Jeotgalibacillus Auranticolor sp. nov., Isolated from Freshwater Collected from Baiyangdian Lake Lake, China

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Abstract

A novel Gram-positive, strictly aerobic, rod-shaped, orange-pigmented bacterial strain, designated R-1-5s-1^T, was isolated from Baiyangdian Lake, China. Strain R-1-5s-1^T grew at 15-37°C (optimum 37°C) and pH 7-11 (optimum pH 8) in Luria-Bertani medium. Based on 16S rRNA gene sequence analysis, strain R-1-5s-1^T was assigned to the genus *Jeotgalibacillus* and showed the closest relationships with *Jeotgalibacillus salarius* ASL-1^T (97.69%), *Jeotgalibacillus alkaliphilus* JC303^T (97.29%), *Jeotgalibacillus marinus* DSM 1297^T (97.15%), *Jeotgalibacillus campisalis* SF-57^T (97.01%), and *Jeotgalibacillus* spp. (≤ 97%). The predominant polar lipids were phosphatidylglycerol and diphosphatidylglycerol; the major cellular fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$, and anteiso-$C_{17:0}$; and the major respiratory quinones were MK-7 and MK-8. The peptidoglycan type of the cell wall was A1a linked via L-lysine as the diamino acid. The G+C content was 43.6%, and the draft genome size of strain R-1-5s-1^T was 3.4 Mbp. Between strain R-1-5s-1^T and the related strain *J. salarius* ASL-1^T, the ANI and dDDH relatedness values were 78.9% and 20.8%, respectively. Phylogenetic, chemotaxonomic, and genotypic analyses revealed that strain R-1-5s-1^T is a novel species in the genus *Jeotgalibacillus*, for which the name *Jeotgalibacillus auranticolor* sp. nov. is proposed. The type strain is R-1-5s-1^T (=CGMCC 1.13567^T=KCTC 43038^T).

Introduction

The genus *Jeotgalibacillus* belongs to the family *Planococcaceae* of the class Bacilli, the original species of which was *Jeotgalibacillus alimentarius*, isolated from jeotgal in Korea by Yoon *et al.* (2001). The *J. alimentarius* strain possesses MK-7 and MK-8 as major respiratory quinones (Yoon *et al*., 2001). Currently, the genus *Jeotgalibacillus* comprises eight species with validly published names according to the *List of Prokaryotic Names with Standing in Nomenclature* website (http://www.bacterio.net/). These members of the genus *Jeotgalibacillus* have been isolated from diverse environments, including salt pan, sandy beach, seawater, and soil (Srinivas *et al*., 2016). We focused on a novel species, the type strain of which, R-1-5s-1^T, was isolated from water collected from Baiyangdian Lake.

Baiyangdian Lake is the largest freshwater lake and most important ecological resource bank in northern China. As an important body of freshwater, Baiyangdian Lake is important for economic development in the surrounding area (Cui *et al*., 2018). The composition, activity, and physiology of the microbial community in water, sediment and aquatic plant of Baiyangdian Lake has been investigated extensively, particularly of functional microorganisms (Du *et al*., 2018; Sun *et al*., 2021; Zhou *et al*., 2020a; Zhou *et al*., 2020b). To assess the community structure and species diversity of culturable bacteria in lakes, we analyzed the microbial populations in Baiyangdian Lake and identified a new species.

Materials And Methods

Samples and isolation

A colony of strain R-1-5s-1^T was isolated from water of Baiyangdian Lake (38.850°N 116.000°E; salinity 0.58 g/L, pH 8.48, temperature 28.08℃, oxygen content 2.28 mg/L), the largest freshwater lake in northern China. The water samples were diluted (10^{-3}–10^{-5}), spread on solid Luria–Bertani (LB) medium (1 L of deionized water containing 10 g of tryptone, 10 g of yeast extract, and 15 g of agar) and incubated for 3 days on LB agar at 28℃. The strain R-1-5s-1^T was isolated from the medium and stored in 50% (v/v) glycerol at -80℃. Two related type strains, *Jeotgalibacillus salarius* ASL-1^T and *Jeotgalibacillus alimentarius* YKJ-13^T, were obtained from the Korean Collection for Type Cultures.

