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Lysinibacillus irui sp. nov., isolated from Iru, fermented African locust beans

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Abstract

A Gram-positive, motile, aerobic, rod-shaped, endospore-forming strain designated IRB4-01^T was isolated from fermented African locust beans (Iru) obtained from Bodija market in the city of Ibadan, southwestern Nigeria, during a screening process from food-related sources. IRB4-01^T grew at 10–50°C (optimum, 35–37°C), pH 6–10 (optimum, pH 7) and in 0–6% NaCl (optimum, 1–3%). Phylogenetic analyses based on 16S rRNA and combined short- and long-read genome sequencing revealed that IRB4-01^T is closely related to *Lysinibacillus cavernae* SYSU K30005^T and *Lysinibacillus boronitolerans* 10a^T. The cell-wall peptidoglycan type was A4α (Lys–Asp), containing the diagnostic diamino acid lysine. The major polar lipids in strain IRB4-01^T were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unidentified phospholipid, while the predominant menaquinone was MK-7. The major cellular fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$. Genomic DNA G+C content was 37.4 mol%, while the digital DNA–DNA hybridization revealed 33.6 and 32.3% relatedness to *L. cavernae* SYSU K30005^T and *L. boronitolerans* 10a^T, respectively. Based on phenotypic, physiological and chemotaxonomic characteristics, as well as genome comparisons, strain IRB4-01^T represents a novel species of the genus *Lysinibacillus*, for which the name *Lysinibacillus irui* sp. nov. is proposed. The type strain is IRB4-01^T (NCIMB 15452^T=LMG 32887^T). Hybrid genome data are provided on the NCBI database using the Bioproject number PRJNA906010 and accession numbers CP113527 and CP113528. Additionally, a representative 16S rRNA sequence is available with the GenBank accession number OQ566940.

DATA SUMMARY

The authors confirm all supporting data, code and protocols have been provided within the article or through supplementary data files. The hybrid genome data are available on the NCBI database under the Bioproject number PRJNA906010 and accession numbers CP113527 and CP113528. A representative 16S rRNA sequence is provided using the GenBank accession number OQ566940.

INTRODUCTION

The genus *Lysinibacillus* was proposed as a new genus within the family *Bacillaceae* by Ahmed *et al.* in 2007 [1]. At the time of writing, 37 different species were included in the List of Prokaryotic names with Standing in Nomenclature (LPSN; https://lpsn.dsmz.de/genus/lysinibacillus) for this genus. The genus is distinguished by the presence of lysine and aspartate in the

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Keywords: biotechnology; food; genome analysis; *Lysinibacillus*.

Abbreviations: Asp, aspartic acid; A T G C h (v/v) (w/v), adenine thymine guanine cytosine hour volume per volume weight per volume; DNA, deoxyribonucleic acid; Gbp, gigabase pairs; G+C content, guanine+cytosine content; GC-MS, gas chromatography-mass spectrometry; Glu, glutamic acid; HPLC, high performance liquid chromatography; Kb, kilobase pairs; LB, Luria-Bertani; Lys, lysine; µm, micrometer; Mb, megabase pairs; mm, millimeter; N2, nitrogen; NCBI, National Center for Biotechnology Information; ncRNA, noncoding ribonucleic acid; rRNA, ribosomal ribonucleic acid percentage; tRNA, transfer ribonucleic acid.

Hybrid genome data are available on the NCBI database under the Bioproject number PRJNA906010 and accession numbers CP113527 and CP113528. A representative 16S rRNA sequence is provided using the accession number OQ566940.

Four supplementary figures and two supplementary tables are available with the online version of this article.

Impact Statement

The identification and description of *Lysinibacillus irui* sp. nov. in this work was based on a whole-genome approach for taxonomic characterization that offers notable phylogenetic precision and the ability to distinguish even the most closely related isolates, thus providing the opportunity to discover and characterize previously elusive isolates. The approach demonstrates reliable fine differentiation of species or genera that would otherwise be difficult to achieve. The novel species proposed in this work is expected to be of interest for future biotechnological applications. The hybrid genome data are available on the NCBI database using the Bioproject number PRJNA906010 and accession numbers CP113527 and CP113528.

peptidoglycan of the cell wall [1]. All previously described species of the genus are motile and mostly aerobic [2, 3]. These Gram-positive, catalase-positive, rod-shaped and spore-forming bacteria are commonly found in soil [1]. They have also been isolated from water [4] and the rhizosphere of plants [2]. In a recent study, several strains were also isolated from food and food production sites [5]. Ecological functions of strains of this genus include nitrogen fixation, phosphorus solubilization, adsorption of toxic metals [6], as well as antimicrobial activity against bacteria and fungi [7]. *Lysinibacillus* strains have potential use as biocontrol, bioremediation and biostimulant agents [7, 8]. Possible applications in surface treatment and crack healing in mortar [9], and in wastewater treatment have been described [10].

