Rhodobacter ruber sp. nov., isolated from a freshwater pond

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Abstract

Strain CCP-1\textsuperscript{T}, isolated from a freshwater pond in Taiwan, is characterized using a polyphasic taxonomy approach. Cells of strain CCP-1\textsuperscript{T} are Gram-stain-negative, aerobic, non-motile, rod-shaped and form dark red colored colonies. Growth occurs at 20–40 °C, at pH 6.5-9 and with 0-0.5% NaCl. Strain CCP-1\textsuperscript{T} contains bacteriochlorophyll \(a\), and shows optimum growth under anaerobic condition by photoheterotrophy, but not by photoautotrophy. 16S rRNA gene sequence similarity indicates that strain CCP-1\textsuperscript{T} is closely related to species within the genus \textit{Rhodobacter} (93.9–96.2% sequence similarity), \textit{Haematobacter} (96.3%) and \textit{Xinfangfangia} (95.5–96.2%). Phylogenetic analyses based on 16S rRNA gene sequences and based on up-to-date bacterial core gene set (92 protein clusters) reveal that strain CCP-1\textsuperscript{T} is affiliated with species in the genus \textit{Rhodobacter}. The average nucleotide identity, average amino acid identity and digital DNA-DNA hybridization identity between strain CCP-1\textsuperscript{T} and \textit{Rhodobacter} species are 71.3–76.3%, 70.4–77.9% and 21.4–23.2%, respectively, supporting that strain CCP-1\textsuperscript{T} is a novel species of the genus \textit{Rhodobacter}. The DNA G + C content is 66.2%. The predominant fatty acid is \(\text{C}_{18:1}\omega7c\) and the major isoprenoid quinone is Q-10. The polar lipids have phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, two uncharacterized aminophospholipids and two uncharacterized phospholipids. On the basis of phenotypic and genotypic properties and phylogenetic inference, strain CCP-1\textsuperscript{T} should represent a novel species of the genus \textit{Rhodobacter}, for which the name \textit{Rhodobacter ruber} sp. nov. is proposed. The type strain is CCP-1\textsuperscript{T} (= BCRC 81189\textsuperscript{T} = LMG 31335\textsuperscript{T}).

Introduction

The genus \textit{Rhodobacter} (type species, \textit{Rhodobacter capsulatus}) proposed by Imhoff et al. (1984) and emended by Srinivas et al. (2007), Wang et al. (2014) and Suresh et al. (2019), belongs to the family \textit{Rhodobacteraceae} of the order \textit{Rhodobacterales} in the class \textit{Alphaproteobacteria} (Imhoff 2005; Pujalte et al. 2014). The genus \textit{Rhodobacter} comprises 16 species with validly published names so far stated on the List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de/genus/rhodobacter). Species of the genus \textit{Rhodobacter} had been isolated from various environmental sources including freshwater, eutrophic freshwater, stagnant water, polluted water, alkaline water, hot spring, stream mud, lagoon sediment, estuarine water and marine water (Arunasri et al. 2008; Eckersley and Dow 1980; Gandham et al. 2018; Imhoff et al. 1985; Khan et al. 2019; Raj et al. 2013; Ramana et al. 2008; Sheu et al. 2020; Srinivas et al. 2007; Subhash and Lee 2016; Suresh et al. 2017, 2020; Venkata Ramana et al. 2009; Xian et al. 2020). Members of the genus \textit{Rhodobacter} are characterized as Gram-stain-negative, motile or non-motile and ovoid to rod shaped. Chemotaxonomically, members of the genus are characterized by \(\text{C}_{18:1}\omega7c\) as the predominant fatty acid, ubiquinone 10 (Q-10) as the major respiratory quinone, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine as the main polar lipids, and the DNA G + C content between 62 to 73 mol% (Imhoff 2005; Pujalte et al. 2014; Suresh et al. 2019; Wang et al. 2014). The present study was carried out to clarify the taxonomic position of a putative novel species belonging to the genus \textit{Rhodobacter}, designated CCP-1\textsuperscript{T}, by a polyphasic taxonomic approach.
Materials And Methods

Bacterial strains and culture conditions

During the characterization of microorganisms present in the freshwater sample of a pond in a crocodile farm (GPS location: 22°33'45" N 120°32'07" E) in the Chaozhou Township of Pingtung County, Taiwan, a water sample (25 °C, pH 6.5, 0% NaCl) was collected on 4 April 2016. The water sample was spread on R2A agar (BD Difco) plates by the standard dilution plating method. After incubation of the plates at 25 °C for 3 days, a novel dark red-pigmented bacterium, designated CCP-1T, was selected and purified as single colonies and subjected for detailed taxonomy analyses. Sub-cultivation was performed on R2A agar at 25 °C for 48–72 h. The isolate was preserved in R2A broth with 20% (v/v) glycerol at -80 °C and also by lyophilization before storing at -80 °C.

The phylogenetic related strains, Rhodobacter tardus CYK-10T (= BCRC 81191T), Rhodobacter flagellatus SYSU G03088T (= KCTC 72354T), Rhodobacter blasticus NCIMB 11576T (= ATCC 33485T), Rhodobacter thermarum YIM 73036T (= KCTC 52712T) and Rhodobacter capsulatus ATCC 11166T (= KACC 15298T) were obtained from culture collections. The five type strains were used as reference strains and evaluated together under identical experimental conditions to those for strain CCP-1T.

Morphological, physiological, and biochemical characteristics

The morphology of bacterial cells was observed by phase-contrast microscopy (DM 2000; Leica) and transmission electron microscopy (H-7500; Hitachi) using cells grown on R2A agar for 3 days at 25 °C. Cellular motility was tested by the hanging drop method (Beveridge et al. 2007). The Gram Stain Set S kit (BD Difco) and the Ryu non-staining KOH method (Powers 1995) were used to perform the Gram reaction. Poly-β-hydroxybutyrate granule accumulation was examined under light microscopy after staining of the cells with Sudan black (Schlegel et al. 1970). Colony morphology was observed on R2A agar under a stereoscopic microscope (SMZ 800; Nikon).

The physiological characteristics of strain CCP-1T and the five reference strains were examined by growing bacteria at various pH values, temperatures and NaCl concentrations. The pH range for bacterial growth was estimated by measuring the optical densities (wavelength 600 nm) of R2A broth cultures. The pH of the medium was adjusted prior to sterilization to pH 4–9 (at intervals of 0.5 pH unit) using the following biological buffers (Breznak and Costilow 2007): citrate/Na₂HPO₄ (pH 4-5.5); phosphate (pH 6-7.5); and Tris (pH 8-9). The temperature range for growth was determined on R2A agar at 4, 10, 15, 20, 25, 30, 35, 37, 40, 45 and 50 °C. To investigate the tolerance to NaCl, R2A broth was prepared according to the formula of the BD Difco medium with NaCl concentration adjusted to 0, 0.5% and 1–5%, w/v (at intervals of 1%). Growth under anaerobic conditions was determined after incubating strain CCP-1T on R2A agar and on R2A agar supplemented with nitrate (0.1% KNO₃) in anaerobic jars by using AnaeroGen.
anaerobic system envelopes (Oxoid) at 25 °C for 15 days. Bacterial growth was studied on R2A, nutrient, Luria-Bertani and trypticase soy agars (all from Difco) under aerobic condition at 25 °C for 15 days. Photoheterotrophic growth under anaerobic conditions was determined after incubation in an Oxoid AnaeroGen system or in 30 ml tubes with a rubber septum under a stream of nitrogen gas in light using minimal medium containing yeast extract (0.3%, w/v), tryptone (0.3%, w/v) or sodium acetate (0.3%, w/v). Photoautotrophic growth was examined under the same conditions but using medium with thiosulfate (0.1%, w/v) or sodium bicarbonate (0.1%, w/v) as previously described by Pfennig and Trüper (1974). For photosynthetic pigment analysis, cell mass from 30 ml culture was extracted and the absorption spectrum of the extract was recorded as described by Biebl et al. (2005).

