Bermanella marisrubri gen. nov., sp. nov., a genome-sequenced gammaproteobacterium from the Red Sea

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A novel heterotrophic, marine, strictly aerobic, motile bacterium was isolated from the Red Sea at a depth of 1 m. Analysis of its 16S rRNA gene sequence, retrieved from the whole-genome sequence, showed that this bacterium was most closely related to the genera *Oleispira*, *Oceanobacter* and *Thalassolituus*, each of which contains a single species, within the class *Gammaproteobacteria*. Phenotypic, genotypic and phylogenetic analyses supported the creation of a novel genus and species to accommodate this bacterium, for which the name *Bermanella marisrubri* gen. nov., sp. nov. is proposed. The type strain of *Bermanella marisrubri* is RED65^T (=CECT 7074^T =CCUG 52064^T).

Molecular biological techniques have demonstrated that members of the class *Gammaproteobacteria* are predominant among the marine bacterioplankton, together with members of the *Alphaproteobacteria* and the *Bacteroidetes* (Giovannoni & Rappé, 2000). Furthermore, the phylogenetic diversity of cultured and uncultured gammaproteobacteria accounts for 25–44% of the global marine bacterioplankton diversity, as revealed by analysis of 16S rRNA gene sequences reported in public databases (Hagström *et al.*, 2002). In previous studies, we have described several novel marine bacteria isolated from seawater collected in the Mediterranean Sea at the Blanes Bay Microbial Observatory (Pinhassi *et al.*, 2006, 2007;

Arahal *et al.*, 2007). In the present study, we describe a novel bacterium, designated strain RED65^T, isolated from a surface-seawater sample collected from the Gulf of Eilat in the northern Red Sea (29° 51' N 34° 94' E) on 24 September 2000.

The sample was inoculated in 0.2-µm-pore-size sterile-filtered seawater (inoculum/filtered seawater, 1:9) and then incubated in the dark for 48 h at 28 °C. For details on the experimental set-up, see Pinhassi & Berman (2003). For strain isolation, 0.1 ml of a 100 × dilution of sample water was spread onto agar plates prepared with seawater samples taken at the Gulf of Eilat. The formulation of the isolation medium was a modification of medium 2216 (ZoBell, 1946) and consisted of 750 ml seawater, 250 ml MilliQ water, 5 g peptone and 1 g yeast extract; for solid medium, 15 g agar was added. Sterilization was performed at 120 °C for 20 min. After primary isolation and purification, strain RED65^T was cultivated at room temperature on the same medium and stored at –80 °C in isolation medium with 25% (v/v) glycerol.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the genome sequence of strain RED65^T are AY136131 and AAQH00000000, respectively.

A scanning electron micrograph of cells of strain RED65^T and maximum-parsimony and maximum-likelihood phylogenetic trees based on almost-complete 16S rRNA gene sequences of strain RED65^T and closely related species are available as supplementary figures with the online version of this paper.

The complete 16S rRNA gene sequence of strain RED65^T was 1533 nt in length. This sequence was compared with public sequences in the EMBL gene databases by using the BLAST program (National Center for Biotechnology Information; <http://ncbi.nlm.nih.gov/>). Related sequences were further analysed using the ARB package (Ludwig *et al.*, 2004; <http://www.arb-home.de>). Sequence alignments were corrected manually using the sequence editor ARB_EDIT. Phylogenetic analysis using various treeing methods (neighbour joining, maximum parsimony and maximum likelihood) and data subsets were performed using the appropriate ARB tools (Ludwig *et al.*, 1998). Fig. 1 shows the tree derived from the neighbour-joining method using Jukes–Cantor evolutionary corrections. Phylogenetic analysis revealed that strain RED65^T was only distantly related to the species shown in Fig. 1: the highest sequence similarity obtained was with *Oceanobacter kriegii* IFO 15467^T (92.1 %); the remaining strains showed less than 91.9 % similarity. Both the high bootstrap values (Fig. 1) and the data from comparisons of the local topologies obtained using several treeing methods (see Supplementary Figs S1 and S2, available in IJSEM Online) serve to confirm that strain RED65^T clusters consistently with *Oleispira antarctica* RB-8^T in the first instance and thereafter with a node formed by *Oceanobacter kriegii* IFO 15467^T and *Thalassolituus oleivorans* MIL-1^T.

