Fermentative degradation of glutarate via decarboxylation by newly isolated strictly anaerobic bacteria

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Abstract. Two strains of new strictly anaerobic, gram-negative bacteria were enriched and isolated from a freshwater (strain WoGl3) and a saltwater (strain CuGl1) anoxic sediment with glutarate as sole energy source. Strain WoGl3 formed spores whereas strain CuGl1 did not. Both strains were rod-shaped, motile bacteria growing in carbonate-buffered, sulfide-reduced mineral medium supplemented with 2% of rumen fluid. Both strains fermented glutarate to butyrate, isobutyrate, CO₂, and small amounts of acetate. With methylsuccinate, the same products were formed, and succinate was fermented to propionate and CO₂. No sugars, amino acids or other organic acids were used as substrates. Molar growth yields (Y_s) were very small (0.5–0.9 g cell dry mass/mol dicarboxylate). Cells of strain WoGl3 contained no cytochromes, and the DNA base ratio was 49.0 ± 1.4 mol% guanine-plus-cytosine. Enzyme activities involved in glutarate degradation could be demonstrated in cell-free extracts of strain WoGl3. A pathway of glutarate fermentation via decarboxylation of glutacetyl-CoA to crotonyl-CoA is suggested which forms butyrate and partly isobutyrate by subsequent isomerization.

Key words: Anaerobic degradation — Dicarboxylic acids — Glutarate — Glutaconyl — CoA decarboxylase

Fermentative degradation of short-chain dicarboxylic acids such as oxalate, malonate, or succinate has been documented. The isolated bacteria grow with these dicarboxylates as sole energy sources and decarboxylate them to the respective fatty acids (Schink and Pfennig 1982; Allison et al. 1985; Smith et al. 1985; Dehning and Schink 1989 a, b; Dehning et al. 1989; Denger and Schink 1990). With oxalate and succinate, the free energy change of this decarboxylation reaction ($\Delta G^{\circ} \approx -30 \text{ kJ} \cdot \text{mol}^{-1}$, Thauer et al. 1977) is conserved in an ion gradient across the membrane which serves as sole energy source for ATP synthesis

(Anantharam et al. 1989; Hilpert et al. 1984). Only little is known about fermentative degradation of long-chain dicarboxylic acids with more than 4 carbon atoms. Glutarate can be used as growth substrate by sulfate-reducing bacteria (Imhoff-Stuckle and Pfennig 1983; Bak and Widdel 1986; Szewzyk and Pfennig 1987; Schnell et al. 1989) and nitrate-reducing pseudomonads (strains K172 and S2; B. Seyfried, personal communication). In both cases, glutarate is oxidized probably in a similar way as in aerobic microorganisms: In *Pseudomonas fluorescens*, glutarate is activated to glutaryl-CoA which is oxidized to glutaconyl-CoA by glutaryl-CoA dehydrogenase and decarboxylated to crotonyl-CoA. The latter is further metabolized by the well-known steps of β -oxidation of fatty acids (Numa et al. 1964).

In methanogenic enrichment culture, glutarate was shown to be degraded completely to methane and CO₂ with butyrate and isobutyrate as intermediates (Stieb 1985).

In this paper, the isolation and characterization of a strictly anaerobic glutarate-fermenting bacterium in pure culture is presented. Enzymes involved in glutarate degradation were measured, and a catabolic pathway is proposed.

Material and methods

Sources of organisms

Enrichment cultures with glutarate as sole organic substrate were inoculated with anoxic sediment of a polluted creek near Konstanz (WoGl), FRG, domestic sewage sludge (KoGl) and the marine enrichment culture with black mud of the North Sea coast near Cuxhaven, FRG, (CuGl).

Desulfovibrio vulgaris strain Marburg and *Methanospirillum hungatei* strain SK were kindly provided by Prof. Dr. R. K. Thauer, Marburg, FRG, and Prof. Dr. F. Widdel, Munich, FRG.

Acetobacterium woodii (DSM 1030) and *Escherichia coli* strain K12 (DSM 498) were obtained from the Deutsche Sammlung von Mikroorganismen GmbH, Braunschweig, FRG.