Activities of catalase, oxidase, DNase, urease, lipase (corn oil), and hydrolysis of starch, casein and lecithin were determined using the methods of Tindall et al. (2007). Chitin hydrolysis was assessed on chitinase-detection agar (Wen et al. 2002) and visualized by the formation of clear zones around the colonies. Hydrolysis of carboxymethyl cellulose (CM-cellulose) was tested as described by Bowman (2000) using R2A agar as the basal medium. Utilization of carbon sources was investigated in a basal medium containing (l−1): 0.4 g KH2PO4, 0.53 g Na2HPO4, 0.3 g NH4Cl, 0.3 g NaCl, 0.1 g MgCl2·6H2O, 0.11 g CaCl2 and 1 ml trace element solution, pH 7.0 (Chang et al. 2004). Positive control tubes were prepared with 2 g yeast extract l−1, while the basal medium was used as a negative control. Substrates were added at a concentration of 0.1% (w/v or v/v). Incubation was prolonged for 15 days at 25 °C under aerobic condition by means of duplicate experiments, and bacterial growth was examined every two days. Additional biochemical tests were performed using API ZYM and API 20NE kits (bioMérieux). Both commercial phenotypic tests were performed according to the manufacturers’ recommendations.

Sensitivity of strain CCP-1T to antibiotics was tested by the disc diffusion method after spreading cell suspensions (0.5 McFarland standard) on R2A agar plates at 25 °C for 3 days as described by Nokhal and Schlegel (1983). The discs (Oxoid) contained the following antibiotics: ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), penicillin G (10 U), rifampicin (5 µg), streptomycin (10 µg), sulfamethoxazole (23.75 µg) plus trimethoprim (1.25 µg) and tetracycline (30 µg).

**Determination of cellular fatty acids, polar lipids and isoprenoid quinones**

The fatty acid profiles of strain CCP-1T and the five phylogenetic related strains were analyzed on cells grown on R2A agar at 25 °C for 3 days. Fatty acid methyl esters were extracted and separated according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0), analyzed by GC (Hewlett-Packard 5890 Series II) and identified by using the RTSBA6.00 database of the microbial identification system (Sasser 1990).

The polar lipids were extracted and analyzed by two-dimensional TLC according to Embley and Wait (1994). Ethanolic molybdophosphoric acid (10%) was used for the detection of the total polar lipids,
ninhydrin for amino lipids, the α-naphthol reagent for glycolipids and the Zinzadze reagent for phospholipids. The isoprenoid quinones were extracted and purified according to the method of Collins (1994) and analyzed by HPLC with a Spherisorb ODS column using methanol/1-chlorobutane (100:10, v/v) as mobile phase (1.5 ml min⁻¹).

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA was isolated using a bacterial genomic DNA purification kit (DP02-150, GeneMark). The 16S rRNA gene was amplified using the universal primer set (27F and 1541R) (Weisburg et al. 1991; Anzai et al. 1997). PCR products were purified using a plus PCR clean up kit (DP04P, GeneMark), and then sequenced using four primers (27F, 520F, 800R and 1541R) (Weisburg et al. 1991; Anzai et al. 1997), the BigDye Terminator Cycle Sequencing kit (Applied Biosystems) with an ABI Prism 3730xl automated DNA analyzer (Applied Biosystems). The novel sequence was compared to those available from the EzBioCloud (Yoon et al. 2017).

Multiple sequence alignments were performed with clustal W (Larkin et al. 2007) and BioEdit software (Hall 1999). Phylogenetic trees were reconstructed by the neighbour-joining (NJ) (Saitou and Nei 1987), maximum-likelihood (ML) (Felsenstein 1981) and maximum-parsimony (MP) (Kluge and Farris 1969) methods using MEGA 7 software (Kumar et al. 2016). In each case bootstrap values were calculated based on 1000 resamplings.

Whole genome analysis, average nucleotide identity and average amino acid identity calculations, digital DNA-DNA hybridization scores, UBCG phylogenetic tree construction and genome comparative analysis

A whole genome sequence was prepared by the Genomics BioSci & Tech. Co., Ltd. (Taipei, Taiwan, ROC) using the Illumina NextSeq sequencer platform and using MultiQC v1.2 for evaluating read quality (Ewels et al. 2016). The whole genome was assembled using SPAdes (version 3.10.1) (Bankevich et al. 2012), and gene prediction and annotation by Prokka pipeline (Seemann 2014). The protein encoding genes were classified into functional categories based on eggNOG (evolutionary genealogy of genes: Nonsupervised Orthologous Groups)-Mapper using precomputed cluster and phylogenies from the eggNOG database as described by Huerta-Cepas et al. (2016, 2017).

The estimated genome-sequence-based digital DNA-DNA hybridization (dDDH) values were calculated as described by Meier-Kolthoff et al. (2013). Average nucleotide identity (ANI) calculations were performed by OrthoANI analysis (Lee et al. 2016). Average amino acid identity (AAI) calculations were performed (http://enve-omics.ce.gatech.edu/). An up-to-date bacterial core gene set (UBCG, concatenated alignment of 92 core genes) and pipeline was utilized for phylogenetic tree construction as described by Na et al. (2018).

For genome comparative analysis, the genome sequences of strain CCP-1T and genome sequences from the genus Rhodobacter including the type strain of type species of the genus, Rhodobacter capsulatus ATCC 11166T, and four type strains isolated from various environments, Rhodobacter tardus CYK-10T,
Rhodobacter flagellatus SYSU G03088T, Rhodobacter blasticus ATCC 33485T and Rhodobacter thermarum YIM 73036T, were annotated by the NCBI Prokaryotic Genome Annotation Pipeline and also submitted to Rapid Annotation of microbial genomes using Subsystem Technology (RAST) as described by Overbeek et al. (2014). Comparative gene content analyses were performed by EDGAR 2.0, an enhanced software platform as described by Blom et al. (2016).

