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Isolation and characterization of a new spore-forming sulfate-reducing bacterium growing by complete oxidation of catechol

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Abstract. A new mesophilic sulfate-reducing bacterium, strain Groll, was isolated from a benzoate enrichment culture inoculated with black mud from a freshwater ditch. The isolate was a spore-forming, rod-shaped, motile, gram-positive bacterium. This isolate was able of complete oxidation of several aromatic compounds including phenol, catechol, benzoate, *p*- and *m*-cresol, benzyl alcohol and vanillate. With hydrogen and carbon dioxide, formate or O-methylated aromatic compounds, autotrophic growth during sulfate reduction or homoacetogenesis was demonstrated. Lactate was not used as a substrate. SO_4^{2-}, SO_3^{2-}, and $S_2O_3^{2-}$ were utilized as electron acceptors. Although strain Groll originated from a freshwater habitat, salt concentrations of up to 30 g · l^{-1} were tolerated. The optimum temperature for growth was 35–37 °C. The G + C content of DNA was 42.1 mol%. This isolate is described as a new species of the genus *Desulfotomaculum*.

Key words: Sulfate reduction – *Desulfotomaculum* – Anaerobic catechol oxidation – Degradation and transformation of aromatic compounds

During anaerobic microbial transformation and degradation of lignin-related aromatic compounds only a few intermediary products, like benzoate or phenolic compounds are formed (Healy and Young 1980; Bache and Pfennig 1981; Kaiser and Hanselmann 1982). Anaerobic degradation of phenolic compounds including catechol has been reported for methanogenic mixed cultures and pure cultures of phototrophic, nitrate-reducing and sulfate-reducing bacteria (for review see Evans and Fuchs 1988). Sulfate-reducing bacteria capable to use several aromatic compounds as the only carbon source were almost exclusively isolated from marine habitats. Only *Desulfobacterium catecholicum, Desulfomonile tiedjei, Desulfotomaculum sapomandens*, the thermophilic *Desulfotomaculum thermobenzoicum* and a few strains of *Desulfococcus multivorans* are able to grow in fresh water media (Szewzyk and Pfennig 1987; De Weerd et al. 1990; Tasaki et al. 1991).

Among the sulfate-reducing bacteria belonging to the genus *Desulfotomaculum* only the two species mentioned above have been reported to metabolize aromatic compounds. While *Desulfotomaculum thermobenzoicum* was able to grow only with benzoate, *Desulfotomaculum sapomandens* could also use phenylpropionate, phenylacetate and 4-hydroxybenzoate (Cord-Ruwisch and Garcia 1985; Tasaki et al. 1991). With respect to the degradation of aromatic compounds strain Groll showed a similar versatility as the marine strains, *Desulfobacterium phenolicum* and *Desulfobacterium anilini* (Bak und Widdel 1986; Schnell et al. 1989).

In the present paper we describe an isolate that utilizes aromatic compounds, fatty acids and alcohols as the only electron donor and carbon source. In addition, strain Groll is able to O-demethylate and decarboxylate aromatic compounds. To our knowledge this is the first report on a sulfate-reducing bacterium growing by O-demethylation of an aromatic compound and subsequent complete decomposition of the benzene nucleus.

Materials and methods

Sources of microorganisms

Strain Groll was isolated from an enrichment culture inoculated with a black mud sample from a small freshwater ditch. Benzoate was used as only carbon source and electron donor. *Desulfotomaculum orientis* (DSM 675) was kindly provided by H. Cypionka, University of Konstanz, FRG.

Media and conditions for cultivation

The freshwater basal medium was prepared according to Pfennig et al. (1981). The bicarbonate-buffered (30 mM) medium was reduced with sulfide (1.5 mM), and contained trace element solution SL 10 (1 ml · l^{-1}), and selenite-tungstate solution (2 ml · l^{-1}), and 5 ml · l^{-1} vitamin solution (Widdel and Pfennig 1984). Na-dithionite (8 mg · l^{-1}) was supplemented. Resazurin (0.4 mg · l^{-1}) was added

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as a redox indicator. The pH value of the medium was adjusted to 7.0–7.2. Sterilized srew cap bottles (50 ml) or serum bottles (50 and 100 ml) were completely filled with medium and sealed with latex or butyl rubber stoppers fixed with aluminium seals. For checking autotrophic growth, the serum bottles contained 20 ml medium and 80 ml gas phase (H_2/CO_2: 80%/20%, v/v).

Substrates and electron acceptors were added from neutralized filter-sterilized or autoclaved stock solutions prior to inoculation by sterile syringes. Bicarbonate-free medium was prepared with 10 mM potassium phosphate, pH 7.2. Strain Groll was incubated at 37 °C, whereas *Desulfotomaculum orientis* was cultivated at 30 °C.

Isolation

A pure culture was obtained by repeated application of the agar dilution method (Pfennig et al. 1981).

