Noncontiguous finished genome sequence and description of *Necropsobacter massiliensis* sp. nov.

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Abstract

Strain FF6^T was isolated from the cervical abscess of a 4-year-old Senegalese boy, in Dakar, Senegal. MALDI-TOF MS did not provide any identification. This strain exhibited a 95.17% 16S rRNA sequence identity with *Necropsobacter rosorum*. Using a polyphasic study including phenotypic and genomic analyses, strain FF6^T was an aero-anaerobic Gram-negative cocobacillus, oxidase positive, and exhibited a genome of 2,493,927 bp (1 chromosome but no plasmid) with a G+C content of 46.2% that coded 2,309 protein-coding and 53 RNA genes. On the basis of these data, we propose the creation of *Necropsobacter massiliensis* sp. nov.

Keywords: Culturomics, genome, *Necropsobacter massiliensis*, Senegal, taxono-genomics

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Introduction

The genus *Necropsobacter* (Christensen *et al.* 2011) was first described in 2011 [1]. At this time, there is only one species with a validly published name [2]. In 2013, five clinical cases of bacteraemia associated with *Necropsobacter rosorum* were reported [3]. Members of the genus *Necropsobacter* were previously associated with the SP group that comprised mainly strains isolated from rabbits, rodents and humans [3]. Because *Necropsobacter rosorum* was the only described species in this genus with no genome available, we first sequenced its genome for genomic comparison [4]. *Necropsobacter massiliensis* strain FF6^T (= Collection de souches de l'Unité des Rickettsies (CSUR) P3511 = Deutsche Sammlung von Mikroorganismen (DSM) = 27814) was isolated from a patient with a cervical abscess hospitalized at Hôpital Principal in Dakar, Senegal. *N. massiliensis* is Gram negative, aeroanaerobic, indole negative, nonmotile, and coccobacillus. This bacterium was cultivated as part of the implementation of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) in Hôpital Principal, Dakar, aiming at improving the routine laboratory identification of bacterial strains in Senegal [5].

The current taxonomic classification of prokaryotes relies on a combination of phenotypic and genotypic characteristics [6,7], including 16S rRNA sequence similarity, G+C content and DNA-DNA hybridization. However, these tools suffer from various drawbacks, mainly as a result of their threshold values, which are not applicable to all species or genera [8,9]. With the development of cost-effective high-throughput sequencing techniques, tens of thousands of bacterial genome sequences have been made available in public databases [9]. Recently we developed a strategy, taxonomogenomics, in which genomic and phenotypic characteristics, notably the MALDI-TOF spectrum, are systematically compared to the phylogenetically closest species with standing in nomenclature [8–10].

Here we present a summary classification and a set of features for *Necropsobacter massiliensis* sp. nov. strain FF6^T, together with the description of the complete genomic sequencing and annotation. These characteristics support the circumscription of the species *Necropsobacter massiliensis.*

Organism Information

Classification and features

Since July 2012, the Hôpital Principal in Dakar, Senegal, has been equipped with a MALDI-TOF (Vitek MS RUO;

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bioMérieux, Marcy l'Etoile, France) to improve the microbiology laboratory work flow by enabling rapid bacterial identification. Isolates that are poorly identified using MALDI-TOF are referred to the URMITE laboratory in Marseille, France, for further identification. Strain FF6^T (Table 1) was isolated by cultivation on 5% sheep's blood–enriched Columbia agar (bioMérieux) from the cervical abscess of a 4-year-old Senegalese boy. Strain FF6^T exhibited a 95.17% 16S rRNA sequence identity with *Necropsobacter rosorum* [1], the phylogenetically closest bacterial species with a validly published name (Fig. 1). These values were lower than the 98.7% 16S rRNA gene sequence threshold recommended by Meier-Kolthoff *et al.* [11] to delineate a new species within phylum *Firmicutes* without carrying out DNA-DNA hybridization.

Different growth temperatures (25°C, 30°C, 37°C, 45°C and 56°C) were tested. Growth was obtained between 37°C and 45°C, with the optimal growth temperature being 37°C. 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 observed between 37°C and 45°C under aerobic and microaerophilic conditions. Colonies were 1 mm in diameter, grey and nonhaemolytic on 5% sheep's blood–enriched Columbia agar (bioMérieux). *Necropsobacter massiliensis* is Gram negative, coccobacillus, not motile, and unable to form spores (Fig. 2). Under electron microscopy, cells had a mean length of 1.5 µm (range, 0.9–2.1 µm) and a mean diameter of 0.4 µm (range, 0.2–0.6 µm) (Fig. 3).

