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Desulfovibrio inopinatus, sp. nov., a new sulfate-reducing bacterium that degrades hydroxyhydroquinone (1,2,4-trihydroxybenzene)

Abstract A new sulfate-reducing bacterium was isolated from marine sediment with hydroxyhydroquinone (1,2,4-trihydroxybenzene) as the sole electron and carbon source. Strain HHQ 20 grew slowly with doubling times of > 20 h and oxidized hydroxyhydroquinone, lactate, pyruvate, ethanol, fructose, and ribose incompletely to acetate and carbon dioxide, with concomitant reduction of sulfate to sulfide. Cells were large, vibrio-shaped, and gram-negative with a G+C content of 49.7 mol%, and contained desulfoviridin. Based on analysis of the 16S rRNA sequence, strain HHQ 20 was found to be related to the genus *Desulfovibrio* but formed a separate line, thus justifying the establishment of a new species within this genus. Hydroxyhydroquinone was the only aromatic compound utilized among numerous hydroxybenzoates, hydroxybenzenes, methoxybenzoates, and methoxybenzenes tested, suggesting that phloroglucinol and resorcinol are not degradation intermediates. Cell-free extracts of strain HHQ 20 did not contain pyrogallol-phloroglucinol transhydroxylase activity. First experiments indicated that this strain uses a new reductive pathway for anaerobic hydroxyhydroquinone degradation.

Key words Anaerobic degradation · Trihydroxybenzenes · Hydroxyhydroquinone · *Pelobacter massiliensis* · *Desulfovibrio* sp.

Introduction

Hydroxyhydroquinone (1,2,4-trihydroxybenzene) is an intermediary product in the aerobic degradation of hydroxylated aromatic compounds such as phloroglucinol, resorcinol, *p*-hydroxybenzoate, and phenol (Chapman and Ribbons 1976; Karasevich et al. 1976; Patel et al. 1990). In some microorganisms, oxygenases have been found that form maleylacetate from hydroxyhydroquinone, which is further reduced to 3-oxoadipate. The pathway is known as the hydroxyhydroquinone variation to the pathway that usually involves catechol as the intermediate (Middelhoven 1993).

Anaerobically fermenting bacteria such as *Eubacterium oxidoreducens*, *Pelobacter acidigallici*, *Pelobacter massiliensis*, and the homoacetogenic *Holophaga foetida* degrade trihydroxybenzenes through the phloroglucinol pathway (Schink and Pfennig 1982; Krumholz et al. 1987; Brune and Schink 1990; Schnell et al. 1991; Kreft and Schink 1993). One or three transhydroxylation reactions transform pyrogallol (1,2,3-trihydroxybenzene) or hydroxyhydroquinone to phloroglucinol (1,3,5-trihydroxybenzene) (Brune and Schink 1990; Brune et al. 1992), which is subsequently reduced to dihydrophloroglucinol by an NADPH-dependent reductase (Samain et al.1986; Brune and Schink 1992) and is further oxidized to three acetate residues.

Sulfate-reducing bacteria degrade a large number of organic substrates including hydroxyben-zenes, hydroxybenzoates, aminobenzoates, trimethoxybenzoates, and several aldehydes (Hansen 1993). In the present paper, we report on the isolation and description of a new sulfate-reducing bacterium that degrades hydroxyhydroquinone as the sole aromatic substrate.

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Material and methods

Sources of organisms

For enrichment and isolation, one marine sediment sample from Etang des Palmes, France, two marine sediment samples from Venice, Italy, two freshwater sediments from a pond and a creek, and anoxic sewage sludge from the municipal treatment plant in Konstanz, Germany, were used.

The following strains were used for comparative studies: *Desulfomicrobium baculatum* (DSM 1741), *Desulfovibrio desulfuricans* (DSM 642), *Desulfovibrio gigas* (DSM 1382), *Desulfobotulus sapovorans* (DSM 2055), *Desulfovibrio sulfodismutans* (DSM 3696), *Acetobacterium woodii* (DSM 2396), and *H. foetida* (DSM 6591).