Results And Discussion

The novel strain CCP-1T was isolated during the characterization of microorganisms present in the freshwater crocodile pond in Taiwan on 4 April 2016 (Supplementary Fig. S1). For strain CCP-1T, the 16S rRNA gene sequence (1431 nucleotides) has been deposited in GenBank under accession number LT852521. Sequence similarity calculations (over 1400 bp) revealed that the novel isolate was related to the genera Rhodobacter (93.9-96.2% sequence similarity), Haematobacter (96.3%) and Xinfangfangia (95.5-96.2%). The closest relatives of strain CCP-1T were Haematobacter massiliensis CCUG 47968T and Haematobacter missouriensis CCUG 52307T (96.3%), followed by Rhodobacter tardus CYK-10T and Xinfangfangiasoli ZQBW1T (96.2%). Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain CCP-1T formed a separate phylogenetic branch cluster with Rhodobacter tardus CYK-10T, Rhodobacter blasticus ATCC 33485T, Rhodobacter thermarum YIM 73036T and Rhodobacter flagellatus SYSU G03088T within the genus Rhodobacter in the neighbour-joining tree (Fig. 1). The overall topologies of the maximum-likelihood and maximum-parsimony trees were similar (Supplementary Figs. S2 and S3). However, although the novel isolate had the highest similarity to Haematobacter massiliensis CCUG 47968T and Haematobacter missouriensis CCUG 52307T, it is obvious from the phylogenetic tree that they belong to different genera. In addition, the novel isolate had a higher similarity to Xinfangfangiasoli ZQBW1T, and two validly published Xinfangfangia species were adjacent in the phylogenetic tree. But, based on the low 16S rRNA gene sequence similarity values and the absence of photosynthesis genes and photosynthesis pigments, Xinfangfangia has been published as a different genus from Rhodobacter (Hu et al. 2018). The absence of photosynthesis genes in genome of Xinfangfangia humi CIP 111625T was also confirmed by Rapid Annotation of microbial genomes using Subsystem Technology (Supplementary Fig. S4B). Furthermore, the presence of photosynthesis genes (Supplementary Fig. S4A), photoheterotrophy and bacteriochlorophyll a (described below) for this novel isolate, indicated that it might be assigned to a novel species of the Rhodobacter.

The whole genome sequence of strain CCP-1T was prepared and assembled and deposited in GenBank under accession number NZ_JAAATW000000000. The estimated genome size was approximately 3.96 Mb, the coverage depth was 263., the number of contigs was 23 and the N50 length was 809392 bp. The genomic G+C content of the DNA was 66.2%. The sequences of the 16S rRNA gene encoded in the genome of strain CCP-1T and that of PCR determined sequence are the same. It contained 3758 protein encoding genes, 3 rRNA genes and 48 tRNA genes. Based on the eggNOG database, the 3758 protein encoding genes were classified into 21 functional categories (Supplementary Table S1). Most of coding
sequences in strain CCP-1\textsuperscript{T} genome are classified as amino acid transport and metabolism (E, 9.4 %), followed by those identified as having roles in general function prediction only (R, 9.4 %), functional unknown (S, 8.6 %), carbohydrate transport and metabolism (G, 6.6 %) and transcription (K, 5.0 %).

In order to further explore the relationships of strain CCP-1\textsuperscript{T} and related species in the genus \textit{Rhodobacter}, an UBCG and pipeline was utilized for phylogenetic tree construction. The phylogenetic tree based on the coding sequences of 92 protein clusters showed that strain CCP-1\textsuperscript{T} formed a distinct phylogenetic lineage in the \textit{Rhodobacter} (Fig. 2), which supported that strain CCP-1\textsuperscript{T} might be assigned to a novel species of the \textit{Rhodobacter}.

Following the proposed minimal standards for the use of genome data for the taxonomy of prokaryotes (Chun et al. 2018), both dDDH and ANI between strain CCP-1\textsuperscript{T} and other related \textit{Rhodobacter} species with whole genome sequence publicly available were determined. Genomic comparison with ANI and dDDH calculations between strain CCP-1\textsuperscript{T} and other related \textit{Rhodobacter} species indicated that 71.3-76.3\% of ANI and 21.4-23.2\% of dDDH, respectively (Supplementary Table S2), were sufficient for the threshold of species-level differentiation, 95-96\% ANI (Richter and Rosselló-Móra 2009) and 70\% dDDH (Goris et al. 2007). In addition, AAI calculations were performed, which gave AAI values of 70.4-77.9\% when strain CCP-1\textsuperscript{T} compared to the related strains (Supplementary Table S2). The calculated AAI values were clearly lower than the AAI threshold (about 90\%) for species demarcation and higher than the threshold (about 60\%) for genus boundary proposed by Rodriguez-R and Konstantinidis (2014). These data warranted the status of strain CCP-1\textsuperscript{T} as a separate species in the genus \textit{Rhodobacter}.

An overview of genome characteristics of strain CCP-1\textsuperscript{T} and five related \textit{Rhodobacter} species is given in Supplementary Table S3. The results of genome comparative analysis showed that most genes were shared in all strains while some genes were not (Table 1). These six strains had genes putatively encoding proteins regarding to photosynthesis including photosystem II-type photosynthetic reaction center (e.g. photosynthetic reaction center L, M, H subunits and putative photosynthetic complex assembly protein), bacterial light-harvesting proteins (e.g. light-harvesting LHI, alpha and beta subunits) and chlorophyll biosynthesis [e.g. protoporphyrin IX Mg-chelatase subunit H (EC 6.6.1.1), Mg-protoporphyrin IX monomethyl ester oxidative cyclase (anaerobic) (EC 1.14.13.81), Mg-protoporphyrin O-methyltransferase (EC 2.1.1.11), chlorophyll \textit{a} synthase ChlG (EC 2.5.1.62), light-independent protochlorophyllide reductase iron-sulfur ATP-binding protein ChlL (EC 1.18.-.-), 2-desacyl-2-hydroxyethyl bacteriochlorophyllide A dehydrogenase BchC, 2-vinyl bacteriochlorophyllide hydratase BchF (EC 4.2.1.-), bacteriochlorophyll synthase BchJ, geranylgeranyl hydrogenase BchP/geranylgeranyl reductase (EC 1.3.1.83) and chlorophyllide reductase subunit BchZ (EC 1.18.-.-)].