Media and growth conditions

All procedures for anaerobic cultivation and isolation were the same as described earlier (Widdel and Pfennig 1981; Pfennig and

Trüper 1981). The mineral salts medium contained 30 mM sodium bicarbonate as buffer and 1 mM sodium sulfide as reducing agent and sulfur source. Trace element solution SL 10 (1 ml/l) and selenite-tungstate solution (1 ml/l) as well as 7-vitamines solution were added to the medium from sterile stock solutions (Widdel and Pfennig 1981; Widdel et al. 1983).

Growth experiments were carried out in 22-ml-screw-cap tubes in which cell turbidity was measured at 600 nm wavelength with a Spectronic 20 spectrophotometer (Milton Roy, Rochester, NY, USA). All cultures were incubated at 30 °C.

Cytological characterization

Gram staining was carried out according to Bartholomew (1962), with *Acetobacterium woodii* and *Desulfovibrio vulgaris* strain Marburg as reference organisms.

Flagella were stained as described by Blenden and Goldberg (1965).

Spore formation was checked for in mineral salts medium with 20% soil extract and 5 mg MnSO_4/l . Alternatively, the latter medium was supplemented with 0.1% tryptone, 5 mM glutarate or 10 mM xylose (modified after Hollaus and Sleytr 1972). Heat resistance of spores was checked for by pasteurization (80 °C for 10 min).

Cytochromes were checked for in cell-free extracts of glutarate-grown cells through redox difference spectra taken with an Uvikon 860 spectrophotometer (Kontron, Zürich, Switzerland).

Chemical analyses

Fatty acids, dicarboxylic acids, methane, and carbon dioxide were assayed by gas chromatography. Fatty acids were determined by a method modified after Platen and Schink (1989). Acetate, butyrate and isobutyrate were separated by increasing the column temperature from 120 °C to 150 °C at a rate of 25 °C/min. Propionate was measured at 120 °C column temperature. All samples were acidified before injection with formic acid (0.5 M final concentration). Dicarboxylic acids were quantified as methyl esters (Metcalf et al. 1966) with a capillary column according to Dehning and Schink (1989b). Conditions were slightly modified: detector and injector temperature 225 °C, column temperature 140 °C (succinate) or 150 °C (glutarate, methylsuccinate), with malonate as internal standard.

The guanine-plus-cytosine content of the DNA was determined after preparation of DNA (Marmur 1961) and thermal denaturation (De Ley et al. 1970). *Escherichia coli* strain K12 was taken as reference.

Protein was determined with the micro-buret method (Zamenhoff 1957).

Enzyme assays

If not indicated otherwise, all enzyme assays were carried out with cell-free extracts prepared from glutarate-grown cells of strain WoG13 under anaerobic conditions (Matthies and Schink 1991). Photometric assays were performed in a spectrophotometer model 100-40 (Hitachi, Tokyo, Japan). A linear relationship of activity and protein concentration was checked for with all enzyme activities.

Glutaryl-CoA synthetase (EC 6.2.1.6) was measured after Brandis-Heep et al. (1983) using glutaryl-CoA instead of succinyl-CoA.

Acyl-CoA: acetate CoA transferase (EC 2.8.3.8) was assayed by the propionyl-CoA: acetate CoA transferase assay described by Hilpert et al. (1984).

Glutaryl-CoA dehydrogenase (EC 1.3.99.7) and *butyryl-CoA dehydrogenase* (EC 1.3.99.2) were determined by a modified succinate dehydrogenase assay (Stams et al. 1984). The reaction mixture contained: potassium phosphate buffer, pH 7.5, 50 mM; potassium hexacyanoferrate (III), 1 mM; phenazine methosulfate, 0.1 mM; glutaryl-CoA or butyryl-CoA, 0.5 mM; cell extract, 0.1–0.25 mg protein · ml⁻¹.

Glutaconyl-CoA decarboxylase (EC 4.1.1.-) was determined by a photometric test modified after Buckel (1986). The assay mixture

contained: potassium phosphate buffer, 50 mM, pH 7.0, 2.5 mM dithioerythritol; auxiliary enzyme mixture (acetyl-CoA: glutamate CoA transferase, crotonase, 3-hydroxybutyryl-CoA dehydrogenase, β -keto-thiolase, phosphate acetyl transferase), 0.2 mg protein · ml⁻¹; coenzyme A, 1 mM; acetyl phosphate, 1 mM; NAD, 10 mM; NaCl, 20 mM; Na₂-glutaconate, 1 mM; cell-free extract, 0.1–0.25 mg protein · ml⁻¹.

