

Dorea phocaeensis sp. nov., a new bacterium isolated from the stool of a healthy 29-year-old male

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Abstract

Dorea phocaeensis strain Marseille-P4003^T (= CSURP4003^T; = CCUG71359^T) is a new species isolated from the stool of a healthy 29-year-old male from France.

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Introduction

Culturomics is the concept of developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once isolated, 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 and genome sequencing, to describe the strain [5,6].

Isolation and growth conditions

In 2017, we isolated from the stool sample of a healthy 29-year-old male an unidentified bacterial strain. A screening was performed by MALDI-TOF MS on a Microflex LT spectrometer

(Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectra (Fig. 1) were imported into MALDI BIOTYPER 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in two databases (Bruker and constantly updated MEPHI databases <https://www.mediterranee-infection.com/urms-data-base/>). The study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 2016-011. The initial growth was obtained 48 h after culture in a Colombia agar enriched with 5% sheep's blood (bioMérieux, Marcy l'Etoile, France) under anaerobic conditions at 37°C.

Phenotypic characteristics

Colonies were white and translucent with a mean diameter of 0.1 mm. Bacterial cells were Gram-negative, rod-shaped, ranging from 0.74 to 1.38 µm in length and from 0.37 to 0.65 µm in width (Fig. 2). Strain Marseille-P4003^T showed negative catalase and oxidase activities. API 50 CH and API ZYM tests were performed at 37°C under anaerobic conditions and the results are described in Table 1. The main characteristics of this strain are summarized in Fig. 3.

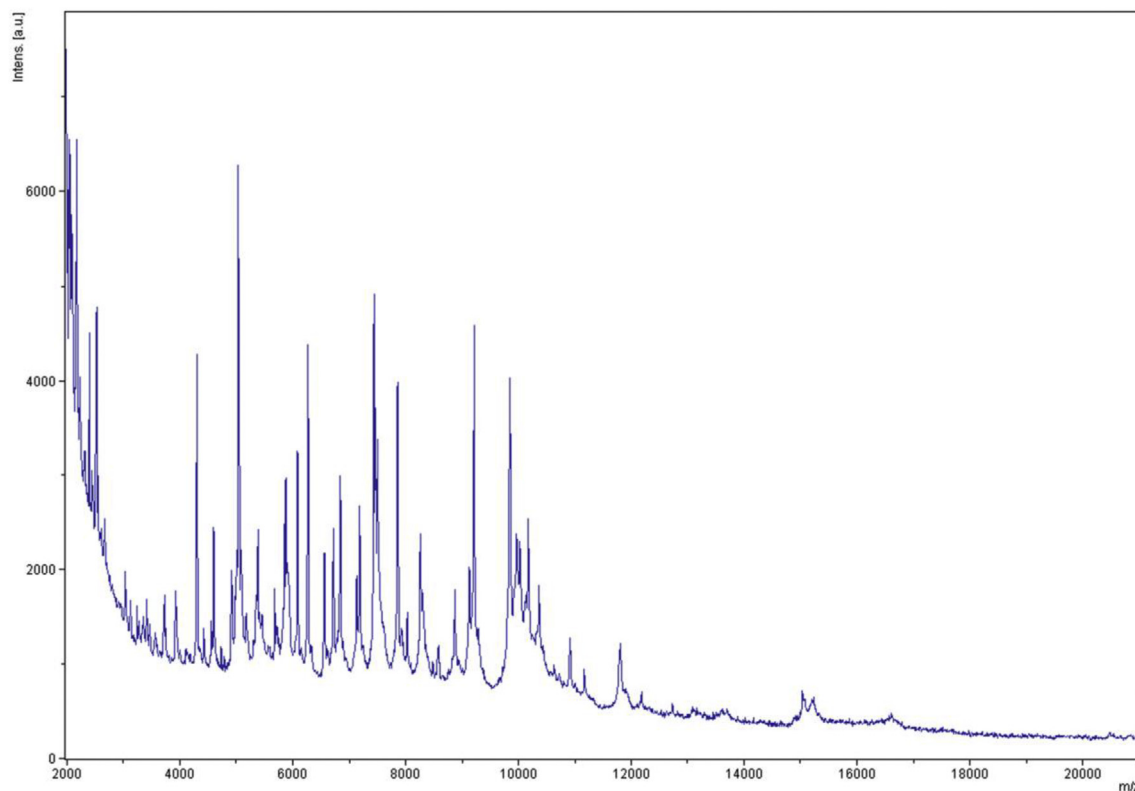


FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

Strain identification

The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification and sequencing were performed using the primer pair rD1 and rP2 (Eurogentec, Angers, France) and

