Lysinibacillus Agricola sp. nov.Isolated from Soil

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Research Article

Keywords: Lysinibacillus agricola sp, nov., soil

Posted Date: February 23rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-222207/v1

Abstract

A Gram-staining-positive, rod-shaped cells, designed strain FJAT-51161^T, was isolated from farmland soil collected from Fujian Province, China. Growth was observed at 25 - 40 °C (optimum 30 °C), pH 7.0-9.0 (optimum 7.0), and NaCl tolerance in the range of 0-7 % (w/v), respectively. Phylogenetic analysis based on the 16S rRNA gene sequences indicated that the strain FJAT-51161^T belonged to the genus *Lysinibacillus*, and had the closest relationship with *Lysinibacillus xylanilyticus* XDB9^T (with 99.0 % 16S rRNA sequence similarity). The digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) values based on the genome sequence analysis between strain FJAT-51161^T and the closest reference strain were 38.0 % for dDDH and 88.7 % for ANI, respectively, lower than the prokaryotic species defined values. Further analysis showed that strain FJAT-51161^T shared the fatty acid profiles such as iso-$C_{15:0}$ (46.7%), iso-$C_{16:0}$ (15.8%), $C_{16:1}$ ω7*c* alcohol (14.0%), anteiso-$C_{15:0}$ (6.9%) with other members of the genus *Lysinibacillus*. The peptidoglycan was L-Lys–D-Asp (type A4α). The main quinone was MK-7 and MK-6. The major polar lipids were diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE). The DNA G+C content is 36.6 mol%. Based on the phenotypic characters and taxono-genomics study, strain FJAT-51161^T is considered to represent a novel *Lysinibacillus* species, for which the name *Lysinibacillus agricola* sp. nov. is proposed. The type strain is FJAT-51161^T (= GDMCC1.2350^T = KCTC XXXXXX^T).

Introduction

The genus *Lysinibacillus* was established and transferred from the genus *Bacillus* by Ahmed et al. in 2007 (Ahmed et al. 2007), which belong to the family Bacillaceae of the phylum Firmicutes. The *Lysinibacillus* species are unique among the family Bacillaceae as they are characterized by a special cell-wall peptidoglycan type of A4α (L-Lys-D-Asp), such as *Lysinibacillus yapensis* isolated from deep-sea sediment of the Yap Trench, Pacific Ocean (Yu et al. 2019), *Lysinibacillus xyleni* sp. nov. from a bottle of xylene (Begum et al. 2016), *Lysinibacillus louembei* sp. nov. from alkaline fermented leaves of cassava (Ouoba et al. 2015), *Lysinibacillus manganicus* sp. nov. isolated from manganese mining soil (Liu et al. 2013). At the time of writing, the genus *Lysinibacillus* consisted of 30 species with validly published names (https://lpsn.dsmz.de/genus/lysinibacillus) with *Lysinibacillus boronitolerans* as the type species (Ahmed et al. 2007). During the survey of *Bacillus*-like species diversity, an endospore-forming, a novel strain FJAT-51161^T was obtained from soil samples and found to have morphological properties consistent with the genus *Lysinibacillus* but differentiate from them. Therefore, we adopted polyphasic taxonomic approach combining with the genome indexes to evaluate the taxonomic position of strain FJAT-51161^T.

Materials And Methods

Sample collection, isolation, and preservation

Strain FJAT-51161^T was isolated from soil sample of farm land, Fujian Province, China. The sample was serially diluted and an aliquot (100 µL) was spread on LB medium. The plate was incubated at 30 ºC for two days. The colonies obtained were repeatedly re-streaked on the same medium until pure colonies were obtained and stored as glycerol suspensions (20%, w/v) at −80 °C and as lyophilized form in skimmed milk (15 %, w/v) at 4 °C.

