**NEW SPECIES**

**Pseudoruminococcus massiliensis** gen. nov., sp. nov., a new bacterium isolated from the human gut microbiota

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**Abstract**

**Pseudoruminococcus massiliensis** strain Marseille-P3876\(^T\) (= CSUR P3876) is a new genus from the family *Ruminococcaceae* that was isolated from the gut microbiota of a healthy Senegalese man. © 2020 The Author(s). Published by Elsevier Ltd.

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**Introduction**

Knowing the different fundamental roles of the microbiota in the physiological and genetic processes in humans is one of this century’s challenges, notably through The Human Microbiome Project [1]. In this sense, to determine the cultivable microbiota diversity of man seems a crucial step. Culturomics, an approach based on the variation of culture conditions, has allowed us to increase the human microbial repertoire, especially through the isolation of several fastidious minority bacterial species of the human gut [2–4]. Here we report the type strain Marseille-P3876, isolated from a faecal transplant specimen using a culturomics approach.

**Isolation and growth conditions**

In March 2017, we collected a fresh stool specimen from a 32-year-old man, who was a faecal transplant donor. The stool was decontaminated with 100% ethanol (volume/volume) [5]. We isolated a bacterial strain that was not identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) and the Biotype 3.0 software against the Bruker database that was continually incremented with the MEPHI database (https://www.mediterranee-infection.com/urms-data-base/) as previously reported [6] (Fig. 1). The stool was pre-incubated for 10 days in an anaerobic blood culture bottle (Becton Dickinson, Le Pont de Claix, France) containing 2 mL sheep blood and 2 mL rumen. Strain Marseille-P3876 was then grown on 5% sheep-blood-enriched Columbia agar (bioMérieux, Marcy l’Étoile, France) for 96 hours at 37°C in an anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France).

**Strain identification**

For identifying this bacterium, the 16S rRNA gene was amplified using the fD1 and rP2 primer pair (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and a 3500xLGenetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France) as previously described [7]. The 16S rRNA nucleotide sequence was assembled and corrected using the CODONCODE ALIGNER software (http://www.codoncode.com).
FIG. 1. MALDI-TOF MS reference spectrum of *Pseudoruminococcus massiliensis* gen. nov., sp. nov. strain Marseille-P3876\(^T\). The reference spectrum was generated by comparison of spectra from 12 individual colonies.

FIG. 2. 16S rRNA-based phylogenetic tree highlighting the position of *Pseudoruminococcus massiliensis* gen. nov., sp. nov. strain Marseille-P3876\(^T\) with regard to other closely related species. GenBank accession numbers are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters. Phylogenetic inference was obtained using the Maximum composite likelihood method and MEGA 6 software. Bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree are indicated at the nodes. The scale bar indicates a 1% nucleotide sequence divergence.
FIG. 3. Transmission electron micrograph of *Pseudoruminococcus massiliensis* gen. nov., sp. nov. strain Marseille-P3876T. A colony was collected from agar and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 1 h at 4°C. A drop of cell suspension was deposited for approximately 5 minutes on glow-discharged formvar carbon film with 400-mesh nickel grids (FCF400–Ni, EMS). The grids were dried on blotting paper and the cells were negatively stained for 10 seconds with 1% ammonium molybdate solution in filtered water at room temperature. Electron micrographs were acquired with a Morgagni 268D (Philips) transmission electron microscope operated at 80 keV. Scales and acquisition settings are shown by the figures.

