Negativicoccus succinicivorans gen. nov., sp. nov., isolated from human clinical samples, emended description of the family Veillonellaceae and description of Negativicutes classis nov., Selenomonadales ord. nov. and Acidaminococcaceae fam. nov. in the bacterial phylum Firmicutes

Hélène Marchandin,1,2 Corinne Teyssier,1 Josiane Campos,2 Hélène Jean-Pierre,1,2 Frédéric Roger,1 Bernard Gay,3 Jean-Philippe Carlier4 and Estelle Jumas-Bilak1

Correspondence
Hélène Marchandin
h-marchandin@chu-montpellier.fr

1Université Montpellier 1, EA 3755, Laboratoire de Bactériologie-Virologie, Faculté de Pharmacie, 15, Avenue Charles Flahault, BP 14491, 34060 Montpellier Cedex 5, France
2Centre Hospitalier et Universitaire de Montpellier, Hôpital Arnaud de Villeneuve, Laboratoire de Bactériologie, 371 Avenue du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France
3Université Montpellier 1 et Université Montpellier 2, UMR 5236-CPBS CNRS, Centre d’études d’agents pathogènes et Biotechnologies pour la Santé, Institut de Biologie, 4, Boulevard Henri IV, CS 69033, 34965 Montpellier Cedex 2, France
4Institut Pasteur, Centre National de Référence des Bactéries Anaérobies et du Botulisme, 25 rue du Dr Roux, 75724 Paris Cedex 15, France

Three strains of a hitherto unknown, Gram-negative, tiny, anaerobic coccus were collected from human clinical samples originating from skin and soft tissues. The three isolates displayed at least 99.9 % identity in their 16S rRNA gene sequences and more than 99.8 % identity in their dnaK gene sequences. The isolates were affiliated to the family Veillonellaceae, the coccobacillus Dialister micraerophilus being the most closely related species, but there was no more than 91.1 % identity in the 16S rRNA gene sequence between this species and the three isolates. Phylogeny based on the 16S rRNA gene confirmed that the three strains represent a novel and robust lineage within the current family Veillonellaceae. A similar genomic structure was demonstrated for the three isolates by PFGE-based analysis. Morphology and metabolic end products, as well as genotypic and phylogenetic data supported the proposal of the novel genus Negativicoccus gen. nov., with the novel species Negativicoccus succinicivorans sp. nov. [type strain ADV 07/08/06-B-1388T (=AIP 149.07T = CIP 109806T = DSM 21255T = CCUG 56017T) as type species]. Phylogenetic analyses based on the 16S rRNA gene sequences of members of the phylum Firmicutes and other phyla indicated that the family Veillonellaceae forms a robust lineage clearly separated from those of the classes ‘Bacill’, ‘Clostridia’, Thermolithobacteria and ‘Erysipelotrichi’ in the phylum Firmicutes. Therefore, we propose that this family is a class-level taxon in the phylum Firmicutes, for which the name Negativicutes classis nov. is proposed, based on the Gram-negative type of cell wall of its members, with the type order Selenomonadales ord. nov. In this order, a novel family, Acidaminococcaceae fam. nov., is proposed and description of the family Veillonellaceae is emended.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and dnaK gene sequences of Negativicoccus succinicivorans gen. nov., sp. nov. ADV 07/08/06-B-1388T are FJ715930 and FJ715931, respectively.

PFGE migration of I-CeuI-restricted DNAs, an ML tree based on 227 partial 16S rRNA gene sequences (1200 nt), showing relationships between members of the family Veillonellaceae, other members of the phylum Firmicutes and other selected phyla, and composition of datasets A and B are available with the online version of this paper.
An aerobic, Gram-negative cocci are currently classified in four genera of the family Veillonellaceae (Garrity & Holt, 2001): Veillonella, Acidaminococcus, Megaphaera and Anaeroglobin (Prévot, 1933; Rogosa, 1969, 1971; Carlier et al., 2002). These four genera can be distinguished by phenotypic and genotypic features, mainly metabolic end products, and 16S rRNA and dnaK gene sequence analysis.

