Description of *'Arabia massiliensis'* gen. nov., sp. nov., *'Gordonibacter massiliensis'* sp. nov., and *'Bacilliculturomica massiliensis'* gen. nov., sp. nov., isolated from a faecal specimen of a 50-year-old Saudi Bedouin woman

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Abstract

We report here the main characteristics of *'Arabia massiliensis'* strain Marseille-P3078^T gen. nov., sp. nov., *'Gordonibacter massiliensis'* Marseille-P2775^T sp. nov. and *'Bacilliculturomica massiliensis'* strain Marseille-P3303 gen. nov., sp. nov. The culturomics approach combined with taxonogenomics was used to characterize these strains, which were all isolated from a faecal specimen of a 50-year-old Saudi Bedouin woman.

Keywords: '*Arabia massiliensis*', '*Bacilliculturomica massiliensis*', '*Gordonibacter massiliensis*', gut microbiota
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Concerning the study of the human gut microbiota content, we isolated in 2016 using a bacterial culturomics approach three bacteria that could not be identified by matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) on a Microflex spectrometer (Bruker Daltonics, Bremen Germany) [1,2]. These strains were isolated from the stool sample of a 50-year-old healthy Bedouin woman living in the Jazan region of Saudi Arabia. This healthy individual provided informed consent. This study was performed in Saudi Arabia after approval from the ethical committee of King Abdulaziz University (Saudi Arabia) and the local ethic committee of the IFR48 (Marseille, France) under numbers 014-CEGMR-2-ETH-P and 09-022, respectively. All three strains failed to be identified by MALDI-TOF MS, and their 16S rRNA gene were sequenced using fD1-rP2 primers as described previously using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France) [3].

The stool sample was preincubated for 7 days at 37°C blood culture bottle (Becton Dickinson Diagnostics, Le Pont-de-Claix, France) supplemented with 3 mL of rumen fluid filter sterilized through a 0.2 µm pore filter (Thermo Fisher Scientific, Villebon-sur-Yvette, France). The strains Marseille-P3078 and Marseille-P2775 were isolated after an initial growth of 48 hours on Columbia agar supplemented with 5% sheep's blood at 37°C under strict anaerobic conditions.

Colonies of strain Marseille-P3078 appeared beige, non-haemolytic, motile and non–spore forming, and were 0.5 mm in size. The cells were Gram negative and small rod-shaped, ranging 1 µm long and 0.7 µm in diameter. The strain did not show oxidase activity but catalase positive activity. The strain Marseille-P3078 had a 16S rRNA gene sequence identity of 94.59% with *Gordonibacter pamelaeae* strain 7-10-1-b (NR_102934), the phylogenetically closest species with standing in nomenclature (Fig. 1). *Gordonibacter pamelaeae* strain 7-10-1-b^T was isolated from the colon of a patient with acute

