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ORIGINAL PAPER

Rhodobacter thermarum sp. nov., a novel phototrophic bacterium isolated from sediment of a hot spring

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Abstract An ovoid to rod-shaped, phototrophic, purple non-sulfur bacterium was isolated from a sediment sample of a hot spring in Tibet, China. Cells of strain YIM 73036^T were Gram-stain negative, non-motile and multiplied by binary fission. Strain YIM 73036^T grew optimally at pH 7.0–7.5 at 37–45 °C. Growth occurred in 0.5–3.5% (w/v) NaCl. Vitamins were not required for growth. The presence of photosynthesis genes *pufL* and *pufM* were shown and photosynthesis pigments were formed. Bacteriochlorophyll α, the bacteriopheophytin and carotenoids were present as photosynthetic pigments. Internal cytoplasmic membranes were of the lamellar type. The organism YIM 73036^T was able to grow chemo-organoheterophically, chemo-lithoautotrophically and photo-organoheterotrophically but photo-lithoautotrophic and fermentative growth were not demonstrated. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that strain YIM 73036^T is closely related to *Rhodobacter blasticus* ATCC 33485^T (96.65% sequence similarity) and clustered with species of the genus *Rhodobacter* of the family *Rhodobacteraceae*. Whole-genome sequence analyses based on the average nucleotide BLAST identity (ANI < 82%) indicated that this isolate belongs to a novel species. The genomic DNA G+C content of organism YIM 73036^T was

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determined to be 66.0 mol%. Strain YIM 73036^T contained Q-10 as the predominant ubiquinone and $C_{18:1}\omega7c$, $C_{18:1}$ $\omega7c$ 11-methyl and $C_{18:0}$ as the major fatty acids. The major polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and unidentified phospholipid. Differential phenotypic and chemotaxonomic properties, together with the phylogenetic distinctiveness, demonstrated that strain YIM 73036^T is distinguishable from other species of the genus *Rhodobacter*. On the basis of the data presented, strain YIM 73036^T is considered to represent a novel species of the genus *Rhodobacter*, for which the name *Rhodobacter thermarum* sp. nov. [type strain YIM 73036^T (= KCTC 52712^T = CCTCC AB 2016298^T)] is proposed.

Keywords *Rhodobacter thermarum* sp. nov. · Sediment sample · Hot spring · Tibet · Phylogenetic analysis

Introduction

The genus *Rhodobacter* belongs to the family *Rhodobacteraceae* of the class *Alphaproteobacteria* in the phylum *Proteobacteria*. Members of this genus are Gram-stain negative and contain bacteriochlorophyll-*a*, as the core of photosynthesis reaction system RC complexes in phototrophic bacteria (Zhang et al. 2016); phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are the major polar lipids of species of the genus *Rhodobacter* (Raj et al. 2013). At the time of writing, the genus *Rhodobacter* comprises 15 recognized species names: *Rhodobacter aestuarii*, *Rhodobacter azotoformans*, *Rhodobacter blasticus*, *Rhodobacter capsulatus*, *Rhodobacter johrii, Rhodobacter maris*, *Rhodobacter megalophilus*, *Rhodobacter ovatus*, *Rhodobacter sphaeroides*, *Rhodobacter veldkampii*, *Rhodobacter vinaykumarii*, *Rhodobacter viridis*, *Rhodobacter sediminis* and the recently described *Rhodobacter lacus* and *Rhodobacter azollae* (Subhash and Lee 2016; Suresh et al. 2017). Species of this genus have been isolated from different environmental samples, such as a brown-coloured microbial mat (Venkata et al. 2009), semi-arid tropical soils (Girija et al. 2010), marine habitats (Venkata et al. 2008; Srinivas et al. 2007), polluted pond sediment (Srinivas et al. 2008), mud of a stream (Raj et al. 2013) and lagoon sediments (Subhash and Lee 2016) and pond (Suresh et al. 2017). The family *Rhodobacteraceae* members, particularly the genus *Rhodobacter* is a very heterogeneous assemblage of phototrophic bacteria with a large number of interspersing chemotrophic bacteria and their evolutionary relationships are not well established. Based on 16S rRNA gene sequence phylogenetic analysis, *Rhodobacter* species are grouped into five monophyletic clusters, each of which comprise one to seven species. In this study, we describe isolation and characterization of a non-motile, Gram-stain negative phototrophic *Alphaproteobacterium*, designated YIM 73036^T, which was isolated from a sediment sample collected at Qucai Geothermal Field, in Tibet hot spring, China. Comparative 16S rRNA gene sequence analysis indicated that this strain is closely related to members of the genus *Rhodobacter*. The aim of the present work was to determine the exact taxonomic position of strain YIM 73036^T by using a polyphasic characterization that included determination of chemotaxonomic and other phenotypic properties and phylogenetic investigation based on 16S rRNA gene sequences and at the genomic level.