Phenotypic, microscopic and growth conditions

Colony morphology was observed on LB medium after 24 h of aerobic incubation under optimal growth conditions. The Gram staining and the KOH lysis test were carried out according to the methods described by Gregersen (1978) and Smibert and Krieg (1994). The size of the cells was determined by transmission electron microscopy (Hitachi, Japan). Endospores were examined according to Schaeffer–Fulton staining method (Murray et al. 1994). Motility was examined on motility agar (Chen et al. 2007). Ten different growth temperatures (10, 15, 20, 25, 30, 37, 45, 50, 55 and 60 °C), six NaCl concentrations (0, 1, 3, 5, 7, and 10 %, w/v) and ten pHs (5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 11.0) were tested. Catalase activity was determined by investigating bubble production with 3 % (v/v) H_2O_2, and oxidase activity was determined using 1 % (v/v) tetramethyl p-phenylenediamine. Cell growth under anaerobic conditions was determined in a CO_2 incubator on anaerobically prepared maintenance media. Other physiological and biochemical characteristics were confirmed using API 20E and API 50CHB strips (BioMérieux, France) following the manufacturer's instructions.

16S rRNA gene sequence and phylogenetic analysis

Genomic DNA was extracted from a single colony of strain FJAT-51161^T grown on LB plates at 30 $^{\circ}$C for 24 h using the bacteria genomic DNA extraction kit (Shanghai Generay Biotech Co., Ltd, China) according to the manufacturer's instructions. 16S rRNA gene was amplified and sequenced using primers and the conditions described previously (Liu et al. 2015). The obtained 16S rRNA gene sequence was compared with available sequences of cultured species at EZBioCloud server (https://www.ezbiocloud.net/) (Yoon et al. 2017a). After multiple alignments of data by CLUSTAL_X (Thompson et al. 1997), phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei 1987), maximum-parsimony (MP) (Fitch 1971) and maximum-likelihood (Felsenstein 1981) methods implemented with MEGA version 7 (Kumar et al. 2016). For all the trees, gaps were treated as complete deletions, evolutionary distances were computed according to the Kimura 2-parameter model (Kimura 1980) and the reliability of each branch was evaluated by bootstrap analysis based on 1000 replications (Felsenstein 1985). The 16S rRNA gene sequences used for the phylogenetic comparisons were shown in the maximum-likelihood phylogenetic tree with their strain designations and accession numbers.

Chemotaxonomy

To investigate chemotaxonomy characters, the peptidoglycan diamino acid test was carried out according to the method described by Schumann (2011). Main quinone was analyzed as described by Collins (1977) using reverse-phase HPLC (Groth et al. 1996). Extraction and analysis of polar lipids by

two-dimensional TLC was performed according to Minnikin et al. (1979). The cellular fatty acid profiles of strain FJAT-51161^T and its closely related strains grown on TSBA medium at 28 °C for 24 h were determined according to the Sherlock Microbial Identification System (MIDI). The fatty acids were separated using an automated GC system (model 7890N; Agilent) and identified with the TSBA6 database of the Microbial Identification System (Sasser 1990).

Genome sequencing and comparison

For determination of Digital DDH (dDDH) and Average Nucleotide Identity (ANI), the genome of FJAT-51161^T, *L. xylanilyticus* DSM 23493^T, *L. macroides* DSM 54^T and *L.contaminans* DSM 25560^T were sequenced by the Beijing Novogene Bioinformatics Technology Co., Ltd (China), with accession number CP067341, LFXJ00000000, LGCI00000000 and LGRV00000000. Other genomes were obtained from NCBI database. Estimation of dDDH was performed using the Genome-to-Genome Distance Calculator (GGDC) (Auch et al. 2010; Meier-Kotloff et al. 2013). The genome files were uploaded to the GGDC 2.0 Web interface (http://ggd-c.dsmz.de/ distcalc2.php) and the Formula 2 was used according to the recommendation for the calculation of dDDH for incomplete genomes. The ANI value was calculated using OrthoANIu algorithm (https://www.ezbiocloud.net/tools/ani) according to the description by Yoon et al. (2017b) at the EzGenome web server (http://www.ezbiocloud.net/ezgenome/ani).

