***Taylorella asinigenitalis* sp. nov., a bacterium isolated from the genital tract of male donkeys (*Equus asinus*)**

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Three bacterial isolates that were phenotypically indistinguishable from *Taylorella equigenitalis* were obtained from the urethral fossae of three male donkeys (*Equus asinus*), one located in the state of California and the other two in the state of Kentucky, USA. Based on results of pulsed-field gel electrophoresis, the isolate from California differed from the two Kentucky isolates, which were the same. Mares bred artificially (California) or naturally (Kentucky) did not show signs of disease, even though infection with the organism was established in those bred naturally. Mares and, uncharacteristically, all three jacks produced antibodies that reacted in the complement fixation test utilized to identify mares recently infected with *T. equigenitalis*. Sequence analysis of DNA encoding the 16S rRNA revealed that the gene sequences of these isolates were virtually identical to each other (>99.8% similarity), but different (97.6% similarity) from those of several confirmed isolates of *T. equigenitalis*. The 16S rDNA sequences of the latter were 100% identical. DNA–DNA hybridization studies revealed a mean hybridization level of 89% between the donkey isolate from California and the donkey isolate from Kentucky. On the other hand, the mean DNA–DNA hybridization level from the donkey isolates with DNA from a strain of *T. equigenitalis* was 23%. The DNA G+C composition was 37.8 mol% for the two donkey isolates, as well as the strain of *T. equigenitalis* used in the hybridization studies. These data support our opinion that micro-organisms isolated from the male donkeys are different from *T. equigenitalis* and it is proposed that they be considered a new species within the genus *Taylorella* and named *Taylorella asinigenitalis* sp. nov. The type strain is strain UCD-1^T (= ATCC 700933^T = LMG 19572^T).

Keywords: *Taylorella asinigenitalis* sp. nov, donkey, genital tract, 16S rRNA sequence

INTRODUCTION

Contagious equine metritis (CEM) is a highly con-

Abbreviations: CEM, contagious equine metritis; CF, complement fixation; IFA, indirect fluorescent antibody; MRVP, methyl red/Voges-Proskauer.

The GenBank accession numbers for the 16S rRNA gene sequence of *T. asinigenitalis* strains UCD-1^T, UK-1 and UK-2 and *T. equigenitalis* strains 10783 and 96-178 are AF067729, AF297174, AF297175, AF297172 and AF297173, respectively.

tagious venereal disease of members of the horse family that is caused by the microaerophilic, Gram-negative coccobacillus *Taylorella equigenitalis* (Crowhurst, 1977; Sugimoto *et al.*, 1983; Taylor *et al.*, 1978; Timoney, 1996). CEM was first described in 1977 in Europe and has since been reported in a number of countries throughout the world. Where present, *T. equigenitalis* occurs as an inhabitant on the external genitalia of stallions (Taylor *et al.*, 1978). The bacterium is usually transmitted venereally to mares. Mares develop an endometritis, from which they

recover. Abortion is a rare sequel to *T. equigenitalis* infection in the mare (Nakashiro *et al.*, 1981). Mares, but not stallions, develop antibodies to the micro-organism, which can be demonstrated during the early convalescent phase of the infection (Benson *et al.*, 1977; Bryans *et al.*, 1979). The carrier state has been demonstrated in both the stallion and the mare (Platt *et al.*, 1978; Platt & Taylor, 1982).