Materials and methods

Isolation and preservation

The sediment sample was collected from Qucai Geothermal Field (pH 7.0, temperature 65.7 °C, 30°39′58.5″N, 91°35′28.8″E) in August 2014. For the isolation, 3 g of sediment sample was taken into a flask with 30 ml sterile water. The flask was kept incubated in a shaker (37 °C, 200 r.p.m., 2 h). The resultant suspension was diluted to 10^{-2} dilution, and 0.5 ml aliquots of the diluted suspension was spread on R2A agar (pH 7.0–7.2) plates with the following composition (0.6 g peptone, 0.6 g yeast extract, 0.6 g glucose, 0.6 g casamino acids, 0.6 g soluble starch, 0.3 g sodium pyruvate, 0.3 g $K_2HPO_4{\cdot}7H_2O$, 0.005 g $MgSO_4{\cdot}7H2O$, 15.0 g agar per litre). The isolation plates were incubated at 45 °C for 1 week. The strain YIM 73036^T was maintained as pure cultures on R2A agar medium at 45 °C, and stored as a glycerol suspension (20%, w/v) at − 80 °C. *R. blasticus* ATCC

33485^T and *Tabrizicola aquatica* RCRI19^T were grown under the same conditions and used as reference strains for comparative taxonomic work.

Phenotypic characterization

Morphological characteristics of strain YIM 73036^T were determined by transmission electron microscopy (JEM-2100; JEOL) after the culture was grown on R2A agar at 45 °C for 2 days. The internal membrane structures were determined from exponentially growing cultures by using a transmission electron microscope (JEM-2100; JEOL) after the cells had been processed (Hanada et al. 2002). Gram staining was carried out by using the standard Gram's reaction and was confirmed by a non-staining procedure (Gregersen 1978). Growth was tested at 20, 25, 37, 45, 50 and 55 °C in R2A broth medium. Tolerance of salt was tested by supplementing R2A broth medium with various concentrations of NaCl ranging from 0.5 to 5% (w/v) at intervals of 0.5%. The pH response was determined in R2A broth medium adjusted between pH 4.0 and 11.0 (with interval of 1.0 pH unit) using buffer system as described by Xu et al. (2005). Nitrogen source utilization tests were carried out as described by Gordon et al. (1974). The ability to utilize various carbon sources, production of acid from sugars and physiological profile of the strain YIM 73036^T were studied using API ZYM, API 20 NE and API 50 CH B/L kits according to the manufacturer's (BioMérieux) instructions.

To test phototrophic growth, the bacterium was incubated in Pfennig medium (Pfennig and Truper 1992), supplemented with sodium pyruvate (0.3%, w/v) and NH_4Cl (0.12%) as carbon and nitrogen sources, respectively, under light exposure (2400 l×) and anaerobic conditions at 30 ± 2 °C. Photo-lithoautotrophic growth was investigated under anaerobic condition and light exposure (2400 l×) with Na_2S (0.5 mM), $Na_2S_2O_3$ (0.5 mM) and $NaHCO_3$ (0.1%, w/v). The bacterium was incubated in aerobic dark conditions in Pfennig medium in the presence of sodium pyruvate (0.3%, w/v) as the only carbon source to determinate chemo-organoheterotrophic growth. After that, the growth was investigated under aerobic and dark conditions with $Na_2S_2O_3$ (0.5 mM) and $NaHCO_3$ (0.1%, w/v) also under anaerobic, dark conditions with pyruvate (0.3%, w/v) in order to determine chemo-lithoautotrophic and fermentative growth, respectively. Vitamin (biotin, niacin, *p*-aminobenzoic acid, thiamine and vitamin B12) requirement was tested by replacing yeast extract with single and also combinations of vitamins as growth factors. Determination of oxidase activity was carried out using 1% (w/v) tetramethyl-*p*-phenylenediamine as described by Kovacs (1956). Catalase activity was tested using 3% (w/v) H_2O_2 by assessing bubble production as the positive result. Other biochemical characteristics including hydrolysis of aesculin, casein, chitin, gelatin and Tweens (20, 40, 60 and 80), H_2S production and nitrate reduction were observed as previously described (MacFaddin 1976; Gonzalez et al. 1978; Smibert and Krieg 1994).

