100	100	90	100	79	26	25	40
DNA	20	100	50	57	3	0	0	40
Egg yolk cleared	0	25	0	0	48	0	0	0
Egg yolk precipitation	20	50	40	14	76	16	0	20

Egg yolk iridescence	0	25	0	0	62	16	0	60
Gelatin	20	50	80	0	3	5	50	20
Starch	0	25	30	100	97	95	100	100
Tween 40 clearing	100	100	10	29	0	0	100	0
Tween 40 precipitation	0	0	0	57	31	5	0	60
Tween 60 clearing	100	75	0	57	0	0	100	0
Tween 60 precipitation	40	50	10	71	21	11	0	60
Tween 80 clearing	0	25	0	57	6	0	0	0
Tween 80 precipitation	0	0	0	14	6	0	25	20
Tyrosine	40	0	0	0	0	0	0	0
Enzymic tests:								
Acid phosphatase	100	100	50	86	93	63	100	100
Alkaline phosphatase	100	100	100	86	66	68	100	60
Chymotrypsin	60	25	0	14	0	0	0	0
Cystine aminopeptidase	20	50	0	0	3	0	0	40
Esterase C_4	100	75	50	29	21	5	25	100
Esterase C_8	100	75	40	14	31	21	0	100
α-Fucosidase	0	50	0	0	0	16	0	0
α-Galactosidase	0	75	10	0	45	11	25	0
β-Galactosidase	40	100	20	57	66	21	50	0
β-Glucosaminidase	20	0	0	0	14	11	0	0
α-Glucosidase	100	100	100	100	100	84	50	60
β-Glucosidase	100	100	80	100	0	21	0	20
β-Glucuronidase	0	0	10	14	0	0	0	0
Leucine aminopeptidase	60	100	80	100	93	95	100	60
Lipase C_{14}	0	25	0	14	0	5	0	20
α-Mannosidase	0	0	0	0	24	16	0	0
Phosphoamidase	80	100	20	0	7	11	100	40
Trypsin	0	0	0	0	0	0	0	40
Valine aminopeptidase	0	75	10	0	3	0	0	0
β-Xylosidase	0	0	0	0	0	11	0	0
Acid from:								
L-Arabinose	0	100	0	57	45	53	0	0
Adonitol	0	0	0	14	7	11	0	0
Cellobiose	100	75	40	71	0	0	0	0
Dulcitol	20	50	0	29	34	37	25	20
Fructose	100	100	100	100	79	89	25	100
Galactose	60	100	20	57	38	16	0	80
Glucose	100	100	100	100	100	100	100	100
Glycerol	80	100	0	57	3	37	50	40
Inositol	40	50	10	0	0	0	0	0
Lactose	100	100	20	71	17	21	50	0
Maltose	100	100	100	100	97	100	25	80
Mannitol	60	100	20	29	0	0	0	0
Mannose	100	100	60	100	10	26	75	100
Melibiose	0	50	0	14	0	5	0	0
Raffinose	0	25	10	43	0	0	50	0
Rhamnose	80	50	0	0	0	16	25	0
Salicin	100	100	50	71	0	32	0	0
Sorbitol	60	50	0	43	3	16	0	0
Sorbose	0	0	0	0	6	16	0	0
Starch	100	100	90	100	97	95	25	60
Sucrose	100	100	100	100	17	74	50	40
Trehalose	100	100	100	86	24	21	50	20
Xylose	60	100	0	43	10	63	0	0
Growth on sole carbon source:								
Sodium citrate	40	0	0	0	0	0	0	0
Sensitivity to antimicrobial agents:								
Ampicillin (0·2 μg ml^{-1})	40	0	80	100	97	74	25	60
Bacitracin (2000 U ml^{-1})	0	75	100	57	100	100	0	100
Cloxacillin (5 μg ml^{-1})	60	75	100	86	100	100	0	100
Colistine (50000 U ml^{-1})	0	0	0	0	0	0	100	0
Erythromycin (2 μg ml^{-1})	100	100	100	100	100	89	75	100

Table 3. (*cont.*)

	Listeria species/ *Strep. faecalis*	*Lactobacillus casei/plantarum*	*Lactobacillus acidophilus/ jensenii*	*Streptococcus* species	*Haemophilus vaginalis*	Unclassified coryneforms	*Haemophilus influenzae*	*Corynebacterium sensu stricto*
Kanamycin (25 μg ml^{-1})	60	0	80	14	100	100	0	80
Lincomycin (2 μg ml^{-1})	0	75	90	86	100	95	0	100
Metronidazole (32 μg ml^{-1})	0	0	0	0	45	0	0	0
Metronidazole (128 μg ml^{-1})	0	0	0	0	86	37	0	0
Nitrofurantoin (100 μg ml^{-1})	0	0	0	0	93	32	75	0
Novobiocin (2 μg ml^{-1})	0	75	90	57	34	95	25	80
Optochin	0	0	0	0	3	0	0	0
Penicillin (0·5 μg ml^{-1})	100	100	100	100	100	100	75	80
Streptomycin (6 μg ml^{-1})	40	0	60	29	93	32	100	100
Tetracycline (0·5 μg ml^{-1})	0	0	40	43	41	84	0	20
Trimethoprim (5 μg ml^{-1})	0	0	0	29	97	11	0	60
Vancomycin (5 μg ml^{-1})	100	0	0	100	100	100	0	100
Growth at:								
pH 4·5	40	100	40	0	0	0	0	0
pH 5·5	100	100	100	57	3	68	75	80
pH 8·0	100	100	60	100	62	95	100	40
4 °C	100	25	0	14	0	0	0	0
20 °C	100	75	10	86	0	11	0	80
30 °C	100	100	90	100	66	100	100	100
45 °C	100	75	50	57	0	5	0	20
After heating at 60 °C for 15 min	100	50	0	0	0	0	0	0
Growth in the presence of:								
0·5 % (v/v) Bile	100	100	100	100	10	100	100	100
0·15 % (w/v) Bile salts	60	100	0	0	0	0	100	0
0·1 % (w/v) Phenol	100	100	100	100	21	100	25	100
0·001 % (w/v) Potassium tellurite	100	100	100	100	41	95	75	100
0·01 % (w/v) Potassium tellurite	100	100	40	100	0	0	25	100
0·05 % (w/v) Potassium tellurite	100	0	0	29	0	0	0	40
2 % (w/v) Sodium chloride	100	100	50	71	10	95	0	100
6·5 % (w/v) Sodium chloride	100	0	0	0	0	0	0	100
10 % (w/v) Sodium chloride	100	0	0	0	0	0	0	20
0·4 % (w/v) Sodium selenite	60	25	0	0	0	0	0	0
0·01 % (w/v) Tetrazolium	100	100	100	100	3	37	50	60
0·1 % (w/v) Tetrazolium	100	25	70	71	0	0	0	0
0·01 % (w/v) Thallous acetate	100	100	100	100	45	42	100	0
0·1 % (w/v) Thallous acetate	60	100	80	100	10	16	100	0
Reduction of:								
0·001 % (w/v) Potassium tellurite	100	100	20	100	7	58	75	100
0·01 % (w/v) Potassium tellurite	100	100	20	100	0	0	25	100
0·05 % (w/v) Potassium tellurite	100	0	0	29	0	0	0	40
0·01 % (w/v) Tetrazolium	80	100	40	100	0	0	50	40
0·1 % (w/v) Tetrazolium	100	0	70	71	0	0	0	0

All strains grew anaerobically. None gave pigmented colonies, produced lysine decarboxylase or phenylalanine deaminase, degraded serum or grew on acetate or malonate as sole carbon source.

