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Fermentation of methoxyacetate to glycolate and acetate by newly isolated strains of *Acetobacterium* sp.

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Abstract. Three strains of new mesophilic homoacetogenic bacteria were enriched and isolated from sewage sludge and from marine sediment samples with methoxyacetate as sole organic substrate in a carbonate-buffered medium under anoxic conditions. Two freshwater isolates were motile, Gram-positive, non-sporeforming rods. The marine strain was an immotile, Gram-positive rod with a slime capsula. All strains utilized only the methyl residue of methoxyacetate and released glycolic acid. They also fermented methyl groups of methoxylated aromatic compounds and of betaine to acetate with growth yields of 6–10 g dry matter per mol methyl group. H_2/CO_2, formate, methanol, hexamethylene tetramine, as well as fructose, numerous organic acids, glycerol, ethylene glycol, and glycol ethers were fermented to acetate as well. High activities of carbon monoxide dehydrogenase (0.4–2.2 U × mg protein^{-1}) were detected in all three isolates. The guanine-plus-cytosine-content of the DNA of the freshwater isolates was 42.7 and 44.4 mol %, with the marine isolate it was 47.7 mol %. The freshwater strains were assigned to the genus *Acetobacterium* as new strains of the species *A. carbinolicum*. One freshwater isolate, strain KoMac1, was deposited with the Deutsche Sammlung von Mikroorganismen GmbH, Braunschweig, under the number DSM 5193.

Key words: Anaerobic ether cleavage – Methoxyacetate – 2-Methoxyethanol – Demethylation – Homoacetogenic bacteria – *Acetobacterium* sp.

Ether linkages are cleaved aerobically usually by oxygenase reactions which hydroxylate one of the vicinal ether carbon atoms and thus transform the ether compound into a comparably unstable hemiacetal structure (Heydeman 1974). Accordingly, phenylmethyl ethers such as lignin monomers are aerobically oxidized to the corresponding phenols and formaldehyde (Axelrod 1956; Cartwright and Smith 1967; Bernhardt et al. 1970; Taylor 1983; Sutherland 1986).

Acetobacterium woodii was the first bacterium reported to be able to cleave phenylmethyl ether linkages in the absence of molecular oxygen, and to ferment the methyl residues, analogous to methanol, with CO_2 to acetate (Bache and Pfennig 1981). Similar findings were made later on with other homoacetogenic bacteria (Krumholtz and Bryant 1985; Frazer and Young 1985, 1986; Mountfort and Asher 1986) and even with an *Enterobacter* strain (Grbic-Galic and LaPat-Polasko 1985). All these bacteria could remove the methyl groups only from aromatic methyl ethers; cleavage of methyl alkyl ethers was never observed.

Another case of anaerobic ether cleavage was observed with polyethylene glycol degradation by *Pelobacter venetianus* (Schink and Stieb 1983). The first degradation intermediate detected was acetaldehyde, suggesting that the terminal hydroxyl group of the polymer is shifted to the subterminal carbon atom, analogous to a diol dehydratase reaction, which in this case transforms the ether linkage again into a hemiacetal bond (Straß and Schink 1986). It was shown by feeding experiments that anaerobic polyethylene glycol degradation depended on a free terminal hydroxyl function, and that terminally methylated polyethylene glycols were not degraded. A different pathway of anaerobic polyethylene glycol degradation was assumed to occur in other fermenting and sulfate-reducing bacteria, however, the ether cleavage reaction was not studied (Dwyer and Tiedje 1986).

In the present study, we chose methoxyacetate as a model substrate of a methylalkylether which cannot be attacked by a diol dehydratase because it does not contain a free alcohol function. New strains of homoacetogenic bacteria were isolated which degraded methoxyacetate, and very slow anaerobic degradation was also found in enrichment cultures with dimethoxyethane.

