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Anaerobic degradation of hydroaromatic compounds by newly isolated fermenting bacteria

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Abstract. Aerobic organisms degrade hydroaromatic compounds via the hydroaromatic pathway yielding protocatechuic acid which is further metabolized by oxygenase-mediated ring fission in the 3-oxoadipate pathway. No information exists on anaerobic degradation of hydroaromatics so far. We enriched and isolated from various sources of anoxic sediments several strains of rapidly growing gram-negative bacteria fermenting quinic (1,3,4,5-tetrahydroxy-cyclohexane-1-carboxylic acid) and shikimic acid (3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid) in the absence of external electron acceptors. Quinic and shikimic acid were the only ones utilized of more than 30 substrates tested. The marine isolates formed acetate, butyrate, and H_2, whereas all freshwater strains formed acetate and propionate as typical fermentation products. Aromatic intermediates were not involved in this degradation. Characterization of the isolates, fermentation balances for both hydroaromatic compounds, and enzyme activities involved in one degradation pathway are presented.

Key words: Anaerobic degradation – Hydroaromatic compounds – Quinic acid – Shikimic acid

Quinic (1) and shikimic (2) acid are important precursors in the biosynthesis of aromatic compounds (Fig. 1) (Minamikawa 1976; Herbert 1981). In vascular plants, these hydroaromatic carboxylates are stored in considerable amounts as building blocks for synthesis of lignin and phenols (Boudet 1973). Especially gymnosperms and woody dicotyledons contain these compounds at high concentrations (on average 2.21 mg quinic and 0.16 mg shikimic acid per g fresh weight; Yoshida et al. 1975). Quinic acid is also widespread as component of many tannins, esterified with various aromatic acids (e.g. Stoeckigt and Zenk 1974; Möller and Herrmann 1983; Nishimura et al. 1984).

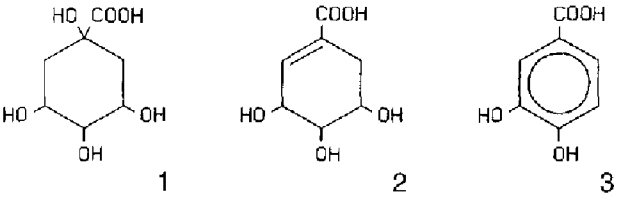

Fig. 1. Structural formulas of quinic (*1*), shikimic (*2*), and protocatechuic (*3*) acid

Oxidative degradation of hydroaromatic compounds by aerobic bacteria and fungi takes the hydroaromatic pathway and yields protocatechuic acid (3) requiring oxygenase-mediated ring fission in the 3-oxoadipate pathway. This pathway has been studied intensively with *Acinetobacter* (Ingledew et al. 1971), *Pseudomonas* (Ingledew and Tai 1972), and several fungi; with *Neurospora crassa* (Geever et al. 1989) and *Aspergillus nidulans* (Beri et al. 1990), also the regulation of this pathway was studied in detail. A hydroaromatic pathway was postulated to occur also in Rhizobiaceae (Parke and Ornston 1984) and streptomycetes (Grund et al. 1990), and was recently demonstrated in the nocardioform actinomycete, *Rhodococcus rhodochrous* (Bruce and Cain 1990).

Little is known about the fate of hydroaromatics in the absence of oxygen. Reduction of the hydroaromatic ring in the presence of suitable electron donors has been studied with lactobacilli (Whiting and Coggins 1971; 1974) and occurs also in the mammalian digestive tracts (Brewster et al. 1978; Wheeler et al. 1979; Martin 1982), but no report exists on degradation of the hydroaromatic ring in anoxic environments.

In this study, we enriched, isolated and characterized several strains of anaerobic, fermenting bacteria from

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Abbreviations: BV, benzyl viologen (1,1'-dibenzyl-4,4'-bipyridinium dichloride); CoA, coenzyme A; CTAB, cetyltrimethylammonium bromide; DCPIP, 2,4-dichlorophenolindophenol; DTT, 1,4-dithiothreitol; MV, methyl viologen, (1,1'-dimethyl-4,4'-bipyridinium dichloride); Tricine, *N*-[tris-(hydroxymethyl)-methyl]-glycine; Tris, tris-(hydroxymethyl)-aminomethane

various anoxic marine and freshwater sediments. These strains degrade hydroaromatics through novel pathways not involving aromatic intermediates.

