Brevilactibacter Soli Sp. Nov., a Novel Member of the Genus *Brevilactibacter* Isolated from Soil in China

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Research Article

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Abstract

A Gram-stain-positive, cocci-shaped, facultatively anaerobic, non-motile bacterial strain, designated YIM S02567^T, was isolated from a forest soil sample collected from Gejiu City, Yunnan Province, south-west PR China. Growth was observed at 10–45°C, at pH 6.0-9.5, in the presence of up to 4.0 % (w/v) NaCl on R2A medium. Through the results of 16S rRNA gene sequence similarity analysis showed that strain YIM S02567^T was most closely related to the type strain of *Brevilactibacter sinopodophylli* (95.38 %) and *Propioniciclava tarda* (94.67%), phylogenetic analysis based on genome data showed that strain YIM S02567^T should be assigned to the genus *Brevilactibacter*. The cell-wall diamino acid was *meso*-diaminopimelic acid. The major cellular fatty acids were identified as anteiso-$C_{15:0}$ and $C_{16:0}$, and the major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, and two unidentified glycolipids. The predominant menaquinone was MK-9(H_4). The genomic DNA G + C content was 71.20 mol%. Based on the polyphasic taxonomic evidence, strain YIM S02567^T is assigned to a novel member of the genus *Brevilactibacter*, for which the name *Brevilactibacter soli* sp. nov., (type strain YIM S02567^T = CCTCC AB 2020128^T = CGMCC 1.18504^T = KCTC 49478^T) is proposed.

Introduction

The family *Propionibacteriaceae* comprises 25 validly named genera (https://lpsn.dsmz.de/family/propionibacteriaceae). This family is classified into the order *Propionibacteriales* in the phylum *Actinobacteria* and is known for its high genomic proportion of DNA G+C content. Members of the genera *Propioniciclava* and *Brevilactibacter*, with the only recognized species *Propioniciclava tarda* WR061^T (Sugawara et al. 2011) and two recognized species *Brevilactibacter sinopodophylli* TEYR-7^T (Zhang et al. 2017) and *Brevilactibacter flavus* VG341^T (Wenning et al. 2020), are propionate-producing bacterial strains within the family *Propionibacteriaceae*. Both *B. sinopodophylli* TEYR-7^T and *B. flavus* VG341^T were rod-shaped, Gram-reaction-positive, facultatively anaerobic, and non-motile bacterial strains. They all have certain characteristics in common, which are Gram-positive, facultatively anaerobic, non-spore-forming, catalase-positive, with MK-9(H_4) as the predominant menaquinone and a high DNA G+C content. Recently we found a cocci-shaped, Gram-reaction-positive and non-motile bacterial strain YIM S02567^T that was isolated from Gejiu City, Honghe Hani and Yi Autonomous Prefecture, Yunnan Province, PR China (23°21'34.20"N, 103°9'42.12"E). The altitude of the sample collection is 1738.0 m. In this study, the morphological, chemical characteristics, and phylogenetic analysis showed that the strain should be classified as representing a novel member of the genus *Brevilactibacter*, for which the name *Brevilactibacter soli* sp. nov., is proposed.

Materials And Methods

Isolation and culture conditions

Strain YIM S02567^T was isolated from a forest soil sample after incubation for 7 days at 30 °C on Nutrient Agar (NA). Colonies of strain YIM S02567^T were picked after incubation for 7 days at 30 °C. Purified YIM S02567^T was routinely maintained on Reasoner's 2A (R2A) agar slants at 4 °C and also preserved as glycerol suspensions (20 %, w/v) at -80 °C. Biomass of YIM S02567^T for chemical and molecular studies was obtained from incubating the isolate on R2A liquid medium at 30 °C for 5 days. The reference type strains of the closely related species, *P. tarda* WR061^T supplemented with vitamin B_{12} (10 µg l^{-1}) and *B. sinopodophylli* TEYR-7^T, were also under similar conditions for comparative analyses.

