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Anaerobic degradation of 1,2-propanediol by a new *Desulfovibrio* strain and *D. alcoholovorans*

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Abstract. A sulfate-reducing bacterium, strain HDv, was isolated from the anoxic soil of a ricefield using lactate as electron donor. Cells were gram-negative, motile, nonsporulating curved rods, with single polar flagella. Substrates were incompletely oxidized to acetate and included glycerol, 1,2- and 1,3-propanediol. Sulfate, sulfite, thiosulfate, elemental sulfur, fumarate, maleate, and malate were utilized as electron acceptors. Pyruvate, fumarate, maleate, malate and dihydroxyacetone were fermented. Desulfoviridin and *c*-type cytochromes were present. The DNA base composition was 66.6 ± 0.3 mol% G+C. The isolate was identified as a *Desulfovibrio* sp.; its metabolic properties were somewhat different from those of previously described *Desulfovibrio* species. Comparative biochemical study of 1,2-propanediol dissimilation by the new isolate and *Desulfovibrio alcoholovorans* showed that NAD-dependent dehydrogenases play a key role in the catabolism of this substrate. The hypothetical pathways of 1,2-propanediol degradation by *Desulfovibrio* spp. are presented.

Key words: Sulfate reduction – *Desulfovibrio* – 1,2-Propanediol degradation – Alcohol and aldehyde dehydrogenase – NADH dehydrogenase

Among sulfate-reducing bacteria, the "classical" *Desulfovibrio* species are known to oxidize typical fermentation products such as hydrogen, ethanol or higher homologs, lactate, pyruvate, and dicarboxylic acids (Hansen 1988; Widdel 1988). However, some *Desulfovibrio* spp. can also grow on amino-acids (Postgate 1984; Stams et al. 1985), sugars (Ollivier et al. 1988), and reduced compounds such as glycerol and 1,3-propanediol (Kremer and Hansen 1987; Nanninga and Gottschal 1987; Oppenberg and Schink 1990; Qatibi et al. 1991b).

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Biochemical studies of glycerol dissimilation in two marine *Desulfovibrio* strains, showed the absence of a NAD(P)-dependent or independent glycerol dehydrogenase, suggesting that, in these microorganisms, glycerol is degraded to acetate *via* glycerol-3-phosphate, dihydroxyacetone phosphate, and part of the glycolytic pathway (Kremer and Hansen 1987). Oppenberg and Schink (1990) suggested a sequence for 1,3-propanediol oxidation to acetate by *Desulfovibrio* strain OttPdl; a possible intermediate is malonyl semialdehyde. Recently, 1,2-propanediol degradation by *Desulfovibrio* strains has been reported (Dwyer and Tiedje 1986; Qatibi et al. 1991a, b). However, to our knowledge, no data are available on the biochemistry of 1,2-propanediol dissimilation in *Desulfovibrio.* The most common pathway encountered in anaerobic degradation of 1,2-propanediol by microorganisms involves, in a first step, the conversion of the substrate to propionaldehyde by a dehydratase, followed by the subsequent oxidation of this compound to propanol and propionate by a NAD-dependent aldehyde dehydrogenase, a CoA-linked NAD-dependent aldehyde dehydrogenase, a phosphate propionyltransferase, and propionate kinase (Toraya et al. 1979). However, degradation of 1,2-propanediol involving NAD-linked alcohol dehydrogenase has been reported for a mutant of *Escherichia coli* cultivated anaerobically with fumarate as electron acceptor (Sridhara et al. 1969).

Studies on the implication of sulfate-reducing bacteria on sulfide toxicity observed in many ricefields of the "Kou Valley" (Burkina Faso) included counting series of these microorganisms using various substrates (acetate, propionate, ethanol, and lactate). The last positive dilutions were retained for detailed studies. Several sulfate-reducing bacteria oxidizing ethanol and lactate incompletely were isolated. Among them, two 1,2-propanediol oxidizers, strains EDv and HDv, exhibited metabolic properties different from those of previously described *Desulfovibrio* species. Because the two strains closely resembled, we report on the characterization of strain HDv. Comparative studies of enzymes involved in the anaerobic degradation of 1,2-propanediol by strain HDv and *Desulfovibrio alcoholovorans* and probable pathways are presented.

Materials and methods

Origin of strains

Strain HDv was isolated from reduced layers (10 to 25 cm) of ricefield soils of the "Kou Valley" in Burkina Faso. Lactate and sulfate were the substrates of enrichment cultures. The temperature in *situ* averaged 33 °C and the pH was near 7. *Methanospirillum hungatei*, DSM864, was purchased from the German Collection of Microorganism, Braunschweig, FRG. *Desulfovibrio alcoholovorans* DSM5433 and *Methanobacterium* sp. were obtained from our culture collection.

