Journal of Microbiology (2022) Vol. 60, No. 10, pp. 977–985
DOI 10.1007/s12275-022-2134-8
eISSN 1976-3794
pISSN 1225-8873

Phenotypic and genomic characteristics of *Brevibacterium zhoupengii* sp. nov., a novel halotolerant actinomycete isolated from bat feces[§]

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(Received Mar 21, 2022 / Revised Jul 22, 2022 / Accepted Jul 26, 2022)

Two strictly aerobic, Gram-staining-positive, non-spore-forming, regular rod-shaped (approximately 0.7 × 1.9 mm) bacteria (HY170^T and HY001) were isolated from bat feces collected from Chongzuo city, Guangxi province (22°20′54″N, 106°49′20″E, July 2011) and Chuxiong Yi Autonomous Prefecture, Yunnan province (25°09′10″N, 102°04′39″E, October 2013) of South China, respectively. Optimal growth is obtained at 25–28°C (range, 4–32°C) on BHI-5% sheep blood plate with pH 7.5 (range, 5.0–10.0) in the presence of 0.5–1.0% NaCl (w/v) (range, 0–15% NaCl [w/v]). The phylogenetic and phylogenomic trees based respectively on the 16S rRNA gene and 845 core gene sequences revealed that the two strains formed a distinct lineage within the genus *Brevibacterium*, most closely related to *B. aurantiacum* NCDO 739^T (16S rRNA similarity, both 98.5%; dDDH, 46.7–46.8%; ANI, 91.9–92.1%). Strain HY170^T contained MK-8(H_2), diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG), galactose and ribose as the predominant menaquinone, major polar lipids, and main sugars in the cell wall teichoic acids, respectively. The *meso*-diaminopimelic acid (*meso*-DAP) was the diagnostic diamino acid of the peptidoglycan found in strain HY170^T. *Anteiso*-$C_{15:0}$ and *anteiso*-$C_{17:0}$ were the major fatty acids (> 10%) of strains HY170^T and HY001, with *anteiso*-$C_{17:1}$A predominant in strain HY170^T but absent in strain HY001. Mining the genomes revealed the presence of secondary metabolite biosynthesis gene clusters encoding for non-alpha poly-amino acids (NAPAA), ectoine, siderophore, and terpene. Based on results from the phylogenetic, chemotaxonomic and phenotypic analyses, the two strains could be classified as a novel species of the genus *Brevibacterium*, for which the name *Brevibacterium zhoupengii* sp. nov. is proposed (type strain HY170^T = CGMCC 1.18600^T = JCM 34230^T).

Keywords: *Brevibacterium zhoupengii*, bat, carotenoid, *Mrp* gene, halotolerant

Introduction

The fact is eventually accepted that bats, the only mammal that can fly, carry a large number of viruses, including deadly viruses like SARS coronavirus, Nipah virus, Hendra virus, and Ebola virus (Shi, 2010; Smith and Wang, 2013). However, bat-associated bacteria were relatively neglected (Muhldorfer, 2013; Afonso and Goydadin, 2018), which might play an important role as components of the microbiota. Some novel bacterial species previously isolated from bat feces in our laboratory belong to the phylum Actinobacteria, such as *Agromyces laixinhei* (Cheng *et al.*, 2021), *Haloactinobacterium kanbiaonis* (Xu *et al.*, 2021), *Microbacterium chengjingii*, *M. fandaimingii* (Zhou *et al.*, 2021), and *Ruania zhangjianzhongii* (Xu *et al.*, 2021). In the present study, we continue to explore bat fecal microbiota, and describe the phenotypic and genomic characterization of a novel species in the genus *Brevibacterium* belonging to phylum Actinobacteria.

The genus *Brevibacterium* is a group of Gram-stain- and catalase-positive, nonsporulating, nonmotile, obligate aerobic or anaerobic-tolerant rod-shaped bacteria, having *meso*-diaminopimelic acid (*meso*-DAP) as diagnostic peptidoglycan, *anteiso*-$C_{15:0}$ and *anteiso*-$C_{17:0}$ as the primary fatty acids, and MK-8(H_2) as major respiratory menaquinone (Collins and Farrow, 1983). As of July 2022, there are 36 validly published species within genus *Brevibacterium* (https://lpsn.dsmz.de/genus/brevibacterium) isolated from various sources, such as milk products, poultry, sediment, soil, oil paintings, clinical specimens, multiple sites on the human body, insects, brown algae, and marine environments. Overall, the genus has quite a few extraordinary features. Genetically, the sequenced ge-

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[§]Supplemental material for this article may be found at https://doi.org/10.1007/s12275-022-2134-8.

nomes have relatively high G + C contents within a range of 55.8–72.4 mol% (Wauters *et al.*, 2001; Deng *et al.*, 2020). Phenotypically, almost all of the members are nonmotile and can tolerate 10% (w/v) or even higher of NaCl, with a few exceptions. Only two (*B. album* and *B. samyangense*) are motile (Lee, 2006; Tang *et al.*, 2008), and *B. anseongense* is the only one reported to be unable to grow in medium with NaCl higher than 1% (Jung *et al.*, 2018). Furthermore, the genus *Brevibacterium*, with a lot of unknowns, also plays a crucial role in industry, producing enzymes, aroma, antimicrobial substances, amino acids (L-lysine and L-glutamic acid), and carotenoids (Onraedt *et al.*, 2005). *Brevibacterium linens*, the type species of genus, first isolated from the surface of ripened cheese in 1910 (Forquin-Gomez and Weimer, 2014) and formally described in 1953 (Breed, 1953), is found to be vital for the hallmark color, flavor, and aroma of smear surface-ripened cheeses (Onraedt *et al.*, 2005). Also of note is that some species of the genus *Brevibacterium* are likely opportunistic pathogens causing bacteremia, brain abscess, endocarditis, meningitis, and osteomyelitis. At least five species (*B. casei*, *B. epidermidis*, *B. iodinum*, *B. otitidis*, and *B. paucivorans*) have been involved in diverse infections affecting either immunocompromised or even immunocompetent people (McCaughey and Damani, 1991; Antoniou *et al.*, 1997; Wauters *et al.*, 2000; Cannon *et al.*, 2005; Kumar *et al.*, 2011; Asai *et al.*, 2019; Joshi *et al.*, 2020).

Here, we propose *Brevibacterium zhoupengii* sp. nov., a novel halotolerant actinomycete of the genus *Brevibacterium* isolated from bat feces, with a carotenoid biosynthetic gene cluster highly similar to that of *B. linens*.