Methylmalonyl-CoA decarboxylase (EC 4.1.1.41) was measured following methylmalonyl-CoA absorption decrease at 232 nm in the presence of phosphate acetyl transferase and arsenate (Hilpert and Dimroth 1983).

Acetate kinase (EC 2.7.2.1.) and *phosphate acetyl transferase* (EC 2.3.1.8) were determined by standard methods (Bergmeyer 1974). β -Ketothiolase (EC 2.3.1.19) and *3-hydroxybutyryl-CoA dehydrogenase* (EC 1.1.1.157) were measured according to Lynen and Ochoa (1953). *Crotonase* (EC 4.2.1.17) was assayed following crotonyl-CoA absorption decrease at 263 nm (Moskowitz and Merrick 1969).

Glutaconate CoA-transferase was determined as described by Buckel (1986).

Chemicals

All chemicals used were of reagent grade quality and obtained from Merck, Darmstadt; Fluka, Neu-Ulm; Sigma, Deisenhofen; Boehringer, Mannheim, and Biomol, Hamburg, FRG. Gases were obtained from Messer-Griesheim, Darmstadt, FRG.

Results

Enrichment and isolation

Mineral salts medium with 10 mM glutarate as sole source of carbon and energy was inoculated with anoxic sediment samples. After 4–7 weeks, turbidity increased and gas production started. The enrichment cultures were transferred several times, and motile spore-forming rods, large refractive rods, and methanogens shaped like *Methanospirillum hungatei* were enriched, but no glutarate-degrading bacterium could be isolated in pure culture. However, after the cultures were incubated with enhanced glutarate concentration (20 mM), long, motile, rod-shaped cells dominated. After more than 10 transfers, the subcultures CuG1, WoG1 and KoG1 grew up within 2–5 days. Addition of 30 mM bromoethane-sulfonate, a specific inhibitor of methanogenesis (Sparling and Daniels 1987), to the medium did not inhibit glutarate degradation, but enriched long, motile rods in the cultures.

Isolation of glutarate-degrading bacteria in pure culture was attempted by diluting the culture in agar shake series. White, lentil-shaped colonies developed within 1–2 weeks. Picked colonies from the last positive tube of a second agar dilution series started growth only if they were transferred into filter-sterilized spent growth medium of the enrichment culture amended with additional 0.5 mM sodium sulfide. Two isolates, WoG13 from freshwater and CuG11 from a marine sediment, were obtained by this procedure. Purity was checked microscopically and by growth tests in complex AC-medium (Difco, Ann Arbor, Mich., USA) in the presence or absence of added glutarate.

Both isolated strains were very similar with respect to morphology, gram reaction, and physiological proper-

ties. Therefore, only strain WoG13 was chosen for detailed characterization.

Morphology and cytology

Both strains were long, rod-shaped cells, $4.5\text{--}6.5\text{ }\mu\text{m} \times 0.8\text{ }\mu\text{m}$ in size (Fig. 1 a, b). Motility was observed only in growing cultures. Cells moved in a twisting and tumbling manner. Flagella staining revealed one subpolarly inserted flagellum (Fig. 1c). Motility was lost after about 6 months of cultivation. Both strains stained gram-negative, and the KOH test (Gregersen 1978) showed the typical reaction of gram-negative bacteria. Part of the cells of strain WoG13 formed spores in well-growing cultures or in colonies in agar. Spores were formed at the terminal part of the cells (Fig. 1a), and survived pasteurization at $80\text{ }^{\circ}\text{C}$ for 10 min. With strain CuG11, spore formation was not observed. The DNA base ratio of strain WoG13 determined by thermal denaturation was $49.0 \pm 1.4\text{ mol}\%$ guanine-plus-cytosine. No cytochromes could be detected in redox difference spectra of crude cell extracts.

