Lactobacillus garii sp. nov., isolated from a fermented cassava product

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Abstract

A novel Gram-positive, catalase negative, rod-shaped strain, FI11369T, was isolated from gari, a traditional West African fermented food derived from cassava. Based on 16S rRNA gene sequence similarity, the closest type strains were Lactobacillus xiangfangensis LMG 26013T (99.4% similarity), Lactobacillus plajomi NBRC 107333T (99.1%), Lactobacillus paraplanatarum DSM 10667T (99.1%), Lactobacillus pentosus DSM 20314T (99.0%), Lactobacillus plantarum subsp. plantarum ATCC 14917T (99.0%), Lactobacillus modestisalitolerans NBRC 107235T (98.9%), Lactobacillus plantarum subsp. argentoratensis DSM 16365T (98.9%) and Lactobacillus daowaensis NCIMB 15183T (98.8%). The genome of strain FI11369T was sequenced and the average nucleotide identity (ANI) was compared with its closest relatives. ANI analysis showed that the closest relative, L. xiangfangensis DSM 27103T, had only a 82.4% similarity. The main fatty acids of FI11369T were saturated C16:0 (18.2%), unsaturated C18:1ω9c (43.8%) and cyclopropane C19:0 cyclo (ω10c and/or ω8c; 22.5%). Based on the genotypic and phenotypic data obtained in this study, a novel Lactobacillus species, Lactobacillus garii sp. nov., with the type strain FI11369T (=NCIMB 151148=DSM 108249), is proposed.

The genus Lactobacillus, which includes more than 200 species, is a taxonomically complex group due to the high level of phenotypic and genotypic diversity [1]. Lactobacilli are Gram-positive, mostly non-motile, catalase-negative, non-sporulate forming and rod-shaped bacteria. Their habitats are nutrient-rich environments such as food, soil, plants, animals and humans [2]. Lactobacilli dominate the microbiota of the vast majority of fermented foods and most studies have focused on their role in food fermentation and prevention of food spoilage [3], as well as their importance in the gut and their applications as probiotics [4].

Gari, a fermented food derived from cassava (Manihot esculenta), is widely consumed in West and Central Africa. The steps to obtain gari include washing and grating fresh cassava roots, followed by fermenting and dewatering at ambient temperature (ca. 30 °C) for up to 72 h. The fermented pressed cake is disintegrated and roasted into gari at ambient temperature (ca. 30 °C) for up to 72 h. The steps to obtain gari include washing and grating fresh cassava roots, followed by fermenting and dewatering at ambient temperature (ca. 30 °C) for up to 72 h. The fermented pressed cake is disintegrated and roasted into gari [5, 6]. In a previous study of the diversity of lactic acid bacteria in gari, Lactobacillus plantarum was the most frequently isolated species, followed by Leuconostoc fallax and Lactobacillus fermentum [6].

