Dialister massiliensis sp. nov., a new bacterium isolated from the human gut

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Abstract

Dialister massiliensis strain Marseille-P5638^T (= CSUR P5638) is a new species from the genus *Dialister* and family *Veillonellaceae* which was isolated from the gut microbiota of a healthy individual.
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The genus *Dialister* belongs to the order *Firmicutes* and includes anaerobic, nonmotile and Gram-negative bacilli [1]. To date, there are only five bacterial species with standing in nomenclature (https://www.bacterio.net/genus/dialister), all exclusively isolated from human samples. Here we report the isolation of a new *Dialister* species from a stool sample of a faecal transplant donor. This strain, Marseille-P5638, was obtained using the culturomics approach [2–4].

Isolation and growth conditions

In November 2017, we collected a fresh stool specimen from a 30-year-old Frenchman, who was a faecal transplant donor. The stool was decontaminated with 100% ethanol (v/v) [5]. The stool was preincubated for 5 days in an anaerobic blood culture bottle (Becton Dickinson, Le Pont de Claix, France) containing 2 mL sheep's blood and 2 mL filter-sterilized rumen. Subsequently, culture suspension was inoculated on 5% sheep's blood–enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) and incubated for 72 hours at 37°C in anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France). Identification of isolated bacterial colonies was attempted by MALDI-TOF MS with a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) and the Biotyper 3.0 software against the Bruker database that was continually incremented with the MEPHI database (https://www.mediterranee-infection.com/urms-database/), as previously reported [6]. Among these, the bacterial strain Marseille-P5638 could not be identified (Fig. 1).

The study was approved by the ethics committee of the Institut Mediterranee-Infection under reference 2016-010. The faecal transplant donor provided written informed consent for participation in this study.

Phenotypic characteristics

Colonies from strain Marseille-P5638 were small, transparent and smooth with a mean diameter of 0.1 to 0.2 mm. Bacterial cells were Gram-negative coccobacilli occurring in pairs or in sets of 4, ranging in length from 0.83 to 1.20 µm and in width from 0.70 to 0.80 µm (Fig. 2). Strain Marseille-P5638 exhibited catalase- and oxidase-negative activities. This strain is non–spore forming. The survival of several nonsporulated

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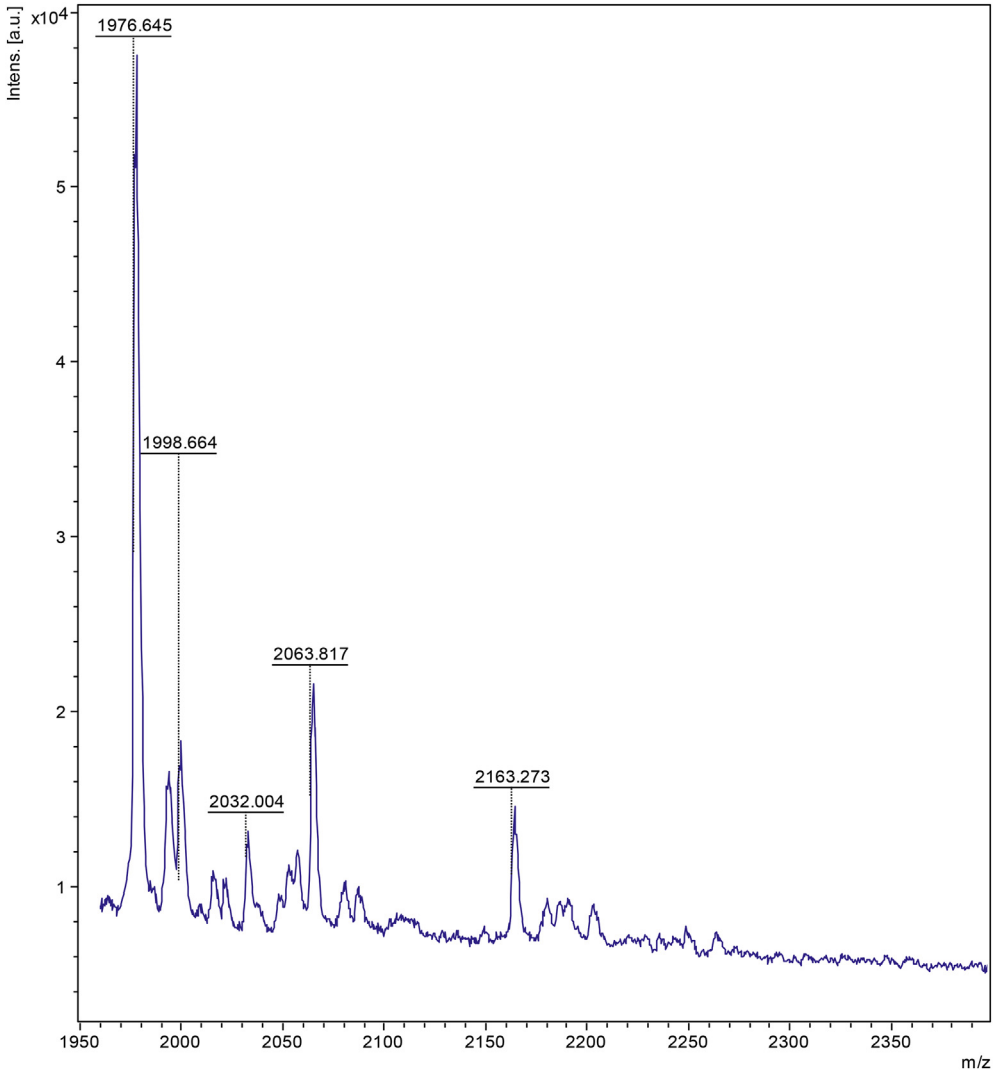

