Description of ‘Synergistetes’ phyl. nov. and emended description of the phylum ‘Deferribacteres’ and of the family Syntrophomonadaceae, phylum ‘Firmicutes’

Estelle Jumas-Bilak,1 Laurent Roudière1 and Hélène Marchandin1,2

1Université Montpellier 1, Laboratoire de Bactériologie-Virologie, EA 3755, Faculté de Pharmacie, 15, Avenue Charles Flahault, BP 14491, 34060 Montpellier Cedex 5, France
2Centre Hospitalier Universitaire de Montpellier, Hôpital Arnaud de Villeneuve, Laboratoire de Bactériologie, 371 Avenue du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France

The number of bacterial phyla has greatly increased in the past decade. Among them, a candidate division named ‘Synergistetes’ was proposed in a phylogenetic study on the global diversity of bacteria. We previously described the genus Jonquetella and suggested that it belonged to this not yet well-delineated candidate phylum. 16S rRNA gene-based phylogeny studies were conducted using four reconstruction methods and 599 sequences forming five datasets were used in an alternative treeing approach. These analyses indicated that the genera Aminiphilus, Aminobacterium, Aminomonas, Anaerobaculum, Dethiosulfovibrio, Jonquetella, Synergistes, Thermanaerovibrio and Thermovirga should be grouped in the same high-level taxon. This taxon was shown to be a phylum-rank lineage in the domain Bacteria and, because of the prior use of the name Synergistes for a genus, the name ‘Synergistetes’ is proposed for this candidate phylum. We also propose an emended delineation of the phylum ‘Deferribacteres’, which is now only represented by the family Deferribacteriaceae. The emended family Syntrophomonadaceae is limited to the genera Pelospora, Syntrophomonas, Syntrophothermus and Thermosyntropha.

The phylum or division is the highest taxonomic rank in the three-domains tree of life. Consequently, the only phylogenetic character shared between members of two phyla is ‘belonging to the domain Bacteria’. A phylum is formed to accommodate a group of bacteria that cannot be aggregated to any taxon, except Bacteria. According to this definition, molecular phylogeny can easily delineate a phylum avoiding the use of controversial similarity thresholds, as done for species delineation. Despite this robust definition and the increasing number of candidate phyla, descriptions or emendation of phyla are published rarely, resulting in the misclassification of species or genera even in recent descriptions. This is the case for bacteria and clones phylogenetically related to Synergistes jonesii and classified either in the phylum ‘Deferribacteres’ or in the family Syntrophomonadaceae (phylum ‘Firmicutes’).

Synergistes jonesii is a Gram-negative, anaerobic, non-spore-forming bacterium isolated from goat rumen that degraded dihydroxypyridine compounds (Allison et al., 1992). When described, Synergistes jonesii was not related to any characterized bacteria, but this initial analysis was compromised by insufficient 16S rRNA gene sequences being available for outgrouping. At the same time, the moderate thermophile Flexistipes sinusarabici was described in the domain Bacteria but no relationship to any known phylum was detected (Fiala et al., 1990). Later, the metal-reducing genera Geovibrio (Caccavo et al., 1996) and Deferribacter (Greene et al., 1997), as well as the nitrate-reducing bacteria of the genus Dentitrovibrio (Myhr & Torsvik, 2000), were grouped together with Flexistipes sinusarabici, to form a separate clade in Bacteria. Both Flexistipes-like and Synergistes-like organisms have been proposed as new phyla in the domain Bacteria by Hugenholtz et al. (1998). Despite their independent deep branching, these two lineages were grouped in the phylum ‘Deferribacteres’ in the Taxonomic Outline of the Prokaryotes (Garrity et al., 2004) and in the NCBI taxonomy database (www.ncbi.nlm.nih.gov). According
to both classifications, Synergistes jonesii remained unclassified as incertae sedis among the ‘Deferrribacteres’.

The family Syntrophomonadaceae was created by Zhao et al. (1993) to group anaerobic, saturated fatty acid-β-oxidizing syntrophic bacteria isolated from anaerobic digester sludge (genera Syntrophomonas and Syntrophospora, now reclassified as Syntrophomonas). Subsequently, bacteria from various environments such as a wastewater treatment lagoon (Menes & Muxi, 2002), activated sludge (Baena et al., 1999; Sekiguchi et al., 2000; Diaz et al., 2007), hot springs (Gorlenko et al., 2004; Sokolova et al., 2002), sulfur mats in a saline environment (Surkov et al., 2001) and a deep marine trench (Takai et al., 1999) were aggregated to the family Syntrophomonadaceae in the phylum ‘Firmicutes’. The family contained 16 genera (Garrity et al., 2004), but most of them did not match the initial description of the family; in particular, syntrophic metabolism with hydrogenotrophic bacteria has only been observed for some of them (Svetlitshnyi et al., 1996; Wu et al., 2006a). In addition, their deep branching in the 16S rRNA gene tree suggested that they might represent a taxon of higher rank than the family.