Physiology

Growth of strains WoG13 and CuG11 occurred only under strictly anaerobic conditions. Strain WoG13 grew well in freshwater medium containing 1 g/l NaCl and 0.4 g/l $\text{MgCl}_2 \cdot 6\text{ H}_2\text{O}$, and no growth was detected in saltwater medium (20 g/l NaCl, 3 g/l $\text{MgCl}_2 \cdot \text{H}_2\text{O}$). Strain CuG11 grew well in both media. Strain WoG13 could also be grown in freshwater medium containing 20 mM potassium phosphate as buffer. Both strains required growth supplines provided in the enrichment medium by partner bacteria. With strain WoG13, reproducible growth was obtained with 2% rumen fluid to the glutarate-containing freshwater mineral medium. With 0.05% yeast extract, 0.1% pig manure extract, or 0.1% casamino acids, similar cell densities were obtained, but growth lag phases increased. No growth was observed with any of these supplines alone. Optimal growth of strain WoG13 was found at $37\text{ }^{\circ}\text{C}$ ($\mu = 0.062$, $t_d = 11\text{ h}$).

Cells grew well within a pH range of 7.1–8.2. No growth was detected below $20\text{ }^{\circ}\text{C}$ or above $37\text{ }^{\circ}\text{C}$, or at $\text{pH} < 6.0$.

Only glutarate, methylsuccinate, and succinate could be used as growth substrates by strain WoG13. Neither sugars, amino acids, organic acids nor other dicarboxylic acids were degraded (Table 1). Butyrate, isobutyrate, CO_2 and small amounts of acetate were formed as products of glutarate and methylsuccinate fermentation. Succinate was fermented to propionate and CO_2 . Strain CuG11 used the same substrates and formed the same products.

A growth curve of strain WoG13 (Fig. 2) illustrates correlation of growth with glutarate consumption and formation of butyrate, isobutyrate, and acetate.

Stoichiometry and growth yields

Stoichiometry of substrate utilization and product formation was determined with both strains, WoG13 and

Fig. 2. Growth of strain WoG13 with glutarate (■): cell density (□) and formation of butyrate (○), isobutyrate (●), and acetate (△)

Fig. 1a–c. Phase contrast photomicrographs of the isolates WoG13 and CuG11. a Vegetative cells, free spores and spore formation of strain WoG13; b vegetative cells of strain CuG11; c vegetative cells of strain WoG13 with stained flagella. Bars equal $10\text{ }\mu\text{m}$

Table 1. Fermentation stoichiometries and growth yields of strains WoGl3 and CuGl1

Substrate	Substrate degraded (μmol)	Optical density reached E ₆₀₀	Cell dry mass formed ^a (mg)	Substrate assimilated ^b (μmol)	Products formed (μmol)				Electron recovery (%)	Molar growth yield (g/mol)
					Butyrate	Isobutyrate	Propionate	Acetate		
<i>Strain WoGl3</i>										
Glutarate	403	0.065	0.365	3.7	210	220	—	5	108	0.91
Methylsuccinate	396 ^c	0.061	0.342	3.5	222	150	—	7	96	0.87
Succinate	396 ^c	0.033	0.185	2.7	—	—	376	22	99	0.47
<i>Strain CuGl1</i>										
Glutarate	429	0.048	0.335	3.4	319	95	—	9	98	0.79
Methylsuccinate	396 ^c	0.040	0.279	2.8	285	119	—	4	103	0.71
Succinate	403	0.045	0.314	4.6	—	—	438	6	111	0.79

Growth experiments were carried out in duplicates in 22 ml screw cap tubes

^a Cell dry matter was calculated *via* cell turbidity using the conversion factors $0.1 \text{ OD}_{600} \cong 25.5 \text{ mg cell dry matter per liter}$ (WoGl3) and $0.1 \text{ OD}_{600} \cong 31.7 \text{ mg cell dry matter per liter}$ (CuGl1), which were obtained by direct gravimetric determinations in 500 ml cultures grown with glutarate

^b Substrate assimilated into cell material was calculated according to the following equations:

^c Substrate supplied

The following substrates were not degraded by strain WoGl3 in the presence of 2% rumen fluid (if not indicated otherwise, substrates were added 20 mM, sugars 5 mM): glutaconate, adipate, pimelate, suberate, malonate, oxalate, fumarate, methylglutarate, L- α -hydroxyglutarate, mesaconate, itaconate, malate, lactate (10), citrate (10), pyruvate (10), glyoxylate, dipicolinate, formate, crotonate, butyrate (10), glucose, fructose, xylose, lactose, sucrose, arabinose, aspartate (10), glutamate (10), histidine, casamino acids (0.1%), ethylene glycol (10), glycerol (10), ethanol (10), cadaverine (10). Degradation of fumarate, crotonate, mesaconate, maleinate, and itaconate was tested also in the presence of 5 or 20 mM formate as external electron donor. No growth was observed with butyrate in a mixed culture experiment with *Methanospirillum hungatei* strain SK