In this study, strain IRB4-01^T is phenotypically and genotypically characterized and identified as representing a novel *Lysinibacillus* species. The hybrid genome data are available on the NCBI database under the Bioproject number PRJNA906010 and accession numbers CP113527 and CP113528. A representative 16S rRNA gene sequence is provided using the GenBank accession number OQ566940. Comparative whole-genome sequencing analyses were conducted to investigate phylogenetic relationships with other species in the genus. In addition, comprehensive physiological, biochemical and chemotaxonomic characteristics were determined.

ISOLATION AND ECOLOGY

Strain IRB4-01^T was isolated from Iru, fermented African locust beans, in Nigeria. Isolation was part of a study collecting *Bacillus* and *Lysinibacillus* strains to screen wild-type strains for biosurfactants production for biotechnological purposes.

A few grams of Iru were obtained in a sterile container from Bodija market in Ibadan, Nigeria (7° 26′ 04.5″ N 3° 54′ 43.4″ E), and stored at 4°C. An amount of 1 g of the sample was diluted in autoclaved distilled water. A volume of 1 ml of dilutions of 10^{-12}, 10^{-10} and 10^{-8} was plated on nutrient agar (NA). The plates were incubated at 37°C for 48 h. Distinct colonies were selected based on colony morphology on plate and were subcultured three times on fresh plates to obtain clear single colonies from one bacterial species [5].

GENOME SEQUENCING AND HYBRID ASSEMBLY

A pure culture was forwarded to Eurofins Genomics Sequencing GmbH (Konstanz, Germany) for whole genome sequencing using an Illumina HiSeq 2500 (2×150 bp, paired-end) benchtop device.

The MagAttract Kit (Qiagen) was used for extracting high molecular weight DNA. The Qubit dsDNA HS Assay Kit (Invitrogen) was used for DNA quantification. Size selection was realized using SPRISelect beads (Beckman Coulter) and DNA was further subjected to long-read sequencing with barcode 91 of the rapid barcoding kit (SQK-RBK110.96) on an R9.4 (FLO-MIN106) MinION flow cell and an Mk1c device (Oxford Nanopore) for 21 h with live fast base-calling using guppy (version 4.2.3) and auto de-multiplexing. This resulted in 15472 passed reads and 0.31 Gbp data for isolate IRB4-01^T. Reads were quality controlled using pycoqc (version 2.5.0.23, https://github.com/a-slide/pycoQC) and kraken (version 1.0) using an 8 GB mini kraken database. Adaptors were trimmed with porechop (version 0.2.4, https://github.com/rrwick/Porechop) and the best 90% of the reads with a minimum length of 3 kb were selected using filtlong (version 0.2.0) resulting in a dataset of 282 Mb containing 11856 reads. Adaptor-clipped Illumina and filtered long-read data were hybrid assembled with Unicycler (version 0.4.8) and annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [11]. The hybrid genome data are provided in the NCBI database with the Bioproject number PRJNA906010 and accession numbers CP113527 and CP113528.

PHYLOGENY BASED ON 16S rRNA AND WHOLE GENOME SEQUENCING

To determine the phylogenetic relationship of strain IRB4-01^T with the closest type strains, a two-in-one complementary approach based on 16S rRNA and whole genome sequences (accession numbers CP113527 and CP113528) was used (Figs 1 and S1, available in the online version of this article). The 16S rRNA gene sequence was extracted from the genome of IRB4-01^T by the Type

