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*

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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 microaerophilus* 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-1388^T (=AIP 149.07^T=CIP 109806^T=DSM 21255^T=CCUG 56017^T) 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 'Bacilli', '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-1388^T 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.

Anaerobic, Gram-negative cocci are currently classified in four genera of the family *Veillonellaceae* (Garrity & Holt, 2001): *Veillonella*, *Acidaminococcus*, *Megasphaera* and *Anaeroglobus* (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 validly 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 succinivorans* 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^T=AIP 149.07^T, 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 (bioMérieux) 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*, *Megasphaera* 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 *rrn* 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. succinivorans* 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-*CeuI*, appeared to be species-specific (Marchandin *et al.*, 2003a; Jumas-Bilak *et al.*, 2005, 2007). The number and sizes of bacterial chromosomes were analysed by PFGE of intact DNAs, and mapping experiments with I-*CeuI* (New England Biolabs) were undertaken as described previously (Marchandin *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 *et al.*, 1993). The three strains possessed four *rrn* operons and displayed a common I-*CeuI* profile that differed from those of related species such as species of the genus *Dialister* (Jumas-Bilak *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

et al., 2003a; Jumas-Bilak *et al.*, 2005). They presented as small cocci of about 0.4 µm 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 *Veillonellaceae* (Jumas-Bilak *et al.*, 2004).

The strains were identified according to the procedures of the VPI Anaerobe Laboratory Manual (Holdeman *et al.*, 1977). Susceptibility to special-potency discs was determined as described by Jousimies-Somer *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 % H₂, 5 % CO₂ and 90 % N₂ (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

Fig. 2. (A) General morphology of *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 *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

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.

Characteristic	<i>Negativicoccus</i>	<i>Megasphaera</i>	<i>Veillonella</i>	<i>Acidaminococcus</i>	<i>Anaeroglobus</i>	<i>Dialister</i>
Cell morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Coccobacilli
Cell size (µm)	0.4	1.7–2.6	0.3–0.5	0.5–1.0	0.5–1.1	0.2–0.4 × 0.3–0.6
Growth in microaerophilic conditions	+	–	–	–	–	±
Nitrate reduction	–	–	+	–	–	–
Ability to ferment carbohydrates*	–	+	–	–	+	–
Fermentation of lactate	–	+	+	–	–	–
Decarboxylation of succinate	+	–	+	–	–	±
Amino acids as main source of energy	–	–	–	+	–	–
Gas production	–	+	+	+	–	–
Metabolic end products	A, P (L)	A, P, B, V, C	A, P	A, B, (P), (L)	A, P, iB, B, iV	A, P, (L), (S)†
DNA G + C content (mol%)	ND	53.1–54.1	40.3–44.4	56 (Bd) or 49.3 (<i>T_m</i>)	51.8	35–36.3 or 45–46
Mean genomic size (Mb)	1.45	1.81 or 2.58	2.18	2.35 or 2.49	1.85	1.34, 1.7 or 1.97
Number of <i>rrn</i> operons	4	4 or 7	4	3 or 6	4	4

*Weak reactions observed for some species were not taken into account.

†Not detected in significant quantities for *Dialister microaerophilus*.

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*, *Chrysiogenetes*, *Cyanobacteria*, *Deferribacteres*, *Ferribacter*, *Fervidomicrobium*, *Fibrobacteres*, *Fusobacteria*, *Gemmanimonadetes*, *Haloanaerobacteriales*, *Lentisphaera*, *Moorella*, *Natroanaerobium*, *Nitrospirae*, *Planctomycetes*, *Poribacteria*, *Proteobacteria*, *Spirochaetae*, *Sulfobacilli*, *Synergistetes*, *Thermi*, *Thermacetogenium*, *Thermodesulfobacterium*, *Thermovernabulae* and *Verrumicrobia*) 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 phylum-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; Kalmokoff *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 *Nega-*

tivicutes 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 megaclassification 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 *Prokaryotae* (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 *Clostridiaceae*, 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 [Ne.ga.ti.vi.coc'cus. 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-1388^T (=AIP 149.07^T=CIP 109806^T=DSM 21255^T=CCUG 56017^T), 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 coccobacilli. 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'ce.ae. 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 *Succinilasticum*.

Gram-negative bacteria. Anaerobic. Non-sporulating. Cocci, or curved or pleiomorphic 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 *Acetonema*, *Acidaminococcus*, *Allisonella*, *Anaeroarcus*, *Anaeroglobus*, *Anaeromusa*, *Anaerosinus*, *Anaerovibrio*, *Centipeda*, *Dendrosporobacter*, *Dialister*, *Megamonas*, *Megasphaera*, *Mitsuokella*, *Pectinatus*, *Pelosinus*, *Phascolarctobacterium*, *Propionispira*, *Propionispora*, *Quinella*, *Schwartzia*, *Selenomonas*, *Sporomusa*, *Sporotalea*, *Succinilasticum*, *Succinispira*, *Thermosinus*, *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.ga.ti.vi.cu'tes. L. adj. *negativus* negative; L. fem. n. *cutis* 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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