The family Veillonellaceae, formerly 'Acidaminococcaceae', is currently classified in the phylum Firmicutes (low-G+C Gram-positive bacteria), class Clostridia, one of the three classes currently recognized in the phylum together with 'Bacilli' and Thermolithobacteria (Sokolova et al., 2007), and in the order Clostridiales, which comprises 19 families (including nine families incertae sedis) (Ludwig et al., 2009). Despite the establishment of a revised road map to the phylum Firmicutes by Ludwig et al. (2009), significant further reorganization is expected in this taxon. For example, Ludwig et al. (2009) also suggested that the family Erysipelotrichaceae should be elevated to the rank of class, for which the name 'Erysipelotrichi' was proposed, but this has not yet been validated published. From a previous high-level phylogenetic study, we proposed an emended description of the family Syntrophomonadaceae, order Clostridiales, and suggested that it should be elevated to a higher rank than that of family (Jumas-Bilak et al., 2009).

The aim of this study was to describe a novel genus and species in the family Veillonellaceae. The relationships among members of the current family Veillonellaceae with other members of the phylum Firmicutes were also explored in order to propose a reappraisal of their taxonomic level.

**Negativicoccus succinicivorans gen. nov., sp. nov., isolated from human clinical samples**

The three strains studied were isolated from three patients hospitalized at the University Hospital of Montpellier, France, between January 2004 and August 2006 (ADV 07/08/06-B-1388, ADV 12/01/04-B-1195 = AIP 150.07 and ADV 03/08/05-B-3158 = AIP 147.07). The three strains were isolated from skin or soft tissue samples. The isolates grew on Columbia sheep blood agar (bioMerieux) after 2 to 3 days incubation at 37 °C in an anaerobic jar with the Anaerogen System (Oxoid Unipath). All isolates were recovered in mixed aerobic/anaerobic flora. These isolates were further submitted to polyphasic investigation.