Medium and growth conditions

Bacteria were enriched and cultivated in a bicarbonate-buffered, sulfide-reduced anoxic mineral medium with 30 mM sulfate; the medium was modified for freshwater, brackish-water, or sea-water salinity (Widdel and Pfennig 1981). The medium was dispensed in 50-ml serum bottles sealed with butyl rubber stoppers, in 1-l infusion bottles sealed with rubber septa with an N_2/CO_2 (90:10) head space, or in 22-ml screw-capped tubes. For determination of the pH optimum, the medium was buffered with 30 mM *N*-[Tris-(hydroxymethyl)-methyl]-glycine and 30 mM 2-morpholinoethane sulfonate. Substrates were added from concentrated stock solutions. Solutions of oxygen-sensitive compounds were prepared anoxically and sterilized by filtration. Growth was recorded by measuring the optical density at 578 nm.

Pure cultures were obtained by repeated serial dilution in agar media as described by Pfennig and Trüper (1981). Purity was checked microscopically in cultures grown in mineral media with 2 mM hydroxyhydroquinone and in media containing 0.05% (w/v) yeast extract, 5 mM glucose, and 5 mM fumarate.

Analytical procedures

Sulfide was determined as described by Cline (1969). Fatty acids and alcohols were analyzed by gas chromatography (Platen and Schink 1987), aromatic compounds by HPLC (Brune and Schink 1990). Protein was quantified with bovine serum albumin as the standard (Bradford 1976).

Cell-free extracts were examined for cytochrome and desulfoviridin content by spectrophotometry in a double-beam spectrometer Uvikon 930 (Kontron, Zürich, Switzerland). The amount of cytochromes was calculated with $\varepsilon = 190\ mM^{-1}\ cm^{-1}$ for the α-band (Chance and Williams 1955). Cytochromes *b* and *c* were differentiated according to Weston and Knowles (1973). Desulfoviridin was also measured fluorimetrically with an LS 50 fluorimeter (Perkin Elmer, Überlingen, Germany).

The G+C content of the DNA was measured by HPLC after enzymatic digestion of the isolated DNA (Cashion et al. 1977; Mesbah et al. 1989). 16S rDNA was analyzed according to Rainey et al. (1992, 1993). The determination of the G+C content and the sequence comparison of 16S rDNA were performed by F. Rainey (DSMZ, Braunschweig, Germany).

For quantification of cell dry mass, a 1-l culture was gassed with CO_2 to strip off hydrogen sulfide, centrifuged at $20{,}000 \times g$ for 40 min, washed with 200 ml 20 mM ammonium acetate (pH 5.0), centrifuged again, and dried to constant weight.

$\Delta G^{\circ\prime}$ values were calculated according to Thauer et al. (1977); hydroxyhydroquinone was caculated according to Mavrovouniotis (1990, 1991). The value for hydroxyhydroquinone ($-358\ kJ\ mol^{-1}$) can only be estimated since the energetic impact of mutual influence of the hydroxy groups on each other is not considered. The deviation should be within the range of the free energy change of pyrogallol to phloroglucinol ($-15.5\ kJ\ mol^{-1}$; Brune and Schink 1990).

Enzyme assays in cell-free extracts

Cells of a 1-l culture in the late exponential growth phase were harvested in an anoxic chamber (Coy, Ann Arbor, Mich., USA) with an N_2/H_2 atmosphere (95:5 v/v), centrifuged at $13{,}000 \times g$ for 20 min, and washed with 250 ml 100 mM anoxic potassium phosphate buffer (pH 7.0). Cell extracts were prepared anoxically by passing resuspended cells two times through a French press cell at 138 MPa. Cell debris was removed by centrifugation at $20{,}000 \times g$ for 20 min.

Enzyme activities were measured anoxically at 25° C, either discontinuously by HPLC or continuously by photometry. Acetate kinase (EC 2.7.2.1.) and phosphotransacetylase (EC 2.3.1.8.) were assayed according to Bergmeyer et al. (1974a,b). Carbon monoxide dehydrogenase (EC 1.2.99.2.) was measured with benzyl viologen according to Diekert and Thauer (1978). Measurement of transhydroxylase was described by Brune and Schink (1990). Reduction of hydroxyhydroquinone with NADH was measured photometrically as the decrease of NADH at a 365-nm wavelength in oxygen-free 100 mM potassium phosphate buffer (pH 7.0).

