

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.

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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-3876, 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 BIOTYPER 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 3500xL Genetic 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>).

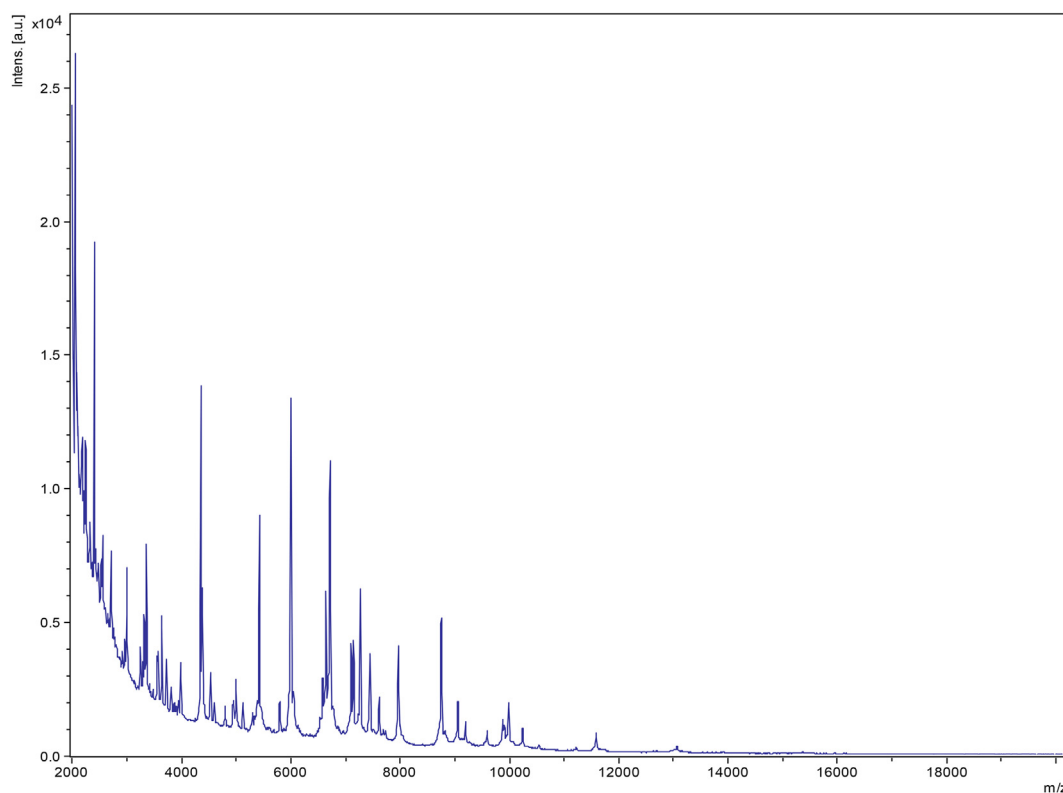


FIG. 1. MALDI-TOF MS reference spectrum of *Pseudoruminococcus massiliensis* gen. nov., sp. nov. strain Marseille-P3876^T. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

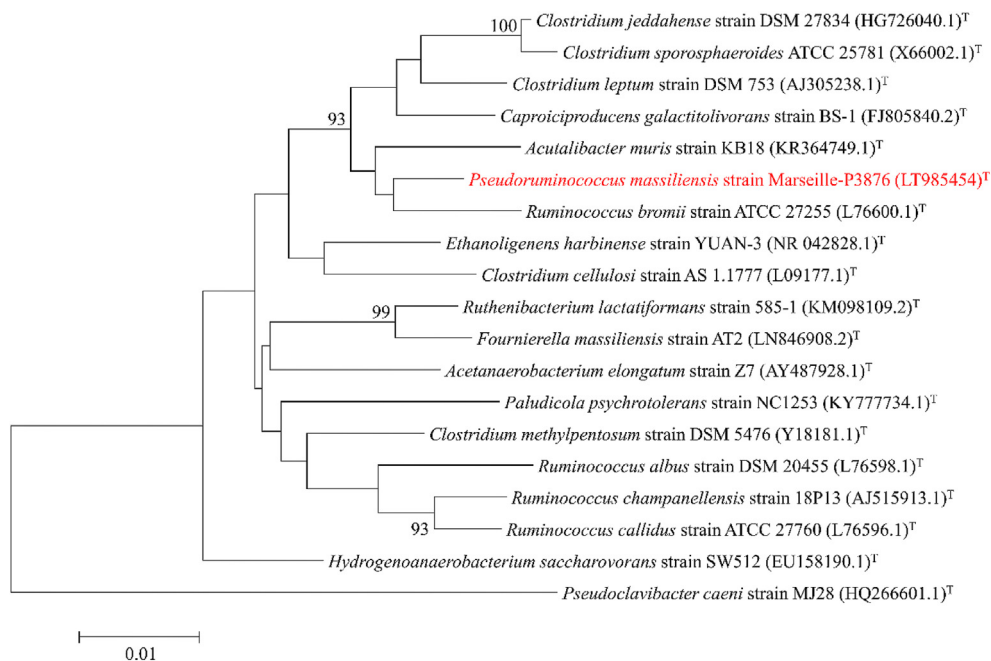


FIG. 2. 16S rRNA-based phylogenetic tree highlighting the position of *Pseudoruminococcus massiliensis* gen. nov., sp. nov. strain Marseille-P3876^T with regard to other closely related species. GenBank accession numbers are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters. 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-P3876^T. 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.