Media and culture conditions

The techniques for cultivation of strict anaerobes described by Macy et al. (1972) were used throughout this study. Strains were grown on a basal medium containing (per liter): KH_2PO_4, 0.25 g; NH_4Cl, 0.30 g; NaCl, 1 g; KCl, 0.50 g; $MgCl_2 \cdot 6 H_2O$, 0.40 g; $CaCl_2 \cdot 2 H_2O$, 0.16 g; resazurin 0.001 g; and 1 ml of a trace element solution (Imhoff-Stuckle and Pfennig 1983). The medium was boiled under a stream of O_2-free N_2, cooled at room temperature and dispensed into Hungate tubes or serum bottles using Hungate's anaerobic technique (1969) as modified by Miller and Wolin (1974) and Balch and Wolfe (1976). After autoclaving (110 °C for 40 min), $NaHCO_3$ and $Na_2S \cdot 9 H_2O$ were added, from autoclaved stock solutions to final concentrations of 0.25% and 0.025%, respectively. Just before inoculation, substrates were supplied from filter-sterilized stock solutions. Filter-sterilized solutions of vitamins according to Balch et al. (1979) or Pfennig et al. (1981) were used to account for vitamin requirements. Coculture experiments were realized as described in Qatibi et al. (1991a).

Unless otherwise indicated, all experiments were conducted at 37 °C and pH 7.0 in a culture medium containing 22 mM sulfate and 20 mM lactate. Growth was monitored by measuring the optical density at 580 nm with a Shimadzu (Kyoto, Japan) UV300 spectrophotometer. The pH range for growth was estimated in a Tris-maleate buffer medium. The pH was adjusted with sterile NaOH solutions. The temperature range for growth was tested from 13 °C to 50 °C. Studies for salt requirements were carried out in media containing from 0 to 3% (w/v) NaCl.

Isolation

Pure cultures were obtained by repeated dilution, using the roll-tube method (Hungate 1969). Purity of isolates was checked in a medium containing 0.25% yeast extract (Difco Laboratories, Detroit, Mich., USA), 0.25% peptone (Difco), 0.25% Biotrypcase (Biomérieux, Craponne, France), and 0.25% glucose. The culture was examined microscopically after three weeks of incubation.

Cell pigment determination and gram staining

About 3 g of cells (wet weight) in 10 ml of 50 mM Tris-HCl buffer (pH 7.6) were sonicated and centrifuged at $30000 \times g$ for 20 min. The cell-free supernatant was separated into the soluble fraction and the particulate fraction by centrifugation at $140000 \times g$ for 1 h. The particulate fraction was resuspended in the previous buffer. Cytochromes were identified by recording the redox difference spectrum (dithionite-reduced minus air-oxidized) of each fraction using a Shimadzu UV300 spectrophotometer.

Gram staining was performed using a standard method with a coloration kit (Sigma Chemicals, St. Louis, Mo., USA). *Escherichia coli* and *Micrococcus luteus* were used as controls.

Enzyme assays

Cells grown on 20 mM lactate, 15 mM 1,2-propanediol, or a mixture of 15 mM 1,2-propanediol and 5 mM glycerol, in the presence of 22 mM sulfate, were harvested at the end of the exponential growth phase, washed three times with 50 mM phosphate buffer (pH 7.5), and sonicated under a stream of N_2. After centrifugation for 15 min at $15000 \times g$ under N_2, the supernatant was transferred into an oxygen-free serum bottle supplemented with 0.5 mM dithiotreitol.

Enzyme activities were measured spectrophotometrically according to the general procedures of Kremer and Hansen (1987) and Kremer et al. (1988). NAD-dependent 1,2-propanediol dehydrogenase (the forward reaction) was determined in 100 mM Tris-HCl (pH 9.0) containing 5 mM NAD and 10 mM 1,2-propanediol; the activity of the backward reaction was assayed in 50 mM Tris-HCl (pH 7.5) containing 0.2 mM NADH and 2 mM glycolaldehyde instead of lactaldehyde, because the substrate for the physiological reduction to 1,2-propanediol is not available commercially. Reactions were started with 1,2-propanediol or glycolaldehyde and the formation or disappearence of NADH was recorded at 340 nm. NADP- and NADPH-dependent dehydrogenase activities were recorded by the same procedure, except that NAD was replaced by NADP and NADH by NADPH. Enzyme activities with glycerol, 1,3-propanediol, ethylene glycol, ethanol, and propanol as substrates were measured in the direction of corresponding aldehydes by following the appearance of NADH at 340 nm as described for 1,2-propanediol dehydrogenase. NAD(P)H-dependent acetaldehyde, and propionaldehyde dehydrogenase were tested as described for glycolaldehyde. Following enzyme activities were measured according to their respective authors: 1,2-propanediol dehydratase (Toraya et al. 1977), lactate dehydrogenase (Stams and Hansen 1982), pyruvate dehydrogenase (Odom and Peck 1981), NAD(P)- or dye-linked CoA-dependent propionaldehyde dehydrogenase (Kremer et al. 1988), phosphate acetyltransferase, acetate kinase, and propionate kinase (Oberlies et al. 1980), NADH and NADPH dehydrogenases (Kremer and Hansen 1987). A unit is expressed as μmole of product formed or substrate consumed per minute ($\mu mol \cdot min^{-1}$). The protein content was determined by the method of Sedmak and Grossberg (1977), using bovine serum albumin as standard. Each experiment was carried out at least in duplicate. All chemicals used were of analytical grade.

