Thiobacimonas profunda gen. nov., sp. nov., a member of the family *Rhodobacteraceae* isolated from deep-sea water

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A bacterial strain, JLT2016^T, was isolated from a sample of South-eastern Pacific deep-sea water. Cells were Gram-stain-negative, aerobic, devoid of flagella, motile by gliding and rod-shaped. Colonies were mucoid and cream. Growth occurred at 1.0–11.0 % (w/v) NaCl, 10–40 °C and pH 4.0–9.0. The major fatty acids were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) (60.5 %), $C_{19:0}$ cyclo $\omega8c$ (10.9 %) and $C_{16:0}$ (9.0 %). The polar lipids included diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and two sphingoglycolipids. The DNA G+C content was 67.1 mol%. The closest relative of strain JLT2016^T was *Salipiger mucosus* A3^T (96.7 % 16S rRNA gene sequence similarity). The results of phylogenetic analyses with different treeing algorithms indicated that this strain belonged to the *Roseobacter* clade in the order *Rhodobacterales*. Based on polyphasic analysis, strain JLT2016^T is considered to represent a novel genus and species, for which the name *Thiobacimonas profunda* gen. nov., sp. nov. is proposed. The type strain is JLT2016^T (=LMG 27365^T=CGMCC 1.12377^T).

Since the first description of *Roseobacter litoralis* and *Roseobacter denitrificans* by Shiba (1991), more than 40 *Roseobacter* clade-affiliated genera with validly published names have been described. Organisms of this clade are adapted to disparate ecological niches and characterized as having a wide range of physiological capacities (Tang *et al.*, 2010; Zech *et al.*, 2009). Members of the marine *Roseobacter* clade (Giovannoni & Rappé, 2000) in the order *Rhodobacterales* (Garrity *et al.*, 2005) have been detected in a large variety of habitats, from coastal regions to deep-sea sediments and from polar ice to tropical latitudes (Wagner-Döbler & Biebl, 2006). However, strains of the *Roseobacter* clade isolated from the deep-sea water column have been little reported (Newton *et al.*, 2010).

In this study, strain JLT2016^T was isolated from a seawater sample that was obtained from a depth of 2571 m in the south-eastern Pacific (106° 29′ W 5° 17′ S) during a cruise in September 2011. A 200 µl sample was spread on marine agar 2216 (MA; BD Difco) and incubated at 25 °C for 6 days. A standard dilution plating technique on MA was used for isolation. The culture was maintained routinely in marine broth 2216 (MB; BD) and was preserved as glycerol suspensions (20 %, v/v) at −80 °C.

Colony morphology and pigmentation were observed using routine cultivation aerobically on MA at 25 °C for 1–2 days. Cell morphology was examined by transmission electron microscopy (H600; Hitachi) with cells grown in MB medium at 25 °C for 24 h (Fig. S1, available in the online Supplementary Material). Gliding motility was determined by the semi-solid agar puncture method (Dong & Cai, 2001). Gram staining was determined on cells grown on MA medium at 25 °C for 24 h, according to the method described by Gerhardt *et al.* (1994).

Salt requirements and the optimum salt concentration were tested in MB medium with a final NaCl concentration of 0, 0.5, and 1.0–12.0 % (w/v) at intervals of 1.0 % (w/v) (at pH 7.8, 25 °C). Growth at various temperatures [4, 10, 20, 25, 30, 35, 40 and 50 °C, at pH 7.8, 2.0 % (w/v) NaCl] was measured in MB medium. Growth at different pH values was determined by adjusting the final pH of MB medium to pH 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 with HCl or NaOH [with 2.0 % (w/v) NaCl, 25 °C]; the following biological buffers were used to adjust the pH: Na_2HPO_4/NaH_2PO_4 for pH 4.0–7.0 and Na_2CO_3/$NaHCO_3$ for pH 8.0–10.0.

Hydrolysis of casein, starch and DNA was determined according to the method described by Smibert & Krieg

Abbreviation: PHA, poly-β-hydroxyalkanoate.

The GenBank accession number for the 16S rRNA gene sequence of strain JLT2016^T is JX397932. The Whole Genome Shotgun project for strain JLT2016^T has been deposited under accession number PRJNA175613.

Two supplementary figures and two supplementary tables are available with the online Supplementary Material.

