CURRENT MICROBIOLOGY Vol. 36 (1998), pp. 283–290

Current Microbiology
An International Journal

Anaerobic Degradation of Glycerol by *Desulfovibrio fructosovorans* and *D. carbinolicus* and Evidence for Glycerol-Dependent Utilization of 1,2-Propanediol

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Received: 1 October 1997 / Accepted: 3 November 1997

Abstract. The degradation of glycerol by *Desulfovibrio carbinolicus* and *Desulfovibrio fructosovorans* was tested in pure culture with sulfate and in coculture with *Methanospirillum hungatei*. *Desulfovibrio carbinolicus* degraded glycerol into 3-hydroxypropionate with the formation of sulfide in pure culture and methane in the coculture. The maximum growth rates were 0.063 h^{-1} in pure culture and 0.014 h^{-1} in coculture (corresponding growth yields: 8.9 and 6.0 g dry weight/mol glycerol). With *D. fructosovorans*, the pathway of glycerol degradation depended upon the terminal electron acceptor. Acetate and sulfide were produced in the presence of sulfate, while 3-hydroxypropionate and methane were formed by the syntrophic association with *M. hungatei*. The maximum growth rates were 0.057 h^{-1} in pure culture and 0.020 h^{-1} in coculture (corresponding growth yields: 8.9 and 6.0 g dry weight/mol glycerol). In a medium containing both glycerol and 1,2-propanediol but no sulfate, *D. carbinolicus* and *D. fructosovorans* degraded both substrates. A drop in the concentration of 1,3-propanediol was observed, and propionate and *n*-propanol production was recorded. Putative biochemical pathways of 1,2-propanediol degradation by *D. carbinolicus* and *D. fructosovorans* indicated that the enzymes involved in this metabolism are present only when the strains are grown on a mixture of 1,2-propanediol and glycerol without sulfate.

More than 100 compounds have been shown to be utilized by one or more sulfate-reducing bacteria (SRB) [7]. Glycerol is used as energy source by several species of the genus *Desulfovibrio* [5, 14, 19, 23–25]. *Desulfovibrio carbinolicus* and *D. fructosovorans* dismuted glycerol to 1,3-propanediol and 3-hydroxypropionate in the absence of sulfate [20, 23]. In the presence of sulfate, *D. carbinolicus* oxidized glycerol to 3-hydroxypropionate [20], but *D. fructosovorans* oxidized it to acetate [23]. Both *D. carbinolicus* and *D. fructosovorans* oxidized 1,3-propanediol to 3-hydroxypropionate [20, 27]. Thus, the only metabolic difference in the degradation of glycerol and 1,3-propanediol between these species is the nature of the end product(s) of glycerol degradation.

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The most common pathway of anaerobic degradation of 1,2-propanediol by non-sulfate-reducing microorganisms involves the conversion of the substrate into propionaldehyde by a dehydratase, followed by the subsequent conversion of propionaldehyde to propanol and propionate by an NAD-dependent alcohol dehydrogenase, a CoA-linked NAD-dependent aldehyde dehydrogenase, a phosphate propionyl transferase, and a propionate kinase [36]. However, a degradation of 1,2-propanediol involving NAD-linked alcohol dehydrogenase was reported for *Desulfovibrio alcoholovorans* and *Desulfovibrio* sp. strain HDv [25]. In contrast to these strains, *D. carbinolicus* and *D. fructosovorans* were unable to use 1,2-propanediol as energy source [20, 26].

In this study we have tested the degradation of glycerol by SRB in the presence of (a) a high potential terminal electron acceptor: sulfate (adenylylsulfate/

HSO_3^- + AMP, $E'^0 = -60$ mV; HSO_3^-/HS^-, $E'^0 = -116$ mV) and (b) a lower potential terminal electron acceptor: a hydrogenotrophic methanogen used as hydrogen scavenger (CO_2/CH_4, $E'^0 = -238$ mV). E'^0 values were obtained from Thauer et al. [34]. In addition, we have demonstrated the anaerobic degradation of 1,2-propanediol by *D. fructosovorans* and *D. carbinolicus*; this process was found to occur in media without sulfate containing both glycerol and 1,2-propanediol. Under these conditions the strains expressed a 1,2-propanediol dehydratase activity.

Materials and Methods

Source of microorganisms. *Desulfovibrio carbinolicus* (DSM 3852), *D. fructosovorans* (DSM 3604), and *Methanospirillum hungatei* (DSM 864) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). *Desulfovibrio* sp. strain DFG (DSM 6133) is a sulfate-reducing bacterium isolated from an anaerobic pilot plant fed with wastewater from a high-strength distillery; this strain can degrade 1,2-propanediol exclusively to propionate in the presence of sulfate.

