Jeotgalibacillus kunyuensis sp. nov., a novel orange-pigmented species with relative genes or gene clusters, isolated from wetland soil in Yantai, China

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Research Article

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Abstract

A Gram-stain-positive, orange-pigmented, rod-shaped, flagellated bacterial strain T12^T, was isolated from wetland soil in Kunyu Mountain Wetland in Yantai, China. This organism was able to grow at 15–40 °C (optimum 37 °C), at 0.0–9.0% NaCl (optimum 2%, w/v) and at pH 5.5–9.0 (optimum 8.5). Phylogenetic analysis based on 16S rRNA indicated that strain T12^T was a member of the family *Planococcaceae* and it represented highly 16S rRNA gene sequence similarity with *Jeotgalibacillus salarius* (97.6%) and the type strain, *Jeotgalibacillus marinus* (96.9%). Genome analysis revealed that the genome size was 3506682 bp and the DNA G+C content was 43.7 mol%. Besides, the genome sequence showed 55.0–74.6% average amino acid identity values and 67.8–74.7% average nucleotide identity values between the strain T12^T and all the related strains. Digital DNA-DNA hybridization of strain T12^T with *Jeotgalibacillus proteolyticus* and *Jeotgalibacillus marinus* demonstrated 19.0% and 20.3% relatedness. Chemotaxonomic analysis showed that the sole quinone was MK-7. The predominant cellular fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol and iso-$C_{14:0}$. The polar lipids consisted of aminolipid, phosphatidylglycerol, diphosphatidylglycerol and two unidentified phospholipids. Based on polyphasic characterization, strain T12^T is considered to represent a novel species, for which the name *Jeotgalibacillus kunyuensis* sp. nov. is proposed. The type strain is T12^T (= KCTC 43296^T = MCCC 1K07171^T).

Introduction

The genus *Jeotgalibacillus*, which belongs to the family *Planococcaceae*, was first established by Yoon (Yoon et al. 2001). There are nine species in this genus at the time of writing: *Jeotgalibacillus marinus* (Ruger 1983, Ruger and Richter 1979), *Jeotgalibacillus alimentarius* (Yoon et al. 2001), *Jeotgalibacillus campisalis* (Yoon et al. 2004), *Jeotgalibacillus salarius* (Yoon et al. 2010), *Jeotgalibacillus soli* (Cunha et al. 2012), *Jeotgalibacillus malaysiensis* (Yaakop et al. 2015), *Jeotgalibacillus terrae* (Srinivas et al. 2016, Chen et al. 2010), *Jeotgalibacillus alkaliphilus* (Srinivas et al. 2016) and *Jeotgalibacillus proteolyticus* (Li et al. 2018). Members of the family *Planococcaceae* were mostly isolated from marine environments or seafood jeotgal. Most of the species were described as Gram-stain-positive; the G+C content was around 39.3–44.0 mol% (Yaakop et al. 2015); the major cellular fatty acids were iso-$C_{15:0}$ and anteiso-$C_{15:0}$ and the MK-7 and MK-8 were the respiratory quinone. In our study, the novel isolate T12^T was isolated from wetland soil, exhibiting similar characteristics as described above. The present study was proposed to demonstrate its taxonomic position throughout a polyphasic analysis.

Materials And Methods

Isolation of strain T12^T

Strain was isolated from wetland soil collected from Kunyu Mountain Wetland in Yantai, Shandong Province, China (121°49'36"E, 37°13'18"N). The wetland soil sample was inoculated on marine agar 2216

(MA, Becton Dickinson) after being diluted in multiple series of gradient with sterile water. Colonies with different phenotypes were obtained after 48h of incubated at 37°C. An orange colony was obtained by repeated purified. Five reference strains: *J. alimentarius* JCM 10872^T, *J. proteolyticus* MCCC 1H00228^T, *J. salarius* KCTC 13257^T, *J. marinus* DSM 1297^T and *J. campisalis* JCM 11810^T, were purchased from their respective strain collection institutions. Above-mentioned strains were preserved in seawater supplemented with 15% (v/v) glycerol at – 80°C for further use.