The 16S rRNA gene and 70 kDa heat-shock protein gene (dnaK) were selectively amplified by PCR as described previously (Carlier et al., 2002; Marchandin et al., 2003b). The dnaK gene sequences were translated to the Hsp70 amino acid sequence using TRANSLATE (www.expasy.ch) to search for diderm insert signature sequences that distinguish Gram-negative bacteria from Gram-positive bacteria (Gupta, 1998). Using LALIGN software (www.expasy.org), the three isolates were shown to share at least 99.9 % of their 16S rRNA gene sequence (GenBank accession numbers FJ715928–FJ715930) and at least 99.8 % of their dnaK gene sequences (FJ715931–FJ715933). Nearly complete 16S rRNA gene sequences (1401 nt) and partial dnaK sequences (about 650 nt) were matched with GenBank and EMBL databases using the BLAST program (Altschul et al., 1997). They displayed a maximum level of identity with members of the genus Dialister within the family Veillonellaceae (Willems & Collins, 1995; Garrity & Holt, 2001), the most closely related known species being Dialister micraerophilus with sequence identity of about 91.1 %. The maximum 16S rRNA gene sequence identity (more than 99.7 %) was observed with sequences of several uncultured bacterial clones from the human skin microbiome (GenBank accession number GQ014591, for example) (Grice et al., 2009) and with uncultured clone rRNA030 from human vaginal epithelium (GenBank accession number AY958803). The highest levels of dnaK gene sequence identity were observed with members of the genera Veillonella, Megaphaera and Dialister, but were no higher than 74.2–76.2 %. Hsp70 proteins lacked the diderm insert observed for Firmicutes, Actinobacteria and Archaea (Gupta, 1998). 16S rRNA gene-based phylogenetic analyses were conducted on sequences selected from the GenBank database to study the relationships between the unknown isolates and species of the current family Veillonellaceae. The sequences were aligned using CLC Sequence Viewer, version 5.11 (Knudsen et al., www.clcbio.com). The regions of uncertain alignment were visually and automatically determined using Gblocks 0.91b software (Castresana, 2000) and then manually removed using Sequence Alignment Editor software (Se-Al Version 2.0a11; http://tree.bio.ed.ac.uk/software/seal/) before reconstruction of phylogenetic trees. Phylogenetic trees were inferred by using distance and maximum-likelihood methods. Neighbour-joining (NJ) and maximum-likelihood (ML) evolutionary trees were reconstructed by using PHYLIP (Felsenstein, 1993) (algorithm F84 as substitution model) and PHYML (algorithm general time-reversible, GTR, as substitution model plus gamma-distribution, plus invariant sites) (Guindon & Gascuel, 2003), respectively. The robustness of the trees was evaluated by bootstrap analysis of 1000 resamplings for NJ and 100 resamplings for ML. The ML 16S rRNA gene-based tree is shown in Fig. 1. The three isolates formed a robust phylogenetic clade supported by high bootstrap values and distinct from other genera in the family Veillonellaceae, in particular from the genus Dialister. These results were congruent with those observed from the NJ tree (data not shown). The DNA G+C content could not be determined, because the very limited growth of the strains in broth media meant that it was not possible to obtain enough biomass for G+C content analysis. We used a genomic approach based on pulsed-field gel electrophoresis (PFGE) to study large-scale chromosome structure, which has been described as a sensitive indicator of phylogenetic relationships between bacteria of the same genus or species (Liu et al., 1999). This approach was previously applied to other members of the family Veillonellaceae and the rrs skeleton, studied after restriction of the DNA with the intron-encoded endonu-
Fig. 1. ML tree based on partial 16S rRNA gene sequences (1211 nt), showing relationships between *N. succinicivorans* and other genera of the family *Veillonellaceae*. *Bacillus subtilis* was used as the outgroup. Numbers at nodes indicate percentage bootstrap support, based on analysis of 100 replicates. Nodes with asterisks varied according the method used to generate the tree. Bar, 0.1 substitutions per site.
clease I-\textit{Ceu}, appeared to be species-specific (Marchandin \textit{et al.}, 2003a; Jumas-Bilak \textit{et al.}, 2005, 2007). The number and sizes of bacterial chromosomes were analysed by PFGE of intact DNAs, and mapping experiments with I-\textit{Ceu} (New England Biolabs) were undertaken as described previously (Marchandin \textit{et al.}, 2003a). The three strains studied possessed a unique chromosome with mean genomic size estimated to be 1.62 Mb. The chromosome migrated in PFGE as a faint band, suggesting a circular topology (Allardet-Servent \textit{et al.}, 1993). The three strains possessed four \textit{rrn} operons and displayed a common I-\textit{Ceu} profile that differed from those of related species such as species of the genus \textit{Dialister} (Jumas-Bilak \textit{et al.}, 2005). The large-scale chromosome structure of the three strains studied and relatives is available as Fig. S1 in IJSEM Online. The three isolates formed circular, convex, translucent and tiny colonies of less than 0.5 mm in diameter on Columbia sheep blood agar plates after 48 h incubation in an anaerobic jar with the Anaerogen System (Oxoid Unipath). The bacteria displayed a Gram-negative stain and were non-sporulating, non-motile and coccoid. Strains ADV 07/08/06-B-1388$^T$ AIP 149.07$^T$ and ADV 12/01/04-B-1195=AIP 150.07 were prepared for electron microscopy as described previously for both ultrathin sections observation and negative staining (Marchandin \textit{et al.}, 2003a; Jumas-Bilak \textit{et al.}, 2005). They presented as small cocci of about 0.4 \textmu m in diameter (Fig. 2A, B) and an outer membrane typical of Gram-negative surface layers was observed in ultrathin sections [Fig. 2B(c)], as described previously for several members of the family \textit{Veillonellaceae} (Jumas-Bilak \textit{et al.}, 2004).

The strains were identified according to the procedures of the VPI Anaerobe Laboratory Manual (Holdeman \textit{et al.}, 1977). Susceptibility to special-potency discs was determined as described by Jousimies-Somer \textit{et al.} (2002). Growth in microaerophilic conditions was tested using Campygen Compact (Oxoid Unipath). Metabolic end products were assayed by quantitative GC as described by Carlier (1985). For further biochemical characterization, the strains were grown anaerobically in trypticase/glucose/yeast extract (TGY) broth and incubated at 37 °C for 72 h in an anaerobic jar containing 5 % H$_2$, 5 % CO$_2$ and 90 % N$_2$ (by vol.). The strains were asaccharolytic; acid was not produced from fructose, glucose, lactose, maltose, mannose and sucrose. The isolates were non-reactive towards conventional biochemical tests (nitrate reduction, nitrite reduction, production of gas, catalase, indole and urease). They grew in microaerophilic conditions. The three strains displayed susceptibility to a bile disk (1 mg), and to