Strain FF6^T was oxidase positive and catalase negative. Using an API ZYM strip (bioMérieux), positive reactions were observed for alkaline phosphatase, esterase, leucine arylamidase, phosphatase acid, α-glucosidase and naphthol-AS-BI-phosphohydrolase. Negative reactions were noted for α-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase, N-acetyl-β-glucosaminidase, lipase, α-chrymotrypsin and cystine arylamidase. Using API 50CH, positive reactions were observed for glycerol, ribose, D-xylose, D-mannose, D-glucose, inositol, N-acetyl glucosamine, D-fructose, D-maltose D-melibiose, D-trehalose, D-saccharose, D-raffinose, starch, potassium 5-ketogluconate, alkaline phosphatase, esterase, leucine arylamidase, phosphatase acid, α-glucosidase and naphthol-AS-BI-phosphohydrolase. Negative reactions were observed for D-mannitol, D-sorbitol, L-xylose, D-adonitol, methyl β-D-xylopyranose, D-melezitose, inulin, α-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase, N-acetyl-β-glucosaminidase, lipase, α-chrymotrypsin and cystine arylamidase. *Necropsobacter massiliensis* strain FF6^T is susceptible to amoxicillin, amoxicillin/clavulanic acid, ceftriaxone, gentamicin, nitrofurantoin, trimethoprim/sulfamethoxazole, rifampicin and ciprofloxacin but resistant to erythromycin, doxycycline and vancomycin. Five species validly published names in the *Pasteurellaceae* family were selected to make a phenotypic comparison with *Necropsobacter massiliensis* (Table 2).

TABLE 1. Classification and general features of *Necropsobacter massiliensis* strain FF6^T

MIGS ID	Property	Term	Evidence code[a]
	Classification	Domain: *Bacteria*	TAS [28]
		Phylum: *Proteobacteria*	TAS [29]
		Class: *Gammaproteobacteria*	TAS [30]
		Order: *Pasteurellales*	TAS [31]
		Family: *Pasteurellaceae*	TAS [31,32]
		Genus: *Necropsobacter*	TAS [1]
		Species: *Necropsobacter massiliensis*	IDA
		(Type) strain: FF6^T	IDA
	Gram stain	Negative	IDA
	Cell shape	Rods	IDA
	Motility	None motile	IDA
	Sporulation	Non–spore forming	NAS
	Temperature range	37–45°C	IDA
	Optimum temperature	37°C	IDA
	pH range; optimum	6.2–7.6; 7	
	Carbon source	Unknown	
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	
MIGS-4	Geographic location	Senegal	IDA
MIGS-5	Sample collection	April 2013	IDA
MIGS-4.1	Latitude	14.6937000	IDA
MIGS-4.1	Longitude	−17.4440600	IDA
MIGS-4.4	Altitude	12 m above sea level	IDA

[a]IDA, inferred from direct assay; TAS, traceable author statement (i.e. a 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 on anecdotal evidence). These evidence codes are from the Gene Ontology project (http://www.geneontology.org/GO.evidence.shtml) [33]. If the evidence is IDA, then the property was directly observed for a live isolate by one of the authors or an expert mentioned in the acknowledgements.

Extended features descriptions

MALDI-TOF protein analysis was carried out as previously described [12,13] using a Microflex LT (Bruker Daltonics, Leipzig, Germany). Twelve individual colonies were deposited on a MTP 384 MALDI-TOF target plate (Bruker). A total of 2 µL of matrix solution (saturated solution of alpha-cyano-4-hydroxycinnamic acid) in 50% acetonitrile and 2.5% trifluoroacetic acid were distributed on each smear and air dried for 5 minutes at room temperature. The 12 individual spectra from strain FF6^T were imported into MALDI BioTyper software 2.0 (Bruker) and analysed by standard pattern matching (with default parameter settings) against the main spectra of 6252 bacterial spectra. The scores previously established by Bruker Daltonics allowing (or not) validating the identification of species compared to the database of the instrument were applied. Briefly, a score $\geq$2.000 with a species with a validly published