Phylogenetic analysis of the 16S rRNA gene and whole genome sequencing

The 16S rRNA of strain T12^T was determined after being amplified by PCR with two universal primers 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') as described previously (Zhou et al. 2016). The acquired 16S rRNA sequence was then compared with other published species using EzBioCloud (Yoon et al. 2017) and NCBI BLAST. Strain T12^T was discovered to be a member of the genus *Jeotgalibacillus* based on the 16S rRNA sequence similarity greater than 95.0% compared with all the genus-type strains. Phylogenetic trees were inferred by the neighbour-joining (Saitou and Nei 1987) and maximum-likelihood (Felsenstein 1981) using the software package MEGA version 7.0 (Kumar et al. 2016) with the Kimura two-parameter model (Kimura 1980). Bootstrap analyses of the phylogenetic trees were performed based on 1000 replicates.

Genome features

The draft genome sequence of strain T12^T was performed using the Illumina HiSeq-PE150 platform. *De novo* assembly of sequenced reads were assembled using the SOAP, Spades, and Abyss software. These operations were all implemented by Beijing Novogene Bioinformatics Technology Co, Ltd. (Beijing, China). The genes involved in metabolic pathways were analysed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (Kanehisa et al. 2016). Protein-encoding regions were identified and annotated with the Rapid Annotations using Subsystems Technology (RAST) server (http://rast.nmpdr.org/rast.cgi) (Aziz 2008) and the UniProtKB/Swiss-Prot (Consortium TU 2019). The secondary metabolites were undertaken by antiSMASH 5.0 (Blin et al. 2019). The DNA G+C content of strain T12^T was determined from the genome sequence.

Genome sequence comparisons between strain T12^T and closely related strains were performed by calculating the average nucleotide identity (ANI) values and using the ANI Calculator EZ Biocloud tool (www.ezbiocloud.net/tools/ani). The average amino acid identity (AAI) calculator was carried out between strain T12^T and its neighbouring species (http://enve-omics.ce.gatech.edu/aai/). Digital DNA–DNA hybridization (dDDH) were calculated using the Genome-to-Genome Distance Calculator (GGDC) version 3.0 (Meier-Kolthoff et al. 2013).

Morphology, physiology, and biochemical analysis

Gram staining was performed as described by Moyes (Moyes et al. 2009). After incubating the bacterium on MA at 37°C for 2 days, the cell morphology and presence of flagella were observed via transmission electron microscope (Jem-1200EX; JEOL). Growth at various NaCl concentrations was investigated in NaCl-free artificial seawater medium supplemented with various concentrations of NaCl (final concentration 0–15.0%, in increments of 0.5%, w/v) (Yang and Cho 2008). Growing status of strain T12^T at different temperatures, ranging from 4°C to 50°C (4, 10, 15, 20, 25, 30, 35, 37, 40, 45 and 50°C), was measured on marine broth 2216 (MB, Becton Dickinson) and the optimum growth temperature was obtained from above. The pH range for growth was determined on MB, adjusted to various pH values (pH 5.5–9.5, at intervals of 0.5 pH units) using the following buffer systems: MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5), and CAPSO (pH 9.0 and 9.5). Anaerobic growth was tested on MA with or without 0.1% $NaNO_3$ under anaerobic conditions at 37°C for 2 weeks. Various biochemical tests, such as oxidase, hydrolysis of cellulose, agar, casein and Tween 80 were tested by procedures as described by Tindall (Tindall et al. 2007). The oxidation and fermentation test of carbohydrates were investigated using the Biolog GEN III Micro Plates and API 50CHB Fermentation Kit (bioMérieux) after growth on MA at 37°C for 2 days. Additional physiological tests were examined using API 20E, API 20NE and API ZYM strips (bioMérieux). Susceptibility to antibiotics was carried out on MA plates with discs (Tianhe) containing various antibiotics for 3 days at 37°C.

