Oleispira antarctica gen. nov., sp. nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water

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The taxonomic characteristics of two bacterial strains, RB-8^T and RB-9, isolated from hydrocarbon-degrading enrichment cultures obtained from Antarctic coastal marine environments (Rod Bay, Ross Sea), were determined. These bacteria were psychrophilic, aerobic and Gram-negative with polar flagella. Growth was not observed in the absence of NaCl, occurred only at concentrations of Na^+ above 20 mM and was optimal at an NaCl concentration of 3–5 % (w/v). The major cellular fatty acids were monounsaturated straight-chain fatty acids. The strains were able to synthesize the polyunsaturated fatty acid eicosapentaenoic acid (20 : 5ω3) at low temperatures. The DNA G+C contents were 41–42 mol%. The strains formed a distinct phyletic line within the γ-*Proteobacteria*, with less than 89·6 % sequence identity to their closest relatives within the *Bacteria* with validly published names. Both isolates exhibited a restricted substrate profile, with a preference for aliphatic hydrocarbons, that is typical of marine hydrocarbonoclastic micro-organisms such as *Alcanivorax*, *Marinobacter* and *Oleiphilus*. On the basis of ecophysiological properties, G+C content, 16S rRNA gene sequences and fatty acid composition, a novel genus and species within the γ-*Proteobacteria* are proposed, *Oleispira antarctica* gen. nov., sp. nov.; strain RB-8^T (=DSM 14852^T=LMG 21398^T) is the type strain.

INTRODUCTION

Hydrocarbon-degrading micro-organisms usually exist in very low abundance in the absence of oil pollution. A pollution event is rapidly followed by a bloom of these micro-organisms, the populations of which expand to nearly complete dominance of the viable microbial community during the period of contamination (Margesin & Schinner, 1999; Harayama *et al.*, 1999). The properties of hydrocarbon compounds depend on the ambient temperature. Short-chain alkanes become less volatile and more water-soluble at low temperatures, whereas longer-chain compounds precipitate under cold conditions as waxes, respectively rendering them bioavailable and inaccessible to microbes. Such behaviour at low temperatures obviously reflects the establishment of specific oil-based marine microbial communities at these temperatures that are somehow different from those observed in a temperate climate. The most important permanently cold habitat is the ocean, since the temperature of more than 90 % of the seawater volume is below 5 °C. Genera that are typically well represented in cold, petroleum-contaminated sites are *Acinetobacter*, *Arthrobacter*, *Mycobacterium*, *Pseudomonas*, *Rhodococcus* and *Sphingomonas*, many of which can grow solely on hydrocarbon compounds and have been previously characterized as petroleum degraders of terrestrial origin (Rosenberg *et al.*, 1992; MacCormack & Fraile, 1997). Although the role of these microbes is evident in the petroleum-degradation process in cold marine environments,

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Abbreviations: PLFA, phospholipid fatty acid; PUFA, polyunsaturated fatty acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of *Oleispira antarctica* strains RB-8^T and RB-9 are respectively AJ426420 and AJ426421.

there are also marine hydrocarbonoclastic bacteria involved in this process. Such micro-organisms of the genera *Alcanivorax* (Yakimov *et al.*, 1998), *Cycloclasticus* (Dyksterhouse *et al.*, 1995), *Marinobacter* (Gauthier *et al.*, 1992) and *Oleiphilus* (Golyshin *et al.*, 2002) represent recently described new genera and families within the *γ-Proteobacteria*. Some of the species, namely *Alcanivorax borkumensis*, '*Cycloclasticus oligotrophus*' and '*Marinobacter arcticus*', were initially isolated from permanently cold marine environments (Yakimov *et al.*, 1998; Button *et al.*, 1998).

