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Roseibacterium beibuensis sp. nov., a Novel Member of *Roseobacter* Clade Isolated from Beibu Gulf in the South China Sea

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Abstract A novel aerobic, bacteriochlorophyll-containing bacteria strain JLT1202r^T was isolated from Beibu Gulf in the South China Sea. Cells were gram-negative, non-motile, and short-ovoid to rod-shaped with two narrower poles. Strain JLT1202r^T formed circular, opaque, wine-red colonies, and grew optimally at 3–4 % NaCl, pH 7.5–8.0 and 28–30 °C. The strain was catalase, oxidase, ONPG, gelatin, and Voges–Proskauer test positive. In vivo absorption spectrum of bacteriochlorophyll *a* presented two peaks at 800 and 877 nm. The predominant cellular fatty acid was $C_{18:1}$ ω7c and significant amounts of $C_{16:0}$, $C_{18:0}$, $C_{10:0}$ 3-OH, $C_{16:0}$ 2-OH, and 11-methyl $C_{18:1}$ ω7c were present. Strain JLT1202r^T contained Q-10 as the major respiratory quinone and the genomic DNA G+C content was 76.3 mol%. Phylogenetic analysis based on 16S rRNA gene sequences of various species with validly published names showed that strain JLT1202r^T fell within the genus *Roseibacterium*, family *Rhodobacteraceae*, sharing the highest similarity with *Roseibacterium elongatum* OCh 323^T (97.9 % similarity), followed by *Dinoroseobacter shibae* DFL 12^T (95.4 % similarity). The phylogenetic distance of *pufM* genes between strain JLT1202r^T and *R. elongatum* OCh 323^T was 9.4 %, suggesting that strain JLT1202r^T was distinct from the only strain of the genus *Roseibacterium*. Based on the variabilities of phylogenetic and phenotypic characteristics, strain JLT1202r^T stands for a novel species of the genus *Roseibacterium* and the name *R. beibuensis* sp. nov. is proposed with JLT1202r^T as the type strain (=JCM 18015^T = CGMCC 1.10994^T).

The GenBank accession number for the 16S rRNA gene sequence of strain JLT1202r^T is JN247667. The *pufLM* gene sequences of strain JLT1202r^T and *R. elongatum* OCh 323^T has been deposited under GenBank accession numbers JQ694098 and JQ694099.

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Introduction

Aerobic anoxygenic phototrophs (AAPs), a bacterial functional group containing bacteriochlorophyll *a* were first discovered by Shiba et al. [28]. Since then these bacteria have been isolated from various habitats, such as hypersaline lake [17], deep-sea hydrothermal vent [37], acidic environments [10], and dinoflagellates [1] in different categories higher taxa, mainly *Alphaproteobacteria*, one known *Betaproteobacteria* [36] and a newly described *Gammaproteobacteria* species [8]. Members of *Roseobacter* clade belonging to the class *Alphaproteobacteria* are abundant in oceanic and halophilic environments [35]. During the past two decades, a variety of bacteria in this clade have been identified, including *Roseobacter* [26], *Rubrimonas* [33], *Roseovarius* [17], *Roseivivax* [32], *Roseinatronobacter* [30], *Roseisalinus* [18], *Dinoroseobacter* [1], *Roseicyclus* [23], and *Roseibacterium*[31].

The genus *Roseibacterium* was previously established by Suzuki et al. [31] and only one recognized species isolated from sand at Monkey Mia, Shark Bay, located on the west coast of Australia, with the name of *Roseibacterium elongatum* [27, 31] is included. During the investigation of

bacterial community diversity in the South China Sea, we isolated the bacteriochlorophyll-containing bacterium because of the attractive pink colonies on agar plates, and then named it as strain JLT1202r^T for further studies. Based on the genetic and physiological similarities and variabilities with *R. elongatum* OCh 323^T, here we describe a novel aerobic bacteriochlorophyll-containing species as a new member of genus *Roseibacterium*.

Materials and Methods

Isolation and Cultivation

Strain JLT1202r^T was previously isolated from surface water in Beibu Gulf (20°37′35″N, 108°50′47″E) during the summer cruise of No. 908 program in July 2006 with the purpose of surveying bacterial community diversity. The original seawater salinity, temperature, and pH were 33 ‰, 32 °C, and 8.13, respectively. A standard dilution plating technique on marine agar 2216 (MA, Difco) was used for isolation and then cultivation was performed on marine broth 2216 (MB, Difco) at 28 °C for 4 days.

Morphological, Physiological, and Biological Tests

Cell morphology was examined by using a transmission electron microscopy (H600; Hitachi). Motility test was performed according to the method described by Dong and Cai [4]. The gram reaction was determined on cells grown on MA at 28 °C for 24 h according to standard procedures [9].

