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Pelospora glutarica gen. nov., sp. nov., a glutarate-fermenting, strictly anaerobic, spore-forming bacterium

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The strictly anaerobic, Gram-negative, spore-forming bacterium strain WoGl3^T had been enriched and isolated in mineral medium with glutarate as the sole source of energy and organic carbon. Glutarate was fermented to a mixture of butyrate, isobutyrate, CO_2 and small amounts of acetate. Strain WoGl3^T grew only with the dicarboxylates glutarate, methylsuccinate and succinate. 16S rDNA sequence analysis revealed an affiliation of strain WoGl3^T to the family *Syntrophomonadaceae*. This monophyletic group is comprised of strain WoGl3^T and the genera *Syntrophomonas*, *Syntrophospora* and *Thermosyntropha*, within the phylum of Gram-positive bacteria with a low DNA G+C content. Overall intra-group 16S rRNA sequence similarities of 89·2–93·9% document a separate phylogenetic status for strain WoGl3^T. Strain WoGl3^T (= DSM 6652^T) is described as the type strain of a new species within a new genus, *Pelospora glutarica* gen. nov., sp. nov.

Keywords: *Pelospora glutarica* sp. nov., anaerobic degradation, dicarboxylic acids, glutarate

INTRODUCTION

Fermentative degradation of short-chain dicarboxylic acids such as oxalate, malonate and succinate has been documented. Several anaerobic bacteria have been isolated that grow with these dicarboxylates as sole sources of organic carbon and energy and decarboxylate them to the respective fatty acids (Schink & Pfennig, 1982; Allison *et al.*, 1985; Smith *et al.*, 1985; Dehning & Schink, 1989a, b; Dehning *et al.*, 1989; Denger & Schink, 1990; Janssen *et al.*, 1996). The decarboxylation energy can be conserved by these micro-organisms through two different mechanisms involving either membrane-bound decarboxylases that act as primary sodium ion pumps or the combined action of a soluble decarboxylase with a dicarboxylate/monocarboxylate antiporter (Dimroth & Schink, 1998).

Under anoxic conditions, glutarate can be utilized as a growth-supporting substrate by sulfate-reducing bacteria (Imhoff-Stuckle & Pfennig, 1983; Bak & Widdel, 1986; Szewzyk & Pfennig, 1987; Schnell *et al.*, 1989) and nitrate-reducing pseudomonads (Anders *et al.*, 1995). In a study on the degradation of higher dicarboxylic acids, we isolated a strictly anaerobic bacterium, strain WoGl3^T, that grew only with glutarate, methylsuccinate or succinate (Matthies & Schink, 1992a). The dicarboxylates were fermented by decarboxylation to the respective fatty acids. A membrane-bound, sodium ion-dependent glutaconyl-CoA decarboxylase was involved in energy conservation during growth with glutarate (Matthies & Schink, 1992b). In the present study, strain WoGl3^T is described as a new species of a new genus, *Pelospora glutarica* gen. nov., sp. nov., on the basis of 16S rDNA sequence comparisons.

METHODS

A pure culture of strain WoGl3^T (= DSM 6652^T) was taken from our laboratory collection. Strain WoGl3^T was originally isolated from an anoxic freshwater sediment under strictly anoxic conditions (Matthies & Schink, 1992a).

The strain was cultivated in a sulfide-reduced, bicarbonate-buffered medium that contained trace element solution SL10, selenite tungstate solution (Widdel *et al.*, 1983), seven-vitamin solution (Widdel & Pfennig, 1981) and 2% rumen fluid under a N_2/CO_2 (90:10) atmosphere. The addition of

The EMBL accession number for the 16S rDNA sequence of *Pelospora glutarica* strain WoGl3^T is AJ251214.