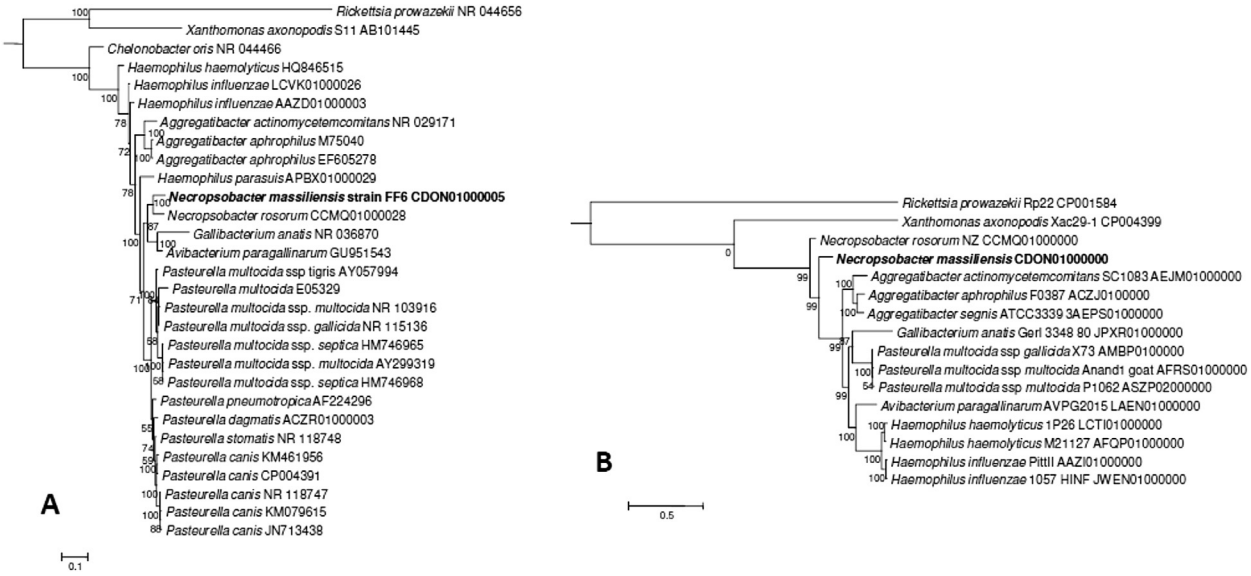

FIG. 1. Phylogenetic trees highlighting position of *Necropsobacter massiliensis* sp. nov. strain FF6 relative to *Pasteurellaceae* type strains. Sequences of 16S rRNA (rrs) gene (A) and concatenated *groEL* and *rpoB* genes (B) were aligned by CLUSTALW, and phylogenetic inferences were obtained from Bayesian phylogenetic analysis. GTR+Ã substitution model was used for rrs-based tree (A) and GTR+Ã, SYM+Ã and GTR+Ã for first, second and third codon position, respectively, for *groEL*/*rpoB* tree (B). GenBank accession numbers of sequences, genomes or shotgun contigs from which gene sequences were extracted are indicated at end. Numbers at nodes are bootstrap values obtained by repeating analysis 100 times to generate majority consensus tree. There were total 1397 (A) and 5814 (1641 for *groEL* and 4173 for *rpoB*) (B) positions in final data set. Scale bar = 10% nucleotide sequence divergence.

name provided allows the identification at the species level; a score of ≥1.700 to <2.000 allows the identification at the genus level; and a score of <1.700 does not allow any identification. Thus, scores ranging from 1.2 to 1.3 were obtained, suggesting that this isolate was not a member of any known species. The reference mass spectrum from strain FF6^T was incremented in our database (Fig. 4). Finally, the gel view showed the spectral differences with other members of the family *Pasteurellaceae* (Fig. 5).

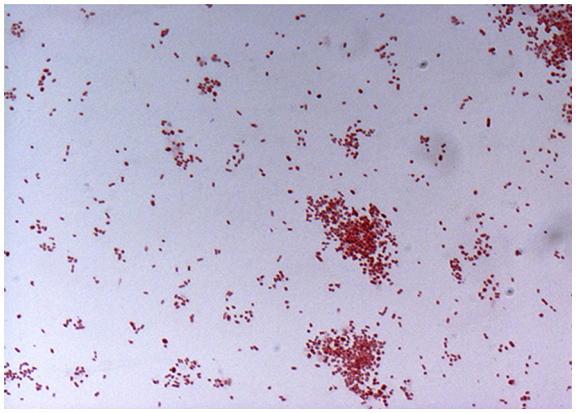

FIG. 2. Gram staining of *Necropsobacter massiliensis* strain FF6^T.

Genome Sequencing Information

Genome project history

The organism was selected for sequencing on the basis of its 16S rRNA similarity, phylogenetic position and phenotypic differences with other members of the family *Pasteurellaceae*, and is part of a study aiming at using MALDI-TOF for the routine identification of bacterial isolates in Hôpital Principal in Dakar [1]. It is the second genome of a *Necropsobacter* species and the first genome of *Necropsobacter massiliensis* sp. nov. A summary of the project information is shown in Table 3. The GenBank accession number is CDON00000000 and consists of 101 contigs. Table 3 shows the project information and its association with MIGS version 2.0 compliance [14]; associated MIGS records are also summarized in Supplementary Table S1.

Growth conditions and genomic DNA preparation

Necropsobacter massiliensis strain FF6^T (= CSUR P3511 = DSM 27814) was grown aerobically on 5% sheep's blood–enriched Columbia agar (bioMérieux) at 37°C. Bacteria grown on four petri dishes were resuspended in 5 × 100 µL of TE buffer; 150 µL of this suspension was diluted in 350 µL TE buffer

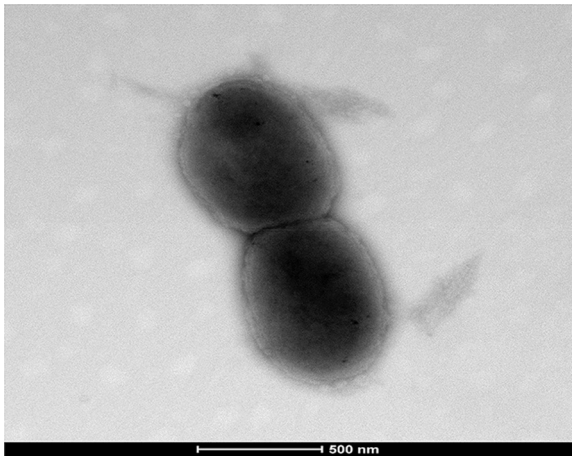

FIG. 3. Transmission electron microscopy of *Necropsobacter massiliensis strain* FF6^T strain. Cells are observed on Tecnai G20 transmission electron microscope operated at 200 keV. Scale bar = 500 µm.