Chemotaxonomic characterisation

To determine the chemotaxonomic characteristics, strain T12^T and the reference strains were cultured in MB at optimum temperature for 2 days. Fatty acid extraction and analyses were performed by Sherlock Microbial Identification System (MIDI) (Sawant et al. 2015) with the TSBA6.0 database. Detection of respiratory quinone was completed with High Performance Liquid Chromatography (HPLC) (Hiraishi et al. 1996). Polar lipid analysis was performed by the Marine Culture Collection of China (MCCC), Xiamen, Fujian Province, China.

Results And Discussion

Phylogenetic analysis

The almost-complete 16S rRNA gene sequence of strain T12^T (1514 bp) was determined and confirmed with the draft genome sequence. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain T12^T belonged to the genus *Jeotgalibacillus*, within the family *Planococcaceae*, sharing highly similarity with *J. salarius* ASL-1^T (97.6%). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain T12^T was in the same cluster as *J. campisalis* SF-57^T, *J. proteolyticus* 22-7^T, *J. marinus* strain 581^T and *J. soli* P9^T in the neighbour-joining tree (Fig. 1), which was also shown in the maximum-likelihood tree (Supplementary Fig. S1). The low 16S rRNA gene sequence similarities and phylogenetic location indicated that strain T12^T should be located within the genus *Jeotgalibacillus* as a novel species.

Genome features

General features of strain T12^T genome were summarized in Supplementary Table S1. The genome size of strain T12^T was 3506682 bp, which contained 4066 predicted genes of 26 scaffolds and the depth coverage of sequencing was 330×. The N50 and N90 value was 933824 bp and 135228 bp. There were 5S rRNAs of 6, tRNAs of 67 and sRNAs of 2. The DNA G+C content of strain T12^T was 43.7 mol%, which was within the range of DNA G+C contents for species of the genus *Jeotgalibacillus* (39–44 mol%) as previously reported.

According to Cluster of Orthologous Groups (COG), the genome contained several genes coding for amino acid transport and metabolism, transcription, carbohydrate transport and metabolism, signal transduction mechanisms, inorganic ion transport and metabolism and a number of unknown functions, which played a role in several aspects of cell live activities (Supplementary Fig. S2).

Strain T12^T contained 2041 genes related to the KEGG metabolic pathway, including metabolism (68.3%), environmental information processing (9.9%), genetic information processing (9.0%), cellular processes (7.5%), human diseases (3.1%) and organismal systems (2.2%) (Supplementary Fig. S3). RAST identified 915 subsystems from the strain T12^T, in which amino acids and derivatives (250) had the highest counts, followed by carbohydrates (194) and protein metabolism (179) (Supplementary Fig. S4).

Furthermore, strain T12^T contained one or more polar or peripheral flagella and 62 genes related to cell motility were found in the genome as well. In agreement with phenotypic data, a flagellar assembly pathway (02040) was found, which annotated genes coding for flagellar related proteins (flagellar basal-body rod protein FlgG; flagellar secretion chaperone FliS; flagellin; chemotaxis protein MotA and MotB). Besides, in carotenoid synthesis, a complete pathway synthesized from phytoene to lycopene was discovered. Among them, genes encoding for carotenoid biosynthesis such as *crt*I was found in strain T12^T, which may be responsible for the phenotypic colour of strain T12^T (Kallscheuer et al. 2019).

The results of the antiSMASH analysis showed that strain T12^T had three biosynthetic gene clusters (BGCs) in its genome including lasso peptide (paeninodin), terpene (carotenoid) and siderophore cluster. The terpene biosynthetic gene cluster, which may be related to the colour pigmentation associated with the bacterial colony (Paniagua-Michel et al. 2012), was detected in most species of the genus *Jeotgalibacillus*.

