***Succinispira mobilis* gen. nov., sp. nov., a succinate-decarboxylating anaerobic bacterium**

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A succinate-decarboxylating anaerobic bacterium, designated strain 19gly1^T, was previously isolated from a mixed culture growing with glycolate. The almost complete sequence of the 16S rRNA gene (1495 nt) was determined for this strain. On the basis of 16S rRNA gene sequence homology, strain 19gly1^T was identified as a member of the *Sporomusa* sub-branch of the 'low G+C' Gram-positive bacteria. Phylogenetic analysis showed that strain 19gly1^T was most closely related to *Succiniclasticum ruminis*, *Phascolarctobacterium faecium* and *Acidaminococcus fermentans*. The use of different algorithms, such as least-squares or neighbour-joining analyses of Jukes–Cantor pairwise distances, or maximum-parsimony or maximum-likelihood analyses of the aligned sequence data, revealed that strain 19gly1^T grouped as the most deeply branching lineage of the strain 19gly1^T–*Succiniclasticum*–*Acidaminococcus*–*Phascolarctobacterium* cluster. The phenotypic characteristics of strain 19gly1^T distinguish it from members of the genera *Succiniclasticum*, *Phascolarctobacterium* and *Acidaminococcus*, and the phylogenetic distances inferred from comparative analysis of the 16S rDNA sequences suggest that strain 19gly1^T is a representative of a new genus. Accordingly, strain 19gly1^T (= DSM 6222^T) is proposed as the type strain of a new species within a new genus, *Succinispira mobilis* gen. nov., sp. nov.

Keywords: *Succinispira mobilis*, Gram-positive anaerobe, succinate decarboxylation, 16S rRNA gene

INTRODUCTION

Propionigenium modestum was the first strictly anaerobic bacterium unequivocally shown to be able to grow by coupling the decarboxylation of succinate to the generation of ATP (Schink & Pfennig, 1982). *Propionigenium modestum* and the related *Propionigenium maris* (Janssen & Liesack, 1995) belong to cluster XIX (Collins *et al.*, 1994) of the 'low G+C' Gram-positive bacteria (*Clostridium* spp. and relatives) and are related to *Fusobacterium* spp. A succinate-decarboxylating *Peptostreptococcus* sp. (Janssen *et al.*, 1996) was found to belong to cluster XIII (Collins *et al.*, 1994) of the 'low G+C' Gram-positive bacteria. However, the majority of the bacterial species known to be able to grow anaerobically on succinate by a decarboxylation reaction, yielding

propionate and CO₂ (or HCO₃⁻), belong to the *Sporomusa* sub-branch of the 'low G+C' Gram-positive bacteria, corresponding to cluster IX of Collins *et al.* (1994). These include *Schwartzia succinivorans* (van Gylswyk *et al.*, 1997), *Selenomonas acidaminovorans* (Guangsheng *et al.*, 1992), *Sporomusa acidovorans* (Ollivier *et al.*, 1985), *Sporomusa termitida* (Breznak *et al.*, 1988), *Sporomusa malonica* (Dehning *et al.*, 1989) and *Succiniclasticum ruminis* (van Gylswyk, 1995). The ability to decarboxylate succinate is also found in other members of the *Sporomusa* sub-branch such as *Veillonella parvula*, which can couple succinate decarboxylation to ATP synthesis (Janssen, 1992), *Selenomonas ruminantium* (Scheifinger & Wolin, 1973) and *Phascolarctobacterium faecium* (Osawa *et al.*, 1992).

A succinate-decarboxylating anaerobic bacterium, designated strain 19gly1^T, was isolated from a mixed culture growing with glycolate (Janssen, 1990, 1991). This strain was clearly able to grow with succinate and a possible sodium-dependent succinate metabolism

Abbreviation: ANGIS, Australian National Genomic Information Service.