16S rRNA gene sequencing

Genomic DNA was extracted and purified using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The complete 16S rRNA gene of R-1-5s-1^T was amplified by polymerase chain reaction (PCR) as described by Niemann *et al*. (1997) using universal primers (27F, 5'-AGAGTTTGATCCTGGCTCA-3'; and 1492R, 5'-GGTTACCTTGTTACGACTT-3') (Hou *et al*., 2018). The PCR products were purified, ligated into a cloning vector according to the standard protocol of the *pEASY*-T1 Cloning Kit (TransGen Biotech Co. Ltd., Beijing, China). To obtain the complete 16S rRNA gene sequence, the corresponding plasmid derivatives were Sanger-sequenced (primers M13F, 5'-TGTAAAACGACGGCCAGT-3'; and M13R, 5'-CAGGAAACAGCTATGACC-3'). The 16S rRNA gene sequence similarity of R-1-5s-1^T was analyzed using the EzBioCloud server (http://www.ezbiocloud.net/) (Yoon *et al*., 2017).

Phylogenetic status and DNA relatedness

The 16S rRNA gene sequences were aligned using ClustalW in MEGA7 software (Tamura *et al*., 2013), and phylogenetic trees were constructed using the neighbor-joining (NJ) (Saitou and Nei, 1987), maximum-likelihood (ML) (Felsenstein, 1981), and maximum-parsimony (MP) (Fitch, 1971) algorithms in MEGA7 software based on 1000 bootstrap replications.

The draft genome was sequenced on an Illumina HiSeq X10 platform(San Diego, CA) using a paired-end 150 bp sequencing strategy. Metabolic pathways were reconstructed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.kegg.jp/) (Kanehisa *et al*., 2016). *De novo* assembly of the reads was performed using Velvet v. 1.2.10 (Zerbino and Birney, 2008).

The genome sequences of strains R-1-5s-1^T and *J. alaries* ASL-1^T were submitted to GenBank under accession numbers SORW00000000 and SORX00000000, respectively. The pair-wise average nucleotide identity (ANI) values were estimated by calculating the average identity value of all BLAST results between every two genomes (Richter *et al*. 2009). Digital DNA-DNA hybridization (dDDH) was evaluated using Genome-to-Genome Distance Calculator v. 2.1 (GGDC, https://ggdc.dsmz.de/) and isolates with < 70% dDDH similarity were regarded as distinct species (Meier and Klenk *et al*., 2013; Goris *et al*., 2007).

Phenotypic characteristics

Cell morphology and size were observed using a JEM-1400 transmission electron microscope (JEOL, Tokyo, Japan) and SU1080 scanning electron microscope (Hitachi, Tokyo, Japan) after 24 h of incubation at 37°C on LB agar. The temperature range for growth was examined in modified LB broth at 4, 15, 20, 25, 30, 37, and 45°C (Gordon RE *et al.,* 1974). The pH range for growth was evaluated in modified LB broth at pH 4–13 (at intervals of 1.0 pH unit) The range of NaCl concentrations for growth was examined in modified LB medium containing 0–15 % (w/v) NaCl (at intervals of 1 %). Gram staining was carried out using a Gram stain kit (Solarbio, Beijing, China). Cell motility was tested on 0.5% agar plates, as described by Xu *et al*. (2013). Oxidase activity was evaluated using an oxidase reagent (bioMérieux, Marcy-l'Étoile, France), and catalase activity was examined by adding 5% (v/v) H_2O_2 to colonies. Growth under anoxic conditions was evaluated in LB broth in an anaerobic tube after incubation for 1 week at 37°C. Starch hydrolysis was tested in LB agar as a modified basal medium (Gordon *et al*., 1974). A physiological analysis of strain R-1-5s-1^T was performed using the API 20NE (test assimilation), API 50CH (test enzyme activities), and API ZYM (test acid production) systems following the manufacturer's instructions (bioMérieux) with incubation at 37°C. The Biolog-Gen-Microplate system was used to evaluate the strain's carbon metabolic capability at 37°C.