(1994). Accumulation of poly-β-hydroxyalkanoate (PHA) granules in cells was determined by using the Nile blue A staining method (Ostle & Holt, 1982). Bacteriochlorophyll *a* production was determined by using the method of Pukall *et al.* (1999). The ability to grow with carbohydrates or amino acids as sole sources of carbon and energy was examined by using Biolog GN_2 microplates (Rüger & Krambeck, 1994). Other physiological and biochemical tests were investigated using the API ZYM, API 20E and API 20NE (bioMérieux) systems according to the manufacturer's instructions, except that the cell suspension was prepared by using sterile 3.2 % (w/v) sea salt (Sigma). The results of the physiological characterization are given in Table 1 and the species description. Tests of antimicrobial agent susceptibility were performed by using the disc-diffusion plate method (Fraser & Jorgensen, 1997) with discs containing ampicillin (10 μg), carbenicillin (100 μg), chloromycetin (5 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), neomycin (30 μg), novobiocin (5 μg), penicillin (10 μg), polymyxin B (300 μg), rifampicin (5 μg), streptomycin (10 μg), tetracycline (30 μg), vancomycin (30 μg). Bacterial cultures (200 μl) were spread on MA plates, discs impregnated with antibiotics were placed on the plate surfaces, and the plates were incubated for three days at 25 °C.

Genomic DNA was extracted using a TIANamp Bacteria DNA kit (Tiangen Biotech) with cells grown in MB medium for two days at 25 °C and subsequently washed and resuspended in TE buffer. The yield and concentration were assessed with a NANODROP 2000 spectrophotometer (Thermo Scientific). The genomic DNA G+C content of strain JLT2016^T was based upon the results from whole genome shotgun sequences obtained by 454 pyrosequencing (Roche 454 GS FLX). The G+C content of strain JL2016^T was 67.1 mol%.

Isoprenoid quinones were extracted from 100 mg of freeze-dried cell material using the two-stage method described by Tindall (1990a b) and analysed by HPLC. Polar lipids were extracted and analysed by two-dimensional TLC following the protocols of Collins *et al.* (1980) and Kates (1986), using Merck silica gel 60F254 plates (10 × 20 cm) and chloroform/methanol/water (65 : 25 : 4, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) in the second dimension. The general detection reagent, sulfuric acid/ethanol (1 : 2, v/v), was also used to detect total polar lipids. Cellular fatty acid analysis was performed on cultures grown on MA at 25 °C for two days and carried out with the method described by Komagata & Suzuki (1987). The MIDI version was Sherlock version 6.0 and the library was TSBA6 6.00. The dominant cellular fatty acids of JLT2016^T were summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) (60.5 %), $C_{19:0}$ cyclo $\omega8c$ (10.9 %), $C_{16:0}$ (9.0 %) and $C_{18:0}$ (5.0 %). The predominant fatty acid component of strains JLT2016^T, *Salipiger mucosus* A3^T and *Pelagibaca bermudensis* HTCC2601^T was summed feature 8 with 60.5 %, 70.6 % and 78.9 %, respectively. However, the dominant fatty acid in *Donghicola xiamenensis* Y-2^T was $C_{19:0}$ cyclo $\omega8c$ (36.5 %). In addition, anteiso-$C_{11:0}$ was only detectable in cells of JLT2016^T, $C_{12:1}$ 3-OH was only detectable in cells of *S. mucosus* A3^T, $C_{14:0}$ and $C_{16:0}$ 2-OH were only detectable in cells of *D. xiamenensis* Y-2^T and iso-$C_{19:0}$ was only just detectable in cells of *Pelagibaca bermudensis* HTCC2601^T (Table 2).

The 16S rRNA gene of strain JLT2016^T was amplified using universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Embley, 1991). The nearly complete 16S rRNA gene sequence of strain JLT2016^T (1426 bp) was compared with sequences available in GenBank by using the EzTaxon-e (Kim *et al.*, 2012) to determine their approximate phylogenetic affiliations. Phylogenetic analyses (with neighbour-joining, maximum-likelihood and maximum-parsimony algorithms) based on 16S rRNA gene sequences were performed as described by Kim *et al.* (1998). To remove alignment regions that contained gaps or were highly divergent, Gblocks (Talavera & Castresana, 2007) was used for generating alignments of rRNA genes, which resulted in the selection of 1365 nt positions. Phylogenetic trees were reconstructed within the software MEGA version 5 (Tamura *et al.*, 2011) after sequence alignment using the CLUSTAL W algorithm embedded into BioEdit (Hall, 1999). Distances and clustering obtained via neighbour-joining (Saitou & Nei, 1987) are shown in Fig. 1. As the phylogenetic tree shows, strain JLT2016^T fell within the *Roseobacter* clade forming a coherent cluster with *Pelagibaca bermudensis* HTCC2601^T (Cho & Giovannoni, 2006) and *S. mucosus* A3^T (Martínez-Cánovas *et al.*, 2004). The maximum-likelihood and maximum-parsimony algorithms gave a similar result. Strain JLT2016^T exhibited 96.7 %, 95.9 % and 95.6 % 16S rRNA gene sequence similarity with *S. mucosus* A3^T, *D. xiamenensis* Y-2^T (Tan *et al.*, 2009) and *Pelagibaca bermudensis* HTCC2601^T, respectively. Based on 16S rRNA gene sequence analysis, the strain is affiliated to the *Roseobacter* clade in the family *Rhodobacteraceae*.