Results

Enrichment and isolation of strain HHQ 20

Enrichment cultures with 2 mM trihydroxybenzenoid substrate (gallate, pyrogallol, phloroglucinol, and hydroxyhydroquinone) and 30 mM sulfate were inoculated with 3 ml sediment or sludge. In salt-water cultures, growth started within 1–3 weeks, and in freshwater cultures within 2–8 weeks. Enrichment cultures with gallate or phloroglucinol grew faster and to higher turbidity than those with pyrogallol or hydroxyhydroquinone. Most enrichment cultures ceased to produce sulfide after two to three passages, and short rods resembling *P. acidigallici* dominated. In enrichments with hydroxyhydroquinone and sediment from Venice, large vibrioid cells were observed. From these cultures, strain HHQ 20 was isolated in two subsequent agar dilution series.

Morphology

Strain HHQ 20 formed pale-reddish, lense-shaped colonies in agar. Cells were curved to spirilloid with rounded ends, 1–1.5 m wide, and 4–12 m long (Fig. 1). They were motile

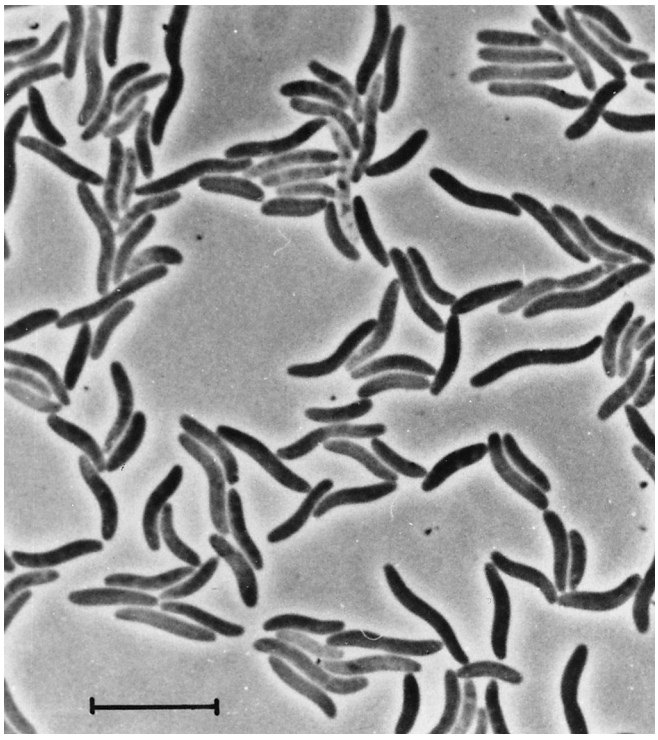

Fig. 1 Phase-contrast photomicrograph of strain HHQ 20 grown with 2 mM hydroxyhydroquinone plus 30 mM sulfate (*bar* 10 m)

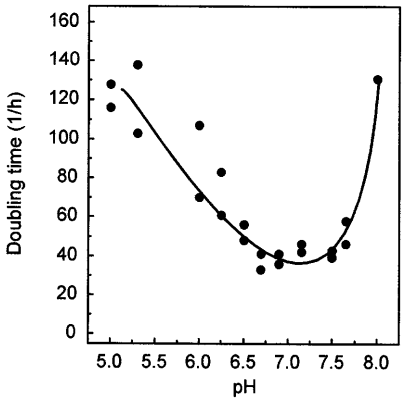

Fig. 2 Doubling times of strain HHQ 20 with hydroxyhydroquinone and sulfate in relation to the prevailing pH

by a single polar flagellum and stained gram-negative. Spores were never formed. No capsules were observed in Indian ink preparations. Sometimes cells contained small dark inclusions, but poly-β-hydroxybutyrate (Jüttner et al. 1975) could not be detected in chloroform extracts. In aging cells, refractile areas reminiscent of plasmolysis became visible. Under nonoptimal growth conditions (e.g., pH 5.5 or 15° C), cells tended to form large aggregates.