To check purity, the isolate was inoculated into media containing 0.1% yeast extract plus 0.1% of Bacto-peptone (Difco), glucose, fructose or lactate. After incubation the media were examined microscopically.

Preparation of dense cell suspensions for determining decarboxylation activity

Cells were harvested anaerobically by centrifugation at 13000 × *g* and 4 °C for 20 min. Cells were washed twice with medium without substrate and electron acceptor. Pellets were suspended in medium with 20 mM sulfate in an anaerobic chamber. For experiments with dense cell suspensions, the reaction mixture (20 ml) contained a bicarbonate-buffered (30 mM), sulfide-reduced medium, 20 mM sulfate, 1–1.5 mM aromatic substrate, and 100–250 µM Na-dithionite. The final protein content was 50 to 80 $\mu g \cdot ml^{-1}$. Assays were performed in 25 ml serum bottles closed with butyl rubber septa. At periodical intervalls 0.5 ml samples were withdrawn by sterile syringes and analyzed.

Analytical procedures

Growth was monitored spectrophotometrically at 578 nm. Oxidation of resazurin was prevented by addition of a few crystals of Na-dithionite.

Substrates and products were measured by gas chromatography using 15% SP1220 on Chromosorb WAW 80/100 (Supelco, Bellefonte, USA); glass column, 2 mm × 3 m; column temperature: 160 °C; equipped with a FI-detector). Aromatic compounds were identified by their characteristic UV-spectra (200–400 nm). Prior to analysis sulfide was precipitated by 100 $\mu l \cdot ml^{-1}$ zinc acetate (100 mM). Separation and quantification of aromatic compounds were performed with high performance liquid chromatography (LKB 2152 (Pharmacia/LKB, Freiburg, FRG) using a RP 18 column (Kontron, Eching, FRG) with UV detection at variable wavelength, and using a solvent system of methanol-water-glacial acetic acid (30%/65%/5%, v/v/v) at a flow rate of 2 ml min^{-1}. The pH was adjusted to 3.0. 10 µl samples were directly injected after sterile filtration.

The guanine plus cytosine content of DNA was determined by the thermal denaturation method (Marmur and Doty 1962). Presence of desulfoviridin was tested by the fluorescence test according to Postgate (1956). cytochromes were characterized by monitoring absorption spectra of Na-dithionite-reduced against airoxidized cell-free extracts. Sulfate was analysed as barium sulfate according to Cypronka and Pfennig (1986). Sulfide was determined photometrically by the methylene blue method (Cline 1969). Protein was determined by the method of Bradford (1976) with bovine serum albumin as standard. Cell dry mass was determined as described by Widdel und Pfennig (1981).

Chemicals

All chemicals were of analytical grade.

Results

Enrichment, isolation and morphology

Cultures containing benzoate as the only carbon source and electron donor were inoculated with black anaerobic mud from a freshwater ditch. After 2 weeks of incubation formation of H_2S and growth of large spore forming rods showing slow motility were observed. After several transfers in liquid media the motility was lost, but cells freshly transferred from solid agar media regained motility. From this enrichment culture a pure culture was isolated by repeated dilution in agar (Pfennig et al. 1981). It was named strain Groll after its origin (Grolland, a suburb of Bremen, FRG).

Cells of the isolate were straight rods with pointed ends (Fig. 1), 1–2.5 µm wide and to 4–7 µm long, occurring singly or in pairs. Formation of spherical and central spores was observed, which caused swelling of the cells (Fig. 1). No gas vacuoles have been observed. Spores tolerated 10 min pasteurization at 85 °C. Cells of the exponential phase stained gram negative. Filamentous growth was sometimes observed when cells were transferred to media containing a new substrate.

In other enrichment cultures with benzoate inoculated with anaerobic mud of several freshwater habitats similar types of spore-forming sulfate-reducing bacteria developed together with *Desulfococcus multivorans*-like cells. For enrichment of *Desulfotomaculum* species benzyl alcohol, catechol or vanillate was found to be a selective substrate, because *Desulfococcus multivorans* strains were unable to use these aromatic compounds.

Pigments und G + C content

Determination of the DNA base ratio of the isolate yielded a content of 42.1 (± 1.6) mol% guanine + cytosine. Cytochromes of the *b*-type were detected and evidence for *c*-type cytochromes were also found.