10 × , 25 µL proteinase K and 50 µL sodium dodecyl sulfate for lysis treatment. This preparation was incubated overnight at 56°C. Extracted DNA was purified using three successive phenol–chloroform extractions and ethanol precipitation at −20°C of minimum 2 hours each. After centrifugation, the DNA was suspended in 65 µL EB buffer. The genomic DNA concentration was measured at 30.06 ng/µL using the Qubit assay with the high sensitivity kit (Life Technologies, Carlsbad, CA, USA).

Genome sequencing and assembly

Genomic DNA of *Necropsobacter massiliensis* FF6^T was sequenced on the MiSeq sequencer (Illumina, San Diego, CA, USA) with the Paired-end and Mate-pair strategies. The paired-end and the 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).

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 1.5 kb. Then 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 single 39-hour runs at 2 × 250 bp.

Total information of 3.89 GB was obtained from a 416 k/mm^2 cluster density with a cluster passing quality control filters of 95.4% (7 899 000 clusters). Within this run, the index representation for *Necropsobacter massiliensis* was determined to

TABLE 2. Differential characteristics of *Necropsobacter massiliensis* strain FF6^T with *Necropsobacter rosorum* [1], *Actinobacillus actinomycetemcomitans* [34,35], *Haemophilus influenzae* [34–36] and *Pasteurella multocida* [34–37]

Character	*Necropsobacter massiliensis*	*Necropsobacter rosorum*	*Actinobacillus actinomycetemcomitans*	*Haemophilus influenzae*	*Pasteurella multocida*
Cell diameter (µm)	0.4	NA	0.5		
Gram stain	−	−	−	−	−
Motility	−	−	−	−	−
Endospore formation	−	NA	−	NA	NA
Production of					
Alkaline phosphatase	+	NA	+	+	Variable
Acid phosphatase	+	NA	+	NA	Variable
Catalase	−	+	+	+	+
Oxidase	+	+	−	+	+
β-Haemolysis	−	−	−	−	−
Urease	−	−	−	+	−
Indole	−	−	NA	+	+
Nitrate reductase	+	+	+	+	+
α-Galactosidase	−	+	NA	−	NA
β-Galactosidase					
α-glucosidase (PNPG)	+	+	NA	−	NA
β-Glucosidase	−	−	NA	−	NA
Esterase	+	NA	Variable	−	Variable
Esterase lipase	−	NA	Variable	NA	Variable
N-acetyl-β-glucosaminidase	−	NA	NA	NA	NA
Utilization of					
D-Fructose	+	−	+	NA	+
D-Mannose	+	+	+	NA	+
D-Xylose	+	+	Variable	+	+
D-Glucose	+	+	+	+	NA
Habitat	Human	Guinea pig	Human	Human	Human and animal

NA, data not available.

