Veillonella montpellierensis sp. nov., a novel, anaerobic, Gram-negative coccus isolated from human clinical samples

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Three strains of a hitherto unknown, Gram-negative, anaerobic coccus were isolated from human samples. At the phenotypic level, the isolates displayed all the characteristics of bacteria belonging to the genus *Veillonella*. Sequence analysis revealed that the three strains shared >99.5% similarity in 16S rDNA sequence and >98.4% similarity in *dnaK* sequence. The three unknown strains formed a separate subclade that was clearly remote from *Veillonella* species of human and animal origin. Based on these results, the three strains were considered to represent a novel species within the genus *Veillonella*, for which the name *Veillonella montpellierensis* is proposed. The type strain of the species is ADV 281.99^T (= CIP 107992^T = CCUG 48299^T).

The genus *Veillonella* consists of small, non-fermentative, anaerobic, Gram-negative cocci. Originally classed in the family *Veillonellaceae* (Rogosa, 1971), the genus *Veillonella* was subsequently included in the family 'Acidaminococcaceae' of the phylum *Firmicutes* (Garrity & Holt, 2001) and the family *Veillonellaceae* was abolished. Serological reactions (Rogosa, 1965) and DNA–DNA hybridization studies led to the individualization of seven species in the genus *Veillonella*: *Veillonella parvula*, *Veillonella atypica*, *Veillonella dispar*, *Veillonella criceti*, *Veillonella ratti*, *Veillonella rodentium* and *Veillonella caviae* (Mays *et al.*, 1982; Rogosa, 1984). Presently, the identification of isolates of *Veillonella* at the genus level is relatively straightforward, whereas the identification of *Veillonella* strains at the species level remains uncertain and inconvenient, owing to the lack of conventional phenotypic and biochemical discriminating tests (Kolenbrander & Moore, 1992). Therefore, molecular methods based on 16S rDNA sequences have been used increasingly to identify *Veillonella* isolates (Sato *et al.*, 1997a, b). These methods have recently been criticised, due to the low level of sequence variation among some *Veillonella*

species, particularly between *V. dispar* and *V. parvula*, and the frequent occurrence of intra-chromosomal heterogeneity between 16S rRNA gene copies in human isolates of *Veillonella* spp. (Marchandin *et al.*, 2003b). Despite difficulties in species identification within the genus *Veillonella*, *V. atypica*, *V. dispar* and *V. parvula* are mainly reported from human flora or from clinical samples during infectious processes (Singh & Yu, 1992; Houston *et al.*, 1997; Liu *et al.*, 1998; Marchandin *et al.*, 2001). The other four species were found only in animals, except for one *V. ratti* isolate, strain ADV 4313.2 (= CIP 107810), which was recovered from human semen and identified after 16S rDNA sequencing (GenBank accession no. AY211542) (H. Marchandin, C. Teyssier, J.-P. Carlier, E. Jumas-Bilak, M. Robert, A.-C. Artigues and H. Jean-Pierre, unpublished results).

During a survey of anaerobic, Gram-negative cocci in human clinical samples, we isolated three nitrate-reducing strains that showed a resistant phenotype when tested with a 10 µg colistin disc (Rosco). The aim of the present study was to determine the taxonomic status of these isolates by using a polyphasic approach.

Bacterial strains and growth conditions

Three strains (designated ADV 281.99^T, ADV 2216.03 and ADV 3198.03) were isolated from clinical samples of three

Abbreviations: EM, electron microscopy; ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence of *Veillonella montpellierensis* ADV 281.99^T (= CIP 107992^T = CCUG 48299^T) is AF473836.