The EMBL accession number for the 16S rRNA gene sequence of strain 19gly1^T is AJ006980.

was found (Janssen, 1991). The strain was characterized phenotypically, but could not be assigned to a known genus. Possible affiliations with *Campylobacter*-like organisms have been discussed, but evidence for inclusion of this strain within the genus *Campylobacter* was unconvincing (Janssen, 1991). We have now used comparative 16S rRNA gene sequence analysis to help classify strain 19gly1^T.

METHODS

Strain and growth conditions. Strain 19gly1^T (= DSM 6222^T) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen. Cultures were grown in the freshwater medium FM of Janssen *et al.* (1997) with 20 mM succinate and 1 g yeast extract l⁻¹ in completely filled screw-capped bottles, at 30 °C.

DNA extraction. Late exponential-phase cultures were harvested by centrifugation and the cells were washed in TE buffer (10 mM Tris, 1 mM EDTA, pH 8 with HCl). Cell pellets were resuspended in 3.5 ml E3T buffer [50 mM EDTA, 50 mM Tris, 0.5% (w/v) Tween 20, 0.5% (w/v) Triton X-100, pH 8.0 with NaOH] and 0.7 mg RNase A (Qiagen), 8 mg lysozyme (Fluka) and 2 mg protease (Qiagen) were added. After 30 min incubation at 37 °C, 1.2 ml GT buffer [3 M guanidine.HCl, 20% (w/v) Tween 20, pH 5.5 with NaOH] was added, followed by further incubation at 50 °C for 15 min. The preparations were cooled to room temperature, 0.1 vol. 5 M NaCl and 2 vol. 100% ethanol were added and mixed well, and the preparations were incubated at -20 °C for 2 h. Genomic DNA was collected by centrifugation at 3000 g and 4 °C for 20 min, air-dried at room temperature, then dissolved in 1 ml TE buffer. Phenol (1 ml) equilibrated to pH 8.0 (Sigma) was added, and the two phases were mixed by briefly vortexing. The two phases were then separated by centrifugation at 13000 g for 5 min, and the aqueous phase was extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1, by vol.), and then with an equal volume of chloroform:isoamyl alcohol (24:1, v/v). The extracted DNA was precipitated after the addition of 0.1 vol. 5 M NaCl and 2 vol. 100% ethanol, washed once in 70% ethanol, and the DNA pellet was dissolved in 50 µl TE buffer after being air-dried.

16S rRNA gene sequence determination. The almost complete 16S rRNA gene was amplified by PCR using the oligonucleotide primers 27f and 1525r (Lane, 1991) in a Progene thermocycler (Techne). The reaction mix (100 µl final volume) contained 10 mM Tris/HCl (pH 9.0 at 25 °C), 50 mM KCl, 1 mM MgCl₂, 0.1% (w/v) Triton X-100, 20 µg BSA, 0.2 mM each of dATP, dCTP, dGTP and dTTP, 100 pmol of each primer (Beckman Instruments) and about 250 ng extracted genomic DNA. The thermal profile for PCR was as follows: (i) 94 °C for 4 min, after which 2.5 U *Taq* DNA polymerase (Promega) was added to each reaction, followed by a further 60 s at 94 °C, then (ii) 35 cycles of 56 °C for 90 s, 72 °C for 120 s and 94 °C for 60 s, followed by (iii) 1 cycle of 56 °C for 90 s and 72 °C for 6 min. The PCR product was purified by gel electrophoresis (1% low-melting point agarose; Progen) and the bands were excised after staining with ethidium bromide and visualization under UV light. The excised gel fragments were heated to 65 °C and TE buffer was added to give a total volume of 400 µl. Phenol (400 µl, pH 8.0) was added and the two phases were mixed by briefly vortexing. The two phases were then separated by centrifugation at 13000 g for 5 min, and the aqueous phases were extracted with an equal volume

Fig. 1. Phylogenetic dendrogram indicating the relationships between strain 19gly1^T and other members of the *Sporomusa* sub-branch of the 'low G+C' Gram-positive bacteria. The dendrogram is based on 16S rRNA gene sequences and was derived from pairwise evolutionary distances by the least-squares method. Scale bar, 10 inferred nucleotide substitutions per 100 nucleotides. Numbers given at the nodes represent bootstrap percentage values (1000 analyses; not all are shown).

