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Petrimonas sulfuriphila gen. nov., sp. nov., a mesophilic fermentative bacterium isolated from a biodegraded oil reservoir

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A mesophilic, anaerobic, fermentative bacterium, strain BN3^T, was isolated from a producing well of a biodegraded oil reservoir in Canada. Cells were Gram-negative, non-motile rods that did not form spores. The temperature range for growth was 15–40 °C, with optimum growth at 37–40 °C. The strain grew with up 4 % NaCl, with optimum growth in the absence of NaCl. Tryptone was required for growth. Yeast extract and elemental sulfur stimulated growth. Growth was also enhanced during fermentation of glucose, arabinose, galactose, maltose, mannose, rhamnose, lactose, ribose, fructose, sucrose, cellobiose, lactate, mannitol and glycerol. Acetate, hydrogen and CO_2 were produced during glucose fermentation. Elemental sulfur and nitrate were used as electron acceptors and were reduced to sulfide and ammonium, respectively. The G+C content of the genomic DNA was 40·8 mol%. Phylogenetic analyses of the 16S rRNA gene sequence indicated that the strain was a member of the phylum '*Bacteroidetes*', distantly related to the genera *Bacteroides* and *Tannerella* (similarity values of less than 90 %). The chemotaxonomic data (fatty acids, polar lipids and quinones composition) also indicated that strain BN3^T could be clearly distinguished from its closest cultivated relatives. This novel organism possesses phenotypic, chemotaxonomic and phylogenetic traits that do not allow its classification as a member of any previously described genus; therefore, it is proposed that this isolate should be described as a member of a novel species of a new genus, *Petrimonas* gen. nov., of which *Petrimonas sulfuriphila* sp. nov. is the type species. The type strain is BN3^T (=DSM 16547^T=JCM 12565^T).

Culture-based methods and recently performed molecular studies have demonstrated that fermentative bacteria are widely distributed in low- and high- temperature oilfields (see Magot *et al.*, 2000 for review; Orphan *et al.*, 2000; Takahata *et al.*, 2000; Bonch-Osmolovskaya *et al.*, 2003). In these extreme ecosystems, moderately thermophilic to hyperthermophilic strains have attracted particular attention and many novel bacterial and archaeal fermentative strains have been described in the last century (Magot *et al.*, 2000). In recent years, novel fermentative thermophiles belonging to the *Thermotogales* (Takahata *et al.*, 2001; L'Haridon *et al.*, 2001, 2002; Miranda-Tello *et al.*, 2004), *Thermococcales* (Miroshnichenko *et al.*, 2001) and *Firmicutes* (Fardeau *et al.*, 2000, 2004; Miranda-Tello *et al.*, 2003) have been characterized.

Some mesophilic bacteria have also been isolated from low-temperature oilfields, and most of them belong to the *Firmicutes* (Magot *et al.*, 1997b; Ravot *et al.*, 1997, 1999). A free-living moderately halophilic member of the phylum '*Spirochaetes*', *Spirochaeta smaragdinae*, has also been isolated from an African oilfield (Magot *et al.*, 1997a). Fermentative

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Growth curves for strain BN3^T at different temperatures and NaCl concentrations are available as supplementary material in IJSEM Online.

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bacteria isolated from oilfields are able to ferment sugars, organic acids or amino acids and most of them share the ability to reduce elemental sulfur or thiosulfate to hydrogen sulfide (Magot *et al.*, 2000). The ecological significance of this feature is not clear but it could be a way to overcome hydrogen, a potent inhibitor of growth produced during fermentation.

On the basis of the comparative analysis of 16S rRNA gene sequences (Paster *et al.*, 1994), members of the genus *Bacteroides* form a relatively coherent cluster within the phylum *Cytophaga–Flexibacter–Bacteroides*. In the latest edition of *Bergey's Manual of Systematic Bacteriology*, this phylum is referred as the phylum '*Bacteroidetes*', and is divided into three classes, the '*Bacteroidetes*', the '*Flavobacteria*' and the '*Sphingobacteria*' (Garrity & Holt, 2001). The genus *Bacteroides* and phylogenetically closely related members of genera *Porphyromonas*, *Dysgonomonas* and *Tannerella* are strictly anaerobic, Gram-negative, chemoheterotrophic bacteria that have been predominantly isolated from oral cavities, the gastrointestinal tract and rumen of warm-blooded animals and other body cavities of humans and animals (Shah & Collins, 1988, 1989; Hofstad *et al.*, 2000; Sakamoto *et al.*, 2002). We isolated a mesophilic fermentative sulfur-reducing bacterium affiliated to the phylum '*Bacteroidetes*' from a biodegraded oil reservoir in Canada. To our knowledge, no members of this phylum have been so far isolated from oil reservoirs.

