Reclassification of 11 Members of the Family Rhodobacteraceae at Genus and Species Levels and Proposal of *Pseudogemmobacter hezensis* sp. nov.

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A novel Gram-stain-negative, aerobic, motile bacterial strain, D13-10-4-6ᵀ, was isolated from the bark sample of *Populus × euramericana*. The strain could grow at 15–35°C, at pH 6–10 and in 0–4% (w/v) NaCl, and the strain tested positive for oxidase and catalase activities. The main polar lipids were phosphatidylmonomethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, and phosphatidylethanolamine. The main respiratory quinone was Q-10, and the predominant fatty acid was C₁₈:₁ω₇c. The phylogenetic analyses showed that the strain belonged to the genus *Pseudogemmobacter* of the family Rhodobacteraceae. The family Rhodobacteraceae is an ecologically diverse group that includes bacteria from aquatic to terrestrial ecosystems. As a consequence, the classification of the family Rhodobacteraceae is difficult, not least when the early taxonomy work relied heavily on 16S rRNA gene analysis. Recently, the taxonomic status of many members of the family has been revised based on the genome analysis; however, there are still some classification conflicts due to the lack of genome sequences and parallel publication time. In this study, phylogenetic trees based on 16S rRNA gene, *gyrB* gene, and 120 concatenated proteins, the average amino acid identity (AAI) and percentage of conserved proteins (POCP) have been used for the analysis of strain D13-10-4-6ᵀ and other members of 15 genera within the family to further clarify their taxonomic relationships. For the data of phylogeny, AAI, and POCP, the taxonomic proposals are (1) reclassification of *Rhodobacter tardus* as the type species of a novel genus, *Stagnihabitans* gen. nov., as *Stagnihabitans tardus* comb. nov.; (2) reclassification of *Tabrizicola alkalilacus*, *Tabrizicola sediminis*, *Tabrizicola algicola* into a novel genus, *Pseudotabrizicola* gen. nov., as *Pseudotabrizicola alkalilacus* comb. nov., *Pseudotabrizicola sediminis* comb. nov., *Pseudotabrizicola algicola* comb. nov.; (3) reclassification of *Rhodobacter sediminicola* into the genus *Cereibacter* as *Cereibacter sediminicola* comb. nov.; (4) reclassification of *Rhodobacter flagellatus*, *Rhodobacter thermarum*, and *Xinfangfangia soli* into the genus *Tabrizicola* as *Tabrizicola flagellatus* comb. nov., *Tabrizicola thermarum* comb. Nov., and...
INTRODUCTION

Populus × euramericana cakers on poplar trees were found in China for many years, and the stem or branch bark of the diseased tree was cracked and exuded frothy fluid. During our investigation of the bacterial diversity in Populus × euramericana caker, strain D13-10-4-6T was isolated from the symptomatic bark of Populus × euramericana caker. The phylogenetic analyses showed that the strain belonged to the genus Pseudogemmobacter of the family Rhodobacteraceae. The family Rhodobacteraceae, described by Garrity et al. (2005), the so-called purple nonsulfur bacteria (Imhoff et al., 1998), is one of the major subdivisions of the class Alphaproteobacteria. It is ecologically and phenotypically diverse, and most of the members of the family have been found in various marine environments, including seawater, sea sediments, sea ice, coastal biofilms, marine animal tissues, and seaweeds (Selje et al., 2004; Buchan et al., 2005; Brinkhoff et al., 2008). At the time of writing, the family included more than 180 genera with validated names.1

The early classification of the genera within the family Rhodobacteraceae relied heavily on the analysis of 16S rRNA gene sequence and resulted in several non-monophyletic genera, for instance, the genus Rhodobacter (Imhoff et al., 1984). The genus Rhodobacter was reclassified by Suresh et al. (2019) and Hördt et al. (2020) based on the genome analysis. The members of the genus were divided into five distinct clades in the 16S rRNA gene-based phylogenetic tree constructed by Suresh et al. (2019). The Cereibacter sphaeroides (formerly Rhodobacter sphaeroides) clade was reclassified into the genus Luteovulum (Suresh et al., 2019) and then transferred into the genus Cereibacter by Hördt et al. (2020). However, Rhodobacter alkalitolerans, which belongs to the C. sphaeroides clade, was not reclassified due to the lack of genomic sequence. At present, the genus Rhodobacter contains 13 species with validated names according to the List of Prokaryotic names with Standing in Nomenclature (LSPN).2 The taxonomic status of the recently described Rhodobacter species, R. thermarum (Khan et al., 2019), R. flagellatus (Xian et al., 2020), R. sediminicola (Suresh et al., 2020), and R. tardus (Sheu et al., 2020), was also not included in the early reclassification of Rhodobacter due to the nearly parallel time of description, causing confusion in the classification of the genus Rhodobacter.

The genus Xinfangfangia, described by Hu et al. (2018), is closely related to the genera of Rhodobacter and Tabrizicola within the family Rhodobacteraceae (Hu et al., 2018). It contains only two species with validated names, namely, Xinfangfangia soli and Xinfangfangia humili (Kämpfer et al., 2019). The genus Pseudogemmobacter described by Suman et al. (2019) contains only one species with validly published names, namely, Pseudogemmobacter bohemicus. While X. humili and P. bohemicus, which were proposed almost simultaneously, share a high 16S rRNA gene sequence similarity of 99.2%. Therefore, the relationship of P. bohemicus and X. Humi needs to be studied.