Salt requirements for growth were examined in MB medium with final NaCl concentration from 0 to 12 % at intervals of 1 % (w/v) (pH 7.8, at 28 °C). pH gradients were tested by adjusting the final values to 4.5, 5.5, 6.5, 7.5, 8.0, 9.0, 9.5, 10.5, 11.5, and 12.5 (2 % NaCl, 28 °C) with 2 N HCl and 10 % NaOH (w/v). Studies for temperature ranges were carried out by setting the incubations at 4, 10, 15, 20, 25, 28, 30, 32, 37, 43, 48, and 55 °C (2 % NaCl, pH 7.8).

Catalase activity was determined from the formation of bubbles after adding a drop of 3 % H_2O_2 solution to a fresh colony [4]. Hydrolysis of casein, starch and Tween 80 were conducted as described by Dong and Cai [4] on MA amended with 1 % substrates. Acid production from D-fructose, D-glucose, and lactose was tested on ZOF medium [19] supplemented with 1 % (w/v) carbohydrate source. Sole carbon sources utilization were carried out (1) using carbon-free MB as a basal medium with the final carbon source concentration at 0.1 % (w/v or v/v) and (2) using the commercial API 20E kit (bioMérieux). Other phenotypic and enzymic characterizations were tested using API 20E, API 20NE, and API ZYM kits (bioMérieux) with reference to the manufacturer's instructions. The production of polyhydroxyalkanoates (PHAs) [2] from bacterial cells were studied under different incubation conditions with the method introduced by previous studies [13, 34]. Both strain JLT1202r^T and *R. elongatum* OCh 323^T were incubated in MB simultaneously and different carbon sources were added to study the ability of PHAs production for JLT1202r^T, with glucose and acetate 2.5 g and glycerol 2 mL/L MB.

Susceptibility to antibiotics of strain JLT1202r^T and *R. elongatum* OCh 323^T were tested using the routine diffusion plate method [4]. Disks were pasted with the following antibiotics (per piece): penicillin G (10 μg), ampicillin (10 μg), kanamycin (30 μg), rifampicin (5 μg), streptomycin (10 μg), neomycin (30 μg), gentamicin (10 μg), tetracycline (30 μg), chloromycetin (5 μg), erythromycin (15 μg), vancomycin (30 μg), lincomycin (2 μg), novobiocin (5 μg), and carbenicillin (100 μg). The diameter of the inhibition zone was determined after incubation at 28 °C for 48 h. The strain was considered to be strong sensitive when the diameter of the inhibition circles >15 mm, intermediate at 6–15 mm and resistant at 5 mm according to Dong and Cai [4].

Both strain JLT1202r^T and *R. elongatum* OCh 323^T were incubated simultaneously on MA at 28 °C under dark for 2, 3, and 4 days in order to compare the variations of cellular fatty acids. The extraction process was performed according to the method described by Komagata and Suzuki [15] and Agilent technologies 6850 Network GC system was used for the identification of different fatty acids components. Cells were collected and completely dried after incubation in MB medium for 48 h for isoprenoid quinones analysis as described by Hirashi et al. [11], and detection was operated on Waters Acquity Ultra Performance LC^{TM} (UPLC)-Q-TOF-MS spectrometer with electrospraying ionization method (Waters, Milford, USA) [24].

Bacteriochlorophyll *a* and Photosynthetic Efficiency

Strain JLT1202r^T and *R. elongatum* OCh 323^T were incubated simultaneously in MB under dark at 28 °C for 48 h. The in vivo absorption spectrum of pigments was detected by Flexstation3 system with full wavelength scanning from 350 to 900 nm, using a 1 nm interval. The photochemical quantum efficiencies (F_v/F_m) of fresh cells were assessed by Satlantic FIRe (fluorescence induction and relaxation) fluorometer system (Canada), in which blue and green light-emitted diodes (LEDs) were incorporated.

16S rRNA Gene, *pufLM* Gene Sequencing and Phylogenetic Analysis

Genomic DNA was extracted with reference to the method of Marmur [21] from cells grown in MB medium for 18–24 h at 28 °C, washed and re-suspended in the buffer

