Massilibacterium senegalense gen. nov., sp. nov., a new bacterial genus isolated from the human gut

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Abstract

Massilibacterium senegalense gen. nov., sp. nov., strain mt8^T, is the type strain of *Massilibacterium* gen. nov., a new genus within the *Bacillaceae* family. This Gram-negative facultative anaerobic rod was isolated from the gut microbiota of a severely malnourished boy. Its phenotypic description is hereby presented with a complete annotation of its genome sequence. This genome is 5 697 950 bp long and contains 5615 protein-coding genes and 178 RNA genes, among which are 40 rRNA genes.

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Keywords: Culturomics, genome, gut microbiota, *Massilibacterium senegalense*, taxonogenomics

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Introduction

The human gut microbiota is a complex and vast ecosystem harbouring eukaryotes, viruses, archaea and bacteria, these being by far the most abundant [1]. Its cell count is estimated to approximately 10^{14}, representing ten times the human somatic cell count, and its collective bacterial genome size is 150 times the size of the human genome [1–4]. The development of metagenomics has allowed a better exploration of gut microbiota by bypassing the noncultivable bacteria problem and unveiling links between altered gut microbiota and several diseases such as obesity, inflammatory bowel disease and irritable bowel syndrome [2]. It has also been demonstrated that the microbiota plays key roles in digestion and in immunologic and metabolic functions [2–4]. Nevertheless, a cultivation approach would be a complementary way to explore the gut microbiome in order to have a better representation of the viable population. In addition, it would allow further knowledge about the gut bacterial repertoire.

A new approach was developed in our laboratory in order to explore as exhaustively as possible the human gut microbiota by multiplying culture conditions with different atmospheres, media and temperatures [5]. This approach, known as culturomics, allowed us to isolate a new member of the *Bacillaceae* family. This family was created by Cohn in 1872 and consists of 52 validated genera (http://www.bacterio.net/). *Bacillus* is the type genus of this family, containing genera that are mostly aerobic or facultative anaerobic, rod-shaped, spore-forming, Gram-positive bacteria. These ubiquitous species are found in many ecosystems—mainly soil but also other environmental and clinical samples. Most *Bacillaceae* species are harmless, but some can be opportunistic pathogens, and *Bacillus anthracis,* the agent of anthrax, is well known to be pathogenic for humans [6].

Bacterial classification is currently based on phylogenetic relationships built on the 16S ribosomal RNA gene, phenotypic and genotypic characteristics including G+C content and DNA-DNA hybridization [7–9]. However, a great breakthrough has been

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made in the last years in the area of genome sequencing, partly due to its decreasing cost. In fact, to this day, almost 70 000 genomes have been sequenced (https://gold.jgi.doe.gov/). With the development of this innovation, we proposed a new concept of bacterial description, including a proteomic description with a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) profile [10,11] alongside a biochemical and genomic description of the new species [12–17].

We describe here a new member of this family, the genus *Massilibacterium,* isolated in the faeces of a patient with kwashiorkor. *Massilibacterium senegalense* is the type species (= CSUR P1510 = DSM 100455) of this new genus.

Materials and Methods

Organism information

As part of a culturomics study of the gut microbiota of children with severe acute malnutrition, a stool sample was collected from a 2-month-old Senegalese boy with kwashiorkor (body mass index, 14 kg/m^2) in April 2014. The patient was not treated with antibiotics at the time of sample collection; the sample was stored at −80°C. This study was authorized by the child's parents and was approved by the Institut Fédératif de Recherche 48 (Faculty of Medicine, Marseille, France) under agreement 09-022.

Strain identification by MALDI-TOF and 16S rRNA sequencing

Using the 18 culture conditions of the culturomics concept, the fecal sample was cultivated, and the obtained colonies were identified by MALDI-TOF as described below [5]. Proteomic analysis of our strain was carried out with MALDI-TOF as previously described [10,11]. A Microflex spectrometer (Bruker Daltonics, Leipzig, Germany) was used with a MTP 96 MALDI-TOF target plate (Bruker) on which 12 individual colonies were deposited. Twelve spectra were thus obtained, imported into MALDI BioTyper 2.0 software (Bruker) and analysed by standard pattern matching (with default parameter settings) against the main spectra of 7567 bacteria. Comparison with the BioTyper database spectra enabled the identification and discrimination of the analysed species from those in the database in accordance with the obtained score: a score >2 with a validated species enabled the identification at the species level, and a score <1.7 did not enable any identification. After a failed identification of the colony with a clean spectrum, it was identified by sequencing the 16S ribosomal RNA as previously described [18]. A threshold of 98.7% similarity level was determined to define a new species without performing DNA-DNA hybridization [19].

Growth conditions

In order to determine the ideal growth condition of *M. senegalense,* different growth temperatures (28, 30, 37, 45 and 56°C) were tested under anaerobic and microaerophilic atmospheres using GENbag anaer and GENbag miroaer systems respectively (bioMérieux, Marcy l'Étoile, France). The strain growth was also tested aerobically with or without 5% CO_2 supplementation.