Media and growth conditions. Hungate's anaerobic techniques [12, 18] were used throughout these experiments. The medium composition was described elsewhere [26]. Substrates were added from freshly prepared, anaerobically autoclaved solutions. *Desulfovibrio* strains were grown in pure culture in the presence of sulfate at 35°C in 100-ml, completely filled serum bottles, sealed with black rubber stoppers. Coculture with *M. hungatei* was grown in the absence of sulfate in 500-ml serum bottles with 200 ml medium under an atmosphere of N_2-CO_2 (80–20%). For cultivation of large amounts of cells and determination of growth yield, 0.01% yeast extract was added to the media. Cocultures were inoculated with 10% (vol/vol) of cultures of *Desulfovibrio* strain and *M. hungatei*. Coculture adaptation was achieved by repeated transfer on appropriate substrates and controlled by the disappearance of substrate and methane production.

Cell material determination. Growth was followed in screw-capped Hungate tubes by measuring optical density at 580 nm in a Bausch and Lomb Spectrophotometer. Dry weight was determined with 2000-ml, screw-capped bottle cultures. Cell material was harvested by centrifugation and washed twice with 50 mM potassium phosphate buffer, pH 7.0. The pellet was dried to constant weight at 80°C.

Analytical techniques. Sulfide was determined spectrophotometrically as colloidal CuS [1]. Methane was measured by gas chromatography (Delsi serie 30; injection temperature, 200°C; column, 3m × 1/4", stainless steel, Porapack Q 80–100 mesh; oven temperature, 190°C; carrier gas, N_2; flow rate, 30 ml/min; detection, flame ionization 250°C). Glycerol and diols were measured by HPLC (column, interaction cation exchange ORH-801 3/8" O.D. × 30 cm, Ion-Exclusion; column temperature, 65°C; detection, differential refractometer, Knauer, Berlin; recorder integrator, Chromatopack C-R3A, Shimadzu, Kyoto; flow rate, 0.8 ml/min). Propionate, acetate, and 3-hydroxypropionate were determined by HPLC with the same column (column temperature, 35°C; detection, UV spectrophotometric detector at 210 nm, SPD6A Module, Shimadzu, Kyoto).

Preparation of cell-free extract. Cells of *D. carbinolicus* and *D. fructosovorans* grown on a mixture of 1,2-propanediol and glycerol (20 mM), in the absence of sulfate, and cells of *Desulfovibrio* sp. DFG grown on 1,2-propanediol (20 mM) in the presence of sulfate were harvested at the end of the exponential growth phase, washed twice with 50 mM potassium phosphate buffer (pH 7.5) containing 2 mM dithiothreitol, and stored under N_2 at 0°C. Cells were disrupted by two passages through a French pressure cell [9]. The broken cell suspension was centrifuged at 18,000 rpm for 30 min; the resulting supernatant was referred to as cell-free extract and used for enzyme assays.