Materials and methods

Media and cultivation techniques

All strains were isolated and routinely cultivated using the anoxic, bicarbonate-buffered, sulfide-reduced mineral medium of Widdel and Pfennig (1984), with modifications and additions described previously (Schnell et al. 1991). For saltwater enrichments, the medium was supplemented with NaCl (20 g/l) and $MgCl_2 \cdot 6H_2O$ (3 g/l). Hydroaromatic and aromatic substrates were added from filter-sterilized, neutralized stock solutions kept under N_2; other substrates were autoclaved or filter-sterilized. 5% inoculum was used routinely. Stock cultures were grown on quinic acid in completely filled 50-ml screw-cap bottles at 30 °C in the dark, stored at 4 °C, and had to be transferred twice monthly.

Enrichment and isolation

Enrichment cultures with quinic acid (5 mM) were inoculated with anoxic sediments from various origins: (1) organic-rich marine black mud from the canals of Venice, Italy, freshwater sediment from (2) a eutrophic pond and (3) a mildly polluted creek near Tübingen, FRG, and (4) anaerobic sludge from a digester of the Tübingen municipal sewage treatment plant. Pure cultures were obtained by repeated application of the agar-shake dilution technique (Widdel and Pfennig 1984). Purity was checked microscopically and by growth tests in complex medium (AC medium, Difco, Detroit, Mich., USA), with or without added quinic acid (5 mM).

Morphological and cytological characterization

Gram staining (Bartholomew 1962), KOH test (Gregersen 1978), and flagella staining (Blenden and Goldberg 1965) were performed using standard techniques. Endospore formation was tested in a special sporulation medium (Matthies et al. 1989).

Oxidase and catalase tests followed standard procedures (Smibert and Krieg 1981). Superoxide dismutase was assayed according to Elstner et al. (1985). The presence of cytochromes was tested by redox difference spectroscopy of air-oxidized minus dithionite-reduced cell extracts (25 mg protein · ml^{-1}).

DNA was isolated after Cashion et al. (1977), and the guanine-plus-cytosine content of the DNA was determined directly by high-pressure liquid chromatography (Mesbah et al. 1989; Tamaoka and Komagata 1984), using phage Lambda DNA as a reference.

Wet mounts for microphotography (Pfennig and Wagener 1986) and electron micrographs of ultra-thin sections (Matthies et al. 1989) were prepared as described.

Physiological characterization

Substrate utilization tests and growth experiments were performed in 25-ml tubes with butyl rubber septa, filled with 10 ml of sulfide-reduced medium, and gassed with N_2/CO_2 (9 : 1). For determination of pH optima, the medium was supplemented with 2-morpholinoethane sulfonic acid (MES) and *N*-[tris-(hydroxymethyl)-methyl]-glycine (TRICINE), 30 mM each, and adjusted to various pH values with NaOH. Turbidity was measured at 578 nm directly in the culture or in 1-cm cuvettes. Growth yields with quinic acid were determined gravimetrically in 1-l batch cultures (Widdel and Pfennig 1981).

Aerobic growth on hydroaromatic compounds was tested in test tubes with homogenously inoculated agar medium, and in Erlenmeyer flasks with liquid medium, incubated statically or on a rotary shaker (150 rpm). The phosphate-buffered mineral medium described by Platen and Schink (1989) was used.

Enzyme assays

Enzyme activities were determined at 25 °C, either by spectrophotometrical (Brune and Schink 1990b) or discontinuous HPLC assays (Brune and Schink 1990a) using freshly prepared French-Press extracts of quinate-grown cells. All tests were performed under N_2 unless indicated otherwise.

Quinate dehydrogenase (= *catabolic shikimate dehydrogenase*, EC 1.1.1.24) was measured following NAD^+ reduction by the respective hydroaromatic substrate (Bruce and Cain 1990).

Shikimate: NADP 3-oxidoreductase (= *anabolic shikimate dehydrogenase*, EC 1.1.1.25) was measured analogously with $NADP^+$; $NAD(P)^+$-independent ("dye-linked") activities with DCPIP as electron acceptor (Cánovas et al. 1968).

Pyruvate synthase (EC 1.2.99.2) and *2-oxobutyrate synthase* (EC 1.2.7.2.) were measured using the 2-oxoglutarate synthase assay previously described in detail (Brune and Schink 1990b), with BV as artificial electron acceptor, and substituting either pyruvate or 2-oxobutyrate (10 mM each) for 2-oxoglutarate.

