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ORIGINAL PAPER

Neotabrizicola shimadae gen. nov., sp. nov., an aerobic anoxygenic phototrophic bacterium harbouring photosynthetic genes in the family *Rhodobacteraceae*, isolated from a terrestrial hot spring

So Muramatsu · Setsuko Hirose · Takao Iino · Moriya Ohkuma · Satoshi Hanada · Shin Haruta

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Abstract A bacteriochlorophyll-containing bacterium, designated as strain N10^T, was isolated from a terrestrial hot spring in Nagano Prefecture, Japan. Gram-stain-negative, oxidase- and catalase-positive and ovoid to rod-shaped cells showed the features of aerobic anoxygenic phototrophic bacteria, i.e., strain N10^T synthesised bacteriochlorophylls under aerobic conditions and could not grow anaerobically even under illumination. Genome analysis found genes for bacteriochlorophyll and carotenoid biosynthesis, light-harvesting complexes and type-2 photosynthetic reaction centre in the chromosome. Phylogenetic analyses based on the 16S rRNA gene sequence and 92 core proteins revealed that strain N10^T was located in a distinct lineage near the type species of the genera *Tabrizicola* and *Xinfangfangia* and some species in the genus *Rhodobacter* (e.g., *Rhodobacter blasticus*). Strain N10^T shared < 97.1% 16S rRNA gene sequence identity with those species in the family *Rhodobacteraceae*. The digital DNA–DNA hybridisation, average nucleotide identity and average amino acid identity values with the relatives, *Tabrizicola aquatica* RCRI19^T (an aerobic anoxygenic phototrophic bacterium), *Xinfangfangia soli* ZQBW^T and *R. blasticus* ATCC 33485^T were 19.9–20.7%, 78.2–79.1% and 69.1–70.1%, respectively. Based on the phenotypic features, major fatty acid and polar lipid compositions, genome sequence and phylogenetic position, a novel genus and species are proposed for strain N10^T, to be named *Neotabrizicola shimadae* (= JCM 34381^T = DSM 112087T). Strain N10^T which is phylogenetically located among aerobic anoxygenic phototrophic bacteria (*Tabrizicola*), bacteriochlorophyll-deficient bacteria (*Xinfangfangia*) and anaerobic anoxygenic phototrophic bacteria (*Rhodobacter*) has great potential to promote studies on the evolution of photosynthesis in *Rhodobacteraceae*.

Keywords AAPB · Aerobic anoxygenic phototrophic bacteria · Bacteriochlorophyll · *Neotabrizicola* · *Neotabrizicola shimadae* · Photosynthetic gene cluster · *Rhodobacteraceae*

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S. Muramatsu · S. Hirose · T. Iino · S. Hanada · S. Haruta (✉)
Department of Biological Sciences, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji, Tokyo 192-0397, Japan
e-mail: sharuta@tmu.ac.jp

T. Iino · M. Ohkuma
Japan Collection of Microorganisms, RIKEN BioResource Center, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

S. Hanada
Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-0856, Japan

Introduction

Bacteriochlorophyll (BChl)-producing aerobic bacteria, known as aerobic anoxygenic phototrophic bacteria (AAPB), have photosynthetic ability but cannot grow phototrophically (Shimada 1995; Yurkov and Hughes 2017). AAPB are widely found in natural environments and have attracted attention due to the ecological importance of their aerobic heterotrophic metabolism (Kolber et al. 2001). Culture-dependent and culture-independent studies have identified AAPB in the phyla *Proteobacteria*, *Acidobacteria* and *Gemmatimonadetes* (Thiel et al. 2018). Some AAPB are phylogenetically closely related to typical anaerobic anoxygenic phototrophic bacteria which produce BChls under anaerobic conditions with light and grow photoheterotrophically and photoautotrophically (Yurkov and Csotonyi 2009). Comparative studies between AAPB and non-AAPB are of considerable interest in understanding the evolutionary diversification of phototrophic organisms and their photosynthetic ability (Yurkov and Hughes 2017).