The phylogenetic analyses conducted to describe most of the genera currently classified in Syntrophomonadaceae, such as Dethiosulfovibrio (Magot et al., 1997), Aminomonas (Baena et al., 1999), Anaerobaculum (Menes & Muxi, 2002), Aminobacterium (Baena et al., 2000), Thermovirga (Dahle & Birkeland, 2006) and Aminophilus (Diaz et al., 2007), did not include Synergistes jonesii. The first report that related Synergistes-like organisms to members of the family Syntrophomonadaceae was the descriptions of Dethiosulfovibrio russensis and Dethiosulfovibrio marinus (Surkov et al., 2001), showing that Aminobacterium, Aminomonas, Anaerobaculum, Dethiosulfovibrio, Thermaerovibrio and Synergistes formed a monophyletic cluster. Recently, the genus Jonquetella was included in this cluster (Jumas-Bilak et al., 2007).

In this study, based on a comparative analysis of 16S rRNA genes, we propose that the genera Aminophilus, Aminobacterium, Aminomonas, Anaerobaculum, Dethiosulfovibrio, Jonquetella, Synergistes, Thermaerovibrio and Thermovirga should be assembled in the same high-level taxon. We also show that this taxon represents a new phylum in the domain Bacteria, for which the name ‘Synergistetes’ is proposed.

Paraphyly of the family Syntrophomonadaceae in the 16S rRNA gene-based phylogeny

Sequences of reference strains of genera representative of the family Syntrophomonadaceae, the phylum ‘Deferrribacteres’, and the main orders of the phylum ‘Firmicutes’, according to the Taxonomic Outline of the Prokaryotes (Garrity et al., 2004) and NCBI taxonomy in the greengenes database (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) (DeSantis et al., 2006), were compared in order to analyse their phylogenetic relationships. The sequences downloaded from the greengenes database were NAST aligned based on the secondary structure and were checked for chimeras using Belloraphon v. 3 (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi). The sequences were also aligned using the CLUSTAL_X program (Thompson et al., 1997) and the alignments were corrected manually to exclude ambiguously aligned regions corresponding to loops variable in length. The most appropriate substitution model determined according to the Akaike information criterion calculated with MODELTEST (v. 3.7) (Posada & Crandall, 1998) was TN93. Evolutionary distances (ED) were analysed by using neighbour-joining with MEGA 4.0 software (Tamura et al., 2007). Maximum-likelihood (ML) phylogenetic analysis was performed using PHYML v. 2.4.6 (Guindon & Gascuel, 2003), the gamma likelihood (ML) phylogenetic analysis was performed using PHYML v. 2.4.6 (Guindon & Gascuel, 2003), the gamma shape parameter being estimated from the dataset. ML bootstrap support was computed using PHYML. Trees based on bayesian inference were reconstructed with MrBayes (v. 3.1.2) (Ronquist & Huelsenbeck, 2003). Four Markov chains were run for 200 000 generations and were sampled every 100 generations. Based on convergence of likelihood scores, the first 500 trees were discarded and a consensus tree was generated using the remaining trees, including posterior probability of clades and branch lengths. Maximum-parsimony (MP) trees were reconstructed and their robustness was evaluated by using bootstrap methods with DNAPARS of the PHYLIP package (Felsenstein, 1984). The phylogenies generated based on the four methods and the two types of alignments were congruent. The ML phylogenetic tree is shown in Fig. 1. Type strains of species of the genera Aminophilus, Aminobacterium, Aminomonas, Anaerobaculum, Dethiosulfovibrio, Jonquetella, Synergistes, Thermaerovibrio and Thermovirga formed the taxonomic framework of a well-separated, deep-branched group supported by high bootstrap values in ED, ML and MP trees and by high posterior probabilities in a Bayesian tree and was named ‘Synergistetes’ clade. S. jonesii within this clade clearly branched out of the phylum ‘Deferrribacteres’ where it was classified (Garrity et al., 2004). The ‘Synergistetes’ clade appeared to be subdivided into five main robust lineages (named A–E; Fig. 1). In order to evaluate the diversity of the ‘Synergistetes’ clade, phylogenetic trees were reconstructed as described previously using a set of 81 sequences representative of the clade. The tree confirmed that the ‘Synergistes’ clade was subdivided into five deep branches, forming five subdivisions (data not shown).