Materials and methods

Sources of organisms

Strains KoMac1 and GöMac1 were enriched and isolated from anoxic digestor sludges of municipal sewage plants at Göttingen and Konstanz, FRG, respectively. Strain RMMac1 was isolated from a saltwater sediment sample taken from Rio Marin in the city of Venice, Italy. An enrichment culture degrading dimethoxyethane (culture WoDME) was obtained with an inoculum from a polluted freshwater creek near Konstanz, FRG.

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Media and growth conditions

All procedures for cultivation and isolation as well as all methods for analysis of metabolic products were essentially as described in earlier papers (Widdel and Pfennig 1981; Schink and Pfennig 1982). The mineral medium for enrichment, isolation and cultivation contained 30 mM sodium bicarbonate as buffer, 1 mM sodium sulfide as reducing agent, the trace element solution SL 10 (Widdel et al. 1983) and a vitamin solution (Pfennig 1978). Freshwater medium contained 0.5 g NaCl and 0.4 g $MgCl_2 \times 6\ H_2O$, saltwater medium 20.0 g and 3.0 g, respectively, per liter. The pH was 7.1–7.3. Growth experiments were carried out at 30°C. For isolation of pure cultures, the agar shake dilution method was applied (Pfennig 1978). Gram staining was carried out after Magee et al. (1975); the Gram type was also checked by the KOH method (Gregersen 1978).

Chemical determinations and chemicals

Sulfide was determined after Cline (1969), nitrite after Procházková (1959), protein after Zamenhoff (1957), carbon monoxide dehydrogenase after Diekert and Thauer (1978). Fatty acids and methoxyacetate were determined by direct gas chromatography on a packed column (Schink and Pfennig 1982), glycolic acid on a glas capillary column after methylation (Dehning and Schink 1989).

All chemicals used were of analytical or reagent grade quality and were obtained from Merck, Darmstadt, Sigma, München, and Fluka, Neu-Ulm, FRG.

Results

Enrichment and isolation

Enrichment cultures with freshwater or saltwater medium containing 10 mM methoxyacetate were inoculated with about 5 ml sediment and sludge samples from various sources, including marine and freshwater sediments, as well as sewage sludge from two municipal sewage plants. Gas production started in all cases after 3–5 weeks, and subcultures were subsequently inoculated with 5 ml culture fluid, usually after 2–3 weeks of incubation. Pure cultures were isolated from these cultures by two subsequent agar shake dilution series with methoxyacetate as substrate.

Freshwater cultures formed yellowish lens-shaped colonies, marine cultures small white colonies in the agar. Pure cultures were finally obtained with the enrichment cultures from two sewage plants (strains KoMac1 and GöMac1) and from Rio Marin, Venice (strain RMMac1). Purity was checked by microscopical examination after growth in minimal medium and in complex medium (AC- medium, Difco, Ann Arbor, Michigan, USA).

Similar enrichment cultures with dimethoxyethane (ethylene glycol dimethyl ether) exhibited gas production only in one case (sediment from a polluted freshwater creek) after 5 months of incubation. The dimethoxyethane-degrading bacteria could be transferred over 16 subcultures with cultivation times of 4–6 weeks every time. Pure cultures could not be isolated. In the enrichment cultures, complete conversion to CH_4 and CO_2 with intermediate formation of acetate could be demonstrated (data not shown).

Cytological properties of pure cultures

The two freshwater strains KoMac1 and GöMac1 were motile rods, 0.9–1.0 × 2.0–4.0 µm in size, with slightly pointed ends (Fig. 1a, b). In older cultures, they tended to form chains of several cells which were sometimes swollen in the middle. Both strains stained Gram-positive; results of the KOH test indicated a Gram-positive cell wall architecture as well. Cells of strain RMMac1 were immotile, rather small rods, 0.5–0.7 × 1–1.5 µm in size, with rounded ends (Fig. 1c). They stained Gram-negative, but both the KOH test as well as electron microscopic examination indicated a Gram-positive cell wall architecture (Fig. 2). In Indian ink preparations, thin slime capsules became visible. No strain formed spores, neither in defined medium nor in a special sporulation medium (Hollaus and Sleytr 1972). Pasteurized cultures did not show growth after more than 6 weeks of incubation. The guanine-plus-cytosine content of the DNA was 42.7 ± 1.0 mol% with strain KoMac1, 44.4 ± 1.0 mol% with strain GöMac1, and 47.8 ± 1.0 mol% with strain RMMac1. No cytochromes could be detected by redox difference spectroscopy of crude cell extracts of either strain.

