Noncontiguous finished genome sequence and description of *Diaminobutyricimonas massiliensis* strain FF2^T sp. nov.

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Abstract

Strain FF2^T was isolated from the blood sample of a 35 year-old febrile Senegalese male, in Dielmo, Senegal. This strain exhibited a 97.47% 16S rRNA sequence identity with *Diaminobutyricimonas aerilata*. The score from MALDI-TOF-MS does not allow any identification. Using a polyphasic study made of phenotypic and genomic analyses, strain FF2^T was Gram-negative, aerobic, motile, rod-shaped, and exhibited a genome of 3,227,513 bp (1 chromosome but no plasmid) with a G+C content of 70.13% that coded 3,091 protein-coding and 56 RNA genes. On the basis of these data, we propose the creation of *Diaminobutyricimonas massiliensis* sp. nov.

Keywords: Culturomics, *Diaminobutyricimonas massiliensis*, genome, taxono-genomics
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Introduction

The *Microbacteriaceae* family is constituted of a large group of rod-shaped or, rarely, coccoid or mycelium-forming *Actinobacteria* [1,2]. In this group, there are 42 genera with validly published names [3]. Members of the family were found in diverse environments, including seawater, seaweed, soil, butter and cow feces but also in human specimens such as urine and human blood as well as in wounds [4–6]. *Diaminobutyricimonas aerilata* is the only species which has been officially validly published within the genus *Diaminobutyricimonas;* it was isolated from an air sample in Korea [7]. The genetic parameters used in the delineation of bacterial species include 16S rRNA sequence identity and phylogeny [8,9], genomic G+C content diversity and DNA-DNA hybridization [10]. These reference tools exhibit limitations, notably because their cutoff values vary across species or genera [11]. Thanks to the introduction of high-throughput sequencing techniques, more than 45 000 bacterial genomes have been fully sequenced and are currently available [12]. We recently proposed to incorporate genomic data into a polyphasic approach to describe new bacterial taxa (taxonogenomics). This strategy combines phenotypic characteristics, notably the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) spectrum, and genomic analysis and comparison [13].

Diaminobutyricimonas massiliensis strain FF2^T (= Collection de souches de l'Unité des Rickettsies (CSUR) P3023 = Deutsche Sammlung von Mikroorganismen (DSM) 27836) is designated as the type strain of *Diaminobutyricimonas massiliensis* sp. nov. This bacterium was isolated from a blood specimen from a 35-year-old febrile Senegalese patient as part of a study aiming at detecting bacterial pathogens associated with fever in malaria-negative patients [14–16].

Diaminobutyricimonas massiliensis is a Gram-positive, obligate aerobe, and motile rod-shaped bacterium.

Here we present a summary classification and a set of features of *Diaminobutyricimonas massiliensis* sp. nov. strain FF2^T (= CSUR P3023 = DSM 27836), together with the description of the complete genome sequencing and annotation. These characteristics support the circumscription of the species *Diaminobutyricimonas massiliensis.*

Organism Information

A blood sample was collected from a 35-year-old febrile Senegalese patient living in Dielmo, Senegal (Table 1). *Diaminobutyricimonas massiliensis* strain FF2^T (Table 1) was isolated

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TABLE 1. Classification and general features of *Diaminobutyricimonas massiliensis* strain FF2^T [17]

MIGS ID	Property	Term	Evidence code[a]
	Classification	Domain: *Bacteria*	TAS [31]
		Phylum: *Actinobacteria*	TAS [2]
		Class: *Actinobacteria*	TAS [32,33]
		Order: *Actinomycetales*	TAS [33,34]
		Family: *Microbacteriaceae*	TAS [35]
		Genus: *Diaminobutyricimonas*	TAS [7]
		Species: *Diaminobutyricimonas massiliensis*	IDA
		(Type) strain: FF2^T	IDA
	Gram stain	Positive	IDA
	Cell shape	Rod	IDA
	Motility	Motile	IDA
	Sporulation	Nonsporulating	IDA
	Temperature range	Mesophile	IDA
	Optimum temperature	37°C	IDA
	pH range; optimum	7.2–6.9; 7.05	
	Carbon source	Unknown	NAS
MIGS-6	Habitat	Human blood	IDA
MIGS-6.3	Salinity	Unknown	
MIGS-22	Oxygen requirement	Aerobic	IDA
MIGS-15	Biotic relationship	Free-living	IDA
MIGS-14	Pathogenicity	Unknown	NAS
MIGS-4	Geographic location	Senegal	IDA
MIGS-5	Sample collection time	October 2012	IDA
MIGS-4.1	Latitude	13.716667	IDA
MIGS-4.1	Longitude	−16.416667	IDA
MIGS-4.4	Altitude	21 m above sea level	IDA

[a]Evidence codes: IDA, inferred from direct assay; TAS, traceable author statement (i.e. direct report exists in the literature); NAS, nontraceable author statement (i.e. not directly observed for the living, isolated sample but based on a generally accepted property for the species or anecdotal evidence). These evidence codes are from the Gene Ontology project (http://www.geneontology.org/GO.evidence.shtml) [36]. If the evidence is IDA, then the property was directly observed for a live isolate by one of the authors or by an expert mentioned in the acknowledgements.

