

'*Collinsella phocaeensis*' sp. nov., '*Clostridium merdae*' sp. nov., '*Sutterella massiliensis*' sp. nov., '*Suttarella timonensis*' sp. nov., '*Enorma phocaeensis*' sp. nov., '*Mailhella massiliensis*' gen. nov., sp. nov., '*Mordavella massiliensis*' gen. nov., sp. nov. and '*Massiliprevotella massiliensis*' gen. nov., sp. nov., 9 new species isolated from fresh stool samples of healthy French patients

S. Ndongo, F. Cadoret, G. Dubourg, J. Delerce, P.-E. Fournier, D. Raoult and J.-C. Lagier

Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, Marseille, France

Abstract

Here we report of summary of the characteristics of '*Collinsella phocaeensis*' strain Marseille-P3245^T sp. nov., '*Clostridium merdae*' strain Marseille-P2953^T, '*Sutterella massiliensis*' strain Marseille-P2435^T sp. nov., '*Suttarella timonensis*' strain Marseille-P3282^T sp. nov., '*Enorma phocaeensis*' Marseille-P3242^T sp. nov., '*Mailhella massiliensis*' strain Marseille-P3199^T gen. nov., sp. nov., '*Mordavella massiliensis*' strain Marseille-P3246^T sp. nov. and '*Massiliprevotella massiliensis*' strain Marseille-P2439^T sp. nov. isolated from fresh stool samples of healthy French patients.

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Corresponding author: J.-C. Lagier, Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, 27 Boulevard Jean Moulin, 13385, Marseille cedex 05, France
E-mail: jclagier@yahoo.fr

In 2015, using the culturomics approach [1], we isolated eight bacteria which could not be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) [1,2]. These species were isolated from the stool samples of healthy French patients. All patients gave informed consent, and this study was approved by the ethics committee of the IFR48 Federative Research Institute under number 09-022. Because the identification of these eight isolates failed, we sequenced their 16S rRNA gene using fD1-rP2 primers as described previously by using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France) [3].

Strain Marseille-P3245^T was isolated after 5 days of pre-incubation at 37°C in an anaerobic 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) and 3 mL of sheep's blood (bioMérieux, Marcy l'Etoile, France). Then isolated colonies of the strain Marseille-P3245^T were obtained by subculturing on 5% sheep's blood agar (bioMérieux) in anaerobic conditions generated by AnaeroGen (bioMérieux) after 72 hours of incubation. The colonies on sheep's blood agar were whitish with a diameter of 1 mm. Cells were Gram-negative bacilli, with a mean width of 2.5 µm and a length of 3.5 to 6.0 µm under electron microscopy. Bacterial cells were nonmotile, nonhaemolytic, non-endospore forming and obligately anaerobic. This strain did not exhibit catalase or oxidase activities. Strain Marseille-P3245^T exhibited a 96.54% sequence similarity with *Collinsella tanakaei* strain JCM 16071 (NR_113273), the phylogenetically closest species with standing in nomenclature (Fig. 1). *Collinsella tanakaei* strain JCM 16071 is a Gram-negative bacillus isolated from human faeces in 2010 [4]. The 16S rRNA gene sequence similarity was <98.7% between strain Marseille-P3245^T and its

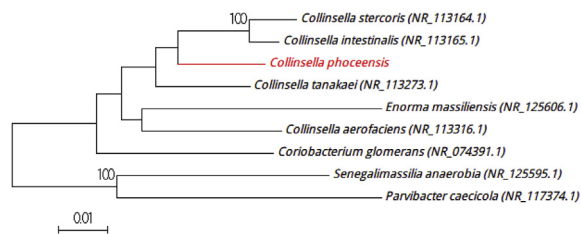


FIG. 1. Phylogenetic tree showing position of ‘*Collinsella phocaensis*’ strain Marseille-P3245^T (red) relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences obtained with Kimura-2 parameter models using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis to generate majority consensus tree 1000 times. Only values >95% are displayed. Scale bar represents 1% nucleotide sequence divergence.

phylogenetically closest species with standing in nomenclature, which putatively classifies it as a new member of the *Collinsella* genus in the *Actinobacteria* phylum [5]. Thus, we propose the creation of the new species ‘*Collinsella phocaensis*’ (pho.cae.en’sis, N.L. adj. fem., from *Phocaea*, the antic name of Phocaea, the Greek city which founded Marseille, France, where the strain was isolated). Marseille-P3245^T is the type strain of the species ‘*Collinsella phocaensis*.’