Along with the advances in whole-genome sequencing technologies, several methods for taxonomic classification at the species and genus levels have been developed. The new standards for species recognition are developed using digital whole-genome comparisons, such as average nucleotide identities (ANIs) (Konstantinidis and Tiedje, 2005) and genome-to-genome-distance calculations (GGDCs) (Richter and Rosselló-Móra, 2009; Meier-Kolthoff et al., 2013). The average amino acid identity (AAI) (Luo et al., 2014; Rodriguez-R and Konstantinidis, 2014) and percentage of conserved proteins (POCPs) (Qin et al., 2014), which are methods of measuring amino acid-level genomic similarity between protein-coding regions, have been used in the delineation of prokaryotic organisms at the genus level. Furthermore, the phylogenetic analysis based on the whole-genome sequence has been recently encouraged for the taxonomy of prokaryotes owing to its robustness and repeatability (Chun et al., 2018). Nowadays, along with those methods, the reclassification of prokaryotes at class (Hördt et al., 2020), order (Orata et al., 2018), family (Li et al., 2021), and genus (Suresh et al., 2019) levels has been done.

In this study, phylogenetic analysis based on the 16S rRNA gene, gyrB gene, and genomes sequence, as well as ANI, AAI, and POCP, was used to confirm the taxonomic relationship of the novel strain and its closely related members (e.g., members of the genus Xinfangfangia and Rhodobacter) in the family Rhodobacteraceae.

MATERIALS AND METHODS

Strain and Culture Conditions

Strain D13-10-4-6T was isolated from the bark sample of Populus × euramericana collected from Heze, Shandong Province, China (34° 82′ N, 115° 46′ E) as previously described (Li et al., 2015). In brief, the bark samples were sterilized for 30 s with 70% ethanol, and then exposed to 4% (v/v) sodium hypochlorite for 3 min. After rinsing in sterile water three times, the samples with 2 ml sterile water were transferred to sterile mortar and

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1https://lpsn.dsmz.de/family/rhodobacteraceae
2https://lpsn.dsmz.de/genus/rhodobacter
ground with a pestle, respectively. The obtained solution was then shaken for 30 min at 30°C. The suspension with a dilution series was spread on tryptic soy agar (TSA, Difco). After 2 days of incubation on TSA plates at 30°C, single colonies were selected and cultured on a new plate, and were then preserved at -80°C with a supplement of 20% (v/v) glycerol.

**Genome Sequencing**

The genomes of the strains D13-10-4-6\(^T\) and X. soli ZQBW\(^T\) were sequenced by Illumina NovaSeq PE150 (Novogene, Co., Ltd., Beijing, China). Low-quality reads in the raw data were filtered by readfq (version 10), then the genome assembly with high-quality reads was performed using SOAPdenovo (version 2.04) (Li et al., 2008; Li et al., 2010), SPAdes (Bankevich et al., 2012), ABySS (Simpson et al., 2009), and then the results were integrated with CISA (Lin and Liao, 2013). The gap of the genome assembly was filled using gapclose (version 1.12).

**Phylogenetic Analysis**

The 16S rRNA gene of strain D13-10-4-6\(^T\) was amplified by the primers 27F/1492R (Lane, 1991). The similarity of the 16S rRNA gene sequence between the strain D13-10-4-6\(^T\) and the validly published bacterial species was determined using EzBio-Cloud’s identify service\(^1\) (Yoon et al., 2017). The 16S rRNA gene sequence of the related strains was obtained from GenBank for the phylogenetic analysis. After multiple sequence alignment with Clustal W, the phylogenetic analysis was carried out using MEGA X by the neighbor-joining, maximum-parsimony methods (Kumar et al., 2018). *Aquidulcibacter paucihalophilus* TH1-2\(^T\) was used as an outgroup. The phylogenetic trees were evaluated by 1,000 bootstrap resamplings.

The gyrB gene sequences of the strain D13-10-4-6\(^T\) were obtained from its genomic sequences according to Altschul et al. (1990), and a 1,050 bp sequence was obtained. The gyrB gene sequences of the related strains were obtained from GenBank or their genome sequences. The phylogenetic trees based on the gyrB gene sequence were constructed using the maximum-likelihood, neighbor-joining, and maximum-parsimony methods as a description of 16S rRNA gene phylogenetic analysis.

Concatenated protein tree has a higher recognition than single phylogenetic marker gene tree (e.g., 16S rRNA and gyrB as a description of 16S rRNA gene phylogenetic analysis. likelihood, neighbor-joining, and maximum-parsimony methods (Kumar et al., 2018). Isoprenoid quinones were extracted from the strain D13-10-4-6\(^T\) as reported by Collins et al. (1977), analyzed by high-performance liquid chromatography (Groth et al., 1997; Du et al., 2013), and confirmed by liquid chromatography/mass spectrometry. After culturing for 2 days in TSB at 30°C, the cells were harvested at exponential phase and used for cellular fatty acids. Cellular fatty acids were extracted as reported by Kuykendall et al. (1988), analyzed using the Sherlock Microbial Identification System (Sasser, 1990).

**Chemotaxonomic Characterization**

The strain D13-10-4-6\(^T\) was shaken for 48 h in a tryptic soy broth (TSB; Difco) at 30°C, then collected by centrifuging at 10,000 rpm for 4 min. The harvested cells were freeze-dried and used to analyze the polar lipid and respiratory quinone. Polar lipids were analyzed by two-dimensional thin-layer chromatography as described by Minnikin et al. (1984). Isoprenoid quinones were extracted from the strain D13-10-4-6\(^T\) and analyzed using OrthoANI (Lee et al., 2016). The GGDC\(^2\) was used to calculate the dDDH values among the novel and its closely related reference strains (Meier-Kolthoff et al., 2013). The analysis of the average AAI and POCP among the strains in this work was carried out with CompareM\(^4\) and a Python script (POCP)\(^7\) (Xu et al., 2020), respectively. The pan-genome analysis was carried out with BPGA (Chaudhari et al., 2016) with default parameters.