Results And Discussion

The colonies of strain FJAT-51161^T were approximate 2 mm in diameter, white-creamy, smooth, opaque circular. The size of cells and presence of flagella were determined by transmission electron microscopy (Hitachi, Japan), with a length ranging from 2.0 μm to 3.37 μm and a diameter ranging from 0.8 μm to 1.12 μm (**Supplementary Fig. S1**). Growth of strain FJAT-51161^T occurred at 25-40 °C (optimum 30 °C), between 0% and 7.0 % NaCl concentration (optimum 0 %) and pH in the range of 7.0-9.0 (optimum 7.0). They produced spherical endospores that lay in terminal position. The results showed that strain FJAT-51161^T could not utilize any carbon source to produce acid in API 50 CHB strip. The hydrolysis of gelatin, V-P test and lysine decarboxylase were positive in API 20E, others were negative. The different characteristics of strain FJAT-51161^T in comparison with its closest phylogenetic neighbors were presented in **Table 1**.

The results of phylogenetic analysis of 16S rRNA gene sequences suggested that strain FJAT-51161^T formed a single branch distinguished from members of the genus *Lysinibacillus* (**Fig. 1**). EZBioCloud server search analysis revealed that strain FJAT-51161^T had high 16S rRNA similarities with the closely related species of *Lysinibacillus xylanilyticus* DSM 23493^T (99.0 % sequence similarity), *Lysinibacillus pakistanensis* NCCP-54^T (98.7 %), *Lysinibacillus macroides* DSM 54^T (98.6 %), *Lysinibacillus fusiformis* NBRC 15717^T (98.1 %), *Lysinibacillus boronitolerans* T-10a^T (98.1 %), *Lysinibacillus sphaericus* KCTC 3346^T (98.0 %), *Lysinibacillus contaminans* FSt3A^T (97.7 %), and *Lysinibacillus parviboronicapiens* BAM-582^T (97.0 %), respectively, other species in the genus *Lysinibacillus* were low than 97.0 %. Therefore, it

was obvious that strain FJAT-51161^T should be a member of the genus *Lysinibacillus*. The phylogenetic position was also confirmed by trees generated using the methods of neighbor-joining (**Supplementary Fig. 2**) and minimum evolution (**Supplementary Fig. 3**).

The peptidoglycan of strain FJAT-51161^T was L-Lys – D-Asp (type A4*α*). The main quinone profiles of strain FJAT-51161^T were MK-7 (58.3 %), MK-6 (29.1 %), MK-5 (6.3 %), and MK-8 (6.3 %). The major polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), one unknown aminolipid, two unknown aminophospholipids and two unknown phospholipids (**Supplementary Fig. S4**). The cellular fatty acid profiles of strain were characterized by high proportions of branched fatty acids, such as iso-$C_{15:0}$ (46.7 %), iso-$C_{16:0}$ (15.8 %), $C_{16:1}$ ω7*c* alcohol (14.0 %), anteiso-$C_{15:0}$ (6.9 %) (**Table 2**), which confirmed the placement of strain FJAT-51161^T in the genus *Lysinibacillus* with iso-$C_{15:0}$ as the major fatty acid (Ahmed et al. 2007).

The genome size of FJAT-51161^T was 5, 381, 280 bp, and the genomic DNA G+C content was 36.0 mol%. Detailed genome features of FJAT-51161^T and closely related members were showed in **Table 3**. The values of dDDH and ANI for stain FJAT-51161^T with its most closely related species *L. xylanilyticus* DSM 23493^T were 38.0 % and 88.7 %, respectively, lower than the recognized cut-off values of *is*DDH >70% and ANI > 95–96% served as a threshold for prokaryotic species delineation (Wayne et al. 1987; Goris et al. 2007; Richter and Rosselló-Móra 2009; Meier-Kolthoff et al. 2013).

