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D. Ricaboni, M. Mailhe, A. Benezech, C. Andrieu, Pierre-Edouard Fournier, et al.. 'Pseudoflavonifractor phocaeensis' gen. nov., sp. nov., isolated from human left colon. *New Microbes and New Infections*, Wiley Online Library 2017, 17, pp.15-17. 10.1016/j.nmni.2016.12.012 . hal-01795971

HAL Id: hal-01795971

<https://hal-amu.archives-ouvertes.fr/hal-01795971>

Submitted on 22 May 2018

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'*Pseudoflavonifractor phocaeensis*' gen. nov., sp. nov., isolated from human left colon

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Abstract

We describe here characteristics of '*Pseudoflavonifractor phocaeensis*' strain Marseille-P3064^T, which was isolated from a human left colon wash sample.

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Keywords: Culturomics, human gut, microbiota, '*Pseudoflavonifractor phocaeensis*', taxonogenomics

Original Submission: 18 November 2016; **Revised Submission:** 7 December 2016; **Accepted:** 7 December 2016

Article published online: 13 December 2016

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We report here the identification of a new member of the human gut microbiome, strain Marseille-P3064^T, which was isolated during a culturomics study [1] focusing on the content of the human gut microbiome along the whole gastrointestinal tract. Samples from human upper and lower gastrointestinal tracts were simultaneously collected from patients who underwent endoscopic examination. The left colon wash sample was collected from a 27-year-old obese patient who provided signed informed consent, and the study was approved by the local ethics committee under number 2016-010.

Strain Marseille-P3064^T growth was obtained on 5% sheep's blood-enriched Columbia agar medium (bioMérieux, Marcy l'Étoile, France) under anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France) after a 21-day enrichment of the fresh left colon sample in an anaerobic blood culture bottle (Becton Dickinson, Pont de Claix, France) added with 5 mL sheep's blood (bioMérieux) and 5 mL of 0.2 µm filtered (Thermo Fisher Scientific, Villebon-sur-Yvette, France) rumen at 37°C. After 48 to 72 hours of anaerobic incubation on 5% sheep's blood-enriched

Columbia agar (bioMérieux), colonies were 0.2 to 0.7 mm in diameter, circular, raised, whitish to translucent and non-haemolytic. Bacterial cells were Gram-negative, motile rods of 0.6 to 1 µm wide by 1.6 to 3.8 µm long. Strain Marseille-P3064^T test did not show catalase or oxidase activity. No growth was obtained after sporulation test (20 minutes at 80°C) under aerobic or microaerophilic (campyGEN; Oxoid) conditions. Growth was obtained only on blood-enriched agar under anaerobic atmosphere at 37°C (no growth at 20, 28, 45 and 55°C).

After three failed identifications by our systematic matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) screening on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [2], the 16S rRNA gene of strain Marseille-P3064^T was sequenced using fD1-rP2 primers as previously described [3] using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Strain Marseille-P3064^T exhibited a 97.22% sequence identity with *Pseudoflavonifractor capillosus* strain ATCC 29799^T (GenBank accession no. AY136666), the phylogenetically closest species with standing in nomenclature (Fig. 1), which putatively classifies strain Marseille-P3064^T as a member of a new species within the genus *Pseudoflavonifractor* in the *Clostridiales* cluster IV within the phylum *Firmicutes*.

Strain Marseille-P3064^T's closest species, *Pseudoflavonifractor capillosus*, is a Gram-negative, strictly anaerobic bacillus that was originally described as *Bacteroides capillosus* in 1908 by Tissier [4]. On the basis of a very high genomic identity with the *Flavonifractor*

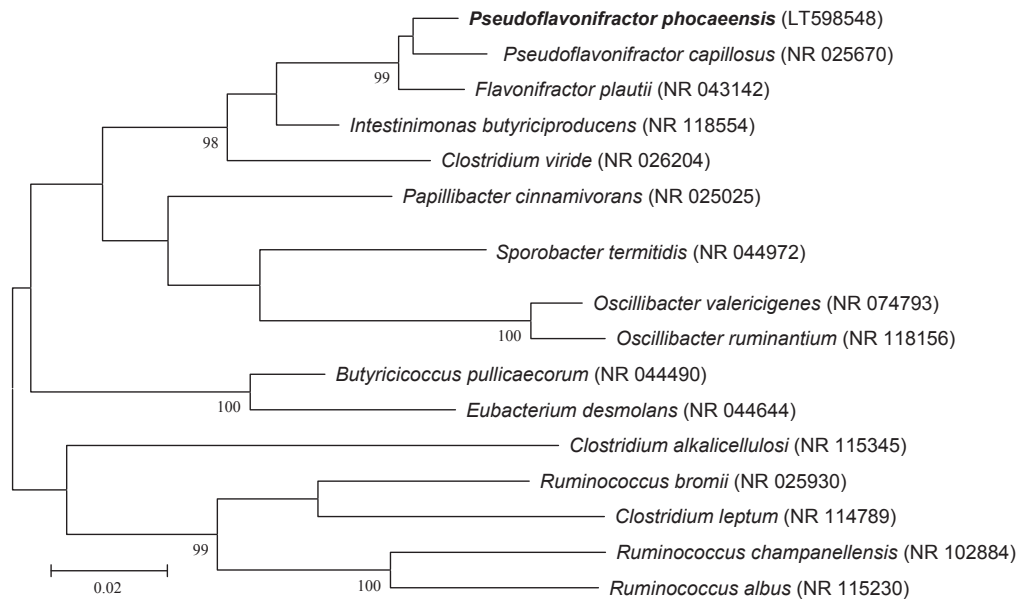


FIG. 1. Phylogenetic tree showing position of '*Pseudoflavonifractor phocaeensis*' strain Marseille-P3064^T relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Only bootstraps scores of at least 90 were retained.

genus (>97%), together with biochemical and physiological similarities, it was moved to a new genus, *Pseudoflavonifractor*, within the phylum *Firmicutes* in 2010 [5]. The creation of a new genus was justified on the basis of different Gram staining together with some different biochemical properties [5].

On the basis of the 16S rRNA gene sequence divergence of strain Marseille-P3064^T with the phylogenetically closest species with standing in nomenclature [6], we propose here the creation of the new species '*Pseudoflavonifractor phocaeensis*' (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), for which strain Marseille-P3064 (= CSUR P3064) is the type strain.

MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of '*Pseudoflavonifractor phocaeensis*' strain Marseille-P3064^T is available online (<http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>).

Nucleotide sequence accession number

The 16S rRNA gene sequence of '*Pseudoflavonifractor phocaeensis*' strain Marseille-P3064^T was deposited in GenBank under accession number LT598548.

Deposit in a culture collection

Strain Marseille-P3064^T was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number P3064.

Acknowledgement

This study was funded by the Fondation Méditerranée Infection.

Conflict of Interest

None declared.

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