CuGl1. The results are presented in Table 1. The amounts of formed products agreed with complete substrate degradation. Small amounts of acetate ($< 0.5 \text{ mM}$) were formed to balance the electron requirement of assimilative metabolism (Table 1). The molar growth yield of strain WoGl3 with both glutarate and methylsuccinate was $0.9 \text{ g dry cell mass} \cdot \text{mol}^{-1}$, and $0.5 \text{ g dry cell mass} \cdot \text{mol}^{-1}$ with succinate. For strain CuGl1, growth yields were nearly the same with all 3 substrates tested ($0.7\text{--}0.8 \text{ g} \cdot \text{mol}^{-1}$).

Enzymes involved in energy metabolism of strain WoGl3

Enzyme assays were carried out with cell-free extracts prepared from cells of strain WoGl3 grown with the respective substrate. Acyl-CoA : acetate CoA transferase, glutaryl-CoA dehydrogenase, glutaconyl-CoA decarboxylase, and butyryl-CoA dehydrogenase were detected at moderate activities, sometimes at less than 20% of *in vivo* activity (Table 2): during exponential growth at a growth rate of 0.033 h^{-1} at 30°C with a molar growth yield coefficient (Y_s) of $0.9 \text{ g} \cdot \text{mol}^{-1}$, the rate of glutarate conversion was about $1200 \text{ nmol/min} \cdot \text{mg protein}$.

The highest acyl-CoA : acetate CoA transferase activities were observed with butyryl-CoA and glutaryl-CoA as substrate, whereas activities with isobutyryl-CoA were considerably lower. Glutaryl-CoA synthetase could not be detected. Glutaryl-CoA dehydrogenase activity was found only with glutaryl-CoA; no dehydrogenase activity was detected with glutarate as substrate. Glutaryl-CoA dehydrogenase activity as well as butyryl-CoA dehydro-

Table 2. Enzymes involved in glutarate fermentation by strain WoGl3

Enzyme	specific activity (nmol/min $\cdot$ mg protein)
Glutaryl-CoA : acetate CoA transferase	1300
Butyryl-CoA : acetate CoA transferase	1700
Isobutyryl-CoA : acetate CoA transferase	540
Glutaryl-CoA dehydrogenase	220 ^a
Glutaconyl-CoA decarboxylase	60
Butyryl-CoA dehydrogenase	300 ^a
Methylmalonyl-CoA decarboxylase	60

All enzyme activities were measured in cell-free extracts of glutarate-grown cells except for methylmalonyl-CoA decarboxylase which was determined with succinate-grown cells.

^a measured with potassium hexacyanoferrate (III) as electron acceptor in the presence of PMS

genase activity measured with potassium hexacyanoferrate (III) and PMS (at catalytic concentrations) could not be enhanced by 0.01% (w/v) Triton X-100.

The glutaconyl-CoA-decarboxylase activity was about 5% of the *in vivo* rate of glutarate conversion. Addition of Triton X-100 (0.01% or 0.1%), ATP (1 mM), thiamine pyrophosphate (1 mM), biotin (0.1 mM), coenzyme B₁₂ (10–20 μM), FAD (50 μM), MgCl₂ (1–7.5 mM) or CaCl₂ (1–2 mM) could not significantly enhance this activity. The decarboxylase activity was in the same range if the original aerobic assay of Buckel (1986) was used. With *Acidaminococcus fermentans*, activities of about 0.2 U/mg protein were

observed with this assay. To ensure a sufficient activity of the auxiliary enzyme mixture in the glutaconyl-CoA decarboxylase assay the activity of glutaconate CoA-transferase was measured as a representative.

In cell-free extracts prepared from methylsuccinate-grown cells, glutaryl-CoA dehydrogenase activity was considerably lower (78 nmol/min · mg protein) than in glutarate-grown cells. Neither isomerization of glutaryl-CoA to methylsuccinyl-CoA nor rearrangement of methylsuccinate to glutarate could be detected in the presence of coenzyme B₁₂ in cell-free extracts of these cells.