Physiology

The strains KoMac1 and RMMac1 grew well in mineral medium containing NaCl from 0.05 up to 2.0% and $MgCl_2 \times 6\ H_2O$ from 0.04 to 0.3 % (w/v), strain GöMac1 grew in medium with 0.05–1.5% NaCl and 0.04–0.4% $MgCl_2 \times 6\ H_2O$. Phosphate inhibited growth of strains

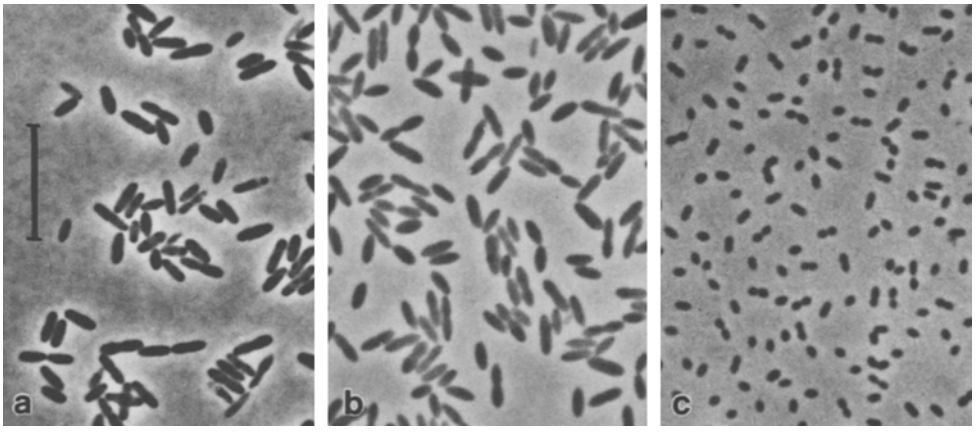

Fig. 1a–c
Phase-contrast photomicrographs of cells of the new isolates. **a** Strain KoMac1, **b** GöMac1, **c** strain RMMac1. Bar equals 10 µm for all three pictures

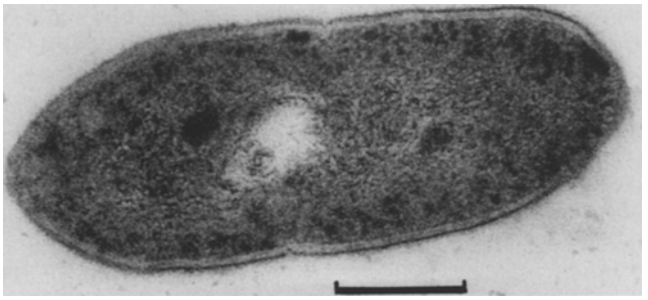

Fig. 2. Electron micrograph of an ultrathin section of cells of strain RMMac1. Bar equals 0.2 μm

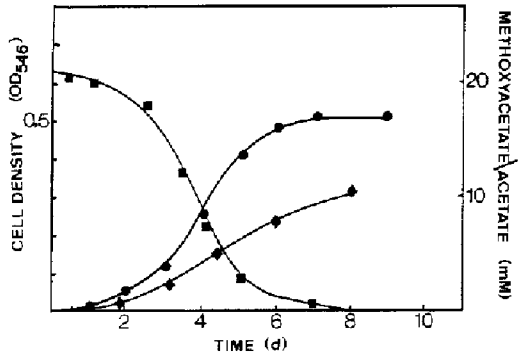

Fig. 3. Fermentation time course of strain KoMac1 growing with methoxyacetate. Experiments were performed at 30°C in half-filled 100 ml culture bottles with rubber septa. Samples were taken at times indicated and the headspaces were flushed with N_2/CO_2 gas mixture. (●) cell density (oD_{540}); (■) methoxyacetate; (◆) acetate