in October 2012 in culture on agar enriched with 5% sheep's blood (bioMérieux, Marcy l'Etoile, France) in aerobic conditions after 48 hours' incubation at 37°C. Strain FF2^T exhibited a 97.47% 16S rRNA sequence identity with *Diaminobutyricimonas aerilata* (GenBank accession number JQ639052), the phylogenetically closest bacterial species with a validly published name (Fig. 1). The value was lower than the 98.8% 16S rRNA gene sequence threshold recommended by Meier-Kolthoff *et al.* [17] to delineate a new species within phylum *Actinobacteria* without carrying out DNA-DNA hybridization. Different growth temperatures (25°C, 30°C, 37°C, 45°C and 56°C) were tested. Growth occurred between 37°C and 45°C, but optimal growth was observed at 37°C after 48 hours' incubation in aerobic conditions. The colonies were 0.8 mm in diameter and exhibited a light yellow colour on agar enriched with 5% sheep's blood (bioMérieux). Growth of the strain was tested under anaerobic and microaerophilic conditions using GENbag anaer and GENbag microaer systems, respectively (bioMérieux) and under aerobic conditions, with or without 5% CO_2. Optimal growth was only obtained under aerobic conditions in the presence of 5% CO_2. No growth was obtained under anaerobic and microaerophilic conditions. The Gram staining was positive in fresh culture (less than 72 hours), but in old culture the bacteria may appear as a mixture of Gram-positive and Gram-negative bacteria rods (Gram variable) or as Gram-negative cells (Fig. 2). The motility test was positive. Cells grown on agar exhibited a mean diameter of 0.5 µm (range, 0.4–0.6 µm) and a mean length of 1.3 µm (range, 0.7–2 µm) under electron microscopy (Fig. 3).

Strain FF2^T was catalase and oxidase positive. Using an API 50CH strip (bioMérieux), a positive reaction was observed only for esculin ferric citrate. Negative reactions were observed for D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, D-trehalose, D-turanose, D-fucose, starch, glycogen, D-maltose, amygdalin, N-acetyl-glucosamine, methyl-αD-glucopyranoside and D-cellobiose. Using an API ZYM strip (bioMérieux), positive reactions were obtained for alkaline phosphatase, esterase, esterase-lipase, leucine arylamidase, acid phosphatase, N-acetyl-β-glucosaminidase and α-glucosidase. Negative reactions were observed for α-chymotrypsin, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, β-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase and α-fucosidase.

Diaminobutyricimonas massiliensis is susceptible to penicillin, ceftriaxone, imipenem, ciprofloxacin, gentamicin, rifampicin, vancomycin and doxycycline, but resistant to erythromycin, nitrofurantoin, metronidazole and trimethoprim/sulfamethoxazole. The phenotypic characteristics of *Diaminobutyricimonas massiliensis* strain FF2^T were compared to those of representative species from the family *Microbacteriaceae,* as summarized in Table 2.

MALDI-TOF protein analysis was carried out as previously described [18,19] using a Microflex LT (Brüker Daltonics,