Chemotaxonomic characterization

The cell wall was prepared, and the peptidoglycan structure was determined as described previously (Schleifer and Kandler, 1972). The cellular fatty acid properties of strain R-1-5s-1^T, *Azospirillum doebereinerae* GSF71^T, and *Azospirillum lipoferum*59b^T were evaluated in parallel using a 6890N gas chromatograph (Agilent Technologies, Santa Clara, CA), and analyzed against the TSBA v. 6.0 database (Liu *et al*., 2016). The polar lipid and respiratory quinone properties of strain R-1-5s-1^T, *J. salarius* R-1-5s-1^T, and ASL-1^T were examined in parallel. Polar lipids were extracted and separated as described previously with modifications and two-dimensional thin-layer chromatography (Kamekura 2020; Tindall *et al*., 2007). Respiratory quinones were extracted and purified as described previously (Collins 1985) and analyzed by high-performance liquid chromatography (Kroppenstedt 1986; Hu *et al*., 2004).

Results And Discussion

16S rRNA gene sequence similarities and phylogenetic analysis

The almost complete 16S rRNA gene sequence of R-1-5s-1^T was 1513 bp. According to EzBioCloud, strain R-1-5s-1^T showed ≤ 97% sequence similarity with *Jeotgalibacillus salarius* ASL-1^T (97.69%), *J. alkaliphilus* JC303^T (97.29%), *J. marinus* DSM 1297^T (97.15%), and *J. campisalis* SF-57^T (97.01%). The sequence similarity of these 16S rRNA genes met the recommended threshold (98.65%) for discriminating two prokaryotic species (Kim *et al*., 2014). Phylogenetic trees constructed by the ML, NJ and MP methods all revealed that strain R-1-5s-1^T belonged to the genus *Jeotgalibacillus* and was most closely related to *J. salarius* ASL-1^T with < 98% sequence similarity (Fig. 1, Fig. 2 and Fig. 3). The ANI and dDDH values between strain R-1-5s-1^T and *J. salarius* ASL-1^T was 78.9% and 20.8%, respectively, lower

than the thresholds recommended for species differentiation (95–96% and 70%, respectively)(Richter *et al*., 2009; Meier and Klenk *et al*., 2013; Goris *et al*., 2007). Based on the ANI and dDDH values, R-1-5s-1^T was proposed as a novel species of the genus *Jeotgalibacillus* (Delcher *et al*., 2007), and the name *Jeotgalibacillus auranticolor* sp. nov. is proposed.

Phenotypic features

Morphological characterization of strain R-1-5s-1^T showed rod-shaped cells that formed orange-pigmented colonies after 48 h of incubation at 37°C on LB agar; the cells were 1.3–2.3 × 0.5–0.7 μm. The strain was orange and rod-shaped. Strain R-1-5s-1^T had peritrichous flagella under transmission electron microscopy (Fig. S1a–b). Strain R-1-5s-1^T formed slightly raised colonies on LB agar and produced pigment. The cells were Gram-positive and were motile peritrichous flagella (Fig. S1b). The API 20NE, API ZYM, and API 50CH test results are shown in Table 1. Strain R-1-5s-1^T differed from *J. salarius* ASL-1^T based on its ability to assimilate D-glucose, D-mannitol, and D-maltose; inability to hydrolyze gelatin; presence of α-galactosidase and naphthol-AS-BI-phosphohydrolase activity; lack of cystine arylamidase activity; and ability to use D-galactose, glycogen, D-mannitol, and D-melibiose to produce acid (Table 1).

