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ORIGINAL PAPER

Pelagibaca abyssi sp. nov., of the family *Rhodobacteraceae*, isolated from deep-sea water

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Abstract A Gram-stain negative, oval-shaped, aerobic, catalase and oxidase-positive bacterium, designated JLT2014^T, was isolated from a deep-seawater sample (obtained at a 2,000 m depth) of the Southeastern Pacific Ocean. The dominant fatty acids were identified as $C_{18:1}\omega7c/C_{18:1}\omega6c$, $C_{16:0}$ and $C_{10:0}$ 3-OH, which altogether represented 60.1 % of the total. The predominant respiratory quinone was identified as Q-10. The G+C content of genomic DNA was determined to be 66.4 mol %. The major polar lipids were identified as phosphatidylethanolamine and diphosphatidylglycerol. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that the novel isolate can be affiliated with the *Roseobacter* clade within the family *Rhodobacteraceae*. Strain JLT2014^T exhibited highest 16S rRNA gene sequence similarity value to *Pelagibaca bermudensis* HTCC2601^T (sequence similarity value: 97.6 %). The DNA–DNA relatedness value between strain JLT2014^T and *P. bermudensis* HTCC2601^T was 46.9 ± 2 %. Based on phenotypic properties and phylogenetic analysis, the name *Pelagibaca abyssi* sp. nov. is proposed, with JLT2014^T(=LMG 27363^T=CGMCC 1.12376^T) as the type strain.

Keywords *Pelagibaca abyssi* sp. nov. · Aerobic · Deep-sea water

Introduction

A large number of strains within the family *Rhodobacteraceae* have been obtained from diverse environments such as seawater, marine dinoflagellates and sediments (Cho and Giovannoni 2006; Biebl et al. 2005; Shiba 1991). Members of the *Roseobacter* clade within the family *Rhodobacteraceae* are ecologically and physiologically diverse, occupying a wide variety of lifestyles (Tang et al. 2010). However, to our knowledge, only a few strains of the *Roseobacter* clade have been isolated from deep-sea water (Oh et al. 2009; Lai et al. 2011). In this study, the taxonomic status of a novel strain isolated from deep-sea water, strain JLT2014^T, has been determined by using phylogenetic, chemotaxonomic and phenotypic analyses. Based on this evidence, strain JLT2014^T is considered to represent a novel member of the genus *Pelagibaca,* which belongs to the *Roseobacter* clade within the family *Rhodobacteraceae.* At the time of writing, the genus *Pelagibaca* contains only one recognized species: *Pelagibaca bermudensis* HTCC2601^T (Cho and Giovannoni 2006). Strain JLT2014^T is thus proposed to

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represent a second species, for which the name *Pelagibaca abyssi* sp. nov. is proposed.

Materials and methods

Bacterial isolation and culture conditions

Strain JLT2014^T was collected from deep-sea water (depth, 2,000 m) of the Southeastern Pacific Ocean (102°W, 3°S). The seawater sample was spread on a plate containing marine agar 2216 (MA; Becton–Dickinson) and then incubated at 25 °C for 1 week. Strain JLT2014^T was purified as single colonies by the direct plating method. The strain was preserved in marine broth 2216 (MB; Becton–Dickinson) at −80 °C supplemented with 15 % (v/v) glycerol.

P. bermudensis HTCC2601^T (JCM 13377, obtained from the Japan Collection of Microorganisms), *Citreicella thiooxidans* CHLG1^T (DSM 10146) and *Salipiger mucescens* A3^T (DSM 16094), both obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), were used as reference strains following growth under comparable conditions.

Morphology and phenotypic characterization

Morphological and physiological characteristics of strain JLT2014^T were investigated using routine cultivation. Cell morphology was studied by using transmission electron microscopy (JEM-1230; JEOL USA). Gliding motility and aerobic/anaerobic culture tests were determined by the semi-solid puncture method (Dong and Cai 2001). The Gram reaction was examined according to standard procedures described by Gerhardt et al. (1994).