\textbf{Fig. 2.} (A) General morphology of \textit{N. succinicivorans} strain ADV 07/08/06-B-1388$^T$ after negative staining. Bars, 500 nm (a) and 100 nm (b). (B) Electron microscopy of ultrathin sections to show ultrastructure of \textit{N. succinicivorans} strain ADV 12/01/04-B-1195; (a, b) general morphology; (c) Gram-negative type cell wall with outer membrane. Bars: 500 nm (a), 100 nm (b, c).
kanamycin (500 μg) and metronidazole (50 μg) disks, and showed resistance to vancomycin (5 μg) and colistin (10 μg) disks (Rosco). Enzymic profiles obtained with Rapid ID 32 A (API, bioMérieux) showed arginine arylamidase activity for all three strains and alkaline phosphatase activity for strains ADV 12/01/04-B-1195 = AIP 150.07 and ADV 03/08/05-B-3158 = AIP 147.07. Major metabolic end products in TGY broth were acetic and propionic acids (2.2–2.4 and 1.2–1.5 mmol l⁻¹, respectively). The strains also produced trace amounts (0.1–0.2 mmol l⁻¹) of 2-hydroxyvaleric acid. In addition, strain ADV 12/01/04-B-1195 = AIP 150.07 produced lactic acid (2.4 mmol l⁻¹). Sodium succinate enhanced the growth of the three strains, whereas lactate and glutamate did not. Subsequent GC analysis revealed that the three strains produced a larger amount of propionate from TGY supplemented with sodium succinate (33.9–47.2 mmol l⁻¹), and a larger amount of 2-hydroxyvaleric acid (2.9–4.1 mmol l⁻¹) was also noted. Characteristics that differentiate the three strains from closely related taxa of the family Veillonellaceae are presented in Table 1.

Based on phenotypic, genotypic and phylogenetic evidence, a new genus, *Negativicoccus* gen. nov., is proposed for the three strains studied with one species, *Negativicoccus succinicivorans* sp. nov. Moreover, it is suggested that the uncultured bacterial clone rRNA030 and several uncultured clones from the human skin microbiome (Grice et al., 2009) probably belong to the species *N. succinicivorans*.

### Proposal to elevate the current family Veillonellaceae to class rank in the phylum Firmicutes, and description of Negativicutes classis nov.

A 16S rRNA gene-based phylogeny was reconstructed using sequences of both cultured and non-cultured representative taxonomic units of different phyla selected according to their length (more than 1200 bp) and quality (fewer than 0.5% of undetermined positions) in the Greengenes database (http://greengenes.lbl.gov), and aligned using NAST program. Different datasets were used. Dataset A represented the phylum *Firmicutes* and included sequences of *Veillonellaceae* analysed in the tree in Fig. 1. The *Firmicutes* sequences were chosen on the basis of the diversity between the different taxonomic units belonging to this phylum according to Hugenholtz and Ludwig classifications in the Greengenes database. A total of 162 sequences were selected to represent the phylum *Firmicutes* sensu stricto, excluding *Tenericutes*. Among them, 62 were selected from uncultured clones and 63 were from type strains of the corresponding species. Dataset B included data from various sources.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Negativicoccus</th>
<th>Megasphaera</th>
<th>Veillonella</th>
<th>Acidaminococcus</th>
<th>Anaeroglobus</th>
<th>Dialister</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Cocci</td>
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</tr>
<tr>
<td>Cell size (µm)</td>
<td>0.4</td>
<td>1.7–2.6</td>
<td>0.3–0.5</td>
<td>0.5–1.0</td>
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<td>0.2–0.4 × 0.3–0.6</td>
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<td>Growth in microaerophilic conditions</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>− ±</td>
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<td>Nitrate reduction</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<td>−</td>
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<tr>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Decarboxylation of succinate</td>
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<td>−</td>
<td>+</td>
<td>−</td>
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<td>±</td>
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<td>Amino acids as main source of energy</td>
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<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Gas production</td>
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<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>ND</td>
<td>53.1–54.1</td>
<td>40.3–44.4</td>
<td>56 (Bd) or 49.3 (Tm)</td>
<td>51.8</td>
<td>35–36.3 or 45–46</td>
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<tr>
<td>Mean genomic size (Mb)</td>
<td>1.45</td>
<td>1.81 or 2.58</td>
<td>2.18</td>
<td>2.35 or 2.49</td>
<td>1.85</td>
<td>1.34, 1.7 or 1.97</td>
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<td>Number of rRN operons</td>
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<td>4 or 7</td>
<td>4</td>
<td>3 or 6</td>
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</tr>
</tbody>
</table>

*Weak reactions observed for some species were not taken into account.
†Not detected in significant quantities for *Dialister micraerophilus*."