Analytical techniques

Volatile fatty acids, organic acids, sugars and alcohols were assayed by high-performance liquid chromatography (H.P.L.C.): pump, Analprep 93 (Touzart et Matignon, Vitry sur Seine, France); flow rate, 0.6 ml/min; injection loop, 20 μl; column, Interaction ORH-801, 300×6.5 mm (Interaction Chemicals, Mountain View, Calif., USA); column temperature, 30 °C; detection, differential refractometer RID-6 A (Shimadzu, Kyoto, Japan); recorder, Chromatopak C-R3A (Shimadzu, Kyoto, Japan).

Sulfide was measured spectrophotometrically as colloidal CuS. Methane was measured by gas chromatography. The procedures used were as reported by Qatibi et al. (1991a).

DNA base composition

DNA was isolated by chromatography on hydroxyapatite (Cashion et al. 1977). The guanine-plus-cytosine content of the DNA was determined by HPLC; non-methylated Lambda virus DNA was used for calibration (Meshbah et al. 1989).

Results

Morphology

After 7 days of incubation at 35 °C, strain HDv formed circular, lens-shaped colonies that were light brown in colour. The isolate was a vibrioid rod, and highly motile by a single polar flagellum. Cells occurred singly or in pairs and were $0.8-1.2 \times 2.2-3.1$ μm in size. In old

cultures, they became spirilloid and lost their motility. The cells stained gram-negative. Spores were not observed.

Physiology

Strain HDv was strictly anaerobic. The optimum growth temperature was 37 °C; no growth was observed above 42 °C or below 13 °C. Optimum growth was observed at pH 6.8 in a range of 5.8–8.0. The isolate grew optimally in a medium without NaCl; growth was completely inhibited at 1% NaCl.

No growth occurred in the absence of vitamins. Vitamins could be replaced by yeast extract. Sulfate, sulfite, thiosulfate, elemental sulfur, fumarate, maleate, malate served as electron acceptors. Fumarate, maleate, and malate were reduced to succinate whereas the other electron acceptors were reduced to sulfide. The isolate did not use nitrate or ferric iron as electron acceptor. The compounds used as energy sources in the presence or absence of sulfate and their respective end products are summarized in Table 1. We presumed that CO_2 was produced from all degradation. No growth was observed on sugars, amino acids, and fatty acids (Table 1). Growth with molecular hydrogen or formate required acetate as carbon source. Malate was a transitory product during fumarate and maleate oxidation.

Studies of the degradation of reduced compounds in coculture with methanogenic hydrogen scavengers showed that small amounts of 3-hydroxypropionate were produced from glycerol degradation in addition to acetate. Such results differ from the exclusive production of acetate from glycerol catabolism in the presence of sulfate (see footnote c of Table 1). Furthermore, as compared to the results obtained in the presence of sulfate, strain HDv was unable to use more than 2.1 mM and 3.0 mM of 1,2- and 1,3-propanediol, respectively in syntrophic association with methanogens; however, the organic end product profile observed from these substrate degradations was the same regardeless of the terminal electron acceptor. Our results differed from those reported for 1,2-propanediol degradation by *Desulfovibrio alcoholovorans* which yielded acetate and propionate when the terminal electron acceptor was sulfate, and only propionate when the acceptor was *Methanospirillum hungatei* (Qatibi et al. 1991a).