Enzyme assays. Enzyme activities were measured at 30°C in 1-ml quartz cuvettes, using a double beam spectrophotometer according to Hensgens et al. [9, 10]. Samples of the extract were withdrawn with a microsyringe to maintain the remaining extract oxygen-free. NAD-dependent 1,2-propanediol dehydrogenase was assayed in 100 mM Tris-HCl (pH 9.0) containing 5 mM NAD and 10 mM 1,2-propanediol or in 50 mM Tris-HCl (pH 7.5) containing 0.2 mM NADH and 2 mM glycolaldehyde instead of lactaldehyde (not commercially available). Reactions were started by adding 1,2-propanediol or glycolaldehyde, and the formation or disappearance of NADH was recorded at 340 nm ($E^{340} = 6.22\ mM^{-1} \cdot cm^{-1}$). NAD-independent 1,2-propanediol dehydrogenase was tested in 50 mM PIPES-KOH (pH 7.5) containing 0.05% Triton X-100, 1.2 mM MTT (3-(4',5'-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide) as electron acceptor, 0.3 mM PMS (phenazine methosulfate), and 10 mM 1,2-propanediol. The reaction was started by adding 1,2-propanediol, and the formation of the reduction product of MTT was recorded at 578 nm ($E^{578} = 13\ mM^{-1} \cdot cm^{-1}$). NAD-dependent propionaldehyde dehydrogenase activity (reverse reaction) was tested in 50 mM Tris-HCl (pH 7.5) containing 0.2 mM NADH and 2 mM propionaldehyde. The reaction was started by adding propionaldehyde, and the disappearance of NADH was recorded at 340 nm. NADH-independent CoA-dependent propionaldehyde dehydrogenase was assayed according to modified procedures described by Kremer et al. [15]; the reaction mixture contained 50 mM Tris-HCl (pH 7.5), 2 mM CoA, 5 mM benzylviologen (BV^{2+}), and 5 mM propionaldehyde. The reaction was started by adding cell-free extract; the propionaldehyde-dependent reduction of BV^{2+} to BV^+ was recorded at 500 nm ($E^{500} = 7.4\ mM^{-1} \cdot cm^{-1}$). 1,2-Propanediol dehydratase activity was assayed according to the procedure of Toraya et al. [36] or in a mixture of 100 μM coenzyme-B_{12}, 10 mM 1,2-propanediol, 50 mM Tris HCl (pH 7.4), and 5 mM NADH. Cell-free extract was incubated in the presence of 1,2-propanediol for 10–15 min at 30°C. The reaction was started by adding NADH, and the disappearance of NADH was recorded at 340 nm. The production of *n*-propanol was also checked. Lactate dehydrogenase was assayed according to Stams and Hansen [31], except that 50 mM Tris-HCl pH 7.5 was used as buffer and 1 mM dichlorophenolindophenol (DCPIP) was used as electron acceptor. The reaction was started by adding cell-free extract, and the reduction of DCPIP was recorded at 600 nm ($E^{600} = 19\ mM^{-1} \cdot cm^{-1}$). Pyruvate dehydrogenase activity was assayed by measuring the pyruvate-dependent reduction of BV^{2+} to BV^+ at 500 nm according to the procedure of Odom and Peck [22]. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 2 mM CoA, 5 mM BV^{2+}, and 20 mM sodium pyruvate. The reaction was started by adding cell-free extract. Phosphate acetyl transferase was tested by following the formation of acetyl-CoA at 233 nm ($E^{233} = 300\ mM^{-1} \cdot cm^{-1}$) as described by Oberlies et al. [21]. The reaction was started by adding acetyl-phosphate. Acetate kinase was tested according to Rose et al. [28]. Propionate kinase was assayed as described by Stams et al. [32]. NADH dehydrogenase was determined by following the procedures described by Kremer and Hansen [14]. NADP- and NADPH-dependent dehydrogenase activities were recorded by the same procedure that NAD- and NADPH-dependent dehydrogenase except that NAD or NADH was replaced by NADP or NADPH, respectively; the formation or disappearance of NADPH was recorded at 340 nm. Enzyme units are expressed as μmols of product formed or substrate consumed per minute ($\mu mol \cdot min^{-1}$). Protein content was determined by the method of

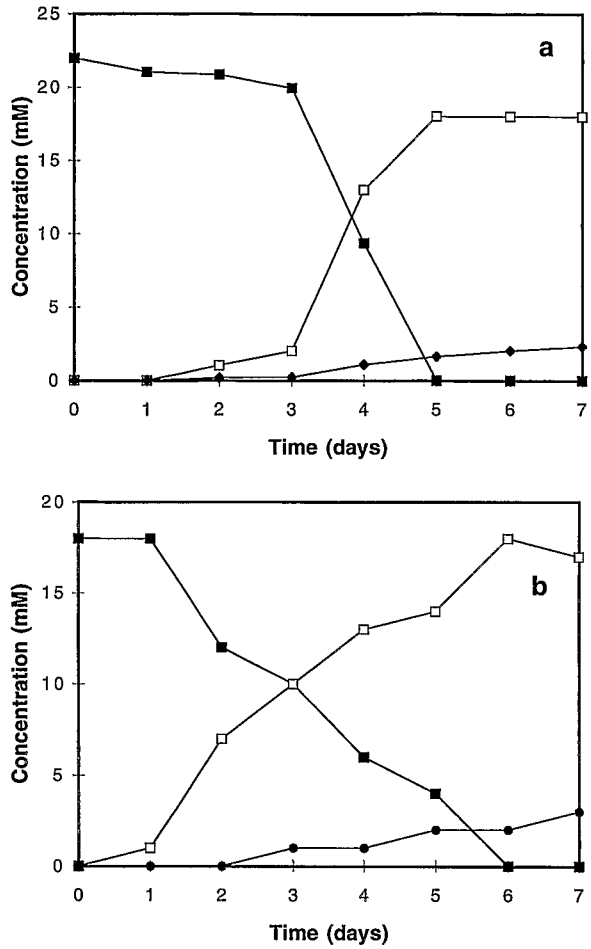

Fig. 1. Dissimilation of glycerol by *Desulfovibrio carbinolicus* in pure culture with sulfate (a) and in coculture with *Methanospirillum hungatei* (b). Symbols: ■, glycerol; □, 3-hydroxypropionate; ◆, sulfide; ●, methane.

Lowry et al. [17], with bovine serum albumin as standard. All experiments were duplicated. Analytical grade chemicals were used.

Results

Degradation of glycerol by *D. carbinolicus* and *D. fructosovorans*. Glycerol degradation by *D. carbinolicus* in the presence of sulfate led to the production of 3-hydroxypropionate and sulfide (Fig. 1a); the maximum growth rate was 0.063 h^{-1}, and the maximum growth yield was 8.9 g dry weight per mol glycerol degraded. In syntrophic association with *M. hungatei* in the absence of sulfate, 3-hydroxypropionate and methane were produced (Fig. 1b); the maximum growth rate was 0.014 h^{-1} and the maximum growth yield was 6.0 g dry weight per mol glycerol degraded (Table 1).