Chemotaxonomy

Biomass used for chemical studies was obtained from cultures grown on R2A agar plates for 3 days at 45 °C. Polar lipids were extracted, separated by two-dimensional TLC and identified using previously described procedures (Minnikin et al. 1979; Collins and Jones 1980). For the cellular fatty acid analysis, strain YIM 73036^T and related type strains were harvested from growth on R2A [Difco (pH 7.0)] plates at 37 °C for 2 days. The cellular fatty acids were extracted, methylated and analyzed by using the Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6) (Sasser 1990). Quinones were isolated as described by Collins et al. (1977) and separated by HPLC (Kroppenstedt 1982). To extract carotenoids, 100 ml of T5 medium in semi-aerobically condition cultures were centrifuged at 9000 rpm for 10 min at 4 °C. After separation of supernatant, the pellet was mixed with acetone-methanol (7:3 v/v) solution containing 0.1% butylhydroxytoluene as antioxidant. The pelleted cells were then frozen in liquid nitrogen and thawed in room temperature to improve the extraction yield. This step was repeated several times and finally was followed by centrifugation at 12,000×*g* for 15 min at 4 °C. The solvent was evaporated under a stream of nitrogen gas and the pigments were dissolved in 10 ml of acetone (containing 0.1% BHT). The identification of carotenoids were performed by comparing retention time, UV–VIS spectra and characteristics of the mass spectra (protonated molecule ([M+H]+). All of the carotenoids were monitored at 450 nm with a UV–visible detector (Naziri et al. 2014). Xcalibur 2.0 SR2

software (copyright Thermo Electron Corporation 1998–2006) was used for data analysis. In order to confirm the presence of bacteriochlorophyll a and pigments, UV–VIS absorption spectra of the strain was measured with a spectrophotometer (Shimadzu UV-1800 Series, Kyoto, Japan). Moreover, the carotenoid composition comparison, was determined by C18-HPLC analysis among strains YIM 73036^T, *R. blasticus* ATCC 33485^T and *T. aquatica* RCRI19^T under similar phototrophical conditions (Ramana et al. 2010).

The genomic DNA G+C contents were determined by HPLC after enzymatic degradation (Mesbah et al. 1989) using *E. coli* strain DH5α as the reference.

Molecular characterization

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene sequences were performed as previously described (Cui et al. 2001; Li et al. 2007). The resulting 16S rRNA gene sequence was compared with available 16S rRNA gene sequences of cultured species from GenBank via the BLAST program and from the EzBioCloud server databases (Yoon et al. 2017a, b). The genetic ability to form a photosynthetic apparatus was tested by the presence of *pufL* and *pufM* genes. PCR amplification of the *pufL* and *pufM* genes was performed using the primer set used previously with the forward primer (67F) 5′-TTC GAC TTY TGG RTN GGN CC-3′ and the reverse primer (781R) 5′-CCA KSG TCC AGC GCC AGA ANA-3′ (Tank et al. 2009). Phylogenetic trees were generated using three tree-making algorithms, neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum parsimony (Fitch 1971) methods in the MEGA version 5.0 software package (Tamura et al. 2011). Kimura's two-parameter model (Kimura 1980) was used to calculate evolutionary distance matrices of the neighbour-joining method and maximum-likelihood method. The topology of the phylogenetic trees was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 resamplings. Average Nucleotide Identity (ANI) was calculated between the draft genome sequence of strain YIM 73036^T and closely related reference type strains by using online software (Yoon et al. 2017a, b).

Genome sequencing, assembly and annotation

Draft genome sequencing of strain YIM 73036^T was performed on a Illumina HiSeq 2000 platform (Illumina, USA) at MajorBio Shang Hai, China. Genome assembly of the raw sequence data generated was performed with SOAPdenovo2 (Luo et al. 2012).

