INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

Ma *et al.*, *Int. J. Syst. Evol. Microbiol.* 2023;73:005747

DOI 10.1099/ijsem.0.005747

MICROBIOLOGY SOCIETY

Dyadobacter chenhuakuii sp. nov., *Dyadobacter chenwenxiniae* sp. nov., and *Dyadobacter fanqingshengii* sp. nov., isolated from soil of the Qinghai-Tibetan Plateau

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Abstract

Six novel bacterial strains, designated CY22^T, CY357, LJ419^T, LJ53, CY399^T and CY107 were isolated from soil samples collected from the Qinghai–Tibetan Plateau, PR China. Cells were aerobic, rod-shaped, yellow-pigmented, catalase- and oxidase-positive, Gram-stain-negative, non-motile and non-spore-forming. All strains were psychrotolerant and could grow at 0 °C. The results of phylogenetic and phylogenomic analyses, based on 16S rRNA gene sequences and core genomic genes, indicated that the three strain pairs (CY22^T/CY357, LJ419^T/LJ53 and CY399^T/CY107) were closely related to members of the genus *Dyadobacter* and clustered tightly with two species with validly published names, *Dyadobacter alkalitolerans* 12116^T and *Dyadobacter psychrophilus* BZ26^T. Values of digital DNA–DNA hybridization between genome sequences of the isolates and other strains from the GenBank database in the genus *Dyadobacter* were far below the 70.0% threshold. The genomic DNA G+C content of these six strains ranged from 45.2 to 45.8%. The major cellular fatty acids of all six strains were *iso*-$C_{15:0}$ and summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). MK-7 was the only respiratory quinone, and phosphatidylethanolamine was the predominant polar lipid for strains CY22^T, LJ419^T and CY399^T. On the basis of the phenotypic, phylogenetic and genomic evidence presented, these six strains represent three novel members of the genus *Dyadobacter*, for which the names *Dyadobacter chenhuakuii* sp. nov., *Dyadobacter chenwenxiniae* sp. nov. and *Dyadobacter fanqingshengii* sp. nov. are proposed. The type strains are CY22^T (= GDMCC 1.3045^T = KCTC 92299^T), LJ419^T (= GDMCC 1.2872^T = JCM 33794^T) and CY399^T (= GDMCC 1.3052^T = KCTC 92306^T), respectively.

INTRODUCTION

The genus *Dyadobacter*, a member of the family *Spirosomaceae* within the phylum *Bacteroidota*, was first described by Chelius and Triplett in 2000 [1] and then amended by Reddy and Garcia-Pichel in 2005 [2]. Members of the genus *Dyadobacter* are characterized as aerobic, catalase- and oxidase-positive, Gram-stain-negative, non-motile, non-spore-forming, rod-shaped and yellow-pigmented. In young cultures cells occur in pairs, and chains of coccoid cells form in old cultures [1, 2]. Most species of the genus can endure temperatures as low as 4 °C. The members have MK-7 as the predominant respiratory quinone and phosphatidylethanolamine (PE) as the dominant polar lipid. The typical fatty acids comprise *iso*-$C_{15:0}$, $C_{16:1}\omega5c$, *iso*-$C_{17:0}$

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Keywords: *Dyadobacter*; phylogenetic analysis; phylogenomic analysis; Qinghai-Tibet Plateau; soil.
Abbreviations: ANI, average nucleotide identity; Csps, cold shock proteins; Dar, dialkylresorcinol; dDDH, digital DNA-DNA hybridization; PE, phosphatidylethanolamine; Usps, universal stress proteins.
The GenBank accession numbers for the 16S rRNA gene sequences of strains CY22^T, CY357, LJ419^T, LJ53, CY399^T and CY107 are OM884033, OM857550, OM857832, OM857630, OM857592 and OM884043, respectively. The GenBank accession numbers for the genome sequences of strains CY22^T, CY357, LJ419^T, LJ53, CY399^T and CY107 are CP098805, JAKFFV000000000, CP094997, JAJTTB000000000, CP098806 and JAKFFR000000000, respectively.
Five supplementary figures and four supplementary tables are available with the online version of this article.