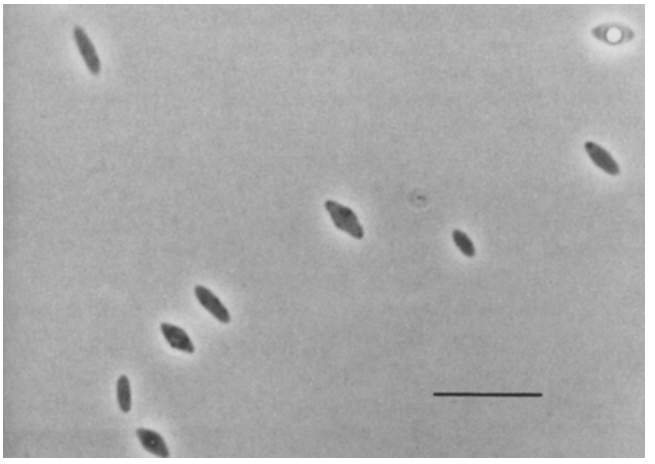


Fig. 1. Phase contrast photomicrograph of *Desulfotomaculum* strain Groll. *Bar* represents 10 µm

Growth conditions and nutrition

Strain Groll grew at 20 to 40 °C, the optimum temperature being at 35–37 °C. The pH range was between 6.0 and 8.0 with an optimum at pH 7.0–7.4. The isolate required no organic supplements. Vitamins were not essential for growth. After transfer to fresh media the isolate required Na-dithionite (100 µM) as additional reductant to initiate growth. Without dithionite rather long lag-phases up to one week were observed.

Strain Groll is a strictly anaerobic sulfate-reducing bacterium, able to grow either chemolithoautotrophically with H_2 as electron donor and CO_2 as only carbon source, or with formate, methoxylated aromatic and several other compounds (Table 1). The isolate was capable to grow with H_2 plus CO_2, formate and methoxylated aromatic compounds in media lacking sulfate by homoacetogenesis as indicated by the production of small amounts of acetate (1–5 mM) if yeast extract was added. Crotonate and pyruvate were completely fermented to acetate. Fatty acids, alcohols, aromatic compounds and other substrates were oxidized completely. Fastest growth occurred with butyrate and butanol with a minimum doubling time of about 12 to 14 h (for benzoate 22–25 h). Increased substrate concentrations resulted in an excretion of acetate. Some aromatic compounds were transformed by O-demethylation or decarboxylation prior to complete oxidation (Fig. 2).

Sodium chloride was not required for growth, although the isolate showed high salt tolerance. Even a sodium chloride concentration of 35 $g \cdot l^{-1}$ was not inhibitory (data not shown). Variations of the initial sulfide concentration (up to 10 mM) in the freshly inoculated media demonstrated that strain Groll was more tolerant to sulfide than other members of the genus (Cord-Ruwisch und Garcia 1985). The maximum OD_{578} was around 0.2 in the presence of 10 mM sulfide added.

Table 1. Organic compounds tested as electron donors and carbon sources in the presence of 10 mM sulfate; added substrate concentrations are given in mM in parentheses

Utilized
H_2 plus CO_2(80%/20%), formate(20), propionate(10), butyrate(5), valerate(5), capronate(3), caprylate(2), caprinate[a](2), isobutyrate(5), 2-methylbutyrate(5), 3-methylbutyrate(5), crotonate(8), ethanol(10), propanol(5), butanol(5), hexanol(5), octanol(2), 2-methyl-1-propanol, 1,3-propanediol(5), pyruvate(10), malate(5), fumarate(5), succinate(5), maleinate(5), benzoate(2), 3-hydroxybenzoate(2), 4-hydroxybenzoate(2), 2-aminobenzoate(2), phenol(2), catechol(2), *m*- und *p*-Cresol(1), 4-hydroxybenzyl-alcohol(1), benzylalcohol(2), 4-hydroxybenzaldehyde(1), benzaldehyde(2), 3,4-dihydroxybenzaldehyde(1), 3,4-dihydroxybenzoate(2), phenylacetate(2), phenylpropionate(2), vanillyl alcohol(1), vanillin(2), vanillate(2), anisol(1), cinnimate(2), ferulate(2)

O-demethylation without further degradation
2,6-dimethoxyphenol(2), 2-methoxybenzoate(2), syringate(2)

Utilized slowly without SO_4^{2-}
Pyruvate(10), crotonate(8), formate(20), H_2 plus CO_2(80%/20%), methoxylated aromatic compunds

Tested but not utilized
Fructose(2), glucose(2), xylose(2), maltose(2), galactose(2), cellobiose(2), methanol(20), methanol(20) plus acetate(2), 2-propanol(5), 2-butanol(5), glycerol(5), cyclohexanol(1), cyclohexanon(1), cyclohexane-carboxylate(2), acetate(10), undecanate(1), laurinate(0.5), myristate(1), palmitate(1.5), stearate(1.5), cysteine(2), glycine(5), aspartate(2), lactate(10), citrate(5), tartrate(5), oxalate(10), malonate(10), adipate(3), 2-hydroxybenzoate(2), phthalate(2), gallate(2), 3-methoxybenzoate(2), 4-methoxybenzoate(2), 2,4-dihydroxybenzoate(2), 2,5-dihydroxybenzoate(2), 3,5-dihydroxybenzoate(2),4-aminobenzoate(2), resorcinol(1), hydroquinone(1), pyrogallol(1), phloroglucinol(1), hydroxyhydroquionone(1), 2-, 3- und 4-aminophenol(0.5), 2,4-diaminophenol(0.5), anilin(0.5), 4-nitrophenol[b](0.5), toluene(0.5), 4-hydroxybiphenyl(0.5), 2-hydroxybiphenyl(0.5), nicotinate(2), phenylalanin(2).

