Parabacteroides bouchesdurhonensis sp. nov., a new bacterium isolated from the stool of a healthy adult

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Abstract

Parabacteroides bouchesdurhonensis strain Marseille-P3763^T (= CSURP3763) is a new species isolated from the stool of a heathy adult.

Keywords: Culturomics, new species, *Parabacteroides bouchesdurhonensis*, stool, taxono-genomics
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Introduction

Culturomics is a concept developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once an isolate is obtained, we used a taxono-genomics approach including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description (Table 1) and genome sequencing, to describe it [5,6].

Isolation and growth conditions

In 2016, we isolated from human stool an unidentified bacterial strain. The study was validated by the ethics committee of IHU Méditerranée Infection under number 2016-011. A screening was performed using MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The spectra obtained (Fig. 1) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated with MEPHI database: https://www.mediterranee-infection.com/urms-database/http://www.mediterraneeinfection.com/article.php?larub=280&titre=urms-database)). The initial growth was obtained after 48 h of culture on Columbia Agar with 5% sheep blood in anaerobic conditions at 37 °C and pH 7.5.

Strain identification

The 16S rRNA gene was sequenced to classify this bacterium. Amplification was done by using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com). Strain *Parabacteroides bouchesdurhonensis* exhibited a 96.68% sequence identity with *Parabacteroides chinchillae* strain JCM 17104 (GenBank accession number NR_113208.1, the phylogenetically closest species with standing in nomenclature (Fig. 2). We consequently classify this strain as a member of a new species within the genus *Parabacteroides*, family *Tannerellaceae*, phylum Bacteroidetes.

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TABLE 1. Description of *Parabacteroides bouchesdurhonensis* according to the digitalized protologue TA00969 on the www.imedea.uib.es/dprotologue website

TAXONUMBER	TA00969
DATE OF THE ENTRY	2019-05-28
DRAFT NUMBER/DATE	001
VERSION	Submitted
TYPE OF DESCRIPTION	New Description
SPECIES NAME	*Parabacteroides bouchesdurhonensis*
GENUS NAME	Parabacteroides
SPECIFIC EPITHET	*Parabacteroides bouchesdurhonensis*
SPECIES STATUS	sp. nov.
SPECIES ETYMOLOGY	bou.ches.du.rho.nen'sis, N.L. neut. adj. bouchesdurhonensis, pertaining to Bouches du Rhône, the name of the French territory where strain Marseille-P3763 was isolated
SUBMITTER	KUETE YIMAGOU EDMOND
E-MAIL OF THE SUBMITTER	edmondkuete@yahoo.fr
DESIGNATION OF THE TYPE STRAIN	Marseille-P3763T
STRAIN COLLECTION NUMBERS	CSURP 3763
16S rRNA GENE ACCESSION NUMBER	LT722681
GENOME ACCESSION NUMBER [RefSeq]	FYCK00000000
GENOME ACCESSION NUMBER [EMBL]	
GENOME STATUS	Complete
GC mol %	40.8
DATA ON THE ORIGIN OF THE SAMPLE FROM WHICH THE STRAIN HAD	BEEN ISOLATED
COUNTRY OF ORIGIN	FRANCE
REGION OF ORIGIN	Bouches du Rhône
DATE OF ISOLATION	2016-03-15
SOURCE OF ISOLATION	STOOL
SAMPLING DATE	2016-03-12
GEOGRAPHIC LOCATION	MARSEILLE
SOURCE OF ISOLATION OF NON-TYPE STRAINS	GUT
GROWTH MEDIUM, INCUBATION CONDITIONS [Temperature, pH, and further information] USED FOR STANDARD CULTIVATION	5% sheep's blood–enriched Columbia agar 37°C PH: 7.5
GRAM STAIN	NEGATIVE
CELL SHAPE	Rod
MOTILITY	Non-motile
SPORULATION (resting cells)	None
LOWEST TEMPERATURE FOR GROWTH	28°C
HIGHEST TEMPERATURE FOR GROWTH	45°C
TEMPERATURE OPTIMUM	37°C
HABITAT	HUMAN