Growth conditions and nutrition

Strain HHQ 20 grew with 2 mM hydroxyhydroquinone in media containing 0.5–2% (w/v) NaCl, with an optimum at 1% NaCl. The doubling time was lowest in the range of pH 6.9–7.2 and approximately threefold higher at pH 5 and pH 8 (Fig. 2). Growth was observed between 15 and 42C, with an optimum at 30° C.

With sulfate as the electron acceptor, strain HHQ 20 degraded hydroxyhydroquinone, lactate, pyruvate, malate, fumarate, ethanol, hydrogen, formate, and two sugars (Table 1). The doubling time with lactate was 20 h, with hydroxyhydroquinone 33 h, with fructose 35 h, and with ethanol 80 h. Hydroxyhydroquinone was the only aromatic compound that sustained growth. All tri- and dihydroxybenzene isomers were also tested for degradation by dense suspensions (OD_{578} = 10) of hydroxyhydroquinone-grown cells. Hydroxyhydroquinone was the only compound degraded within 3 h. After several transfers, growth with hydroxyhydroquinone could be reproduced only in the presence of 0.1% (w/v) yeast extract, whereas growth with lactate was always reproducible in mineral medium.

No growth was found with H_2 and CO_2 in the absence of sulfate. Instead of sulfate, thiosulfate (10 mM) or sulfite (2 mM) could be reduced. No growth occurred with lactate plus nitrate (10 mM), sulfite (5 mM), sulfur, or under air. Lactate, pyruvate, fumarate, malate, fructose, or hydroxyhydroquinone were not fermented in the absence of sulfate.

Cultures fed repeatedly with lactate at 10 mM increments reached an optical density (OD_{578}) of 0.9 at a sulfide concentration of 23 mM, but cells still exhibited normal motility.

Table 1 Compounds tested as substrates for growth of strain HHQ 20 with 28 mM sulfate as electron acceptor. Concentrations (in mM) in the culture medium are given *in parentheses*

Utilized substrates:
Lactate (10), pyruvate (10), malate (10), fumarate (10)
Ethanol (10)
H_2 plus acetate (10)
Formate (10) plus acetate (10)
D-(+)-fructose (5), D-(–)-ribose (5)
Hydroxyhydroquinone (2)
Substrates tested, but not utilized:
Succinate (10), citrate (10), glutarate (10), malonate (10)
Acetate (10), propionate (10), butyrate (10), valerate (10), caproate (5)
Methanol (10), propanol (10), butanol (10)
D-(+)-glucose (5), D-(+)-xylose (5), L-(+)-arabinose (5), D-(+)-maltose, D-(+)-cellobiose, inositol (5)
Methylglyoxal (2), acetone (5), acetol (5)
Benzoate (2), 2-hydroxybenzoate (2), 3-hydroxybenzoate (2), 4-hydroxybenzoate (2), 2,3-dihydroxybenzoate (2), 2,4-dihydroxybenzoate (2), 2,5-dihydroxybenzoate (2), 2,6-dihydroxybenzoate (2), 3,4-dihydroxybenzoate (2), 3,5-dihydroxybenzoate (2), phenol (2), resorcinol (2), catechol (2), hydroqzinone (2), pyrogallol (2), phloroglucinol (2), gallate (2), anisol (2), 3-hydroxyanisol (2), 4-hydroxyanisol (2), 2-methoxyhydroquinone (2), 3,4,5-trimethoxybenzoate (2), phenylacetate (2), phenylpropionate (2), nicotinate (2)

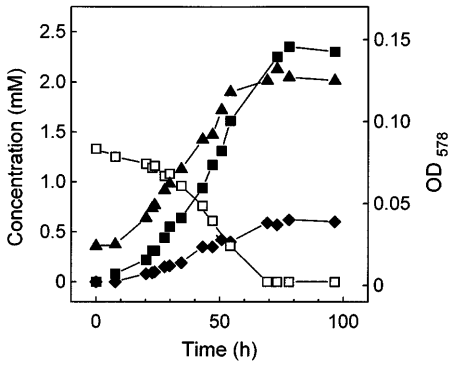

Fig. 3 Time course of sulfate-dependent hydroxyhydroquinone degradation by strain HHQ 20. □ Hydroxyhydroquinone, ▲ OD_{578}, ■ acetate, ◆ sulfide