Propionyl-CoA: acetate CoA transferase (EC 2.8.3.1.) and *butyryl-CoA: acetate CoA transferase* (EC 2.8.3.-) were determined by discontinuous HPLC analysis of the assay mixture containing Tris-HCl buffer (100 mM, pH 7.3), acetyl-CoA (0.5 mM), and the respective carboxylic acid (10 mM).

3-Hydroxybutyryl-CoA dehydratase (EC 4.2.1.55) was measured analogously, following crotonyl-CoA (0.5 mM) conversion to 3-hydroxybutyryl-CoA, or by a spectrophotometric assay (Platen et al. 1990).

Butyryl-CoA dehydrogenase (EC 1.3.99.2) was assayed spectrophotometrically at 365 nm. The assay mixture contained K-phosphate buffer (100 mM, pH 7.0), NADH (0.3 mM), and crotonyl-CoA (1 mM).

3-Hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.35), *acetoacetyl-CoA thiolase* (EC 2.3.1.9), *phosphotransacetylase* (EC 2.3.1.8) and *acetate kinase* (EC 2.7.2.1) were measured as described (Brune and Schink 1991).

Carbon monoxide dehydrogenase (EC 1.2.99.2) was determined following reduction of BV upon addition of carbon monoxide to the prereduced assay mixture (Diekert and Thauer 1978). *Hydrogenase* (EC 1.18.99.1) was measured analogously.

Protocatechuate 3,4-dioxygenase (EC 1.13.1.3.) and *catechol 1,2-dioxygenase* (EC 1.13.1.1) were assayed spectrophotometrically (Stanier and Ingraham 1954; Hayaishi et al. 1957).

Analytical methods

Acetate, propionate, and butyrate were assayed by gas chromatography with flame-ionization detection (Platen and Schink 1987), hydrogen by gas chromatography with thermal conductivity detection (Matthies et al. 1989). Coenzyme A esters were analyzed by HPLC on a reversed phase system (Brune and Schink 1991). Nitrite formation was assayed after Procházková (1959), sulfide formation half-quantitatively with the assay of Cord-Ruwisch (1985). Protein was quantitated using the micro-protein assay of Bradford (1976) with bovine serum albumin as standard.

Chemicals

D(-)-Quinic acid (1R, 3R, 4S, 5R)-1,3,4,5-tetrahydroxy-cyclohexane-1-carboxylic acid] was obtained in purum quality (>98%) from Aldrich, Steinheim, FRG; D(-)-shikimic acid [(3R, 4S, 5R)-3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid] in >97% quality was from Fluka, Neu-Ulm, FRG; both were used without further purification. Commercial sources of all other chemicals (analytical grade or highest available purity) and gases were as previously published (Brune an Schink 1990b).

Results

Enrichment cultures

All enrichments with quinic acid showed turbidity and gas production after incubation for 2–3 days. Subcultures transferred to fresh medium grew within less than 1 day. After several transfers, uniform bacterial populations had developed. The respective dominating morphotypes were isolated by repeated agar-dilution series, and retransferred into liquid medium.

All marine isolates were non-motile coccoid rods or cocci, and grew rapidly with quinic acid (doubling time 1.5–2 h), forming acetate, butyrate, and hydrogen as fermentation products. The freshwater isolates were motile, rod-shaped bacteria of various sizes, growing slightly slower than the saltwater strains (doubling time ~2.5 h), and forming only acetate and propionate as fermentation products; no hydrogen was detected. All strains formed large, lens-shaped, cream-coloured colonies in the agar medium. The strains VenChi2 (marine inoculum 1) and GolChi1 (freshwater inoculum 2) were selected for further characterization.

Enumeration of quinate-degrading bacteria in the original samples by serial dilution in anoxic liquid medium revealed very low numbers (10^2 10^3 cells per ml) of quinate degraders in the inoculum sediment material.