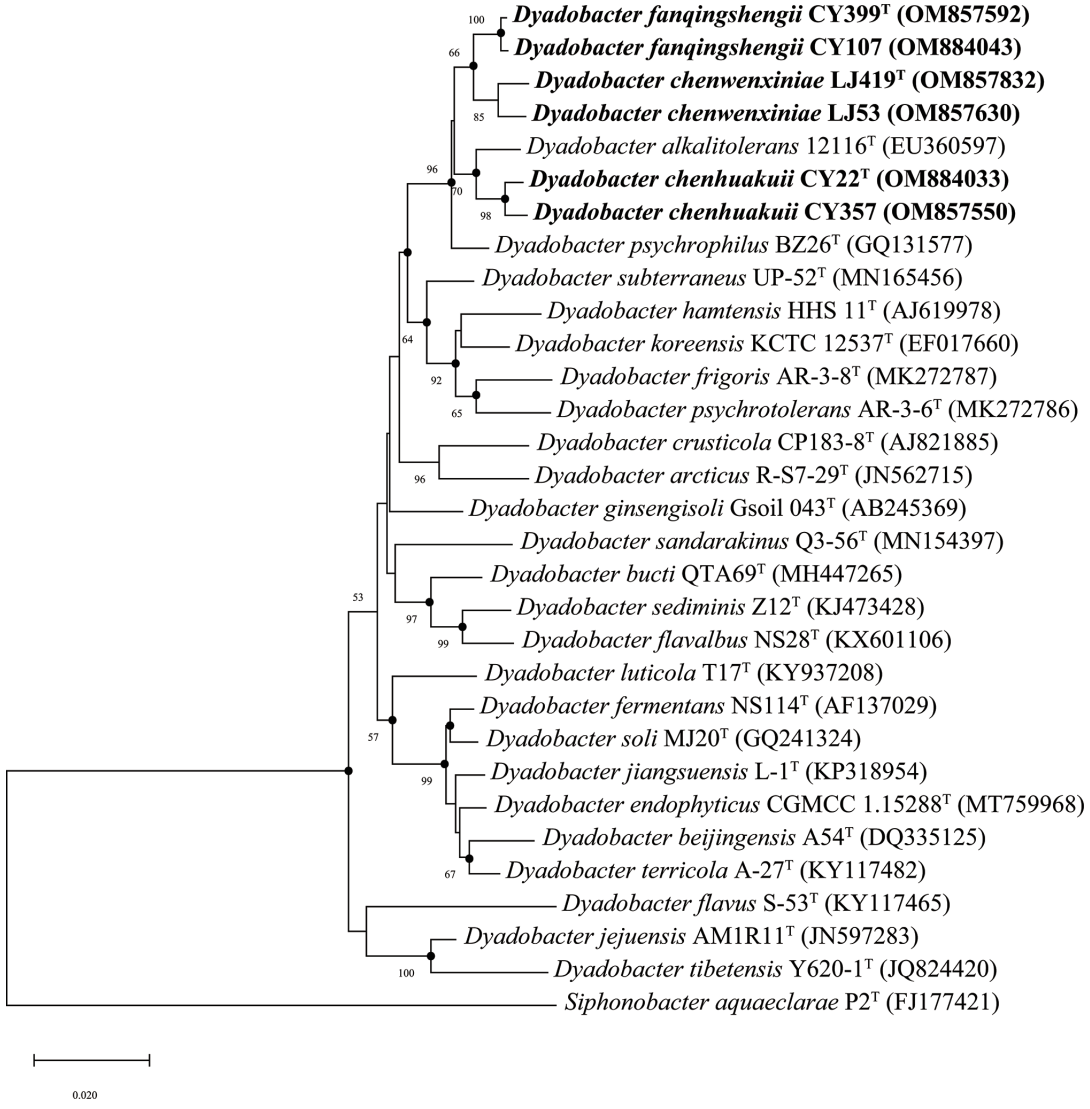

Fig. 1. Neighbor-joining tree based on 16S rRNA gene sequences to show the phylogenetic position of the isolates among all the members of the genus *Dyadobacter* with validly published names. Numbers at nodes indicate bootstrap percentages (based on 1000 replications); only values exceeding 50% are shown. The sequence of *Siphonobacter aquaeclarae* P2^T (FJ177421) was used as an outgroup. GenBank accession numbers are given in parentheses. Novel strains from the present study are highlighted in bold type. Bar, 0.020 substitutions per nucleotide position. Filled circles indicated that the nodes of the tree are also supported by both the minimum-evolution algorithm and the maximum-likelihood algorithm.

3-OH and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). The DNA G+C content approximately ranges from 40.0 to 51.3%. At the time of writing, the genus *Dyadobacter* comprises 24 species with validly published names (https://lpsn.dsmz.de/genus/dyadobacter) originally isolated from various natural environments including plant materials [1, 3, 4], soil [2, 5–12], water [13–15], desert sand [16], sediment [17–20] and glacial ice [21, 22].

The Qinghai–Tibet Plateau has unique microbial resources due to its special ecological environment. In this study, we further revealed the distribution of microorganisms in the Qinghai–Tibet Plateau by investigating the diversity of soil bacteria. As a subproject, a polyphasic taxonomic characterization was conducted for six previously uncharacterized strains, CY22^T, CY357, LJ419^T, LJ53, CY399^T and CY107, isolated from soil samples collected from the Qinghai–Tibetan Plateau (33°25′25″ N 96°22′38″ E, 33°25′25″ N 96°22′40″ E and 32°49′21″ N 97°11′56″ E).

SAMPLING AND ISOLATION

The Qinghai–Tibetan Plateau is the youngest geological structural unit on earth, with a vast planation surface, basins and mountains. In this study, the bacterial diversity in the soil of the Qinghai–Tibetan Plateau was investigated. Soil samples