Strain FI11369T was isolated as part of a study to sequence the whole genomes of micro-organisms present in African fermented foods. In this study, a sample of gari, produced in the suburb of Pokuase, Accra (Ghana) was collected and preserved at 4 °C until further processing in the UK. The sample of gari was homogenized in PBS and 100 µl of a 10−5 dilution of the homogenate were plated on MRS (Oxoid) agar medium [7]. Plates were incubated for 48 h at 37 °C and 10 out of 194 colonies were picked based on different morphology and sub-cultured for three rounds until pure cultures were obtained. A pure culture of strain FI11369T, the only selected colony with irregular edges, was obtained after three rounds of sub-culturing. DNA from strain FI11369T was extracted using the cetyltrimethylammonium bromide-based extraction protocol [8] and the genome was sequenced. Libraries were obtained using the Nextera XT DNA library Prep kit (Illumina) according to manufacturer instructions and sequenced for 150 cycles using the Illumina NextSeq platform at the Quadram Institute Bioscience (QIB; Norwich, UK). The 887 369 reads generated were quality trimmed with BBBDuk (version 38.68) to
at least a quality of Q21. Cleaned reads were assembled with SPAdes (version 3.11.1) [9] and annotated using PATRIC [10]. The genome was assembled into 158 contigs, with an N50 of 57125 bp. The 16S rRNA gene sequence (accession no. MK011005) was extracted from the genome assembly (accession no. QWZQ00000000; size 2972171 bp). The sequence was compared with all the type strains present in the nucleotide collection database from the National Center for Biotechnology Information (NCBI). Sequence similarity with the closest type strains was calculated using the pairwise nucleotide sequence alignment for taxonomy tool of EzBioCloud [11]. The highest similarity was found with the type strains of *Lactobacillus xiangfangensis* (99.4% similarity), *Lactobacillus paraplantarum* (99.1%), *Lactobacillus pentosus* (99.0%), *Lactobacillus plantarum* subsp. *plantarum* (99.0%), *Lactobacillus modestisalitolerans* (98.9%), *Lactobacillus plantarum* subsp. *argentoratensis* (98.9%), *Lactobacillus daowaiensis* (98.8%), *Lactobacillus fabifermentans* (98.6%), *Lactobacillus nangangensis* (98.6%), *Lactobacillus daolensis* (98.5%), *Lactobacillus pingfangensis* (98.5%), *Lactobacillus herbarum* (98.5%), *Lactobacillus mudanjangensis* (98.0%), *Lactobacillus donghensis* (98.1%) and *Lactobacillus songbeiensis* (98.1%). All sequences were aligned using CLUSTAL_W version 2.1 [12]. A 16S rRNA gene phylogeny was reconstructed using the maximum-likelihood method with the Jukes–Cantor model [13] incorporated into Geneious version 11.1.3 (Biomatters) (Fig. 1). Branching support was estimated with 1000 bootstrap replicates. In addition, the 16S rRNA gene was amplified by PCR using primers AMP_F (5'–GAG AGT TTG ATY CTG GCT CAG-3') and AMP_R (5'–AAG GAG GTG ATC CAR CCG CA-3') and sequenced by a Sanger sequencing service (Eurofins Genomics Germany GmbH, Germany). The partial 16S rRNA gene sequence (accession no. MN817919) obtained by Sanger sequencing was identical to the 16S rRNA gene sequence extracted from the genome.

To reconstruct a well-supported phylogenomic tree, we extracted the following single-copy marker genes from 18 type strains using AMPHORA2 [14]: rfr, infC, nusA, pgk, pyrG, rplA, rplC, rplD, rplE, rplF, rplK, rplL, rplM, rplP, rpsL, rpsT, rpmA, rpoB, rpsB, rpsC, rpsE, rpsL, rpsJ, rpsK, rpsM, rpsS, smpB and tsf. Sequences were aligned using RAxML version 8.2.12 [15]. Single protein alignments were concatenated with the script from phylogenomics-tools (github.com/kbseah/phylogenomics-tools). Positions of the alignment that had more than 75% gaps were removed using Geneious version 11.1.3 (www.geneious.com). The resulting alignment was then used for maximum-likelihood phylogenomic reconstruction with FastTree version 2.1.11 [16] (Fig. 2).

The average nucleotide identity (ANI) of strain FI113697 was compared to the type strains that were closest based on our phylogenomic reconstruction, which included: *L. xiangfangensis*, *L. plajomi*, *L. paraplantarum*, *L. pentosus*, *L. plantarum* subsp. *plantarum*, *L. modestisalitolerans*, *L. plantarum* subsp. *argentoratensis*, *L. daowaiensis*, *L. argentoratensis*.
The DNA G+C content of FI11369\textsuperscript{T} is 48.3 mol%, similar to the 46.6 mol% G+C of FI11369\textsuperscript{T} represents a novel species. The DNA G+C content (70 % for dDDH [18–20]). Thus, we confirmed that strain梭菌\textsuperscript{T} is used as an outgroup. The related species. Tree reconstructed with an alignment spanning 6923 aa using the approximately-maximum-likelihood method implemented in FastTree 2.1.11.