ANI values between strain T12^T and the type strains of recognized *Jeotgalibacillus* species were less than 74.9% (Supplementary Table S2), significantly lower than the threshold value of 95% for species and genus (Richter and Rossello-Mora 2009). AAI values (55.0–74.6%) were lower than 75% with all the closely related species, well below the proposed cut-off values for genus delineation in the family *Planococcaceae*. The dDDH relatedness comparison with the draft genome for strain T12^T showed low percentage (19.0–20.6%) similarities with all the related species and were much lower than the boundary

(70.0%) for species identification. ANI, AAI and dDDH analysis results suggest that strain T12^T is a new species of the genus *Jeotgalibacillus*.

Morphology and Phenotypic characterization

Cells were Gram-stain-positive, flagellated, rod-shaped (0.4–0.6 μm in width, 1.6–3.4 μm in length) (Supplementary Fig. S5). Colonies were circular to slightly irregular, flat to raised, smooth, orange coloured and 0.8–1.4 mm in diameter after incubation on MA for 2 days at 37°C. Strain T12^T had one or more polar or peritrichous flagella, which was similar to the reference strains. The novel strain was found to grow at a temperature range of 15–40°C (optimum temperature 37°C), pH 5.5–9.0 (optimum pH of 8.5) and NaCl tolerance of 0.0–9.0% (optimum 2%, w/v). It could grow at 0% NaCl condition, which was similar to the related strains. Strain T12^T differed from the closely related strain *J. salarius* ASL-1^T with respect to numerous phenotypic characteristics, such as lipase (C14), alkaline phosphatase, trypsin and α-chymotrypsin activities. Strain T12^T showed negative for H_2S, starch, alginate, cellulose, oxidase and catalase. Besides, it was sensitive to penicillin, ampicillin cefazolin, amikacin, gentamicin, erythromycin, norfloxacin, ciprofloxacin, compound sulfamethoxazole and chloramphenicol. Other characteristics of strain T12^T are presented in the species description and Table 1.

Table 1 Differential phenotypic characteristics of strain T12^T and other closely related members of the genus *Jeotgalibacillus*.

Characteristic	1	2	3	4	5	6
Oxidase	-	+	-	-	+	+
Growth range (optimum)						
NaCl (w/v, %)	0–9% (2%)	0–20% (3–12%)	0–15% (2–5%)	0–10% (2%)	0–7% (3–3.5%)	0–18% (2%)
Temperature (°C)	15–40 °C (37 °C)	10–45 °C (30–35 °C)	4–39 °C (30 °C)	10–40 °C (33 °C)	5–30 °C (12–23 °C)	4–40 °C (30 °C)
Hydrolysis of						
Gelatin	+	+	-	+	+	-
Starch	-	-	+	-	-	-
Acids from						
Mannitol	-	+	+	-	-	+
D-Mannose	-	-	-	-	+	-
Enzyme activity						
Esterase (C4)	+	+	-	+	-	+
β-Galactosidase	+	+	+	+	-	-
α-Glucosidase	+	+	-	-	-	-
Predominant menaquinone	MK-7	MK-7, MK-8	MK-7	MK-7	MK-7	MK-7, MK-8
Major fatty acid	iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol, iso-$C_{14:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$	anteiso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol, iso-$C_{14:0}$	anteiso-$C_{15:0}$, iso-$C_{15:0}$	anteiso-$C_{15:0}$, iso-$C_{15:0}$	anteiso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol
DNA G+C content (%)	43.7	44	41.8	41.6	39.3	42.9

Strains: 1, strain T12^T; 2, *J. alimentarius* JCM 10872^T; 3, *J. campisalis* JCM 11810^T; 4, *J. proteolyticus* 1H00228^T; 5, *J. marinus* DSM 1297^T; 6, *J. salarius* KCTC 13257^T. +, Positive; -, negative; w, weak. All data from this study except DNA G+C contents of the related strains, which were from the original species description: 2 (Yoon *et al.* 2010, Yoon *et al.* 2001), 3 (Cunha *et al.* 2012, Yaakop *et al.* 2015, Yoon *et al.* 2010), 4 (Li *et al.* 2018), 5 (Yoon *et al.* 2010, Yoon *et al.* 2001), 6 (Yoon *et al.* 2010).