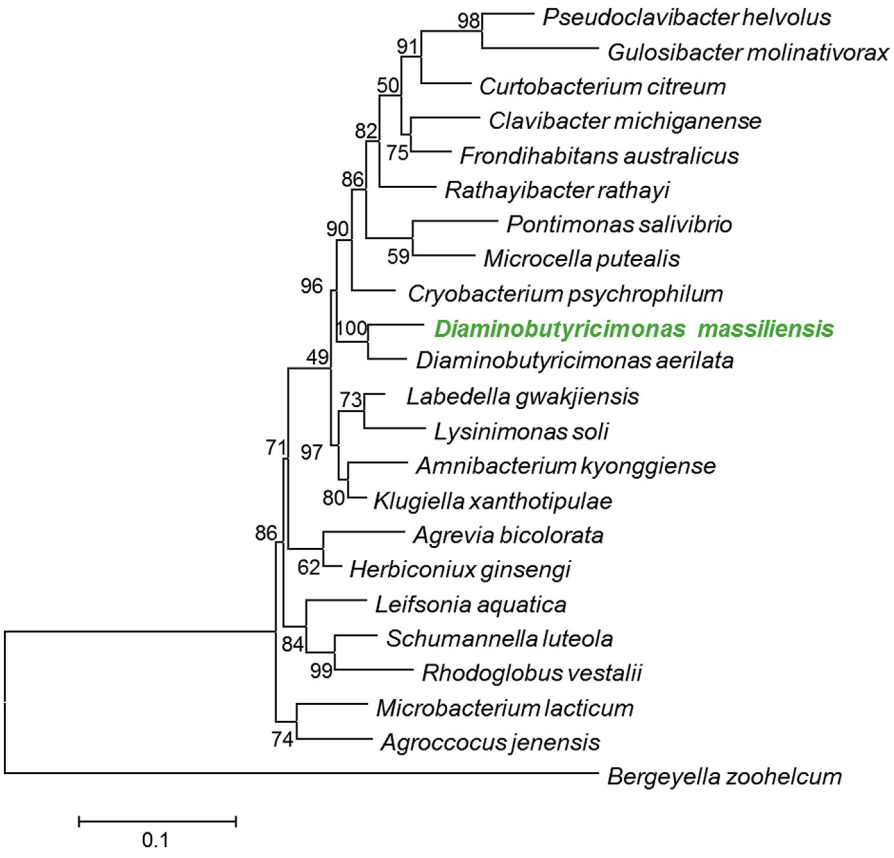


FIG. 1. Phylogenetic tree highlighting position of *Diaminobutyricimonas massiliensis* sp. nov., strain FF2^T, relative to other type strains within family *Microbacteriaceae*. Strains and their corresponding GenBank accession numbers for 16S rRNA are: *Pseudoclavibacter helvolus* strain DSM 20419, X77440; *Gulosibacter molinativorax* strain ON4, AJ306835; *Curtobacterium citreum* strain DSM 20528, X77436; *Clavibacter michiganensis* strain DSM 7483, X77434; *Frondihabitans australicus* strain E1HC-02, DQ525859; *Rathayibacter rathayi* strain DSM 7485, X77439; *Pontimonas salivibrio* strain CL-TW6, JQ639087; *Microcella putealis* strain CV2T, AJ717388; *Cryobacterium psychrophilum* strain DSM 4854, AJ544063; *Diaminobutyricimonas massiliensis* strain FF2^T, HG315674; *Diaminobutyricimonas aerilata* strain 6408J-67, JQ639052; *Labedella gwakjiensis* strain KSW2–17, DQ533552; *Lysinimonas soli* strain SGM3–12, JN378395; *Amnibacterium kyonggiense* strain KSL51201–037, FJ527819; *Klugiella xanthotipulae* strain 44C3, AY372075; *Agrevia bicolorata* strain VKM Ac-1804, AF159363; *Herbiconiux ginsengi* strain wged11, DQ473536; *Leifsonia aquatica* strain DSM 20146, D45057; *Schumannella luteola* strain KHIA, AB362159; *Rhodoglobus vestalii* strain LV3, AJ459101 *Microbacterium lacticum* strain DSM20427, X77441; *Agroccocus jenensis* strain DSM 9580, X92492; *Bergeyella zoohelcum* strain D658, M93153. Sequences were aligned with CLUSTALW, and phylogenetic inferences obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. *Bergeyella zoohelcum* strain D658 was used as outgroup. Scale bar = 10% nucleotide sequence divergence.

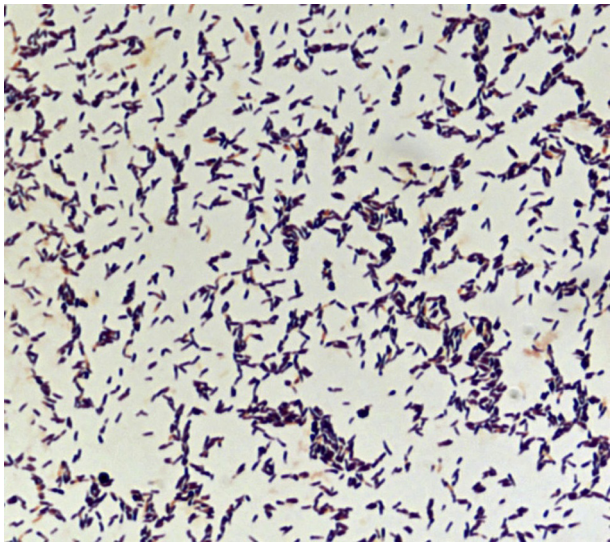


FIG. 2. Gram staining of *Diaminobutyricimonas massiliensis* strain FF2^T.

Leipzig, Germany). Twelve individual colonies were deposited on an MTP 384 MALDI-TOF target plate (Brüker). A total of 2 µL of matrix solution (saturated solution of alpha-cyano-4-hydroxycinnamic acid) in 50% acetonitrile and 2.5% trifluoro-acetic acid was distributed on each smear and air dried for 5 minutes at room temperature. The 12 individual spectra from strain FF2^T were imported into the MALDI BioTyper software (version 2.0; Brüker) and analysed by standard pattern matching (with default parameter settings) against the main spectra of 6252 bacterial spectra. The scores previously established by Brüker Daltonics to validate whether the species could be compared to the database of the instrument were applied. Briefly, a score $\geq$2.000 with a species with a validly published name provided allows identification at the species level; a score of $\geq$1.700 and <2.000 allows identification at the genus level; and a score of <1.700 does not allow any identification. A score ranging between 1.3 and 1.4 was obtained for strain FF2^T and did not allow any identification. We added the spectrum from strain FF2^T to our database (Fig. 4). Finally, the gel view showed spectral differences with other members of the family *Microbacteriaceae* (Fig. 5).

