Curr Microbiol (2012) 64:385–391
DOI 10.1007/s00284-012-0081-z

Oceaniovalibus guishaninsula gen. nov., sp. nov., A Marine Bacterium of the Family *Rhodobacteraceae*

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Published online: 26 January 2012

Abstract The alphaproteobacterial strain JLT2003^T was isolated from surface seawater off the coast of Guishan island, Taiwan. The strain was Gram negative, ovoid or coccoid, non-motile and formed pink colonies on marine agar 2216 (MA; DIFCO) medium. The dominant fatty acids were $C_{18:1}\omega7c$, cyclo $C_{19:0}\omega8c$, and $C_{16:0}$. The polar lipid profile consisted of diphosphatidylglycerol and phosphatidylglycerol. The major respiratory ubiquinone was Q-10. The DNA G+C content was 62.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that the strain was most closely related to *Pontibaca methylaminivorans* GRP21^T with 94.8% similarity. The isolate was distinguishable from members of the family *Rhodobacteraceae* based on phenotypic and biochemical characteristics. On the basis of the taxonomic data presented, strain JLT2003^T is considered to represent a novel species of a new genus, for which the name *Oceaniovalibus guishaninsula* gen. nov., sp. nov. is proposed. The type strain of *Oceaniovalibus guishaninsula* is JLT2003^T (=JCM 17765^T = CGMCC 1.10827^T).

The GenBank accession number for the 16S rRNA gene sequence of strain JLT2003^T is HQ638975.

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Introduction

The number of genera within the family *Rhodobacteraceae* [12] has increased considerably in recent years. At the time of writing, more than 80 genera have been identified. Over three quarters of the species in the family originate from marine environments, such as seawater, sediment, marine algae, invertebrates, vertebrates, hypersaline microbial mats, and coastal biofilm [3]. Many novel genera of this clade have been described recently, for example *Agaricicola* [4], *Celeribacter* [17], *Gaetbulicola* [35], *Hwanghaeicola* [19], and *Pontibaca* [20].

Materials and Methods

Bacterial Strains Isolation and Cultivation

During a survey of the biodiversity of bacteria off the coast of Guishan island, with a hydrothermal vent nearby, Taiwan, a novel strain, designated JLT2003^T, was isolated from a surface seawater sample collected during the cruise in September 2009 after using a standard dilution-to-extinction culturing method. After incubation on RO plates [36] at 30°C for 2 weeks, strain JLT2003^T was purified as single colonies. The culture was maintained routinely on RO plates and was preserved as glycerol suspensions (15%, v/v) at −80°C.

Phenotypic and Chemical Characterization

Morphological, physiological, and biochemical characteristics of strain JLT2003^T were investigated using routine cultivation aerobically on RO medium or MA at 30°C for 3 days. Cell morphology and motility were examined by

high-resolution confocal microscopy (BX61; OLYMPUS) with cells grown for 3 days. Cell motility was determined by transmission electron microscopy (JEM-1230; JEOL USA) after negatively stained with 1% (w/v) phosphotungstic acid. The Gram staining was determined as described by Gerhardt et al. [13].

Oxidase activity was determined using Bactident Oxidase strips (MERCK) according to Smibert and Krieg [31] and catalase activity was tested using 3% H_2O_2 [8]. Nitrite and nitrate reduction were tested in RO medium by growing the strain in the presence of NO_2^- and NO_3^-, respectively [8]. The presence of poly-β-hydroxybutyrate (PHB) granules were determined by Nile blue A staining [27]. Hydrolysis of casein, starch, gelatin, Tween 80 and cellulose, and urease activity were tested as described by Cowan and Steel [7]. H_2S production was tested on RO medium supplemented with 0.05% L-cysteine, with a strip of 5–10% lead acetate impregnated paper as indicator placed in the neck of the tube [5, 26]. Methyl red and Voges–Proskauer reaction tests were performed by using methyl red and Barritt's reagent [2, 26]. The presence of bacteriochlorophyll a was tested according to Pukall [28]. Glucose fermentation was tested using the fermentation medium reported by Leifson [24]. The results are summarized in Table 1. Growth on sole carbon sources and nitrogen sources was examined by using Microlog GN2 plates (Biolog) according to the procedures of Garland [11]. Other physiological and biochemical tests were performed with the API ZYM (bioMérieux) systems according to the manufacturer's instructions.

