Genome sequence and description of *Bacteroides bouchesdurhonensis* sp. nov., a new anaerobic bacterium isolated from the human gut

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Abstract

Bacteroides bouchesdurhonensis sp. nov., strain Marseille-P2653[T] (= CSUR; P2653=DSM103120) is a new bacterial species belonging to the *Firmicutes* phylum in the family *Bacteroidaceae* that was isolated from the human gut microbiota.
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Introduction

It is important to decipher the bacterial diversity involved in normal and pathogenic functions [1]. To reveal the microbiota diversity in human gut, the culturomics approach, based on diverse culture conditions, was designed to isolate uncultured species and to complement 16S rRNA metagenomics [2−4]. Furthermore, a new taxonomic strategy named taxono-genomics was developed to include the analysis of complete genome sequences in combination with phenotypic character-istics [5]. Herein, we report a short description of strain Marseille-P2653[T] that was isolated from the human gut microbiota.

Isolation and growth conditions

Strain Marseille-P2653[T] was isolated from a stool sample collected from a healthy volunteer; 0.5g of the stool specimen was diluted ten times in phosphate-buffered saline solution (Life Technologies, Carlsbad, CA, USA). Then, 50 μL of each dilution was directly spread on 5% sheep blood agar (Bio-Mérieux, Marcy l'Etoile, France) and incubated in anaerobic conditions for 48 h. The isolated colonies were purified by subculturing on the same culture medium. The pure colonies obtained could not be identified by matrix-assisted laser desorption/ionization−time-of-flight mass spectrometry (MALDI-TOF MS). The Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) was used to perform the identification as previously described by Seng *et al.* [6]. Spectra obtained (Fig. 1) were imported and compared against the Bruker database that was continually updated with the MEPHI database [1].

Phenotypic characteristics

Strain Marseille-P2653[T] colonies were white-beige, circular, convex, raised and haemolytic with a mean diameter of 1−1.5 mm after 48 h of incubation. Bacterial cells were Gram-negative bacilli, non-motile and non-spore-forming with a diameter of 0.5−0.7 by 1.5−2.5 μm (Fig. 2). Strain Marseille-P2653[T] exhibited catalase activity but no oxidase activity. Using the API ZYM gallery, positive reactions were observed for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase,
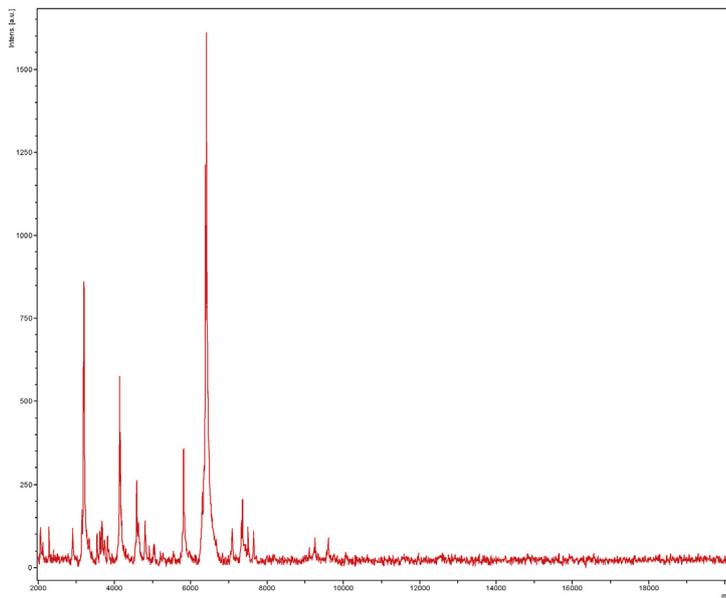
FIG. 1. MALDI-TOF MS reference spectrum of *Bacteroides bouchesdurhonensis* sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.
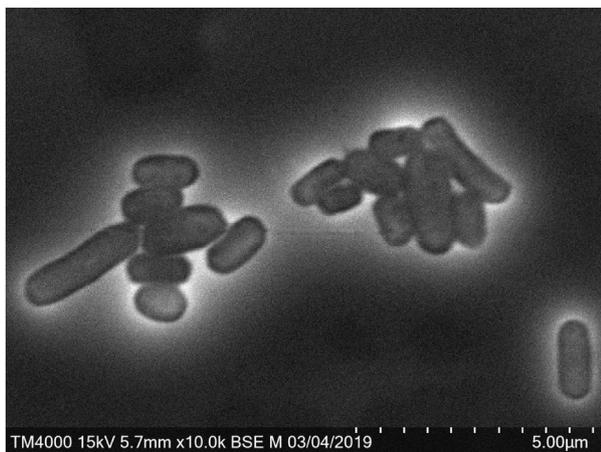
FIG. 2. Scanning electron microscopy (SEM) of stained *Bacteroides bouchesdurhonensis* sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 min and treated with 1% phosphotungstic acid (PTA) aqueous solution (pH 2.0) for 2 min to increase SEM image contrast. The slide was gently washed in water; air-dried and examined in a tabletop SEM (Hitachi TM4000) approximately 60 cm high and 33 cm wide to evaluate bacterial structure. Scales and acquisition settings are shown in figures.