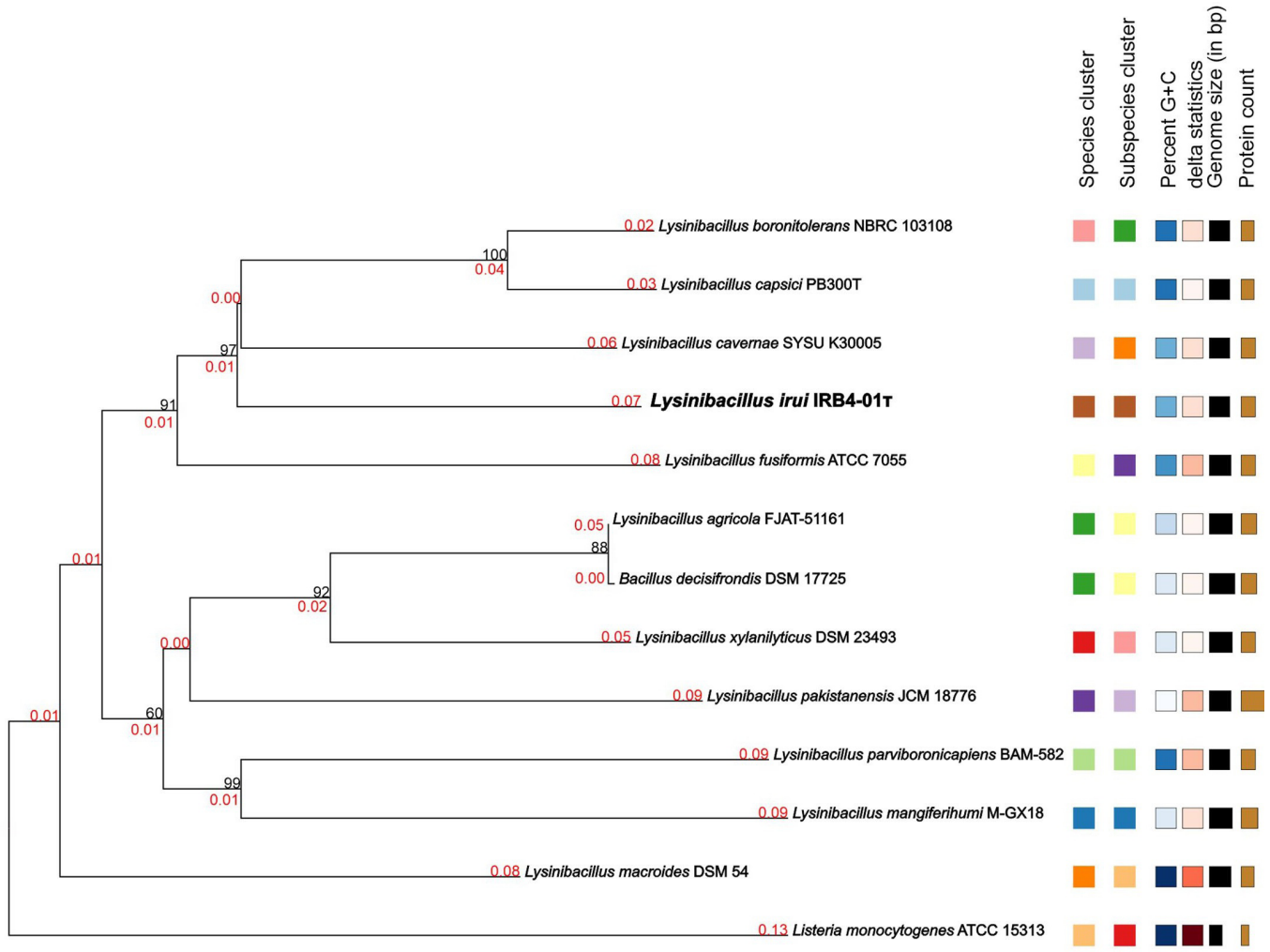

Fig. 1. Phylogenetic tree derived with FastME 2.1.6.1 [36] from GBDP (Genome BLAST Distance Phylogeny) distances calculated from genome sequences. The branch lengths are scaled in terms of the GBDP distance formula d5. Here, the red numbers are branch length values and the black numbers are branch support. The tree was rooted at the midpoint [33].

Strain Genome Server (TYGS) using RNAmmer [12] and the sequence was compared with the 16S rRNA gene sequences of all type strains available in the TYGS database using BLAST [13]. In addition, the genome of IRB4-01^T, based on a hybrid *de novo* assembly of short- and long-read sequencing, was subjected to TYGS for comparison with all type strain genomes available in the the TYGS database [14]. In this way, the best matching type strains (based on the bitscore) were identified for the strain IRB4-01^T genome and subsequently the precise distances were calculated using the Genome BLAST Distance Phylogeny approach under the algorithm 'coverage' and distance formula d5 [15]. TYGS identification revealed that strain IRB4-01^T represents a novel species as it does not belong to any species found in the TYGS database. TYGS provides a truly whole-genome-based method for phylogeny and classification by comparing the query genome with database of all type strain genomes, based on the technique of Genome-to-Genome Distance Calculator [15], which is followed by inference of phylogenetic trees, with high branch support [16, 17].