**Phenotypic Characterization**

Growth conditions of the strain D13-10-4-6\(^T\) were determined at different temperature, pH, and salinity levels according to the method described by Li et al. (2016). The growth temperature was set at 4, 10, 15, 20, 25, 28, 30, 37, 41, and 45°C. The pH values for growth were adjusted to various pH values (pH 4.0–11.0, at intervals of 1.0 pH unit) by the buffers (Delory and King, 1945; Gomori, 1955) citrate and Na\(_2\)HPO\(_4\) buffer (pH 4.0–5.0), phosphate buffer (pH 6.0–7.0), Tris buffer (pH 8.0–9.0), and Na\(_2\)HPO\(_4\)/NaOH (pH 10.0–11.0). The salinity was determined in the range of 0–9% (w/v, intervals of 1%). Gram staining was performed according to the method described by Jenkins et al. (2003). To examine the anaerobic growth, the strain was incubated on TSA plates at 30°C for 1 week in an anaerobic jar (Li et al., 2016). The activities of catalase and oxidase were determined by the methods described by Smibert and Krieg (1994). Enzymatic activity, carbon source utilization, and acid production were performed by API ZYM, API 20 NE, API 50 CH, API 20E, and API 20NE (Letunic and Bork, 2021).

**Phylogenomic Metric Calculations**

Average nucleotide identity (ANI is a measure of similarity between two genomic sequences, which is a useful tool to differentiate bacterial species in common with DNA-DNA hybridization (DDH) (Goris et al., 2007; Richter and Rosselló-Móra, 2009). The ANI values among the novel strain D13-10-4-6\(^T\) and its closely related reference strains (*P. bohemicus* Cd-10\(^T\), *X. humili* IMT-291\(^T\)) were determined using OrthoANI (Lee et al., 2016). The GGDC\(^2\) was used to calculate the dDDH values among the novel and its closely related reference strains (Meier-Kolthoff et al., 2013). The analysis of the average AAI and POCP among the strains in this work was carried out with CompareM\(^4\) and a Python script (POCP)\(^7\) (Xu et al., 2020), respectively. The pan-genome analysis was carried out with BPGA (Chaudhari et al., 2016) with default parameters.

**Supplementary Material**

- Supplementary Table 1

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\(^1\)https://www.ezbiodcloud.net/identify

\(^2\)https://github.com/Ecogenomics/GtdbTk

\(^4\)https://github.com/2015qyliang/POCP

\(^7\)https://github.com/dparks1134/CompareM
RESULTS AND DISCUSSION

Genome Information

The genome of strains D13-10-4-6T and X. soli ZQBWt were sequenced and analyzed. In total, 63 contigs with a total sequence length of 4,683,906 bp for the strain X. soli ZQBWt were obtained, which was predicted to have 4,455 protein-coding genes, 47 tRNA genes, 3 rRNA genes, and 3 other RNA genes. The DNA G + C content was 67.6%. The strain D13-10-4-6T genome produced 66 contigs with a total sequence length of 4,605,234 bp, which was predicted to have 4,206 protein-coding genes, 45 tRNA genes, 3 rRNA genes, and 3 other RNA genes. The DNA G + C content of the strain D13-10-4-6T was 62.9%, which was similar to P. bohemicus Cd-10T (63.2%).

Phylogenetic Analyses

In this study, we have constructed phylogenetic trees based on the 16S rRNA gene, gyrB gene, and concatenated proteins (Figures 1–3) for representative members of the family Rhodobacteraceae encompassing 15 genera. The main groups clustering with the members of Rhodobacteraceae in 16S rRNA gene-based tree, gyrB gene-based tree, and concatenated proteins-based tree are almost consistent. The strains D13-10-4-6T, P. bohemicus Cd-10T, and X. humi IMT-291T form one monophyletic group to in turn form Pseudogemmobacter clade with strong bootstrap support in all three phylogenetic trees (Figures 1–3), which is far removed from the branch of X. soli (the type species of the genus Xinfangfangia). X. humi IMT-291T forms a distinct branch from the strains D13-10-4-6T and P. bohemicus Cd-10T in the Pseudogemmobacter clade. The results suggested that X. humi IMT-291T should be a species belonging to the genus Pseudogemmobacter, although P. bohemicus Cd-10T and X. humi IMT-291T were published almost simultaneously and shared 99.26% 16S rRNA gene sequence similarity with each other. The strain D13-10-4-6T forms a distinct branch from P. bohemicus Cd-10T and X. humi IMT-291T in all phylogenetic trees, and it has the highest 16S rRNA gene sequence similarity to P. bohemicus Cd-10T (97.6%) and X. humi IMT-291T (97.4%), and shares a less than 97% sequence similarity with all other validly published species. The results indicate that the strain D13-10-4-6T should belong to a novel species of the genus Pseudogemmobacter.

Several genera are non-monophyletic, such as Rhodobacter, Tabrizicola, and Xinfangfangia. In most of the cases, the 16S rRNA gene-based tree shows its low discriminatory power. For instance, the species of the genus Tabrizicola are divided into two branches in the 16S rRNA gene-based tree, but they are clustered into three distinct branches, not least T. aquatica RCR19T (type species of the genus) and T. piscis K13M18T are grouped together with R. thermarum YIM 73036T, R. flagellatus SYSU G03088T, and X. soli ZQBWt in trees based on gyrB gene and concatenated proteins tree with strong support.