Pigments

The soluble fraction of strain HDv exhibited the characteristic absorption bands of *c*-type cytochrome with

Table 1. End products of substrates used as electron donors by strain HDv, in the presence or absence of 22 mM sulfate. We presumed that CO_2 was produced from all degradations

Substrate[a]	$+ SO_4^{2-}$		$- SO_4^{2-}$	
	Growth	End products	Growth	End products
$H_2 + CO_2$	+	n.r	–	n.r
Formate	+*	n.r	–	n.r
Ethanol	+	Acetate	–	n.r
1-Propanol	+	Propionate	–	n.r
1-Butanol	+	Butyrate	–	n.r
1-Pentanol	+	Valerate	–	n.r
Ethylene glycol	(+)	Glycolaldehyde	–	n.r
1,2-Propanediol	+	Acetate	–	n.r
1,3-Propanediol	+*	3-H.P[b]	–	n.r
Glycerol	+	Acetate[c]	–	n.r
DHA	+	Acetate	+	Acetate
Lactate	+	Acetate	–	n.r
Pyruvate	+	Acetate	+	Acetate
Fumarate	+	Acetate + succinate	+	Acetate + succinate
Maleate	+	Acetate + succinate	+	Acetate + succinate
Malate	+	Acetate + succinate	+	Acetate + succinate
Succinate	+*	Acetate	–	n.r

Legends: DHA, dihydroxyacetone; 3-H.P, 3-hydroxypropionate; +, substrate used as electron donor with good growth; +*, substrate used as electron donor with weak growth (OD $\leqq 0.07$); (+), substrate used as electron donor without growth; –, substrate not fermented; n.r, not recorded

[a] Substrates were tested at concentrations of 10 or 20 mM

[b] In addition of 3-hydroxypropionate, 0.2 to 0.5 mM of acetate were also detected

[c] When the terminal electron acceptor was *Methanospirillum hungatei* instead of sulfate, 12 mM of glycerol were converted to 11.0 mM acetate and 0.8 mM 3-hydroxypropionate; when sulfate was replaced by *Methanobacterium* sp., 10.8 mM acetate and 1.8 mM 3-hydroxypropionate were produced from the degradation of 12 mM glycerol

Substrates tested but not utilized by strain HDv were: acetate, propionate, butyrate, methanol, 2-propanol, 2-butanol, benzoate, citrate, ribose, glyceraldehyde, arabinose, xylose, glucose, fructose, galactose, sorbose, rhamnose, sucrose, lactose, melibiose, raffinose, starch, cellulose, amylose, pectin, choline, serine, glutamate, aspartate, alanine, glycine, proline, threonine, lysine, betaine, tyrosine, valine, leucine, phenylalanine, isoleucine, cystein, gelatin

maxima at 419, 523, and 553 nm. The oxidized extract showed the cytochrome Soret peak at 408 nm. The cytochrome was not reduced by sodium ascorbate, which indicated that it probably had a low midpoint redox potential. The spectrum showed a strong absorption band at 628 nm and a weaker one at 582 nm, characteristic of desulfoviridin (see Postgate 1984; Widdel 1988).

DNA base composition

The guanine-plus-cytosine content of the DNA was 66.6 $\pm$ 0.3 mol% (average of three determinations).

Enzymes involved in 1,2-propanediol degradation

Cell-free extracts of 1,2-propanediol-grown cells of the two strains tested contained significant specific activities of lactate dehydrogenase, pyruvate dehydrogenase, propionate kinase and acetate kinase but relatively weak specific activities of phosphate acetyltransferase (Table 2). However, whereas significant specific activity of 1,2-propanediol dehydrogenase was found in the crude cell extract of strain HDv, we measured relatively weak activity in cell extract of *Desulfovibrio alcoholovorans* grown on the same substrate [about one hundred fold lower as compared to the one found in strain HDv (Table 2)].

Attempts to detect coenzyme B_{12}-dependent 1,2-propanediol dehydratase activity in cell-free extracts of strain HDv grown on 1,2-propanediol, or 1,2-propanediol plus glycerol were unsuccessful (Table 2). The dehydratase activity found in crude cell extracts of *D. alcoholovorans* grown on 1,2-propanediol in the presence of sulfate was very low, about 0.015 unit $\cdot$ mg^{-1} protein (Table 2). Such result is consistent with the small amounts of propionate produced in the culture medium (1 to 2 mM).

NAD-linked CoA-dependent propionaldehyde dehydrogenase activities were not detected in the two strains tested. NADP-linked CoA-dependent propionaldehyde dehydrogenase activities measured in the crude extracts of the two strains were not significant: $\leq 10^{-4}$ unit $\cdot$ mg^{-1} protein for strain HDv and $\leq 10^{-3}$ unit $\cdot$ mg^{-1} protein for *D. alcoholovorans*. However, the presence of very active alcohol dehydrogenase may render difficult the detection of such activity (see Kremer et al. 1988). We could observed significant dye-linked CoA-dependent propionaldehyde dehydrogenase activities in cell extracts of the two *Desulfovibrio* strains. With benzylviologen as electron acceptor, the specific activity of CoA-dependent propionaldehyde dehydrogenase measured was 0.44 unit $\times$ mg^{-1} protein for *Desulfovibrio alcoholovorans* and 0.21 unit $\cdot$ mg^{-1} protein for strain HDv.