In the latter part of 1997, the urethral fossa of a donkey in the state of California cultured positive for an organism that resembled *T. equigenitalis* in that it was a microaerophilic, Gram-negative coccobacillus that grew only on media used for the isolation of *T. equigenitalis*. The bacterium grew more slowly than known strains of *T. equigenitalis* and reacted weakly with fluorescent labelled antibody to *T. equigenitalis* in an indirect fluorescent antibody (IFA) test. Serum from the jack contained antibodies that reacted to *T. equigenitalis* antigen in the complement fixation (CF) test. Shortly thereafter, very similar isolates were obtained from the urethral fossae of two male donkeys in the state of Kentucky. These isolates (designated UK-1 and UK-2) displayed the same phenotypic characteristics as the Californian isolate (UCD-1^T). Sera from both Kentucky jacks were also positive in the CF test for antibodies to *T. equigenitalis*. None of the donkey isolates were associated with the development of signs of disease in mares bred naturally (Kentucky) or artificially (California) to infected jacks, although the micro-organism was cultured from mares bred by natural service. There was no contact or other association between the culture-positive jack in California and the two jacks in Kentucky. The two Kentucky jacks, however, were from the same farm.

The characteristics of the micro-organisms isolated differed in certain respects from those of known reference strains of *T. equigenitalis* (Taylor *et al.*, 1978). The isolates had slower growth rates, gave a weak positive reaction in the IFA test and did not cause apparent disease in mares. In light of these differences, together with the demonstration of a serological response to *T. equigenitalis* in all three jacks, it was decided to determine the exact taxonomic position of the donkey isolates by means of an integrated genotypic and phenotypic taxonomic study.

METHODS

Source of isolates. The sources of the isolates used in this study are presented in Table 1. The donkey isolates were obtained from the urethral fossa.

Phenotypic tests. All strains were cultivated for 48 h at 37 °C in an environment containing 5–7% CO₂ in air on Eugon agar (Difco) supplemented with 10% (v/v) laked horse blood and containing trimethoprim and clindamycin (ECA) (Timoney *et al.*, 1982). Media listed below were inoculated from colonies growing on ECA medium and then incubated in 5–7% CO₂ in air at 37 °C for varying lengths of time. Tests were performed and results were interpreted as described previously (Finegold & Baron, 1986).

Oxidase and catalase tests were performed on colonies

growing on ECA medium. Motility was assessed by observation of wet mounts prepared from colonies formed after 48 h incubation.

Assessment of acid production from carbohydrates was determined by inoculation of broth containing carbohydrate (1%, w/v), bromocresol purple (Purple Broth Base; Difco) and filter-sterilized horse serum (1%, v/v); the broth was incubated for 7 d. Carbohydrates used included glucose, lactose, maltose, mannitol, sucrose and xylose. The results of the methyl red/Voges-Proskauer (MRVP) test (Difco) were determined after incubation for 48 h. Christensen's urea agar (Difco) was used to assess the presence of urease after incubation for 7 d, and for the determination of aesculin hydrolysis. An indole spot test (Anaerobe Systems) was used to determine whether indole was produced from tryptophan. Reduction of nitrate was assessed following inoculation and incubation of nitrate broth (Difco) for 7 d. Growth on MacConkey agar (Hardy Diagnostics) was measured following inoculation and incubation for 48 h. To determine whether growth enhancement by haemin and/or NAD occurred, paper disks containing these substances (Difco) were placed on the surface of tryptose agar (Difco) that had been streaked with the isolate under test. Plates were incubated for 48 h.

Growth under various atmospheric conditions [anaerobic (90% N₂, 5% H₂, 5% CO₂); air/10% CO₂; air; and microaerophilic (6% O₂, 14% CO₂)] was measured following incubation at 37 °C for 7 d. Growth at various temperatures was assessed following incubation at 24, 37 and 42 °C for 48 h under microaerophilic conditions.

The presence of various preformed enzymes was determined by utilizing a commercially available multi-compartment system (API ZYM; bioMérieux-Vitek). The tests were run as recommended by the manufacturer.

Fatty acid analysis. Fatty acids were determined with an automated GC system (Microbial Identification System; Midi) as recommended by the manufacturer. Extracts were prepared from isolates grown on ECA for 48 h.

