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Labedella phragmitis sp. nov. and *Labedella populi* sp. nov., two endophytic actinobacteria isolated from plants in the Taklamakan Desert and emended description of the genus *Labedella*

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ABSTRACT

Two novel strains, designated 11W25H-1^T and 8H24J-4-2^T, were isolated from surface-sterilized plant tissues collected from the Taklamakan Desert in the Xinjiang Uygur Autonomous Region, China. The strains were characterized by a polyphasic approach in order to clarify their taxonomic positions. They were Gram-stain positive, aerobic, non-motile, non-spore-forming and rod-shaped. The 16S rRNA gene sequences of the strains showed highest similarities with *Labedella gwakjiensis* KCTC 19176^T (99.2% and 98.9%, respectively) and *Labedella endophytica* CPCC 203961^T (98.9% and 99.0%, respectively). The sequence similarity between strains 11W25H-1^T and 8H24J-4-2^T was 99.4%. Phylogenetic analyses based on 16S rRNA gene sequences and single-copy phylogenetic marker genes (pMGs) showed that the two strains belonged to the genus *Labedella* and formed a separate cluster from the closest species *L. gwakjiensis* KCTC 19176^T and *L. endophytica* CPCC 203961^T. Genomic analyses, including average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH), clearly separated the strains from each other and from the other species of the genus *Labedella* with values below the thresholds for species delineation. The two strains showed chemotaxonomic characteristics and phenotypic properties in agreement with the description of the genus *Labedella* and also confirmed the differentiation from the closest species. The data demonstrated that strains 11W25H-1^T and 8H24J-4-2^T represented two novel species of the genus *Labedella*, for which the names *Labedella phragmitis* sp. nov. (type strain 11W25H-1^T=JCM 33144^T=CGMCC 1.16700^T) and *Labedella populi* sp. nov. (type strain 8H24J-4-2^T=JCM 33143^T=CGMCC 1.16697^T) are proposed.

Introduction

The genus *Labedella* is affiliated to the family *Microbacteriaceae* [30,38] of the order *Micrococcales* and was first proposed by Lee with *Labedella gwakjiensis* as the type species [20]. At the time of writing, the genus *Labedella* comprises two validly named species (www.bacterio.net/labedella.html), *Labedella gwakjiensis* isolated from a dried seaweed sample [20] and *Labedella endophytica* isolated from a surface-sterilized stem of *Anabasis elatior* [40].

Members of the genus *Labedella* are typically Gram-stain positive, aerobic, non-motile, aerobic, and non-spore-forming. Branching or mycelium formation does not occur [20]. Cells of *L. gwakjiensis* are rod-shaped, while cells of *L. endophytica* are coccoid [40], but the description of the genus *Labedella* has not been emended. Species in *Labedella* are catalase-positive, oxidase-negative mesophilic actinobacteria. The chemotaxonomic markers characterizing the genus *Labedella* include ornithine as the diagnostic diamino acid, MK-10 and MK-11 as the predominant menaquinones, with a relatively high quantity of MK-9 in some species, iso- and anteiso-branched components (anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$) as major fatty acids and phosphatidylglycerol and diphosphatidylglycerol as the major lipids [20,40], as well as a DNA G+C content between 68.0 mol% and 69.2 mol% [40].

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Based on the cell wall peptidoglycan type and major menaquinone compositions, the genus *Labedella* is well differentiated from other genera of the family *Microbacteriaceae* [20].

The 16S rRNA gene similarity of the two validly named species in the genus *Labedella* (99.0%) is above the threshold for the definition of a new species [16]. However, the DNA–DNA hybridization (DDH) value between them (27.4%) is lower than 70%, which is the threshold for the delineation of new species [32]. The presence of highly conserved 16S rRNA genes in different species is not particularly uncommon, and DDH is accepted as the gold standard in prokaryote taxonomy. With the rapid progress in genome sequencing, the replacement of DDH with pairwise genome-sequence derived similarity has been proposed [5,12] and computational methods for genome comparison and taxonomic differentiation have been utilized [18]. Average nucleotide identity (ANI) and digital DDH (dDDH) have been most widely used to calculate the relatedness between genome sequences of the strains and the type strain of a species [3].

In the present study, we report the identification and classification of strains 11W25H-1^T and 8H24J-4-2^T, which were isolated from surface-sterilized plant tissues collected from the Taklamakan Desert in the Xinjiang Uygur Autonomous Region, China. The taxonomic positions of the two isolates were determined through phylogenetic analysis, a physiological test and chemotaxonomic characterizations.

Materials and methods

Isolation and culture conditions

Strains 11W25H-1^T and 8H24J-4-2^T were isolated respectively from the surface-sterilized flowers of reed (*Phragmites australis*) and a surface-sterilized branch of *Populus euphratica*, both of which were collected from different sampling sites (38°15′23.17″N, 80°47′50.43″E and 38°27′57.91″N, 80°10′40.59″E, respectively) in the Taklamakan Desert in the Xinjiang Uygur Autonomous Region, China. The plant tissues were washed in running tap water and subjected to a five-step surface sterilization procedure, as described by Qin et al. [33]. After drying in a laminar flow hood for 2 days, the surface-sterilized plant tissues were ground into powders and distributed on twelve different isolation media in order to isolate the actinobacterial strains. All the isolation media were supplemented with 1% plant tissue extract. In addition, nalidixic acid (20 mg L^{-1}), cycloheximide (50 mg L^{-1}) and potassium dichromate (50 mg L^{-1}) were added to the media to prevent the growth of Gram-stain negative bacteria and fungi. After incubation at 30 °C for 4–8 weeks, the colonies were selected carefully and streaked onto International Streptomyces Project (ISP) 2 agar [37] in order to obtain pure isolates. Strain 11W25H-1^T was isolated on chitin medium [containing, L^{-1} distilled water: 1.0 g chitin, 0.7 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.3 g KH_2PO_4, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.001 g $ZnSO_4$, 0.001 g $MnCl_2$, 15.0 g agar, pH 8.5], and strain 8H24J-4-2^T was isolated on modified Gauze's medium no. 2 [containing, L^{-1} distilled water: 1.0 g glucose, 0.5 g peptone, 0.5 g NaCl, 0.3 g tryptone, 15.0 g agar, pH 8.5]. The purified strains were preserved in 20% (v/v) autoclaved glycerol at −80 °C.

16S rRNA gene sequencing and phylogenetic trees

Genomic DNA was extracted using the method described by Li et al. [22]. The 16S rRNA gene was amplified from total DNA by PCR with universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [6]. The cloning and sequencing of the 16S rRNA gene were performed according to Li et al. [21]. The almost complete 16S rRNA gene sequence was compiled with SeqMan software (DNASTAR) [39] and compared against the available sequences of type species using EzBioCloud's Identify service [43]. According to the blast result of the 16S rRNA gene sequence, the corresponding sequences of closely related type species were retrieved from the GenBank database using the EzBioCloud server. Multiple alignments were made using the CLUSTAL_X tool in MEGA version 7.0 [19]. Evolutionary distances were calculated using the Kimura two-parameter model [17]. Phylogenetic trees were constructed with the neighbor-joining [35], maximum likelihood [7] and maximum parsimony [10] methods using MEGA version 7.0. The topologies of the evolutionary trees were evaluated by bootstrap analysis based on 1000 replications [8].

