

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

T. Takakura<sup>1</sup>, R. Francis<sup>2,3</sup>, H. Anani<sup>2,4</sup>, S. Naud<sup>2,3</sup>, D. Raoult<sup>2,3</sup> and J. Y. Bou Khalil<sup>2</sup>

1) Hitachi High-Technologies Corporation, Analytical & Medical Solution Business Group, Ibaraki-ken, Japan, 2) Institut Hospitalo-Universitaire Méditerranée-Infection, 3) Aix-Marseille Université, Institut de Recherche pour le Développement (IRD), UMR Microbes Evolution Phylogeny and Infections (MEPHI) and 4) Aix Marseille Université, Institut de Recherche pour le Développement (IRD), Service de Santé des Armées, AP-HM, UMR Vecteurs Infections Tropicales et Méditerranéennes (VITROME), Marseille, France

## Abstract

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

© 2019 The Author(s). Published by Elsevier Ltd.

**Keywords:** Culturomics, *Dorea phocaeensis*, taxono-genomics

**Original Submission:** 24 July 2019; **Accepted:** 30 August 2019

**Article published online:** 7 September 2019

**Corresponding author:** J.Y. Bou Khalil, MEPHI, Institut Hospitalo-Universitaire Méditerranée Infection, 19–21 Boulevard Jean Moulin, 13005, Marseille Cedex 05, France.