Materials and Methods

Purpose of isolation

Our laboratory has a long term interest in investigating the intestine (via fecal samples) microbiome diversity of wild animals such as *Equus kiang* (Huang *et al.*, 2019), *Marmota himalayana* (Liu *et al.*, 2015), *Pantholops hodgsonii* (Bai *et al.*, 2016), and yak (Ge *et al.*, 2021) to identify potentially pathogenic microbial species and dissect the process of disease transmission. Recently, bats have attracted global attention because of their zoonotic association with severe acute respiratory syndrome coronaviruses (SARS-CoV and SARS-CoV-2) (Hu *et al.*, 2021). Thus far, the prevalence and abundance of pathogenic bacteria in bats and their potential public health significance have largely been neglected (Muhldorfer, 2013; Afonso and Goydadin, 2018), and we are curious if bat fecal samples contain any novel bacteria alongside the viruses.

Isolation and culture conditions of the strains

During investigating the microbial diversity in bat feces, two strains (HY001, HY170^T) were isolated from fecal samples of bats (*Taphozuos* spp. and *Rousettus* spp.), which were collected from the Chongzuo city, Guangxi province (22°20′54″N, 106°49′20″E, July 2011) and Chuxiong Yi Autonomous Prefecture, Yunnan province (25°09′10″N, 102°04′39″E, October 2013), respectively. Sample collection, storage, transportation and strain isolation were similar to what was previously described (Cheng *et al.*, 2021; Huang *et al.*, 2022). In brief, bat feces from the same location were pooled together, fully ground, well mixed on a 1.5 ml tube rotator, and 150 µl of each mixed sample (1.0 g diluted with 1.0 ml 0.85% [w/v] NaCl solution) was immediately plated onto the brain-heart infusion (BHI) plates supplemented with 5% sheep blood (BHIB). After aerobic incubation at 28°C for 24–96 h, selected bacterial colonies were subjected to preliminary characterization by PCR amplification and sequencing of 16S rRNA gene sequence analysis. Strains HY170^T and HY001, undergoing an identical procedure as other colonies, were purified on BHIB agar and preserved at -80°C in BHI broth supplemented with glycerol (20%, v/v).

16S rRNA gene sequences and phylogenetic analysis

To determine the phylogenetic positions of strains HY170^T and HY001, their 16S rRNA genes were amplified with the universal primers 27F and 1492R, cloned by using the *pEASY*-T3 cloning kit, sequenced as before (Huang *et al.*, 2022), and deposited in GenBank with accession numbers MH-915557 and OL752407, respectively. The newly generated 16S rRNA gene sequences were compared with others by EzBioCloud's identification service (https://www.ezbiocloud. net) (Yoon *et al.*, 2017a) to locate their taxonomic position (phylum, Actinobacteria; class, Actinomycetia; order, Brevibacteriales; family, Brevibacteriaceae; genus, *Brevibacterium*). Multiple alignment of the 16S rRNA gene sequences of the type strains in the genus *Brevibacterium* was performed using the CLUSTAL W program (Thompson *et al.*, 1994) and phylogenetic analysis using the software package MEGA X (https://www.megasoftware.net/), by the neighbor-joining (NJ) (Saito and Nei, 1987), maximum parsimony (MP) (Fitch, 1971) and maximum likelihood (ML) (Felsenstein, 1981) algorithms with a bootstrap analysis of 1,000 replicates (Felsenstein, 1985).

Whole-genome sequencing and phylogenomic analysis

Strain HY170^T was sequenced by single molecule real-time (SMRT) technology (McCarthy, 2010) on the Pacific Biosciences (PacBio) sequencing platform to obtain its whole genome sequence without gaps, which was assembled using *de novo* and analyzed using the Hierarchical Genome Assembly Process (HGAP4) application (Castanera *et al.*, 2020). Meanwhile, strain HY001 was sequenced on the Illumina HiSeq TM2000 platform and assembled using VELVET (Zerbino and Birney, 2008). The genomic sequences of strains HY170^T and HY001 were deposited in GenBank with accession numbers CP088298 and JAJNND000000000, the other available whole genomes within the genus of *Brevibacterium* (listed in Supplementary data Table S1) were obtained from the NCBI sequence read archive (https://www.ncbi.nlm.nih.gov/genome), and *Sediminivirga luteola* CGMCC 1.12785^T served as outgroup. Gene annotation, including the protein-coding DNA sequences (CDSs), tRNAs, and rRNAs was processed by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP; https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). Gene calling and annotation was performed using the RAST (Rapid Annotation using Subsystem Technology) server (https://rast.nmpdr.org/) and the SEED viewer framework

(Aziz *et al.*, 2008; Overbeek *et al.*, 2014). The clusters of orthologous groups of proteins (COG) database was used for functional gene annotation by a BLAST search (Galperin *et al.*, 2015) with the minimal alignment length percentage larger than 40% (E-value less than 1e-5). Secondary metabolite biosynthesis gene clusters in bacterial genomes were identified, annotated and analyzed by antiSMASH bacterial version (https://antismash.secondarymetabolites.org/#!/start) (Medema *et al.*, 2011). Furthermore, digital DNA-DNA hybridization (dDDH) (http://ggdc.dsmz.de/ggdc.php; with a threshold of 70%) and average nucleotide identity (ANI) (ANI Calculator | Ezbiocloud.net; with a threshold of 95–96%) have been widely used for bacterial species delineation, with percentage calculations using Formula 2 (Auch *et al.*, 2010) and the OrthoANIu algorithm (Yoon *et al.*, 2017b), respectively.

To further verify the taxonomic position of strains HY170^T and HY001 in the genus *Brevibacterium*, a bio-clustering tool software, CD-HIT (Fu *et al.*, 2012) was used to cluster amino acids from 31 whole genomes (Supplementary data Table S1, including strains HY170^T and HY001) by a protein sequence identity threshold of 0.4. A phylogenomic tree was rebuilt with the NJ method using FastTree (Price *et al.*, 2009) after the extracted core genes were concatenated and aligned by MAFFT. Then, the phylogenomic tree was visualized by Dendroscope 3 (version 3.5.10, 14 Nov 2018) (Huson and Scornavacca, 2012), and modified with Interactive Tree of Life (https://itol.embl.de/).

Phenotypic and biochemical characterization

Bacterial growth requirement was tested in BHI broth for 7 days in various temperatures (4, 10, 15, 20, 25, 28, 30, 32, 35, 37, 40, 42, or 45°C), pH (4.0–11.0, 0.5 units intervals) and salt concentrations (0.5–22.0% [w/v] NaCl, with an interval of 0.5%). Culture characteristics were determined on different media, such as BHI, BHIB, Reasoner's 2A, Nutrient, MacConkey, and Tryptic soy agar. Growth on identical plates was also assessed for up to one week under microaerophilic (a 5% [v/v] CO_2 incubator) or anaerobic condition (anaerobic chamber: 80.0% N_2, 10.0% CO_2, and 10.0% H_2). Gram staining was performed using a Gram-staining kit (Baso) (Austrian, 1960), and motility was tested via observing the spreading growth of cells inoculated by piercing into BHI semisolid (0.3% agar) medium in test tubes (Xu *et al.*, 2013). Morphology of strain HY170^T was observed under a light microscope (RVL2-K, Echo) and a transmission electron microscope (HT7700, Hitachi). Catalase activity of strains was tested with a drop of 3% H_2O_2 (v/v) solution, and 1% (w/v) tetramethyl-p-phenylenediamine was used to determine the oxidase activity of fresh cells. Further biochemical characteristics (carbon utilization, acid production, and enzyme activities) were obtained using the identification system of API 50CH strips (with API 50 CHB medium), API 20NE and API ZYM according to the manufacturers' instructions (bioMérieux) (Irakli *et al.*, 2011).