The optimum temperature, pH and NaCl concentration for growth were examined by monitoring the OD_{600} in MB medium. The effect of temperature was explored at 4, 10, 15, 20, 25, 30, 35, 40, 45, 50 °C (pH 8, 8.0 % NaCl). The pH range studied was from 5.0 to 11.0, in increments of one pH unit (25 °C, 8.0 % NaCl), by using the following buffers to adjust the pH: Na_2HPO_4/NaH_2PO_4 for pH 5.0–7.0 and $Na_2CO_3/NaHCO_3$ for pH 8.0–11.0. Medium pH was checked after autoclaving. The NaCl concentration ranged from 0 to 13.0 % (w/v), at intervals of 1.0 % (with incubation at 25 °C, pH 8).

Catalase activity was tested based on the formation of bubbles after adding a drop of 3 % H_2O_2 solution to a fresh bacterial colony (Dong and Cai 2001). Hydrolysis of casein (5 % skimmed milk, w/v), starch (0.2 %, w/v) and DNA (0.2 %, w/v) were determined as described by the methods of Smibert and Krieg (1994). The presence of bacteriochlorophyll *a* was tested by the method of Pukall et al. (1999). The intracellular accumulation of polyhydroxyalkanoates (PHAs) was tested by Nile blue A staining after the strain was cultivated in MB at 25 °C for 2 days (Ostle and Holt 1982). Sole carbon-source utilization was performed using Biolog GN2 microplates (Rüger and Krambeck 1994). Other phenotypic and enzymatic characterizations of strain JLT2014^T were conducted using API 20E, API 20NE and API ZYM kits (bioMérieux). The inoculum was prepared by suspending cells in sterile water after the strain was cultivated in MB at 25 °C for 2 days. Physiological saline, 0.9 % (w/v) NaCl solution, was added to all suspension media for API test strips. API 20E and API 20NE test strips were read after 24 h of incubation at 25 °C, while API ZYM test strips were read after 4 h of incubation at 25 °C. The results of API 20E, API 20NE and API ZYM test strips were read according to the manufacturer's instructions. Antimicrobial susceptibility was tested by using the disk-diffusion plate method (Fraser and Jorgensen 1997). Susceptibility was tested to the following 15 antimicrobial agents (amounts per disc): 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).

Chemotaxonomy

The G+C content of genomic DNA was determined by the HPLC method of Mesbah et al. (1989) after DNA extraction and purification according to the method of Marmur (1961). Isoprenoid quinones were extracted by using the two-stage method described by Tindall (1990a, b) and analysed by HPLC. The cellular fatty acid composition was analyzed by the procedures described by Komagata & Suzuki (1987) after cells were grown on MA at 25 °C for 2 days, at which points cells were considered to be in the exponential