Whole genome sequencing and analysis of DNA G+C content

For the analysis of genome relatedness, genomic DNA of strains 11W25H-1^T and 8H24J-4-2^T together with reference strains *L. gwakjiensis* KCTC 19176^T and *L. endophytica* CPCC 203961^T was extracted following the method of Marmur [25]. The quality and concentration of DNA were determined by agarose electrophoresis and the Qubit Fluorometer, respectively. Whole genome sequencing was carried out on a BGISEQ-500 platform (insert size, 200–400 bp; read length, paired-end 100 bp) at BGI, Shenzhen, China. After filtering adapter contaminations and low-quality reads with SOAPnuke [2], *de novo* assembly of the high-quality reads was performed with IDBA-UD (version 1.1.3) [31] and scaffolds shorter than 500 bp were filtered. The genome size and scaffold N50 were provided by the QUAST tool (version 4.6.3) [28]. The DNA G+C content was determined directly from the draft genome sequence, and the sequence has been deposited in the GenBank/EMBL/DDBJ. The protein coding genes were annotated by GeneMarkS+ (version 4.6) based on the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The carbohydrate-active enzyme coding genes were annotated by HMMER searching [9] against the Carbohydrate-Active enZYmes database (http://www.cazy.org/) [23].

Genome comparison and single-copy phylogenetic marker gene analysis

In order to provide support for the classification of strains 11W25H-1^T and 8H24J-4-2^T as two novel species of genus *Labedella*, the average nucleotide identity (ANI) value was calculated using the online ANI calculator (https://www.ezbiocloud.net/tools/ani). The digital DNA–DNA hybridization (dDDH) value was calculated via the online Genome to Genome Distance Calculator (http://ggdc.dsmz.de/ggdc.php) [26]. The result was based on recommended formula 2 (identities/HSP length), which is independent of genome length and is thus robust against the use of incomplete draft genomes.

To verify the evolutionary position of strains 11W25H-1^T and 8H24J-4-2^T, genomes of closely related species were recruited to construct a phylogenic tree. The genome sequences of strains 11W25H-1^T and 8H24J-4-2^T together with reference strains *L. gwakjiensis* KCTC 19176^T and *L. endophytica* CPCC 203961^T were obtained in the present study, whereas the genomes of eight closely related species of related genera in the family *Microbacteriaceae* were already available, namely *Diaminobutyricimonas aerilata* DSM 27393^T (assemble accession no. GCF_002797715.1), *Cryobacterium psychrotolerans* CGMCC 1.5382^T (assemble accession no. GCF_900101115.1), *Cryobacterium luteum* CGMCC 1.11210^T (assemble accession no. GCF_900110125.1), *Cryobacterium roopkundense* RuG17^T (assemble accession no. GCF_000764165.1), *Cryobacterium levicorallinum* CGMCC 1.11211^T (assemble accession no. GCF_900113585.1), *Cryobacterium flavum* CGMCC 1.11215^T (assemble accession no. GCF_900103805.1), *Mycetocola miduiensis* CGMCC 1.11101^T (assemble accession no. GCF_900115155.1)

and *Agrococcus lahaulensis* DSM 17612^T (assemble accession no. GCF_000425105.1). All assembly scaffolds were predicted by Prodigal (version 2.6.3) [14] with default parameters. Complete sets of 40 universal single-copy phylogenetic marker genes (pMGs) were extracted from genomes using the fetchMG tool [27] and used to build phylogenetic trees by MEGA 7.0 with the neighbor-joining and maximum likelihood methods.

Morphological, physiological and biochemical analyses

The cultural, physiological and biochemical characteristics of strains 11W25H-1^T and 8H24J-4-2^T together with the two reference strains were tested under the same conditions. Gram staining was determined using the method described by Magee et al. [24]. Cell morphology was observed under light microscopy (model BH 2; Olympus) and transmission electron microscopy (JEM-1400; JEOL) after incubation on tryptic soy agar (TSA; BD) at 30 °C for 2–3 days. Growth was evaluated on ISP 2 agar (BD), ISP 4 agar (BD), nutrient agar (NA; BD), Reasoner's 2A (R2A) agar (BD), Luria-Bertani (LB) agar and TSA (BD) after incubation at 30 °C for 15 days. Colony colors were observed according to color chips from the ISCC-NBS color chart standard [15]. Growth at different temperatures (4, 10, 15, 20, 25, 28, 30, 32, 35, 37, 40, 45, 50 and 55 °C) was investigated on TSA. NaCl tolerance for growth was tested on TSA adjusted to various NaCl concentrations in the range 0–10% (w/v; intervals of 1%) at 30 °C. The pH range (pH 4.0–12.0 at intervals of 1 pH unit) for growth was determined at 30 °C on TSA with the buffer systems of Xu et al. [42]. The ability to grow anaerobically was tested on TSA using the AnaeroPack (Mitsubishi Gas Chemical Company, Inc.) at 30 °C for 15 days, according to the manufacturer's instructions.

Oxidase activity was assessed via oxidation of 1% tetramethyl-*p*-phenylenediamine [1]. Catalase activity was determined by assessing production of bubbles in 3% (v/v) H_2O_2. H_2S production, milk coagulation and peptonization, gelatin liquefaction and hydrolysis of Tween 20, Tween 40 and Tween 80 were examined as described by Gonzalez et al. [11]. Hydrolysis of CM-cellulose and starch was tested using R2A medium (BD) as the basal medium, supplemented with a final concentration of 1% (w/v) starch and cellulose, respectively. The enzyme activities and biochemical characteristics were tested by using the API ZYM (bioMérieux) and API 20NE (bioMérieux) kits according to the manufacturer's recommendations. Carbon source utilization and sensitivity to antimicrobial compounds were tested using Biolog GEN III MicroPlates following the manufacturer's instructions, and acid production from carbon sources was tested by using the API 50CH (bioMérieux) system.

Chemotaxonomic analyses

Cellular fatty acid profiles of the two strains 11W25H-1^T and 8H24J-4-2^T were comparatively analyzed with the reference strains *L. gwakjiensis* KCTC 19176^T and *L. endophytica* CPCC 203961^T. All strains were harvested on TSA after incubation at 30 °C for 2 days, when bacterial growth reached the late-exponential stage [36]. The cellular fatty acids were saponified, methylated and extracted according to the standard protocol described by Sasser [36]. Fatty acid methyl esters were analyzed by gas chromatograph (Agilent 7890B) with the Sherlock Microbial Identifications System (version 6.2; MIDI), and identified automatically by the MIDI RTSBA 6 database. Biomass for other chemotaxonomic investigations was harvested after incubation in ISP 2 broth at 28 °C and 180 r.p.m. for 4 days. Menaquinone components were extracted as described by Collins et al. [4] and identified by HPLC (LC-20AD; Shimadzu) coupled to a single quadrupole mass spectrometer (LCMS-2020; Shimadzu), as previously described [13]. Polar lipids were extracted according to the method of Minnikin et al. [29] and analyzed by two-dimensional TLC on silica gel 60 F_{254} plates (Merck). The solvent systems of the first and second dimension were chloroform–methanol–water (64:27:5, by vol) and chloroform–methanol–acetic acid–water (80:18:12:5, by vol), respectively. Individual lipids were identified by spraying with appropriate detection reagents: phosphomolybdic acid for detection of total lipids, ninhydrin for aminolipids, molybdenum blue for phospholipids, and anisaldehyde for glycolipids. Analysis of peptidoglycan amino-acid composition was carried out by the Identification Service of the DSMZ.