**Fig. 2.** Phylogenomic tree based on the concatenated \( frr, intC, nuaA, pgk, pyrG, rplA, rplC, rplD, rplE, rplF, rplK, rplL, rplM, rplN, rplP, rpsL, rpsT, rpmA, rpmB, rpsB, rpsC, rpsE, rplI, rpsK, rpsM, rpsS, smpB \) and \( t\)s\( f\) gene sequences showing the relationship of strain FI11369\textsuperscript{T} and the related \textit{Lactobacillus} species. Tree reconstructed with an alignment spanning 6923 aa using the approximately-maximum-likelihood method implemented in FastTree 2.1.11. **Leuconostoc mesenteroides** subsp. **mesenteroides** ATCC 8293\textsuperscript{3} was used as an outgroup. The node labels represent SH-like branching support values. Bar, 0.1 substitutions per aa position.
on Columbia blood agar with 5% sheep blood. Pyruvate utilization was tested in broth medium as previously described [24]. Tellurite tolerance was tested on MRS agar supplemented with 0.02% of potassium tellurite. Carbon source utilization by strain FI11369 T and the closest relative L. xiangfangensis DSM 27103 T (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) was examined using the API 50 CH system (bioMérieux) following the manufacturer’s instructions. Strips were incubated at 30 °C and readings were made after 48 h (Table 2). Whole-cell fatty acids were analysed by gas chromatography of fatty acids.

Table 1. ANI values (%) and dDDH prediction values (%) between Lactobacillus garii sp. nov. and its closely related species

| Species            | Strain      | Accession No. | ANI   | dDDH |
|--------------------|-------------|---------------|-------|------|
| L. xiangfangensis  | LMG 26013   | JQCL00000000  | 82.4  | 23.8 |
| L. plajomi         | NBRC 107333 | BJDZ00000000  | 81.6  | 22.1 |
| L. plantarum subsp. argenteratensis | DSM 16365 | CP032751      | 81.1  | 21.2 |
| L. paraflavus      | DSM 10667   | CP032744      | 80.8  | 20.9 |
| L. modestialitolerans | NBRC 107235 | AYGX00000000  | 80.6  | 21.2 |
| L. pentosus        | DSM 20314   | AZCU01000001  | 80.6  | 25.1 |
| L. plantarum subsp. plantarum | ATCC 14917 | ACGZ00000000  | 80.4  | 20.6 |
| L. herbarum        | TCF032-E4   | LFE01000047   | 80.1  | 38.8 |
| L. fabifermans     | DSM 21115   | AYGX00000000  | 80.1  | 21.2 |
| L. daohiensis      | NCIMB 15181 | BJD00000000   | 80.0  | 20.4 |
| L. dongliensis     | NCIMB 15184 | BJD00000000   | 80.0  | 20.4 |
| L. nangangensis    | NCIMB 15186 | BJD00000000   | 79.9  | 20.0 |
| L. daowaiensis     | NCIMB 15183 | BJD00000000   | 79.9  | 20.4 |
| L. sengbeiensis     | NCIMB 15189 | BJD00000000   | 79.7  | 20.4 |
| L. mutilangiensis  | DSM 28402   | BJD00000000   | 79.7  | 19.6 |
| L. pingfangensis   | NCIMB 15187 | BJD00000000   | 79.7  | 20.2 |

Table 2. Distinctive features of the carbohydrate fermentation profiles of strain FI11369 T and closest phylogenetically related species

Strains: 1. FI11369 T (data from this study); 2. Lactobacillus xiangfangensis DSM 27103 T (data from this study); 3. Lactobacillus plajomi NBRC107333 T [22]; 4. Lactobacillus modestialitolerans NBRC 107235 T [22]. +, Positive; −, negative; w, weak reaction; d, delayed (>72h).

| Carbon source | 1 | 2 | 3 | 4 |
|---------------|---|---|---|---|
| d-Xylose      | w | w | − | − |
| d-Adonitol    |   | w | − | − |
| d-Galactose   | w | − | + | + |
| Amygdalin     | + | − | − | − |
| Arbutin       | + | − | − | − |
| Aesculin      | + | d | w | w |
| Salicin       | + | − | + | + |
| Lactose       | − | − | − | + |
| Melibiose     | − | − | − | + |
| Raffinose     | − | − | − | + |
| l-Arabitol    | − | − | + | − |
| Gluconate     | − | − | + | + |