However, the most obvious features are that strain CCP-1\textsuperscript{T} possessed genes putatively encoding proteins related to lipopolysaccharide related cluster involved in cell wall and capsule e.g. tetraacyldisaccharide 4'-kinase (EC 2.7.1.130), 3-deoxy-D-manno-octulosonic-acid transferase (EC 2.-.-.-), TldE protein (part of TldE/TldD proteolytic complex), inositol monophosphatase family protein, enoyl-CoA hydratase (EC
4.2.1.17), dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases) and murein endopeptidase; related to Phd-Doc, YdcE-YdcD toxin-antitoxin (programmed cell death) systems involved in regulation and cell signaling e.g. prevent host death protein (Phd antitoxin), programmed cell death antitoxin MazE like and programmed cell death toxin MazF like; related to ectoine biosynthesis and regulation involved in stress response e.g. L-ectoine synthase (EC 4.2.1.-); related to inorganic sulfur assimilation involved in sulfur metabolism e.g. sulfate and thiosulfate import ATP-binding protein CysA (EC 3.6.3.25), sulfate adenylyltransferase, dissimilatory-type (EC 2.7.7.4)/adenylylsulfate kinase (EC 2.7.1.25), phosphoadenylnyl-sulfate reductase [thioredoxin] (EC 1.8.4.8)/adenylylnyl-sulfate reductase [thioredoxin] (EC 1.8.4.10), 3’(2’),5’-bisphosphate nucleotidase (EC 3.1.3.7), oxidoreductase probably involved in sulfite reduction, sulfite reductase [NADPH] hemoprotein beta-component (EC 1.8.1.2) and ferredoxin; related to urea carboxylase and allophanate hydrolase cluster involved in metabolism of amino acid and derivatives e.g. allophanate hydrolase (EC 3.5.1.54), allophanate hydrolase 2 subunit 1 (EC 3.5.1.54), allophanate hydrolase 2 subunit 2 (EC 3.5.1.54), lactam utilization protein LamB and biotin carboxyl carrier protein; related to tricarballylate utilization involved in organic acid metabolism e.g. TcuA (flavoprotein used to oxidize tricarballylate to cis-aconitate) and TcuB (works with TcuA to oxidize tricarballylate to cis-aconitate); related to erythritol utilization involved in sugar alcohol metabolism e.g. erythritol kinase EryA (EC 2.7.1.27), erythritol phosphate dehydrogenase EryB, possible D-erythulose 4-phosphate dehydrogenase EryC (EC 1.1.1.1.-), erythritol transcriptional regulator EryD and predicted erythritol ABC transporter 1 (ATP-binding component 1); and related to L-fucose utilization involved in monosaccharide metabolism e.g. L-fucose mutarotase, L-fuco-beta-pyranose dehydrogenase (EC 1.1.1.122), L-fuconolactone hydrolase, L-fuconate dehydratase (EC 4.2.1.68) and 2-keto-3-deoxy-L-fuconate dehydrogenase. But the other five strains, *Rhodobacter tardus* CYK-10\(^T\), *Rhodobacter flagellatus* SYSU G03088\(^T\), *Rhodobacter blasticus* ATCC 33485\(^T\), *Rhodobacter thermarum* YIM 73036\(^T\) and *Rhodobacter capsulatus* ATCC 11166\(^T\), did not have these related genes. Instead, these five strains had genes putatively encoding proteins with respect to benzoate degradation involved in aromatic compound metabolism such as benzoate transport protein. But the novel strain CCP-1\(^T\) did not have these related genes.

Additionally, concerning iron acquisition and metabolism, sulfur, potassium, nitrogen, nucleoside and nucleotide, amino acid and derivatives, protein, DNA, lipid, aromatic compound and carbohydrate metabolism, all strains showed highly diverse distribution pattern among them. Furthermore, the percentage of genes of strain CCP-1\(^T\) shared with the type species of the genus was estimated. Strain CCP-1\(^T\) showed 1935 genes (51.5%) shared with *Rhodobacter capsulatus* ATCC 11166\(^T\) while the rest of 1823 genes (48.5%) were different (Supplementary Fig. S5A). When strain CCP-1\(^T\) and another four strains were analyzed together, it can be found that there are 1906 genes in common, which is about 50.7% of the total number of genes of strain CCP-1\(^T\). For strain CCP-1\(^T\), there are 879 genes present as strain CCP-1\(^T\) specific genes, accounting for about 23.4% (Supplementary Fig. S5B). Because strain CCP-1\(^T\), *Rhodobacter tardus* CYK-10\(^T\), *Rhodobacter flagellatus* SYSU G03088\(^T\), *Rhodobacter blasticus* ATCC 33485\(^T\), *Rhodobacter thermarum* YIM 73036\(^T\) and *Rhodobacter capsulatus* ATCC 11166\(^T\) are isolated
from freshwater crocodile pond, freshwater lotus pond, hot spring, eutrophic freshwater, sediment of a hot spring and stagnant water, respectively. In order to adapt to complex microbial ecosystem, they may develop different cellular regulation, toxic resistance, stress response and metabolic activities.

Cells of strain CCP-1^T are non-motile and rod-shaped (Supplementary Fig. S6). The presence of bacteriochlorophyll a in strain CCP-1^T is typically indicated by maximum of 765 nm in the absorption spectrum (Supplementary Fig. S7). Strain CCP-1^T was resistant to sulfamethoxazole plus trimethoprim, and sensitive to chloramphenicol, kanamycin, nalidixic acid, novobiocin, rifampicin, streptomycin, tetracycline, gentamicin, ampicillin and penicillin G. Detailed results from the phenotypic and biochemical analyses of strain CCP-1^T are provided in the species description, Table 2 and Supplementary Table S4.

The major cellular fatty acid (> 70%) of strain CCP-1^T was C_{18:1} w7c. The cellular fatty acids of strain CCP-1^T were composed of C_{18:1} w7c (73.5%), C_{18:1} w7c 11-methyl (6.9%), C_{18:0} 3-OH (6.3%), C_{18:0} (4.3%), C_{16:0} (2.2%), C_{10:0} 3-OH (2.1%) and summed feature 7 (C_{19:1} w6c and/or C_{19:0} cyclo w10c; 1.7%). The complete fatty acid composition was shown in Table 3. The fatty acid compositions of the six strains were similar, with only limited differences in the proportions of some fatty acids. The major cellular fatty acid of these strains was C_{18:1} w7c, which is consistent with those reported for the genus Rhodobacter (Khan et al. 2019; Pujalte et al. 2014; Suresh et al. 2020; Wang et al. 2014; Xian et al. 2020). Strain CCP-1^T exhibited a complex polar lipid profile consisting of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), two uncharacterized aminophospholipids (APL1 and APL2) and two uncharacterized phospholipids (PL1 and PL2) (Supplementary Fig. S8). Strain CCP-1^T contained PE, PG and PC as predominant polar lipids; the possession of PE, PG and PC as the common major polar lipids is consistent with previous description of species of Rhodobacter (Pujalte et al. 2014; Raj et al. 2013; Subhash and Lee 2016; Suresh et al. 2017; Wang et al. 2014). Strain CCP-1^T had Q-10 as the major respiratory quinone (Supplementary Fig. S9); in line with members of the genus Rhodobacter (Imhoff et al. 1984; Pujalte et al. 2014).

Phenotypic examination revealed many common traits between the novel strain and the five reference strains. However, strain CCP-1^T could be clearly differentiated from these five closest relatives by different properties listed in Table 2. Based on the phylogenetic, phenotypic, chemotaxonomic and biochemical data, it can be concluded that strain CCP-1^T represents a novel member of the genus Rhodobacter, for which the name Rhodobacter ruber sp. nov. is proposed.