GENOME FEATURES AND PHYLOGENETIC ANALYSIS BASED ON HYBRID SEQUENCING

The genome assembly of strain IRB4-01^T resulted in the closed chromosome of 4.4 Mb and one plasmid of 250 Kb (Fig. 2). A prediction of (*n*=4732) total genes, (*n*=4524) coding genes, (*n*=37) rRNA genes, (*n*=5) ncRNA genes and (*n*=106) tRNA genes was determined using NCBI PGAP. All information can be found in the NCBI database using the Bioproject number PRJNA906010 and accession numbers CP113527 and CP113528. The genomic G+C content was 37.4mol%, which is within the value (35.9–43.2mol%) of all *Lysinibacillus* strains [3], and close to the G+C contents of *L. boronitolerans* 10a^T (37.6%) and *L. carvernae* SYSU K30005^T (37.1%). The average nucleotide identity (ANI) was calculated by JSpeciesWS (http://jspecies.ribohost.

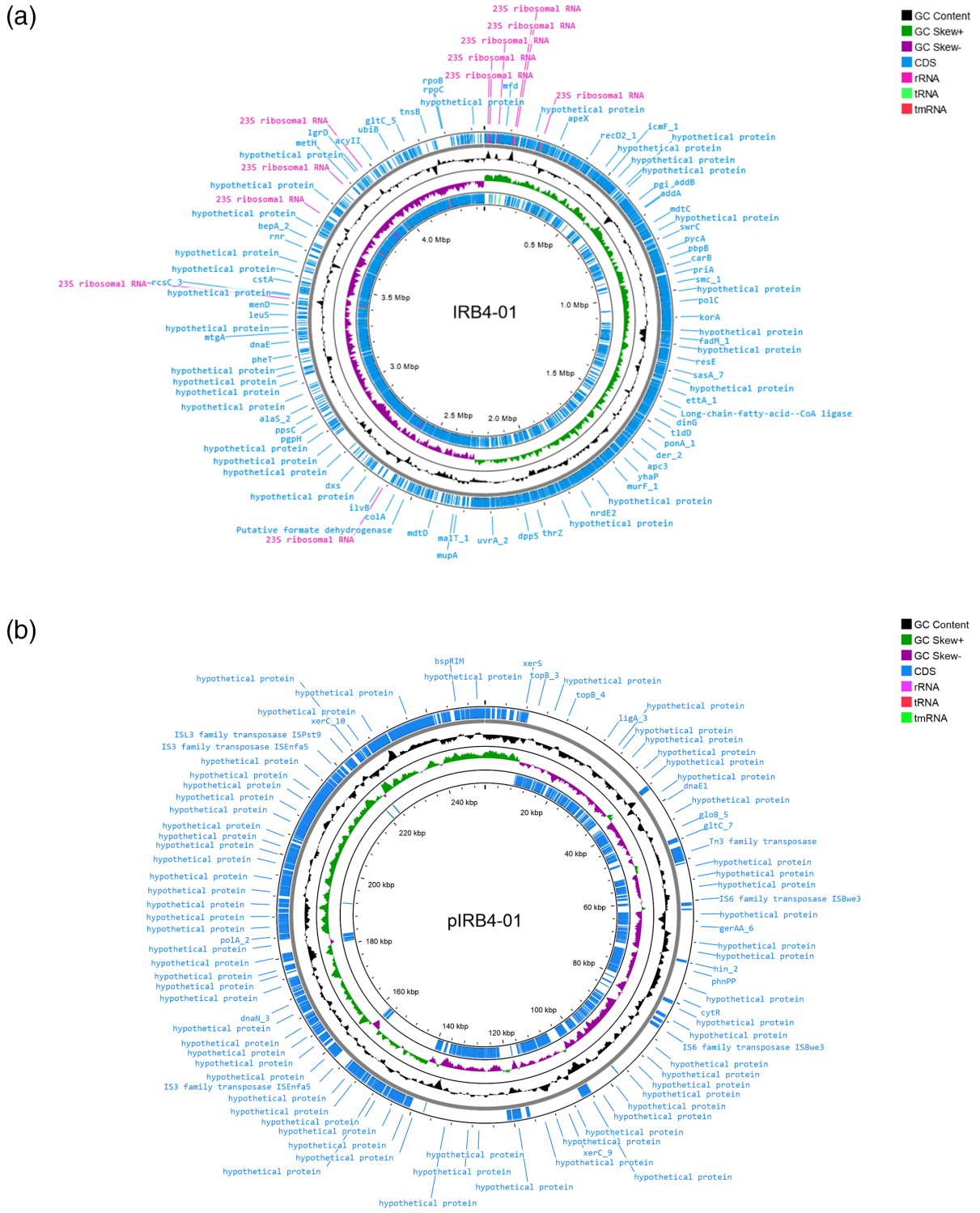

Fig. 2. Chromosome (a) and plasmid (b) of Lysinibacillus *irui* IRB4-01^{T}. CDS, coding sequences; tRNA, transfer RNA; rRNA, ribosomal RNA; tmRNA, transfer-messenger RNA; GC skew+/–, overabundance or lack of GC nucleotide. Image based on hybrid assembly, annotation with PROKKA, generated using Proksee [37].

com/jspeciesws/#analyse) [18] and the digital DNA–DNA hybridization (dDDH) was calculated by Genome-to-Genome Distance Calculator web server version 3.0 (https://ggdc.dsmz.de/ggdc.php) [15, 19]. The ANIb analyses showed high congruence to species available on the genome server, but below the suggested threshold for identical species (<0.998). The dDDH approach, known for more reliable results compared to ANIb, revealed a lower concordance value; the best match was 33.6% identity to *L. cavernae* SYSU K30005^T, 32.3% to *L. boronitolerans* 10a^T and 32.2% to *L. capsici* PB300^T, revealing clear genotypic disparities between strain IRB4-01^T and the most closely related species. The phylogenetic analysis confirmed that the strain represented a novel species within the genus *Lysinibacillus* (Fig. 1).

GENOME ANALYSIS

The hybrid assembly revealed that the 16S rRNA gene was present in 12 copies within the chromosome (Fig. 2). Six copies were present in the forward direction and six in the reverse direction. Seven copies showed 100 % identity, while the other showed some point mutations, aligning all 12 sequences [20]. Three sequences showed an identical single base, T-to-C, substitution at position 1300. One copy showed three substitutions: C-to-A at position 1296; G-to-A at position 1331; C-to-T at position 1350. The remaining copy showed four substitutions: C-to-A at position 1314; A-to-T at position 1317; A-to-G at position 1318; A-to-G at position 1336. Additionally, this copy showed an insertion (G) at position 1313. Overall, the alignment of all copies showed a pairwise identity of 99.8%.