Morphologic, biochemical and antibiotics susceptibility tests

The phenotypic characteristics (Gram staining, sporulation, motility, catalase, oxidase) were analysed as previously described [20]. Antibiotic susceptibility testing was performed using the disk diffusion method according to EUCAST 2015 recommendations (http://www.eucast.org/). Using API 20NE, API ZYM and API 50CH strips, we investigated the biochemical characteristics of the strain according to the manufacturer's instructions (bioMérieux). Electronic microscopy was performed with detection Formvar-coated grids which were deposited on a 40 μL bacterial suspension drop and incubated at 37°C for 30 minutes. Then followed a 10-second incubation on ammonium molybdate 1%. The grids were dried on blotting paper and finally observed with a Tecnai G20 transmission electron microscope (FEI Company, Limeil-Brevannes, France).

Genomic DNA preparation

M. senegalense strain mt8^T was cultured on 5% sheep's blood–enriched Columbia agar (bioMérieux) at 37°C aerobically. Bacteria grown on three petri dishes were resuspended in 4 × 100 μL of Tris-EDTA (TE) buffer. Then 200 μL of this suspension was diluted in 1 mL TE buffer for lysis treatment that included a 30-minute incubation with 2.5 μg/μL lysozyme at 37°C, followed by an overnight incubation with 20 μg/μL proteinase K at 37°C. Extracted DNA was then purified using three successive phenol–chloroform extractions and ethanol precipitations at −20°C overnight. After centrifugation, the DNA was resuspended in 160 μL TE buffer.

Genome sequencing and assembly

Genomic DNA (gDNA) of *M. senegalense* was sequenced on the MiSeq Technology (Illumina, San Diego, CA, USA) with the mate pair strategy. The gDNA was barcoded in order to be mixed with 11 other projects with the Nextera Mate Pair sample prep kit (Illumina). gDNA was quantified by a Qubit assay with a high sensitivity kit (Thermo Fisher Scientific, Waltham, MA, USA) to 66.2 ng/μL. The mate pair library was prepared with 1 μg of gDNA using the Nextera mate pair Illumina guide. The gDNA sample was simultaneously fragmented and tagged with a mate pair junction adapter. The

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pattern of the fragmentation was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USAUSA) with a DNA 7500 lab chip. The DNA fragments ranged in size from 1 to 11 kb, with an optimal size at 3.927 kb. No size selection was performed, and 505 ng of tagmented fragments were circularized. The circularized DNA was mechanically sheared to small fragments with an optimal at 597 bp on the Covaris device S2 in microtubes (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent Technologies, Santa Clara, CA, USA), and the final concentration library was measured at 59.2 nmol/L. The libraries were normalized at 2 nM and pooled. After a denaturation step and dilution at 15 pM, the pool of libraries was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. An automated cluster generation and sequencing run was performed in a single 39-hour run in a 2 × 251 bp read length.

Genome annotation and analysis

Open reading frames (ORFs) were predicted using Prodigal [21] with default parameters, but the predicted ORFs were excluded if they were spanning a sequencing gap region (contain N). The predicted bacterial protein sequences were searched against the Clusters of Orthologous Groups (COGs) database using BLASTP (*E* value 1e-03, coverage 0.7 and identity percentage of 30%). If no hit was found, then it was searched against the NR database using BLASTP with an *E* value of 1e-03, coverage 0.7 and identity percentage of 30%, and if the sequence length was smaller than 80 amino acids, we used an *E* value of 1e-05. The tRNAScanSE tool [22] was used to find tRNA genes, whereas ribosomal RNAs were found by using RNAmmer [23]. Lipoprotein signal peptides and the number of transmembrane helices were predicted using Phobius [24]. ORFans were identified if all the BLASTP performed did not give positive results (*E* value smaller than 1e-03 for ORFs with sequence size larger than 80 amino acids or *E* value smaller than 1e-05 for ORFs with sequence length smaller 80 amino acids). Such parameter thresholds have already been used in previous studies to define ORFans.

Genomes were automatically retrieved from the 16S RNA tree using Xegen software (Phylopattern) [25]. For each selected genome, the complete genome sequence, proteome genome sequence and Orfeome genome sequence were retrieved from the FTP site of the National Center for Biotechnology Information (NCBI). All proteomes were analysed with proteinOrtho [26]. Then for each couple of genomes a similarity score was computed. This score is the mean value of nucleotide similarity between all couple of orthologues between the two genomes studied (average genomic identity of orthologous gene sequences, AGIOS) [27]. An annotation of the entire proteome was performed to define the distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins (using the same method as for the genome annotation). To evaluate the genomic similarity among studied *Bacillus* strains, we determined two parameters: digital DNA-DNA hybridization (dDDH), which exhibits a high correlation with DNA-DNA hybridization (DDH) [28,29], and AGIOS [27], which was designed to be independent from DDH. Annotation and comparison processes were performed in the Multi-Agent software system DAGOBAH [30] that included Figenix [31] libraries that provide pipeline analysis.