TABLE 1. Description of *Pseudoruminococcus massiliensis* gen. nov., sp. nov. According to the digital protologue TA00765 available at http://imedea.uib-csic.es/dprotologue/

| Taxonumber | TA00765 |
|------------|---------|
| Date of the entry | 2018-10-16 |
| Draft number/Date | 002 |
| Version | Draft |
| Species name | *Pseudoruminococcus massiliensis* |
| Genus name | *Pseudoruminococcus* |
| Specific epithet | *massiliensis* |
| Species status | sp. nov. |
| Species etymology | *Pseudoruminococcus* (Pseu.do.Ru.mi.no.co.'cus. Gr. adj. pseudes false; N.L. masc. n. Ruminococcus bacterial generic name; N.L. masc. n. *Pseudoruminococcus* false Ruminococcus) and (mas.si.li.en'sis. L. masc. adj. mas.sili.en'sis of Massilia, the ancient Roman name for Marseille, where the strain was isolated). |
| Submitter | AFOUDA Pamela |
| E-mail of the submitter | afoudapamela@yahoo.fr |
| Designation of the type strain | strain Marseille-P3876 |
| Strain collection numbers | CSUR P3876 LT985454 OLPR00000000 |
| 16S rRNA gene accession number | LT985454 |
| Genome accession number [EMBL] | |
| Genome status | Draft |
| Genome size | 2 428 410 bp |
| GC mol % | 37.6 |
| Data on the origin of the sample from which the strain had been isolated | France Marseille 2017-03-06 Human gut 2017-02-17 Columbia agar supplemented with 3% sheep blood, 37°C for 96 hours of incubation |
| Country of origin | France |
| Region of origin | Marseille |
| Date of isolation | 2017-03-06 |
| Source of isolation | Human gut |
| Sampling date | 2017-02-17 |
| Growth medium, incubation conditions [Temperature, pH, and further information] used for standard cultivation | Columbia agar supplemented with 3% sheep blood, 37°C for 96 hours of incubation |
| Gram stain | Negative |
| Cell shape | Coccos |
| Cell size (length or diameter) | 0.6 × 0.45(μm) |
| Motility | Non-motile |
| Colony morphology | Grey, smooth |
| Temperature range | 37°C |

Continued
Strain Marseille-P3876\textsuperscript{T} exhibited 92.01% 16S rRNA sequence similarity with *Ruminococcus bromii* strain ATCC 27255\textsuperscript{T} (GenBank accession number L76600), the closest phylogenetically related species with standing in nomenclature (Fig. 2). Consequently, we considered strain Marseille-P3876\textsuperscript{T} as a member of a new genus within the family *Ruminococcaceae* in the phylum *Firmicutes*, for which we propose the name *Pseudoruminococcus*.

### Phenotypic characteristics

Colonies were transparent and smooth with a mean diameter of 0.2–1.5 mm. Bacterial cells were Gram-negative cocci and measured 0.6 × 0.45 (Fig. 3). Catalase and oxidase activities were negative for strain Marseille-P3876. Characteristics of the strain are summarized in Table 1. Strain Marseille-3876 differs

**TABLE 1. Continued**

| Taxonumber   | TA00765 |
|--------------|---------|
| Temperature optimum | 37°C    |
| Lowest pH for growth | 6.5     |
| Highest pH for growth | 7       |
| Relationship to O\textsubscript{2} | Anaerobe |
| O\textsubscript{2} conditions for strain testing | Aerobiosis, Anaerobiosis, Microaerophilic |
| Oxidase      | Negative |
| Catalase     | Negative |

**FIG. 4.** Heatmap generated with OrthoANI values calculated using the OAT software between *Pseudoruminococcus massiliensis* gen. nov., sp. nov. strain Marseille-P3876\textsuperscript{T} and other closely related species with standing in nomenclature.
from its most closely related species by: cell length, Gram stain, motility, endospore formation, indole production, DNA G + C content, acid phosphatase activities and glucose assimilation. Differences also exist in relation to enzymatic reactions such as: arginine dihydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, β-N-acetyl glucosaminidase, glutamic acid decarboxylase, alanine arylamidase, alkaline phosphatase, arginine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase (see Supplementary material, Table S1).

**Genome sequencing**

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Hilden, Germany) and the EZ1 DNA Tissues kit (Qiagen) 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 [9]. The assembly was performed using SPAdes [9] and TRIMMOMATIC [10]. GAPCLOSER [11] was used to reduce gaps. Then, scaffolds <800 bp and those with a depth value <25% of the mean depth were removed (identified as possible contaminants). The best assembly was selected by using different criteria (number of scaffolds, N50, number of N).