Based on the morphological, phenotypic and genotypic distinctiveness (G+C content, 16S rRNA gene sequence and taxono-genomics (dDDH and ANI)), strain FJAT-51161^T can be considered to represent a novel species within the genus *Lysinibacillus*, for which the name *Lysinibacillusagricola* sp. nov. is proposed.

Description of *Lysinibacillus agricola*sp. nov.

Lysinibacillus agricola (a.gri'co.la L. n. ager field; L. suff. -cola (from L. n. incola) a dweller, inhabitant; L. masc. n. agricola field dwelling)

Cells are aerobic Gram-positive, motile and rod-shaped bacterium with rounded ends. Cells have a length ranging from 2.0 μm to 3.37 μm and a diameter ranging from 0.8 μm to 1.12 μm. Cells are motile by means of lateral flagella. On LB plate, the colony diameter is about 1-2 mm, white-creamy, smooth, and opaque. Round endospores are located at terminal position. Growth of strain FJAT-51161^T is achieved aerobically between 25 and 40 °C (optimum 30 °C), between pH 7.0-9.0 (optimum 7.0), and NaCl (w/v) concentration in the range of 0 % to 7.0 % (optimum 0 %). It could not grow at 10% NaCl (w/v). Catalase and oxidase are positive. In API 50CHB strip, strain FJAT-51161^T could not utilize any carbon source to produce acid. In API 20E, hydrolysis of gelatin, Voges–Proskauer test and lysine decarboxylase were positive, others were negative. The main quinone was MK-7. The major polar lipids were diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE). The peptidoglycan of strain FJAT-

51161^T was L-Lys–D-Asp (type A4*α*). The main quinone was MK-7 and MK-6. The predominant fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$ and $C_{16:1}$ *ω7c* alcohol.

The type strain of the species FJAT-51161^T (=GDMCC1.2350^T = KCTC XXXXX^T) was isolated from soil in Fujian Province, China. The G+C content of the genome is 36.6 mol %.

Abbreviations

dDDH, digital DNA-DNA hybridization; ANI, Average Nucleotide Identity; DPG, diphosphatidylglycerol; PE, phosphatidyl ethanolamine

Declarations

Footnote

The GenBank accession numbers for the genome sequence of species *Lysinibacillus agricola* FJAT-51161^T, was CP067341.

Author statements

This work was financially supported by Fujian Academy of Agricultural Sciences (GJPY2019003).

Compliance with ethical standards

Conflicts of interest – The authors declared that they had no conflict of interest.

Ethical statement–This article did not contain any studies with animals performed by any of the authors.

References

Ahmed I, Yokota A, Yamazoe A, Fujiwara T (2007) Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillusfusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillussphaericus* comb. nov. Int J Syst Evol Microbiol 57: 1117–1125

Auch AF, von Jan M, Klenk HP, 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

Begum MA, Rahul K, Sasikala C, Ramana CV (2016) *Lysinibacillus xyleni* sp. nov., isolated from a bottle of xylene. Arch Microbiol 198: 325–332

Chen YG, Cui XL, Pukall R, Li HM, Yang YL, Xu LH, Wen ML, Peng Q, Jiang CL (2007) *Salinicoccuskunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine in Yunnan, south-west China. Int J Syst Evol Microbiol 57: 2327–2332

Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol 100: 221–230

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: 783–789

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20: 406–416

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57: 81–91

Gregersen T (1978) Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl MicrobiolBiotechnol* 5: 123–127

Groth I, Schumann P, Weiss N, Martin K, Rainey FA (1996) *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. Int J Syst Bacteriol 46: 234–239

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Liu H, Song Y, Chen F, Zheng S, Wang G (2013) *Lysinibacillus manganicus* sp. nov., isolated from manganese mining soil. Int J Syst Evol Microbiol 63: 3568–3573