The growth of strain Marseille-P2953^T was obtained after a 7-day incubation period in a 5% sheep’s blood–enriched anaerobic blood culture bottle enriched with filter-sterilized rumen (Becton-Dickinson Diagnostics) at 37°C. Then subculture was performed on 5% sheep’s blood–enriched Columbia agar (bioMérieux) at 37°C under anaerobic atmosphere generated by AnaeroGen (bioMérieux). The colonies were beige, translucent and nonhaemolytic with about 1 to 2 mm in diameter on blood-enriched agar (bioMérieux). Gram staining highlighted rod-shaped Gram-positive bacilli. It is an obligate anaerobic, motile and spore-forming, with a mean diameter and length of 0.5 µm and 3.0 µm respectively observed under electron microscopy. The cells of strain Marseille-P2953^T were flagellate rods. Catalase and oxidase tests were negative. Strain Marseille-P2953^T exhibited a 97.8% 16S rRNA gene sequence identity with *Clostridium sporosphaeroides* strain DSM 1294 (GenBank accession no. NZ_KB911066), the phylogenetically closest species with standing in nomenclature (Fig. 2), which putatively classifies it as a member within the family *Clostridiaceae* in the *Firmicutes* phylum. *Clostridium sporosphaeroides* DSM 1294 is a Gram-positive strict anaerobic and was isolated from the environment [6]. The 16S rRNA gene sequencing of strain Marseille-P2953^T diverged by more than 2.3% from the other members of the genus *Clostridium*, which allowed us to

delineate the bacterial genera without carrying out DNA–DNA hybridization [5]. From these results, we propose the creation of the new species, ‘*Clostridium merdae*’ sp. nov. (mer’dae, L. gen. adj. merdae, ‘of faeces,’ referring to the source where the strain Marseille-P2953^T was isolated). Marseille-P2953^T is the type strain of the new species ‘*Clostridium merdae*.’

The initial growth of strain Marseille-P2435^T was obtained after a 3-day incubation period in a 5% sheep’s blood– and filter-sterilized rumen-enriched anaerobic blood culture bottle (Becton-Dickinson Diagnostics) at 37°C. Strain Marseille-P2435^T was isolated by subculture in a 5% sheep’s blood–enriched Columbia agar (bioMérieux) at 37°C under strict anaerobic conditions generated by AnaeroGen (bioMérieux). Strain Marseille-P2435^T is a Gram-negative bacillus, non-spore forming and nonmotile (4.0 × 4–10 µm). It presents neither catalase nor oxidase activities. On the agar plates, the colonies were slightly translucent, greyish and about 0.5 to 1 mm in diameter after 3 days of culture on 5% sheep’s blood agar. The strain Marseille-P2435^T had a 16S rRNA gene sequence identity of 97.94% with *Sutterella stercoricanis* strain 5BAC4 (NR_025600), the phylogenetically closest species with standing in nomenclature (Fig. 3), which potentially classifies it as a member of a new genus within the family *Sutterellaceae* in the *Proteobacteria* phylum. *Sutterella stercoricanis* strain 5BAC4 is a Gram-negative, rod-shaped bacterium which was isolated from canine faeces [7]. Strain Marseille-P2435^T shows a 16S rRNA gene sequence divergence of >1.3% with the phylogenetically closest species with a validly published name and standing in nomenclature [5]. Therefore, we propose the creation of the new species ‘*Sutterella massiliensis*’ (ma.ssi.li.en’sis, N.L. adj. fem., from *Massili*, the antic name of Marseille, France, where the strain was isolated). Marseille-P2435^T is the type strain of the species ‘*Sutterella massiliensis*.’