α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-fucosidase. Lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase and α-mannosidase were negative. Using the API 20 NE system, cells are positive for nitrate reduction, L-arginine, urease, esculin hydrolysis, gelatine hydrolysis, β-galactosidase and for assimilation of malate and trisodium citrate. Indole formation, mannitol and *N*-acetyl-glucosamine were negative. An API 50CH strip positive reactions were obtained for glycerol, D-fructose, L-arabinose, D-xylose, D-galactose, D-ribose, D-glucose, D-mannose, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-arabinose, D-saccharose, D-trehalose, amidon and glycogen. erythritol, D-sorbitol, L-xylose, methyl-βD-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, *N*-acetyl-glucosamine, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, amygdalin D-lactose, D-melibiose, inulin, D-melezitose D-raffinose, xylitol, gentiobiose, D-turanose, D-tagatose, L-fucose, potassium 2-ketogluconate and potassium 5-ketogluconate. Negative reactions were found for D-adonitol D-lyxose, D-fucose, D-arabitol and potassium gluconate.

Fatty acid methyl ester analysis

Cellular fatty acid methyl ester (FAME) analysis was performed by gas chromatography/mass spectrometry (GC/MS) as described by Sasser [7]. Two samples were prepared with about 70 mg of bacterial biomass per tube harvested from several culture plates. GC/MS analyses were carried out as described elsewhere [8]. The major fatty acids found for this

strain were branched structures: 12-methyl-tetradecanoic acid (39%), 3-hydroxy-15-methyl-hexadecanoic acid (20%) and 13-methyl-tetradecanoic acid (12%). Many other branched structures were also described. Several specific 3-hydroxy fatty acids were also detected.

Strain identification

For phylogenetic classification of this bacterium, the 16S rDNA gene was amplified using the primer pair fD1 and rP2 (Euro-gentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France) as previously described [9]. The 16S rRNA

nucleotide sequence was assembled and corrected using CODONCODE ALIGNER software (http://www.codoncode.com).