Functional annotation performed with Rapid annotation using Subsystems Technology (RAST) via the KBASE platform [21], showed that the genome of IRB4-01^T has 264, 22 and 30 genes associated with carbohydrate, nitrogen and phosphorus metabolism, respectively. Also, 29 genes are involved in regulation and signalling, while 82 genes are associated with DNA metabolism. Further details on gene functions are available in the annotated gnome via NCBI accession number CP113527.

Genome mining for bioactive secondary metabolites using antiSMASH 6.0 [22] showed that IRB4-01^T possesses five biosynthetic gene clusters, including non-ribosomal peptide synthetase, polyketide synthase, terpene and ribosomally-produced and post-translationally modified peptides. A genome-scale metabolic model constructed based on the RAST-annotated genome using the KBase implemented Build Metabolic Model App based on the ModelSEED Pipeline for genomes [23] predicted a final model that includes 1182 reactions and 1204 associated compounds, involving 1016 genes.

PHYSIOLOGY AND CHEMOTAXONOMY

Colony morphology was examined from distinct colonies after 48 h of growth on LB agar. Gram-staining and investigation of spore formation were carried out using the method described by Smibert and Krieg [24]. Bubble production in 3% (v/v) hydrogen peroxide was used to determine catalase activity, while oxidase activity was determined using oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine [25]. Determination of the tolerable temperature range for growth was performed in LB medium at 4–50°C. Growth at different pH was determined with LB medium adjusted with 4M HCl or NaOH solutions and cell growth for 48 h at 37°C. Tolerance to NaCl was tested with LB medium containing 0–10% (w/v) NaCl. Cell motility was determined in LB medium containing 0.4% (w/v) agar and confirmed with microscopy. Anaerobic growth was investigated in LB medium supplemented with nitrate, in a serum flask sealed with crimp seal, and residual oxygen was removed by purging sterile N_2 gas through the medium via filters for 5 min [26]. Other physiological and biochemical characterizations were achieved using API 50 CH, API ZY, API 20NE (bioMerieux) and Biolog GEN III kits, according to the manufacturers instructions.