Nucleotide sequence accession numbers

The Whole Genome Shotgun project of strain YIM 73036^T has been deposited at DDBJ/EMBL/GenBank under the accession QMJY00000000. Genomes for the reference strains were downloaded from NCBI.

Results and discussion

Phenotypic characteristics

The colonies of strain YIM 73036^T were small with a convex surface. The bacterium produces glassy-colored colonies on marine agar medium with 0.5% NaCl at 30 °C. The colonies change to pink over time. Strain YIM 73036^T was found to be Gram-stain negative. The isolate YIM 73036^T was able to grow on T5, R2A and marine agar media. Cells of the organism YIM 73036^T were rod shaped with 0.7–1.1 × 1.8–3.4 μm (supplementary Fig. S1). The strain showed growth at 20–55 °C, optimum growth at temperature 37–45 °C. Growth for strain YIM 73036^T was observed at pH 6.0–8.5 (optimum, 7.0–7.5). The novel isolate was positive for oxidase and catalase activities. Strain YIM 73036^T was positive for hydrolysis of Tween 20, but negative for milk coagulation, hydrolysis of chitin, gelatin and Tweens (40, 60, 80), and also H_2S production tests. The bacterium was able to grow chemo-organoheterophically [aerobic, dark, in the presence of pyruvate (0.3% w/v)], chemo-lithoautotrophically [aerobic, dark, $Na_2S_2O_3$ (0.5 mM) and $NaHCO_3$ (0.1% w/v)] (very weak) and photo-organoheterotrophic growth [anaerobic conditions, in the light 2400 l×, pyruvate (0.3% w/v)] but photo-lithoautotrophic growth [anaerobic, light 2400 l×, Na_2S (0.5 mM), $Na_2S_2O_3$ (0.5 mM) and $NaHCO_3$ (0.1%, w/v)] and fermentative growth [anaerobic, dark, pyruvate (0.3%, w/v)] do not occur.

During photosynthetic growth (Imhoff et al. 1984), the colour of the cell suspension of both strains YIM

73036^T and *R. blasticus* ATCC 33485^T were yellow–brown. The whole-cell extract absorption spectrum (Fig. S4) of strain YIM 73036^T showed maximam absorptions at 375, 480, 681, 759 and 846 nm, confirming the presence of bacteriochlorophyll *a* and pigments (Girija et al. 2010). According to the LC–MS data at 450 nm, the major caretenoid of strain YIM 73036^T in the semi-aerobically growth is spheroidenone (Chi et al. 2015). Also HPLC analysis determined that strains YIM 73036^T and the closely related species *R. blasticus* ATCC 33485^T showed the same pattern for the major carotenoids (Fig.S5) implying the presence of spheroidene and sphoroidenone as major carotenoids (Girija et al. 2010). Interestingly we didn't observe any peak for pigments in *T. aquatica* RCRI19^T in this condition. Lamellar type of internal cytoplasmic membranes were present (Fig S1).

In the API ZYM system, the isolate displayed positive results for activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, *β*-galactosidase, α-glucosidase and *β*-glucosidase. Applying API 20NE kit *β*-galactosidase and *β*-glucosidase were positive but negative results were obtained for indole production, fermentation (glucose), arginine dihydrolase, urease, and hydrolysis of gelatin. The reduction of nitrate was negative. Arabinose, glucose, mannose, manitol, maltose and adipic acid are assimilated but *N*-acetyl-glucosamine, capric acid, potassium gluconat, malate, trisodium citrate, and phenylacetic acid are not assimilated. According to API 50CH kit results, the bacterium can produce acid from D-glucose, D-fructose, D-sorbitol, L-arabinose, salicine, D-saccharose (sucrose), D-lyxose, D-xylose, D-mannitol, esculin, D-fucose, methyl-α-D-monno pyranoside, D-lactose (bovine origin), D-trehalose, D-melezitose, and D-cellobiose, but not from glycerol, D-arabinose, erythritol, D-tagatose, L-lyxose, D-adonitol, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, methyl-α-D-mannapyanoside, L-rhamnose, dulcitol, inositol, *N*-acetylglucosamine, amygdalin, arbutin, D-celiobiose, D-maltose, D-melibiose, inulin, D-raffinose, amidon (starch), glycogen, xylitol, gentiobiose, D-turanose, L-fucose, L-arabitol, potassium gluconate, potassium 2-ketogluconate, D-ribose, methyl-*β*-D-xylopyranoside, D-galactose, methyl-α-D-glucopyranoside, potassium 5-keto gluconate, and D-arabitol. Selective differentiating characteristics with closely related type strains and the genus *Rhodobacter* species are listed in Tables 1 and S3.