Strain JLT2016^T and the strains representing species of the closely related genera *Salipiger*, *Donghicola* and *Pelagibaca* were positive for leucine arylamidase and esterase (C4) activity and for the Voges–Proskauer reaction. Furthermore, all strains were negative for the hydrolysis of starch and DNA; H_2S and indole production; the assimilation of D-mannose, capric acid, malic acid and trisodium citrate; acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose and amygdalin; activity of lipase (C14), cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and β-fucosidase. However, only JLT2016^T was positive for acid production from melibiose, and activity of alkaline phosphatase, α-chymotrypsin and acid phosphatase. All strains, except for JLT2016^T, were positive for the hydrolysis of urea. More details of the physiological characterizations are given in Table 1 and in the genus and species descriptions below. Strain JLT2016^T could be clearly distinguished from its closest phylogenetic neighbours, *S. mucosus* A3^T and *Pelagibaca bermudensis*

Table 1. Characteristics that distinguish strain JLT2016^T from the type strains of species of the closely related genera *Salipiger*, *Donghicola* and *Pelagibaca*

Strains: 1, JLT2016^T; 2, *S. mucescens* A3^T; 3, *D. xiamenensis* Y-2; 4, *Pelagibaca bermudensis* HTCC2601^T. +, Positive; −, negative; ND, no data available. The data for *S. mucescens* A3^T, *D. xiamenensis* Y-2^T and *Pelagibaca bermudensis* HTCC2601^T were obtained from this study, unless stated otherwise, in parallel tests with strain JL2016^T under identical conditions.

Characteristic	1	2	3	4
Source of isolation	Sea water	Hypersaline soil*	Sea water†	Sea water‡
Cell morphology	Rods	Rods*	Ovoid rods†	Short rods‡
Cell size (width × length) (μm)	0.2–0.4 × 1.2–1.4	0.75–1 × 2–2.5*	0.5–1.2 × 1.0–2.0†	0.6–1.1 × 1.2–2.3‡
Gliding motility	+	−*	−†	−‡
Pigment	−	−*	Beige†	−‡
Growth conditions				
Temperature range (°C)	10–30	20–40*	18–37†	10–40‡
Optimum temperature (°C)	25	ND*	25†	30–33‡
NaCl concentration range (%)	2–11	0.5–20*	0.5–12†	0.25–15‡
Optimum NaCl concentration (%)	1–12	9–10*	1–3†	3‡
pH Range	6–9	6–10*	ND†	5.0–10.5‡
Optimum pH	8	ND*	7.0†	8.5‡
PHA*	−	−*	ND†	+‡
Exopolysaccharide production*	+	+*	−†	−‡
Nitrate to nitrite	−	−	+	+
Hydrolysis of:				
Aesculin	+	+	−	+
Casein	−	+	+	−
Urea	−	+	+	+
Enzymic activity (API ZYM)				
Alkaline phosphatase, α–chymotrypsin, acid phosphatase	+	−	−	−
Esterase lipase (C8)	+	−	+	−
Naphthol-AS-BI-phosphohydrolase, α-glucosidase	+	+	−	+
Valine arylamidase	+	+	+	−
Acid production from (API 20E):				
Melibiose	+	−	−	−
Arabinose	+	−	−	+
Assimilation of (API 20NE):				
N-Acetylglucosamine, L-Arabinose, potassium gluconate	−	+	−	−
Adipic acid, D-mannose, maltose	−	+	−	+
L-Arginine	−	−	+	+
Phenylacetic acid	−	−	+	−
Utilization as sole carbon sources				
Glycerol	−	−*	ND†	+‡
D-Glucose	+	−*	−†	+‡
D-Fructose	−	−*	−†	+‡
Cellobiose	−	−*	−†	+‡
Trehalose, L-rhamnose	−	ND*	ND†	+‡
Susceptibility to:				
Ampicillin	+	+*	−†	+‡
Carbenicillin	−	+*	−†	+‡
Kanamycin	−	+*	+†	−‡
Polymyxin	−	−*	+†	ND‡
DNA G+C content (mol%)	67.1	64.5*	62.4†	65.4‡

*Data taken from Martínez-Cánovas *et al.* (2004).
†Data taken from Cho & Giovannoni (2006).
‡Data taken from Tan *et al.* (2009).

