

***Listeria ilorinensis* sp. nov. isolated from cow milk cheese in Nigeria**

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Abstract

During microbial assessment of cow milk cheese products in the city of Ilorin, Nigeria, a *Listeria*-like isolate was detected which could not be assigned to any known species. Whole-genome sequence analyses against all currently known 26 *Listeria* species confirmed that this isolate constitutes a new taxon within the *Listeria* genus, with highest similarity to *Listeria costaricensis* (average nucleotide identity BLAST of 82.66%, *in silico* DNA-DNA hybridization of 28.3%). Phenotypically, it differs from *L. costaricensis* by the inability to ferment D-sucrose, L-fucose and starch. The absence of haemolysis and *Listeria* pathogenic islands suggest that this novel species is not pathogenic for humans and animals. The name *Listeria ilorinensis* sp. nov. is proposed, with the type strain CLIP 2019/01311^T (=CIP 111875^T = DSM 111566^T).

Main text

The genus *Listeria* is a member of the phylum Bacillota, consisting of Gram-positive, low G+C content, oxidase-negative and typically catalase-positive rod-shaped bacteria [1]. Currently, 26 species have been described, 18 of which reported only in the last decade, as a result of the increasing use of genomics in taxonomic classification. Two major groups can be distinguished [2, 3]: (i) *Listeria sensu stricto*, comprising 9 species (*L. monocytogenes* [4], *L. innocua* [5], *L. welshimeri* [6], *L. seeligeri* [6], *L. ivanovii* [7], *L. marthii* [8], *L. cossartiae* [9], *L. farberi* [9] and *L. immobilis* [9]) and (ii) *Listeria sensu lato*, containing 17 species (*L. grayi* [10], *L. rocourtiae* [11], *L. weihenstephanensis* [12], *L. aquatica* [13], *L. cornellensis* [13], *L. fleishchmannii* [13], *L. floridensis* [13], *L. grandensis* [13], *L. riparia* [13], *L. booriae* [14], *L. newyorkensis* [14], *L. costaricensis* [15], *L. goaensis* [16], *L. thailandensis* [17], *L. valentina* [18], *L. rustica* [9] and *L. portnoyi* [9]). All species are non-pathogenic except *L. monocytogenes* and *L. ivanovii* [19], which can cause disease in humans and other animals. *L. monocytogenes* represents an important concern for food safety and public health, due to its high case-fatality rate [2].

In October 2018, a *Listeria*-like bacterium was isolated from a cow milk cheese product (locally called “Wara”) obtained at the Emir’s market in Ilorin, Kwara state, Nigeria (Supplementary Figure 1), during a microbiological quality control of milk products for the presence of *Listeria* spp.. Briefly, cow milk cheese samples (5 grams) were aseptically collected using sterile sample bottles containing peptone water and transported to the laboratory on ice pack. Samples were homogenized by vortexing and incubated aerobically at 37 °C for 18 - 24 h. Two mL of the sample were then added to 18 mL of Fraser Broth Base (Himedia, Mumbai, India) for enrichment and incubated in aerobic conditions at 37 °C for 24 h. 10 µL of the enriched culture was then streaked on HiCrome Listeria Agar (Himedia, Mumbai, India) plates and incubated at 37 °C for 24 h. A blue-green (indicative of β -glucosidase activity) large size colony was isolated (CLIP 2019/01311, originally named

MP30) and sent to the World Health Organization Collaborating Centre *Listeria*, Institut Pasteur, Paris, France for identification and further characterization.

Species identification using the MALDI-TOF mass spectrometry MicroFlex LT system and the MBT library DB-8326 (Bruker Daltonics, Bremen, Germany) [20] was inconclusive (scores < 2.0, ranging between 1.54 and 1.84, only indicative of *Listeria* sp.), and the isolate CLIP 2019/01311^T subjected to whole-genome sequencing.

GENOME SEQUENCE AND ANALYSES

Genomic DNA was extracted using the NucleoSpin Tissue purification kit (Macherey-Nagel, Düren, Germany) from 0.9 mL Brain heart infusion (BHI, Difco, France) cultures grown overnight at 35°C. DNA libraries were prepared using the Nextera XT DNA Sample kit (Illumina, California, USA) and sequenced with Illumina NextSeq 500 using a 2 × 150 bp paired-end run. Raw reads (175X coverage) were trimmed with fqCleaner v.3.0 (Alexis Criscuolo, Institut Pasteur) as previously described [21] and assembled with SPAdes v.3.11 [22] with the automatic *k-mer*, *--only-assembler* and *--careful* options. Contigs longer than 300 bp were annotated with Prokka v.1.14 [23] and plasmids were screened with using MOB-suite v.2.0.1 [24].

The draft assembly (57 contigs, N50 of 154 kb) met the quality standards for taxonomic determination [25], with a total length of 3.03 Mb and an average G+C content of 41.3 mol%, similar to other members of *Listeria* genus (2.10-3.5 Mb, 35.9-43.6 mol% G+C). 2,900 coding DNA sequences, 54 tRNAs, 1 tmRNA, 3 rRNA genes and 2 CRISPR-Cas systems were annotated. No plasmids were detected.