Strain Marseille-P2653^T exhibited a 96.5% 16S rRNA similarity with *Bacteroides faecis* strain MAJ27 (GenBank accession number NR_113067.1), the phylogenetically closest species with standing in nomenclature (Fig. 3). We consequently proposed to classify strain Marseille-P2653^T as a new species within the genus *Bacteroides* in the phylum *Firmicutes*.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) and then sequenced on a MiSeq sequencer (Illumina Inc, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and
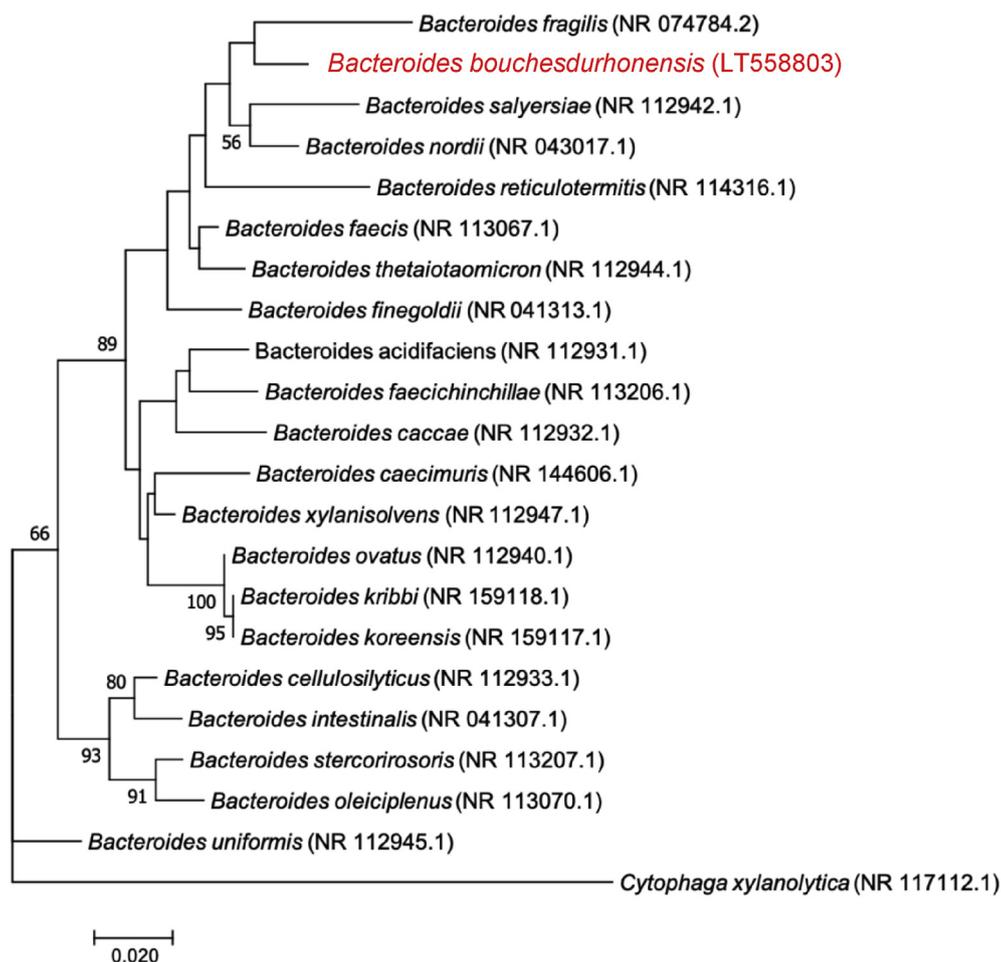
FIG. 3. Phylogenetic tree highlighting the position of *Bacteroides bouchesdurhonensis* sp. nov., with regard to other closely related species. GenBank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inference were obtained using the Maximum likelihood method and the MEGA 7 software. Bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree are indicated at the nodes. The scale bar indicates a 2% nucleotide sequence divergence.
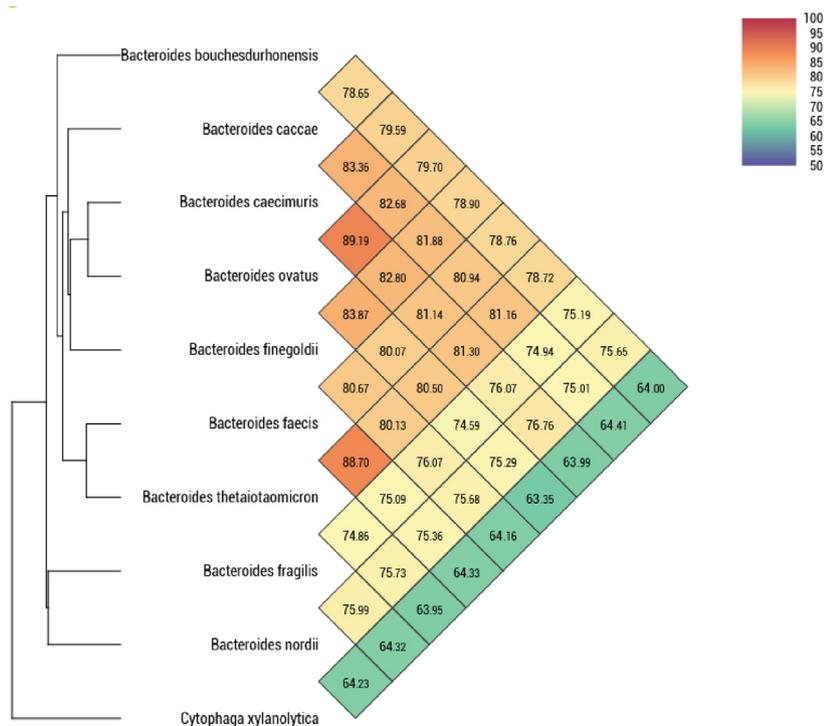
FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Bacteroides bouchesdurhonensis* sp. nov., and other closely related species with standing in nomenclature.