After growth for two weeks at 37 °C each culture was transferred three times in fresh media containing the same substrate. Autotrophic growth was examined through seven repeated transfers.
[a] Utilized slowly within six weeks
[b] Not utilized but reduced to 4-aminophenol, if other electron donors were available

Stoichiometry of substrate oxidation

The stoichiometry of benzoate, protocatechuate, vanillate, phenol, catechol, butanol and butyrate oxidation was determined for *Desulfotomaculum* strain Groll. Results are shown in Table 2. All aromatic compounds tested were completely oxidized to CO_2, although acetate was produced at increased concentrations of benzoate, butyrate and butanol. If grown with initial high butanol concentrations significant amounts of butyrate were formed besides acetate. The molar ratios between substrate oxidized and sulfide produced coincided with the following equations.

Catechol

$$C_6H_6O_2 + 3.25\ SO_4^{2-} + 3\ H_2O \rightarrow 6\ HCO_3^- + 3.25\ HS^- + 2.75\ H^+$$
$$\Delta G^{o\prime} = -259\ kJ \cdot mol\ catechol^{-1} \quad (1)$$

Phenol

$$C_6H_6O + 3.5\ SO_4^{2-} + 3\ H_2O \rightarrow 6\ HCO_3^- + 3.5\ HS^- + 2.5\ H^+$$
$$\Delta G^{o\prime} = -212\ kJ \cdot mol\ phenol^{-1} \quad (2)$$

Protocatechuate

$$C_7H_5O_4^- + 3.25\ SO_4^{2-} + 4\ H_2O \rightarrow 7\ HCO_3^- + 3.25\ HS^- + 2.75\ H^+$$
$$\Delta G^{o\prime} = -259\ kJ \cdot mol\ protocatechuate^{-1} \quad (3)$$

Vanillate

$$C_8H_7O_4^- + 4\ SO_4^{2-} + 4\ H_2O \rightarrow 8\ HCO_3^- + 4\ HS^- + 3\ H^+$$
$$\Delta G^{o\prime} = -359\ kJ \cdot mol\ vanillate^{-1} \quad (4)$$

Benzoate

$$C_6H_5COO^- + 3.75\ SO_4^{2-} \rightarrow 7\ HCO_3^- + 3.75\ HS^- + 2.25\ H^+$$
$$\Delta G^{o\prime} = -186\ kJ \cdot mol\ benzoate^{-1} \quad (5a)$$

$$C_6H_5COO^- + 2.75\ SO_4^{2-} + 4\ H_2O \rightarrow CH_3COO^- + 5\ HCO_3^- + 2.75\ HS^- + 2.25\ H^+$$
$$\Delta G^{o\prime} = -139\ kJ \cdot mol\ benzoate^{-1}. \quad (5b)$$

Table 2. Results of stoichiometric measurements with *Desulfotomaculum* sp. strain Groll on benzoate, catechol, phenol, vanillate, protocatechuate, butyrate and butanol as electron donor and carbon source, and 10 mM sulfate as electron acceptor

Substrate given and utilized [mM]	Acetate excreted [mM]	Cell dry weight formed [mg · l^{-1}]	Substrate consumed for cell material[a] [mM]	Substrate oxidized by sulfate reduction	mol HS formed per mol substrate oxidized	Growth yield: g dry weight per mol substrate oxidized
Benzoate						
1.7	0	42.2	0.23	1.47	3.0	27.0
2.1	1.1	43.2	0.24	1.86	3 8	27.9
Catechol						
1.8	0	32.1	0.20	1.60	3.19	23.2
2.4	0	39.4	0.25	2.15	2.74	18.3
Phenol						
1.9	0	29.7	0.16	1.74	3.56	15.9
Vanillate						
2.2	0	49.6	0.26	1.94	3.56	25.6
Protocatechuate						
2.4	0	41.1	0.27	2.13	3.19	19.3

[a] Substrate consumed for cell material was calculated according to Schnell et al. (1989):
Benzoate:
$17\,C_6H_5COO^- + HCO_3^- + 71\,H_2O \rightarrow 30\,\langle C_4H_7O_3\rangle + 18\,OH^-$; thus 0.0055 mmol benzoate are required for 1.0 mg cell dry weight.
Catechol:
$17\,C_6O_2 + 2\,HCO_3^- + 40\,H_2O \rightarrow 26\,\langle C_4H_7O_3\rangle + 2\,OH^-$; thus 0.0063 mmol catechol are required for 1.0 mg cell dry weight

The $\Delta G^{\circ\prime}$ values (for pH 7.0) were calculated from the data of Thauer et al. (1977) and Kaiser and Hanselmann (1982).