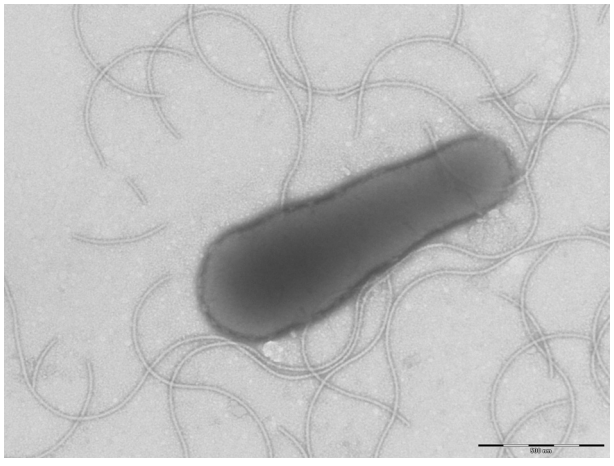


FIG. 3. Transmission electron microscopy of *Diaminobutyricimonas massiliensis* strain FF2^T. Cells are observed on Tecnai G20 operated at 200 keV. Scale bar = 500 nm.

Genome Sequencing Information

Genome project history

Strain FF2^T was selected for sequencing on the basis of its phylogenetic position and 16S rRNA similarity to other members of the family *Microbacteriaceae*. It was the first sequenced genome of a *Diaminobutyricimonas* species and the first genome of *Diaminobutyricimonas massiliensis* sp. nov. The GenBank accession number is CCSB00000000 and consists of 39 large contigs. Table 3 shows the project information and its association with MIGS version 2.0 compliance [20].

Growth conditions and DNA isolation

Diaminobutyricimonas massiliensis sp. nov., strain FF2^T (= CSUR P3023 = DSM 27836), was grown on Columbia agar enriched with 5% sheep's blood (bioMérieux) at 37°C in an aerobic atmosphere. Bacteria grown on five plates were collected and resuspended in 500 µL of Tris-EDTA buffer 10×. A total of 100 µL of this solution was then completed with 400 µL TE buffer 10×, 25 µL proteinase K, and 50 µL sodium dodecyl sulfate, then incubated overnight at 56°C for complete cells lysis. The next day, DNA was extracted using a phenol-chloroform protocol. The final DNA extract was resuspended in 65 µL EB buffer. The genomic DNA concentration was measured at 35.8 ng/µL using the Qubit assay with the high sensitivity kit (Life Technologies, Carlsbad, CA, USA).

Genome sequencing and assembly

Genomic DNA of *Diaminobutyricimonas massiliensis* was sequenced on the MiSeq sequencer (Illumina, San Diego, CA, USA) using two sequencing strategies: paired end and mate pair. The paired-end and mate-pair strategies were barcoded in order to be mixed, respectively, with ten other genomic projects prepared with the Nextera XT DNA sample prep kit (Illumina) and 11 other projects with the Nextera Mate-Pair sample prep kit (Illumina).

The genomic DNA was diluted to 1 ng/µL to prepare the paired-end library. The tagmentation step fragmented and tagged the DNA with an optimal size distribution at 4.5 kb. Then

TABLE 2. Differential phenotypic characteristics of *Diaminobutyricimonas massiliensis* strain FF2^T (data from this study), *Labedella gwakjiensis* strain KSW2–17 [37], *Diaminobutyricimonas aerilata* strain 6408J-67 [7], *Lysinimonas soli* strain SGM3–12 [38] and *Cryobacterium psychrophilum* strain DSM 4854 [39].

Property	*D. massilensis*	*D. aerilata*	*L. gwakjiensis*	*L. soli*	*C. psychrophilum*
Cell diameter (μm)	0.4–0.6	0.5–0.6	0.3–0.4	0.4–0.5	0.5–0.7
Gram stain	+	+	+	+	+
Motility	+	+	–	–	–
Endospore formation	–	–	–	–	–
Oxygen requirement	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Production of:					
Alkaline phosphatase	+	+	–	–	NA
Acid phosphatase	+	–	–	–	NA
Catalase	+	–	+	–	+
Oxidase	+	–	–	+	NA
Nitrate reductase	–	–	–	–	–
Urease	–	–	–	–	NA
α-Galactosidase	–	–	+	+	NA
β-Galactosidase	–	+	+	+	NA
β-Glucuronidase	–	–	–	–	NA
α-Glucosidase	+	+	+	+	NA
β-Glucosidase	–	+	+	–	NA
Esterase	+	–	–	+	NA
Esterase lipase	+	+	+	+	NA
Acid from:					
Ribose	–	–	–	–	–
Mannose	–	+	+	+	+
D-Fructose	–	+	+	NA	+
D-Glucose	–	+	+	+	+
Habitat	Human blood	Air sample	Dried seaweed	Soil	Soil

NA, data not available.

limited-cycle PCR amplification (12 cycles) completed the tag adapters and introduced dual-index barcodes. After purification on AMPure XP beads (Beckman Coulter, Fullerton, CA, USA), the libraries were then normalized on specific beads according to the Nextera XT protocol (Illumina). Normalized libraries were pooled into a single library for sequencing on the MiSeq. The pooled single-strand library was loaded onto the reagent cartridge and then onto the instrument, along with the flow cell. Automated cluster generation and paired-end sequencing with dual index reads were performed in a single 39-hour run at 2 × 250 bp.