Nextera XT Paired End (Illumina), as previously described [8]. The assembly was performed using a pipeline containing several softwares (Velvet [10], Spades [11] and Soap Denovo [12], on trimmed data (MiSeq and Trimmomatic [13]) or untrimmed data (only MiSeq). GapCloser was used to reduce assembly gaps. Scaffolds of <800 bp and scaffolds with a depth value <25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of N).

The genome of strain Marseille-P2653[T] was 5 306 073 bp long with a 39.8 mol% G+C content. The degree of genomic similarity between strain Marseille-P2653[T] and closely related species was estimated using the OrthoANI software [14]. OrthoANI values among closely related species (Fig. 4) ranged from 64.00% between *Bacteroides bouchesdurhonensis* and *Cythophaga xylanolytica* to 89.19% between *Bacteroides caecimuris* and *Bacteroides ovatus*. When *Bacteroides bouchesdurhonensis* was compared with these closely related species, values ranged from 63.35% with *Cytophaga xylanolytica* to 83.87% with *Bacteroides ovatus*.

Conclusion

By referring to the unique phenotypic features, including MALDI-TOF spectrum, a 16S rRNA sequence divergence >1.3

TABLE 1. Description of *Bacteroides bouchesdurhonensis* sp. nov., according to the digitalized protologue TA00841 at the www.imedea.uib.es/dprotologue website

Species name	*Bacteroides bouchesdurhonensis*
Genus name	*Bacteroides*
Specific epithet	*bouchesdurhonensis*
Species status	sp. nov
Species etymology	*Bacteroidesbouchesdurhonensis* (bou.ches.du.rho.nen'sis, N.L. mas. adj. bouchesdurhonensis, referring to Bouches-du-Rhône, the name of the French department where strain Marseille-P2653T was isolated)
Designation of the type strain	Marseille-P2653
Strain collection numbers	CSUR P2653=DSM103120
16S rRNA gene accession number	LT558803
Genome accession number [refseq]	NZ_FTLV00000000
Genome status	Draft
Genome size	5 306 073 bp
GC mol %	39.8
Data on the origin of the sample from which the strain had been isolated	
Country of origin	France
Region of origin	Marseille
Date of isolation	2016-03-07
Source of isolation	Human stool
Sampling date	2016-02-20
Gram stain	Negative
Cell shape	Rod
Motility	Non-motile
Sporulation (resting cells)	None
Colony morphology	white-beige, circular, raised, convex and haemolytic with a mean diameter about 1−1.5 mm after 48 h incubation.
Temperature optimum	37°C
pH optimum	7
Relationship to O$_2$	Anaerobe
Oxidase	Negative
Catalase	Negative

% and an OrthoANI value >95% with the phylogenetically closest species with standing in nomenclature, we formally propose strain Marseille-P2653[T] as the type strain of *Bacteroides bouchesdurhonensis* sp. nov., a new species within the genus *Bacteroides*.

Description of *Bacteroides bouchesdurhonensis* sp. nov.

Bacteroides bouchesdurhonensis (bou.ches.du.rho.nen'sis, N.L. mas. adj. *bouchesdurhonensis*, referring to Bouches-du-Rhône, the name of the French department where strain Marseille-P2653[T] was isolated). The characteristics of the species are given in Table 1. The type strain is Marseille-P2653[T] (=CSUR P2653 =DSM103120).

Nucleotide sequence accession number. The 16S rRNA gene and genome sequences were deposited in GenBank under Accession numbers LT558803 and NZ_FTLV00000000.1, respectively.

Deposit in a culture collection

Strain Marseille-P2653[T] was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR) under number P2653[T] and DSMZ collection under number DSM103120.

MALDI-TOF-MS spectrum

The MALDI-TOF-MS spectrum of '*Bacteroides bouchesdurhonensis*' strain Marseille-P2653[T] is available online at: http://backup.mediterranee-infection.com/article.php?larub=280&titre=urms-database.

Conflicts of interest

None to declare.

Funding sources

Ethics and consent

The study was approved by the ethics committee of the Institut Fédératif de Recherche 48 under reference 2016-010. The patient gave his approval and consent for participating in this study.

Acknowledgements

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