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# Fermentation of 2-methoxyethanol by *Acetobacterium malicum* sp. nov. and *Pelobacter venetianus*

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**Abstract.** Anaerobic bacteria degrading 2-methoxyethanol were enriched from freshwater sediments, and three strains were isolated in pure culture. Two of them were Gram-positive non-spore-forming rods and grew strictly anaerobically by acetogenic fermentation. Optimal growth occurred at 30° C, initial pH 7.5–8.0. 2-Methoxyethanol and 2-ethoxyethanol were fermented to acetate and corresponding alcohols. Hydrogen plus carbon dioxide, formate, acetoin, L-malate, lactate, pyruvate, fructose, and methoxyl groups of 3,4,5-trimethoxybenzoate and 3,4,5-trimethoxycinnamate were fermented to acetate. 1,2-Propanediol was fermented to acetate, propionate, and propanol. Strain MuME1 was described as a new species, *Actetobacterium malicum*. It had a DNA base composition of 44.1 mol% guanine plus cytosine. The third strain, which was identified as *Pelobacter venetianus*, fermented 2-methoxyethanol to methanol, ethanol, and acetate.

**Key words:** 2-Methoxyethanol – 2-Ethoxyethanol – L-Malate – Hydrogen – *Acetobacterium malicum* – *Pelobacter venetianus* – Acetogenic – Anaerobic – Fermentation – Degradation

2-Methoxyethanol (Methyl Cellosolve, ethyleneglycol monomethylether) is a compound which has been used widely as an organic solvent since ca. 1956. To our knowledge 2-methoxyethanol is a xenobiotic compound, but it is known that 2-methoxyethanol can be degraded completely to methane plus carbon dioxide under anaerobic conditions by an undefined mixture culture (Tanaka et al. 1986).

In the present study 2-methoxyethanol-degrading anaerobes were enriched, and three strains were isolated in pure culture. Two strains were acetogenic nonspore-forming bacteria that fermented not only 2-methoxyethanol and 2-ethoxyethanol but also L-malate, three compounds that are not utilized by known species of the genus *Acetobacterium*. The new strains are described as a new species of the genus *Acetobacterium*, *A. malicum* sp. nov. The third strain using 2-methoxyethanol was identified as *Pelobacter venetianus* (Schink and Stieb 1983). Metabolic difference between *A. malicum* and *P. venetianus* concerning fermentation of 2-methoxyethanol is described.

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## Materials and methods

### *Strains and sources of organisms*

Strains MuME1 and WoME1 were isolated in pure culture from enrichment cultures inoculated with freshwater sediments of ditches in Wollmatingen, Konstanz, FRG. Strain LiME1 was isolated in pure culture from an enrichment culture inoculated with freshwater sediment of a ditch in Litzelstetten, Konstanz, FRG.

*Acetobacterium woodii* strain NZva16, *A. wieringae* strain C (DSM 1911), and *A. carbinolicum* strain WoPropl (DSM 2925), *Pelobacter venetianus* strain GraPEG1 (DSM 2394), and *P. carbinolicus* strain GraBd1 (DSM 2380) were kindly provided by E. Kayser from the Konstanz culture collection.

### *Media and cultivation*

The basal freshwater medium contained per liter distilled water: 0.2 g $KH_2PO_4$, 0.3 g $NH_4Cl$, 0.4 g $MgCl_2 \cdot 6H_2O$, and 0.15 g $CaCl_2 \cdot 2H_2O$. The following sterile stock solutions were added to the autoclaved cooled basal medium (per l): 40 ml sodium bicarbonate solution (2.5 g/40 ml), 3 ml of a sodium sulfide solution (12 g/100 ml), 1 ml of a trace element solution SL10 (Widdel et al. 1983), 1 ml of a selenite/tungstate solution (Tschech and Pfennig 1984), and 1 ml of a 7-vitamin solution (Widdel and Pfennig 1981). Substrates were added from sterile neutralized stock solutions before inoculation. The pH of the medium was adjusted to 7.3–7.5 in enrichment and isolation experiments, but to 7.8–8.0 after pH-optimum experiments. Pure cultures were obtained by repeated application of the agar deep dilution method described by Pfennig (1978).