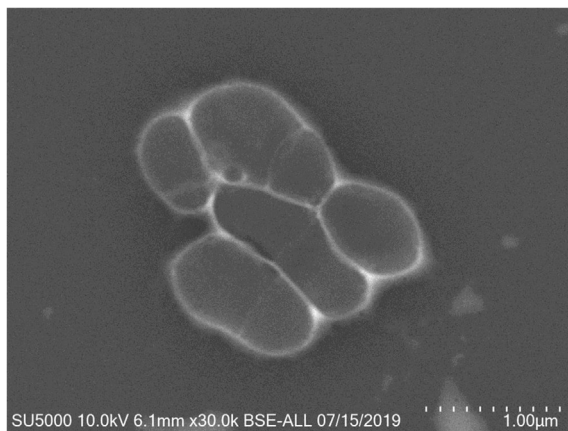


FIG. 2. Scanning electron micrograph of *Dorea phocaeensis* strain Marseille P4003^T using the Schottky Field Emission Scanning Electron Microscope SU5000 from Hitachi. Scale bar and acquisition settings are shown on the original micrograph.

the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary 3500xL Genetic Analyzer capillary sequencer (ThermoFisher, Saint-Aubin, France) respectively, as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CODONCODE ALIGNER software (<http://www.codoncode.com>). Strain Marseille-P4003^T exhibited a 98.23% sequence identity with *Dorea formicigenerans* strain ATCC 27755 (GenBank accession number NR044645), the phylogenetically closest species with standing in nomenclature (Fig. 4). Consequently, *Dorea phocaeensis* was classified as a new member of the genus *Dorea*, family *Lachnospiraceae*, phylum Firmicutes, with the strain Marseille P4003^T as the type strain of the new species *Dorea phocaeensis*.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit, then sequenced using MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit (Illumina), as previously described [9]. Genome assembly was performed with a pipeline incorporating different softwares (SPADES [10]),

TABLE 1. Phenotypic characterization of *Dorea phocaeensis* based on the analytical profile index: API 50 CH and API ZYM

(A)			
Bacteria: <i>Dorea phocaeensis</i>			
api 50 CH			
Test	Results (+/-)	Test	Results (+/-)
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-rhamnose	-	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	+/-

(B)	
Bacteria: <i>Dorea phocaeensis</i>	
api ZYM	
Test	Results (+/-)
Control	-
Alkaline phosphatase	-
Esterase (C 4)	-
Esterase Lipase (C 8)	-
Lipase (C 14)	-
Leucine arylamidase	-
Valine arylamidase	-
Cystine arylamidase	-
Trypsine	-
α-chymotrypsine	-
Acid phosphatase	-
Naphtalo-AS-BI-phosphohydrolase	+
α-galactosidase	-
β-galactosodase	-
β-glucuronidase	-
α-glucosidase	-
β-glucosidase	-
N-acetyl-β-glucosaminidase	-
α-mannosidase	-
α-fucosidase	-

DIGITAL
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 2019-07-18
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 002
 Submitted
SPNA *Dorea phocaeensis*
GENA *Dorea*
SPEP *Dorea phocaeensis*
SPST sp. nov.
SPTY *pho.ca.een'sis*, L. masc. adj., *phocaeensis*, 'of Phocaea,' the Latin name of Phokaia, the ancient Ionian city whose colony founded the city of Marseille
SUBM RANIA FRANCIS
EMSU raniagfrancis@gmail.com
TYPE Marseille P4003
COLN CSURP4003T
16SR LT934499
GARE NZ_OEPZ00000000
GSIZ 2467622
GGCM 43.2
COUN France
REGI France
SOUR Human gut
DATS 2017-01-10
CULT Colombia agar enriched with 5% sheep's blood
GRAM NEGATIVE
CSHA rod
CSIZ 1.06
MOTI nonmotile
SPOR none
COLM white, translucent, diameter of 0.1mm
TEMO 37
OREL anaerobe
OXID negative
CATA negative