Phylogenetic analyses were performed based on 16S rRNA gene sequences and on the concatenated deduced amino acid sequences of 99 core genes present in all *Listeria* species, defined using Roary v.3.12 [26] and a BLASTP identity cut-off of 80%. Sequences were aligned using MUSCLE v.3.8 [27]. Maximum likelihood phylogenetic trees were inferred by using IQ-Tree v.1.6 [28] and the best fit substitution models (TPM3u+F+I+G4 for 16S rRNA and LG+F+I+G4 for the concatenated core genome, as estimated using ModelFinder [29]) and visualized in MEGA v.7.0 [30].

Both 16S rRNA gene sequence (Figure 1) and core genome (Figure 2) analyses showed highest sequence similarity to the species *L. costaricensis*. The 16S rRNA gene sequence similarity, which lacks resolution for species delimitation, was of 99.8% with *L. costaricensis*, above the proposed cut-off (98.7-99.0%; [31]) below which strains do not belong to the same species.

The average nucleotide identity (ANI) shared with other *Listeria* species was determined using the enveomics package [32], using the BLASTN settings defined as in JSpecies v1.2.1 and Goris *et al.* [33]. Amino acid identities (AAI) were determined using the enveomics package [32] using default parameters. Dendrograms based on the unweighted pair group method with arithmetic mean (UPGMA) method were obtained from the distance matrixes using Bionumerics v.7.6 (Applied Maths, Belgium).

Isolate CLIP 2019/01311^T shared less than 95% ANI, the proposed genomic cut-off for species identification [33], with all known species (Figure 3, Supplementary Table 1). The closest species was *L. costaricensis* (two-way ANI of 82.66% $\pm$ 4.91%, based on 13,448 genome fragments). Analyses based on the deduced proteomes also showed highest identities

with *L. costaricensis* (two-way AAI of $95.47 \pm 9.32\%$, based on 2241 orthologous proteins), higher than proposed cut-off for genus delineation of 60%.

The average *in silico* DNA–DNA hybridization similarities (isDDH) were calculated using the GGDC 2.1 web server using formula 2 (sum of all identities found in high-scoring segment pairs [HSP] divided by overall HSP length) [35].

The isDDH between *L. ilorinensis* and *L. costaricensis* was of 28.3% [25.9-30.8%], below the species delineation cut-off of 70% [35].

These results confirmed that the isolate CLIP 2019/01311^T constitute a novel species within *Listeria* genus, for which the name *Listeria ilorinensis* is proposed.

Genomic comparisons performed using Roary, as described above, showed that CLIP 2019/01311^T harbors 641 proteins absent in the other 35 *Listeria* type and reference strains, including proteins involved in cell wall/membrane/envelop biogenesis ($n=21$), replication and repair ($n=17$), transcription ($n=12$), carbohydrate metabolism and transport ($n=12$), amino acid metabolism and transport ($n=9$), energy production and conversion ($n=9$). and 441 hypothetical proteins.

PHENOTYPIC CHARACTERIZATION

CLIP 2019/01311^T was then characterized at the phenotypic level as described below. Results are summarized in Table 1. Unless stated otherwise, all inocula were grown on Brain Heart Infusion broth (BHI; Becton Dickinson, USA) at 37 °C for 24 h.

The isolate was grown on Rapid'*L.mono* agar (Bio-Rad, France) and Agar *Listeria* according to Ottaviani and Agosti (ALOA; bioMérieux, France) plates at 30 °C and 37 °C for 24 h. On Rapid'*L.mono*, colonies were white [no phosphatidylinositol-specific phospholipase C (PI-

PLC) activity] surrounded by a yellow halo (D-xylose fermentation). On ALOA agar, colonies were blue (β -glucosidase activity) without halo (no PI-PLC activity).

Gram staining was performed with the Color Gram 2 kit (bioMérieux), according to the manufacturer's instructions. Catalase and oxidase activities were determined using the API ID Color Catalase kit (bioMérieux) and the Bactident Oxidase test strips (Merck Millipore, France), respectively, according to the manufacturer's instructions. Respiratory characteristics were determined on tubes containing meat liver agar (Bio-Rad) at 30 °C after 24 h, following the manufacturer's instructions. The presence of capsule was determined using the India ink staining. Growth characteristics were determined on BHI agar and broth at 22, 37 and 42 °C for 7 days and at 4 °C for 10 days. Growth was considered positive if there was an increase in cell number of at least 1.0 log (cfu.mL⁻¹).

L. ilorinensis CLIP 2019/01311^T colonies were Gram-positive bacilli without endospores or capsule, facultative anaerobic and oxidase-negative. CLIP 2019/01311^T was able to grow at all tested temperatures, except at 4 °C. Contrarily to all *Listeria* species except *L. costaricensis*, *L. ilorinensis* CLIP 2019/01311^T was catalase-negative, consistent with the absence of catalase genes in its genome.

Motility was tested by stab-inoculation in U-shaped glass tubes containing tryptic soy semi-solid agar (Bio-Rad), followed by incubation at 22 and 37 °C for 7 days in aerobic conditions. *L. monocytogenes* ATCC 19115 and *L. costaricensis* CLIP 2016/0682^T were used as positive controls and *L. booriae* CIP 111022^T, *L. thailandensis* CLIP 2015/00305^T and *L. valentina* CLIP 2019/00642^T as negative controls.

Similar to *L. costaricensis*, *L. ilorinensis* CLIP 2019/01311^T was motile at 37 °C but not at 22°C.