In pure culture with sulfate, *D. fructosovorans* oxidized glycerol to acetate (Fig. 2a). The maximum growth rate was 0.057 h^{-1}, and the maximum growth yield was 8.9 g dry weight per mol glycerol degraded. *Desulfovibrio fructosovorans* transferred reducing equivalents, most likely as hydrogen (and possibly also as formate), to *M. hungatei*, but produced 3-hydroxypropionate (Fig. 2b), which is more reduced than acetate; small amounts of 1,3-propanediol were also detected. When 20 mM of sulfate was added to the coculture after 8 days of incubation, neither sulfide nor acetate was produced (Fig. 2b). On the basis of the results reported in Table 1, the stoichiometry of the degradation of glycerol by the two cocultures is described by the following equation ($\Delta G'^0$ values were calculated from data of Thauer et al. [34]):

$$\text{(I) } CH_2OHCHOHCH_2OH + 0.25\ HCO_3^- \rightarrow CH_2OHCH_2COO^- + 0.25\ CH_4 + 0.75\ H_2O + 0.75H^+ \quad \Delta G'^0 = -115\ \text{kJ/mol}$$

Effect of the presence of glycerol on the utilization of 1,2-propanediol by *D. carbinolicus* and *D. fructosovorans*. *Desulfovibrio carbinolicus* and *D. fructosovorans*, which cannot use 1,2-propanediol as sole energy source [20, 26], were cultivated on glycerol without sulfate, in the presence or absence of 1,2-propanediol. On glycerol and 1,2-propanediol without sulfate, both substrates were degraded, and approximately 50% of 1,2-propanediol was utilized (Table 2). The addition of 1,2-propanediol to glycerol-containing medium induced a decrease in 1,3-propanediol concentration but not in that of 3-hydroxypropionate, which remained unchanged in the presence or absence of 1,2-propanediol. Propionate and *n*-propanol production by both strains was observed only when 1,2-propanediol was added as energy source. *D. carbinolicus* produced two times more *n*-propanol than propionate, and *D. fructosovorans* five times more *n*-propanol than propionate. *D. fructosovorans* produced two times less propionate than *D. carbinolicus* (Table 2).

In experiments with *D. fructosovorans*, an SRB fermenting fructose [23], adding glycerol in a fructose-containing medium inhibited ethanol production and increased acetate concentration from fructose fermentation (Table 3).

Enzymes involved in 1,2-propanediol degradation. Unless otherwise indicated, enzyme activities were determined in cell-free extracts of *Desulfovibrio* sp. DFG grown on 1,2-propanediol in the presence of sulfate, and in cell-free extracts of *D. carbinolicus* and *D. fructosovorans* grown on a mixture of 1,2-propanediol and glycerol, in the absence of sulfate.

Cell-free extracts of *Desulfovibrio* sp. DFG, *D. carbinolicus*, and *D. fructosovorans* exhibited significant specific activities for lactate dehydrogenase, pyruvate

Table 1. Stoichiometry[a] and yield of *D. carbinolicus* and *D. fructosovorans* grown in syntrophic association with *M. hungatei* on sulfate-free medium

Glycerol degraded (mM)	OD (580 nm)	Cells (mg/L)	Yield (g/mol)	Cells formed[b] expressed as acetate (mM)	3-Hydroxypropionate (mM)	Methane (mM)	Electron recovery (%)
D. carbinolicus							
4.17	0.07	25.0	6.0	0.65	3.55	1.10	97
D. fructosovorans							
5.00	0.06	30.0	6.0	0.78	4.36	1.54	101

[a]Bicarbonate present.
[b]Calculated from the cells (mg/L) and the following equation: 17 CH_3COO^- + 11 H_2O → 8 $C_4H_7O_3$ + 2HCO_3^- + 15 OH^- [34].