Phylogenetic analysis

The 16S rRNA gene sequence of strain YIM S02567^T was amplified with the universal primers PA (5'-CAGAGTTTGATCCTGGCT-3') and PB (5'-AGGAGGTGATCCAGCCGCA-3') following a described method previously (Feng et al. 2020). The PCR product was cloned to pEASY-Blunt Cloning Kit vector (TransGen Biotech) according to the manufacturer's instructions and sequenced by Tsingke Company (Beijing, PR China). The obtained 16S rRNA gene sequence was analyzed on the EzBioCloud server (https://www.ezbiocloud.net/) (Yoon et al. 2017). Phylogenetic analysis was carried out based on the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981), and maximum-parsimony (Fitch 1971) methods, and taxonomic position of strain YIM S02567^T was determined by using the MEGA 7 software package (Sudhir et al. 2016). The type strain sequences of all type species belonging to the *Propionibacteriaceae* were selected to calculate a phylogenetic tree. Evolutionary distance matrices of phylogenetic trees were calculated according to Kimura's two-parameter model (Kimura 1980). Bootstrap analysis was performed with 1000 replications (Felsenstein 1985).

Genome sequencing and evaluation

Whole-genome sequencing of strain YIM S02567^T was performed using a paired-end sequencing method with the Hiseq X platform (Illumina) at Majorbio Bio-pharm Technology Company, Shanghai, PR China. Reads of each data set were filtered, and high-quality reads were assembled using SOAPdenovo2 (Luo et al. 2012). Contigs with lengths greater than 500 bp were kept for gene prediction by applying GLIMMER 3.0 (Delcher et al. 1999). The DNA G + C content of the genome was calculate based on the genome sequences. Based on genomic data, JSpecies (http://jspecies.ribohost.com/jspeciesws/#analyse) was used to calculate the average nucleotide identity (ANI) (Richter et al. 2016). And a phylogenomics tree closely related type species of the *Propionibacteriaceae* was reconstructed utilizing the supermatrix method (Zhi et al. 2017).

Morphological, physiological, and biochemical features

For transmission electron microscopy (JEM-2100, jeol) observations, strain YIM S02567^T was cultured for 5 days at 30 °C on R2A agar. The motility of cells was observed by phase-contrast microscopy (Leica DM2000) on R2A agar (Nam et al. 2008). The Gram reaction was carried out according to the Gram Stain Solution kit (Solarbio Biotechnology) and Gregersen (Gregersen 1978) by using 4 % (w/v) KOH for cell

lysis. The growth of the strain under anaerobic conditions was assessed by incubating inoculated R2A plates in a Gas Pak anaerobic system at 30 °C for 5 days. Catalase activity was determined by the production of bubbles after the addition of a drop of 3 % (v/v) H_2O_2 to the cells and oxidase activity was evaluated by using the bioMérieux oxidase reagent according to the manufacturer's instructions. Growth at different temperatures (0, 4, 10, 15, 20, 25, 30, 35, 37, 40, 45, 50 °C) and NaCl tolerance (0-10%, w/v, at intervals of 1% unit) were determined on R2A plates. The pH range and optimum (4.0-10.0, at intervals of 0.5 pH unit) for growth were tested on R2A broth. pH was adjusted using the buffer system described by Xu *et al*. (Xu et al. 2005). Hydrogen sulfide production and hydrolysis of cellulose, starch and Tweens (20, 40, and 80), were performed as described by Gonzalez *et al.* (Gonzalez et al. 1978). Strain YIM S02567^T was cultivated anaerobically at 30 °C for 1 week in modified peptone-yeast extract-glucose (PYG) medium (Hopebiol Biotechnology), supplemented with a total of 10 g·l^{-1} glucose. The fermentation products were analyzed by UPLC-MS (LTQ Orbitrap XL) as described previously (Han et al. 2013). In the following experiments, the two reference type strains of related species were cultivated under the same conditions except for *P. tarda* WR061^T using the same medium supplemented with vitamin B_{12} (10 μg l^{-1}). The Biolog Gen III Micro Plate system was used to test sole source utilization according to the manufacturer's instructions. Acid production from carbohydrates, enzyme activities and other physiological properties were determined by using the API 50CH, API 20NE and API ZYM test strips (bioMérieux) according to the manufacturer's instructions.