Classical serotyping was performed against somatic (O; I to XV) and flagellar (H; A to E) antigens as described by commercial anti-factor sera (I to IX and A to D antigens; Denka Seiken, Tokyo, Japan) and reference anti-factor sera from the World Health Organization Collaborating Centre *Listeria* (X to XV and E antigens; Institut Pasteur, Paris).

L. ilorinensis CLIP 2019/01311^T did not agglutinate with any anti-factor sera. PCR-serogrouping determined by multiplex PCR and *in silico* PCR from the draft genome as described previously [37, 38], was positive for the *prs* gene (PCR serogroup L, typical of non-*L. monocytogenes* *Listeria* species and rare *L. monocytogenes* serotypes).

Haemolysis capacity was assessed by stabbing isolates into Columbia agar plates (bioMérieux) containing 5% defibrinated blood horse, as described in the *Bacteriological Analytical Manual* (BAM)[39], followed by incubation for 24 h at 37 °C. The Christie, Atkins, Munch-Petersen (CAMP) test was performed as previously described, by streaking isolates and controls horizontally on Columbia agar containing 5% defibrinated sheep blood (bioMérieux), together with *Rhodococcus equi* NCTC 1621, which was streaked vertically. *L. ivanovii* ATCC 19119 and *L. seeligeri* ATCC 35967 were used as positive and negative controls, respectively. Plates were incubated at 37 °C for 24 h and examined for haemolysis in the zone of influence of the vertical streaks.

L. ilorinensis CLIP 2019/01311^T haemolysis and CAMP tests were negative, consistent with the absence of any *Listeria* pathogenicity islands within its draft genome, as observed in other *Listeria sensu lato* species.

Nitrate reduction was determined by inoculation in nitrate broth (bioMérieux) and incubation at 37 °C for 5 days, using *L. booriae* CIP 111022^T and *L. monocytogenes* ATCC 19115 as a positive and negative controls, respectively.

Similar to all *Listeria sensu stricto* and some *Listeria sensu lato* (*L. grayi*, *L. floridensis*, *L. goaensis* and *L. costaricensis*), *L. ilorinensis* CLIP 2019/01311^T was not able to reduce nitrate (red-violet colour developed only after zinc powder addition, indicative of unreduced nitrate).

Carbohydrate fermentation profiles were determined with API *Listeria* and the API50CH system (bioMérieux), following manufacturer's recommendations, using *L. monocytogenes* ATCC 19115, *L. booriae* CIP 111022^T, *L. costaricensis* CLIP 2016/0682^T, *L. thailandensis* CLIP 2015/00305^T and *L. valentina* CLIP 2019/00642^T as controls. After incubation at 37 °C for 24 h, fermentation was observed for the following substrates: amygdalin, D-arabitol, arbutin, aesculin, N-acetylglucosamine, D-cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, D-lactose, D-maltose, D-mannose, methyl- α -D-glucopyranoside, D-ribose, salicin, D-trehalose, xylitol and D-xylose (Table 1).

The production of acetoin from glucose fermentation (Voges-Proskauer test) was performed using API 20E strips (bioMérieux), recorded after incubation at 37 °C for 24 h. *L. monocytogenes* ATCC 19115, *L. booriae* CIP 111022^T, *L. costaricensis* CLIP 2016/0682^T, *L. thailandensis* CLIP 2015/00305^T and *L. valentina* CLIP 2019/00642^T were used as controls. Similarly to all *Listeria sensu stricto* species and a few *Listeria sensu lato* (*L. costaricensis*, *L. valentina*, *L. thailandensis* and *L. aquatica*), the Voges-Proskauer test was positive for *L. ilorinensis* CLIP 2019/01311^T (production of pink-to-ruby red colour).

Unweighted pair group method with arithmetic mean (UPGMA) analysis of all species phenotypic traits based on the Dice similarity coefficient showed that, phenotypically, *L. ilorinensis* is also the closest to *L. costaricensis*, differing from the later only by its inability to ferment D-sucrose, L-fucose and starch.

Finally, susceptibility to antimicrobials was determined with the disk diffusion method on Mueller–Hinton agar plates (Bio-Rad), following the recommendations from the European Committee on Antibiotic Susceptibility Testing (EUCAST, <https://www.eucast.org/>). The following antibiotic discs (Bio-Rad) were used: ampicillin (10 µg), amoxicillin (25 µg), penicillin G (6 µg), cefotaxime (30 µg), imipenem (10 µg), gentamicin (15 µg), kanamycin (30 µg), streptomycin (10 µg), erythromycin (15 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), moxifloxacin (5 µg), tetracycline (30 µg), rifampicin (30 µg), clindamycin (2 µg), chloramphenicol (30 µg), sulphonamides (200 µg), trimethoprim (5 µg), fosfomycin (50 µg), vancomycin (30 µg) and fusidic acid (10 µg). The diameters of inhibition zones were measured with automatic reader Scan 4000 (Interscience, France). *L. ilorinensis* isolates were sensitive to all tested antibiotics, except to cefotaxime and nalidixic acid (no inhibition halo). Genes conferring resistance towards antibiotics or tolerance towards antiseptics were not detected.

DESCRIPTION OF *LISTERIA ILORINENSIS* SP. NOV.

Listeria ilorinensis (i.lo.ri.nen'sis. N.L. fem. adj. *ilorinensis*, “from Ilorin”, the region where the type strain was isolated).