Molecular characterization of the strains

The mol % GC was determined for five strains (Table 5). The *H. vaginalis* strains (cluster 8) and one strain of cluster 9 (unclassified coryneforms) had % GC values between 41·8 and 43·4. Strain ITG 900 (cluster 9), however, differed from the other organisms by about 10 mol % GC, making a genetic relationship highly unlikely.

Table 6 gives the percentage homology of DNA from 14 coryneform strains to DNA from *H. vaginalis* ATCC 14018 at 60 °C and at the more stringent temperature of 68 °C. Table 6

(*continued on p.* 389)

Table 4. *Distribution of positive characters to the minor clusters*

	Lactobacillus cellobiosus/ fermentum	*Streptococcus sanguis*	*Corynebacterium haemolyticum/ pyogenes*	*Cellulomonas flavigena*	*Haemophilus aphrophilus*	*Erysipelothrix rhusiopathiae*	*Arthrobacter globiformis*	*Corynebacterium pseudodiphtheriticum*	*Corynebacterium bovis/flavidum*
Cluster no.	3	5	7	10	11	13	14	15	17
No. of strains	2	3	2	2	3	2	2	3	2
Colonial characters:									
Margin entire	2	1	2	2	3	2	2	3	1
Elevation convex	2	1	1	2	3	0	2	3	2
Elevation 'fried egg'	0	0	0	0	0	2	0	0	0
Colonies pigmented	0	0	0	2	0	0	0	0	0
Colonies translucent	2	3	1	2	1	2	0	0	0
Colonies leave impression in agar	0	1	1	0	0	2	0	0	0
Browning of blood	0	3	1	0	3	2	2	3	1
α-Haemolysis	0	0	0	0	0	0	0	1	1
β-Haemolysis	1	3	2	0	0	2	2	0	0
Homogeneous growth in broth	2	0	2	2	0	2	2	3	2
Micromorphological and staining characters:									
Rods formed	2	1	3	2	3	2	2	3	2
Motility	0	0	1	1	0	0	2	0	0
Flagella	0	0	0	1	0	0	1	0	0
Growth requirements:									
Anaerobic growth	2	0	2	0	3	2	1	1	2
Growth on:									
Nutrient agar	2	3	2	2	2	0	2	3	2
Peptone starch dextrose agar	2	3	2	1	3	2	2	3	2
Biochemical tests:									
Arginine decarboxylase	2	0	0	0	0	0	0	0	1
Benzidine	0	0	0	2	2	0	2	0	2
Catalase	0	0	0	2	1	0	2	3	2
Hippurate hydrolysis	0	0	1	0	0	0	0	1	2
H_2O_2 inhibition	0	1	1	0	3	0	0	1	0
H_2S	0	0	0	1	1	2	2	2	0
Litmus milk acid	2	3	2	2	0	2	1	0	1
Litmus milk alkaline	0	0	0	0	2	0	1	3	1
Litmus milk coagulation	1	3	2	1	1	2	0	3	1
Litmus milk digestion	1	1	0	0	1	0	0	2	0
Litmus milk peptonization	1	1	1	1	1	0	0	3	0
Litmus milk reduction	0	3	2	1	0	2	0	0	1
Methyl red	2	3	2	2	3	1	0	0	2
NH_3 from peptone	0	0	0	2	3	0	2	1	1
$NO_3 \rightarrow NO_2$	0	1	0	2	3	0	1	3	0
$NO_3 \rightarrow N_2$	0	0	0	0	1	0	0	1	0
Ornithine decarboxylase	0	0	0	0	0	0	0	0	0
Oxidase	0	0	1	1	0	0	1	1	0
Phenylalanine deaminase	0	0	0	0	2	0	0	1	0
Urease	0	0	0	0	0	0	0	1	0
Voges–Proskauer	0	0	0	0	0	0	0	0	2
Degradation tests:									
Aesculin	1	1	0	2	0	0	1	0	0
Casein	0	3	1	1	0	0	0	1	1
DNA	2	1	2	0	0	2	2	2	0
Egg yolk cleared	0	0	1	0	0	0	0	0	0
Egg yolk precipitation	1	0	2	0	0	0	0	0	1

Table 4. (*cont.*)

	Lactobacillus cellobiosus/*fermentum*	*Streptococcus sanguis*	*Corynebacterium haemolyticum*/*pyogenes*	*Cellulomonas flavigena*	*Haemophilus aphrophilus*	*Erysipelothrix rhusiopathiae*	*Arthrobacter globiformis*	*Corynebacterium pseudodiphtheriticum*	*Corynebacterium bovis*/*flavidum*
Egg yolk iridescence	0	0	1	0	0	0	0	0	1
Gelatin	0	0	1	1	2	1	2	2	0
Serum digestion	0	0	1	0	0	0	1	2	0
Starch	0	3	2	2	3	0	2	1	2
Tween 40 clearing	1	0	0	1	3	2	0	0	1
Tween 40 precipitation	0	0	1	0	0	0	2	2	1
Tween 60 clearing	1	0	0	1	3	0	0	0	1
Tween 60 precipitation	0	0	2	1	0	0	2	1	2
Tween 80 clearing	1	0	0	1	0	0	1	0	1
Tween 80 precipitation	0	0	0	0	0	0	2	3	1
Tyrosine	0	0	0	0	0	0	0	3	1
Enzymic tests:									
Acid phosphatase	2	1	2	1	3	2	2	3	2
Alkaline phosphatase	2	2	2	2	3	2	2	3	2
Chymotrypsin	0	2	0	2	0	2	0	0	1
Cystine aminopeptidase	1	1	2	1	0	0	1	1	0
Esterase C_4	2	1	2	2	2	2	2	3	2
Esterase C_8	2	1	2	2	0	2	2	3	2
α-Fucosidase	0	1	2	0	0	0	0	0	0
α-Galactosidase	2	1	2	1	1	0	2	0	0
β-Galactosidase	2	3	2	2	2	0	1	0	2
β-Glucosaminidase	0	0	1	2	0	2	0	0	0
α-Glucosidase	0	2	2	2	2	0	2	0	1
β-Glucosidase	1	3	0	2	0	0	2	1	2
β-Glucuronidase	0	1	1	2	0	0	0	0	0
Lipase C_{14}	0	0	0	0	0	0	0	3	1
α-Mannosidase	0	1	0	0	0	0	1	0	0
Phosphoamidase	1	0	0	0	0	1	1	1	1
Trypsin	0	0	1	0	0	0	2	2	1
Valine aminopeptidase	1	1	0	0	0	0	0	1	0
β-Xylosidase	1	1	0	2	0	0	2	0	0
Acid from:									
L-Arabinose	1	3	2	2	1	1	1	0	0
Cellobiose	1	0	0	1	0	0	0	0	0
Dulcitol	2	2	0	0	1	0	0	0	0
Fructose	2	3	2	1	3	2	1	0	2
Galactose	2	2	2	2	2	0	0	0	2
Glucose	2	3	2	2	3	3	1	0	2
Glycerol	0	1	1	1	2	0	0	1	0
Inositol	1	0	1	0	1	0	0	2	1
Lactose	2	3	2	1	3	2	0	0	1
Maltose	2	3	2	2	3	2	0	0	2
Mannitol	1	0	0	1	1	0	0	0	0
Mannose	2	3	2	2	3	1	0	0	2
Melibiose	2	1	0	1	0	0	0	0	0
Raffinose	2	2	0	0	2	0	0	0	0
Salicin	1	0	0	2	2	0	0	0	2
Sorbitol	0	0	1	0	1	0	0	0	0
Sorbose	0	0	0	0	0	0	0	0	0
Starch	0	3	2	2	3	0	0	0	1
Sucrose	1	3	1	2	3	0	1	0	2
Trehalose	1	0	2	1	2	0	0	0	2
Xylose	1	3	1	2	3	0	0	0	0