Cells of strain IRB4-01^T were 2.0–7.0 µm long and 1.0–1.6 µm wide, Gram-positive, rod-shaped (Fig. S2), motile, with subterminal oval endospores (Fig. S2). Cells grew at 10–50°C (optimum, 35–37°C). Growth was also observed at pH 6–10, with an optimum achieved at pH 7. IRB4-01^T grew in medium containing 0–6% NaCl (optimum, 1–3%). No growth occurred under anaerobic conditions. Strain IRB4-01^T was determined to be catalase-positive and oxidase-positive, like its close relatives *L. cavernae* SYSU K30005^T and *L. boronitolerans* 10a^T. Other differential characteristics between IRB4-01^T and its closest relatives in the genus *Lysinibacillus* are shown in Table 1.

Determination of enzymatic activity using the API ZYM kit showed strong activity for esterase, esterase lipase and chymotrypsin, while intermediate activity was observed for acid phosphatase and naphthol-AS-BI-phosphohydrolase. In contrast, no activity was detected for esterase lipase and acid phosphatase in *L. carvanae*, and weak activity was reported in *L. boronitolerans*. However, weak activity was observed in IRB4-01^T for alkaline phosphatase, leucine arylamidase and cystine arylamidase.

Results for the API 50 CH kit were obtained from active growth on the indicated substrates as a measure of turbidity (API 50 CH AUX), with mostly negative results, and positive results recorded only for *N*-acetylglucosamine and gluconate. Acid production from these substrates (API 50 CH B) was negative for all compounds after 5 days of incubation at 37°C. The results are shown in Table S1. Further characterization with the Biolog GENIII system showed positive results for fructose-6-PO_4, L-aspartic acid, L-pyroglutamic acid, bromo-succinic acid, α-hydroxy-butyric acid, β-hydroxy-D,L-butyric acid, α-keto-butyric acid, Tween

Table 1. Differential characteristics of strain IRB4-01^T and related species of the genus *Lysinibacillus*

Characteristics	**IRB4-01^T**	**SYSU K30005^T*L. cavernae***	**10a^T*L. boronitolerans***
Cell size (μm)	2.0–7.0	ND	3.0–5.0
Spore shape and position	O, S	S	R/O, T, B
Motility	+	+	+
Anaerobic growth	–	ND	ND
pH range (optimum) for growth	6–10 (7)	6–9 (7)	5.5–9.5 (7–8)
Temperature range (optimum) for growth (°C)	10–50 (35–37)	15–37 (28)	16–45 (35–37)
NaCl tolerance range (optimum) for growth (%, w/v)	6 (1–3)	0–7 (3.5)	5 (ND)
API ZYM assays:			
Esterase lipase	+	–	W
Leucine arylamidase	W	–	W
Acid phosphatase	+	–	W
Acid phosphatase	+	–	+
API 20NE assays:			
Gelatin hydrolysis	+	–	–
Malate	+	–	+
Glucose	–	+	ND
Biolog GENIII assay:			
β-Methyl-D-glucoside	–	+	–
N-Acetyl-D-glucosamine	+/-	+	–
N-Acetyl-β-D-mannosamine	–	+	–
N-Acetyl-D-galactosamine	–	+	–
myo-Inositol	–	+	–
Glycerol	–	+	–
Fructose-6-PO_4	+	–	+
Serine	+/-	–	–
Glycyl-L-proline	+	–	+
L-Alanine	+/-	+	–
L-Aspartic acid	+	–	+
L-Glutamic acid	–	+	+
L-Pyroglutamic acid	+	–	+
L-Serine	+/-	+	+
Citric acid	–	+	–
Bromo-succinic acid	+	–	+
α-Hydroxy-butyric acid	+	–	+
β-Hydroxy-D,L-butyric acid	+	–	+
α-Keto-butyric acid	+	–	+

Continued

Table 1. Continued

Characteristics	IRB4-01^T	SYSU K30005^T*L. cavernae*	10a^T*L. boronitolerans*
Propionic acid	–	–	+
L-Malic acid	–	+	+
Tween 40	+	–	+
α-Keto-butyric acid	+	–	+
Sodium lactate	+	–	+

O, oval; T, terminal; S, subterminal; R, round; B, bulging; ND, not determined; W, weak. Data for characteristics of *L. cavernae* SYSU K30005^T were obtained from Kan *et al*. [38] and data for *L. boronitolerans* 10a^T were obtained from Ahmed *et al*. [1] and Burkett-Cadena *et al*. [2].