Cells are straight, Gram-stain-positive, non-spore-forming and non-capsulate rods. Facultative anaerobic, catalase-negative and oxidase-negative. Colonies are opaque, yellow-pigmented, with a flat shape and entire margin on BHI. On ALOA, colonies are blue centred without white halo, typical of *Listeria* species. Growth occurs at 22-42 °C, with optimal growth at 37

°C. Motile at 37 °C, but not at 4 °C nor 22 °C. Negative for haemolysis, nitrate and nitrite reduction. Voges-Proskauer test positive (production of acetoin). Absence of arylamidase, α -mannosidase and PI-PLC activities. After 24h, acid is produced from N-acetylglucosamine, aesculin, amygdalin, D-arabitol, arbutin, D-cellobiose, D-galactose, D- gentiobiose, glucose, D-fructose, D-mannose, D-maltose, methyl- α -D-glucopyranoside, D-lactose, D-ribose, salicin, D-trehalose, xylitol and D-xylose. Acid is not produced from starch, D-arabinose, L-arabinose, L-arabitol, D-adonitol, dulcitol, erythritol, D-fucose, L-fucose, glycerol, glycogen, glucose-1-phosphate, inositol, inulin, D-lyxose, D-mannitol, D-melezitose, D-melibiose, methyl- α -D-mannopyranoside, methyl- β -D-xylopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, D-raffinose, L-rhamnose, D-sucrose, D-sorbitol, L-sorbose, D-tagatose, D-turanose, and L-xylose. It can be differentiated from other *Listeria* species by the simultaneous absence of catalase activity and the inability to ferment D-sucrose.

The type strain CLIP 2019/01311^T was isolated in October 2018 from a cow milk cheese sample collected in a local market in Ilorin, Nigeria. The genomic DNA G+C content of the type strain is 41.3 mol%. The type strain is deposited at the Leibniz Institute DSMZ – German Collection of Microorganisms (DSM 111566^T) and the Collection of Institut Pasteur (CIP 111875^T). The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and draft genome of strain CLIP 2019/01311^T are MZ723504 and CAJYCN01, respectively. All sequence data is available under BioProject PRJEB45215.

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Conflicts of interest

The authors declare no conflict of interests.

Abbreviations

AAI	Average amino acid identity
ANI	Average nucleotide identity
BAM	Bacteriological Analytical Manual
BHI	Brain heart infusion
BLAST	Basic local alignment search tool
CAMP	Christie, Atkins, Munch-Petersen test
isDDH	<i>In silico</i> DNA–DNA hybridization
N50	Minimum contig length covering 50% of the genome
PCR	Polymerase chain reaction
PI-PLC	phosphatidylinositol-specific phospholipase C
UPGMA	Unweighted pair group method with arithmetic mean

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Legends to figures

Figure 1. Maximum likelihood phylogeny based on the 16S rRNA gene. Distance estimation was obtained by the model of TPM3u+F+I+G4 [29], based on an alignment of 1,116 positions. Selected members of *Brochothrix* genus were used as outgroup. Branch lengths represent the number of nucleotide substitutions per site and bootstrap percentages of 1,000 replicates are shown. GenBank accession numbers are provided in brackets. *Listeria ilorinensis* sp. nov. is highlighted in bold.

Figure 2. Maximum likelihood phylogenetic analysis based on the concatenated amino acid sequences of 99 core genes present in all 35 *Listeria* type and reference strains. Distance estimation was obtained by the LG+F+I+G4 model [29], based on an alignment of 28,722 positions. Branch lengths represent the number of amino acid substitutions per site and bootstrap percentages of 1,000 replicates are shown. GenBank accession numbers are provided in brackets. *Listeria ilorinensis* sp. nov. is highlighted in bold.

Figure 3. UPGMA clustering based on the genomic average nucleotide difference (ANI). The vertical dashed bar represents the proposed 95% ANIb species cut-off that correlates with the 70% DNA-DNA hybridization threshold [33]. Scale bar represents the percentage of similarity. *Listeria ilorinensis* sp. nov. is highlighted in bold.

1 **Table 1. Biochemical characteristics of species of the genus *Listeria*.** Data is based on previous studies [8, 9, 13, 14, 16, 40, 41] and this study.

2 *Listeria ilorinensis* sp. nov. is highlighted in grey. Species are sorted by their phylogenetic position shown in Figure 2.