of phenol:chloroform:isoamyl alcohol (25:24:1, by vol.), and then with an equal volume of chloroform:isoamyl alcohol (24:1, v/v). Ammonium acetate (10 M, 100 µl) and 1 ml 100% ethanol were then added to the aqueous phase which was incubated at -20 °C for 16 h. The samples were then centrifuged at 13000 g for 15 min at 4 °C and the resultant pellet was washed by adding 500 µl 70% ethanol and centrifuging again. The pellet, consisting of the PCR product, was then air-dried. The purified PCR product was sequenced by using ABI PRISM Dye Terminator technology (Applied Biosystems) at the Australian Genome Research Facility at the Walter and Eliza Hall Institute of Medical Research (Parkville, Victoria, Australia). The sequencing oligonucleotide primers were 27f, 519r, 530f, 907r, 926f and 1525r (Lane, 1991). The data were assembled and edited using the software package SEQUENCHER version 3.1RC4 (Gene Codes Corporation).

Comparative sequence analysis. The almost complete 16S rRNA gene sequence was compared with known 16S rRNA gene sequences by carrying out a BLAST search (Altschul *et al.*, 1990) in the GenBank database (Benson *et al.*, 1997). The 16S rRNA gene sequence of strain 19gly1^T was then aligned against homologous sequences of selected members (see Fig. 1) of cluster IX (Collins *et al.*, 1994) obtained from GenBank using the alignment program PILEUP implemented in the ANGIS (Australian National Genomic Information Service) system (Littlejohn *et al.*, 1996). This alignment was

then manually checked and corrected, and regions of uncertain alignment were eliminated, using the 16S rRNA eubacterial mask of Lane (1991) as a guide, with the software SEQAPP version 1.9a169 (D. G. Gilbert, Biology Department, Indiana University, Bloomington, IN, USA). Further analyses were restricted to the unambiguously aligned regions totalling 1232 positions corresponding to nucleotide positions 101–205, 220–452, 481–859, 862–1000, 1037–1127, 1145–1262 and 1265–1431 of the *Escherichia coli* 16S rRNA gene (numbering according to Brosius *et al.*, 1978).

Evolutionary analyses were carried out using the Wisconsin Package version 8.1.0 (Genetics Computer Group, Madison, WI, USA) and the EGCG package (Peter Rice, Sanger Centre, Cambridge, UK), implemented in the ANGIS system. Evolutionary distances between pairs of micro-organisms were determined using the Jukes & Cantor (1969) equation implemented in the EDNADIST program and a tree estimating the phylogenetic relationships was derived using the EFITCH program of the same package employing a least-squares algorithm (Fitch & Margoliash, 1967), with a random-order input of sequences and the global rearrangement option. Trees were represented graphically using the software TREEVIEWPPC version 1.4 (R. D. M. Page, Division of Environmental & Evolutionary Biology, University of Glasgow, Glasgow, UK). The significance of the nodes was tested by bootstrap analysis generating evolutionary distances and using neighbour-joining to produce 1000 trees, and then compiling a consensus tree, using the programs ESEQBOOT, EDNADIST, ENEIGHBOR and ECONSENSE. Maximum-likelihood and maximum-parsimony analyses of the sequence data and neighbour-joining analysis of the evolutionary distances employed the programs EDNAML, EDNAPARS and ENEIGHBOR, respectively.

