Arch Microbiol (1987) 147:163–168

Archives of Microbiology

Complete oxidation of catechol by the strictly anaerobic sulfate-reducing *Desulfobacterium catecholicum* sp. nov.*

R. Szewzyk and N. Pfennig

Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz, Federal Republic of Germany

Abstract. From an anaerobic enrichment culture with vanillate as substrate, a catechol-degrading lemon-shaped nonsporing sulfate-reducing bacterium, strain NZva20, was isolated in pure culture. Growth occurred in defined, bicarbonate-buffered, sulfide-reduced freshwater medium with catechol as sole electron donor and carbon source. Catechol was completely oxidized to CO_2 with an average growth yield of 31 g cell dry mass per mol of catechol, corresponding to 9.5 g cell dry mass per mol of sulfate reduced. Further substrates utilized as electron donors and carbon sources were resorcinol, hydroquinone, benzoate and several other aromatic compounds, hydrogen plus carbon dioxide, formate, lactate, pyruvate, alcohols including methanol, dicarboxylic acids, acetate, propionate and higher fatty acids up to 18 carbon atoms. Instead of sulfate, sulfite, thiosulfate, dithionite or nitrate served as electron acceptors. Nitrate was reduced to ammonium. Strain NZva20 is the first bacterium in which the complete oxidation of organic substrates is linked to the ammonification of nitrate. Elemental sulfur was not utilized as electron acceptor. In the absence of an electron acceptor slow growth occurred on pyruvate or fumarate. The G + C content of the DNA of strain NZva20 was 52.4 mol%. Cytochromes were present. Desulfoviridin could not be detected. Strain NZva20 is described as type strain of a new species, *Desulfobacterium catecholicum* sp. nov.

Key words: Catechol – Hydroquinone – Resorcinol – Methanol – Complete oxidation – Sulfate reduction – Dissimilatory reduction of nitrate to ammonium – *Desulfobacterium catecholicum*

Catechol (1,2-dihydroxybenzene) was shown to be an important intermediary product of anaerobic degradation of plant material (Simpson et al. 1969; Kaiser and Hanselmann 1982). Degradation of catechol under anaerobic conditions in mud samples or enrichment cultures was found to be slow (Chmielowski et al. 1965; Healy and Young 1978) or even impossible (Kaiser and Hanselmann 1982). The first catechol-degrading culture in defined medium was obtained by Szewzyk et al. 1985. Catechol was quantitatively degraded to acetate, CH_4 and CO_2 by their methanogenic enrichment culture. The authors failed, however, to isolate the catechol-degrading bacteria in pure culture or in defined methanogenic coculture. Bache and Pfennig (1981) reported on the fermentation of methoxyl groups of aromatic compounds by *Acetobacterium woodii*. In two enrichment cultures with vanillate as substrate they observed not only demethoxylation but also complete degradation of the aromatic ring with simultaneous sulfide production. Slow growth occurred when these cultures were transferred into medium with sulfate as electron acceptor and catechol as sole electron donor and carbon source. In the present study the sulfate-reducing bacterium strain NZva20 was isolated in pure culture from one of these catechol-degrading enrichment cultures. The strain which completely oxidized catechol and many other organic substrates to CO_2 is described as a new species of the genus *Desulfobacterium, D. catecholicum* sp. nov.

Materials and methods

Sources of organisms

The catechol-degrading enrichment culture was kindly provided by R. Bache. The original enrichment culture with vanillate as substrate had been inoculated with anoxic mud from the Delaware Bay north-east of Nelson, New Zealand.

Medium and growth conditions

Strain NZva20 grew best in fresh water medium of the following composition (values in g/l): KH_2PO_4, 0.2; NH_4Cl, 0.25; NaCl, 1.0; $MgCl_2 \cdot 6\,H_2O$, 0.4; KCl, 0.5; $CaCl_2 \cdot 2H_2O$, 0.15. Sodium bicarbonate, sodium sulfide, trace element solution SL9, selenite-tungstate solution (Tschech and Pfennig 1984), and vitamin solution (Pfennig 1978) were added to the autoclaved cooled medium from sterile stock solutions as described in detail by Widdel and Pfennig (1981a). The medium was dispensed into 50 ml screw cap bottles. Substrates were added from sterile stock solutions before inoculation. Na-dithionite was added either from a sterile stock solution or as dry crystals, to a final concentration of about 100 µmol/l.