Growth yields and stoichiometry of substrate oxidation

A growth curve showing cell density, hydroxyhydroquinone degradation, and concomitant sulfide and acetate production is shown in Fig. 3; the stoichiometry of substrate conversion to acetate, cell matter, and sulfide is shown in Table 2. The growth curve shows exponential growth only during the first 50 h and changes to linear growth afterwards. Molar growth yields were 15 g with hydroxyhydroquinone, 7.5–8 g with lactate and ethanol, and 32 g with fructose. Substrate conversions and growth yields agreed with the following equations and reaction energetics ($\Delta G^{\circ\prime}$ values calculated for CO_2 and H_2S in the gaseous state:

Table 2 Growth yields and stoichiometry of sulfate-dependent oxidation of some substrates by strain HHQ 20. Electron balances compare electrons recovered in the products including cell matter with those provided with the substrate. Cell mass formed was calculated via cell density (OD). An OD_{578} of 1.0 equaled 246 mg dry mass l^{-1}. Substrate assimilation was calculated as follows:

$17\ C_6H_6O_3 + 33\ H_2O \rightarrow 24 < C_4H_7O_3 > + 6\ CO_2$
$17\ C_3H_6O_3 \rightarrow 12 < C_4H_7O_3 > + 3\ CO_2 + 9\ H_2O$
$17\ C_2H_6O + 14\ CO_2 \rightarrow 12 < C_4H_7O_3 > + 9\ H_2O$
$17\ C_6H_{12}O_6 \rightarrow 24 < C_4H_7O_3 > + 6\ CO_2 + 18\ H_2O$

Substrate	Substrate utilized (mM)	Accetate produced (mM)	Sulfide produced (mM)	Cell mass formed (mg l^{-1})	Electron-balance (%)	Growth yield (g dry mass mol^{-1})
Hydroxyhydroquinone	1.8	3.3	1.6	27.3	99	15.1
Lactate	10.0	9.6	4.3	74.8	103	7.5
Ethanol	2.5	2.2	1.4	19.7	96	7.9
Fructose	5.0	9.1	4.3	162	113	32.4

$C_6H_6O_3 + H_2O + SO_4^{2-} \rightarrow 2\ C_2H_3^- + 2\ CO_2 + H_2S$
$\Delta G^{\circ\prime} \cong -221.4$ kJ
(mol hydroxyhydroquinone)$^{-1}$

$2\ C_3H_5O_3^- + 2\ H^+ + SO_4^{2-} \rightarrow 2\ C_2H_3O_2^- + 2\ CO_2 + H_2S + 2\ H_2O$
$\Delta G^{\circ\prime} = -87.8$ kJ
(mol lactate)$^{-1}$

$2\ C_2H_6O + SO_4^{2-} \rightarrow 2\ C_2H_3O_2^- + H_2S + 2\ H_2O$
$\Delta G^{\circ\prime} = -69.3$ kJ (mol ethanol)$^{-1}$

$C_6H_{12}O_6 + {}_4^{2-} \rightarrow 2\ C_2H_3O_2^- + 2\ CO_2 + H_2S + 2\ H_2O$
$\Delta G^{\circ\prime} = -375.6$ kJ (mol fructose)$^{-1}$

Pigments and G+C content

Dithionite-reduced *minus* air-oxidized redox difference spectra of cell-free extracts exhibited absorption maxima at 552, 522, and 419 nm, as is typical of *b*- and *c*-type cytochromes. The major part of cytochromes was found in the cytoplasmic fraction and was identified as *c*-type cytochrome after extraction with acetone/HCl. Air-oxidized extract exhibited an absorption maximum at 627 nm, which is typical of desulfoviridin. Excitation with short-wave light (365 nm) in alkaline solution resulted in the emission of red light (731 nm). The DNA base ratio of strain HHQ 20 was 49.7 ± 0.5 mol% guanine+cytosine.