Chemotaxonomic characterization

For the following assays, all the tested strains were harvested in exponential growth phase under the same conditions (BHIB plates [1.0% NaCl, w/v; pH 7.5], aerobically at 28°C for 2 days). Cellular fatty acids were extracted and identified following the standard protocols of the Sherlock Microbial Identification System (MIDI) (Sasser, 1990). High performance liquid chromatography (HPLC) was used to extract, purify, and analyze the respiratory isoprenoid quinones (Komagata and Suzuki, 1988). Polar lipids were analyzed using two-dimensional thin-layer chromatography (2D TLC) after hydrolysis with 6 M HCl at 100°C for 18 h (Staneck and Roberts, 1974; Harper and Davis, 1979). Peptidoglycan amino acid composition was measured with a Hitachi-8900 high speed amino acid analyzer after hydrolyzing the cell-wall, and whole-cell sugars were examined according to the method of Hasegawa *et al.* (1983). Bacterial carotenoid extracts were analyzed at 450 nm using an UPLC system with DAD detector (UPLC, U3000; Thermo Scientific). The analytical conditions were as follows, UPLC: column, YMC Carotenoid S-3 μm (150 × 4.6 mm); column temperature, 40°C; flow rate, 1.0 ml/min; injection volume, 2 μl; solvent system (MeOH: MTBE:H_2O = 20:75:5); gradient program, 100:0 V/V at 0 min, 39:61 V/V at 15 min, 0:100 V/V at 25 min, 100:0 V/V at 25.1 min, 100:0 V/V at 30 min. Data were acquired on the U3000 UPLC (Thermo Scientific), and processed using chromeleon 7.2 CDS (Thermo Scientific) (Meléndez-Martínez *et al.*, 2010; Irakli *et al.*, 2011).

Results and Discussion

Phylogenetic and phylogenomic analyses

The near full-length 16S rRNA gene (1,499 bp) of strain HY170^T was most closely related to that of strain HY001 (100%), *B. aurantiacum* NCDO 739^T (98.5%), *B. antiquum* VKM Ac-2118^T (97.8%) and *B. celere* KMM 3637^T (97.6%), with < 97.5% to other members of the genus *Brevibacterium*, via the EzBioCloud database service. In the NJ phylogenetic tree (Fig. 1), our isolates (HY170^T and HY001) clustered with *B. aurantiacum* NCDO 739^T and *B. antiquum* VKM Ac-2118^T, respectively, with bootstrap values of 61% and 93%. This cluster was adjacent to another cluster comprising *B. celere* KMM 3637^T and other species of *Brevibacterium*, and these findings were also supported by the ML and MP analysis (Supplementary data Figs. S1 and S2).

The phylogenomic tree (Fig. 2) based on 845 core genes illustrated that our isolates were closest to *B. aurantiacum* ATCC 9175^T (= NCDO 739^T) and *B. antiquum* DSM 21545^T (= VKM Ac-2118^T), further proving that the isolates belong to genus *Brevibacterium*. The topologies of the phylogenetic/phylogenomic trees (Figs. 1, 2; Supplementary data Figs. S1 and S2) were consistent to the DNA relatedness between strains, i.e., *B. aurantiacum* JCM 2590^T had almost identical G + C content, the highest 16S rRNA similarity and scores of dDDH and ANI with our isolates (Supplementary data Table S1), followed by *B. antiquum* JCM 13317^T. Thus, *B. aurantiacum* JCM 2590^T (= ATCC 9175^T = NCDO 739^T), *B. antiquum* JCM 13317^T (= DSM 21545^T = VKM Ac-2118^T) and *B. celere* JCM 13521^T (= KMM 3637^T) were chosen and purchased from Japan Collection of Microorganisms for direct comparison in subsequent phenotypic and biochemical analyses.