Morphological and cytological characterization

Figure 2 shows phase contrast photomicrographs of strains VenChi2 and GolChi1. Cells of strain VenChi2 were non-motile, coccoid rods (0.8–1.0 × 1.0–1.5 µm). Strain GolChi1 formed more slender, short rods (0.6 to 0.7 × 1.5–2.5 µm) propelled rapidly by a polar flagellum (Fig. 2C). Electron micrographs of ultra-thin sections showed a typical gram-negative cell wall structure with both strains (not shown), confirming the results of Gram staining and KOH test. Spore formation was never observed, neither in defined media nor in special sporulation media. Strains VenChi2 and GolChi1 had rather different DNA base ratios. Results of the microbiological characterization are summarized in Table 1.

Table 1. Microbiological characterization of strains VenChi2 and GolChi1

strain:	VenChi2	GolChi1
Gram-type	Negative	Negative
DNA G + C ratio (mol%)	35.7 ± 0.1	61.6 + 0.2
Motility	–	+
Flagellation		Polar monotrichous
Growth under air	–	+
Oxidase	–	–
Catalase	–	–
Superoxide dismutase	–	+
Cytochromes	–	–
pH Optimum	6.5–8.0	7.0–7.5
pH Limits	6.0/9.0	6.0/8.0
Temp. optimum[a] (°C)	30	37

[a] not growth at 45 °C

Physiological characterization

Strain GolChi1 grew only in freshwater media, while strain VenChi2 grew well in saltwather and also in brackish media containing at least 7 g NaCl and 0.7 g $MgCl_2$ per 1. No unusual features with respect to pH and temperature optimum were detected (Table 1). Both strains tolerated phosphate buffer (50 mM) without a decrease in growth yield. They did not require a reductant for growth, and could be cultivated in non-reduced mineral media. However, in agar-deep suspension under air, strain VenChi2 did not grow directly at the agar surface; a 10-mm deep clear zone developed. Strain GolChi1 differed significantly with respect to oxygen sensitivity, growing homogenously throughout the agar suspension and, if not agitated, even in an Erlenmeyer flask under air. Superoxide dismutase test was positive only with GolChi1.

No substrates other than quinic or shikimic acid supported growth. No external electron acceptors were reduced, neither with propionate, lactate, nor quinate as electron donors. Table 2 lists the substrates which did not support growth.

No additional growth factors were required by either strain for growth in the vitamin-supplemented medium.

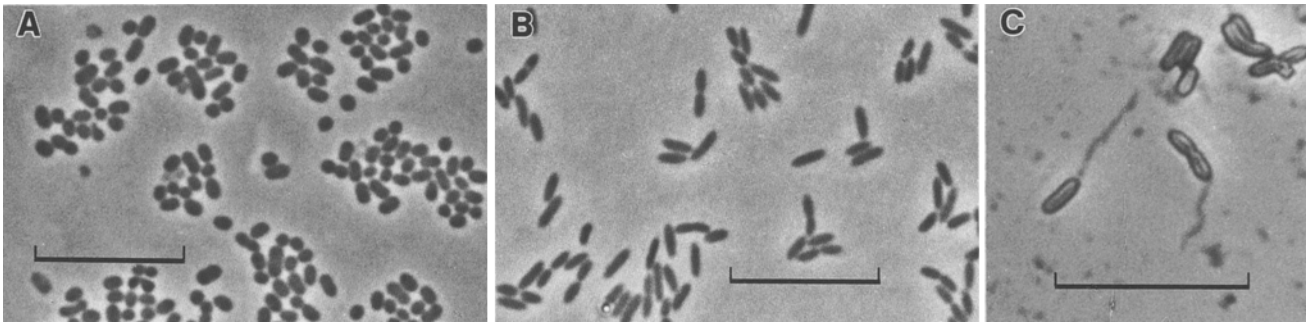

Fig. 2A–C. Phase contrast photomicrographs of strains VenChi2 **(A)** and GolChi1 **(B)**. Flagella stain of strain GolChi1 **(C)**. *Bar* equals 10 µm

Table 2. Compounds tested which did not support growth of strains VenChi2 and GolChi2. Substrate concentrations were 5 or 10 mM (2 or 5 mM for aromatic compounds)

D-Glucose, D-fructose, D-xylose, D-ribose, D-cellobiose, D-lactose, D-erythrose, D-mannitol, meso-erythritol, glycerol
Citrate, pyruvate, L-lactate, DL-malate, fumarate, L-tartrate, sorbate (4 mM), glycolate, ethanol
L-Threonine, L-aspartate, L-glycine, L-alanine
Crotonate, 2-hydroxybutyrate, 3-hydroxybutyrate, 4-hydroxybutyrate, 2-oxobutyrate
Protocatechuate, trimethoxycinnamate, resorcinol, 3,4,5-trimethoxybenzoate, phloroglucinol, gallate
Electron acceptors (10 mM): Nitrate, sulfate, thiosulfate, sulfur, ferric iron (Fe^{3+})

Yeast extract up to 0.2% (w/v) had no influence on yield or rate of growth on quinic acid. Higher concentrations were increasingly inhibitory. Neither strain showed significant growth with yeast extract alone.