In this paper, the isolation and morphological, phenotypic, genetic and chemotaxonomic characterization of an aerobic hydrocarbonoclastic bacterium are reported. Strains were obtained from sea-water samples collected from Rod Bay (Ross Sea, Antarctica) during an Italian scientific expedition during the Antarctic summer of 1999–2000. Two of these bacterial strains, RB-8^T and RB-9, represent a novel genus, designated *Oleispira* gen. nov.

METHODS

Isolation. Two isolates, RB-8^T and RB-9, were obtained from superficial sea-water samples collected in the inlet Rod Bay (Ross Sea; 74°41·753′S, 164°07·188′E) during an Italian oceanographic expedition in the Antarctic summer of 1999–2000. Enrichment cultures were made by supplementing 20 ml samples with crude oil (Arabian light; 0·1 %, v/v) and nutrients [$(NH_4)H_2PO_4$; 0·2 %, w/v], which were maintained for 2 months at 4 °C until transported for further work in the laboratory. Replicate 10-fold dilutions of the primary enrichment were made in 10 ml ONR7a mineral medium (Dyksterhouse *et al.*, 1995) supplemented with sterile crude Arabian light oil (0·1 %, v/v). The tubes were incubated in the dark at 4 °C until turbidity changes due to bacterial growth ceased (approx. 8 weeks). Positive tubes representing the highest dilution (10^{-4}) were subsequently plated on solid ONR7a mineral medium supplemented with tetradecane and single colonies were obtained after 15 days incubation at 4 °C.

Nutritional and growth characteristics. Basal medium ONR7a supplemented with *n*-tetradecane was used throughout these studies unless stated otherwise. Growth under anaerobic conditions was determined by testing growth of strain RB-8^T on ONR7a supplemented with 0·5 % acetate and 0·25 % $NaNO_3$ (w/v) at 12 °C in an anaerobic chamber (5 % CO_2, 7 % H_2 and 88 % N_2). Routine tests, like Gram staining and agarase, amylase, catalase, gelatinase, lipase and oxidase activity, were carried out as described by Smibert & Krieg (1981). Arginine dihydrolase, lysine decarboxylase, urease and ornithine decarboxylase activities and accumulation of poly-*β*-hydroxybutyrate were determined using tests developed for marine bacteria (Baumann & Baumann, 1981). The isolates were further tested for their ability to oxidize different carbon sources using Gram-negative MicroPlates (Biolog) according to the manufacturer's instructions. Data were analysed using the software package provided by Biolog.

To determine the salinity and temperature ranges for growth, ONR7a medium supplemented with *n*-tetradecane was prepared by adjusting the concentration of NaCl (0·01–2·00 M, i.e. 0·06–12·00 %, w/v) and incubating cultures at 1, 2, 4, 10, 15, 20, 25 and 30 °C. Tubes containing 10 ml medium were inoculated with 0·5 ml cells taken from late-exponential-phase cultures grown at 10 °C. Growth was measured by OD_{600} for up to 20 days. Growth was considered to have occurred if the OD_{600} increased by more than 20 %. Five replicate test cultures of each strain were analysed after three serial transfers under identical conditions.

Electron microscopy. Mid-exponential-phase cells of isolate RB-8^T were prepared for ultrastructural analysis by transmission electron microscopy. Briefly, vegetative cells were sedimented and fixed in 5 % glutaraldehyde, buffered with 50 mM PBS (Sigma), pH 7·1. Negative-staining, shadow-casting, embedding and ultrathin sectioning was done according to methods described previously (Yakimov *et al.*, 1998; Golyshina *et al.*, 2000).

Cellular fatty acid analysis. Lipids were extracted by a modified Bligh–Dyer procedure and fatty acid methyl esters were generated and analysed by GC, as described previously (Vancanneyt *et al.*, 1996).