Utilization of carbon sources and electron acceptors was tested in 20 ml screw cap tubes. Use of $H_2$ plus $CO_2$ was tested in 20 ml tubes that contained 10 ml inoculated medium and were sealed with butyl rubber stoppers. Growth was followed in the test tubes by turbidity measurements at 600 nm in a Bausch & Lomb Spectronic 70 spectrophotometer. All growth tests were carried out in duplicates at 28° C unless indicated otherwise. Time course experiments were carried out using half-filled 120 ml serum bottles which were shaken at a frequency of 90 rpm at an amplitude of 2 cm at 28° C. Optical density in the bottle was measured after sampling using a 1 cm cuvette.

### *Analytical procedures*

Volatile fatty acids were determined using a Perkin-Elmer gas chromatograph with FID, with a 2 m PEG 6000/

**Table 1**
Substrates tested for growth of 2-methoxyethanol-degrading strains of *Acetobacterium malicum* compared with *A. woodii*, *A. wieringae*, and *A. carbinolicum*

| Substrate (mM used in this study) | *A. malicum* strain | | *A. woodii*[a,c] | *A. wieringae*[b,c] | *A. carbinolicum*[c] |
|---|---|---|---|---|---|
| | MuME1 | LiME1 | | | |
| $H_2/CO_2$ (80/20, v/v) | + | + | + | + | + |
| Formate (10) | +w | +w | + | + | + |
| Methanol (5; 10) | − | − | + | − | + |
| Trimethoxybenzoate (2) | + | + | + | − | + |
| Trimethoxycinnamate (2) | + | + | + | − | + |
| Ethanol (10) | − | − | − | − | + |
| Propanol (10) | − | − | − | − | + |
| Butanol (10) | − | − | − | − | + |
| Pentanol (10) | − | − | − | − | + |
| 1,2-Propanediol (5) | + | + | + | + | + |
| 2,3-Butanediol (10) | − | − | + | − | + |
| Acetoin (5) | + | + | + | + | + |
| Glycerol (10) | + | + | + | + | + |
| Fumarate (10) | − | − | − | −* | −* |
| L-Malate (5) | + | + | − | − | − |
| Oxaloacetate (5) | − | − | +* | +* | +* |
| Lactate (10) | + | + | +w | + | + |
| Pyruvate (10) | + | + | + | − | + |
| Glucose (10) | − | − | − | − | + |
| Fructose (2) | + | − | + | + | + |
| Ethyleneglycol (10) | − | + | + | + | + |
| Tetraethyleneglycol (10) | − | − | −* | −* | −* |
| PEG 200 (1 g/l) | − | − | −* | −* | −* |
| 2-Methoxyethanol (10) | + | + | −* | −* | −* |
| Methoxyacetate (10) | − | − | − | −* | −* |
| Glycollate (10) | − | − | − | −* | −* |
| 2-Ethoxyethanol (10) | + | + | −* | −* | −* |
| Ethanolamine (10) | − | − | −* | +* | −* |
| Choline (5) | + | − | − | +w* | −* |
| Trimethylamine (10) | − | − | −* | −* | −* |
| Betaine (10) | + | + | +* | +* | + |
| Glycine (10) | − | − | −* | −* | −* |

*Abbreviation*: PEG, polyethyleneglycol
*Symbols*: +w, weak growth; *, data in this study.
[a] Data from Balch et al. (1977), Bache and Pfennig (1981)
[b] Data from Braun and Gottschalk (1982). Growth tests were carried out in defined medium in the presence of 1 g/l yeast extract
[c] Data from Eichler and Schink (1984)

Shimalite TPA (30 − 60 mesh) column, at oven temperature of 120° C, injector and detector temperature of 140° C. Before injection 0.45 ml of the culture supernatant was acidified with 0.05 ml of 1 M phosphoric acid. Alcohols and acetaldehyde were determined using the same gas chromatograph with a 2 m Porapak QS (100 − 120 mesh) column, at oven temperature of 140° C, injector and detector temperature of 160° C, without acidifying the supernatant.

Sulfide was determined photometrically by the methylene blue method (Cline 1969). Nitrate was determined by the salicylic acid-nitration method (Cataldo et al. 1975) after sulfide was removed by precipitation with zinc acetate and centrifugation as described by Seitz and Cypionka (1986).

Cytochromes were surveyed according to Seitz and Cypionka (1986) using a double beam spectrophotometer (Shimadzu UV300).

Cell dry mass was determined as described by Klemps et al. (1985). Substrate assimilated into cell material was calculated via acetate according to the following equation:

$$17\,CH_3COO^- + 11\,H_2O \rightarrow$$
$$8\,\langle C_4H_7O_3\rangle + 2\,HCO_3^- + 15\,OH^- \ .$$

Cell dry weight of strain MuME1 was calculated by means of cell density using the conversion factor 0.1 $OD_{600}$ = 18.3 mg dry weight per liter, which was obtained by direct determination in 500 ml cultures grown with 2-methoxyethanol.