**Description of Rhodobacter ruber sp. nov.**

*Rhodobacter ruber* (ru'ber. L. masc. adj. ruber red, referring to the red color of colonies)

Cells are Gram-stain-negative, aerobic, non-motile and rod-shaped. Cells grow well on R2A agar, but not on nutrient agar, trypticase soy agar and LB agar. Cells are approximately 0.5-0.7 mm in width and 1.2-1.8 mm in length after 3 days of incubation on R2A agar at 25 °C. Colonies are dark red colure, convex, round
and smooth with entire edges. Growth occurs at 20-40 °C (optimum, 25 °C), at pH 6.5-9 (optimum, pH 8) and with 0-0.5% NaCl (optimum, 0%). Growth occurs under anaerobic conditions by photoheterotrophy, but not by photoautotrophy. Bacteriochlorophyll \(a\) is present. Poly-b-hydroxybutyrate accumulation is observed. Positive for oxidase and catalase activities. Negative for hydrolysis of starch, casein, chitin, CM-cellulose, DNA, corn oil and lecithin. In the API 20NE tests, positive reactions for nitrate reduction, aesculin hydrolysis and b-galactosidase (PNPG) activity. In the API ZYM kit alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, b-galactosidase (ONPG), a-glucosidase and b-glucosidase activities are present. Growth under aerobic condition is positive on: D-glucose, D-fructose, D-mannose, D-cellobiose, dextrin, D-trehalose, L-rhamnose, L-arabinose, sucrose, D-raffinose, \(N\)-acetyl-D-glucosamine, D-mannitol, adonitol, D-sorbitol, Tween 20, Tween 40, Tween 60, L-proline, L-histidine and L-aspartic acid. The predominant quinone is Q-10. Major cellular fatty acid is \(C_{18:1}w7c\). Phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, two uncharacterized aminophospholipids and two uncharacterized phospholipids are present in the polar lipid profile. The DNA G+C content is 66.2%.

The type strain is CCP-1\(^T\) (=BCRC 81189\(^T\) =LMG 31335\(^T\)) isolated from a freshwater crocodile pond in the Chaozhou Township of Pingtung County, Taiwan. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the whole genome of \textit{Rhodobacter ruber} CCP-1\(^T\) are LT852521 and NZ_JAAATW000000000, respectively.

### Abbreviations

\textit{eggNOG}, evolutionary genealogy of genes: Nonsupervised Orthologous Groups; UBCG, up-to-date bacterial core gene set; ANI, average nucleotide identity; AAI, average amino acid identity; dDDH, digital DNA-DNA hybridization; RAST, Rapid Annotation of microbial genomes using Subsystem Technology; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; APL, uncharacterized aminophospholipid; PL, uncharacterized phospholipid; Q-10, ubiquinone-10.

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The authors declare that there are no conflicts of interest.

### References
Anzai Y, Kudo Y, Oyaizu H (1997) The phylogeny of genera *Chryseomonas*, *Flavimonas*, and *Pseudomonas* supports synonymy of these three genera. Int J Syst Bacteriol 47: 249-251

Arunasri K, Venkata Ramana V, Sproer C, Sasikala Ch, Ramana ChV (2008) *Rhodobacter megalophilus* sp. nov., a phototroph from the Indian Himalayas possessing a wide temperature range for growth. Int J Syst Evol Microbiol 58:1792-1796

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prielbelski AD, Pyshkin AV, Sirotkin AV, Vyakhni N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455-477

Beveridge TJ, Lawrence JR, Murray RGE (2007) Sampling and staining for light microscopy. In: Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM, Snyder LR et al. (editors). Methods for General and Molecular Bacteriology, 3rd ed. Washington, DC: American Society for Microbiology, pp. 19-33

Biebl H, Allgaiier 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

Blom J, Kreis J, Spänig S, Juhre T, Bertelli C, Ernst C, Goesmann A (2016) EDGAR 2.0: an enhanced software platform for comparative gene content analyses. Nucleic Acids Res 44:W22-W28

Bowman JP (2000) Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. Int J Syst Evol Microbiol 50:1861-1868

Breznak JA, Costilow RN (2007) Physicochemical factors in growth. In: Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM, Snyder LR et al. (editors). Methods for General and Molecular Bacteriology, 3rd edn. Washington, DC: American Society for Microbiology, pp 309-329

Chang SC, Wang JT, Vandamme P, Hwang JH, Chang PS, Chen WM (2004) *Chitinimonas taiwanensis* gen. nov., sp. nov., a novel chitinolytic bacterium isolated from a freshwater pond for shrimp culture. Syst Appl Microbiol 27:43-49

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, Meyer SD, Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461-466

Collins MD (1994) Isoprenoid quinones. In: Goodfellow M, O'Donnell AG (editors). Chemical Methods in Prokaryotic Systematics. Chichester: Wiley, pp 265-309

Eckersley K, Dow CS (1980) *Rhodopseudomonas blastica* sp. nov.: a member of the *Rhodospirillaceae*. J Gen Microbiol 119:465-473
Embley TM, Wait R (1994) Structural lipids of eubacteria. In: Goodfellow M, O’Donnell AG (editors). Chemical Methods in Prokaryotic Systematics. Chichester: Wiley, pp 121-161

Ewels P, Magnusson M, Lundin S, Käller M (2016) MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32:3047-3048

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368-376

Gandham S, Lodha T, Chintalapati S, Chintalapati VR (2018) *Rhodobacteralkalitolerans* sp. nov., isolated from an alkaline brown pond. Arch Microbiol 200:1487-1492

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81-91

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

Hu Q, Zhang L, Hang P, Zhou X-Y, Jia WB, Li SP, Jiang JD (2018) *Xinfangfangia soli* gen. nov., sp. nov., isolated from a diuron-polluted soil. Int J Syst Evol Microbiol 68:2622-2626

Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, von Mering C, Bork P (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol Biol Evol 34:2115-2122

Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattei T, Mende DR, Sunagawa S, Kuhn M, Jensen LJ, von Mering C, Bork P (2016) eggNOG4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic Acids Res 44:D286-D293

Imhoff JF, Trüper HG, Pfennig N (1984) Rearrangement of the species and genera of the phototrophic "purple nonsulfur bacteria". Int J Syst Bacteriol 34:340-343

Imhoff JF, Trüper HG, Pfennig N (1985) *Rhodobacter veldkampii*, a new species of phototrophic purple nonsulfur bacteria. Int J Syst Bacteriol 35:115-116

Imhoff JF (2005) Genus *Rhodobacter*. In: Brenner DJ, Krieg NR, Staley JT and Garrity GM (editors). Bergey's Manual of Systematic Bacteriology, 2nd ed. New York: Springer, pp. 161-167

Khan IU, Habib N, Xiao M, Li MM, Xian WD, Hejazi MS, Tarhriz V, Zhi XY, Li WJ (2019) *Rhodobacter thermarum* sp. nov., a novel phototrophic bacterium isolated from sediment of a hot spring. Antonie Van Leeuwenhoek 112:867-875
Kluge AG, Farris FS (1969) Quantitative phyletics and the evolution of anurans. Syst Zool 18:1-32

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870-1874

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, other authors (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947-2948

Lee I, Ouk Kim Y, Park SC, Chun J (2016) OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100-1103

Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60

Na SI, Kim YO, Yoon SH, Ha SM, Baek I, Chun J (2018) UBCG: Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol 56:280-285