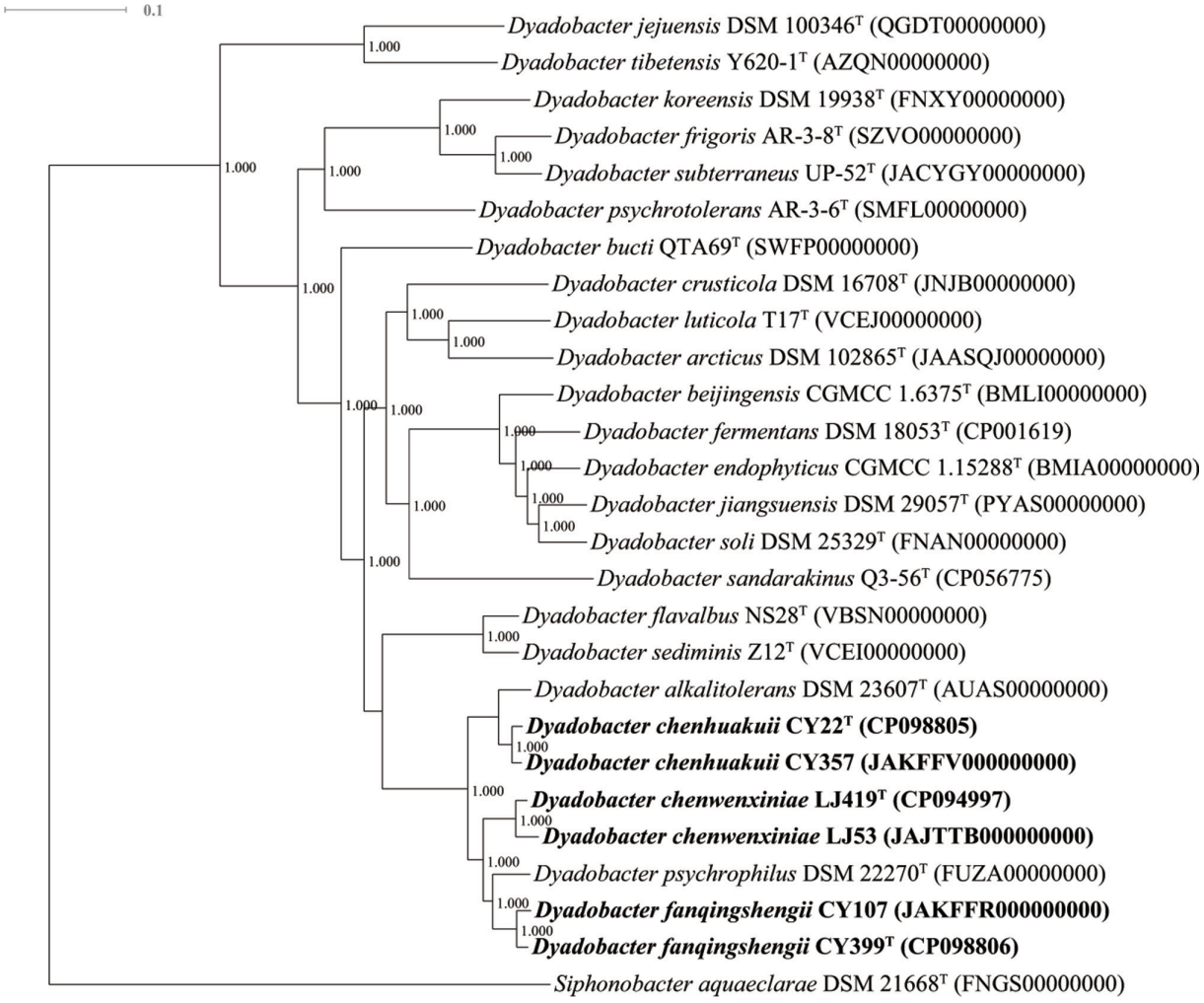

Fig. 2. Phylogenomic tree based on 1527 core genomic genes annotated from genome sequences of the isolates and other strains from the GenBank database in the genus *Dyadobacter*. Numbers on the tree indicate each split in the tree support values with the Shimodaira–Hasegawa test calculated for 1000 resamples. *Siphonobacter aquaeclarae* DSM 21668^T was used as an outgroup. Novel strains from the present study are highlighted in bold type. Bar, 0.1 substitutions per nucleotide position.

were collected in July 2017. After removing the rhizomes, eight soil cores (15 cm in depth, 7 cm in diameter) were collected in a 10 m^2 area of each grassland plot. Then the eight soil cores were mixed and sieved through a 2 mm mesh to remove apparent roots and stones. A total of 75 soil samples were collected at different geographical locations in the Yushu Tibetan Autonomous Prefecture of Qinghai Province, PR China. Each soil sample was kept in a 50 ml sterile tube, stored in a cooler with ice packs before transported to our laboratory and preserved at −80 °C until use. After being diluted serially with sterile water, soil samples were plated on Reasoner's 2A (R2A) agar (Oxoid) plates and incubated at 28 °C for 5 days. Pure cultures were obtained by randomly selecting single colonies and transferring them onto new R2A agar plates under the same conditions. The isolates were routinely stored at −80 °C in R2A broth supplemented with glycerol (20%, v/v) for further tests.

PHYLOGENETIC AND PHYLOGENOMIC ANALYSES

The 16S rRNA genes of these six strains were amplified by PCR using two universal primers, 27F and 1492R [23], which were also used for subsequent cloning and sequencing. The purified PCR products were ligated into pEASY-T3 cloning vectors and then transformed into competent *Escherichia coli* DH5α cells [24]. The nearly complete 16S rRNA gene sequences were obtained by picking positive clones and sequencing. Each 16S rRNA gene sequence obtained was uploaded to the NCBI GenBank to search for similar sequences via the Basic Local Alignment Search Tool. All the 16S rRNA gene sequences showed high similarities to those of species within the genus *Dyadobacter*. The resulting similarities were below the 98.7% threshold for categorizing the strains into different bacterial species [25]. The closest relative of these six strains was *Dyadobacter psychrophilus* BZ26^T, with

Table 1. Average nucleotide identity (ANI; bold type, lower left) and digital DNA–DNA hybridization (dDDH; upper right) values between the isolates and phylogenetically related type strains in the genus *Dyadobacter*

Strains: 1, CY22^T; 2, CY357; 3, LJ419^T; 4, LJ53; 5, CY399^T; 6, CY107; 7, *D. alkalitolerans* CCTCC AB 207176^T; 8, *D. psychrophilus* CGMCC 1.8951^T.