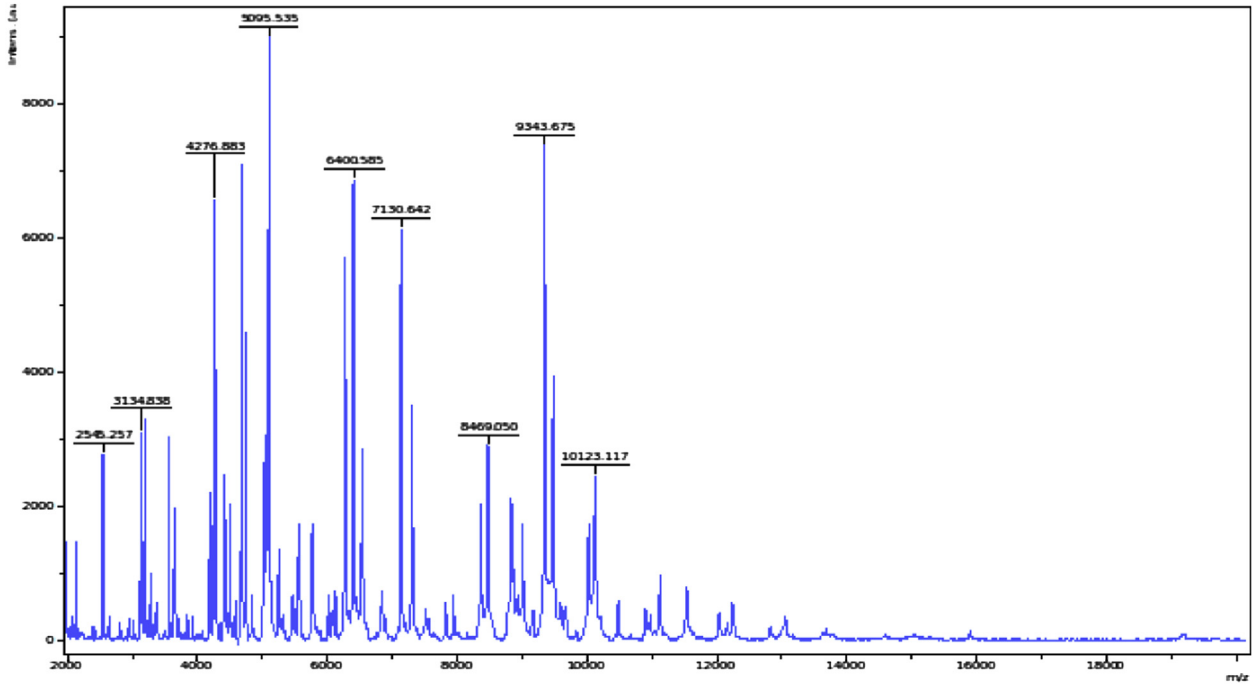

FIG. 4. Reference mass spectrum from *Necropsobacter massiliensis* strain FF6^T. Spectra from 12 individual colonies were compared and reference spectrum was generated.

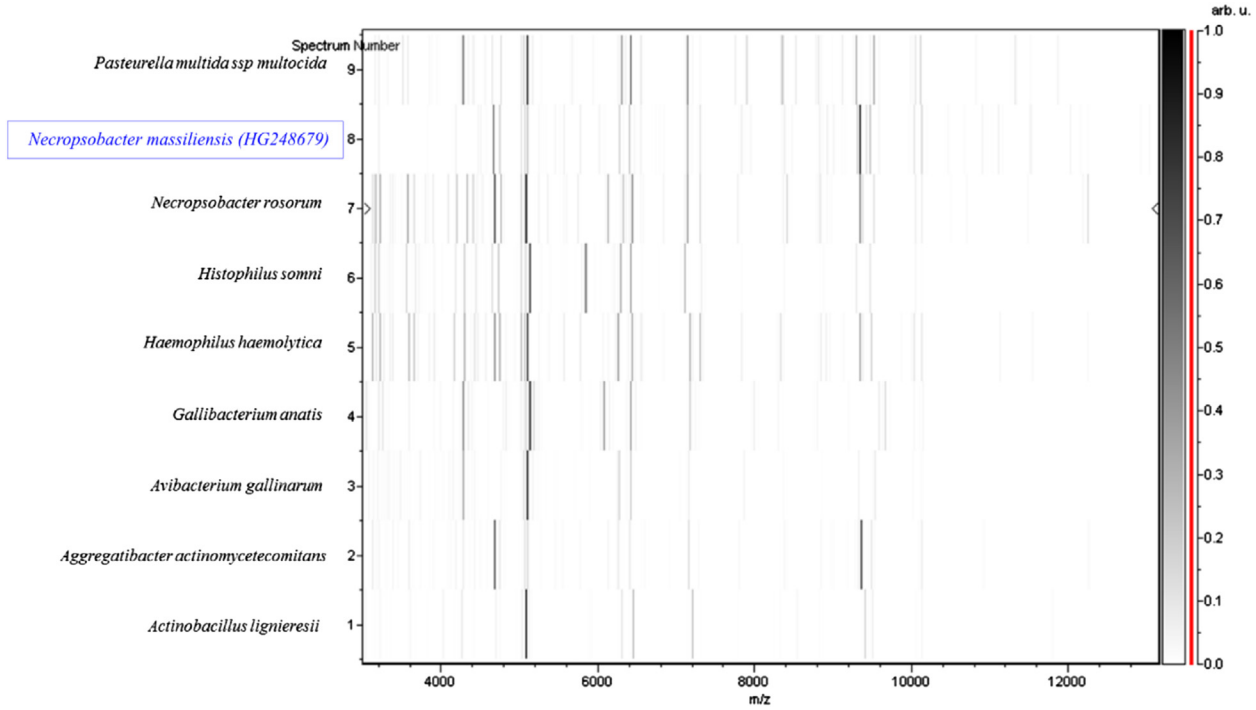

FIG. 5. Gel view comparing *Necropsobacter massiliensis* strain FF6^T to members of family *Pasteurellaceae*. Gel view displays raw spectra of all loaded spectrum files arranged in pseudo-gel-like look. X-axis records m/z value; left y-axis displays running spectrum number originating from subsequent spectra loading. Peak intensity is expressed by greyscale scheme code. Colour bar and right y-axis indicating relation between colour peak is displayed; peak intensity indicated arbitrary units. Displayed species are indicated at left.