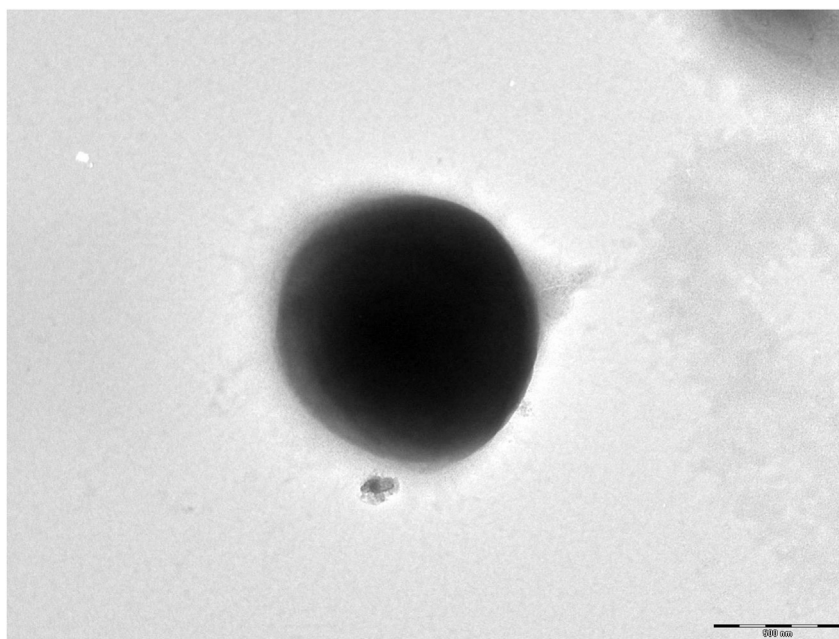


TABLE 1. Description of *Pseudoruminococcus massiliensis* gen. nov., sp. nov. According to the digital protologue TA00765 available at <http://imedea.uib-csic.es/dprotologue/>

Taxonnumber	TA00765
Date of the entry	2018-10-16
Draft number/Date	002
Version	Draft
Species name	<i>Pseudoruminococcus massiliensis</i>
Genus name	<i>Pseudoruminococcus</i>
Specific epithet	<i>massiliensis</i>
Species status	sp. nov.
Species etymology	<i>Pseudoruminococcus</i> (Pseu.do.Ru.mi.no.co.'cus. Gr. adj. <i>pseudes</i> false; N.L. masc. n. <i>Ruminococcus</i> bacterial generic name; N.L. masc. n. <i>Pseudoruminococcus</i> false <i>Ruminococcus</i>) and (mas.si.li.en'sis. L. masc. adj. <i>massiliensis</i> of <i>Massilia</i> , 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
16S rRNA gene accession number	LT985454
Genome accession number [EMBL]	OLMR00000000
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	
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 5% sheep blood, 37°C for 96 hours of incubation
Gram stain	Negative
Cell shape	Coccus
Cell size (length or diameter)	0.6 × 0.45(μm)
Motility	Non-motile
Colony morphology	Grey, smooth
Temperature range	37°C

Continued

TABLE I. Continued

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

Strain Marseille-P3876^T exhibited 92.01% 16S rRNA sequence similarity with *Ruminococcus bromii* strain ATCC 27255^T (GenBank accession number L76600), the closest phylogenetically related species with standing in nomenclature (Fig. 2). Consequently, we considered strain Marseille-P3876^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 I. Strain Marseille-3876 differs