Liu B, Liu GH, Sengonca C, Schumann P, Che JM, Zhu YJ, Wang JP (2015) *Bacillus wuyishanensis* sp. nov., isolated from rhizosphere soil of a medical plant, Prunella vulgaris, in the Wuyi mountain of China. Int J Syst Evol Microbiol 65: 2030–2035

Meier-Kotloff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14: 60

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal K, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2: 233–241

Ouoba LI, Vouidibio Mbozo AB, Thorsen L, Anyogu A, Nielsen DS, Kobawila SC, Sutherland JP (2015) *Lysinibacillus louembei* sp. nov., a spore-forming bacterium isolated from Ntoba Mbodi, alkaline

fermented leaves of cassava from the Republic of the Congo. Int J Syst Evol Microbiol 65: 4256–4262

Richter M, Rosselló-Mora R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106: 19126–19131

Saitou N, 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. USFCC News Lett 20: 1–6

Schumann P (2011) Peptidoglycan structure. Methods Microbiol 38: 101-129

Smibert RM, Krieg NR (1994) Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by Gerhardt P, Murray RGE, Wood WA, Krieg NR. Washington DC: American Society for Microbiology.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876–4882

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the ad hoc Committee on Reconciliation of Approaches to Bacterial Systematics. Int J Syst Bacteriol 37: 463–464

Yu L, Tang X, Wei S, Qiu Y, Xu X, Xu G, Wang Q, Yang Q (2019) Isolation and characterization of a novel piezotolerant bacterium Lysinibacillus yapensis sp. nov., from deep-sea sediment of the Yap Trench, Pacific Ocean. J Microbiol 57: 562-568

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

Yoon SH, Ha SM, Lim JM, Kwon SJ, Chun J (2017b) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie van Leeuwenhoek. 110: 1281–1286

Tables

Table 1 Characteristics used to distinguish strain FJAT-51161^T from the type strains of phylogenetically related species.

1, FJAT-51161^T; 2, *Lysinibacillus xylanilyticus* DSM 23493^T; 3, *Lysinibacillus macroides* DSM 54^T; 4, *Lysinibacillus boronitolerans* T-10a^T; 5, *Lysinibacillus contaminans* DSM 25560^T; 6, *Bacillus subtilis* DSM 10^T. ND, no data. The data was from this study, except taxon 4 was from Ahmed *et al.* (2007).

Characteristics	1	2	3	4	5	6
Spore shape	round	round	round	round	round	Ellipsoidal
pH range	7.0-9.0	5-9	7.0-9.0	5.5-9.5	6.5-10.5	5.5-9.0
pH optimal	7	7	7	7	7-8	7
Temp range (°C)	25-40	10-40	10-45	16-45	15-45	5-55
Temp optimal (°C)	30	30	30	35-37	30	30
Nitrate reduction	-	-	-	-	-	+
Urease activity	-	-	-	+	-	+
Hydrolysis of gelatin	+	+	-	-	+	+
Voges–Proskauer test	+	-	+	-	+	+
Arginine dihydrolase	-	-	-	+	-	-
Lysine decarboxylase	+	-	-	-	-	-
Polar lipid[#]	PE, DPG	PG, PE, DPG	PG, PE, DPG	DPG, PG	PG, PE, DPG	ND
MK	7	7	7	7	7, 6	7
Cell-wall peptidoglycan	L-Lys–D-Asp	L-Lys–D-Asp	L-Lys–D-Asp	L-Lys–D-Asp	L-Lys–D-Asp	*meso*-DAP
DNA G+C content (mol %)	41	37.2	38.2	36.5	37.3	42.9

[#] DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine.

Table 2 Fatty acids profiles of strain FJAT-51161^T and its related species

1, FJAT-51161^T; 2, *Lysinibacillus xylanilyticus* DSM 23493^T; 3, *Lysinibacillus macroides* DSM 54^T; 4, *Lysinibacillus boronitolerans* T-10a^T; 5, *Lysinibacillus contaminans* DSM 25560^T; 6, *Bacillus subtilis* DSM 10^T.