FIG. 1. MALDI-TOF MS reference spectrum of *Dialister massiliensis* sp. nov. strain Marseille-P5638^T. Reference spectrum was generated by comparison of spectra from 12 individual colonies.

bacterial species to stool disinfection with ethanol has previously been described [5,7,8]. Characteristics of the strain are summarized in Table 1. Strain Marseille-P5638 differed from closely related species with validly published names in terms of cell length, growth temperature, major fatty acid methyl ester composition, DNA G + C content, alkaline phosphatase and glutamic acid decarboxylase activities (Supplementary Table S1).

Strain identification

To identify strain Marseille-P5638, the 16S rRNA gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France), as previously described [9], and sequenced using the Big Dye Terminator v1.1 Cycle Sequencing Kit and a 3500xLGenetic Analyzer capillary sequencer (Thermo-Fisher, Saint-Aubin, France). The 16S rRNA nucleotide sequence was assembled and corrected using the CodonCode Aligner software (https://www.codoncode.com/).

Strain Marseille-P5638 exhibited a 95.99% sequence identity with *Dialister succinatiphilus* strain YIT 11850^T (GenBank accession no. AB370249), the closest phylogenetically related species with standing in nomenclature (Fig. 3). We consequently classified this strain as type strain of a new species within the genus *Dialister* (family *Veillonellaceae*, phylum *Firmicutes*).

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Hilden, Germany) with the EZ1 DNA tissue kit (Qiagen) and then sequenced on a MiSeq sequencer (Illumina, San Diego, CA,

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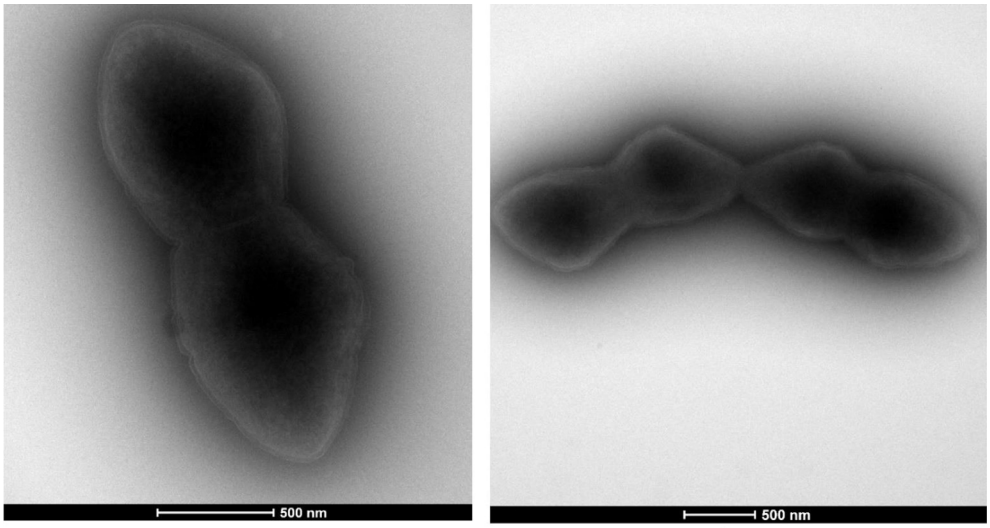

FIG. 2. Transmission electron micrograph of *Dialister massiliensis* sp. nov. strain Marseille-P5638^T (a) Bacterial cells in pairs. (b) Bacterial cells in quadruplet. Colony was collected from agar and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 1 hour at 4°C. Drop of cell suspension was deposited for approximately 5 minutes on glow-discharged formvar carbon film with 400 mesh nickel grids (FCF400−Ni, EMS). Grids were dried on blotting paper and cells were negatively stained for 10 seconds with 1% ammonium molybdate solution in filtered water at room temperature. Electron micrographs were acquired with a Tecnai G^{20} Cryo (FEI Company, Limeil-Brévannes, France) transmission electron microscope operated at 200 keV. Scale bar represents 500 nm.