different patients. Strain ADV 281.99^T was isolated in 1999 from gastric fluid of a newborn. It was recovered together with *Gardnerella vaginalis*, *Escherichia coli* and serotype III group B *Streptococcus*. Strains ADV 2216.03 and ADV 3198.03 were isolated in 2003 from amniotic fluid samples from two women (28 and 21 years old, respectively) who were hospitalized in the obstetric surgical unit of Montpellier University Hospital. Isolate ADV 2216.03 was recovered together with coagulase-negative *Staphylococcus*, *Lactobacillus*, α -haemolytic *Streptococcus* and *Ureaplasma urealyticum*. Isolate ADV 3198.03 was recovered together with serotype III group B *Streptococcus*. Cultures were performed on Columbia sheep blood agar, incubated for 4 days in an anaerobic jar with the AnaeroGen system (Oxoid Unipath). The three isolates were anaerobic, Gram-negative cocci that were identified presumptively as *Veillonella* sp. on the basis of their capacity to reduce nitrate.

Strains ADV 281.99^T, ADV 2216.03 and ADV 3198.03 were studied further in comparison with strains of each of the seven species currently described within the genus *Veillonella*. *V. atypica* ATCC 17744^T, *V. dispar* ATCC 17748^T, *V. parvula* ATCC 17745, *V. ratti* ATCC 17746^T and *V. rodentium* ATCC 17743^T were purchased from the American Type Culture Collection (ATCC), Manassas, VA, USA. *V. caviae* DSM 20738^T and *V. criceti* DSM 20734^T were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany, and *V. parvula* CIP 60.1 from the Collection de l'Institut Pasteur (CIP), Paris, France.

16S rDNA analysis and phylogeny

16S rDNA was amplified from genomic DNA as described previously (Carlier *et al.*, 2002). The 16S rDNA PCR product was sequenced directly on an Applied Biosystems automatic sequencer (Genome Express). A partial 16S rDNA sequence of 1409 nt was determined previously for strain ADV 281.99^T (GenBank accession no. AF473836; Marchandin *et al.*, 2003a) and partial 16S rDNA sequences of 1388 and 1374 nt were determined in this study for isolates ADV 2216.03 and ADV 3198.03, respectively. As 16S rDNA sequences of *V. rodentium* and *V. caviae* were not available, we determined almost-complete 16S rDNA sequences for the type strains of these two species. GenBank accession numbers for 16S rDNA sequences determined in this work are as follows: *V. caviae* DSM 20738^T, AY355140; *V. rodentium* ATCC 17743^T, AY514996; strain ADV 2216.03, AY355141; and strain ADV 3198.03, AY355142.

Alignment of the 16S rDNA sequences by using LALIGN software (www.expasy.ch) showed >99.5 % similarity between the sequences of strains ADV 281.99^T, ADV 2216.03 and ADV 3198.03. This indicated that the three isolates were closely related and could belong to the same species. BLAST analysis showed that the tested organisms were related most closely to the currently uncharacterized *Veillonella* sp. strain 2001-112662 (GenBank accession no. AY244769), with 99.3–99.6 % sequence similarity. This

suggested that this strain might represent the fourth member of the novel taxon. Highest similarity was found with members of the genus *Veillonella*, from 92.5 % with *V. criceti* DSM 20734^T to 94.7 % with *V. atypica* ATCC 17744^T. The 16S rDNA sequences of strains ADV 281.99^T, ADV 2216.03 and ADV 3198.03 were aligned against sequences of representative strains and clones retrieved from GenBank by using the DIALIGN program (Morgenstern, 2002). The resulting 1309 nt alignment was checked manually and used for construction of phylogenetic trees. Evolutionary trees were inferred by using the maximum-likelihood (ML) (Olsen *et al.*, 1994), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (NJ) (Saitou & Nei, 1987) methods from the PHYLIP suite of programs (Felsenstein, 1993). The F84 algorithm (Kishino & Hasegawa, 1989) was used to generate evolutionary distance matrices for the NJ method structure; the evolutionary distance tree obtained for members of the genus *Veillonella* is shown in Fig. 1. Robustness of trees was evaluated by bootstrap analysis of 1000 resamplings by using the SEQBOOT and CONSENSE programs from the PHYLIP package (Felsenstein, 1993). It is evident from the 16S rDNA-based phylogeny that strains ADV 281.99^T, ADV 2216.03 and ADV 3198.03, together with *Veillonella* sp. strain 2001-112662, formed a distinct, monophyletic unit. All three treeing algorithms supported the taxonomic integrity of this clade. The monophyly of this group was supported strongly by the high bootstrap value of 100 % of the ML analysis, as well as by parsimony and NJ analyses (96 and 100 %, respectively) (Fig. 1). A previously published phylogenetic analysis