O-demethylation of aromatic compounds

Strain Groll was able to O-demethylate several aromatic compounds. The specifity of O-demethylation was tested with mono-methoxybenzoate isomers and *Desulfotomaculum orientis* as reference strain. Strain Groll O-demethylated only 2-methoxybenzoate, whereas *Desulfotomaculum orientis* utilized only the para-isomer. However, both strains O-demethylated vanillin, vanillate, vanillyl alcohol, anisol, ferulate, 2-methoxyphenol, 2,6-dimethoxyphenol and syringate. Besides this transformation also decarboxylation of protocatechuate to catechol occurred. The same high decarboxylation activity was observed with catechol-grown cells of strain Groll, but not with cells grown with benzoate, phenol, butyrate or butanol. 4- or 3-hydroxybenzoate or other aromatic acids were not decarboxylated and cells grown with these substrates showed no decarboxylation activity of protocatechuate. Aromatic aldehydes were rapidly oxidized to the corresponding acids by strain Groll. Vanillin was oxidized to vanillate which was O-demethylated and decarboxylated to catechol prior to complete oxidation (Fig. 3). Benzyl alcohol was rapidly metabolized without detection of intermediates in the culture fluid. Vanillyl alcohol was first O-demethylated before oxidation of the alcohol group occurred. Concentrations up to 0.5 mM were completely oxidized. At higher concentrations protocatechyl alcohol was excreted, which was not further metabolized. *Desulfotomaculum orientis* showed only O-demethylation of vanillyl alcohol (data not shown).

Degradation of catechol and phenol

Growth of strain Groll with catechol and phenol as only electron donor was only possible in bicarbonate-buffered media, whereas benzoate and butanol allowed also growth in phosphate-buffered media without bicarbonate. The complete oxidation of 2 mM phenol by strain Groll was more than two times slower than of 2 mM catechol, which was completely oxidized within 8 days. Degradation of catechol was studied with dense cell suspensions (50–80 µg protein/ml) of benzoate- and catechol-grown cells of strain Groll. Benzoate-grown cells of strain Groll degraded benzoate and 4-hydroxybenzoate without any lag-phase, whereas degradation of 3-hydroxybenzoate (> 60 h), catechol (> 105 h), protocatechuate (60 h), and phenol (> 190 h) occurred after different prolonged lag-phases. In the case of protocatechuate degradation, catechol was identified as an intermediate by HPLC-analysis.

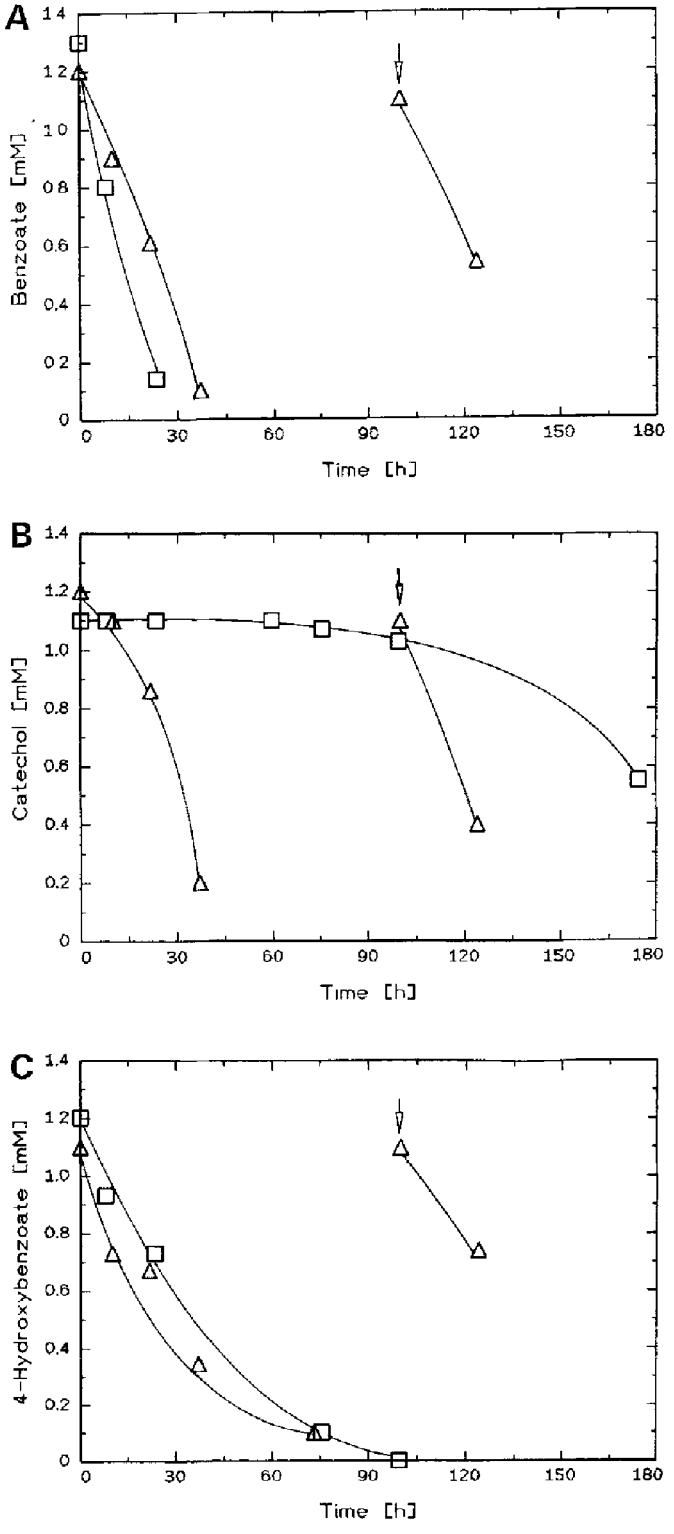
Catechol-grown cells degraded the same substrates without any lag-phases, except the degradation of phenol, which only occurred after a lag-pase of more than 190 h. During protocatechuate degradation catechol was formed as an intermediate. As shown in Fig. 2A–F, catechol-grown cells rapidly degraded catechol, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, and in addition transformed protocatechuate to catechol, whereas phenol was utilized only rather slowly (data not shown).