TABLE 1. Description of *Dialister massiliensis* sp. nov. according to digital protologue TA00779

Characteristic	Value
Taxonumber	TA00779
Date of entry	31 October 2018
Draft number/date	001
Version	Draft
Species name	*Dialister massiliensis*
Genus name	*Dialister*
Specific epithet	*massiliensis*
Species status	sp. nov.
Species etymology	*massiliensis* (mas.si.li.en'sis, L. masc. adj. *massiliensis*, 'of Massilia,' ancient Roman name for Marseille, where strain was isolated)
Submitter	AFOUDA Pamela
E-mail of submitter	afoudapamela@yahoo.fr
Designation of type strain	Strain Marseille-P5638
Strain collection number	CSUR P5638
16S rRNA gene accession number	LT996173
Genome accession number (EMBL)	LT996885
Genome status	Draft
Genome size	2 320 000 bp
GC mol%	48.4
Data on origin of sample from which strain had been isolated	
Country of origin	France
Region of origin	Marseille
Date of isolation	6 November 2017
Source of isolation	Human gut
Sampling date	20 October 2017
Growth medium, incubation conditions (temperature, pH and further information) used for standard cultivation	Columbia agar supplemented with 5% sheep's blood, 37°C for 72 hours of incubation
Gram stain	Negative
Cell shape	Rod
Cell size (length or diameter)	0.83−1.20 × 0.70−0.80 µm
Motility	Nonmotile
Colony morphology	Transparent, smooth
Temperature range	28−45°C
Temperature optimum	37°C
Lowest pH for growth	6
Highest pH for growth	7.5
Highest NaCl concentration for growth	0.5
Relationship to O_2	Anaerobe
O_2 conditions for strain testing	Aerobiosis, anaerobiosis, microaerophilic
Oxidase	Negative
Catalase	Negative

Digital prologue available at http://imedea.uib-csic.es/dprotologue/.

USA) with the Nextera Mate Pair sample prep and Nextera XT Paired End kits (Illumina), as previously described [10]. To improve the sequence assembly, a second genomic sequencing was performed using the MinIon sequencer using the SQK-LSK108 kit (Oxford Nanopore Technologies, Oxford, UK). The combination of these two technologies enabled us to obtain a single scaffold as the assembly. The assembly was performed with a pipeline incorporating several software packages (Spades [11] and Trimmomatic [12] for trimmed data). GapCloser [13] was used to reduce gaps.