In crude cell extracts of strain HDv, we observed high NAD-dependent dehydrogenase activity with several aldehydes and alcohols including glycerol as substrate (Table 3). However, the activities measured with polyols and glycolaldehyde were lower than those found with ethanol or propanol and their corresponding aldehydes as substrates. Furthermore, the NAD-dependent-glycerol dehydrogenase measured was higher with 1,2-propanediol plus glycerol-grown cells than with 1,2-propanediol-grown cells.

In crude extracts of 1,2-propanediol-grown cells of *D. alcoholovorans*, only propionaldehyde dehydrogenase activities were measured at values similar to those found in strain HDv (Table 3). Ethanol or propanol dehydrogenases were significant but about ten fold lower than those found in cell extract of strain HDv (Table 3). Specific dehydrogenase activities with polyols as substrates measured in cells of *D. alcoholovorans* were below 0.03 unit $\cdot$ mg^{-1} protein regardeless of the cultivation conditions. This represents 1% or less of the values found in strain HDv (Table 3). The dehydrogenase activities measured in cell extracts of *D. alcoholovorans* with polyols as substrates represented about 5 to 10% of those found when substrate was ethanol or propanol, regardeless of the cultivation conditions; only 20 to 25% of the activities measured with acetaldehyde or propionaldehyde as substrate were recovered when they were replaced by glycolaldehyde (Table 3).

Table 2. Enzyme activities (μmol $\cdot$ min^{-1} $\times$ mg^{-1} protein) in cell-free extracts of cells of strain HDv and *Desulfovibrio alcoholovorans* grown on 1,2-propanediol in the presence of sulfate

Enzyme	Specific activity	
	Strain HDv	*D. alcoholovorans*
1,2-Propanediol dehydratase	not detected	≤0.017
Propionaldehyde dehydrogenase[a]	≤0.0001	≤0.001
Propionate kinase	1.88	2.08
1,2-Propanediol dehydrogenase	1.88	0.016
Lactate dehydrogenase	0.32	1.92
Pyruvate dehydrogenase	4.07	1.04
Phosphate acetyltransferase	0.06	≤0.015
Acetate kinase	1.60	1.20
NADH dehydrogenase	3.31	1.93
NADPH dehydrogenase	1.22	0.60

[a] CoA-dependent NAD-linked propionaldehyde dehydrogenase
The electron acceptors used were: NAD for 1,2-propanediol and propionaldehyde, DCPIP (Dichlorophenolindophenol) for lactate, benzylviologen for pyruvate, and MTT [3-(4′,5′-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide] for NADH or NADPH dehydrogenase activities, respectively. The references are indicated in the text. Data are mean values of at least two determinations

Table 3. Specific NAD-dependent dehydrogenase activity (μmol · min^{-1} · mg^{-1} protein) with different alcohols and polyols as substrates in crude cell-free extracts of *Desulfovibrio* strain HDv and *D. alcoholovorans* grown on 1,2-propanediol or 1,2-propanediol plus glycerol in the presence of sulfate

Substrate[a]	1,2-Propanediol grown cells of		1,2-Propanediol + glycerol grown cells of	
	Strain HDv	*D. alcoholovorans*	Strain HDv	*D. alcoholovorans*
Ethanol	3.56	0.311	2.37	0.22
Acetaldehyde	6.95	1.05	5.03	0.41
Propanol	3.63	0.312	5.15	0.22
Propionaldehyde	1.37	1.02	1.77	0.38
Ethylene glycol	3.10	0.024	4.55	0.017
Glycolaldehyde	2.60	0.282	2.27	0.088
1,2-Propanediol	1.88	0.016	2.04	0.022
1,3-Propanediol	2.11	0.027	2.63	0.022
Glycerol	1.64	0.015	2.33	0.016

[a] The electron acceptor was NAD^+ for alcohol dehydrogenase activity assays (tested in a 100 mM Tris-HCl buffer, pH 9.0). The electron donor was NADH for aldehyde dehydrogenase activity assays (tested in a 50 mM Tris-HCl buffer, pH 7.5). About 1 to 5% of the dehydrogenase activities were recovered when NAD or NADH were replaced by NADP or NADPH, respectively

The following specific activities (unit · mg^{-1} protein) were found in cell-free extracts of lactate-grown cells of strain HDv: lactate dehydrogenase 0.794, pyruvate dehydrogenase 0.075, phosphate acetyltransferase ≤0.01, acetate kinase 0.04, ethanol and propanol dehydrogenase ≤0.011, NADH dehydrogenase 0.278, and NADPH dehydrogenase 0.292. NAD(P)-dependent dehydrogenase activities with polyols and aldehydes as substrates were not detected in lactate-grown cells.