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 platforms	MiSeq
MIGS-31.2	Fold coverage	141×
MIGS-30	Assemblers	CLC 7
MIGS-32	Gene calling method	Prodigal
	Locus Tag	Not indicated
	GenBank ID	CDON00000000
	GenBank date of release	26 March 2015
	GOLD ID	Gp0102103
	BIOPROJECT	PRJEB4626
	Source material identifier	DSM 27814
	Project relevance	MALDI-TOF implementation in Dakar

MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

be 7.02% and to present 529 002 reads 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 were circularized. The circularized DNA was mechanically sheared to small fragments on the Covaris S2 device in microtubes (Covaris, Woburn, MA, USA).The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent) with an optimal peak at 672 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. *Necropsobacter massiliensis* strain FF6^T was determined to be 6.86%. The 639 775 reads were filtered according to the read qualities.

Genome annotation

Open reading frame (ORF) prediction was carried out using Prodigal [15] 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 [16] and the Clusters of Orthologous Groups (COGs) databases using BLASTP. tRNAs, rRNAs, signal peptides and transmembrane helices were identified using tRNAscan-SE 1.21 [17], RNAmmer [18], SignalP [19] and TMHMM [20], respectively. Artemis [21] was used for data management, whereas DNA Plotter [22] 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 nonredundant (nr) or identified if their BLASTP E value was lower than 1e-03 for alignment lengths greater than 80 amino acids. PHAST was used to identify, annotate and graphically display prophage sequences within bacterial genomes or plasmids [23].

To estimate the nucleotide sequence similarity at the genome level between *Necropsobacter massiliensis* and another ten members of the *Pasteurellaceae* family, we determined the average genomic identity of orthologous gene sequences (AGIOS) parameter as follows: orthologous proteins were detected using the Proteinortho software [24] (with the following parameters: E-value 1e-5, 30% percentage identity, 50% coverage and 50% algebraic connectivity) and genomes compared two by two. 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. Genomes from the genus *Necropsobacter* and closely related genera were used for the calculation of AGIOS values. The script created to calculate AGIOS values was named MAGi (Marseille Average genomic identity) and is written in perl and bioperl modules. GGDC analysis was also performed using the GGDC Web server (http://ggdc.dsmz.de) as previously reported [25,26].

Here, we compared the genome sequences of *Necropsobacter massiliensis* strain FF6^T (GenBank accession number CDON00000000) with those of *N. rosorum* strain P709T (CCMQ00000000), *Pasteurella multocida* subsp. *multocida* strain Pm70 (AE004439), *Haemophilus influenzae* strain Rd KW20 (L42023), *Haemophilus ducreyi* strain 35000HP (AE017143), *Histophilus somnus* strain 129PT (CP000436), *Haemophilus parasuis* strain SH0165 (CP001321), *Haemophilus parainfluenzae* strain T3T1 (FQ312002) and *Aggregatibacter aphrophilus* strain NJ8700 (CP001607).

Genome properties

The genome of *Necropsobacter massiliensis* strain FF6^T is 2 493 927 bp long with a 46.2% G+C content (Fig. 6). Of the 2363 predicted genes, 2309 were protein coding genes and 54 were RNA genes including 1 complete rRNA operon. A total of 1838 genes (77.7%) were assigned a putative function. A total of 210 were identified as ORFans (9.09%). The remaining genes were annotated as hypothetical proteins. The properties and the statistics of the genome are presented in Table 4. The distribution of genes into COGs functional categories is summarized in Table 5.