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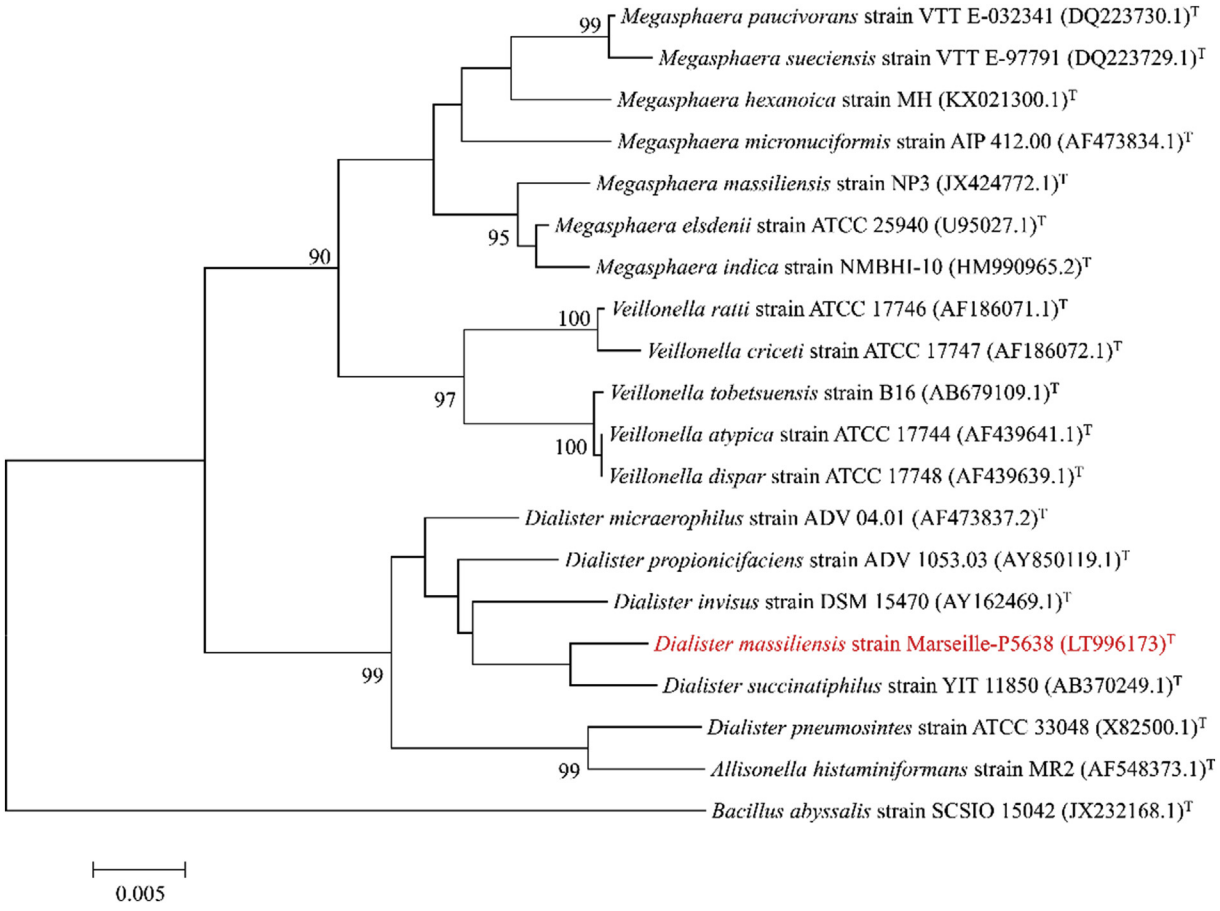

FIG. 3. Phylogenetic tree showing position of *Dialister massiliensis* sp. nov., strain Marseille-P5638^T, relative to other phylogenetically close neighbours. GenBank accession numbers of 16S ribosomal RNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters; phylogenetic inferences were obtained by maximum composite likelihood method and MEGA 6 software. Bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree are indicated at nodes. Only bootstrap values $\geq$ 90% were retained. Scale bar indicates 0.5% nucleotide sequence divergence.

The genome of strain Marseille-P5638 is 2 320 000 bp long with a 48.4 mol% G + C content. The degree of genomic similarity of strain Marseille-P5638^T with closely related species was estimated by OrthoANI [14]. OrthoANI values among closely related species (Fig. 4) ranged from 63.32% between *Dialister succinatiphilus* and *Veillonella tobetsuensis* to 87.52% between *Veillonella atypica* and *Veillonella tobetsuensis*. When *Dialister massiliensis* was compared to these closely related species, values ranged from 63.12% with *Veillonella tobetsuensis* to 73.96% with *Dialister succinatiphilus*.

Conclusion

Strain Marseille-P5638^T, exhibiting several phenotypic and genomic differences, as well as a 16S ribosomal RNA sequence divergence of >1.3% and OrthoANI value of <95% with the closest phylogenetically related species with standing in nomenclature, is consequently proposed as the type strain of the new species *Dialister massiliensis* sp. nov.

Description of *Dialister massiliensis* sp. nov.

Dialister massiliensis (mas.si.li.en'sis, L. masc. adj. *massiliensis* from Massilia, the ancient Roman name for Marseille, where the strain was isolated).

Cells are anaerobic, Gram negative, oxidase and catalase negative, and are nonmotile rods. Colonies are small, transparent and smooth, with a diameter ranging from 0.1 to 0.2 mm on 5% sheep's blood–enriched Columbia agar. The growth temperature ranges from 28 to 45°C after 72 hours of incubation, with an optimal growth temperature at 37°C. Cells range in size from 0.83 to 1.20 µm in length and 0.70 to 0.80 µm in width.