Sequencing and analysis of DNA encoding the 16S rRNA. The sequence of the DNA encoding the 16S rRNA was determined by direct sequencing of PCR products obtained using appropriate primers (see below) (Eden *et al.*, 1991). Plate-grown organisms were suspended in distilled water and placed in a boiling-water bath for 10 min. Cellular debris was removed by centrifugation (5000 g, 5 min, room temperature). The supernatant, containing the template, was used with appropriate primers to amplify the 16S rDNA. The PCR mixture was as follows: 5 µl template, 0.2 µM each primer, 50 mM KCl, 10 mM Tris/HCl (pH 8.3), 2 mM MgCl₂, 5% (v/v) DMSO, 150 µM each dNTP and 2.5 U *Taq* DNA polymerase (Amplitaq; Perkin Elmer) in a total volume of 100 µl. An initial 5 min denaturation at 94 °C was followed by 35 cycles of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 2 min. The resulting PCR products were purified twice by filtration (Centricon-100; Amicon). Approximately 90 ng purified PCR product was labelled for sequencing with the ABI PRISM dRhodamine Terminator Cycle Sequencing Ready Reaction kit according to the manufacturer's instructions (Perkin Elmer). Sequencing gels were run on an ABI PRISM 377 automated DNA sequencer (Perkin Elmer). Sequence analysis and generation of consensus sequences were performed using a commercially available software package (SEQUENCHER 3.0; Gene Codes). Both strands of DNA were sequenced.

Table 1. Strains, geographical origin and host source of *Taylorella* strains used

Strain	Geographical origin	Host source	Identification
96-178	Kentucky, USA	Horse	<i>T. equigenitalis</i>
48987	The Netherlands	Horse	<i>T. equigenitalis</i>
10783	The Netherlands	Horse	<i>T. equigenitalis</i>
C89-490	Maryland, USA	Horse	<i>T. equigenitalis</i>
ECMO	UK	Horse	<i>T. equigenitalis</i>
94-344	Maryland, USA	Horse	<i>T. equigenitalis</i>
81-1173	Kentucky, USA	Horse	<i>T. equigenitalis</i>
92-972	Maryland, USA	Horse	<i>T. equigenitalis</i>
C85-150	Maryland, USA	Horse	<i>T. equigenitalis</i>
C82-534	Kentucky, USA	Horse	<i>T. equigenitalis</i>
98-079	New Jersey, USA	Horse	<i>T. equigenitalis</i>
MO3056	Missouri, USA	Horse	<i>T. equigenitalis</i>
C82-535	Kentucky, USA	Horse	<i>T. equigenitalis</i>
202	Japan	Horse	<i>T. equigenitalis</i>
98-2581	Kentucky, USA	Horse	<i>T. equigenitalis</i>
UCD-1 ^T	California, USA	Donkey	<i>T. asinigenitalis</i>
UK-1	Kentucky, USA	Donkey	<i>T. asinigenitalis</i>
UK-2	Kentucky, USA	Donkey	<i>T. asinigenitalis</i>

The primers used were designed to match the sequences of rDNA from *T. equigenitalis* strain NCTC 11184^T (Bleumink-Pluym *et al.*, 1993), *Escherichia coli* K-12 (GenBank accession no. V00348) and UCD-1^T. The primer sequences were as follows [the letter designations refer to forward (F) or reverse (R); numbers correspond to the 5' base designation of the 16S rRNA sequence used for *E. coli*]: 8F, 5'-AGA GTT TGA TCC TGG CTC AG-3'; 515F, 5'-GTG CCA GCA GCC GCG GTA A-3'; 935F, 5'-ACA AGC GGT GGA GCA TGT GG-3'; 1328F, 5'-CAT GAA GTC GGA ATC GCT AG-3'; 1492R, 5'-GGT TAC CTT GTT ACG ACT T-3'; 1098R, 5'-AGG GTT GCG CTC GTT GCG-3'; 617R, 5'-ATG CAG TTC CCA GGT TGA GC-3'; and 297R, 5'-GTG TGG CTG GTC ATC CTC TC-3'.