Figure 3 shows typical growth curves of both strains on quinic or shikimic acid. Growth rates were almost identical on both substrates (t_d = 1.85 h; μ = 0.375 h^{-1} for strain VenChi2; t_d = 2.65 h; μ = 0.22 h^{-1} for strain GolChi3). If precultivated on the respective substrates, lag phases were short (1–2 h). However, quinate-grown precultures exhibited a lag phase of 10–12 h if transferred to medium with shikimate as substrate.

Fermentation balance

Table 3 shows electron and carbon balances of quinic and shikimic acid fermentation by strains VenChi2 and GolChi1. The strains differed with respect to the fermentation products. While strain VenChi2 formed butyrate, acetate, and small amounts of propionate and H_2, strain GolChi1 formed only acetate and propionate. Since CO_2 was not quantitated, only the electrons and not the carbon in substrate and products are balanced with strain VenChi2. With strain GolChi1, the substrate carbon atoms were completely recovered in the fermentation products indicating that there was not net CO_2 formation.

With both strains, growth yields on quinate were linearly dependent on the substrate concentration up to 10 mM quinic acid; at higher concentrations, the medium pH was shifted beyond the pH optimum of the culture.

Enzyme activities

Table 4 lists the enzyme activities detected in crude extracts of strain VenChi2. Quinate and shikimate were both oxidized with NAD^+; activity with $NADP^+$ and

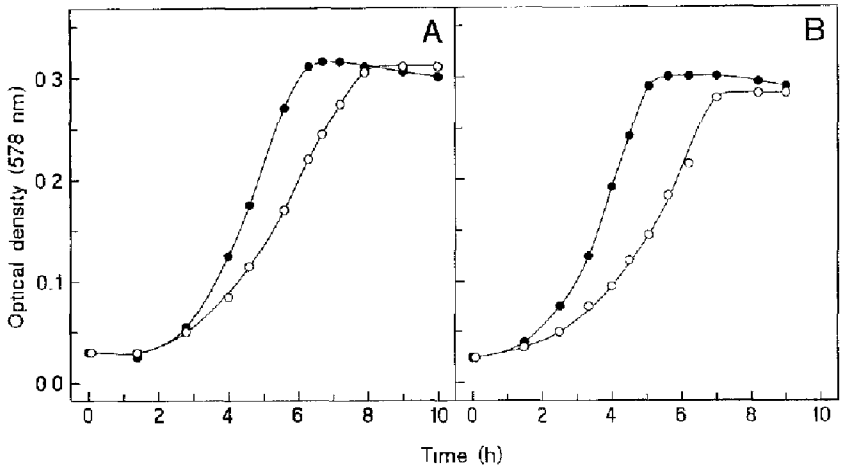

Fig. 3. Growth curves of strains VenChi2 (●) and GolChi1 (○) on quinic (**A**) and shikimic acid (**B**), 5 mM each

Table 3. Carbon and electron balance and growth yields of quinic and shikimic acid fermentation by strains VenChi2 and GolChi1

Substrate	Cell matter formed[a] (µg)	Substr. consumed (µmol)			Products formed (µmol)				Growth yield (g/mol)	Carbon recovery (%)	Electron recovery (%)
		total	assim.[b]	degraded	Acetate	Propionate	Butyrate	H_2			
Strain VenChi2											
Quinate	527	50.0	3.1	46.9	68	5	37	18	10.5	91	106
Shikimate	517	50.0	3.1	46.9	57	5	44	4	10.4	92	106
Strain GolChi1											
Quinate	758	50.0	4.4	45.6	126	23	–	–	15.2	100	104
Shikimate	716	50.0	4.3	45.7	124	25	–	–	14.3	101	105

[a] Calculated from increase in optical density using the experimentally determined coefficients of 198 and 290 mg cell dry matter per 1 of a quinate-grown suspension at OD_{578} = 1 for strains VenChi2 and GolChi1, respectively