Analysis of 16S rDNA base sequence

On the basis of 16S rDNA sequence comparison, strain HHQ 20 was placed within the δ-subclass of the Proteobacteria (Fig. 4). It showed a close relationship to other members of the *Desulfovibrio*naceae, possibly forming a separate line. There was no significant sequence similarity to that of the pyrogallol-fermenting bacterium *P. acidigallici.*

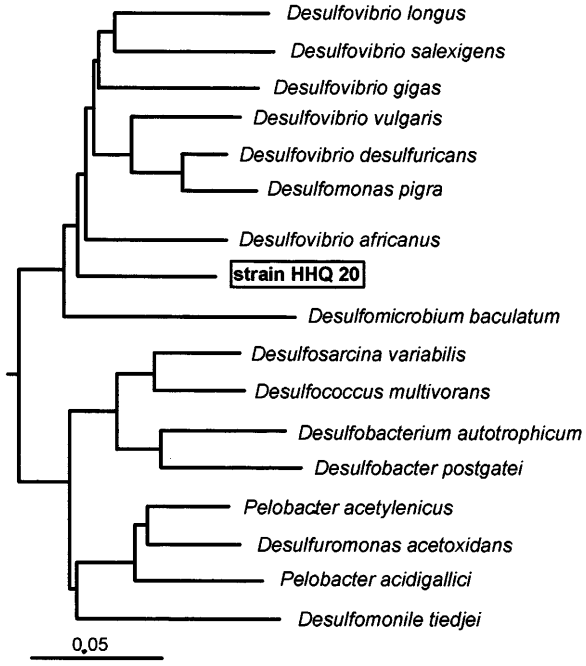

Fig. 4 Phylogenetic dendrogram based on a comparison of 16S rRNA-encoding gene sequences indicating the position of strain HHQ 20 within the radiation of members of the -subclass of the Proteobacteria. The root was determined by inclusion of *Escherichia coli* as an outgroup organism. The *bar* represents 5 nucleotide changes per 100 nucleotides

Enzyme activities

Cell-free extract of hydroxyhydroquinone-grown cells exhibited acetate kinase activity of 1.2 U (mg protein)$^{-1}$. No activity was found after growth with ethanol or lactate. Phosphotransacetylase activity was 44 mU (mg protein)$^{-1}$ in extracts of ethanol-grown cells, while it was below 3 mU (mg protein)$^{-1}$ after growth with hydroxyhydroquinone or lactate. No carbon monoxide dehydrogenase activity was found under any growth conditions.

Cell-free extracts of hydroxyhydroquinone-grown cells were incubated oxygen-free with 1 mM hydroxyhydroquinone, 1 mM hydroxyhydroquinone plus 1 mM 1,2,3,5-tetrahydroxybenzene, or 1 mM pyrogallol plus 1,2,3,5-tetrahydroxybenzene. No decrease of these substrates was observed within 7 h, indicating that no transhydroxylase activity was present. In assays with cell-free extracts incubated with hydroxyhydroquinone (2 mM) and NADH (0.3 mM), a rapid decrease of absorbance at 365 nm was observed. No activity was measured when any of the three components were left out or when the crude extract was

heated for 5 min at 95° C prior to use. he specific activity of NADH oxidation was 410 mU (mg protein)$^{-1}$, which is approximately ten times as high as the in vivo turnover rate of the substrate [44 mU (mg protein)$^{-1}$].

Growth tests with other sulfate reducers and homoacetogenic bacteria

Desulfomicrobium baculatum, *Dv. desulfuricans*, *Dv. gigas*, *Desulfobotulus sapovorans*, and *Dv. sulfodismutans* were tested for utilization of di- or trihydroxybenzenes, each at a concentration of 2 mM. No growth was observed in any case. Good growth with benzoate and little growth with pyrogallol had been reported earlier for strain Cat 2 (Schnell et al. 1989). In our study, strain Cat 2 grew well with benzoate (2 mM), but not at all with pyrogallol or hydroxyhydroquinone. *A. woodii* was found to grow with 2-methoxy-hydroquinone, forming an equimolar amount of hydroxyhydroquinone that was not further degraded. *H. foetida* did not use hydroxyhydroquinone or 2-methoxy-hydroquinone.

Discussion

Strain HHQ 20 was isolated from a marine sediment with hydroxyhydroquinone as the sole carbon and electron source. The strain is of interest for several reasons: (1) it uses hydroxyhydroquinone and hexoses as substrates, (2) it degrades hydroxyhydroquinone obviously by a new reductive pathway, and (3) it represents a new and unusual member of the genus *Desulfovibrio*.