Phylogenetic analysis. The SeqLab graphical interface supplied with the Wisconsin Package (Genetics Computer Group software package) was used to construct a neighbour-joining tree. Bootstrap analysis was performed using 1000 replicates. The GenBank accession numbers for the 16S rDNA sequences used to construct the phylogenetic tree are as follows: M22508 (*Alcaligenes faecalis* ATCC 8750^T); M22509 (*Alcaligenes xylosoxidans* ATCC 15173); U04947 (*Bordetella avium* ATCC 35086^T); U04948 (*Bordetella bronchiseptica* ATCC 19395^T); U04950 (*Bordetella pertussis* ATCC 9797^T); Y11890 (*Pelistega europaea* LMG 10982^T); AF067729 (*Taylorella asinigenitalis* ATCC 700933^T); and X68645 (*T. equigenitalis* NCTC 11184^T). *Leptothrix mobilis* Feox-1^T (X97071) was used as the outgroup (Vandamme *et al.*, 1998).

DNA-DNA hybridization. Caesium chloride-purified DNA was obtained from bacterial cells (Wilson, 1994). DNA-DNA hybridizations were performed with photobiotin-labelled probes in microplate wells in 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7) in 50% (v/v) formamide and using an HTS7000 Bio Assay Reader (Perkin Elmer) (Ezaki *et al.*, 1989). The hybridization temperature was 37 °C.

DNA base composition. Caesium chloride-purified DNA was enzymically degraded into nucleosides (Mesbah *et al.*, 1989).

The nucleoside mixture obtained was then separated by reverse-phase HPLC (SymmetryShield C8 column thermostatically regulated to 37 °C; Waters). The solvent used was 0.02 M NH₄H₂PO₄ (pH 4.0) with 1.5% (v/v) acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as the calibration reference.

Pulsed-field gel electrophoresis (PFGE) analysis. PFGE was used to determine relatedness among isolates of *Taylorella* (CHEF-DR III system; Bio-Rad) (Finney, 1987). Conditions for DNA isolation and digestion with endonucleases (*NotI*, *ApaI* and *NaeI*) were as recommended by the manufacturer (New England Biolabs). Plugs were incubated overnight at 37 °C with 20 U restriction enzyme in 150 µl restriction enzyme buffer. Gels were 1.2% (w/v) agarose in Tris/borate/EDTA buffer, pH 8.3 (45 mM Tris, 45 mM boric acid, 1 mM EDTA) and were run at 6 V cm⁻¹, 22 h, 14 °C, with a linearly ramped pulse time varying from 5 to 50 s (*NotI*, *NaeI*) or 3 to 10 s (*ApaI*) at an angle of 120°.

RESULTS AND DISCUSSION

Phenotypic characteristics

Cells of strains UCD-1^T, UK-1 and UK-2 were Gram-negative, non-motile coccobacilli. They were catalase- and oxidase-positive, did not produce acid in broth containing carbohydrates, did not reduce nitrate, did not hydrolyse aesculin and were MRVP-, urease- and indole-negative. They did not grow on MacConkey agar or ECA medium incubated in air or anaerobically. They grew more slowly at 24 than at 37 °C and did not grow at 42 °C. Strains UCD-1^T, UK-1 and UK-2 were somewhat slower growing than *T. equigenitalis*. UCD-1^T was less viscid in consistency than UK-1, UK-2 or *T. equigenitalis*. Their growth on tryptose agar was not stimulated by haemin or NAD. All of the strains of *T. equigenitalis* tested (Table 1) and the three strains isolated from donkeys contained the same or very

Table 2. Enzyme profiles of *T. equigenitalis* and *T. asinigenitalis*

+, Maximum intensity (5+); w, weak (2–3+); TR, trace (+); –, no reaction. Strains 96-178, UCD-1^T, UK-1 and UK-2 were positive for alkaline phosphatase and leucine arylamidase, but gave no reaction for lipase (C-14), trypsin, chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase.