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**Table 1. Characteristics differentiating the genus Negativicoccus from related genera of anaerobic Gram-negative bacteria**

Data were taken from Rogosa (1984), Holt et al. (1994), Carlier et al. (2002), Marchandin et al. (2003a) and Jumas-Bilak et al. (2007). The phylogenetically related genus *Allisonella* was not included due to its unique phenotypic characteristics. Briefly, this ovoid-shaped, Gram-negative bacterium uses histidine decarboxylation as the sole source of energy since carbohydrates and organic acids are not used. Lysine can also be used but does not permit bacterial growth. The G + C content of the DNA is 46.8 mol% (Garner et al., 2002). A, Acetic acid; P, propionic acid; L, lactic acid; B, butyric acid; V, valeric acid; C, caproic acid; iB, isobutyric acid; iV, isovaleric acid; S, succinic acid; ND, not detected.

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[Link to the article](http://ijs.sgmjournals.org)
sequences of the phyla Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes and Chlamydiae. The composition of the two datasets is available as Table S1 in IJSEM Online. Datasets representative of other described bacterial phyla (Chlorobi, Chloroflexi, Chrysioengnetes, Cyanobacteria, Deferribacteres, Ferribacter, Fervidimicrobiun, Fibrobacteres, Fusobacteria, Gemmamimonadetes, Halanaeobacteriales, Lentinisphaera, Morella, Natoanerobium, Nitrospirae, Planctomycetes, Poribacteria, Proteobacteria, Spirochaetae, Sulfo-bacilli, Synergistetes, Thermi, Thermacetogenium, Thermo-desulfo bacterium, Thermovornabulae and Verrucomicrobia) corresponded to sequences selected in datasets 1, 2, 3, 4 and 5 used for the description of the phylum Synergistetes (Jumas-Bilak et al., 2009). Phylogenetic analyses were conducted by three methods, NJ, ML and Bayesian inference, as described previously (Jumas-Bilak et al., 2009). Phylogenetic trees were reconstructed from the datasets A + B (Fig. S2 in IJSEM Online) and from datasets A + 1, A + 2, A + 3, A + 4 and A + 5 (data not shown). When compared with phylogeny-level taxa of each dataset, members of the family Veillonellaceae always aggregated to the phylum Firmicutes, whereas each described phylum formed an independent branch (Fig. S2 and data not shown). The same relationship between Veillonellaceae and Firmicutes or other phyla was observed whatever the phylogenetic method employed (data not shown). This provided strong evidence that on the basis of the 16S rRNA gene sequence Veillonellaceae belongs to the phylum Firmicutes.

However, members of the family Veillonellaceae are unified in a deep-branching clade, suggesting that Veillonellaceae is a taxon higher than family. The tree reconstructed with the datasets A + B (Fig. S2) showed that the clade corresponding to Veillonellaceae formed a robust lineage in the Firmicutes (bootstrap values of 99% and 927% in ML and NJ analyses, respectively, and posterior probability of 0.99 for Bayesian inference). Although very low significance of the relative order of branches connecting the Firmicutes classes was observed, the Veillonellaceae lineage was clearly separated from the classes ‘Bacilli’, Thermolithobacteria and ‘Erysipelotrichi’ as well as from the class ‘Clostridia’, the polyphyletic class where the family Veillonellaceae is currently classified. Therefore, class rank is proposed for the current family Veillonellaceae. Moreover, the class ‘Clostridia’ should probably be split into several taxa of various taxonomic levels and some of them should probably be elevated to a higher rank than that of class, as suggested by Ludwig et al. (2009). The new class is characterized by a major ultrastructural character, since all its members harbour a typical Gram-negative cell wall structure with an outer membrane as observed in electron microscopy (Bladen & Mergenhagen, 1964; Kamio & Takahashi, 1980; Kalmkoff et al., 2000; Males et al., 1984; Rogosa, 1969; Jumas-Bilak et al., 2007). With reference to this structure, we propose the name Negativicutes for the novel class.