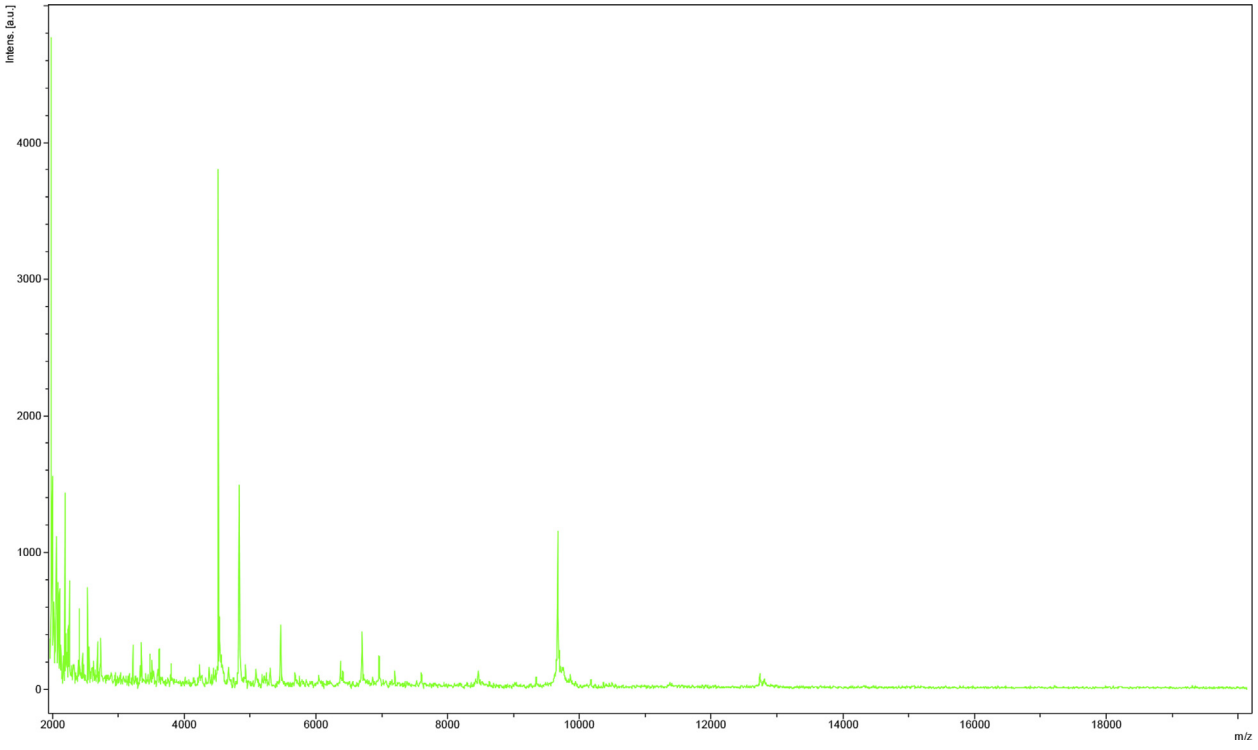

FIG. 1. MALDI-TOF MS Reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

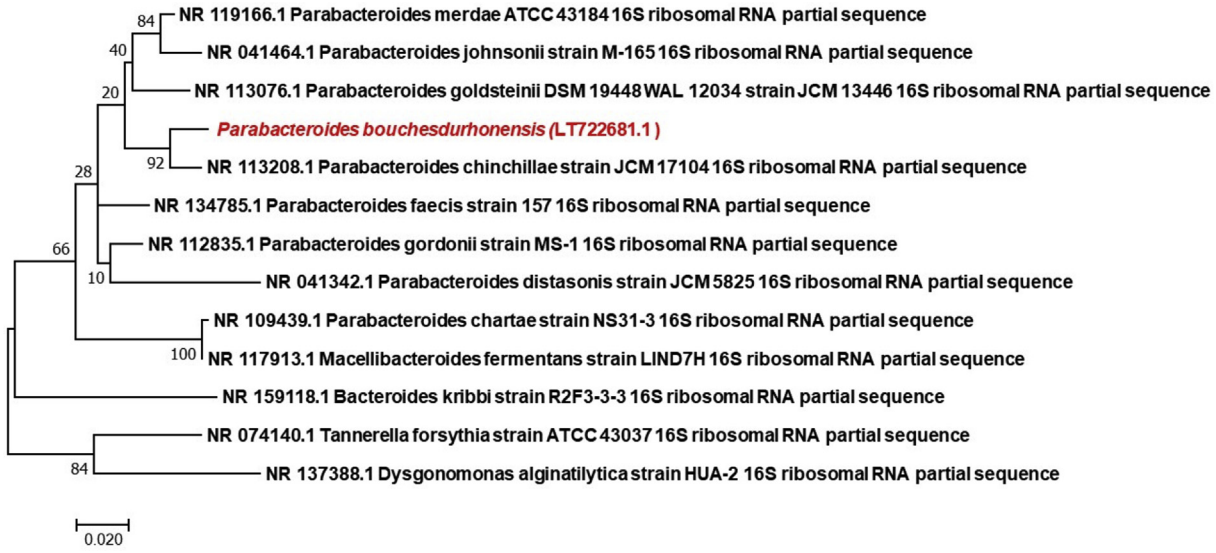

FIG. 2. Phylogenetic tree showing the position of *Parabacteroides bouchesdurhonensis* strain Marseille-P3763^T relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using MUSCLE v3.8.31 with default parameters and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. The scale bar indicates a 5% nucleotide sequence divergence.

Phenotypic characteristics

Colonies were beige in colour and circular in shape with a mean diameter of 1 mm. Bacterial cells were Gram-negative, rod-shaped, ranging in length from 0.4 to 0.8 µm and in width from 0.7 to 1.2 µm and non-motile (Fig. 3). Strain Marseille-P3763^T showed catalase-positive and oxidase-negative activities. Characteristics of the strain are summarized in Table 1. API 50CH and API ZYM tests were performed at 37 °C under anaerobic conditions and the results are summarized in Table 2.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit and then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera XT Paired end (Illumina), as previously described [9]. The assembly was performed with a pipeline incorporating different softwares (VELVET [10], SPADES [11] and SOAP DENOVO [12]) on trimmed (TRIMMOMATIC [13]) or raw data. GAPCLOSER was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value <25% of the mean depth were removed. The best assembly was selected by using different criteria (17 scaffolds, 19 contigs). The genome of strain Marseille-P3763^T is 3.7321 Mb long with a 40.8 mol% G + C content and contains 3024 predicted genes. The degree