Chemotaxonomical characteristics

The polar lipid profile consists of PG, diphosphatidylglycerol(DPG), PE, PC, two unidentified phospholipids and two unidentified aminophospholipids (Fig. S3). The cellular fatty acids (> 1% of total fatty acids) of strain YIM 73036^T consisted of $C_{18:1}\omega7c$ (50.9%), $C_{18:1}$ *ω7c* 11-methyl (19.6%), $C_{18:0}$ (10.8%), $C_{16:0}$ (7.4%), $C_{18:0}$ 3–OH (4.9%) and $C_{10:0}$ 3–OH (2.5%) (Table S1). The predominant cellular fatty acids particularly $C_{18:1}\omega7c$ and $C_{18:1}$ *ω7c* 11-methyl were also reported in other members of the genus *Rhodobacter*. In the present study, the fatty acid ($C_{18:1}$ *ω7c*) was predominant in the novel isolate, *Rhodobacter thermarum* YIM 73036^T and *R. blasticus* CGMCC 1.3365^T which was consistent with the previous study, in *Rhodobacter sediminis* N1^T, *R. capsulatus* KACC 15298^T and *Rhodobacter viridis* KCTC 15167^T (Subhash and Lee 2016). Subhash and Lee (2016) also reported the dominant fatty acids ($C_{16:1}$ *ω6c*/$C_{16:1}$ *ω7c*) in *R. sediminis* N1^T, *R. capsulatus* KACC 15298^T and *R. viridis* KCTC 15167^T which were not detected in *R. thermarum* YIM 73036^T and *R. blasticus* CGMCC 1.3365^T in the present study. Also in the present study, $C_{18:1}$ *ω7c* 11-methyl was a major component in *R. thermarum* YIM 73036^T, in a small amount in *R. blasticus* CGMCC 1.3365^T, while in the earlier study, not detected or in trace amount in *R. sediminis* N1^T, *R. capsulatus* KACC 15298^T and *R. viridis* KCTC 15167^T (Subhash and Lee 2016).

Detailed fatty acid profiles of strain YIM 73036^T and the two closely related reference type strains are given in Table S1. The major respiratory lipoquinone was ubiquinone-10 (Q-10), which is consistent with those reported for the genus *Rhodobacter*. The DNA G+C contents of YIM 73036^T were determined to be 66.0 mol%.

Molecular characteristics

Based on 16S ribosomal gene sequence and comparison to 16S rRNA genes in the EzTaxon-e server, strain YIM 73036^T is closely related to *R. blasticus* ATCC 33485^T (96.65%) and *T. aquatica* RCRI19^T (95.85%). The ANI values between the draft genome

Table 1 Key taxonomic characteristics differentiating strain YIM 73036^T from the reference type strains, *R. blasticus* CGMCC 1.3365^{T}and *T. aquatica* KCTC 23724^T

Characteristic	1	2	3
Cell shape	Ovoid to rod-shaped	Ovoid to rod-shaped	Rod-shaped
Cell width (μm)	0.7–1.1	0.6–0.8	0.9
Cell length (μm)	1.8–3.4	1.0–2.5	1.3–3
Colour of cells	Brown	Orange–brown	Cream
Suspension			
Internal membrane system	+	+	−
Growth in 3% (w/v) NaCl	+	−	+
Optimal pH	7.0–7.5	6.5–7.5	7.0
Optimum temperature (°C)	37–45	30–37	37–45
pufL and *pufM* genes	+	+	+
Phototrophic growth	+	+	−
Photosynthetic pigments	Bacteriochlorophyl II α	Bacteriochlorophyl II α	−
β-Caroten production	+	+	−
Utilization of:			
Dulicitol	−	+	−
D-Sorbitol	+	+	−
D-Glucose	+	+	−
D-Mannitol	+	+	−
Glycerol	−	+	−
DNA G+C contents (mol%)	66.0	65.3	65.9