In addition to phylogenetic evidence, the cell wall structure confirmed the separation of the proposed class Negativicutes from other members of the phylum Firmicutes. In this class, a robust lineage consistently recovered from the diverse phylogenetic analyses, including diverse datasets and diverse methods (Fig. S2 and data not shown), groups all the members of the current family Veillonellaceae. This lineage, supported by high bootstrap values (92% and 801% in ML and NJ analyses, respectively, and posterior probability of 0.98 for Bayesian inference), was described as the order Selenomonadales ord. nov. on the basis of the name of the first characterized genus in the taxon. Besides Selenomonadales ord. nov., a second robust lineage including six clones might well correspond to another order-level taxon in the class Negativicutes. The intra-order tree topology reveals the presence of two phylogenetically distinct and robust lineages, which are also consistently recovered from phylogenetic analyses. We propose the name Acidaminococcaceae fam. nov. for one of these clades (Fig. S2) supported by bootstrap values of 100% and 928% in ML and NJ analyses, respectively, and posterior probability of 1.00 for Bayesian inference. We also propose an emended description of the family Veillonellaceae for the second clade (Fig. S2) supported by bootstrap values of 100% and 1000% in ML and NJ analyses, respectively, and posterior probability of 0.93 for Bayesian inference.

Other branches at the subclass level could not be robustly aggregated and remain as Selenomonadales incertae sedis, but they may well represent several novel bacterial taxa. Further reappraisal will be warranted when their taxonomic position becomes more clearly delineated as new sequence data and/or data from analysis of other phylogenetic markers becomes available. The Gram-positive/Gram-negative dichotomy appears as a structural character with evolutionary significance since it has been generally confirmed by molecular phylogeny based on 16S rRNA sequences, in spite of some exceptions (Woese, 1987), but also on the basis of Hsp70 sequence signatures (Gupta, 1998). The major significance of the cell wall structure was also highlighted in the bacterial mega classification proposed by Cavalier-Smith (2002). Gram-positive cell wall structure appears in only two eubacterial phyla, Firmicutes and Actinobacteria. The Firmicutes were proposed before the genetic era as a division in the kingdom Procraytae (Gibbons & Murray, 1978) along with the divisions Gracilicutes (Gram-negative bacteria) and Mollicutes (bacteria without peptidoglycan). The Firmicutes comprised three class-level taxa, including bacteria with different cell wall structure: ‘Bacilli’, ‘Clostridia’ and Mollicutes (Ludwig & Klenk, 2001). A proposal to transfer the Mollicutes to the phylum Tenericutes on the basis of their unique phenotypic properties and the general low support by alternative markers (Ludwig & Schleifer, 2005) led to the characterization of the class ‘Erysipelotrichi’ to accommodate wall-forming Gram-positive organisms of the family Erysipelotrichaceae previously classified with the Mollicutes in the phylum Firmicutes (Ludwig & Schleifer, 2005). The classes ‘Bacilli’ and Thermolithobacteria include Gram-positive type organisms and thus the paraphyletic
class 'Clostridia' is currently the only class in the phylum Firmicutes grouping both Gram-positive and Gram-negative taxa. The question of the belonging of Gram-negative members of the 'Clostridia' to the phylum Firmicutes remains open. Gram-negative members of the family Syntrophomonadaceae, class 'Clostridia', were recently reclassified in the phylum Synergistetes (Jumas-Bilak et al., 2009). On the contrary, we showed here that the novel class Negativicutes could not be excluded from the phylum Firmicutes on the basis of 16S rRNA gene phylogeny. The proposed Gram-negative class Negativicutes is more closely related to Gram-positive bacteria of the phylum Firmicutes than to any other Gram-negative bacteria. Moreover, the lack of the diderm insert was observed in all representatives of the Negativicutes for which Hsp70 sequences are available in the GenBank database, and confirmed in this study in the proposed genus Negativicoccus. The inclusion in the phylum Firmicutes of other bacteria with a well-documented Gram-negative structure, such as Thermohalobacter (Cayol et al., 2000), Caloranaerobacter (Wery et al., 2001) and Caminicella (Alain et al., 2002), warrants further investigation for a better understanding of the evolutionary relationships between Gram-positive and Gram-negative bacteria. Currently classified in the family Clostridiales, probable reclassification of these genera has already been evoked by Ludwig et al. (2009), but has still to be studied.

Description of Negativicoccus gen. nov.

Negativicoccus [Neg.at.i.vi.coc’c.us. L. adj. negativus negative; N.L. masc. n. coccus (from Gr. masc. n. kokkos) grain or berry; N.L. masc. n. Negativicoccus coccus with a typical Gram-negative cell wall structure with an outer membrane observed by electron microscopy].