Discussion

Physiological acpects

To our knowledge the newly isolated strain Groll is the first sulfate-reducing bacterium growing by O-deme-

thylation and subsequent complete mineralization of aromatic compounds. It is also the first freshwater strain of sulfate-reducing bacteria capable of mineralizing phenol and cresols. Table 1 shows that strain Groll utilizes more than 20 different aromatic compounds. With regard to the utilization of aromatic compounds this freshwater isolate is as versatile as the marine isolates, *Desulfobacterium anilini* (Schnell et al. 1989) and *Desulfobacterium phenolicum* (Bak und Widdel 1986).



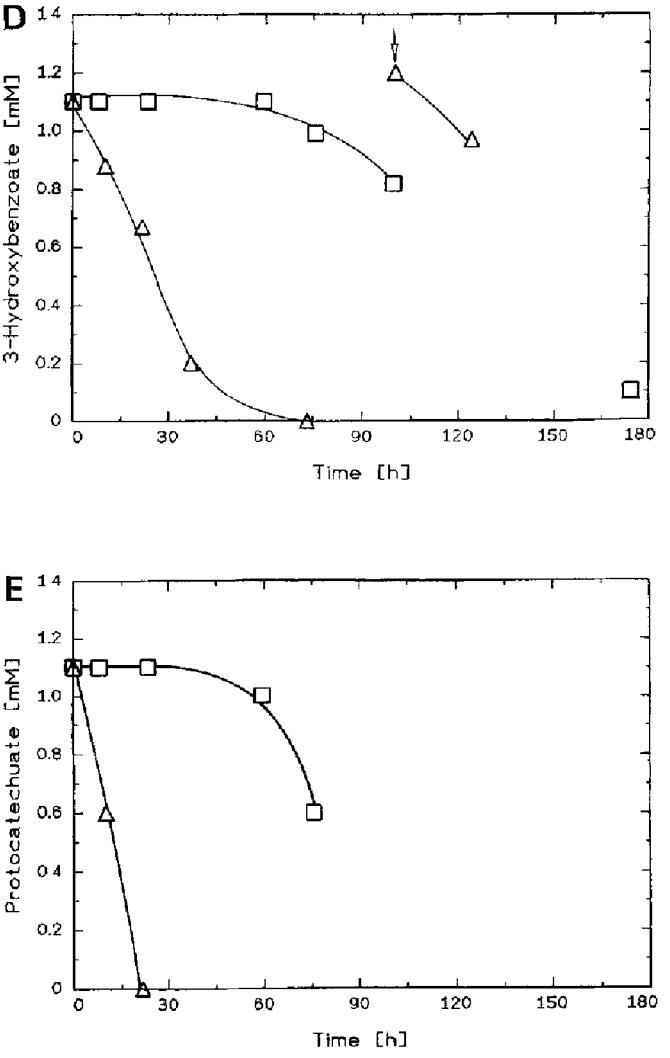


Fig. 2. Degradation of benzoate (**A**), catechol (**B**), 4-hydroxybenzoate (**C**), 3-hydroxybenzoate (**D**) and decarboxylation of protocatechuate (**E**) in dense cell suspensions of strain Groll pregrown with catechol (△) and benzoate (□)
↓ substrate added