40, acetic acid, formic acid, sodium lactate, guanidine HCl, nalidixic acid, lithium chloride, potassium tellurite, aztreonam and sodium butyrate (Tables 1 and S2). Strain IRB4-01^T showed several distinguishing phenotypic characteristics from its close relative *L. cavernae* SYSU K30005^T and less differentiating phenotypes from *L. boronitolerans* 10a^T under Biolog GENIII assay conditions. While IRB4-0^T lacked the ability to metabolize L-glutamic acid, propionic acid and L-malic acid, these carbon sources were utilized by *L. boronitolerans* 10a^T.

ANTIBIOTIC SUSCEPTIBILITY AND RESISTANCE PREDICTION

Antibiotic substances were used to screen for putative antibiotic susceptibilities according to the EUCAST guidelines (version 12.0) for *Bacillus* species. By applying the zone diameter breakpoints for the agar diffusion method, the following results were obtained: ciprofloxacin – susceptible; levofloxacin – susceptible; vancomycin – susceptible; imipenem – susceptible; meropenem – resistant; erythromycin – susceptible; clindamycin – resistant; linezolid – susceptible.

The hybrid assembled sequence data was further used to identify genes encoding for antibiotic resistance. For this approach, the ResFinder version 4.1 [27] and the Resistance Gene Identifier [28], strict hits only, databases were used. The resistance genes *clbA* (87.54% identity) and *msrG* (72.35% identity) were identified. These genes could cause decreased therapeutic effects of macrolide, lincosamide, streptogramin, phenicol and oxazolidinone antibiotics. With low identities (32–41%), three genes (*vanY*, *vanT* and *vanW*) of a glycopeptide resistance gene cluster were found. Further, the *qacJ* (41.12% identity) and *qacG* (47.47 % identity) genes were detected, which are described as unspecified antibiotic efflux pumps.

CELLULAR FATTY ACIDS, RESPIRATORY QUINONES, POLAR LIPIDS AND PEPTIDOGLYCAN STRUCTURE

Cellular fatty acids were converted to fatty acid methyl esters prior to analysis using the method of [29, 30] with slight modifications. Separation and detection of the fatty acid methyl ester mixture were performed using Sherlock Microbial Identification System (MIS; MIDI, Microbial ID). Fatty acid names and percentages were calculated by the MIS Standard Software (Microbial ID), and the identities of the fatty acids were confirmed by a GC-MS-based analysis. Respiratory quinones were extracted using hexane, and purified samples were analysed by HPLC [31, 32]. Polar lipids were extracted from freeze-dried cell material using the modified method of Bligh and Dyer [33]. Separation and detection of the polar lipids was achieved by two-dimensional silica gel thin-layer chromatography [34].

The major fatty acids of strain IRB4-01^T were iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$, and these correspond to the major fatty acids in closest relatives *L. cavernae* SYSU K30005^T and *L. boronitolerans* 10a^T, although with variations in the proportions of the fatty acids (Fig. S3). The proportion of iso-$C_{15:0}$ in IRB4-01^T (37.14%) was lower than in *L. cavernae* SYSU K30005^T (52.9%) but higher than that of *L. boronitolerans* 10a^T (31.8%); while the proportion of iso-$C_{16:0}$ was higher in IRB4-0^T (13.68%) than in both *L. cavernae* SYSU K30005^T (7.1%) and *L. boronitolerans* 10a^T (11.2%) (Table 2). The following menaquinones (MK) were detected as respiratory quinones, MK 6(7.1%), MK 7(92.5%) and MK 8(0.4%). The major polar lipids of strain IRB4-01^T included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and an unidentified phospholipid (PL) (Fig. S4). The polar lipids were similar to those of *L. cavernae* SYSU K30005^T and *L. boronitolerans* 10a^T, which also included DPG, PE, PG and an unidentified PL.