Physiology

Strain HHQ 20 resembles classical *Desulfovibrio*-like sulfate reducers that oxidize pyruvate, lactate, malate, fumarate, and ethanol incompletely to acetate and carbon dioxide (Widdel and Bak 1992). Growth, especially with ethanol, was unusually slow, and this strain could never have been selected in a batch enrichment culture with these substrates. We obtained this strain by enrichment with hydroxyhydroquinone, an unusual aromatic compound that was known to be degraded anaerobically only by a fermenting bacterium, *P. massiliensis* (Schnell et al. 1991). Growth of strain HHQ 20 under optimal conditions with hydroxyhydroquinone (t_d =33 h) is definitively faster than that reported for *P. massiliensis* (t_d =50 h; Schnell et al. 1991) and explains why this sulfate reducer outcompetes the fermenting bacterium in enrichments with sulfate present.

Strain HHQ 20 is a pronounced specialist that used hydroxyhydroquinone as the only aromatic compound among a wide range of different substrates tested. Obviously, this substrate poses some specific problems for anaerobic degradation, and this may be the reason why only few specialists developed suitable degradation pathways. Utilization of aromatic compounds by sulfate reducers is so far known for some generalists such as *Desulfobacterium phenolicum* (Bak and Widdel 1986), *Desulfobacterium catecholicum* (Szewzyk and Pfennig 1987), and *Desulfobacterium anilini* (Schnell et al. 1989), which, however, do not oxidize hydroxyhydroquinone.

Little is known about the natural occurrence of hydroxyhydroquinone, but it is probably not as widespread as phloroglucinol or gallic acid, which are present in several plant tissue constituents such as flavonols, anthocyans, tannin, or lignin. Hydroxyhydroquinone has been found in fungi and some sponges (Cimino et al. 1974; Von Ardenne and Steglich 1974; Jägers et al. 1981) and may be formed during aerobic degradation of lignin. Like pyrogallol, it is highly unstable in the presence of oxygen (Windholz et al. 1983); formation of oxygen-derived reaction products may be partly responsible for its assumed antimicrobial effect (Wratten and Meinwald 1981). Enrichment cultures with hydroxyhydroquinone showed growth less frequently than those with phloroglucinol. This finding may be attributed to the rare occurrence of hydroxyhydroquinone in anoxic environments.

Utilization of sugars is still an unusual capacity among the sulfate-reducing bacteria. Few sulfate reducers grow with carbohydrates, mostly oxidizing monosaccharides such as fructose or glucose (Widdel 1988; Zellner et al. 1989; Trinkerl et al. 1990; Van Niel et al. 1996). Strain HHQ 20 oxidized ribose and fructose, the latter with a doubling time of 35 h, which is approximately half as fast as *Desulfovibrio fructosovorans* (Ollivier et al. 1988). In contrast to strain HHQ 20, *Dv. fructosovorans* is able to ferment fructose to acetate and succinate.

Strain HHQ 20 was unusually tolerant towards sulfide: the cells were still actively motile at a sulfide concentration of 23 mM. Many autotrophic sulfate reducers stop growth at 5–10 mM sulfide, whereas the heterotrophic *Desulfovibrio* species are less sensitive (Brysch et al. 1987). There are only a few exceptional situations in nature in which such high sulfide concentrations could arise. In marine sediments, the concentration of free hydrogen sulfide is in the range of 10–100 M due to precipitation of iron sulfides. In certain salt lakes (e.g., in Lake Mahoney, Canada) at 7 m depth, a free hydrogen sulfide concentration of 7 mM has been measured (Overmann 1991). To our knowledge, higher free sulfide concentrations in natural habitats have not been reported in the literature.