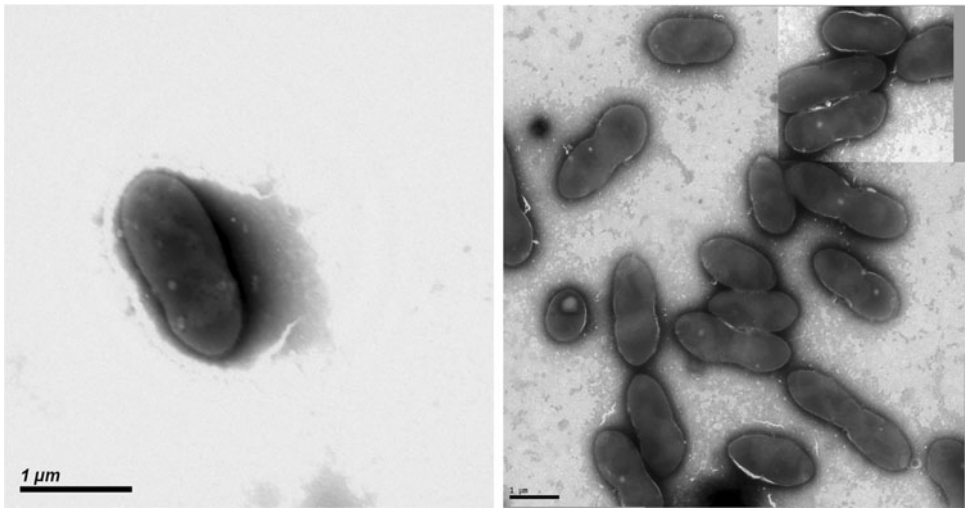

Fig. 1 Electron micrography of negatively stained cells of strain JLT1202r^T observed by transmission electron microscope. *Bar* 1 μm

for 16S rRNA gene, *pufLM* gene sequence analysis and genomic G+C content detection. The purity of the final obtained DNA was assessed by A_{280}/A_{260} and A_{230}/A_{260} values with NANODROP 2000 spectrophotometer [12]. The 16S rRNA gene was amplified with universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [6]. The *pufLM* genes of strain JLT1202r^T and *R. elongatum* OCh 323^T were PCR-amplified using primers *pufL* forward (5′-CTKTTCGACTTCTGGGTSGG-3′) and *pufM* reverse (5′-CCATSGTCCAGCGCCAGAA-3′) [14, 22]. The 16S rRNA gene and *pufLM* gene sequences of strain JLT1202r^T and *R. elongatum* OCh 323^T were compared with those related available at the GenBank database by using the BLAST program online (http://www.ncbi.nlm.nih.gov/, NCBI), the EzTaxon server 2.1(http://147.47.212.35:8080/) [3] and the LPSN website (http://www.bacterio.cict.fr/) to determine its approximate phylogenetic affiliation. Phylogenetic trees were constructed based on 16S rRNA gene and *pufM* gene sequences by using the neighbor-joining [25] and maximum-parsimony algorithms methods [5, 7] with the MEGA software package [16].

The genomic DNA G+C content was determined by the midpoint value (T_m) of the thermal denaturation curve [20], using DNA from *Escherichia coli* K-12 as a standard.

Results and Discussion

Colonies of strain JLT1202r^T were wine-red, uniformly opaque, circular and 0.7–1.1 mm in diameter after cultivation on MA at 28 °C for 3 days. The strain was gram-negative, non-motile, without flagella, short-ovoid to rod-shaped and two narrower poles. Single cell was 1.0- to 2.2-μm long and 0.7- to 0.9-μm wide, divided by binary fission (Fig. 1). The salinity, pH and temperature range for growth of JLT1202r^T were 0–10 % (optimum 3–4 %), 6.5–9.5 (optimum 7.5–8.0) and 15–43 °C (optimum 28–30 °C), respectively.

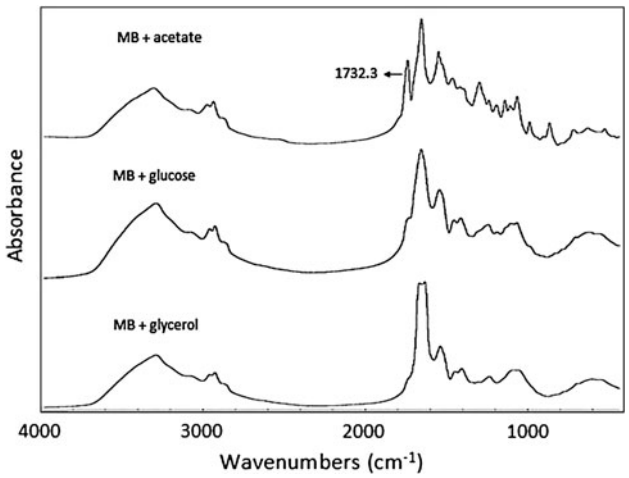

Fig. 2 FTIR spectra from dry cells of strain JLT1202r^T incubated with acetate (*above*), glucose (*middle*), and glycerol (*below*) with MB as a basal medium. The signal peak (1,732.3 cm^{-1}) indicated the formation of PHA

Strain JLT1202r^T showed positive reactions of aerobic nitrate reduction and Voges–Proskauer test, while hydrolysis of starch, Tween 80 and casein were negative. No acid was produced from D-glucose or lactose except D-fructose. A variety of substrates could be used as sole carbon sources for JLT1202r^T, such as acetate, glycerol, and glucose. Strain JLT1202r^T could not produce PHAs in MB aerobically in the dark, but strong ability of PHAs formation was found in MB with additional acetate compared to glucose and glycerol (Fig. 2). Strain JLT1202r^T was sensitive to rifampicin, chloromycetin, vancomycin, novobiocin and carbenicillin, and resistant to penicillin G, streptomycin,

Table 1 Differentiation of physiological and biochemical characteristics between strain JLT1202r^T and *R. elongatum* strain OCh 323^T

