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NOTE

# Phylogenetic analysis of *Formivibrio citricus*, *Propionivibrio dicarboxylicus*, *Anaerobiospirillum thomasii*, *Succinimonas amylolytica* and *Succinivibrio dextrinosolvens* and proposal of *Succinivibrionaceae* fam. nov.

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**The phylogenetic position of Gram-negative, strictly anaerobic, non-spore-forming bacteria, representing four different genera, was determined by analysis of their 16S rDNA sequences. *Formivibrio citricus* and *Propionivibrio dicarboxylicus* are members of the β-subclass of the class *Proteobacteria*. While *Formivibrio citricus* stands phylogenetically isolated, *Propionivibrio dicarboxylicus* is moderately related to members of the genus *Rhodocyclus*. *Succinimonas amylolytica* and *Succinivibrio dextrinosolvens* are members of the γ-subclass of the class *Proteobacteria* in which they, together with members of the genus *Anaerobiospirillum* and *Ruminobacter amylophilus*, form a separate line of descent. This phylogenetic group is described as *Succinivibrionaceae* fam. nov.**

**Keywords:** *Succinivibrionaceae*, *Formivibrio citricus*, *Propionivibrio dicarboxylicus*, *Anaerobiospirillum thomasii*, *Succinimonas amylolytica*, *Succinivibrio dextrinosolvens*

Of all validly described 732 prokaryotic genera, about 87% have been subjected to phylogenetic analysis by determination of the complete or almost complete 16S rDNA sequence of at least one representative species. Some of the genera which have not yet been included in such studies embrace non-cultured, obligate symbiotic species, but the type species of the majority of the remaining genera should be easily accessible for sequence analysis. It appears desirable to gain a more complete coverage of 16S rDNA diversity of recognized species, not only for unravelling the course of gene evolution, phylogeny and systematics but also for better assessment of community analysis, probe development and identification purposes. In this paper we report on the phylogenetic position of the type species of the monospecific genera *Formivibrio*, *Propionivibrio*, *Succinimonas* and *Succinivibrio*. These organisms are Gram-negative, strictly anaerobic organisms, displaying different morphologies, physiologies and isolation sources.

The EMBL accession numbers for the 16S rDNA sequences reported in this paper are Y17599–Y17603.

Organisms were cultured as recommended in the DSMZ catalogue of strains (1998). DNA isolation, PCR amplification and sequence analysis of 16S rDNA of *Anaerobiospirillum thomasii* DSM 11806$^T$, *Formivibrio citricus* DSM 6150$^T$, *Propionivibrio dicarboxylicus* DSM 5885$^T$, *Succinimonas amylolytica* DSM 2873$^T$ and *Succinivibrio dextrinosolvens* DSM 3072$^T$ were done as described by Rainey *et al.* (1996). The sequences, which were deposited in EMBL under accession numbers Y17599–17603, were aligned with sequences of the 16S rDNA database of the DSMZ, using the ae2 editor (Maidak *et al.*, 1996). Similarity values were transformed into phylogenetic distance values that compensate for multiple substitutions at any given site in the sequence (Jukes & Cantor, 1969). The algorithm of DeSoete (1983) and the neighbour-joining method contained in the PHYLIP package (Felsenstein, 1993) were used in the construction of phylogenetic dendrograms. All analyses were done on a SUN SparcII workstation.

Analysis of the 16S rDNA indicates that all four type species are members of the class *Proteobacteria*. The branching pattern obtained by the application of