Strain Marseille-P3282^T was isolated under the same conditions as ‘*Sutterella massiliensis*’ strain Marseille-P2435^T. After 72 hours of incubation, the colonies were light grey, non-haemolytic and nontranslucent, and exhibited a diameter of 1 to 2 mm on 5% blood-enriched agar (bioMérieux). Cells from this strain were Gram-negative bacilli, and were mobile and non-spore forming. Under electron microscopy, bacterial cells exhibited a diameter of 2.5 to 3.0 µm and a length of 3.7 to 6.0 µm. Catalase and oxidase activities were negative. The strain Marseille-P3282^T had a 16S rRNA gene sequence identity of 95.34% with *Sutterella stercoricanis* strain 5BAC4 (NR_025600), the phylogenetically closest species with standing in nomenclature (Fig. 3). This divergence of the 16S rRNA gene sequence >1.3% with its phylogenetically closest species with a validly published name standing in nomenclature classifies

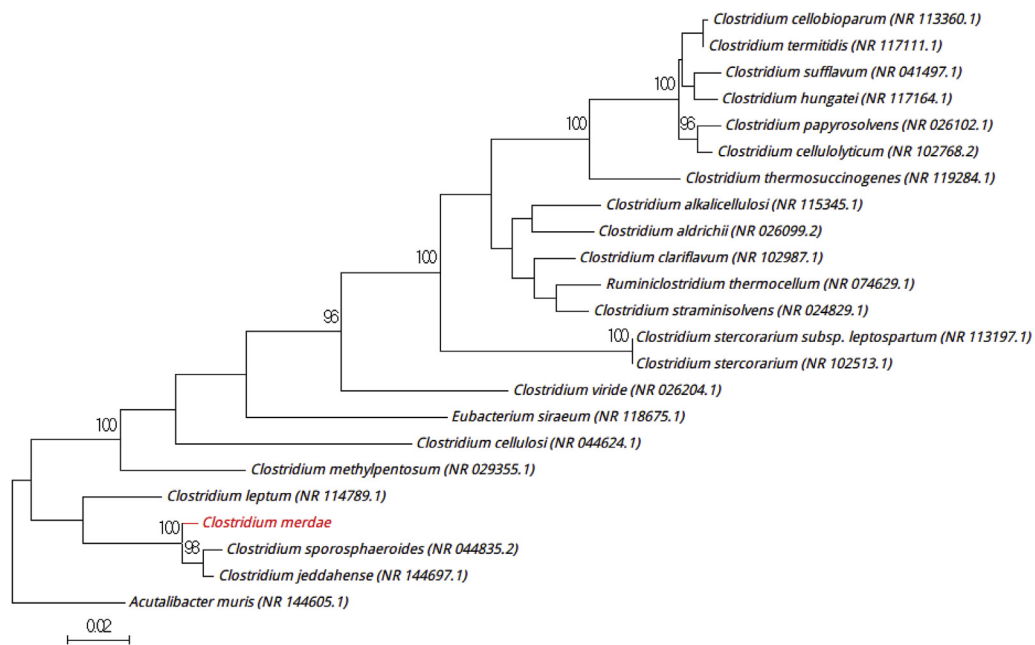


FIG. 2. Phylogenetic tree highlighting position of 'Clostridium merdae' strain Marseille-P2953^T (red) relative to other phylogenetically close members of family Clostridiaceae. Alignment and phylogenetic inferences were performed as described in Fig. 1. Scale bar represents 2% nucleotide sequence divergence.

Marseille-P3282 as a second new *Sutterella* species in the Sutterellaceae family [5]. Consequently, we propose the creation of the new species 'Sutterella timonensis' (ti.mo.nen'sis, N.L. adj. fem., from *Timone*, the name of the main hospital in Marseille, France, where the strain was isolated). Marseille-P3282^T is the type strain of the species 'Sutterella timonensis.'

Strain Marseille-P3242^T was isolated by directly seeding the fresh stool on 5% sheep's blood–enriched Columbia agar under anaerobic conditions generated by AnaeroGen after 72 hours of incubation at 37°C. Strain Marseille-P3242^T presented light nonhemolytic grey colonies, with a diameter of 0.5 to 1 mm on agar plates. The bacteria appeared as bacilli arranged in pairs or long chains with a length and diameter between 2.0 to 3.0 μm and 5.0 to 6.5 μm respectively under electron microscopy. Strain Marseille-P3242^T is an obligately anaerobic bacterium