It can be seen from the trees based on the 16S rRNA gene, gyrB gene, and concatenated proteins that Rhodobacter and Tabrizicola are two closely related non-monophyletic genera. The members of the genus Tabrizicola are observed in three clades, clades A, B, and C, which are labeled in Figures 1–3. Clade A, formed by Tabrizicola alkalilacus DJC, Tabrizicola sediminis DRYC-M-16T, and Tabrizicola algicola ETT8T, is next...
FIGURE 2 | Neighbor-joining phylogenetic trees based on partial gyrB gene sequences showing the position of strain D13-10-4-6T and reference strains. Bootstrap values over 70% (expressed as percentages of 1,000 replications) are shown. The scale bar corresponds to 0.05 substitutions per nucleotide site. Filled circles indicate branches recovered by maximum-likelihood method and open circles at branches recovered by the maximum-parsimony method.
FIGURE 3 | Phylogenetic tree among strain D13-10-4-6T and reference strains based on a concatenated alignment of 120 ubiquitous single-copy proteins. *Aquidulcibacter paucihalophilus* TH1-2T was used as an outgroup. The scale bar corresponds to 0.1 substitutions per amino acid position.
to the Gemmobacter clade and is far removed from two other Tabrizicola clades with a strong support in 16S rRNA gene-based, gyrB gene-based, and concatenated proteins-based trees. These results suggest that clade A should belong to a novel genus of the family Rhodobacteraceae. Tabrizicola clade B, grouped by T. aquatica RCR119T (type species of the genus), T. piscis K13M18T, R. thermarum YIM 73036T, R. flagellatus SYSU G03088T, and X. soli ZQBWTT, is a monophyletic cluster found in trees based on the gyrB gene and concatenated proteins with a strong support, indicating that R. thermarum YIM 73036T, R. flagellatus SYSU G03088T, and X. soli ZQBWTT should be transferred to the genus Tabrizicola. Tabrizicola fusiformis SY72T, located in clade B in the 16S rRNA gene-based tree, is clustered together with Tabrizicola oligotrophica KMS-5T to form clade C in both trees based on the gyrB gene and concatenated proteins, demonstrating that T. fusiformis SY72T and T. oligotrophica KMS-5T may be a novel genus of the family Rhodobacteraceae.

In the 16S rRNA gene-based tree, Cereibacter clade include R. alkaliotolerans JA916T, R. sediminicola JA983T), and members of the genus Cereibacter, except for Cereibacter changlensis JA139T (the type species). The species C. changlensis JA139T is observed in the Gemmobacter clade, which is similar to the results reported by Suresh et al. (2015). While C. changlensis JA139T is grouped in the Cereibacter clade and is located at the edge of the clade in trees inferred from the gyrB gene and concatenated proteins, indicating that it should belong to the genus Cereibacter, which is consistent to the results described by Suresh et al. (2015). R. sediminicola JA983T is clustered in the Cereibacter clade in all the trees based on the 16S rRNA gene, gyrB gene, and concatenated proteins, indicating that they should be transferred to the genus Cereibacter.

The genus Rhodobacter proposed by Imhoff et al. (1984) contains 13 species with validated names according to the LSPN. In trees based on the 16S rRNA gene, gyrB gene, and concatenated protein, R. tardus CYK-10T forms one distinct branch from other clades, suggesting that it should belong to a novel genus of the family Rhodobacteraceae. Three members (R. sediminicola JA983T, R. thermarum YIM73036T, R. flagellatus SYSU G03088T) are clustered into the Tabrizicola clade and Cereibacter clade, respectively. The other eight members of the genus Rhodobacter are temporarily classified into the genus Rhodobacter because of the absence of genome sequence of R. azollae, R. lacus, R. alkaliotolerans, and R. sediminis, although they are grouped into two clusters in the 16S rRNA gene tree.

Genomic, Chemotaxonomic, and Physiological Analysis of the Novel Strain

The ANI values between the strain D13-10-4-6T and its three closely related strains range from 74.4 to 81.2%, which are lower than the recommended ANI species boundary cutoff value (95–96%). The dDHH values between the strain D13-10-4-6T and its closely related strains are 19.7–24.3%, lower than the threshold for species (70%). Those data indicate that the strain D13-10-4-6T should belong to a novel species of the genus Pseudogemmobacter. Besides, P. bohemicus Cd-10T and X. humi IMT-291T share a 99.26% 16S rRNA gene sequence similarity, while their ANI and dDHH values are 79.1 and 22.1%, respectively (Table 1), which are lower than the species boundary cutoff values. Therefore, P. bohemicus Cd-10T and X. humi IMT-291T should belong to a different species of the genus Pseudogemmobacter.

The polar lipids of the strain D13-10-4-6T are phosphatidyldimonomethylethanolamine (PEM), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylcholine (PC), an unidentified phospholipid (PL), and six unidentified lipids (L) (Supplementary Figure 4). The presence of PE in the strain D13-10-4-6T is a useful characteristic to distinguish it from P. bohemicus and X. soli. The presence of DPG and absence of PC in the strain D13-10-4-6T are important characteristics to differentiate it from X. humi. The respiratory quinones detected in the strain D13-10-4-6T are Q-10 (91.3%) and Q-9 (8.7%), which are similar to P. bohemicus Cd-10T and X. humi IMT-291T. X. soli contains the only respiratory quinone of Q-10, which is different from the strains D13-10-4-6T, P. bohemicus Cd-10T, and X. humi IMT-291T. The phenotypic characterization of the strain D13-10-4-6T is listed in Table 2 and in the species description.

The predominant fatty acids of the strain D13-10-4-6T are C18:1ω7c (81.1%), C16:0 (5.4%), and C18:0 (4.1%). The detailed and differential fatty acids data of strain D13-10-4-6T and its related species are listed in Table 3. The percentage of C18:1ω7c in the novel strain can be used to distinguish it from P. bohemicus Cd-10T and X. humi IMT-291T. The absence of 11-methyl C18:1ω7c in the strain D13-10-4-6T is a useful characteristic to differentiate it from X. soli ZQBWTT.

Phylogenomic Metric Analysis

Average AAI is one of the well-established methods to separate prokaryotic genera (Luo et al., 2014; Rodriguez-R and Konstantinidis, 2014). It is proposed to be 65% AAI value for genera delineation of Bacteria and Archaea (Konstantinidis et al., 2017). However, the category thresholds of AAI for genus delineation are variable in many genera. For example, the value of 70% AAI is used to separate the genus Geomonas from the other genera of the family Geobacteraceae (Xu et al., 2020), and a range of 64.6–77.0% AAI is used to delineate different genera of the family Geobacteraceae (Xu et al., 2019). In this study, the values of AAI among 52 type strains from 15 related genera of the family Rhodobacteraceae have been determined and are listed in Table 4 and Supplementary Table 2. It can be seen from Table 4 that a gradient of 63.5–75.3% and 74.2–98.7% AAI values is found among the different clade (genera) and in the same clade of the family Rhodobacteraceae. Those data are consistent to the results of phylogeny based on concatenated proteins.