The G + C contents of the DNA were kindly determined by Dr. Flossdorf (GBF, Braunschweig, FRG) using the CsCl-gradient-ultracentrifugation-method (Flossdorf 1983).

### *Other methods*

Gram-staining was carried out according to Magee et al. (1975). Gram-type tests using KOH were carried out as described by Gregersen (1978).

The API 20A system (for identification of anaerobic bacteria) was used according to the prescription of API System S.A., Montalieu Vercieu (France).

Growth tests in aerobic-anaerobic-gradient agar were carried out using tubes which were half filled with a sodium sulfide-reduced agar medium containing 10 mM 2-methoxyethanol and 1 mg/l resazurin under air with butyl rubber stoppers.

## Results

### *Enrichment and isolation*

Freshwater medium containing 10 mM 2-methoxyethanol was inoculated with freshwater sediments obtained near

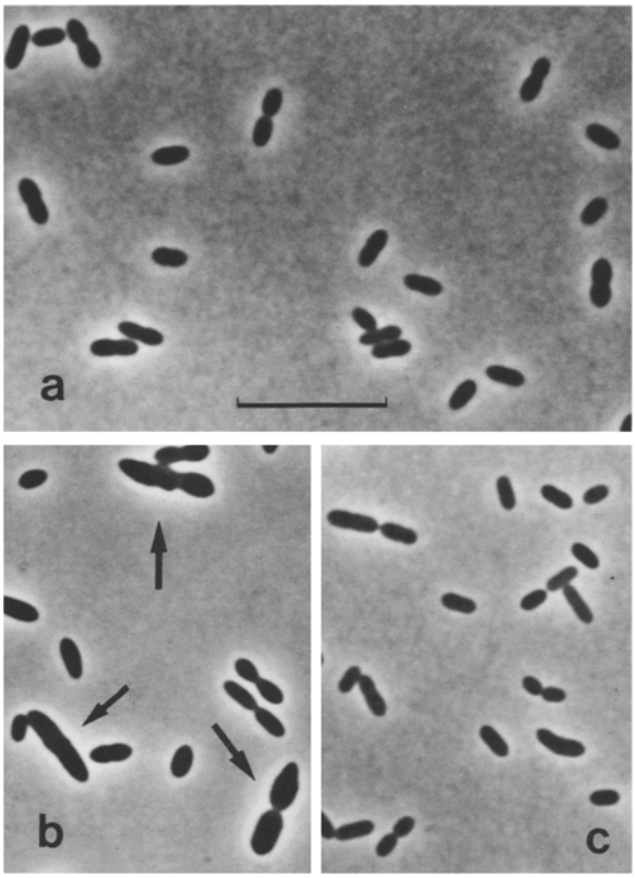


**Fig. 1a–c.** Phase-contrast photomicrographs of the new isolates. Strain MuME1 was grown on 5 mM L-malate (**a**) and 10 mM 2-methoxyethanol (**b**). Arrows indicate abnormal swollen cells of strain MuME1. Strain LiME1 was grown on 10 mM 2-methoxyethanol (**c**). Bar represents 10 μm

Konstanz. Bacterial growth was observed within 2 weeks. Transfer of the cultures to fresh media shortened the lag phase to 2 or 3 days. Pure cultures were obtained from single white colonies after repeated application of agar deep dilution method using 10 mM 2-methoxyethanol as a substrate.

Strains WoME1 and MuME1 were isolated in this way. Strain LiME1 was purified similarly using 10 mM 2-methoxyethanol, however, no growth was obtained if colonies were isolated into liquid medium with 10 mM 2-methoxyethanol. If 10 mM acetoin was used, isolated colonies grew well. Afterwards there was no difficulty to grow the strain in liquid media containing 10 mM 2-methoxyethanol.