Chemotaxonomy

The major polar lipids of strain T12^T were phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and phosphatidylcholine (PC) (Supplementary Fig. S6); these were different from *J. proteolyticus* 22-7^T (aminophospholipid, phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol) (Li et al. 2018). The predominant cellular fatty acids of strain T12^T were summed iso-$C_{15:0}$ (28.4%), anteiso-$C_{15:0}$ (19.6%), $C_{16:1}$ω7c alcohol (13.0%) and iso-$C_{14:0}$ (10.1%), which were similar to those of the related strains and the detailed information was shown in Table 2. The sole quinone of strain T12^T was identified as MK-7, which was similar to that of the other members of the genus *Jeotgalibacillus*. These chemotaxonomic data further confirm that strain T12^T belongs to the genus *Jeotgalibacillus*.

Table 2 Cellular fatty acid compositions of strain T12^T and the closest relatives.

Fatty acid	1	2	3	4	5	6
Saturated fatty acids						
$C_{10:0}$	–	–	TR	1.99	–	–
$C_{14:0}$	1.31	TR	1.65	1.05	1.19	1.54
$C_{16:0}$	2.30	TR	2.94	1.68	2.44	5.37
$C_{18:0}$	TR	TR	1.63	TR	1.37	3.67
Unsaturated						
$C_{16:1}\omega7c$ alcohol	**13.00**	5.01	**18.32**	4.98	9.10	**14.22**
$C_{16:1}\omega11c$	2.24	2.04	2.06	2.84	1.84	2.02
$C_{18:1}\omega9c$	TR	–	1.25	–	TR	2.51
Branched fatty acids						
iso-$C_{14:0}$	**10.10**	1.60	**16.26**	1.47	8.83	9.09
iso-$C_{15:0}$	**28.40**	**52.22**	3.20	**15.98**	**19.61**	8.57
anteiso-$C_{15:0}$	**19.63**	**14.65**	**31.05**	**39.15**	**31.19**	**30.62**
iso-$C_{16:0}$	6.56	TR	7.23	2.04	6.08	4.69
iso-$C_{17:0}$	1.63	2.17	TR	4.61	1.54	TR
anteiso-$C_{17:0}$	3.92	2.08	4.13	8.44	4.88	5.72
iso-$C_{17:1}\omega10c$	1.06	8.10	TR	2.66	1.33	TR
Summed Feature3[a]	TR	TR	TR	1.04	TR	TR
Summed Feature4[b]	2.34	5.06	3.97	5.36	4.83	3.51
Summed Feature8[c]	TR	TR	TR	TR	TR	2.22

Strains: 1, strain T12^T; 2, *J. alimentarius* JCM 10872^T; 3, *J. campisalis* JCM 11810^T; 4, *J. proteolyticus* 1H00228^T; 5, *J. marinus* DSM 1297^T; 6, *J. salarius* KCTC 13257^T. All data were taken from this study. Major components are indicated with bold text. TR, Traces (< 1.0%); -, not detected. Fatty acids amounting to < 1.0% of the total fatty acids in both strains are not shown.

[a]Summed feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$

[b]Summed feature 4, iso-$C_{17:1}$I and/or anteiso-$C_{17:1}$B

[c]Summed feature 8, $C_{18:1}$ω7c and/or $C_{18:1}$ω6c

Conclusion

According to polyphasic taxonomy analyses, strain T12^T should be assigned to the genus *Jeotgalibacillus* as a new species, for which the name *Jeotgalibacillus kunyuensis* sp. nov. is proposed.