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Ma et al. Reclassification of Family Rhodobacteraceae

**TABLE 1** Average nucleotide identity (ANI), digital DNA–DNA hybridization (dDDH) values among D13-10-4-6T, *P. bohemicus* Cd-10T, *X. humi* IMT-291T, and *Xinfangfangia soli* ZQBW7T.

| Strain | D13-10-4-6T | Cd-10T | IMT-291T |
|--------|-------------|--------|-----------|
|        | ANI | dDDH | ANI | dDDH | ANI | dDDH |
| strain D13-10-4-6T | 100 |       | 100 |       | 100 |       |
| *Pseudogemmobacter bohemicus* Cd-10T | 81.2 | 24.3 | 79.1 | 22.1 | 74.7 | 19.4 |
| *Xinfangfangia humi* IMT-291T | 78.3 | 22.0 | 76.7 | 20.3 | 74.4 | 19.7 |
| *Xinfangfangia soli* ZQBW7T | 74.4 | 19.7 |       |       |       |       |

**TABLE 2** Differential characteristics of strain D13-10-4-6T and closely related reference strains.

| Characteristic | 1 | 2 | 3 | 4 |
|----------------|---|---|---|---|
| Cell shape     | Ovoid to rod-shaped | Ovoid to rod-shaped | Rod-shaped | Rod-shaped |
| Colour of colonies | Creamy white | Creamy white to Beige | Beige | Light yellow |
| Optimum pH     | 7.0–8.0 | 7.0–8.0 | 5.5–6.5 | 7.0–8.0 |
| Optimum temperature (°C) | 25–30 | 28 | 20–28 | 30 |
| Growth in max NaCl (% w/v) | 4 | 1 | 2 | 2 |
| Reduction of nitrate, indole production | – | + | – | – |
| Utilization of: |   |   |   |   |
| D-Glucose, D-mannose, D-mannitol | + | + | – | – |
| L-Arabinose | + | – | + | – |
| D-Maltose | – | + | – | – |
| L-Rhamnose, N-acetyl-glucosamine | + | – | – | – |
| Enzyme activities: |   |   |   |   |
| Lipase (C14) | W | + | – | – |
| Valine arylamidase | W | + | – | – |
| Cystine arylamidase | – | + | – | – |
| α-Chymotrypsin | – | + | – | – |
| α-Glucoamidase | + | – | – | + |
| N-acetyl-β-glucosaminidase | + | – | – | – |
| Trypsin | – | + | – | – |
| Hydrolysis from: |   |   |   |   |
| Aesculin | + | – | – | – |
| Gelatin | – | – | + | – |
| Predominant polar lipids | PME, DPG, PE, PG | PE, PME, PG, PC, PC, PC, PME | PE, PME, PG, PC, PC, PC, PME |
| G + C content (%) | 62.9 | 63.2 | 66.5 | 67.0 |

1, strain D13-10-4-6T; 2, *Pseudogemmobacter bohemicus* Cd-10T (data from Suman et al., 2019); 3, *Xinfangfangia humi* IMT-291T (data from Kämpfer et al., 2019); 4, *Xinfangfangia soli* ZQBW7T (data from this study). +, positive; -, negative; W, weakly positive.

The *Pseudogemmobacter* clade, including strains D13-10-4-6T, *P. bohemicus* Cd-10T and *X. humi* IMT-291T, has 77.4–81.8% AAI values among each other and shows 63.8–73.6% AAI values among the members of other clades in this study (Table 4 and Figure 4), which is consistent to the results of phylogeny based on the 16S rRNA gene, gyrB gene, and concatenated proteins (Figures 1–3). The *Cereibacter* clade, including *R. sediminicola* JA983T and members of the genus *Cereibacter*, has 75.8–98.7% AAI values among the members of the clade and 65.2–72.7% AAI values among the members from the other clades in this work, indicating that *R. sediminicola* should be transferred to the genus *Cereibacter* (Table 4 and Supplementary Figure 1). Similarly, the AAI values within the *Tabrizicola* clade A and *Tabrizicola* clade B can also distinguish them from the other strains (Table 4 and Supplementary Figures 2, 3).

Percentage of conserved protein is another method for genus delineation of prokaryote, and the value for genera delineation of POCP is proposed to be 50% (Qin et al., 2014). While the thresholds of POCP for genus delineation are also variable in many genera. The value of 65% POCP was used to separate the genus *Geomonas* from the other genus of the family *Geobacteraceae* (Xu et al., 2020), and most of the POCP values within the *Roseobacter* group comparisons were greater than 50% of the family *Rhodobacteraceae* (Wirth and Whitman, 2018). In this work, we also calculated the POCP values among the 52
type strains of 15 related genera of the family Rhodobacteraceae (Supplementary Table 2). The results show that a gradient of 41.7–68.1% POCP, except the values between, was found among the different clade (genera) of the family Rhodobacteraceae, and 56.5–88.6% among the species of the same clade. Therefore, it is hard to use the same thresholds for genus delineation because they show a broad range of values from both intragenus and intergeneric. But for several clades, it is useful to distinguish one group from the others, for instance, members of the Pseudogemmobacter clade show the values of POCP from 61.0 to 62.9% among each other and have 41.7–59% POCP values among members from the other clades (Table 4 and Figure 4). The same goes for the Cereibacter clade and Tabrizicola clade A (Table 4 and Supplementary Figures 1, 2).

The POCP values in this study are not always consistent to the phylogenetic analysis, as exemplified by the Tabrizicola clade B. Members of the Tabrizicola clade B show 45.2–68.1% POCP values among each other, and 65.9–85.1% POCP values among the members of other clades, respectively (Table 4). The POCP values can distinguish Tabrizicola clade B from other related members except for T. oligotrophica KMS-5T, Fusovulum blasticum DSM 2131T, and Gemmobacter aestuarii CC-PW-75T (Supplementary Figure 3). The POCP values between X. soli ZQBW and other members in Tabrizicola clade B (Supplementary Figure 3). Due to X. soli ZQBW forming a stable clade within Tabrizicola clade B and forming a distinct clade with T. oligotrophica KMS-5T, F. blasticum DSM 2131T, and G. aestuarii CC-PW-75T, we analyzed the pan-genome of Tabrizicola clade B in a supplementary analysis.