Strains	1	2	3	4	5	6	7	8
1	–	78.2	25.8	25.7	26.6	26.4	54.3	26.2
2	**97.6**	–	25.6	25.6	26.2	26.3	54.4	26.1
3	**82.5**	**82.3**	–	79.0	28.9	28.8	25.8	28.0
4	**82.3**	**82.3**	**97.5**	–	28.7	28.7	25.8	28.0
5	**83.2**	**83.0**	**85.0**	**84.9**	–	84.8	26.1	31.2
6	**83.1**	**83.0**	**85.0**	**84.9**	**98.3**	–	26.1	31.2
7	**94.0**	**93.9**	**82.3**	**82.6**	**82.9**	**82.9**	–	25.7
8	**83.1**	**82.9**	**84.3**	**84.2**	**86.4**	**86.3**	**82.7**	–

Digital DNA–DNA hybridization values were calculated using Formula 2.

similarities of 98.13–98.43%. Among the isolates, pairwise internal 16S rRNA gene similarities were 99.45% (strains CY22^T and CY357), 99.00% (strains LJ419^T and LJ53) and 99.93% (strains CY399^T and CY107). Pairwise similarities between any two novel type strains were 97.82–98.50%. Therefore, as per the preliminary identification based on 16S rRNA sequences, these six isolates represent three novel species.

For further phylogenetic and phylogenomic analyses of these six isolates, the 16S rRNA gene and whole-genome sequences of all species with validly published names in the genus *Dyadobacter* were accessed from the NCBI GenBank database. Phylogenetic trees based on the 16S rRNA gene sequences were reconstructed using the MEGA X software package with three tree-making algorithms, neighbor-joining [26], minimum-evolution [27] and maximum-likelihood [28]. Multiple alignments were performed using Clustal_W; the reliability of the tree topologies was estimated based on 1000 bootstrap replications [29], and the evolutionary distances were generated using Kimura's two-parameter calculation model [30]. The phylogenetic tree based on the neighbor-joining method (Fig. 1) indicated that these six isolates were closely related to the genus *Dyadobacter* and grouped tightly in a cluster with two species with validly published names, *Dyadobacter alkalitolerans* 12116^T and *Dyadobacter psychrophilus* BZ26^T. In this cluster, these six strains formed three novel clades: strain pairs CY22^T/CY357 (clade I), LJ419^T/LJ53 (clade II) and CY399^T/CY107 (clade III). This relationship was also supported by another similar topology generated by the minimum-evolution algorithm (Fig. S1, available in the online version of this article). The maximum-likelihood tree (Fig. S2) seemed to be slightly unstable compared with the neighbor-joining and minimum-evolution phylogenetic trees, as the strain pair LJ419^T/LJ53 was not tightly bound in clade II.

To further confirm the taxonomic status, a whole-genome-based phylogenetic tree was reconstructed. Genomic DNA of all six strains was extracted using a commercially available genomic DNA extraction kit (Wizard Genomic DNA Purification Kit, Promega) following the manufacturer's instructions. The purity and quantity of genomic DNA were assessed using NanoDrop and Qubit. The whole genomes of strains CY22^T, LJ419^T and CY399^T were sequenced using single-molecule real-time sequencing technology on the Pacific Biosciences sequencing platform [31]. The low-quality reads were filtered out. Subsequently, Hierarchical Genome Assembly Process (HGAP) *de novo* assembly analysis was used to package one contig with no gaps. Meanwhile, draft genomes of the other strains CY357, LJ53 and CY107 were obtained using a HiSeq TM2000 platform (Illumina). The phylogenomic tree was reconstructed based on 1527 core genomic genes [32] from the genome sequences (including those of six novel strains, an outgroup and 20 available strains with validly published names in the genus *Dyadobacter*), and the tree support values were calculated by the Shimodaira–Hasegawa test on 1000 resamples. Finally, the phylogenomic tree is presented in Fig. 2, which shows similar phylogenetic relationships to those based on the 16S rRNA gene sequences. According to the analyses, *D. alkalitolerans* CCTCC AB 207176^T and *D. psychrophilus* CGMCC 1.8951^T were selected as the reference strains, purchased from the China Centre for Type Culture Collection (CCTCC) and China General Microbiological Culture Collection Centre (CGMCC), respectively.

GENOME COMPARISON

All genome sequences have been submitted to the GenBank database and annotated by the NCBI Prokaryotic Genome Annotation Pipeline. The whole-genome lengths for CY22^T, LJ419^T and CY399^T were 5989234 bp (45.8% DNA G+C content; one plasmid; 5114 genes), 7171767 bp (45.2% DNA G+C content; no plasmids; 6153 genes) and 6251631 bp (45.2% DNA

Table 2. Differential phenotypic characteristics between the isolates and phylogenetically related type strains of species of the genus *Dyadobacter*

Strains: 1, CY22^T; 2, CY357; 3, LJ419^T; 4, LJ53; 5, CY399^T; 6, CY107; 7, *D. alkalitolerans* CCTCC AB 207176^T; 8, *D. psychrophilus* CGMCC 1.8951^T. +, Positive; –, negative; w, weakly positive reaction. All data were taken from the present study except for the DNA G+C contents of the reference strains.