of genomic similarity of strain Marseille-P3763^T with closely related species was estimated using the ORTHOANI software [14]. Values among closely related species (Fig. 4) ranged from 69.68% between *Parabacteroides distasonis* and *Parabacteroides chartae* to 90.65% between *Parabacteroides johnsonii* and *Parabacteroides merdae*. When the isolate was compared with these closely related species, values ranged from 70.54% with *Parabacteroides chartae* to 78.30% with *Parabacteroides chinchillae*.

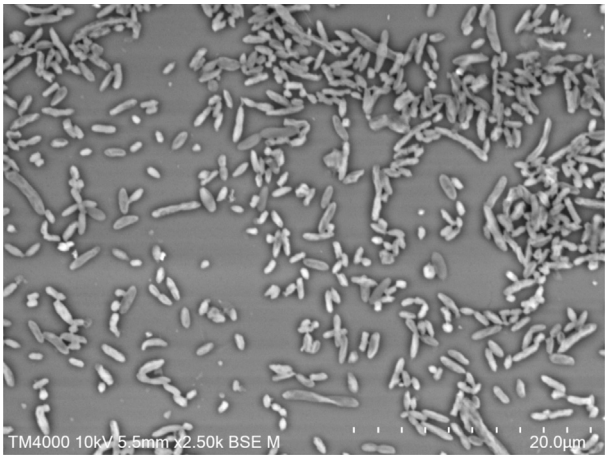

FIG. 3. Electron micrograph of *Parabacteroides bouchesdurhonensis* strain Marseille-P3763^T was acquired with a Hitachi TM4000Plus tabletop scanning electron microscope.

TABLE 2. Phenotypic characterization of *Parabacteroides bouchesdurhonensis* based on the biochemical tests: Profile Index: (A) API 50 CH, (B) API ZYM

Bacteria: Parabacteroides bouchesdurhonensis

Test	Results (+/–)	Test	Results (+/-)
(A) API 50 CH			
Control	–	Esculine	-
Glycerol	+	Salicine	+
Erythrol	+	D-cellobiose	-
D-arabinose	+	D-maltose	+
L-arabinose	+	D-lactose	+
D-ribose	+	D-melibiose	+
D-xylose	+	D-saccharose	+
L-xylose	+	D-trehalose	+
D-adonitol	+	Inuline	+
Methyl-βD-xylopyranoside	+	D-melezitose	+
D-galactose	+	D-raffinose	+
D-glucose	+	Amidon	+
D-fructose	+	Glycogene	+
D-mannose	+	Xylitol	+
L-sorbose	+	Gentibiose	+
L-rhammose	+	D-turanose	+
Dulcitol	+	D-lyxose	+
Inositol	+	D-tagatose	+
D-mannitol	+	D-fucose	+
D-sorbitol	+	L-fucose	+
Methyl-αD-mannopyranoside	+	D-arabitol	+
Methyl-αD-glucopyranoside	+	L-arabitol	+
N-acetylglucosamine	+	Potassium gluconate	+
Amygdaline	+	Potassium 2-cetogluconate	–
Arbutine	+	Potassium 5-cetogluconate	+