Fig. 1. Neighbour-joining tree based on nearly complete 16S rDNA sequences (1309 nt), showing relationships between *V. montpellierensis* strains ADV 281.99^T, ADV 2216.03 and ADV 3198.03 and representatives of the genus *Veillonella*. *Dialister pneumosintes* ATCC 33048^T was the outgroup organism. Bootstrap values higher than 60 % are indicated at corresponding nodes. Bar, 0.01 substitutions per site.

placed strain ADV 281.99^T in an intermediate position among members of the genus *Veillonella* (Marchandin *et al.*, 2003a). In the present study, the clade that contains strain ADV 281.99^T is more deeply branched. This discordance could be explained by the sequences sampled; particularly, the sequences of *V. caviae* and *V. rodentium* and those of strains ADV 2216.03, ADV 3198.03 and *Veillonella* sp. 2001-112662 have been included in the present trees. Nevertheless, the branching of strains ADV 281.99^T, ADV 2216.03, ADV 3198.03 and *Veillonella* sp. 2001-112662, relative to other *Veillonella* strains, is supported by a good bootstrap value of 83 % in the NJ analysis, which is better than that obtained in the previous tree (67 %), and by the congruence observed between the three phylogenetic methods.

16S rDNA sequence similarity values of strains ADV 281.99^T, ADV 2216.03 and ADV 3198.03 with other members of the genus *Veillonella* are appropriate for determining intragenetic relationships for species definition (Stackebrandt & Goebel, 1994) and indicate that these three strains represent a novel species within the genus *Veillonella*. However, recent results showed that 16S rDNA-based approaches were not suitable for the identification of *V. dispar* and *V. parvula*, owing to the close genetic relationship observed for these two species and to the relatively high level of intra-chromosomal heterogeneity between the four 16S rRNA gene copies observed in human isolates of *Veillonella* spp. Furthermore, this heterogeneity impaired the phylogenetic placement of a clinical strain in the tree (Marchandin *et al.*, 2003b). As a consequence and despite the robustness of the 16S rDNA phylogeny we obtained here, we chose to analyse another genetic marker.

***dnaK* sequence analysis and phylogeny**

The 70 kDa heat-shock protein gene (*dnaK*) was generally present as one copy in bacterial genomes and showed higher interspecies variability than the 16S rRNA gene. These two characteristics should avoid the pitfalls of the 16S rDNA-based taxonomy of the genus *Veillonella*. A 700 bp *dnaK* fragment was amplified and sequenced as described previously (Marchandin *et al.*, 2003b) for strains ADV

281.99^T, ADV 2216.03, ADV 3198.03, *V. caviae* DSM 20738^T, *V. criceti* DSM 20734^T, *V. parvula* ATCC 17745, *V. ratti* ATCC 17746^T and *V. rodentium* ATCC 17743^T. Sequences were deposited in GenBank under accession numbers AY353717, AY353715, AY353716, AY353714, AY353713, AY514999, AY514998 and AY514997, respectively. Sequence data obtained were aligned with those of *V. atypica* ATCC 17744^T, *V. dispar* ATCC 17748^T and *V. parvula* CIP 60.1 (previously deposited in databases) and were submitted to phylogenetic analysis, as described above. Phylogenetic trees obtained by analysis of 396 nt revealed that the overall topology was congruent with that of 16S rDNA-based trees except for the relative branching of *V. caviae*, which was supported by a very low bootstrap value in the *dnaK* tree. The three strains described here formed a distinct lineage, supported by a high bootstrap value, within the genus *Veillonella* (Fig. 2). Pairwise similarity analysis showed low levels of *dnaK* sequence similarity between strain ADV 281.99^T and other *Veillonella* species, from 79 % with *V. ratti* ATCC 17746^T to 86.4 % with *V. dispar* ATCC 17748^T, whereas strains ADV 281.99^T and ADV 2216.03 were identical in *dnaK* sequence. The *dnaK* sequence of strain ADV 3198.03 shared only 98.4 % similarity with those of strains ADV 281.99^T and ADV 2216.03. Regarding the sequence similarity level of 99.7 % between the two strains of *V. parvula*, the affiliation of the three strains in a single species is questionable. However, as no higher than 96 % similarity was observed between *dnaK* sequences of the type strains of the related species *V. parvula* and *V. dispar*, inclusion of strain ADV 3198.03 in the same species as strains ADV 281.99^T and ADV 2216.03 appeared reasonable.