Growth at various NaCl concentrations was investigated on MA medium with final NaCl concentrations of 0, 0.5, and 1–12%, at intervals of 1% (at 30°C and pH 7.8). Growth at various temperatures (4, 16, 20, 25, 30, 35, and 40°C, at pH 7.8, 2% NaCl) was measured on MA medium. Growth at different pH values was determined by adjusting the final pH of MA medium to 4, 5, 6, 7, 8, 9, and 10 with HCl or NaOH (2% NaCl, 30°C). Tests of antimicrobial

Table 1 Differential characteristics of strain JLT2003^T and other related taxa within the family *Rhodobacteraceae*

Characteristic	1	2	3	4	5	6	7
Morphology	Oviod or cocci	Oviod rods	Rods	Rods	Rods	Short rods	Cocci or rods
Source	Seawater	Coastal sediment	Hypersaline water	Seawater	Seawater	Seawater	Seawater
Colony color	Pink	Creamy white	Beige	Pink	Greyish yellow	Beige	Ivory
Optimum temperature (°C)	20–30	30	30–35	43	37	30	ND
Optimum NaCl (%)	4–5	2–3	ND	2	ND	2.5–3.0	2
Optimum pH	6–9	7–8	ND	8.5	7–8	10	7–8
DNA G+C content (mol%)	62.3	64.8–65.6	70	69.1	67	61.7	59.7
Nitrate reduction	+	+	−	+	−	−	+
Urease	+	+	+	−	+	+	−
Arginine dihydrolase	−	−	−	ND	−	−	−
β-galactosidase	+	−	+	+	+	−	+
Hydrolysis of:							
Aesculin	−	−	+	ND	+	−	−
Starch	+	−	+	ND	−	ND	−
Casein	−	−	ND	+	−	ND	−
Tween 80	−	ND	+	ND	−	+	+
Sole carbon source:							
L-Arabinose	+	−	ND	+	+	−	+
D-Cellobiose	+	ND	+	ND	+	−	+
D-Mannitol	+	−	+	+	ND	−	ND
D-Glucose	+	−	−	+	+	−	+
Acid from glucose	−	+	ND	−	+	+	+
Major polar lipids	PG, DPG	PC, PG, AL, PL, L1–L3	ND	ND	PG, DPG, PE, PL, GL	PC, PG, PE	PC, PE, PG, AL

+ Positive; − negative; *ND* not determined

Strains: 1. strain JLT2003^T; 2. *Pontibaca methylaminivorans* GRP21^T [20]; 3. *Maribius salinus* CL-SP27^T [3]; 4. *Tranquillimonas alkanivorans* A34^T [15]; 5. *Pseudoruegeria aquimaris* SW-255^T [34]; 6. *Maritimibacter alkaliphilus* HTCC2654^T [23]; 7. *Donghicola eburneus* SW-277^T [33]. All strains are positive for catalase and oxidase activities and the major quinine is ubiquinone Q-10

susceptibility were performed by using the disk-diffusion plate (Kirby–Bauer) method according to Fraser and Jorgensen [10] and Andrews [1].

Cellular Fatty Acid and Polar Lipids Analysis

Cellular fatty acid analysis was carried out as described by Komagata and Suzuki [21]. The fatty acid composition on the growth phase of strain JTL2003^T was tested (see Table 3) according to Kim [20]. Polar lipids were texted by two-dimensional thin-layer chromatography according to Collins et al. [6], using Merck silica gel 60F_{254} plates (10 by 20 cm) and chloroform–methanol–water (65:25:4, vol/vol) in the first dimension and chloroform–methanol–acetic acid–water (80:12:15:4, vol/vol) in the second dimension. Lipids were revealed by spraying with 10% molybdophosphoric acid in ethanol, followed by heating at 150°C for 3–5 min.

G+C Content and Isoprenoid Quinones Analysis

The genomic DNA G+C content of strain JLT2003^T was estimated from the midpoint value (T_m) of the thermal denaturation profile, as described by Mandel et al. [25]. Isoprenoid quinones were analyzed according to Hiraishi et al. [16] by using a UPLC–Q-TOF–MS spectrometer and electrospraying ionization [30].