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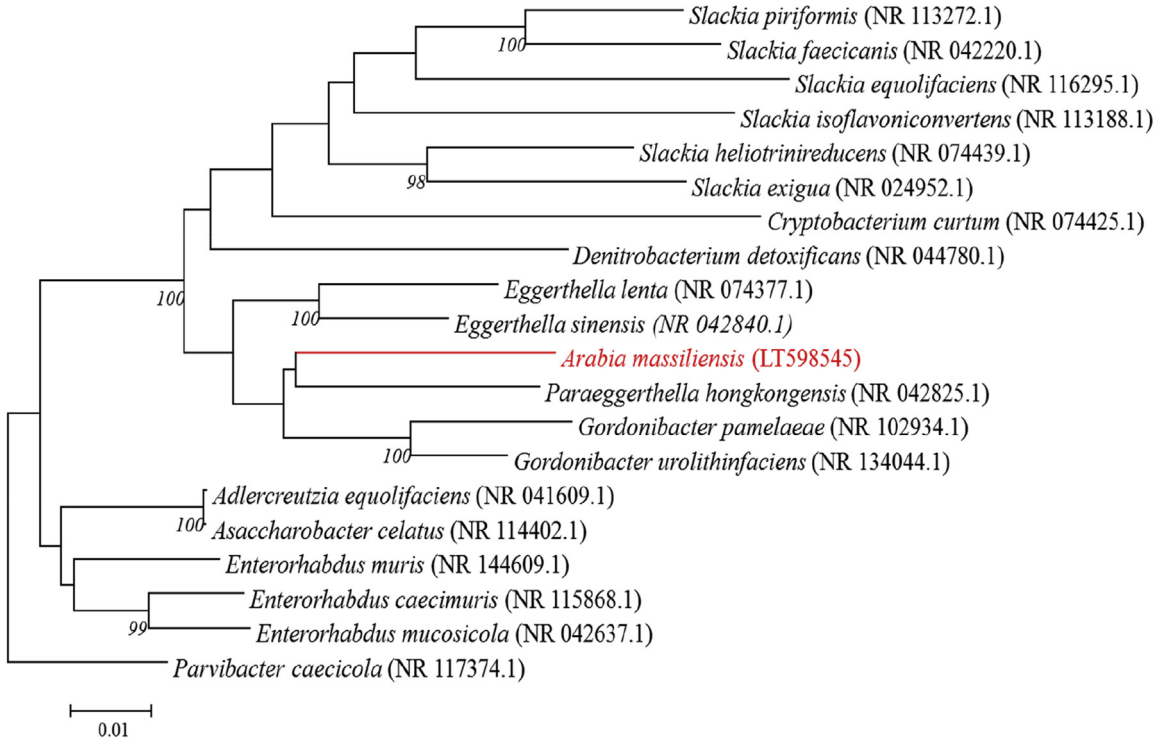

FIG. 1. Phylogenetic tree showing positions of *Arabia massiliensis* strain Marseille-P3078^T relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained by Kimura two-parameter models using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Scale bar indicates 1 to 2% nucleotide sequence divergence.

Crohn disease. This isolate formed small, pale white, semi-translucent colonies on solid cultivation media. The strain was catalase positive, obligately anaerobic, non–spore forming, Gram stain positive and short rod/coccobacilli [4]. This similarity of <95% led us to putatively classify Marseille-P3078 as a new member in the *Eggerthellaceae* family of the *Firmicutes* phylum [5]. Therefore, we propose the creation of the new genus *'Arabia'* (A.ra.bia, NL gen. fem., from Arabia, the region, Saudi Arabia, where the sample were collected). *'Arabia massiliensis'* is the type strain of the new genus *'Arabia.'* Marseille-P3078 ^{T} is the type strain of the species *'Arabia massiliensis'* (ma.ssi.lien′sis, L. adj. fem., *massiliensis* from 'Massilia,' the antic name of Marseille, France, where the strain was isolated).

Strain Marseille-P2775 presents beige colonies, non-haemolytic, motile and non–spore forming, with a 1 mm size. The cells were Gram and negative and rod-shaped, ranging 1.2 µm long and 0.5 µm in diameter. The strain did not show oxidase activity but catalase positive activity. The 16S rRNA gene sequence shows an identity of 95% with *Arabia massiliensis* and 97.19% with *Gordonibacter urolithinfaciens* strain CEBAS 1/15P (NR_134044), the phylogenetically closest species with standing in nomenclature (Fig. 2). *Gordonibacter urolithinfaciens* strain CEBAS 1/15PT, capable of metabolizing ellagic acid to urolithins, which was isolated from healthy human faeces and characterized by determining phenotypic, biochemical and molecular methods, was obligately anaerobic, non–spore forming, Gram stain positive and short rod/coccobacilli [6]. This similarity of <98.65% led us to putatively classify Marseille-P2775 as a new member in the genus *Gordonibacter,* in the *Eggerthellaceae* family of the *Firmicutes* phylum [5]. Therefore, we propose the creation of the new species *'Gordonibacter massiliensis'* (ma.ssi.lien′sis, L. adj. neut., *massiliensis* from 'Massilia,' the antic name of Marseille, France, where the strain was isolated). Marseille-P2775^T is the type strain of the new species *'Gordonibacter massiliensis.'*

Strain Marseille-P3303 was isolated after an initial growth of 72 hours on Columbia agar supplemented with 5% sheep's blood at 37°C under strict anaerobic conditions, after the stool specimen was preincubated for 30 days in an anaerobic blood culture bottle enriched with 37 g/L of Difco marine broth (Becton Dickinson Diagnostics) at 37°C. It was then subcultured on 5% sheep's blood–enriched agar (bioMérieux, Marcy l'Etoile, France). The colonies appeared ochre, non-haemolytic, nonmotile and spore forming, with a 2 mm size.

