INTERNATIONAL JOURNAL OF SYSTEMATIC BACTERIOLOGY, July 1997, p. 818–824 Vol. 47, No. 3
0020-7713/97/$04.00+0

Dethiosulfovibrio peptidovorans gen. nov., sp. nov., a New Anaerobic, Slightly Halophilic, Thiosulfate-Reducing Bacterium from Corroding Offshore Oil Wells

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A strictly anaerobic thiosulfate-reducing bacterium was isolated from a corroding offshore oil well in Congo and was designated strain SEBR 4207^T. Pure culture of the strain induced a very active pitting corrosion of mild steel, with penetration rates of up to 4 mm per year. This constitutes the first experimental evidence of the involvement of thiosulfate reduction in microbial corrosion of steel. Strain SEBR 4207^T cells were vibrios (3 to 5 by 1 μm), stained gram negative, and possessed lateral flagella. Spores were not detected. Optimum growth occurred in the presence of 3% NaCl at pH 7.0 and 42°C. Strain SEBR 4207^T utilized peptides and amino acids, but not sugars or fatty acids. It fermented serine, histidine, and Casamino Acids, whereas arginine, glutamate, leucine, isoleucine, alanine, valine, methionine, and asparagine were only used in the presence of thiosulfate. Peptides were fermented to acetate, isobutyrate, isovalerate, 2-methylbutyrate, H_2, and CO_2. The addition of either thiosulfate or sulfur but not sulfate increased peptide utilization, growth rate, and biomass; during growth, H_2S was produced and a concomitant decrease in H_2 was observed. The addition of either thiosulfate or sulfur also reversed H_2 inhibition. 16S rRNA sequence analysis indicates that strain SEBR 4207^T is distantly related to members of the genus *Thermoanaerobacter* (83% similarity). Because the phenotypic and phylogenetic characteristics cannot be assigned to any described genus, strain SEBR 4207^T is designated as a new species of a new genus, *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov. Strain SEBR 4207^T has been deposited in the Deutsche Sammlung von Mikroorganismen und zellkulturen GmbH (= DSM 11002).

In 1989, Elf Congo experienced corrosion of the first 5 km of a 23-km main subsea pipeline that transported sour oil (i.e., H_2S-containing petroleum) produced from the Emeraude oil field. The corroded segment was replaced, but it corroded again a year later. The whole line was then replaced and operated under a specifically designed biocide treatment regimen. The preliminary examination of the corroded iron showed that the breakthrough was due to bacterial pitting corrosion, with an unusually high penetration rate of about 1 cm per year. Chemical analysis of the pipeline water revealed the presence of up to 0.5 mM thiosulfate. It is likely that the thiosulfate was produced as a result of oxidation of the H_2S naturally present in the oil field ecosystem by oxygen that is introduced in the pipelines during processing (10, 15, 22). The corrosion of pipelines was suspected to be due not only to sulfate but also to thiosulfate reduction by sulfate-reducing bacteria (SRB), because computer modeling had shown that thiosulfate reduction could induce the pitting corrosion of steel at higher rates than sulfate reduction (13).

Since the pipeline was under a biocide treatment regimen during our microbiological investigations, the production fluids from several wellheads upstream of the line were collected and analyzed. Besides different SRB species (42), several strains of non-sulfate-, thiosulfate-reducing anaerobic bacteria were isolated. We report here on the characterization of one of these strains that induced intense pitting corrosion of mild steel in the presence of thiosulfate. This strain represents a new genus of the domain *Bacteria*, *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov.

(Part of this work was presented at the Annual Meeting of the American Society of Microbiology, New Orleans, La., 19 to 23 May 1996 [29].)

MATERIALS AND METHODS

Sample collection and sample source. Strain SEBR 4207^T was isolated from the Emeraude oil field, which is an offshore oil-producing well (BB325, Congo). Water samples were aseptically collected as described previously (2). The *in situ* temperature of the oil field was 38°C, and the total salinity was 52 g/liter.

Bacterial counts. Acridine orange direct counting was used to determine the total number of bacterial cells in the sample (21). Cultivable bacteria were enumerated by the three-tube most-probable-number (MPN) procedure. A saline solution was used for the preparation of all culture media in order to mimic the composition of the reservoir water. It contained (per liter) 13 mg of $SrCl_2$, 3.907 g of $MgCl_2 \cdot 6H_2O$, 0.148 g of Na_2SO_4, 2.021 g of $CaCl_2 \cdot 2H_2O$, 31.841 g of NaCl, 1.336 g of KCl, 0.054 g of NH_4Cl, 3.030 g of $NaHCO_3$, and 0.001 g of $FeCl_2 \cdot 2H_2O$. Half-strength tryptone-yeast extract-glucose (TYG) medium (HSTYG) was used to grow fermentative anaerobes (5). SRB detection kits (Labège) were used for enumerating sulfate-reducing bacteria (27). Methanogenic bacteria were grown with either sodium acetate (10 mM), methanol (40 mM), or H_2-CO_2 (2 bars [2×10^5 Pa]) as the substrate.

