NEW SPECIES

Description of ‘Beduinibacterium massiliense’ gen. nov., sp. nov., ‘Massilimaliae massiliensis’ gen. nov., sp. nov., ‘Provencibacterium massiliense’ gen. nov., sp. nov. and ‘Oscilibacter massiliensis’ sp. nov., isolated from a faecal specimen of a 19-year-old healthy Saudi Arabian Bedouin by culturomics

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Abstract

We report here the main characteristics of ‘Beduinibacterium massiliense’ strain Marseille-P3337T gen. nov., sp. nov., ‘Massilimaliae massiliensis’ Marseille-P2963T gen. nov., sp. nov., ‘Provencibacterium massiliense’ Marseille-P2780T gen. nov., sp. nov. and ‘Oscilibacter massiliensis’ Marseille-P2778T sp. nov., all isolated from the stool of a Bedouin from Saudi Arabia. We used a bacterial culturomics approach combined with taxonogenomics.

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Keywords: ‘Beduinibacterium massiliense’, human microbiota, ‘Massilimaliae massiliensis’, ‘Oscilibacter massiliensis’, ‘Provencibacterium massiliense’

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Concerning the study of the human gut microbiota content, we isolated in 2016, using a bacterial culturomics approach, four bacteria that could not be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [1,2]. These species were isolated from stools from a Saudi Arabian Bedouin. The donor provided written informed consent, and the study was validated by the ethics committee of the IFR48 Federative Research Institute under number 09-022. All the 16S rRNA genes of these four strains were sequenced using fD1-rP2 primers as described previously using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France) [3].

The stool was preincubated for 15 days at 37°C in an anaerobic atmosphere in a culture bottle containing Columbia agar liquid medium (bioMérieux, Marcy l’Etoile, France). After an initial growth of 72 hours on Columbia agar supplemented with 5% sheep’s blood at 37°C under strict anaerobic conditions, strain Marseille-P3337 was isolated. The colonies appeared beige, non-haemolytic, motile and non-spore forming, and were with 1 to 2 mm in size. Bacterial cells were motile, Gram-positive, rods/coccobacilli, ranging from 1.8 to 2 μm in length and 0.4 to 0.6 μm/0.5 μm in diameter. The strain did not show catalase or oxidase activity. The 16S rRNA gene sequence presents an identity of 90.44% with Christensenella minuta strain YT 12065 (NR_112900), the phylogenetically closest species with standing in nomenclature (Fig. 1), which was initially isolated from human faeces [4]. Christensenella minuta is a strictly anaerobic, nonmotile, non-spore-forming, Gram-negative bacterium presenting characteristics to
be short and straight rod with tapered ends. This similarity of <95% leads us to putatively classify Marseille-P3337 as a new member in the Christensenellaceae family of the order Clostridiales in the Firmicutes phylum [5]. Therefore, we propose the creation of the new genus 'Beduinibacterium' (Be.dui.ni.bac.ter'ium, L. gen. neut., composed of Beduini, for ‘Bedouin,’ the people from who the bacterium was isolated, and bacterium). 'Beduinibacterium massiliense' is the type strain of the new genus 'Beduinibacterium.' Marseille-P3337 $^{T}$ is the type strain of the species ‘Beduinibacterium massiliense’ (ma.ssi.lien’se, L. adj. neut., from massiliense, referring to Massilia, the antic name of Marseille, France, where the strain was isolated).

Concerning the identification of strain Marseille-P2963, the stool was preincubated for 10 days at 37°C in anaerobic atmosphere in a culture bottle containing blood-enriched Columbia agar liquid medium (bioMérieux) supplemented with 5 mL of

![Phylogenetic tree showing position of 'Beduinibacterium massiliense' strain Marseille-P3337$^{T}$ relative to other phylogenetically close neighbours.](image1)

Sequences were aligned using CLUSTALW and phylogenetic inferences obtained by Kimura two-parameter models 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. Scale bar indicates 1–2% nucleotide sequence divergence.

![Phylogenetic tree showing position of 'Massimilaliae massiliensis' Marseille-P2963$^{T}$ relative to other phylogenetically close neighbours.](image2)

Alignment and phylogenetic inferences were done as described for Fig. 1.

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rumen fluid filter-sterilized through a 0.2 μm pore filter (Thermo Fisher Scientific, Villebon-sur-Yvette, France). After an initial growth of 48 hours on Columbia agar supplemented with 5% sheep’s blood at 37°C under strict anaerobic conditions, strain Marseille-P2963 was isolated. The colonies appeared beige, nonhaemolytic, nonmotile and non-spore forming, 0.5 mm in size. The cells were Gram negative, and small rod shaped, length of 1.6 μm and width of 0.5 μm. The strain did not show catalase or oxidase activity. The 16S rRNA gene sequence presents an identity of 91.83% with Clostridium methylpentosum strain R2 (NR_029355), the phylogenetically closest species with standing in nomenclature (Fig. 2). Clostridium methylpentosum was obligately anaerobic and has a distinctive morphology. Indeed, its cells are rods bent in the shape of rings with the ends slightly overlapping [6]. This similarity of <95% leads us to putatively classify Marseille-P2963 as a new member of the Clostridiaceae family, order Clostridiales, phylum Firmicutes [5]. Therefore, we propose the creation of the new genus ‘Massilimaliae’ (Massili, for Massilia, the antic name of Marseille, France, where the strain was isolated, and Maliae, referring to the Malian nationality of the grower). ‘Massilimaliae massiliensis’ (massiliensis, L. adj. neut., from Massilensis, ‘to Massilia’, the antic name of Marseille, France, where the strain was isolated) is the type strain of the new genus ‘Massilimaliae.’ Marseille-P2963 T is the type strain of the species.