Total information of 3.89 Gb was obtained from a 654 K/mm^2 cluster density with a cluster passing quality control filters of 93.7% (12 204 000 clusters). Within this run, the index

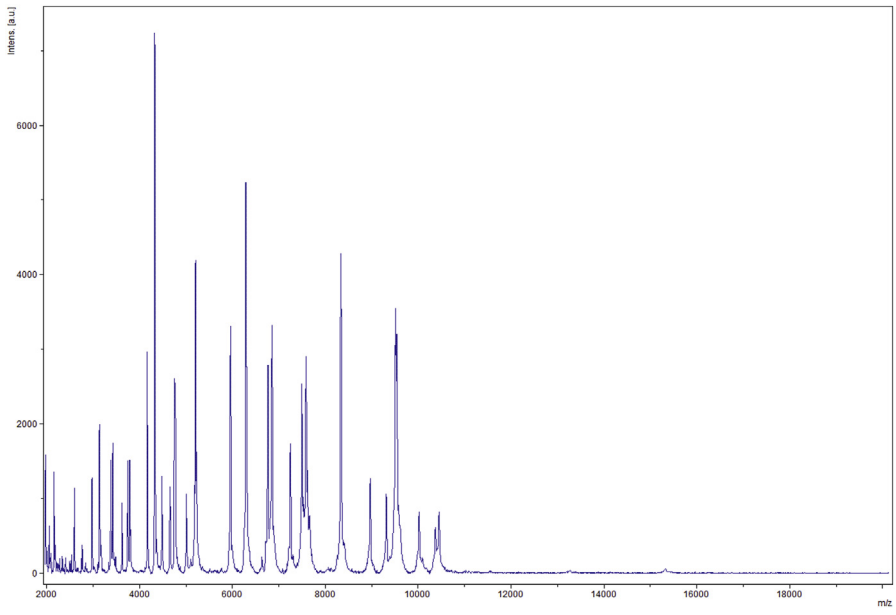


FIG. 4. Reference mass spectrum from *Diaminobutyricimonas massiliensis* strain FF2^T. This reference spectrum was generated by comparison of 12 individual colonies.

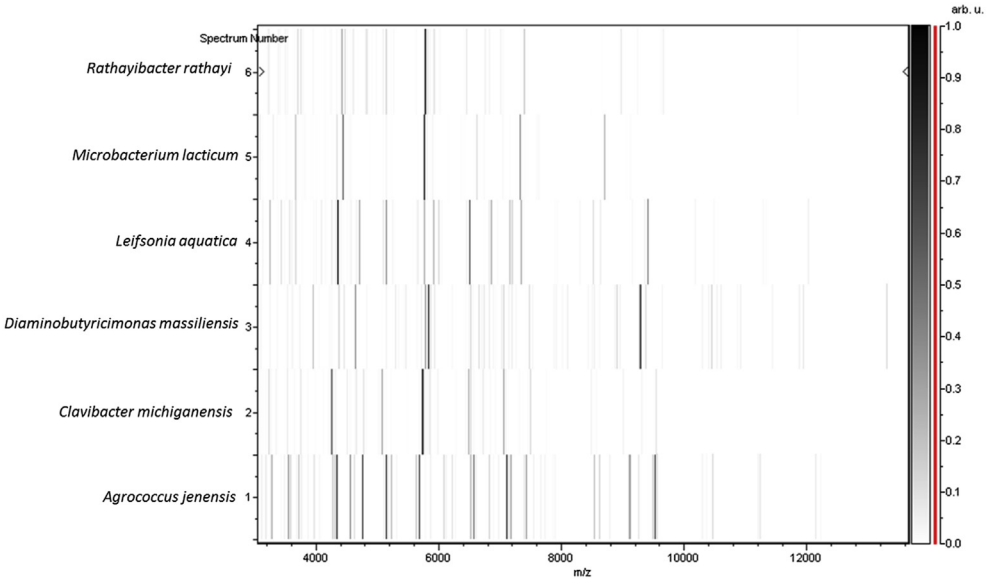


FIG. 5. Gel view comparing *Diaminobutyricimonas massiliensis* strain FF2^T spectrum to other members of family *Microbacteriaceae*. Gel view displays raw spectra of all loaded spectrum files arranged in pseudo-gel-like look. X-axis records m/z value. Left y-axis displays running spectrum number originating from subsequent spectra loading. Peak intensity is expressed by greyscale scheme code. Colour bar and right y-axis indicate relation between colour peak is displayed; peak intensity in arbitrary units. Displayed species are indicated at left.

representation for *Diaminobutyricimonas massiliensis* was determined to 4.72%. The 539 968 reads were filtered according to the read qualities.

The mate-pair library was prepared with 1 µg of genomic DNA using the Nextera mate-pair Illumina guide. The genomic DNA sample was simultaneously fragmented and tagged with a mate-pair junction adapter. The profile of the fragmentation was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) with a DNA 7500 labchip. The DNA fragments ranged in size from 1 kb up to 6.6 kb, with an optimal size at 4.5 kb. No size selection was performed, and 368 ng of tagmented fragments was circularized. The circularized DNA was mechanically sheared to small fragments on the Covaris device S2 in microtubes (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent Technologies) with an optimal peak at 504 bp. The libraries were normalized at 2 nM and pooled. After a denaturation step and dilution at 10 pM, the pool of libraries was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. Automated cluster generation and sequencing run were performed in a single 39-hour run at 2 × 250 bp. *Diaminobutyricimonas massiliensis* was determined to 9.49%. The 1 884 171 reads were filtered according to the read qualities.

