**Abstract**

A gram-staining-positive, rod-shaped bacterium, designated strain FJAT-51161T 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-51161T belonged to the genus *Lysinibacillus*, and had the closest relationship with *Lysinibacillus xylanilyticus* XDB9T (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-51161T and the closest reference strain were 38.0% for dDDH and 88.7% for ANI, respectively, lower than the prokaryotic species delineation values. Further analysis showed that strain FJAT-51161T shared the fatty acid profiles such as iso-C15:0 (46.7%), iso-C16:0 (15.8%), C16:1 ω7c alcohol (14.0%), anteiso-C15:0 (6.9%) with other members of the genus *Lysinibacillus*. As the peptidoglycan contained the amino acids alanine, lysine, glycine and aspartic acid, the type A4α was deduced as found in the closest relatives of strain FJAT-51161T. The peptidoglycan of strain FJAT-51161T was L-Lys–D-Asp (type A4α). The major 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-51161T is considered to represent a novel *Lysinibacillus* species, for which the name *Lysinibacillus agricola* sp. nov. is proposed. The type strain is FJAT-51161T (GDMCC1.2350T = KCTC 43326T).

**Keywords** *Lysinibacillus agricola* sp. nov. · Soil

**Abbreviations**

dDDH  Digital DNA–DNA hybridization  
ANI  Average nucleotide identity  
DPG  Diphosphatidylglycerol  
PE  Phosphatidyl ethanolamine

**Introduction**

The genus *Lysinibacillus* was established and transferred from the genus *Bacillus* by Ahmed et al. (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 novel strain was isolated from soil samples and was found to have morphological properties...
consistent with the genus *Lysinibacillus*. Therefore, we adopted polyphasic taxonomic approach combining with the genome indexes to evaluate the taxonomic position of strain FJAT-51161T.

### Materials and methods

#### Sample collection, isolation, and preservation

Strain FJAT-51161T 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 °C 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), 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 pH values (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) H2O2, and oxidase activity was determined using 1% (v/v) tetramethyl p-phenylenediamine. Cell growth under anaerobic conditions was determined in a CO2 incubator on anaerobic 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-51161T grown on LB plates at 30 °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 X (Kumar et al. 2018). 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 quinine 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-51161T 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 7890 N, 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 genomes of FJAT-51161T, *L. xylanilyticus* DSM 23493T, *L. macroides* DSM 54T and *L. contaminans* DSM 25560T were sequenced by the Beijing Novogene Bioinformatics Technology Co., Ltd (China), with accession number CP067341, LFJX0000000, 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-Kolthoff et al. 2013). The genome files were uploaded to the GGDC 2.0 Web interface (http://ggdc.dsmz.de/distcalc2.php) and the formula two 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-51161T 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 to 3.37 μm and a diameter ranging from 0.8 to 1.12 μm (Supplementary Fig. S1). The results showed that strain FJAT-51161T 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-51161T in comparison with its closest phylogenetic neighbors are presented in Table 1.

The results of phylogenetic analysis of 16S rRNA gene sequences suggested that strain FJAT-51161T formed a single branch distinguished from those of other members of the genus Lysinibacillus (Fig. 1). EZBioCloud server search analysis revealed that strain FJAT-51161T had high 16S rRNA similarities with the type strains of Lysinibacillus xylanilyticus DSM 23493T (99.0% sequence similarity), Lysinibacillus pakistanensis NCCP-54T (98.7%), Lysinibacillus macroides DSM 54T (98.6%), respectively, other species in the genus Lysinibacillus were lower than 98.1%. Therefore, it was obvious that strain FJAT-51161T should be a member of the v genus Lysinibacillus. The phylogenetic position was also confirmed by trees generated using the methods of neighbor-joining (Supplementary Fig. 2) and maximum parsimony (Supplementary Fig. 3).