Using API 20NE, Rapid ID 32A API and API ZYM galleries, positive reactions were observed for L-arginine, glutamic acid,

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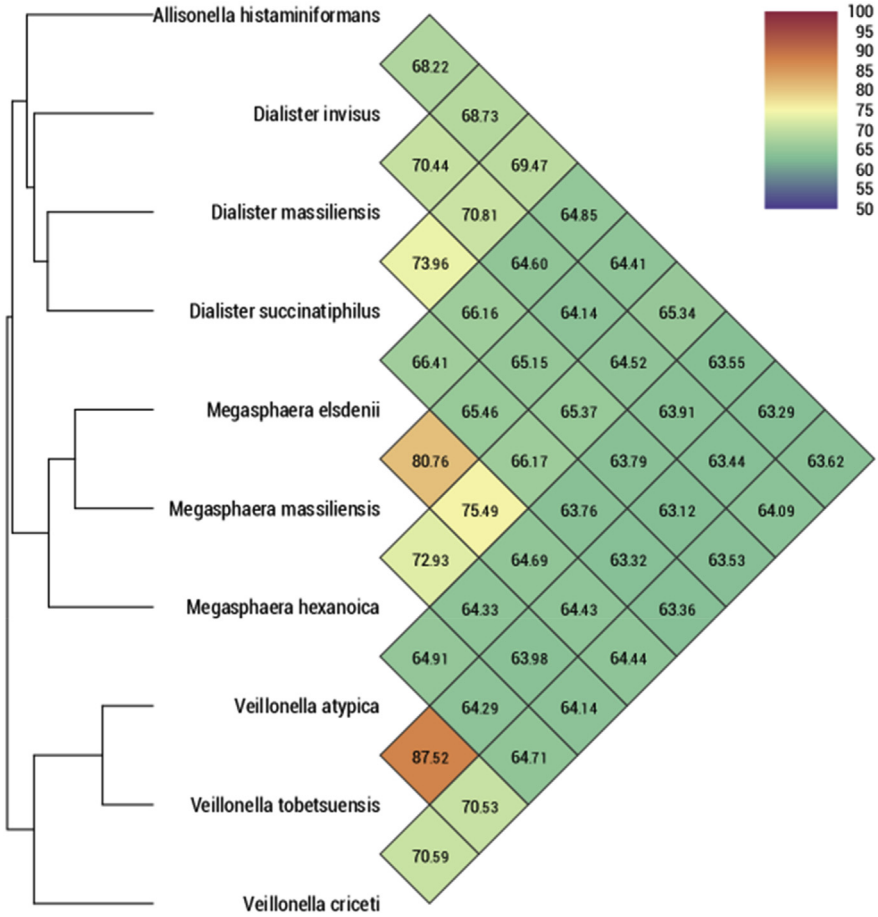

FIG. 4. Heat map generated by OrthoANI values calculated by OAT software between *Dialister massiliensis* sp. nov. strain Marseille-P5638^T and other closely related species with standing in nomenclature.

alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative reactions were observed for β-galactosidase, potassium nitrate (nitrate reductase), L-tryptophan (indole formation), D-glucose (fermentation and assimilation), urease, esculin ferric citrate, gelatin hydrolysis, L-arabinose (assimilation), D-mannose (assimilation), D-mannitol (assimilation), *N*-acetylglucosamine (assimilation), D-maltose (assimilation), potassium gluconate (assimilation), capric acid (assimilation), adipic acid (assimilation), malic acid (assimilation), trisodium citrate (assimilation), phenylacetic acid (assimilation), 4-nitrophenyl-αD-galactopyranoside, 4-nitrophenyl-βD-galactopyranoside, 4-nitrophenyl-βD-galactopyranoside-6-phosphate-2CHA, 4-nitrophenyl-αD-glucopyranoside, 4-nitrophenyl-βD-glucopyranoside, 4-nitrophenyl-αL-arabinofuropyranoside, 4-nitrophenyl-βD-glucuronide, 4-nitrophenyl-*N*-acetyl-βD-glucosaminide, D-mannose (fermentation), D-raffinose (fermentation), 4-nitrophenyl-αL-fucopyranoside, 2-naphtyl-phosphate, L-arginine-β-naphthylamide, L-proline-β-naphthylamide, L-leucyl-L-glycine-β-naphthylamide, L-phenylalanine-β-naphthylamide, L-leucine-β-naphthylamide, pyroglutamic β-naphthylamide acid, L-tyrosine-β-naphthylamide, L-alanyl-L-alanine-β-naphthylamide, L-glycine-β-naphthylamide, L-histidine-β-naphthylamide, L-glutamyl-L-glutamic β naphthylamide acid, L-serine-β-naphthylamide, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

The major fatty acids are $C_{18:1n9}$, $C_{16:0}$, and $C_{18:0}$. The G + C content of the genome is 48.4%. The type strain Marseille-P5638^T (= CSUR P5638) was isolated from the stool specimen of a healthy 30-year-old Frenchman.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT996173 and LT996885, respectively.

Deposit in a culture collection

Strain Marseille-P5638^T was deposited in Collection de Souches de l'Unité des Rickettsies collection under number CSUR P5638.

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Conflict of Interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2020.100657.

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