Whole-genome sequencing was carried out by the J. Craig Venter Institute through the Gordon and Betty Moore Foundation Initiative in Marine Microbiology (<https://research.venterlinstitute.org/moore/>). Genome sequencing revealed a DNA G + C content of 44.0 mol%. The genome size of strain RED65^T is approximately 3.53 Mbp (3314 putative open reading frames). Pulsed-field gel electrophoresis analysis of endonuclease digests of genomic DNA

from *Oleispira antarctica* RB-8^T and *T. oleivorans* MIL-1^T gave estimated genome sizes of 2.0 and 2.2 Mbp, respectively (Yakimov *et al.*, 2003, 2004). The DNA G + C content of *Oleispira antarctica* RB-8^T (41.6 mol%) is relatively close to that of strain RED65^T, but that of *T. oleivorans* MIL-1^T (53.6 mol%) differs by almost 10 units; that of *Oceanobacter kriegii* 197^T (56.1 mol%) exceeds the G + C content of RED65^T by an even greater margin, being at a level that is usually regarded as a threshold for genus separation (Trüper & Schleifer, 2006).

Strain RED65^T was subsequently investigated using previously described methods for phenotypic characterization (Macián *et al.*, 2001, 2005). Optical microscopy of bacterial cultures on wet mounts showed that cells were motile, thin, flexuous spirilla. To elaborate the cell morphology, cells were grown at 21 °C in marine broth 2216 (Difco) until early exponential phase (24–48 h incubation), fixed with glutaraldehyde and filtered onto 0.2-µm-pore-size polycarbonate filters (Nuclepore). Samples were subjected to sequential ethanol dehydration, critical-point drying with CO₂ and coating with silver before being viewed in a Hitachi S-3500N scanning electron microscope. Cells of strain RED65^T appear as thin spirilla, 0.3–0.6 µm in diameter and 1.8–4.5 µm in length (Fig. 2). In some cells, the curvature was less well defined. Budding could be seen at the tips of some cells, and coccoid bodies could be observed in older cultures (Supplementary Fig. S3).

On marine agar 2216 (MA; Difco), strain RED65^T produced regular, translucent, non-pigmented, tiny colonies that did not swarm or luminesce. Notably, colonies appeared to grow immediately below, rather than on top of, the agar surface. Strain RED65^T was able to grow on salt tolerance agar (tryptone, 1 %, w/v; yeast extract, 0.3 %, w/v; agar, 1.5 %, w/v) with the addition of Na⁺ (as 2 % NaCl,

Fig. 1. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences of strain RED65^T and closely related species. Bootstrap percentages (based on 1000 resamplings) >60 % are shown at branching points. Sequence accession numbers are given in parentheses. Bar, 1 estimated substitution per 100 bases.

Fig. 2. Scanning electron micrograph of cells (in the exponential growth phase) of strain RED65^T immobilized on a 0.2-μm-pore-size polycarbonate filter. Bar, 2 μm.

w/v). However, strain RED65^T has a specific requirement for Na⁺, as no growth was obtained when Na⁺ was replaced with K⁺. The salinity range that supported growth on diluted MA or in MA supplemented with NaCl, as reported by Macián *et al.* (2005), was 0.9–12 % (w/v) total salts, and thus RED65^T is a slight halophile. *Oleispira antarctica*, *Oceanobacter kriegii* and *T. oleivorans* also have a requirement for Na⁺, but they exhibit lower ranges of salinity for growth (Table 1).

Strain RED65^T is mesophilic, growing on MA from 15 to 42 °C, but not at 4 or 45 °C. In contrast, *Oleispira antarctica* is markedly psychrophilic. *Oceanobacter kriegii* and *T. oleivorans* are also mesophilic but have lower optimal temperatures than does strain RED65^T.

Strain RED65^T was oxidase- and catalase-positive and was unable to grow under anaerobic conditions, either through glucose fermentation or by nitrate respiration. The same results were reported for *Oleispira antarctica*, *Oceanobacter kriegii* and *T. oleivorans* except that, in the case of *Oleispira antarctica*, nitrate was used for anaerobic respiration (Baumann *et al.*, 1972; Bowditch *et al.*, 1984; Yakimov *et al.*, 2003, 2004). Strain RED65^T was negative for arginine dihydrolase, ornithine decarboxylase and indole production from tryptophan. It hydrolysed Tween 80 and lecithin but not alginate, agar, casein, DNA or starch. The strain was unable to grow in marine broth 2216 (Difco) with 12 % (w/v) gelatin. Other enzyme activities of the strain were tested using the API ZYM (bioMérieux) gallery: the tests were performed according to the manufacturer's instructions, except that the cells were suspended in a 3.5 % sea salts solution before they were added to the API ZYM strips, and the strips were incubated for 20 h at 26 °C. The results are shown in the species description.