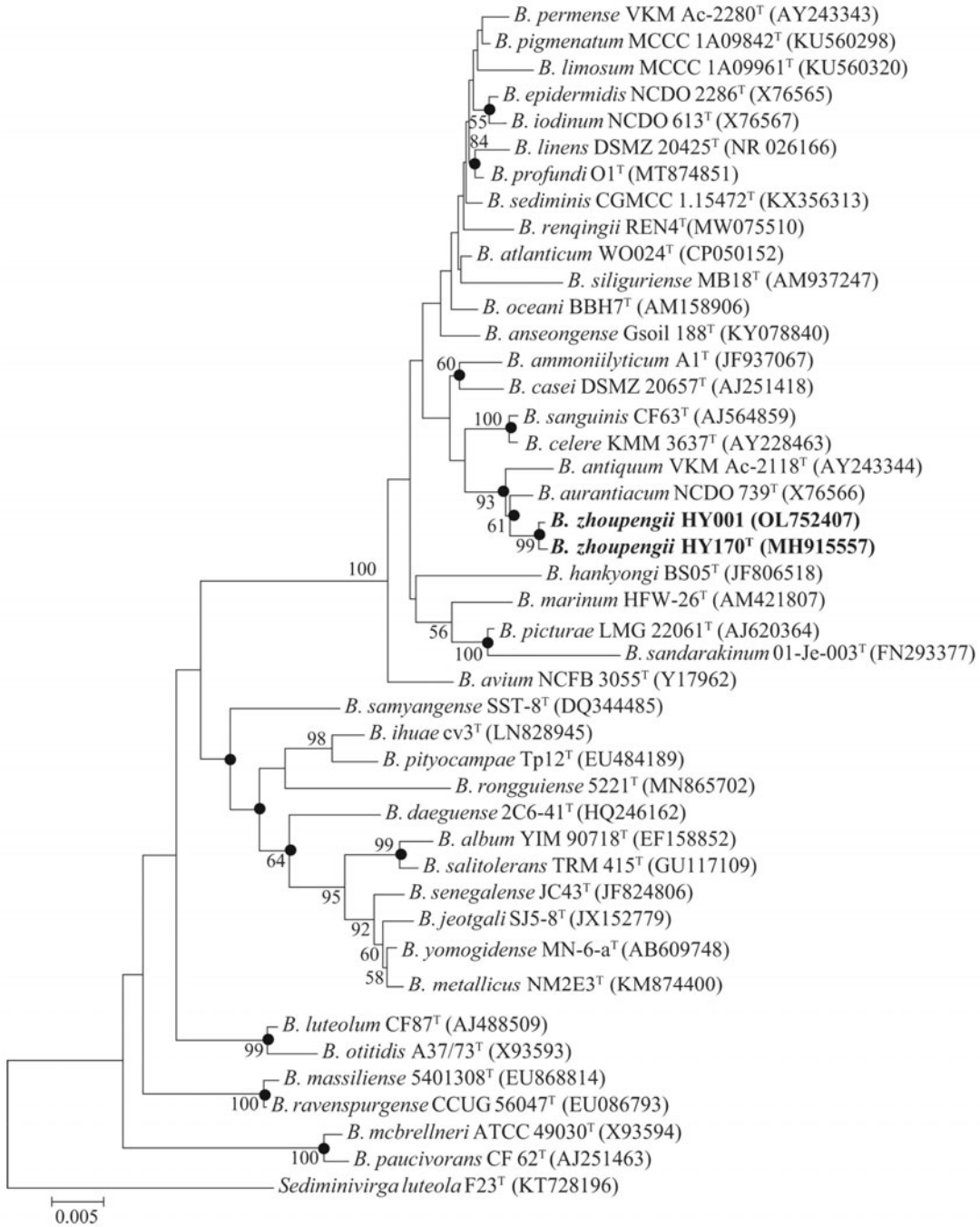

Fig. 1. NJ phylogenetic tree based on nearly full length 16S rRNA gene sequences revealing the position of *Brevibacterium zhoupengii* sp. nov. Solid circles indicate the nodes supported by both maximum-likelihood and maximum-parsimony analyses. Numbers on the tree indicate bootstrap values calculated for 1,000 subsets for branch points > 50%. Bar, 0.005 substitutions per nucleotide position. *Sediminivirga luteola* F23^T serving as outgroup.

The dDDH and ANI values between strains HY170^T and HY001 were 93.4% and 99.2%, respectively, indicating that they belong to the same species. However, they showed dDDH and ANI scores far below the threshold level (dDDH, 70%; ANI, 95–96%) when comparing to their closely related species and other available type strains of genus *Brevibacterium* (Supplementary data Table S1), demonstrating that they could be assigned to a novel species of *Brevibacterium*.

Whole genome characteristics and functional gene annotation

The genome of strain HY170^T was comprised of a circular chromosome of 4,408,116 bp (N_{50} 4,408,116 bp), containing 12 rRNA genes, 49 tRNA genes, and 3,894 predicted CDSs, respectively. Likewise, the draft genome of strain HY001 assembled from 8 contigs was 4,310,443 bp (N_{50} 939,788) long, including 7 rRNA genes, 49 tRNA genes and 4,042 predicted

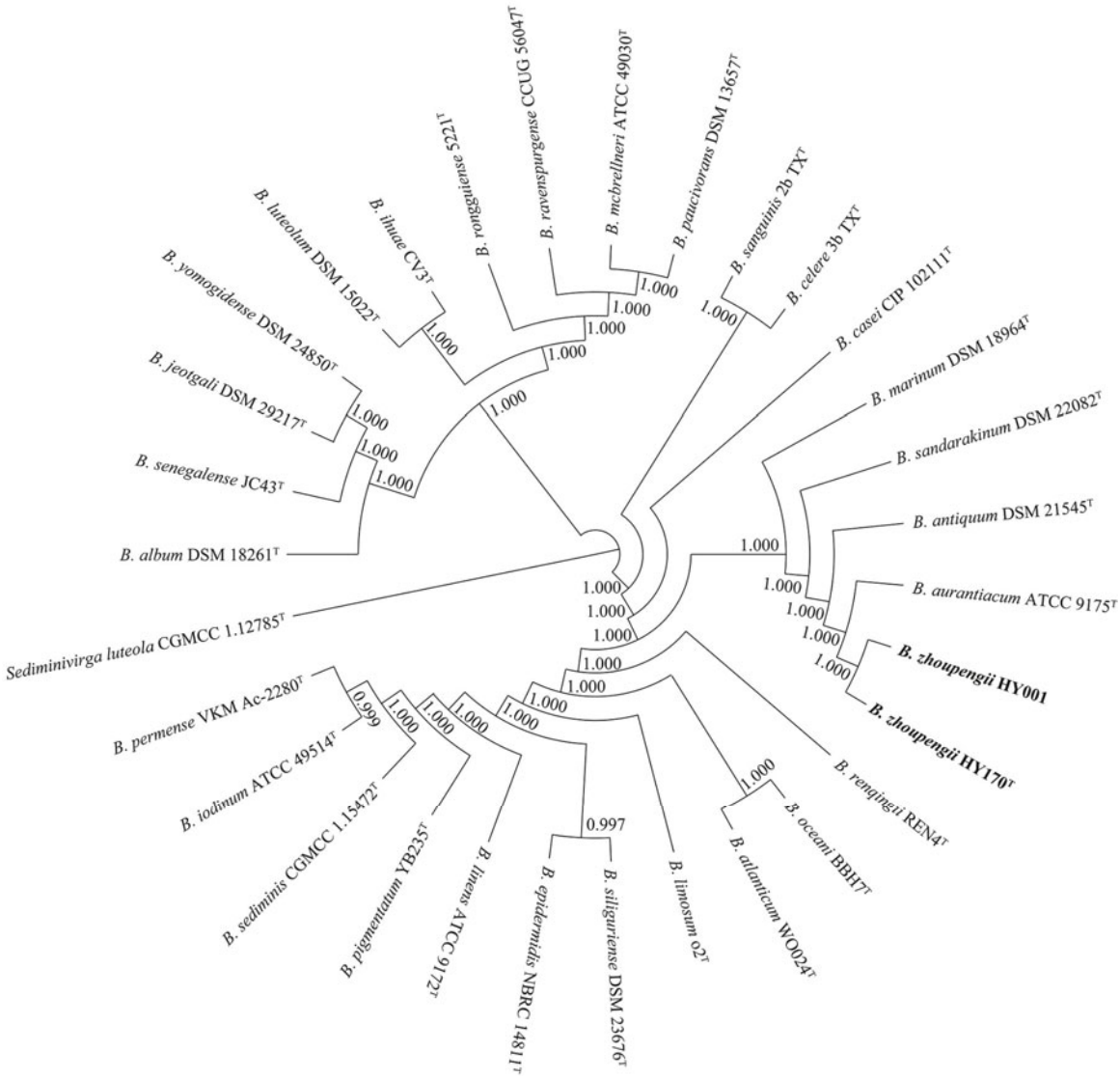

Fig. 2. Neighbor-joining phylogenomic tree based on 845 core genes of analysed strains. Numbers on the tree indicate each split in the tree support values with the Shimodaira-Hasegawa test calculated for 1,000 resamples. *Sediminivirga luteola* CGMCC 1.12785^{T} was used as an outgroup.