Results

Strain identification and phylogenetic analyses

Strain mt8^T (Table 1) was first isolated in January 2015 after 30-day preincubation in a blood culture bottle with sheep's blood and cultivation on 5% sheep's blood–enriched Colombia agar in an aerobic atmosphere at 37°C. MALDI-TOF displayed an identification score under 1.7 for strain mt8^T, suggesting that the obtained spectra was not matched to any spectra in our database. The 16S ribosomal RNA sequence (accession no. LN828943) of strain mt8^T showed a 93% nucleotide sequence similarity with *Bacillus halodurans,* which is the phylogenetically closest species with a validly published name (Fig. 1). Consequently, as this 16S rRNA nucleotide sequence similarity was lower than the threshold of 95% recommended by Stackebrandt and Ebers [19] to delineate a new genus, it was classified as a new genus called *Massilibacterium,* type species *Massilibacterium senegalense* strain mt8^T. The reference spectrum for strain mt8^T (Fig. 2) was thus incremented in our database and then compared to other known species of the genus *Bacillus.* The differences exhibited are shown in the obtained gel view (Fig. 3).

TABLE 1. Classification and general features of *Massilibacterium senegalense* strain mt8^T

Property	Term
Current classification	Domain: *Bacteria* Phylum: *Firmicutes* Class: *Bacilli* Order: *Bacillales* Family: *Bacillaceae* Genus: *Massilibacterium* Species: *Massilibacterium senegalense* Type strain: mt8
Gram stain	Negative
Cell shape	Rod
Motility	Motile
Sporulation	sporulating
Temperature range	Mesophilic
Optimum temperature	37°C

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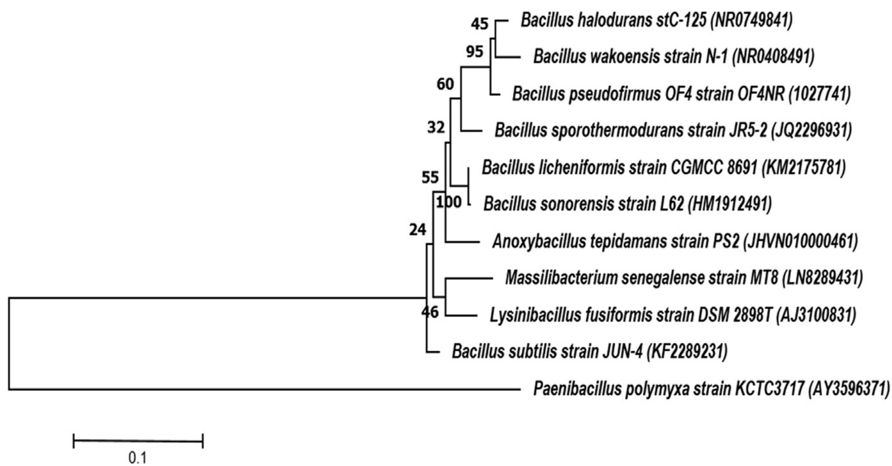

FIG. 1. Phylogenetic tree highlighting the position of *Massilibacterium senegalense* strain mt8^T relative to other close strains. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using the maximum-likelihood method within the MEGA6. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1,000 times to generate a majority consensus tree. *Paenibacillus polymyxa* strain KCTC3717 was used as an outgroup. The scale bar represents a 1% nucleotide sequence divergence.