Chemotaxonomy

Biomass for quantitative cellular fatty acid analysis of the strain YIM S02567^T was obtained from R2A agar after incubation for 5 days at 30 ℃. The two reference type strains of related species were cultivated under the same conditions for chemotaxonomy characterization except for *P. tarda* WR061^T using the same medium supplemented with vitamin B_{12} (10 μg l^{-1}). Cellular fatty acids were extracted, methylated by Sasser (Sasser 1990) and identified according to the standard protocol of the $_{MIDI}$/Hewlett Packard Microbial Identification System (Sherlock version 6.1, midi database: TSBA6) (Toru et al. 1983). The diamino acids were performed by using TLC as described by Staneck and Roberts (Staneck and Roberts 1974). Sugars of whole-cell hydrolysates were determined by following the method of Tang *et al*. (Tang et al. 2009). The respiratory quinones were extracted with chloroform/methanol (2:1, v/v) from lyophilized cells and purified on TLC as described by Collins *et al*. (Collins et al. 1977). The purified extracts were analyzed by reverse-phase HPLC (Groth et al. 1996) with a C18 column (25 cm×4.6 mm, 5 μm). Polar lipids were extracted as described by Minnikin *et al.* (Minnikin et al. 2010), and separated on two-dimensional silica-gel TLC and identified by staining with molybdophosphoric acid, molybdenum blue, ninhydrin and *α*-naphthol to detect total lipids, phospholipids, aminolipids and glycolipids, respectively (Minnikin et al. 1984).

Results And Discusses

Phylogeneticcharacteristics

An almost-complete 16S rRNA gene sequence (1501 bp) was obtained from strain YIM S02567^T. Results from the EzBioCloud server indicated that the closest hit to the validly published species was *B. sinopodophylli* TEYR-7^T (with 95.38 % similarity), while the type species of the genus *B. flavus* VG341^T (94.69 % similarity) and *P. tarda* WR061^T (94.67 % similarity). In the phylogenetic tree based on the maximum-likelihood algorithm (Fig. 1), strain YIM S02567^T formed a tight phyletic group with *P. tarda* WR061^T with a bootstrap value of 76 % and belonged to a cluster of species including *P. tarda* WR061^T, *B. sinopodophylli* TEYR-7^T, and *B. flavus* VG341^T. The overall topology of the neighbour-joining and maximum parsimony tree (Fig. S3 and Fig. S4, available in the online Supplementary Material) was essentially the same as that of the maximum-likelihood tree. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM S02567^T belonged to the family *Propionibacteriaceae*, therefore *B. sinopodophylli* TEYR-7^T and *P. tarda* WR061^T were used as references for the subsequent related tests.

Genomecharacteristics and analysis

The genome assembly size of strain YIM S02567^T had a total size of 3,796,070 bp with N50 length of 405,848 bp. The genomic DNA G + C content was 71.20 mol%. A total of 3,583 protein-encoding genes, three rRNA genes and 45 tRNAs genes were predicted. The number of genes assigned to COGs was 2928 and the number of genes assigned to KEGG was 1423. Other genome characteristics of strain YIM S02567^T and other members in the family *Propionibacteriaceae* were summarized in Table S1. The ANIb values between YIM S02567^T and the closely related type strain were 76.2 % (*B. sinopodophylli* TEYR-7^T), 73.3 % (*B. flavus* VG341^T) and 72.2 % (*P. tarda* WR061^T), respectively. This indicates that strain YIM S02567^T is closer to the genus *Brevilactibacter*. A phylogenomics tree closely related type species of the family *Propionibacteriaceae* based on the supermatrix method was shown in Fig. 2. Strain YIM S02567^T formed a coherent and monophyletic clade closed to the genus *Brevilactibacter*, which supported that strain YIM S02567^T represents a novel species of the genus *Brevilactibacter*.

According to genome prediction for the secondary metabolite synthesis analysis, it showed that the genome of strain YIM S02567^T had gene clusters associated with lanthipeptide and terpene. Lanthipeptide contains the noncanonical amino acid lanthionine and antibacterial activities and anti-HIV activity were exhibited (Ferir et al. 2013). This must be an exciting substance. The result suggested that strain YIM S02567^T may be beneficial for the research of lanthipeptide biosynthesis gene clusters that contributes to the development of anti-HIV.