*General properties of strains MuME1 and LiME1*

In aerobic-anaerobic-gradient agar tubes the strains MuME1 and LiME1 grew only in lower part in which resazurin was still reduced (colorless; from 1–2 cm below the agar surface down to the bottom). This indicated that the strains were strictly anaerobic. When strains MuME1 and LiME1 were grown on 5 mM L-malate, cells were ellipsoidal rods, $1.0-1.3 \times 1.8-4.0$ μm in size, with slightly pointed ends; cells occurred singly or in pairs (Fig. 1a). If they were grown with 10 mM 2-methoxyethanol, strain MuME1 tended to swell abnomally (Fig. 1b), while strain LiME1 grew normally (Fig. 1c). Both strains stained Gram-positive, and the Gram-types were positive. Spores were never observed. No growth occurred in pasteurized enrichment cultures (80°C, 10 min) with 2-methoxyethanol even after 8 weeks of incubation. Catalase was not detected in intact cells of each strain. No cytochromes were detected in cell-free extracts of each strain.

Both strains showed optimal growth at 30°C, initial pH 7.5–8.0. Strain MuME1 grew at 28–35°C, initial pH 7.0–9.0. Strain LiME1 grew at 20–33°C, initial pH 6.5–8.5.

Under the optimal condition, doubling times of strains MuME1 and LiME1 growing on 2-methoxyethanol were 22 h and 9.3 h, respectively. Doubling times of strains MuME1 and LiME1 growing on L-malate were 7.5 h and 5.2 h, respectively.

Figure 2a shows growth and fermentation of 2-methoxyethanol by strain MuME1. This strain accumulated acetaldehyde temporarily as an intermediate. With strain LiME1 similar results were obtained, however, acetaldehyde could not be detected as an intermediate.

In the presence of 2-methoxyethanol (10 mM), nitrate (10 mM), sulfate (10 mM), sulfite (1–10 mM), thiosulfate (10 mM) and sulfur (excess) were tested as electron acceptors

**Table 2.** Substrate utilization by *Pelobacter venetianus* strain WoME1

| |
|---|
| Substrate utilized: |
| Acetoin (10 mM), Ethanolamine (10 mM), Choline (10 mM), Polyethyleneglycol 200 (1 g/l), Tetraethyleneglycol (10 mM), 2-Methoxyethanol (10 mM) |
| Substrate utilized only in coculture with a hydrogen-scavenging bacterium: Ethanol (10 mM), Propanol (10 mM), Butanol (10 mM) |
| Substrate utilized only in the presence of acetate (10 mM): 1,2-Propanediol (10 mM), 1,3-Butanediol (10 mM), Glycerol (10 mM) |

*Acetobacterium woodii* strain NZva16 was used as a hydrogen-scavenging bacterium.
The following substrates were not utilized: methanol (10 mM), isopropanol (10 mM), 2-ethoxyethanol (10 mM), ethyleneglycol (10 mM), diethyleneglycol (10 mM), triethyleneglycol (10 mM), polyethyleneglycol 300 (1 g/l), polyethyleneglycol 400 (1 g/l), polyethyleneglycol 1000 (1 g/l), polyethyleneglycol 1540 (1 g/l), polyethyleneglycol 4000 (1 g/l), polyethyleneglycol 6000(1 g/l), polyethyleneglycol 20000 (1 g/l), 1,3-propanediol (10 mM), 1,2-butanediol (10 mM), 1,3-butanediol (10 mM), 1,4-butanediol (10 mM), 2,3-butanediol (10 mM), 1,2-dimethoxyethane (10 mM), methoxyacetate (10 mM), gallate (2 mM), diacetyl (10 mM), Brij 58 (1 g/l), Triton X-100 (1 g/l), Triton X-114 (1 g/l), Tween 80 (1 g/l), yeast extract (1 g/l), peptone (1 g/l), casamino acids (1 g/l), formate (10 mM), acetate (10 mM), glycolate (10 mM), lactate (10 mM), oxalate (10 mM), citrate (10 mM), malonate (10 mM), glyoxylate (10 mM), L-tartrate (10 mM), succinate (10 mM), oxaloacetate (10 mM), threonine (10 mM), glycine (10 mM), methionine (10 mM), serine (10 mM), fructose (2 mM), sorbose (2 mM). The following substrates were not utilized in tests using API 20A System: glucose, mannitol, lactose, saccharose, maltose, salicin, D-xylose, L-arabinose, cellobiose, mannose, melizitose, raffinose, sorbitol, rhamnose, and trehalose.
The following substrates were not utilized even in the presence of elemental sulfur: $H_2/CO_2$ (80/20, v/v) plus acetate (10 mM), pyruvate (10 mM), fumarate (10 mM), DL-malate (10 mM), ethanol (10 mM), propanol (10 mM), butanol (10 mM)