G+C content and genome size estimation. The G+C contents of DNA isolated from strains RB-8^T and RB-9 were determined directly by HPLC with a Nucleosil 100-5 C18 column (Macherey-Nagel) according to a method described previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). Purified non-methylated lambda phage DNA (Sigma) was used as a control. PFGE separation of digests of the DNA by endonucleases *Asc*I, *Pac*I, *Pme*I and *Sfi*I (New England Biolabs) was performed using the Gene Navigator electrophoresis device (Pharmacia) with switch times ramped between 2 and 64 s at 6 V cm^{-1} (Shizuya *et al.*, 1992). Cells of RB-8^T were examined for plasmids using the Large Construct kit (Qiagen). DNA extracts obtained were later analysed by gel electrophoresis.

16S rRNA gene sequence determination and analysis of phylogenetic relationships. Genomic DNA was prepared from 5 ml late-exponential-phase cells using the CTAB preparative protocol for bacterial genomic DNA isolation, as described previously (Yakimov *et al.*, 1998). Genomic DNA was resuspended in 50 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8) and stored frozen (−20 °C) until the 16S rRNA genes were amplified. PCR amplification of the 16S rRNA genes was performed with an ABI 9600 (PE Applied Biosystems) using the forward primer 16F27 (5′-AGAGTTTGATCMTGGCTCAG-3′) and the reverse primer 16R1492 (5′-TACGGYTACCTTGTTACGACTT-3′). Amplified products were purified with QIAquick PCR purification columns (Qiagen). Direct sequence determination of the purified rRNA genes was carried out using an automated DNA sequencer model 377 (Applied Biosystems) and Prism Ready Reaction dideoxy terminator sequencing kit, according to the protocols of the manufacturer (PE Applied Biosystems). Nucleotide sequences of the 16S rDNA were obtained by sequencing both template strands at least twice and were initially aligned with the Ribosomal Database Project (RDP) database (Maidak *et al.*, 2001) by means of the automatic alignment function of the RDP phylogeny inference package (PHYLIP) interface. The Se-Al sequence alignment editor version 1.0α1 (Rambaut, 1996) was subsequently used to refine the alignments. To make multiple bootstrapped datasets, alignments were exported as PHYLIP 3.5 interleaved file types to run the SEQBOOT program. The robustness of the topology of phylogenetic trees was evaluated by bootstrap analysis with 1000 replications. Evolutionary distances were calculated from pair-wise sequence similarities with Kimura's two-parameter model for nucleotide change, the multiple dataset option and a transition/transversion ratio of 2·0 using the DNADIST program available with PHYLIP version 3.573c (Felsenstein, 1993). The NEIGHBOR program was used to construct phylogenetic trees from evolutionary distance matrices by the neighbour-joining method. Random input order of sequences and multiple outgroups rooting with the 16S rDNA sequences of *Alcanivorax borkumensis* and *Cycloclasticus pugetii* were used to avoid potential bias introduced by the order of sequence

addition. The resulting tree files were analysed by the CONSENSE program to provide confidence estimates for phylogenetic tree topologies and to make a majority-rule consensus tree.