Morphological, physiological, and biochemical characterization

Cells of strain YIM S02567^T were Gram-staining positive, non-motile, facultatively anaerobic, cocci-shaped, 0.5-0.7 μm in diameter (Fig. S1) and formed orange colonies. Growth of YIM S02567^T was observed at 10-45 ℃ (optimum temperature, 20-25 ℃) and at pH 6.0-9.5 (optimum, pH 8.5-9.0). The tolerance to NaCl was up to 4.0 % (w/v) with optimum growth in the presence of 2.0 % (w/v) NaCl. Tests for catalase activity, hydrolysis of starch, hydrolysis of gelatin, and aesculin ferric citrate were positive,

however, those for nitrate reduction, oxidase activity, indole production, H_2S production, hydrolysis of cellulose were negative. Strain YIM S02567^T produced propionic acid, acetic acid and lactic acid from PYG medium supplemented with 10 g·l^{-1} glucose after one week of cultivation under anaerobic conditions. Other physiological and biochemical characteristics of strain YIM S02567^T and a comparison of special results with closely related species were listed in Table 1 and the species description.

Chemotaxonomic characteristics

The cellular fatty acid profile of strain YIM S02567^T was characterized by the predominance of anteiso-$C_{15:0}$ and $C_{16:0}$. The closely related species contained the same major fatty acid (anteiso-$C_{15:0}$), but strain YIM S02567^T also included the major fatty acid $C_{16:0}$ (14.91 %). The cellular fatty acid profiles of strain YIM S02567^T and its closely related strains are given in Table 2. The predominant menaquinone of strain YIM S02567^T was determined to be menaquinone MK-9(H_4), and the whole cell-wall diamino acid was analyzed to be *meso*-diaminopimelic acid, which was all the same as those of the closely related species reported. The whole-cell sugars detected were glucose, mannose, ribose, galactose, arabinose and rhamnose. Polar lipids of strain YIM S02567^T included diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), two unidentified glycolipids (GL1, GL2), and an unidentified lipid (L1) (Fig. S2). Two type species of the genus *Brevilactibacter* also contained DPG, PG, GL1, and GL2 as similar polar lipids. In addition, they had different polar lipids (Fig. S2).

Genealogical taxonomy conclusion

Strain YIM S02567^T shared < 97% similarities of 16S rRNA gene sequence and showed cocci-shaped distinguished from related valid described species in a cluster of the phylogenetic tree. The phylogenomics tree indicated that strain YIM S02567^T formed a coherent with the genus *Brevilactibacter*. Furthermore, strain YIM S02567^T showed differences from closely related type species on nitrate reduction, enzyme activities, and major cellular fatty acids, assimilation of several carbon resources, respiratory quinone, polar lipids and type of diamino acid. Based on morphological, physiological, phylogenetic and biochemical features, strain YIM S02567^T can be considered as a novel member of the genus *Brevilactibacter*, for which the name *Brevilactibacter soli* sp. nov. is proposed.

DESCRIPTION OF *BREVILACTIBACTER SOLI* SP. NOV.

Brevilactibacter soli (So'li. L. neut. gen. n. soli of soil, the source of the type strain).

Cells are cocci-shaped, facultatively anaerobic, Gram-staining positive, non-motile bacterial strain, 0.5-0.7 μm in diameter. Colonies are orange, smooth, and convex on R2A medium. The temperature range for growth is 10-45 °C (optimum at 20-25 °C). The pH range for growth is 6.0-9.5 (optimum pH 8.5-9.0). NaCl tolerance is up to 4.0 % (w/v) with optimal growth at 2.0 % (w/v) NaCl. Positive for catalase activity, hydrolysis of starch, hydrolysis of gelatin, Tweens 40 and aesculin ferric citrate, however, negative for nitrate reduction, oxidase activity, indole production, H_2S production, hydrolysis of cellulose. Propionic