Results and discussion

16S rRNA gene sequencing and phylogenetic tree analyses

16S rRNA gene sequence analysis is a primary tool for delineation of novel bacterial species. The almost full-length 16S rRNA gene sequences of strain 11W25H-1^T (1482 nucleotides; GenBank accession number MK333282) and strain 8H24J-4-2^T (1478 nucleotides; GenBank accession number MK333281) were determined and subjected to comparative analysis. The 16S rRNA gene sequence similarity between these two strains was 99.4%. The 16S rRNA gene sequences of strains 11W25H-1^T and 8H24J-4-2^T exhibited highest similarities to *L. gwakjiensis* KCTC 19176^T (99.2% and 98.9%, respectively) and *L. endophytica* CPCC 203961^T (98.9% and 99.0%, respectively). Both strains showed less than 97.3% 16S rRNA gene sequence similarities to other genera in the family *Microbacteriaceae*. The phylogenetic tree based on 16S rRNA gene sequences, generated by using the neighbor-joining algorithm (Fig. 1) showed that strains 11W25H-1^T and 8H24J-4-2^T formed a statistically well-supported cluster with one another. This cluster was clearly separated from the neighbor cluster formed by closest relatives *L. gwakjiensis* KCTC 19176^T and *L. endophytica* CPCC 203961^T. The branching pattern was also confirmed in maximum parsimony and maximum likelihood trees (Fig. 1), and the stable phylogenetic relationship was supported by high bootstrap values. The two strains have not yet been described as already recognized species within the genus *Labedella*, and the data in the present study suggested that they could represent two novel species within the genus.

Whole genome sequencing and G+C content

The assembled draft genome of strain 11W25H-1^T (accession number RZNB00000000) was 3,524,121 bp and consisted of 18 contigs. The N50 length was 499,867 bp. All contigs were larger than 542 bp, and the largest was 990,466 bp. A total of 3,292 genes were predicted, which mainly included 3187 protein coding genes and 51 RNA genes. The predicted non-coding RNA consisted of 45 tRNAs, 3 rRNAs and 3 ncRNAs. In addition, a total of 117 carbohydrate-active enzyme coding genes were conserved in strain 11W25H-1^T (Table S1). The assembled draft genome of strain 8H24J-4-2^T (accession number RZNC00000000) was 3,417,745 bp and consisted of 15 contigs. The N50 length was 633,627 bp. All contigs were larger than 615 bp, and the largest was 1,200,390 bp. A total of 3,231 genes were predicted, which mainly included 3116 protein coding genes and 51 RNA genes. The predicted non-coding RNA consisted of 45 tRNAs, 3 rRNAs and 3 ncRNAs. In addition, a total of 100 carbohydrate-active enzyme coding genes were conserved in strain 8H24J-4-2^T (Table S1). The DNA G+C contents of strains 11W25H-1^T and 8H24J-4-2^T were 69.1 mol% and 68.9 mol%, respectively, which were in the G+C content range reported for species within the genus *Labedella* (68.0–69.2 mol%) [40]. The G+C contents of strains *L. gwakjiensis* KCTC 19176^T (69.2 mol%) and *L. endophytica* CPCC 203961^T (69.4 mol%) in this study were slightly different from reported data, which might be due to the different technical approaches applied.

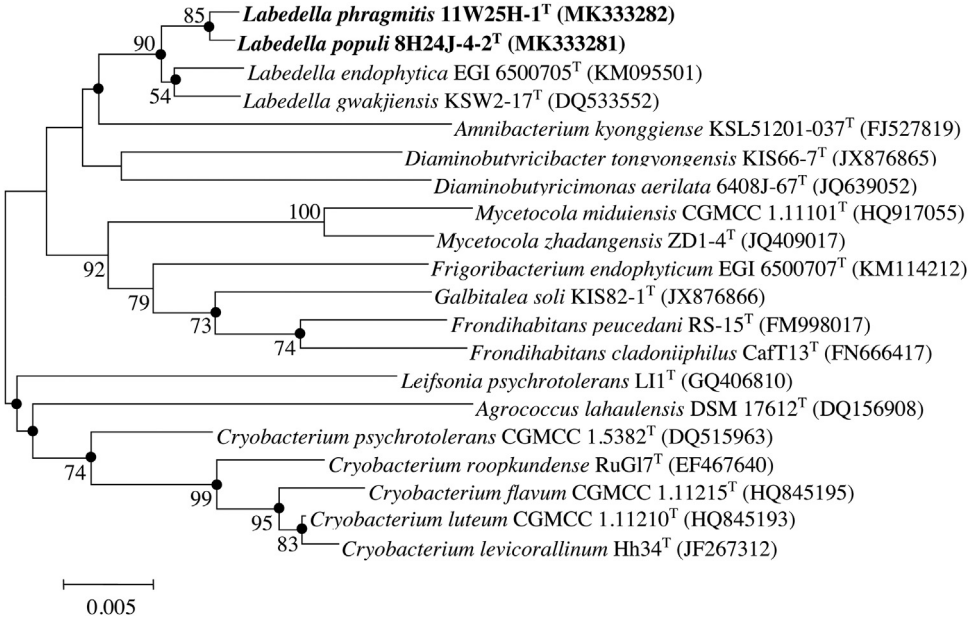

Fig. 1. Neighbor-joining phylogenetic tree on the basis of 16S rRNA gene sequences showing the phylogenetic position of strains 11W25H-1^T and 8H24J-4-2^T with the type species of the genus *Labedella* and other related species. Numbers at nodes refer to bootstrap values (based on 1000 replicates; only values >50% are shown at branch points). Filled circles indicate that the corresponding nodes were also obtained in both the maximum likelihood and maximum parsimony trees. Bar, 5 nt substitutions per 1000 nt.

Genome comparison and single-copy phylogenetic marker gene analysis

The ANI value between strains 11W25H-1^T and 8H24J-4-2^T was 85.6%. The ANI values comparing the two strains to the other species in the genus *Labedella* were lower than 81% (Table 1). These data were much lower than the recommended cut-off value of 95–96% [34] for classifying strains as different species. The dDDH value between strains 11W25H-1^T and 8H24J-4-2^T was 29.7%. The resulting dDDH values between the two strains and the other species in the genus *Labedella* ranged from 23.4% to 23.7%. The species delineation threshold value for DDH is accepted at 70% [41]. These data indicated that the two strains differed considerably from each other and other species within the genus *Labedella*.