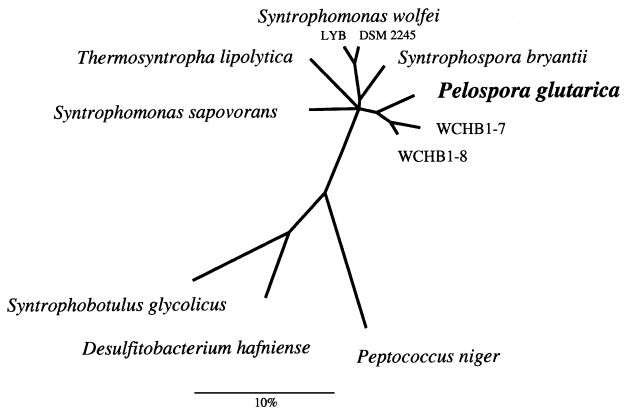

Fig. 1. Phylogenetic tree reflecting the relationships of strain WoGl3^T and representatives of the family *Syntrophomonadaceae*. *Syntrophobotulus glycolicus*, *Desulfitobacterium hafniense* and *Peptococcus niger* are shown as outgroup references. The tree is based on the results of an optimized maximum-parsimony analysis of a data set of about 16000 small subunit rRNA sequences. The tree topology was evaluated and corrected by performing maximum-parsimony, maximum-likelihood and distance matrix analyses of various data sets applying the software tools of the ARB program package (Ludwig & Strunk, 1997). The multifurcation indicates that a common significant relative branching order could not be found. The scale bar indicates 10% estimated sequence divergence. The references for the organisms/sequences shown in the tree are: Ludwig *et al.* (1990), Zhao *et al.* (1990), Christiansen & Ahring (1996), Friedrich *et al.* (1996), Svetlitshnyi *et al.* (1996), Dojka *et al.* (1998) and EMBL accession no. AF022249.

this small amount of rumen fluid was required in order to achieve reproducible growth. Details of cultivation and characterization are given in the original description (Matthies & Schink, 1992a).

In vitro amplification and sequence analysis of rDNA were performed as described previously (Springer *et al.*, 1992). Nearly complete 16S rRNA sequences (homologous to *Escherichia coli* positions 8–1542) used for phylogenetic analyses were fitted into an alignment of about 16000 homologous full or partial primary structures available in public databases by using the respective automated tools of the ARB software package (Ludwig & Strunk, 1997). Distance matrix, maximum-parsimony and maximum-likelihood methods were applied for tree construction as implemented in the ARB software package. Different data sets varying with respect to the selection of outgroup reference organisms (sequences), as well as alignment positions, were analysed.

RESULTS AND DISCUSSION

The physiological properties of strain WoGl3^T, the mechanism of energy conservation and the enzymes involved in glutarate fermentation by this organism have been documented in detail before (Matthies & Schink, 1992a, b, c). It ferments glutarate roughly according to the following fermentation equation:

$$2\ \text{glutarate}^{2-} + 2H^+ \rightarrow \text{butyrate}^- + \text{isobutyrate}^- + 2CO_2.$$

Other taxonomically relevant features of its physiology are summarized in the species description at the end of this section.

16S rDNA sequence analysis

According to the results of comparative 16S rDNA sequence analysis, strain WoGl3^T could clearly be assigned to the family *Syntrophomonadaceae* (Zhao *et al.*, 1993). Although the members of the *Syntrophomonadaceae* are phylogenetically representatives of the phylum of Gram-positive bacteria with a low DNA G+C content, this family contains organisms that exhibit both negative (*Syntrophomonas*, *Thermosyntropha* and strain WoGl3^T) (Zhao *et al.*, 1990; Svetlitshnyi *et al.*, 1996; Matthies & Schink, 1992a) and positive (*Syntrophospora*) (Stieb & Schink, 1985; Zhao *et al.*, 1990) Gram-staining behaviour. To date, all members of the family are strict anaerobes, and most of them are syntrophic heterotrophs. A careful

Table 1. Similarity matrix based on 16S rDNA gene sequences of strain WoGl3^T and its closest relatives

Percentage similarity between the various sequences shown in Fig. 1 is given.