Characteristic	1	2	3	4	5	6	7	8
Colony colour	yellow	yellow	yellow	yellow	yellow	yellow	light yellow	yellow
Temperature range (optimum) (°C)	0–30 (25)	0–30 (25)	0–30 (25)	0–30 (25)	0–30 (25)	0–30 (25)	0–30 (25)	0–30 (20)
pH range (optimum) for growth	6.0–11.0 (9.0)	6.0–11.0 (9.0)	6.0–11.0 (9.0)	6.0–11.0 (9.0)	6.0–11.0 (8.0)	6.0–11.0 (8.0)	6.0–11.0 (8.0)	6.0–11.0 (8.0)
Maximum NaCl tolerance (%, w/v)	2.5	2.5	1.0	1.0	1.0	1.0	1.5	1.0
Assimilation of L-arabinose	+	+	–	–	w	w	+	–
Nitrate reduction	–	–	–	–	–	–	+	–
β-glucuronidase activity	+	+	–	–	w	+	–	+
Acid production from: (API 50CH)								
Amygdalin	–	–	+	+	–	–	+	+
Glycerol	+	–	–	–	–	–	–	–
Inositol	–	–	–	–	–	–	–	+
L-xylose	+	+	+	+	–	–	–	+
Melezitose	–	–	+	+	–	–	–	+
Ribose	+	+	+	+	–	–	–	+
Methyl β-D-xyloside	–	–	+	+	–	–	+	+
DNA G+C content (%)	45.8	45.7	45.2	45.3	45.2	45.2	46.3	48.9

G+C content; one plasmid; 5273 genes), respectively. Detailed genomic differences between the isolates and phylogenetically related type strains (*D. alkalitolerans* CCTCC AB 207176^T and *D. psychrophilus* CGMCC 1.8951^T) are shown in Table S1.

The average nucleotide identity (ANI) was assessed using an online ANI calculator (https://www.ezbiocloud.net/tools/ani) and evaluated with an interspecies value of 95.0–96.0% [33]. The digital DNA–DNA hybridization (dDDH) was estimated using the DDH web software (https://ggdc.dsmz.de/) with a species threshold of 70.0% [34]. The ANI and dDDH values between the isolates and phylogenetically related type strains are listed in Table 1. The dDDH values of genome sequences within each novel clade were higher than the threshold, being 78.2% (clade I), 79.0% (clade II) and 84.8% (clade III). In contrast, the values between each novel clade and phylogenetically related type strains were much lower than the 70.0% threshold. Moreover, the values of dDDH between genome sequences of the type strains (CY22^T, LJ419^T and CY399^T) and other strains (a total of 20 strains) available in the GenBank database for the genus *Dyadobacter*, were also less than 70.0% (Table S2), indicating that these type strains represent three novel species of the genus *Dyadobacter*. Likewise, the ANI values between the isolates and other members of the genus *Dyadobacter* were far below 95.0% but greater than 96.0% within each novel clade (Tables 1 and S2). According to the ANI and dDDH values, strain pairs CY22^T/CY357, LJ419^T/LJ53 and CY399^T/CY107 were confirmed to represent three different novel species of the genus *Dyadobacter*.

AntiSMASH (https://antismash.secondarymetabolites.org/) analysis was used to search for the secondary metabolite gene clusters [35]. All six isolates were estimated to contain potential pigment synthesis clusters, which were most similar to two known clusters, flexirubin and carotenoid. The production of flexirubin pigment is due to a generalized dialkylresorcinol (Dar) structure and the alkyl substituents of this Dar moiety [36]. The search results revealed that the genomes of our isolates and the reference strains contain a core biosynthetic gene (*DarB*, 1143 nt) of 3-oxoacyl-[acyl-carrier-protein] synthase III. For the novel type strains, strain CY22^T encoded WP_235159548.1 (locus tag: NFI80_25095, positive strand: 5979649–5980791), strain LJ419^T encoded WP_234656639.1 (locus tag: MUK70_30725, positive strand: 7161925–7163067) and strain CY399^T encoded WP_234615459.1 (locus tag: NFI81_26205, positive strand: 6241752–6242894). The annotated genomes of the novel type strains were found to contain the related genes encoding cold shock proteins (Csps) and universal stress proteins (Usps), which are likely to mediate various stress responses. Csps are small nucleic acid-binding proteins ranging from 65 to 75 amino acids in length [37] and play a major regulatory role in the physiology of adaptation to low temperatures [38]. CY22^T, LJ419^T and CY399^T were predicted to carry several Csp-encoding genes (Table S3), and there were no significant differences between our isolates and the reference strains. Usps are probably important in responses to a large variety of stress conditions [39]. Nine putative Usp-encoding genes were

Table 3. Cellular fatty acid compositions (percentages of totals) of the isolates and phylogenetically related type strains in the genus *Dyadobacter*

Strains: 1, CY22^T; 2, CY357; 3, LJ419^T; 4, LJ53; 5, CY399^T; 6, CY107; 7, *D. alkalitolerans* CCTCC AB 207176^T; 8, *D. psychrophilus* CGMCC 1.8951^T. All data were obtained during the present study. Bold type indicates the major fatty acids (>10.0%); TR, trace amount (<1.0%); –, not detected.