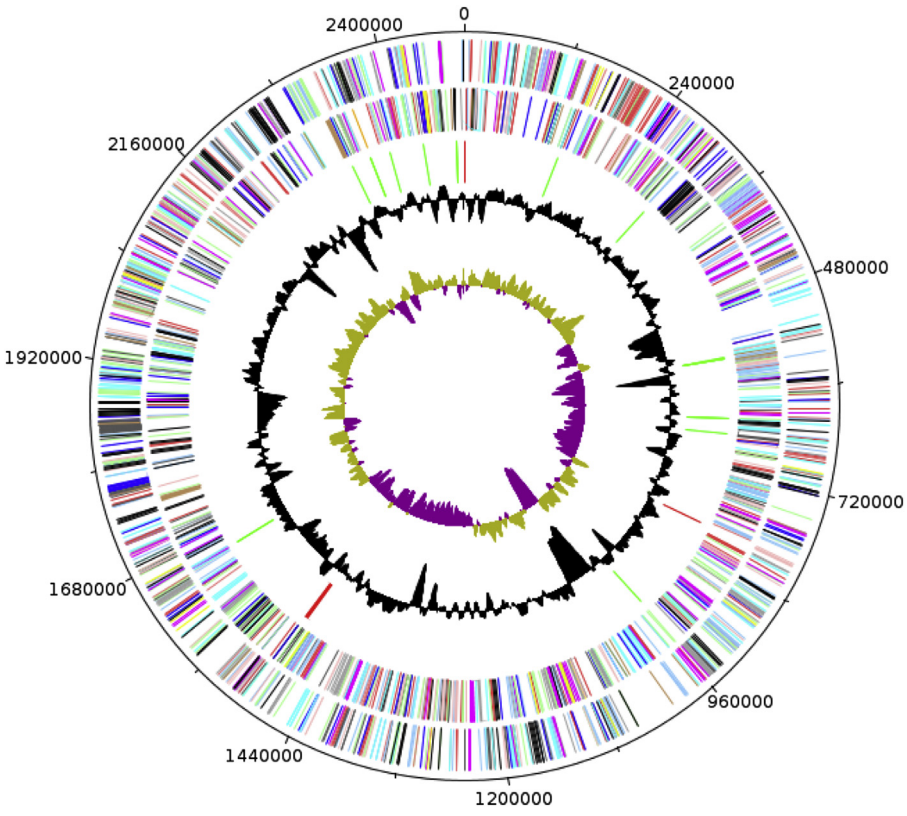

FIG. 6. Graphical circular map of *Necropsobacter massiliensis* strain FF6^T chromosome. From outside in, outer two circles shows open reading frames oriented in forward (coloured by COGs categories) and reverse (coloured by COGs categories) direction, respectively. Third circle marks indicate rRNA gene operon (green) and tRNA genes (red). Fourth circle shows G+C% content plot. Innermost circle shows GC skew, with purple indicating negative values and olive positive values.

Insights From Genome Sequence

Extended insights

The draft genome of *Necropsobacter massiliensis* (2.49 Mb) has a lower size than that of *N. rosorum* (2.52 Mb) but a larger size than those of *P. multocida* (2.25 Mb), *H. influenzae* (1.83 Mb), *H. ducreyi* (1.69 Mb), *H. somnus* (2.00 Mb), *H. parasuis* (2.26 Mb), *H. aphrophilus* (2.31 Mb) and *H. parainfluenzae* (2.08 Mb). The G+C content of *Necropsobacter massiliensis* (46.2%) was lower than that of *N. rosorum* (48.9%) but higher than those of *P. multocida* (40.40%), *H. influenzae* (38.15%), *H. ducreyi* (38.22%), *H. somnus* (37.20%), *H. parasuis* (39.99%), *H. aphrophilus* (42.23%) and *H. parainfluenzae* (39.57%). Because it has been suggested in the literature that the G+C content deviation is at most 1% within species, these data are an additional argument for the creation of a new taxon [27].

The protein-coding genes of *Necropsobacter massiliensis* is larger than those of *P. multocida, H. influenzae, H. ducreyi, H. somnus, H. parasuis, H. aphrophilus* and *H. parainfluenzae* (2012, 1603, 1717, 1791, 2021, 2218 and 1975, respectively) but smaller than that of *N. rosorum* (2311). However, the distribution of genes into categories was similar in all compared genomes. In addition, *Necropsobacter massiliensis* shared 2012, 1603, 1717, 1791, 2021, 1975, 2301 and 2218 orthologous genes with *P. multocida, H. influenzae, H. ducreyi, H. somnus, H. parasuis, H. aphrophilus* and *H. parainfluenzae,* respectively. Among species with standing in nomenclature, AGIOS values ranged from 66.32 between *N. rosorum* and *H. ducreyi* to 98.71% between *P. multocida* and *H. parainfluenzae* (Table 6). When

TABLE 4. **Genome information**

Attribute	Value	% of total
Genome size (bp)	2 493 927	
DNA coding (bp)	2 230 337	89.4
DNA G+C (bp)	1 151 339	46.2
DNA scaffolds	43	
Total genes	2363	100
Protein coding genes	2309	97.7
RNA genes	54	
Pseudo genes	Not indicated	
Genes in internal clusters	130	5.63
Genes with function prediction	1838	77.7
Genes assigned to COGs	2035	88.1
Genes with Pfam domains	75	3.24
Genes with signal peptides	210	9.09
Genes with transmembrane helices	561	24.3
CRISPR repeats	3	

COGs, Clusters of Orthologous Groups.

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

Code	Value	Percentage	Description
J	152	6.58	Translation, ribosomal structure and biogenesis
A	1	0.04	RNA processing and modification
K	100	4.33	Transcription
L	127	5.50	Replication, recombination and repair
B	0	0.00	Chromatin structure and dynamics
D	26	1.13	Cell cycle control, cell division, chromosome partitioning
V	21	0.91	Defense mechanisms
T	29	1.26	Signal transduction mechanisms
M	117	5.07	Cell wall/membrane biogenesis
N	0	0.00	Cell motility
U	37	1.60	Intracellular trafficking and secretion
O	86	3.72	Posttranslational modification, protein turnover, chaperones
C	113	4.89	Energy production and conversion
G	182	7.88	Carbohydrate transport and metabolism
E	152	6.58	Amino acid transport and metabolism
F	55	2.38	Nucleotide transport and metabolism
H	86	3.72	Coenzyme transport and metabolism
I	44	1.91	Lipid transport and metabolism
P	112	4.85	Inorganic ion transport and metabolism
Q	8	0.35	Secondary metabolites biosynthesis, transport and catabolism
R	198	8.58	General function prediction only
S	172	7.45	Function unknown
—	197	8.53	Not in COGs

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

compared to other species, *Necropsobacter massiliensis* exhibited AGIOS values ranging from 67.15 with *H. ducreyi* to 84.44 with *N. rosorum*. We obtained similar results using the GGDC software, as dDDH values ranged from 0.201 to 0.281 between studied species and were 0.275 between *N. rosorum*. These values confirm the status of *Necropsobacter massiliensis* as a new species.