Nokhal TH, Schlegel HG (1983) Taxonomic study of Paracoccus denitrificans. Int J Syst Bacteriol 33:26-37

Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards R, Gerdes S, Parrello B, Shukla M, Vonstein V, Wottam AR, Xia F, Stevens R (2014) The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acid Res 42:D206-D214

Pfennig N, Trüper HG (1974) The phototrophic bacteria. In: Buchanan RE and Gibbons NE (editors). Bergey’s Manual of Systematic Bacteriology, 8th ed. Baltimore: Williams & Wilkins, pp. 24-75

**Powers EM (1995)** Efficacy of the Ryu nonstaining KOH technique for rapidly determining Gram reactions of food-borne and waterborne bacteria and yeasts. Appl Environ Microbiol 61:3756-3758

Pujalte MJ, Lucena T, Ruuira MA, Arahal DR, Macián MC (2014) The family Rhodobacteraceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F et al. (editors). The Prokaryotes-Alphaproteobacteria and Betaproteobacteria, 4th ed. Berlin: Springer, pp. 439-512

Raj PS, Ramaprasad EV, Vaseef S, Sasikala C, Ramana C (2013) Rhodobacter viridis sp. nov., a phototrophic bacterium isolated from mud of a stream. Int J Syst Evol Microbiol 63:181-186

Ramana VV, Sasikala Ch, Ramana ChV (2008) Rhodobacter maris sp. nov., a phototrophic alphaproteobacterium isolated from a marine habitat of India. J Syst Evol Microbiol 58:1719-1722

Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126-19131

Rodriguez-R LM, Konstantinidis KT (2014) Bypassing cultivation to identify bacterial species. Microbe Magazine 9:111-118
Saitou N, Nei M (1987) The neighbor-joining method: a new method for constructing phylogenetic trees. Mol Biol Evol 4:406-425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. Newark, DE: MIDI Inc

Schlegel HG, Lafferty R, Krauss I (1970) The isolation of mutants not accumulating poly-b-hydroxybutyric acid. Arch Mikrobiol 71:283-294

Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068-2069

Sheu C, Li ZH, Sheu SY, Yang CC, Chen WM (2020) Tabrizicola oligotrophica sp. nov. and Rhodobacter tardus sp. nov., two new species of bacteria belonging to the family Rhodobacteraceae. Int J Syst Evol Microbiol 70:6266-6283

Srinivas TN, Kumar PA, Sasikala C, Ramana C, Imhoff JF (2007) Rhodobacter vinaykumarii sp. nov., a marine phototrophic alphaproteobacterium from tidal waters, and emended description of the genus Rhodobacter. Int J Syst Evol Microbiol 57:1984-1987

Subhash Y, Lee SS (2016) Rhodobacter sediminis sp. nov., isolated from lagoon sediments. Int J Syst Evol Microbiol 66:2965-2970

Suresh G, Dhanesh Kumar, Krishnaiah A, Sasikala Ch, Ramana ChV (2020) Rhodobacter sediminicola sp. nov., isolated from a fresh water pond. Int J Syst Evol Microbiol 70:1294-1299

Suresh G, Lodha TD, Indu B, Sasikala Ch, Ramana ChV (2019) Taxogenomics resolves conflict in the genus Rhodobacter: a two and half decades pending thought to reclassify the genus Rhodobacter. Front Microbiol 10:2480

Suresh G, Sailaja B, Ashif A, Bharti PD, Sasikala Ch, Ramana ChV (2017) Description of Rhodobacter azollae sp. nov. and Rhodobacterlacus sp. nov. Int J Syst Evol Microbiol 67:3289-3295

Tindall BJ, Sikorski J, Smibert RA, Krieg NR (2007) Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM, Snyder LR et al. (editors). Methods for General and Molecular Bacteriology, 3rd. Washington, DC: American Society for Microbiology, pp 330-393

Venkata Ramana V, Anil Kumar P, Srinivas TN, Sasikala Ch, Ramana ChV (2009) Rhodobacter aestuarii sp. nov., a phototrophic alphaproteobacterium isolated from an estuarine environment. Int J Syst Evol Microbiol 59:1133-1136

Wang D, Liu H, Zheng S, Wang G (2014) Paenirhodobacter enshiensis gen. nov., sp. nov., a non-photosynthetic bacterium isolated from soil, and emended descriptions of the genera Rhodobacter and Haemotobacter. Int J Syst Evol Microbiol 64:551-558
Weisburg WG, Burns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697-703

Wen CM, Tseng CS, Cheng CY, Li YK (2002) Purification, characterization and cloning of a chitinase from Bacillus sp. NCTU2. Biotechnol Appl Biochem 35:213-219

Xian WD, Liu ZT, Li MM, Liu L, Ming YZ, Xiao M, Salam N, Li MJ (2020) Rhodobacter flagellatus sp. nov., a thermophilic bacterium isolated from a hot spring. Int J Syst Evol Microbiol 70:1541-1546

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613-1617

Tables

Table 1. Comparison of the presence and absence of selected genes among Rhodobacter ruber CCP-1\textsuperscript{T} and five type strains of the genus Rhodobacter.
| Genes putatively encoding                                      | 1 | 2 | 3 | 4 | 5 | 6 |
|--------------------------------------------------------------|---|---|---|---|---|---|
| **Photosynthesis**                                           |   |   |   |   |   |   |
| Photosystem II-type photosynthetic reaction center :         |   |   |   |   |   |   |
| Photosynthetic reaction center L, M, H subunits              | + | + | + | + | + | + |
| Putative photosynthetic complex assembly protein              | + | + | + | + | + | + |
| Bacterial light-harvesting proteins :                        |   |   |   |   |   |   |
| Light-harvesting LHI, alpha subunit and beta subunit         | + | + | + | + | + | + |
| Chlorophyll biosynthesis                                     | + | + | + | + | + | + |
| **Cofactors and vitamins**                                   |   |   |   |   |   |   |
| Ubiquinone biosynthesis                                      | + | + | + | + | - | + |
| Coenzyme B12 biosynthesis                                    | + | - | - | + | - | + |
| Riboflavin to FAD                                             | + | + | + | + | - | + |
| Molybdenum cofactor biosynthesis                             | + | - | + | - | + | - |
| Pterin carbinolamine dehydratase                             | + | + | + | + | + | - |
| Flavodoxin                                                   |   | - | + | + | + | + |
| **Cell wall and capsule**                                    |   |   |   |   |   |   |
| Lipopolysaccharide related cluster                           | + | - | - | - | - | - |
| Lipopolysaccharide assembly                                  | + | - | + | + | + | - |
| Sialic acid metabolism                                       | + | - | + | + | + | + |
| CMP-\(N\)-acetylneuraminate biosynthesis                     |   | - | + | + | + | + |
| Legionaminic acid biosynthesis                               |   | - | + | + | + | + |
| **Virulence, disease and defense**                           |   |   |   |   |   |   |
| Tolerance to colicin E2                                       | + | - | - | + | - | - |
| Mercuric reductase, mercury resistance operon                 | + | + | + | + | - | - |
| Multidrug resistance efflux pumps                            | - | - | + | + | + | + |
| Arsenic resistance                                           | - | - | - | + | + | + |
| **Transposable elements**                                    |   |   |   |   |   |   |
| TnilB NTP-binding protein, TniA putative transposase, \(Mg^{2+}\) chelatase family protein/ComM-related protein, plasmid replication protein RepA, segregation and condensation protein B | + | + | - | - | - | - |
## Membrane transport