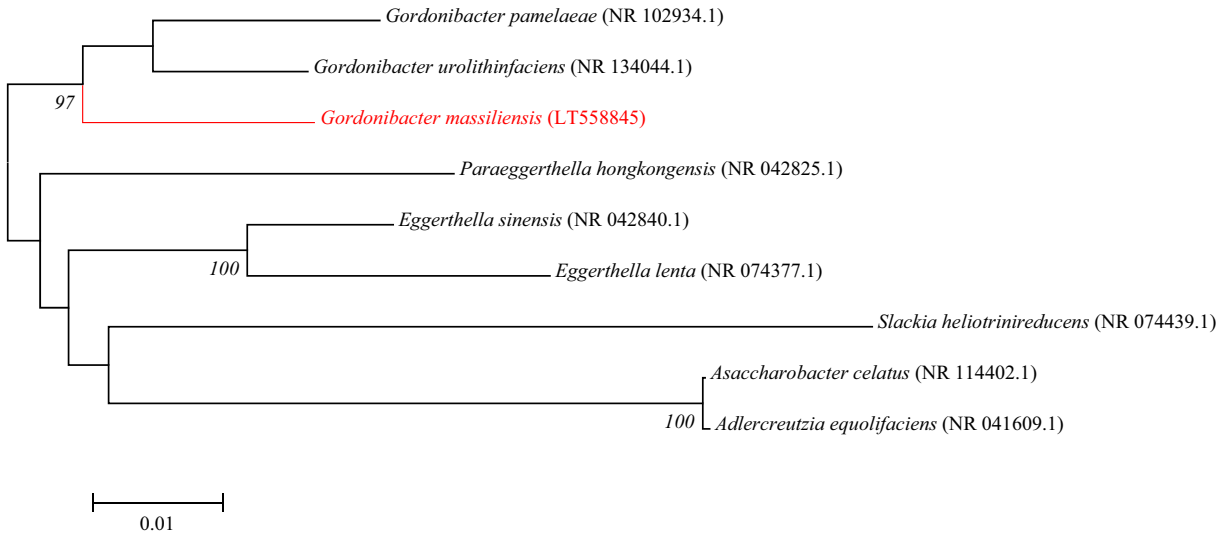

FIG. 2. Phylogenetic tree showing position of *Gordonibacter massiliensis* Marseille-P2775^T relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were done as described for Fig. 1.

The cells were Gram positive and rod shaped, ranging 2 to 5 µm long and 0.6 µm wide. The strain did not show catalase or oxidase activity. The 16S rRNA gene sequence showed an identity of 92.52% with *Anaerovorax odorimutans* strain NorPut1 (NR_028911), the phylogenetically closest species with standing in nomenclature (Fig. 3). The strictly anaerobic, Gram-positive, non-spore-forming bacterium strain NorPut1T ferments putrescine to acetate, butyrate, molecular hydrogen and ammonia [7]. This similarity of <95% led us to putatively classify Marseille-P3303 as a new member in the *Clostridiaceae* family of