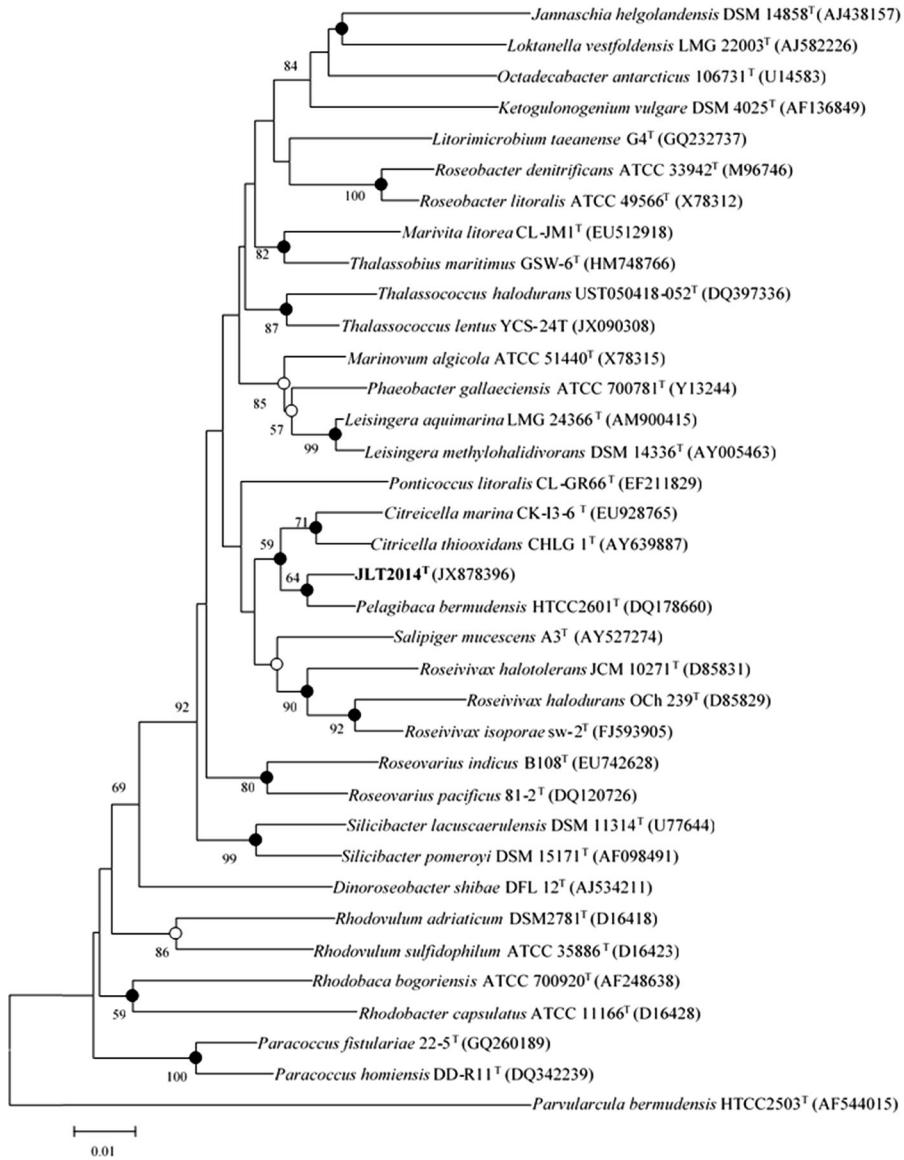

Fig. 1 Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences showing the relationship between strain JLT2014^T and representatives of the family *Rhodobacteraceae*. Only values >50 % are shown (1,000 resamplings) at nodes. *Parvularcula bermudensis* HTCC 2503^T was used as an outgroup. Filled and open circles indicate corresponding nodes recovered reproducibly by all treeing methods or by two treeing methods, respectively. *Bar* 0.01 substitutions per nucleotide position

growth phase. Fatty acids were identified using the Microbial Identification System (MIDI) Sherlock version 6.0 and the TSBA6 6.00 library. Polar lipids were extracted using a chloroform/methanol system and analysed using two-dimensional thin-layer chromatography (TLC), as described previously (Collins et al. 1980; Kates 1986).

Phylogenetic analysis

Genomic DNA was prepared using a TIANamp Bacteria DNA Kit (Tiangen Biotech, DP302). Purity was assessed by the A_{280}/A_{260} and A_{230}/A_{260} ratios (Johnson 1994) with a NANODROP 2000 spectrophotometer (Thermo Scientific, USA). The 16S rRNA gene was amplified by PCR using universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Embley 1991). The 16S rRNA gene sequence of strain JLT2014^T and the sequences of related taxa were obtained from the GenBank database (NCBI) by using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) to ascertain the sequence similarities between the strains (Kim et al. 2012). Sequences alignment was performed with Bioedit 7.0.9 (Hall 1999) and phylogenetic trees were generated by MEGA 5 software using the neighbour-joining,

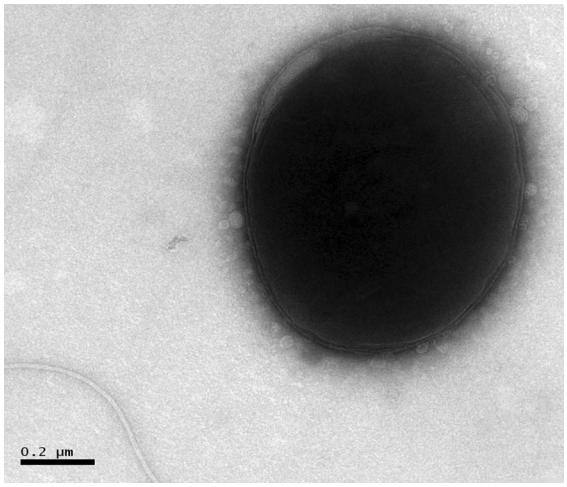

Fig. 2 Transmission electron micrograph of negatively stained cells of strain JLT2014^T. *Bar* 1 µm

maximum-parsimony and maximum-likelihood algorithm methods (Tamura et al. 2011).