High NADH and NADPH dehydrogenase activities were present in 1,2-propanediol- and lactate-grown cells of strain HDv. However, the NADH dehydrogenase was about ten fold higher during growth on 1,2-propanediol (Table 2) or a mixture of 1,2-propanediol plus glycerol (data not shown) than during growth on lactate. Similarly, NADPH dehydrogenase activity increased about 5-fold.

The NADH and NADPH dehydrogenase activities measured in *D. alcoholovorans* were lower than those found in strain HDv. However, NADH dehydrogenase activities found in the crude extracts of the two strains were always higher than NADPH dehydrogenase activities (Table 2).

NADP could replace partially NAD as electron acceptor of alcohol or aldehyde dehydrogenases but only 1 to 5% of the NAD-dependent dehydrogenase activity was recovered. NADPH could replace partially NADH, with similar low recovery of dehydrogenase activity (data not shown).

Discussion

The classification of the new isolates of sulfate-reducing bacteria has been traditionally established on the basis of chemical data such as the G+C content of genomic DNA, and the presence of particular pigments, in addition to phenotypic characters such as morphology and nutrition physiology (Postgate 1984; Widdel 1988; Widdel and Pfennig 1984). Later, genetical data such as 16S rRNA sequences, rRNA-DNA and DNA-DNA hybridizations were added to improve the existing classification, generating new taxonomic affiliations (Devereux et al. 1989, 1990; Fowler et al. 1986; Nazina et al. 1987; Widdel 1988).

The new isolate, strain HDv, is a strictly anaerobic, Gram-negative, sulfate-reducing bacterium. This mesophilic vibrio does not sporulate, is unable to oxidize acetate, propionate, or butyrate, and performs an incomplete oxidation of pyruvate and lactate to acetate and CO_2. Based on these characteristics, strain HDv can be related to genera *Desulfovibrio* and *Desulfomicrobium* (Pfennig et al. 1981; Widdel 1988; Rozanova et al. 1988). Since desulfoviridin is present in the soluble extract of the isolate in addition of *c*-type cytochrome, it can be definitively affiliated to the genus *Desulfovibrio* (Widdel 1988). Morphological and physiological characteristics of *Desulfovibrio* strain HDv are similar to those of the species *D. desulfuricans*, and *D. vulgaris* (Widdel and Pfennig 1984), *D. alcoholovorans* (Qatibi et al. 1991b), *D. fructosovorans* (Ollivier et al. 1988), and *D. carbinolicus* (Nanninga and Gottschal 1987). Strain HDv differs from the *Desulfovibrio* species other than *D. alcoholovorans*, and *D. desulfuricans* strain DG2, by its ability to use 1,2-propanediol as substrate. In contrast to *D. desulfuricans*, strain HDv is unable to utilize choline or nitrate as energy source or electron acceptor, respectively. The isolate differs from *D. vulgaris* by its ability to ferment fumarate or malate. The most distinctive characteristics between *Desulfovibrio* strain HDv, *D. vulgaris*, and *D. desulfuricans* is that the G+C mol% of strain HDv's DNA is 5% and 10% higher than those reported for *D. vulgaris* and *D. desulfuricans*, respectively (Widdel 1988). Unlike *D. fructosovorans*, the isolate does not use fructose. The differences between *Desulfovibrio* strain HDv and *D. carbinolicus* are: (i) *D. carbinolicus* is nonmotile; (ii) in the presence of sulfate, *D. carbinolicus* converts glycerol to 3-hydroxypropionate, and ethylene glycol to acetate, whereas the new isolate oxidizes glycerol to acetate and degrades ethylene glycol to glycolaldehyde,

without growth. In contrast to *D. fructosovorans* and *D. carbinolicus*, strain HDv cannot ferment glycerol and ethylene glycol. *Desulfovibrio* strain HDv can be distinguished from *D. alcoholovorans* by their respective end products of the degradation of glycerol, 1,2- and 1,3-propanediol in the presence of sulfate or in coculture with a methanogen. The main distinctive properties between these *Desulfovibrio* spp. are the inability of strain HDv to produce propionate from 1,2-propanediol degradation, whatever the terminal electron acceptor and to oxidize the 3-hydroxypropionate transitory formed during 1,3-propanediol degradation. Morever, *D. alcoholovorans* differs from strain HDv in being able to convert ethylene glycol to acetate. Furthermore, in contrast to *D. alcoholovorans*, the new isolate ferments dihydroxyacetone and uses fumarate or malate as electron acceptor.