acid, acetic acid and lactic acid are produced anaerobically from PYG medium supplemented with 10 $g \cdot l^{-1}$ glucose. In API ZYM test strips, it is positive for acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-*AS*-*BI*-phosphohydrolase, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-glucosidase, and *β*-glucosidase, weakly positive for alkaline phosphatase, valine arylamidase, cystine arylamidase and chymotrypsin, and negative for lipase (C14), trypsin, *N*-acetyl-*β*-glucosaminidase and *α*-mannosidase, and *β*-fucosidase activities. In the API 20NE test strips, positive for D-glucose fermentation, hydrolysis of esculin, gelatin, and hydrolysis of 4-nitrophenyl *β*-D-galactopyranoside, negative for nitrate reduction, indole production, arginine dihydrolase, and urea. Weakly positive for assimilation of D-glucose, D-mannose and positive for assimilation of L-arabinose, D-maltose and malic acid, negative for assimilation of adipic acid, capric acid, phenylacetic acid, D-mannitol, *N*-acetylglucosamine, potassium gluconate, and trisodium citrate. In API 50CH test strips, acid is produced from L-arabinose, D-xylose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, D-sorbitol, methyl *α*-D-glucopyranoside, amygdalin, arbutin, aesculin ferric citrate, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, raffinose, starch, glycogen, gentiobiose, D-turanose, and produced weakly from glycerol, D-arabinose, D-ribose, L-xylose, D-galactose, L-sorbose, *N*-acetyl-D-glucosamine, D-melezitose, xylitol, D-lyxose, D-tagatose, L-fucose, D-arabitol, L-arabitol, potassium 2-keto gluconate, potassium 5-keto gluconate, but not from erythritol, D-adonitol, methyl *β*-D-xylopyranoside, dulcitol, inositol, methyl *α*-D-mannopyranoside, inulin, D-fucose, D-potassium gluconate. In the Biolog Gen III Micro Plate system, the following carbon sources have positive reactions: propionic acid, acetic acid, acetoacetic acid, *α*-D-lactose, D-maltose, D-raffinose, D-turanose, D-gluconic acid, glucuronamide, but negative reactions for methyl *β*-D-glucoside, D-salicin, inosine, D-serine, D-arabitol, D-aspartic acid, D-serine, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-pyroglutamic acid, L-serine, D-gluconic acid, mucic acid, quinic acid, D-saccharic acid, *P*-hydroxy-phenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, D-malic acid, L-malic acid, bromo-succinic acid, *γ*-amino-butyric acid, *α*-hydroxy-butyric acid, *α*-keto-butyric acid. The cell-wall diamino acid is *meso*-diaminopimelic acid and the predominant menaquinone is MK-9(H_4). The cellular polar lipids are DPG, PG, GL1, GL2, and L1. The whole-cell sugars contain glucose, mannose, ribose, galactose, arabinose and rhamnose. The major cellular fatty acids contain anteiso-$C_{15:0}$ and $C_{16:0}$.

The type strain YIM S02567^T (=CCTCC AB 2020128^T=CGMCC 1.18504^T=KCTC 49478^T) was isolated from a soil sample collected from Gejiu City, Honghe Hani and Yi Autonomous Prefecture, Yunnan Province, PR China. The genomic DNA G + C content was 71.20 mol%. The GenBank accession numbers for the 16S rRNA gene sequence and genomic sequence are MW032491 and JACYOT000000000, respectively.

Declarations

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Tables

Table 1. Comparison of phenotypic and biochemical characteristics of strain YIM S02567^T and other closely referenced species.

Characteristics	1	2*	3	4
Cell morphology	Cocci	Rods	Rods	Irregular rods
Temperature range for growth (°C)	10-45	6-40	15-37	20-37
pH range for growth	6.0-9.5	5.5-8.5	6.0-9.0	5.3-8.0
NaCl tolerance (%)	0-4	0-5	0-3	0-0.5
Catalase activity	+	+	+	+
Oxidase activity	-	+	-	+
Hydrolysis of starch	+	-	+	-
Reduction of nitrate	-	-	+	+
urease	-	-	+	-
gelatin	+	-	-	+
Hydrolysis of:				
D-glucose	w	ND	+	-
L-arabinose	+	ND	-	-
D-mannose	w	ND	-	-
D-mannitol	-	ND	+	-
D-maltose	+	ND	+	-
malic acid	+	ND	+	-
Enzyme activities:				
alkaline phosphatase	w	ND	-	w
valine arylamidase	w	ND	-	w
cystine arylamidase	w	ND	-	w
α-galactosidase	+	ND	-	+
β-glucuronidase	+	ND	-	+
α-mannosidase	-	ND	-	+
Acid production from:				
D-arabinose	w	-	-	w
L-arabinose	+	-	-	-