The pan-genome analysis was used in the classification of bacteria (Awan et al., 2018; Suresh et al., 2019). The amount of core genes was sensitive to heterogeneous in the core- and pan-genome analysis (Inglin et al., 2018; Suresh et al., 2019). The core gene numbers within were Tabrizicola clade B were considerably higher than those within the Tabrizicola clade B and T. oligotrophica KMS-5T, F. blasticum DSM 2131T, and G. aestuarii CC-PW-75T (Table 5), indicating that the

| TABLE 3 | Cellular fatty acid profiles of strain D13-10-4-67 and closely related type strains. |
|-----------------------------------------------|
| Fatty acid | 1 | 2 | 3 | 4 |
| C18:0 | 4.1 | 26.3 | 2.5 | 3.1 |
| C16:0 | 5.4 | 19.9 | 10.5 | 3.5 |
| C16:1 w7c | 1.5 | 2.9 | NA | – |
| C10:0 3-OH | 2.4 | NA | 2.8 | 0.9 |
| C11:0 3-OH | 2.6 | NA | NA | 1.4 |
| 11-methyl C18:1 w7c | – | NA | 22.7 | 2.3 |
| C15:0 20c | 81.1 | 50.3 | 58.8 | 85.2 |

1, strain D13-10-4-67; 2, Pseudogemmobacter bohemicus Cd-10T (data from Suman et al., 2019); 3, Xinfangfanga humi IMT-291T (data from Kämpfer et al., 2019); 4, Xinfangfanga soli ZQBW (data from this study). NA, not available; –, not detected.

| TABLE 4 | Average amino acid identity (AAI) values and percentage of conserved protein (POCP) values of all strains for the intragenus and intergeneric comparisons. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Organism | Intragenus | Intergeneric | Intragenus | Intergeneric |
| Pseudogemmobacter clade | 77.4–81.8 | 63.8–73.6 | 61.0–62.9 | 41.7–59.0 |
| Cereibacter clade | 75.8–96.7 | 65.2–72.7 | 60.9–88.6 | 44.6–62.7 |
| Rhodobacter clade | 79.5–90.1 | 64.2–75.3 | 70.4–79.3 | 41.9–65.5 |
| Rhodobacter tardus | 100 | 63.5–70.9 | 100 | 42.8–61.9 |
| Tabrizicola clade A | 84.6–87.0 | 63.9–73.2 | 70.4–73.3 | 45.6–65.7 |
| Tabrizicola clade B | 79.5–86.7 | 64.2–74.4 | 65.9–85.1 | 45.2–68.1 |
| Tabrizicola clade C | 76.18 | 65.0–74.0 | 67.4 | 45.9–67.8 |
| Gemmobacter | 69.2–93.0 | 64.7–73.26 | 51.0–77.7 | 42.2–67.8 |
| Cypionella | 100 | 63.8–74.0 | 100 | 42.7–63.4 |
| Falcigemmobacter | 100 | 63.8–66.6 | 79.7 | 42.7–52.2 |
| Falaihrhodobacter | 100 | 64.6–69.6 | 100 | 41.7–51.5 |
| Fusovulum | 100 | 65.0–74.4 | 100 | 48.2–68.1 |
| Haematobacter | 92.68 | 63.8–68.1 | 81.8 | 44.0–53.2 |
| Paenirhodobacter | 100 | 64.7–78.4 | 100 | 45.7–63.8 |
| Pheavulum | 74.2 | 64.7–72.4 | 69.2 | 45.2–65.5 |
| Pseudorhodobacter clade | 78.3–83.5 | 63.9–71.5 | 61.9–73.2 | 44.3–67.2 |
| Sinirhodobacter | 74.4–96.1 | 63.5–75.3 | 56.5–88.3 | 44.3–63.8 |

Pseudogemmobacter clades include strain D13-10-4-67, Pseudogemmobacter bohemicus Cd-10T, and Xinfangfanga humi IMT-291T. Cereibacter clades include Rhodobacter sediminicola JA983T and members of the genus Cereibacter. Rhodobacter clades include Rhodobacter maris JA276T, Rhodobacter aestuarii JA296T, Rhodobacter capsulatus DSM 1710T, and Rhodobactervindinis JA737T. Tabrizicola clade A includes Tabrizicola alkaliicus DJC4T, Tabrizicola sediminis DRYC-M-16T, and Tabrizicola algicola ETT8T. Tabrizicola clade B includes Xinfangfanga soli ZQBW, Rhodobacter flagellatus SYSU G03088T, Rhodobacter thermarum YIM 73036T, Tabrizicola aquatic RCRI19T, and Tabrizicola piscis K13M18T. Tabrizicola clade C includes Tabrizicola fusiformis SY72T and Tabrizicola oligotrophica KMS-5T.
FIGURE 4 | The relationship of AAI and POCP between strain D13-10-4-6T, P. bohemicus Cd-10T, X. humi IMT-291T, and the related strains in the family Rhodobacteraceae. The dots inside the dashed line represent the values between strain D13-10-4-6T, P. bohemicus Cd-10T, and X. humi IMT-291T, and those outside represent the values between the three strains and strains in the related genera of the family Rhodobacteraceae. A total of 52 genomes were included in this analysis.