TABLE 3. Project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	High-quality draft
MIGS-28	Libraries used	Paired end and mate pair 9 kb library
MIGS-29	Sequencing platform	Illumina MiSeq
MIGS-31.2	Fold coverage	95×
MIGS-30	Assemblers	Newbler version 2.5.3
MIGS-32	Gene calling method	Prodigal
	Locus tag	Not indicated
	GenBank ID	CCSB00000000
	GenBank date of release	8 September 2013
	GOLD ID	Gp0102104
	BioProject ID	PRJEB4274
MIGS-13	Source material identifier	DSM 27836
	Project relevance	Study of causes of nonmalarial fever

Genome annotation

Open reading frame (ORF) prediction was performed using Prodigal [21] with default parameters. We removed the predicted ORFs if they spanned a sequencing gap region. Functional assessment of protein sequences was performed by comparing them with sequences in the GenBank [22], and Clusters of Orthologous Groups (COGs) databases using BLASTP. tRNAs, rRNAs, signal peptides and transmembrane helices were identified using tRNAscan-SE 1.21 [23], RNAmmer [24], SignalP [25] and TMHMM [26], respectively. Artemis [27] was used for data management, while DNA Plotter [28] was used for visualization of genomic features. In-house perl and bash scripts were used to automate these routine tasks. ORFans were sequences which have no homology in a given database, i.e. in a nonredundant (nr) or identified if their BLASTP E value was lower than $1e^{-03}$ for alignment lengths greater than 80 amino acids. If alignment lengths were smaller than 80 amino acids, we used an E value of $1e^{-05}$.

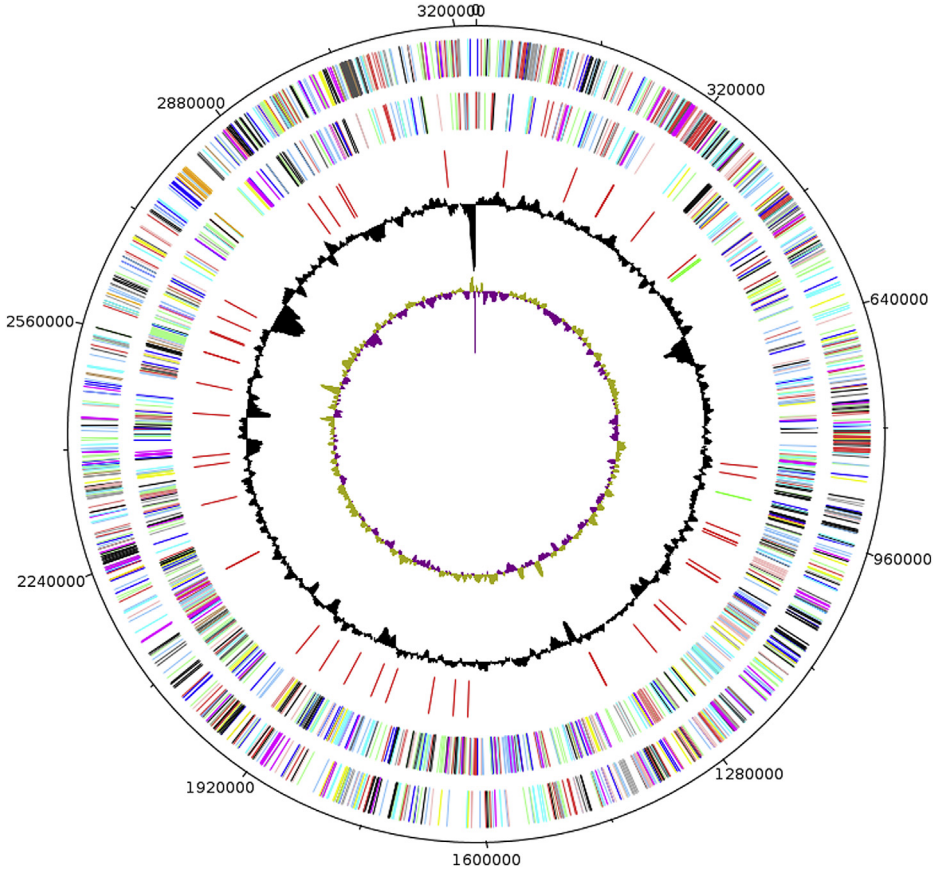
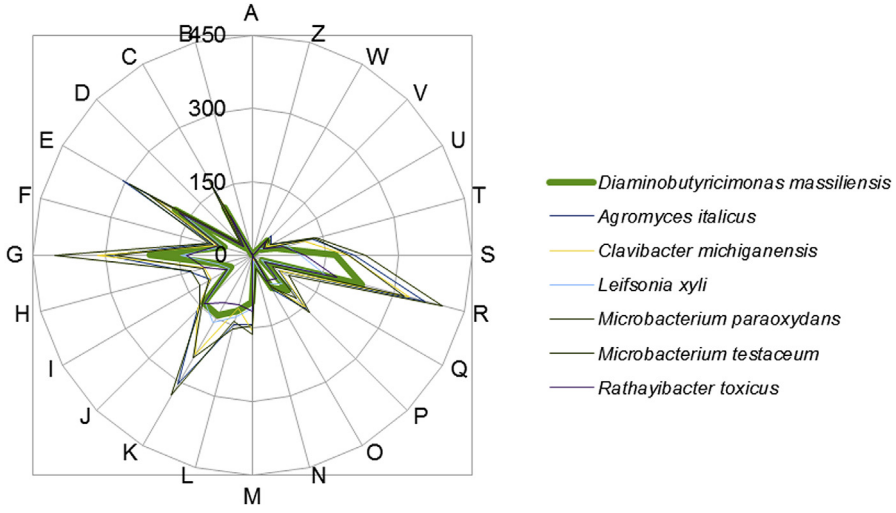