According to the single-copy pMGs analysis, strains 11W25H-1^T and 8H24J-4-2^T formed a highly significant cluster with a high bootstrap value, and this cluster was separated from the closely related species within the genus *Labedella* (Fig. 2). The identical topology was recovered regardless of the tree construction methods applied (Fig. 2), which confirmed the result obtained from the phylogenetic analysis of the 16S rRNA gene sequence. Thus, strains 11W25H-1^T and 8H24J-4-2^T should be assigned to two novel species in the genus *Labedella*.

Morphological, physiological and biochemical characteristics

Both strains were Gram-stain positive, aerobic, non-spore-forming, non-motile, rod-shaped, catalase-positive and oxidase-negative. Cells of strains 11W25H-1^T and 8H24J-4-2^T were 0.6–0.8 × 1.1–1.7 μm and 0.6–0.8 × 0.9–1.5 μm in size, respectively, after incubation for 2 days at 30 °C on TSA (Fig. S1). Colonies of both strains grown on TSA for 3 days at 30 °C were circular, convex, entire, and 0.4–0.7 mm in diameter. Both strains displayed good growth on ISP 2 agar, LB agar, NA, R2A agar and TSA, and moderate growth on ISP 4 agar. Strain 11W25H-1^T was pale yellow and able to grow at 10–40 °C (optimum 28–30 °C), pH 6.0–10.0 (optimum pH 7.0–8.0) and in the presence of 0–6% (w/v) NaCl (optimum 0–2% (w/v) NaCl), while strain 8H24J-4-2^T was light yellow and able to grow at 10–40 °C (optimum 30–32 °C), pH 6.0–10.0 (optimum pH 7.0–8.0) and in the presence of 0–7% (w/v) NaCl (optimum 0–2% (w/v) NaCl). The physiological and biochemical characteristics of strains 11W25H-1^T and 8H24J-4-2^T are summarized in the species descriptions and Table S2. The comparison of selective characteristics of the two strains and the reference strains is given in Table 2. The two strains shared the main phenotypic properties of the genus *Labedella* including: (i) Gram-stain positive, aerobic, non-spore-forming, non-motile and rod-shaped; (ii) positive for catalase and gelatin liquefaction; (iii) negative for oxidase, milk coagulation and peptonization, indole production, nitrate reduction and hydrolysis of Tween 80 and CM-cellulose. The other species of the genus *Labedella*, including strains 11W25H-1^T and 8H24J-4-2^T, can be differentiated on the basis of the characteristics shown in Table 2.

Table 1
ANI and dDDH values (%) of strains 11W25H-1^T and 8H24J-4-2^T with other type species in the genus *Labedella*. Strains: 1, 11W25H-1^T; 2, 8H24J-4-2^T; 3, *L. gwakjiensis* KCTC 19176^T; 4, *L. endophytica* CPCC 203961^T.

Species name	ANI		dDDH	
	1	2	1	2
1	–	85.6	–	29.7
2	85.6	–	29.7	–
3	80.9	80.6	23.7	23.6
4	81.0	80.6	23.6	23.4

Chemotaxonomic characteristics

The major fatty acids (>10% of the total fatty acids) detected in strain 11W25H-1^T mainly consisted of anteiso-$C_{15:0}$ (49.5%), iso-$C_{16:0}$ (25.3%) and anteiso-$C_{17:0}$ (11.1%), while those found in strain 8H24J-4-2^T were anteiso-$C_{15:0}$ (59.4%), anteiso-$C_{17:0}$ (18.1%) and iso-$C_{16:0}$ (15.2%). The two strains and reference strains shared similar fatty acid profiles, but some quantitative differences in certain fatty acids distinguished strains 11W25H-1^T and 8H24J-4-2^T distinctly from each other and from the other recognized species of the genus *Labedella* (Table 3). Menaquinone profiles of strain 11W25H-1^T, strain 8H24J-4-2^T and the reference strains are shown

Table 2
Differential characteristics between strain 11W25H-1^T, strain 8H24J-4-2^T and the reference strains. Strains: 1, 11W25H-1^T; 2, 8H24J-4-2^T; 3, *L. gwakjiensis* KCTC 19176^T; 4, *L. endophytica* CPCC 203961^T. All comparative data are taken from the present study unless indicated otherwise.

Characteristic	1	2	3	4
Morphology	Rod	Rod	Rod[a]	Coccoid[b]
Colony color	Pale yellow	Light yellow	Pale greenish yellow	Light greenish yellow
NaCl tolerance range (%, w/v)	0–6	0–7	0–5	0–5
pH range for growth	6–10	6–10	5–10	5–10
Growth on ISP 4 agar	w	w	+	–
H_2S production	–	–	+	–
Glucose fermentation (API 20 NE)	–	+	–	–
Hydrolysis of:				
Starch	+	+	+	–
Tween 20	+	+	–	+
Enzyme activity (API ZYM):				
Alkaline phosphatase	–	–	–	w
Esterase (C4)	–	+	+	+
α-Fucosidase	–	–	+	–
α-Mannosidase	–	–	–	+
Trypsin	w	–	w	+
Valine arylamidase	–	w	w	–
Assimilation of (API 20 NE):				
Malic acid	–	–	+	–
Utilization of (Biolog GN III MicroPlate):				
N-Acetyl-D-galactosamine	–	–	+	–
N-Acetyl-β-D-mannosamine	+	–	+	w
L-Arginine	–	–	+	w
Bromosuccinic acid	–	–	+	–
D-Fructose-6-phosphate	w	–	–	–
Gelatin	–	+	–	–
p-Hydroxy-phenylacetic acid	+	–	–	–
α-Keto-glutaric acid	w	–	–	w
D,L-Malic acid	–	–	+	–
3-Methyl glucose	w	–	+	–
Methyl pyruvate	–	+	+	+
D-Raffinose	+	–	+	+
L-Rhamnose	–	–	+	–
D-Serine	w	–	w	–
L-Serine	+	–	+	–
D-Sorbitol	+	+	–	–
Acid production from (API 50CH):				
N-Acetylglucosamine	–	w	+	+
D-Arabinose	–	–	–	w
L-Fucose	+	w	–	w
Gentiobiose	+	–	–	+
D-Melibiose	–	w	w	w
Potassium gluconate	–	–	–	w
Potassium 5-ketogluconate	–	–	–	w
D-Raffinose	–	w	+	+
L-Rhamnose	–	–	w	–
Sensitivity to (Biolog GN III MicroPlate):				
Aztreonam	R	R	R	S
Guanidine HCl	S	S	R	R
Rifamycin SV	S	S	R	R
DNA G+C content (mol%)	69.1	68.9	69.2	69.4