16S rRNA Gene Analysis and PCR Amplification

Genomic DNA was extracted in accordance with the method of Rainey et al. [29] from cells grown on RO medium for 2 days at 30°C and subsequently washed and resuspended in TE buffer [37]. Purity was assessed by A_{280}/A_{260} and A_{230}/A_{260} ratios [18], and phylogenetic analysis based on 16S rRNA gene sequences was performed as described by Ying et al. [32]. The 16S rRNA gene was amplified with universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [9]. The 16S rRNA gene sequence of strain JLT2003^T was compared with those available from the GenBank database by using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi; NCBI) to determine approximate phylogenetic affiliation. Phylogenetic analysis was performed using BioEdit [14] and phylogenetic trees were constructed by using the neighbor-joining and maximum-parsimony methods within the MEGA software [22].

Results and Discussion

Phenotypic Properties

Strain JLT2003^T was Gram negative, ovoid or coccoid, non-motile, 0.9- to 1.2-μm long and 0.8- to 1.0-μm wide (Fig. 1). Colonies were pink, 0.5–1.5 mm in diameter, uniformly circular, convex, and opaque on RO or MA medium. Cells grew at 16–40°C, optimally at 20–30°C. Growth occurs at pH 4–10 (optimum 6–9) and at 0.5–12% NaCl (optimum 4–5%).Catalase and oxidase positive. Strain JLT2003^T was susceptible to kanamycin, gentamicin, tetracycline, medemycin, polymyxin B, vancomycin, cephalothin, and azteonam. The DNA G+C content of strain JLT2003^T was 62.3 mol%.

The dominant fatty acids are $C_{18:1}\omega7c$, cyclo $C_{19:0}$, and $C_{16:0}$. During the cultivation the percentage of cyclo19:0ω8c increased until the seventh day but then decreased slightly, while the predominant fatty acid ($C_{18:1}\omega7c$) decreased with length of cultivation. The fatty acid 15:0 3-OH and 11-methyl 18:1ω7c was only detected at early exponential growth (day 2) (Tables 2, 3). The polar lipid profile consisted of diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) (Fig. 2). The major respiratory ubiquinone was Q-10.

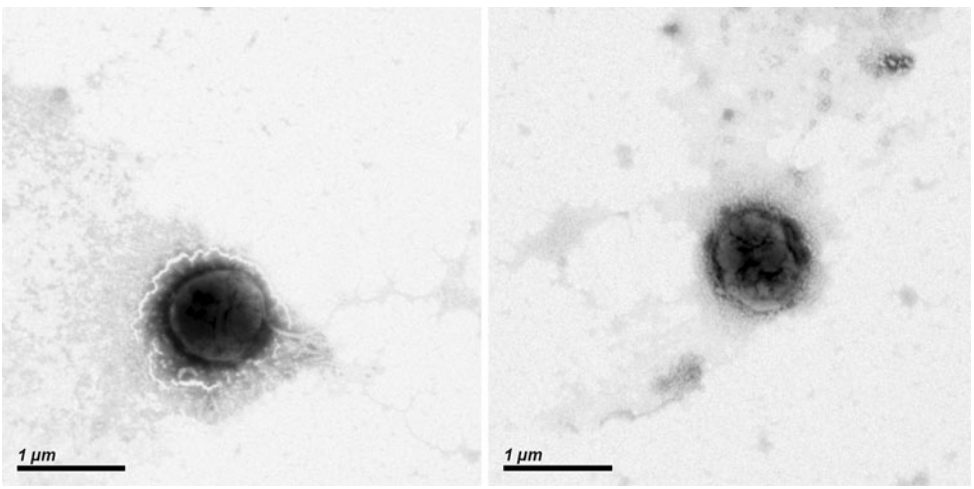

Fig. 1 Micrographs of negatively stained cells of strain JLT2003^T observed using transmission electron microscopy

Table 2 Cellular fatty acids of strains JLT2003^T and the type strains of related species