RESULTS AND DISCUSSION

Phenotypic and chemotaxonomic characterization

Two strains, RB-8^T and RB-9, were isolated from serial dilutions of an enrichment culture set up from superficial sea-water samples collected in the inlet Rod Bay (Ross Sea, Antarctica; 74°41·753′S, 164°07·188′E), and amended with crude Arabian light oil as the sole carbon source. Both strains formed visible colonies after 5–6 days incubation on ONR7a agar supplemented with *n*-tetradecane at 4 °C. The two strains exhibited almost identical phenotypic features. The isolates were aerobic, Gram-negative, non-spore-forming, catalase- and oxidase-positive, motile vibrioid to spiral organisms. Cells grew actively on ONR7a agar supplemented with acetate or pyruvate (2 %, w/v), forming pale-cream colonies, whereas alkane-grown cells formed transparent, slightly yellowish colonies. Sodium nitrate could serve as the sole source of nitrogen. Nitrate was reduced to nitrite under facultatively anaerobic conditions. Strains exhibited strong 'Tweenase' activity, although agarase, amylase, arginine dihydrolase, gelatinase and aesculinase activities were not detected. The strains were not able to grow on α-, β- or γ-hydroxybutyrates and no accumulation of poly-β-hydroxybutyrate was detected. The isolates share many traits with the recently described genera of marine hydrocarbonoclastic bacteria *Alcanivorax*, *Marinobacter* and *Oleiphilus*, including isolation from a marine environment, purely respiratory metabolism (i.e. lack of fermentative metabolism), relatively restricted nutritional profiles, with a strong preference for aliphatic hydrocarbons, and other phenotypic traits. Strains RB-8^T and RB-9 grew in liquid and solid media supplemented with aliphatic hydrocarbons with chain lengths between C_{10} and C_{18} and their fatty alcohols and acids, as well as some other compounds, such as Tweens 20, 40 and 80. Using the procedure reported by Smits *et al.* (1999), which was used successfully for *Alcanivorax borkumensis* (Smits *et al.*, 2002) and *Oleiphilus messinensis* (Golyshin *et al.*, 2002), a gene for alkane hydroxylase/alkane monooxygenase, the key enzymes of alkane catabolism, was not detected (data not shown), which suggests a certain novelty in the structure of those enzymes.

Table 1. Phenotypic characteristics that differentiate the genus *Oleispira* gen. nov. from the related genera *Oceanobacter*, *Marinomonas* and *Marinobacterium*

Data from Bowditch *et al.* (1984) and this study. +, Positive for all strains; −, negative for all strains; ND, not determined; d, differs among strains. Members of all four genera are negative for utilization of galacturonate and glucuronate.

Characteristic	*Oleispira*	*Oceanobacter*	*Marinomonas*	*Marinobacterium*
Morphology	Helical	Rods	Rods	Rods
Optimal temperature (°C)	2–4	25–32	20–25	37
Growth at 30 °C	−	+	+	−
NaCl concentration for growth: (%)				
Optimum	3·0–5·0	0·5–8·0	0·7–3·5	0·6–2·9
Maximum	10·7	8·0	ND	11·6
Nitrate reduction to nitrite	+	−	d	d
Gelatin liquefaction	−	−	d	d
Lipase	+	+	−	−
Utilization of:				
D-Glucose	−	−	+	+
D-Fructose	−	+	+	d
D-Mannose	−	−	d	−
Cellobiose	−	−	d	+
D-Mannitol	−	+	+	ND
Glycerol	−	−	+	−
Gluconate	−	−	+	ND
Succinate	−	d	+	−
L-Aspartate	−	−	d	+
L-Glutamate	−	d	+	+
DL-Alanine	−	−	+	+
Hexadecane	+	ND	−	ND
p-Hydroxybenzoate	−	+	+	+
Poly-β-hydroxybutyrate accumulation	−	+	−	−
DNA G+C content (mol%)	41–42	54–56	46–49	55

The phenotypic characteristics that differentiate the novel Antarctic isolates from related genera are summarized in Table 1. In contrast with these genera, which are characterized by mesophilic behaviour, RB-8^T and RB-9 showed better growth at temperatures below 25 °C, with a broad growth temperature optimum between 1 and 15 °C and a maximum growth temperature of about 27–28 °C. The minimal growth temperature was estimated to be −6·8 °C using the Ratkowsky square-root temperature growth model (Ratkowsky *et al.*, 1983). The isolates were stenohaline, exhibiting optimal growth in the presence of sea water. Growth occurred at NaCl concentrations of 0·02–2·00 M, with an optimum between 0·5 and 0·9 M NaCl. They exhibited poor growth at NaCl concentrations below 0·25 M and above 1·0 M (half- and double-strength sea water, respectively).