D-ribose	w	w	-	-
D-xylose	+	+	-	-
L-xylose	w	-	-	-
D-galactose	w	+	-	+
D-mannose	+	+	-	+
L-sorbose	w	-	-	-
L-rhamnose	+	-	-	-
methyl *α*-D-mannopyranoside	-	-	-	w
amygdalin	+	-	-	+
raffinose	+	w	-	+
starch	+	-	+	-
glycogen	+	-	+	-
xylitol	w	-	-	-
D-lyxose	w	-	-	-
L-fucose	w	-	-	-
D-arabitol	w	+	-	w
L-arabitol	w	-	-	-
D-potassium gluconate	-	-	-	w
potassium 2-keto gluconate	w	-	-	-
DNA G+C content (mol%)	71.2	67.7	71.2	69.3-69.5

*Data taken from Wenning *et al* (Wenning et al. 2020).

Strain: 1, YIM S02567^T, 2*, *Brevilactibacter flavus* VG341^T, 3, *Brevilactibacter sinopodophylli* TEYR-7^T, 4, *Propioniciclava tarda* WR061^T. +, Positive, -, negative, w, weakly positive, ND, no data available. Results of enzyme activities were from API 20NE and API ZYM test kits. Results of producing acid by using carbon sources were from the API 50CH kits. All data of strains (1, 2 and 4) from this report except for morphological characteristics and DNA G+C content.

Table 2. The cellular fatty acid profiles of strain YIM S02567^T and its closely phylogenetic related strains of the genus *Brevilactibacter* and *Propioniciclava*.

Fatty acid	1	2*	3	4
iso-$C_{14:0}$	9.82	ND	6.94	9.30
$C_{14:0}$	1.62	ND	-	1.57
anteiso-$C_{15:0}$	**38.83**	**54**	**36.71**	**54.27**
iso-$C_{16:0}$	5.27	ND	4.27	**10.76**
$C_{16:0}$	**14.91**	8	4.13	4.96
anteiso-$C_{17:0}$	2.30	5	-	-
$C_{17:1}\omega 8c$	3.25	ND	**10.61**	-
$C_{17:1}\omega 6c$	1.46	ND	7.05	-
$C_{17:0}$	8.25	6	**10.17**	-
$C_{18:1}\omega 9c$	2.44	ND	1.26	-
$C_{18:0}$	2.03	ND	-	4.50
Summed Feature 3	2.25	ND	1.72	-
Summed Feature 8	1.39	ND	-	-

*Data taken from Wenning *et al* (Wenning et al. 2020).

Strains: 1, YIM S02567^T, 2*, *Brevilactibacter flavus* VG341^T, 3, *Brevilactibacter sinopodophylli* TEYR-7^T, 4, *Propioniciclava tarda* WR061^T. All data of strains (1, 2 and 4) are obtained from the present study. Values are percentages of total fatty acids and compounds lower than 1 % were not shown, major components (>10 %) are indicated with bold text, ND, no data, -, not detected. Summed feature 3, $C_{16:1}\omega 7c$ or $C_{16:1}\omega 6c$, summed feature 8: $C_{18:1}\omega 7c$.