Characteristics of the genus are as described below for the type and only species N. succinicivorans. Can be differentiated from other genera of the family Veillonellaceae by metabolic end products, and 16S rRNA gene and dnaK sequencing.

The type species is Negativicoccus succinicivorans.

Description of Negativicoccus succinicivorans sp. nov.

Negativicoccus succinicivorans (suc.ci.ni.ci.vo’rans. N.L. n. acidum succinicum succinic acid; L. part. adj. vorans devouring; N.L. part. adj. succinicivorans succinic acid-devouring).

Cells are coccoid (0.4 μm in diameter), Gram-negative, non-motile, non-sporulating. Colonies on Columbia blood agar are very tiny, less than 0.5 mm in diameter after 48 h, circular, convex and translucent. Anaerobic and micro-aerophilic. Unreactive in most conventional biochemical tests. Asaccharolytic. Arginine arylamidase activity is noted and inconstant alkaline phosphatase activity is observed.

Metabolic end products are acetic and propionic acids and trace amounts of 2-hydroxyvaleric acid. Lactic acid may be produced. Growth is enhanced by sodium succinate.

The type strain is strain ADV 07/08/06-B-1388T (=AIP 149.072=CIP 109806T=DSM 21255T=CCUG 56017T), and a reference strain is ADV 12/01/04-B-1195=AIP 150.07=CIP 109807=DSM 21256=CCUG 56016. Found in human clinical samples and the human skin microbiome.

Emended description of the family Veillonellaceae Rogosa 1971

The family Veillonellaceae includes the recognized genera Allisonella, Anaeroglobus, Dialister, Megasphaera, Veillonella and the proposed genus Negativicoccus.

The description is as described by Rogosa (1971) with the following modifications. Gram-negative bacteria. Cocci or cocobacilli. Anaerobic or microaerophilic. Gas may or may not be produced.

The type genus is Veillonella Prévot 1933.

Description of the family Acidaminococcaceae

Acidaminococcaceae (A.ci.dam.in.o.coc.ca’ceae. N.L. masc. n. Acidaminococcus type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. Acidaminococcaceae the Acidaminococcus family).

The family Acidaminococcaceae includes the recognized genera Acidaminococcus, Phascolarctobacterium, Succinispira and Succiniclamicum.

Gram-negative bacteria. Anaerobic. Non-sporulating. Cocci, or curved or pleomorphic rods. Motile or not. Carbohydrates are not fermented (with the exception of some Acidaminococcus fermentans strains for which weak fermentation of glucose is observed). Growth with succinate or propionate production is observed or not. Amino acids are used as energy source or not.

The type genus is Acidaminococcus Rogosa 1969.

Description of Selenomonadales ord. nov.

Selenomonadales (Se.le.no.mo.na.da’les. N.L. fem. n. Selenomonas -adis type genus of the order as the first characterized genus; -ales ending to denote an order; N.L. fem. pl. n. Selenomonadales the Selenomonas order).

The description is the same as for the class Negativicutes. The order includes the validated genera Acetotema, Acidaminococcus, Allisonella, Anaerococcus, Anaeroglobus, Anaeromusa, Anaerominus, Anaerovibrio, Centipida, Dendrosporobacter, Dialister, Megamonas, Megasphaera, Mitsuokella, Pectinatus, Pelosinus, Phascolarctobacterium, Propionispira, Propionispina, Quinella, Schwartzia, Selenomonas, Sporomusa, Sporotalea, Succiniclasticum, Succinispira, Thermostinus, Veillonella and Zymophilus,
and the proposed genera Anaerospora, Desulfosporomusa, Psychrosinus and Negativicoccus.

The type genus is Selenomonas von Prowazek 1913.

Description of Negativicutes classis nov.

Negativicutes (Ne.gai.ti.vi.cu’tes. L. adj. negativus negative; L. fem. n. curis skin; N.L. fem. pl. n. Negativicutes division with cells bounded by skin with two concentric lipid bilayers, the cytoplasmic membrane and an outer membrane, to indicate Gram-negative type of cell wall in the Firmicutes division).

The class Negativicutes is defined in phylogenetic terms on the basis of 16S rRNA gene phylogeny. Members of the class harbour a typical Gram-negative cell wall structure with an outer membrane observable by electron microscopy.

Type order is Selenomonadales.

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References