Cell morphology and ultrastructural analysis

For ultrastructural analysis, mid-exponential-phase cells of isolate RB-8^T were shadow-cast (Fig. 1a, c) or embedded and ultrathin-sectioned (Fig. 1b), as described in Methods. Cells appeared characteristically vibrioid to spiral, with a cell length of 2–5 μm and a cell width of 400–800 nm. They were motile and possessed a single polar flagellum about 5 μm in length (Fig. 1a, c; white arrowheads). One distinguishing morphological feature was the drumstick-like thickening of one or both ends of the cell (Fig. 1a, b; opposing, solid arrowheads). As can be seen in the ultrathin sections (Fig. 1b), the cell wall, which exhibited a Gram-negative appearance, was more electron-dense at this terminal region of the cell. The cytoplasm exhibited no significant features, apart from an occasional polarly located nucleoplasm (Fig. 1b; asterisks), and no storage granules, either electron-dense or translucent, were observed.

Fatty acid and DNA base composition

As revealed by MIDI analysis, the most abundant non-polar fatty acids of the Antarctic strains were C18 : 1 (32·0 %), C16 : 1 (29·9 %) and C16 : 0 (23·9 %) (Table 2). Such a profile is typical of marine γ-*Proteobacteria* belonging to the genus *Oceanospirillum* and other related genera. Interestingly, no 3-hydroxy fatty acids, which have been detected in the majority of these bacteria, were found in either isolate. Strains RB-8^T and RB-9 exhibited very similar phospholipid fatty acid (PLFA) profiles, with the major constituents (>92 % total fatty acids) including mono-unsaturated 14 : 1, 16 : 1 and 18 : 1 and saturated 16 : 0 fatty acids (Table 3). Growth of the Antarctic strains at different temperatures affected the level of unsaturated fatty acids (55·2 % at 20 °C and up to 68·3 % at 4 °C). Such temperature-dependent changes in saturated/unsaturated fatty acid proportions is indicative of homeostatic adaptation of the cellular membrane in terms of its viscosity (Kaneda, 1991; Russell & Nichols, 1999). During cultivation