Characteristics	<i>Lmo</i>	<i>Lcs</i>	<i>Lma</i>	<i>Lin</i>	<i>Lfr</i>	<i>Lws</i>	<i>Liv</i>	<i>Lim</i>	<i>Lse</i>	<i>Lgy</i>	<i>Lth</i>	<i>Lva</i>	<i>Laq</i>	<i>Lfo</i>	<i>Lfc</i>	<i>Lgo</i>	<i>Lil</i>	<i>Lco</i>	<i>Lru</i>	<i>Lpo</i>	<i>Lcn</i>	<i>Lny</i>	<i>Lro</i>	<i>Lwp</i>	<i>Lgd</i>	<i>Lbo</i>	<i>Lri</i>	
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	
Motility	+	+	+	+	+	+	+	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	
Voges–Proskauer	+	+	+	+	+	+	+	+	+	+	+	+	v	–	–	–	+	+	–	–	–	–	–	–	–	–	–	
Nitrate reduction	–	–	–	–	–	–	–	–	–	v	+	+	+	–	+	–	–	–	+	+	+	+	+	+	+	+	+	
Haemolysis	+	–	–	–	–	–	+	–	+	–	–	–	–	–	–	+	(α)	–	–	–	–	–	–	–	–	–	–	
Phosphatidylinositol-specific phospholipase C (PI-PLC)	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
D-Arylamidase	–	–	–	+	+	v	v	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
α-Mannosidase	+	+	+	+	+	+	–	–	–	v	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	+	+	
Acidification from:																												
D-Arabitol	+	+	+	+	+	+	+	+	+	+	–*	–*	–	–	+	+	+	+	(+)	(+)	–	–	–	+	v	+	–	
L-Arabinose	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	+	–	v	+	–	–	–	+	+	
L-Fucose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	
D-Galactose	v	–	–	–	–	–	v	–	–	+	–	–	–	+	–	–	+	+	+	+	–	+	+	–	–	+	+	
D-Glucose	v!	+	v!	v!	+	+	v!	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Glucose 1-phosphate	–	–	–	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Glycerol	v	+	–	+	–	+	+	v	+	v	(+)	(+)	v	–	+	(+)	–	–	–	–	v	+	+	+	–	(+)	v	
Inositol	–	–	–	–	–	–	–	–	–	–	(+)	(+)	v	–	v	–	–	–	–	–	–	–	–	–	–	v!	v	
Inulin	v!	–	–	v!	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
D-Lactose	+	+	+	+	+	+	+	v	+	+	–	–	–	+	+	+	+	+	+	(+)	(+)	+	+	v!	–	+	+	
D-Lyxose	v	–	–	v	–	v	–	–	–	v	–	(+)	v	+	–	–	–	–	–	–	–	–	–	–	–	–	–	
D-Maltose	+	+	+	+	+	+	+	v	+	+	–	–	–	+	+	+	+	+	+	–	+	+	+	+	+	+	+	
D-Mannitol	–	–	–	–	–	–	–	–	–	+	–	–	–	–	v	–	–	–	+	+	–	+	+	+	–	+	v	
D-Melezitose	v	–	–	v	–	v	v	v	v	–	–	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–	–	
D-Melibiose	v!	–	v	v	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	–	–	+	–	–	–	v!	v	
Methyl α-D-glucopyranoside	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+	
Methyl α-D-mannopyranoside	v!	+	–	–	+	nd	–	–	nd	+	–	–	–	–	v	(+)	–	–	–	–	–	–	–	–	–	–	–	
L-Rhamnose	+	–	–	v	+	v	–	–	–	–	+	+	+	+	+	+	–	–	+	+	–	v	+	+	–	+	+	
D-Ribose	–	v	–	–	–	–	+	+	–	+	+	+	+	–	+	–	+	+	–	–	+	+	+	–	+	v	v	
D-Saccharose (sucrose)	v!	–	–	+	–	+	+	v	+	–	–	–	–	–	v	–	–	+	–	–	–	–	–	–	–	–	–	
L-Sorbose	v!	–	v!	v!	–	–	v!	–	–	v!	(+)	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–	–	
D-Tagatose	–	–	–	–	–	+	–	–	–	–	+	v!	+	–	–	–	–	–	–	–	–	–	–	–	–	v!	–	
D-Turanose	–	–	+	v	–	–	–	–	–	–	–	–	–	–	v	–	–	–	–	–	–	–	–	–	–	–	–	
D-Xylose	–	–	–	–	–	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

3

4 All species/strains are positive for aesculin and acid production from N-acetylglucosamine, amygdalin, arbutin, D-cellobiose, D-fructose, D-mannose and
5 salicin. All species/strains are negative for nitrite reduction and acid production from D-adonitol, D-arabinose, glycogen, methyl β -D-xylopyranoside,
6 potassium 2-ketogluconate, and D-raffinose. Some data on this table differ from previously published data. This table should be taken as the new reference for
7 these phenotypic criteria.

8 Species: *Lmo*, *L. monocytogenes*; *Lcs*, *L. cossartiae*; *Lma*, *L. marthii*; *Lin*, *L. innocua*; *Lfr*, *L. farberii*; *Lws*, *L. welshimeri*; *Liv*, *L. ivanovii*; *Lim*, *L. immobilis*;
9 *Lse*, *L. seeligeri*; *Lgy*, *L. grayi*; *Lth*, *L. thailandensis*; *Lva*, *L. valentina*; *Laq*, *L. aquatica*; *Lfo*, *L. floridensis*; *Lfc*, *L. fleischmannii*; *Lgo*, *L. goaensis*; *Lil*, *L.*
10 *ilorinensis* sp. nov.; *Lco*, *L. costaricensis*; *Lru*, *L. rustica*; *Lpo*, *L. portnoyi*; *Lcn*, *L. cornellensis*; *Lny*, *L. newyorkensis*; *Lro*, *L. rocourtiae*; *Lwp*, *L.*
11 *weihenstephanensis*; *Lgd*, *L. grandensis*; *Lbo*, *L. booriae*; *Lri*, *L. riparia*.

12 Notation: +, positive; (+), weakly positive; –, negative; v, variable between strains and/or replicates; v!, variable between studies (possibly due to differences
13 in incubation times and temperatures); *, based on API 50 CH (positive on API Listeria); nd, not determined.