that is nonmotile, non–spore forming and Gram-negative. Cells are negative for oxidase and catalase. The strain Marseille-P3242^T had a 16S rRNA sequence identity of 96.17% with 'Enorma massiliensis' strain phI (NR_125606), the phylogenetically closest species with standing in nomenclature (Fig. 4). 'Enorma massiliensis' strain phI is a Gram-positive bacillus, obligately anaerobic and nonmotile, and was isolated from the human faecal flora [8]. Given the 96.17% similarity level of the 16S rRNA gene sequence of strain Marseille-P3242^T with 'Enorma massiliensis' of <98.7%, we propose that it may be a representative strain of a new species within the *Enorma* genus in the *Actinobacteria* phylum [5]. Thus, we propose the creation of the new species 'Enorma phocaeensis' (pho.cae.en'sis, N.L. adj. fem., from *Phocaea*, the antic name of Phocaea, the Greek city

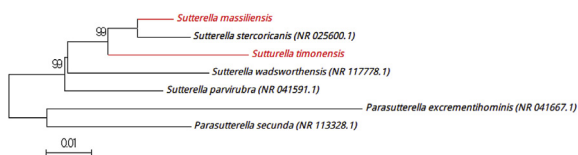


FIG. 3. Phylogenetic tree showing positions of 'Sutterella massiliensis' strain Marseille-P2435^T and 'Sutterella timonensis' Marseille-P3282^T (red) relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were performed as described in Fig. 1. Scale bar represents 1% nucleotide sequence divergence.

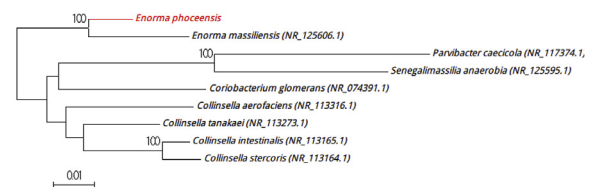


FIG. 4. Phylogenetic tree showing position of *Enorma phocaeensis* Marseille-P3242^T (red) relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were performed as described in Fig. 1. Scale bar represents 1% nucleotide sequence divergence.

which founded Marseille, France, where the strain was isolated). Marseille-P3242^T is the type strain of the species ‘*Enorma phocaensis*.’

The first isolation of the strain Marseille-P3199^T occurred after direct inoculation of fresh stool on 5% sheep’s blood–enriched Columbia agar, without enrichment, into blood bottles. Surface colonies on sheep’s blood agar after 72 hours of incubation under anaerobic atmosphere were circular, translucent and grayish, and approximately 1 mm in diameter. Strain Marseille-P3199^T is a Gram-negative bacillus with a mean diameter and length of 3.5 µm and 15 to 30 µm respectively. It is strictly anaerobic, motile and non–spore forming. Cells were negative for catalase and oxidase. The test of sulfate reducing was positive. Furthermore, the strain Marseille-P3199^T had a 16S rRNA gene sequence identity of 89.13% with *Desulfovibrio oxamicus* strain DSM 1925 (NR_043567) [9], the phylogenetically closest species with standing in nomenclature (Fig. 5). A similarity of 16S rRNA gene sequence <95% between our strain and the phylogenetically closest species with standing in nomenclature lead us to putatively classify Marseille-P3199 as a new member of the *Desulfovibrionaceae* family in the *Proteobacteria* phylum [5]. Thus, we propose the creation of the new genus *Mailhella* (mail.hel’la, L. gen. fem., from *Mailhe*, for the infectious disease resident Morgane Mailhe, who works in

Timone Hospital, Marseille, France). Marseille-P3199^T is the type strain of the species ‘*Mailhella massiliensis*’ (ma.ssi.li.en’sis, N.L. adj. fem., from *Massili*, the antic name of Marseille, France, where the strain was isolated).