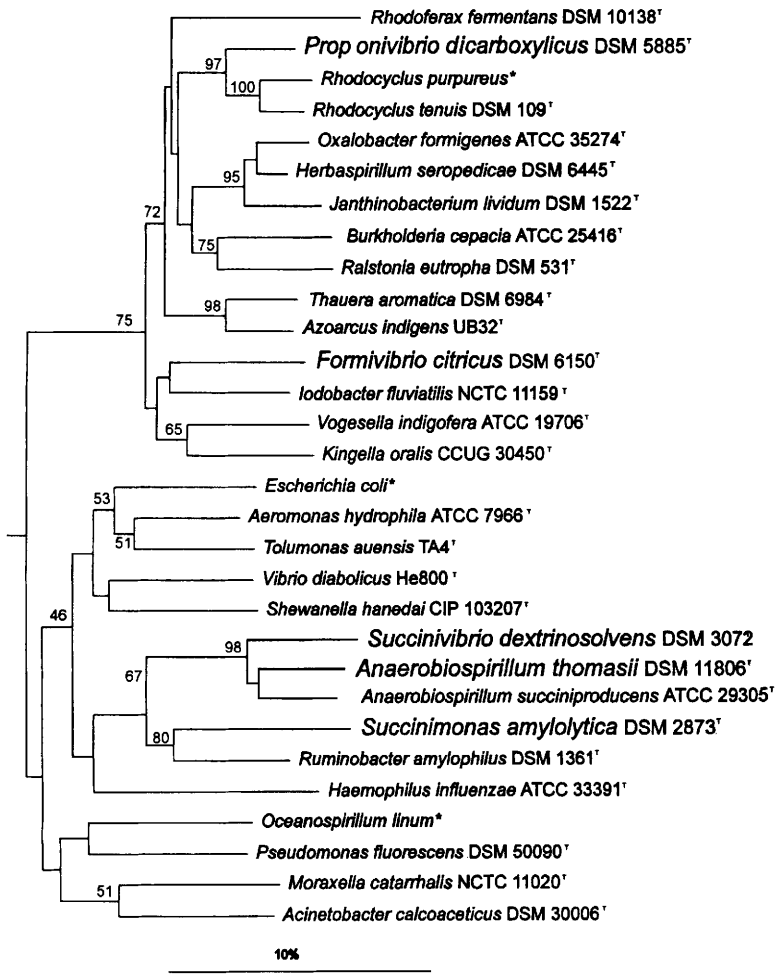


***Fig. 1.*** 16S rDNA sequence based phylogenetic dendrogram showing the phylogenetic position of some Gram-negative, anaerobic and carbohydrate-fermenting bacteria among their phylogenetically closest relatives. The dendrogram was reconstructed from evolutionary distance values (Jukes & Cantor, 1969), using the algorithm of DeSoete (1983). Bootstrap values (expressed as percentages of 500 replications) of 45% or more, are indicated at the branch points. Organisms investigated in this study are printed in larger letters. Bar, 10 inferred nucleotide substitutions per 100 nucleotides. *, Strain numbers were not indicated for sequences that were retrieved from RDP (Maidak *et al.*, 1996). Accession numbers are J01965 for *E. coli*, and M22365 for *O. linum*. Accession numbers for the other reference strains can be obtained from EMBL under species name and/or strain number.

different treeing algorithms did not change their phylogenetic affiliations (Fig. 1). Two species, *Formivibrio citricus* and *Propionivibrio dicarboxylicus*, are members of the $\beta$-subclass, while the other three species, *Anaerobiospirillum thomasii*, *Succinimonas amylolytica* and *Succinivibrio dextrinosolvens*, branch among members of the $\gamma$-subclass. None of the species branch within the radiation of species of another genus, which is indicative of the phylogenetic validity of the four genera to which the species investigated in this study are affiliated.

### *Formivibrio citricus* (Tanaka *et al.* 1991)

This anaerobic species has been described for strains that ferment certain organic acids, such as citrate, mesaconate and pyruvate to acetate and formate, while S-citramalate is fermented to acetate, formate and hydrogen; the G+C content of DNA is 61 mol% (Tanaka *et al.*, 1991). Relationship of *F. citricus* to the genera *Lachnospira*, *Ilyobacter*, *Oxalobacter* and *Acetivibrio*, which also contain Gram-negative formate-producing species, was excluded because of differences in the pattern of utilized substrates and the G+C content of DNA. Like the other four genera, *Formivibrio* was included into the family *Bacteroidaceae*. Analysis of 16S rDNA has clearly indicated that none of these genera are members of *Bacteroidaceae*. While *Oxalobacter formigenes* is related to members of the $\beta$-subclass of *Proteobacteria*, e.g. *Herbaspirillum* and *Janthinobacterium* (Fig. 1), *Ilyobacter*, *Lachnospira* and *Acetivibrio* are phylogenetic members of the *Clostridium–Bacillus* subphylum of Gram-positive bacteria.