Finally, molecular and phylogenetic data obtained for 16S rDNA and *dnaK* sequences were congruent and suggested that the three clinical strains characterized herein represent a novel species within the genus *Veillonella*. A more complete phenotypic analysis of the strains was undertaken.

Colony and cell morphology

The three strains grew on Columbia blood agar at 37 °C under anaerobic conditions, forming small colonies of

Fig. 2. Neighbour-joining tree based on partial *dnaK* sequences (396 nt), showing relationships between *V. montpellierensis* strains ADV 281.99^T, ADV 2216.03 and ADV 3198.03 and representatives of the genus *Veillonella*. *Megasphaera elsdenii* ATCC 25940^T was the outgroup organism. Numbers at nodes indicate percentages of bootstrap support, based on analysis of 1000 resampled datasets. Bar, 0.02 substitutions per site.

1–3 mm diameter, which were smooth, opaque and greyish-white. The three isolates were Gram-negative, non-motile, non-sporulating, tiny cocci that were spherical or slightly elongated and were organized singly, in pairs or occasionally in short chains. General morphology of the cells was observed after negative staining as described previously (Marchandin *et al.*, 2003a), confirming that the cells were spherical, either single or in short chains of two to four cells. Most cells were 0.3–0.5 µm in diameter. The convoluted nature of the cell surface and the diplococcal shape of the cells were observed (Fig. 3a) and were in accordance with the observations of Bladen & Mergenhagen (1964) for other *Veillonella* species. Ultrastructure of thin sections was also observed by electron microscopy (EM) (Fig. 3b) and revealed typical Gram-negative surface layers, which consisted of an outer membrane, a thin peptidoglycan layer and a convoluted cytoplasmic membrane (Fig. 3c), as described previously for several representatives of the family 'Acidaminocaccae', e.g. *Veillonella* spp. (Bladen & Mergenhagen, 1964), *Megasphaera* spp. (Marchandin *et al.*, 2003a), *Selenomonas ruminantium* (Kamio & Takahashi, 1980; Kalmokoff *et al.*, 2000) and *Centipeda periodontii* (Males *et al.*, 1984).

Biochemical characteristics

The three strains displayed susceptibility to discs that contained 1 mg kanamycin or 4 µg metronidazole and resistance to discs that contained 5 µg vancomycin or 10 µg colistin (Rosco). These characteristics were also observed for *V. ratti* (strain ATCC 17746^T and clinical strain ADV 4313.2), whereas strains of the other six *Veillonella* species were found to be susceptible to the 10 µg colistin disc. Further characterization by conventional biochemical tests (Holdeman *et al.*, 1977) and determination of metabolic end products (Carrier, 1985) were performed. The main phenotypic characteristics corresponded to those of the genus *Veillonella* as described by Rogosa (1971). Gas production was noted. Presence of catalase was strain-dependent, as only one of the three strains displayed catalase activity. Urease and cytochrome oxidase activities were not detected. Gelatin was not liquefied and milk was not modified. Indole

and desulfovibrin were not produced. Aesculin was not hydrolysed. Lactate and succinate were fermented to propionate. Acid was not produced from aesculin, arabinose, cellobiose, galactose, glucose, glycerol, inositol, inulin, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, ribose, sucrose, salicin, sorbitol, trehalose or xylose. The major metabolic end products in TGY broth (Carrier *et al.*, 2002) were acetic and propionic acids (8.5 and 6.9 mmol l⁻¹, respectively, for strain ADV 281.99^T, 5.5 and 3.6 mmol l⁻¹ for strain ADV 2216.03 and 5.5 and 4.6 mmol l⁻¹ for strain ADV 3198.03).