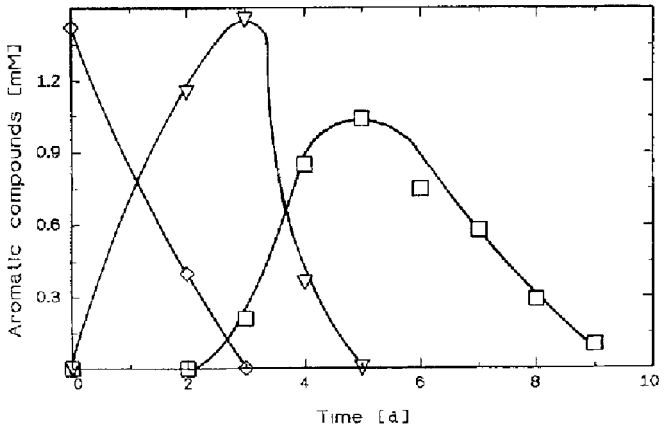


Fig. 3. Time course of vanillin transfromation via vanillate to catechol by strain Groll.
◇, vanillin; ▽, vanillate, □, catechol

Strain Groll is a completely oxidizing sulfate-reducing bacterium, as shown by the stoichiometry of substrate oxidation (Table 2). However, with increased concentrations of butyrate, butanol and benzoate strain Groll produced acetate. Strain Groll oxidized 1 mol substrate to 1 mol acetate. These findings are in agreement with butyrate oxidation by *Desulfobacterium autotrophicum* which is also a complete oxidizer (Schauder et al. 1986). Therefore, it is likely that strain Groll activates butyrate, and perhaps benzoate, by CoA transfer from acetyl-CoA formed from butyryl-CoA by β-oxidation. With increased butanol concentrations (>2 mM) substrate oxidation was incomplete (Table 2), and from 1 mol butanol oxidized two moles acetate were formed. At butanol concentrations higher than 7 mM also butyrate was produced from butanol. This formation of butyrate indicates that the alcohol-oxidizing enzymes were not affected by high sulfide concentrations.

Because of the decarboxylation of protocatechuate by catechol grown cells and the CO_2-dependence of catechol utilization, a carboxylation of catechol to protocatechuate as initial reaction of catechol metabolism by strain Groll is assumed. A reductive dehydroxylation of catechol to phenol as postulated for methanogenic consortia by Balba and Evans (1980) is probably not involved in catechol degradation by strain Groll. We propose a carboxylation of catechol to protocatechuate similar to the carboxylation of phenol to 4-hydroxybenzoate as demonstrated for denitrifying bacteria (Tschech and Fuchs 1989), because our results with strain Groll were in good agreement with the postulated pathway for anaerobic catechol oxidation since all catechol-oxidizing strains are able to utilize protocatechuate, 3-hydroxybenzoate, 4-hydroxybenzoate and benzoate (Szewzyk and Pfennig 1987; Schnell et al. 1989).

The results with strain Groll show that sulfate-reducing bacteria are able to participate in the bioconversion of lignin monomers. The ability to transform aromatic compounds is similiar to that of *Acetobacterium woodii* (Bache and Pfennig 1981) and homoacetogenic *Clostridium* species (Lux et al. 1990).

Taxonomy

We propose to place strain Groll in the genus *Desulfotomaculum*, because the isolate is a spore-forming rod-shaped sulfate-reducing bacterium. Strain Groll differs from other mesophilic strains of this genus with respect to the range of substrates utilized (Widdel 1992). In particular, strain Groll can be distinguished from the other mesophilic members of the genus *Desulfotomaculum* except *Desulfotomaculum orientis* by its ability to grow autotrophically with H_2 plus CO_2 or formate, to use propionate and catechol, and by its G + C content.

Description of Desulfotomaculum sp. strain Groll

Morphology. Straight or slightly curved rods, 1.5–2.5 µm in diameter and 4.5–6.5 µm long, with pointed ends. Spores are spherical and located in the center of cells, causing swelling. Slightly motile. Gram stain of cells in the exponential phase is negative.

Culture conditions. Strict anaerobe. The temperature optimum is 35–37 °C, the pH optimum is between 6.9 and 7.2. Addition of NaCl or vitamins to the medium is not required for growth. Addition of 100 µM Na-dithionite favours initiation of growth.

Growth substrates. Electron donors utilized with sulfate as the electron acceptor: H_2, formate, propionate, butyrate, valerate, capronate, caprylate, *iso*-butyrate, 2-methylbutyrate, 3-methylbutyrate, crotonate, ethanol, propanol, butanol, hexanol, octanol, 2-methyl-1-propanol, 1,3-propanediol, pyruvate, malate, fumarate, succinate, maleinate, benzoate, 3- and 4-hydroxybenzoate, 2-aminobenzoate, phenol, catechol, 3,4-dihydroxybenzoate, phenylacetate, phenylpropionate, aromatic alcohols and aldehydes, methoxylated aromatic compounds. Substrate oxidation is usually complete leading to CO_2, but at high substrate concentrations of butyrate, butanol and benzoate acetate may accumulate.