The occurence of 3-hydroxypropionate as additional end product of glycerol degradation only when methanogens were used as terminal electron acceptor, may be due to the inability of these bacteria to decrease the hydrogen threshold concentration to values allowing substrate oxidation to acetate exclusively. Previous coculture experiments indicated that the hydrogen threshold concentration may be a key factor of the formation of end products more reduced than acetate from fructose catabolism in *D. fructosovorans* (Cord-Ruwish et al. 1986), as well as from 1,2- and 1,3-propanediol dissimilation in *D. alcoholovorans* (Qatibi et al. 1991a). The reasons of the inability of *Desulfovibrio* strain HDv to use more than 2 to 3 mM of 1,2- and 1,3-propanediol in syntrophic association with hydrogen-utilizing methanogens are not known.

Preliminary studies of enzymes involved in 1,2-propanediol degradation by cells of *Desulfovibrio* strain HDv and *D. alcoholovorans* grown on this substrate give first hints on its degradative pathway by *Desulfovibrio* strains. Cell-free extract of *Desulfovibrio* strain HDv contained sufficient activities of 1,2-propanediol dehydrogenase, lactate dehydrogenase, pyruvate dehydrogenase, phosphate acetyltransferase, and acetate kinase to assume their involvement in 1,2-propanediol catabolism. Hence, lactaldehyde, lactate, pyruvate, and acetyl CoA would play a central role in such metabolic pathway (Fig. 1). The high pyruvate dehydrogenase activity suggests that it is not only involved in cell carbon assimilation but also in the catabolism. Occurence of propionate and acetate as end products and the relatively low 1,2-propanediol dehydrogenase specific activities measured in *D. alcoholovorans* indicate that, part of this substrate may be degraded via lactaldehyde and another pathway involving: a 1,2-propanediol dehydratase, a CoA-dependent aldehyde dehydrogenase not linked to NAD, a phosphate propionyl transferase, and a propionate kinase. The enzyme activities measured in *D. alcoholovorans* are consistent with such a catabolic pathway that could be somewhat similar to those found in some genera of Enterobacteriaceae and Propionibacteriaceae (Toraya et al. 1979; Ichikawa et al. 1985). The weak polyol dehydrogenase activity observed could explain the relatively low specific growth rate reported by Qatibi et al.

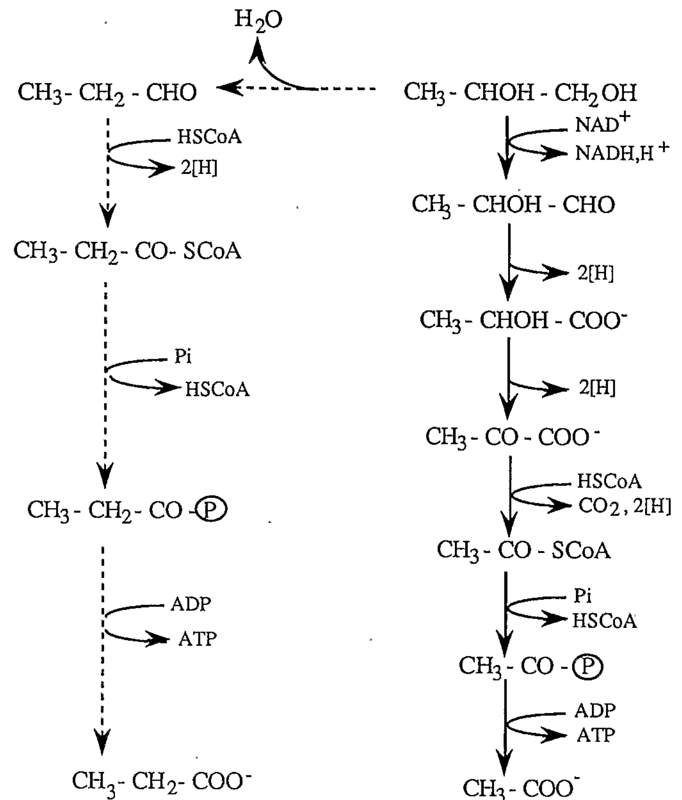

Fig. 1. Proposed pathways of 1,2-propanediol degradation by *Desulfovibrio* strain HDv and *D. alcoholovorans*. *Full lines:* hypothetical pathway of strain HDv. Full and *dotted lines:* hypothetical pathway of *D. alcoholovorans*. *Dotted lines:* hypothetical pathway of *D. desulfuricans* strain DG2 (not supported by enzymatic measurements)

(1991a) for *D. alcoholovorans* cultivated on 1,2-propanediol ($0.09\ h^{-1}$) or 1,3-propanediol ($0.086\ h^{-1}$) as compared to those obtained with glycerol ($0.22\ h^{-1}$). The high specific growth rate observed with glycerol as substrate suggests that the first step of this compound degradation would be rather a phosphorylation than a dehydrogenation.