The DNA–DNA hybridization experiments were conducted using the initial renaturation rate method (De Ley et al. 1970). Hybridization was determined in four replicates.

Results and discussion

The 16S rRNA gene sequence comparisons to representative bacteria with validly published names indicated that strain JLT2014^T was affiliated with the genus *Pelagibaca* within the family *Rhodobacteraceae* (Fig. 1). In neighbour-joining, maximum-likelihood and maximum-parsimony trees, strain JLT2014^T formed the same relationships with *P. bermudensis* HTCC2601^T and *C. thiooxidans* CHLG1^T (Fig. 1; Fig. S1). The phylogenetic tree and sequence similarity values data indicated that the strain JLT2014^T is most closely related to species affiliated with the *Roseobacter* clade: *P. bermudensis* HTCC2601^T (sequence similarity, 97.6 %), *C. thiooxidans* CHLG1^T (sequence similarity, 97.4 %) and *S. mucescens* A3^T (sequence similarity, 96.4 %). The GenBank accession number for the 16S rRNA gene sequence (1426 nt) of strain JL2014^T is JX878396.

Strain JLT2014^T was found to be aerobic, Gram-stain negative, ovoid, non-motile, 0.9–1.2 µm long and 0.8–1.0 µm wide (Fig. 2). After growth on MA medium 2 days, cells of strain JLT2014^T formed white, uniformly circular, convex and opaque colonies. Growth occurred at 4–40 °C (optimum, 25 °C), pH 6.0–9.0 (optimum, 8.0) and with 1.0–13.0 % (w/v) of NaCl (with good growth across a broad range of 5.0–12.0 %). PHA granules were not produced and bacteriochlorophyll *a* was absent.

Strain JLT2014^T was found to be susceptible to ampicillin, carbenicillin, chloromycetin, erythromycin, gentamicin kanamycin, neomycin, novobiocin, penicillin, rifampicin, streptomycin and tetracycline, but resistant to lincomycin and polymyxin B. The major quinone of strain JLT2014^T was identified as ubiquinone Q-10. The G+C content of genomic DNA of strain JLT2014^T was determined to be 66.4 mol %. DNA–DNA relatedness value of strain JLT2014^T with *P. bermudensis* HTCC2601^T was 46.9 ± 2 % and with *C. thiooxidans* CHLG1^T was 29.4 ± 3 %, respectively. These values are well below the threshold value of 70 % that is recommended for delineation of bacterial genospecies (Wayne et al. 1987).

The differences of physiological characterization between JLT2014^T and other related type strains of genera in family *Rhodobacteraceae* are given in Table 1 and other characters are given in the species description. Although JLT2014^T shares several characteristics with *P. bermudensis* HTCC2601^T, including positive for assimilation of gelatin and negative for activities of trypsin and α-chymotrypsin, strain JLT2014^T can be distinguished from *P. bermudensis* HTCC2601^T by characteristics such as the reduction of nitrate and activities of urease and DNase. Sole carbon-source utilization determined using Biolog GN2 microplates is listed in Table S1.

As seen from Table 2, the dominant fatty acids in strain JLT2014^T were identified as $C_{18:1}\omega7c/C_{18:1}\omega6c$, $C_{16:0}$, $C_{10:0}$3-OH. When compared with *P. bermudensis* HTCC2601^T, *C. thiooxidans* CHLG1^T and *S. mucescens* A3^T, strain JLT2014^T did not contain $C_{10:0}$, $C_{15:1}\omega6c$, $C_{17:1\text{anteiso}}\ \omega9c$ $C_{16:1}\omega7c$, $C_{16:1}\omega6c$, $C_{18:1}\omega9c$, $C_{12:0}$3-OH and $C_{12:1}$3-OH. In addition, $C_{9:0}$, $C_{16:0\ \text{N alcohol}}$, anteiso-$C_{15:0}$, $C_{14:1}\omega5c$, $C_{16:1}\omega5c$, $C_{18:3}\omega6c$, iso-$C_{13:0}$3-OH and $C_{10:0}$3-OH were found in JLT2014^T. The polar lipids of strain JL2014^T were identified as phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, three unidentified phospholipids and an unknown glycolipid. The major polar lipids of *P. bermudensis* HTCC2601^T were identified as