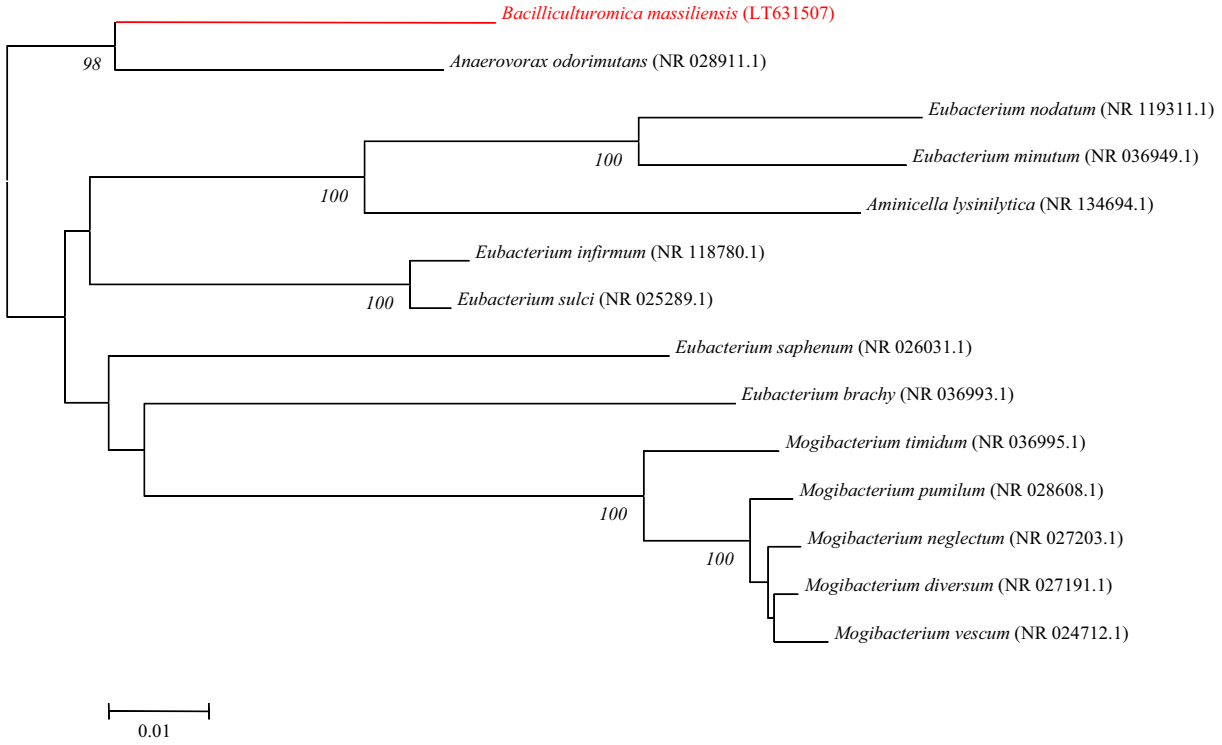

FIG. 3. Phylogenetic tree showing position of *Bacilliculturomica massiliensis* strain Marseille-P3303^T relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were done as described for Fig. 1.

Firmicutes [5]. Therefore, we propose the creation of the new genus *'Bacilliculturomica'* (Ba.ci.lli.cul.tu.ro.mi'ca, L. gen. fem., comprising *bacilli,* 'rod-shaped bacterium,' and *culturomica,* to refer to the culturomics laboratory in Marseille, where the strain was isolated). *'Bacilliculturomica massiliensis'* is the type strain of the new genus *'Bacilliculturomica.'* Marseille-P3303^T is the type strain of the species *'Bacilliculturomica massiliensis'* (ma.ssi.lien'sis, L. adj. fem., *massiliensis* from 'Massilia,' the antic name of Marseille, France, where the strain was isolated).

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 numbers

The 16S rRNA gene sequence was deposited in GenBank under accession numbers *'Arabia massiliensis'* strain Marseille-P3078^T (LT598545), *'Gordonibacter massiliensis'* Marseille-P2775^T (LT558845) and *'Bacilliculturomica massiliensis'* strain Marseille-P3303^T (LT631507).

Deposit in a culture collection

The strains were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under numbers P3078 (*'Arabia massiliensis'* strain Marseille-P3078^T), P2775 (*'Gordonibacter massiliensis'* strain Marseille-P2775^T) and P3303 (*'Bacilliculturomica massiliensis'* strain Marseille-P3303^T).

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Conflict of Interest

None declared.

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