Strain Marseille-P3246^T was isolated under the same conditions as ‘*Mordavella massiliensis*’ strain Marseille-P3199^T, from fresh stools seeded within 5 minutes after specimen collection. After 72 hours of incubation on petri dishes of 5% sheep’s blood–enriched Columbia agar, the colonies were whitish, circular and nonhaemolytic, with an average diameter of 0.5 mm. Gram staining showed Gram-negative colonies, occurring singly or in long chains of rods. Observed under the electron microscope, the cells presented a mean diameter and length of 2 µm and 6 to 12 µm respectively. Catalase and oxidase activities were negative. Strain Marseille-P3246^T exhibited a 16S rRNA gene sequence identity of 94.27% with *Clostridium oroticum* strain DSM 1287 (NR_117130), the phylogenetically closest species with standing in nomenclature (Fig. 6). *Clostridium oroticum* strain DSM 1287 is a Gram-positive rod-shaped bacteria that is anaerobic and nonmotile, as described by Cato *et al.* [10]. This phylogenetic analysis, based on 16S rRNA, showed that strain Marseille-P3246 presented a similarity <98.7% with the other phylogenetically closest species, which lead us to putatively classify Marseille-P3246 as a new genus of the *Lachnospiraceae* family in the

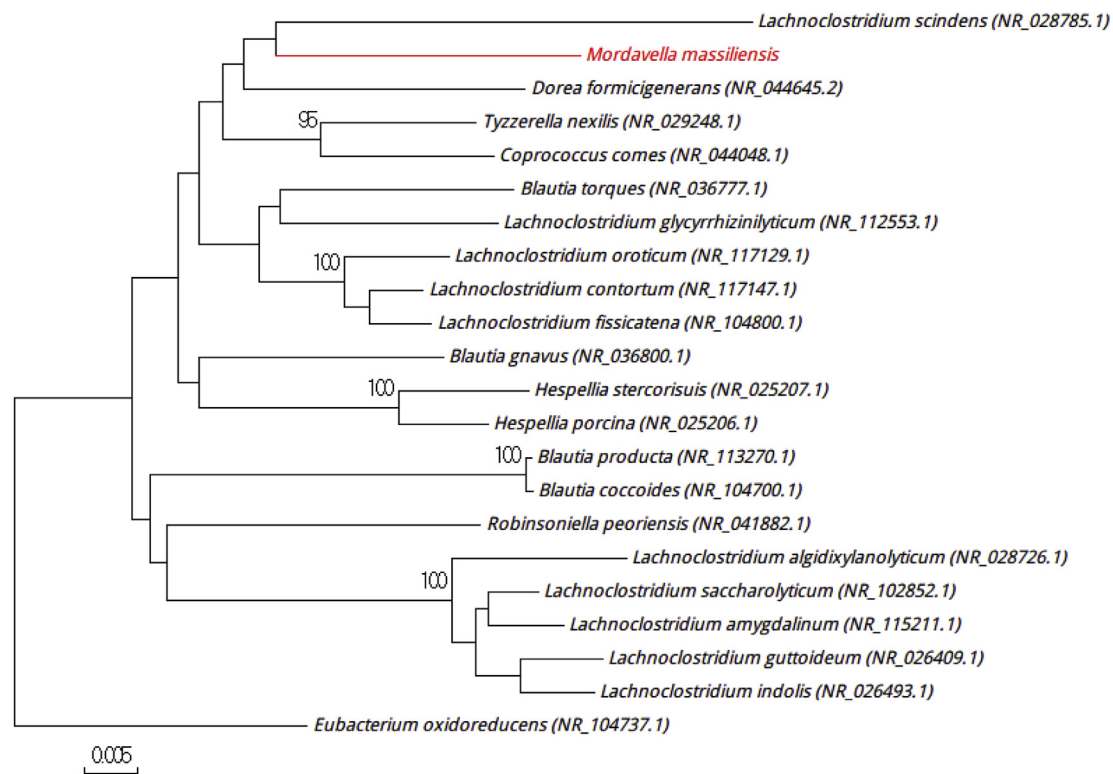


FIG. 5. Phylogenetic tree showing position of ‘*Mordavella massiliensis*’ strain Marseille-P3246^T (red) relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were performed as described in Fig. 1. Scale bar represents 0.5% nucleotide sequence divergence.