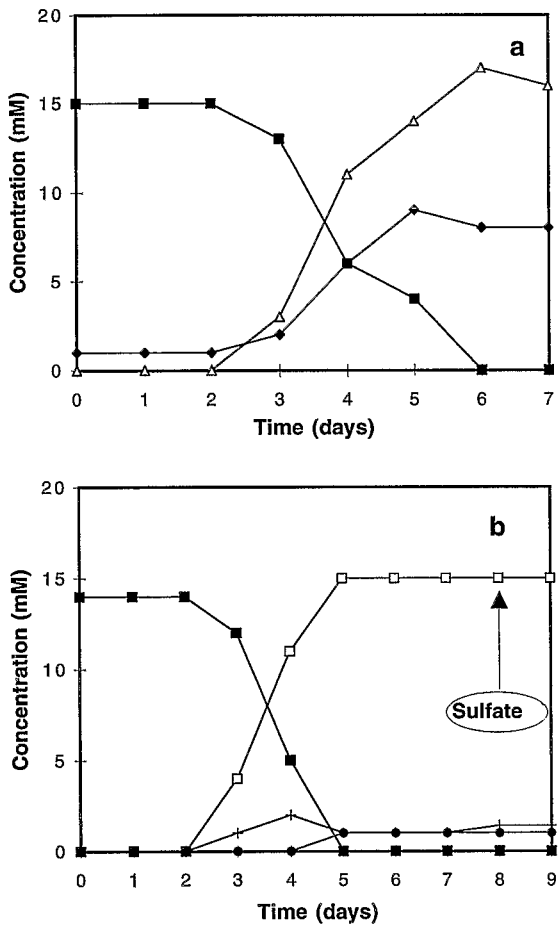

Fig. 2. Dissimilation of glycerol by *Desulfovibrio fructosovorans* in pure culture with sulfate (a) and in coculture with *Methanospirillum hungatei* (b). Arrow: addition of sulfate in the coculture at the 8th day. Symbols: ■, glycerol; □, 3-hydroxypropionate; △, acetate; +, 1,3-propanediol; ◆, sulfide; ●, methane.

dehydrogenase, propionate kinase, and acetate kinase, but relatively weak specific activities for phosphate acetyltransferase (Table 4). However, specific lactate dehydrogenase activities measured in extracts of these strains were relatively low compared with those reported for *D. alcoholovorans* [27].

Table 2. Effect of glycerol on the utilization of 1,2-propanediol by *D. carbinolicus* and *D. fructosovorans* grown on sulfate-free medium[a]

	D. carbinolicus		*D. fructosovorans*	
Products (mM)	−1,2-Propanediol	+1,2-Propanediol	−1,2-Propanediol	+1,2-Propanediol
1,3-Propanediol	12.8	8.4	11.2	9.0
1,2-Propanediol	—	10.4	—	10.2
3-Hydroxypropionate	9.4	9.8	10.5	10.3
n-Propanol	—	9.2	—	6.8
Propionate	—	1.8	—	3.7

[a]Initial concentrations of glycerol and 1,2-propanediol, 20 mM. Final concentration of glycerol, 0 mM.

Table 3. Effect of glycerol on the fermentation of fructose by *D. fructosovorans* grown on sulfate-free medium[a]

	Products (mM)				
Substrates (mM)	Acetate	Succinate	Ethanol	1,3-Propanediol	3-Hydroxypropionate
Fructose	2.0	4.6	1.5	0.0	0.0
Glycerol	0.0	0.0	0.0	4.6	5.0
Fructose + glycerol	3.2	4.5	0.0	5.0	5.1

[a]Initial concentration, glycerol 10 mM; fructose 5 mM. Final concentration of glycerol and fructose, 0 mM.

A relatively low pyruvate dehydrogenase activity, compared with that reported for *D. alcoholovorans*, suggested that in the strains studied, this enzyme was probably involved only in cell carbon assimilation, since we never observed acetate production from 1,2-propanediol by strain DFG, or from a mixture of glycerol and 1,2-propanediol by *D. carbinolicus* and *D. fructosovorans*.

Weak specific activities of NAD-dependent 1,2-propanediol dehydrogenase were measured in cell-free extracts of strain DFG, *D. carbinolicus*, and *D. fructosovorans*. These results are similar to those reported for *D. alcoholovorans* grown on 1,2-propanediol in the presence of sulfate (Table 4). However, unlike *D. alcoholovo-*

Table 4. Comparison of the enzyme activities (μmol · min^{-1} · mg^{-1} protein)[a] in cell-free extracts of *D. alcoholovorans*,[b] *Desulfovibrio* sp. strain DFG,[c] *D. carbinolicus* and *D. fructosovorans*

Growth medium	1,2-Propanediol[c] + sulfate		Glycerol + 1,2-Propanediol	
Enzymes	*D. alcoholovorans*	Strain DFG	*D. carbinolicus*	*D. fructosovorans*
1,2-Propanediol dehydratase	0.015	0.124	0.134	0.105
NAD-dependent CoA-linked propionaldehyde dehydrogenase	ND[d]	0.001	0.002	0.001
NAD-independent CoA-linked propionaldehyde dehydrogenase (BV-linked)	0.44	1.14	1.05	1.08
NAD-dependent propionaldehyde dehydrogenase	1.02	2.15	1.17	0.95
Propionate kinase	2.08	1.62	1.55	1.83
NAD-dependent 1,2-propanediol dehydrogenase	0.016	0.012	0.018	0.002
NAD-independent 1,2-propanediol dehydrogenase (MTT-linked)	—	ND	ND	ND
Lactate dehydrogenase (DCPIP-linked)	1.92	0.32	0.21	0.36
Pyruvate dehydrogenase (BV-linked)	1.04	0.84	0.56	0.33
Phosphate acetyltransferase	<0.015	0.01	0.009	0.007
Acetate kinase	1.20	0.92	0.75	0.64
NADH dehydrogenase (MTT-linked)	1.93	0.85	0.45	0.37
NADPH dehydrogenase (MTT-linked)	0.60	0.23	0.11	0.10