The class *Alphaproteobacteria* is a representative group that includes AAPB. Of more than 300 genera within *Alphaproteobacteria*, 41 genera contain AAPB, and this number is increasing with the continuous discovery of AAPB (Thiel et al. 2018). Bacteria of the genus *Tabrizicola* in the family *Rhodobacteraceae* in the class *Alphaproteobacteria* were firstly described as non-AAPB in 2013 (Tarhriz et al. 2013). Recently, BChl-production ability and photosynthetic gene cluster were reported for bacteria in this genus (Tarhriz et al. 2019; Han et al. 2020). Photosynthetic gene cluster is composed of approximately 40 genes for photosynthetic reaction centre, light-harvesting complexes, BChl and carotenoid biosynthesis and regulatory factors (Zsebo & Hearst 1984). The description of the genus *Tabrizicola* has been emended as "some species of this genus produce bacteriochlorophyll *a* under aerobic, heterotrophic conditions", and the type species, *T. aquatica*, is defined as an aerobic anoxygenic phototrophic bacterium (Tarhriz et al. 2019). Bacteria of the genus *Tabrizicola* is phylogenetically related to some bacteria of the genera *Xinfangfangia* and *Rhodobacter* (Hu et al. 2018; Suresh et al. 2019; Hördt et al. 2020). Bacteria of the genus *Xinfangfangia* does not have photosynthesis-related genes (Hu et al. 2018), and BChl-production ability was not reported. *Rhodobacter* is one of the most well-known groups of anoxygenic phototrophic bacteria (Imhoff 2015); the type species, *Rhodobacter capsulatus* was originally reported in 1907 (Molisch 1907). The genus *Rhodobacter* consists of phylogenetically diverse species, and their reclassification is often proposed (Suresh et al. 2019; Hördt et al. 2020). Phylogeny of the photosynthetic genes is actively studied to explain the patchy distribution of phototrophy in the family *Rhodobacteraceae* (Zheng et al. 2011; Imhoff et al. 2018; Imhoff et al. 2019; Brinkmann et al. 2018; Liu et al. 2019). To draw an elaborate picture of the evolutionary diversification of phototrophs and their photosynthetic ability in *Rhodobacteraceae*, it is desirable to acquire bacteria located at a phylogenetic lineage connecting anaerobic anoxygenic phototrophs, non-phototrophs and aerobic anoxygenic phototrophs.

In this study, we isolated a BChl-containing bacterium, strain N10^T, from a hot spring in Japan. Cells produced BChl under aerobic conditions and could not grow under anaerobic conditions even with light. Phylogenetic analysis based on 16S rRNA gene sequences suggested that the isolate belonged to the family *Rhodobacteraceae* and was distantly related to the genera *Tabrizicola*, *Xinfangfangia* and *Rhodobacter*. The aim of this study was to determine the taxonomic position of strain N10^T by polyphasic taxonomic analyses, and a novel genus and species are proposed for this aerobic anoxygenic phototrophic bacterium in the family *Rhodobacteraceae*.

Materials and methods

Sample collection and isolation

Microbial mats developed in hot spring water at Nakabusa Hot Springs, Nagano, Japan (36° 23′ 20″ N, 137° 44′ 52″ E) (Fig. S1) were collected. The temperature and pH of the hot spring water at the sampling site were 30 °C and pH 8.0, respectively. The greenish microbial mats are dominated by oxygenic phototrophs (Everroad et al. 2012). A piece of the mats was collected using a sterilized tweezers and brought to the laboratory in ice. Approximately 0.5 g of the sample suspended in 10 ml of sterile distilled water were aseptically homogenized on ice using POLYTRON PT10/35 (KINEMATICA, Switzerland), directly spread on 1/10 diluted PE agar plate

