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-P3337^T gen. nov., sp. nov., '*Massilimaliae massiliensis*' Marseille-P2963^T gen. nov., sp. nov., '*Provencibacterium massiliense*' Marseille-P2780^T gen. nov., sp. nov. and '*Oscilibacter massiliensis*' Marseille-P2778^T sp. nov., all isolated from the stool of a Bedouin from Saudi Arabia. We used a bacterial culturomics approach combined with taxonogenomics.

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, nonhaemolytic, 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

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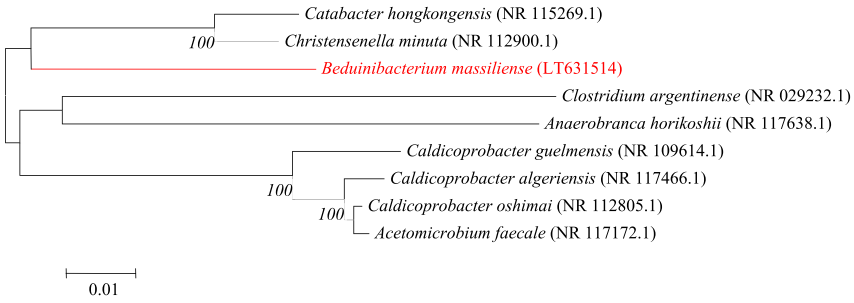

FIG. 1. Phylogenetic tree showing position of '*Beduinibacterium massiliense*' strain Marseille-P3337^T relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW and phylogenetic inferences obtained by Kimura two-parameter models using maximum-likelihood method within 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.

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

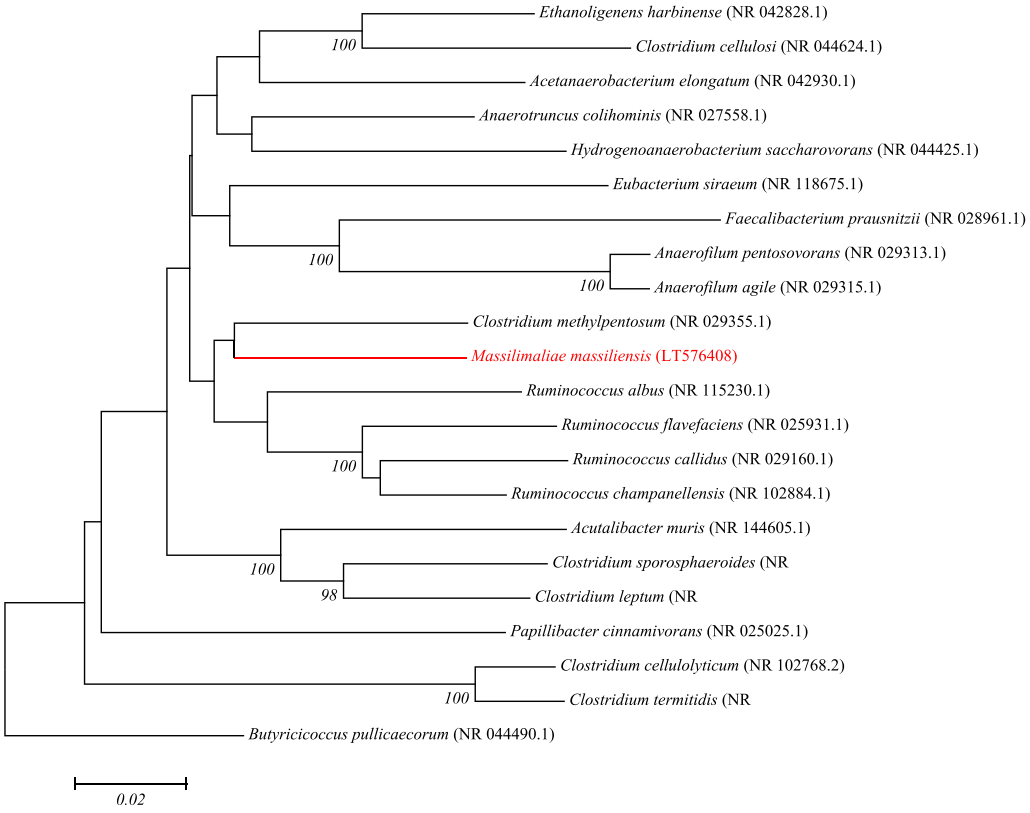

FIG. 2. Phylogenetic tree showing position of '*Massilimaliae massiliensis*' Marseille-P2963^T relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were done as described for Fig. 1.

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*' (Ma.ssi.li.ma'liae, L. gen. fem., composed of *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*' (ma.ssi.lien'sis, L. adj. neut., from *Massiliensis,* '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 H_2-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].