As the peptidoglycan contained the amino acids alanine, lysine, glycine and aspartic acid, the type A4α was deduced as found in the closest relatives of strain FJAT-51161T (Lee et al. 2010). So, the peptidoglycan of strain FJAT-51161T was L-Lys–D-Asp (type A4α) (Supplementary Fig. S4). The main quinone profiles of strain FJAT-51161T 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. S5). The cellular fatty acid profiles of strain were characterized by high proportions of branched fatty acids, such as iso-C15:0 (46.7%), iso-C16:0 (15.8%), C16:1ω7c alcohol (14.0%), anteiso-C15:0 (6.9%) (Table 2), which confirmed the placement of strain FJAT-51161T in the genus Lysinibacillus with iso-C15:0 as the major fatty acid (Ahmed et al. 2007).

The genome size of FJAT-51161T was 5,381,280 bp, and the genomic DNA G+C content was 36.0%. Detailed genome features of FJAT-51161T and closely related members were showed in Table 3. The values of dDDH and ANI for strain FJAT-51161T with its most closely related species L. xylanilyticus DSM 23493T were 38.0 and 88.7%, respectively, lower than the recognized cutoff values of isDDH > 70% and ANI > 95–96% served as a

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

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

1 FJAT-51161T, 2 Lysinibacillus xylanilyticus DSM 23493T, 3 Lysinibacillus macroides DSM 54T, 4 Lysinibacillus boronitolerans T-10aT, 5 Lysinibacillus contaminans DSM 25560T

The data were from this study, except taxon four was from Ahmed et al. (2007)

aDPG diphosphatidylglycerol, PG phosphatidylglycerol, PE phosphatidylethanolamine
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-51161T can be considered to represent a novel species within the genus *Lysinibacillus*, for which the name *Lysinibacillus agricola* sp. nov. is proposed.

**Description of Lysinibacillus agricola sp. nov.**

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

Aerobic gram-positive, motile and rod-shaped bacterium with rounded ends, cells size is approximate 0.8~1.12 × 2.0~3.37 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-51161T 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 0–7.0% (optimum 0%). It could not grow at 10% NaCl (w/v). Catalase and oxidase are positive. In API 50CHB strip, strain FJAT-51161T cannot utilize any carbon source to produce acid. In API 20E, hydrolysis of gelatin, Voges–Proskauer test and lysine decarboxylase are positive, others were negative. The main quinone is MK-7. The major polar lipids are diphosphatidylglycerol (DPG) and phosphatidyl-lethanolamine (PE). The peptidoglycan was L-Lys–D-Asp (type A4α). The main quinone is MK-7 and MK-6. The predominant fatty acids are iso-C₁₅:₀, iso-C₁₆:₀ and C₁₆:₁ ω7c alcohol. The G+C content of the genome is 36.6%.

The type strain of the species FJAT-51161T (GDMCC1.2350T = KCTC 43326T) was isolated from soil in Fujian Province, China.
Table 2 Fatty acids profiles of strain FJAT-51161T and its related species