(Hanada et al. 1995; Hirose et al. 2016) and aerobically cultivated at 30 °C in the dark. 1/10 diluted PE agar solidified with Bacto-Agar (1.5%, w/v) (Thermo Fisher Scientific, USA) contains (per litre, pH 7.5) 0.05 g each of sodium glutamate, sodium succinate, sodium acetate, yeast extract (FUJIFILM Wako Pure Chemical, Japan), Casamino acids, sodium thiosulfate and ammonium sulfate, 0.2 ml of vitamin mixture (Hanada et al. 1995), 5 ml of 1 mol/l phosphate buffer and 5 ml of a basal salt solution (Hanada et al. 1995). PE medium was originally developed by Hanada et al. (1995) as a phototrophic bacteria enrichment medium and the diluted versions have been widely used for isolation of aerobic and anaerobic anoxygenic phototrophic bacteria (Hirose et al. 2016). Colonies containing bacteriochlorophylls (BChls) were fluorescently detected with an imaging system: colonies on Petri dishes were illuminated with LED light at wavelengths of 375 nm and 590 nm, and the infrared fluorescence from BChl-containing colonies was observed using a CCD camera with a long-pass filter (> 850 nm) (Edmund Optics, USA) (Zeng et al. 2014). Colonies with BChl fluorescence were picked for isolation. Isolation was performed using the standard dilution plating technique at 30 °C under aerobic conditions in the dark with 1/10 diluted PE agar medium (Hanada et al. 1995; Hirose et al. 2016). Among four BChl-producing isolates in total six isolates, strain N10^T which stably and strongly showed BChl-fluorescence was selected and used for further experiments. The purified strain was routinely cultured on 1/5 diluted PE agar (Hirose et al. 2016) or Reasoner's 2A (R2A; FUJIFILM Wako Pure Chemical, Japan) (Reasoner and Geldreich 1985) agar solidified with 1.5% Bacto-Agar under aerobic dark conditions. R2A medium had been used for cultivation of the reference strains such as *Rhodobacter blasticus* (Hu et al. 2018). *R. blasticus* NBRC 16437^T (= ATCC 33485^T) was obtained from NBRC (Biological Resource Center, NITE, Japan) and cultivated under the same conditions for strain N10^T.

Morphology and physiology

Morphological characteristics were examined using a phase-contrast microscope (Eclipse E600; Nikon, Japan). The cellular morphology was also observed by transmission electron microscopy (TEM) based on a rapid freezing and freeze-fixation method, performed at Tokai Electron Microscopy (Japan). For sample preparation for TEM, cells were frozen in liquid propane at − 175 °C and the frozen samples were substituted with 2% (v/v) glutaraldehyde, 1% (w/v) tannic acid in ethanol and 2% water at − 80 °C. After dehydration with ethanol at room temperature, the samples were infiltrated with propylene oxide and embedded in resin (Quetol-812, Nisshin EM, Japan). Ultra-thin section (70 nm) was prepared using an ultramicrotome (Ultracut UCT, Leica, Austria), stained with 2% (v/v) uranyl acetate and lead stain solution and observed using a TEM (JEM-1400Plus, JEOL, Japan). Gram staining was performed using the FAVOR-G kit (Nissui Pharmaceutical, Japan). Growth was tested on R2A agar at 4, 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C. Salt tolerance was assessed in 1/5 diluted PE broth adjusted to 0%, 1%, 2% and 3% NaCl (w/v). Growth at various pH values (pH 5.0–9.0 at 1.0 pH intervals) was determined in 1/5 diluted PE broth. Anaerobic growth was tested using the AnaeroPack system (Mitsubishi Gas Chemical, Japan) on 1/5 diluted PE agar under illumination [photo-organoheterotrophic conditions, 2000 lx (tungsten light)] and on R2A agar in the dark (fermentative conditions). Anaerobic growth tests were also performed in screw-capped glass tubes fully filled with 1/5 diluted PE and R2A broth.

The in vitro absorption spectrum was obtained as follows: cells were harvested after a week of cultivation at 30 °C in 1/5 diluted PE broth under aerobic dark conditions and pigments were extracted from cells using acetone:methanol (7:2, v/v) (Tarhriz et al. 2019). The absorption spectrum in the range of 350–850 nm was determined using the UV-2600 spectrophotometer (Shimadzu, Japan).

Oxidase activity was detected using an oxidase reagent kit (BioMérieux, France). Catalase activity was determined by gas production using 3% H_2O_2 (w/v). Enzyme activities were evaluated using the API ZYM system (BioMérieux). The carbon source utilisation pattern was investigated using a GEN III MicroPlate (Biolog, USA).

Molecular phylogenetic analysis

Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). PCR amplification and sequencing of the 16S rRNA gene were performed as described previously (Hirose et al.

2016). The 16S rRNA gene sequence was compared with the sequence data from GenBank using the BLAST program. Phylogenetic trees based on 16S rRNA gene sequences were constructed with MEGA version 7.0 (Kumar et al. 2016) using the neighbour-joining (Saitou and Nei 1987) and maximum-likelihood (Felsenstein 1981) methods. The Kimura two-parameter model (Kimura 1980) was used to calculate evolutionary distances. The topology of the phylogenetic trees was evaluated using the bootstrap resampling method (Felsenstein 1985) with 1000 replicates.