[b] Assimilated substrate was calculated using the equation $17\,C_7H_{12}O_6 \rightarrow 28\,\langle C_4H_7O_3\rangle + 7\,CO_2 + 4\,H_2O$ for quinate; i.e. 5.9 µmol quinate were assimilated into 1 mg dry cell matter

"dye-linked" activities were <2% of the NAD^+-dependent activity. No aromatic intermediates such as protocatechuic acid or catechol were detected by HPLC in the hydroaromatic dehydrogenase assays. In addition, neither protocatechuate 3,4-dioxygenase nor catechol 1,2-dioxygenase activity was detectable in cell extracts.

Pyruvate and 2-oxobutyrate were both oxidatively decarboxylated with BV as electron acceptor indicating the presence of ferredoxin-coupled pyruvate and 2-oxobutyrate synthases. Propionyl-CoA : acetate and butyryl-CoA : acetate CoA transferase activities were present, as well as all enzyme activities necessary for butyrate and acetate formation from crotonyl-CoA (Table 4).

Most enzyme activities measured with strain VenChi2 were more than sufficient to support the specific in vivo acitivity of quinate fermentation, which was calculated to be 1.2 $\mu mol \cdot min^{-1} \cdot mg\ protein^{-1}$. For strain GolChi1, the activity was 0.57 $\mu mol \cdot min^{-1} \cdot mg\ protein^{-1}$. Comparable specific activities though were found only for hydroaromatic dehydrogenases and phosphotransacetylase. Dye-linked pyruvate and 2-oxobutyrate oxidoreductase, the enzymes of acetate formation from crotonyl-CoA, and viologen-dependent carbon monoxide dehydrogenase, however, were found only at very low levels (20–50 $nmol \cdot min^{-1} \cdot mg\ protein^{-1}$). No hydrogenase activity was detected.

Discussion

Physiology and biochemistry

The isolated stains are highly specialized strictly fermentative bacteria thriving only on quinic or shikimic acid as sole source of carbon and energy. They are unable to grow on aromatic compounds or on any other organic compound tested, and employ a hitherto unknown pathway for fermentative breakdown of hydroaromatic compounds which is entirely different from the hydroaromatic pathway present in many aerobic bacteria and fungi.

The initial reactions in fermentative hydroaromatic degradation were identical with those found in the aerobic hydroaromatic pathway (Fig. 4). Specificity of the hydroaromatic dehydrogenases for NAD^+ is a rather unusual feature among prokaryotes, which have mostly "dye-linked" dehydrogenases that were recently shown to be membrane-associated pyrroloquinoline quinone-coupled enzymes (van Kleef and Duine 1988). Exceptions are the dehydrogenase in *Lactobacillus plantarum* (Whiting and Coggins 1974) and *Rhodococcus rhodochrous* (Bruce and Cain 1990), which are NAD^+ linked.

Dehydroshikimate dehydratase, however, the enzyme responsible for aromatization to protocatechuate in the hydroaromatic pathway, was not detected in our strains, and no aromatic intermediates were formed in the hydroaromatic dehydrogenase assays. Rapid growth in the absence of molecular oxygen, and absence of protocatechuate and catechol dioxygenase, both key enzymes for the respective branch of the 3-oxoadipate pathway, support the conclusion that no aromatization of hydroaromatic to aromatic compounds occurs. In addition, the existing data on anaerobic protocatechuate or catechol degradation in enrichment cultures all indicate that such a process is extremely slow and thus highly unlikely to underly the rapid metabolism of hydroaromatics by our strains.

Reversal of the aldol condensations occurring in shikimate biosynthesis, leading to a C_3- and a C_4-product, is more probable. Depending on the particular cleavage sites, successive aldoytic and/or hydrolytic cleavages would yield e.g. pyruvate and 2,3-dihydroxybutyrate. Unfortunately, the latter is not commercially available, and neither erythrose (erythrose-4-phosphate is the bio-