The key enzyme of succinate fermentation, methylmalonyl-CoA decarboxylase, was detected in succinate-grown cells (60 nmol/min · mg protein) but not in glutarate-grown cells.

Enzymes involved in assimilatory metabolism of strain WoGl3

Enzyme activities of β -oxidation, thiolytic fatty acid cleavage, and of phosphate acetyl transferase and acetate kinase were detected in cell-free extracts (Table 3). The activities were sufficient to provide the redox equivalents for synthesis of cell matter from glutarate (calculated minimum activity: 9.2 nmol/min · mg protein).

Discussion

Physiology

In the present study, we describe two strains of glutarate-fermenting bacteria enriched and isolated from anoxic freshwater and saltwater sediment samples. Strain WoGl3 appeared to be adapted to low salt concentrations as typical of a freshwater sediment. Its high growth

temperature optimum (37 °C) indicates that this bacterium is also adapted to the intestinal tract of warm-blooded animals. The isolate from marine sediment behaved more like an estuary organism tolerating salt concentrations up to 2% NaCl and 0.3% MgCl₂ (w/v).

Strain WoGl3 was described here in more detail and is the first strictly anaerobic bacterium fermenting glutarate in pure culture. This strain is very specialized and grows only with dicarboxylic acids, decarboxylating them to the respective fatty acids. Glutarate and methylsuccinate fermentation formed butyrate and isobutyrate whereas succinate was fermented to propionate. Thus, besides oxalate, malonate, and succinate, also the dicarboxylic acid glutarate can be fermented via decarboxylation. This is possible although three methylene groups separate the two carboxylic groups of glutarate and decrease the inductive effect onto each other. Thus, decarboxylation of glutarate has to be prepared through activation and by increasing the electron-attracting effect at the β -carbon atom. Measurements of enzyme activities in cell-free extracts of strain WoGl3 led to the pathway of glutarate degradation shown in Fig. 3.

Activation of glutarate to glutaryl-CoA is carried out by a CoA transferase rather than by a glutaryl-CoA synthetase enzyme. The enzyme catalyzing the CoA transfer reaction probably uses acetyl-CoA as CoA source. The pool of acetyl-CoA is replenished by CoA transfers from butyryl-CoA and isobutyryl-CoA to acetate releasing the fermentation products butyrate and isobutyrate. It is not clear yet whether one single acyl-CoA:acetate CoA transferase catalyzes three reactions with different acyl residues, or whether two or three different enzymes are involved. Oxidation of glutaryl-CoA to glutaconyl-CoA polarizes the linkage between the carbon atoms 4 and 5 and allows subsequent decarboxylation to crotonyl-CoA. The same reaction steps are active also in aerobic glutarate degradation (Numa et al. 1964). Crotonyl-CoA is reduced to butyryl-CoA with the reducing equivalents released by glutaryl-CoA dehydrogenase. The electron transfer might be mediated by an electron carrier of a redox potential in the range of that of the crotonyl-CoA/butyryl-CoA couple ($E^{\circ} = -126$ mV, Gustafson et al. 1986) and of the glutaconyl-CoA/glutaryl-CoA couple which is assumed to be in the same range. The physiological electron carrier

Table 3. Enzymes involved in assimilatory metabolism of strain WoGl3

Enzyme	Specific activity (nmol/min · mg protein)
Crotonase	84
3-Hydroxybutyryl-CoA dehydrogenase	490
β -Ketothiolase	280
Phosphate acetyl transferase	46
Acetate kinase	170

Fig. 3. Pathway of fermentative glutarate degradation by strain WoGl3. Numbers in circles refer to the following enzymes: (1) glutaryl-CoA:acetate CoA transferase, (2) glutaryl-CoA dehydrogenase, (3) glutaconyl-CoA decarboxylase, (4) butyryl-CoA dehydrogenase, (5) butyryl-CoA:isobutyryl-CoA mutase, (6) butyryl-CoA:acetate CoA transferase, (7) isobutyryl-CoA:acetate CoA transferase

is not known yet. Formation of isobutyrate as fermentation side product proceeds via isomerization of butyryl-CoA to isobutyryl-CoA through butyryl-CoA:isobutyryl-CoA mutase and CoA transfer to acetate as mentioned above. This rearrangement is characterized in more detail in a separate paper (Matthies and Schink 1992); the slight increase in entropy of the total reaction through this isomerization may be the only advantage for the bacterium.