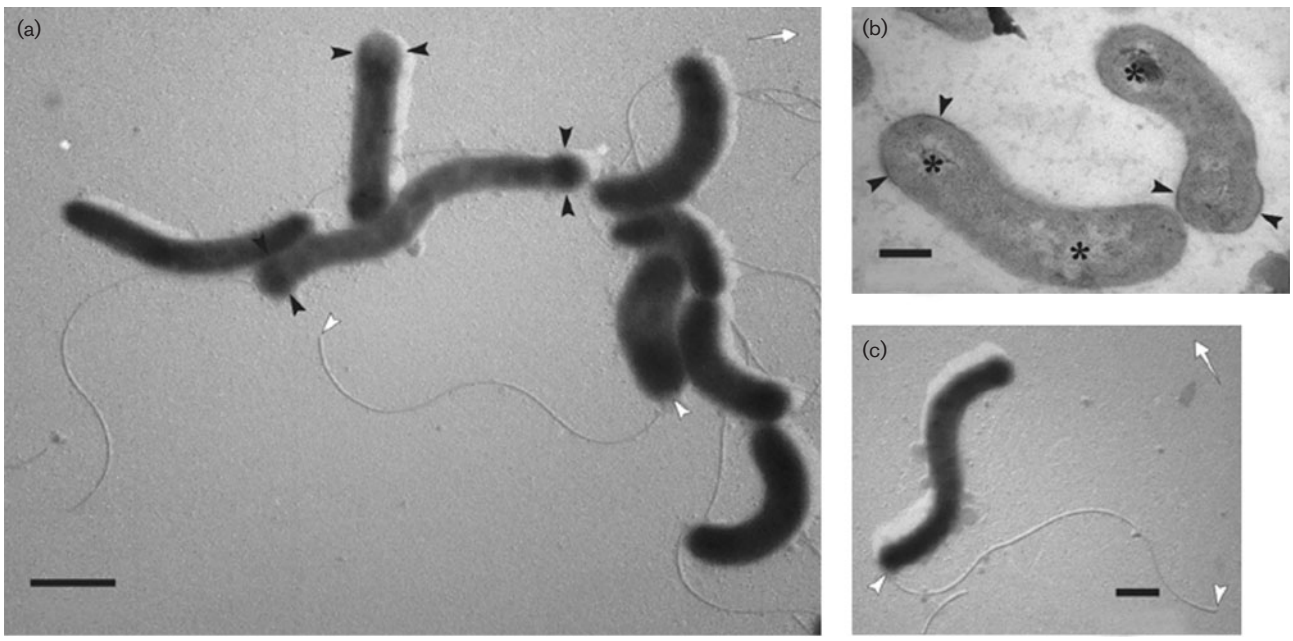

Fig. 1. Electron micrographs of *Oleispira antarctica* gen. nov., sp. nov. (a) General survey view of cell morphology; bar, 1·1 μm. Shadow-cast cells showing monopolar, monotrichous flagellation; white arrowheads indicate the ends of a single flagellum. Opposing black arrowheads point to the drumstick-like thickening of the apical cell endings. (b) Ultrathin-sectioned cells showing polarly oriented nucleoplasm (asterisks) and electron-dense regions within the cell wall of the cell endings (opposing black arrowheads); bar, 250 nm. (c) Habitus of a monopolar, monotrichous flagellated cell; bar, 600 nm. White arrows (a, c) indicate the shadowing direction.

Table 2. Cellular fatty acid composition of strains RB-8^T and RB-9

Fatty acids are designated as total number of carbon atoms with the position of the double bond indicated from the methyl end (ω system). The prefix 'ai-' indicates anteiso fatty acids. Values are mean percentages of total fatty acid content from RB-8^T and RB-9; standard deviations are given in parentheses.

Fatty acid	Content (%)
Saturated fatty acids	
C12 : 0	1·2 (0·7)
C13 : 0	1·4 (0·4)
C14 : 0	1·2 (0·2)
C15 : 0	0·3 (0·1)
C16 : 0	23·9 (2·2)
ai-C17 : 0	1·5 (0·5)
C17 : 0	0·5 (0·1)
C18 : 0	1·2 (0·4)
Sum	31·2
Unsaturated fatty acids	
C14 : 1ω11	0·2 (0·1)
C16 : 1ω9	6·8 (0·6)
C16 : 1ω7*c*	22·4 (1·2)
C16 : 1ω7*t*	0·7 (0·2)
C17 : 1ω7	0·2
C18 : 2ω6,9	6·3 (0·8)
C18 : 1ω9	29·7 (1·9)
C18 : 1ω7*c*	2·0 (0·3)
C18 : 1ω7*t*	0·3 (0·05)
C20 : 1ω9	0·2
Sum	68·8

Table 3. Phospholipid fatty acid profile of *Oleispira antarctica* gen. nov., sp. nov. in response to different cultivation temperatures

tr, Fatty acids present at trace levels (<0·2 % of total fatty acids). Other abbreviations are the same as in Table 2. Standard deviations are given in parentheses.

Fatty acid	Content (%)	
	4 °C	20 °C
Saturated fatty acids		
C12 : 0	0·7 (0·1)	1·4 (0·2)
C13 : 0	tr	3·2 (0·3)
C14 : 0	3·2 (0·6)	5·3 (0·8)
C15 : 0	tr	2·4 (0·2)
C16 : 0	27·8 (2·1)	32·5 (2·9)
Sum	31·7	44·8
Unsaturated fatty acids		
C14 : 1ω11	8·4 (1·8)	2·4 (0·3)
C16 : 1ω7/ω9	49·6 (2·9)	49·9 (3·8)
C17 : 1ω7	2·1 (0·5)	0·5 (0·1)
C18 : 1ω7/ω9	6·6 (0·9)	2·4 (0·4)
C20 : 5ω3	1·6 (0·3)	tr
Sum	68·3	55·2

at low temperatures, the strains synthesized the polyunsaturated fatty acid (PUFA) eicosapentaenoic acid, 20 : 5ω3*c* (up to 1·6 % total PLFA), which was not detected in the PLFA profile at 20 °C. Thus, the Antarctic isolates had non-constitutive PUFA synthesis, induced by low cultivation temperature. Current studies are examining the specific synthesis pathways of PUFA formation in these bacteria.