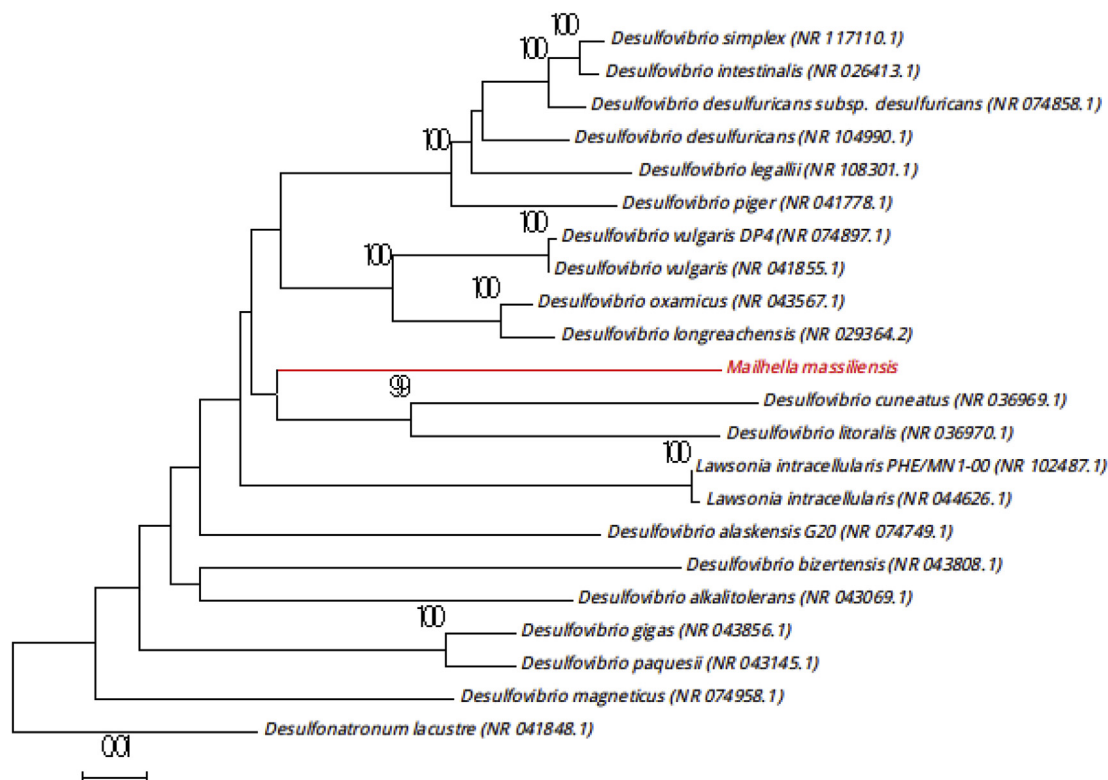


FIG. 6. Phylogenetic tree showing position of ‘*Mailhella massiliensis*’ strain Marseille-P3199^T (red) relative to other phylogenetically close neighbours. Alignment and phylogenetic inferences were performed as described in Fig. 1. Scale bar represents 1% nucleotide sequence divergence.

Firmicutes phylum [5]. The name proposed for this new genus is *Mordavella* (*mor.da.ve'lla*, L. gen. fem., from *Mordav*, for the infectious diseases residents Morgane Mailhe and Davide Ricaboni, who worked in Timone Hospital, Marseille, France). Marseille-P3246^T is the type strain of the species ‘*Mordavella massiliensis*’ (*ma.ssi.li.en'sis*, N.L. adj. fem., from *Massili*, the antic name of Marseille, France, where the strain was isolated).

‘*Massiliprevotella massiliensis*’ strain Marseille-P2439^T was isolated by culture on 5% sheep’s blood agar (bioMérieux) under anaerobic conditions after 48 hours, preceding a preincubation of the stool specimens in a blood culture bottle, with an addition of 5 mL of sheep rumen for 7 days. Surface colonies on sheep’s blood agar were beige, circular and transparent, 1 to 1.5 mm in diameter. Strain Marseille-P2439^T is a Gram-negative bacillus, motile and non-spore forming. The catalase and oxidase tests were negative. Observed under electron microscopy, the bacterial cells were polymorphic. Strain Marseille-P2439^T (LT223577) showed a 16S rRNA sequence identity of 91% with *Prevotella fusca* strain JCM17724 (NZ_CP012074), the phylogenetically closest species with standing in nomenclature (Fig. 7), which putatively classifies it as a member of a new genus within the family *Prevotellaceae* in the *Firmicutes* phylum. *Prevotella fusca* strain JCM17724 is a Gram-negative bacillus, obligately anaerobic and nonmotile, and was isolated from the human oral cavity [11].