Sensitivity to antimicrobial agents:									
Ampicillin (0·2 μg ml^{-1})	1	3	1	0	0	2	1	1	0
Bacitracin (2000 U ml^{-1})	2	3	2	2	0	0	2	3	2
Cloxacillin (5 μg ml^{-1})	0	3	2	2	0	2	2	3	2
Colistine (50000 U ml^{-1})	0	0	0	0	2	0	0	0	0
Erythromycin (2 μg ml^{-1})	2	3	2	2	2	2	2	2	2
Kanamycin (25 μg ml^{-1})	2	0	2	1	3	0	2	2	2
Lincomycin (2 μg ml^{-1})	2	3	2	1	0	2	2	1	1
Metronidazole (32 μg ml^{-1})	0	0	0	1	3	0	0	0	0
Metronidazole (128 μg ml^{-1})	0	0	0	0	3	0	0	0	0
Nitrofurantoin (100 μg ml^{-1})	0	1	0	0	3	0	0	0	0
Novobiocin (2 μg ml^{-1})	0	2	2	2	0	0	2	3	2
Optochin	0	0	0	1	0	0	0	0	0
Penicillin (0·5 μg ml^{-1})	2	3	2	2	1	2	2	3	1
Streptomycin (6 μg ml^{-1})	1	2	2	2	2	0	2	2	2
Tetracycline (0·5 μg ml^{-1})	0	2	0	1	0	0	1	0	0
Trimethoprim (5 μg ml^{-1})	0	1	0	2	1	0	1	0	0
Vancomycin (5 μg ml^{-1})	0	3	2	2	0	0	2	3	2
Growth at:									
pH 4·5	2	0	0	0	0	0	0	0	0
pH 5·5	2	3	0	0	0	0	1	2	2
4 °C	0	1	0	1	0	0	2	0	0
20 °C	0	2	1	2	0	2	2	3	2
45 °C	1	1	0	0	0	0	0	2	1
After heating at 60 °C for 15 min	1	0	0	2	0	2	1	0	1
Growth in the presence of:									
0·5 % (v/v) Bile	2	3	1	2	3	2	2	3	2
0·15 % (w/v) Bile salts	0	0	0	0	3	0	0	0	0
0·1 % (w/v) Phenol	2	2	2	0	2	2	0	2	2
0·001 % (w/v) Potassium tellurite	2	3	2	2	1	2	2	3	2
0·01 % (w/v) Potassium tellurite	2	2	1	0	1	2	0	3	2
0·05 % (w/v) Potassium tellurite	0	0	0	0	0	0	0	2	2
2 % (w/v) Sodium chloride	1	0	1	0	0	2	2	3	2
6·5 % (w/v) Sodium chloride	0	0	0	0	0	0	0	3	2
10 % (w/v) Sodium chloride	0	0	0	0	0	0	0	0	2
0·4 % (w/v) Sodium selenite	0	0	0	0	1	0	0	1	1
0·01 % (w/v) Tetrazolium	2	3	1	1	3	2	0	2	2
0·1 % (w/v) Tetrazolium	0	2	0	0	0	2	0	1	1
0·01 % (w/v) Thallous acetate	2	3	0	0	3	2	1	1	0
0·1 % (w/v) Thallous acetate	2	3	0	0	2	0	0	1	0
Reduction of:									
0·001 % (w/v) Potassium tellurite	2	3	2	2	1	2	2	3	2
0·01 % (w/v) Potassium tellurite	2	2	1	0	1	2	0	3	2
0·05 % (w/v) Potassium tellurite	0	0	0	0	0	0	0	2	2
0·01 % (w/v) Tetrazolium	2	0	1	1	3	0	0	2	1
0·1 % (w/v) Tetrazolium	0	1	0	0	0	2	0	1	0

All strains grew at pH 8·0 and at 30 °C and produced leucine aminopeptidase. None showed enhanced growth with CO_2, required X- or V-factor, gave a positive result in the CAMP test, produced indole, produced lysine decarboxylase, produced acid from adonitol or rhamnose, or grew on acetate, citrate or malonate as sole carbon source.

also lists the strains which showed less than 4 % homology at 60 °C to *H. vaginalis* ATCC 14018. With the exception of *H. vaginalis* T66, all strains of *H. vaginalis* tested showed a high proportion of shared nucleotide sequences with the type strain. Strains from the unclassified coryneform cluster (cluster 9) all showed less than 56 % homology at 60 °C. Table 7 shows the results of hybridization studies with radiolabelled DNA from three strains which showed little or no homology with strain ATCC 14018. These three strains belonged to three genetically different groups as shown by reciprocal hybridization experiments.

Strains ITG 75 and T66 showed 45 to 61 % homology to each other, although both

Table 5. *DNA base composition (mol % GC) of five strains*

Strain designation	Mol % GC
H. vaginalis ATCC 14018	41·8
H. vaginalis CDC 1412	42·1
H. vaginalis Edmunds T66	43·0
Coryneform (cluster 9) ITG 75	43·4
Coryneform (cluster 9) ITG 900	33·7

Table 6. *Percentage homology of DNA from strains of Haemophilus vaginalis and from other organisms to DNA from H. vaginalis* ATCC 14018

Reassociations were performed in 0·42 M-NaCl for 16 h.