Utilization of electron donors and carbon sources was tested in 20 ml screw cap tubes. Utilization of H_2 plus CO_2 was tested in 20 ml tubes that contained 10 ml inoculated medium and were sealed with butyl rubber stoppers. Growth was followed in the test tubes by turbidity measurements at 578 nm in a Bausch and Lomb/Spectronic 7 spectrophotometer. All growth tests were carried out at least in duplicates at 28° C unless indicated otherwise.

* Affectionately dedicated to Professor Ralph S. Wolfe on the occasion of his 65th birthday

Offprint requests to: R. Szewzyk

Isolation, purity control and stock cultures

Pure cultures were obtained by repeated application of the agar deep dilution method described by Pfennig et al. (1981). Dithionite was added to each tube after inoculation to a final concentration of 100 µmol/l (Pfennig et al. 1981). The tubes were gassed with a mixture of 90% N_2 and 10% CO_2, sealed anaerobically with butyl rubber stoppers and incubated at 28° C. After six weeks of incubation several colonies were picked with a sterile Pasteur pipette and suspended in defined mineral medium. These cultures were checked for purity by microscopic control, by inoculation in Difco AC-medium and in a medium containing sulfate, fumarate, pyruvate and yeast extract. The best growing culture was chosen for further investigations. Stock cultures of strain NZva20 were kept at room temperature, since storage at lower temperatures led to inactivation of the cells. Stock cultures were transferred every 3–4 weeks.

Pigment analyses, DNA base composition

Desulfoviridin was determined as described by Postgate (1956). For the analyses of cytochromes, benzoate grown cells were washed, resuspended in anoxic phosphate buffer (50 mmol/l, pH 7.0), and broken by subsonic treatment. Cytochromes were identified after centrifugation in the cell free supernatant by recording reduced minus oxidized difference spectra with a Shimadzu UV300 spectrophotometer.

The DNA base composition was determined by the thermal denaturation method (Marmur and Doty 1962).

Chemical determinations

Utilization of catechol and other aromatic compounds was followed by recording their UV-absorption spectra between 200 and 340 nm in a Shimadzu spectrophotometer. Formation of alcohols and fatty acids was detected by gas chromatography as described by Widdel and Pfennig (1981a). Sulfide was determined photometrically by the methylene blue method (Cline 1969). In samples for the determination of nitrate, nitrite and ammonium, sulfide was removed by precipitation with zinc acetate as described by Seitz and Cypionka (1986). In the supernatant, nitrate was determined after Cataldo et al. (1975), nitrite by the method of Freier (1964) and ammonium as described by Chaney and Marbach (1962).

Enzyme activities

All enzyme assays were carried out with unbroken, benzoate-grown cells. The cells were washed and resuspended in anoxic phosphate buffer. To all enzyme assays hexadecyltrimethylammonium bromide (CTAB, 0.0125% w/v, final conc.) was added to permeabilize the cell membranes. CO-dehydrogenase activity was measured photometrically by following the rate of methyl viologen reduction with carbon monoxide as described by Diekert and Thauer (1978). 2-Oxoglutarate-ferredoxin-oxidoreductase was measured photometrically as described by Brandis-Heep et al. (1983). Instead of ferredoxin and mercaptoethanol, benzyl viologen and dithionite were used as electron acceptor and reducing agent respectively. A Zeiss PM4 spectrophotometer was used for all enzyme assays.

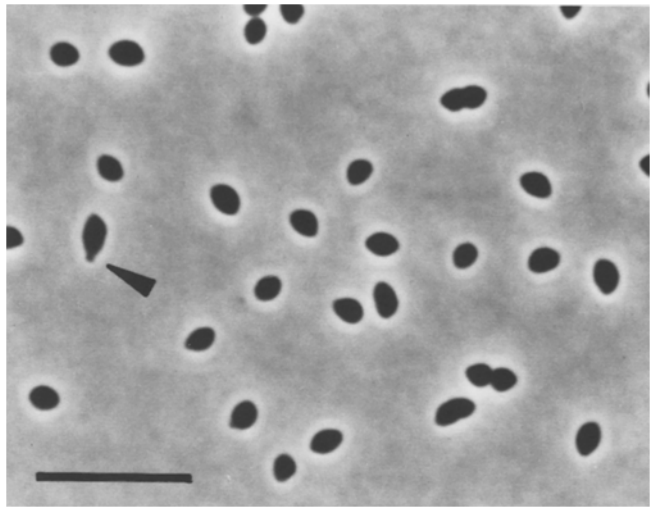

Fig. 1. Phase contrast photomicrograph of strain NZva20 grown on benzoate and sulfate. Cells occasionally formed little buds (see arrow). Bar equals 10 µm