Table 1. Substrates tested for growth with the new strains KoMac1, GöMac1, and RMMac1. All tests were carried out in mineral medium with 0.05% (w/v) yeast extract

Substrate degraded	KoMac1	GöMac1	RMMac1
H_2/CO_2	+	+	+
Formate	+	+	−
Hexamethylene tetramine	+	−	−
Methanol	+	+	+
Methoxyacetate	+	+	+
Vanillate	+	+	+
Syringate	+	+	−
Sinapinate	+	+	−
Trimethoxybenzoate	+	+	−
Betaine	+	+	+
Ethanol	±	−	−
Lactate	+	+	−
Pyruvate	+	+	+
D,L-Malate	+	+	+
Fumarate	−	−	+
Fructose	+	+	−
Glycerol	+	+	−
Triacetin	+	+	−
Ethylene glycol	+	+	−
Diethylene glycol	+	+	−
Triethylene glycol	+	−	−
Ethoxyethanol	+	+	−

No growth was found with the following substrates: Dimethoxyethane, diethoxyethane, 1-propanol, 1-butanol, glucose, arabinose, xylose, glycolate, glycine, ethanolamine, trimethylamine, citrate, D-erythritol

KoMac1 and RMMac1 at > 30 mM, of strain GöMac1 at > 10 mM concentration. Yeast extract was not required for growth, but all strains grew faster and to higher cell densities in the presence of yeast extract. Optimal growth with methoxyacetate was found at 30°C with the following growth rates and doubling times. Strains GöMac1 and KoMac1: $\mu = 0.049\ h^{-1}$, $t_d = 14$ h; strain RMMac1: $\mu = 0.037\ h^{-1}$, $t_d = 19$ h. The temperature limits of growth were 15 and 34°C, the pH optima 6.8–7.0, and the pH ranges from 6.0–8.0 with the freshwater isolates and 6.6–7.4 with strain RMMac1.

The substrates utilized by the various strains are listed in Table 1. Besides those substrates used by many other homoacetogenic bacteria, methoxyacetate and betaine were found to be used as growth substrates. The methoxylated aromatic compounds were fermented to the corresponding phenols. UV spectrophotometric examination indicated that the acrylic acid side chain of sinapic acid was reduced to trihydroxyphenylpropionic acid. Hexamethylene tetramine was used as a formaldehyde-yielding substrate; many homoacetogenic bacteria ferment formaldehyde to acetate (Schink 1987). Neither nitrate, sulfate, sulfite, thiosulfate, nor sulfur was reduced during growth with methoxyacetate. Strain KoMac1 appeared to be the metabolically most versatile isolate. In Fig. 3, a growth curve of this strain growing with methoxyacetate is shown. Similar results were obtained with the other isolates.

Acetate was the main or sole fermentation product in all cases. Fermentation balances and growth yields were measured with all isolates. In Table 2, the data obtained with strain KoMac1 are presented. Similar results were obtained with the other strains as well. The amounts of products formed indicated that the substrates were fermented completely. The methyl groups of all methoxyaromatics, methoxyacetate, and betaine were fermented with CO_2 to acetate, in the same manner as methanol, but the cell yields obtained were by nearly 50% higher than with methanol.

Carbon monoxide dehydrogenase activity was detected in crude extracts of cells of all three isolates grown with methoxyacetate. The specific activities (μmol CO oxidized per min and mg protein) were 1.1 with strain KoMac1, 2.2 with strain GöMac1, and 0.37 with strain RMMac1.