Genome sequencing and analyses

The complete genome of strain N10^T was sequenced by Bioengineering Lab. (Sagamihara, Japan) with GridION X5 (Oxford Nanopore Technologies, UK) and DNBSEQ-G400 (MGI, China). The sequences were assembled using Unicycler version 0.4.7 (Wick et al. 2017). Genes were annotated using Prokka version 1.13 (Seemann 2014) and DFAST version 1.2.4. (Tanizawa et al. 2018). From the genomic data of strain N10^T and related species, 92 core gene sequences were extracted using the Up-to-date Bacterial Core Gene (UBCG) tool (Na et al. 2018). Concatenated amino acid sequences were prepared using the UBCG pipeline. A phylogenetic tree was constructed with MEGA version 7.0 (as described above) using the maximum-likelihood method. The average nucleotide identity (ANI) and digital DNA-DNA hybridisation (dDDH) values of strain N10^T with its phylogenetic neighbours were calculated using the OrthoANI calculator (Lee et al. 2016) and the Genome-to-Genome Distance Calculator (GGDC 2.1; http://ggdc.dsmz.de/distcalc2.php) (Meier-Kolthoff et al. 2014), respectively. Average amino acid identity (AAI) values were calculated using AAI-Matrix (http://enve-omics.ce.gatech.edu/g-matrix/).

Chemotaxonomic characterization

The compositions of respiratory quinone, fatty acids and polar lipids were analysed by Techno Suruga Laboratory (Shizuoka, Japan). Cells for analyses were incubated at 30 °C on R2A agar under aerobic conditions in the dark for a week. Quinones were identified by HPLC as described previously (Hamada et al. 2010). Cellular fatty acids were identified using the Sherlock Microbial Identification System (version 6.0) with the TSBA6 database (MIDI, USA) (Sasser 2001). Polar lipid analysis was performed using TLC methods (Minnikin et al. 1979).

Results

Morphology

Colonies of strain N10^T grown on R2A agar under aerobic dark conditions were beige, whereas those grown on 1/5 diluted PE agar were slightly purple. Cells were ovoid to rod-shaped, 0.7–1.0 µm in diameter and 1.3–1.8 µm in length (Fig. S2), non-motile and Gram-stain-negative (Fig. S3). TEM image indicated that cells of strain N10^T had no lamellar internal membrane (Fig. 1), which are typically observed in *Rhodobacter* sp. (Imhoff 2015).

Phylogenetic analysis based on 16S rRNA gene sequences

The nearly complete 16S rRNA gene sequence of strain N10^T was obtained (1389 bp). BLAST analysis of the 16S rRNA gene sequence revealed that the highest sequence identities were 96.6% with *Tabrizicola piscis* K13M18^T, 97.1% with *Rhodobacter blasticus* ATCC 33485^T and 97.1% with *Xinfangfangia soli* ZQBW^T. The maximum-likelihood tree based on 16S rRNA gene sequences demonstrated that strain N10^T was distantly located at a distinct

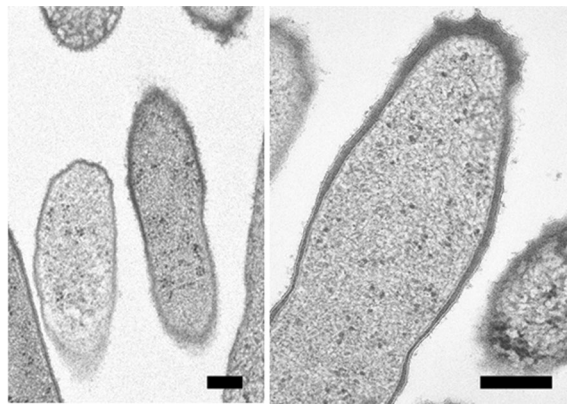

Fig. 1 Transmission electron micrographs of strain N10^T. Cells aerobically grown at 30 °C in 1/5 diluted PE medium in the dark were collected at the growing phase for analysis. Scale bars represent 200 nm

branch near three lineages containing the type species of *Tabrizicola* (i.e., *Tabrizicola aquatica* RCRI19^T), *Xinfangfangia* (i.e., *X. soli* ZQBW^T) and some *Rhodobacter* species such as *R. blasticus* ATCC 33485^T (Fig. 2). This phylogenetic position of strain N10^T was supported by phylogenetic analysis using the neighbour-joining method (Fig. S4).