Nucleotide sequence accession numbers. The GenBank accession numbers for sequences used as references in phylogenetic analyses are as follows: *Acidaminococcus fermentans* AO, X77951; *Acidaminococcus fermentans* VR4^T, X78017; *Arthrobacter globiformis*, M23411; *Bacillus subtilis*, X60646; *Burkholderia cepacia*, M22518; *Clostridium quercicolum*, M59110; *Dialister pneumosintes*, X82500; *Escherichia coli*, J01695; *Megasphaera cerevisiae*, L37040; *Megasphaera elsdenii*, M26493; *Phascolarctobacterium faecium* ACM 3679^T, X72865; *Phascolarctobacterium faecium* ACM 3680, X72867; *Phascolarctobacterium faecium* ACM 3681, X72866; *Quinella ovalis*, M62701; *Schwartzia succinivorans*, Y09434; *Selenomonas ruminantium* GA-192^T, M62702; *Selenomonas ruminantium* HD4, M62703; *Sporomusa paucivorans*, M59117; *Sporomusa silvacetica*, Y09976; *Succiniclasticum ruminis*, X81137; *Veillonella atypica*, X84007; *Veillonella dispar*, X84006; and *Veillonella parvula*, X84005.

RESULTS AND DISCUSSION

Phylogenetic analysis

An almost complete 16S rRNA gene sequence (1495 nt) was determined for strain 19gly1^T. A BLAST search revealed that the sequence of the 16S rRNA gene of strain 19gly1^T was most similar to those of various members of the *Sporomusa* sub-branch of the 'low G + C' Gram-positive bacteria, corresponding to cluster IX of Collins *et al.* (1994). The cell wall structure of strain 19gly1^T was found to be of a Gram-negative type with an outer membrane (Janssen, 1991), con-

sistent with that of other members of the *Sporomusa* sub-branch of the 'low G + C' Gram-positive bacteria, which also have Gram-negative cell wall types, despite belonging to a lineage of the Gram-positive bacteria (Woese, 1987).

A comparison with representatives of this group by comparative analysis of the primary sequence of their 16S rRNA genes (Fig. 1) shows that strain 19gly1^T is most closely related to *Succiniclasticum ruminis* (van Gylswyk, 1995), *Phascolarctobacterium faecium* (Del Dot *et al.*, 1993) and *Acidaminococcus fermentans* (Rogosa, 1984; Cook *et al.*, 1994). The use of different algorithms, such as least-squares or neighbour-joining analyses of Jukes–Cantor pairwise distances, or maximum-parsimony or maximum-likelihood analyses of the aligned sequence data, revealed that the overall topologies of the trees varied slightly. Least-squares and neighbour-joining methods yielded the same tree topologies. Maximum-parsimony and maximum-likelihood analyses grouped the *Clostridium quercicolum*–*Sporomusa* spp. cluster as a sister group of the *Veillonella*–*Megasphaera*–*Selenomonas* cluster, which is reflected in the low bootstrap value (79%) of the branching point between the 19gly1^T–*Succiniclasticum*–*Acidaminococcus*–*Phascolarctobacterium* and the *Clostridium quercicolum*–*Sporomusa* spp. clusters.

In all the analyses, strain 19gly1^T grouped as the most deeply branching lineage of the 19gly1^T–*Succiniclasticum*–*Acidaminococcus*–*Phascolarctobacterium* cluster. Bootstrap analysis shows that the branching order is not certain, and so the exact evolutionary relationship of strain 19gly1^T with the genera *Succiniclasticum*, *Acidaminococcus* and *Phascolarctobacterium* remains unresolved. The 16S rRNA gene sequence similarities between strain 19gly1^T and its relatives, based on the 1231 nt positions examined, are 88.8% to *Succiniclasticum ruminis*, 90.9–91.2% to *Phascolarctobacterium faecium* strains and 91.2% to *Acidaminococcus fermentans* strains.

Comparison with related taxa

The phenotypic characteristics of strain 19gly1^T (Janssen, 1991) distinguish it from members of the genera *Succiniclasticum*, *Phascolarctobacterium* and *Acidaminococcus* (Table 1). The phylogenetic distances inferred from the 16S rRNA gene sequences, together with the phenotypic differences, suggest that strain 19gly1^T is a representative of a new genus distinct from *Succiniclasticum*, *Phascolarctobacterium* and *Acidaminococcus*. Accordingly, we propose strain 19gly1^T (= DSM 6222^T) as the type strain of a new species within a new genus, *Succinispira mobilis* gen. nov., sp. nov. Since there is only one strain available in pure culture, we describe the genus, species and strain together here, realising that further strains and species will allow a definition of specific and generic characteristics. The phenotypic characteristics are based on a previous study (Janssen, 1991).