| System                                         | + | + | - | - | - | - |
|------------------------------------------------|---|---|---|---|---|---|
| AttEFGH ABC transport system                   |   |   |   |   |   |   |
| Tricarboxylate transport system                 |   |   | + | + | - | - |
| ABC transporter for α-glucosides                |   | - | - | + | - | + |
| ABC transporter alkylphosphonate                |   | - | + | + | + | + |
| Multi-subunit cation transporter                |   | - | + | + | + | + |
| TRAP transporter                                |   | - | + | + | + | + |
| Transport nickel and cobalt                     |   | - | + | + | + | + |
| Protein and nucleoprotein secretion system, type IV |   | - | + | - | - | - |
| Protein secretion system, type VI               |   | - | + | - | - | - |

## Regulation and cell signaling

| System                                                                 | + | + | - | - | - | - |
|------------------------------------------------------------------------|---|---|---|---|---|---|
| Phd-Doc, YdcE-YdcD toxin-antitoxin (programmed cell death) systems     |   |   |   |   |   |   |
| Toxin-antitoxin replicon stabilization system                           |   |   | + | + | + | - |
| Murein hydrolase regulation and cell death                             |   | + | - | - | - | + |
| Global two-component regulator PrrBA in *Proteobacteria*              |   | - | + | - | - | + |

## Stress response

| System                                                                 | + | + | - | - | - | - |
|------------------------------------------------------------------------|---|---|---|---|---|---|
| Ectoine biosynthesis and regulation                                     |   |   |   |   |   |   |
| Synthesis of osmoregulated periplasmic glucans                          |   |   | + | - | + | + |
| NADPH:quinone oxidoreductase 2                                           |   | + | - | + | - | + |
| Cluster containing glutathione synthetase                               |   | + | - | - | - | + |
| Osmoregulation                                                          |   | - | + | + | - | - |
| Redox-dependent regulation of nucleus processes                         |   | - | - | - | - | + |
| Rubrerythrin                                                            |   | - | + | - | - | + |
| Flavohaemoglobin                                                        |   | - | + | - | - | - |
| Bacterial hemoglobin                                                    |   | - | + | + | - | - |

## Respiration

| System                                                                 | + | + | - | - | - | - |
|------------------------------------------------------------------------|---|---|---|---|---|---|
| Terminal cytochrome oxidase, terminal cytochrome d ubiquinol oxidase   |   |   |   |   |   |   |
| Carbon monoxide oxidation                                              |   |   | + | + | + | + |
| F0F1-type ATP synthase                                                 |   |   | - | + | - | - |
| Terminal cytochrome O ubiquinol oxidase | - | + | - | - | - |
| NiFe hydrogenase maturation | - | + | - | + | - |
| Na\(^+\) translocating NADH-quinone oxidoreductase | - | - | - | - | + |
| Na\(^+\) translocating decarboxylase | - | - | - | - | + |

**Sulfur metabolism**

| Inorganic sulfur assimilation | + | - | - | - | - |
| Galactosylceramide and sulfatide metabolism | + | + | + | - | + |
| Organic sulfur assimilation: alkanesulfonate utilization | + | - | - | + | - |
| Organic sulfur assimilation: taurine utilization | - | - | - | + | + |
| Sulfur oxidation | - | - | + | - | + |

**Potassium metabolism**

| Hyperosmotic potassium uptake | + | - | + | + | + |
| Glutathion-regulated potassium-efflux system | - | - | - | - | + |

**Iron acquisition and metabolism**

| Heme, hemin uptake and utilization system | + | + | - | + | - |
| Iron B12 siderophore hemin, ABC transporter | + | + | + | - | + |
| Ferrous iron transporter EfeUOB, low-pH-induced | - | - | - | - | + |
| Siderophore enterobactin | - | - | - | - | + |

**Nitrogen metabolism**

| Nitrate and nitrite ammonification | + | - | + | - | + |
| Nitrogen fixation | - | - | - | + | - |
| Nitrilase | - | + | + | - | - |
| Denitrifying reductase gene clusters | + | + | + | + | - |

**Metabolism of nucleoside and nucleotide**

| Xanthine metabolism | + | - | + | - | + |
| Xanthine dehydrogenase subunits | - | + | + | - | + |

**Metabolism of amino acid and derivatives**

| Urea carboxylase and allophanate hydrolase cluster | + | - | - | - | - |
| Methionine degradation | - | + | + | + | - |
| Metabolism                                      | - | - | - | - | + |
|------------------------------------------------|---|---|---|---|---|
| **Valine, leucine degradation, HMG-CoA synthesis and HMG-CoA metabolism** |   |   |   |   |   |
| **Indole-pyruvate oxidoreductase complex**     | - | + | - | - | + |

**Protein metabolism**

|                                | + | + | + | - | + |
|--------------------------------|---|---|---|---|---|
| **Universal GTPase**           |   |   |   |   |   |
| **Translational elongation factors bacterial** | + | + | + | - | + |
| **N-linked glycosylation in Bacteria** | + | + | - | + | - |

**DNA metabolism**

|                                  | + | - | + | + | + | - |
|----------------------------------|---|---|---|---|---|---|
| **DNA ligases**                  |   |   |   |   |   |   |
| **CRISPR-associated protein Cas1, CRISPR-associated helicase Cas3, CRISPR-associated protein (Cse1 family, Cse3 family, Cse4 family and Cas5e family)** | - | - | - | + | - | + |

**Type I restriction-modification**

|                                   | - | + | + | + | - | - |

**Lipid metabolism**

|                               | + | - | - | + | - | - |
|------------------------------|---|---|---|---|---|---|
| **Cardiolipin synthesis**    |   |   |   |   |   |   |

**Metabolism of aromatic compounds**

|                                    | + | + | - | - | - | - |
|------------------------------------|---|---|---|---|---|---|
| **Chloroaromatic degradation pathway** |   |   |   |   |   |   |
| **N-heterocyclic aromatic compound degradation** | + | + | + | - | - | - |
| **Aromatic amin catabolism**       | + | + | - | - | - | - |
| **Benzoate degradation**           | - | + | + | + | + | + |
| **Homogentisate pathway of aromatic compound degradation** | - | - | + | - | + | - |
| **Central meta-cleavage pathway of aromatic compound degradation** | - | - | + | - | + | - |