Genome-based analysis

The complete genome of strain N10^T consisted of a chromosome (4,154,010 bp) and four plasmids (119,227 bp, 70,477 bp, 32,096 bp and 16,643 bp). The DNA G+C content was 66.6 mol%. The genome encodes 4,242 protein-coding genes, 52 tRNAs and 6 rRNAs. The phylogenetic tree based on 92 single-copy core genes (UBCG) in the chromosome showed that strain N10^T formed a separate branch from the lineage of *T. aquatica*, *X. soli* and *R. blasticus* (Fig. S5). The ANI and dDDH values between strain N10^T and *T. aquatica* RCRI19^T, *R. blasticus* ATCC 33485^T and *X. soli* ZQBW^T were 78.2%, 78.3% and 79.1%, respectively (for ANI), and 19.9%, 20.1% and 20.7%, respectively (for dDDH), which were all significantly lower than the cut-off values for discriminating bacterial species (95–96% ANI, 70% dDDH) (Rodriguez-R and Konstantinidis 2014; Goris et al. 2007). *T. aquatica* RCRI19^T, *R. blasticus* ATCC 33485^T and *X. soli* ZQBW^T shared 72.4–83.3% of AAI; however, the AAI values between strain N10^T and *T. aquatica* RCRI19^T, *R. blasticus* ATCC 33485^T and *X. soli* ZQBW^T were 69.3%, 70.1% and 69.1%, respectively. Therefore, strain N10^T could be differentiated at the genus level from *Tabrizicola*, *Xinfangfangia* and *Rhodobacter*.

The genome of strain N10^T lacked the *cbbS* and *cbbL* genes that encode ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO). Strain N10^T had the photosynthetic gene cluster, i.e., genes for BChl (*bch*) and carotenoid (*car*) biosynthesis, light-harvesting complexes and type-2 photosynthetic reaction centre (*pufL*, *pufM* and *puhA*) in the chromosome. PufLM sequence is a great tool for the phylogenetic analysis of anoxygenic phototrophic bacteria (Imhoff et al. 2018). As observed in the phylogenetic trees of 16S rRNA gene (Figs. 2 and S4) and core proteins