Results

Isolation and culture

Although obtained from an estuarine sediment sample the catechol-degrading enrichment culture grew only in mineral media with NaCl concentrations up to 85 mmol NaCl/l. Since catechol was toxic for the bacteria at concentrations higher than 1 mM, only 0.5 mM catechol was added repeatedly to the enrichment cultures. Within 3–4 weeks 1.5–2 mM catechol were completely degraded, and stoichiometric amounts of sulfide were produced. Isolation was attempted in agar deep dilution series with catechol as substrate and sulfate as electron acceptor. Microscopic control of the brownish colonies obtained in the agar deep cultures revealed that in all colonies flexibacteria were present in addition to the catechol-degrading bacteria. Similar observations were made by Widdel and Pfennig (1977), during isolation of *Desulfotomaculum acetoxidans*. These authors obtained pure cultures after lowering the redox potential by the addition of dithionite to the agar deep cultures. In the present study the addition of dithionite (100 µM) to the agar deep cultures also led to pure cultures of the catechol-degrading bacteria.

Morphology and biochemical characteristics

The pure cultures of strain NZva20 consisted of oval to lemon shaped cells, 1.3–1.8 µm wide and 2.2–2.8 µm long (Fig. 1), which sometimes formed little buds (see arrow). Cells were surrounded by slime and stained Gram-negative. Motility or spores were never detected. The guanine plus cytosine content of the DNA of strain NZva20 was 52.4 ± 0.5 mol% as determined by the thermal denaturation method. Cytochromes were present, but were not analysed in detail. Desulfoviridin could not be detected.

Physiological properties

Strain NZva20 is a strict anaerobic sulfate-reducing bacterium, able to grow either chemolithoautotrophically with H_2 as electron donor and CO_2 as sole carbon source or

Table 1. Compounds tested as electron donors and carbon sources with the sulfate-reducing bacterium NZva20. Concentrations (in mmol/l) in the culture are given in parentheses. The culture medium contained 20 mmol sulfate/l

Substrates utilized:
Catechol (0.5), resorcinol (0.5), hydroquinone (0.5);
Benzoate (2), 4-OH-benzoate (2), protocatechuate (2), 2-aminobenzoate (2);
Phloroglucinol (1), pyrogallol (1), cyclohexanecarboxylate (3);
H_2 plus CO_2, formate (7);
Methanol (10), ethanol (10), propanol (10), butanol (10);
Acetate (10), propionate (10), butyrate (5), caproate (4), dodecanoate (1), tetradecanoate (1), hexadecanoate (1), octadecanoate (1);
Lactate (10), pyruvate (10), malate (5), fumarate (10), glutarate (5), pimelate (2), glutamate (3)
Substrates tested but not utilized:
Phenol (0.5), cyclohexanol (2), 2-OH-benzoate (1), 2,3 di-OH-benzoate (1), nicotinate (1), gallate (1);
Aspartate (2), glucose (2), fructose (2), adipate (2)

chemoorganotrophically with a large number of organic compounds. Strain NZva20 required the addition of growth factors. No growth was obtained after two successive transfers into medium without vitamin solution. The temperature optimum of strain NZva20 was 28° C, the pH optimum at 6.9 – 7.1.

Electron donors. Substrates tested for growth are listed in Table 1. In addition to catechol several other aromatic compounds were utilized. Of all aromatic substrates benzoate allowed fastest growth, with a minimum doubling time of 88 h (μ_{max} = 0.0079 h^{-1}). Phenylpropionate, protocatechuate, 2-aminobenzoate, and phloroglucinol were degraded slower than catechol. From all non-aromatic substrates tested as electron donors and carbon sources, only adipate, aspartate, glucose, and fructose were not degraded by strain NZva20. Fastest growth occurred with ethanol, lactate, pyruvate, fumarate, dodecanoate or tetradecanoate with a minimum doubling time of about 30 h (μ_{max} = 0.023 h^{-1}). Only very slow growth was observed with propanol or pimelate. Autotrophic growth with H_2, CO_2 and sulfate was obtained in seven consecutive batch cultures without decrease in growth rate.