Taxa: 1 YIM 73036^T (KY608089); 2 *R. blasticus* CGMCC 1.3365^T; 3 *Tabrizicola aquatic* KCTC 23724^T; +, positive; −, negative. All data for were from this study, except for genomic G+C contents which were taken from related literatures Tarhriz et al. (2013). All strains are Gram-stain-negative and having *pufL* and *pufM* genes. All strains can produce *β*-glucosidase, *β*-galactosidase and α-glucosidase. All strains, applying API 20NE kit, negative results are obtained for indole production, arginine, dihydrolase, urease, and hydrolysis of gelatin. Also negative for mannose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid. All strains are negative in motility, slime production, utilization of formate, tartrate, ethanol, thiosulfate. All strains are able to utilize glucose and pyruvate

of strain YIM 73036^T and the reference type strains were found to be below 95% (Supplementary Table S2), showed that the isolate YIM 73036^T belongs to a novel species. The phylogenetic trees generated using methods based on neighbour-joining, maximum-parsimony and maximum-likelihood algorithms showed that strain YIM 73036^T formed a distinct phylogenetic lineage within the genus *Rhodobacter* (Fig. 1 and supplementary Figs. S6 and S7 respectively).

The morphological features, chemotaxonomic properties and 16S rRNA gene sequence similarity profiles clearly indicated that strain YIM 73036^T is a member of the genus *Rhodobacter*. Strain YIM 73036^T exhibited several physiological and chemotaxonomic characteristics of the genus *Rhodobacter*: containing bacteriochlorophyll-*a* and PG, PE and PC which are the major polar lipids of species of the genus *Rhodobacter* (Raj et al. 2013). Characteristics that differentiate the strain (YIM 73036^T) from related type strains of the genus *Rhodobacter* include differences in growth conditions (temperature and pH ranges for growth), utilization of carbon and nitrogen sources, as well as the proportions of some fatty acids (Tables 1 and S1). The fatty acid profiles of strain YIM 73036^T and related reference type strains were similar, but there were differences in the proportions of some fatty acids. In particular, strain YIM 73036^T was characterized by having a considerable amount of $C_{18:1}\omega7c$11-methyl (19.6%) and $C_{18:0}$ (10.8%) which were minor components (2.0%) and (3.5%), respectively, in closely related reference strain *R. blasticus*