FIGURE 1

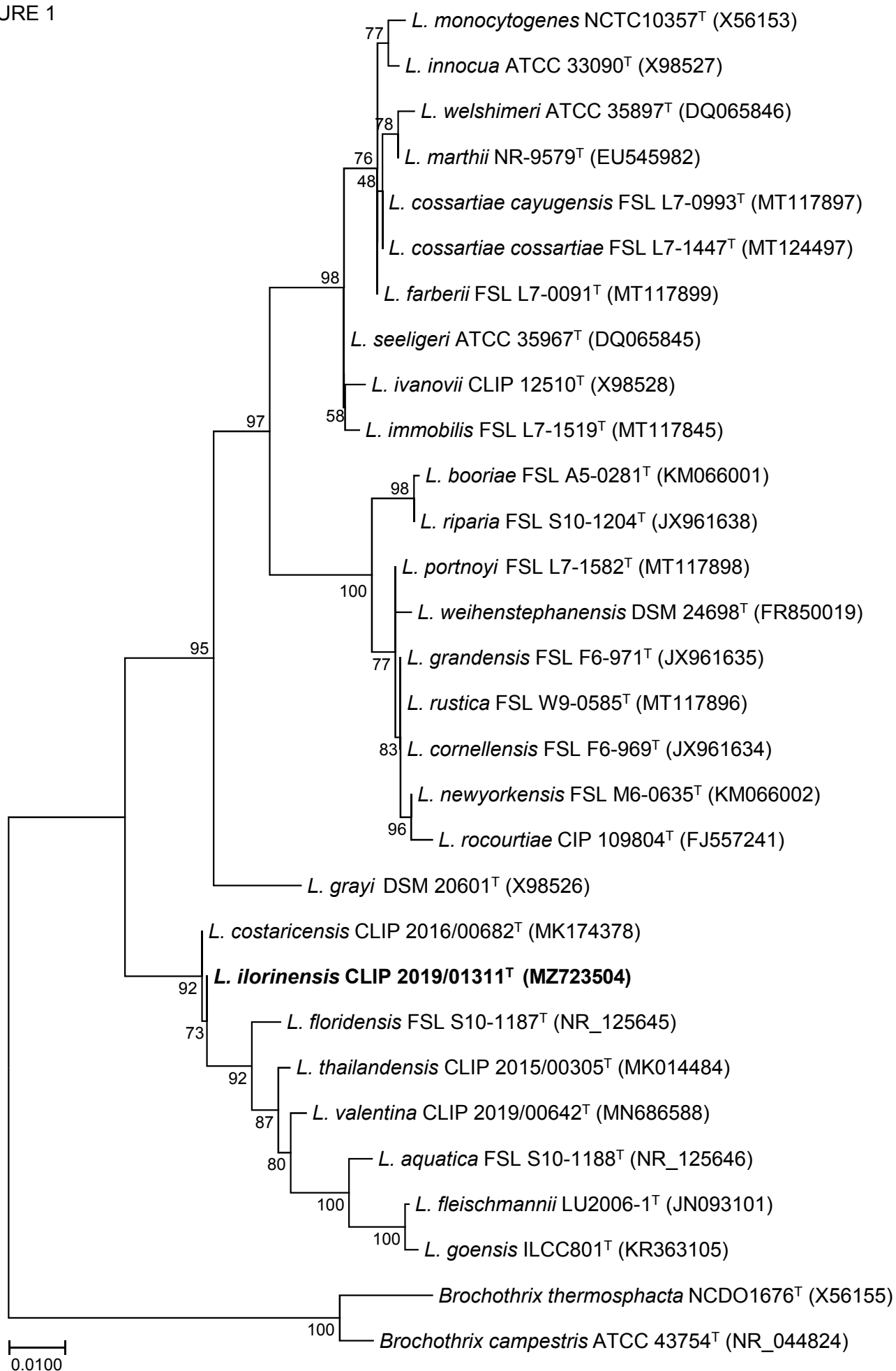


FIGURE 2