Description of Jeotgalibacillus kunyuensis **sp. nov.**

Jeotgalibacillus kunyuensis (kun.yu.en'sis. N.L. masc. adj. *kunyuensis* of or belonging to Kunyu Mountain Wetland, China, the geographical origin of the type strain of the species)

Cells are Gram-stain-positive, catalase-negative, oxidase-negative and rod-shaped (0.4–0.6 μm wide and 1.6–3.4 μm long). Colonies are circular to slightly irregular, orange coloured, flat to raised and 0.8-1.4 mm in diameter on MA plates after 2 days of incubation at 37°C. Growth occurs in the range of 15–40°C and pH 5.5–9.0, with optimal at 37°C and pH 8.5. The optimal NaCl concentration is 2% (w/v). Cells degrade gelatin and but not agar, starch, H_2S, alginate or cellulose. In the API system, alkaline phosphatase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase, esterase (C4), esterase lipase (C8) and lipase (C14) are present, but acid phosphatase, α-galactosidase, β-glucuronidase, β-glucosaccharase, leucine arylamidase and N-acetyl-β-glucosaminidase are absent. The sole quinone is MK-7. Predominant fatty acids (> 10%) were observed as iso-$C_{15:0}$ (28.4%), anteiso-$C_{15:0}$ (19.6%), $C_{16:1}$ω7c alcohol (13.0%) and iso-$C_{14:0}$ (10.1%). The polar lipids consist of aminolipid, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine and two unidentified phospholipids. The DNA G+C content is 43.7 mol%. The strain T12^T was sensitive to penicillin, ampicillin cefazolin, amikacin, gentamicin, erythromycin, norfloxacin, ciprofloxacin, compound sulfamethoxazole and chloramphenicol.

The type strain T12^T (= KCTC 43296^T = MCCC 1K07171^T) was isolated from wetland soil in Kunyu Mountain Wetland in Yantai, Shandong Province, China.

The GenBank accession number for the 16S rRNA gene sequence of strain T12^T is MW527064 and the number for the whole genome sequence is JACNMS000000000.

Abbreviations

AAI Average Amino Acid Identity

AL aminolipid

ANI Average Nucleotide Identity

BGCs Biosynthetic Gene Clusters

COG Cluster of Orthologous Groups

dDDH digital DNA-DNA Hybridization

DPG diphosphatidylglycerol

GGDC Genome-to-Genome Distance Calculator

HPLC High Performance Liquid Chromatography

KCTC Korean Collection for Type Cultures

KEGG Kyoto Encyclopedia of Genes and Genomes

MA Marine agar 2216

MB Marine broth 2216

MEGA Molecular Evolutionary Genetics Analysis

MIDI Microbial Identification System

MCCC Marine Culture Collection of China

PC phosphatidylcholine

PG phosphatidylglycerol

RAST Rapid Annotations using Subsystems Technology

Declarations

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Authors' contributions HNJ drafted the manuscript. BXW, STY, HNJ and YM performed isolation, deposition and identification. STY, BXW and MJZ performed genome analysis. STY and YXZ revised the manuscript. YXZ designed all the experiments and supervised the manuscript.

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Data availability

All data generated or analysed during this study are included in this published article, its supplementary information files and GenBank/EMBL/DDBJ. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T12^T is MW527064 and the number for the whole genome sequence is JACNMS000000000. Supplementary figures and Supplementary tables are available with the online version of this paper.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest Authors declare that there is no conflict of interest.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T12^T is MW527064 and the number for the whole genome sequence is JACNMS000000000.