Fatty acids	1	2	3	4[a]	5	6
$C_{12:0}$	0.16	0	0	0	0	0
iso-$C_{13:0}$	0.1	0.4	0	0	0	0
$C_{14:0}$	0.6	0.9	0.4	0.4	0.7	0.9
iso-$C_{14:0}$	4.7	1.55	2.6	1.7	5.6	1.5
$C_{15:0}$	0	0	0	0.5	0	0
anteiso-$C_{15:0}$	6.9	8.0	7.4	21.4	3.14	39.2
iso-$C_{15:0}$	46.7	58.2	45.9	31.8	35.4	15.3
iso-$C_{15:1}$ ω9*c*	0	0.5	0	0	0.2	0
$C_{16:0}$	1.9	1.85	2.7	1.8	2.4	8.3
$C_{16:0}$ 2OH	0.1	0	0	0	0	0
$C_{16:0}$ 3OH	0.12	0	0	0	0	0
iso-$C_{16:0}$	15.8	1.8	12.19	11.2	11.5	4.6
iso-C1$_{6:1}$ H	0	0	0	0	0.2	0
$C_{16:1}$ ω11*c*	2.3	2.7	5.3	2.7	6.4	1.0
$C_{16:1}$ ω7*c* alcohol	14.0	7.0	10.1	7.6	24.9	0
anteiso-$C_{17:0}$	1.8	2.7	3.1	11.1	0.8	13.1
iso-$C_{17:0}$	3.3	3.4	6.1	5.5	1.9	10.0
anteiso-$C_{17:1}$ ω9*c*	0	0	0	0	0	0.6
iso-$C_{17:1}$ ω10*c*	0.6	6.3	2.0	1.3	3.2	0.6
$C_{17:1}$ ω9*c*	0	0	0	0	0.8	0
$C_{18:0}$	0.19	0.8	0.4	0	0.6	2.7
$C_{18:1}$ ω9*c*	0	0.4	0.4	0	0.5	1.7
Summed Feature 3 *	0.19	0.1	0	0	0.2	0
Summed Feature 4 *	0.5	3.1	1.6	2.8	1.5	0

Fatty acids	1	2	3	4[a]	5	6
Summed Feature 8 *	0	0.2	0	0	0	0

* Summed Feature 3, $C_{16:1}$ ω6c and/or $C_{16:1}$ ω7*c*; Summed Feature 4, anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I; Summed Feature 8, $C_{18:1}$ ω6*c* and/or $C_{18:1}$ ω7*c*. [a] The data were got from the paper by Ahmed *et al.* (2007).

Table 3 The 16S rRNA similaritis, ANI, AAI, POCP and dDDH values of strain FJAT-51161^T with its closely related species

Species	Strain no	Acession number*	16S rRNA similarities(%)	ANI (%)	dDDH(%)
FJAT-51161^T	FJAT-51161^T	CP067341			
Lysinibacillus xylanilyticus	DSM 23493^T	LFXJ00000000	99.0	88.7	38.0
Lysinibacillus pakistanensis	NCCP-54^T	BBDJ00000000	98.7	82.8	28.1
Lysinibacillus macroides	DSM 54^T	LGCI00000000	98.6	79.9	25.5
Lysinibacillus fusiformis	NBRC 15717^T	CP010820	98.1	80.5	25.1
Lysinibacillus boronitolerans	T-10a^T	JPVR00000000	98.1	80.0	24.7
Lysinibacillus sphaericus	KCTC 3346^T	AUOZ00000000	98.0	80.1	25.7
Lysinibacillus contaminans	DSM 25560^T	LGRV00000000	97.7	77.9	23.4
Lysinibacillus parviboronicapiens	BAM-582^T	PYWI00000000	97.0	80.5	25.8