Enrichment, isolation, and growth conditions. Strain SEBR 4207^T was purified by streaking the inoculum from the last positive dilution of HSTYG tube on HSTYG agar plates (2.5% Noble agar; Difco Laboratories, Detroit, Mich.) in an anaerobic chamber (La Calhène, Vélizy, France) followed by incubation at 30°C under anaerobic conditions. For subsequent studies, a different medium (DP) was used. Medium DP contained (per liter) 1 g of NH_4Cl, 0.3 g of K_2HPO_4, 0.3 g of KH_2PO_4, 3.0 g of $MgCl_2 \cdot 6\ H_2O$, 0.1 g of $CaCl_2 \cdot 2H_2O$, 30 g of NaCl, 1.0 g

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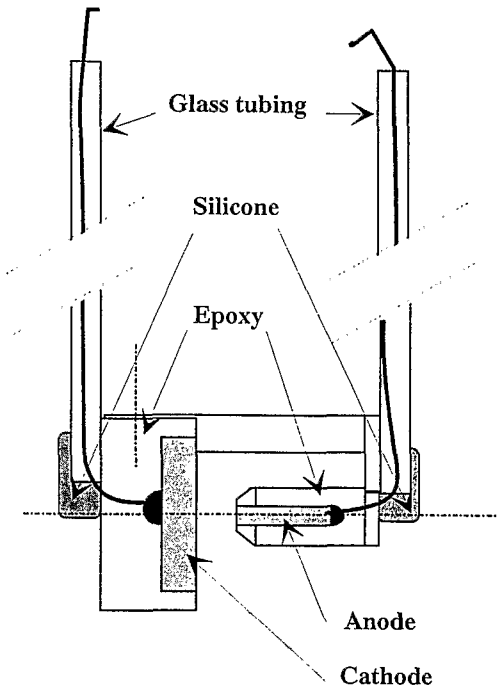

FIG. 1. Electrodes used in corrosion experiments.

of KCl, 0.5 g of cysteine-HCl, 0.5 g of CH_3COONa, 1.0 g of yeast extract (Difco Laboratories), 5.0 g of bio-Trypticase (bioMérieux, Craponne, France), 10 ml of a trace mineral element solution (1), and 1 mg of resazurin. The pH was adjusted to 7.3 with 10 M KOH. The medium was boiled under a stream of O_2-free N_2 gas and cooled to room temperature. Five milliliters of the medium was distributed into Hungate tubes under a stream of N_2-CO_2 (80:20) and autoclaved for 45 min at 110°C. Prior to inoculation, 0.1 ml of $Na_2S \cdot 9H_2O$ (from a 2% sterile anaerobic stock solution) and 0.1 ml of Na_2CO_3 (from a sterile 10% anaerobic stock solution) were injected into the tubes. Thiosulfate (0.1 ml from a sterile 1 M anaerobic stock solution) was added to the medium when required.

Corrosion experiments. The influence of strain SEBR 4207^T on the pitting corrosion of mild steel was evaluated by measuring the free-flowing current between the anode and the cathode of a simulated pit. This simulated pit consisted of a small anode kept 6 mm from a larger cathode (Fig. 1). The surface ratio of the electrodes was about 160 to 1. The anode and the cathode were preconditioned to ensure their adequate functioning as previously described (6, 7). A 100-μA galvanostatic current was applied between the anode and an auxiliary anode connected together (to avoid an excessive current on the anode) and the cathode. Mean current densities were 122 $\mu A/cm^2$ on the anode and 20 $\mu A/cm^2$ on the cathode. The preconditioning was maintained as long as necessary for the bacterial biofilm to develop on the electrodes. Then, after the preconditioning was stopped, a zero amperemeter was used to measure the current flowing between the anode and the cathode. Once expressed in millimeters per year (mm/y), this current represents the actual penetration rate inside the pit. The medium composition, the electrochemical setup, and the sterilizing procedure have been described previously (7).

pH, temperature, and sodium chloride ranges for growth. Growth studies were performed with medium DP. For pH studies, prereduced media that had been dispensed into Hungate tubes were adjusted to the desired pH with $NaHCO_3$ or Na_2CO_3 (added from 10% sterile anaerobic stock solutions). Growth of SEBR 4207^T was tested at temperatures ranging from 20 to 45°C. To determine the salt requirement for growth, sodium chloride was either weighed directly into tubes (for concentrations greater than 1%) or injected from a 10% sterile stock solution (for concentrations less than 1%) to obtain the desired concentrations.

Substrate utilization. Substrate utilization tests were performed with modified DP medium containing 20 mM thiosulfate and bio-Trypticase at 1 g/liter. Substrates were injected into prereduced sterile anaerobic medium from sterile stock solutions. The substrates tested were carbohydrates at 20 mM (arabinose, fructose, galactose, glucose, lactose, maltose, mannose, rhamnose, ribose, sucrose, sorbose, trehalose, and xylose) and fatty acids at 10 mM (acetate, propionate, butyrate, lactate, and citrate). Amino acids were tested at 10 mM in either the presence or the absence of thiosulfate (alanine, arginine, asparagine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, and valine).