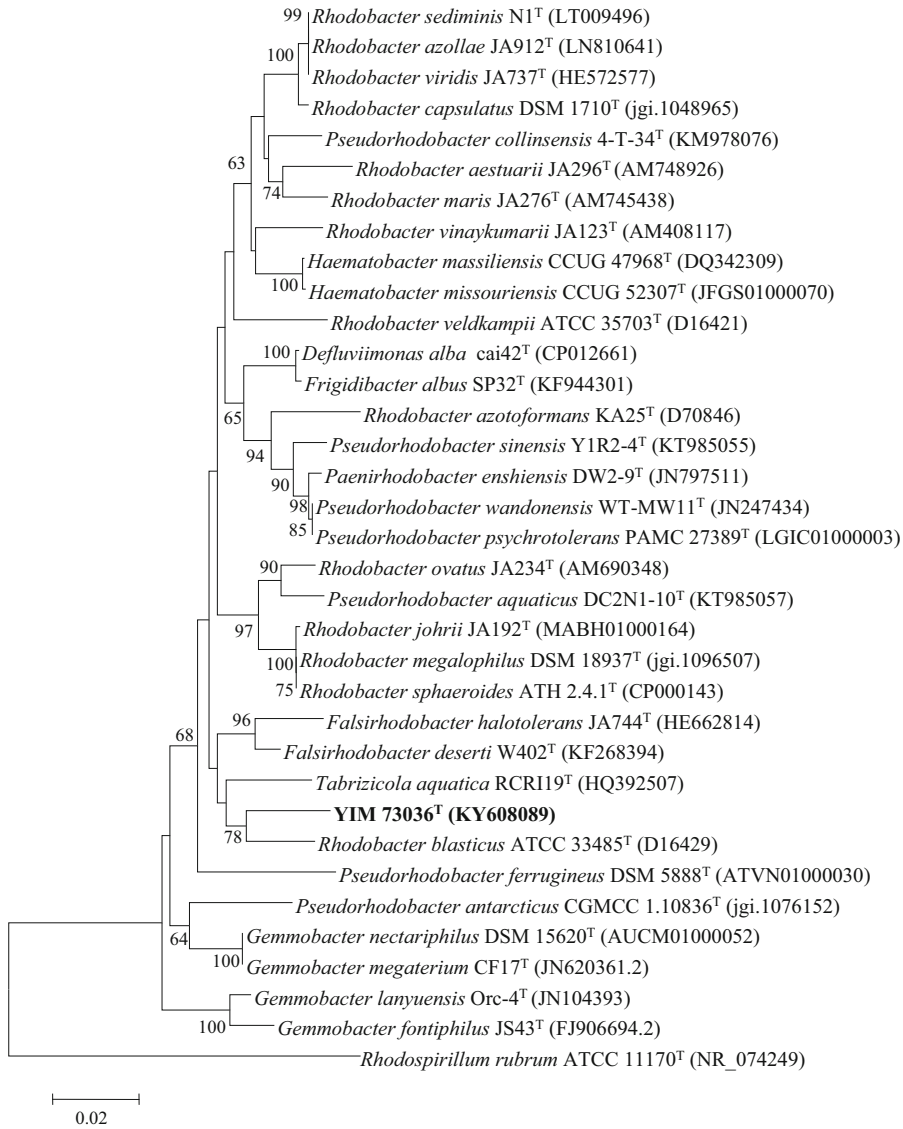

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain YIM 73036^T with related members of the family *Rhodobacteraceae*. Bootstrap percentages based on 1000 resamplings are listed at the nodes. Only bootstrap values above 50% are shown at branch points. Asterisks indicate branches that were also recovered in the maximum-parsimony and maximum likelihood trees. 16S rRNA gene sequence of *Rhodospirillum rubrum* ATCC 11170^T (NR_074249) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position

CGMCC 1.3365^T (Supplementary Table S1). The polar lipid profile of YIM 73036^T was similar to that of *R. blasticus* CGMCC 1.3365^T in that PG, DPG, PE, PC and unidentified phospholipid were the major polar lipids, but it could be distinguished from the reference strain *R. blasticus* CGMCC 1.3365^T by the nature and proportion of the differences between the other polar lipids (Fig. S3). In particular, two unidentified aminophospholipids were detected in strain YIM 73036^T, which were absent from the closely related reference type strain *R. blasticus* CGMCC 1.3365^T. Based on these properties, strain YIM 73036^T merit classification as a novel species of the genus *Rhodobacter*, for which the name *Rhodobacter thermarum* sp. nov. is proposed.

Description of *Rhodobacter thermarum* sp. nov.

Rhodobacter thermarum (ther.ma'rum. L. gen. pl. n. *thermarum* of hot springs).

Cells are Gram-stain negative and ovoid to rod-shaped (0.7–1.1 × 1.8–3.4 μm in size) and multiply by binary fission. The colonies are small with a convex surface. The bacterium produces glassy-colored colonies on marine agar medium with 0.5% NaCl at 30 °C. The colonies change to pink over time. Growth occurs at 20–55 °C, pH 6.0–8.0 and in the presence of up to 3.5% (w/v) NaCl. Positive for catalase and oxidase activities. Hydrolyzes Tween 20, but not casein, starch, or Tweens 40, 60 and 80. Bacteriochlorophyll-*a*, bacteriopheophytin and spheroidene and spheroidenone are the photosynthetic pigments. Internal cytoplasmic membranes are of the lamellar type. The predominant respiratory quinone is ubiquinone Q-10 and the G+C content of the genomic DNA of the type strain is 66.0 mol%. The major cellular fatty acids (> 10%) are $C_{18:1}\omega7c$,$C_{18:1}$ ω 7*c* 11-methyl and $C_{18:0}$. The polar lipids consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, unidentified phospholipids and aminophospholipids.

The type strain YIM 73036^T (= KCTC 52712^T = CCTCC AB 2016298^T) was isolated from a sediment sample collected from a hot spring in western Tibet, China. The GenBank accession number of the 16S rRNA gene sequence of strain YIM 73036^T is KY608089, and its draft genome sequence accession number is QMJY00000000. The taxon number of the strain in the digital protologue is TA00508.

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Author contributions IUK, NH, MX and WJL conducted this study. IUK, NH, MSH, VT and MX performed the experiments. XYZ and WJL supervised the experiments. IUK, NH, MML, and WDX wrote the manuscript. All of the authors assisted in writing the manuscript, discussed the results and commented on the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no direct or indirect conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals.

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