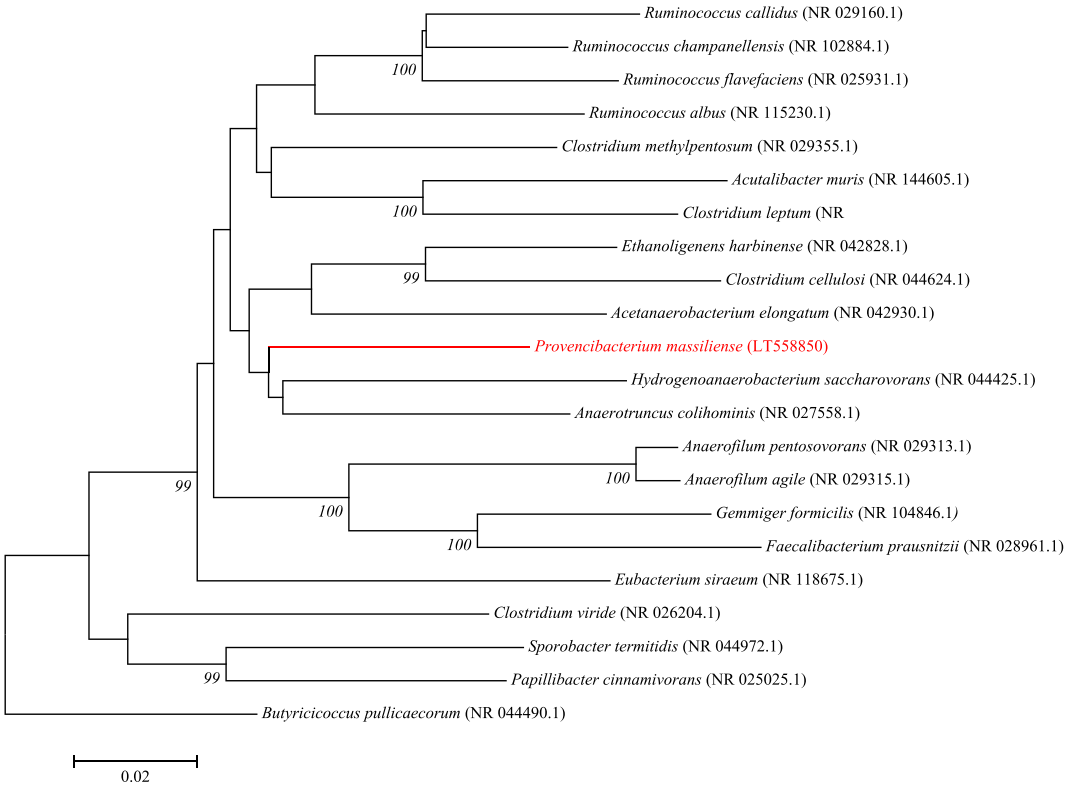

FIG. 3. Phylogenetic tree showing position of '*Provencibacterium massiliense*' Marseille-P2780^{T} relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were done as described for Fig. 1.

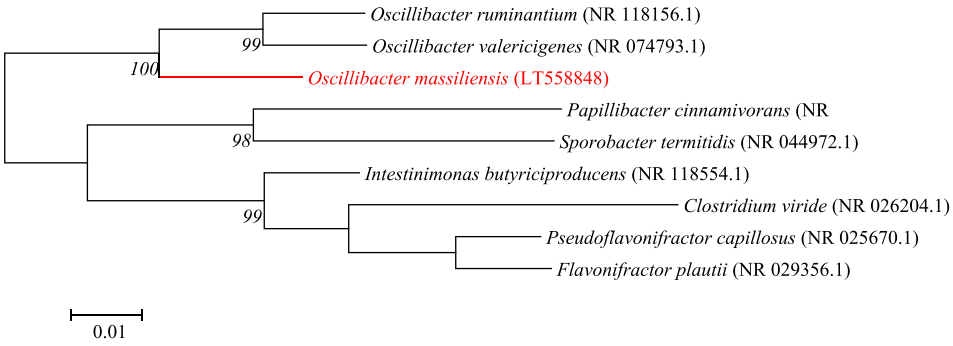

FIG. 4. Phylogenetic tree showing position of '*Oscilibacter massiliensis*' Marseille-P2778^T relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were done as described for Fig. 1.

Therefore, we propose the creation of the new genus '*Provencibacterium*' (Pro.ven.ci.bac.te'rium, L. gen. neut., composed of *Provenci,* for Provence, the region of France where the strain was isolated, and *bacterium*). '*Provencibacterium massiliense*' (ma.ssi.lien'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 '*Provencibacterium.*' Marseille-P2780^T is the type strain of the species '*Provencibacterium 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 filter-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-P2778 had a 16S rRNA gene sequence identity of 95.65% with *Oscilibacter valericigenes* strain Sjm 18-20 (NR_074793), the phylogenetically closest species with standing in nomenclature (Fig. 4). *Oscilibacter valericigenes,* a mesophilic, strictly anaerobic bacterium, was isolated from the alimentary canal of a Japanese *Corbicula* clam. Cells were Gram-negative, nonsporulating, straight to slightly curved rods, and were motile with oscillatory movements by means of peritrichous flagella [8]. This similarity of <98.65% leads us to putatively classify Marseille-P2778 as a new member in the *Oscillospiraceae* family of the order *Clostridiales* of the phylum *Firmicutes* [5]. Therefore, we propose the creation of the new species '*Oscilibacter massiliensis*' (ma.ssi.lien'sis, L. adj. neut., *Massiliensis,* 'to Massilia,' the antic name of Marseille, France, where the strain was isolated). Marseille-P2778^T is the type strain of the species '*Oscilibacter massiliensis.*'

MALDI-TOF MS spectra

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 massiliense*' strain Marseille-P3337^T (LT631514), '*Massilimaliae massiliensis*' stain Marseille-P2963^T (LT576408), '*Provencibacterium massiliense*' stain Marseille-P2780^T (LT558850) and '*Oscilibacter massiliensis*' stain Marseille-P2778^T (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 massiliense*' strain Marseille-P3337^T), P2963 ('*Massilimaliae massiliensis*' Marseille-P2963^T), P2780 ('*Provencibacterium massiliense*' Marseille-P2780^T) and P2778 ('*Oscilibacter massiliensis*' Marseille-P2778^T).

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Conflict of interest

None declared.

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