Chemotaxonomic characteristics

The chemotaxonomic analysis results supported those of the phylogenetic analysis. Cellular fatty acid analysis revealed that the dominant fatty acids (> 5% of the total fatty acids) were iso-$C_{15:0}$ (41.92%), anteiso-$C_{15:0}$ (17.92%), iso-$C_{17:0}$ (6.51%), and anteiso-$C_{17:0}$ (8.94%). The percentages of other fatty acids are listed in Table 2. The major phospholipids of strain R-1-5s-1^T were phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) (Fig. S2a–b), similar to the related type strain *J. salarius* ASL-1^T (Fig. 2c). In addition, the fatty acids of strain R-1-5s-1^T were different from those of the reference strains in composition and proportion. There were similarities between R-1-5s-1^T and the reference strains. The major menaquinones of this strain were MK-7 and MK-8, typical of the genus *Jeotgalibacillus* (Yoon *et al*., 2001; Srinivas *et al*., 2016; Cui *et al*., 2018; Zhou *et al*., 2020). R-1-5s-1^T had cell wall acetyl-type bacteria A1a linked via L-Lys (Groth *et al*., 1996). The chemotaxonomic findings for strain R-1-5s-1^T were similar to those of type strains of *Jeotgalibacillus* species.

Conclusion

The phylogenetic, morphologic, and chemotaxonomic results show that strain R-1-5s-1^T is affiliated with genus *Jeotgalibacillus*. However, the strain can be distinguished from other *Jeotgalibacillus* species by several phenotypic differences, such as the temperature and pH range; gelatin; use of D-glucose, D-mannitol, D-maltose, D-galactose, glycogen, and gelatin; and fatty acid composition (Table 2). Based on these findings, strain R-1-5s-1^T represents a novel species of the genus *Jeotgalibacillus*, for which the name *Jeotgalibacillus auranticolor* sp. nov. is proposed.

Description of *Jeotgalibacillus auranticolor* sp. nov.

Jeotgalibacillus auranticolor (*auranti'color*, N.L. neut. n. *Aurantium*, generic name of the orange; L. masc. n. *color*, color, tin; N.L. adj. *auranticolor*, referring to orange colored colnoy)

Cells are Gram-positive, aerobic, rod-shaped, flagellum-motile, and 1.3–2.3 × 0.5–0.7 μm in size. The colony was orange, oxidase-negative, catalase-positive, and hydrolyzed starch, and 1–2 mm in diameter after culture on LB agar at 37℃ for 48 h. Growth occurs at 15–37℃ (optimum 37℃), pH 7.0–11.0 (optimum 8.0), and in 5% (w/v) NaCl on LB medium. R-1-5s-1^T has flagella, and cell wall acetyl type bacteria A1a linked directly via L-Lys. In the API 20NE test, no indole is formed and aescin is hydrolyzed. Cells are positive for urease; negative for arginine dihydrolase, nitrate reduction, and glucose fermentation; and do not assimilate N-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, trisodium citrate, phenylacetic acid, or adipic acid. By the API ZYM test, cells are positive for esterase (C4) and esterase lipase (C8), weakly positive for alkaline phosphatase, negative for L-arabinose, lipase (C14), acid phosphatase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, and D-mannose. By the API 50CH test, cells can use glycerol, D-glucose, D-fructose, and esculin ferric citrate to produce acid. By Biolog-Gen□-Microplate tests, cells can use as carbon sources dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, D-melibiose, β-methyl-D-glucoside, D-salicin, α-D-glucose, D-fructose, D-galactose, D-mannitol, glycerol, L-alanine, L-glutamic acid, L-pyroglutamic acid, pectin, D-galacturonic acid, L-malic acid, Tween 40, acetoacetic acid, and acetic acid. The cells can also use, albeit less efficiently, L-arginine, L-aspartic acid, L-serine, and inosine. The predominant polar lipids are PG and DPG; the major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$, and anteiso-$C_{17:0}$. The major menaquinones are MK-7 and MK-8.

The draft genome of strain R-1-5s-1^T is 3.4 Mb, and the G+C content is 43.6%. The type strain, R-1-5s-1^T (=CGMCC 1.13567^T=KCTC 43038^T), was isolated from Baiyangdian Lake, Hebei, China. The GenBank accession numbers for the 16S rRNA gene and whole-genome sequences of strain R-1-5s-1^T are MK641361 and SORW00000000, respectively.