The production water sample used to isolate the novel strain was collected in January 2001 from the Pelican Lake oilfield, which is located in the Western Canadian Sedimentary Basin (Canada). The reservoir, located at 400 m depth, has an *in situ* fluid temperature of about 18–20 °C. The main productive zone is the Wabiskaw formation, a laterally continuous sandstone which is part of the lower cretaceous Mannville Group of Alberta. The heavy oils from Pelican Lake field are derived from the same source rock, deposited in a marine environment, but have undergone varying degrees of biodegradation leading to heavy viscous oils ranging from 9 to 15° API (Riediger *et al.*, 1999). Samples were taken through sampling valves located on the well head into sterile 1 litre steel bottles, after flushing the lines for at least 15 min. The bottles were filled completely with the oil/water/gas mixture under pressure and sealed to maintain anoxic conditions. The bottles were kept at 4 °C during their transport back to the laboratory. Water was separated from crude oil by decantation at room temperature and then transferred into vials that were sealed with butyl rubber stoppers. The vials containing production waters, with an overlying oil phase, were stored at 4 °C until use.

Enrichment and isolation were performed in the nitrate broth medium (Difco) generally used for the enumeration of heterotrophic denitrifying bacteria. Single colonies were isolated by using the roll-tube technique (Hungate, 1969) with the same medium solidified with 2 % (w/v) purified agar (Difco). The YTS medium used as basal medium contained, per litre of distilled water: 1·0 g NaCl, 0·4 g $MgCl_2.6H_2O$, 0·075 g $CaCl_2$, 0·25 g NH_4Cl, 0·2 g KH_2PO_4, 0·5 g KCl, 1 g yeast extract, 3 g tryptone, 2 g sulfur and 0·5 mg resazurin. The YTS medium was prepared under strictly anoxic conditions according to Widdel & Bak (1992) and was supplemented with $NaHCO_3$ (3·4 g l^{-1}), 1 ml vitamin solution l^{-1} (Widdel & Bak, 1992) and 1 ml trace element solution l^{-1} (Widdel & Bak, 1992). Sodium sulfide (1·5 mM) was used as reducing agent. GYTS medium, consisting of YTS medium supplemented with glucose (20 mM), was used routinely to grow the novel isolate and to study its physiology. The medium was dispensed into 120 ml vials (with 40 ml medium) that were sealed with butyl rubber stoppers and screw caps. The final pH was 7·2 and the gas phase was N_2/CO_2 (80 : 20, v/v).

Physiological studies were performed with agitation. To determine the NaCl range for growth, NaCl concentrations were varied while maintaining the concentrations of other inorganic components. Sugars, organic acids and alcohols were tested as possible carbon sources in the YTS medium. Possible electron acceptors were tested in the GYTS medium without sulfur.

Growth was monitored by removing samples from culture vessels and measuring the OD_{600} using a Shimadzu UV-1601 spectrophotometer. Sulfide production was quantified colorimetrically by the methylene blue method (Cline, 1969). Acetate, lactate and ethanol were assayed by using enzymic detection kits (R-biopharm). Hydrogen and CO_2 in the headspace of the vials were measured with a gas chromatograph (Varian 3800) equipped with a Porapak Q (80/100 mesh; Millipore) steel column and a thermal conductivity detector. Temperatures for the column, injection port and detector were 35, 110 and 130 °C, respectively. The carrier gas was helium at a flow rate of 30 ml min^{-1}. Determination of short-chain organic acids was performed by HPLC using a Hamilton PRP-X300 column [eluant, H_2SO_4 1 mM/acetonitrile (90 : 10, v/v); flow rate, 1·5 ml min^{-1}; column temperature, 65 °C] and a UV detector at 210 nm. Nitrate and nitrite were measured by ionic chromatography (Metrohm) on a Metrosep Anion Dual 2 column (240 × 4·0 mm) equipped with a suppressor module (eluant, 1·8 mM Na_2CO_3, 1·7 mM $NaHCO_3$; flow rate, 1 ml min^{-1}; room temperature) and conductivity detection. Ammonium was detected by ionic chromatography (Metrohm) on a 250 × 4 mm Metrosep Cation 1-2 column (eluant, 4 mM tartaric acid, 1 mM dipicolinic acid; flow rate, 1 ml min^{-1}; room temperature) and conductivity detection. An Olympus AX-70 microscope was routinely used for observation and to obtain photomicrographs. Cell morphology and flagellation were observed using a model JEM 100 CX II (JEOL) electron microscope with an acceleration voltage of 80 kV. For negative staining, 20 µl cell suspension fixed with 2 % (w/v) glutaraldehyde was dropped on Formvar/carbon-coated grids (400 mesh) and stained with 4 % (w/v) uranyl acetate.