Fatty acids (percentages of totals)	1	2	3	4	5	6	7	8
$C_{16:0}$	3.1	4.0	4.4	–	–	3.7	4.5	2.9
$C_{16:0}$ 3-OH	TR	1.8	2.2	2.1	1.9	1.7	1.9	2.2
$C_{16:1}\omega5c$	**11.8**	**11.9**	9.6	**11.7**	**11.6**	9.1	8.8	**14.5**
iso-$C_{15:0}$	**24.7**	**24.7**	**11.8**	**10.1**	**16.6**	**13.8**	**20.8**	**17.7**
iso-$C_{15:0}$ 3-OH	2.2	2.0	2.8	2.6	2.4	2.5	2.8	2.5
iso-$C_{15:1}$G	TR	1.0	7.9	8.6	4.7	6.4	2.2	2.3
iso-$C_{17:0}$ 3-OH	8.3	7.5	8.5	8.5	9.8	**10.1**	8.5	7.1
anteiso-$C_{15:0}$	1.3	1.3	TR	TR	1.0	TR	1.6	1.0
Summed Feature 3*	**42.9**	**41.8**	**46.3**	**49.3**	**45.3**	**45.3**	**41.8**	**43.7**
Summed Feature 4*	–	TR	TR	TR	1.0	1.4	TR	TR
Summed Feature 9*	1.0	TR	1.2	1.3	1.4	1.8	1.3	TR

*Summed Feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, Summed Feature 4 comprises *iso*-$C_{17:1}$I and/or *anteiso*-$C_{17:1}$B; and Summed Feature 9 comprises 10-methyl $C_{16:0}$ and/or *iso*-$C_{17:1}\omega9c$.

estimated to be present in the genome of CY22^T, which is similar to the number for *D. psychrophilus* CGMCC 1.8951^T (12 related genes), indicating that CY22^T can survive cold stress as a result of living at high altitudes. The related Usps of the three novel type strains and the reference strains are listed in Table S4. Csps and Usps may help strains resist and adapt to harsh environments.

PHENOTYPIC CHARACTERISTICS

Growth at different temperatures (0, 4, 15, 20, 25, 28, 30, 32 and 35 °C) was examined in R2A broth for 5 days, followed by the determination of the temperature range and the optimum temperature by monitoring the McFarland values. The pH range (3.0–12.0, at 1.0 pH unit intervals) and the optimum pH were determined in R2A broth [buffer systems: Na_2HPO_4/citric acid (pH ≤7.0), Tris/HCl (pH 8.0–9.0) and Na_2CO_3/$NaHCO_3$ (pH ≥10.0)]. The maximum NaCl tolerance was identified after incubating the isolates in R2A broth supplemented with NaCl (0–5.0%, w/v, 0.5% intervals) for up to a week. Growth of these six isolates was observed at 0–30 °C (optimally at 25 °C) and pH 6.0–11.0 (optimally at pH 8.0–9.0). Strain pairs LJ419^T/LJ53 and CY399^T/CY107 tolerated up to 1.0% NaCl, while the highest NaCl tolerance concentration for strain pair CY22^T/CY357 was 2.5%.

Growth characteristics of these six strains were examined at 25 °C on various media, including R2A agar, tryptone soya agar (TSA), charcoal agar (CA), nutrient agar (NA), brain heart infusion (BHI) agar with/without 5% (v/v) sheep blood, Luria–Bertani (LB) agar, de Man, Rogosa and Sharpe (MRS) agar, MacConkey agar (MCA) and marine agar (MA). All six strains grew well on R2A and CA agar plates, slowly on TSA, NA, LB and BHI media, while no growth was observed on MRS, MCA and MA plates. Within 3 days of plating, circular, smooth, convex and yellow-pigmented colonies appeared on R2A agar plates (Fig. S3). The morphology was also observed under a light microscope and a transmission electron microscope. Transmission electron microscopic images are presented in Fig. S4. Cells occurred in pairs in young cultures (48 h of growth) and formed chains of coccoid cells in old cultures (72 h of growth). Gram-reaction was assessed using a Gram-staining kit, and cell motility was assessed using R2A medium with 0.5% agar (w/v). Oxidase and catalase activities were examined using a commercial oxidase reagent and 3% H_2O_2 (v/v), respectively. Cells of all six strains were Gram-stain-negative, non-motile and catalase- and oxidase-positive. Flexirubin-type pigment was detected using KOH solution (20%, w/v) [40]. After adding 20% KOH solution, the colour of the colonies changed from yellow to orange, indicating that all six strains produce a flexirubin-type pigment.