Abbreviations

ANI Average nucleotide identity

dDDH digital DNA-DNA hybridization

MK-7 menaquinone-7

MK-8 menaquinone-8

ML maximum-likelihood

MP maximum parsimony

NJ neighbor-joining

PG phosphatidylglycerol

DPG diphosphatidylglycerol

Declarations

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Conflicts of interest

The authors have declared that no competing interests exist.

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Tables

Table 1. Differential phenotypic characteristics of strain R-1-5s-1^T and related species of the genus *Jeotgalibacillus*

Characteristic	1	2	3
Temperature range (optimum) °C	15–37 (37)	(30)[a]	(30–35)[b]
pH range (optimum)	7–11 (8)	7–8[a]	7–8[b]
API 20NE			
Gelatin	-	+	+
4-Nitrophenyl-*β*-d-galactopyranoside	+	w	+
d-Glucose	+	-	-
d-Mannitol	+	-	+
d-Maltose	+	-	+
Enzyme activity (API ZYM)			
Valine arylamidase	w	+	w
Cysteine arylamidase	-	+	-
Trypsin	w	+	-
α-Chymotrypsin	w	+	+
α-Galactosidase	+	-	w
Naphthol-AS-BI-phosphohydrolase	+	w	+
β-Galactosidase	+	-	+
Acid production (API 50 CH)			
d-Galactose, glycogen	+	-	-
d-Mannitol, d-melibiose	+	-	+
d-Maltose, d-saccharose, d-trehalose, amidon, d-turanose	+	w	+
Inulin, d-raffinose	-	w	-
DNA G+C content (mol%)	43.6	41.8	44[b]
Data sources: a, Yoon *et al.*, 2010; b, Yoon *et al.*, 2001.			

Strains: 1, R-1-5s-1^T; 2, *J. salarius* ASL-1^T; 3, *J. alimentarius* YKJ-13^T. +, Positive; -, negative; w, weakly positive. Data are from this study, unless indicated otherwise.

Table 2. Dominant fatty acids of strain R-1-5s-1^T and related reference strains

Fatty acids	1	2	3
Straight chain			
$C_{16:0}$	3.4	1.6	1.5
Branched			
Iso-$C_{14:0}$	2.0	5.7	2.9
Iso-$C_{15:0}$	41.9	6.1	52.2
Anteiso-$C_{15:0}$	17.9	42.8	21.4
Iso-$C_{16:0}$	3.7	9.6	1.8
Anteiso-$C_{17:0}$	8.9	20.0	3.7
Iso-$C_{17:0}$	6.5	1.2	2.9
Unsaturated			
Iso-$C_{17:1}\omega 10c$	1.2	TR	3.2
$C_{16:1}\omega 11c$	2.9	1.5	1.8
$C_{18:1}\omega 9c$	1.1	TR	1.9
Summed features*			
Summed feature 4	1.8	2.4	3.9
Summed features*: 4,iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B			

Strains: 1, R-1-5s-1^T; 2, *J. salarius* ASL-1^T; 3, *J. alimentarius* YKJ-13^T. -, Not detected; TR, < 1.0%.

All data are from this study.