Light and electron microscopy. Light microscopy was performed as previously described (9). Exponentially grown cells were negatively stained with 1% sodium phosphotungstic acid (pH 7.2). For preparation of thin sections, exponentially grown cells were centrifuged and fixed for 1 h in 3% (wt/vol) glutaraldehyde prepared in HCl-cacodylate buffer (pH 6.0) containing 0.7 M sucrose and fixed again overnight in 1% (wt/vol) osmium tetroxide prepared in 0.75 M sucrose. The cells were then washed and embedded in 2% agarose and stained with 4% uranyl acetate. The agar was cut into small cubes, dehydrated in acetone, and embedded in Araldite. Thin sections were stained with 5% uranyl acetate for 20 min and with 2% lead citrate for 10 min. Electron microphotographs were taken with a Hitachi model H600 electron microscope at an accelerating voltage of 75 kV.

Cytochrome analysis. Cell extracts were examined for the presence of cytochrome and desulfoviridin as already reported (33).

Analytical techniques. Unless otherwise indicated, experiments were conducted in duplicate. Growth was measured at 580 nm by inserting tubes directly into a Shimadzu (Kyoto, Japan) UV-160A spectrophotometer. Sulfide was determined photometrically as collodial CuS according to the method of Cord-Ruwisch (12). H_2 and fermentation products (alcohols and fatty acids) were measured as described previously (17).

Determination of G+C content. DNA was extracted and purified by chromatography on hydroxyapatite. The guanine-plus-cytosine (G+C) content was determined by high-performance liquid chromatography by the method of Mesbah et al. (31). Nonmethylated lambda DNA (Sigma) was used as a standard (8).

16S rRNA sequence studies. Amplification of the 16S rRNA from semipurified DNA followed by purification of the amplified product was performed as described previously (26, 38). The sequence was determined with an ABI automated DNA sequencer by using a Prism dideoxy terminator cycle sequencing kit and the protocols recommended by the manufacturer (Applied Biosystems Inc.). The 12 primers used for sequencing have been described previously (38). The 16S ribosomal DNA sequence was manually aligned with reference sequences of various members of the domain *Bacteria* by using the alignment editor ae2. Reference sequences were obtained from the Ribosomal Database Project (25, 30). A phylogenetic analysis was performed with various programs implemented as part of the PHYLIP package (19) as described below. The pairwise evolutionary distances based on 1,245 unambiguous bases were determined by the method of Jukes and Cantor (23), and dendrograms were constructed from evolutionary distances by the neighbor-joining method. A transversion analysis was performed with the program DNAPARS. Tree topology determined with 100 data sets was examined by running a script file consisting of the following programs: SEQBOOT, DNADIST, FITCH, and CONSENSE. Programs available in the Molecular Evolutionary Genetic Analysis (MEGA) package, version 1 (24), were also used in the analysis. All of the programs except MEGA were run on a Sun Sparc workstation. Mega was run on a Compaq Contura 410CX IBM-compatible notebook computer.

Nucleotide sequence accession number. The nucleotide sequence of the 16S rRNA of strain SEBR 4207^T has been deposited in the GenBank database under accession no. U52817.

RESULTS

Bacterial counts in BB325 wellhead sample. At the time of our investigation, the new oil pipeline was treated by frequent biocide injections at elevated concentration. Since this treatment drastically reduced the bacterial numbers within the line, we collected water samples upstream of the corroded pipe and from the non-biocide-treated producing wellheads whose tubings were actively corroded.

The bacterial community (Table 1) was exclusively composed of strict anaerobes. The MPN counts showed that most bacterial cells enumerated under the epifluorescence microscope were cultivable. Fermentative bacteria outnumbered

TABLE 1. Bacterial counts in well BB325 water sample

Sampling group	Bacterial count (no. of cells/ml)
Epifluorescence microscopy	4.5×10^3
Aerobes or facultative anaerobes	ND[a]
Sulfate-reducing bacteria (SRB test kits)	9.5×10^2
Methanogens[b]	1.0×10^3
Fermentative anaerobes	2.5×10^3

[a] ND, not detected.

[b] Methanogens comprise hydrogenotrophs (0.5×10^3/ml) and methylotrophs (0.5×10^3/ml).

FICHE DESCRIPTIVE

Auteur(s) : Magot M., Ravot G., Campaignolle X., **Ollivier B.,** Patel B.K.C., **Fardeau M.L.**, Thomas P., Crolet J.L., **Garcia J.L.**

Titre original : *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov., a new anaerobic, slightly halophilic, thiosulfate-reducing bacterium from corroding offshore oil wells.

Revue : International Journal of Systematic Bacteriology 1997, **47**, 818-824.

Titre en Français : *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov., une nouvelle bactérie anaérobie, modérée halophile, thiosulfato-réductrice isolée d'un puits pétrolier offshore corrodé.