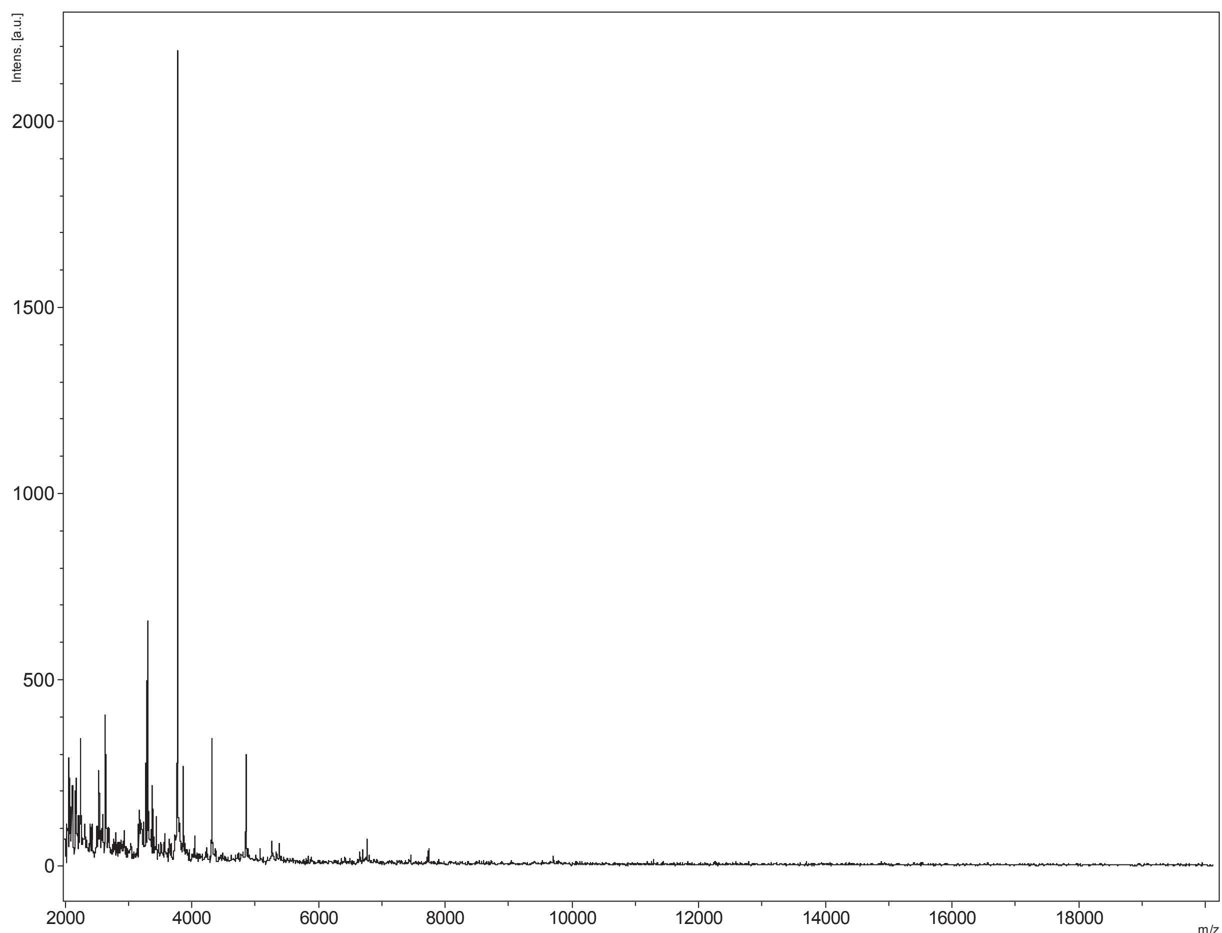

FIG. 2. Reference mass spectrum from *Massilibacterium senegalense* strain mt8^T. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

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Phenotypic description

Growth of strain mt8^T was observed between 28 and 45°C on 5% sheep's blood–enriched Columbia agar, and optimal growth was achieved at 37°C after 24 hours' incubation in aerobic conditions. Poor growth occurred under microaerophilic and anaerobic conditions. Cells were motile and sporulating. Colonies were irregular white colonies with a mean diameter of 5 mm on blood-enriched Colombia agar. The Gram staining (Fig. 4) showed Gram-negative rods. Using electron microscopy, the rods had a mean diameter of 1.8 μm and a length of 5.9 μm (Fig. 5).

Catalase and oxidase activities were negative for strain mt8^T. Using API ZYM, positive reactions were observed for esterase (C4) and acid phosphatase. Reactions for alkaline phosphatase, esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase, N-acetyl-β-glucosaminidase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-fucosidase and α-mannosidase were negative. Using API 20NE, nitrate reduction and esculin hydrolysis were observed. All other reactions were negative, including indole formation and urease. An API 50CH strip showed positive reactions for N-acetyl-glucosamine, amygdalin, arbutin, salicin, D-maltose, D-fructose, inulin, D-mannose, D-sucrose and D-raffinose. Negative reactions were recorded for glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-βD-xylopyranoside, D-glucose, D-galactose, D-lactose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, esculin ferric citrate, D-cellobiose, D-melibiose, D-trehalose, D-melezitose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-

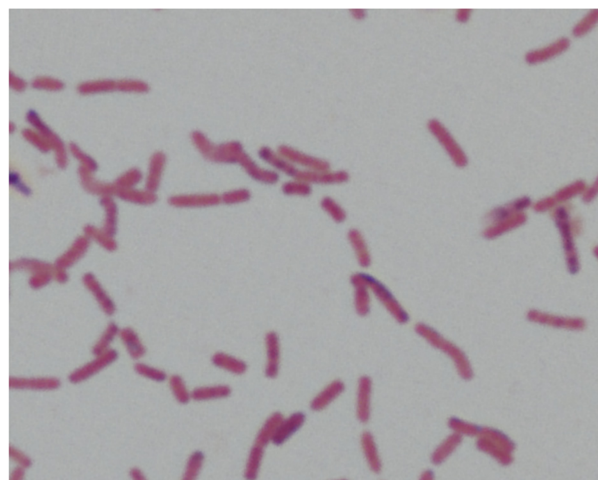

FIG. 4. Gram staining of *Massilibacterium senegalense* strain mt8^T.