Note: +, positive; –, negative; w, weakly positive; R, resistant; S, susceptible. Reference data were taken from: a, Lee [20]; b, Wang et al. [40].
All strains are aerobic and Gram-stain positive. All strains display good growth on ISP 2 agar, LB agar, NA, R2A agar and TSA. All strains are positive for the following characteristics: activities of acid phosphatase, catalase, esterase lipase (C8), leucine arylamidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and naphthol-AS-BI-phosphohydrolase; hydrolysis of aesculin and gelatin; assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose and gluconate; utilization of L-aspartic acid, dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, α-D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, α-D-glucose, D-mannose, D-fructose, D-galactose, L-fucose, inosine, D-mannitol, myo-inositol, glycerol, L-alanine, L-glutamic acid, L-pyroglutamic acid, pectin, D-gluconic acid, L-lactic acid, Tween 40, acetoacetic acid and acetic acid as sole sources of carbon and energy; acid production from amygdalin, L-arabinose, glycerol, D-melezitose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, arbutin, aesculin, salicin, D-cellobiose, D-maltose, D-lactose, sucrose and D-turanose; sensitivity to fusidic acid, D-serine, troleandomycin, minocycline, lincomycin, niaproof 4, vancomycin and tetrazolium blue. All strains are negative for the following characteristics: presence of milk coagulation and peptonization, nitrate reduction, indole production and arginine dihydrolase; activities of oxidase, lipase (C14), cysteine arylamidase, α-chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase and urease; hydrolysis of Tween 80 and CM-cellulose; assimilation of caprate, adipate, citric acid and phenylacetate; utilization of *N*-acetyl-neuraminic acid, D-fucose, D-arabitol, D-glucose-6-phosphate, D-aspartic acid, glycyl-L-proline, L-histidine, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, glucuronamide, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methyl ester, citric acid, γ-amino-butryric acid, α-hydroxy-butyric acid, β-hydroxy-DL-butyric acid, α-keto-butyric acid, propionic acid and formic acid as sole sources of carbon and energy; acid production from erythritol, D-ribose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbose, dulcitol, inositol, D-sorbitol, inulin, starch, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol and potassium 2-ketogluconate; sensitivity to 1% sodium lactate, tetrazolium violet, nalidixic acid, lithium chloride, potassium tellurite, sodium bromate and sodium butyrate.

in Fig. S2. The major menaquinones of strains 11W25H-1^T and 8H24J-4-2^T contained MK-12 and MK-11, while those of the two reference strains were MK-11, MK-12 and MK-10 (Table S3). The major polar lipids of both strains 11W25H-1^T and 8H24J-4-2^T comprised diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), an unidentified phospholipid (PL1), an unidentified glycolipid (GL1), and unidentified lipids (Ls), which were almost consistent with those of the reference strains (Fig. S3). The amino acids in the

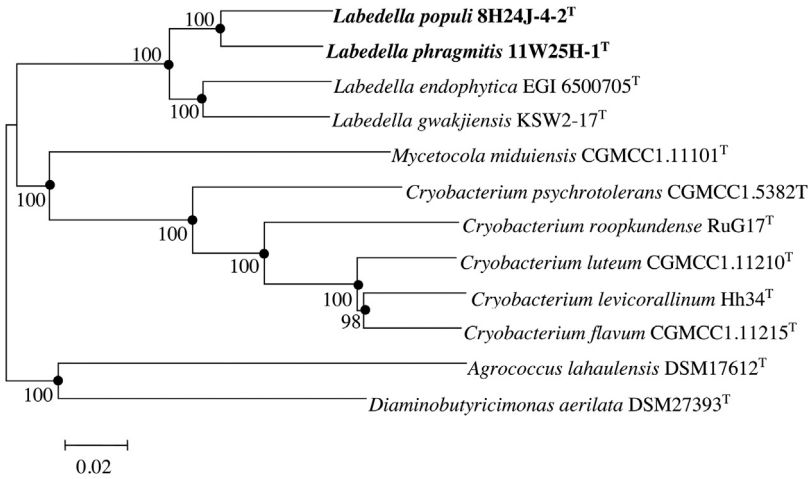

Fig. 2. Neighbor-joining phylogenetic tree based on single-copy phylogenetic marker genes indicating the phylogenetic position of strains 11W25H-1^T and 8H24J-4-2^T with the type species of the genus *Labedella* and other related species. Numbers at nodes refer to bootstrap values (based on 1000 replicates; only values >50% are shown at branch points). Filled circles indicate that the corresponding nodes were also obtained in the maximum likelihood tree. Bar, 2 nt substitutions per 100 nt.

Table 3
Cellular fatty acid compositions (%) of strain 11W25H-1^T, strain 8H24J-4-2^T and the reference strains. Strains: 1, 11W25H-1^T; 2, 8H24J-4-2^T; 3, *L. gwakjiensis* KCTC 19176^T; 4, *L. endophytica* CPCC 203961^T. All strains were cultured simultaneously on TSA at 30 °C for 2 days. All data are from this study.

Fatty acid	1	2	3	4
iso-$C_{14:0}$	3.1	0.7	3.5	2.8
iso-$C_{15:0}$	4.7	3.1	7.3	6.7
anteiso-$C_{15:0}$	**49.5**	**59.4**	**46.9**	**52.3**
iso-$C_{16:0}$	**25.3**	**15.2**	**26.2**	**22.0**
$C_{16:0}$	1.1	1.2	1.4	1.1
iso-$C_{17:0}$	0.6	0.8	1.7	1.9
anteiso-$C_{17:0}$	**11.1**	**18.0**	**10.2**	**10.9**

Note: Only fatty acids >1% of the total fatty acids are shown. Values are percentages of total fatty acids. Major fatty acids (>10%) are indicated in bold type.

cell-wall peptidoglycan of strains 11W25H-1^T and 8H24J-4-2^T contained ornithine, alanine, glycine and glutamic acid, which shared the same compositions as those of the reference strains.

Taxonomic conclusions

In conclusion, the characteristics of strains 11W25H-1^T and 8H24J-4-2^T were consistent with the description of the genus *Labedella,* but they were different from the reference strains in phenotypic, genomic and chemotaxonomic properties. Therefore, it was appropriate to conclude that strains 11W25H-1^T and 8H24J-4-2^T should be assigned to the genus *Labedella* as two new species, for which the names *Labedella phragmitis* sp. nov. and *Labedella populi* sp. nov., respectively, are proposed.

Emended description of the genus *Labedella* Lee 2007

The description of the genus *Labedella* is as given previously by Lee [20] with the following modifications. Cells are rods or cocci. The major menaquinones are MK-12, MK-11 and/or MK-10. Genomic DNA G+C contents are between 68.9 and 69.4 mol%.

Description of *Labedella phragmitis* sp. nov.

Labedella phragmitis (phrag.mi'tis. L. gen. n. *phragmitis* of *Phragmites*, referring to the reed plant, from where the type strain was isolated).

Cells are Gram-stain positive, aerobic, non-spore-forming, non-motile, and rod-shaped (approximately 0.6–0.8 × 1.1–1.7 μm in size) after incubation for 2 days at 30 °C on TSA. The colonies on TSA after 3 days incubation at 30 °C are pale yellow, entire, convex and circular (0.4–0.7 mm in diameter). Strain 11W25H-1^T displays good growth on ISP 2 agar, LB agar, NA, R2A agar and TSA, and moderate growth on ISP 4 agar. Growth is observed at 10–40 °C, pH 6.0–10.0 and in the presence of 0–6% (w/v) NaCl. Optimum growth occurs at 28–30 °C, pH 7.0–8.0 and in 0–2% (w/v) NaCl. Catalase and gelatin liquefaction are positive, whereas milk coagulation and peptonization, H_2S production and oxidase are negative. Hydrolyzes Tween 20, Tween 40 and starch, but not Tween 80 and CM-cellulose. The results of the biochemical and physiological characteristics of strain 11W25H-1^T are shown in Table S2. The peptidoglycan contains ornithine as the diagnostic diamino acid. Major fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The predominant menaquinones are MK-12 and MK-11. The polar lipids contain DPG, PG, PL1, GL1 and six Ls. The genomic G+C content is 69.1 mol%.

