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