Samples of formation water collected at the well head of a

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producing well in the Pelican Lake oilfield were used for the inoculation (10 %, v/v) of nitrate broth medium. After 3 weeks of incubation at 20 °C, most-probable number enumerations (triplicate tubes) indicated the presence of 10^5 cultivable denitrifiers ml^{-1}. The last positive dilution was used to isolate the dominant cultivable species. After 4 weeks of incubation at 30 °C, several translucent colonies that developed in the solidified medium were randomly picked and subcultured in liquid medium. Analysis of the RFLP profiles of the 16S rRNA genes (Jeanthon *et al.*, 1999) showed that all the patterns were identical. One strain, designated strain $BN3^T$, was chosen for further characterization. Since weak growth was observed in nitrate broth medium, several media were tested to obtain higher growth yields. GYTS medium was finally routinely used to grow the novel isolate.

Cells were short rods, about $0{\cdot}7\text{–}1\times1{\cdot}5\text{–}2$ μm in size depending on the growth phase (shown in Supplementary Fig. A in IJSEM Online); some longer cells ($0{\cdot}5\times4$ μm) were observed in old cultures. The cells stained Gram-negative (Murray *et al.*, 1994) and occurred singly or in pairs. They were non-motile and no flagella were observed by negative staining. No spores were formed.

Unless otherwise stated, two separate growth experiments were performed in duplicate in GYTS medium at 37 °C. Strain $BN3^T$ grew between 15 and 40 °C, with optimum growth at 37–40 °C (Supplementary Fig. B); no growth was observed at 10 or 45 °C. The isolate grew in the presence of NaCl concentrations up to 4 %, with optimum growth in the absence of NaCl (Supplementary Fig. C); no growth was observed at 5 % NaCl. Under the optimal conditions for growth in GYTS medium, the doubling time of the novel organism was around 110 min and the final growth concentration reached $1{\cdot}5\times10^9$ cells ml^{-1}.

Strain $BN3^T$ is a strictly anaerobic, chemo-organotrophic organism. Growth was prevented when air was used as the gas phase. No growth was observed in the absence of organic compounds. Tryptone was required for growth (no growth was observed in GYTS medium without tryptone). Yeast extract, glucose and sulfur stimulated growth (weak and slow growth was observed when one of these compounds was omitted from GYTS medium). When yeast extract, tryptone and elemental sulfur were present (medium YTS), strain $BN3^T$ fermented the following compounds: glucose, arabinose, galactose, maltose, mannitol, mannose, rhamnose, lactose, ribose, fructose, sucrose (all at 20 mM), cellobiose (10 g l^{-1}), lactate (15 mM) and glycerol (10 mM). Acetate, hydrogen and CO_2 were produced during glucose fermentation. Weak growth occurred with fumarate (10 mM), pyruvate (10 mM) and Casamino acids (5 g l^{-1}). Acetate (15 mM), formate (40 mM), butyrate (10 mM), propionate (10 mM), methanol (30 mM), peptone (2 g l^{-1}), ethanol (1 g l^{-1}), propanol (1 g l^{-1}), butanol (1 g l^{-1}), toluene (1·5 mM), sorbose (20 mM) and cellulose (10 g l^{-1}) were not used. Strain $BN3^T$ reduced elemental sulfur to H_2S. Sulfide production started weakly in the exponential phase of growth and increased during the stationary phase; the final concentration of sulfide was dependent on the substrates used. Sulfide production exceeded 2 mM (as final concentration) when glucose, fructose, galactose, sucrose, mannose or ribose were added to the YTS medium. Sulfide production was lower than 2 mM when other substrates were used. Thiosulfate (20 mM), sulfate (20 mM) and cystine (2 g l^{-1}) were not used as electron acceptors. Nitrate (10 mM) was used as electron acceptor and ammonium was formed as the result of nitrate reduction.

Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried cell material using the two-stage method described by Tindall (1990a, b). Respiratory quinones were extracted using methanol : hexane (Tindall, 1990a, b) and the polar lipids were extracted by adjusting the remaining methanol : 0·3 % aqueous NaCl phase (containing the cell debris) to give a chloroform : methanol : 0·3 % aqueous NaCl mixture (1 : 2 : 0·8, by vol.). The extraction solvent was stirred overnight and the cell debris was pelleted by centrifugation. Polar lipids were recovered into the chloroform phase by adjusting the chloroform : methanol : 0·3 % aqueous NaCl mixture to a ratio of 1 : 1 : 0·9 (by vol.). Respiratory lipoquinones were separated into their different classes (menaquinones and ubiquinones) by TLC on silica gel (Macherey-Nagel art. no. 805 023), using hexane : *tert*-butylmethylether (9 : 1, v/v) as solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out on an LDC Analytical HPLC (Thermo Separation Products) fitted with a reverse phase column (Macherey-Nagel; 2 × 125 mm, 3 μm, RP_{18}) using methanol as the eluant. Respiratory lipoquinones were detected at 269 nm.

Examination of the respiratory lipoquinone composition of strain $BN3^T$ indicated that the major respiratory quinones present were menaquinones. The predominant quinone was MK-8, with smaller amounts of MK-7 and MK-9. Whereas those members of the *Cytophaga–Flavobacterium–Sphingobacterium* subgroups produce either MK-6 or MK-7 as the major menaquinones (Nakagawa & Yamasato, 1993; Oyaizu & Komagata, 1981), it is not unusual to find not only longer chain (>MK-9), but also more than one isoprenologue in significant amounts in the *Bacteroides–Prevotella–Porphyromonas–Tannerella* subgroup (Shah & Collins, 1980; Sakamoto *et al.*, 2002). It should be noted that some of the closest relatives of strain $BN3^T$, namely *Tannerella forsythensis*, *Bacteroides merdae* and *Bacteroides distasonis* (see below), produce menaquinones with 9–12 isoprenologues. *T. forsythensis* produces MK-9 to MK-12, with MK-10 and MK-11 predominating, whereas *B. merdae* produces MK-9 and MK-10 (in equal amounts) and MK-10 predominates in *B. distasonis*. Since 16S rRNA gene similarities between the type strains of these three species are >95 %, these data are indicative of the fact that the taxonomy of this group (including members of the genus *Dysgonomonas*) warrants

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further taxonomic rearrangement. The fact that strain BN3[T] differs from all its closest 16S rRNA relatives in producing MK-8 as the predominant menaquinone may be taken as indicative of its unique evolutionary and taxonomic status within this group.

Polar lipids were separated by two-dimensional silica gel TLC (Macherey-Nagel art. no. 818 135). The first direction was developed in chloroform : methanol : water (65 : 25 : 4, by vol.) and the second in chloroform : methanol : acetic acid : water (80 : 12 : 15 : 4, by vol.). Total lipid material and specific functional groups were detected using dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate-Schiff (α-glycols), Dragendorff reagent (quaternary nitrogen) and anisaldehyde-sulfuric acid (glycolipids).

The major polar lipids of strain BN3[T] comprised phosphatidylethanolamine, an unidentified phospholipid, two unidentified aminophospholipids, three unidentified phosphoglycolipids, a glycolipid, an aminolipid and two additional uncharacterized lipids (Fig. 1). Interpretation of the polar lipid pattern detected in strain BN3[T] is not easy, given the fact that little work has been carried out on the polar lipid composition of the *Bacteroides–Flavobacterium–Cytophaga* group. However, based on the data presented here and that currently available in the literature, it appears that a number of trends allow the delineation of the group, and further differentiation within the group. In particular, among the major phospholipids, ethanolamine phosphate head-groups seem to predominate and amino-acid-based lipids have also been reported in this group (Batrakov *et al.*, 1998, 1999, 2000; Kawazoe *et al.*, 1991; Pitta *et al.*, 1989). In addition to some of the common features in the polar lipid patterns, it should be noted that, of the few strains examined, the presence or absence of phosphatidylcholine or phosphatidylinositol may be significant (Batrakov *et al.*, 1998, 1999, 2000; Godchaux & Leadbetter, 1980, 1983, 1984; Kawazoe *et al.*, 1991; Le Bach & White, 1969; Naka *et al.*, 2003; Pitta *et al.*, 1989; Rizza *et al.*, 1970).