Figures

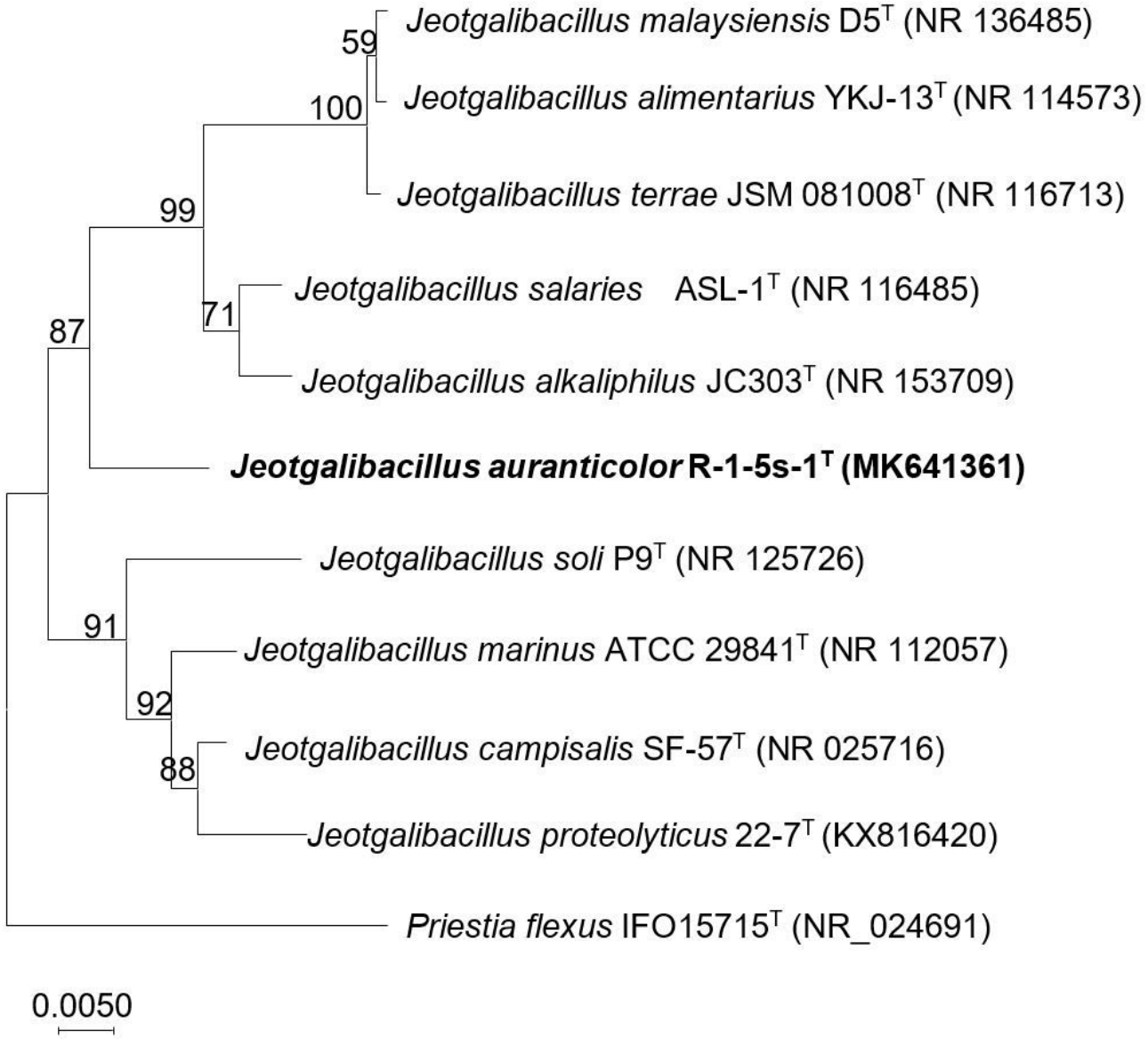

Figure 1

Phylogenetic tree using the neighbour-joining method based on the 16S rRNA genes sequence, showing the strain R-1-5s-1^T belong to genus *Jeotgalibacillus* and the reference strain was *J. salarius* ASL-1^T (bootstrap values is 76%). GenBank accession numbers are given behind strains. Bootstrap values above 50% based on 1000 replicates are shown at branch nodes. Bar, 0.005 substitutions per nucleotide.