Mots-clés matières (10 au plus) : *Dethiosulfovibrio peptidovorans* - Halophilie - Anaérobiose - Taxonomie - Réduction du thiosulfate- Pétrole - Corrosion

Résumé en Français :
(150 mots maximum)

Plan de classement : Monde végétal et Animal - Fermentations

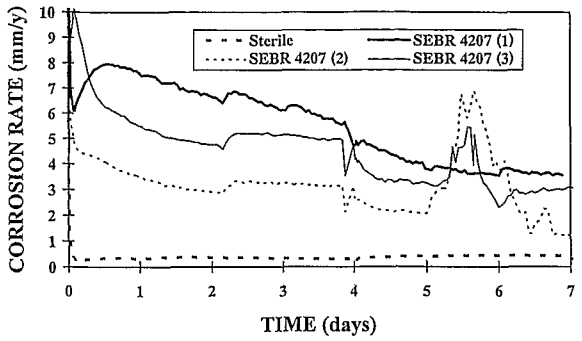

FIG. 2. Corrosion rates induced by strain SEBR 4207^T in three replicated experiments (experiment numbers given in parentheses).

both sulfate-reducing and methanogenic bacteria. Seven different fermentative strains were purified, and six of them were shown to reduce sodium thiosulfate to hydrogen sulfide (28), including the recently described novel species *Haloanaerobium congolense* (37). Two SRB strains (42) and two methanogens, including the new species *Methanoplanus petrolearius* (34), were also isolated. None of these isolates displayed characteristics common to the described species (34, 37, 42).

Enrichment and isolation. Strain SEBR 4207^T was purified from the last positive MPN HSTYG tube by three consecutive streakings on HSTYG agar plates, followed by incubation at 30°C in an anaerobic chamber. After 7 days of incubation, colonies 2 mm in diameter developed. The colonies were white and round with smooth edges. A single colony (designated as strain SEBR 4207^T) was picked and transferred into HSTYG liquid medium.

Corroding activity of strain SEBR 4207^T. Figure 2 shows the pit penetration rates (in millimeters per year) versus time after the preconditioning for one experimental cell kept sterile and three independent experimental cells inoculated with strain SEBR 4207^T. Under sterile conditions, the penetration rate decreased to a fraction of millimeters per year within a few hours of coupling, after the preconditioning current was stopped. In the presence of strain SEBR 4207^T, a stable galvanic current of 130 to 350 $\mu A/cm^2$ was measured. When expressed as a penetration rate, this corresponded to 325 to 865 mg of iron per dm^2 per day, or 1.5 to 4.0 mm/y (Fig. 2). These differences were suspected to result from differences in biofilm formation. These values are far above the accepted industrial limit of 0.1 mm/y for mild steel corrosion.

Morphology. Strain SEBR 4207^T was a vibrio measuring 3 to 5 μm by 1 μm (Fig. 3). Electron microscopy of negatively stained cells indicated the presence of from one up to five lateral flagella (Fig. 4). Electron microscopy of thin sections of cells of strain SEBR 4207^T exhibited a multilayered cell wall ultrastructure with an outer membrane typical of gram-negative bacteria (Fig. 5).

Growth characteristics. Strain SEBR 4207^T grew from 20 to 45°C, with an optimum temperature for growth at 42°C. No growth was observed at 50°C (Fig. 6a). It required NaCl for growth, with an optimum of 3% NaCl. No growth occurred at 11% NaCl (Fig. 6b). The optimum pH for growth was 7.0, with no growth at pH 5.5 and 8.8.

Substrate utilization and physiological characteristics. Strain SEBR 4207^T utilized peptides in the form of bio-Trypticase and Bacto Peptone and amino acids in the form of Casamino Acids as its sole carbon and energy sources. Proteins such as gelatin or casein, carbohydrates (arabinose, fructose, galactose, glucose, lactose, maltose, mannose, rhamnose, ribose, sucrose, sorbose, trehalose, and xylose), and fatty acids (acetate, butyrate, propionate, citrate, and lactate) were not utilized. End products formed from peptide utilization included acetate, isobutyrate, isovalerate, 2-methylbutyrate, CO_2, and H_2. Serine and histidine were fermented, whereas arginine, glutamate, leucine, isoleucine, alanine, valine, methionine, and asparagine were only oxidized in the presence of thiosulfate as an electron acceptor. Lysine, proline, threonine, and glycine were not used in either the presence or the absence of thiosulfate. Serine was fermented to acetate. In the presence of thiosulfate, alanine was oxidized to acetate, leucine to 3-methylbutyrate, isoleucine to 2-methylbutyrate, valine to isobutyrate, asparagine to acetate, and methionine to propionate. Although yeast extract was not required for utilization of peptides and Casamino Acids, its presence improved growth. In contrast, yeast extract was required for the use of any single amino acid as an energy source. Strain SEBR 4207^T used elemental sulfur and thiosulfate, but not sulfate, as electron acceptors with peptides. Strain SEBR 4207^T did not perform thiosulfate or elemental sulfur disproportionation in the presence of acetate or yeast extract as a carbon source. Moreover, growth did not occur on H_2 plus thiosulfate and acetate as the carbon source. The presence of either elemental sulfur or thiosulfate increased the biomass and the growth rate (Fig. 7). In addition, H_2S was produced with a concomitant decrease in H_2 when thiosulfate or sulfur was present. H_2 inhibited the growth of strain SEBR 4207^T, but this inhibition could be partially reversed by the addition of thiosulfate and sulfur. Overall, the presence of thiosulfate had a much greater effect on growth than sulfur did. Strain SEBR 4207^T was unable to use carbohydrates and volatile fatty acids as carbon and energy sources with yeast extract and/or thiosulfate. The cells did not contain cytochrome or desulfoviridin.