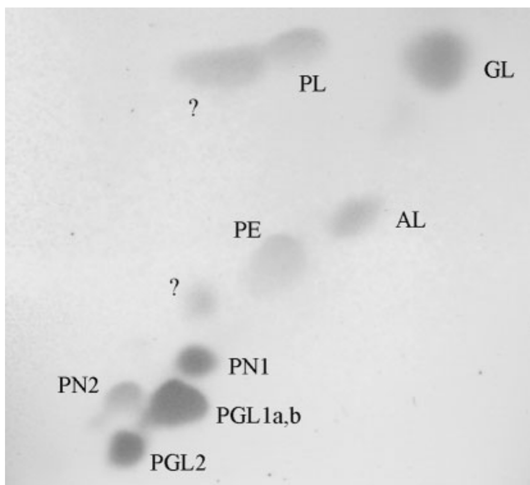

Fig. 1. Two-dimensional chromatogram showing the different polar lipids of strain BN3[T]. ?, Unidentified lipid; PL, phospholipid; PE, phosphatidylethanolamine; AL, aminolipid; PN1, aminophospholipid 1; PN2, aminophospholipid 2; PLG1, phosphoglycolipid 1 [resolving into a major (a) and a minor (b) spot]; PLG2, phosphoglycolipid 2; GL, glycolipid.

Fatty acids were analysed as the methyl ester derivatives prepared from 10 mg dry cell material. Cells were subjected to differential hydrolysis in order to detect ester-linked and non-ester-linked (amide-bound) fatty acids (B. J. Tindall, unpublished). Fatty acid methyl esters were analysed by gas chromatography using a 0·2 μm × 25 m non-polar capillary column and flame-ionization detection. The run conditions were: injection and detector port temperature 300 °C, inlet pressure 60 kPa, split ratio 50 : 1, injection volume 1 μl, with a temperature program from 130 to 310 °C at a rate of 4 °C min^{-1}. The carrier gas was hydrogen.

The fatty acids of strain BN3[T] comprised a complex mixture of straight-chain, branched (iso- and anteiso-) and hydroxylated fatty acids (Table 1). Major cellular fatty acids were anteiso-15 : 0, anteiso-13 : 0, iso-15 : 0 and 15 : 0. Among the hydroxy fatty acids, 3-OH iso-16 : 0, 3-OH iso 17 : 0 and 2-OH 17 : 0 predominated. Differential hydrolysis indicated that 2-OH 17 : 0, 3-OH iso-16 : 0 and 3-OH iso 17 : 0 were presumptively amide-linked, as well as ester-linked in smaller proportions. The presence of a complex pattern of fatty acids including straight-chain, branched (iso- and anteiso-) and hydroxylated fatty acids is a characteristic found in many species within the *Bacteroides–Flavobacterium–Cytophaga–Sphingobacterium* group (Daneshvar *et al.*, 1991; Fautz *et al.*, 1979; Mayberry, 1980; Miyagawa & Suto, 1980; Miyagawa *et al.*, 1979; Moore *et al.*, 1994; Oyaizu & Komagata, 1981; Sakamoto *et al.*, 2002; Shah & Collins, 1980, 1983; Steyn *et al.*, 1998; Urakami & Komagata, 1986). A detailed survey of the fatty acid patterns within this group is outside the scope of the present work, but it is obvious that features such as the presence or absence of significant amounts of unsaturated fatty acids or the relative abundance of iso- and anteiso-branched fatty acids of differing chain length can be used to differentiate different subgroups. This aspect has been emphasized in the work of Moore *et al.* (1994) and Shah & Collins (1980, 1983, 1988, 1989, 1990). Similarly, the distribution of 2- and 3-OH hydroxy fatty acids may be additional differential criteria.