Cellular fatty acid analysis

Cellular fatty acid composition was analysed by GC according to Veys *et al.* (1989). Briefly, strains were grown anaerobically in 10 ml TGYH (Carrier *et al.*, 2002) for 48–72 h and methyl esters were chromatographed on a fused-silica capillary column (25 m × 0.25 mm ID) coated with 5% methyl phenyl silicone. The cellular fatty acid composition of the three clinical strains was very similar to that of other species of the genus *Veillonella* (Table 1). It is also noteworthy that all species studied contained a unidentified compound, which eluted between 17:0 iso and Δ -cis-9, 10-methylenehexadecanoate (17:0 Δ -cyclic 9, 10) (Table 1). With the exception of *V. caviae* DSM 20738^T, the only notable difference with the other type strains consisted of a smaller percentage of 13:0 and a higher amount of cis-9-octadecenoic acid (18:1 ω 9cis). Also, levels of 18:0 that were observed for these new isolates tended to be slightly higher than those obtained for the other species.

Notwithstanding the absence of clearly distinct phenotypic characteristics between the different species of the genus *Veillonella*, it is clear from the genotypic data that strains ADV 281.99^T, ADV 2216.03, ADV 3198.03 and probably *Veillonella* sp. 2001-112662 are members of a novel species of the genus *Veillonella*. It is therefore proposed that these strains should be classified in the genus *Veillonella* as *Veillonella montpellierensis* sp. nov. Considering that the three strains in our study were the only anaerobic bacteria that were recovered from the three corresponding clinical

Fig. 3. Ultrastructure of strain ADV 281.99^T. (a) General morphology after negative staining; (b) general morphology after EM of ultrathin sections; (c) cell wall and membranes after EM of ultrathin sections. Bars: (a) 166 nm; (b) 666 nm; (c) 200 nm.

Table 1. Relative content (%) of cellular fatty acids of *V. montpellierensis* and other *Veillonella* species

Reference strains: 1, *V. atypica* ATCC 17744^T; 2, *V. caviae* DSM 20738^T; 3, *V. criceti* DSM 20734^T; 4, *V. dispar* ATCC 17748^T; 5, *V. ratti* ATCC 17746^T; 6, *V. rodentium* ATCC 17743^T; 7, *V. parvula* ATCC 17745. Fatty acid nomenclature: unsaturated fatty acids, the position of the double bond can be located by counting from the methyl (ω) end of the carbon chain; *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively.

Fatty acid	<i>V. montpellierensis</i>			1	2	3	4	5	6	7
	ADV 281.99 ^T	ADV 2216.03	ADV 3198.03							
11:0	2.3	2.5	4	1.1	1.4	2	0.8	2.1	1.6	1.8
12:0	7	9	8.8	3.8	4	1.4	3.8	4.5	5	4.9
13:0	6.3	7	11.5	20.4	7.3	16.3	23.5	20	25.3	24
14:0 iso	—	—	—	0.6	1.2	—	1.5	0	—	1.9
14:0	5	5.4	5.4	5.7	3.1	1.2	4.9	2	9.2	6.5
15:0	6.3	5.5	11	17.7	7	12.7	7	10.3	11.6	12
14:0 2-OH	—	—	—	0.3	—	0.3	—	1	—	—
16:1 ω 9 <i>c</i>	8.4	7.5	5.9	5.2	10.6	4.2	7.7	4.8	5.5	5.2
16:0	12.9	13.2	11.1	7.5	10.3	3.7	8.4	4.6	6.7	7
Unknown*	20.7	17.1	21.7	25.7	29.9	48.9	25.6	36.9	17	22.3
17:0	2	1.7	3.7	2.4	2.5	2.6	2.5	2.4	4.2	4.1
18:1 ω 9 <i>c</i>	17.4	18.2	13.9	4.3	14.3	2.8	8	6.1	6.7	6.2
18:1 ω 9 <i>t</i>	2	1.6	3	—	1.9	1.2	—	—	—	—
18:0	11.1	10.9	6.3	2.7	6.2	2.1	5.7	5.8	6.7	3.9