Discussion

Physiology and taxonomy of the new isolates

In the present study, anaerobic degradation of methylalkyl ether compounds is documented for the first time. Three strains of homoacetogenic bacteria are described which ferment methoxyacetate with carbon dioxide to glycolate and acetate, according to the following equation (all calculations of free energy changes after Thauer et al., 1977; Dimroth 1983):

$$4\ CH_3OCH_2COO^- + 2\ CO_2 + 2\ H_2O \rightarrow 4\ HOCH_2COO^- + 3\ CH_3COO^- + 3H^+ ;$$

$$\Delta G'_0 = -85.2\ \text{kJ per mol methoxyacetate} .$$

The free energy change of this reaction is considerably higher than that of the analogous fermentation of methanol and CO_2 to acetate and water ($\Delta G'_0 = -55.5$ kJ per mol methanol).

Table 2. Stoichiometry of substrate conversion and growth yields of strain KoMac1 with various substrates

Substrate degraded	Amount of substrate supplied (μmol)	Cell dry matter formed[a] (mg)	Acetate assimilated[b] (μmol)	Acetate formed (μmol)	Growth yield (g/mol)	Electron recovery (%)
Formate	440	0.52	10.6	103.4	1.2	104
Hexamethylene tetramine	44	1.5	30.8	94.4	5.7	95
Methanol	110	0.72	14.7	65.3	6.5	97
Methoxyacetate	220	1.9	39.2	1237.6	8.6	101
Vanillate	110	1.05	19.8	61.6	9.5	99
Syringate	110	2.3	47.1	132	20.8	109
Sinapinate	110	2.1	42.9	114.4	19	95
Trimethoxybenzoate	110	3.1	63.8	206	27.9	109
Betaine	220	2.16	44	144.5	9.8	114
Lactate	220	2.16	44	320.5	9.8	110
Pyruvate	220	2.35	48.4	226	10.7	100
D,L-Malate	220	1.44	28.6	142.3	13.0	104
Fructose	44	1.18	24.2	108.5	26.8	101
Glycerol	220	2.18	28.6	151.1	6.5	
Triacetin	110	2.06	41.8	464.2	18.7	97
Ethylene glycol	220	0.98	19.8	261.1	4.5	102
Diethylene glycol	220	1.83	37.4	530.2	8.3	103
Triethylene glycol	220	2.18	52.8	675.4	9.9	
Ethoxyethanol	220	0.85	17.4	259.6	3.9	101

All growth experiments were carried out in screw cap tubes containing 22 ml mineral medium with 0.05% yeast extract

[a] Cell dry matter values were calculated via cell density using an experimentally determined conversion factor (0.1 oD_{540} = 29.7 mg dry matter/l)

[b] Acetate assimilated was calculated according to the formula: $17\ CH_3COO^- + 17\ H^+ \rightarrow 8\ \langle C_4H_7O_3\rangle + 2\ CO_2 + 6\ H_2O$

The product of betaine fermentation was not identified. From the stoichiometry of acetate formation, it appears that only one methyl group per substrate molecule was fermented and therefore dimethylglycine was formed as demethylated product:

$$4\ (CH_3)_3N^+CH_2COO^- + 2\ CO_2 + 2\ H_2O \rightarrow$$
$$4\ (CH_3)_2N^+HCH_2COO^- + 3\ CH_3COO^- + 3\ H^+ .$$

A similar methyl group fermentation pattern was found with *Eubacterium limosum* (Müller et al. 1981) and *Acetobacterium carbinolicum* (Eichler and Schink 1984).

The newly isolated homoacetogens differ from those of the species described so far by their ability to demethylate methoxyacetate to glycolate. Our freshwater isolates resemble *A. carbinolicum* most among the described types of homoacetogens, also with respect to triacetin utilization (Emde and Schink 1987), and we suggest that they should be grouped with this species as new biotypes. The taxonomic status of the morphologically atypical marine isolate RMMac1 would require further studies. Strain KoMac1 was deposited with the Deutsche Sammlung für Mikroorganismen GmbH, Braunschweig, under the number DSM 5193.