Although the chemical diversity of the members of the phylum '*Bacteroidetes*' may be taken as an indication that this group is rapidly evolving, the combination of high levels of sequence divergence and chemical heterogeneity in a group is indicative of immature taxonomies (B. J. Tindall, unpublished). Thus, the chemical heterogeneity in the genera *Bacillus*, *Pirellula* and *Methanogenium* (Minnikin & Goodfellow, 1981; Koga *et al.*, 1993; Sittig & Schlesner, 1993; Schlesner *et al.*, 2004) may be taken to correlate well with the hypothesis that they are rapidly

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Table 1. Cellular fatty acid composition of strain BN3[T] determined by differential analysis to detect ester-linked and non-ester-linked (amide-bound) fatty acids

Values are percentages of total fatty acids. Method 1 releases ester-linked fatty acids only, whereas method 2 releases both ester-linked and non-ester-linked fatty acids. ND, Not detected.

Fatty acid	Method 1	Method 2
11 : 0 anteiso	0·13	0·17
11 : 0	0·08	0·08
12 : 0 iso	1·02	0·95
13 : 0 iso	1·85	1·68
13 : 0 anteiso	8·60	7·91
13 : 0	0·72	0·66
14 : 0 iso	4·99	4·56
14 : 0	0·34	0·31
15 : 0 iso	8·39	7·40
15 : 0 anteiso	37·05	33·90
15 : 0	15·73	14·52
3-OH 14 : 0 iso	0·29	0·26
16 : 0 iso	2·90	2·65
16 : 1ω7*c*	0·00	0·15
16 : 0	1·84	1·91
3-OH 15 : 0 iso	0·20	0·74
2-OH 15 : 0	0·18	0·41
3-OH 15 : 0	0·00	0·74
17 : 0 iso	4·27	4·11
17 : 0 anteiso	1·74	1·63
17 : 1ω8*c*	0·12	0·18
17 : 0	4·71	4·34
3-OH 16 : 0 iso	0·75	2·07
3-OH 16 : 0	0·26	0·27
18 : 0 iso	0·24	0·28
18 : 0 anteiso	0·00	0·08
18 : 0	0·44	0·48
3-OH 17 : 0 iso	0·79	2·38
2-OH 17 : 0	1·47	4·49
3-OH 17 : 0	0·20	0·37
19 : 0 anteiso	0·11	0·23
20 : 0 iso	0·60	ND

evolving (Liesack *et al.*, 1992; Rouviere *et al.*, 1992; Stackebrandt, 1988). The alternative interpretation is that the chemical and genetic diversity of the group may be simply reduced by creating a number of genera which are chemically and genetically more homogeneous. This point has been discussed by Schlesner *et al.* (2004), but is also evident in other publications (Blotevogel *et al.*, 1992; Hezayen *et al.*, 2002; Stöhr *et al.*, 2001; Wainø *et al.*, 1999; Zellner *et al.*, 1999).

DNA was isolated after disruption of cells using a French pressure cell (Thermo Spectronic) and purified by hydroxyapatite chromatography (Cashion *et al.*, 1977). The DNA was hydrolysed with P1 nuclease and the nucleotides were dephosphorylated with bovine alkaline phosphatase (Mesbah *et al.*, 1989). The G+C content of the DNA of strain BN3[T] was 40·8 mol% as determined by using the HPLC method (Tamaoka & Komagata, 1994).

The 16S rRNA gene of the isolate was amplified as described previously (L'Haridon *et al.*, 1998). PCR products were sequenced by the BigDye Terminator version 3.1 method as recommended by the manufacturer. The nearly complete sequence (1413 bp) of the 16S rRNA gene was directly sequenced on both strands with a 3730 XL sequencer (Applied Biosystems). The sequence was submitted to the GenBank database of the NCBI (http://www.ncbi.nih.gov) using the BLAST program. It was manually aligned against its closest relatives using FastAlign version 3.0 of the ARB program package (http://www.arb-home.de). All phylogenetic trees were constructed by using the ARB package. Distance trees were constructed by using neighbour-joining algorithms (Saitou & Nei, 1987) with the Jukes–Cantor correction (Jukes & Cantor, 1969). Parsimony and maximum-likelihood trees were constructed using the PHYLIP package (Felsenstein, 1993) and fastDNAmL software (Olsen *et al.*, 1994), respectively. The robustness of distance and parsimony tree topologies was evaluated by using a bootstrap analysis after 100 samplings.