Electron acceptors. With benzoate as substrate, utilization of different electron acceptors was tested with strain NZva20. Instead of sulfate, sulfite (2 mmol/l), thiosulfate (10 mmol/l), dithionite (2 mmol/l) or nitrate (3 mmol/l) were utilized. Nitrate was reduced to ammonium. Nitrite could not be detected as an intermediate reduction product. Elemental sulfur was not reduced, its addition to cultures with sulfate as electron acceptor retarded growth.

With *nitrate as electron acceptor* the range of oxidizable substrates was tested. In addition to all the substrates utilized with sulfate (see Table 1) aspartate and 2-OH-benzoate were oxidized with nitrate. The specific growth yields obtained with nitrate as electron acceptor were always higher than with sulfate. With pyruvate as substrate the

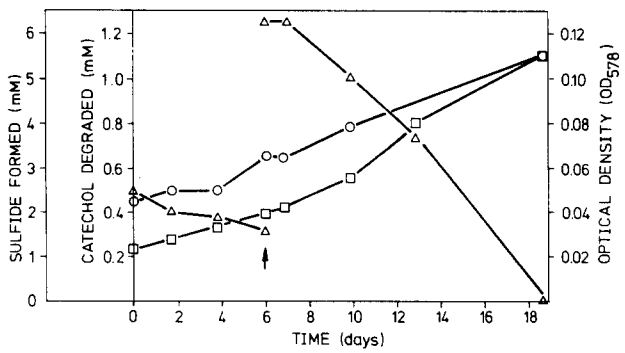

Fig. 2. Time course of catechol oxidation by strain NZva20. The experiment was performed in a 500 ml bottle sealed with a Bellco rubber septum. At times indicated samples for analysis were removed by a syringe and the removed volume was replaced by sterile N_2/10% CO_2 gas mixture. (△) Catechol, (□), sulfide, (○) OD_{578}. ↑ Time when culture was spiked with additional catechol (1 mM)

growth yields were 11.5 and 25.7 g dry cell mass per mol of sulfate or nitrate reduced respectively. Nevertheless initiation of growth was more rapid and consistent with sulfate as electron acceptor. After transfer into fresh medium with nitrate lag phases of several days occurred and the cells often grew in clumps or on the surface of the culture bottles.

To assure that sulfate reduction and nitrate reduction were really capacities of one and the same bacterium, the following agar deep cultures were carried out. Cells of strain NZva20, pregrown for several passages with benzoate and sulfate were diluted out in parallel agar deep cultures containing benzoate (2 mmol/l) as substrate and either nitrate (3 mmol/l) or sulfate (3 mmol/l) as electron acceptor. In both dilution series exactly the same number of colonies developped, indicating that the sulfate-reducing bacteria were able to grow on nitrate as well. The same was true if benzoate/nitrate pregrown cells of strain NZva20 were diluted out in the same way. All colonies picked out of the four agar deep cultures were able to grow in liquid cultures with benzoate as substrate and sulfate or nitrate as electron acceptor. Again growth with sulfate was faster than with nitrate. This was even true for cells, pregrown and diluted out with nitrate as electron acceptor.

In the absence of an electron acceptor pyruvate and fumarate were fermented very slowly. The following other substrates that were tested in the absence of an electron acceptor could not be fermented: Lactate, ethanol, pyrogallol and phloroglucinol.

Quantitative measurements of catechol and butyrate oxidation

Growth of strain NZva20 with catechol as electron donor and carbon source and sulfate as electron acceptor was followed in a 500 ml bottle culture (Fig. 2). 1.5 mmol catechol/l were completely consumed within 3 weeks. Neither volatile fatty acids nor alcohols could be detected as intermediary or end products. 4.25 mmol sulfide/l and 49 mg dry cell mass/l were formed. The amount of catechol assimilated into cell material ($C_4H_7O_3$) was calculated using the following equation:

$$8.5\ C_6H_6O_2 + HCO_3^- + 20\ H_2O \rightarrow 13\ <C_4H_7O_3> + OH^-.$$

Thus 6.34 mmol catechol are assimilated into 1 g dry cell mass. Since 49 mg cell mass were formed, 0.31 mmol of

catechol were used for assimilation and the remaining 1.19 mmol were oxidized. The molar ratio of catechol oxidized (1.19 mmol) to sulfate reduced to sulfide (4.25 mmol) of 1:3.57 is in good agreement with the ratio of 1:3.25 obtained by the following reaction for complete oxidation of catechol:

$$4\,C_6H_6O_2 + 13\,SO_4^{2-} + 12\,H_2O \rightarrow 24\,HCO_3^- + 13\,HS^- + 11\,H^+.$$