The unclassified ‘Deferrribacteres’ and Caldithrix abyssi in association with uncultured organisms formed a deep-branched lineage of ‘Deferrribacteres’ and ‘Synergistes’ clade. The phylum ‘Deferrribacteres’ appeared to be limited to the current family Deferrribacteriaceae. The genera Anaerobrancia, Caldithrix, Carboxydovilla, Pelospora, Syntrophomonas, Syntrophothermus, Thermaerobacter and Thermonautropha, which currently belong to the family Syntrophomonadaceae, were remote from the ‘Synergistetes’ clade, whatever the treeing method and alignment.
used. However, the relative branching of these genera was supported by low bootstrap values and varied according to the method used. Therefore, the 16S rRNA gene-based phylogeny clearly split the members of the current family Syntrophomonadaceae into at least two unrelated groups.

A major ultrastructural character, cell-wall structure, supported the separation of the ‘Synergistes’ clade from other members of the current family Syntrophomonadaceae. The members of the ‘Synergistes’ clade had a typical Gram-negative cell-wall structure with an outer membrane, as observed using electron microscopy (Allison et al., 1992; Baena et al., 1999, 2000; Dahle & Birkeland, 2006; Magot et al., 1997; Menes & Muxi, 2002; Surkov et al., 2001; Zavarzina et al., 2000). In contrast, other members of Syntrophomonadaceae possessed a Gram-positive type cell-wall structure without an outer membrane but with a thin peptidoglycan layer (Cayol et al., 1995; Gorlenko et al., 2004; Huang et al., 1998; Sokolova et al., 2002; Sekiguchi et al., 2000; Svetlitskhyi et al., 1996; Takai et al., 1999). The thinness of this layer could explain the negative reaction to Gram stain observed for these bacteria. Among them, members of the genus Syntrophomonas showed variable responses to Gram staining and presented with Gram-positive type or unusual cell-wall ultrastructure (Wu et al., 2006a, b; McInerney et al., 1981).

Finally, the cell-wall structure, which was considered to be a characteristic of high significance in bacterial evolution (Gupta, 1998; Cavalier-Smith, 2002), supported the argument for the paraphyly of the current family Syntrophomonadaceae. Current members of the family Syntrophomonadaceae other than those belonging to the ‘Synergistes’ clade also formed a deep-branched clade, but was supported by low bootstrap values. The family should be probably elevated to a higher rank than that of the family and also split into several taxa, for which the relationships with members of the phylum Firmicutes remain to be explored. The genera Syntrophomonas, Syntrophothermus and Thermosyntropha shared syntrophy as a functional character and, together with the genus Pelospora, formed a robust phylogenetic group (Fig. 1) and might represent the ‘core family’ Syntrophomonadaceae.

The ‘Synergistes’ clade is a phylum-level taxon for which the name ‘Synergistetes’ is proposed

The typical inter-phyla 16S rRNA gene sequence differences ranged from 20 to 25% (Hugenholtz et al., 1998).

The ‘Synergistes’ clade displayed less than 75% 16S RNA gene similarity with the sequences of representative type strains of genera in the ‘Firmicutes’, ‘Deferribacteres’ and other phyla. We used a phylogenetic approach in order to confirm the phylum rank of the ‘Synergistes’ clade. A bacterial phylum was defined from a phylogenetic point of view by Hugenholtz et al. (1998) as a group of sequences that are reproducibly monophyletic and are not affiliated to other groups at the phylum level. Approaches that include various methods and datasets have been recommended for the description of phylogenetic relationships between high-level taxa (Dalevi et al., 2001). In particular, multiple sets of outgroup sequences have to be used to test the monophyly of the ingroup. This approach was used in this study and five datasets were analysed as follows.

The sequences used for the construction of datasets 1–5 are given in Supplementary Table S1 (available in IJSEM Online). Each set of sequences contained a constant ingroup, formed by 29 sequences from cultured or uncultured organisms of the candidate phylum. Twenty-one sequences of cultured or uncultured members of the phylum ‘Deferribacteres’ formed constant outgroups in each dataset. Each dataset displayed other division-level outgroups that varied according to the dataset. The aligned sequences were downloaded from the greengenes database.