The 16S rRNA gene sequence analyses placed strain BN3[T] as a member of the phylum '*Bacteroidetes*' having *T. forsythensis* (88 % similarity) and *B. merdae* (87 % similarity) as its closest cultivated relatives. However, very high similarities (99·6 %) were shared between the 16S rRNA gene sequence of strain BN3[T] and those of environmental clones retrieved from a dechlorinating consortium (GenBank accession no. AJ488088) and bovine rumen (AB003390). Phylogenetic trees were generated using three methods. Bootstrap values from 100 samplings confirmed the affiliation of the novel strain to a clade that also included the sequences of these environmental clones (Fig. 2).

Due to its fermentative metabolism, strain BN3[T] resembles species of the genus *Bacteroides* and differs from its closest phylogenetic relatives such as *Tannerella* or *Porphyromonas* (Sakamoto *et al.*, 2002). However, the predominance of MK-8 as menaquinone is a feature that distinguishes the novel isolate from all its phylogenetic relatives (Table 2). In addition, the 16S rRNA gene sequences of strain BN3[T] and its relatives are very different (more than 10 % distance). To our knowledge, this is the first report of the isolation of a member of the phylum '*Bacteroidetes*' from an oilfield. Several authors have however reported the presence of members of the *Cytophaga–Flavobacterium–Bacteroides* group in oil-polluted environments (von Wintzingerode *et al.*, 1999; LaPara *et al.*, 2000; Teske *et al.*, 2002; Elshahed *et al.*, 2003) and a possible indirect role in hydrocarbon metabolism has been suggested (Elshahed *et al.*, 2003). In a previous study on the Pelican lake oilfield (A. Grabowski, O. Nercessian, F. Fayolle, D. Blanchet & C. Jeanthon, unpublished), 16S rRNA gene sequences very closely related to that of strain BN3[T] were retrieved from cultures grown in the presence of acetate as sole carbon

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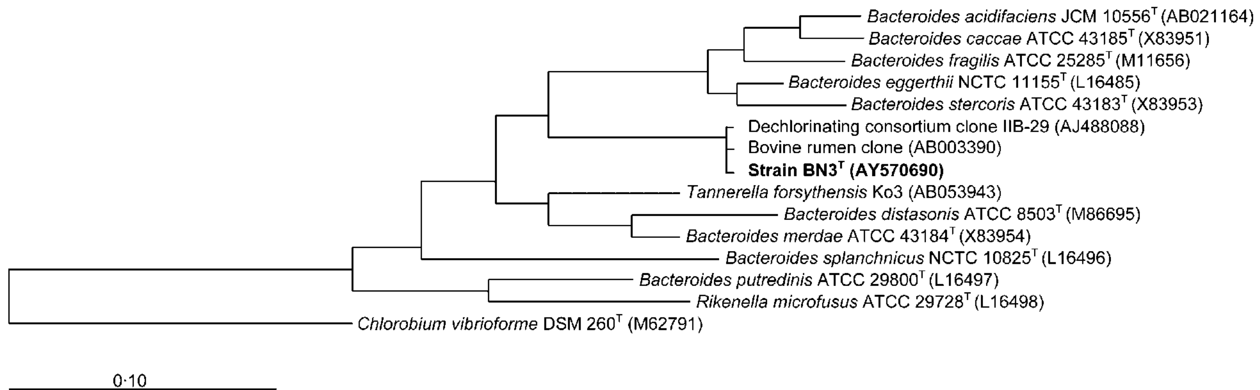

Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain BN3^T within the *Bacteroides* subgroup of the phylum '*Bacteroidetes*'. The topology shown was obtained using the maximum-likelihood method using *Chlorobium vibrioforme* as outgroup. The scale bar represents 0·01 changes per nucleotide position.

and energy source. This suggests that strains closely related to strain BN3^T could grow in the absence of tryptone, glucose, yeast extract and sulfur. Since we demonstrated that these compounds were required or stimulatory for growth of strain BN3^T, it could suggest that strain BN3^T could have another metabolism *in situ*, in cooperation with or dependent on that of other micro-organisms.

Based on a combination of 16S rRNA gene sequence, chemotaxonomic and physiological data, we propose that strain BN3^T be placed into a novel genus for which we propose the name *Petrimonas*, and as a novel species, *Petrimonas sulfuriphila*.