During oxidation of butyrate with sulfate, acetate was formed as intermediate product which was slowly oxidized lateron. 4.5 mmol butyrate/l gave rise to 28 mg dry cell mass/l and 9.5 mmol sulfide/l. 0.24 mmol butyrate were assimilated into the cell material according to the equation:

$$8.5\,C_4H_7O_2^- + 9.5\,H_2O + 6\,HCO_3^- \rightarrow 10\,<C_4H_7O_3> + 14.5\,OH^-.$$

The molar ratio of the remaining butyrate oxidized (4.26 mmol) to sulfide formed (9.5 mmol) of 1:2.23 agrees well with the ratio of 1:2.5 for complete oxidation of butyrate according to the following equation:

$$2\,C_4H_7O_2^- + 5\,SO_4^{2-} \rightarrow 8\,HCO_3^- + 5\,HS^- + H^+.$$

Quantitative measurements of acetate oxidation with nitrate

Oxidation of acetate with nitrate as electron acceptor was followed after three successive passages in acetate/nitrate medium in a 500 ml culture, containing 11 mmol acetate/l. Nitrate was added repeatedly in amounts of 2–3 mmol/l to give a final concentration of 7 mmol nitrate/l. 6.8 mmol acetate/l and 5.8 mmol nitrate/l were utilized within 4 weeks. 30 mg dry cell mass/l and 5.3 mmol ammonium/l were formed. Nitrite could not be detected as intermediate product of nitrate reduction. The amount of acetate assimilated into cell material was calculated using the following equation:

$$17\,C_2H_3O_2^- + 11\,H_2O \rightarrow 8\,<C_4H_7O_3> + 2\,HCO_3^- + 15\,OH^-.$$

Thus 0.6 mmol acetate were assimilated into 30 mg cell material. The remaining 6.2 mmol acetate were oxidized with 5.8 mmol nitrate being reduced to ammonium (ratio 1:0.94). These results agree well with the reaction for a complete oxidation of acetate according to the following equation (ratio 1:1):

$$C_2H_3O_2^- + NO_3^- + H_2O + H^+ \rightarrow 2\,HCO_3^- + NH_4^+.$$

Enzymes involved in acetate oxidation

Cells of strain NZva20, cultivated with benzoate and sulfate as substrates, contained CO-dehydrogenase activity. No activity of the 2-oxoglutarate-ferredoxin-oxidoreductase could be detected.

Discussion

Physiology

Degradation of catechol was so far only reported for undefined methanogenic enrichment cultures (Healy and Young 1978, 1979; Balba et al. 1979; Szewzyk et al. 1985). The sulfate reducing bacterium strain NZva20 described in this paper is the first anaerobic bacterium in pure culture, able to degrade catechol. Growth occurred in defined mineral medium supplemented only with vitamins. Quantitative determinations of substrates and products revealed that catechol, butyrate and acetate were completely oxidized to CO_2. The reducing equivalents from dissimilated substrates were recovered in sulfide, formed from sulfate or in ammonium formed from nitrate. No acetate was found at the end of growth on all other substrates used, indicating complete oxidation of these compounds, too. Besides catechol many other aromatic compounds were used by strain NZva20, including resorcinol and hydroquinone, but not phenol. This was surprising because highly enriched methanogenic cultures of catechol or hydroquinone-degrading bacteria described so far were not able to degrade resorcinol, but most of them degraded phenol (Szewzyk et al. 1985). In contrast all defined cocultures of resorcinol-degrading bacteria with a *Campylobacter* species were not able to degrade catechol, hydroquinone or phenol (Tschech and Schink 1985). Two different degradation pathways have been proposed for these aromatic compounds: direct reduction to cyclohexanedione for resorcinol (Tschech and Schink 1985) and reductive dehydroxylation to phenol for catechol and hydroquinone (Szewzyk et al. 1985). Since strain NZva20 degraded all three hydroxybenzenes but not phenol, either another degradation pathway is present in this sulfate reducer or strain NZva20 used both pathways side by side, lacking a phenol uptake system.