Fatty acid	1	2	3	4	5	6	7
$C_{12:0}$	1.15	3.1	–	–	0.5	0.2	–
$C_{16:0}$	22.72	15.8	4.6	9.1	1.4	15.3	13.6
$C_{17:0}$	0.99	0.7	1.3	–	0.9	0.5	1.3
$C_{18:0}$	12.16	2.1	5.4	1.6	6.6	0.5	9.2
$C_{10:0}$ 3-OH	2.07	2.6	3.5	–	2.9	0.6	–
$C_{12:0}$ 3-OH	1.66	3.6	–	2.3	–	–	–
$C_{16:0}$ 2-OH	–	–	–	–	0.6	27.3	–
$C_{18:1}\omega7c$	43.39	51.1	65.3	56.2	72.9	17.3	61.6
11-Methyl $C_{18:1}$ $\omega7c$	0.92	0.9	3.4	–	2.8	19.6	–
Cyclo $C_{19:0}\omega8c$	7.14	16.1	8.2	26.0	5.9	4.8	5.2
ECL 11.799	–	–	2.3	–	2.8	2.6	0.9

– Not detected/not reported; *ECL* equivalent chain length

Strains: 1. strain JLT2003^T; 2. *Pontibaca methylaminivorans* GRP21^T [20]; 3. *Maribius salinus* CL-SP27^T [3]; 4. *Tranquillimonas alkanivorans* A34^T [15]; 5. *Pseudoruegeria aquimaris* SW-255^T [34]; 6. *Maritimibacter alkaliphilus* HTCC2654^T [23]; 7. *Donghicola eburneus* SW-277^T [33]. Fatty acid compositions were analyzed in this study with cells grown on tryptic soy agar for 2 days

Table 3 Cellular fatty acids of strain JLT2003^T grown on tryptic soy agar harvested after different lengths of time

Fatty acid	Time (days)					
	2	3	4	6	7	8
10:0 3-OH	2.07	2.44	3.49	4.54	2.58	6.28
12:0	1.15	1.08	1.22	1.56	1.11	3.63
12:0 3-OH	1.66	2.83	2.10	1.85	2.26	4.53
14:0	1.20	2.62	ND	1.5	2.78	3.27
15:0 3-OH	1.69	ND	ND	ND	ND	ND
16:0	22.72	28.35	25.21	22.96	26.52	25.02
17:0	0.99	1.40	1.48	1.34	1.13	1.21
18:1ω9c	0.93	1.86	1.26	1.02	3.16	1.56
18:0	12.16	16.79	8.66	5.34	15.30	11.66
11-Methyl 18:1ω7c	0.92	ND	ND	ND	ND	ND
19:0 Cyclo ω8c	7.14	13.23	19.84	26.76	24.69	21.19
Summed feature 3[a]	0.57	0.70	0.87	1.2	0.78	0.98
Summed feature 8[b]	43.39	28.71	35.87	31.93	19.70	18.63

ND not detected

Fatty acids are listed using standard abbreviations (number of carbon atoms:number of double bonds). Values are percentages of total fatty acids

[a] 16:1ω7c/16:1ω6c

[b] 18:1ω7c

Additional phenotypic properties are given in Table 1 and in the genus and species descriptions below.

Phylogenetic Analysis

Phylogenetic analysis of the 16S rRNA gene sequences showed that strain JLT2003T was affiliated with the "*Rhodobacteraceae*" (Fig. 3), most closely related to species of various genera: *Pontibaca methylaminivorans* GRP21^T (94.8% similarity), *Maribius salinus* CL-SP27^T (94.5%), *Tranquillimonas alkanivorans* A34^T (94.4%), *Pseudoruegeria aquimaris* SW-255^T (94.1%), *Maritimibacter alkaliphilus* HTCC2654^T (94.0%), and *Donghicola eburneus* SW-277^T (94.0%).

In conclusion, on the basis of phylogenetic analysis of the 16S rRNA gene sequence and phenotypic characteristics, strain JLT2003^T is considered to represent a novel species of a new genus and species, for which the name *Oceaniovalibus guishaninsula* gen. nov., sp. nov. is proposed.