CDSs (Supplementary data Table S2). DNA G + C content of strains HY170^{T} and HY001 was 62.6% and 62.7%, respectively, which was close to that of the three reference strains (*B. aurantiacum* ATCC 9175^{T}, *B. antiquum* DSM 21545^{T}, and *B. celere* JCM 13521^{T}) and was within the range (55.8–72.4 mol%) of the genus *Brevibacterium* (Chen *et al.*, 2016). As shown in Supplementary data Table S2, subsystem feature counts of strains HY170^{T} and HY001 were 1,531 and 1,614, slightly higher than the reference strains and most of them were involved in activities for Amino Acids and Derivatives (335 [21.9%], 330 [20.4%]), Carbohydrates (174 [11.4%], 264 [16.4%]) and Protein Metabolism (177 [11.6%], 172 [10.7%]). In addition, mining the genomes revealed the presence of some secondary metabolite biosynthesis gene clusters, including those encoding for non-alpha poly-amino acids (NAPAA), ectoine, siderophore, and terpene. Of note, the presence of several biosynthetic gene clusters presumably encoding ulleungmycin and desferrioxamine, reveals the potential of our isolates in antibiotic biosynthesis (Supplementary data Table S3). Furthermore, strains HY001 and HY170^{T} carry a terpene-related secondary metabolism regions on their the genomes which is respectively 85% and 92% similar to the carotenoid biosynthetic gene cluster (MIBiG accession, BGC0000636) in *B. linens* (AF139916) (Krubasik and Sandmann, 2000) (Supplementary data Table S3 and Fig. S3B), respectively. However, the results showed that the novel strains under our culturing and testing conditions were unable to produce any of the eight carotenoids used as calibration standard (Supplementary data Fig. S4), raising a possibility that carotinoid(s) other than the eight tested might confer colonies of the orange color. The impact of containing 30–50% more or less genes for carbohydrates between our isolates (Supplementary data Table S2) remains to be investigated.

Direct test and comparison of morphological, physiological, and biochemical characteristics with reference strains

Strains HY170^T and HY001 were Gram-stain-positive, strictly aerobic, non-motile and rod-shaped (approximately 0.7 × 1.9 mm, Supplementary data Fig. S5). Our isolates and *B. aurantiacum* JCM 2590^T grew between 4 and 32°C (optimum, 25–28°C), while the other two (*B. antiquum* JCM 13317^T and *B. celere* JCM 13521^T) managed to grow beyond 32°C (≥ 35°C). Optimal pH for growth was 7.5, but the strains proliferated between pH 5–10. Colonies of strains HY170^T and HY001 grown on BHIB agar were circular (with a diameter of ~1 mm), convex and changed color from opaque and creamy (day 2) to orange (day 5) when incubating at 28°C (Supplementary data Fig. S3A). The isolates grew also on BHI agar, Reasoner's 2A agar, Nutrient agar and Tryptic soy agar but not on MacConkey agar. Our isolates could tolerate NaCl up to 15.0% (w/v), 0.5–1.0% optimal, similar to their

Table 1. **Differential characteristics of *Brevibacterium zhoupengii* sp. nov. and the type strains of closely related species**
All data are from this study unless indicated.

	B. zhoupengii HY001*Characteristics*	*B. zhoupengii* HY170^T	*B. antiquum* JCM 13317^T	*B. aurantiacum* JCM 2590^T	*B. celere* JCM 13521^T
Colony colour	Creamy[a]	Creamy[a]	Orange	Pale-yellow	White
Isolation source	Feces[b]	Feces[c]	Sediments	Cheese	Brown alga
Temperature (°C)					
Range	4–32	4–32	4–37	4–32	10–40
Optimum	25–28	25–28	25	25	28
NaCl (w/v, %)					
Range	0–15	0–15	0–20	0–15	0–15
Optimum	0.5–1.0	0.5–1.0	1.0–2.0	1.0	1.0–2.0
pH					
Range	5–10	5–10	5–9	5–9	5–10
Optimum	7.5	7.5	7–7.5	7–7.5	8.5
API ZYM results					
Alkaline phosphatase	w	+	–	w	+
Esterase lipase (C8)	+	+	–	+	+
Leucine arylamidase	+	+	w	+	+
Cystine arylamidase	+	–	–	–	–
Acid phosphatase	+	+	–	–	+
α-Glucosidase	–	–	–	–	+
API 50 CH results					
Glycerol	–	–	+	+	+
D-Arabinose	–	–	–	–	+
D-Ribose	–	–	+	–	–
D-Xylose	w	w	+	+	–
D-Galactose	w	w	w	+	–
D-Glucose	w	w	+	+	–
D-Fructose	–	–	+	+	–
L-Rhamnose	+	–	–	–	–
Salicin	–	–	+	+	–
D-Maltose	–	–	–	–	+
D-Trehalose	–	–	–	–	+
D-Turanose	–	–	–	–	+
L-Fucose	–	–	–	–	+
Potassium 5-ketogluconate	+	+	+	+	–
API 20NE results					
Urea	+	+	+	–	–
Gelatin	w	+	+	–	+
D-Glucose	+	+	–	–	+
N-acetyl-glucosamine	–	–	–	–	+
D-Maltose	–	–	–	–	+
Potassium gluconate	+	+	–	+	+
Adipic acid	+	+	–	–	–
Malic acid	+	+	–	+	+
Trisodium citrate	+	+	–	+	+
Phenylacetic acid	+	+	–	–	+

[a] Culture for 2 days; [b] *Taphozous* spp. bat; [c] *Rousettus* spp. bat; +, positive; w, weakly positive; –, negative.

closest relatives and the majority of genus *Brevibacterium* exhibiting halotolerant capability. The presence of multiple resistance and pH (*mrp*) gene cluster (Supplementary data Fig. S6) and genes for Na^+/H^+ antiporter-associated proteins (below in brackets) in the four type strains might explain the sodium tolerance and pH homeostasis (Wang *et al.*, 2019); Na^+/H^+ antiporter-associated proteins in HY170^T (WP_231441931.1, WP_231446240.1, WP_231443555.1, WP_231443664.1, WP_231443551.1 and WP_231443548.1), in *B. aurantiacum* ATCC 9175^T (WP_069600513.1, WP_101583342.1, WP_101584787.1, WP_009882258.1, WP_101584789.1 and WP_096157730.1), in *B. antiquum* DSM 21545^T (WP_232488493.1, WP_198395160.1, WP_198395750.1, WP_198395751.1, WP_198395752.1 and WP_198395753.1) and in *B. celere* JCM 13521^T (WP_113902932.1, WP_113903004.1, WP_113903003.1 and WP_113903002.1). It would be interesting to dissect the role of *mrpC* present in the other three type strains in contrast to its absence in *B. celere* JCM 13521^T (Supplementary data Fig. S6). Differential biochemical characteristics between our isolates and the representative strains of the closest phylogenetically related *Brevibacterium* species are depicted in Table 1. Among other interesting and relatively discriminatory biochemical differences, strains HY170^T and HY001 were able to utilize adipic acid but unable to utilize glycerol as carbon source, in striking contrast to all their three closest relatives.