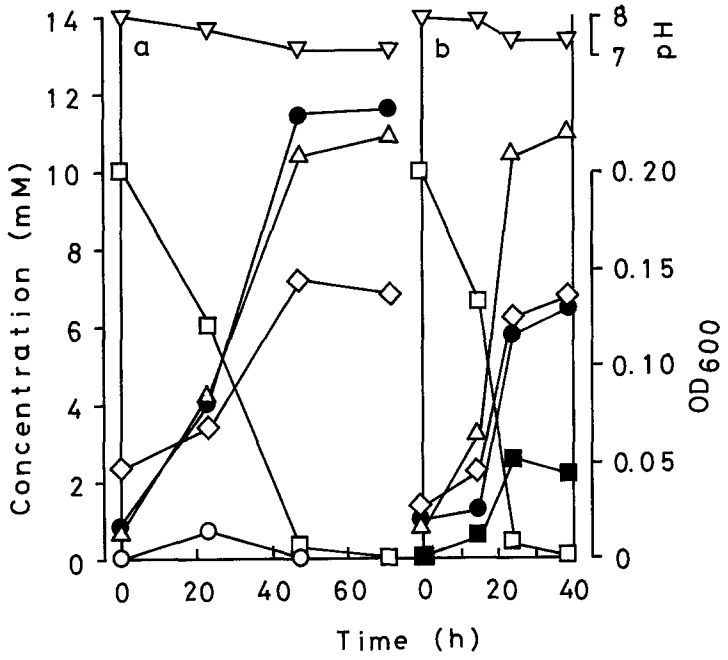


**Fig. 2**
Time course of fermentation of 2-methoxyethanol by strain MuME1 (**a**) and WoME1 (b). Half-filled serum bottles (120 ml) were used for cultivation. Gas phase was 100% nitrogen. The bottles were shaken at a frequency of 90 rpm at an amplitude of 2 cm at 28° C. $OD_{600}$ was measured using a 1 cm cuvette. Symbols: ▽ pH; □ 2-methoxyethanol; ● acetate; △ methanol; ○ acetyldehyde; ■ ethanol; ◇ $OD_{600}$

with strains MuME1 and LiME1. None of the electron acceptors was reduced by either strain. The mol % G + C of strains MuME1 and LiME1 were 44.1 and 43.9, respectively.

These characteristics as well as the substrates utilized (Table 1) indicate that these bacteria are members of the genus *Acetobacterium*. The species problem is treated in the Discussion.

*General properties of strain WoME1*

Strain WoME1 grew only in the colorless part of the aerobic-anaerobic-gradient agar tubes indicating strict anaerobiosis. When strain WoME1 was grown on 5 mM acetoin or 10 mM 2-methoxyethanol, cells were rods, $0.5-0.9 \times 0.9-10$ µm in size; cells tended to elongate and to form chains of two or more cells. Cells stained Gram-negative and the Gram-type was negative. Spores were never observed. Pasteurized enrichment cultures did not yield growth even after 8 weeks of incubation. Catalase activity was detected with intact cells. Cytochromes could not be detected in cell-free extract of strain WoME1.

Indole was not formed from tryptophan. Gelatin and urea were not hydrolyzed. Optimal growth occurred at 35° C; the temperature limits were 15° C and 39° C. The pH-optimum was at pH 8.0; the pH limits were pH 6.0 and 8.5.

Under optimal conditions, the doubling time of strain WoME1 growing on 2-methoxyethanol (10 mM) or acetoin (5 mM) was 6.1 h or 11 h, respectively.

Figure 2b shows growth and substrate consumption, product formation, and pH-change during fermentation of 2-methoxyethanol by strain WoME1. Characteristically ethanol is formed as a fermentation product in addition to methanol and acetate.

Strain WoME1 grew with 2-methoxyethanol (10 mM) in the presence of elemental sulfur, which was reduced to hydrogen sulfide in the stationary phase. Fermentation products were methanol and acetate, but no ethanol. Addition of sulfur did not increase the growth yield with 2-methoxyethanol.

In the presence of 2-methoxyethanol, nitrate (10 mM), sulfate (10 mM), sulfite (1 mM), or thiosulfate (10 mM) were tested as electron acceptors with strain WoME1. None of the compounds was reduced. The mol% G + C of strain WoME1 was 53.1.

On the basis of these characteristics as well as the substrates fermented (Table 2), strain WoME1 was identified as a member of the species *Pelobacter venetianus* (Schink and Stieb 1983).