Figures

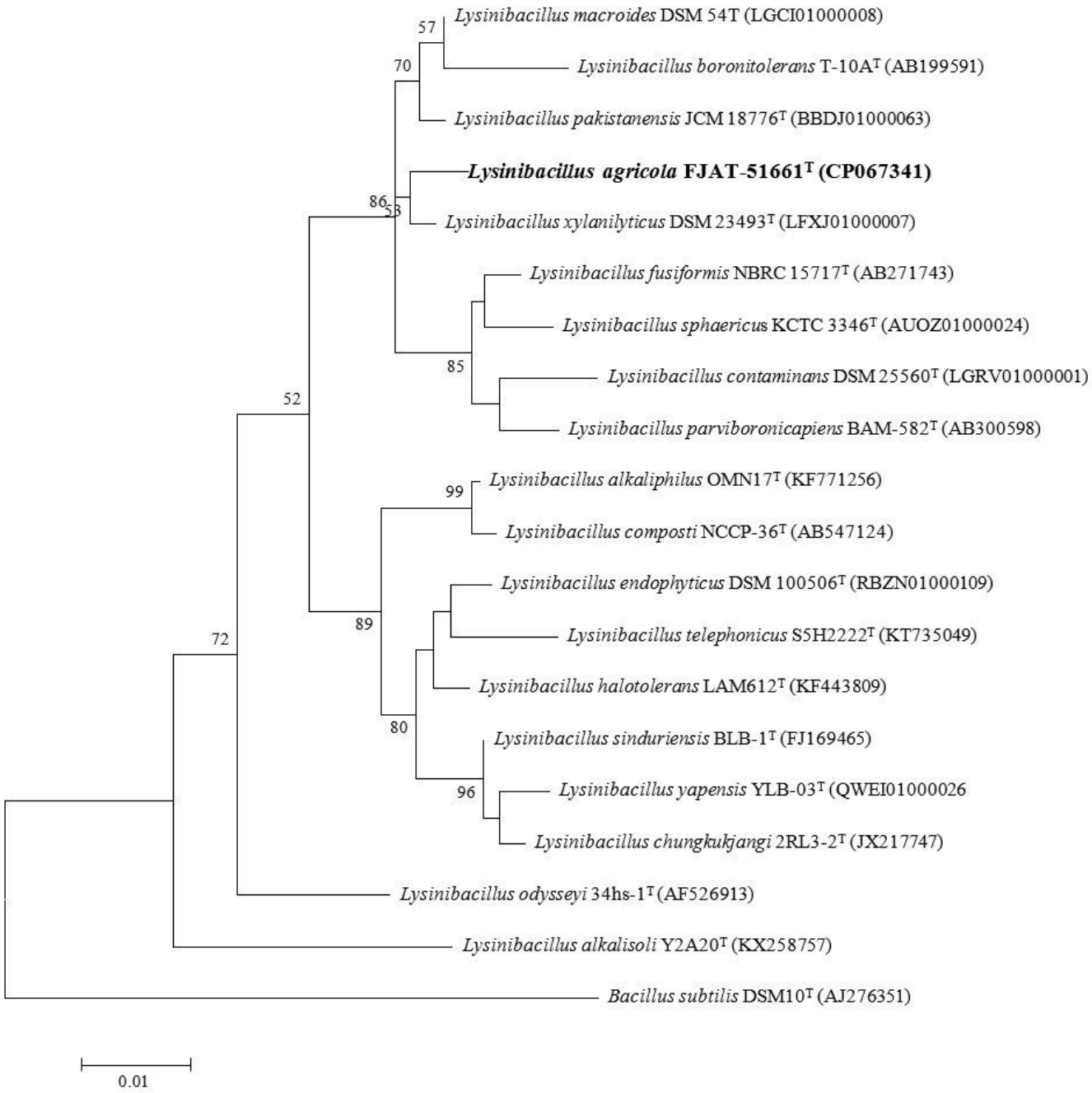

Figure 1

Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequence of strain FJAT-51161T and closely related species within the genus Lysinibacillus. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

Supplementary Files

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