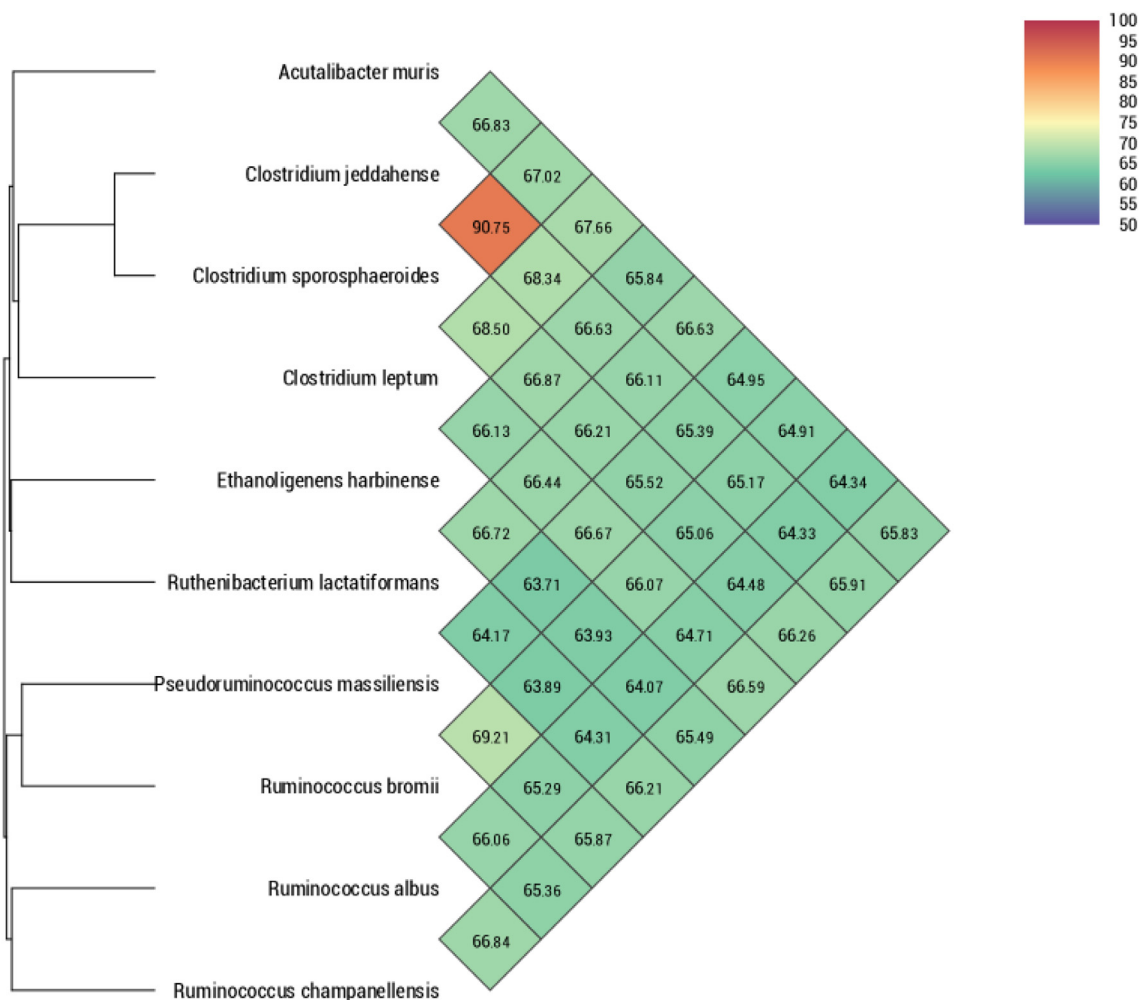


FIG. 4. Heatmap generated with ORTHOANI values calculated using the OAT software between *Pseudoruminococcus massiliensis* gen. nov., sp. nov. strain Marseille-P3876^T and other closely related species with standing in nomenclature.

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 EZI biorobot (Qiagen, Hilden, Germany) and the EZI 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 [8]. 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 jeddahense* and *Clostridium sporosphaeroides*. When strain Marseille-P3876 was compared with these closely related species, values ranged from 63.93% with *Ethanoligenens harbinense* to 69.21% with *Ruminococcus bromii*.

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 μ m 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 (assimilation), D-mannitol (assimilation), N-acetylglucosamine (assimilation), D-maltose (assimilation), potassium gluconate (assimilation), capric acid (assimilation), adipic acid (assimilation), malic acid (assimilation), trisodium citrate (assimilation), phenylacetic acid (assimilation), 4-nitrophenyl- α -D-galactopyranoside, 4-nitrophenyl- β -D-galactopyranoside, 4-nitrophenyl- β -D-galactopyranoside-6-phosphate-2CHA, 4-nitrophenyl- β -D-glucopyranoside, 4-nitrophenyl- α -L-arabinofuropyranoside, 4-nitrophenyl- β -D-glucuronide, 4-nitrophenyl-N-acetyl- β -D-glucosaminide, D-mannose (fermentation), D-raffinose (fermentation), 4-nitrophenyl- α -L-fucopyranoside, 2-naphthylphosphate, L-arginine- β -naphthylamide, L-proline- β -naphthylamide, L-leucyl-L-glycine- β -naphthylamide, L-phenylalanine- β -naphthylamide, L-leucine- β -naphthylamide, pyroglutamic β -naphthylamide acid, L-tyrosine- β -naphthylamide, L-alanyl-L-alanine- β -naphthylamide, L-glycine- β -naphthylamide, L-histidine- β -naphthylamide, L-glutamyl-L-glutamic β -naphthylamide acid, L-serine- β -naphthylamide, alkaline phosphatase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, α -galactosidase, β -glucuronidase, β -glucosidase, 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-P3876^T was deposited in the CSUR collection under number CSUR P3876.

Acknowledgements

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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>.

Conflicts of interest

None to declare.

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Ethics and consent

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

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