Figures

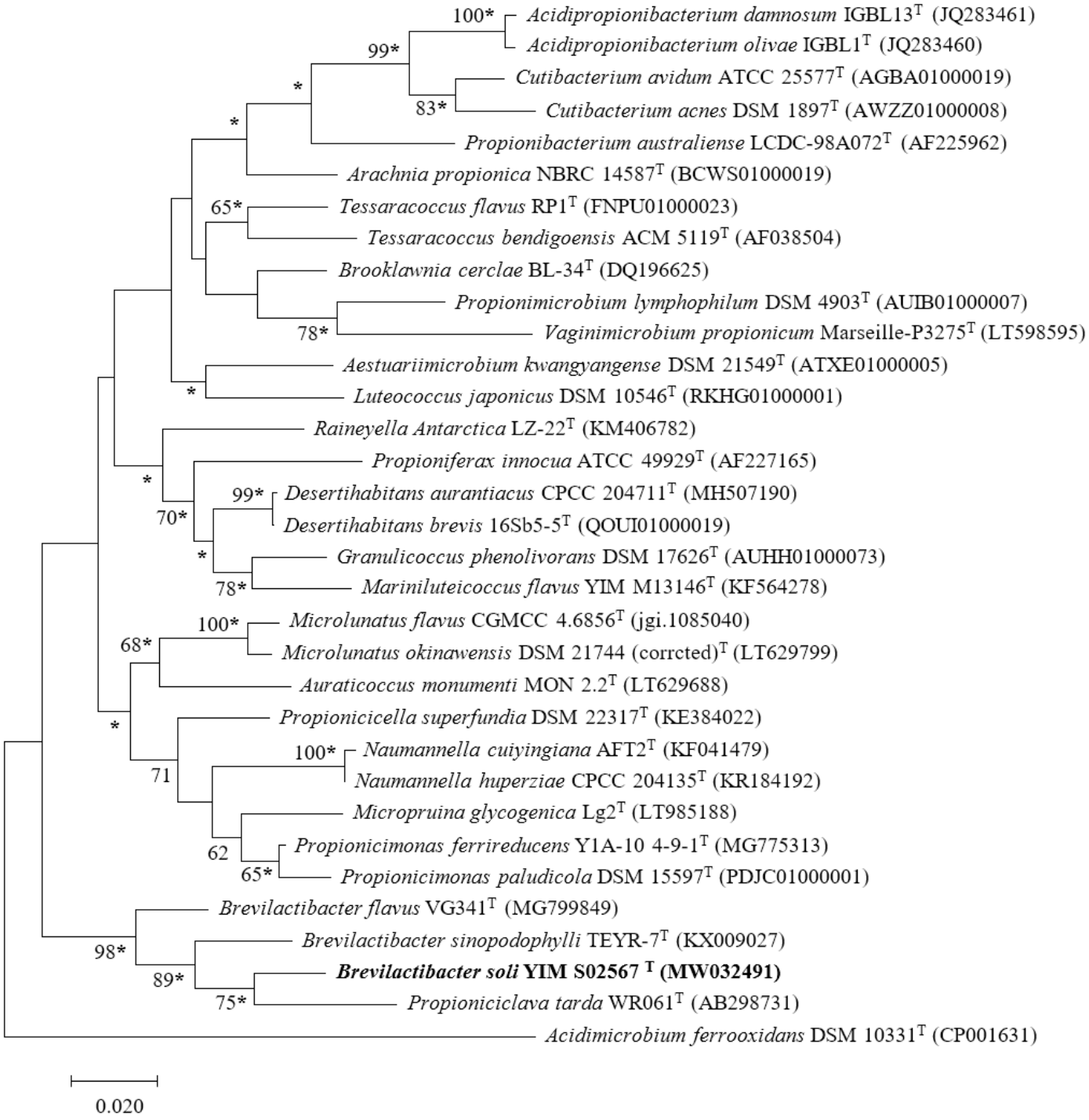

Figure 1

The maximum-likelihood phylogenetic tree based on 16S rRNA gene sequence showing the position of strain YIM S02567T and all type species of the family Propionibacteriaceae as well as strain B. sinopodophylli TEYR-7T with the highest 16S rRNA gene similarity. Acidimicrobium ferrooxidans DSM 10331T was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) of above 60 % are shown at the nodes of branches. Asterisks indicate identical clades in the phylogenetic

trees that are recovered using the maximum-likelihood and maximum-parsimony methods. Bar, 0.02 represents substitution per nucleotide position.