G+C content. The G+C content of isolate SEBR 4207^T was 56 mol%.

16S rRNA sequence analysis. Using 12 primers, we determined 1,505 nucleotide bases from positions 8 to 1542 (*Escherichia coli* numbering of Winker and Woese [44] of the 16S rRNA gene of strain SEBR 4207^T). A phylogenetic analysis performed with representatives of the domain *Bacteria* revealed that strain SEBR 4207^T was a member of the subdivision containing gram-positive bacteria with DNA G+C content less than 55 mol% and clustered with members of the clostridial group. A recent phylogenetic analysis of the 16S

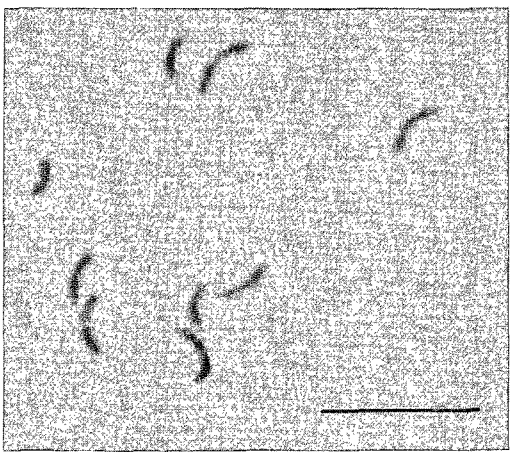

FIG. 3. Phase-contrast photomicrograph of strain SEBR 4207^T showing vibrioid cells. Bar, 10 μm.

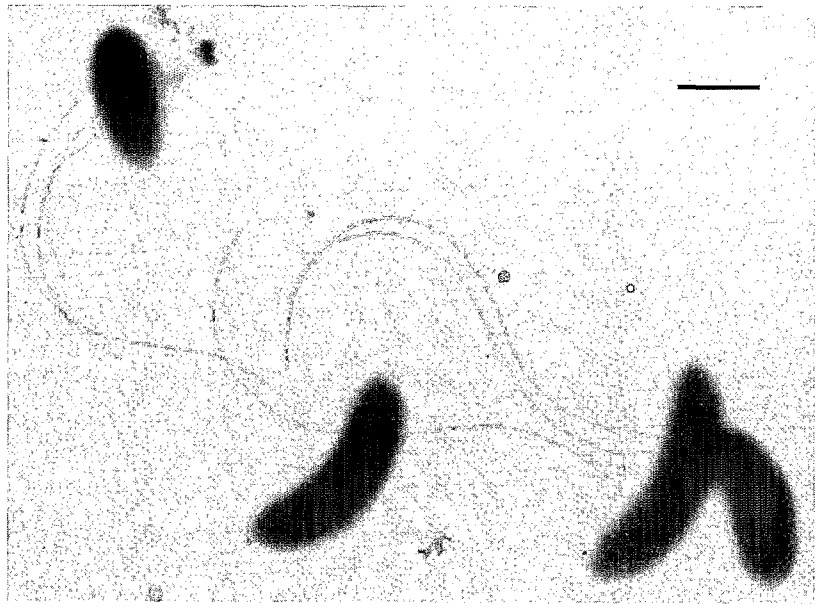

FIG. 4. Electron micrograph of negative-stained culture of strain SEBR 4207^T showing lateral flagellation. Bar, 1 μm.

rRNAs of the members of the clostridial group revealed that there are at least 19 defined clusters and several lines of descent. This led to the creation of five new genera and 11 new species combinations to accommodate phylogenetically distinct organisms (11). Phylogenetic analysis revealed that strain SEBR 4207^T was peripherally related to cluster V, consisting of the family *Thermoanaerobacteriaceae*, with a similarity value of 83%. A bootstrap value of 53 was obtained from a 100 data sets, indicating a poor relationship between strain SEBR 4207^T and cluster V. Several data sets, which included different representatives from the various clusters of the subdivision containing gram-positive bacteria with DNA G+C content less than 55 mol%, consistently placed strain SEBR 4207^T as a member of a novel line of descent at the periphery of cluster V and the genus *Dictyoglomus* (39). Transversion analysis did not affect the position of strain SEBR 4207^T in the phylogenetic tree. The evolutionary distances separating strain SEBR 4207^T and its relative and the dendrogram derived from these distances are depicted in Table 2 and Fig. 8, respectively. The G+C content of the 16S rRNA gene was 56 mol%.

DISCUSSION

The bacterial flora of oil field production water transported in an actively corroded undersea oil pipeline was shown to be composed of only strict anaerobes, which appear to commonly dominate such ecosystems (2, 20). Most of the bacteria that compose this consortium were cultivated (Table 1), although it is generally considered that very few bacteria from the natural environment are cultivable under laboratory conditions. Similar observations were recently reported from another oil field facility (32). Fermentative, thiosulfate-reducing anaerobic bacteria exhibited significant populations in the oil field studied (Table 1), a situation which seems to be common in oil field reservoirs (14, 20, 36).