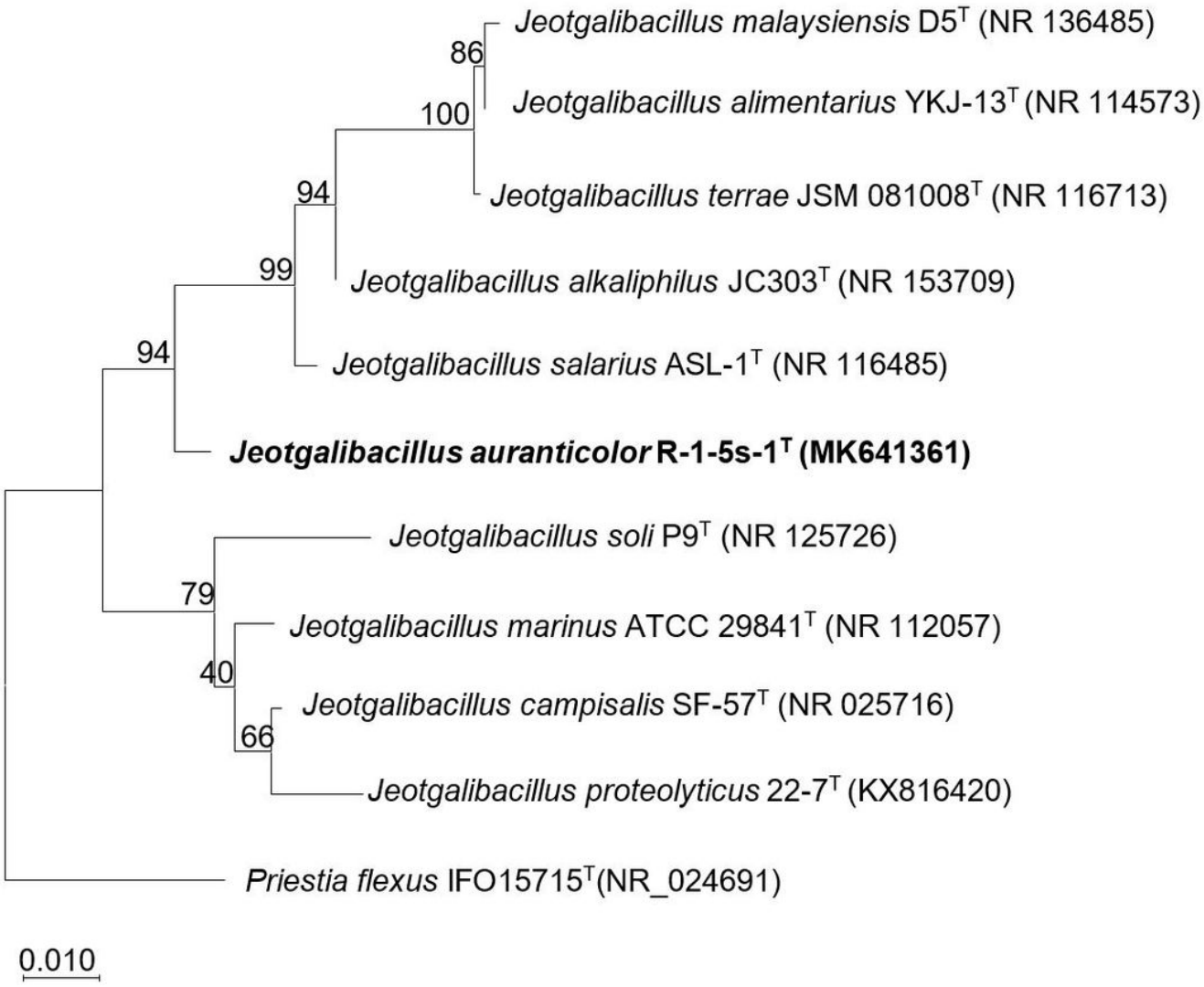

Figure 2

Phylogenetic tree using the maximum-likelihood method based on the 16S rRNA genes sequence, showing the strain R-1-5s-1^T belong to genus *Jeotgalibacillus* and the reference strain was *J. salarius* ASL-1^T. GenBank accession numbers are given behind strains. Bootstrap values above 50% based on 1000 replicates are shown at branch nodes. Bar, 0.01 substitutions per nucleotide.