*Unknown compound that eluted between 17:0 iso and 17:0 Δ (cyclic 9, 10).

samples and that *Veillonella* sp. strain 2001-112662 was isolated from the blood culture of a female patient who presented with septic shock, the novel species may have pathogenic potential.

Description of *Veillonella montpellierensis* sp. nov.

Veillonella montpellierensis (mont.pel.li.er.en'sis. N.L. fem. adj. *montpellierensis* pertaining to Montpellier in the south of France, where the type strain and two other strains supporting the description of the species were isolated).

Cells are coccoid (0.3–0.5 μ m in diameter) and occur singly, in pairs or in short chains. Gram-negative, non-motile, non-sporulating and with a convoluted surface. Colonies on Columbia blood agar are 1–3 mm in diameter and appear smooth, opaque and greyish-white. Strictly anaerobic and oxidase-negative. Nitrate is reduced. Gas is produced. Major metabolic end products are acetic and propionic acids. Major cellular fatty acids are 12:0, 13:0, 14:0, 15:0, 16:1 ω 9*cis*, 16:0, 18:1 ω 9*cis* and 18:0. Strains contained an unidentified compound, which eluted between 17:0 iso and Δ -*cis*-9, 10-methylenehexadecanoate (17:0 Δ -cyclic 9, 10). Can be differentiated from other species of the genus *Veillonella* by 16S rDNA and *dnaK* sequencing.

The type strain is ADV 281.99^T (=CIP 107992^T=CCUG 48299^T) and reference strains are ADV 2216.03 and ADV 3198.03. Found in human clinical samples.

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References

- Bladen, H. A. & Mergenhagen, S. E. (1964). Ultrastructures of *Veillonella* and morphological correlation of an outer membrane with particles associated with endotoxic activity. *J Bacteriol* **88**, 1482–1492.
- Carlier, J.-P. (1985). Gas chromatography of fermentation products: its application in diagnosis of anaerobic bacteria. *Bull Inst Pasteur* **83**, 57–69.
- Carlier, J.-P., Marchandin, H., Jumas-Bilak, E., Lorin, V., Henry, C., Carrière, C. & Jean-Pierre, H. (2002). *Anaeroglobus geminatus* gen. nov., sp. nov., a novel member of the family Veillonellaceae. *Int J Syst Evol Microbiol* **52**, 983–986.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Department of Genetics, University of Washington, Seattle, USA.
- Garrity, G. M. & Holt, J. G. (2001). Taxonomic outline of the *Archaea* and *Bacteria*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 155–166. Edited by D. R. Boone & R. W. Castenholz. New York: Springer.
- Holdeman, L. V., Cato, E. P. & Moore, W. E. C. (editors) (1977). *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, VA: Virginia Polytechnic Institute and State University.