The sequence of 1,2-propanediol degradation by *D. alcoholovorans* is summarized in Fig. 1. Part of this pathway can be also the one used by *D. desulfuricans* strain DG2 described by Dwyer and Tiedje (1986), which produce exclusively propionate from 1,2-propanediol dissimilation.

Energy substrates used by the most *Desulfovibrio* species are degraded via NAD-independent enzymes (see Postgate 1984). However, a role for NAD(P)-dependent catabolic enzymes was recently established in some sulfate-reducing bacteria (Stams et al. 1984; Kremer and Hansen 1987; Kremer et al. 1988). In this study, we have also demonstrated that during the growth of *Desulfovibrio* strain HDv on 1,2-propanediol or 1,2-propanediol and glycerol, NAD-dependent dehydrogenases play a key role (Table 3). The role of these NAD(P)-dependent dehydrogenases and NAD(P)H-dehydrogenases in alcohol degradation is discussed in detail by Kremer and Hansen (1987), Kremer et al. (1988) and Kremer (1989).

In contrast to the results reported by Kremer and Hansen (1987), we report a high NAD-dependent glycerol dehydrogenase activity during the growth of *Desulfovibrio* strain HDv on 1,2-propanediol. The increase of the NAD-dependent glycerol dehydrogenase specific activity in 1,2-propanediol plus glycerol-grown cell extracts (Table 3) suggests a possible competition between a glycerol kinase and a NAD-dependent dehydrogenase for initiation of glycerol catabolism in *Desulfovibrio* strain HDv.

The NAD-dependent ethanol and propanol dehydrogenase activities measured in 1,2-propanediol-grown cells of *D. alcoholovorans* are always higher than those of polyols, and they decrease in presence of high concentration of polyols (Table 3). This relatively high specificity for primary monovalent alcohols suggests the existence in *D. alcoholovorans*, of only one type of alcohol dehydrogenase weakly active on polyols. The characteristics of this enzyme will be similar to those of the component I of NAD-linked alcohol dehydrogenase reported by Sridhara et al. (1969) in a mutant of *Escherichia coli*. In contrast, the NAD-dependent dehydrogenase of *Desulfovibrio* strain HDv seems to be not specific (Table 3). Furthermore this (these) alcohol and aldehyde dehydrogenase(s) present in strain HDv is (are) an inducible enzyme(s).

Propionate formation from 1,2-propanediol is a common metabolic property of species of genera *Lactobacillus* (Schütz and Radler 1984), *Klebsiella*, *Citrobacter* (Toraya et al. 1978; 1979), *Acetobacterium* (Eichler and Schink 1985), and *Propionibacterium* (Ichikawa et al. 1985). In these organisms, a coenzyme B_{12}-dependent 1,2-propanediol dehydratase is involved in 1,2-propanediol degradation. The lack of this common enzyme could explain the inability of strain HDv to use 1,2-propanediol in the absence of sulfate and to produce propionate in its presence or in coculture with methanogenic bacteria. This is consistent with the quasi stoichiometric conversion of 1,2-propanediol to acetate observed with strain HDv (Table 1). The weak dehydratase activity observed in *D. alcoholovorans* could explain its inability to ferment 1,2-propanediol (or ethylene glycol) in absence of external electron acceptor and the slow growth in coculture experiments [specific growth rate: 0.005 h^{-1} (Qatibi et al. 1991a)]. An intriguing question is why propanol is not produced in coculture experiments if very active NADH-linked propionaldehyde dehydrogenase can be detected in vitro. In *D. alcoholovorans*, the pathway of 1,2-propanediol degradation depends upon a competition between the dehydratase and the dehydrogenase catalyzing the initial step of this substrate catabolism. The mecanism of regulation of the shift toward the "dehydratation" or the "dehydrogenation" pathway needs to be investigated.

Our results suggest that in the two *Desulfovibrio* strains tested, phosphate propionyltransferase and propionate kinase may in fact be the same as phosphate acetyltransferase and acetate kinase, respectively.

On the account of all the differences mentioned above, it is unlikely that *Desulfovibrio* strain HDV is a mutant or a subspecies of *D. alcoholovorans*. Hence, this isolate cannot be ascribed to any *Desulfovibrio* species so far described. DNA-DNA hybridization experiments between *Desulfovibrio* strain HDv and the most closely related *Desulfovibrio* species will be performed soon to determine its taxonomic affiliation.

Strain HDv has been deposited in the German Collection of Microorganisms, Braunschweig, FRG under the collection number DSM 6830.

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