Decarboxylation of glutarate to butyrate and isobutyrate can obviously support the whole energy metabolism of strain WoG13:

(calculated after Thauer et al. 1977)

The small free energy change of this reaction would allow synthesis of only one third of an ATP, taking into account that irreversible ATP synthesis in the living cell requires 60–80 kJ · mol⁻¹ (Thauer et al. 1977). With this assumption, one can calculate from the measured growth yield (Table 1) Y_{ATP} to 2.7 g dry cell mass/mol ATP which is rather low compared to that of other anaerobic bacteria (5–15 g dry cell mass/mol ATP; Stouthamer 1979). The low growth rate of 0.033 h⁻¹ ($t_d = 21$ h) at 30 °C may be the main reason for this low Y_{ATP} value: The part of the total energy budget that has to be spent in maintenance metabolism is comparably high at low growth rates (Pirt 1965).

The free energy change of fermentative glutarate degradation ($\Delta G^\circ = -25.4 \text{ kJ} \cdot \text{mol}^{-1}$) does not allow ATP synthesis by substrate-linked phosphorylation. There is also no indication of an electron transport chain involved in energy metabolism of this bacterium. In the last few years, two different mechanisms of energy conservation were described for bacteria growing by decarboxylation of dicarboxylic acids to the respective fatty acids. The succinate-fermenting bacterium *Propionigenium modestum* couples the membrane-associated methylmalonyl-CoA decarboxylase reaction with sodium ion transport across the cytoplasmic membrane (Hilpert et al. 1984). The Na⁺ gradient is used directly for ATP synthesis by a Na⁺-dependent ATPase (Laubinger and Dimroth 1988). In *Oxalobacter formigenes*, the exergonic decarboxylation of oxalate to formate drives an electrogenic antiporter system which exchanges oxalate for formate. The decarboxylating enzyme localized in the cytoplasm consumes one mol of protons per mol oxalate thus building up a transmembrane proton gradient which drives ATP synthesis (Anantharam et al. 1989). In strain WoG13, glutacetyl-CoA decarboxylase appears to be the key enzyme in glutarate catabolism and is probably directly involved in energy conservation. Unfortunately, the activity of this enzyme in cell-free extracts was very low and could not be enhanced by various additions to the assay mixture. Obviously, the decarboxylase is very unstable in crude cell-free extracts and nearly 95% of the present decarboxylase became inactive during the

preparation of the cell-free extracts. The biochemistry of this decarboxylation as well as the mechanism of energy conservation will be subject of further studies in our laboratory.

Taxonomy

The new glutarate-fermenting isolate strain WoG13 is a strictly anaerobic, gram-negative, sporeforming bacterium. With respect to gram reaction, DNA guanine-plus-cytosine content and the small range of substrates used, our isolate resembles the species *Oxalobacter formigenes* (Allison et al. 1985), *Oxalobacter vibrioformis* (Dehning and Schink 1989a) and *Malonomonas rubra* (Dehning and Schink 1989b) which all ferment exclusively dicarboxylic acids. However, strain WoG13 differs from these species by their inability to form spores, and to use glutarate, methylsuccinate or succinate as energy and carbon source. In addition, the *Oxalobacter* species are in most cases short rods or vibroid cells, and *Malonomonas rubra* differs by its very high cytochrome content.

At present, the genus *Sporomusa* is the only genus which includes Gram-negative, sporeforming bacteria. Within this genus, six species have been described which have guanine-plus-cytosine values in the range of 42–48 mol%. Two species, *S. termitida* and *S. malonica*, can use dicarboxylic acids as substrates: they decarboxylate succinate and malonate (Breznak et al. 1988; Dehning et al. 1989). However, our isolate differs from these and the four other species (Möller et al. 1984; Ollivier et al. 1985; Hermann et al. 1987) by its non-acetogenic metabolism and the small range of substrates used. Thus, with its physiological properties strain WoG13 does not fit with any of the genera mentioned. For correct assignment of this new isolate to a taxonomic entity, 16S rRNA analysis has to be carried out.

Strain WoG13 was deposited with the Deutsche Sammlung von Mikroorganismen GmbH, Braunschweig, under the number DSM 6652.

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