Other phenotypic tests for the isolates and their phylogenetically related neighbours were performed using API 20NE, API ZYM and API 50 CH (with 50 CHB/E medium) strips following the manufacturer's (bioMérieux) instructions with slight modifications. All the isolates and reference strains tested positive for aesculin hydrolysis, assimilation of D-glucose, D-mannose, *N*-acetylglucosamine and maltose but negative for urease, gelatinase and indole production. Differential phenotypic characteristics between the isolates and phylogenetically related type strains in the genus *Dyadobacter* are listed in Table 2. Compared with the reference strains, all the isolates and *D. psychrophilus* CGMCC 1.8951^T tested negative for nitrate reduction, whereas *D. alkalitolerans* CCTCC AB 207176^T was positive. All the isolates within each novel clade were distinguished by L-arabinose

assimilation, and the results were positive in clade I (similar to the reference strain *D. alkalitolerans* CCTCC AB 207176^T), weakly positive in clade III and negative in clade II (similar to the reference strain *D. psychrophilus* CGMCC 1.8951^T). The other detailed characteristics are presented in the species descriptions.

CHEMOTAXONOMIC ANALYSIS

Cells were grown at 25 °C up to the exponential phase and were used for chemotaxonomic analysis. The detection indexes of fatty acids, polar lipid profiles and respiratory quinones were included. The cellular fatty acids were extracted using the Sherlock Microbial Identification System (MIDI) and analysed by gas chromatography [41]. Cellular fatty acid compositions (percentages of totals) of the isolates and phylogenetically related type strains of members of the genus *Dyadobacter* are presented in Table 3. The major cellular fatty acids (>10.0%) of strains isolated in this study were *iso*-$C_{15:0}$ and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). The respiratory quinones in CY22^T, LJ419^T and CY399^T were analysed by high-performance liquid chromatography, and polar lipid profiles were determined by two-dimensional thin-layer chromatography (TLC) [42, 43]. For the TLC, the first dimension comprised chloroform/methanol/distilled water (65:25:4), and the second dimension comprised chloroform/glacial acetic acid/methanol/distilled water (80:18:12:5). The only respiratory quinone was MK-7, and the predominant polar lipid was PE, similar to those in other members of the genus *Dyadobacter* with validly published names. Detailed polar lipid profiles are shown in Fig. S5 and summarized in the species descriptions.

On the basis of the phenotypic, phylogenetic and genomic evidence presented, these six strains represent three novel members of the genus *Dyadobacter*, for which the names *Dyadobacter chenhuakuii* sp. nov., *Dyadobacter chenwenxiniae* sp. nov. and *Dyadobacter fanqingshengii* sp. nov. are proposed.

DESCRIPTION OF *DYADOBACTER CHENHUAKUII* SP. NOV.

Dyadobacter chenhuakuii (chen.hua.kui'i. N.L. gen. n. *chenhuakuii* of Huakui Chen, an academician of the China Academy of Sciences, for his contributions to soil microbiology, especially in symbiotic nitrogen fixation)

Cells grow well on R2A, CA and BHI (with 5% sheep blood, v/v) agar plates, slowly on TSA, NA, LB and BHI media and display absolutely no growth on MRS, MCA and MA plates. Cells are aerobic, Gram-stain-negative, non-motile, non-spore-forming and rod-shaped, approximately 0.9 µm wide and 2.0 µm long (Fig. S4a). Colonies on R2A agar plates appear circular, smooth, convex and yellow-pigmented after 3 days. Growth is observed at 0–30 °C (optimally at 25 °C), pH 6.0–11.0 (optimally at pH 9.0) and with 0–2.5% NaCl (optimally without NaCl). Cells are positive for catalase, oxidase, alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, hydrolysis of aesculin and assimilation of glucose, L-arabinose, mannose, *N*-acetylglucosamine and maltose; negative for lipase (C14), α-chymotrypsin, β-fucosidase, nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase, assimilation of mannitol, potassium gluconate, capric acid, adipic acid, malate, citrate and phenylacetic acid; and weakly positive for esterase (C4), esterase lipase (C8), cystine arylamidase and trypsin. The API 50 CH (with 50 CHB/E medium) test indicates acid production when the following are used as carbon sources: glycerol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, galactose, glucose, fructose, mannose, methyl α-mannoside, methyl α-D-glycoside, *N*-acetylglucosamine, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, synanthrin, raffinose, dextrinose, turanose, lyxose and L-fucose. The major cellular fatty acids (>10.0%) are *iso*-$C_{15:0}$, $C_{16:1}\omega5c$ and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). MK-7 is the only respiratory quinone. The predominant polar lipids are PE, one unidentified phospholipid, four unidentified aminolipids and nine unidentified lipids.

The type strain, *Dyadobacter chenhuakuii* CY22^T (= GDMCC 1.3045^T = KCTC 92299^T), was isolated from soil collected from the Qinghai–Tibetan Plateau (PR China). The genomic DNA of the type strain has a G+C content of 45.8%. CY357 is an additional strain. The GenBank accession numbers for the 16S rRNA gene sequences of strains CY22^T and CY357 are OM884033 and OM857550, respectively. The GenBank accession numbers for the genome sequences of strains CY22^T and CY357 are CP098805 and JAKFFV000000000, respectively.

DESCRIPTION OF *DYADOBACTER CHENWENXINIAE* SP. NOV.