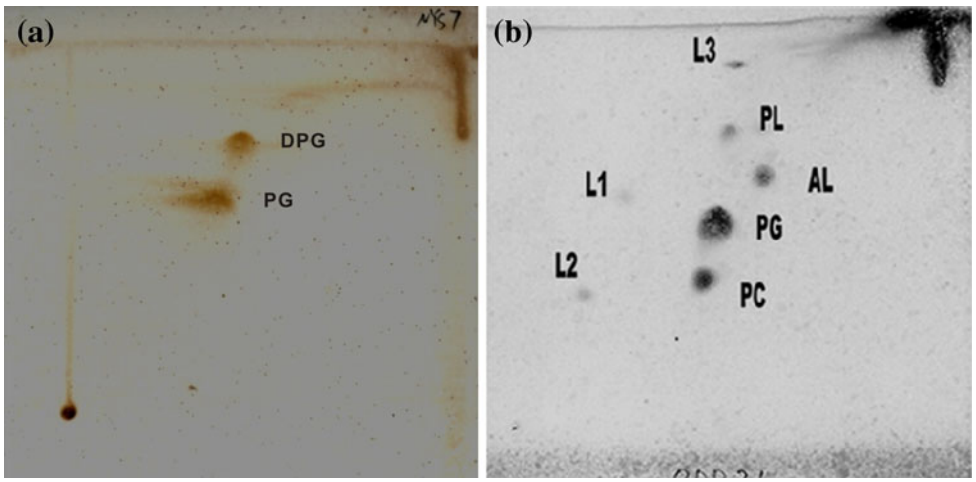

Fig. 2 Two-dimensional TLC of polar lipids of strain *O. guishaninsula* JLT2003^T (**a**) and *P. methylaminivorans* GRP21^T (**b**) detection with 10% molybdophosphoric acid in ethanol, followed by heating at 150°C for 3 min. *PC* Phosphatidylcholine, *PE* phosphatidylethanolamine, *PG* phosphatidylglycerol, *AL* unknown aminolipid, *PL* unknown phospholipid, *L1–3* unknown polar lipids that were not stainable with any of the specific spray reagents applied to detect a phosphate group, an amino group or a sugar moiety

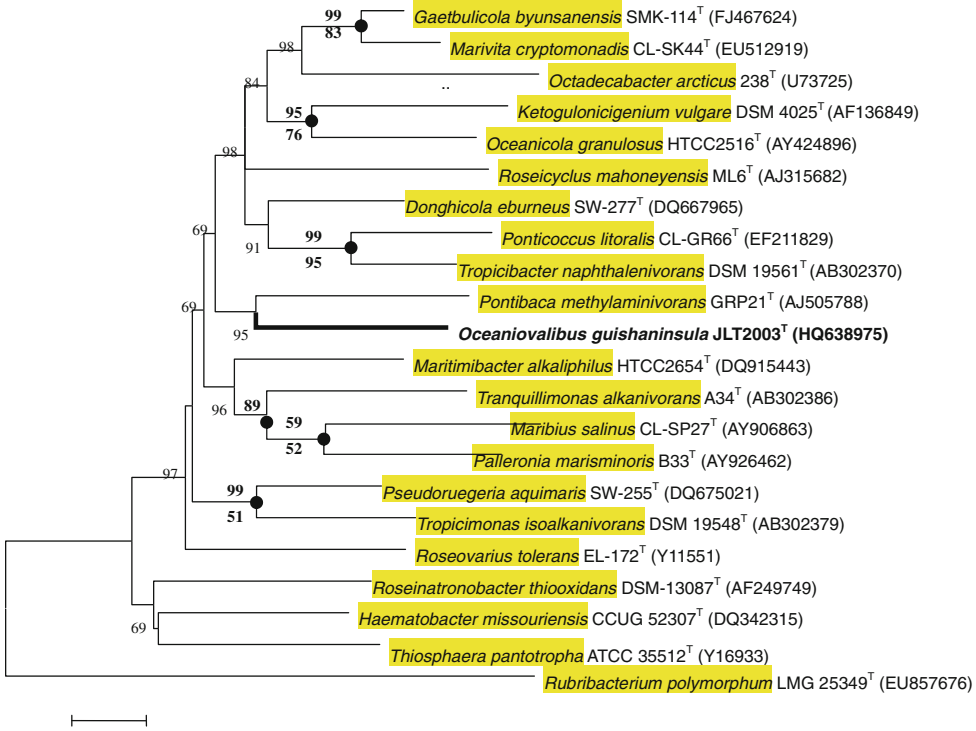

Fig. 3 Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences showing the relationship between strain JLT2003^T and representatives of the order *Rhodobacterales*. Bootstrap percentages from neighbor-joining (*above nodes*) and maximum-parsimony (*below nodes*) analyses based on 1,000 replications are shown at nodes; only values >50% are shown. *Rubribacterium polymorphum* LMG 25349^T was used to root the tree. *Filled circles* indicate nodes recovered reproducibly by using the two treeing methods. *Bar* 0.01 substitutions per nucleotide position

Description of *Oceaniovalibus* gen. nov.