Fig. 3. Scanning electron microscope image of strain FI11369 T cells after 48 h incubation at 30 °C in MRS broth. Bar, 1 µm.
acid methyl esters (GC-FAME) using the Sherlock microbiological identification method [25, 26] after growth of the strain in trypticase soy medium for 2 days at 28 °C. Peptidoglycan was isolated from cells grown in MRS broth for 10 h at 30 °C and its structure and cell-wall sugar composition was studied after total hydrolysis (100 °C, 4 N HCl, 16 h) and partial hydrolysis (4 N HCl, 45 min, 100 °C) as previously described [27]. Both analyses were performed by the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures identification service. The main fatty acids (>10%) of FI11369 T were saturated C_{16:0} (18.2%), unsaturated C_{18:1 \omega9c} (43.8%) and cyclopropane C_{19:0 \omega10c} (22.5%) (Table 3). The total hydrolysate of the peptidoglycan of strain FI11369 contained muramic acid (Mur) and the amino acids diaminopimelic acid (Dpm), alanine (Ala) and glutamic acid (Glu) in a molar ratio of 1.6:0.9:1.6:1. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of meso-Dpm. The partial hydrolysate contained peptides M-Glu, Ala-Glu, Glu-Dpm, Dpm-Ala, Glu-Dpm-Ala and Glu-Dpm-Ala-Dpm. These data strongly suggested that the peptidoglycan type of the strain was A1γ meso-Dpm direct.

### Table 3. Comparative fatty acid compositions of strains FI11369 T and the closely related Lactobacillus species

| Fatty acid | Strain | 1  | 2  | 3  | 4  |
|------------|--------|----|----|----|----|
| Saturated: |        |    |    |    |    |
| C_{14:0}   | 0.7    | 0.8| 6.6| ND |    |
| C_{16:0}   | 18.2   | 20.0| 11.4| 13.0|  |
| C_{18:0}   | 2.9    | 2.5| ND | ND |    |
| C_{19:0 iso}| 2.0    | ND | ND | ND |    |
| Unsaturated: |      |    |    |    |    |
| C_{16:1 \omega7c and/or \omega6c} | 1.1 | 0.9| 2.6| ND |    |
| C_{18:1 \omega9c} | 43.8 | 28.1| 36.1| 51.5| |
| C_{18:1 \omega6c and/or \omega7c} | 8.8 | 6.9| 9.7| ND |    |
| Cyclopropane: |      |    |    |    |    |
| C_{19:0 cyclo \omega10c and/or \omega6} | 22.5 | 40.8| 33.7| 35.6| |

### DESCRIPTION OF LACTOBACILLUS GARII SP. NOV.

*Lactobacillus garii* (ga’ri.i. N.L. gen. n. garii of gari).

Cells of strain FI11369 T are Gram-positive, non-motile, non-spore-forming, catalase-negative, straight rod-shaped, 1–2 μm long, and usually occur in pairs or in short chains. They are facultative anaerobes. Colonies grown aerobically on MRS agar at 30 °C for 48 h are irregular and umbonate. Strain FI11369 T grows at 12–40 °C (weakly at 6 and 42 °C, optimum at 30–37 °C), at pH range 4.0–8.8 (optimum at pH 6) and with 0–8 % NaCl (delayed growth at 10 %, optimum in the absence of NaCl supplementation). Gas is not produced from glucose. Only D-lactate is synthesized from glucose. Acid is produced from D-ribose, D-xylene, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, D-sorbitol, N-acetyl glucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, sucrose, trehalose and gentiobiose. Acid is not produced from glycerol, erythritol, D-arabinose, L-arabinose, L-xylose, D-adonitol, β-D-xylopyranoside, L-sorbose, dulcitol, inositol, α-D-mannopyranoside, α-D-glucopyranoside, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate and 2- or 5-keto-glucuronate. Strain FI11369 T is positive for α-haemolytic activity, bile–aesculin test and Voges–Proskauer test and negative for pyruvate utilization, tellurite tolerance, hippurate hydrolysis, pyrroldonylarylamidase production, deamination of arginine, H₂S production, nitrate and nitrite reduction, urease production and gelatin hydrolysis. Cellular fatty acids mainly comprised saturated C_{16:0}, unsaturated C_{18:1 \omega9c} and cyclopropane C_{19:0 cyclo (\omega10c and/or \omega6)}.

Cells contain meso-diaminopimelic acid in their cell-wall peptidoglycan. The peptidoglycan type is A1γ meso-Dpm.
direct. The genome size of the type strain is 2972171 bp and the DNA G+C content is 48.3 mol%.

The type strain, F111369T (＝NCIMB 15148＝DSM 108249), was isolated in the UK from gari produced in Ghana. The GenBank accession numbers of the 16S rRNA gene and the genome sequence of F111369T are MN817919 and QWZQ00000000, respectively.

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Conflicts of interest
The authors declare no conflicts of interest.

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