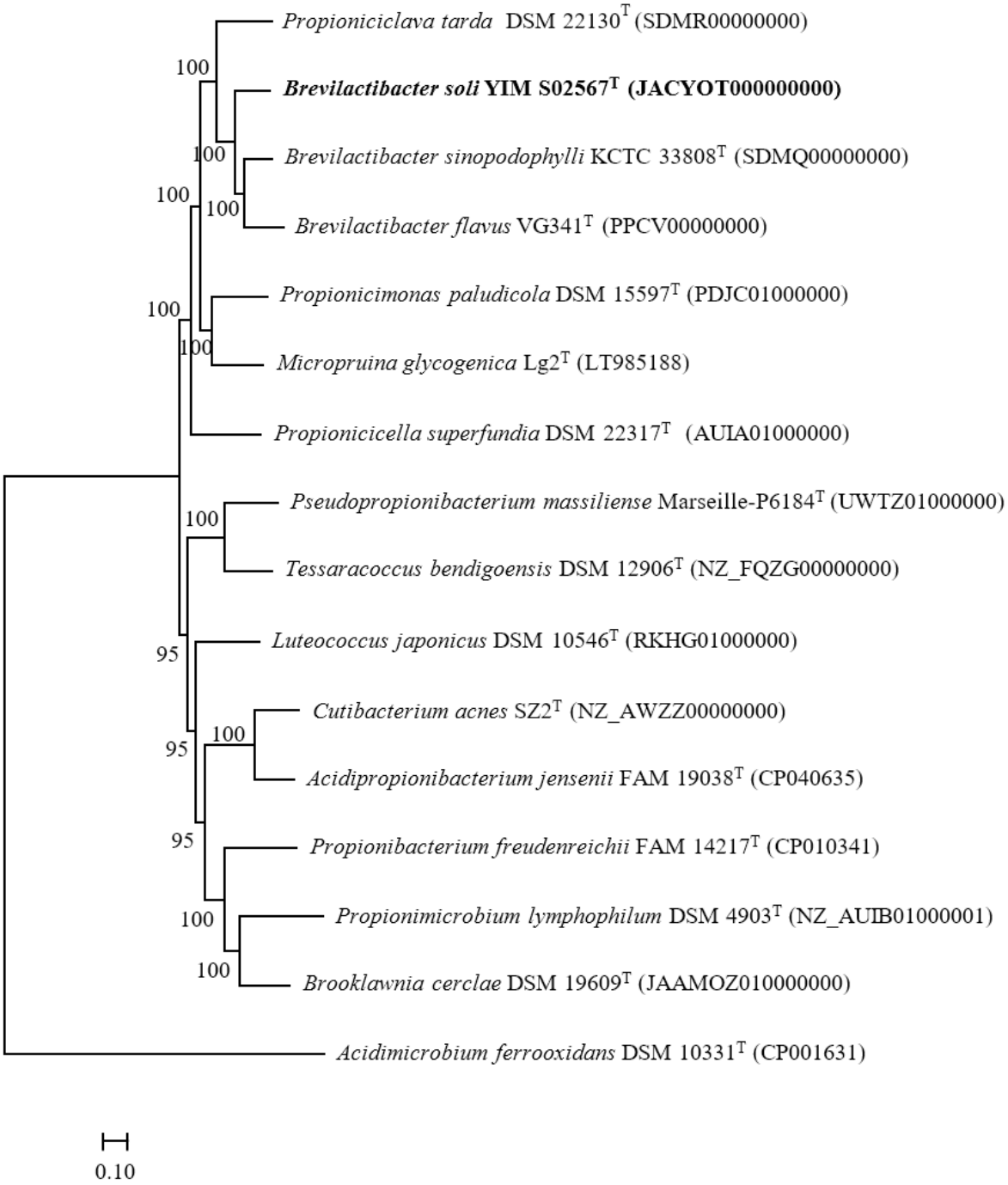

Figure 2

Phylogenomics tree YIM S02567T and closely related species of the family Propionibacteriaceae based on the supermatrix method (Number of single-copy orthogroups: 406). Bootstrap values (≥70 %) based

on 1000 resamplings are given at the nodes. Acidimicrobium ferrooxidans DSM 10331T was used as an outgroup. Bars, the number of substitutions per site.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementarymaterials.pdf