Table 1 Differential characteristics of strain JLT2014^T and type strains of three related taxa within the family *Rhodobacteraceae*

Characteristic	1	2	3	4
Source	Sea water	Sea water	Sea water	Hypersaline soil
Oxygen requirement	Strict aerobe	Facultative anaerobe	Aerobe	Strict aerobe
Optimum temperature (°C)	25	10–40	25	20–40
Nitrate reduction	−	+	−	−
DNase	+	−	−	−
Urease	−	+	+	+
Enzymic activity (API ZYM)				
Acid/Alkaline phosphatase	+	−	+	−
Esterase lipase (C8)	w$^+$	−	+	−
Valine arylamidase	w$^+$	−	+	+
Cystine arylamidase	+	−	+	−
Trypsin	−	−	+	−
α-Chymotrypsin	−	−	+	−
Acid production from (API 20E)				
L-arginine, L-lysine, L-ornithine	−	+	+	−
L-arabinose	−	+	−	−
Indole production	w$^+$	−	−	−
Voges–Proskauer test	−	+	+	+
Assimilation of (API 20NE)				
Gelatin	+	+	−	+
N-Acetylglucosamine	−	−	−	+
D-Mannitol	−	+	+	+
D-Glucose	−	−	+	+
Adipic acid	−	+	−	+
Phenylacetic acid	−	−	+	−
Potassium gluconate	−	−	−	+
DNA G+C mol %	66.4	64.5*	67.5–69.2*	64.5*

Data for the reference strains in table lines 1–4 were obtained from Martínez-Cánovas et al. (2004), Sorokin et al. (2005) and Cho & Giovannoni (2006)

* Data for the reference strains were obtained from Martínez-Cánovas et al. (2004), Sorokin et al. (2005) and Cho & Giovannoni (2006)

Strains 1, JLT2014^T; 2, *Pelagibaca bermudensis* HTCC2601^T; 3, *Citreicella thiooxidans* HLG1^T; 4, *Salipiger mucescens* A3^T

Data of reference strains obtained from this study unless otherwise indicated. All strains were Gram-stain-negative and positive for oxidase and catalase activities. The major quinine was ubiquinone Q-10. From the result of tests in API 20NE, API 20E and API ZYM, all strains were negative for assimilation of D-mannose, trisodium citrate, malic acid, capric acid; hydrolysis of casein, starch, D-glucose, D-sorbitol, L-rhamnose, L-tryptophan, D-melibiose, amygdalin, sucrose, inositol; activity of lipase (C14), α-galactosidase, β-galactosidase, β–fucosidase, β-glucuronidase, β-glucosidase, α-mannosidase, *N*-acetyl-β-glucosaminidase; nitrate reduction and H_2S production. All species were positive for esculin, leucine arylamidase, α-glucosidase, naphtol-AS-BI- phosphohydrolase. +, Positive; −, negative; w$^+$ weakly positive

phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol, along with a small amount of an unidentified phospholipid and phosphatidylcholine. However, only strain JLT2014^T was found to contain an unknown glycolipid (Fig. S2).

Based on the data presented above, strain JLT2014^T is considered to represent a novel species of the genus *Pelagibaca* in the family *Rhodobacteraceae*, for which the name *Pelagibaca abyssi* sp. nov. is proposed.