Chemotaxonomic characteristics

The patterns of the major polar lipids (DPG, PG), predominant menaquinone (MK-8[H_2]) and main fatty acids (*anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$) in our isolates were consistent with other species of the genus *Brevibacterium*. The major polar lipids of strain HY170^T were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), an unidentified glycolipid (GL) and two unidentified lipids (L1, L2) (Supplementary data Fig. S7). MK-8(H_2) was the predominant menaquinone. Additionally, all the five strains tested in parallel shared *anteiso*-$C_{15:0}$ and *anteiso*-$C_{17:0}$ as major fatty acids (Supplementary data Table S4), with both amounting to around 40% in *B. celere* JCM 13521^T, a result similar to that originally reported (Ivanova *et al.*, 2004). The major sugars in the cell-wall teichoic acids of strain HY170^T were galactose and ribose (Supplementary data Fig. S8A). Moreover, strain HY170^T contained *meso*-diaminopimelic acid (*meso*-DAP) as the diagnostic diamino acid of the peptidoglycan (Supplementary data Fig. S8B), which is a typical feature of the *Brevibacterium* species, readily seen in the closely related species (Gavrish *et al.*, 2004; Ivanova *et al.*, 2004).

Taxonomic conclusion

Strains HY170^T and HY001 isolated from bat feces collected in south China were closest to *B. aurantiacum* ATCC 9175^T (Figs. 1 and 2) according to the topologies of phylogenetic and phylogenomic trees based on the 16S rRNA gene sequence and core gene analysis, and their genomic relatedness with other species were less than the proposed thresholds for species delineation by their dDDH and ANI values (Supplementary data Table S1), indicating that they represent a novel species belonging to genus *Brevibacterium.* This distinctiveness was also supported by the different profiles from their closely related species in biochemical tests (Table 1). However, the range of growth temperature, pH, and NaCl of strains HY170^T and HY001 is similar to other related strains in the genus *Brevibacterium*. Moreover, the major polar lipids (DPG, PG), fatty acids (*anteiso*-$C_{15:0}$ and *anteiso*-$C_{17:0}$), respiratory quinone (MK-8[H_2]) and diagnostic diamino acid (*meso*-DAP) of strain HY170^T were congruent to their closest species of the genus *Brevibacterium.* Of note, mining the genomes of strains HY170^T and HY001 for secondary metabolite biosynthesis gene clusters using the antiSMASH server revealed the presence of several putative biosynthetic gene clusters, such as infrastructures for ulleungmycin and desferrioxamine E, implying their potential in antibiotic biosynthesis. Additionally, the two strains' genomes have carotenoid biosynthetic gene clusters highly similar (85–92%) to that of *B. linens.* Galactose and ribose were the primary cell wall sugars for strain HY170^T judged by the results from the 2D TLC analysis of its cell-wall (Supplementary data Fig. S8A). Based on the results of phenotypic, phylogenetic, genotypic analyses and chemotaxonomic data, we conclude that strains HY170^T and HY001 represent a novel species of the genus *Brevibacterium*, for which the name *Brevibacterium zhoupengii* sp. nov. is proposed.

Description of *Brevibacterium zhoupengii* sp. nov.

Brevibacterium zhoupengii (zhou.peng´i.i. N.L. gen. n. *zhoupengii* of Peng Zhou, a researcher in Wuhan Institute of Virology, Chinese Academy of Sciences, for his contribution to taxonomic analysis of viruses isolated from bats)

Cells are strictly aerobic, Gram-stain- and catalase-positive, oxidase-negative, non-motile, regular rod-shaped (approximately 0.7 × 1.9 mm). Growth in brain heart infusion (BHI) broth occurs at 4–32°C (optimum, 25–28°C), at pH 5.0–10.0 (optimum, 7.5) and with 0–15% NaCl (w/v) (optimum, 0.5–1.0%); best and fastest growth on BHIB agar, with decent growth on BHI agar, Reasoner's 2A agar, Nutrient agar and Tryptic soy agar, but not on MacConkey agar. Colonies on BHIB agar at 28°C are circular (with a diameter of ~1 mm), convex and opaque, and change color from creamy to orange after 2–5 days of incubation. *Anteiso*-$C_{15:0}$ and *anteiso*-$C_{17:0}$ are the major fatty acids (> 10%), with variable amount of *anteiso*-$C_{17:1}$A and *anteiso*-$C_{17:1}\omega 9c$ between strains. The polar lipids include diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), an unidentified glycolipid (GL) and two unidentified lipids (L1, L2). MK-8(H_2) is the predominant menaquinone. The type strain contains *meso*-DAP as the diagnostic diamino acid in the cell-wall peptidoglycan, and galactose and ribose in the whole-cell extracts. Using API 50 CH, acid is produced from D-xylose (weakly), D-galactose (weakly), D-glucose (weakly), esculin ferric citrate and potassium 5-ketogluconate, but not from amidon, amygdalin, arbutin, dulcitol, D-adonitol, D-arabinose, D-arabitol, D-cellobiose, D-fructose, D-fucose, D-lactose, D-lyxose, D-maltose, D-mannose, D-mannitol, D-melezitose, D-melibiose, D-raffinose, D-ribose, D-saccharose, D-sorbitol, D-tagatose, D-trehalose, D-turanose, erythritol, gentiobiose, glycerol, glycogen, inositol, inulin, L-arabinose, L-arabitol, L-fucose, L-sorbose, L-xylose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, *N*-acetyl-

glucosamine, potassium 2-ketogluconate, potassium gluconate, salicin or xylitol, and variable from L-rhamnose. With API ZYM, positive for acid phosphatase, alkaline phosphatase (HY001 weakly), esterase (C4), esterase lipase (C8), leucine arylamidase, and naphthol-AS-BI-phosphohydrolase, negative for lipase (C14), *N*-acetyl-*β*-glucosaminidase, trypsin, valine arylamidase, *α*-chymotrypsin, *α*-fucosidase, *α*-galactosidase, *α*-glucosidase, *α*-mannosidase, *β*-galactosidase, *β*-glucosidase or *β*-glucuronidase, and variable for cystine arylamidase. In the API 20NE kit, positively assimilate urea, esculin ferric citrate, gelatin (HY001 weakly), D-glucose, potassium gluconate, adipic acid, malic acid, phenylacetic acid, and trisodium citrate, but not capric acid, D-maltose, D-mannitol, D-mannose, L-arabinose, L-arginine, L-tryptophane, *N*-acetyl-glucosamine, potassium nitrate and 4-nitrophenyl-*β*-D-galactopyranoside.

Acknowledgements

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

We would like to express our appreciation to Professors Zhengli Shi and Peng Zhou (Wuhan Institute of Virology, Chinese Academy of Sciences) for sharing the bat fecal samples.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethical Statement

The ethical practice was approved by Ethical Committee of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention (NO: ICDC-2016004).