Description of *Petrimonas* gen. nov.

Petrimonas (Pe.tri.mo′nas. L. fem. n. *petra* rock, stone; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Petrimonas* stone monad).

Cells are straight, Gram-negative rods. Spores are not formed. Strictly anaerobic. Mesophilic. Carbohydrates and some organic acids are fermented. Major respiratory quinones are menaquinones; the predominant quinone is MK-8, with smaller amounts of MK-7 and MK-9. The major polar lipids are phosphatidylethanolamine, an unidentified phospholipid, two unidentified aminophospholipids, three unidentified phosphoglycolipids, a glycolipid, an aminolipid and two additional uncharacterized lipids. The fatty acids comprise both straight-chain and branched fatty acids, in addition to which both 2-OH and 3-OH fatty acids are present. Major cellular fatty acids are anteiso-15 : 0, anteiso-13 : 0, iso-15 : 0 and 15 : 0. Among the hydroxy fatty acids, 3-OH iso-16 : 0, 3-OH iso 17 : 0 and 2-OH 17 : 0 predominate. About one-third of each of all three appear to be amide-linked. On the basis of the 16S rRNA gene analysis, the genus *Petrimonas* is most closely related to the genera *Bacteroides* and *Tannerella* within the phylum *Bacteroidetes*. The type species is *Petrimonas sulfuriphila*.

Table 2. Differential characteristics of *Petrimonas sulfuriphila* gen. nov., sp. nov. and some related taxa

Data for related taxa were taken from Sakamoto *et al.* (2002).

Characteristic	*Petrimonas sulfuriphila*	*Tannerella*	*[Bacteroides] distasonis*	*[Bacteroides] merdae*	*Bacteroides sensu stricto*	*Porphyromonas*
Metabolism*	F	NF	F	F	F	NF†
Major end-products‡	A	A, B, IV, P, PA	A, S	A, S	A, S	A, B, IV, P, PA, S
Major cellular fatty acids	15 : 0 anteiso	15 : 0 anteiso	15 : 0 anteiso	15 : 0 anteiso	15 : 0 anteiso	15 : 0 iso§
Ratio of 15 : 0 anteiso to 15 : 0 iso	4·5	22·8–95·2	4·1	6·3	2·1–5·4	<1
Predominant menaquinone(s)	MK-8	MK-10, MK-11	MK-10	MK-9, MK10	MK-10, MK-11	MK-9, MK-10
G+C content (mol%)	40·8	44–48	43–45	43–46	40–48	40–55
Habitat	Oil reservoir	Periodontal pockets	Faeces	Faeces	Faeces	Oral cavities

*F, Fermentative; NF, non-fermentative.

†Some species are weakly saccharolytic.

‡A, Acetic acid; B, butyric acid; IV, isovaleric acid; P, propionic acid; PA, phenylacetic acid; S, succinic acid.

§*Porphyromonas catoniae* contains approximately equal amounts of 15 : 0 iso and anteiso as the predominant fatty acids.

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Description of *Petrimonas sulfuriphila* sp. nov.

Petrimonas sulfuriphila (sul.fu.ri.phi′la. L. n. *sulfur* sulfur; Gr. adj. *philos* loving; N.L. fem. adj. *sulfuriphila* sulfur-loving, indicating that sulfur stimulates growth).

Cells (0·7–1 × 1·5–2 μm) occur singly or in pairs. Chemo-organotroph. The temperature range for growth is 15–40 °C and the optimum is 37–40 °C at pH 7·2. The NaCl concentration range is 0–4 % with an optimum at 0 %. Tryptone is required for growth. Yeast extract and elemental sulfur stimulate growth. Glucose, arabinose, galactose, maltose, mannose, rhamnose, lactose, ribose, fructose, sucrose, lactate, mannitol, glycerol and cellobiose are fermented. Acetate, hydrogen and CO_2 are produced during glucose fermentation. Elemental sulfur is reduced to sulfide and nitrate is reduced to ammonium. The G + C content of the DNA of the type strain is 40·8 mol% (HPLC).

The type strain, BN3^T (= JCM 12565^T = DSM 16547^T), was isolated from an oilfield well head in the Western Canadian Sedimentary Basin (Canada).

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