**E-mail:** [boukhaliljacques@gmail.com](mailto:boukhaliljacques@gmail.com)

## 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].

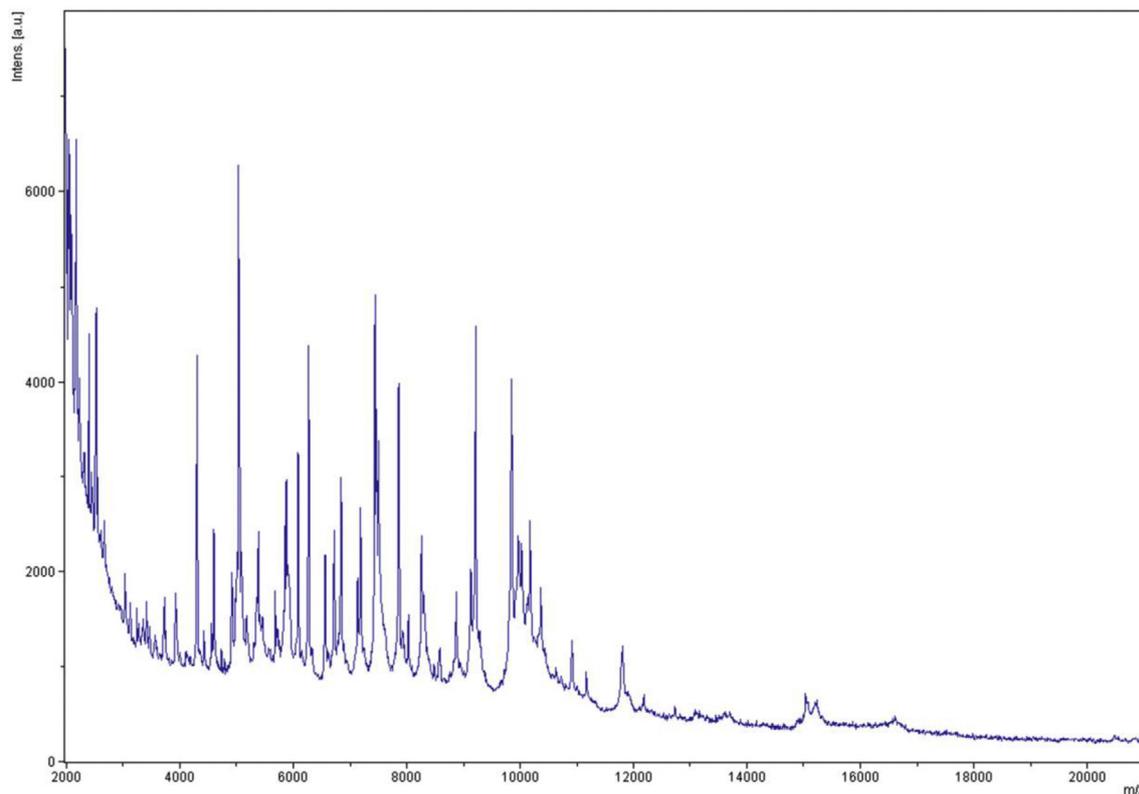
## Isolation and growth conditions

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

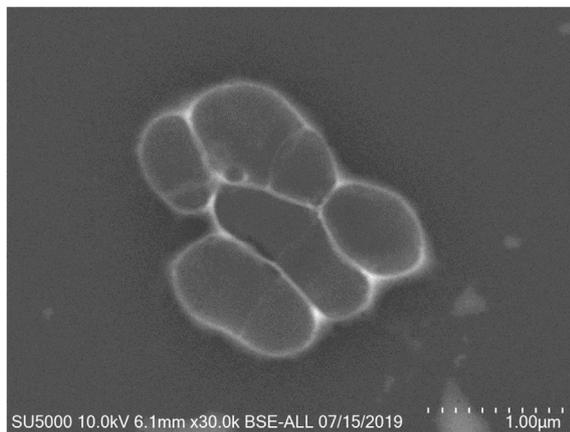
Colonies were white and translucent with a mean diameter of 0.1 mm. Bacterial cells were Gram-negative, rod-shaped, ranging from 0.74 to 1.38 µm in length and from 0.37 to 0.65 µm in width (Fig. 2). Strain Marseille-P4003<sup>T</sup> showed negative catalase and oxidase activities. API 50 CH and API 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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

<b>(A)</b>			
<b>Bacteria: <i>Dorea phocaeensis</i></b>			
<b>api 50 CH</b>			
<b>Test</b>	<b>Results (+/-)</b>	<b>Test</b>	<b>Results (+/-)</b>
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>(B)</b>	
<b>Bacteria: <i>Dorea phocaeensis</i></b>	
<b>api ZYM</b>	
<b>Test</b>	<b>Results (+/-)</b>
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  
PROTOLOGUE

**TXNR** TA01017  
 2019-07-18  
 2019-07-18  
 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

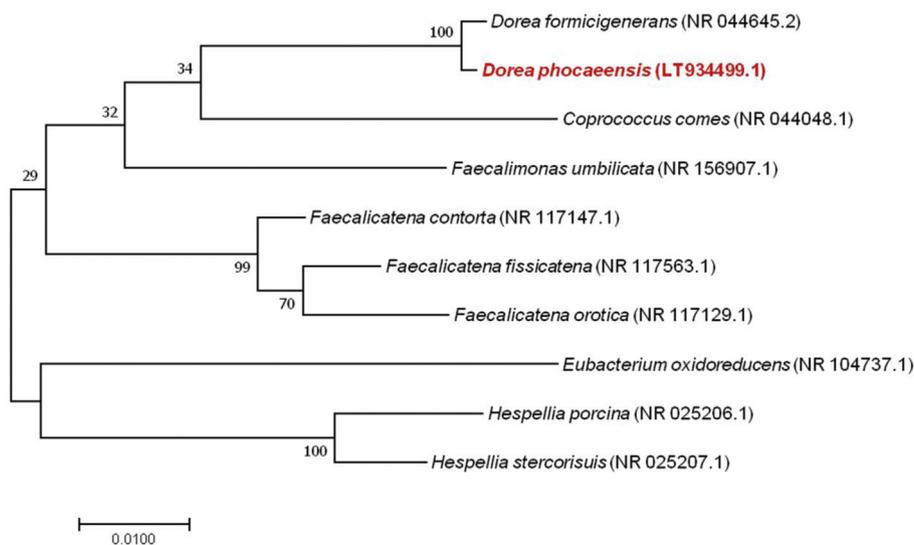
1 / 1

**FIG. 3.** Description of *Dorea phocaeensis* strain Marseille P4003<sup>T</sup> according to the digital protologue TA01017 on the [www.imedea.uib.es/dprotologue](http://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<sup>T</sup> 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<sup>T</sup> 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.