Table 2. Cellular fatty acid compositions of strain JL2016^T and type strains of the closely related genera *Salipiger*, *Donghicola* and *Pelagibaca*

Strains: 1, JLT2016^T; 2, *S. mucescens* A3^T; 3, *D. xiamenensis* Y-2^T; 4, *Pelagibaca bermudensis* HTCC2601^T. Values are percentages of total fatty acids. −, Not detected. All data for *S. mucescens* A3^T, *D. xiamenensis* Y-2^T and *Pelagibaca bermudensis* HTCC2601^{T}were obtained from this study in parallel tests with strain JL2013^T under identical conditions.

Fatty acid	1	2	3	4
Straight-chain				
$C_{10:0}$	0.5	−	−	−
$C_{12:0}$	3.3	−	1.8	−
$C_{14:0}$	−	−	3.0	−
$C_{16:0}$	9.0	10.7	12.5	6.8
$C_{18:0}$	5.0	−	3.8	2.1
$C_{18:1}\omega 9c$	−	−	1.1	−
Cyclo				
$C_{19:0}$ cyclo $\omega 8c$	10.9	5.2	23.8	−
Hydroxy				
$C_{10:0}$ 3-OH	4.7	−	1.1	−
$C_{12:0}$ 3-OH	−	−	5.0	3.9
$C_{12:1}$ 3-OH	−	2.9	−	−
$C_{13:0}$ 2-OH	−	−	0.4	−
$C_{16:0}$ 2-OH	−	−	14.5	−
$C_{16:1}$ 2-OH	−	−	1.0	−
$C_{18:1}$ 2-OH	−	−	2.1	−
Methyl ester				
11-Methyl $C_{18:1}\omega 7c$	4.1	5.3	−	2.9
Branched-chain				
anteiso-$C_{11:0}$	0.6	−	−	−
anteiso-$C_{12:0}$	1.5	−	−	−
iso-$C_{17:0}$	−	−	0.4	−
iso-$C_{19:0}$	−	−	−	5.4
Summed features*				
3	−	−	1.7	−
5	−	−	0.6	−
8	60.5	76.0	27.5	78.9

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 3, $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$; Summed feature 5, $C_{18:2}\omega 6,9c$ and/or ante-$C_{18:0}$; Summed feature 8, $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$.

HTCC2601^T, by cell size; and motility; temperature and NaCl concentration range for growth; activity of alkaline phosphatase, α-chymotrypsin, acid phosphatase and esterase lipase (C8); acid production from melibiose; and polar lipid composition (e.g. the presence of sphingoglycolipid) (Fig. S2). Characteristics that distinguish strain JLT2016^T from other strains in the phylogenetic tree are compared in Tables S1 and S2. Based on these traits, strain JLT2016^T cannot be classified as a member of any of the known genera within the *Roseobacter* clade.

On the basis of phenotypic properties and genotypic characteristics along with evidence from chemotaxonomic and phylogenetic analysis, strain JLT2016^T is considered to represent a novel species of a novel genus, for which the name *Thiobacimonas profunda* gen. nov., sp. nov. is proposed.

Description of *Thiobacimonas* gen. nov.

Thiobacimonas (Thi.o.ba.ci.mo′nas. L. fem. n. *baca* berry; Gr. fem. n. *monas* a unit, monad; N.L. fem. n. *Thiobacimonas* sulfur oxidation berry monad).

Cells are Gram-stain-negative, devoid of flagella, motile by gliding, rod-shaped. PHA granules are not produced and bacteriochlorophyll *a* is absent. Catalase and phosphatase are positive. The predominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and two sphingoglycolipids. The type species is *Thiobacimonas profunda*.

Description of *Thiobacimonas profunda* sp. nov.

Thiobacimonas profunda (pro.fun′da. L. fem. adj. *profunda* deep).