Concerning strain Marseille-P2780, the stool was preincubated for 3 days at 37°C in anaerobic atmosphere in a culture bottle containing Columbia agar liquid medium (bioMérieux) supplemented with 5 mL of rumen fluid filter-sterilized through a 0.2 μm pore filter (Thermo Fisher Scientific). Strain Marseille-P2780 grew after an initial culture of 48 hours on Columbia agar supplemented with 5% sheep’s blood at 37°C under strict anaerobic conditions. The colonies appeared ochre, nonhaemolytic and nonmotile, 1 to 1.5 mm in size. The cells were Gram negative, rod shaped and curved, 3.5 μm long and 0.4 to 0.6 μm wide. The strain did not show catalase or oxidase activity. The 16S rRNA gene sequence presents an identity of 90.82% with Hydrogenoanaerobacterium saccharovorans strain SW512 (NR_044425), the phylogenetically closest species with standing in nomenclature (Fig. 3). Hydrogenoanaerobacterium saccharovorans is a strictly anaerobic bacterial strain, isolated from a laboratory-scale H2-producing upflow anaerobic sludge blanket (UASB) reactor. Marseille-P2780 is Gram stain negative and nonmotile, and did not form spores [7]. This similarity of <95% leads us to putatively classify Marseille-P2780 as a new member in the Ruminococcaceae family of the order Clostridiales in the phylum of Firmicutes [5].

![Figure 3](image-url)

**FIG. 3.** Phylogenetic tree showing position of ‘Provencibacterium massiliense’ Marseille-P2780T relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were done as described for Fig. 1.
Therefore, we propose the creation of the new genus 'Provenci-
bacterium' (Pro.ven.ci.bac.te-rium, L. gen. neut., composed of
Provence, for Provence, the region of France where the strain was
isolated, and bacterium). 'Provenci-bacterium massiliense' (mas.sil-
ien'se, L. adj. neut., Massiliense, 'to Massilia,' the antic name of
Marseille, France, where the strain was isolated) is the type strain
of the new genus 'Provenci-bacterium.' Marseille-P2780T is the type
strain of the species 'Provenci-bacterium massiliense.'

For the identification of strain Marseille-P2778, the stool was
preincubated for 3 days at 37°C in anaerobic atmosphere in a
culture bottle containing blood-enriched Columbia agar liquid
medium (bioMérieux) supplemented with 5 mL of rumen fluid
filtrate-sterilized through a 0.2 μm pore filter (Thermo Fisher
Scientific). Strain Marseille-P2778 was isolated after an initial
growth of 48 hours on Columbia agar supplemented with 5%
sheep's blood at 37°C under strict anaerobic conditions. The
colonies appeared beige, nonhaemolytic, nonmotile and non-
spore forming, with a size of 1 mm. The cells were Gram
negative and rod shaped, 3.4 μm long and 0.7 μm wide. The
strain did not show catalase or oxidase activity. The strain
Marseille-P2780 had a 16S rRNA gene sequence identity of
95.65% with Oscillobacter valericigenes strain Sjm 18-20
(NR_074793), the phylogenetically closest species with standing
in nomenclature (Fig. 4).

The MALDI-TOF MS spectra of these species are available
online (http://mediterranee-infection.com/article.php?laref=256
&titre=urms-database).

Nucleotide sequence accession number

The 16S rRNA gene sequences were deposited in GenBank
under the following accession numbers: 'Beduinibacterium mas-
silense' strain Marseille-P3337T (LT631514), 'Massilimaliae mas-
silensis' stain Marseille-P2963T (LT576408), 'Provenci-bac-
terium massiliense' stain Marseille-P2780T (LT558850) and 'Osci-
lobacter massiliensis' stain Marseille-P2778T (LT558848).

Deposit in a culture collection

The strains were deposited in the Collection de Souches de
l'Unité des Rickettsies (CSUR, WDCM 875) under numbers
P3337 ('Beduinibacterium massilense' strain Marseille-P3337T),
P2963 ('Massilimaliae massilensis' Marseille-P2963T), P2780
('Provenci-bacterium massiliense' Marseille-P2780T) and P2778
('Oscillobacter massiliensis' Marseille-P2778T).

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Conflict of interest

None declared.
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