*Fermentation balance and growth yields of strains MuME1 and LiME1*

The stoichiometry of substrate utilization and product formation was measured with strains MuME1 and LiME1. The results obtained with strain MuME1 are presented in Table 3. The fermentation balances of substrates that have not been considered previously are given here:

L-Malate,

$$2\,[OOCCH_2CHOHCOO]^{2-} + 2\,H_2O \rightarrow 3\,CH_3CCO^- + 2\,HCO_3^- + H^+;$$

Alcoxyethanols,

$$4\,ROCH_2CH_2OH + 2\,HCO_3^- \rightarrow 4\,ROH + 5\,CH_3COO^- + 3\,H^+ .$$

The amounts of products formed by strain MuME1 (see Table 3) were in good agreement with these fermentation balances. Growth yields were determined with all substrates. Fructose gave the highest growth yield. With strain LiME1 basically the same results were obtained with the exception of 2-ethoxyethanol. This compound was degraded nearly according to the following equation by strain LiME1:

$$C_2H_5OCH_2CH_2OH + 0.5\,H_2O + 1.22\,HCO_3^- \rightarrow 0.28\,C_2H_5OH + 2.33\,CH_3COO^- + 1.11\,H^+ .$$

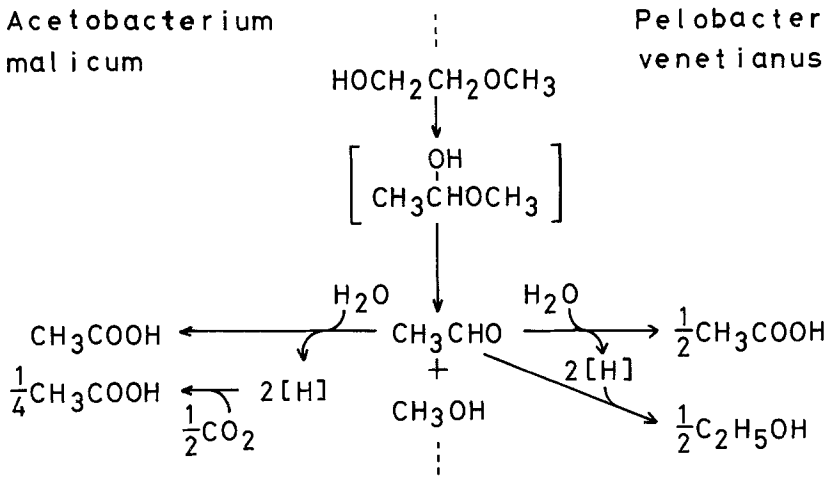


**Fig. 3**
Hypothetical pathways for fermentation of 2-methoxyethanol by *Acetobacterium malicum* (left branch) and *Pelobacter venetianus* (right branch)

**Table 3.** Stoichiometry of fermentation by *Acetobacterium malicum* strain MuME1

| Substrate | Anmount of substrate supplied (μmol) | Acetate assimilated (μmol) | Products formed | Amounts of products formed (μmol) | Growth yield (g/mol) | Carbon recovery[a] (%) |
|---|---|---|---|---|---|---|
| $H_2/CO_2$ | 332 | 8.0 | Acetate | 71 | 1.17 | 95 |
| Formate[b] | 200 | 7.2 | Acetate | 50 | 1.75 | 114 |
| Trimethoxybenzoate[c] | 40 | 19.2 | Acetate | 73 | 23.25 | 102 |
| Trimethoxycinnamate[c] | 40 | 12.8 | Acetate | 84 | 15.50 | 107 |
| 1,2-Propanediol | 100 | 9.5 | Acetate | 22 | | |
| | | | + Propanol | 10 | | |
| | | | + Propionate | 66 | 4.60 | 85 |
| Acetoin | 100 | 16.3 | Acetate | 240 | 7.90 | 102 |
| Glycerol[d] | 200 | 12.2 | Acetate | 100 | 2.95 | – |
| L-Malate | 100 | 20.4 | Acetate | 142 | 9.90 | 108 |
| Lactate | 200 | 18.2 | Acetate | 280 | 4.40 | 99 |
| Pyruvate | 200 | 22.7 | Acetate | 194 | 5.50 | 86 |
| Fructose | 40 | 30.1 | Acetate | 102 | 36.50 | 109 |
| 2-Methoxyethanol | 200 | 14.6 | Acetate | 240 | | |
| | | | + Methanol | 160 | 3.55 | 96 |
| 2-Ethoxyethanol | 200 | 12.2 | Acetate | 258 | | |
| | | | + Ethanol | 220 | 2.95 | 109 |
| Choline[d] | 100 | 29.5 | Acetate | 220 | 14.30 | – |
| Betaine[d] | 200 | 18.2 | Acetate | 126 | 4.40 | – |