Characteristics	JLT1202r^T	OCh 323^T
Cell size (μm)[a]	0.7–0.9 × 1.0–2.2	0.5–0.8 × 1.6–10.0
Colony color	Wine-red	Pink
NaCl range (%)[a]	0–10	0.5–7.5
DNA G+C content (mol%)[a]	76.3	68.1
Nitrate reduction[a]	+	−
V–P[a]	+	−
PHA in MB	−	w
Bchl *a* in vivo peaks (nm)	800, 877	802, 878
F_v/F_m	0.67	0.72
Hydrolysis of[a]		
Gelatin	+	−
Utilization of		
D-Glucose	+	w
Maltose	+	w
Acetate	+	−
Pyruvate	w	−
Enzyme activities (API ZYM)		
Aklaline phosphatase	w	+
Esterase (C4)	+	w
Esterase lipase (C8)	w	+
Cystine aminopeptidase	w	−
β-Glucosidase	w	−
Acid production from[a]		
D-Fructose	+	−
Antibiotic sensitivity		
Penicillin G	+	w
Streptomycin	+	w
Neomycin	w	−
Chloromycetin	−	w
Novobiocin	−	w
Carbenicillin	−	w
Vancomycin	−	w

Roseibacterium elongatum OCh 323^T was described as D-glucose utilization negative and Bchl *a* in vivo peaks at 800 and 879 nm by Suzuki et al. [31]

[a] Data of strain OCh 323^T from Suzuki et al. [31]. Other data were all from this study. Both the strains were urease, catalase, oxidase and ONPG reaction positive, indole and H_2S production negative. Hydrolysis of starch and Tween 80 and acid production from D-glucose and lactose were negative of the strains. D-arabinose, DL-malate, citrate, succinate and ethanol could not be used as sole carbon source for two strains. Both strains showed positive reaction of leucine aminopeptidase, while lipase (C14), trypsin, chymotrypsin, α-galactosidase, *β*-galactosidase, *β*-glucuronidase, α-mannosidase, *β*-fucosidase, and *N*-acetyl-*β*-glucosaminidase were reactions were negative. Antibiotic sensitivity of ampicillin, kanamycin, gentamicin, tetracycline, and erythromycin were weak, rifampicin negative and lincomycin positive. Both strains contained Q-10 as the major respiratory quinone. + positive, − negative, *w* weak reaction, *ND* not determined

and lincomycin. Details for biochemical and physiological characteristics are given in Table 1.

The predominant cellular fatty acids were $C_{18:1}$ *ω*7c for strain JLT1202r^T, and $C_{16:0}$, $C_{18:0}$, $C_{10:0}$ 3-OH, $C_{16:0}$ 2-OH, and 11-methyl $C_{18:1}$ *ω*7c were also present. The relative content of major fatty acid components changed under different cell growth phases. The cellular fatty acids comparison between strain JLT1202r^T and the related strain *R. elongatum* OCh 323^T were shown in Table 2.

Cells of JLT1202r^T grown aerobically under dark had absorption peaks in the near-infrared region at 800 and 877 nm while strain *R. elongatum* OCh 323^T presented two peaks at 802 and 878 nm (Fig. 3). The 800- to 802-nm band indicated the presence of light-harvesting complex 2 (LH2) and the 877- to 878-nm band suggested the existence of light-harvesting complex 1 (LH1), which is common in other phototrophic bacteria [14]. Bacteriochlorophyll *a* of strain JLT1202r^T were completely inhabited under full exposure to light (∼10,000 lux), which was consistent with former studies by Shioi and Doi [29] in *Roseobacter denitrificans* and other AAPs [35]. A sudden rise in fluorescence in vivo from an initial value (F_0) to the maximum value (F_m) following the FIRe

Table 2 Fatty acid compositions (%) of strain JLT1202r^T and the related strain OCh 323^T

Fatty acid	JLT1202r^T			OCh 323^T		
	Day 2	Day 3	Day 4	Day 2	Day 3	Day 4
$C_{10:0}$	–	–	–	–	–	0.9
$C_{12:0}$	–	–	–	–	–	1.2
$C_{14:0}$	0.7	–	1.6	0.8	–	1.8
$C_{16:0}$	8.5	10.2	17.6	12.3	14.4	19.4
$C_{17:0}$	–	–	1.1	–	–	0.8
$C_{18:0}$	4.8	3.6	16.2	2.3	1.7	8.3
$C_{19:0}$	–	–	1.6	–	–	
$C_{20:0}$	–	–	6.2	–	–	–
$C_{18:1}$ *ω*7c	81.7	79.7	45.5	73.8	74.3	57.0
$C_{18:1}$ *ω*9c	–	–	2.9	–	–	
$C_{10:0}$ 3-OH	1.8	4.4	1.2	3.3	3.2	1.4
$C_{16:0}$ 2-OH	1.2	2.1	1.4	2.5	2.5	2.2
10-Methyl $C_{17:0}$	–	–	0.6	–	–	–
11-Methyl $C_{18:1}$ *ω*7c	1.4	–	1.0	1.3	1.1	1.5
$C_{19:0}$ cyclo *ω*8c	–	–	2.3	–	–	2.0
Summed feature 3	–	–	0.7	2.3	2.8	2.5
Summed feature 7	–	–	–	1.5	–	1.0