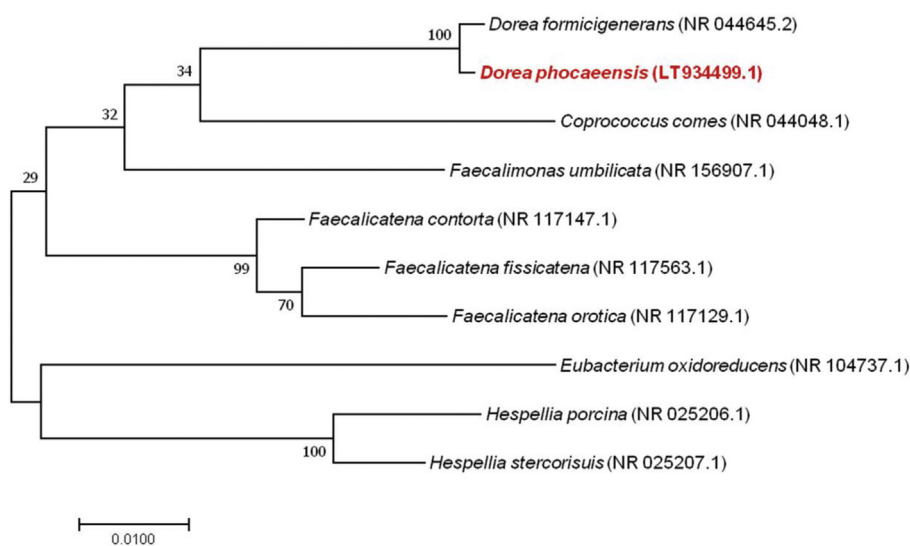
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FIG. 3. Description of *Dorea phocaeensis* strain Marseille P4003^T according to the digital protologue TA01017 on the www.imedea.uib.es/dprotologue website.

on trimmed (TRIMMOMATIC [11]) 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 (number of scaffolds: 12, number of contigs: 12). The genome of strain Marseille-P4003^T is 2 467 622 bp long with a 43.2 mol% G+C

FIG. 4. Phylogenetic tree showing the position of *Dorea phocaeensis* strain Marseille P4003^T relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequence alignment 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 1000 times to generate a majority consensus tree.



content. The degree of genomic similarity of Marseille-P4003^T with closely related species was estimated using the ORTHOANI software [12]. Values among closely related species (Fig. 5) ranged from 66.46% between *Eubacterium oxidoreducens* and

Faecalicatena orotica to 76.47% between *Faecalicatena orotica* and *Faecalicatena fissicatena*. When the isolate was compared with these closely related species, values ranged from 67.82% with *Eubacterium oxidoreducens* to 72.70% with *Dorea formicigerans*.



Heatmap generated with OrthoANI values calculated from the OAT software. Please cite Lee et al. 2015.

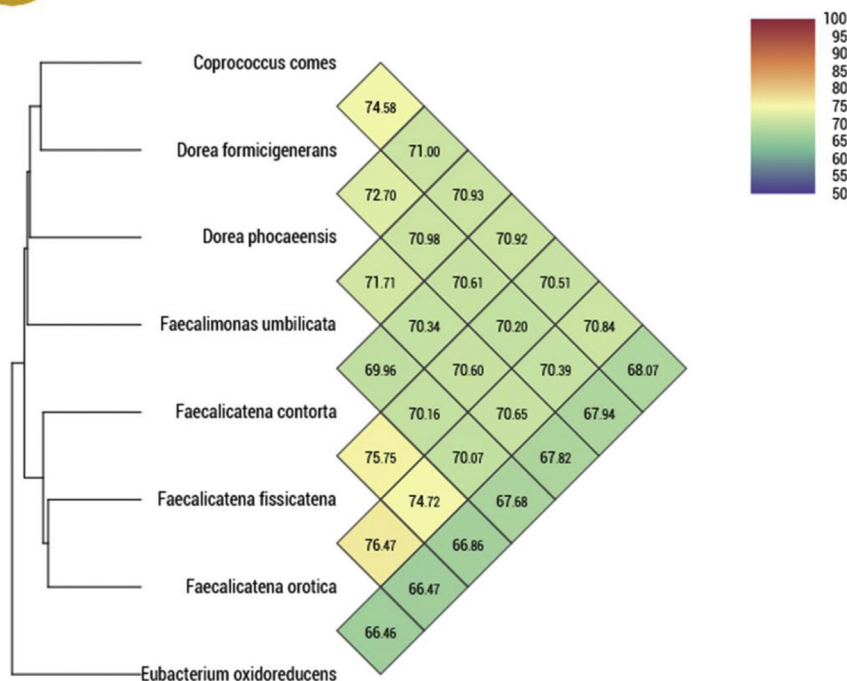


FIG. 5. Heatmap generated with ORTHOANI values calculated using the OAT software between *Dorea phocaeensis* and other closely related species with standing in nomenclature.

Conclusion

Strain Marseille-P4003^T exhibits a 16S rRNA sequence divergence <98.65 % with its phylogenetically closest species with standing in nomenclature, so is consequently proposed as the type strain of the new species *Dorea phocaeensis* sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT934499 and NZ_OEPZ00000000, respectively.

Deposit in culture collections

Strain Marseille-P4592^T was deposited in two different strain collections under number = CSURP4003^T; = CCUG71359^T.

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Conflict of interest

The authors have no conflict of interest to declare.

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