Table 2 Cellular fatty acid profiles of strain JLT2014^T and type strains of three related taxa within the family *Rhodobacteraceae*

Fatty acid	1	2	3	4
Straight-chain				
$C_{9:0}$	3.2	–	–	–
$C_{10:0}$	–	3.0	2.2	–
$C_{12:0}$	3.1	16.4	2.3	–
$C_{14:0}$	3.1	5.9	–	–
$C_{16:0}$	14.4	16.5	11.7	10.7
$C_{16:0\ N\ alcohol}$	1.3	–	–	–
$C_{18:0}$	7.0	11.6	3.6	–
Branched saturated acids				
Anteiso-$C_{15\ :\ 0}$	2.2	–	–	–
Unsaturated acids				
$C_{14:1}\omega5c$	1.3	–	–	–
$C_{15:1}\omega6c$	–	–	2.1	–
$C_{15:1}\omega5c$	–	–	4.3	–
$C_{16:1}\omega5c$	1.2	–	–	–
$C_{17:1anteiso}\ \omega9c$	–	–	4.0	–
$C_{18:1}\omega9c$	–	2.9	–	–
$C_{18:1}\omega7c$ 11-methyl	3.2	1.9	–	5.3
$C_{18:3}\omega6c$	1.4	–	–	–
$C_{19:0cyclo}\ \omega8c$	10.2	–	4.1	5.2
Hydroxy fatty acids				
$C_{10:0}$3-OH	13.3	–	–	–
$C_{12:0}$3-OH	–	2.8	4.3	–
$C_{12:1}$3-OH	–	–	–	2.9
$C_{14:0}$2-OH	tr	–	–	–
Iso-$C_{13:0}$3-OH	1.1	–	–	–
Summed features[a]				
Summed feature 3	–	–	1.5	–
Summed feature 8	32.4	39.0	60.0	76.0

[a] Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$. Summed feature 8 comprises $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$

Strains 1, JLT2014^T; 2, *Pelagibaca bermudensis* HTCC2601^T; 3, *Citreicella thiooxidans* HLG1^T; 4, *Salipiger mucescens* A3^T. Fatty acid compositions were analyzed in this study with cells grown on marine agar 2216 for 2 days

Values are percentages of total fatty acids. –, not detected or no data. *tr* values are less than 1.0 %

Description of *Pelagibaca abyssi* sp. nov

Pelagibaca abyssi (a.bys'si. L. gen. n. *abyssi* of immense depths, living in the depth of the ocean).

Cells are Gram-stain negative, ovoid or coccoid, non-motile and aerobic. Cells are 0.9–1.2 μm long and 0.8–1.0 μm wide. Cells form the white, uniformly circular, convex and opaque colonies on MA medium. The bacterium is capable of growing in 1.0–13.0 % (w/v) NaCl (good growth over the range 5.0–12.0 %), at 4–40 °C (optimum, 25 °C) and at pH 6.0–9.0 (optimum, pH 8.0). Negative for hydrolysis of starch, casein and urea. With the API 20E test, positive for gelatinase, negative for nitrate reduction, H_2S production, hydrolysis of 2-nitrophenyl-β-D-galactopyranoside and Voges–Proskauer test. Indole is weakly produced. The following substrates cannot be utilized for growth: D-glucose, D-mannose, D-sorbitol, L-rhamnose, L-ornithine, L-arginine, L-tryptophan, D-melibiose, L-arabinose, amygdalin, sucrose, citric acid or inositol. In assays with the API ZYM system, acid phosphatase, alkaline phosphatase, esterase (C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, cystine arylamidase and α-glucosidase activities are present, esterase lipase (C8) and valine arylamidase are weakly present but trypsin, α-chymotrypsin, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. According to the API 20NE test, esculin activity is present, but PNPG, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid and phenylacetic acid are absent. The predominant fatty acids are $C_{18:1}\omega7c/C_{18:1}\omega6c$, $C_{16:0}$ and $C_{10:0}$ 3-OH. The predominant isoprenoid quinone is Q-10. The major polar lipids include phosphatidylethanolamine and diphosphatidylglycerol. The G+C content of genomic DNA of the type strain is 66.4 mol %.

The type strain JL2014^T (=LMG 27363^T=CGMCC 1.12376^T) was isolated from deep-sea water in southeastern Pacific Ocean. The GenBank accession number for the 16S rRNA gene sequence of strain JLT2014^T is JX878396.