	Reassociation temperature	
Source of unlabelled DNA	60 °C	68 °C
H. vaginalis CDC 1412	85	95
H. vaginalis Ellsw.	102	104
H. vaginalis ITG 879	83	80
Corynebacterium vaginale Pease 646	81	NT
H. vaginalis ITG 130	76	75
H. vaginalis ITG 836	72	NT
H. vaginalis ITG 145	81	83
H. vaginalis Edmunds T66	47	37
Coryneform (cluster 9) ITG 75	55	37
Coryneform (cluster 9) ITG 131	29	19
Coryneform (cluster 9) ITG 296	22	18
Coryneform (cluster 9) ITG 900	2	0
Coryneform (cluster 9) ITG 137	4	0
Coryneform (cluster 9) ITG 833	0	0

NT, Not tested.

The following strains showed less than 4 % homology at 60 °C: *Actinobacillus actinomycetemcomitans* NCTC 9709, *Actinomyces viscosus* BLW46, *Bifidobacterium adolescentis* ATCC 15703, *Bifidobacterium breve* ATCC 15700, *Bifidobacterium infantis* ATCC 15697, *Branhamella catarrhalis* ITG 1642, *Brevibacterium* sp. NCTC 11084, *Corynebacterium diphtheriae* NCTC 3984, *Corynebacterium haemolyticum* NCTC 8452, *Corynebacterium pyogenes* NCTC 5225, *Corynebacterium xerosis* NCTC 9755, Coryneform sp. P87, *Haemophilus aphrophilus* NCTC 5886, *Haemophilus influenzae* NCTC 7279, *Haemophilus vaginalis*-like organism RFS52, *H. vaginalis*-like organism RFS88, *Lactobacillus acidophilus* A1, *Lactobacillus casei* NCDO 243, *Lactobacillus fermentum* F1, *Lactobacillus jensenii* ATCC 25258, *Propionibacterium jensenii* NCIB 5960.

Table 7. *Percentage homology of DNA from selected strains of Haemophilus vaginalis and related strains to DNA from some coryneform organisms*

Reassociations were performed at 60 °C for 16 h in 0·42 M-NaCl.

	Source of radiolabelled DNA		
Source of unlabelled DNA	Coryneform ITG 75	*H. vaginalis* Edmunds T66	Coryneform ITG 900
H. vaginalis ATCC 14018	62	59	4
H. vaginalis CDC 1412	43	33	2
H. vaginalis Ellsw.	53	40	0
H. vaginalis ITG 145	60	49	NT
H. vaginalis Edmunds T66	45	100	0
Coryneform ITG 75	100	61	0
Coryneform ITG 131	26	23	1
Coryneform ITG 900	0	0	100
Coryneform ITG 137	NT	0	81

NT, Not tested.

strains exhibited similar values of reassociation when DNA of these strains was hybridized with radiolabelled DNA of *H. vaginalis* ATCC 14018. Strain ITG 900 yielded very low sequence relationship values for all organisms tested, with the exception of strain ITG 137.

DISCUSSION

The different taxonomic approaches used in this study yielded similar results. The numerical classification of the test strains was only affected in a minor way by the coefficients of association employed (D_P, S_J and S_{SM}) while the test error of 6·5 % was well within the guideline of Sneath & Johnson (1972). Congruence between results based on different numerical techniques is a hallmark of a good taxonomy (Austin & Colwell, 1977; Goodfellow *et al.*, 1978) and means that the numerical classification can be interpreted with confidence.

The genetic and numerical data strongly support the view that *H. vaginalis* is a good species but it should not be retained in the genus *Haemophilus* (Lapage, 1974). It is also clear that this taxospecies has little in common with other genera with which it is occasionally associated. Moreover, the classification of *H. vaginalis* either in the genus *Corynebacterium* or in *Haemophilus* is not supported by antigenic (Vice & Smaron, 1973; Smaron & Vice, 1974) or biochemical (Lapage, 1974; Greenwood & Pickett, 1979) data or, in the former case, by the results of DNA base composition studies (Minnikin *et al.*, 1978). The latter technique can be effective in helping to clarify the relationships between poorly classified strains since bacteria with DNA differing by more than 10 mol % GC should not be classified in the same genus (Bradley & Mordarski, 1976; Bradley, 1980; Priest *et al.*, 1980). Thus, the loose association noted between *H. vaginalis* and cluster 7 (*Corynebacterium haemolyticum/pyogenes*) in the D_P and S_{SM}/UPGMA analyses is probably more apparent than real, for the former have a DNA base composition in the range of 41 to 43 mol % GC, whereas the value for *C. pyogenes* is 58 % (Cummins *et al.*, 1974). In the S_J/UPGMA analysis little affinity was noted between cluster 7 and *H. vaginalis* which suggests that the higher similarities in the earlier analyses were based on factors such as negative correlation.

Haemophilus vaginalis showed very little polynucleotide sequence homology with *Actinomyces*, *Bifidobacterium* or *Propionibacterium*. Strains in these genera have mol % GC values of 60 to 73 (Johnson & Cummins, 1972; Slack, 1974), 57 to 66 (Sebald *et al.*, 1965; Gasser & Mandel, 1968; Scardovi *et al.*, 1969; Rogosa, 1974) and 59 to 66 (Moore & Holdeman, 1974), respectively. *Eubacterium* is currently a 'catch-all' in need of revision but most eubacteria, unlike *H. vaginalis*, produce butyric as well as acetic acid as fermentation end-products (Holdeman & Moore, 1974; Holdeman *et al.*, 1975; Moss & Dunkelberg, 1969; Akerlund & Mårdh, 1974).

There is also little ground for considering *H. vaginalis* to be related to established Gram-negative taxa. Thus, *H. vaginalis* strains are unambiguously Gram-positive on inspissated serum (Zinnemann & Turner, 1963), form walls, septa (Reyn *et al.*, 1966) and citrate synthase (Jones & Weitzman, 1971) of a Gram-positive character and do not contain ketodeoxyoctonate (S. Morse, personal communication). In contrast, Criswell *et al.* (1971) found a trilaminar cell wall with low mucopeptide content with 11 to 14 amino acids but no teichoic acids in the type strain. In the present study, the *H. vaginalis* strains were uniformly or partially Gram-positive in early-exponential phase on Loeffler's inspissated serum and had an antibiotic sensitivity pattern found in Gram-positive bacteria. It is clear that the matter of Gram reaction awaits further study.

The clustering of most of the unclassified genital coryneforms in one phenon closely related to *H. vaginalis* was unexpected. However, hybridization experiments (Table 7) with three reference strains from cluster 9 revealed at least three genetically different groups within this cluster. Each of these strains exhibited less than 4 % polynucleotide sequence homology with any of the reference strains tested, including the *Bifidobacterium* strains and two so-called *H. vaginalis*-like strains described by Bailey *et al.* (1979). This reflects their lack of relatedness to any of these organisms. Although strains ITG 75 and T66 both showed about 60 % homology with *H. vaginalis* ATCC 14018, they did not share a high degree of sequence homology with each other, and even less with strain ITG 900. The latter, isolated from a penile ulcer, showed a significant degree of homology only to strain ITG 137, isolated

from the vagina. These strains were clearly not related either to the *H. vaginalis* strains or to the other unclassified coryneforms tested. The phenotypic numerical analysis obviously failed to recognize this differentiation among the strains of cluster 9. Due to difficulties in lysing the organisms, too few strains of cluster 9 were used in the hybridization experiments to conclude whether some of them should be incorporated in a new species of the same genus as *H. vaginalis* or whether they constitute aberrant strains. Additional data on a larger number of strains should clarify this problem.