Table 1. Comparison of phenotypic characteristics of strain 19gly1^T and members of the species *Succiniclasicum ruminis*, *Phascolarctobacterium faecium* and *Acidaminococcus fermentans*

Data from Rogosa (1984), Janssen (1991), Osawa *et al.* (1992), Del Dot *et al.* (1993), Cook *et al.* (1994), van Gylswyk (1995). +, Positive; (+), stimulates growth; –, negative; w, weak growth by some strains; ND, no data.

Characteristic	19gly1 ^T	<i>Succiniclasicum ruminis</i>	<i>Phascolarctobacterium faecium</i>	<i>Acidaminococcus fermentans</i>
Cell shape	Curved rod	Short rod	Pleomorphic rod	Coccus
Motility	+	–	–	–
G + C (mol %)	36	52	41–42	55–57
Propionate production	+	+	+	–
Growth with:				
Succinate	+	+	(+)	–
Carbohydrates	–	–	–	w
Amino acids	+	–	ND	+

Description of *Succinispira* gen. nov.

Succinispira [Suc.ci.ni.spi'ra. L. n. *succinum* amber; M.L. n. *acidum succinicum* succinic acid (derived from amber); Gr. n. *spira* coil, spiral; M.L. fem. n. *Succinispira* succinate-utilizing, spiral-shaped bacterium].

The type species is *Succinispira mobilis*. The genus and species descriptions are combined below until further species are described.

Description of *Succinispira mobilis* sp. nov.

Succinispira mobilis (mo'bi.lis. L. adj. *mobilis* movable, motile).

The cells are curved rods, 0.5 µm in diameter and 2–10 µm long. Longer cells can be spirals of up to 3 or 4 turns with an amplitude of 1.5–2.0 µm and a wavelength of 3–4 µm. Endospores are not formed. The cells are highly motile with laterally inserted flagella. Cytochromes are not formed. Gram-staining gives a negative result. Aminopeptidase activity is absent and the cell wall structure appears to be of a Gram-negative type with an outer membrane. Colonies in agar-deep cultures are white and lens-shaped. Liquid cultures grow with a uniform turbidity. The genomic DNA has a G+C ratio of 36 mol %, determined by thermal denaturation. Comparative analysis of the 16S rRNA gene suggests a close phylogenetic relationship with the genera *Succiniclasicum*, *Phascolarctobacterium* and *Acidaminococcus* within the *Sporomusa* sub-branch of the 'low G+C' Gram-positive bacteria. The species displays a chemo-organotrophic fermentative metabolism. Pyruvate, citrate, oxaloacetate, maleate, succinate, fumarate, glutamate and aspartate are fermented, whereas carbohydrates and alcohols are not utilized. Formate, acetate, propionate, malate, CO₂ and H₂ are

the fermentation end-products, depending on the substrate. Hydrogenase activity is present. Sulfate, sulfur, thiosulfate, nitrate and fumarate are not used as terminal electron acceptors. Aesculin hydrolysis, urea hydrolysis, indole formation from L-tryptophan and sulfide formation from L-cysteine are all negative. The organism is a strict anaerobe. Media containing a suitable reductant are required for growth. Growth factors are required and can be supplied by the addition of yeast extract. The range of pH for growth is 6.7–8.5, with an optimum of 7.4–7.7. The optimum growth temperature is 37 °C. No growth is possible at 40 °C. NaCl at 0.2 g l⁻¹ is sufficient for growth. Growth is possible at NaCl concentration of 19 g l⁻¹, but not at 24 g l⁻¹. The type strain, 19gly1^T (= DSM 6222^T), was isolated from a mixed culture growing on glycolate, originally obtained from a pond receiving effluent from an anaerobic solids digester.

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