| Fatty acids | 1 | 2 | 3 | 4<sup>a</sup> | 5 |
|-------------|---|---|---|-------------|---|
| C<sub>12:0</sub> | 0.16 | 0 | 0 | 0 | 0 |
| iso-C<sub>13:0</sub> | 0.1 | 0.4 | 0 | 0 | 0 |
| C<sub>14:0</sub> | 0.6 | 0.9 | 0.4 | 0.4 | 0.7 |
| iso-C<sub>14:0</sub> | 4.7 | 1.55 | 2.6 | 1.7 | 5.6 |
| C<sub>15:0</sub> | 0 | 0 | 0 | 0.5 | 0 |
| anteiso-C<sub>15:0</sub> | 6.9 | 8.0 | 7.4 | 21.4 | 3.14 |
| iso-C<sub>15:0</sub> | 46.7 | 58.2 | 45.9 | 31.8 | 35.4 |
| iso-C<sub>12:1 ω9c</sub> | 0 | 0.5 | 0 | 0 | 0.2 |
| C<sub>16:0</sub> | 1.9 | 1.85 | 2.7 | 1.8 | 2.4 |
| C<sub>16:0 2OH</sub> | 0.1 | 0 | 0 | 0 | 0 |
| C<sub>16:0 3OH</sub> | 0.12 | 0 | 0 | 0 | 0 |
| iso-C<sub>16:0</sub> | 15.8 | 1.8 | 12.19 | 11.2 | 11.5 |
| iso-C<sub>16:1 H</sub> | 0 | 0 | 0 | 0 | 0.2 |
| C<sub>16:1 ω11c</sub> | 2.3 | 2.7 | 5.3 | 2.7 | 6.4 |
| C<sub>16:1 ω7c alcohol</sub> | 14.0 | 7.0 | 10.1 | 7.6 | 24.9 |
| anteiso-C<sub>17:0</sub> | 1.8 | 2.7 | 3.1 | 11.1 | 0.8 |
| iso-C<sub>17:0</sub> | 3.3 | 3.4 | 6.1 | 5.5 | 1.9 |
| anteiso-C<sub>17:1 ω9c</sub> | 0 | 0 | 0 | 0 | 0 |
| iso-C<sub>17:1 ω10c</sub> | 0.6 | 6.3 | 2.0 | 1.3 | 3.2 |
| C<sub>17:1 ω9c</sub> | 0 | 0 | 0 | 0 | 0.8 |
| C<sub>18:0</sub> | 0.19 | 0.8 | 0.4 | 0 | 0.6 |
| C<sub>18:1 ω7c</sub> | 0 | 0.4 | 0.4 | 0 | 0.5 |
| Summed feature 3<sup>a</sup> | 0.19 | 0.1 | 0 | 0 | 0.2 |
| Summed feature 4<sup>b</sup> | 0.5 | 3.1 | 1.6 | 2.8 | 1.5 |
| Summed feature 8<sup>c</sup> | 0 | 0.2 | 0 | 0 | 0 |

<sup>a</sup>Summed feature 3, C<sub>16:1 ω6c</sub> and or C<sub>16:1 ω7c</sub>
<sup>b</sup>Summed feature 4, anteiso-C<sub>17:1</sub> B and/or iso-C<sub>17:1</sub> I
<sup>c</sup>Summed feature 8, C<sub>18:1 ω6c</sub> and/or C<sub>18:1 ω7c</sub>
<sup>d</sup>The data were got from the paper by Ahmed et al. (2007)

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

| Species | Strain no. | Accession no. | 16S rRNA similarities (%) | ANI (%) | dDDH (%) |
|---------|------------|--------------|---------------------------|---------|----------|
| FJAT-51161T | FJAT-51161T | CP067341 | 99.0 | 88.7 | 38.0 |
| Lysinibacillus xylanilyticus | DSM 23493T | LFXI00000000 | 98.7 | 82.8 | 28.1 |
| Lysinibacillus pakistanensis | NCCP-54T | BBDJ00000000 | 98.6 | 79.9 | 25.5 |
| Lysinibacillus macroides | DSM 54T | LGCI00000000 | 98.1 | 80.5 | 25.1 |
| Lysinibacillus fusiformis | NBRC 15717T | CP010820 | 98.1 | 80.5 | 25.1 |
| Lysinibacillus boronitolerans | T-10aT | JPV00000000 | 98.1 | 80.0 | 24.7 |
| Lysinibacillus sphaericus | KCTC 3346T | AUOZ00000000 | 98.0 | 80.1 | 25.7 |
| Lysinibacillus contaminans | DSM 25560T | LGRV00000000 | 97.7 | 77.9 | 23.4 |
| Lysinibacillus parviboronicapiens | BAM-582T | PYWI00000000 | 97.0 | 80.5 | 25.8 |

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02394-4.

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

Declarations

Conflict of interest The authors declared that they had no conflict of interest.

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

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