Biochemistry of anaerobic methylether cleavage

The chemistry of anaerobic phenylmethyl ether cleavage by homoacetogenic bacteria is not yet understood. Ether linkages are too stable to be split by a simple hydrolysis reaction. Studies on kinetics and yields of growth of *A. woodii* on phenylmethyl ether compounds and on methanol indicated that free methanol is not an intermediate (Tschech and Pfennig 1984). Degradation experiments with *Eubacterium limosum* on ^{18}O-labelled methoxybenzoates demonstrated recently that the ether oxygen stays with the aromatic ring and is not released into acetate or water (deWeerd et al. 1988). This supports the concept that the ether linkage is not cleaved by a hydrolysis reaction but that the methyl residue is transferred directly to a methyl carrier such as coenzyme B_{12}, possibly as a methyl radical. A similar chemistry of ether linkage cleavage must be involved in methoxyacetate degradation by our new isolates.

It is open at present whether all the demethylation reactions carried out by our new isolates are catalyzed by the same enzyme system. From the fermentation stoichiometries and growth yields it is obvious that methyl groups bound to nitrogen or ether oxygen give about 50% higher growth yields than methanol, in close agreement with the free energy changes of these reactions (see above). This rules out again a formation of free methanol as intermediate in demethylation and indicates that methanol, unlike the linked methyl groups, has to be transferred to a methyl carrier by an energy-consuming system. Cross-feeding experiments with cell suspensions grown with either methyl substrate indicated that separate demethylating activities had to be induced for methanol, vanillic acid, methoxyacetate, and betaine, respectively (data not shown). However, since these specificities may as well be due to different substrate uptake systems, these results cannot rule out that a common enzyme system may be responsible for the respective demethylation and methyl group transfer reactions.

Anaerobic demethylation of methoxyacetate by our new isolates opens a further pathway for methanogenic degradation of methoxy ethanol, an important industrial solvent. In anoxic sludge degrading this substrate, methoxyacetate was found and was assumed to be an intermediate product in a degradation pathway leading from methoxyethanol via 2-methoxyacetate and glycolate to methane plus carbon dioxide, however, bacteria catalyzing such a reaction sequence could not be cultivated (Tanaka et al. 1986). Instead, 2-methoxyethanol-degrading homoacetogenic and other fer-

menting bacteria isolated from this culture were found to use a different pathway similar to that of anaerobic polyethylene glycol degradation: they formed acetate and free methanol, probably via 1-methoxyethanol as intermediate (Tanaka and Pfennig 1988). Our new isolates prove that the pathway first suggested is possible as well, and perhaps is used in the same culture to a minor extent. It is worth emphasizing that in both hypothetical pathways coenzyme B_{12} plays a key role in ether destabilization or ether cleavage.

The freshwater strain KoMac1 was deposited with the Deutsche Sammlung von Mikroorganismen GmbH, Braunschweig, under the number DSM 5193.

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References

Axelrod J (1956) The enzymic cleavage of aromatic ethers. Biochem J 63:634–639

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

Bernhardt F-H, Staudinger H, Ulrich V (1970) Eigenschaften einer p-Anisat-O-Demethylase im zellfreien Extrakt von *Pseudomonas* species. Hoppe-Seyler's Z Physiol Chem 351:467–478

Cartwright NJ, Smith ARW (1967) Bacterial attack on phenolic ethers. An enzyme system demethylating vanillic acid. Biochem J 102:826–841

Cline JD (1969) Spectrometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14:454–458

Dehning I, Schink B (1989) *Malonomonas rubra* gen. nov. sp. nov., a microaerotolerant anaerobic bacterium growing by decarboxylation of malonate. Arch Microbiol 151:427–433

DeWeerd KA, Saxena A, Nagle DP, Suflita JM (1988) Metabolism of the ^{18}O methoxy substituent of 3-methoxybenzoic acid and other unlabelled methoxybenzoic acids by anaerobic bacteria. Appl Environ Microbiol 54:1237–1242