Enzyme	96-178	UCD-1 ^T	UK-1	UK-2
Esterase (C-4)	TR	TR	TR	TR
Esterase lipase (C-8)	TR	TR	TR	TR
Leucine arylamidase	+	+	+	+
Valine arylamidase	W	TR	W	W
Cystine arylamidase	TR	TR	TR	W
Acid phosphatase	W	W	W	W
Naphthol-AS-BI-phosphohydrolase	W	TR	W	W

Table 3. Fatty acid content of *T. equigenitalis* and *T. asinigenitalis*

Values (%) shown are for those fatty acids that were present in amounts > 1%; TR, trace amount (< 1%); ND, not detected. Summed feature 3: 12:0 ALDE, 14:0 3-OH/16:1 iso I or any combination. Summed feature 4: 16:1 ω 7c/15:0 2-OH, 15:0 iso 2-OH/16:1 ω 7c or any combination. Summed feature 6: 18:2 ω 6,9/18:0 ANTE, 18:0 ANTE/18:2 ω 6,9c or any combination. Summed feature 7: 18:1 ω 9c/12:0 ω 7c, 18:1 ω 7c/12:0 ω 9c or any combination.

Fatty acid	96-178	UCD-1 ^T	UK-1
10:0	ND	TR	TR
12:0	TR	1.06	TR
14:0	TR	1.14	TR
15:1 ω 8c	ND	1.60	TR
16:0	30.64	29.37	20.05
16:0 3-OH	TR	ND	TR
17:0	ND	ND	TR
18:1 ω 9c	TR	1.82	1.73
18:0	7.77	6.85	13.15
19:0 10-methyl	1.17	1.64	2.74
20:1 ω 9c	ND	ND	TR
20:1 ω 9t	TR	TR	2.22
Summed feature 3	7.84	5.45	4.19
Summed feature 4	1.45	1.79	TR
Summed feature 6	TR	1.40	TR
Summed feature 7	47.87	46.49	51.68

similar preformed enzyme profiles (Table 2; only the results for *T. equigenitalis* strain 96-178 are shown).

Cellular fatty acid analysis

Fatty acids from *T. equigenitalis* were compared with those of two of the donkey strains (Table 3). With the exception of minor differences in some of the fatty acids (e.g. 18:0), the profiles of *T. equigenitalis* and the donkey isolates were similar. The characteristics shown are in agreement with those reported for *T.*

Fig. 1. PFGE separation of *NotI*-digested genomic DNA obtained from *T. equigenitalis* strains 48987 (lane A), 92-972 (B), 98-079 (C), M03056 (D), C82-535 (E) and 202 (F) and *T. asinigenitalis* strains UCD-1^T (G), UK-1 (H) and UK-2 (I). Numbers on the left indicate the migration positions of molecular mass standards (kb).

equigenitalis (Neill *et al.*, 1984; Sugimoto *et al.*, 1982; Vandamme *et al.*, 1998).

PFGE analysis

PFGE analysis of DNA obtained from the three donkey strains revealed that the two strains from jacks in Kentucky (UK-1, UK-2) were the same, but they differed from the strain from the jack in California (UCD-1^T) (Fig. 1). In addition, PFGE analysis showed that the strains of *T. equigenitalis*, although of different geographical origin, were very similar to each other, but distinct from the donkey isolates. Identical groupings were obtained regardless of the restriction enzyme used.

Sequence analysis of 16S rRNA

The sequences of approximately 1500 bp of rDNA were determined for the three donkey strains and for

Fig. 2. A phylogenetic tree based on sequence analysis of the 16S rDNA showing the positions of *T. equigenitalis*, *T. asinigenitalis* and various members of the β -Proteobacteria. The name of the micro-organism is followed by the strain and the accession number of the 16S rDNA sequence. Bar, 0.01 nucleotide substitutions per position. Bootstrap values (%) are indicated at each node.