Thiosulfate is produced from chemical oxidation of sulfide (10, 15, 22). It is common in aquatic environments and has also recently been detected in oil fields. It has been hypothesized from models (13, 16) and field observations (14, 28) that its presence in oil fields may increase the risk of biocorrosion of oil pipelines. We have therefore initiated studies of the isola-

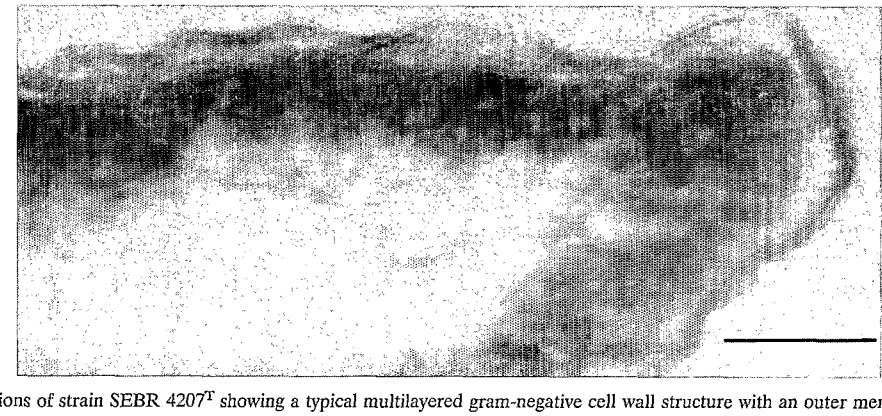

FIG. 5. Thin sections of strain SEBR 4207^T showing a typical multilayered gram-negative cell wall structure with an outer membrane. Bar, 0.1 μm.

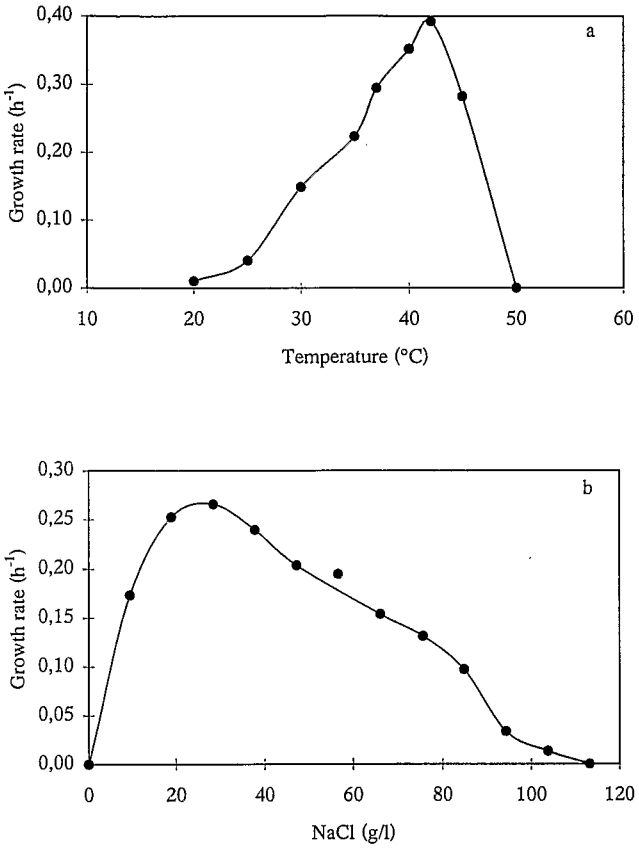

FIG. 6. (a) Effect of temperature on the growth of strain SEBR 4207^T cultured in medium DP and incubated at various temperatures. (b) Effect of NaCl concentration on the growth of strain SEBR 4207^T cultured in medium DP containing sodium chloride concentrations ranging from 0 to 115 g/liter and incubated at 42°C.

tion of anaerobic thiosulfate-reducing bacteria from oil field environments undergoing active corrosion. Strain SEBR 4207^T was isolated from an African oil field in Congo and was observed to use thiosulfate, but not sulfate, as an electron acceptor. One important characteristic of this strain, when tested in appropriate in vitro experiments, was that it induced corrosion rates into mild steel at least 1 order of magnitude higher than the industrial design limit. This corroding activity was similar to or higher than that recorded for sulfate-reducing bacteria in similar experiments (6). This paper thus shows for the first time that thiosulfate-, non-sulfate-reducing bacteria, as previously suspected, do have a significant corrosive activity.

Strain SEBR 4207^T also used elemental sulfur as an electron acceptor. The reduction of elemental sulfur to sulfide is not an obligate physiological trait for strain SEBR 4207^T, and therefore it is different from the mesophilic sulfur-respiring bacteria, viz. *Desulfuromonas acetoxidans*, *Desulfurella acetivorans*, and *Desulfovibrio* sp. (35), or the thermophilic members of domains *Archaea* and *Bacteria* (3, 40, 41). The metabolism of this isolate is unique among the mesophilic sulfur reducers because it resembles to some degree that of the hyperthermophilic members of the domain *Archaea* when proteinaceous compounds are used in the presence of elemental sulfur as an electron acceptor (4). Strain SEBR 4207^T possesses unique phenotypic characteristics, because it utilizes only a limited range of substrates, which include amino acids and peptides, but not proteins such as gelatin and casein, carbohydrates, or volatile fatty acids.