Conclusions

On the basis of phenotypic, phylogenetic and genomic analyses, we formally propose the creation of *Necropsobacter massiliensis* sp. nov. that contains strain FF6^T. The strain was isolated from a cervical abscess of a 4-year-old Senegalese boy.

Taxonomic and nomenclatural proposals: description of *Necropsobacter massiliensis* strain FF6^T sp. nov.

Necropsobacter massiliensis (mas•il•ien'sis, L. gen., fem. n. *massiliensis,* of Massilia, the Latin name of Marseille, where this strain was characterized). On 5% sheep's blood–enriched Columbia agar (BioMérieux), colonies were 1 mm in diameter and grey. Cells are Gram negative and not motile, with a mean diameter of 0.4 µm (range, 0.2–0.6 µm) and a mean length of 1.5 µm (range, 0.9–2.1 µm). Catalase test was negative and oxidase test was positive. Positive reactions were observed for glycerol, ribose, D-xylose, D-mannose, D-glucose, inositol, N-acetyl glucosamine, D-fructose, D-maltose D-melibiose, D-trehalose, D-saccharose, D-raffinose, starch, potassium 5-ketogluconate, alkaline phosphatase, esterase, leucine arylamidase, phosphatase acid, α-glucosidase, naphthol-AS-BI-phosphohydrolase and α-glucosidase. Negative reactions were observed for D-mannitol, D-sorbitol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-melezitose, inulin, α-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase, N-acetyl-β-glucosaminidase, lipase, α-chrymotrypsin, cystine arylamidase, α-galactosidase, β-glucuronidase, α-mannosidase, α-fucosidase, N-acetyl-β-glucosaminidase, lipase, α-chrymotrypsin and cystine arylamidase.

Necropsobacter massiliensis strain FF6^T is susceptible to amoxicillin, amoxicillin/clavulanic acid, ceftriaxone, gentamicin, nitrofurantoin, rifampicin, trimethoprim/sulfamethoxazole and ciprofloxacin but resistant to erythromycin, doxycycline and vancomycin. The G+C content of the genome is 46.2%. The 16S rRNA and genome sequences of *N. massiliensis* strain FF6^T (= CSUR P3511 = DSM 27814) are deposited in GenBank under accession numbers HG428679 and CDON00000000, respectively. The type strain, FF6^T, was isolated from a cervical abscess of a 4-year-old Senegalese boy hospitalized in Hôpital Principal in Dakar, Senegal.

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

	N. massiliensis	*P. multocida.* subsp. *multocida*	*H. influenzae*	*H. ducreyi*	*H. somnus*	*H. parasuis*	*H. parainfluenzae*	*N. rosorum*	*A. aphrophilus*
N. massiliensis	2311[a]	71.37	70.61	67.15	71.73	68.01	71.29	84.44	72.12
P. multocida. subsp. *multocida*	1508	2012[a]	72.86	69.26	73.13	69.78	98.71	70.73	72.45
H. influenzae	1305	1271	1603[a]	69.89	72.96	70.15	72.81	69.71	73.69
H. ducreyi	1137	1107	1013	1717[a]	69.74	73.04	69.26	66.32	68.61
H. somnus	1306	1261	1124	1010	1791[a]	69.88	73.12	71.00	71.88
H. parasuis	1335	1290	1165	1068	1140	2021[a]	69.63	67.23	69.35
H. parainfluenzae	1523	1869	1275	1119	1270	1307	1975[a]	70.68	72.40
N. rosorum	1730	1514	1323	1111	1236	1335	1528	2301[a]	71.90
A. aphrophilus	1514	1407	1239	1065	1183	1204	1412	1463	2218[a]

Necropsobacter massiliensis FF6^T; *Necropsobacter rosorum; Pasteurella multocida* subsp. *multocida* Pm70; *Haemophilus influenzae* Rd KW20; *Haemophilus ducreyi* 35000HP; *Haemophilus somnus* 129PT; *Haemophilus parasuis* SH0165; HPI^g, *Haemophilus parainfluenzae* T3T1; *Aggregatibacter aphrophilus* NJ8700.
AGIOS, average genomic identity of orthologous gene sequences.
[a]Numbers of proteins per genome.

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Conflict of Interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nmni.2015.09.007.

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