Bacteria: Parabacteroides bouchesdurhonensis

Test	Results (+/–)
(B) API ZYM	
Control	–
Alkaline phosphatase	+
Esterase (C 4)	+
Esterase lipase (C 8)	+
Lipase (C 14)	–
Leucine arylamidase	+
Valine arylamidase	–
Cystine arylamidase	+
Trypsine	–
α-chymotrypsine	–
Acid phosphatase	+
Naphthalo-AS-BI-phosphohydrolase	+
α-galactosidase	+
β-galactosidase	+
β-glucuronidase	–
α-glucosidase	+
β-glucosidase	–
N-acetyl-β-glucosaminidase	+
α-mannosidase	–
α-fucosidase	+

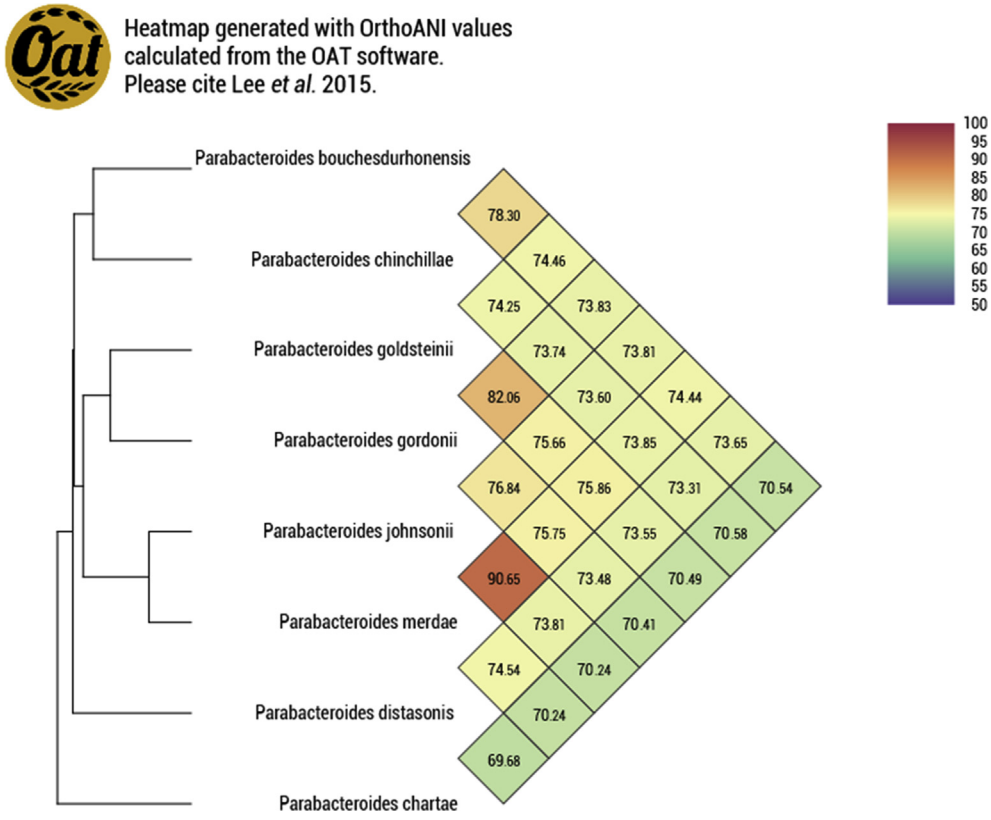

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between genus species and other closely related species with standing in nomenclature.

Conclusion

Strain *Parabacteroides bouchesdurhonensis* exhibits a 16S rRNA sequence divergence <98.65% and an OrthoANI value <95% with its phylogenetically closest species with standing in nomenclature, together with unique phenotypic features. It is consequently proposed as the type strain of the new species: *Parabacteroides bouchesdurhonensis* sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession number LT722681 and FYCK00000000, respectively.

Deposit in culture collections

Strain Marseille-P3763T was deposited in the collections under number CSURP3763.

Conflict of interest

None to declare.

Acknowledgements

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