[a]In the absence of sulfate, *D. carbinolicus* and *D. fructosovorans* degrade glycerol to a mixture of 1,3-propanediol and 3-hydroxypropionate with no or weak growth. Consequently, enzyme activities involved in glycerol dismutation to 1,3-propanediol and 3-hydroxypropionate cannot be carried out.
[b]Data from Ouattara et al. [29].
[c]1,2-Propanediol is degraded exclusively to propionate (data not shown).
[d]ND, not detected.

rans, which produces approximately ten times more acetate than propionate from 1,2-propanediol in the presence of sulfate [32], only propionate was produced by strain DFG from 1,2-propanediol in the presence of sulfate. On the other hand, no 1,2-propanediol dehydrogenase activity was observed for the three strains when NAD was replaced by MTT as electron acceptor. When NAD was replaced by NADP as electron acceptor, less than 5% of this activity was recovered (data not shown).

High specific activity of coenzyme B_{12}-dependent 1,2-propanediol dehydratase was measured in the cell-free extracts of strain DFG. The low activity of the dehydratase reported for *D. alcoholovorans* (0.015 unit · mg^{-1} protein) and the higher activity measured in cell-free extract of strain DGF (0.124 unit · mg^{-1} protein) (Table 4) were not consistent with the physiological data of both strains. Furthermore, high dehydratase activities were found in cell-free extracts of *D. carbinolicus* (0.134 unit · mg^{-1} protein) and *D. fructosovoranws* (0.105 unit · mg^{-1} protein). This raised several questions: Why was 1,2-propanediol not used as energy source? Why was only little propionate produced when glycerol was added as additional energy source in the culture medium (Table 2)? Why was 1,2-propanediol not fermented in the absence of sulfate by strain DFG, which, like *D. carbinolicus* and *D. fructosovorans*, exhibited a high dehydratase activity?

The specific activities of NAD-independent CoA-linked propionaldehyde dehydrogenase with benzylviologen as electron acceptor were higher than those of NAD-dependent CoA-linked propionaldehyde dehydrogenase in cell-free extracts of strain DFG, *D. carbinolicus*, and *D. fructosovorans*. Furthermore, the specific activities of NAD-independent CoA-linked propionaldehyde dehydrogenase in these three strains were higher than those reported for *D. alcoholovorans* (Table 4). This probably explained the very active pathway of exclusive production of propionate from 1,2-propanediol by strain DFG in sulfate-containing medium (results not shown), but did not explain the very low production of propionate by *D. carbinolicus* and *D. fructosovorans*.

A very low specific activity of NAD-dependent CoA-linked propionaldehyde dehydrogenase was observed in cell-free extracts of strain DFG, *D. carbinolicus*, and *D. fructosovorans*. However, as described by Kremer et al. [15], the presence of very active alcohol dehydrogenase as measured in cell-free extracts of the three tested strains may render difficult the detection of such an activity. NADPH could replace NADH as electron donor for propionaldehyde dehydrogenase, but less than 10% activity was recovered. Furthermore, specific activities of NAD-dependent propionaldehyde dehydrogenase found in cell-free extracts of strain DFG grown on 1,2-propanediol in the presence of sulfate (2.15 unit · mg^{-1} protein) or reported for *D. alcoholovorans* grown in the same condition (1.02 unit · mg^{-1} protein) were similar to those detected in cell-free extracts of *D. carbinolicus* (1.17 unit · mg^{-1} protein) and *D. fructosovorans* (0.95 unit · mg^{-1} protein) grown on a mixture of 1,2-propanediol and glycerol without sulfate. Unlike *D.*

carbinolicus and *D. fructosovorans*, which produced propanol from 1,2-propanediol (Table 2), n-propanol was not produced, neither in syntrophic association of strain DFG with *M. hungatei* in the absence of sulfate (results not shown), nor in coculture with *D. alcoholovorans* and *M. hungatei* without sulfate [27], whereas a very active NAD-dependent propionaldehyde dehydrogenase was detected in cell-free extracts.

A significant NADH dehydrogenase activity was measured in cell-free extracts of strain DFG, *D. carbinolicus* and *D. fructosovorans*, but it was lower than that reported for *D. alcoholovorans*. NADPH dehydrogenase activities measured in the three tested strains were four times lower than those measured with NADH as electron acceptor (Table 4). The role of these NAD(P)-dependent dehydrogenases and NAD(P)H-dehydrogenases in alcohol and diol metabolism was discussed in detail by Kremer and Hansen [14], Kremer et al. [10], and Hensgens et al. [9, 10].