Utilization of sugars, alcohols and organic acids as sole carbon and energy sources was analysed on basal medium agar [50 mM Tris/HCl, pH 7.5, 19 mM NH₄Cl, 0.33 mM K₂HPO₄ · 3H₂O and 0.1 mM FeSO₄ · 7H₂O in half-strength

Table 1. Phenotypic characteristics that serve to differentiate strain RED65^T from the type strains of closely related species

Strains: 1, RED65^T (data from this study); 2, *Oleispira antarctica* RB-8^T (data from Yakimov *et al.*, 2003); 3, *Oceanobacter kriegii* 197^T (Baumann *et al.*, 1972; Bowditch *et al.*, 1984; Sakane & Yokota, 1994); 4, *T. oleivorans* MIL-1^T (Yakimov *et al.*, 2004). +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4
Cell shape	Thin spirilla	Vibrioid to spiral	Straight rods	Vibrioid to spiral
Number of (polar) flagella	1	1	1	1–4
Poly-β-hydroxybutyrate accumulation	+	–	+	–
Anaerobic growth on nitrate	–	+	–	–
Salt range (% w/v)	1–12	0.5–10.7	0.5–8	0.5–5.7
Temperature range (°C)	15–42	1–25	10–35	4–30
Optimal temperature (°C)	25–30	2–4	20–26	20–25
Lecithin hydrolysis	+	–	–	–
Growth on:				
D-Fructose, D-glucose, citrate, 2-ketoglutarate, lactate, L-pyruvate, DL-β-hydroxybutyrate, propionate, D-mannitol	–	–	+	–
Malate, fumarate	w	–	+	–
Butyrate	+	+	+	–
Succinate	+	–	+	–
L-Alanine, γ-aminobutyric acid, putrescine, betaine	–	–	+	–
Major non-polar fatty acids	16:1, 16:0, 18:1	18:1, 16:1, 16:0	16:1, 18:1, 16:0	16:1, 16:0, 18:1
Major 3-hydroxy fatty acid	10:0	None	12:0	12:0
DNA G + C content (mol%)	44.0	41.6	56.1	53.2

artificial seawater solidified with 1.3 % (w/v) purified agar (Oxoid) (Baumann & Baumann, 1981)]. Amino acids and amines were tested as sole carbon, nitrogen and energy sources on basal medium agar without NH_4Cl . Compounds were added at 2 g l^{-1} . Positive control plates were prepared with yeast extract (5 g l^{-1}), while negative control media consisted of basal medium agar. Growth was monitored for 12 days. Of the 55 compounds tested, only a few organic acids enabled growth of strain RED65^T: acetate, butyrate, fumarate (weak), malate (weak) and succinate. Compared with strain RED65^T, *Oleispira antarctica* and *T. oleivorans* had narrower ranges of utilizable resources whereas *Oceanobacter kriegii* had the greatest nutritional versatility (Table 1). Biolog GN2 MicroPlates were also employed according to the manufacturer's instructions except that the suspension medium was supplemented with 1.5 % (w/v) NaCl. Again, few positive results were obtained. Strain RED65^T gave strong responses to Tweens 40 and 80 and weak responses to pyruvic acid methyl ester and succinic acid.

The cellular fatty acid composition of strain RED65^T (grown on marine agar at 30 °C) was determined by GLC at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany), using a method described previously (Kämpfer & Kroppenstedt, 1996). Polar lipid and analysis of strain RED65^T was carried out by the Identification Service of the DSMZ and Dr B. J. Tindall (DSMZ). The cellular fatty acid pattern, in decreasing order of abundance, was 16:1 ω 7c/15:0 iso 2-OH (38.03 %), 16:0 (20.26 %), 18:1 ω 9c (12.06 %), 10:0 3-OH (6.54 %), 14:0 (5.42 %), 18:1 ω 7c (4.97 %), 10:0 (4.58 %), unknown fatty acid with an equivalent chain-length of 11.799 (3.72 %), 18:0 (2.73 %), 12:0 (1.12 %), 16:1 2-OH (0.30 %) and 16:0 iso (0.28 %). Important chemotaxonomic differences were identified between strain RED65^T and its closest neighbours (Table 1).