G+C content and genome size

The strains had G+C contents of 41–42 mol% (mean 41·6±0·6; n=5). The G+C contents of the amplified rRNA gene sequences from strains RB-8^T and RB-9 were 52·44–52·54 mol%. These relatively low 16S rRNA G+C contents are consistent with metabolic responsiveness at low environmental temperatures (Woese *et al.*, 1991). As revealed by PFGE analysis of endonuclease digests of genomic DNA of isolate RB-8^T, the size of the genome was about 2·0 Mbp. No plasmids were visualized.

Phylogenetic analysis

Near-complete 16S rDNA sequences (1438 bp) of isolates RB-8^T and RB-9 were determined. The strains were phylogenetically very similar (99·5 % identity), with six mismatches detected along the sequenced 16S rDNA fragments. Initial sequence comparison against the 16S rRNA sequences available in GenBank and the RDP (Altschul *et al.*, 1997; Maidak *et al.*, 1997; Pearson & Lipman, 1988) indicated that the strains belong to the *γ-Proteobacteria*. Subsequently, the sequences were aligned manually against those of representatives of the *γ-Proteobacteria*, as described in Methods. Depending on the method of analysis, the Antarctic isolates were phylogenetically most closely related to a number of marine bacteria that included species of the genera *Oceanobacter* (Satomi *et al.*, 2002), *Marinobacterium* and *Marinomonas*. In all cases, none of the bacterial species with validly published names showed more than 90 % sequence similarity (89·6 and 89·7 % 16S rDNA sequence similarity to *Oceanobacter kriegii* and *Marinomonas vaga*, respectively). It was obvious that the isolates formed an independent phyletic line within the *γ-Proteobacteria* with a rather uncertain phylogenetic position, clustering either with the *Marinomonas vaga* group or the *Oceanobacter kriegii* lineage (Satomi *et al.*, 2002), with bootstrap values of less than 50 % in both cases (Fig. 2). Using the approach of Anzai *et al.* (2000), by eliminating the hypervariable regions at positions 70–100, 181–219, 447–487, 1004–1036, 1133–1141 and 1446–1456 (in the *Escherichia coli* numbering system) from the analysis, placement of the genus *Oleispira* within this cluster was more evident (Fig. 2). It is interesting that a close phylogenetic relationship was found between the novel strains and the non-identified environmental clones Arctic95B-13, Arctic95B-7 and Arctic95B-17, recovered from bacterioplankton assemblages of the Arctic Ocean