The type strain, 11W25H-1^T (=JCM 33144^T=CGMCC 1.16700^T, taxonumber TA00886) was isolated from surface-sterilized reed flowers collected from the Taklamakan Desert in the Xinjiang Uygur Autonomous Region, China.

Description of *Labedella populi* sp. nov.

Labedella populi (po'pu.li. L. gen. n. *populi* of *Populus*, the poplar tree from which the type strain was isolated).

Cells are Gram-stain positive, aerobic, non-spore-forming, non-motile, and rod-shaped (approximately 0.6–0.8 × 0.9–1.5 μm in size) after incubation for 2 days at 30 °C on TSA. The colonies on TSA after 3 days incubation at 30 °C were light yellow, entire, convex and circular (0.4–0.7 mm in diameter). Strain 8H24J-4-2^T displays good growth on ISP 2 agar, LB agar, NA, R2A agar and TSA, and moderate growth on ISP 4 agar. Growth is observed at 10–40 °C, pH 6.0–10.0 and in the presence of 0–7% (w/v) NaCl. Optimum growth occurs at 30–32 °C, pH 7.0–8.0 and in 0–2% (w/v) NaCl. Catalase and gelatin liquefaction are positive, whereas milk coagulation and peptonization, H_2S production and oxidase are negative. Hydrolyzes Tween 20, Tween 40 and starch, but not Tween 80 and CM-cellulose. The results of the biochemical and physiological characteristics of strain 8H24J-4-2^T are shown in Table S2. The peptidoglycan contains ornithine as the diagnostic diamino acid. Major fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. The predominant menaquinones are MK-12 and MK-11. The polar lipids contain DPG, PG, PL1, GL1 and seven Ls. The genomic G+C content is 68.9 mol%.

The type strain, 8H24J-4-2^T (=JCM 33143^T=CGMCC 1.16697^T, taxonumber TA00887) was isolated from a surface sterilized stem of *Populus euphratica* collected from the Taklamakan Desert in the Xinjiang Uygur Autonomous Region, China.

Taxonumber	TA00886	TA00887
Species name	*Labedella phragmitis*	*Labedella populi*
Genus name	*Labedella*	*Labedella*
Specific epithet	*phragmitis*	*populi*
Species status	sp. nov.	sp. nov.
Species etymology	phrag.mi'tis. L. gen. n. *phragmitis* of *Phragmites*, referring to reed plant, from where the type strain was isolated	po′pu.li. L. gen. n. *populi* of *Populus*, the poplar tree from which the type strain was isolated
Authors	Fei-Na Li, Qin-Pei Lu, Shui-Lin Liao, Tao Jin, Wen-Jun Li, Cheng-Hang Sun	Fei-Na Li, Qin-Pei Lu, Shui-Lin Liao, Tao Jin, Wen-Jun Li, Cheng-Hang Sun
Title	*Labedella phragmitetis* sp. nov. and *Labedella populi* sp. nov., two endophytic actinobacteria isolated fromplants in the Taklamakan Desert and emended description of the genus *Labedella*	*Labedella phragmitetis* sp. nov. and *Labedella populi* sp. nov., two endophytic actinobacteria isolated fromplants in the Taklamakan Desert and emended description of the genus *Labedella*
E-mail of the submitter	hbulifeina@126.com	hbulifeina@126.com
Designation of the type strain	11W25H-1	8H24J-4-2
Strain collection numbers	=JCM 33144 =CGMCC 1.16700	=JCM 33143 =CGMCC 1.16697
16S rRNA gene accession number	MK333282	MK333281
Genome accession number [RefSeq]	RZNB00000000	RZNC00000000
Genome status	Draft	Draft
Genome size	3,524,121bp	3,417,745bp
GC mol %	69.1	68.9
Country of origin	China	China
Region of origin	Xinjiang Uyghur Autonomous Region	Xinjiang Uyghur Autonomous Region
Date of isolation	2016-11-14	2016-11-28
Source of isolation	Flowers of *Phragmites australis*	Branch of *Populus euphratica*
Sampling date	2016-10-01	2016-10-01
Geographic location	Taklamakan Desert	Taklamakan Desert
Latitude	38°15′23.17Ñ	38°27′57.91Ñ
Longitude	80°47′50.43Ë	80°10′40.59Ë
Number of strains in study	1	1
Growth medium, incubation conditions [temperature, pH, and further information] used for standard cultivation	Good growth occurs on ISP 2 agar, Luria-Bertani (LB) agar, nutrient agar (NA), R2A agar and tryptic soy agar (TSA) at 30 °C and at pH 7.0	Good growth occurs on ISP 2 agar, Luria-Bertani (LB) agar, nutrient agar (NA), R2A agar and tryptic soy agar (TSA) at 30 °C and at pH 7.0
Conditions of preservation	In 20% (v/v) glycerol and stored at −80 °C	In 20% (v/v) glycerol and stored at −80 °C
Gram stain	Positive	Positive
Cell shape	Rod	Rod
Cell size (length or diameter)	Cells are approximately 0.6–0.8 μm in width and 1.1–1.7 μm in length, after incubation for 2 days at 30 °C on TSA	Cells are approximately 0.6–0.8 μm in width and 0.9–1.5 μm in length, after incubation for 2 days at 30 °C on TSA
Motility	Non-motile	Non-motile
Colony morphology	Colonies are pale yellow, entire, convex, circular and 0.4–0.7 mm in diameter on TSA after 3 days incubation at 30 °C	Colonies are light yellow, entire, convex, circular and 0.4–0.7 mm in diameter on TSA after 3 days incubation at 30 °C
Temperature range	10–40 °C	10–40 °C
Lowest temperature for growth	10 °C	10 °C
Highest temperature for growth	40 °C	40 °C
Temperature optimum	28–30 °C	30–32 °C
Lowest pH for growth	6.0	6.0
Highest pH for growth	10.0	10.0
pH optimum	7.0–8.0	7.0–8.0
pH category	Neutrophile	Neutrophile
Lowest NaCl concentration for growth	0%	0%
Highest NaCl concentration for growth	6.0%	7.0%
Salinity optimum	0–2.0%	0–2.0%
Salinity category	Mild halophile (optimum 1–6% NaCl)	Mild halophile (optimum 1–6% NaCl)
Relationship to O2	Aerobe	Aerobe
Positive tests with BIOLOG	Utilization of acetic acid, acetoacetic acid, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, L-alanine, L-aspartic acid, D-cellobiose, dextrin, D-fructose, L-fucose, D-galactose, gentiobiose, D-gluconic acid, α-D-glucose, L-glutamic acid, glycerol, p-hydroxy-phenylacetic acid, inosine, L-lactic acid, α-D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, myo-inositol, pectin, L-pyroglutamic acid, D-raffinose, D-salicin, L-serine, D-sorbitol, stachyose, sucrose, D-trehalose, D-turanose and Tween 40, and sensitivity to fusidic acid, D-serine, troleandomycin, rifamycin SV, minocycline, lincomycin, guanidine HCl, niaproof 4, vancomycin and tetrazolium blue	Utilization of acetic acid, acetoacetic acid, *N*-acetyl-D-glucosamine, L-alanine, D-cellobiose, dextrin, D-fructose, L-fucose, D-galactose, gelatin, gentiobiose, α-D-glucose, D-gluconic acid, L-glutamic acid, glycerol, inosine, L-lactic acid, α-D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, methyl pyruvate, myo-inositol, pectin, L-pyroglutamic acid, D-salicin, D-sorbitol, stachyose, sucrose, D-trehalose, D-turanose and Tween 40, and sensitivity to fusidic acid, D-serine, troleandomycin, rifamycin SV, minocycline, lincomycin, guanidine HCl, niaproof 4, vancomycin and tetrazolium blue