All data were from this study

Summed feature 3 $C_{16:1}$ *ω*6c and $C_{16:1}$ *ω*7c, *summed feature 7* un 18.846 and $C_{19:1}$ *ω*6c, – not detected

operating instructions. F_v was proposed as the balance of F_m and F_0. The maximum photochemical quantum efficiency (F_v/F_m) of photosystem II for strain JLT1202r^T and *R. elongatum* OCh 323^T were 0.67 and 0.72, respectively, suggesting that strain *R. elongatum* OCh 323^T possessed higher light energy conversion efficiency under dark incubation (Fig. 3).

The 16S rRNA and *pufM* gene sequences of strain JLT1202r^T were determined as 1,389 and 726 bp after cutting off the exogenous sequences for phylogenetic analyses. 16S rRNA gene sequence of strain JLT1202r^T was aligned with those of related species in the family *Rhodobacteraceae* and the strain was related most closely to *R. elongatum* OCh 323^T (97.9 % similarity) [31], followed by *Dinoroseobacter shibae* DFL 12^T (95.4 % similarity) [1], and quite far from other species, such as *Roseisalinus antarcticus* EL-88^T (94.5 % similarity), *Jannaschia helgolandensis* Hel 10^T (94.4 % similarity), and *Salinihabitans flavidus* ISL-46^T (93.1 % similarity) based on neighbor-joining and maximum-parsimony methods with high coherence and stability (Fig. 4). The *pufM* gene sequence of strain *R. elongatum* OCh 323^T was 750 bp after slicing the nucleotide sequences of the forward *pufL* gene. Nucleic acid sequences alignment of *pufM* genes between strain JLT1202r^T and *R. elongatum* OCh 323^T was 90.6 % similarity with *Jannaschia* sp. CCS1 (80.6 % similarity) and *Dinoroseobacter shibae* DFL 12^T (80.0 % similarity) based on the two treeing algorithms methods (Fig. 5). According to the 6 and 15 % average distance for species and genus level determination by *pufM* gene proposed by Zeng et al. [38], strain JLT1202r^T and *R. elongatum* OCh 323^T are considered to be two separate species in one genus. The G+C content of strain JLT1202r^T is 76.3 mol%.

Based on the data present, strain JLT1202r^T is considered to represent a novel species of the exist genus *Roseibacterium*, belonging to *Roseobacter* clade of the family *Rhodobacteraceae*, and for which the name *R. beibuensis* sp. nov. is proposed.

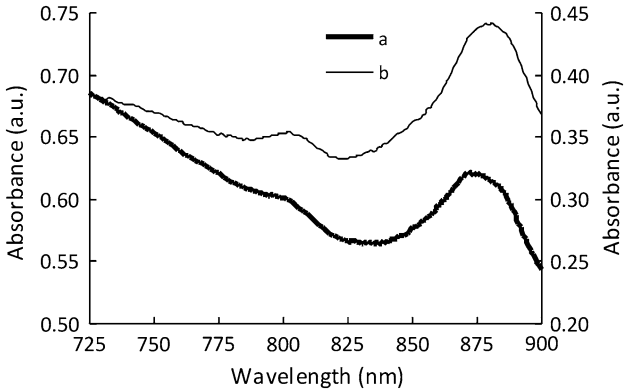

Fig. 3 In vivo absorption spectrum of photosynthetic apparatus and pigmentation in aerobically grown cells of strain JLT1202r^T and *R. elongatum* OCh 323^T. *a* Strain JLT1202r^T (*bold line*, *left y* axis), *b R. elongatum* OCh 323^T (*thin line*, *right y* axis)