Biochemistry

Anaerobic degradation of pyrogallol and phloroglucinol proceeds through the phloroglucinol pathway as shown for *E. oxidoreducens* (Krumholz and Bryant 1986; Krumholz et al. 1987), *P. acidigallici* (Schink and Pfennig 1982; Brune and Schink 1992), *P. massiliensis* (Brune et al. 1992), *H. foetida* (Kreft and Schink 1993), and is true perhaps also for *Db. catecholicum* (Szewzyk and Pfennig 1987). *P. massiliensis* is the only bacterium able to degrade all three trihydroxybenzene isomers via the same

pathway; it converts hydroxyhydroquinone by a sequence of three transhydroxylations to phloroglucinol (Brune et al. 1992). Strain HHQ 20 did not utilize pyrogallol or phloroglucinol and was phylogenetically only distantly related to *P. acidigallici*. The phylogenetic position of *P. massiliensis* is unknown, but it can be assumed to be close to that of *P. acidigallici*. *H. foetida* is only tentatively grouped within the Proteobacteria. This bacterium can degrade both trihydroxybenzenes and the methoxylated derivatives (Liesack et al. 1994).

Strain HHQ 20 obviously uses a different degradation pathway not involving phloroglucinol, and no transhydroxylase activity could be shown in cell-free extracts. Instead, there was a high activity of an NADH-dependent hydroxyhydroquinone reductase. The product of this reduction is still unknown. The fact that strain HHQ 20 forms only two acetate residues per substrate molecule and has no acetate-degrading carbon monoxide dehydrogenase activity indicates that the six-carbon ring structure is decomposed to two acetate and two one-carbon moieties, rather than three acetate residues as is typical for all other pathways of anaerobic degradation of aromatics (Schink et al. 1992; Fuchs et al. 1994). Future research in our laboratory will have to elucidate this fourth, so far unknown degradation pathway.

Taxonomy

Strain HHQ 20 is a typical member of the family Desulfovibrionaceae (Devereux et al. 1990): cells were gram-negative, large, motile vibrios containing cytochrome *c* and desulfoviridin; they oxidized substrates such as pyruvate, lactate, malate, fumarate, and ethanol incompletely to acetate and carbon dioxide, as is typical of this family (Widdel and Bak 1992). Autotrophic growth was not found, and the G+C content of 49.7 mol% was within the range of 48–65 mol% reported for this family. With respect to size and morphology, the cells of strain HHQ 20 are similar to those of *Dv. gigas* and *Desulfovibrio giganteus*.

These properties match well with the results of 16Sr-RNA sequence analysis, which placed strain HHQ 20 within the -subclass of the Proteobacteria in close relation to *Desulfovibrio* species. Strain HHQ 20 is most similar to *Desulfovibrio africanus* and *Desulfomicrobium baculatum*, which are two of five deep lineages forming the family Desulfovibrionaceae (Devereux et al. 1989, 1990). The other lineages are formed by (1) *Desulfovibrio salexigens* and *Dv. desulfuricans* strain El Agheila, (2) *Dv. desulfuricans* ATCC 27774, *Desulfomonas pigra*, and *Desulfovibrio vulgaris* strain Hildenborough, and (3) *Dv. gigas*. The degradation of trihydroxybenzenes seems to be unusual among the Desulfovibrionaceae or other *Desulfovibrio* species. All five bacteria tested failed to grow with any of these compounds. Therefore, we suggest establishing a new species for strain HHQ 20 that forms a separate line within the family Desulfovibrionaceae (Fig. 4). A formal description of the new species follows.

Desulfovibrio inopinatus sp. nov. in.o.pi.na'tus. L. adj. *inopinatus* unusual, unexpected, referring to utilization of an aromatic compound by a member of the genus *Desulfovibrio*.

Vibrioid cells, 1–1.5 m wide and 4–12 m long, gram-negative, nonsporing, with one single polar flagellum. Strictly anaerobic chemoheterotroph reducing sulfate, thiosulfate, or sulfite and oxidizing H_2 or formate. Ethanol, lactate, pyruvate, malate, fumarate, hydroxyhydroquinone, fructose, and ribose are oxidized incompletely to acetate and CO_2. No other substrates utilized. Growth requires mineral media with sulfide as reductant. Growth is optimal in brackish-water or salt-water medium, with at least 10 g NaCl and 1.5 g $MgCl_2$ per l. pH range 5.0–8.0, optimum at pH 6.9–7.2. Temperature range 15–42C; optimum at 30C. Cells contain cytochrome *c* and desulfoviridin.

DNA base ratio: 49.7 mol% G+C.

Habitat: Anoxic marine and brackish sediments.

Type strain: HHQ 20, deposited as DSM 10711 with the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany).

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