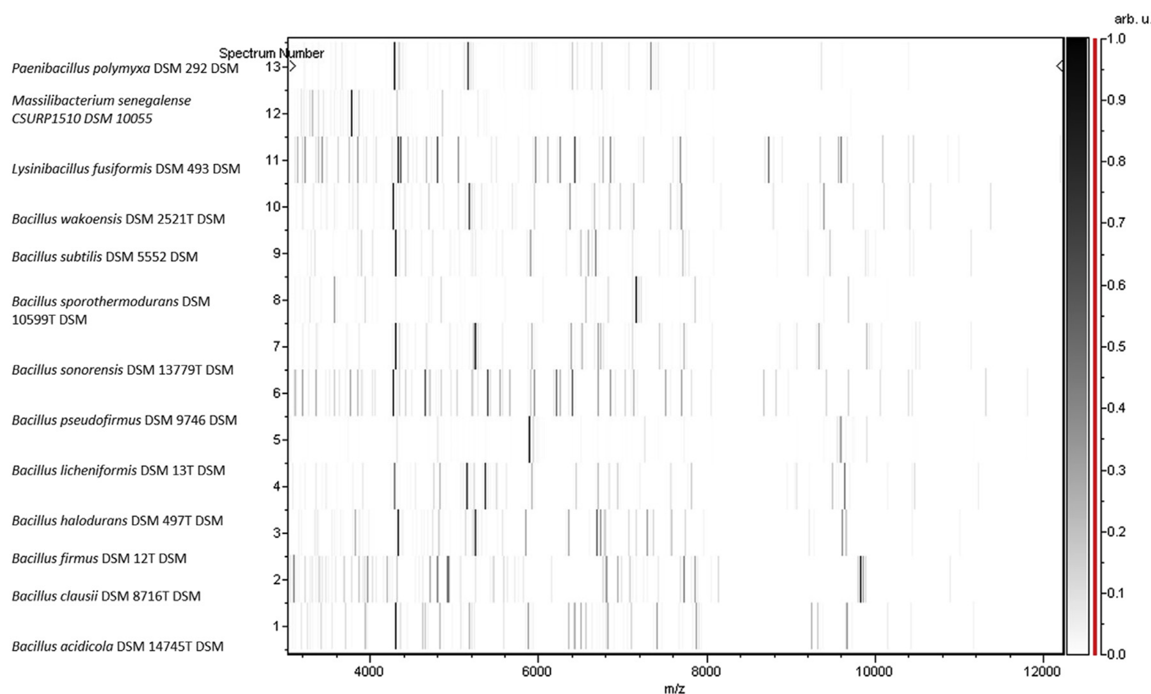

FIG. 3. Gel view comparing *Massilibacterium senegalense* strain mt8^T to other species within the Bacillaceae family. The gel view displays the raw spectra of loaded spectrum files arranged in a pseudo-gel like look. The x-axis records the m/z value. The left y-axis displays the running spectrum number originating from subsequent spectra loading. The peak intensity is expressed by a Gray scale scheme code. The color bar and the right y-axis indicate the link between the color in which a peak is displayed and the peak intensity in arbitrary units. Displayed species are indicated on the left.

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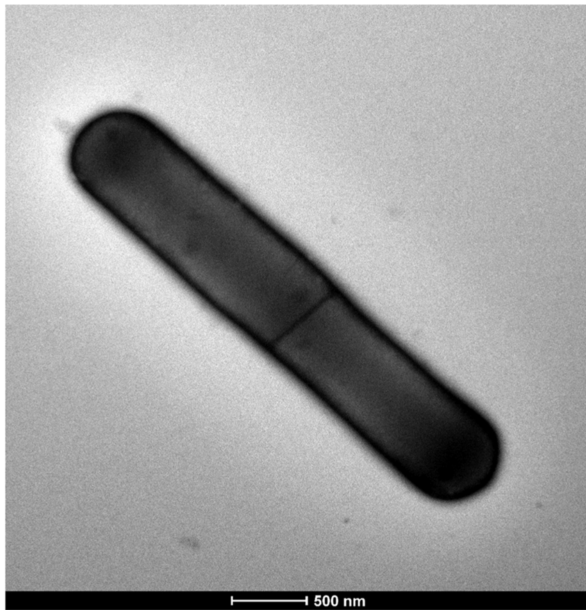

FIG. 5. Transmission electron microscopy of *Massilibacterium senegalense* strain mt8^T using Tecnai G20 transmission electron microscope (FEI Company). Scale bar = 500 nm.

arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate.

Cells were susceptible to imipenem, doxycycline, rifampicin, vancomycin, amoxicillin, ceftriaxone, gentamicin (500 μg), trimethoprim/sulfamethoxazole, erythromycin, ciprofloxacin and gentamicin (15 μg) but were resistant to metronidazole.

Table 2 shows the differences between the biochemical features of close relatives of *Massilibacterium senegalense* strain mt8^T, members of the *Bacillaceae* family.

Genome properties

The genome of *M. senegalense* strain mt8^T genome (accession no. CTRN00000000) was 5 697 950 bp long with a 35.67% G+C content (Fig. 6, Table 3). It was composed of ten scaffolds and 12 contigs. There were 5793 predicted genes, among which 5615 were protein-coding genes and 178 RNAs (14 5S rRNA genes, 16 16S rRNA genes, ten 23S rRNA genes and 138 tRNA genes). A total of 4262 genes were assigned a putative function, and 208 genes were identified as ORFans. The remaining 386 genes were annotated as hypothetical proteins. Using ARG-ANNOT [32], no resistance genes were found. The properties and statistics of the genome are summarized in Table 3, and the gene distribution into COGs functional categories is presented in Table 4.