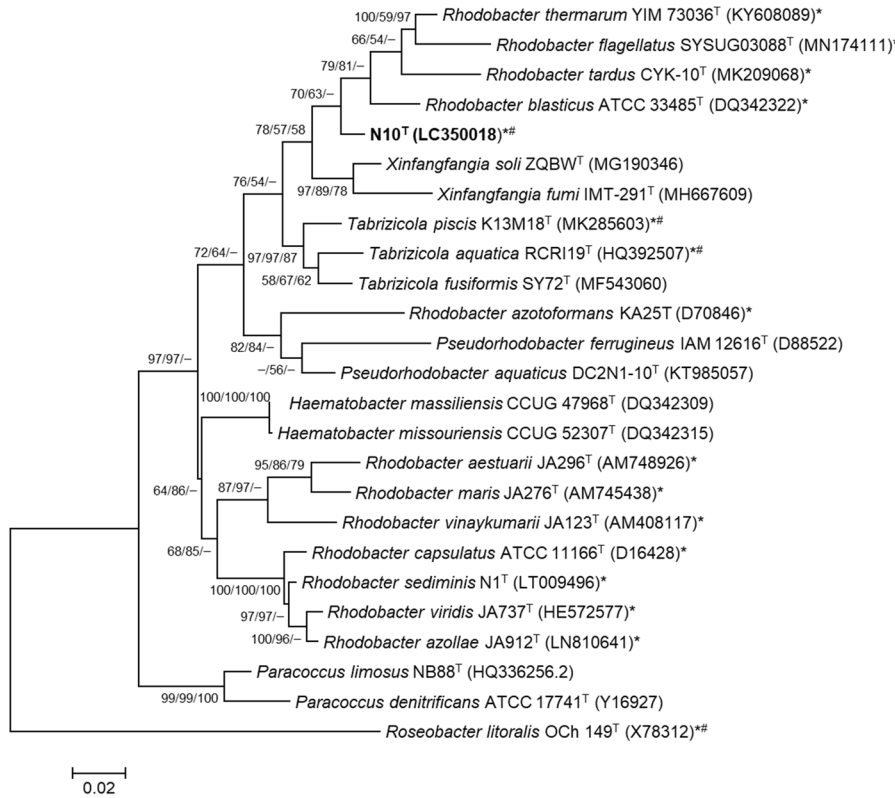

Fig. 2 Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strain N10^T and closely related species. *Roseobacter litoralis* OCh114^T (GenBank accession no. X78312) was added as an outgroup. Bootstrap values calculated from 1000 replications and the percentages (over 50%) from three algorithms are shown at the branch nodes (maximum-likelihood/neighbour joining/unweighted pair group method with arithmetic mean). Bar represents nucleotide substitutions per site. Bacteria known to produce BChl were marked with an asterisk (*) beside the accession numbers and among them, aerobic anoxygenic phototrophic bacteria were additionally marked with a hash (#)

(Fig. S5), PufLM amino acid sequence of strain N10^T was closely related to those from a group of *R. blasticus* (e.g., *Rhodobacter thermarum* and *Rhodobacter flagellates*) and *Tabrizicola* (e.g., *T. aquatica* and *T. piscis*) but distantly related to those from the type species of *Rhodobacter*, i.e., *R. capsulatus* (data not shown). *R. capsulatus* and its phylogenetic relatives lacked the *acsF* gene that encodes the oxygen-dependent type of magnesium-protoporphyrin IX monomethyl ester cyclase for BChl biosynthesis and had the *bchE* gene for the oxygen-independent type (Boldareva-Nuianzina et al. 2013), but strain N10^T had both the genes, *acsF* and *bchE*, in the photosynthetic gene cluster, as with *R. blasticus*, *R. thermarum*, *R. flagellates*, *T. aquatica* and *T. piscis*.

Phenotypic and physiological characterization

Strain N10^T grew aerobically at 10–45 °C, at pH 7.0–8.0 and with the addition of 0–1% NaCl (w/v). Fermentative growth was not observed on R2A agar in the dark. Strain N10^T did not grow in 1/5 diluted PE and R2A media under anaerobic light conditions. The in vitro absorption spectrum of strain N10^T (Fig. S6) showed absorption peaks at approximately 360, 600 and 770 nm, indicating the presence of BChl *a*, and an absorption at approximately 480 nm, indicating the presence of carotenoids, which were similar to the spectra of phototrophic relatives (Eckersley and Dow 1980; Tarhriz et al. 2019).

Strain N10^T was found to be oxidase- and catalase-positive. According to the API ZYM assay results, cells were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase and *N*-acetyl-*β*-glucosaminidase, and negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, *α*-chymotrypsin, *β*-glucuronidase, *α*-mannosidase and *α*-fucosidase. In the GEN III MicroPlate, strain N10^T was found to be positive for glucuronamide, Tween 40, acetoacetic acid, propionic acid and acetic acid, and negative for dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, *α*-D-lactose, D-melibiose, *β*-methyl-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-*β*-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl neuraminic acid, *α*-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, *myo*-inositol, glycerol, D-glucose-6-PO_4, D-fructose-6-PO_4, D-aspartic acid, D-serine, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, D-saccharic acid, *p*-hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, *α*-keto-glutaric acid, D-malic acid, L-malic acid, bromo-succinic acid, *γ*-amino-butyric acid, *α*-hydroxybutyric acid, *β*-hydroxy- D,L-butyric acid, *α*-keto-butyric acid and formic acid.

Chemotaxonomic characteristics

The sole respiratory quinone was found to be ubiquinone-10, which is a typical characteristic of bacteria of the genera *Tabrizicola*, *Xinfangfangia*, and *Rhodobacter* (Imhoff 2015; Tarhriz et al. 2013; Hu et al. 2018). Strain N10^T had $C_{19:0}$ cyclo ω8*c*, $C_{18:1}$ ω7*c* 11-methyl and summed feature 8 ($C_{18:1}$ ω6*c* and/or $C_{18:1}$ ω7*c*) as the major fatty acids (> 20%) and $C_{18:0}$ and $C_{18:0}$ 3OH as the minor fatty acids (> 1%) (Table S1). The polar lipids of strain N10^T mainly comprised phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), a glycolipid (GL), an unidentified phospholipid (PL), an unidentified amino lipid (AL) and eight unidentified polar lipids (UL1 − UL8) (Fig. S7).