Electron acceptors. Sulfate, sulfite, thiosulfate and CO_2 serve as electron acceptors, whereas nitrate and sulfur do not.

Biochemical characteristics. Cells contains cytochromes; desulfoviridin is not present. The mol% G + C of DNA is 42.1 ± 1.6 (thermal denaturation).

Source. Black sediment of a freshwater ditch. The strain was deposited in the Deutsche Sammlung von Mikroorganismen (DSM), Braunschweig, FRG under No. 7213.

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References

Bache R, Pfennig N (1981) Selective isolation of *Acetobacterium woodii* on methoxylated aromatic acids and determination of growth yields. Arch Microbiol 130: 255–262

Bak F, Widdel F (1986) Anaerobic degradation of phenol and phenol derivatives by *Desulfobacterium phenolicum* sp. nov. Arch Microbiol 146: 177–180

Balba MT, Evans WC (1980) The methanogenic biodegradation of catechol by a microbial consortium: evidence for the production of phenol through *cis*-benzenediol. Biochem Soc Trans 8: 452–453

Bradford MM, (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Cline E (1969) Spectrophotometric determination of hydrogen-sulfide in natural waters. Limnol Oceanogr 14: 454–458

Cord-Ruwisch R, Garcia JL (1985) Isolation and characterization of an anaerobic benzoate-degrading spore-forming sulfate-reducing bacterium, *Desulfotomaculum sapomandens* sp nov. FEMS Microbiol Lett 29: 325–330

Cypionka H, Pfennig N (1986) Growth yields of *Desulfotomaculum orientis* with hydrogen in chemostat culture. Arch Microbiol 143: 396–399

DeWeerd KA, Mandelco L, Tanner RS, Woese CR, Suflita JM (1990) *Desulfomonile tiedjei* gen nov. and sp. nov., a novel anaerobic, dehalogenating, sulfate-reducing bacterium. Arch Microbiol 154: 23–30

Evans WC, Fuchs G (1988) Anaerobic degradation of aromatic compounds. Ann Rev Microbiol 42: 289–317

Healy JB, Young LY (1980) Anaerobic degradation of eleven aromatic compounds to methane. Appl Environ Microbiol 38: 84–89

Kaiser J-P, Hanselmann KW (1982) Fermentative metabolism of substituted mono-aromatic compounds by a bacterial community from anaerobic sediments. Arch Microbiol 133: 185–194

Lux MF, Keith E, Hsu T, Drake HL (1990) Biotransformation of aromatic aldehydes by acetogenic bacteria. FEMS Microbiol Lett 67: 73–78

Marmur J, Doty P (1962) Determination of the base composition of desoxyribonucleic acid from its thermal denaturation temperature. J Mol Biol 5: 109–118

Pfennig N, Widdel F, Trüper HG (1981) The dissimilatory sulfate-reducing bacteria. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes, vol 1. Springer, New York Berlin Heidelberg, pp 926–944

Postgate JR (1956) Cytochrome C_3 and desulfoviridin; pigments of the anaerobic *Desulphovibrio desulphuricans*. J Gen Microbiol 14: 545–572

Schauder R, Eikmanns B, Thauer RK, Widdel F, Fuchs G (1986) Acetate oxidation to CO_2 in anaerobic bacteria via a novel pathway not involving reactions of the citric acid cycle. Arch Microbiol 145: 162–172

Schnell S, Bak F, Pfennig N (1989) Anaerobic degradation of aniline and dihydroxybenzenes by newly isolated sulfate-reducing bacteria and description of *Desulfobacterium anilini*. Arch Microbiol 152: 556–563

Szewzyk R, Pfennig N (1987) Complete oxidation of catechol by strictly anaerobic sulfate-reducing *Desulfobacterium catecholicum* sp. nov. Arch Microbiol 147: 163–168

Tasaki M, Kamagata Y, Nakamura K, Mikami E (1991) Isolation and characterization of a thermophilic benzoate-degrading, sulfate-reducing bacterium, *Desulfotomaculum thermobenzoicum* sp nov. Arch Microbiol 155: 348–352

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

Tschech A, Fuchs G (1989) Anaerobic degradation of phenol via carboxylation to 4-hydroxybenzoate: in vitro study of isotope exchange between $^{14}CO_2$ and 4-hydroxybenzoate. Arch Microbiol 152: 594–599

Widdel F (1992) The genus *Desulfotomaculum*. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes, 2nd edn, vol 1. Springer, New York Berlin Heidelberg, pp 1792–1799

Widdel F, Pfennig N (1981) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. isolation of new sulfate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov., sp. nov. Arch Microbiol 129: 395–400

Widdel F, Pfennig N (1984) Dissimilatory sulfate- or sulfur-reducing bacteria. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 1. Williams and Wilkins, Baltimore London, pp 663–679