Genome comparison

The genomic characteristics (size, percentage of G+C content, protein-coding genes and total number of genes) were used to compare strain mt8^T with the genome of closely related species (Table 5). The size of *Massilibacterium senegalense* strain mt8^T (5.69 Mb) is larger than *Bacillus wakoensis* strain N_1, *Lysinibacillus fusiformis* strain DSM 2898^T, *Bacillus halodurans* strain C-125, *Bacillus pseudofirmus* strain OF4, *Anoxybacillus tepidamans* strain PS2 and *Bacillus smithii* strain 7_3_47FAA (5.53, 4.84, 4.2, 3.86, 3.36 and 3.24 Mb respectively). The G+C content of *M. senegalense* (35.6%) is smaller than those of *B. halodurans, A. tepidamans, B. smithii, B. pseudofirmus, B. wakoensis* and *L. fusiformis* (43.7, 43.0, 40.7, 40.3, 38.3 and 37.6%). The gene content of *M. senegalense* (5793) is bigger than the gene content of *L. fusiformis, B. wakoensis, B. halodurans, B. pseudofirmus, A. tepidamans* and *B. smithii* (4764, 4460, 4076, 3841, 3400 and 3235 respectively) (Table 5). There are more protein-coding genes (5615) in the genome of *M. senegalense* than in the genomes of *L. fusiformis, B. wakoensis, B. halodurans, B. pseudofirmus, A. tepidamans,* and *B. smithii* (4548, 3912, 3903, 3704, 3245 and 2832 respectively). The distribution of genes into COGs categories was similar in all compared genomes (Fig. 7, Table 4). *M. senegalense* also shared 1368, 1244, 1263, 1318, 1321 and 1231 orthologous genes with *B. pseudofirmus, L. fusiformis, B. wakoensis, A. tepidamans, B. halodurans,* and *B. smithii* respectively (Table 6). Among species with standing in nomenclature, AGIOS values ranged from 63.43 to 70.67% among compared species except *M. senegalense.* When compared to other species, the AGIOS values ranged from 65.40% with *B. halodurans* to 66.37% with *A. tepidamans* (Table 6). To evaluate the genomic similarity among studied *Bacillaceae* strains, we determined two parameters, dDDH, which exhibits a high correlation with DDH [28,29], and AGIOS [27], which was designed to be independent from DDH (Table 7).

Conclusion

Given the 93% similarity level to *Bacillus halodurans* for the 16S rRNA sequence of strain mt8^T, its MALDI-TOF spectrum and the analysis of its annotated genome, we created a new genus, *Massilibacterium. Massilibacterium senegalense* is the type strain.

Description of *Massilibacterium* gen. nov.

Massilibacterium (from Massilia, Marseille's old Roman and Greek name; Marseille is the city in which the strain was isolated).

Facultative anaerobic rod-shaped bacteria. Gram negative. Optimal growth in aerobic conditions at 37°C. Catalase and oxidase negative. Nitrates were reduced into nitrites. Negative for indole formation. β-Glucosidase positive. Urease negative. The type species is *Massilibacterium senegalense* strain mt8^T. Habitat is human gut.

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TABLE 2. Differential characteristics of *Massilibacterium senegalense* strain mt8T, *Bacillus halodurans* DSM 497, *Bacillus acidicola* DSM 14745T, *Bacillus wakoensis* DSM 2521T, *Bacillus hemicellulosilyticus* DSM 16731T, *Bacillus cellulosilyticus* DSM 2522T, *Bacillus akibai* ATCC 43226T, *Bacillus mannanilyticus* DSM 16130T, *Bacillus okuhidensis* DSM 13666T, *Bacillus sonorensis* DSM 13779T [33–37]

Property	*Massilibacterium senegalense*	*Bacillus halodurans*	*Bacillus acidicola*	*Bacillus wakoensis*	*Bacillus hemicellulosilyticus*	*Bacillus cellulosilyticus*	*Bacillus akibai*	*Bacillus mannanilyticus*	*Bacillus okuhidensis*	*Bacillus sonorensis*
Cell diameter (μm)	1.7–1.9	0.5–0.6	1.0–1.6	0.5–0.8	0.3–0.5	0.6–0.8	0.6–0.8	0.6–0.8	0.5–1.0	1.0
Oxygen requirement	+	+	+	+	+	+	+	+	+	+
Gram stain	−	NA	+	+	+/−	+	+	+/−	+/−	+
Salt requirement	−	+	−	−	−	−	−	−	−	−
Motility	+	NA	+	+	+	+	+	+	+	+
Endospore formation	+	+	+	+	+	+	+	+	+	+
Indole	−	NA	−	−	−	−	−	−	NA	NA
Production of:										
Alkaline phosphatase	−	NA	NA	NA	NA	NA	NA	NA	NA	NA
Catalase	−	NA	+	+	+	+	+	+	+	+
Oxidase	−	NA	−	−	−	−	−	−	+	NA
Nitrate reductase	+	−	−	+	−	+	+	−	+	+
Urease	−	NA	NA	NA	NA	NA	NA	NA	NA	NA
β-Galactosidase	−	NA	NA	NA	NA	NA	NA	NA	NA	NA
N-acetyl-glucosamine	+	+	NA	NA	NA	NA	NA	NA	+	NA
Acid from:										
L-Arabinose	−	+	−	−	−	−	+	−	+	+
Ribose	−	−	+	−	−	−	−	−	NA	NA
Mannose	+	+	+	+	+	+	+	+	−	NA
Mannitol	−	+	+	+	+	−	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	NA	NA
D-Glucose	−	+	+	+	+	+	+	+	NA	+
D-Fructose	+	+	+	+	+	+	+	+	NA	NA
D-Maltose	+	+	+	+	+	+	+	+	NA	NA
D-Lactose	−	+	+/−	−	+	+	−	−	+	NA
Habitat	Human gut	Soil	Acidic peat bogs	Industry	Industry	Industry	Industry	Industry	Water	Desert

NA, not available.