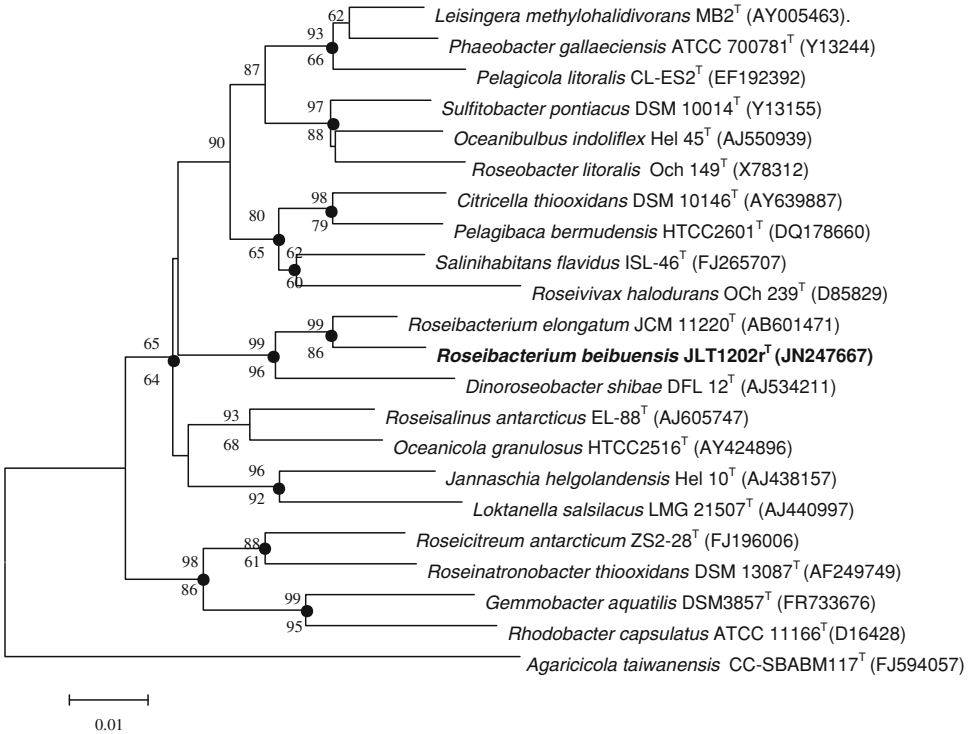

Fig. 4 Neighbor-joining phylogenetic tree for strain JLT1202r^T, based on 16S rRNA gene sequence analysis. Bootstrap values were based on 1,000 replications and percentages (*above* 50 %) from neighbor-joining (*above nodes*), and maximum-parsimony (*below nodes*) are shown. *Filled circles* indicate nodes recovered reproducibly by using the two treeing methods. *Bar* 0.01 nucleotide substitutions per nucleotide position

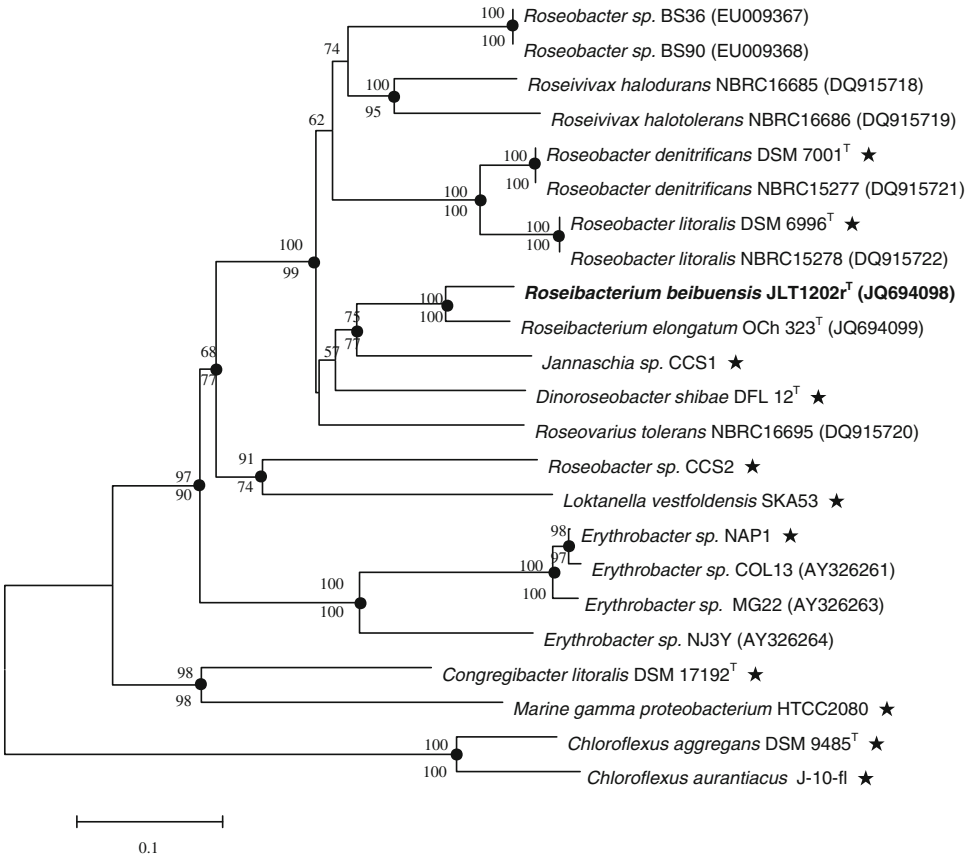

Fig. 5 Neighbor-joining tree for strains JLT1202r^T, *R. elongatum* OCh 323^T and other related taxa based on *pufM* gene sequences available from GenBank database. Bootstrap values were based on 1,000 replications and percentages (*above* 50 %) from neighbor-joining (*above nodes*) and maximum-parsimony (*below nodes*) are shown. *Black star symbols* represented the *pufM* gene sequences from whole genome sequences. *Filled circles* indicate nodes recovered reproducibly by using the two treeing methods. *Bar* 0.1 nucleotide substitutions per nucleotide position

Description of *Roseibacterium beibuensis* sp. nov.