The isolation of strain SEBR 4207^T extends the known diversity of microorganisms involved in amino acid and peptide degradation and emphasizes the importance of thiosulfate or sulfur reducers in the oxidation of peptides and amino acids. Thiosulfate dramatically improves the utilization of amino acids and peptides by members of the genus *Thermoanaerobacter* (18) in a manner similar to that of strain SEBR 4207^T. Interestingly, *Thermoanaerobacter* species are the nearest phylogenetic relatives of strain SEBR 4207^T, although the distance separating them is very large (83% similarity). In addition, strain SEBR 4207^T is a mesophile, utilizes a very limited range of substrates, and has G+C content of 56 mol%, traits which clearly differentiate strain SEBR 4207^T from members of all known bacteria, including members of the genus *Thermoanaerobacter*.

Strain SEBR 4207^T stains gram-negative and has a gram-negative cell wall ultrastructure, but is phylogenetically related to members of the subdivision containing gram-positive subdivision bacteria with DNA G+C content of less than 55%. This discrepancy between phenotype and genotype is not new in this subdivision. For example, the closest relatives of strain SEBR 4207^T are the members of the genus *Thermoanaerobacter*. All members of this genus are gram-positive, except *Thermoanaerobacter ethanolicus*, which possesses a gram-negative cell wall (43). Members of *Dictyoglomus*, which are also distant phylogenetic relatives of strain SEBR 4207^T and *Thermoanaerobacter* species, possess gram-negative cell wall (39). Interestingly, the common phenotypic element binding strain SEBR 4207^T with *Thermoanaerobacter* and *Dictyoglomus* is their obligate anaerobic nature.

Strain SEBR 4207^T is an anaerobic, slightly halophilic vibrio but does not utilize sulfate as an electron acceptor, and despite its morphology and ability to utilize thiosulfate, it cannot be described as a member of the sulfate-reducing bacteria. Strain SEBR 4207^T is also phylogenetically distinct from all sulfate-reducing bacteria and members of the family *Thermoanaerobacteriaceae*. Because strain SEBR 4207^T cannot be assigned to any known bacterial genera, we propose that it be designated a member of a new genus, *Dethiosulfovibrio peptidovorans*, gen. nov., sp. nov.

Description of *Dethiosulfovibrio* gen. nov. *Dethiosulfovibrio* (De.thi.o.sul.fo.vi'bri.o. L. pref. *de*, from; Gr. n. *thios*, sulfur; L. n. *sulfur*, sulfur; *thiosulfo*, thiosulfate; L. v. *vibrio*, to vibrate;

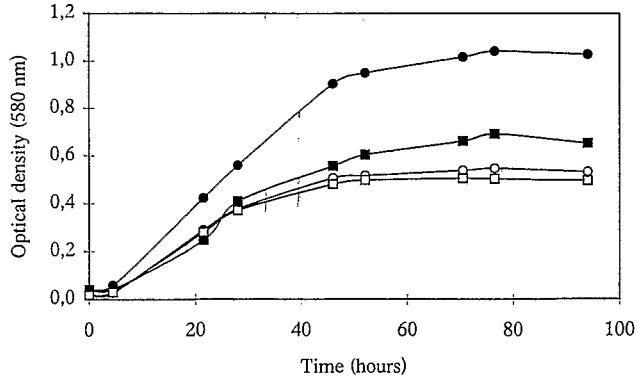

FIG. 7. Effect of different electron acceptors on the growth of strain SEBR 4207^T cultured on medium DP. ■, elemental sulfur, 2%; ●, thiosulfate, 20 mM; □, sulfate, 20 mM. Medium DP without any electron acceptors added acted as a control (○).

TABLE 2. Evolutionary distance matrix determined from a comparison of the 16S rRNA sequence of strain SEBR 4207^T and related genera by the method of Jukes and Cantor (23)[a]

Organism	Evolutionary distance of sequence from that of organism:														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 Strain SEBR 4207															
2 *Thermoanaerobacter thermocopriae*	83.0														
3 *Thermoanaerobacter acetoethylicus*	82.7	94.2													
4 *Thermoanaerobacter ethanolicus*	83.4	94.8	97.0												
5 *Thermoanaerobacter kivui*	83.8	94.2	96.1	97.1											
6 *Dictyoglomus thermophilum*	80.3	85.5	85.1	85.8	85.1										
7 *Desulfotomaculum thermobenzoicum*	81.8	86.8	87.1	88.2	87.9	82.5									
8 *Desulfotomaculum thermosapovorans*	81.3	85.2	85.4	86.1	85.7	81.0	89.4								
9 *Desulfotomaculum australicum*	81.3	86.3	86.4	87.6	87.6	82.9	94.6	89.3							
10 *Thermoanaerobacterium thermosaccharolyticum*	80.2	86.3	87.1	87.6	87.4	81.5	86.0	84.3	85.4						
11 *Thermoanaerobacterium thermosulfurigenes*	79.8	86.0	87.4	87.4	87.3	81.8	86.3	84.5	85.4	98.1					
12 *Moorella thermoacetica*	81.6	87.9	88.2	89.1	88.8	83.5	89.4	88.6	88.8	86.9	87.1				
13 *Syntrophomonas bryantii*	80.1	84.9	85.3	85.9	85.4	81.6	85.6	84.1	85.7	84.2	84.5	86.9			
14 *Syntrophomonas wolfei*	80.2	85.3	85.6	86.1	85.8	81.9	85.3	84.4	86.2	83.8	83.9	86.9	94.4		
15 *Selenomonas ruminantium*	79.5	82.1	82.5	83.7	82.5	79.9	83.6	85.2	83.0	82.7	82.8	83.1	83.5	83.1	
16 *Megasphaera elsdenii*	80.2	81.0	81.7	82.9	82.7	79.4	81.9	83.0	81.9	82.7	82.3	83.8	81.0	81.3	87.9