TABLE 5 | Pan-genomic analysis of Tabrizicola clade B and the related strains.

| Clade                                      | Organism name                                   | No. of core genes | No. of other genes |
|--------------------------------------------|------------------------------------------------|-------------------|--------------------|
| All eight strains                          | Rhodobacter flagellatus SYSU G03088T            | 1,656             | 1,892              |
|                                            | Rhodobacter thermarum YIM 73036T               | 1,656             | 1,873              |
|                                            | Tabrizicola aquatica RCRI19T                    | 1,656             | 2,112              |
|                                            | Tabrizicola piscis K13M18T                      | 1,656             | 2,522              |
|                                            | Xinfangfangia soli ZQBW T                       | 1,656             | 2,877              |
|                                            | Tabrizicola oligotrophica KMS-5T                | 1,656             | 2,087              |
|                                            | Fuscovulumlasticum DSM 2131T                    | 1,656             | 1,896              |
|                                            | Gemmobacter estuarii CC-PW-75T                  | 1,656             | 1,933              |
| Tabrizicola clade B                        | Rhodobacter flagellatus SYSU G03088T            | 2,144             | 1,442              |
|                                            | Rhodobacter thermarum YIM 73036T               | 2,144             | 1,414              |
|                                            | Tabrizicola aquatica RCRI19T                    | 2,144             | 1,651              |
|                                            | Tabrizicola piscis K13M18T                      | 2,144             | 2,126              |
|                                            | Xinfangfangia soli ZQBW T                       | 2,144             | 2,571              |

relationships of *Tabrizicola* clade B and the three species were heterogeneous and the *Tabrizicola* clade B should belong to a genus different from the three species. The pan-genome analysis reinforces the results of gyrB and concatenated protein phylogenetic trees. In conclusion, *Tabrizicola* clade B should belong to the same genus.
CONCLUSION

Phylogenetic trees based on the 16S rRNA gene, gyrB gene, and concatenated alignment of 120 ubiquitous single-copy proteins were constructed to clarify the relationship of the members from the 15 closely related genera within the family Rhodobacteraceae. The AAI, POCIP, and ANI analyses as well as chemotaxonomic and physiological tests were also performed and used as supplementary evidence. On the basis of the data obtained, the taxonomic proposals were (1) reclassification of R. tardus as the type species of a novel genus, Stagnihabitans gen. nov., as Stagnihabitans tardus comb. nov.; (2) reclassification of T. alkalilacus, Tabrizicola sediminis, and Tabrizicola algicola into a novel genus, Pseudotabrizicola gen. nov., as Pseudotabrizicola alkalilacus comb. nov., Pseudotabrizicola sediminis comb. nov., Pseudotabrizicola algicola comb. nov.; (3) reclassification of Rhodobacter sediminicola into the genus Cereibacter as Cereibacter sediminicola comb. nov.; (4) reclassification of Rhodobacter flagellatus, Rhodobacter thermarum, and X. soli into the genus Tabrizicola as Tabrizicola flagellatus comb. nov., Tabrizicola thermarum comb. nov., and Tabrizicola soli comb. nov.; (5) reclassification of X. humi into the genus Pseudogemmobacter as Pseudogemmobacter humicola comb. nov.; and (6) classification of strain D13-10-4-6T as a novel species of the genus Pseudogemmobacter, for which the name Pseudogemmobacter hezensis sp. nov. is proposed; the type strain is D13-10-4-6T (= CFCC 12033T = KCTC 82215T).

Description of Pseudogemmobacter hezensis Sp. nov.

Pseudogemmobacter hezensis (he.zen’sis. N.L. masc./fem. adj. hezensis, of Heze, a city in Shandong Province, China, where the organism was isolated).

Cells are Gram-stain-negative, aerobic, non-motile, catalase- and oxidase-positive, ovoid to rod-shaped, 1.6–2.0 μm in length and 0.8–1.0 μm in width. Colonies are creamy white, circular, smooth, with entire margins after incubation for 2 days at 28°C on TSA. The strain can grow at 15–37°C (optimum, 25–30°C), at pH 6–10 (optimum, pH 7–8). Growth occurs at a concentration of 0–4% (w/v) NaCl. It is positive for the activity of alkaline phosphatase, esterase lipase (C8), esterase (C4), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, N-acetyl-β-glucosaminidase; weakly positive for lipase (C14), β-galactosidase; negative for cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-galactosidase, α-mannosidase, and α-fucosidase (API ZYM). It is negative for reduction of nitrate to nitrogen, reduction of nitrate to nitrite, indole production, gelatin hydrolysis, and the activity of urease, arginine dihydrolase; positive for the utilization of glucose, D-mannose, L-arabinose, D-mannitol, N-acetyl-glucosamine, malic acid (API 20 NE). Acid is produced from L-arabinose, D-xylene, D-galactose, D-fructose, L-rhamnose, D-lyxose, D-fucose, L-fucose; weakly positive for erythritol, D-arabinose, D-ribose, and L-sorbose (API 50 CH). The polar lipids are PME, DPG, PE, PG, PC, PL1, and six unidentified lipids (L). The respiratory quinones are Q-10 and Q-9. The predominant fatty acids are C18:1ω7c. The type strain is D13-10-4-6T (= CFCC 12033T = KCTC82215T), isolated from the bark samples of Populus × euramericana in Shandong Province, China. The DNA G + C content is 62.9%.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and genome sequences for strain D13-10-4-6T is MT036106 and JABJXT000000000, respectively.

Description of Pseudogemmobacter humi Comb. nov.

Pseudogemmobacter humi (hu’mi. L. gen. fem. n. humi, of/from soil, the isolation source of the type strain).

Basonym: Xinfangangia humi (Kämpfer et al., 2019).
The description of Pseudogemmobacter humi is the same as that given for X. humi by Kämpfer et al. (2019). The type strain is IMT-291T (= LMG 30636T = CIP 111625T = CCM 8858T).

Emended Description of the Genus Pseudogemmobacter

The description as given by Suman et al. (2019) remains correct except that the species are positive or negative for catalase and nitrate reductase.

Description of C. sediminicola Comb. nov.

Cereibacter sediminicola (se.di.mi.ni’co.la. L. neut. n. sedimen, sediment; L. masc./fem. n. incola, dweller; from L. masc./fem. n. sediminicola, dweller of sediments).