16S rDNA similarity indicates that the presently recognized nearest phylogenetic neighbour of *F. citricus* is *Iodobacter fluviatilis*. However, statistical significance of the branching point and the similarity value of 92·2% are rather low. The moderate degree of genomic relatedness is expressed by overall low phenotypic similarity, as the latter species is a facultative anaerobic, chemo-organotrophic Gram-negative rod with a DNA G+C content of 50–52 mol%.

### *Propionivibrio dicarboxylicus* (Tanaka *et al.* 1990)

This maleate-fermenting, propionic-acid-forming and anaerobic rod-shaped organism was described as a new taxon because of the presence of significant phenotypic differences from other anaerobic propionic-acid-producing taxa, such as the genera

*Anaerovibrio*, *Propionispira*, *Pectinatus* and *Selenomonas* (Tanaka *et al.*, 1990). As judged from the phylogenetic position of *Propionivibrio dicarboxylicus* among members of the β-subclass of *Proteobacteria* the taxonomic delimitation from *Anaerovibrio*, *Pectinatus*, *Propionispira* and *Selenomonas* is justified as these four genera are members of the *Clostridium–Bacillus* subphylum of descent (H. Hippe, unpublished data; Hespell *et al.*, 1992).

*Propionivibrio dicarboxylicus* is moderately related to members of the genus *Rhodocyclus*, i.e. *Rhodocyclus purpureus* and *Rhodocyclus tenuis*, with which it shares 16S rDNA sequence similarity of 93·9 and 95·0%, respectively. A high bootstrap value of 97% supports the branching. In addition to morphology, anaerobic growth, possession of cytochrome $c_{551}$, requirement for vitamin $B_{12}$ and a similar DNA base composition, these organisms share the utilization of fumarate and L-malate; succinate is utilized by *Propionivibrio dicarboxylicus* and *Rhodocyclus tenuis* (Trüper & Imhoff, 1992). Whether *Propionivibrio dicarboxylicus* represents a descendant of a common ancestor that acquired genes for the photosynthetic apparatus in the *Rhodocyclus* line of descent or a mutant lacking bacteriochlorophyll *a* requires further investigation.

### *Succinivibrio dextrinosolvens* (Bryant and Small 1956) *Anaerobiospirillum thomasii* (Malnick 1997) and *Succinimonas amylolytica* (Bryant *et al.* 1958)

*Succinivibrio dextrinosolvens* and *Succinimonas amylolytica* are anaerobic and motile species that inhabit the rumen of sheep and cattle in which they play an ecologically important role as starch digesters, producing acetic and succinic acids as the main fermentation end products from carbohydrates. Differences between the two species are observed in the diet on which they generate high cell numbers, morphology and in the minor fermentation end products (Hespell, 1992). Phylogenetically both species are not members of *Bacteroidaceae* to which they were affiliated originally but they are members of the same subline of descent within the γ-subclass of *Proteobacteria*. This subline also contains members of the genus *Anaerobiospirillum*, i.e. *Anaerobiospirillum succiniproducens* (Davis *et al.*, 1976), and *Anaerobiospirillum thomasii* (Malnick, 1997) and *Ruminobacter amylophilus* (Hamlin & Hungate, 1956; Stackebrandt & Hippe, 1986) which ferment a variety of carbohydrates but differ phenotypically slightly from the other two starch-fermenting species in morphology and some physiological properties (Hespell, 1992; Malnick, 1997). In contrast to the rumen strains, the *Anaerobiospirillum* species were isolated from the throat and the colon of dogs (*Anaerobiospirillum succiniproducens*) and from faeces of humans, cats and dogs (*Anaerobiospirillum thomasii*). The binary 16S rDNA similarity values for the type species of the four genera range between 87·1 and 93·6%, the latter value obtained for the pair *Anaerobiospirillum succiniproducens* and *Anaerobiospirillum thomasii* (Fig. 1). The physiological and morphological relatedness between *Succinivibrio dextrinosolvens* and *Anaerobiospirillum succiniproducens* has been discussed in the original description of *Anaerobiospirillum* (Davis *et al.*, 1976). The three species share a 16S rDNA idiosyncrasy in that part of the helical region and the loop region between positions 456–473 (*E. coli* nomenclature) are missing which are present in the other members of the *Succinivibrio* lineage and in neighbouring lineages.