If grown with butyrate, acetate was formed as an intermediate oxidation product and excreted in significant amounts into the medium. After complete degradation of butyrate the excreted acetate was oxidized. Most likely, the rate-limiting step in complete oxidation of organic substrates is the oxidation of acetate and not the reactions leading to acetyl-CoA. Intermediate formation of acetate from organic substrates has also be found for other strains of completely oxidizing sulfate reducers (Widdel and Pfennig 1981b; Imhoff-Stuckle and Pfennig 1983; Laanbroeck et al. 1984). In contrast to strain NZva20 further oxidation of acetate was found to be extremely slow in some cases.

Oxidation of acetate by completely oxidizing sulfate reducers may proceed via two different pathways. For *Desulfobacter* species it has been shown that acetate was oxidized via the citric acid cycle (Brandis-Heep et al. 1983; Gebhardt et al. 1983; Schauder et al. 1986). In many other sulfate reducing bacteria, which lack a complete citric acid cycle and 2-oxoglutarate-ferredoxin-oxidoreductase, a reversal of the acetate synthesis from 2 CO_2 was found to be used for complete acetate oxidation (Schauder et al. 1986). Since cells of strain NZva20 contained CO-dehydrogenase activity and no activity of the 2-oxoglutarate-ferredoxin-oxidoreductase it is reasonable to conclude that strain NZva20 oxidized acetate via the novel C_1-pathway and not via the citric acid cycle.

In good agreement with the operation of the novel acetate oxidation pathway is the capacity of strain NZva20 to utilize methanol as an electron donor substrate. Four strains of methanol-oxidizing sulfate reducing bacteria have been described so far (Braun and Stolp 1985; Klemps et al. 1985; Nanninga and Gottschal 1986). Only one of them, *Desulfotomaculum orientis*, was capable of utilizing methanol as sole electron donor and carbon source (Klemps et al. 1985).

The most striking feature of strain NZva20 was its ability to couple complete oxidation of all organic substrates used

to the ammonification of nitrate. Many facultatively anaerobic fermentative bacteria are able to reduce nitrate to ammonium. They use this reaction mainly as electron sink, instead of reducing intermediates of substrate oxidation. In this way additional substrate level phosphorylation via the acetate kinase reaction is possible. But none of these bacteria is capable of completely oxidizing organic substrates. Dissimilatory reduction of nitrate to ammonia has been found in certain sulfate-reducing bacteria (Widdel and Pfennig 1982; McCready et al. 1983; Keith and Herbert 1983; Mitchell et al. 1986; Seitz and Cypionka 1986); they all belong to the genera *Desulfobulbus* or *Desulfovibrio* which carry out incomplete substrate oxidation. Besides NZva20 none of the complete oxidizing species of sulfate reducing bacteria of the genera *Desulfobacter, Desulfobacterium, Desulfotomaculum, Desulfosarcina* or *Desulfonema* has been found to grow with nitrate as electron acceptor (Widdel and Pfennig 1984; Widdel 1987). Therefore, strain NZva20 is the first anaerobic bacterium, in which the complete oxidation of organic substrates is linked to the ammonification of nirate.

Strain NZva20 was growing rather slowly on all substrates tested. This was not due to an insufficient supply with growth factors because addition of 0.2% pepton, tryptone or yeast extract did not enhance the growth rate on e.g. benzoate as substrate. It appears that strain NZva20 is a typical representative of the slow growing autochthonous microflora of anaerobic sediments. This point of view is underlined by the wide variety of substrates and electron acceptors used by strain NZva20.