Roseibacterium beibuensis (bei.bu.en′sis. N.L. masc. adj. beibuensis pertaining to Beibu Gulf in the South China Sea, the place where the type strain was isolated).

Cells are gram-negative, non-motile, short-ovoid to rod-shaped, no flagella. Wine-red, smooth, opaque colonies form on MA at 28 °C after incubation aerobically for 3 days. The strain is nitrate reduction, catalase, oxidase, urease, and Voges–Proskauer reaction positive, negative for indole production, hydrolysis of starch and Tween 80. Growth occurs at 15–43 °C, 0–10 % NaCl, pH 6.5–9.0 with optimal growth at 28–30 °C, 3–4 % NaCl, pH 7.5–8.0. Bacteriochlorophyll is synthesized under aerobic, dark condition with two in vivo absorption peaks at 800 and 877 nm. Several regular carbon sources can be used as sole substrate, such as D-glucose, acetate, glycerol, and maltose. The major cellular fatty acids were $C_{18:1}$ ω7c, $C_{16:0}$, $C_{18:0}$, $C_{10:0}$ 3-OH, $C_{16:0}$ 2-OH, and 11-methyl $C_{18:1}$ ω7c. The major respiratory quinone is Q-10 and genomic DNA G+C content is 76.3 mol%.

The type strain, JLT1202r^T (=JCM 18015^T = CGMCC 1.10994^T) was isolated from the surface water of the Beibu Gulf in the South China Sea.

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References

1. Biebl H, Allgaier M, Tindall BJ, Koblizek M, Lünsdorf H, Pukall R, Wagner-Döbler I (2005) *Dinoroseobacter shibae* gen nov., sp. nov., a new aerobic phototrophic bacterium isolated from dinoflagellates. Int J Syst Evol Microbiol 55:1089–1096
2. Chen GQ (2009) A microbial polyhydroxyalkanoates (PHA) based bio- and materials industry. Chem Soc Rev 38:2434–2446
3. Chun J, Lee JH, Jung Y, Kim M, Kim S, Kim BK, Lim YW (2007) EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. Int J Syst Evol Microbiol 57:2259–2261
4. Dong XZ, Cai MY (2001) Determinative manual for routine bacteriology. Scientific Press, Peking, pp 353–412
5. Eck RV, Dayhoff MO (1966) In: Dayhoff MO (ed) Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Spring, pp 161–169
6. Embley TM (1991) The linear PCR reaction: a simple and robust method for sequencing amplified rRNA genes. Lett Appl Microbiol 13:171–174
7. Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416