The most appropriate substitution model for all datasets was general time-reversible (GTR) plus gamma-distribution, plus invariant sites. For each dataset, ED, ML, MP and Bayesian phylogenies were reconstructed as described above except for the ED trees, which were reconstructed by using neighbour-joining in the ARB environment (Ludwig et al., 2004). The ML trees corresponding to the five datasets are shown with collapsed branches in Fig. 2(a–e). The complete ML trees are shown in Supplementary Fig. S1(a–e) in IJSEM Online. In all of the phylogenetic reconstructions, the ingroup sequences representing the ‘Synergistes’ clade formed a cluster at the phylum level, as its branching was as deep as those of recognized phyla. The cluster was monophyletic and could not be related to a previously described phylum, particularly to ‘Deferribacteres’ or to ‘Firmicutes’. The ingroup tested here corresponded to a phylum-level clade, supported by bootstrap values of >95% or Bayesian posterior probability values of 1.0, independent of the composition of the dataset. Each clade corresponding to a known phylum was supported by good confidence values. Lower clade confidence values were observed at the higher nodes.
indicating lack of statistical significance of the inter-phyla branching order. The branching order also varied according to the phylogenetic method used. As described previously (Hugenholtz et al., 1998), this unsolved deep multifurcation suggested the limits of the 16S rRNA-based phylogeny resolution but could also reflect a true biological explosive radiation corresponding to the phylum radiation in Bacteria. The ‘Synergistetes’ clade was rooted at this radiation confirming its phylum rank.

The name proposed for the new phylum is Synergistetes because of the precedence of the genus Synergistes as proposed by Jumas-Bilak et al. (2007). It includes Synergistia classis nov., which corresponds to subdivision A, the first robust subdivision after the phylum root in the lineage of the genus Synergistes. The order Synergistales ord. nov. and the family Synergistaceae fam. nov. correspond to the next two internal nodes to the species Synergistes jonesii. In the current family Syntrophomonadaceae (Garrity et al., 2004), only the representatives of the genera Aminobacterium, Anaerobaculum, Aminiphilus, Dethiosulfovibrio, Thermovirga, Thermaanaerovibrio and Aminomonas were related to the phylum ‘Synergistes’. The multi-dataset approach also confirmed the monophyly and the phylum rank of the family Deferribacteriaceae, the sole representative of the phylum ‘Deferribacteres’.

**Description of Synergistetes phyl. nov.**

Synergistetes (Syn.er.gis’t.et’es. N.L. masc. n. Synergistes -is a genus included in the phylum; N.L. fem. pl. n. Synergistetes the phylum of the genus Synergistes).

The phylum is defined on the basis of 16S rRNA gene phylogeny of 11 type strains corresponding to eight recognized genera (Aminobacterium, Aminomonas, Anaerobaculum, Dethiosulfovibrio, Januettella, Synergistes, Thermovirga, Thermaanaerovibrio). Includes anaerobic, Gram-negative, rod-shaped bacteria isolated from humans, animals and terrestrial and oceanic habitats. Amino-acid degrading bacteria.

Type order: Synergistales ord. nov.

**Description of Synergistia classis nov.**

Synergistia (Syn.er.gis’t.i.a. N.L. n. Synergistes type genus of the type order of the class; suff. -ia ending proposed by Gibbons and Murray and by Stackebrandt et al. to denote a class; N.L. fem. pl. n. Synergistia the Synergistales class).

The description is the same as that for the phylum Synergistetes.

**Type order: Synergistales ord. nov.**

**Description of Synergistiales ord. nov.**

Synergistiales (Syn.ner.gis’t.a.les. N.L. masc. n. Synergistes type genus of the order; -ales ending to donate an order; N.L. fem. pl. n. Synergistales the order of the genus Synergistes).

The description is the same as for the genus Synergistes.

**Type family: Synergistaceae.**

**Description of Synergistaceae fam. nov.**

Synergistaceae (Syn.ner.gis’t.a.ceae. N.L. fem. n. Synergistes type genus of the family; -aceae ending to donate a family; N.L. fem. n. Synergistaceae family of the genus Synergistes).

The description is the same as for the genus Synergistes.

**Type genus: Synergistes**

**Emended description of the phylum Deferribacteres Garrity and Holt 2001**

Includes the genera Deferribacter, Denitrovibrio, Flexistipes, Geovibrio and Mucispirillum.

**Emended description of the family Syntrophomonadaceae Zhao et al. 1993**

Includes the genera Pelospora, Syntrophomonas, Syntrophothermus and Thermosyntrophus.

**References**


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