Taxonomy

The newly isolated bacterium, strain NZva20, cannot clearly be classified with one of the existing genera of sulfate reducing bacteria. By its morphology and its ability to completely oxidize a wide range of substrates, including aromatic compounds, strain NZva20 resembles *Desulfococcus multivorans*. However, strain NZva20 can be clearly distinguished from *D. multivorans* by its ability to grow autotrophically with H_2 plus CO_2, to use fumarate and malate and by lacking Desulfoviridin. Since it is impossible to affiliate all newly isolated sulfate reducing bacteria according to their genetic relationships, a new genus, *Desulfobacterium,* has been proposed for all recently isolated rod-shaped to coccoid sulfate reducers that completely oxidize a wide variety of substrates (Widdel 1986). Many morphological and physiological properties of strain NZva20 agree well with the characteristics of the genus *Desulfobacterium* and strain NZva20 is therefore affiliated to this genus. As strain NZva20 differs from all *Desulfobacterium* species described so far by its G + C content of 52 mol%, its ability to oxidize catechol and by alternatively coupling complete oxidation of organic substrates to ammonification of nitrate, it appears justified to establish a new species, *Desulfobacterium catecholicum* sp. nov. The genus *Desulfobacterium* was recently described by Bak and Widdel (1986).

Desulfobacterium catecholicum

ca.te.cho'li.cum. M.L. adj. catecholicum pertaining to catechol (1,2-dihydroxy-benzene). *Desulfobacterium catecholicum:* a sulfate reducing bacterium oxidizing catechol.

Cells oval to lemon-shaped, 1.3–1.8 μm wide and 2.2–2.8 μm long, singly or in pairs, surrounded by slime. Gram-negative, nonsporing, non motile. Strictly anaerobic, chemoorganotrophic, facultatively chemolithoautotrophic. Sulfate, sulfite, thiosulfate, dithionite or nitrate serve as electron acceptors. Nitrate reduced to ammonium. Elemental sulfur not reduced. Catechol, resorcinol, hydroquinone, benzoate, 4-OH-benzoate, protocatechuate, 2-aminobenzoate, phloroglucinol, pyrogallol, cyclohexane-carboxylate, H_2 plus CO_2, formate, methanol, ethanol, propanol, butanol, acetate, propionate, butyrate, caproate, dodecanoate, tetradecanoate, hexadecanoate, octadecanoate, lactate, pyruvate, malate, fumarate, glutarate, pimelate, glutamate completely oxidized to CO_2. Not used: phenol, cyclohexanol, 2-OH-benzoate, 2,3 di-OH-benzoate, nicotinate, gallate, aspartate, adipate, glucose, fructose. Pyruvate and lactate slowly fermented.

Addition of growth factors and of a reducing agent (100 μM dithionite) is necessary. No growth in medium with NaCl concentrations exceeding 85 mmol/l. pH-optimum: 6.9–7.1, temperature optimum: 28° C.

Cells contain cytochromes, desulfoviridin is not present. Carbon monoxide dehydrogenase activity demonstrated.

The mol% G + C of the DNA is 52.4 ± 0.5 (thermal denaturation). Type strain: DSM 3882 (strain NZva20, Nelson, NZ.), deposited in the Deutsche Sammlung von Mikroorganismen, Göttingen.

Acknowledgements. The authors are indepted to Friedhelm Bak and Hans-Jürgen Seitz for helpful discussions. We thank Waltraud Dilling for the determination of the G + C content of the DNA. This work was supported by the Deutsche Forschungsgemeinschaft.

References

Bache R, Pfennig N (1981) Selective isolation of *Acetobacterium woodii* on methoxylated aromatic acids and determination of growth yields. Arch Microbiol 130:255–261

Bak F, Widdel F (1986) Anaerobic degradation of indolic compounds by sulfate-reducing enrichment cultures, and description of *Desulfobacterium indolicum* gen. nov. sp. nov. Arch Microbiol 146:170–176

Balba MT, Clarke NA, Evans WC (1979) The methanogenic fermentation of plant phenolics. Biochem Soc Trans 7:1115–1116

Brandis-Heep A, Gebhardt NA, Thauer RK, Widdel F, Pfennig N (1983) Anaerobic acetate oxidation to CO_2 by *Desulfobacter postgatei*. 1. Demonstration of all enzymes required for the operation of the citric acid cycle. Arch Microbiol 136:222–229

Braun M, Stolp H (1985) Degradation of methanol by a sulfate reducing bacterium. Arch Microbiol 142:77–80

Cataldo DA, Haroon M, Schrader LE, Young VL (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant Anal 6:71–80

Chaney AL, Marbach EP (1962) Modified reagents for the determination of urea and ammonia. Clin Chem 8:130–132