References

Afonso, E. and Goydadin, A.C. 2018. Molecular detection of *Anaplasma phagocytophilum* DNA in the lesser horseshoe bat (*Rhinolophus hipposideros*) guano. *Epidemiol. Infect.* **146**, 1253–1258.

Antoniou, S., Dimitriadis, A., Polydorou, F., and Malaka, E. 1997. *Brevibacterium iodinum* peritonitis associated with acute urticaria in a CAPD patient. *Perit. Dial. Int.* **17**, 614–615.

Asai, N., Suematsu, H., Yamada, A., Watanabe, H., Nishiyama, N., Sakanashi, D., Kato, H., Shiota, A., Hagihara, M., Koizumi, Y., *et al.* 2019. *Brevibacterium paucivorans* bacteremia: case report and review of the literature. *BMC Infect. Dis.* **19**, 344.

Auch, A.F., von Jan, M., Klenk, H.P., and Göker, M. 2010. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand. Genomic Sci.* **2**, 117–134.

Austrian, R. 1960. The Gram stain and the etiology of lobar pneumonia, an historical note. *Bacteriol. Rev.* **24**, 261–265.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., *et al.* 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* **9**, 75.

Bai, X., Xiong, Y., Lu, S., Jin, D., Lai, X., Yang, J., Niu, L., Hu, S., Meng, X., Pu, J., *et al.* 2016. *Streptococcus pantholopis* sp. nov., isolated from faeces of the Tibetan antelope (*Pantholops hodgsonii*). *Int. J. Syst. Evol. Microbiol.* **66**, 3281–3286.

Breed, R. 1953. The *Brevibacteriaceae* fam. nov. of order *Eubacteriales*. *Rias. Commun. VI Congr. Int. Microbiol. Roma* **1**, 13–14.

Cannon, J.P., Spandoni, S.L., Pesh-Iman, S., and Johnson, S. 2005. Pericardial infection caused by *Brevibacterium casei*. *Clin. Microbiol. Infect.* **11**, 164–165.

Castanera, R., Ruggieri, V., Pujol, M., Garcia-Mas, J., and Casacuberta, J.M. 2020. An improved melon reference genome with single-molecule sequencing uncovers a recent burst of transposable elements with potential impact on genes. *Front. Plant Sci.* **10**, 1815.

Chen, P., Zhang, L., Wang, J., Ruan, J., Han, X., and Huang, Y. 2016. *Brevibacterium sediminis* sp. nov., isolated from deep-sea sediments from the Carlsberg and Southwest Indian Ridges. *Int. J. Syst. Evol. Microbiol.* **66**, 5268–5274.

Cheng, Y., Bai, Y., Huang, Y., Yang, J., Lu, S., Jin, D., Pu, J., Zheng, H., Li, J., Huang, Y., *et al.* 2021. *Agromyces laixinhei* sp. nov. isolated from bat feces in China. *J. Microbiol.* **59**, 467–475.

Collins, M.D., Farrow, J.A., Goodfellow, M., and Minnikin, D.E. 1983. *Brevibacterium casei* sp. nov. and *Brevibacterium epidermidis* sp. nov. *Syst. Appl. Microbiol.* **4**, 388–395.

Deng, T., Lu, H., Qian, Y., Chen, X., Yang, X., Guo, J., Sun, G., and Xu, M. 2020. *Brevibacterium rongguiense* sp. nov., isolated from freshwater sediment. *Int. J. Syst. Evol. Microbiol.* **70**, 5205–5210.

Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Biol.* **20**, 406–416.

Forquin-Gomez, M.P. and Weimer, B.C. 2014. Brevibacterium. *In* Batt, C.A. and Tortorello, M.L. (eds.), Encyclopedia of Food Microbiology, 2nd edn., vol. 1, pp. 324–330. Academic Press, San Diego, USA.

Fu, L., Niu, B., Zhu, Z., Wu, S., and Li, W. 2012. CD-HIT: accelerated for clustering the next-generation sequencing data. *Bioinformatics* **28**, 3150–3152.

Galperin, M.Y., Makarova, K.S., Wolf, Y.I., and Koonin, E.V. 2015. Expanded microbial genome coverage and improved protein family annotation in the COG database. *Nucleic Acids Res.* **43**, D261–D269.

Gavrish, E.L., Krauzova, V.I., Potekhina, N.V., Karasev, S.G., Plotnikova, E.G., Altyntseva, O.V., Korosteleva, L.A., and Evtushenko, L.I. 2004. Three new species of brevibacteria–*Brevibacterium antiquum* sp. nov., *Brevibacterium aurantiacum* sp. nov. and *Brevibacterium permense* sp. nov. *Mikrobiologiia* **73**, 218–225.

Ge, Y., Jin, D., Lai, X.H., Yang, J., Lu, S., Huang, Y., Zheng, H., Zhang, X., and Xu, J. 2021. *Vagococcus zengguangii* sp. nov., isolated from yak faeces. *J. Microbiol.* **59**, 1–9.

Harper, J.J. and Davis, G.H.G. 1979. Two-dimensional thin-layer chromatography for amino acid analysis of bacterial cell walls. *Int. J. Syst. Evol. Microbiol.* **29**, 56–58.

Hasegawa, T., Takizawa, M., and Tanida, S. 1983. A rapid analysis for chemical grouping of aerobic Actinomycetes. *J. Gen. Appl. Microbiol.* **29**, 319–322.

Hu, B., Guo, H., Zhou, P., and Shi, Z.L. 2021. Characteristics of SARS-CoV-2 and COVID-19. *Nat. Rev. Microbiol.* **19**, 141–154.

Huang, Y., Wang, X., Yang, J., Lu, S., Lai, X.H., Jin, D., Pu, J., Huang, Y., Ren, Z., Zhu, W., *et al.* 2019. *Nocardioides yefusunii* sp. nov., isolated from *Equus kiang* (Tibetan wild ass) faeces. *Int. J. Syst. Evol. Microbiol.* **69**, 3629–3635.

Huang, Y., Yang, J., Lu, S., Lai, X.H., Jin, D., Zhou, J., Zhang, S., Huang, Q., Lv, X., Zhu, W., *et al.* 2022. Morphological and genomic char-

acteristics of two novel halotolerant actinomycetes, *Tomitella gaofuii* sp. nov. and *Tomitella fengzijianii* sp. nov. isolated from bat faeces. *Syst. Appl. Microbiol.* **45**, 126294.

Huson, D.H. and Scornavacca, C. 2012. Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. *Syst. Biol.* **61**, 1061–1067.

Irakli, M.N., Samanidou, V.F., and Papadoyannis, I.N. 2011. Development and validation of an HPLC method for the simultaneous determination of tocopherols, tocotrienols and carotenoids in cereals after solid-phase extraction. *J. Sep. Sci.* **34**, 1375–1382.