[a] See Materials and Methods for details. The sequences used in the analysis were obtained from the Ribosomal Database Project, version 5 (25), and from EMBL for *Desulfotomaculum thermosapovorans* (accession no. Z26315). Only 1,245 unambiguous nucleotide positions were used in the analysis.

M.L. masc. n. vibrio that vibrates, a generic name; M.L. masc. n. *Dethiosulfovibrio*, a vibrio that reduces thiosulfate). Cells are gram-negative vibrios, mesophilic, neutrophilic, and slightly halophilic strict anaerobes that use peptides and amino acids, but not sugars, as the sole carbon and energy sources. The organism uses elemental sulfur and thiosulfate, but not sulfate, as electron acceptors. Growth is inhibited by hydrogen. Cells do not contain cytochrome or desulfoviridin.

Description of *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov. *Dethiosulfovibrio peptidovorans* (pep.ti.do.vo'rans. Gr. adj. *peptos*, cooked; L. v. *voro*, to devour; M.L. part adj. *peptidovorans*, devouring peptides). Cells are gram-negative vibrios, 3 to 5 μm by 1 μm. Growth occurs at temperatures between 20 and 45°C (optimum 42°C) and at pH values between 5.5 and 8.8 (optimum 7.0). The organism is slightly halophilic; growth requires NaCl (optimum, 3%), but no growth occurs at 11% NaCl. The organism is a strictly anaerobic bacterium using peptides and amino acids as its sole carbon and energy sources.

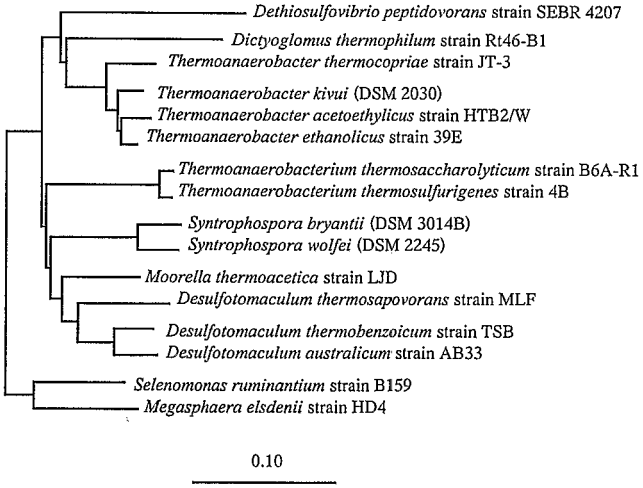

FIG. 8. Phylogenetic position of strain SEBR 4207^T among members of the subdivision containing gram-positive bacteria with DNA G+C content of less than 55 mol%.

The organism ferments serine and histidine. *D. peptidovorans* uses alanine, arginine, asparagine, glutamate, isoleucine, leucine, methionine, and valine only in the presence of thiosulfate as an electron acceptor. The organism produces acetate, isobutyrate, isovalerate, 2-methylbutyrate, CO_2, and H_2 from peptides. Yeast extract is not required, but improves growth on Casamino Acids and peptides. Yeast extract is required for the use of a single amino acid. The organism uses elemental sulfur and thiosulfate, but not sulfate, as electron acceptors with peptides. The organism does not perform disproportionation of thiosulfate or elemental sulfur. The presence of either sulfur or thiosulfate increases the biomass and the growth rate. In addition, H_2S is produced with a concomitant decrease in H_2 when thiosulfate or sulfur is present. Growth is inhibited by hydrogen, but this inhibition is partially reversed by the addition of thiosulfate. Cells do not contain cytochrome or desulfoviridin. The organism is unable to utilize proteins (gelatin and casein), carbohydrates (arabinose, fructose, galactose, glucose, lactose, maltose, mannose, rhamnose, ribose, sucrose, sorbose, trehalose, and xylose), fatty acids (acetate, propionate, butyrate, citrate, and lactate) as its sole carbon and energy source with added yeast extract, bio-Trypticase, and/or thiosulfate.

The DNA base composition (G+C content) is 56 mol%. *D. peptidovorans* lives in an oil-producing well in Africa. The type strain is SEBR 4207 (=DSM 11002).

ACKNOWLEDGMENTS

We thank C. E. Hatchikian for helpful discussions, O. Arnauld and C. Lanau for technical assistance, and P. A. Roger for critical comments on the manuscript.

This work was supported by grants from Elf Aquitaine (G.R. and X.C.) and in part from the Australian Research Council (B.K.C.P.).

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