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-P2653T (= 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 taxonogenomics was developed to include the analysis of complete genome sequences in combination with phenotypic characteristics [5]. Herein, we report a short description of strain Marseille-P2653T that was isolated from the human gut microbiota.

Isolation and growth conditions

Strain Marseille-P2653T 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 (Biomérieux, 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-P2653T 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 μm and 1.5–2.5 μm (Fig. 2). Strain Marseille-P2653T 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,
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-xyllose, D-galactose, D-ribose, D-glucose, D-mannose, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-arabinose, D-saccharose, D-trehalose, amion and glycogen. erythritol, D-sorbitol, L-xyllose, methyl-β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, N-acetyl-glucosamine, methyl-D-mannopyranoside, methyl-D-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 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer capillary sequencer (Thermo Fisher, 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-P2653T 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-P2653T 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...
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-P2653T was 5 306 073 bp long with a 39.8 mol% G+C content. The degree of genomic similarity between strain Marseille-P2653T 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 Cytophaga 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

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**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 |
|-----------------------|--------------------------------|
| Specific epithet      | bouchesdurhonensis             |
| Species status        | sp. nov                        |
| Species etymology     | Bacteroides bouchesdurhonensis (bou.ches.dur.hon.en’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 | Marseille-P2653T |
| Country of origin     | France                         |
| Region of origin      | Marselle                       |
| 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 O₂    | Anaerobe                       |
| Oxidase               | Negative                       |
| Catalase              | Negative                       |

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% and an OrthoANI value >95% with the phylogenetically closest species with standing in nomenclature, we formally propose strain Marseille-P2653T 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-P2653T was isolated). The characteristics of the species are given in Table 1. The type strain is Marseille-P2653T (=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-P2653T was deposited in the Collection de Souches de l’Unité des Rickettsies (CSUR) under number P2653T and DSMZ collection under number DSM103120.

MALDI-TOF-MS spectrum

The MALDI-TOF-MS spectrum of ‘Bacteroides bouchesdurhonensis’ strain Marseille-P2653T is available online at: http://backup.mediterranee-infection.com/article.php?larub=280&titre=urms-database.

Conflicts of interest

None to declare.

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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.

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