FIG. 6. Distribution of functional classes of predicted genes of *Diaminobutyricimonas massiliensis* strain FF2^T along with other genomes belonging to *Microbacteriaceae* family according to clusters of orthologous groups of proteins.

FIG. 7. Graphical circular map of *Diaminobutyricimonas massiliensis* strain FF2 ^{T} chromosome. From outside in, outer two circles show open reading frames oriented in forward (coloured by COGs categories) and reverse (coloured by COGs categories) directions, respectively. Third circle marks rRNA gene operon (green) and tRNA genes (red). Fourth circle shows G+C% content plot. Innermost circle displays GC skew, with purple and olive indicating negative and positive values, respectively.



PHAST was used to identify, annotate and graphically display prophage sequences within bacterial genomes or plasmids [29].

To estimate the nucleotide sequence similarity at the genome level between *Diaminobutyricimonas massiliensis* and another six members of the family of *Microbacteriaceae,* we determined the average genomic identity of orthologous gene sequences (AGIOS) parameter as follows: orthologous proteins were detected using the Proteinortho software (with the following parameters: E value $1e^{-5}$, 30% identity, 50% coverage and algebraic connectivity of 50%) [30] and genomes compared two by two (Fig. 6). After fetching the corresponding nucleotide sequences of orthologous proteins for each pair of genomes, we determined the mean percentage of nucleotide sequence identity using the Needleman-Wunsch global alignment algorithm. The script created to calculate AGIOS values was named MAGi (Marseille Average genomic identity) and was written in perl and bioperl modules.

Genome properties

The genome of *Diaminobutyricimonas massiliensis* strain FF2^T is 3 227 513 bp long with a 70.13% G+C content (Fig. 7). Of the 3147 predicted genes, 3091 were protein-coding genes and 56 were RNAs. A total of 1937 genes (62.66%) were assigned a putative function. The properties and statistics of the genome are summarized in Table 4. The distribution of genes into COGs functional categories is presented in Table 5.

Insights From the Genome Sequence

Extended insights

We compared the genome from *Diaminobutyricimonas massiliensis* strain FF2^T to those of other members of the *Microbacteriaceae* family, including *Agromyces italicus* strain DSM 16388 (GenBank accession number ATXF00000000), *Clavibacter michiganensis* strain NCPPB 382 (AM711867), *Leifsonia xyli* strain CTCB07 (AE016822), *Microbacterium paraoxydans* strain 77MFTsu3.2 (AQYI00000000), *Microbacterium testaceum* strain StLB037 (AP012052) and *Rathayibacter toxicus* strain DSM 7488 (AUDF00000000). The draft genome of *Diaminobutyricimonas massiliensis* (3.22 Mb) is larger than that of *L. xyli* and *R. toxicus* (2.58 and 2.3 Mb, respectively) but is smaller than those of *C. michiganensis, M. paraoxydans* and *M. testaceum* (3.39, 3.47 and 3.98 Mb, respectively). The G+C content of *Diaminobutyricimonas massiliensis* (70.13%) is higher than those of *L. xyli, M. paraoxydans* and *R. toxicus* (67.70%, 69.50% and 61.50%, respectively) but lower than those of *A. italicus, M. testaceum* and *C. michiganensis* (70.2%, 70.30% and 72.53%, respectively). The gene content of *Diaminobutyricimonas massiliensis* (3147 genes) is larger than that of *L. xyli* and *R. toxicus* (2373 and 2188 genes, respectively) and smaller than those of *C. michiganensis, A. italicus, M. paraoxydans* and *M. testaceum* (3167, 3399, 3403 and 3727 genes, respectively). The AGIOS values between *D. massiliensis* and other members of the family *Microbacteriaceae* ranged from 68.85% with *R. toxicus* to 73.55% with *C. michiganensis* (Table 6).

TABLE 4. Nucleotide content and gene count levels of genome

Attribute	Value	% Of total[a]
Genome size (bp)	3 227 513	
DNA coding (bp)	2 925 192	90.63
DNA G+C (bp)	2 263 455	70.13
DNA scaffolds	ND	
Total genes	3147	100
Protein-coding genes	3091	98.22
RNA genes	56	
Pseudo genes	31	1.0
Gens in internal clusters	215	6.955
Genes with function prediction	1937	62.66
Genes assigned to COGs	2233	72.24
Genes with Pfam domains	172	5.56
Genes with peptide signals	186	6.017
Genes with transmembrane helices	846	27.36
ORFan genes	780	25.23
CRISPR repeats	4	

COGs, Clusters of Orthologous Groups; ND, not determined.
[a]Total is based on either genome size (in base pairs) or total number of protein coding genes in annotated genome.