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References

Biebl H, Allgaier M, Tindall BJ, Koblizek M, Lünsdorf H, Pukall R, Wagner-Döbler, (2005) *Dinoroseobacter shibae* gen. nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. Int J Syst Evol Microbiol 55:1089–1096

Cho JC, Giovannoni SJ (2006) *Pelagibaca bermudensis* gen. nov., sp. nov., a novel marine bacterium within the *Roseobacter* clade in the order *Rhodobacterales*. Int J Syst Evol Microbiol 56:855–859

Collins MD, Goodfellow M, Minnikin DE (1980) Fatty acid isoprenoid quinone and polar lipid composition in the classification of *Curtobacterium* and related taxa. J Gen Microbiol 118:29–37

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Dong XZ, Cai MY (2001) Determinative manual for routine bacteriology. Scientific Press, Peking, pp 353–412

Embley TM (1991) The linear PCR reaction: a simple and robust method for sequencing amplified rRNA genes. Lett Appl Microbiol 13:171–174

Fraser SL, Jorgensen JH (1997) Reappraisal of the antimicrobial susceptibilities of *Chryseobacterium* and *Flavobacterium* species and methods for reliable susceptibility testing. Antimicrob Agents Chemother 41:2738–2741

Gerhardt P, Murray R, Wood WA, Krieg NR (1994) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Johnson JL (1994) Similarity analysis of DNAs. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Method for general and molecular bacteriology. American Society for Microbiology press, Washington, DC, pp 655–681

Kates M (1986) Techniques of lipidology: isolation, analysis and identification of lipids. In: Burdon RH, van Knippenberg PH (eds) Laboratory techniques in biochemistry and molecular biology. Elsevier, Amsterdam

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Komagata K, Suzuki K (1987) Lipid and cell-wall analysis in bacterial systematics. Methods Microbiol 19:161–206

Lai Q, Zhong H, Wang J, Yuan J, Sun F, Wang L, Zheng Z, Shao Z (2011) *Roseovarius indicus* sp. nov., isolated from deep-sea water of the Indian Ocean. Int J Syst Evol Microbiol 61:2040–2044

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. J Mol Biol 3:208–218

Martínez-Cánovas MJ, Quesada E, Martínez-Checa F, del Moral A, Béjar V (2004) *Salipiger mucescens* gen. nov., sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium isolated from hypersaline soil, belonging to the *α-Proteobacteria*. Int J Syst Evol Microbiol 54:1735–1740

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Bacteriol 39:159–167

Oh YS, Lim HJ, Cha IT, Im WT, Yoo JS, Kang UG, Rhee SK, Roh DH (2009) Roseovarius halotolerans sp. nov., isolated from deep seawater. Int J Syst Bacteriol 59(11):2718–2723

Ostle AG, Holt JG (1982) Nile blue A as a fluorescent stain for poly-β-hydroxybutyrate. Appl Environ Microbiol 44: 238–241

Pukall R, Buntefuss D, Frühling A, Rohde M, Kroppenstedt RM, Burghardt J, Lebaron P, Bernard L, Stackebrandt E (1999) *Sulfitobacter mediterraneus* sp. nov., a new sulfite-oxidizing member of the alpha-proteobacteria. Int J Syst Bacteriol 49:513–519

Rüger HJ, Krambeck HJ (1994) Evaluation of the BIOLOG substrate metabolism system for classification of marine bacteria. Syst Appl Microbiol 17:281–288

Shiba T (1991) *Roseobacter litoralis* gen. nov., sp. nov., and *Roseobacter denitrificans* sp. nov., aerobic pink-pigmented bacteria which contains bacteriochlorophyll a. Syst Appl Microbiol 14:140–145

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular microbiol. American Society for Microbiology, Washington, DC, pp 611–654

Sorokin DY, Tourova TP, Muyzer G (2005) *Citreicella thiooxidans* gen. nov., sp. nov., a novel lithoheterotrophic sulfur-oxidizing bacterium from the Black Sea. Syst Appl Microbiol 28:679–687

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5:molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Tang K, Huang HZ, Jiao NZ, Wu CH (2010) Phylogenomic analysis of marine *Roseobacters*. PLoS One 5:e11604

Tindall BJ (1990a) A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. Syst Appl Microbiol 13:128–130

Tindall BJ (1990b) Lipid composition of *Halobacterium lacusprofundi*. FEMS Microbiol Lett 66:199–202

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464