15 other strains of *T. equigenitalis*, which were then compared to that published for *T. equigenitalis* NCTC 11184^T (GenBank accession no. X68645) (Table 1). The DNA sequences from the donkey strains were almost identical (> 99.8%) to each other, but they differed (97.6% similarity) from those of strains of *T. equigenitalis*. *T. equigenitalis* appears to be homogeneous with respect to the 16S rDNA sequence, since all 15 strains sequenced (Table 1), together with the published sequence (NCTC 11184^T), were 100% identical.

Phylogenetic analysis, G + C content and DNA–DNA hybridization

It has been determined previously that *T. equigenitalis* belongs to the β -Proteobacteria (Vandamme *et al.*, 1998). A phylogenetic tree was constructed utilizing the sequence of the 16S rDNA of strain UCD-1^T, *T. equigenitalis* and various Gram-negative bacteria be-

longing to this subclass (Fig. 2). The data show that strain UCD-1^T and *T. equigenitalis* form a distinct cluster, more similar to *Pelistega europaea* than to other members of this subclass, a finding consistent with that of others (Vandamme *et al.*, 1998).

The G + C contents of UCD-1^T, UK-1 and 96-178 were identical, at 37.8 mol%, which is similar to that reported previously for *T. equigenitalis* (36.1 mol%) (Taylor *et al.*, 1978).

DNA from UCD-1^T hybridized to DNA from UK-1 to a mean level of 89%. DNA from *T. equigenitalis* strain 96-178 hybridized to DNA from UCD-1^T to a level of 23% and from UK-1 to a level of 24%. These data imply that the isolates from donkeys represent a species within the genus *Taylorella*.

Taxonomic position of the donkey isolates

All three donkey isolates were similar to *T. equigenitalis* reference strains in terms of overall biochemical reactivity pattern, DNA base ratio and whole-cell fatty acid composition. Immunological cross-reactions and comparative 16S rDNA sequence analysis demonstrated that these organisms were closely related. However, the absence of high DNA–DNA hybridization levels and the phenotypic differences (the donkey isolates had slower growth rates, gave a weak positive reaction in the IFA test and did not cause apparent disease in mares) indicate that it is appropriate to classify the donkey isolates as a new species in the genus *Taylorella*, for which the name *Taylorella asinigenitalis* sp. nov. is proposed.

Description of *Taylorella asinigenitalis* sp. nov.

Taylorella asinigenitalis (a.si.ni.ge.ni.ta'lis. L. masc. n. *asinus* donkey; L. adj. *genitalis* genital; M.L. adj. *asinigenitalis* pertaining to donkey genitalia).

The bacterium is a Gram-negative, non-motile coccobacillus. Colonies are approximately 1 mm in diameter, entire, convex, opaque, tan in colour and sometimes viscid in consistency after growth on ECA medium for 48 h. Catalase- and oxidase-positive, asaccharolytic, does not reduce nitrate, does not hydrolyse aesculin and MRVP-, urease- and indole-negative. Does not grow on MacConkey agar or ECA medium incubated in air or anaerobically at 37 °C. Grows microaerophilically and in air with 5–7% CO₂ at 37 °C, but not at 24 or 42 °C. Is not stimulated by haemin or NAD. Major enzymes present are alkaline phosphatase and leucine arylamidase. Major fatty acids are 16:0, 18:0, 19:0 10-methyl and summed features 3, 4 and 7. Does not appear to produce disease in jacks or mares, although it is contagious and induces an antibody response in infected animals. The G + C content is 37.8 mol%. The type strain of *T. asinigenitalis* is UCD-1^T (= ATCC 700933^T = LMG 19572^T). Strains UK-1 and UK-2 have been deposited in the BCCM/LMG Culture Collection under accession numbers LMG 19573 and LMG 19574, respectively.