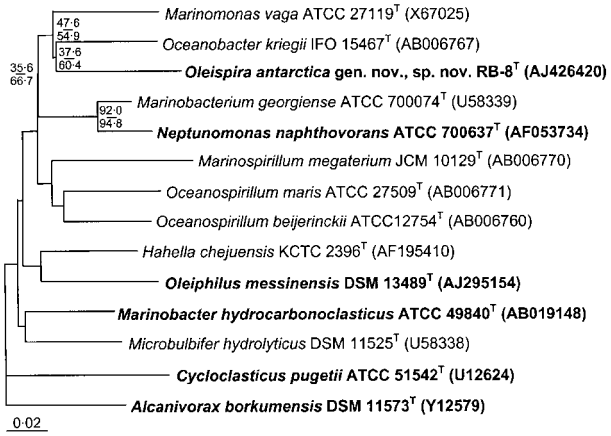

Fig. 2. Estimated phylogenetic position of *Oleispira antarctica* gen. nov., sp. nov. RB-8^T among the most closely related representatives of the *γ-Proteobacteria*, derived from 16S rRNA gene sequence comparisons. The tree, based on 1430 nt positions, was constructed by the neighbour-joining method and nucleotide substitution rates (K_{nuc} values) and computed by Kimura's two-parameter model. *Alcanivorax borkumensis* and *Cycloclasticus pugetii* were included as the multiple outgroup. Hydrocarbonoclastic marine bacteria are shown in bold. Numbers at nodes, related to the positioning of *Oleispira antarctica*, indicate the percentage occurrence in 1000 bootstrapped trees: upper values were obtained after comparison of full sequences, whereas the lower ones were obtained using the approach of Anzai *et al.* (2000). Bar, genetic distance of 0·02 (K_{nuc}).

(Bano & Hollibaugh, 2002) (respectively 98·5, 98·4 and 98·3 % 16S rDNA sequence similarity).

It is apparent from the phenotypic properties and differences in 16S rRNA gene sequences that the hydrocarbon-degrading Antarctic strains RB-8^T and RB-9, isolated from sea-water samples collected in Rod Bay (Ross Sea, Antarctica), cannot be assigned to any previously recognized bacterial genus and represent a novel species within a new genus, *Oleispira antarctica* gen. nov., sp. nov.

Description of *Oleispira* gen. nov.

Oleispira (O.le.i′spi.ra. L. n. *oleum* oil; Gr. fem. n. *spira* a spire; N.L. fem. n. *Oleispira* an oil-degrading, spiral-shaped organism).

Gram-negative, vibrioid to spiral cells, 2·0–5·0 μm long by 0·4–0·8 μm wide, motile by a single polarly inserted, long (>5 μm) flagellum. Chemoheterotroph with strong preference for aliphatic carbon substrates. Aerobic. Able to grow under anaerobic conditions by nitrate reduction. Oxidase and catalase are present. Ammonia and nitrate may serve as nitrogen sources. The narrow range of growth-supporting substrates is restricted to aliphatic hydrocarbons, Tweens and volatile fatty acids. Uptake of common carbohydrates or amino acids as sole carbon sources for growth is detected in a very narrow spectrum. Stenohaline, requires Na^+ ions, exhibiting optimal growth in the presence of 3–5 % (w/v) NaCl. Psychrophilic growth, with optimal growth temperature of 2–4 °C. The major cellular fatty acids are monounsaturated fatty acids. The type and only species of the genus is *Oleispira antarctica.*

Description of *Oleispira antarctica* sp. nov.

Oleispira antarctica (ant.arc′ti.ca. N.L. fem. adj. *antarctica* of the Antarctic, where the organism was isolated).

In addition to the traits reported for the genus, the species is able to grow at 1–25 °C, is negative for hydrolysis of starch, casein, lecithin, alginate and agar and uses the carbon sources shown in Table 1. Colonies on ONR7a supplemented with tetradecane are opaque, unpigmented or slightly yellow. The G+C content of the type strain is 41·6 mol%. Principal fatty acids detected are C18:1 (32·0 %), C16:1 (29·9 %) and C16:0 (23·9 %). Under low cultivation temperatures, strains are able to synthesize polyunsaturated eicosapentaenoic acid (20:5ω3*c*). According to the 16S rRNA sequence, the isolates belong to the *γ-Proteobacteria.* Phylogenetically, forms an independent phyletic line with a rather uncertain position, clustering either with the *Marinomonas vaga* group or the *Oceanospirillum kriegii* lineage. No bacterial species with validly published names show more than 90 % sequence identity.

The type strain is RB-8^T (=DSM 14852^T=LMG 21398^T), isolated from superficial sea-water samples collected in the inlet Rod Bay (Ross Sea, Antarctica).

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