Oceaniovalibus (Oceaniovalibus Arbitrary Name from oceani n. ocean; ovalibus adj. oval)

Cells are Gram negative, non-motile, ovoid or coccoid. PHB granules are not produced, and bacteriochlorophyll a is absent. Colonies are circular, convex, opaque, and pink formed on RO or MA medium. Catalase and oxidase positive. Nitrate is reduced but nitrite is not. The dominant fatty acids are $C_{18:1}\omega7c$, cyclo $C_{19:0}$, and $C_{16:0}$. The predominant respiratory ubiquinone is Q-10.

The type species is *Oceaniovalibus guishaninsula.*

Description of *Oceaniovalibus guishaninsula* sp. nov.

Oceaniovalibus guishaninsula (n. guishaninsula guishan Island)

The type strain exhibits the following properties in addition to those given in the genus description. Cells are

0.8–1.0 × 0.9–1.2 μm. Colonies (about 0.8–1.0 mm in diameter) are circular, smooth, convex, opaque, and pink. Growth occurs at 16–40°C (optimum 20–30°C), at pH 4–10 (optimum 6–9) and at 0.5–12% NaCl (optimum 4–5%). Positive for hydrolysis of starch and nitrate reduction. Negative for aesculin, casein, and gelatin hydrolysis. The Voges–Proskauer test is negative, the methyl red test is positive. Indole and H_2S are not produced. In tests with Biolog GN2 microplates, the following carbon substrates are utilized: L-arabinose, D-cellobiose, D-glucose, D-fructose, sucrose, D-raffinose, D-mannitol, D-gluconic, mono-methyl-succinate, D, L-lactic acid, γ-amino butyric acid, 2-amino-ethanol, glycerol, glucose-6-phosphate, m-inositol, starch, L-alanine, L-proline, maltose, L-rhamnose, and D-galactose are weakly utilized. The following carbon substrates are not utilized: α-cyclodextrin, dextrin, Tween 40 and 80, D-arabitol, L-fucose, α-D-lactose, D-mannose, D-sorbitol, D-trehalose, xylitol, acetic acid, cis-aconitic acid, citric acid, formic acid, D-glucosaminic acid, α-keto-glutaric acid, malonic acid, succinic acid, succinamic acid, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, L-leucine, L-ornithine, L-serine, L-threonine, inosine, and uridine. Acid is produced from sucrose, D-fructose, D-raffinose, D-cellobiose, L-arabinose, m-inositol, maltose, D-galactose, L-rhamnose, but not from D-glucose, L-threonine, L-alanine, and sodium pyruvate. Sensitive to kanamycin, gentamicin, tetracycline, medemycin, polymyxin B, vancomycin, cephalothin, azteonam, but was resistant to penicillin. According to API ZYM tests, α-glucosidase, β-glucosidase, alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), ONPG, naphthol-AS-BI-phosphohydrolase are positive. The predominant fatty acid is $C_{18:1}\omega7c$, other significant fatty acids are cyclo $C_{19:0}$, $C_{16:0}$, $C_{18:0}$, $C_{12:0}$ 3-OH, $C_{10:0}$ 3-OH, $C_{12:0}$, 11-methyl $C_{18:1}$ $\omega7c$, and $C_{17:0}$. The DNA G+C content of the type strain is 62.3 mol%. The type strain, JLT2003^T (=JCM 17765^T = CGMCC 1.10827^T), was isolated from surface seawater off the coast of Guishan island, Taiwan.

Acknowledgments The authors would like to thank Professor Henglin Cui, School of Food & Biological Engineering, Jiangsu University for his valuable help. This study was supported by the 973 program (2011CB808800), the NSFC project (91028001, 41076063), SOA project (201105021); MELRS0940.

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