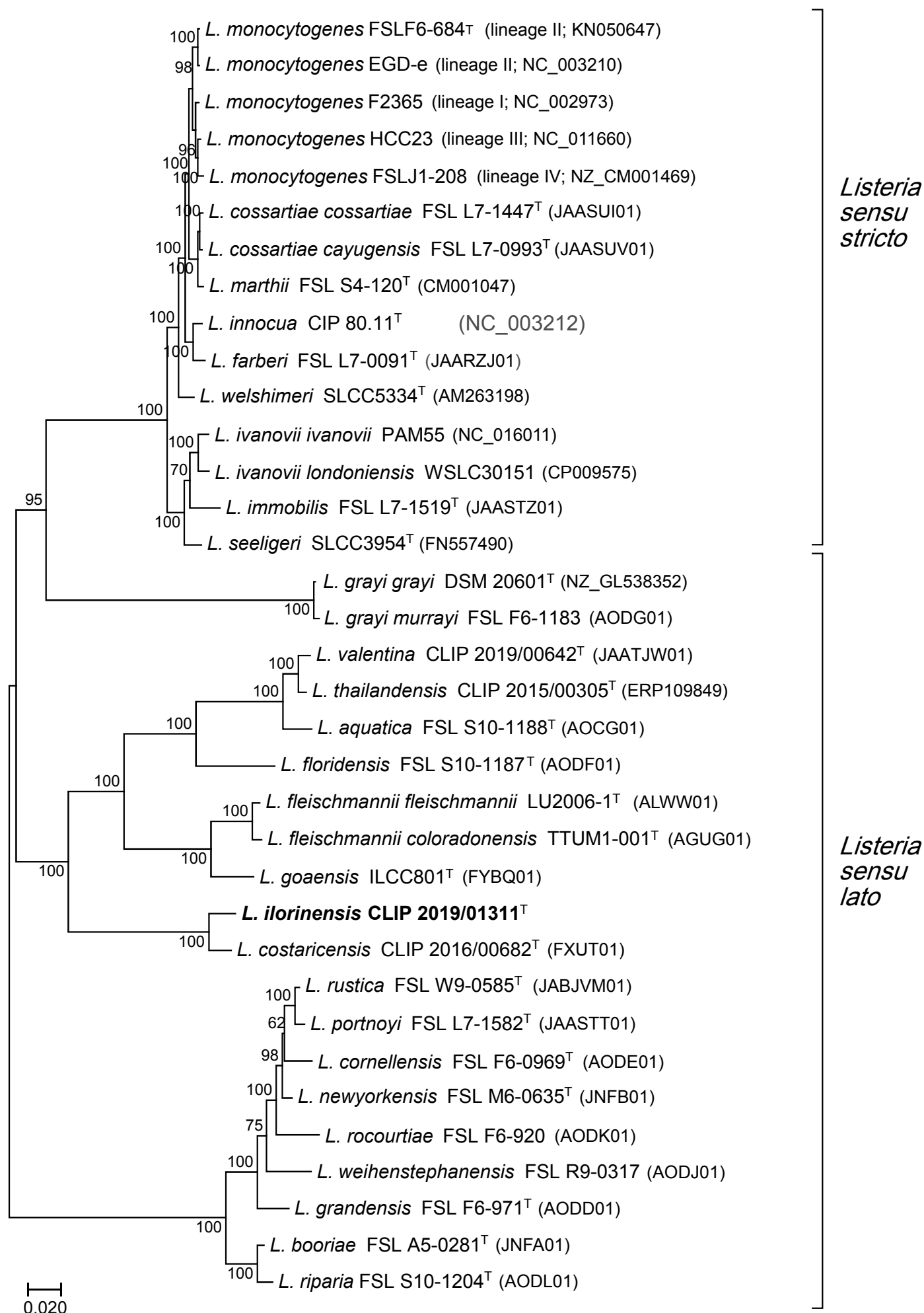
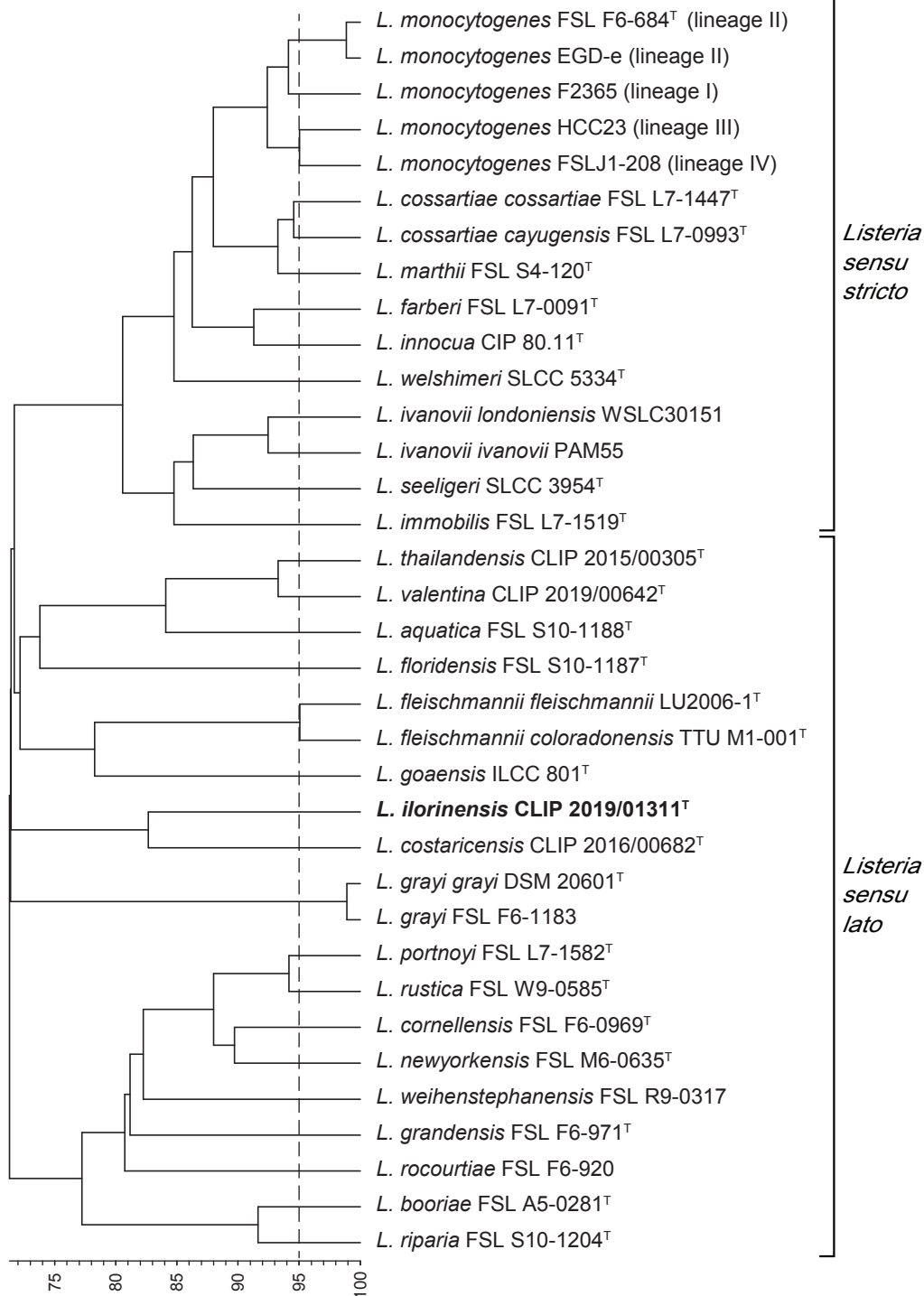