Experiments were carried out in 20 ml tubes which were completely filled. Growth on $H_2/CO_2$ was tested in half-filled tubes under an atmosphere of 80% $H_2$/20% $CO_2$.
[a] Carbon recovery in pure culture was calculated after the given equations of fermentation (see Text)
[b] Yeast extract (0.5 g/l) was added to increase reproducibility
[c] Stoichiometry concerning methoxyl groups
[d] Details were not studied

## Discussion

### *Taxonomy of strain MuME1 and LiME1*

The morphological, physiological and biochemical characteristics of strains MuME1 and LiME1 show that these strains are members of the genus *Acetobacterium*. The strains differ, however, from all *Acetobacterium* species described so far (see Table 1) by their capacity to ferment 2-methoxyethanol, 2-ethoxyethanol and L-malate, as well as their inability to ferment methanol, oxaloacetate and ethyleneglycol (MuME1). On the basis of these differences, it appears justified to consider the alcoxyethanol- and malate-fermenting strains as a new species of the genus *Acetobacterium*, *A. malicum* sp. nov.

### *Physiology*

All our methanogenic enrichment cultures on 2-methoxyethanol yielded bacteria that belonged to either one of the two types of bacteria described in the present paper. Apparently there are rarely other metabolic types of bacteria that ferment 2-methoxyethanol.

Syntrophy with methanogenic bacteria is not required for the degradation of 2-methoxyethanol and the presence of methanogens did not alter the fermentation pathway in cases of *Acetobacterium malicum* strains MuME1 and LiME1. Coculture with methanogens is purely commensalistic in the sence that the fermentation products methanol and acetate are further metabolized to methane and

$CO_2$ by *Methanosarcina barkeri* that was regularly present in methanogenic enrichment cultures.

The second bacterium capable of fermenting 2-methoxyethanol was *Pelobacter venetianus*. In this case, however, coculture with hydrogen-scavenging bacteria did alter the kind of fermentation products, since ethanol was not formed in their presence. The same results were obtained by Schink and Stieb (1983) using defined cocultures.

2-Methoxyethanol was fermented by *Acetobacterium malicum* strains MuME1, LiME1, and *Pelobacter venetianus* strain WoME1, but 1,2-dimethoxyethane was not fermented by any strain (the results with strains MuME1 and LiME1 are not shown). This result suggests that a terminal hydroxy group is necessary for the degradation. The necessity was pointed out also by Strass and Schink (1986) in the degradation of polyethyleneglycol by *Pelobacter venetianus*.

The first step for the fermentation of 2-methoxyethanol is considered to be hydroxy group transfer mediated by $B_{12}$ coenzyme (Toraya and Fukui 1982) with formation of acetaldehyde half-acetal (Strass and Schink 1986). The half-acetal could be degraded to methanol and acetaldehyde; acetaldehyde is then oxidized to acetate. The reducing equivalents are utilized to reduce carbon dioxide to acetate by *Acetobacterium malicum* strains MuME1 and LiME1, or to reduce acetaldehyde to ethanol by *Pelobacter venetianus* strain WoME1 (Fig. 3).

L-Malate was completely fermented to acetate by *Acetobacterium malicum* strains MuME1 and LiME1. A hypothetical pathway for degradation of L-malate is as follows: L-malate → oxaloacetate → pyruvate → acetate. Among two hypothetical intermediates, pyruvate was fermented to acetate by the two strains, but oxaloacetate was not utilized by them for an unknown reason.

In a previous study (Tanaka et al. 1986) it was proposed that 2-methoxyethanol was degraded through two hypothetical pathways as follows: pathway I, 2-methoxyethanol → methoxyacetate → glycollate →→→ methane plus carbon dioxide; pathway II, 2-methoxyethanol → ethyleneglycol → ethanol plus acetate → acetate → methane plus carbon dioxide. In the present study, three strains of bacteria were isolated which used two different pathways, both similar to pathway II with acetaldehyde as an intermediate. Using defined cocultures or pure cultures, no evidence could be obtained for the operation of pathway I.

*A. malicum sp. nov.*

ma'li.cum. M.L.adj. *malicum* pertaining to malic acid. *Acetobacterium malicum:* an acetogenic bacterium fermenting malic acid.