The peptidoglycan was isolated, and its structure was studied using published protocols [35]. The total hydrolysate (100°C, 4M HCl, 16 h) of the peptidoglycan contained muramic acid (Mur) and the amino acids lysine (Lys), aspartic acid (Asp), alanine (Ala) and glutamic acid (Glu). Quantification of amino acids by GC/MS of *N*-heptafluorobutyric amino acid isobutyl esters resulted in the following molar ratio: 1.8 Ala:0.5 Asp:0.5 Lys:1.0 Glu. The identity of all amino acids was confirmed by agreement in the

Table 2. Fatty acid profiles of strain IRB4-01^T and the type strains of related species of the genus *Lysinibacillus*

Fatty acids	IRB4-01^T	*L. cavernae* SYSU K30005^T	*L. boronitolerans* 10a^T
iso-$C_{15:0}$	37.14	52.9	31.8
anteiso-$C_{15:0}$	13.78	10.1	21.4
$C_{16:1}$ ω7*c* alcohol	9.73	3.5	–
iso-$C_{16:0}$	13.68	7.1	11.2
$C_{16:1}$ ω11*c*	1.93	–	2.7
iso-$C_{17:0}$	8.15	–	5.5
anteiso-$C_{17:0}$	7.89	–	11.1
Summed feature 4*	2.26	1.5	2.8

–, Not detected. Fatty acids comprising <0.5% of the total are not shown.
*Summed feature 4 comprises iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B. Summed features are fatty acids that cannot be resolved reliably from another fatty acid using chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total by GC-MS using the MIDI system.

gas chromatographic retention time with those of authentic standards and by characteristic mass spectrometric fragment ions of the derivatives. The analysis of enantiomers of the total hydrolysate showed the presence of D-Asp, L-Lys and D-Glu. From these data, the occurrence of the peptidoglycan type A4α L-Lys–D-Asp was concluded. Chemotaxonomic analyses including cell-wall peptidoglycan, polar lipids, respiratory quinones and cellular fatty acids showed the considerable similarity of strain IRB4-01^T to its closest relatives and sufficient differences to propose that it represents a novel species in the genus *Lysinibacillus*. Based on the above findings, strain IRB4-01^T represents a novel species of the genus *Lysinibacillus*, and the name *Lysinibacillus irui* sp. nov. is proposed.

DESCRIPTION OF *LYSINIBACILLUS IRUI* SP. NOV.

Lysinibacillus irui (i'ru.i. N.L. neut. n. *iruum*, iru, Nigerian fermented locust beans; N.L. neut. *irui*, of iru).

The cells are Gram-positive, motile, aerobic, rods, which are 2–7 µm long, forming subterminal oval endospores. Colonies on LB agar at 37°C after 48 h are 4.5–5.1 mm, smooth, circular, slightly raised, colourless with entire margins. Grows at 10–50°C (optimum, 35–37°C), pH 6–10 (optimum, pH 7) and in NaCl 0–6% (optimum, 1–3%). Strain IRB4-01^T is catalase-positive and oxidase-positive. Strong enzymatic activity is observed for esterase, esterase lipase and chymotrypsin. In addition, strain IRB4-01^T was positive for urease, gelatin hydrolysis, gluconate, malate and *N*-acetylglucosamine (API 20NE). The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0.}$ The G+C content of the genomic DNA of the type strain is 37.4mol%. In total, 4732 genes are identified, including 4524 coding genes, 37 rRNA genes, five ncRNA genes and 106 tRNA genes. Hybrid genome data are provided in the NCBI database with Bioproject number PRJNA906010 and accession numbers CP113527 and CP113528.

Strain IRB4-01^T (NCIMB 15452^T=LMG 32887^T) is the type strain, isolated from Iru, fermented African locust beans in Nigeria (culture collection numbers NCIMB 15452 and LMG 32887).

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Author contributions
S.O.A. conceptualized and carried out the investigation, formal analysis, data curation, and drafted the manuscript. B.N. substantially contributed to the conceptualization, data curation, analysis and the drafting of the manuscript. M.A.F. performed part of the investigation and data curation. M.H. contributed to the interpretation of data. L.L. significantly contributed to the conceptualization, data analysis, and editing of the manuscript. R.H. contributed to the conceptualization, interpretation of data, and editing of manuscript. All authors read and approved the final manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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