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REFERENCES

- Benson, J. A., Dawson, F. L. M., Durrant, D. S., Edwards, P. T. & Powell, D. G. (1977). Serological response in mares affected by contagious equine metritis. *Vet Rec* **102**, 277–280.
- Bleumink-Pluym, N. M. C., van Dijk, L., van Vliet, A. H. M., van der Giessen, J. W. B. & van der Zeijst, B. A. M. (1993). Phylogenetic position of *Taylorella equigenitalis* determined by analysis of amplified 16S ribosomal DNA sequences. *Int J Syst Bacteriol* **43**, 618–621.
- Bryans, J. T., Darlington, R. W., Smith, B. & Brooks, R. R. (1979). Development of a complement fixation test and its application to diagnosis of contagious equine metritis. *J Equine Med Surg* **3**, 467–472.
- Crowhurst, R. C. (1977). Genital infection in mares. *Vet Rec* **100**, 476.
- Eden, P. A., Schmidt, T. M., Blakemore, R. P. & Pace, N. R. (1991). Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. *Int J Syst Bacteriol* **41**, 324–325.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Finegold, S. M. & Baron, E. J. (1986). *Bailey and Scott's Diagnostic Microbiology*. St Louis, MO: C.V. Mosby.
- Finney, M. (1987). Pulsed-field gel electrophoresis. In *Current Protocols in Molecular Biology*, pp. 2.5.9–2.5.17. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Swidman, J. A. Smith & K. Struhl. New York: John Wiley.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nakashiro, H., Naruse, M., Sugimoto, C., Isayama, Y. & Kuniyasu, C. (1981). Isolation of *Haemophilus equigenitalis* from an aborted equine foetus. *Natl Inst Anim Health Q* **21**, 184–185.
- Neill, S. D., O'Brien, J. J., McMurray, C. H. & Blanchflower, W. J. (1984). Contagious equine metritis – use of gas liquid chromatography in identifying the causal agent. *Equine Vet J* **16**, 430–434.
- Platt, H. & Taylor, C. E. D. (1982). Contagious equine metritis. In *Medical Microbiology*, vol. 1, pp. 49–46. Edited by C. S. F. Easmon & J. Jeljaszowicz. London: Academic Press.
- Platt, H., Atherton, J. G. & Simpson, D. J. (1978). The experimental infection of ponies with contagious equine metritis. *Equine Vet J* **10**, 153–159.
- Sugimoto, C., Miyagawa, E., Mitani, K., Nakazawa, M. & Isayama, Y. (1982). Cellular fatty acid composition of *Haemophilus equigenitalis*. *J Clin Microbiol* **15**, 791–794.
- Sugimoto, C., Isayama, Y., Sakazaki, R. & Kuramochi, S. (1983). Transfer of *Haemophilus equigenitalis* Taylor *et al.* 1978 to the genus *Taylorella* gen. nov. as *Taylorella equigenitalis* comb. nov. *Curr Microbiol* **9**, 155–162.
- Taylor, C. E. D., Rosenthal, R. O., Brown, D. F. J., Lapage, S. P., Hill, L. R. & Legros, R. M. (1978). The causative organism of contagious equine metritis 1977: proposal for a new species to be known as *Haemophilus equigenitalis*. *Equine Vet J* **10**, 136–144.
- Timoney, P. J. (1996). Contagious equine metritis. *Comp Immunol Microbiol Infect Dis* **19**, 199–204.
- Timoney, P. J., Shin, S. J. & Jacobson, R. H. (1982). Improved selective medium for isolation of the contagious equine metritis organism. *Vet Rec* **111**, 107–108.
- Vandamme, P., Segers, P., Ryll, M. & 8 other authors (1998). *Pelistega europaea* gen. nov., sp. nov., a bacterium associated with respiratory disease in pigeons: taxonomic structure and phylogenetic allocation. *Int J Syst Bacteriol* **48**, 431–440.
- Wilson, K. (1994). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.5. Edited by F. A. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.