Rod-shaped cells, $1.0-1.3 \times 1.8-4.0$ μm, with slightly pointed ends, single or in pairs. Motile. No spore formation. Gram-positive.

Chemoorganotroph or autotroph. L-Malate, 2-methoxyethanol, 2-ethoxyethanol, ethyleneglycol, acetoin, lactate, pyruvate, fructose, and formate as well as methoxyl groups of methoxylated aromatic compounds utilized for growth and fermented to acetate. 1,2-Propanediol fermented to propanol, propionate, and acetate.

Autotrophic growth occurs on $H_2/CO_2$ with acetate as a fermentation product. Sulfate, sulfite, thiosulfate, elemental sulfur, or nitrate not reduced. No cytochromes. Strictly anaerobic. Optimal initial pH 7.5–8.0, no growth at initial pH 6.0 and 9.5. Optimal temperature: 30°C, no growth at 16°C and 40°C. Habitats: anoxic mud of freshwater ditches.

DNA base ratio: 44.1 mol% G + C.

Type strain: MuME1, DSM 4132; deposited in Deutsche Sammlung von Mikroorganismen, Göttingen, FRG.

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## References

Bache R, Pfennig N (1981) Selective isolation of *Acetobacterium woodii* on methoxylated aromatic acids and determination of growth yields. Arch Microbiol 130:255–261

Balch WE, Schoberth S, Tanner RS, Wolfe RS (1977) *Acetobacterium*, a new genus of hydrogen-oxidizing, carbon-dioxide-reducing, anaerobic bacteria. Int J Syst Bacteriol 27:355–361

Braun M, Gottschalk G (1980) *Acetobacterium wieringae* sp. nov., a new species producing acetic acid from molecular hydrogen and carbon dioxide. Zbl Bakt Hyg, I Abt Orig C3:368–376

Cataldo DA, Haroon M, Schrader LE, Young VL (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal 6:71–80

Cline E (1969) Spectrophotometric determination of hydrogen-sulfide in natural waters. Limnol Oceanogr 14:454–458

Eichler B, Schink B (1984) Oxidation of primary aliphatic alcohols by *Acetobacterium carbinolicum* sp. nov., a homoacetogenic anaerobe. Arch Microbiol 140:147–152

Flossdorf J (1983) A rapid method for the determination of the base composition of bacterial DNA. J Microbiol Meth 1:305–311

Gregersen T (1978) Rapid method for distinction of Gram-negative from Gram-positive bacteria. Eur J Appl Microbiol Biotechnol 5:123–127

Klemps R, Cypionka H, Widdel F, Pfennig N (1985) Growth with hydrogen, and further physiological characteristics of *Desulfotomaculum* species. Arch Microbiol 143:203–208

Magee CM, Rodeheaver G, Edgerton RF (1975) A more reliable Gram staining techniques for diagnosis of surgical infections. Am J Surg 130:341–346

Pfennig N (1978) *Rodocyclus purpureus* gen. nov. and sp. nov., a ringshaped vitamin $B_{12}$-requiring member of the family Rhodospirillaceae. Int J Syst Bacteriol 28:283–288

Schink B, Stieb M (1983) Fermentative degradation of polyethyleneglycol by a strictly anaerobic, Gram-negative, nonsporeforming bacterium, *Pelobacter venetianus* sp. nov. Appl Environ Microbiol 45:1905–1913

Seitz H-J, Cypionka H (1986) Chemolithotrophic growth of *Desulfovibrio desulfuricans* with hydrogen coupled to ammonification of nitrate or nitrite. Arch Microbiol 146:63–67

Strass A, Schink B (1986) Fermentation of polyethylene glycol via acetaldehyde in *Pelobacter venetianus*. Appl Microbiol Biotechnol 25:37–42

Tanaka K, Mikami E, Suzuki T (1986) Methane fermentation of 2-methoxyethanol by mesophilic digesting sludge. J Ferment Technol 64:305–309

Toraya T, Fukui S (1982) Diol dehydrase. In: Dolphin D (ed) $B_{12}$ vol 2. John Wiley and Sons, New York, pp 233–262

Tschech A, Pfennig N (1984) Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. Arch Microbiol 137:163–167

Widdel F, Pfennig N (1981) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of new sulfate-reducing bacteria enriched with acetate from saline sediments. Description of *Desulfobacter postgatei* gen. nov., sp. nov. Arch Microbiol 129:395–400

Widdel F, Kohring GW, Mayer F (1983) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonema magnum* sp. nov. Arch Microbiol 134:286–294

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