Discussion

Whatever the terminal electron acceptor, *D. carbinolicus* degraded glycerol to 3-hydroxypropionate. On the other hand, *D. fructosovorans* degraded glycerol to acetate in the presence of sulfate, and 3-hydroxypropionate in coculture with *M. hungatei*. Thus, the terminal electron acceptor influenced the dissimilative pathway of glycerol by *D. fructosovorans*. *Methanospirillum hungatei* was probably unable to ensure sufficient hydrogen transfer [2] to allow the activity of the enzymes of the acetate synthesis pathway. A similar phenomenon was observed during glutamate fermentation by *Acidaminobacter hydrogenoformans* in pure culture or in coculture with *Methanobrevibacter arboriphilus* [30]. In *D. fructosovorans*, 3-hydroxypropionate was not an intermediary product of glycerol degradation into acetate. This was shown by adding an early stationary phase of *D. fructosovorans* culture to the coculture of *D. fructosovorans* with *M. hungatei* and observing that 3-hydroxypropionate was not used. 3-Hydroxypropionate was also produced during the degradation of 1,3-propanediol by *D. fructosovorans* in the presence of sulfate [26]; it could not be degraded because of the lack of appropriate enzymes (unpublished data).

We assume that the pathway of glycerol to acetate by *D. fructosovorans* is the same as described by Kremer and Hansen [14] and involves glycerol-3-phosphate, dihydroxyacetone phosphate, glyceraldehyde phosphate, etc. A step that is difficult to couple to the reduction of protons (instead of adenylylsulfate/sulfite ($E'^0 = -60$ mv)) is the dehydrogenation of glycerol-3-phosphate to dihydroxyacetone phosphate ($E'^0 = -190$ mv) [34]. In a culture with a pH_2 of 10 Pa, the redox potential of H^+/H_2 is approximately -300 mv. The only way of reducing protons with the electrons of glycerol-3-phosphate under these conditions would be to pump up the electrons to a lower redox potential with the aid of the proton motive force or by maintaining a very high substrate/product ratio in the reaction. This does not appear to happen. In the coculture and in the absence of sulfate, glycerol is dehydrated first to 3-hydroxypropionaldehyde and then oxidized to 3-hydroxypropionate, but why does the pure culture not produce a mixture of acetate and 3-hydroxypropionate (Fig. 2a)?

Adding 1,2-propanediol to cultures of *D. carbinolicus* and *D. fructosovorans* in a medium containing glycerol but no sulfate decreased the concentration of 1,3-propanediol but not that of 3-hydroxypropionate. Moreover, propionate and *n*-propanol production was observed only when 1,2-propanediol was added as an energy source. However, production of *n*-propanol was much higher than that of propionate, which might be produced by a non-specific acetaldehyde dehydrogenase [36]. Propionate formation from 1,2-propanediol is a common metabolic property of species of genera *Lactobacillus* [29], *Klebsiella*, *Citrobacter* [35, 36], *Acetobacterium* [4], and *Propionibacterium* [13]. In these organisms, a coenzyme B_{12}-dependent 1,2-propanediol dehydratase is involved in 1,2-propanediol degradation. Glycerol and 1,2-propanediol dehydratases were inducible in *Klebsiella pneumoniae* growing on glycerol [6]. Probably, the 1,2-propanediol dehydratase we measured in cell-free extracts of *D. carbinolicus* and *D. fructosovorans* growing on a mixture of 1,2-propanediol and glycerol was a nonspecific inducible glycerol dehydratase, since *D. carbinolicus* and *D. fructosovorans* cannot ferment 1,2-propanediol.

It would, however, be desirable to try to demonstrate the presence of significant levels of glycerol dehydratase in cells that have been grown on other substrates in the absence of glycerol, and to check whether 1,2-propanediol is degraded in combination with substrates other than glycerol. Recently a *Desulfovibrio* strain IsBd was described to ferment only S-enantiomer among optical isomers of 1,2-propanediol into propionate and 1-propanol, but a racemic mixture did not allow growth [33]. These results strongly suggested that the fermentation of S-1,2-propanediol was inhibited by R-enantiomer [33].