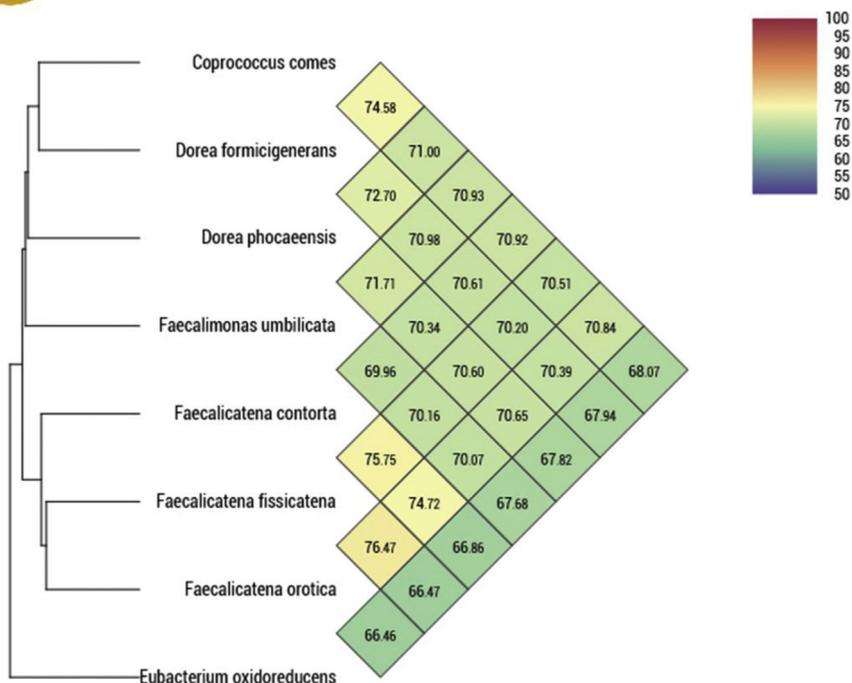


content. The degree of genomic similarity of Marseille-P4003<sup>T</sup> with closely related species was estimated using the ORTHOANI software [12]. Values among closely related species (Fig. 5) ranged from 66.46% between *Eubacterium oxidoreducens* and

*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 formicigenerans*.



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<sup>T</sup> 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<sup>T</sup> was deposited in two different strain collections under number = CSURP4003<sup>T</sup>; = CCUG71359<sup>T</sup>.

## Acknowledgements

We sincerely thank Taku Sakazume, Takashi Irie, Yusuke Ominami, Kyoko Imai, Shigeki Matsubara, Akiko Hisada, and all the Hitachi Team in Japan for the collaborative study we are conducting together between Hitachi High-Technologies Corporation and the Institut Hospitalo-Universitaire Méditerranée-Infection, and for the installation and services on the Schottky Field Emission Scanning Electron Microscope SU5000 in our facility.

## Conflict of interest

The authors have no conflict of interest to declare.

## Funding sources

The study was supported by the Méditerranée Infection foundation, the French National Research Agency under the

programme *Investissements d'avenir*, reference ANR-10-IAHU-03 and by Région Provence Alpes Côte d'Azur and European funding FEDER PRIM1.

## References

- [1] Lagier J-C, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* 2012;18:1185–93.
- [2] Lagier JC, Hugon P, Khelaifia S, Fournier PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. *Clin Microbiol Rev* 2015;28:237–64.
- [3] Lagier JC, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. *Nat Microbiol* 2016;1:16203.
- [4] Lagier J-C, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. *Clin Microbiol Rev* 2015;28:208–36.
- [5] Fournier PE, Lagier JC, Dubourg G, Raoult D. From culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. *Anaerobe* 2015;36:73–8.
- [6] Ramasamy D, Mishra AK, Lagier JC, Padhmanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. *Int J Syst Evol Microbiol* 2014;64:384–91.
- [7] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009;49:543–51.
- [8] Morel A-SS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta J-PP, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. *Eur J Clin Microbiol Infect Dis* 2015;34:561–70.
- [9] Diop A, Khelaifia S, Armstrong N, Labas N, Fournier P-E, Raoult D, et al. Microbial culturomics unravels the halophilic microbiota repertoire of table salt: description of *Gracilibacillus massiliensis* sp. nov. *Microb Ecol Heal Dis* 2016;27.
- [10] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–77.
- [11] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 2014;30:2114–20.
- [12] Lee I, Kim YO, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100–3.