The genome of strain Marseille-P3876 was 2 428 410 bp long with a 37.6 mol% G + C content. The degree of genomic similarity of strain Marseille-P3876 T with closely related species was estimated using the ORTHOANI software [12]. ORTHOANI values among closely related species (Fig. 4) ranged from 63.93% between *Ethanoligenens harbinense* and *Ruminococcus bromii* to 90.75% between *Clostridium sporosphaeroides* and *Ethanoligenens harbinense*. The degree of genomic similarity of strain Marseille-P3876 with closely related species with standing in nomenclature, Table S1).

**Conclusion**

On the basis of unique phenotypic features, including MALDI-TOF spectrum, a 16S rRNA sequence divergence >5% and an ORTHOANI value <80.5% with the closest phylogenetically related species with standing in nomenclature, we formally proposed strain Marseille-P3876 T as the type strain of *Pseudoruminococcus massiliensis* gen. nov., sp. nov. (Table 1), a new genus within the family Ruminococcaceae.

**Description of Pseudoruminococcus massiliensis gen. nov., sp. nov**

*Pseudoruminococcus* (Pseu.do.Ru.mi.no.co. 'cus. Gr. adj. pseudes false; N.L. masc. n. Ruminococcus bacterial generic name; N.L. masc. n. *Pseudoruminococcus* false Ruminococcus) and (mas.si-li.en'sis. L. masc. adj. massiliensis of Massilia, the ancient Roman name for Marseille, where the strain was isolated).

Cells are strict anaerobic, Gram-negative, non-motile cocci with a size of 0.6 × 0.45 μm and do not show oxidase and catalase activities. Growth was observed at 37°C after 96 hours of incubation and colonies were transparent and smooth, with a diameter ranging from 0.2 to 1.5 mm on 5% sheep-blood-enriched Columbia agar.

Using API 20NE, Rapid ID 32A API and API ZYM galleries, positive reactions were observed for L-arginine, 4-nitrophenyl-α-D-glucopyranoside, glutamic acid, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, β-galactosidase and α-glucosidase. Negative reactions were observed for β-galactosidase, potassium nitrate (nitrate reductase), L-tryptophan (indole formation), D-glucose (fermentation and assimilation), urease, esculin ferric citrate, gelatin hydrolysis, L-arabinose (assimilation), D-mannose (fermentation), D-mannitoli (assimilation), N-acetylglucosamine (assimilation), D-maltose (assimilation), potassium gluconate (assimilation), capric acid (assimilation), adipic acid (fermentation), malic acid (fermentation), trisodium citrate (assimilation), phenylacetic acid (fermentation), 4-nitrophenyl-α-D-galactopyranoside, 4-nitrophenyl-β-D-galactopyranoside, 4-nitrophenyl-β-D-glucopyranoside-6-phosphate-2CHA, 4-nitrophenyl-β-D-glucopyranoside, 4-nitrophenyl-α-D-arabinofuranoside, 4-nitrophenyl-β-D-glucuronide, 4-nitrophenyl-N-acetyl-β-D-glucosaminide, D-mannose (fermentation), D-raffinose (fermentation), D-glucose (fermentation), C8), naphthol-AS-BI-phosphohydrolase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

The G + C content of the genome is 37.6%. The type strain Marseille-P3876 T (≡ CSUR P3876) was isolated from the stool specimen of a 32-year-old Senegalese man who was a faecal transplant donor.
**Nucleotide sequence accession number**

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT985454.1 and OLMR00000000.1, respectively.

**Deposit in culture collections**

Strain Marseille-P3876T was deposited in the CSUR collection under number CSUR P3876.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2019.100645.

**Conicts of interest**

None to declare.

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**Ethics and consent**

The study was approved by the ethics committee of the Institut Mediterranée-Infection under reference 2016-010. The faecal transplant donor gave informed and signed consent for participating in this study.

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