Negative tests with BIOLOG	Utilization of *N*-acetyl-D-galactosamine, *N*-acetyl-neuraminic acid, γ-amino-butyric acid, D-arabitol, L-arginine, D-aspartic acid, bromosuccinic acid, citric acid, formic acid, D-fucose, L-galactonic acid lactone, D-galacturonic acid, gelatin, D-glucose-6-phosphate, glucuronamide, D-glucuronic acid, glycyl-L-proline, L-histidine, α-hydroxy-butyric acid, β-hydroxy-DL-butyric acid, α-keto-butyric acid, D-lactic acid methyl ester, D-malic acid, L-malic acid, methyl pyruvate, mucic acid, propionic acid, quinic acid, L-rhamnose and D-saccharic acid, and sensitivity to 1 % sodium lactate, tetrazolium violet, nalidixic acid, lithium chloride, potassium tellurite, aztreonam, sodium butyrate and sodium bromate	Utilization of *N*-acetyl-D-galactosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-neuraminic acid, γ-amino-butyric acid, D-arabitol, L-arginine, D-aspartic acid, bromosuccinic acid, citric acid, formic acid, D-fructose-6-phosphate, D-fucose, D-galacturonic acid, L-galactonic acid lactone, D-glucose-6-phosphate, glucuronamide, D-glucuronic acid, glycyl-L-proline, L-histidine, α-hydroxy-butyric acid, β-hydroxy-DL-butyric acid, p-hydroxy-phenylacetic acid, α-keto-butyric acid, α-keto-glutaric acid, D-lactic acid methyl ester, D-malic acid, L-malic acid, 3-methyl glucose, mucic acid, propionic acid, quinic acid, D-raffinose, L-rhamnose, D-saccharic acid, D-serine and L-serine, and sensitivity to 1 % sodium lactate, tetrazolium violet, nalidixic acid, lithium chloride, potassium tellurite, aztreonam, sodium butyrate and sodium bromate
Variable tests with BIOLOG	Weakly positive for utilization of D-fructose-6-phosphate, α-keto-glutaric acid, 3-methyl glucose and D-serine	Weakly positive for utilization of L-aspartic acid
Positive tests with API	API ZYM: Esterase lipase (C8), α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase API 20NE: Aesculin hydrolysis, β-galactosidase, gelatin hydrolysis, and assimilation of *N*-acetylglucosamine, L-arabinose, gluconate, D-glucose, maltose, D-mannitol and D-mannose	API ZYM: Esterase (C4), esterase lipase (C8), α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and leucine arylamidase API 20NE: Aesculin hydrolysis, β-galactosidase, gelatin hydrolysis, glucose fermentation, and assimilation of *N*-acetylglucosamine, L-arabinose, gluconate, D-glucose, maltose, D-mannitol and D-mannose
Negative tests with API	API ZYM: *N*-Acetyl-β-glucosaminidase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C4), α-fucosidase, β-glucuronidase, lipase (C14), α-mannosidase and valine arylamidase API 20NE: Arginine dihydrolase, glucose fermentation, indole production, nitrate reduction, urease, and assimilation of adipate, caprate, citric acid, malic acid and phenylacetate	API ZYM: *N*-Acetyl-β-glucosaminidase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, α-fucosidase, β-glucuronidase, lipase (C14), α-mannosidase and trypsin API 20NE: Arginine dihydrolase, indole production, nitrate reduction, urease, and assimilation of adipate, caprate, citric acid, malic acid and phenylacetate
Variable tests with API	API ZYM: Weakly positive for acid phosphatase and trypsin	API ZYM: Weakly positive for acid phosphatase, naphthol-AS-BI-phosphohydrolase and valine arylamidase
Acid formation from carbohydrates (all positive)	Aesculin, amygdalin, L-arabinose, arbutin, D-cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, D-glucose, glycerol, D-lactose, D-maltose, D-mannitol, D-mannose, methyl α-D-glucopyranoside, salicin, sucrose, D-trehalose, D-turanose and D-xylose	Aesculin, L-arabinose, arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melezitose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, salicin, sucrose, D-trehalose, D-turanose and D-xylose
ACID FORMATION FROM CARBOHYDRATES (variable)	Weakly positive for D-melezitose and methyl α-D-mannopyranoside	Weakly positive for *N*-acetylglucosamine, amygdalin, L-fucose, glycerol, D-melibiose and D-raffinose
Acid formation for carbohydrates (all negative)	*N*-Acetylglucosamine, D-adonitol, D-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, glycogen, inositol, inulin, D-lyxose, D-melibiose, methyl β-D-xylopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, starch, D-tagatose, xylitol and L-xylose	D-Adonitol, D-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, gentiobiose, glycogen, inositol, inulin, D-lyxose, methyl β-D-xylopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, starch, D-tagatose, xylitol and L-xylose
Oxidase	Negative	Negative
Catalase	Positive	Positive
Positive tests	Gelatin liquefaction, and hydrolysis of Tween 20, Tween 40 and starch	Gelatin liquefaction, and hydrolysis of Tween 20, Tween 40 and starch
Negative tests	Milk coagulation and peptonization, H_2S production, and hydrolysis of Tween 80 and CM-cellulose	Milk coagulation and peptonization, H_2S production, and hydrolysis of Tween 80 and CM-cellulose
Quinone Type	MK-12 and MK-11	MK-12 and MK-11
Major fatty acids	anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$	anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$
Peptidoglycan type	The peptidoglycan contains ornithine as the diagnostic diamino acid	The peptidoglycan contains ornithine as the diagnostic diamino acid
Phospolipid pattern or diagnostic phospolipid	Diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG)	Diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG)
Glycolipids	One gycolipid	One gycolipid

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.syapm.2019.126004.

References

[1] Cappuccino, J.G., Sherman, N. 2002 Microbiology: a laboratory manual, 6th edn., Benjamin Cummings Pearson Education, San Francisco.