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Figures

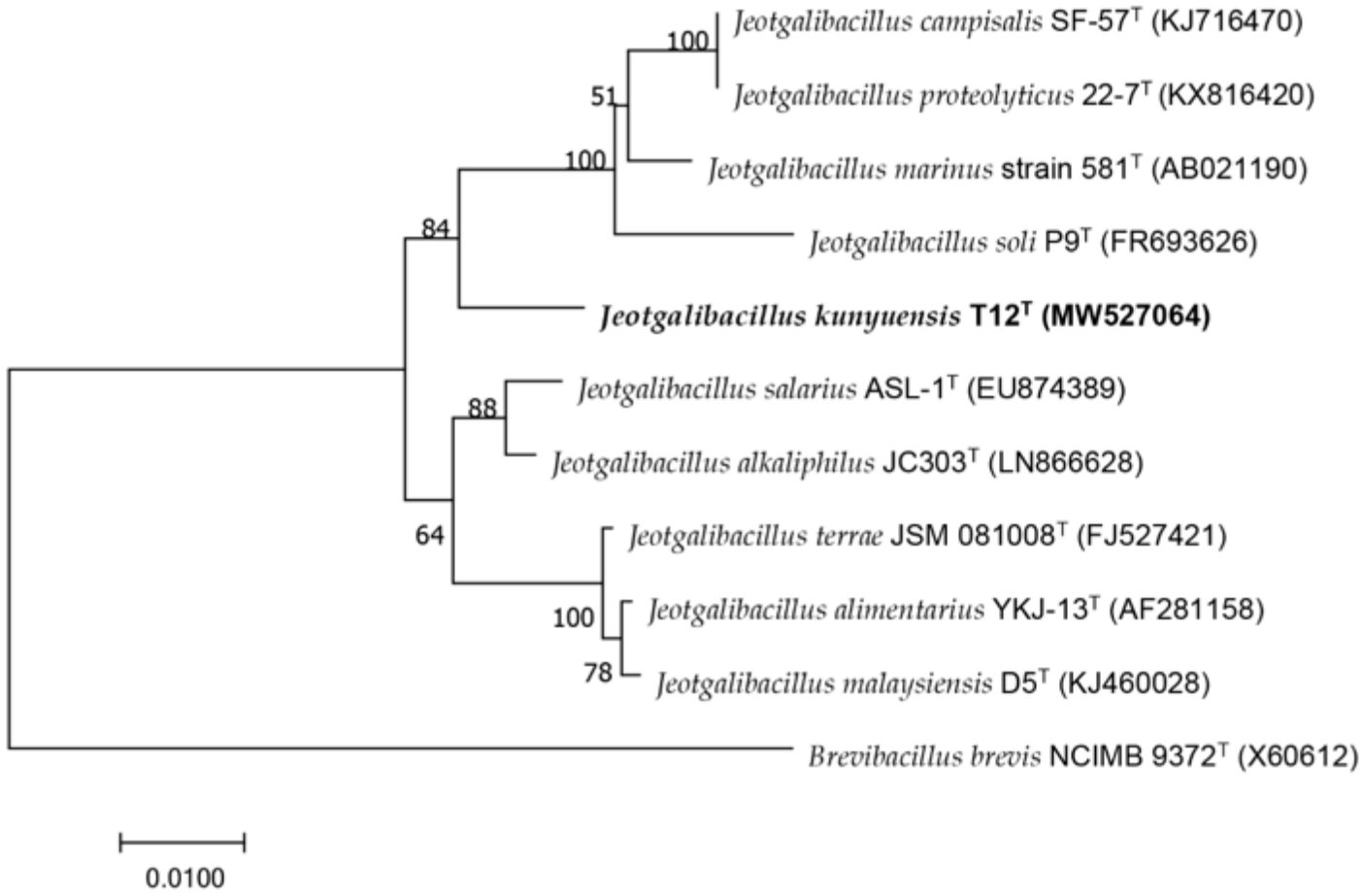

Figure 1

Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain T12^T and other related taxa. Bar, 0.01 substitution per nucleotide position. Bootstrap values were evaluated with 1000 replicates and values above 50% are shown.

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