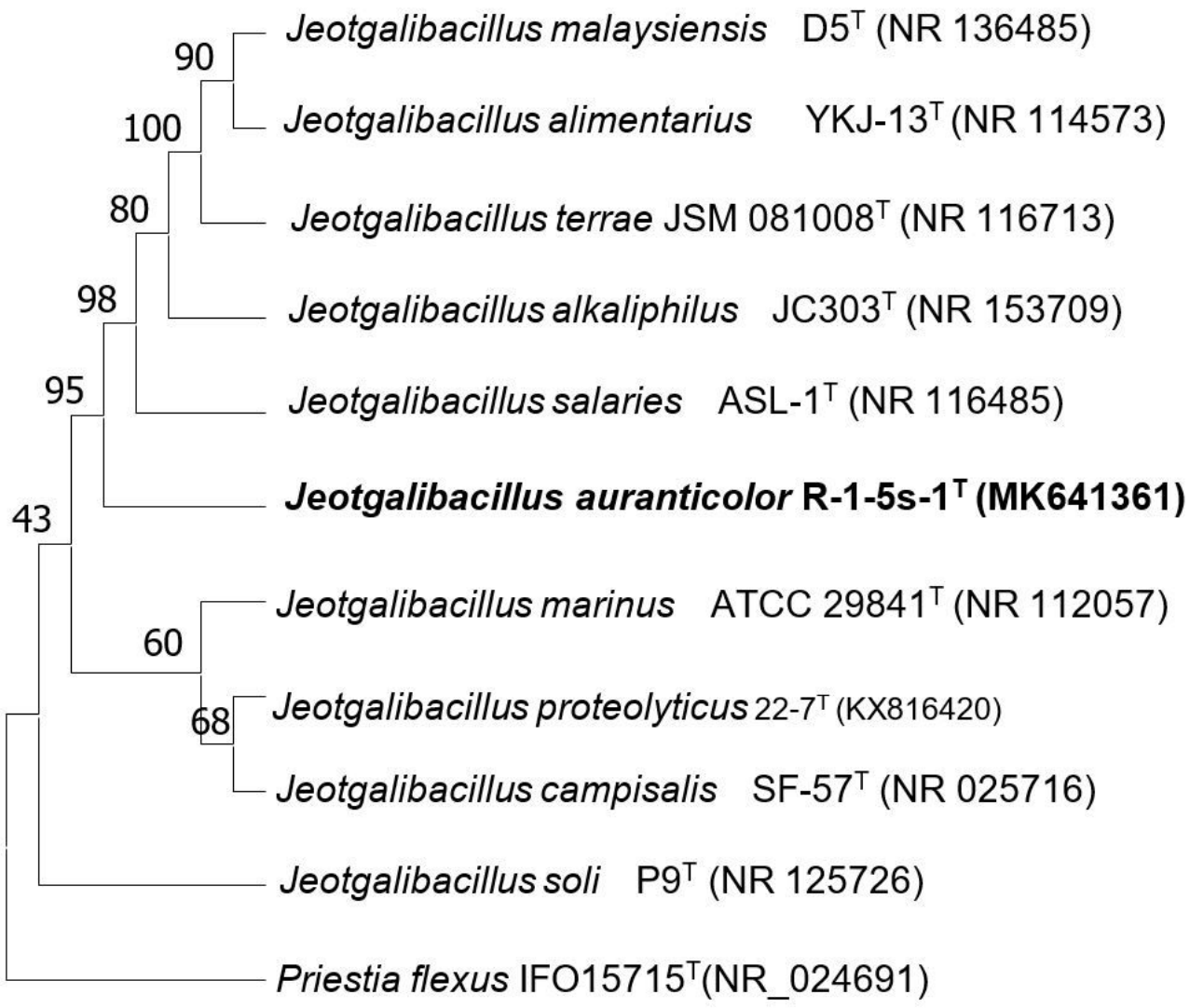

Figure 3

Phylogenetic tree using the maximum-parsimony method based on the 16S rRNA genes sequence, showing the strain R-1-5s-1^T belong to genus *Jeotgalibacillus* and the reference strain was *J. salarius* ASL-1^T. GenBank accession numbers are given behind strains. Bootstrap values above 50% based on 1000 replicates are shown at branch nodes. Bar, 10 substitutions per nucleotide.

Supplementary Files

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

- Supp.Fig.docx