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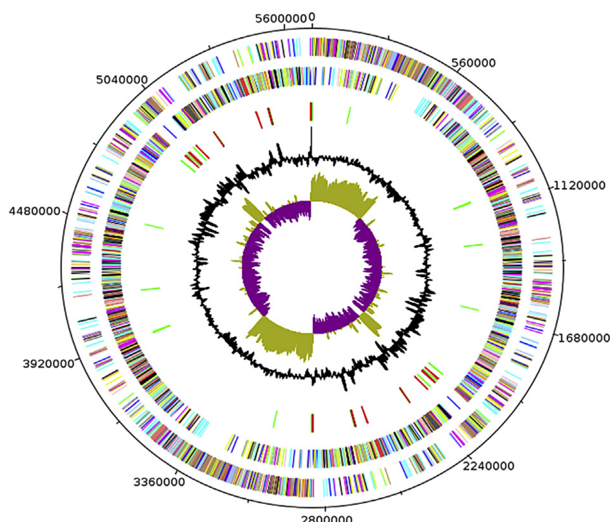

FIG. 6. Graphical circular map of chromosome. From outside to center: genes on forward strain coloured by COGs categories (only gene assigned to COGs), RNA genes (tRNAs green, rRNAs red), G+C content and G+C skew.

Description of *Massilibacterium senegalense* strain mt8^T gen. nov., sp. nov.

Massilibacterium senegalense (se.ne.gal.e′n.sis. L. gen. masc., meaning originating from Senegal, the country from which the stool sample was collected).

Cells are sporulating, motile and facultative anaerobic, Gram-negative, rod-shaped bacilli with a mean diameter of 1.8 μm and a length of 5.9 μm. Colonies were 5 mm diameter white irregular colonies on 5% sheep's blood–enriched Colombia agar. Catalase and oxidase negative.

Positive reactions were observed for esterase (C4) and acid phosphatase. Nitrate reduction and aesculin hydrolysis were positive. N-acetyl-glucosamine, amygdalin, arbutin, salicin, D-maltose, D-fructose, inulin, D-mannose, D-sucrose and D-raffinose were metabolized. Cells were susceptible to imipenem, doxycycline, rifampicin, vancomycin, amoxicillin, ceftriaxone, gentamicin (500 μg), trimethoprim/sulfamethoxazole, erythromycin, ciprofloxacin and gentamicin (15 μg) but were resistant to metronidazole.

The G+C content of the genome is 35.67%. The 16S rRNA gene sequence and whole genome shotgun sequence of *M. senegalense* strain mt8^T are deposited in GenBank under accession numbers LN828943 and CTRN01000000 respectively.

TABLE 4. **Number of genes associated with 25 general COGs functional categories**

Code	Value	% of total[a]	Description
J	298	10.63	Translation
A	0	0.0	RNA processing and modification
K	334	11.91	Transcription
L	406	14.48	Replication, recombination and repair
B	2	0.07	Chromatin structure and dynamics
D	68	2.43	Cell cycle control, mitosis and meiosis
Y	0	0.0	Nuclear structure
V	80	2.85	Defense mechanisms
T	252	8.99	Signal transduction mechanisms
M	224	7.99	Cell wall/membrane biogenesis
N	132	4.71	Cell motility
Z	0	0.0	Cytoskeleton
W	0	0.0	Extracellular structures
U	98	3.50	Intracellular trafficking and secretion
O	190	6.78	Posttranslational modification, protein turnover, chaperones
C	248	8.84	Energy production and conversion
G	140	4.99	Carbohydrate transport and metabolism
E	370	13.20	Amino acid transport and metabolism
F	138	4.92	Nucleotide transport and metabolism
H	226	8.06	Coenzyme transport and metabolism
I	190	6.78	Lipid transport and metabolism
P	286	10.20	Inorganic ion transport and metabolism
Q	92	3.28	Secondary metabolites biosynthesis, transport and catabolism
R	602	21.47	General function prediction only
S	432	15.41	Function unknown
—	424	7.31	Not in COGs

COGs, Clusters of Orthologous Groups database.
[a]Total is based on total number of protein-coding genes in annotated genome.

TABLE 3. **Nucleotide content and gene count levels of the genome**

	Genome (total)	
Attribute	Value	% of total[a]
Size (bp)	5 697 950	100
G+C content (%)	2 034 168	35.7
Coding region (bp)	4 442 019	77.95
Total genes	5793	100
RNA genes	178	3.07
Protein-coding genes	5615	96.92
Genes with function prediction	4262	73.57
Genes assigned to COGs	3838	66.25
Genes with peptide signals	210	3.62
Genes with transmembrane helices	504	8.70
CRISPR repeats	0	0
ORFan genes	208	3.59
Genes associated with PKS or NRPS	13	0.22
No. of antibiotic resistance genes	0	0

COGs, Clusters of Orthologous Groups database; CRISPR, clustered regularly interspaced short palindromic repeat.
[a]Total is based on either size of genome (bp) or total number of protein-coding genes in annotated genome.