Dyadobacter chenwenxiniae (chen.wen.xin'i.ae. N.L. gen. n. *chenwenxiniae* of Wenxin Chen, an academician of the China Academy of Sciences, for her contributions to soil microbiology and bacterial taxonomy)

Cells grow well on R2A and CA agar plates, slowly on TSA, NA, LB and BHI media, while no growth occurs on MRS, MCA and MA plates. Cells are aerobic, Gram-stain-negative, non-motile, non-spore-forming and rod-shaped, approximately 0.9 µm wide and 2.3 µm long (Fig. S4d). Within 3 days of growth, colonies on R2A agar plates appear circular, smooth, convex and yellow-pigmented. Growth is observed at 0–30 °C (optimally at 25 °C), pH 6.0–11.0 (optimally at pH 9.0) and with 0–1.0% NaCl (optimally without NaCl). Cells are positive for catalase, oxidase, alkaline phosphatase, leucine arylamidase, valine arylamidase,

acid phosphatase, naphthol-AS-BI phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, hydrolysis of aesculin and assimilation of glucose, mannose, *N*-acetylglucosamine and maltose; negative for lipase (C14), α-chymotrypsin, β-glucuronidase, β-fucosidase, nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase, assimilation of L-arabinose, mannitol, potassium gluconate, capric acid, adipic acid, malate, citrate and phenylacetic acid; weakly positive for esterase (C4), esterase lipase (C8), cystine arylamidase and trypsin. The API 50 CH (with 50 CHB/E medium) test indicates acid production when the following are used as carbon sources: D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, methyl β-D-xyloside, galactose, glucose, fructose, mannose, methyl α-D-mannoside, methyl α-D-glycoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, synanthrin, melezitose, raffinose, dextrinose, turanose, lyxose and L-fucose. The major cellular fatty acids (>10.0%) are *iso*-$C_{15:0}$ and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). MK-7 is the only respiratory quinone. The predominant polar lipids are PE, three unidentified phospholipids, three unidentified aminolipids, one unidentified aminoglycolipid, eight unidentified lipids and two unidentified glycolipids.

The type strain, *Dyadobacter chenwenxiniae* LJ419^T (= GDMCC 1.2872^T = JCM 33794^T), was isolated from soil collected from the Qinghai–Tibetan Plateau (PR China). The genomic DNA of the type strain has a G+C content of 45.2%. LJ53 is an additional strain. The GenBank accession numbers for the 16S rRNA gene sequences of strains LJ419^T and LJ53 are OM857832 and OM857630, respectively. The GenBank accession numbers for the genome sequences of strains LJ419^T and LJ53 are CP094997 and JAJTTB000000000, respectively.

DESCRIPTION OF *DYADOBACTER FANQINGSHENGII* SP. NOV.

Dyadobacter fanqingshengii (fan.qing.sheng'i.i. N.L. gen. n. *fanqingshengii* of Qingsheng Fan, one of the pioneers of agricultural microbiology in China, for his contributions to the field of soil microbiology)

Cells grow well on R2A and CA agar plates, slowly on TSA, NA, LB and BHI media, while no growth occurs on MRS, MCA and MA plates. Cells are aerobic, Gram-stain-negative, non-motile, non-spore-forming and rod-shaped, approximately 0.8 µm wide and 2.5 µm long (Fig. S4g). Within 3 days of growth, colonies on R2A agar plates appear circular, smooth, convex and yellow-pigmented. Growth is observed at 0–30 °C (optimally at 25 °C), pH 6.0–11.0 (optimally at pH 8.0) and with 0–1.0% NaCl (optimally without NaCl). Cells are positive for catalase, oxidase, alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β glucosaminidase, α-mannosidase, hydrolysis of aesculin and assimilation of glucose, mannose, *N*-acetylglucosamine and maltose; negative for lipase (C14), α-chymotrypsin, β-fucosidase, nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase, assimilation of mannitol, potassium gluconate, capric acid, adipic acid, malate, citrate and phenylacetic acid; weakly positive for esterase (C4), esterase lipase (C8), cystine arylamidase, trypsin, β-glucuronidase and assimilation of L-arabinose. The API 50 CH (with 50 CHB/E medium) test indicates acid production when the following are used as carbon sources: D-arabinose, L-arabinose, D-xylose, galactose, glucose, fructose, mannose, methyl α-mannoside, methyl α-D-glycoside, *N*-acetylglucosamine, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, synanthrin, raffinose, dextrinose, turanose, lyxose and L-fucose. The major cellular fatty acids (>10.0%) are *iso*-$C_{15:0}$, $C_{16:1}\omega5c$ and summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$). MK-7 is the only respiratory quinone. The predominant polar lipids are PE, one unidentified phospholipid, four unidentified aminolipids, nine unidentified lipids and two unidentified glycolipids.

The type strain, *Dyadobacter fanqingshengii* CY399^T (= GDMCC 1.3052^T = KCTC 92306^T), was isolated from soil collected from the Qinghai–Tibetan Plateau (PR China). The genomic DNA of the type strain has a G+C content of 45.2%. CY107 is an additional strain. The GenBank accession numbers for the 16S rRNA gene sequences of strains CY399^T and CY107 are OM857592 and OM884043, respectively. The GenBank accession numbers for the genome sequences of strains CY399^T and CY107 are CP098806 and JAKFFR000000000, respectively.

Funding information
This work was supported by grants from National Key R and D Program of China (2019YFC1200501 and 2019YFC1200505) and Research Units of Discovery of Unknown Bacteria and Function (2018RU010).

Conflicts of interest
The authors declare that they have no conflict of interest.

Ethical statement
The ethical practice was approved by Ethical Committee of the National Institute for Communicable Disease Control (# ICDC 2016004).

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