- Houston, S., Taylor, D. & Rennie, R. (1997). Prosthetic valve endocarditis due to *Veillonella dispar*: successful medical treatment following penicillin desensitization. *Clin Infect Dis* **24**, 1013–1014.
- Kalmokoff, M. L., Austin, J. W., Whitford, M. F. & Teather, R. M. (2000). Characterization of a major envelope protein from the rumen anaerobe *Selenomonas ruminantium* OB268. *Can J Microbiol* **46**, 295–303.
- Kamio, Y. & Takahashi, H. (1980). Outer membrane proteins and cell surface structure of *Selenomonas ruminantium*. *J Bacteriol* **141**, 899–907.
- Kishino, H. & Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in *Hominoidea*. *J Mol Evol* **29**, 170–179.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Kolenbrander, P. E. & Moore, L. V. H. (1992). The genus *Veillonella*. In *The Prokaryotes*, 2nd edn, pp. 2034–2047. Edited by H. G. Balows, M. Trüper, W. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Liu, J. W., Wu, J. J., Wang, L. R., Teng, L. J. & Huang, T. C. (1998). Two fatal cases of *Veillonella* bacteremia. *Eur J Clin Microbiol Infect Dis* **17**, 62–64.
- Males, B. M., Berthold, P., Dougherty, P. A. & Listgarten, M. A. (1984). Helical flagellation in *Centipeda periodontii*, a Gram-negative, anaerobic bacillus from periodontitis lesions. *J Gen Microbiol* **130**, 185–191.
- Marchandin, H., Jean-Pierre, H., Carrière, C., Canovas, F., Darbas, H. & Jumas-Bilak, E. (2001). Prosthetic joint infection due to *Veillonella dispar*. *Eur J Clin Microbiol Infect Dis* **20**, 340–342.
- Marchandin, H., Jumas-Bilak, E., Gay, B., Teyssier, C., Jean-Pierre, H., Siméon de Buochberg, M., Carrière, C. & Carlier, J.-P. (2003a). Phylogenetic analysis of some *Sporomusa* sub-branch members isolated from human clinical specimens: description of *Megasphaera micronuciformis* sp. nov. *Int J Syst Evol Microbiol* **53**, 547–553.
- Marchandin, H., Teyssier, C., Siméon de Buochberg, M., Jean-Pierre, H., Carrière, C. & Jumas-Bilak, E. (2003b). Intra-chromosomal heterogeneity between the four 16S rRNA gene copies in the genus *Veillonella*: implications for phylogeny and taxonomy. *Microbiology* **149**, 1493–1501.
- Mays, T. D., Holdeman, L. V., Moore, W. E. C., Rogosa, M. & Johnson, J. L. (1982). Taxonomy of the genus *Veillonella* Prévot. *Int J Syst Bacteriol* **32**, 28–36.
- Morgenstern, B. (2002). A simple and space-efficient fragment-chaining algorithm for alignment of DNA and protein sequences. *Appl Math Lett* **15**, 11–16.
- Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. (1994). fastDNAm: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput Appl Biosci* **10**, 41–48.
- Rogosa, M. (1965). The genus *Veillonella*. IV. Serological groupings, and genus and species emendations. *J Bacteriol* **90**, 704–709.
- Rogosa, M. (1971). Transfer of *Veillonella* Prévot and *Acidaminococcus* Rogosa from *Neisseriaceae* to *Veillonellaceae* fam. nov., and the inclusion of *Megasphaera* Rogosa in *Veillonellaceae*. *Int J Syst Bacteriol* **21**, 231–233.
- Rogosa, M. (1984). Anaerobic Gram-negative cocci. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 680–685. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sato, T., Matsuyama, J., Sato, M. & Hoshino, E. (1997a). Differentiation of *Veillonella atypica*, *Veillonella dispar* and *Veillonella parvula* using restricted fragment-length polymorphism analysis of 16S rDNA amplified by polymerase chain reaction. *Oral Microbiol Immunol* **12**, 350–353.
- Sato, T., Sato, M., Matsuyama, J. & Hoshino, E. (1997b). PCR-restriction fragment length polymorphism analysis of genes coding for 16S rRNA in *Veillonella* spp. *Int J Syst Bacteriol* **47**, 1268–1270.
- Singh, N. & Yu, V. L. (1992). Osteomyelitis due to *Veillonella parvula*: case report and review. *Clin Infect Dis* **14**, 361–363.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Veys, A., Callewaert, W., Waelkens, E. & Van den Abbeele, K. (1989). Application of gas-liquid chromatography to the routine identification of nonfermenting gram-negative bacteria in clinical specimens. *J Clin Microbiol* **27**, 1538–1542.