The numerical data are in keeping with previous studies which indicated that the genera *Corynebacterium*, *Lactobacillus*, *Listeria* and *Streptococcus* are heterogeneous. There is a strong case for restricting the genus *Corynebacterium* to bacteria that have *meso*-diaminopimelic acid, arabinose and galactose in their walls (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), a DNA base composition in the range 48 to 59 % mol GC, dihydrogenated menaquinones with eight or nine isoprene units and relatively low molecular weight mycolic acids (22 to 38 carbons) (Minnikin *et al.*, 1978). Typical strains of *Corynebacterium bovis*, *C. diphtheriae*, *C. pseudotuberculosis* and *C. xerosis*, representatives of which were recovered in cluster 16, are covered by this definition but *C. betae*, *C. haemolyticum*, *C. hoagii* and *C. pyogenes* are not.

Corynebacterium betae strains have ornithine in their walls (Keddie & Cure, 1978), lack mycolic acids and contain unsaturated menaquinones with nine isoprene units (Minnikin *et al.*, 1978); they should probably be transferred to the genus *Curtobacterium* (Keddie, 1978). Another niche is also required for strains of *C. haemolyticum* and *C. pyogenes* which have lysine, rhamnose and glucose in their peptidoglycan (Cummins & Harris, 1956), lack mycolic acids (Goodfellow *et al.*, 1976) and can be clearly distinguished from other coryneform bacteria in numerical phenetic surveys (Jones, 1978). In turn, *C. hoagii* has a wall chemotype IV, contains mycolic acids and dihydrogenated menaquinones with eight isoprene units, but unlike true corynebacteria it has a mol % GC of 68·8 (Keddie & Cure, 1978; Minnikin *et al.*, 1978) and should be included in comparative studies to determine its relationship with strains classified in the genera *Caseobacter* (Crombach, 1978*a*, *b*) and *Rhodococcus* (Goodfellow & Alderson, 1977). The *C. flavidum* and *C. minutissimum* strains should also be studied further, for in previous studies they were classified with strains in *Corynebacterium sensu stricto* (Jones, 1975, 1978; Keddie & Cure, 1978; Minnikin *et al.*, 1978).

The *Lactobacillus* reference strains were recovered as three distinct clusters which corresponded closely to the subgenera proposed by Orla-Jensen (1919) and extended by Rogosa & Sharpe (1959). Wilkinson & Jones (1977) not only obtained a similar clustering of lactobacilli but also recovered their streptococci in two distinct but related clusters. The relatively high overall similarity found between *Lactobacillus casei*, *Lactobacillus plantarum* and *Streptococcus faecalis* was also noted in the numerical classification of Jones (1975). In an examination of the protein homologies of the malic enzymes and aldolases of rods and cocci of lactic acid and related bacteria, London & Kline (1973) found a close affinity between *L. casei* and *S. faecalis*; they considered that shape was a relatively unimportant character that should not be used to separate groups considered to be closely related on other criteria.

The sharp separation of *Listeria denitrificans* from *Listeria grayi* and *Listeria monocytogenes* is in line with a number of studies based on a representative set of taxonomic criteria (Stuart & Pease, 1972; Stuart & Welshimer, 1974; Jones, 1975; Collins *et al.*, 1979). The sharp separation of *Listeria* and *Erysipelothrix*, and the recovery of *Arthrobacter*, *Brochothrix*, *Cellulomonas* and *Kurthia*, is in keeping with earlier numerical phenetic findings (Jones, 1978). The close relationship found between *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus* (Kilian, 1976) is also supported by the present numerical data.

The properties of the *H. vaginalis* strains cannot readily be compared with those described in earlier studies based on different media and methods. The present results were not always in agreement with earlier findings, particularly concerning acid production from sugars, antibiotic sensitivity patterns and the detection of β-haemolysis. The latter is a particularly

difficult test to interpret with variable results being reported on media containing blood from the same source (Leopold, 1953; Gardner & Dukes, 1955; Lutz *et al.*, 1956; Lapage, 1961; Redmond & Kotcher, 1963), the variable response probably being due to factors such as the basal medium, concentration of blood, thickness of agar, time of incubation and gaseous environment. However, several workers have reported that human and rabbit blood are better than sheep blood for detecting diffuse haemolysis (Edmunds, 1960*a*, *b*; Park *et al.*, 1968; Greenwood & Pickett, 1979), although Malone *et al.* (1975) found sheep blood satisfactory. In the present study, haemolysis was found to be one of the least reproducible tests and difficulty was experienced in distinguishing between diffuse β-haemolysis and weak α-haemolysis. Over 90 % of the strains showed a diffuse β-haemolysis after 48 h on rabbit blood and, infrequently, on horse blood.

The sugar fermentation data are in good agreement with previous findings (Dukes & Gardner, 1961; Edmunds, 1962; Dunkelberg *et al.*, 1970*a*, *b*; Greenwood & Pickett, 1979) although differences exist in the proportions of strains producing acid from arabinose, galactose and rhamnose. Contrary to the description of *H. vaginalis* in the current edition of *Bergey's Manual of Determinative Bacteriology* (Lapage, 1974), some strains produced acid from dulcitol, lactose, sorbitol and trehalose thereby confirming the observations of earlier workers (Malone *et al.*, 1975; Greenwood & Pickett, 1979) with respect to the latter two sugars.

The results of the antibiotic sensitivity studies are also in keeping with recent observations (Smith & Dunkelberg, 1977; Pheifer *et al.*, 1978) although minimal inhibitory concentrations are not comparable due to the different media and methods used. However, many of the *H. vaginalis* strains were susceptible to both streptomycin and tetracycline, in contrast to the description of the species given by Lapage (1974). With the exception of a few strains in cluster 9 the *H. vaginalis* isolates alone were susceptible to metronidazole which is generally only active against strict anaerobes (Sutter & Finegold, 1976).

In contrast to previous studies (Tarlinton & D'Abrera, 1967; Vickerstaff & Cole, 1969; Dunkelberg *et al.*, 1970*a*, *b*; Greenwood & Pickett, 1979) most of the *H. vaginalis* strains degraded casein and around half were active on litmus milk. The greater activity in these tests may have been due to the longer periods of incubation used. About half of the isolates exhibited lipase activity on egg yolk agar but, contrary to the report of Dunkelberg *et al.* (1970*a*, *b*), none grew in the presence of 0·01 % (w/v) potassium tellurite and most were inhibited by 0·001 % (w/v), a result in good agreement with an earlier report (Smith *et al.*, 1977) where strains were inhibited by minimal inhibitory concentrations of 1·56 to 12·5 mg potassium tellurite l^{-1}. Most of the isolates hydrolysed hippurate, a test considered by Greenwood & Pickett (1979) to have value in the recognition of *H. vaginalis*. Nearly all the strains produced acid phosphatase while the distribution of positive results in the glycosidase tests suggested that they might be of value in the recognition of biovars. The glycosidase tests are simple, accurate and give quick results and, together with serotyping (Edmunds, 1962), may be of value in ecological and epidemiological surveys.