Our study showed that 1,2-propanediol was dehydrated into propionaldehyde and reduced to propanol by an NAD-dependent alcohol dehydrogenase in *D. carbinolicus* and *D. fructosovorans*. It was converted into propionate by an NAD-independent CoA-linked propionaldehyde dehydrogenase, a phosphate propionyl transfer-

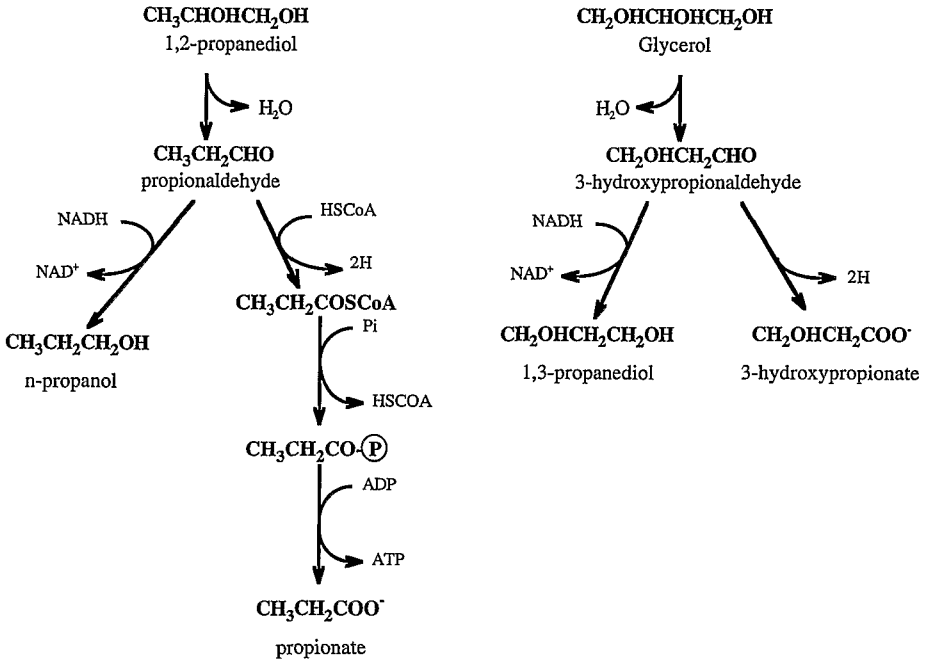

Fig. 3. Hypothetical pathway for the degradation of 1,2-propanediol by *D. carbinolicus* and *D. fructosovorans*. The proposed pathway of 1,2-propanediol can be used by *Desulfovibrio* sp strain DFG except that *n*-propanol is not produced (the hypothetical pathway for the dismutation of glycerol by *D. carbinolicus* and *D. fructosovorans* was established only from physiological data). P in a circle is a phosphate group.

ase, and a propionate kinase in *D. carbinolicus*, *D. fructosovorans*, and *Desulfovibrio* sp. strain DFG as described in *Propionibacterium freudenreichii* [11] and *K. pneumoniae* [36]. High-specific activities of the dehydratase, NAD-independent CoA-linked propionaldehyde dehydrogenase, and propionate kinase measured in cell-free extracts of *D. carbinolicus*, *D. fructosovorans*, and *Desulfovibrio* sp. strain DFG were in agreement with such a metabolism (Fig. 3).

1,2-Propanediol degradation involving NAD-linked 1,2-propanediol dehydrogenase was recently reported in *Desulfovibrio* species [8–10, 25]. We observed weak specific activities of NAD-dependent propanediol dehydrogenase in cell-free extracts of *D. carbinolicus*, *D. fructosovorans*, and strain DFG. Our results were similar to those reported for *D. alcoholovorans* grown on 1,2-propanediol in the presence of sulfate (Table 4). However, unlike *D. alcoholovorans*, which produces approximately 10 times more acetate than propionate from 1,2-propanediol in the presence of sulfate [27], only propionate was produced by strain DFG.

The addition of 1,2-propanediol to the glycerol-containing medium led to a lower final 1,3-propanediol concentration. Probably 1,2-propanediol supplied an alternative acceptor of reducing equivalents in the form of propionaldehyde, resulting in the formation of propanol (Fig. 3). This assumes that reducing equivalents in the form of NADH reduced 3-hydroxypropionaldehyde into 1,3-propanediol [16]. A similar process was observed during the co-fermentation of fructose and glycerol by *D. fructosovorans*. In this case, glycerol seemed to compete with ethanol production: in the presence of glycerol, ethanol formation from fructose ceased and acetate was formed instead (Table 3). This led to the assumption that the reoxidation of NADH occurred in the last step of 1,3-propanediol production, as observed with *Citrobacter freundii* and *Aerobacter aerogenes* [3]. For these strains it was found that glycerol was converted first to 3-hydroxypropionaldehyde, which was then reduced to 1,3-propanediol. We propose a hypothetical pathway for the fermentation of glycerol into 1,3-propanediol and 3-hydroxypropionate by *D. carbinolicus* and *D. fructosovorans* (Fig. 3). It is based on our data and the literature on glycerol metabolism by aerobic bacteria for which enzymes are well known.

ACKNOWLEDGMENTS

We thank B. Ollivier and A. Ouattara for helpful discussions, and T.A. Hansen and P.A. Roger for critical comments and improving the manuscript.

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