Diekert GB, Thauer RK (1978) Carbon monoxide oxidation by *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. J Bacteriol 136:597–606

Dimroth K (1983) Inkremente zur Berechnung der Bildungsenthalpien und der freien Bildungsenthalpien. In: D'Ans Lax Taschenbuch für Chemiker und Physiker. Bd. 2, Organische Verbindungen. 4. ed. Springer, Berlin Heidelberg New York, pp 997–1006

Dwyer DF, Tiedje JM (1986) Metabolism of polyethylene glycol by two anaerobic bacteria, a *Desulfovibrio desulfuricans* and a *Bacteroides* sp. Appl Environ Microbiol 52:852–856

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

Emde R, Schink B (1987) Fermentation of triacetin and glycerol by *Acetobacterium* sp. No energy is conserved by acetate excretion. Arch Microbiol 149:145–148

Frazer AC, Young LY (1985) A Gram-negative anaerobic bacterium that utilizes O-methyl substituents of aromatic acids. Appl Environ Microbiol 49:1345–1347

Frazer AC, Young LY (1986) Anaerobic C_1 metabolism of the O-methyl-^{14}C-labeled substituent of vanillate. Appl Environ Microbiol 51:84–87

Grbic-Galic D, LaPat-Polasko L (1985) *Enterobacter cloacae* DG-6: a strain that transforms methoxylated aromatics under aerobic and anaerobic conditions. Curr Microbiol 12:321–324

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

Heydeman MT (1974) Growth of soil bacteria on diethyl ether. J Gen Microbiol 81:IX–X

Hollaus F, Sleytr V (1972) On the taxonomy and fine structure of some hyperthermophilic saccarolytic clostridia. Arch Mikrobiol 86:129–146

Krumholtz LR, Bryant MP (1985) *Clostridium pfennigii* sp. nov. uses methoxyl groups of mono-benzenoids and produces butyrate. Int J Syst Bacteriol 35:454–456

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

Mountfort DO, Asher RA (1986) Isolation from a methanogenic ferulate degrading consortium of an anaerobe that converts methoxyl groups of aromatic acids to volatile fatty acids. Arch Microbiol 144:55–61

Müller E, Fahlbusch K, Walther R, Gottschalk G (1981) Formation of N,N-dimethylglycine, acetic acid, and butyric acid from betaine by *Eubacterium limosum*. Appl Environ Microbiol 42:439–445

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

Procházková L (1959) Bestimmung der Nitrate im Wasser. Z Anal Chem 167:254–260

Schink B (1987) Ecology of C_1-metabolizing anaerobes. In: van Verseveld HW, Duine JA (eds) Microbial growth on C_1 compounds. Martinus Nijhoff Dordrecht, The Netherlands, pp 81–85

Schink B, Pfennig N (1982) Fermentation of trihydroxybenzenes by *Pelobacter acidigallici* gen. nov. sp. nov., a new strictly anaerobic, non-sporeforming bacterium. Arch Microbiol 133:195–201

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

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

Sutherland JB (1986) Demethylation of veratrole by cytochrome P-450 in *Streptomyces setonii*. Appl Environ Microbiol 52:98–100

Tanaka K, Pfennig N (1988) Fermentation of 2-methoxyethanol by *Acetobacterium malicum* sp. nov. and *Pelobacter venetianus*. Arch Microbiol 149:181–187

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

Taylor BF (1983) Aerobic and anaerobic catabolism of vanillic acid and some other methoxy-aromatic compounds by *Pseudomonas* sp. strain PN-1. Appl Environ Microbiol 46:1286–1292

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180

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 environments. 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

Zamenhoff S (1957) Preparation and assay of deoxyribonucleic acid from animal tissue. In: Colowick SP, Kaplan NO (eds) Methods in enzymology, vol 3. Academic Press, New York, pp 694–704

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