In conclusion, it is clear that *H. vaginalis* forms a good taxospecies which shows little similarity to established Gram-positive and Gram-negative genera. It should be included in a new genus which possibly also contains some strains classified in cluster 9; we support the name *Gardnerella vaginalis*, as proposed by Greenwood & Pickett (1979) in honour of H. L. Gardner who was the first to describe the vaginitis associated with the organism.

The authors are indebted to S. R. Pattyn and K. K. Holmes for helpful advice, to G. Alderson for help with the computing, to B. Ward for running the model E centrifuge and to colleagues (Tables 1 and 2) for generously providing strains. The work in Seattle was supported by grant AI-100191 from the National Institute of Allergy and Infectious Diseases. P. Piot was partly supported by a NATO fellowship.

REFERENCES

AKERLUND, M. & MÅRDH, P.-A. (1974). Isolation and identification of *Corynebacterium vaginale* (*Haemophilus vaginalis*) in women with infections of the lower genital tract. *Acta obstetrica gynecologica scandinavica* **53**, 85–90.

AMIES, C. R. & GARABEDIAN, M. (1963). The bacteriology of human vaginitis. *Canadian Journal of Public Health* **54**, 50.

AUSTIN, B. & COLWELL, R. R. (1977). Evaluation of some coefficients for use in numerical taxonomy of microorganisms. *International Journal of Systematic Bacteriology* **27**, 204–210.

BAILEY, R. K., VOSS, J. L. & SMITH, R. F. (1979). Factors affecting isolation and identification of *Haemophilus vaginalis* (*Corynebacterium vaginale*). *Journal of Clinical Microbiology* **9**, 65–71.

BERGQUIST, L. M. & SEARCY, R. L. (1963). A micromethod for detection of utilization of phenylalanine by microorganisms. *American Journal of Clinical Pathology* **39**, 544–545.

BRADLEY, S. G. (1980). DNA reassociation and base composition. In *Microbiological Classification and Identification*, pp. 11–26. Edited by M. Goodfellow & R. G. Board. London: Academic Press.

BRADLEY, S. G. & MORDARSKI, M. (1976). Association of polydeoxyribonucleotides of deoxyribonucleic acids from nocardioform bacteria. In *The Biology of the Nocardiae*, pp. 310–336. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.

BROWN, W. J. (1974). Modification of the rapid fermentation tests for *Neisseria gonorrhoeae*. *Applied Microbiology* **27**, 1027–1030.

BUISSIÈRE, J., FOUCARD, A. & COLOBERT, L. (1967). Usage de substrats synthétiques pour l'étude de l'équipement enzymatique de microorganismes. *Comptes rendus hebdomadaire des séances de l'Académie des sciences* **264D**, 415–417.

CASMAN, E. P. (1947). A noninfusion blood agar base for neisseriae, pneumococci and streptococci. *American Journal of Clinical Pathology* **17**, 281–285.

CHRISTIE, R., ATKINS, N. E. & MUNCH-PETERSEN, E. (1944). A note on a lytic phenomenon shown by group B streptococci. *Australian Journal of Experimental Biology and Medical Science* **22**, 193–197.

COLLINS, M. D., JONES, D., GOODFELLOW, M. & MINNIKIN, D. E. (1979). Isoprenoid quinone composition as a guide to the classification of *Listeria*, *Brochothrix*, *Erysipelothrix* and *Caryophanon*. *Journal of General Microbiology* **111**, 453–457.

COWAN, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd edn. Cambridge: Cambridge University Press.

CRISWELL, B. S., MARSTON, J. H., STENBACK, W. A., BLACK, S. H. & GARDNER, H. L. (1971). *Haemophilus vaginalis* 594, a gram-negative organism? *Canadian Journal of Microbiology* **17**, 865–869.

CRISWELL, B. S., STENBACK, W. A., BLACK, S. H. & GARDNER, H. L. (1972). Fine structure of *Haemophilus vaginalis*. *Journal of Bacteriology* **109**, 930–932.

CROMBACH, W. H. J. (1978*a*). DNA base ratios and DNA hybridization studies of coryneform bacteria, mycobacteria and nocardiae. In *Coryneform Bacteria*, pp. 161–179. Edited by I. J. Bousfield & A. G. Callely. London: Academic Press.

CROMBACH, W. H. J. (1978*b*). *Caseobacter polymorphus* gen.nov., sp.nov., a coryneform bacterium from cheese. *International Journal of Systematic Bacteriology* **28**, 354–366.

CROSA, J. H., BRENNER, D. J. & FALKOW, S. (1973). Use of a single strand specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *Journal of Bacteriology* **115**, 904–911.

CUMMINS, C. S. & HARRIS, H. (1956). The chemical composition of the cell wall in some Gram-positive bacteria and its possible value as a taxonomic character. *Journal of General Microbiology* **14**, 583–600.

CUMMINS, C. S., LELLIOTT, R. A. & ROGOSA, M. (1974). *Corynebacterium* Lehmann and Neumann 1896. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 602–617. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

CURE, G. L. & KEDDIE, R. M. (1973). Methods for the morphological examination of aerobic coryneform bacteria. In *Sampling – Microbiological Monitoring of Environments*, pp. 123–135. Edited by R. G. Board & D. W. Lovelock. London: Academic Press.

DEIBEL, R. H. & EVANS, I. B. (1960). Modified benzidine test for the detection of cytochrome-containing respiratory systems in microorganisms. *Journal of Bacteriology* **79**, 356–360.

DUKES, C. D. & GARDNER, H. L. (1961). Identification of *Haemophilus vaginalis*. *Journal of Bacteriology* **81**, 277–283.

DUNKELBERG, W. E. (1977). *Corynebacterium vaginale*. *Sexually Transmitted Diseases* **4**, 69–75.

DUNKELBERG, W. E. & MCVEIGH, I. (1969). Growth requirements of *Haemophilus vaginalis*. *Antonie van Leeuwenhoek* **35**, 129–145.

DUNKELBERG, W. E., SKAGGS, R. & KELLOGG, D. S. (1970*a*). A study and new description of *Corynebacterium vaginale* (*Haemophilus vaginalis*). *American Journal of Clinical Pathology* **53**, 370–377.

DUNKELBERG, W. E., SKAGGS, R. & KELLOGG, D. S. (1970*b*). Method for isolation and identification of *Corynebacterium vaginale* (*Haemophilus vaginalis*). *Applied Microbiology* **19**, 47–52.