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TABLE 5. Genome comparison of species closely related to *Massilibacterium senegalense* strain mt8[T]

Organism	INSDC	Size (Mb)	G+C%	Protein-coding genes	Total genes
Massilibacterium senegalense strain mt8[T]	CTRN00000000.1	5.69	35.6	5615	5793
Bacillus pseudofirmus strain OF4	CP001878.2	3.86	40.3	3704	3841
Lysinibacillus fusiformis strain DSM 2898[T]	CP010820.1	4.84	37.6	4548	4764
Bacillus wakoensis strain N_1	BAUT00000000.1	5.53	38.3	3912	4460
Anoxybacillus tepidamans strain PS2	JHVN00000000.1	3.36	43.0	3245	3400
Bacillus halodurans strain C-125	BA000004.3	4.2	43.7	3903	4076
Bacillus smithii strain 7_3_47FAA	ACWF00000000.1	3.24	40.7	2832	3235

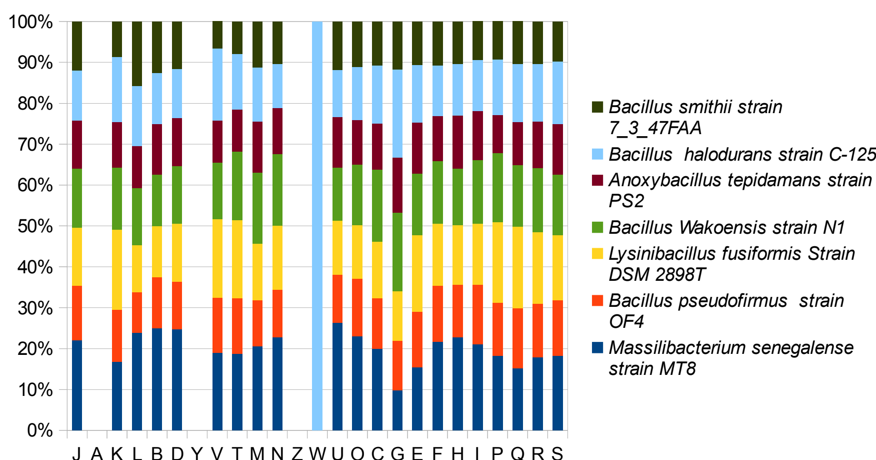

FIG. 7. Distribution of functional classes of predicted genes according to clusters of orthologous groups of protein.

TABLE 6. Number of orthologous proteins shared between genomes (upper right)[a]

	Bacillus pseudofirmus	*Lysinibacillus fusiformis*	*Massilibacterium senegalense*	*Bacillus wakoensis*	*Anoxybacillus tepidamans*	*Bacillus halodurans*	*Bacillus smithii*
B. pseudofirmus	4335	1496	1368	1868	1615	1959	1457
L. fusiformis	64.28	4767	1244	1356	1456	1464	1325
M. senegalense	65.99	65.59	5615	1263	1318	1321	1231
B. wakoensis	70.67	64.46	66.35	4576	1495	1819	1358
A. tepidamans	65.38	64.71	66.37	65.28	3463	1611	1456
B. halodurans	68.48	63.43	65.40	68.03	65.66	4066	1425
B. smithii	65.06	65.14	65.95	64.98	68.03	65.00	3294

[a]Average percentage similarity of nucleotides corresponding to orthologous proteins shared between genomes (lower left) and numbers of proteins per genome.

TABLE 7. Pairwise comparison of *Massilibacterium senegalense* strain mt8[T] with other species using GGDC, formula 2 (DDH estimates based on identities/HSP length)[a] (upper right)

	Bacillus pseudofirmus	*Lysinibacillus fusiformis*	*Massilibacterium senegalense*	*Bacillus wakoensis*	*Anoxybacillus tepidamans*	*Bacillus halodurans*	*Bacillus smithii*
B. pseudofirmus	100%	29.1% ± 2.54	26.3% ± 2.55	21.2% ± 2.59	21.9% ± 2.53	27.5% ± 2.56	25.7% ± 2.54
L. fusiformis		100%	29.5% ± 2.54	26.1% ± 2.54	23.2% ± 2.53	30.8% ± 2.54	31.9% ± 2.54
M. senegalense			100%	25% ± 2.54	21% ± 2.54	24.4% ± 2.55	30.4% ± 2.54
B. wakoensis				100%	19.5% ± 2.53	23.3% ± 2.56	28.3% ± 2.53
A. tepidamans					100%	22.2% ± 2.53	21.6% ± 2.55
B. halodurans						100%	25.9% ± 2.54
B. smithii							100%

DDH, DNA-DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; HSP, high-scoring segment pairs.
[a]Confidence intervals indicate inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test data sets (which are always limited in size). These results are in accordance with the 16S rRNA (Fig. 3) and phylogenomic analyses as well as GGDC results.

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The type strain mt8^T (= CSUR P1510 = DSM 100455) was isolated from the stool of a young Senegalese boy with kwashiorkor.

Conflict of Interest

None declared.

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