Taxon	1	2	3	4	5	6	7	8	9	10	11
1. Clone WCHB1-8	100										
2. Clone WCHB1-7	96·9	100									
3. *Pelospora glutarica* WoGl3^T	93·5	93·9	100								
4. *Syntrophospora bryantii* DSM 3014B^T	92·3	92·9	91·9	100							
5. *Syntrophomonas wolfei* DSM 2245^T	90·5	91·0	90·3	93·0	100						
6. *Syntrophomonas wolfei* LYB	90·0	90·4	89·6	91·7	97·6	100					
7. *Syntrophomonas sapovorans* DSM 3441^T	92·4	92·3	91·3	92·8	91·6	91·2	100				
8. *Thermosyntropha lipolytica* JW/VS-265^T	89·4	89·8	89·2	90·6	89·0	89·9	91·3	100			
9. *Syntrophobotulus glycolicus* FlGlyR^T (= DSM 8271^T)	84·8	84·3	84·8	84·7	83·5	83·2	83·9	83·0	100		
10. *Desulfitobacterium hafniense* DCB-2^T (= DSM 10664^T)	85·0	84·5	85·3	84·1	84·0	83·2	84·8	84·1	90·6	100	
11. *Peptococcus niger* DSM 20475^T	81·7	82·0	81·1	80·7	81·4	80·4	80·0	80·6	81·4	82·0	100

phylogenetic analysis of the sequence data, applying different treeing methods and filters (Ludwig *et al.*, 1998), showed a separate status of strain WoGl3^T among the other related species (Fig. 1). There is no closely related cultured organism known so far. The overall sequence similarity values for strain WoGl3^T and the remaining (cultured) members of the family are 89·2–91·9 % (Table 1). The highest overall sequence similarities (93·5–93·9 %) were found for strain WoGl3^T and the sequences from two cloned 16S rDNA fragments (clones WCHB1-7 and WCHB1-8) retrieved from soil samples from an aquifer contaminated with chlorinated solvents (Dojka *et al.*, 1998). The relationship to species of *Syntrophospora* and other syntrophically fermenting bacteria, as well as to species of *Desulfitobacterium* and to *Peptococcus niger*, was significantly lower (Table 1). A closer relationship of *Syntrophomonas wolfei* strains and *Syntrophospora bryantii* was clearly supported in all phylogenetic analyses (Fig. 1). However, no significant relative branching order could be found for the two subclusters and *Syntrophomonas sapovorans* or *Thermosyntropha lipolytica*. Given the clear affiliation to the *Syntrophomonadaceae* and the only moderate intra-family relationships, it is proposed to place strain WoGl3^T in a new genus, *Pelospora* gen. nov., as *Pelospora glutarica* gen. nov., sp. nov.

Description of *Pelospora* gen. nov.

Pelospora (Pe.lo.spo′ra. Gr. masc. n. *pelos* mud; M.L. fem. n. *spora* a spore-former; *Pelospora* a spore-forming bacterium originating from mud).

Strictly anaerobic bacterium forming spores. So far only one species, the type species *Pelospora glutarica*, has been described.

Description of *Pelospora glutarica* sp. nov.

Pelospora glutarica (glu.ta′ri.ca. M.L. n. *glutarica* referring to glutarate, from M.L. n. *acidum glutaricum* glutaric acid, as the key substrate of this species).

Long, rod-shaped cells, 4·5–6·5 × 0·8 μm in size, motile by one subpolar flagellum, Gram-negative staining, formation of terminal oval spores. Chemoorganotrophic, fermentative metabolism. Contains no cytochromes. Glutarate, methylsuccinate and succinate are the only substrates. No growth with more than 30 different substrates tested such as sugars, organic acids, alcohols, amino acids or other dicarboxylic acids. Products of glutarate and methylsuccinate fermentation are butyrate, isobutyrate and CO_2; succinate is decarboxylated to propionate.

Growth rate with glutarate at 37 °C, $\mu = 0{\cdot}062\ h^{-1}$. pH optimum for growth is 7·1–8·2; no growth below pH 6·0. Temperature optimum 37 °C; no growth below 20 °C or above 37 °C. Growth in medium containing salt concentrations of 0·1 % NaCl and 0·04 % $MgCl_2.6H_2O$ (w/v); no growth in salt-water medium at 2 % NaCl and 0·3 % $MgCl_2.6H_2O$ (w/v). G+C content of DNA of the type strain is 49·0 ± 1·4 mol %.

Habitat: anoxic freshwater sediment. The type strain, strain WoGl3^T, has been deposited in the DSMZ as DSM 6652^T.

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