EDMUNDS, P. N. (1960*a*). *Haemophilus vaginalis*: morphology, cultural characters and viability. *Journal of Pathology and Bacteriology* **79**, 273–284.

EDMUNDS, P. N. (1960*b*). The growth requirements of *Haemophilus vaginalis*. *Journal of Pathology and Bacteriology* **80**, 325–335.

EDMUNDS, P. N. (1962). The biochemical, serological and haemagglutinating reactions of '*Haemophilus vaginalis*'. *Journal of Pathology and Bacteriology* **83**, 411–422.

GARABEDIAN, M. S. (1969). A study of *Haemophilus vaginalis* Gardner and Dukes. *Canadian Journal of Medical Technology* **31**, 144–150.

GARDNER, H. L. & DUKES, C. D. (1954). New etiologic agent in non-specific bacterial vaginitis. *Science* **120**, 853.

GARDNER, H. L. & DUKES, C. D. (1955). *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified 'non-specific' vaginitis. *American Journal of Obstetrics and Gynecology* **69**, 962–976.

GASSER, F. & MANDEL, M. (1968). Deoxyribonucleic acid base composition of the genus *Lactobacillus*. *Journal of Bacteriology* **96**, 580–588.

GOODFELLOW, M. & ALDERSON, G. (1977). The actinomycete genus *Rhodococcus*: a home for the '*rhodochrous*' complex. *Journal of General Microbiology* **100**, 99–122.

GOODFELLOW, M., COLLINS, M. D. & MINNIKIN, D. E. (1976). Thin-layer chromatographic analysis of mycolic acid and other long-chain components in whole-organism methanolysates of coryneform and related taxa. *Journal of General Microbiology* **96**, 351–358.

GOODFELLOW, M., ORLEAN, P. A. B., COLLINS, M. D. & ALSHAMAONY, L. (1978). Chemical and numerical taxonomy of strains received as *Gordona aurantiaca*. *Journal of General Microbiology* **109**, 57–68.

GORDON, R. E. & MIHM, J. M. (1957). A comparative study of some strains received as nocardiae. *Journal of Bacteriology* **73**, 15–27.

GREENWOOD, J. R. & PICKETT, M. J. (1979). Salient features of *Haemophilus vaginalis*. *Journal of Clinical Microbiology* **9**, 200–204.

HOLDEMAN, L. V. & MOORE, W. E. C. (1974). *Eubacterium*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 641–657. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

HOLDEMAN, L. V., CATO, E. P. & MOORE, W. E. C. (editors) (1975). *Anaerobe Laboratory Manual*, 3rd edn. Blacksburg, Virginia: Anaerobe Laboratory, Virginia Polytechnic Institute and State University.

HUCKER, G. L. & CONN, H. J. (1923). Methods of Gram staining. *Technical Bulletin, New York State Agricultural Experiment Station*, no. 93.

JOHNSON, J. L. & CUMMINS, C. S. (1972). Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical propionibacteria and strains of *Arachnia propionica*. *Journal of Bacteriology* **109**, 1047–1066.

JONES, D. (1975). A numerical taxonomic study of coryneform and related bacteria. *Journal of General Microbiology* **87**, 52–96.

JONES, D. (1978). An evaluation of the contributions of numerical taxonomic studies to the classification of coryneform bacteria. In *Coryneform Bacteria*, pp. 13–46. Edited by I. J. Bousfield & A. G. Callely. London: Academic Press.

JONES, D. & WEITZMAN, P. D. J. (1971). Taxonomic significance of citrate synthase. *Journal of General Microbiology* **69**, xi.

KEDDIE, R. M. (1978). What do we mean by coryneform bacteria? In *Coryneform Bacteria*, pp. 1–12. Edited by I. J. Bousfield & A. G. Callely. London: Academic Press.

KEDDIE, R. M. & BOUSFIELD, I. J. (1980). Cell wall composition in the classification and identification of coryneform bacteria. In *Microbiological Classification and Identification*, pp. 167–188. Edited by M. Goodfellow & R. G. Board. London: Academic Press.

KEDDIE, R. M. & CURE, G. L. (1978). Cell wall composition of coryneform bacteria. In *Coryneform Bacteria*, pp. 47–83. Edited by I. J. Bousfield & A. G. Callely. London: Academic Press.

KILIAN, M. (1974). A rapid method for the differentiation of *Haemophilus* strains. The porphyrin test. *Acta pathologica et microbiologica scandinavica* **B82**, 835–842.

KILIAN, M. (1976). A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. *Journal of General Microbiology* **93**, 9–62.

KILIAN, M. & BULOW, P. (1976). Rapid diagnosis of Enterobacteriaceae. I. Detection of bacterial glycosidases. *Acta pathologica et microbiologica scandinavica* **84B**, 245–251.

KOVÁCS, N. (1928). Eine vereinfachte Methode zum Nachweis der Indolbildung durch Bakterien. *Zeitschrift für Immunitätsforschung und experimentelle Therapie* **55**, 311–316.

KOVÁCS, M. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature, London* **178**, 703.

LAPAGE, S. P. (1961). *Haemophilus vaginalis* and its role in vaginitis. *Acta pathologica et microbiologica scandinavica* **52**, 34–54.

LAPAGE, S. P. (1974). *Haemophilus vaginalis* Gardner and Dukes 1955. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 368–370. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

LECHEVALIER, M. P. & LECHEVALIER, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *International Journal of Systematic Bacteriology* **20**, 435–444.

LE MINOR, L. & BEN HAMIDA, F. (1962). Avantages de la recherche de la beta-galactosidase sur celle de la fermentation de lactose en milieu complexe dans le diagnostic bactériologique, en particulier des Enterobacteriaceae. *Annales de l'Institut Pasteur* **102**, 267–277.

LEOPOLD, S. (1953). Heretofore undescribed organism isolated from the genitourinary system. *United States Armed Forces Medical Journal* **4**, 263–266.

LONDON, J. & KLINE, K. (1973). Aldolase of lactic acid bacteria: a case history in the use of an enzyme as an evolutionary marker. *Bacteriological Reviews* **37**, 453–478.

LUTZ, A., GROOTTEN, O. & WURCH, T. (1956). Etude des caractères culturaux et biochimiques de bacilles du type '*Hemophilus hemolyticus vaginalis*'. *Revue d'immunologie* **20**, 132–138.

MALONE, B. H., SCHREIBER, M., SCHNEIDER, N. J. & HOLDEMAN, L. V. (1975). Obligately anaerobic strains of *Corynebacterium vaginale* (*Haemophilus vaginalis*). *Journal of Clinical Microbiology* **2**, 272–275.

MANDEL, M., SCHILDKRAUT, C. L. & MARMUR, J. (1968). Use of CsCl density gradient analysis for determining the guanine plus cytosine content of DNA. *Methods in Enzymology* **12B**, 184–195.

MINNIKIN, D. E., GOODFELLOW, M. & COLLINS, M. D. (1978). Lipid composition in the classification and identification of coryneform and related taxa. In *Coryneform Bacteria*, pp. 85–160. Edited by I. J. Bousfield & A. G. Callely. London: Academic Press.