Ivanova, E.P., Christen, R., Alexeeva, Y.V., Zhukova, N.V., Gorshkova, N.M., Lysenko, A.M., Mikhailov, V.V., and Nicolau, D.V. 2004. *Brevibacterium celere* sp. nov., isolated from degraded thallus of a brown alga. *Int. J. Syst. Evol. Microbiol.* **54**, 2107–2111.

Joshi, S., Misra, R., Kirolikar, S., and Mushrif, S. 2020. Catheter-related *Brevibacterium casei* bloodstream infection in a child with aplastic anaemia. *Indian J. Med. Microbiol.* **38**, 226–228.

Jung, M.S., Quan, X.T., Siddiqi, M.Z., Liu, Q., Kim, S.Y., Wee, J.H., and Im, W.T. 2018. *Brevibacterium anseongense* sp. nov., isolated from soil of ginseng field. *J. Microbiol.* **56**, 706–712.

Komagata, K. and Suzuki, K.I. 1988. 4 lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol.* **19**, 161–207.

Krubasik, P. and Sandmann, G. 2000. A carotenogenic gene cluster from *Brevibacterium linens* with novel lycopene cyclase genes involved in the synthesis of aromatic carotenoids. *Mol. Gen. Genet.* **263**, 423–432.

Kumar, V.A., Augustine, D., Panikar, D., Nandakumar, A., Dinesh, K.R., Karim, S., and Philip, R. 2011. *Brevibacterium casei* as a cause of brain abscess in an immunocompetent patient. *J. Clin. Microbiol.* **49**, 4374–4376.

Lee, S.D. 2006. *Brevibacterium samyangense* sp. nov., an actinomycete isolated from a beach sediment. *Int. J. Syst. Evol. Microbiol.* **56**, 1889–1892.

Liu, S., Jin, D., Lan, R., Wang, Y., Meng, Q., Dai, H., Lu, S., Hu, S., and Xu, J. 2015. *Escherichia marmotae* sp. nov., isolated from faeces of *Marmota himalayana*. *Int. J. Syst. Evol. Microbiol.* **65**, 2130–2134.

McCarthy, A. 2010. Third generation DNA sequencing: Pacific Biosciences' single molecule real time technology. *Chem. Biol.* **17**, 675–676.

McCaughey, C. and Damani, N.N. 1991. Central venous line infection caused by *Brevibacterium epidermidis*. *J. Infect.* **23**, 211–212.

Medema, M.H., Blin, K., Cimermancic, P., Jager, V.D., Zakrzewski, P., Fischbach, M.A., Weber, T., Takano, E., and Breitling, R. 2011. antiSMASH: Rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res.* **39**, W339–W346.

Meléndez-Martínez, A.J., Escudero-Gilete, M.L., Vicario, I.M., and Heredia, F.J. 2010. Study of the influence of carotenoid structure and individual carotenoids in the qualitative and quantitative attributes of orange juice color. *Food Res. Int.* **43**, 1289–1296.

Muhldorfer, K. 2013. Bats and bacterial pathogens: a review. *Zoonoses Public Health* **60**, 93–103.

Onraedt, A., Soetaert, W., and Vandamme, E. 2005. Industrial importance of the genus *Brevibacterium*. *Biotechnol. Lett.* **27**, 527–533.

Overbeek, R., Olson, R., Pusch, G.D., Olsen, G.J., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Parrello, B., Shukla, M., *et al.* 2014. The seed and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res.* **42**, D206–D214.

Price, M.N., Dehal, P.S., and Arkin, A.P. 2009. FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Mol. Biol, Evol.* **26**, 1641–1650.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc., Newark, Delaware, USA.

Shi, Z. 2010. Bat and virus. *Protein Cell.* **1**, 109–114.

Smith, I. and Wang, L.F. 2013. Bats and their virome: an important source of emerging viruses capable of infecting humans. *Curr. Opin. Virol.* **3**, 84–91.

Staneck, J.L. and Roberts, G.D. 1974. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl. Environ. Microbiol.* **28**, 226–231.

Tang, S.K., Wang, Y., Schumann, P., Stackebrandt, E., Lou, K., Jiang, C.L., Xu, L.H., and Li, W.J. 2008. *Brevibacterium album* sp. nov., a novel actinobacterium isolated from a saline soil in China. *Int. J. Syst. Evol. Microbiol.* **58**, 574–577.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680.

Wang, X., Yang, J., Lu, S., Lai, X.H., Jin, D., Pu, J., Niu, L., Zhu, W., Liang, J., Huang, Y., *et al.* 2019. *Paraliobacillus zengyii* sp. nov., a slightly halophilic and extremely halotolerant bacterium isolated from Tibetan antelope faeces. *Int. J. Syst. Evol. Microbiol.* **69**, 1426–1432.

Wauters, G., Charlier, J., Janssens, M., and Delmée, M. 2001. *Brevibacterium paucivorans* sp. nov., from human clinical specimens. *Int. J. Syst. Evol. Microbiol.* **51**, 1703–1707.

Wauters, G., Van Bosterhaut, B., Avesani, V., Cuvelier, R., Charlier, J., Janssens, M., and Delmée, M. 2000. Peritonitis due to *Brevibacterium otitidis* in a patient undergoing continuous ambulatory peritoneal dialysis. *J. Clin. Microbiol.* **38**, 4292–4293.

Xu, M., Dai, Y., Huang, Y., Yang, J., Lai, X.H., Jin, D., Lu, S., Zhou, J., Zhang, S., Bai, Y., Jiao, Y., Qiao, L., Jiang, Y., and Xu, J. 2021. Identification of *Haloactinobacterium kanbiaonis* sp. nov. and *Ruania zhangjianzhongii* sp. nov., two novel species of the family *Ruaniaceae* isolated from faeces of bats (*Hipposideros* spp.). *Int. J. Syst. Evol. Microbiol.* **71**. doi: 10.1099/ijsem.0.004953.

Xu, Y., Xu, X., Lan, R., Xiong, Y., Ye, C., Ren, Z., Liu, L., Zhao, A., Wu, L.F., and Xu, J. 2013. An O island 172 encoded RNA helicase regulates the motility of *Escherichia coli* O157:H7. *PLoS ONE* **8**, e64211.

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

Yoon, S.H., Ha, S.M., Lim, J., Kwon, S., and Chun, J. 2017b. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* **110**, 1281–1286.

Zerbino, D.R. and Birney, E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* **18**, 821–829.

Zhou, J., Huang, Y., Yang, J., Lai, X.H., Jin, D., Lu, S., Pu, J., Zhang, S., Zhu, W., Xu, M., *et al.* 2021. *Microbacterium chengjingii* sp. nov. and *Microbacterium fandaimingii* sp. nov., isolated from bat faeces of *Hipposideros* and *Rousettus* species. *Int. J. Syst. Evol. Microbiol.* **71**. doi: 10.1099/ijsem.0.004858.