Discussion

The phylogenetic trees based on the 16S rRNA gene (Figs. 2 and S4) and core proteins (Fig. S5) showed that strain N10^T was located at a distinct branch, which was related to *Tabrizicola*, *Xinfangfangia* and some *Rhodobacter* species in the family *Rhodobacteraceae*. The characteristics differentiating strain N10^T from these genera are summarised in Tables 1 and 2. The ANI and dDDH values between strain N10^T and *T. aquatica* RCRI19^T, *R. blasticus* ATCC 33485^T and *X. soli* ZQBW^T were lower than the thresholds used for prokaryotic species delineation (Meier-Kolthoff et al. 2013). *Tabrizicola*, *Xinfangfangia* and a group of *Rhodobacter* containing *R. blasticus* shared AAI values of 72–83% (Hördt

Table 1 Genomic features of strain N10^T and closely related type strains

	1	2	3	4
Genome size (Mbp)	4.15	3.71	3.87	4.68
G+C content (mol%)	66.6	66.4	66.4	67.6
No. of coding seq	4,242	3,604	3,846	4,470
No. of tRNAs	52	53	49	47
No. of rRNAs	6	6	3	3
pufL and *pufM* genes	+	+	+	−
cbbS and *cbbL* genes	−	+	−	−
acsF and *bchE* genes	+	+	+	−

Strains: 1, N10^T (this study); 2, *Rhodobacter blasticus* ATCC 33485^T (CP020470); 3, *Tabrizicola aquatica* RCRI 19^T (GCA_002900975); 4, *Xinfangfangia soli* ZQBW^T (GCA_015999335). Symbols: +, positive; −, negative

et al. 2020), and the AAI values between strain N10^T and these type strains were 69.1–70.1%, indicating that strain N10^T could be differentiated at the genus level from *Tabrizicola*, *Xinfangfangia* and *Rhodobacter*. Moreover, strain N10^T had $C_{19:0}$ cyclo ω8*c* and $C_{18:1}$ ω7*c* 11-methyl as major fatty acids and a glycolipid as a major polar lipid, which were not detected as major components in the closely related species (Table 2). Therefore, strain N10^T is considered to represent a novel species in a novel genus in the family *Rhodobacteraceae*, for which the name *Neotabrizicola shimadae* gen. nov., sp. nov. is proposed.

Strain N10^T had photosynthesis-related genes, synthesised BChl *a* under aerobic conditions and did not grow under anaerobic conditions even with light, indicating that strain N10^T was categorised as an aerobic anoxygenic phototrophic bacterium (Shimada 1995; Yurkov and Hughes 2017). Strain N10^T lacked complex intercellular membrane structure (Fig. 1), similar with *T. aquatica* (Tarhriz et al. 2019). Within the microbial mats dominated by oxygenic phototrophs in water stream, strain N10^T heterotrophically grows mainly by oxygen respiration. Strain N10^T could not utilize many organic compounds including D-glucose and sucrose, similar to *Xinfangfangia* sp. (Kämpfer et al. 2019) but different from *Tabrizicola* sp. (Sheu et al. 2020) and *R. blasticus* (Imhoff 2015). The growth of strain N10^T may be partially supported by energy supply through photophosphorylation (Beatty 2002) in the mats. Positive reaction of *N*-acetyl-*β*-glucosaminidase of strain N10^T could differentiate between strain N10^T and *Xinfangfangia* sp. (Hu et al. 2018; Kämpfer et al. 2019).

Table 2 Differentiating characteristics of strain N10^T from closely related type strains

	1	2	3	4
Cell shape	Ovoid to rod	Ovoid to rod	Rod	Rod
Colony colour*	Beige, purple	Faint pink	Colourless, cream	Light yellow
Growth at 10 °C	+	−	−	+
Growth at 45 °C	+	−	+	−
Growth in 3% NaCl	−	−	+	−
Phototrophic growth	−	+	−	−
Bacteriochlorophylls	+	+	+	−
Major fatty acid	$C_{19:0}$ cyclo ω8*c*, $C_{18:1}$ ω7*c* 11-methyl, $C_{18:1}$ ω6*c* and/or $C_{18:1}$ ω7*c*	$C_{18:1}$ ω7*c*	$C_{18:1}$ ω7*c*	$C_{18:1}$ ω7*c*
Polar lipids**	GL	diPG, PME	diPG	PME
Isolation source	Hot spring	Pond	Lake	Soil