TABLE 5. Number of genes associated with general COGs functional categories

Code	Value	Percentage[a]	Description
J	141	4.56	Translation, ribosomal structure and biogenesis
A	1	0.03	RNA processing and modification
K	142	4.59	Transcription
L	118	3.82	Replication, recombination and repair
B	0	0.00	Chromatin structure and dynamics
D	18	0.58	Cell cycle control, cell division, chromosome partitioning
V	42	1.36	Defense mechanisms
T	51	1.65	Signal transduction mechanisms
M	97	3.14	Cell wall/membrane biogenesis
N	13	0.42	Cell motility
U	21	0.68	Intracellular trafficking and secretion
O	78	2.52	Posttranslational modification, protein turnover, chaperones
C	112	3.62	Energy production and conversion
G	210	6.79	Carbohydrate transport and metabolism
E	183	5.92	Amino acid transport and metabolism
F	59	1.91	Nucleotide transport and metabolism
H	72	2.33	Coenzyme transport and metabolism
I	50	1.62	Lipid transport and metabolism
P	103	3.33	Inorganic ion transport and metabolism
Q	23	0.74	Secondary metabolites biosynthesis, transport and catabolism
R	233	7.54	General function prediction only
S	170	5.50	Function unknown
—	296	9.57	Not in COGs

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

Conclusion

On the basis of phenotypic, phylogenetic and genomic analyses, we formally propose the creation of *Diaminobutyricimonas*

TABLE 6. Numbers of orthologous proteins shared between genomes (upper right) and AGIOS values (lower left)

	D. massiliensis	*A. italicus*	*C. michiganensis*	*L. xyli*	*M. paraoxydans*	*M. testaceum*	*R. toxicus*
D. massiliensis	3091[a]	72.99	73.55	72.96	70.61	70.83	68.85
A. italicus	1405	3376[a]	73.34	73.70	71.66	71.86	68.98
C. michiganensis	1347	1356	2983[a]	73.21	71.29	71.34	68.98
L. xyli	1045	1081	1072	2028[a]	70.61	70.97	69.34
M. paraoxydans	1351	1442	1397	1059	3514[a]	75.30	67.08
M. testaceum	1368	1515	1455	1074	1687	3676[a]	67.24
R. toxicus	1050	1068	1105	953	1072	1119	2188[a]

AGIOS, average genomic identity of orthologous gene sequences.
Diaminobutyricimonas massiliensis strain FF2^T; *Agromyces italicus* strain DSM 16388; *Clavibacter michiganensis* strain NCPPB; *Leifsonia xyli* strain CTCB07; *Microbacterium paraoxydans* strain 77MFTsu3.2; *Microbacterium testaceum* strain StLB037; *Rathayibacter toxicus* strain DSM 7488.
[a]Numbers of proteins per genome.

massiliensis sp. nov. that contains the strain FF2^T as type strain. This bacterial strain was isolated from a blood specimen from a 35-year-old Senegalese man with unexplained fever.

Description of *Diaminobutyricimonas massiliensis* sp. nov.

Diaminobutyricimonas massiliensis (mas•si•li•e'n.sis. L. masc. n. *massiliensis,* of Massilia, the Latin name for Marseille, France, where type strain FF2^T was characterized).

Colonies are 0.8 mm in diameter and light yellow on agar enriched with 5% sheep's blood. Cells are Gram positive, rod shaped, motile and aerobic, with a mean diameter and length of 0.5 and 1.3 µm, respectively. Optimal growth was observed after 48 hours on agar enriched with 5% sheep's blood at 37°C in an aerobic atmosphere supplemented with 5% CO_2. *Diaminobutyricimonas. massiliensis* is positive for catalase, oxidase, alkaline phosphatase, N-acetyl-β-glucosaminidase, esterase, esterase-lipase, leucine arylamidase, acid phosphatase, α-glucosidase and esculin ferric citrate. Negative reactions were observed for D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, D-trehalose, D-turanose, D-fucose, starch, glycogen, D-maltose, amygdalin, N-acetyl-glucosamine, methyl-αD-glucopyranoside, D-cellobiose, α-chymotrypsin, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, β-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase and α-fucosidase. *Diaminobutyricimonas massiliensis* is susceptible to penicillin, ceftriaxone, imipenem, ciprofloxacin, gentamicin, rifampicin, vancomycin and doxycycline but resistant to erythromycin, nitrofurantoin, metronidazole and trimethoprim/sulfamethoxazole. The G+C content of the genome is 70.13%. The 16S rRNA and genome sequences are deposited in GenBank under accession numbers HG315674 and CCSB00000000, respectively.

The type strain FF2^T (= CSUR P3023 = DSM 27836) was isolated from the blood of a Senegalese man with unexplained fever.

Conflict of Interest

None declared.

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