The description of the species is as that for the genus, with the following additions. Grows on MA in the form of convex, mucoid, circular, unsmooth, opaque and creamy colonies. Cells are 0.2–0.4 μm in width and 1.2–1.4 μm in length. The temperature range for growth is 10–30 °C (optimum, 25 °C), the pH range for growth is pH 6.0–9.0 (optimum, pH 8.0) and the NaCl concentration range for growth is 1.0–12.0 % (w/v) (optimum 2.0–11.0 %). Gelatin and aesculin are hydrolysed, but Tweens 40 and 80, starch, casein, urea and DNA are not. Indole or H_2S production is not detected. Positive for the Voges–Proskauer reaction. Nitrate and nitrite are not reduced. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-chymotrypsin and α-glucosidase are present, but lipase (C14), cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. According to the API 20E tests, acid is formed from melibiose and arabinose, but not from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose or amygdalin. The following substrates are oxidized as carbon sources from the results of Biolog GN2: Tween 40, L-alanyl-glycine, L-arabinose, dextrin, α-D-glucose, lactulose, maltose, melibiose, D-psicose, monomethyl succinate, D-glucosaminic acid, propionic acid and sebacic acid. Susceptible to ampicillin, tetracycline, rifampicin, chloramphenicol, erythromycin, streptomycin, neomycin, gentamicin, benzylpenicillin and novobiocin, and resistant to carbenicillin, kanamycin, lincomycin, vancomycin and polymyxin. Major fatty acids are summed feature 8 ($C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$), $C_{19:0}$ cyclo $\omega 8c$, $C_{16:0}$ and $C_{18:0}$ (5.0 %). In addition to the lipids listed in the genus description, minor amounts of another unidentified glycolipid and unidentified polar lipids are detectable. The respiratory quinone is ubiquinone Q-10.

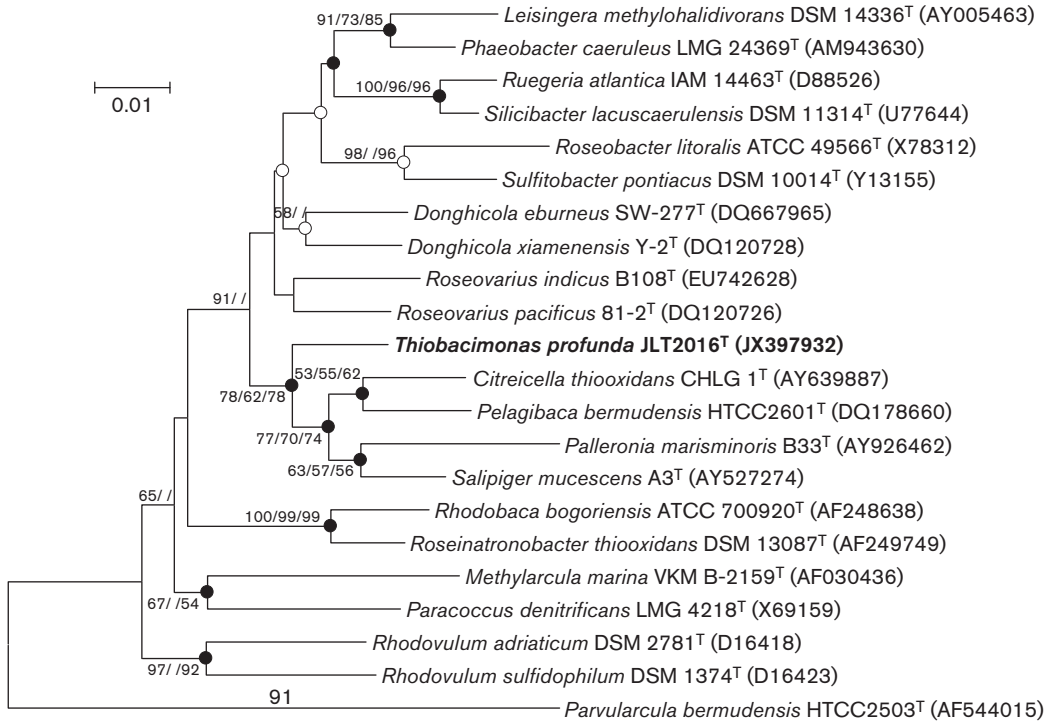

Fig. 1. Neighbour-joining phylogenetic tree showing the relationships between strain JLT2016T and representatives of the family *Rhodobacteraceae* based on 16S rRNA gene sequences. Bootstrap percentages (above 50 %) from the neighbour-joining/maximum-parsimony/maximum-likelihood approach are shown (1000 replications). *Parvularcula bermudensis* HTCC2503^T was used as an outgroup to define the root of the tree. Filled and open circles indicate corresponding nodes recovered reproducibly by all treeing methods or by two treeing methods, respectively. Bar 0.01 nt substitutions per nucleotide position.

The type strain is JLT2016^T (=LMG 27365^T=CGMCC 1.12377^T), isolated from a sample of south-eastern Pacific deep-sea water. The genomic DNA G+C content of the type strain is 67.1 mol%.

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