Data of column 1 were obtained in this study. In column 2, physiological data were obtained in this study and others were originated from Imhoff (2015). Data in column 3 were originated from Tarhriz et al. (2013), Tarhriz et al. (2019) and Sheu et al. (2020) and those in column 4 were originated from Hu et al. (2018)

Strains: 1, N10^T; 2, *Rhodobacter blasticus* NBRC 164737^T; 3, *Tabrizicola aquatica* RCRI 19^T; 4, *Xinfangfangia soli* ZQBW^T. Symbols: +, positive; −, negative

*Under oxic dark conditions

**GL, glycolipid; diPG, diphosphatidylglycerol; PME, phosphatidylmethylethanolamine. All strains in this table possess phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC)

N-acetyl-*β*-glucosaminidase activity may help to obtain organic compounds from other bacterial cells in the mats (Jørgensen et al. 2003).

As estimated by phylogenetic analysis (Brinkmann et al. 2018), the photosynthetic ability was vertically evolved (e.g., *Tabrizicola*) (Tarhriz et al. 2019) and frequently lost (e.g., *Xinfangfangia*) (Hu et al. 2018; Kämpfer et al. 2019) in *Rhodobacteraceae*. The phylogenetic trees shown in Fig. 2, Fig. S4 and Fig. S5 suggest that strain N10^T is located at the transition between aerobic anoxygenic phototrophic bacteria, *Tabrizicola* and typical anoxygenic phototrophic bacteria, *Rhodobacter* sp. such as *R. blasticus*. Further phylogenetic and biochemical studies on strain N10^T and the relatives could provide beneficial knowledges about the evolutionary diversification of phototrophic organisms and their photosynthetic ability.

Description of *Neotabrizicola* gen. nov.

Neotabrizicola (Ne.o.ta.b.ri.zi'co.la. Gr. masc. adj. *neos* new; N.L. fem. n. *Tabrizicola* a bacterial genus name: N.L. fem. n. *Neotabrizicola* a new *Tabrizicola*).

Cells are Gram-stain-negative and ovoid to rod-shaped. The respiratory quinone is ubiquinone-10. The major fatty acids are $C_{19:0}$ cyclo ω8*c*, $C_{18:1}$ ω7*c* 11-methyl and summed feature 8 ($C_{18:1}$ ω6*c* and/or $C_{18:1}$ ω7*c*). The polar lipids are phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and glycolipid. The genus *Neotabrizicola* belongs to the family *Rhodobacteraceae* within the class *Alphaproteobacteria*. The type species is *Neotabrizicola shimadae*.

Description of *Neotabrizicola shimadae* sp. nov.

Neotabrizicola shimadae (shi.ma'dae. N.L. gen. n. *shimadae*, of Shimada, named after Dr. Keizo Shimada, Professor Emeritus, Tokyo Metropolitan University, Tokyo, Japan, in recognition of his many contributions to the biology of aerobic anoxygenic phototrophic bacteria).

Displays the following properties in addition to those given in the genus description. Cells are 0.7–1.0 µm in diameter and 1.3–1.8 µm in length, non-motile and obligate aerobe. Colonies are beige and purple and oxidase- and catalase-positive. Growth occurs at 10–45 °C, pH 7.0–8.0 and with 0–1% (w/v) NaCl. Extracted pigments from cells show absorption peaks at approximately 480 nm and 770 nm, corresponding to carotenoids and BChl *a*, respectively. Growth does not occur under anaerobic conditions even with light. In addition to the major fatty acids mentioned in the genus description, $C_{18:0}$ and $C_{18:0}$ 3OH are present in relatively low proportions. The G+C content of the genomic DNA of the type strain is 66.6 mol%.

The type strain, N10^T (=JCM 34381^T=DSM 112087^T) was isolated from a hot spring sample from Nagano Prefecture, Japan. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N10^T is LC350018. The genome sequence data of this strain are publicly available under the GenBank/EMBL/DDBJ accession numbers, CP069370 (chromosome) and CP069371–CP069374 (four plasmids).

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Authors' contribution SM, SHn and SHr planned the research. SM and SHi carried out experiments. SM, TI and SHr analysed the data and drafted the manuscript. MO and SHn supervised the research and SHn provided the research funding. All authors proofread the manuscript.

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Declarations

Conflict of interests The authors declare that there are no conflicts of interest.

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