FIGURE 3



***Listeria ilorinensis* sp. nov. isolated from cow milk cheese in Nigeria**

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SUPPLEMENTAL MATERIAL

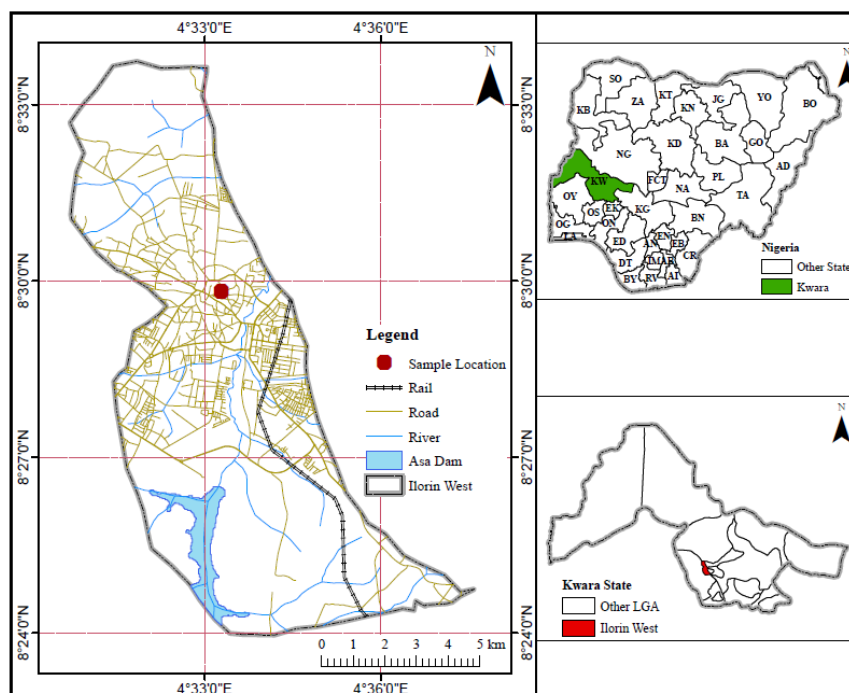


Figure S1. Sampling site location (Ilorin, Kwara State, Nigeria).

Table S1. Pairwise whole-genome average nucleotide identity (ANI) between *L. ilorensis* sp. nov. CLIP 2019/01311^T and other *Listeria* spp.

isolate	Two-way ANI
<i>L. costaricensis</i> CLIP 2016/00682 ^T	82.66%
<i>L. floridensis</i> FSL S10-1187 ^T	71.75%
<i>L. valentina</i> CLIP 2019/00642 ^T	71.71%
<i>L. aquatica</i> FSL S10-1188 ^T	71.67%
<i>L. thailandensis</i> CLIP 2015/00305 ^T	71.63%
<i>L. fleischmannii fleischmannii</i> LU2006-1 ^T	71.56%
<i>L. fleischmannii coloradonensis</i> TTU M1-001 ^T	71.50%
<i>L. seeligeri</i> SLCC 3954 ^T	71.49%
<i>L. ivanovii ivanovii</i> PAM55	71.48%
<i>L. goaensis</i> ILCC 801 ^T	71.44%
<i>L. welshimeri</i> SLCC 5334 ^T	71.41%
<i>L. cossartiae cossartiae</i> FSL L7-1447 ^T	71.39%
<i>L. innocua</i> CIP 80.11 ^T	71.36%
<i>L. ivanovii londoniensis</i> WSLC30151	71.35%
<i>L. cossartiae cayugensis</i> FSL L7-0993 ^T	71.34%
<i>L. immobilis</i> FSL L7-1519 ^T	71.33%
<i>L. monocytogenes</i> FSL F6-684 ^T	71.32%
<i>L. farberii</i> FSL L7-0091 ^T	71.26%
<i>L. grayi grayi</i> DSM 20601 ^T	71.12%
<i>L. grayi murrayi</i> FSL F6-1183	71.10%
<i>L. marthii</i> FSL S4-120 ^T	70.84%
<i>L. portnoyi</i> FSL L7-1582 ^T	70.79%
<i>L. newyorkensis</i> FSL M6-0635 ^T	70.74%
<i>L. cornellensis</i> FSL F6-0969 ^T	70.73%
<i>L. grandensis</i> FSL F6-971 ^T	70.73%
<i>L. riparia</i> FSL S10-1204 ^T	70.73%
<i>L. booriae</i> FSL A5-0281 ^T	70.71%
<i>L. rocourtiae</i> FSL F6-920	70.68%
<i>L. weihenstephanensis</i> FSL R9-0317	70.64%
<i>L. rustica</i> FSL W9-0585 ^T	70.61%