8. Fuchs BM, Spring S, Teeling H, Quast C, Wulf J, Schattenhofer M, Yan S, Ferriera S, Johnson J, Glökner FO, Amann R (2007) Characterization of a marine gammaproteobacterium capable of aerobic anoxygenic photosynthesis. Proc Natl Acad Sci 104:2891–2896
9. Gerhardt P, Murray RGE, Wood WA, Krieg NR (1994) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, DC
10. Hiraishi A, Matsuzawa Y, Kanbe T, Wakao N (2000) *Acidisphaera rubrifaciens* gen. nov., sp. nov., an aerobic bacteriochlorophyll-containing bacterium isolated from acidic environments. Int J Syst Evol Microbiol 50:1539–1546
11. Hirashi A, Ueda Y, Ishihara J (1998) Quinone profiling of bacterial communities in natural and synthetic sewage activated sludge for enhanced phosphate removal. Appl Environ Microbiol 64:992–998
12. 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
13. Kansiz M, Billman-Jacobe H, McNaughton D (2000) Quantitative determination of the biodegradable polymer poly(β-hydroxybutyrate) in a recombinant *Escherichia coli* strain by use of mid-infrared spectroscopy and multivariative statistics. Appl Environ Microbiol 66:3415–3420
14. Koblížek M, Béjà O, Bidigare RR, Christensen S, Benitez-Nelson B, Vetriani C, Kolber MK, Falkowski PG, Kolber ZS (2003) Isolation and characterization of *Erythrobacter* sp. strains from the upper ocean. Arch Microbiol 180:327–338
15. Komagata K, Suzuki K (1987) Lipid and cell wall analysis in bacterial systematics. Methods Microbiol 19:161–206
16. Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment briefings. Bioinformatics 5:150–163
17. Labrenz M, Collins MD, Lawson PA, Tindall BJ, Schumann P, Hirsch P (1999) *Roseovarius tolerans* gen. nov., sp. nov., a budding bacterium with variable bacteriochlorophyll *a* production from hypersaline Ekho Lake. Int J Syst Bacteriol 49:137–147
18. Labrenz M, Lawson PA, Tindall BJ, Collins MD, Hirsch P (2005) *Roseisalinus antarcticus* gen. nov., sp. nov., a novel aerobic bacteriochlorophyll *a*-producing α-proteobacterium isolated from hypersaline Ekho Lake, Antarctica. Int J Syst Evol Microbiol 55:41–47
19. Lemos ML, Toranzo AE, Barja JL (1985) Modified medium for the oxidation–fermentation test in the identification of marine bacteria. Appl Environ Microbiol 49:1541–1543
20. Mandel M, Igambi L, Bergenda J, Dodson ML, Scheltge E (1970) Correlation of melting temperature and cesium chloride buoyant density of bacterial deoxyribonucleic acid. J Bacteriol 101:333–338
21. Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. J Mol Biol 3:208–218
22. Nagashima KVP, Hiraishi A, Shimada K, Matsuura K (1997) Horizontal transfer of genes coding for the photosynthetic reaction centers of purple bacteria. J Mol Evol 45:131–136
23. Rathgeber C, Yurkova N, Stackebrandt E, Schumann P, Beatty JT, Yurkov V (2005) *Roseicyclus mahoneyensis* gen. nov., sp. nov., an aerobic phototrophic bacterium isolated from a meromictic lake. Int J Syst Evol Microbiol 55:1597–1603
24. Romano I, Lama L, Nicolaus B, Poli A, Gambacorta A, Giordano A (2006) *Halomonas alkaliphila* sp. nov., a novel halotolerant alkaliphilic bacterium isolated from a salt pool in Campania (Italy). J Gen Appl Microbiol 52:339–348
25. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
26. Shiba T (1991) *Roseobacter litoralis* gen. nov., sp. nov., and *Roseobacter denitrificans* sp. nov., aerobic pink-pigmented bacteria which contain bacteriochlorophyll *a*. Syst Appl Microbiol 14:140–145
27. Shiba T, Shioi Y, Takamiya K, Sutton DC, Wilkinson CR (1991) Distribution and physiology of aerobic bacteria containing bacteriochlorophyll *a* on the east and west coasts of Australia. Appl Environ Microbiol 57:295–300
28. Shiba T, Simidu U, Taga N (1979) Distribution of aerobic bacteria which contain bacteriochlorophyll *a*. Appl Environ Microbiol 38:43–45
29. Shioi Y, Doi M (1988) Control of bacteriochlorophyll accumulation by light in an aerobic photosynthetic bacterium, *Erythrobacter* sp. OCh 114. Arch Biochem Biophys 266:470–477
30. Sorokin DYu, Tourova TP, Kuznetsov BB, Bryantseva IA, Gorlenko VM (2000) *Roseinatronobacter thiooxidans* gen. nov., sp. nov., a new alkaliphilic aerobic bacteriochlorophyll *a*-containing bacterium isolated from a soda lake. Mikrobiologiya 69:89–97
31. Suzuki T, Mori Y, Nishimura Y (2006) *Roseibacterium elongatum* gen. nov., sp. nov., an aerobic, bacteriochlorophyll-containing bacterium isolated from the west coast of Australia. Int J Syst Evol Microbiol 56:417–421
32. Suzuki T, Muroga Y, Takahama M, Nishimura Y (1999) *Roseivivax halodurans* gen. nov., sp. nov. and *Roseivivax halotolerans* sp. nov., aerobic bacteriochlorophyll-containing bacteria isolated from a saline lake. Int J Syst Bacteriol 49:629–634
33. Suzuki T, Muroga Y, Takahama M, Shiba T, Nishimura Y (1999) *Rubrimonas cliftonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-containing bacterium isolated from a saline lake. Int J Syst Bacteriol 49:201–205
34. Xiao N, Jiao NZ (2011) Formation of polyhydroxyalkanoate in aerobic anoxygenic phototrophic bacteria and its relationship to carbon source and light availability. Appl Environ Microbiol 77:7445–7450
35. Yurkov V, Csotonyi JT (2009) New light on aerobic anoxygenic phototrophs. In: Hunter CN, Daldal F, Thurnauer MC, Beatty JT (eds) The purple phototrophic bacteria. Springer, Dordrecht, pp 31–55
36. Yurkov VV, Csotonyi JT (2003) Aerobic anoxygenic phototrophs and heavy metalloid reducers from extreme environments. In: Pandalai SG (ed) Recent research developments in bacteriology, vol 1. Transworld Research Network, Trivandrum, pp 247–300
37. Yurkov VV, Krieger S, Stackebrandt E, Beatty JT (1999) *Citromicrobium bathyomarinum*, a novel aerobic bacterium isolated from deep-sea hydrothermal vent plume waters that contains photosynthetic pigment–protein complexes. J Bacteriol 181:4517–4525
38. Zeng YH, Chen XH, Jiao NZ (2007) Genetic diversity assessment of anoxygenic photosynthetic bacteria by distance-based grouping analysis of *pufM* sequences. Lett Appl Microbiol 45:639–645