Table 4. Enzyme activities in cell extracts of quinate-grown strain VenChi2

Enzyme	Specific activity [$\mu mol \cdot min^{-1} \cdot mg^{-1}$ protein]
Quinate dehydrogenase (NAD^+)	17.6
Shikimate dehydrogenase (NAD^+)	28.6
Pyruvate synthase (BV)	1.61
2-Oxobutyrate synthase (BV)	0.42
Propionyl-CoA : acetate CoA transferase	0.303
Butyryl-CoA dehydrogenase (NADH)	3.54
Butyryl-CoA : acetate CoA transferase	0.500
3-Hydroxybutyryl-CoA dehydratase (HPLC)	2.86
(photometic)	1.69
3-Hydroxybutyryl-CoA dehydrogenase (NAD^+)	3.66
Acetoacetyl-CoA thiolase	1.31
Phosphotransacetylase	14.9
Acetate kinase	16.0
Hydrogenase (BV)	1.67
CO dehydrogenase	n.d.[a]

[a] not detectable

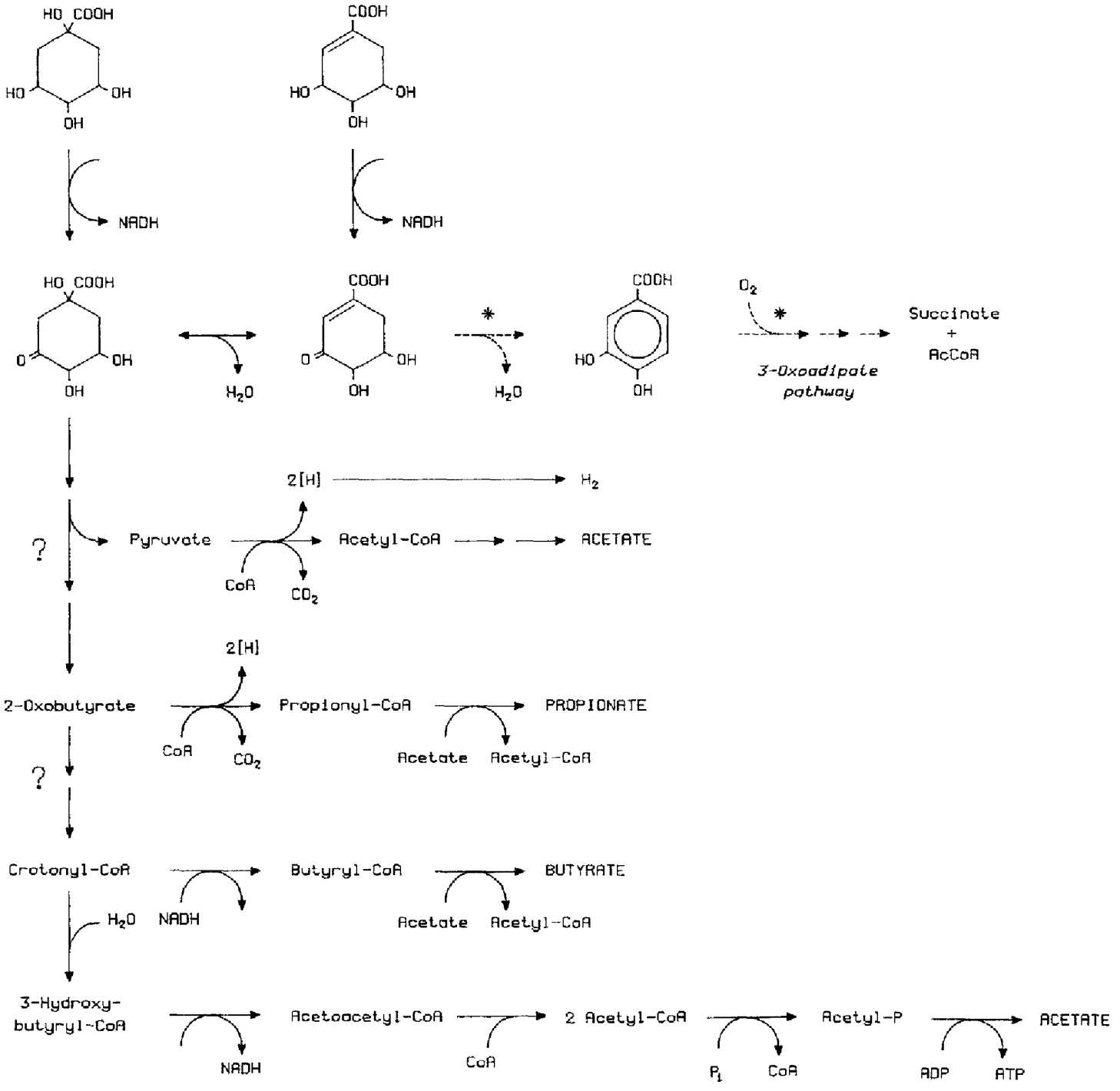

Fig. 4. Pathway proposed for fermentative degradation of hydroaromatic compounds by strain VenChi2. (?) marks reactions not yet understood, (*) are key enzymes of the aerobic hydroaromatic pathway not present in the fermentative strains

synthetic precursor of hydroaromatics in the shikimate pathway) nor 2-oxobutyrate, both isoelectronic with 2,3-dihydroxybutyrate, was a growth substrate for either strain.