### Evolutionary considerations

The rather low degree of relatedness of physiologically very similar organisms, originating from a common ancestor and occupying a similar ecological niche, is of interest from an evolutionary point of view. It may indicate that the ancestor settled in an anaerobic carbohydrate-rich environment, which may have remained unchanged until today. Thus, while similar nutritional pressure may have maintained similar physiological response, the underlying genes and gene products were constantly subjected to evolutionary changes. Using the temporal calibration value of 1% sequence divergence of 16S rDNA per 50 million years (Ochman & Wilson, 1987), enterobacteria, such as *Escherichia coli* and *Salmonella typhimurium*, sharing 97·1% 16S rDNA sequence similarity, evolved from a common ancestor about 145 million years ago. This time point corresponds by and large with the occurrence of animals. Using the same calibration value, the evolution of *Ruminobacter amylophilus* and relatives would have occurred at the end of the Precambrian about 500–600 million years ago, an epoch in which the first vertebrates had evolved. It could thus be argued that the ancestor of these prokaryotic species may have evolved in anaerobic niches composed of starch-rich plant material from where they occupied the gut system of animals and later the rumen of ruminants, which, according to the fossil record, occurred in the Eozoic era about 53 million years ago. This hypothesis would be supported by the isolation of both anaerobic non-animal-associated starch-fermenting bacteria as well a greater variety of organisms which branch at the bases of or within the *Anaerobiospirillum*, *Ruminobacter*, *Succinivibrio* and *Succinimonas* line of descent.

As measured by the similarity values of 16S rDNA of the most unrelated members of the *Succinivibrio* cluster, the phylogenetic depth of this cluster is slightly lower than those found among members of other families of the γ-subclass of the class *Proteobacteria*, e.g. *Enterobacteriaceae* (around 93% similarity), *Vibrionaceae* (around 92%), *Pasteurellaceae* (91%), *Chromatiaceae* (around 88 %) and *Ectothiorhodospiraceae* (around 88%). The phylogenetic distinctness of the *Succinivibrio* cluster from other lineages of the subclass, the phylogenetic coherency and the presence of common taxonomic traits let us propose the

description of a new family, *Succinivibrionaceae* fam. nov. *Succinivibrio* has priority over the other genera on the basis of the date of description.

### Description of *Succinivibrionaceae* fam. nov. Hippe and Stackebrandt

*Succinivibrionaceae* (Suc.ci.ni.vib.ri.o.na'ce.ae. M.L. masc. n. *Succinivibrio* type genus of the family; *-aceae* ending to denote a family; M.L. fem. pl. n. *Succinivibrionaceae* the *Succinivibrio* family).

Gram-negative short, or oval to long, or curved to helical, rods. Motile (*Succinivibrio*, *Anaerobiospirillum and Succinimonas*) or non-motile (*Ruminobacter*). Do not form endospores. Chemo-organotrophic and strictly anaerobic. Glucose and other carbohydrates are fermented with the production of succinate and acetate; low amounts of formate and lactate may be produced. $CO_2$ uptake positive, gas not produced. Catalase-negative. Nitrate is not reduced. Quinones not detected (*Anaerobiospirillum* not tested), cytochromes not found in *Ruminobacter amylophilus*, the only species tested (G. Gottschalk, personal communication). Major fatty acids are saturated (35–66%) and unsaturated (19–59%) straight-chained, even-numbered fatty acids, and 16:0 and 18:0 3-OH fatty acids (4–11%); iso- and anteiso-branched fatty acids, cyclopropane fatty acids and odd-numbered fatty acids are absent (Moore *et al.*, 1994). Isolated from the rumen of sheep and cattle and from faeces of humans, cats and dogs and from the colon of dogs (*Anaerobiospirillum*). The DNA G+C content ranges from 39 to 44 mol% (data available for *Anaerobiospirillum* and *Ruminobacter*). Phylogenetically a member of the $\gamma$-subclass of the class *Proteobacteria*. The type genus is *Succinivibrio* Bryant and Small 1956, 22.

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