Chmielowski J, Grossmann A, Labuzek S (1965) Biochemical degradation of some phenolics during methane fermentation. Zeszyty Naukowe Politechniki Śląskiej 133:97–123

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

Diekert GB, Thauer RK (1978) Carbon monoxide oxidation by *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. J Bacteriol 136:597–606

Freier RK (1964) Wasseranalyse. de Gruyter, Berlin

Gebhardt NA, Linder D, Thauer RK (1983) Anaerobic acetate oxidation to CO_2 by *Desulfobacter postgatei*. 2. Evidence from

^{14}C-labelling studies for the operation of the citric acid cycle. Arch Microbiol 136:230–233

Healy JB, Young LY (1978) Catechol and phenol degradation by a methanogenic population of bacteria. Appl Environ Microbiol 35:216–218

Healy JB, Young LY (1979) Anaerobic degradation of eleven aromatic compounds to methane. Appl Environ Microbiol 38: 84–89

Imhoff-Stuckle D, Pfennig N (1983) Isolation and characterization of a nicotinic acid-degrading sulfate-reducing bacterium, *Desulfococcus niacini* sp. nov. Arch Microbiol 136:194–198

Kaiser JP, Hanselmann KW (1982) Aromatic chemicals through anaerobic microbial conversion of lignin monomers. Experientia 38:167–175

Keith SM, Herbert RA (1983) Dissimilatory nitrate reduction by a strain of *Desulfovibrio desulfuricans*. FEMS Microbiol Lett 18:55–59

Klemps R, Cypionka H, Widdel F, Pfennig N (1985) Growth with hydrogen, and further physiological characteristics of *Desulfotomaculum* species. Arch Microbiol 143:203–208

Laanbroeck HJ, Geerlings L, Sijtsma L, Veldkamp H (1984) Competition for sulfate and ethanol among *Desulfobacter, Desulfobulbus,* and *Desulfovibrio* species isolated from intertidal sediments. Appl Environ Microbiol 47:329–334

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

McCready RGL, Gould WD, Cook FD (1983) Respiratory nitrate reduction by *Desulfovibrio* sp. Arch Microbiol 135:182–185

Mitchell GJ, Jones JG, Cole JA (1986) Distribution and regulation of nitrate and nitrite reduction by *Desulfovibrio* and *Desulfotomaculum* species. Arch Microbiol 144:35–40

Nanninga HJ, Gottschal JC (1986) Isolation of a sulfate reducing bacterium growing with methanol. FEMS Microbiol Ecol 38:125–130

Pfennig N (1978) *Rhodocyclus purpureus* gen. nov. and sp. nov., a ringshaped vitamin B_{12}-requiring member of the family Rhodospirillaceae. Int J Syst Bacteriol 28:283–288

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 I. Springer, Berlin Heidelberg New York, pp 926–940

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

Simpson FY, Jones GA, Wolin EA (1969) Anaerobic biodegradation of some bioflavonoids by microflora of the rumen. Can J Microbiol 15:972–974

Seitz H-J, Cypionka H (1986) Chemolithotrophic growth of *Desulfovibrio desulfuricans* with hydrogen coupled to ammonification of nitrate or nitrite. Arch Microbiol 146:63–67

Szewzyk U, Szewzyk R, Schink B (1985) Methanogenic degradation of hydroquinone and catechol via reductive dehydroxylation to phenol. FEMS Microbiol Ecol 31:79–87

Tschech A, Pfennig N (1984) Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. Arch Microbiol 137:163–167

Tschech A, Schink B (1985) Fermentative degradation of resorcinol and resorcyclic acids. Arch Microbiol 143:52–59

Widdel F (1987) Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In: Zehnder ABJ (ed) Environmental microbiology of anaerobes, chapter 10. John Wiley, New York London

Widdel F, Pfennig N (1977) A new anaerobic, sporing, acetate-oxidizing, sulfate-reducing bacterium, *Desulfotomaculum* (emend.) *acetoxidans*. Arch Microbiol 112:119–122

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

Widdel F, Pfennig N (1981b) Sporulation and further nutritional characteristics of *Desulfotomaculum acetoxidans*. Arch Microbiol 129:401–402

Widdel F, Pfennig N (1982) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. II. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen. nov. sp. nov. Arch Microbiol 131:360–365

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

Received September 26, 1986/Accepted December 2, 1986