Strains VenChi2 and GolChi1 differ with respect to the fate of the aliphatic cleavage products. The fermentation balances of strain VenChi2 with quinate and shikimate (Table 3) agree with the following equation for quinic acid:

$$10\,C_7H_{11}O_6^- + 4\,H_2O \rightarrow$$
$$12\,CH_3COO^- + CH_3CH_2COO^-$$
$$+ 8\,CH_3(CH_2)_2COO^- + 11\,H^+ + 11\,CO_2 + 5\,H_2$$

The products are typical of butyric acid fermentation. Propionate was always found as a minor by-product. It is probably not formed through classical propionic acid fermentation, but by oxidative decarboxylation von 2-oxobutyrate (Fig. 4). Cell extracts of strain VenChi2

possessed a viologen-dependent 2-oxobutyrate synthase (Buchanan 1969) at considerable activities (Table 4). Propionate was formed in a similar manner by suspensions of lactate-grown *Clostridium homopropionicum* cells incubated with 2-hydroxybutyrate (Dörner and Schink 1990).

2-Oxobutyrate could be the product of 2,3-dihydroxybutyrate dehydratase, a reaction resembling tartrate dehydratase (EC 4.2.1.32), dihydroxyacid dehydratase (EC 4.2.1.9), or 3-dehydroquinate dehydratase (EC 4.2.1.10). After reduction to 2-hydroxybutyrate, crotonyl-CoA could be formed by CoA activation and α-elimination of water, a sequence analogous to the acrylyl-CoA pathway of propionate fermentation (Wallhöfer and Baldwin 1967). All enzymes necessary to convert crotonyl-CoA to acetate and butyrate were present at high activities (Fig. 4; Table 4).

The fermentation balance of stain GolChi1 with quinate and shikimate (Table 3) indicates that in this case hydroaromatics are fermented without significant formation of CO_2:

$$10\,C_7H_{11}O_6^- + 7.2\,H_2O \rightarrow$$
$$25.4\,CH_3COO^- + 5.5\,CH_3CH_2COO^- + 20.9\,H^+ + 2.7\,CO_2$$

Two explanations are possible: Either no decarboxylation is involved in the pathway, or strain GolChi1 possesses a homoacetogenic type of metabolism reassimilating CO_2 with the electrons released in the oxidative steps. However, carbon monoxide dehydrogenase activity was detected only at very low levels.

Fermentative degradation of hydroaromatic compounds represents no energetical problem. Quinate and shikimate are at the same redox level as sugars, and comparable ATP yields would be expected. However, the respective growth yields obtained indicate that both strains do not form more than 1–2 ATP per substrate (Stouthamer 1979). Further studies on the metabolic pathways are necessary to clarify these points.

Taxonomy

Both isolates are gram-negative, non-sporeforming bacteria with a fermentative metabolism. They share the so far unique ability to fermentatively degrade hydroaromatic compounds, and are specialized on these substrates only. However, they differ considerably with respect to many morphological, cytological, and physiological traits.

To date there are no other bacteria known that fermentatively degrade hydroaromatic compounds. This reaction appears to represent the only metabolic activity common to both isolates. Both strains are anaerobic bacteria sensu stricto, i.e. they do not employ oxygen as electron acceptor; however, strain GolChi1 proved to be microaerotolerant, superoxide dismutase was positive with this strain. Considering that no easy affiliation with existing genera of anaerobic bacteria (Krieg and Holt 1984) is possible, both strains have to be established as new species as soon as data on their relation to other genera on the basis of rRNA analysis are available.

Strains VenChi2 and GolChi1 were deposited with the Deutsche Sammlung von Mikroorganismen, Braunschweig, under the numbers DSM 6831 and DSM 6832, respectively.

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