MOORE, W. E. C. & HOLDEMAN, L. V. (1974). *Propionibacterium*. In *Bergey's Manual of Determina-*

tive Bacteriology, 8th edn, pp. 633–641. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

MOSS, C. W. & DUNKELBERG, W. E. (1969). Volatile and cellular fatty acids of *Haemophilus vaginalis*. *Journal of Bacteriology* **100**, 544–546.

ORLA-JENSEN, S. (1919). *The Lactic Acid Bacteria*. Copenhagen: Andr. Fred. Hort & Sons.

PARK, C. H., FAUBER, M. & COOK, C. B. (1968). Identification of *Haemophilus vaginalis*. *American Journal of Clinical Pathology* **49**, 590–593.

PHEIFER, T. A., FORSYTH, P. S., DURFEE, M. A., POLLOCK, H. M. & HOLMES, K. K. (1978). Nonspecific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. *New England Journal of Medicine* **298**, 1429–1434.

PITCHER, D. G. & NOBLE, W. C. (1978). Aerobic diphtheroids of human skin. In *Coryneform Bacteria*, pp. 265–287. Edited by I. J. Bousfield & A. G. Callely. London: Academic Press.

PRIEST, F. G., GOODFELLOW, M. & TODD, C. (1980). The genus *Bacillus*: a numerical analysis. In *Aerobic Endospore-forming Bacteria: Classification and Identification*. Edited by R. C. W. Berkeley & M. Goodfellow. London: Academic Press (in the Press).

REDMOND, D. L. & KOTCHER, E. (1963). Cultural and serological studies on *Haemophilus vaginalis*. *Journal of General Microbiology* **33**, 77–87.

REYN, A., BIRCH-ANDERSEN, A. & LAPAGE, S. P. (1966). An electron microscope study of thin sections of *Haemophilus vaginalis* (Gardner and Dukes) and some possibly related species. *Canadian Journal of Microbiology* **12**, 1125–1136.

RHODES, M. E. (1958). The cytology of *Pseudomonas* spp. as revealed by a silver-plating staining method. *Journal of General Microbiology* **18**, 639–648.

ROGOSA, M. (1974). *Bifidobacterium*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 669–676. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

ROGOSA, M. & SHARPE, M. E. (1959). An approach to the classification of the lactobacilli. *Journal of Applied Bacteriology* **22**, 329–340.

SCARDOVI, V., TROVATELLI, L. D., CROCIANI, F. & SGORBATI, B. (1969). Bifid bacteria in bovine rumen. New species of the genus *Bifidobacterium*: *B. globosum* n.sp. and *B. minimale* n.sp. *Archiv für Mikrobiologie* **68**, 278–294.

SEBALD, M., GASSER, F. & WERNER, H. (1965). Teneur GC% et classification. Application au groupe des bifidobactéries et à quelques genres voisins. *Annales de l'Institut Pasteur* **109**, 251–269.

SHAW, C. & CLARKE, P. H. (1955). Biochemical classification of Proteus and Providencia cultures. *Journal of General Microbiology* **13**, 155–161.

SLACK, J. M. (1974). *Actinomyces* Harz 1877. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 660–667. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

SMARON, M. F. & VICE, J. L. (1974). Analysis of *Corynebacterium vaginale* by an immunodiffusion technique. *Applied Microbiology* **27**, 469–474.

SMITH, R. F. & DUNKELBERG, W. E. (1977). Inhibition of *Corynebacterium vaginale* by metronidazole. *Sexually Transmitted Diseases* **4**, 20–21.

SMITH, R. F., VOSS, J. L. & BAILEY, R. K. (1977). Tellurite reduction test to aid in the recognition of *Corynebacterium vaginale*. *Journal of Clinical Microbiology* **5**, 375–377.

SNEATH, P. H. A. (1957). The application of computers to taxonomy. *Journal of General Microbiology* **17**, 201–226.

SNEATH, P. H. A. (1968). Vigour and pattern in taxonomy. *Journal of General Microbiology* **54**, 1–11.

SNEATH, P. H. A. & JOHNSON, R. (1972). The influence on numerical taxonomic similarities of errors in microbiological tests. *Journal of General Microbiology* **72**, 377–392.

SNEATH, P. H. A. & SOKAL, R. R. (1974). *Numerical Taxonomy. The Principles and Practice of Numerical Classification*. San Francisco: W. H. Freeman.

SOKAL, R. R. & MICHENER, C. D. (1958). A statistical method for evaluating systematic relationships. *Kansas University Science Bulletin* **38**, 1409–1438.

STEERS, E., FOLZ, E. L. & GRAVES, B. S. (1959). An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiotics and Chemotherapy* **9**, 307–311.

STUART, C. A., VAN STRATUM, E. & RUSTIGIAN, R. (1945). Further studies on urease production by *Proteus* and related organisms. *Journal of Bacteriology* **49**, 437–444.

STUART, M. R. & PEASE, P. E. (1972). A numerical study of the relationships of *Listeria* and *Erysipelothrix*. *Journal of General Microbiology* **73**, 551–565.

STUART, S. E. & WELSHIMER, H. J. (1974). Taxonomic re-examination of *Listeria* Pirie and transfer of *Listeria grayi* and *Listeria murrayi* to a new genus *Murraya*. *International Journal of Systematic Bacteriology* **24**, 177–186.

SUTTER, V. L. & FINEGOLD, S. M. (1976). Susceptibility of anaerobic bacteria to 23 antimicrobial agents. *Antimicrobial Agents and Chemotherapy* **10**, 736–752.

TARLINTON, M. N. & D'ABRERA, V. ST. E. (1967). Identity of a disputed *Haemophilus*-like organism in non-specific vaginitis. *Journal of Pathology and Bacteriology* **93**, 109–118.

VICE, J. L. & SMARON, M. F. (1973). Indirect fluorescent-antibody method for the identification of *Corynebacterium vaginale*. *Applied Microbiology* **25**, 908–916.

VICKERSTAFF, J. M. & COLE, B. C. (1969). Characterization of *Haemophilus vaginalis*, *Corynebacterium cervicis*, and related bacteria. *Canadian Journal of Microbiology* **15**, 587–594.

WILKINSON, B. J. & JONES, D. (1977). A numerical taxonomic survey of *Listeria* and related bacteria. *Journal of General Microbiology* **98**, 399–421.

WISHART, D. (1968). *A Fortran II Program for Numerical Taxonomy*. St. Andrews: University of St Andrews.

WURCH, T. & LUTZ, A. (1955). Etude du contenu vaginal dans 500 cas de leucorrhées; cytologie–microbiologie. *Revue de gynécologie et d'obstétrique* **50**, 289–294.

ZINNEMANN, K. & TURNER, G. C. (1963). The taxonomic position of '*Haemophilus vaginalis*' (*Corynebacterium vaginale*). *Journal of Pathology and Bacteriology* **85**, 213–219.