[2] Chen, Y., Chen, Y., Shi, C., Huang, Z., Zhang, Y., Li, S., Li, Y., Ye, J., Yu, C., Li, Z., Zhang, X., Wang, J., Yang, H., Fang, L., Chen, Q. (2018) SOAPnuke: a MapReduce acceleration supported software for integrated quality control and preprocessing of high-throughput sequencing data. GigaScience 7, 1–6.
[3] Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., da Costa, M.S., Rooney, A.P., Yi, H., Xu, X.W., De Meyer, S., Trujillo, M.E. (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int. J. Syst. Evol. Microbiol. 68, 461–466.
[4] Collins, M.D., Pirouz, T., Goodfellow, M., Minnikin, D.E. (1977) Distribution of menaquinones in actinomycetes and corynebacteria. J. Gen. Microbiol. 100, 221–230.
[5] Colston, S.M., Fullmer, M.S., Beka, L., Lamy, B., Gogarten, J.P., Graf, J. (2014) Bioinformatic genome comparisons for taxonomic and phylogenetic assignments using *Aeromonas* as a test case. MBio 5, e02136.
[6] DeLong, E.F. (1992) Archaea in coastal marine environments. Proc. Natl. Acad. Sci. U.S.A. 89, 5685–5689.
[7] Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.
[8] Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
[9] Finn, R.D., Clements, J., Eddy, S.R. (2011) HMMER web server: interactive sequence similarity searching. Nucleic Acids Res. 39, W29–W37.
[10] Fitch, W.M. (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst. Biol. 20, 406–416.
[11] Gonzalez, C., Gutierrez, C., Ramirez, C. (1978) *Halobacterium vallismortis* sp. nov., an amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. Can. J. Microbiol. 24, 710–715.
[12] Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., Tiedje, J.M. (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int. J. Syst. Evol. Microbiol. 57, 81–91.
[13] Guo, L., Tuo, L., Habden, X., Zhang, Y., Liu, J., Jiang, Z., Liu, S., Dilbar, T., Sun, C. (2015) *Allosalinactinospora lopnorensis* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae* isolated from soil. Int. J. Syst. Evol. Microbiol. 65, 206–213.
[14] Hyatt, D., Chen, G.L., Locascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J. (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11, 119.
[15] Kelly, K.L. 1964 Inter-Society Color Council-National Bureau of Standards Color Name Charts illustrated with Centroid Colors, US Government Printing Office, Washington, DC.
[16] Kim, M., Oh, H.S., Park, S.C., Chun, J. (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int. J. Syst. Evol. Microbiol. 64, 346–351.
[17] Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. J. Mol. Evol. 16, 111–120.
[18] Klenk, H.P., Göker, M. (2010) *En route* to a genome-based classification of *Archaea* and *Bacteria*? Syst. Appl. Microbiol. 33, 175–182.
[19] Kumar, S., Stecher, G., Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
[20] Lee, S.D. (2007) *Labedella gwakjiensis* gen. nov., sp. nov., a novel actinomycete of the family *Microbacteriaceae*. Int. J. Syst. Evol. Microbiol. 57, 2498–2502.
[21] Li, F.N., Tuo, L., Pan, Z., Guo, M., Lee, S.M., Chen, L., Hu, L., Sun, C.H. (2017) *Aureimonas endophytica* sp. nov., a novel endophytic bacterium isolated from *Aegiceras corniculatum*. Int. J. Syst. Evol. Microbiol. 67, 2934–2940.
[22] Li, W.J., Xu, P., Schumann, P., Zhang, Y.Q., Pukall, R., Xu, L.H., Stackebrandt, E., Jiang, C.L. (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China) and emended description of the genus *Georgenia*. Int. J. Syst. Evol. Microbiol. 57, 1424–1428.
[23] Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., Henrissat, B. (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 42, D490–D495.
[24] Magee, C.M., Rodeheaver, G., Edgerton, M.T., Edlich, R.F. (1975) A more reliable gram staining technic for diagnosis of surgical infections. Am. J. Surg. 130, 341–346.
[25] Marmur, J. (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3, 208–218.
[26] Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Göker, M. (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14, 60.
[27] Mende, D.R., Sunagawa, S., Zeller, G., Bork, P. (2013) Accurate and universal delineation of prokaryotic species. Nat. Methods 10, 881–884.
[28] Mikheenko, A., Valin, G., Prjibelski, A., Saveliev, V., Gurevich, A. (2016) Icarus: visualize for *de novo* assembly evaluation. Bioinformatics 32, 3321–3323.
[29] Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., Parlett, J.H. (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J. Microbiol. Methods 2, 233–241.
[30] Park, Y.H., Suzuki, K., Yim, D.G., Lee, K.C., Kim, E., Yoon, J., Kim, S., Kho, Y.H., Goodfellow, M., Komagata, K. (1993) Suprageneric classification of peptidoglycan group B actinomycetes by nucleotide sequencing of 5S ribosomal RNA. Antonie Van Leeuwenhoek 64, 307–313.
[31] Peng, Y., Leung, H.C., Yiu, S.M., Chin, F.Y. (2012) IDBA-UD: a *de novo* assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28, 1420–1428.
[32] Pikuta, E.V., Hoover, R.B., Bej, A.K., Marsic, D., Whitman, W.B., Krader, P.E., Tang, J. (2006) *Trichococcus patagoniensis* sp. nov., a facultative anaerobe that grows at −5 °C, isolated from penguin guano in Chilean Patagonia. Int. J. Syst. Evol. Microbiol. 56, 2055–2062.
[33] Qin, S., Wang, H.B., Chen, H.H., Zhang, Y.Q., Jiang, C.L., Xu, L.H., Li, W.J. (2008) *Glycomyces endophyticus* sp. nov., an endophytic actinomycete isolated from the root of *Carex baccans* Nees. Int. J. Syst. Evol. Microbiol. 58, 2525–2528.
[34] Richter, M., Rosselló-Móra, R. (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl. Acad. Sci. U.S.A. 106, 19126–19131.
[35] Saitou, N., Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
[36] Sasser, M. 1990 Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101, MIDI Inc., Newark, DE.
[37] Shirling, E.B., Gottlieb, D. (1966) Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16, 313–340.
[38] Stackebrandt, E., Rainey, F.A., Ward-Rainey, N.L. (1997) Proposal for a new hierarchic classification system, *Actinobacteria classis* nov. Int. J. Syst. Bacteriol. 47, 479–491.
[39] Swindell, S.R., Plasterer, T.N. (1997) SEQMAN. Contig assembly. Methods Mol. Biol. 70, 75–89.
[40] Wang, H.F., Zhang, Y.G., Cheng, J., Hozzein, W.N., Liu, W.H., Li, L., Chen, J.Y., Guo, J.W., Zhang, Y.M., Li, W.J. (2015) *Labedella endophytica* sp. nov., a novel endophytic actinobacterium isolated from stem of *Anabasis elatior* (C. A. Mey.) Schischk. Antonie Van Leeuwenhoek 107, 95–102.
[41] Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt, E., Starr, M.P., Truper, H.G. (1987) Report of the *ad hoc* committee on reconciliation of approaches to bacterial systematics. Int. J. Syst. Bacteriol. 37, 463–464.
[42] Xu, P., Li, W.J., Tang, S.K., Zhang, Y.Q., Chen, G.Z., Chen, H.H., Xu, L.H., Jiang, C.L. (2005) *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family '*Oxalobacteraceae*' isolated from China. Int. J. Syst. Evol. Microbiol. 55, 1149–1153.
[43] Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., Chun, J. (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol. 67, 1613–1617.