The low levels of 16S rRNA gene sequence similarity between RED65^T and all other established species lend strong support to the consideration of this strain as a member of a separate genus and species. Furthermore, this strain can be readily distinguished from its closest relatives on the basis of a significant number of phenotypic and genotypic traits, as identified in this work. Thus, strain RED65^T represents a novel genus and species, for which the name *Bermanella marisrubri* gen. nov., sp. nov. is proposed.

Description of *Bermanella* gen. nov.

Bermanella (Ber.ma.nel'la. N.L. fem. dim. n. *Bermanella* named after the aquatic microbial ecologist Dr Tom Berman).

Gram-negative, strictly aerobic, chemo-organotrophic bacteria. Oxidase- and catalase-positive. Cells are motile, thin spirilla. Gas vesicles are not observed. Poly- β -hydroxybutyrate granules are produced. Slightly halophilic; no growth without seawater or the addition of combined marine salts

to the medium. Mesophilic. Do not ferment carbohydrates, as determined on anaerobic Hugh & Leifson O/F medium (Difco) with half-strength artificial seawater (Baumann & Baumann, 1981). Stenotrophic; the only carbon sources that serve as single carbon sources are organic acids. The genus is affiliated to the class *Gammaproteobacteria*. The type species is *Bermanella marisrubri*.

Description of *Bermanella marisrubri* sp. nov.

Bermanella marisrubri (ma.ris.ru'bri. L. n. *mare* sea; L. adj. *ruber* -bra -brum red; N.L. gen. n. *marisrubri* of the Red Sea).

Has the characteristics described below in addition to those given in the genus description. Cells are 0.3–0.6 μm in diameter and 1.8–4.5 μm in length. Does not reduce nitrate to nitrite or gas. Requires at least 1 % (w/v) NaCl or marine salts and tolerates up to 12 % (w/v) salts. Growth occurs at 15–42 °C; no growth at 4 or 45 °C. Hydrolyses Tween 80 and lecithin. Does not hydrolyse gelatin (no growth in medium), alginate, agar, casein, DNA or starch. Negative for arginine dihydrolase, ornithine decarboxylase and indole production from tryptophan. Utilizes acetate, butyrate and succinate as sole carbon and energy sources. Weak growth is obtained on fumarate, malate and L-glutamate. Growth is absent with the following: D-ribose, L-arabinose, D-xylose, D-glucose, D-fructose, trehalose, D-galactose, D-mannose, L-rhamnose, maltose, cellobiose, sucrose, lactose, melibiose, salicin, amygdalin, N-acetyl-D-glucosamine, glycerol, D-mannitol, *myo*-inositol, D-sorbitol, D-gluconate, propionate, pyruvate, D-glucuronate, D-galacturonate, D-saccharate, citrate, 2-ketoglutarate, D-glycerate, lactate, *trans*-aconitate, DL- β -hydroxybutyrate, L-leucine, L-tyrosine, L-arginine, glycine, L-serine, L-ornithine, L-threonine, L-alanine, γ -aminobutyric acid, L-citrulline, L-histidine, L-aspartate, L-lysine, L-sarcosine, betaine and putrescine. Intensities (0, negative; 1–5, increasing activity) recorded with the API ZYM gallery are as follows: alkaline phosphatase, 5; esterase (C4), 2; esterase lipase (C8), 3; lipase (C14), 3; leucine arylamidase, 5; valine arylamidase, 2; cystine arylamidase, 1; trypsin, 1; α -chymotrypsin, 1; acid phosphatase, 3; naphthol-AS-BI-phosphohydrolase, 1; α - and β -galactosidase, 0; β -glucuronidase, 0; α - and β -glucosidase, 0; N-acetyl- β -glucosaminidase, 0; α -mannosidase, 0; α -fucosidase, 0. The only substrates in Biolog GN2 MicroPlates giving clear positives are Tweens 40 and 80. Weak positive responses are obtained for pyruvic acid methyl ester and succinic acid. The cellular fatty acids, in decreasing order of abundance, are 16:1 ω 7c/15:0 iso 2-OH, 16:0, 18:1 ω 9c, 10:0 3-OH, 14:0, 18:1 ω 7c, 10:0, unknown fatty acid with an equivalent chain-length of 11.799, 18:0, 12:0, 16:1 2-OH and 16:0 iso. The polar lipid profile consists of phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, a phosphoaminolipid (that divides into two spots) and a glycolipid. The DNA G + C content of the type strain is 44.0 mol%.

The type strain, RED65^T (=CECT 7074^T =CCUG 52064^T), was isolated from surface water collected from the Red Sea.

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