**Carbohydrate metabolism**

|                                        | + | + | - | + | - | - |
|----------------------------------------|---|---|---|---|---|---|
| **Di- and oligosaccharide: sucrose and lactose utilization** |   |   |   |   |   |   |
| **Di- and oligosaccharide: melibose, maltose, maltodextrin and galactose utilization** | - | + | + | - | + | - |
| **Organic acid: tricarballyte utilization** | + | - | - | - | - | - |
| **Organic acid: 2-methylcitrate to 2-methylaconitate metabolism cluster** | - | - | + | + | + | - |
| **Organic acid: propionyl-CoA to succinyl-CoA module** | + | - | + | + | + | + |
| **Organic acid: malonate decarboxylase** | - | - | - | - | - | + |
| **Fermentation of lactate**            | - | - | - | + | - | + |
| Metabolic Pathway                                                                 | Presence/Activity |
|---------------------------------------------------------------------------------|-------------------|
| Fermentation of mixed acid                                                      | - - - - +         |
| Fermentation: acetoin and butanediol metabolism                                 | + + - - -         |
| CO₂ fixation: CO₂ uptake and carboxysome                                         | - - + + - +       |
| CO₂ fixation: photorespiration (oxidative C2 cycle) and Calvin-Benson cycle      | + - + + - +       |
| Sugar alcohol: erythritol utilization                                           | + - - - - -       |
| Sugar alcohol: mannitol utilization and inositol catabolism                     | + + + + + -       |
| Polysaccharide: alpha-amylase locus                                             | + + + - + -       |
| Monosaccharide: L-fucose utilization                                            | + - - - - -       |
| Monosaccharide: D-galactonate catabolism, D-gluconate and ketogluconate metabolism | + + + + + -       |

Strains: 1, CCP-1<sup>T</sup>; 2, *Rhodobacter tardus* CYK-10<sup>T</sup>; 3, *Rhodobacter flagellatus* SYSU G03088<sup>T</sup>; 4, *Rhodobacter blasticus* ATCC 33485<sup>T</sup>; 5, *Rhodobacter thermarum* YIM 73036<sup>T</sup>; 6, *Rhodobacter capsulatus* ATCC 11166<sup>T</sup>. +, Present; -, absent.

**Table 2.** Differential characteristics of *Rhodobacter ruber* CCP-1<sup>T</sup> and the type strains of related *Rhodobacter* species.
| Characteristic                        | 1                     | 2                      | 3                      | 4                     | 5                      | 6                      |
|--------------------------------------|-----------------------|------------------------|------------------------|-----------------------|------------------------|------------------------|
| Isolation source                     | freshwater crocodile pond | freshwater lotus pond | hot spring             | eutrophic freshwater | sediment of a hot spring | stagnant water         |
| Colony pigmentation                  | dark red              | white                  | light purple           | red                   | white                  | red                    |
| Motility                             | -                     | -                      | +                      | -                     | -                      | +                      |
| Temperature range for growth (°C)    | 20-40 (25)            | 20-35 (25)             | 25-50 (40)             | 25-37 (30)            | 20-50 (37-40)          | 15-40 (30)             |
| (optimum)                            |                       |                        |                        |                       |                        |                        |
| pH range for growth (optimum)        | 6.5-9 (8)             | 6-7.5 (7)              | 7-8 (7)                | 7-8 (7)               | 6-8 (7)                | 6-8 (6-7)              |
| NaCl range for growth (%)            | 0-0.5                 | 0-0.5                  | 0-1                    | 0-2                   | 0-3                    | 0-2                    |
| Nitrate reduction                    | +                     | -                      | +                      | -                     | -                      | -                      |
| **Enzymatic activities (API ZYM):**  |                       |                        |                        |                       |                        |                        |
| Alkaline phosphatase                 | +                     | +                      | +                      | -                     | +                      | +                      |
| C4 esterase                          | +                     | +                      | +                      | +                     | +                      | -                      |
| C8 esterase lipase                   | +                     | +                      | +                      | +                     | +                      | -                      |
| C14 lipase                           | -                     | -                      | -                      | +                     | +                      | -                      |
| Valine arylamidase                   | +                     | -                      | +                      | +                     | +                      | -                      |
| Cystine arylamidase                  | -                     | -                      | +                      | +                     | +                      | -                      |
| Trypsin                              | -                     | -                      | +                      | -                     | +                      | -                      |
| a-Chymotrypsin                       | -                     | -                      | +                      | -                     | -                      | -                      |
| Acid phosphatase                     | +                     | +                      | +                      | -                     | -                      | +                      |
| Naphthol-AS-BI-phosphohydrolase      | +                     | +                      | +                      | -                     | +                      | -                      |
| a-Galactosidase                      | -                     | +                      | -                      | -                     | -                      | -                      |
| b-Galactosidase                      | +                     | +                      | +                      | -                     | +                      | -                      |
| b-Glucosidase                        | +                     | +                      | +                      | -                     | +                      | -                      |
| DNA G+C content (%)                  | 66.2                  | 66.0                   | 67.7                   | 66.5                  | 66.8                   | 66.5                   |
Strains: 1, CCP-1T; 2, *Rhodobacter tardus* CYK-10T; 3, *Rhodobacter flagellatus* SYSU G03088T; 4, *Rhodobacter blasticus* ATCC 33485T; 5, *Rhodobacter thermarum* YIM 73036T; 6, *Rhodobacter capsulatus* ATCC 11166T. All data from this study except the G+C content from NCBI database. +, Positive reaction; -, negative reaction. All strains are positive for activities of oxidase, leucine arylamidase and a-glucosidase. All strains are negative for Gram staining; indole production; D-glucose acidification; activities of urease, b-glucuronidase, N-acetyl-b-glucosaminidase, a-mannosidase and a-fucosidase.

**Supplementary Table 3.** Cellular fatty acid composition of *Rhodobacter ruber* CCP-1T and the type strains of related *Rhodobacter* species.

| Fatty acid       | 1   | 2   | 3   | 4   | 5   | 6   |
|------------------|-----|-----|-----|-----|-----|-----|
| **Straight chain** |     |     |     |     |     |     |
| C16:0            | 2.2 | 8.0 | 6.6 | 3.7 | 6.4 | 6.4 |
| C18:0            | 4.3 | 3.4 | 15.3| 5.3 | 9.9 | 2.8 |
| **Hydroxy**      |     |     |     |     |     |     |
| C10:0 3-OH       | 2.1 | 5.8 | 3.2 | 2.2 | 3.3 | 4.4 |
| C18:0 3-OH       | 6.3 | 4.4 | -   | 6.8 | 5.9 | 3.9 |
| **Unsaturated**  |     |     |     |     |     |     |
| C18:1 w7c        | 73.5| 64.8| 62.3| 75.3| 56.3| 68.8|
| C18:1 w7c 11-methyl | 6.9 | 5.9 | 9.5 | 3.6 | 15.2| -   |
| **Summed feature** |   |     |     |     |     |     |
| 3                | -   | 4.6 | -   | -   | -   | 10.6|
| 7                | 1.7 | -   | -   | -   | -   | -   |

For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (w) end of the carbon chain. *cis* isomer is indicated by the suffix c. *Summed features* are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 comprises C16:1 w7c and/or C16:1 w6c. Summed feature 7 comprises C19:1 w6c and/or C19:0 cycloo w10c.