Basonym: Rhodobacter sediminicola (Suresh et al., 2020).
The description of C. sediminicola is the same as that given for R. sediminicola by Suresh et al. (2020). The type strain is JA983T (= KCTC 15782T = NBRC 113843T).

Description of Stagnihabitans Gen. nov.

Stagnihabitans (Sta.gni.ha’bi.tans. L. neut. n. stagnum, a small area of water, pond; L. pres. part. Habitans, an inhabitant; N.L. masc. n. Stagnihabitans, an inhabitant of pond water).

Cells are Gram-stain-negative, aerobic, non-motile, oxidase-positive, catalase-negative, ovoid to rod-shaped and divide by binary fission, sometimes forming chains. The predominant respiratory quinone is Q-10. The major cellular fatty acid is C18:1ω7c. PE, PG, and PC are the major polar lipids. The DNA G + C content is 66%. The member of the genus is separated from Rhodobacter based on the 16S rRNA, gyrB and concatenated protein phylogenetic trees, genome comparison. The type species is S. tardus comb. nov.

Description of S. tardus Comb. nov.

Stagnihabitans tardus (tar’dus. L. masc.adj. tardus, slow, referring to the slow growth of the organism).

Basonym: Rhodobacter tardus (Sheu et al., 2020).
The description of *S. tardus* is the same as that given for *R. tardus* by Sheu et al. (2020). The type strain is CYK-10\(^T\) (= BCRC 81191\(^T\) = LMG 31336\(^T\)).

**Description of Pseudotabrizicola Gen. nov.**

*Pseudotabrizicola* (Pseu.do.ta.bri.zi.co.la. Gr. masc./fem. adj. pseudéś, false; N.L. fem. n. *Tabrizicola*, a bacterial generic name; N.L. fem. n. *Pseudotabrizicola*, false *Tabrizicola*).

Cells are Gram-stain-negative, aerobic, non-motile, catalase- and oxidase-positive, rod-shaped. PG, DPG, PE, and PC are the major polar lipids. The predominant respiratory quinone is Q-10. The major cellular fatty acids are usually iso-C\(_{18:0}\), C\(_{18:1}\) \(ω_7\)c, and/or C\(_{18:1}\) \(ω_6\)c. The DNA G + C content is 62.9–64.4%. Members of the genus are separated from *Tabrizicola* based on the 16S rRNA, gyrB, and concatenated proteins phylogenetic trees, genome comparison. The type species is *P. sediminis* comb. nov.

**Description of *P. sediminis* Comb. nov.**

*Pseudotabrizicola sediminis* (se.di’mi.nis. L. gen. net. n. sediminis, of a sediment).  
Basonym: *Tabrizicola sediminis* (Liu et al., 2019).  
The description of *P. sediminis* is the same as that given for *Tabrizicola sediminis* by Liu et al. (2019). The type strain is DRYC-M-16\(^T\) (= CGMCC 1.13881\(^T\) = KCTC 72105\(^T\)).

**Description of *P. alkalilacus* Comb. nov.**

*Pseudotabrizicola alkalilacus* (al.ka.li.la’cus. N.L. neut. n. alkalilacus of saltwort, alkalii; L. masc. n. lacus, a lake; N.L. gen. masc. n. alkalilacus of analkaline lake).  
Basonym: *Tabrizicola alkalilacus* (Phurbu et al., 2019).  
The description of *Pseudotabrizicola alkalilacus* is the same as that given for *T. alkalilacus* by Phurbu et al. (2019). The type strain is DJC\(^T\) (= CICC 24242\(^T\) = KCTC 62105\(^T\)).

**Description of *P. algicola* Comb. nov.**

*Pseudotabrizicola algicola* (al.gi’co.la. L. fem. n. algae, an alga; L. masc./fem. suff.-cola, dweller; from L. masc./fem. n. incola an inhabitant; N.L. masc./fem. n. algicola an inhabitant of algae).  
Basonym: *Tabrizicola algicola* (Park et al., 2020).  
The description of *P. algicola* is the same as that given for *Tabrizicola algicola* by Park et al. (2020). The type strain is ETT8\(^T\) (= KCTC 72206\(^T\) = JCM 31893\(^T\) = MCC 4339\(^T\)).

**Description of *T. flagellatus* Comb. nov.**

*Tabrizicola flagellatus* (fla.ge.la’tus. L. masc. part. adj. flagellatus, flagellated).  
Basonym: *Rhodobacter flagellatus* (Xian et al., 2020).  
The description of *T. flagellatus* is the same as that given for *Rhodobacter flagellatus* by Xian et al. (2020). The type strain is SYSU G03088\(^T\) (= CGMCC 1.16876\(^T\) = KCTC 72354\(^T\)).

**Description of *T. thermarum* Comb. nov.**

*Tabrizicola thermarum* (ther.ma’rum. L. gen. fem. pl. n. thermarum, of hot springs).  
Basonym: *Rhodobacter thermarum* (Khan et al., 2019).  
The description of *T. thermarum* is the same as that given for *Rhodobacter thermarum* by Khan et al. (2019). The type strain is YIM 73036\(^T\) (= KCTC 52712\(^T\) = CCTCC AB 2016298\(^T\)).

**Description of *T. soli* Comb. nov.**

*Tabrizicola soli* (so’li. L. neut. n. soli of soil, the source of the type strain).  
Basonym: *Xinfangfangia soli* (Hu et al., 2018).  
The description of *T. soli* is the same as that given for *X. soli* by Hu et al. (2018). The type strain is ZQBW\(^T\) (= KCTC 62102\(^T\) = CCTCC AB 2017177\(^T\)).

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI—MT036106, JABIX'T00000000, JAEACP00000000.

**AUTHOR CONTRIBUTIONS**

CP and YL designed the experiments, provided the methods, and revised the manuscript. TM finished the manuscript and completed most of the experiments. HX helped to reconstructed and analyzed the gene trees. DB finished the fatty acid profiles. CL and MY collected the samples. All authors read and approved the final version of the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.849695/full#supplementary-material
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