Strain Marseille-P2439^T exhibits a 16S rRNA gene sequence divergence >5% with its phylogenetically closest species with a validly published name with standing in nomenclature [5]. Consequently, we formally propose the creation of the new genus ‘*Massiliprevotella*’ (by similarity with the *Prevotella* genus), and strain Marseille-P2439^T is the type strain of the new species ‘*Massiliprevotella massiliensis*’ gen. nov., sp. nov. (*ma.si.li.e'n.sis*, L. gen. masc. n., from *massiliensis*, ‘of Massilia,’ the Latin name of Marseille where the strain was first isolated).

MALDI-TOF MS spectra

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

Nucleotide sequence accession number

The 16s r RNA gene sequence was deposited in GenBank under the following accession numbers: ‘*Collinsella phocaeensis*’ strain Marseille-P3245^T (LT598576), ‘*Clostridium merdae*’ strain Marseille-P2953^T (LT732642), ‘*Sutterella*

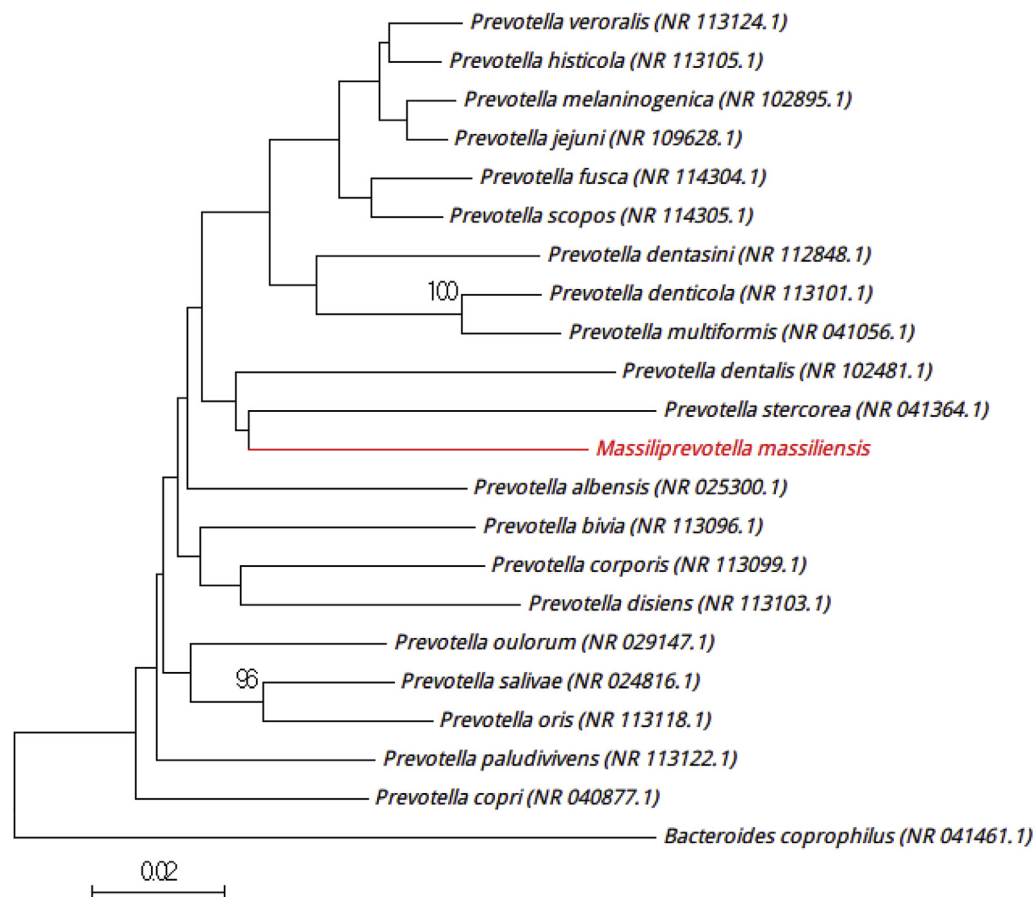


FIG. 7. Phylogenetic tree showing position of 'Massiliprevotella massiliensis' strain Marseille-P2439^T (red) relative to other phylogenetically close members of family Prevotellaceae. Scale bar represents 2% nucleotide sequence divergence.

massiliensis' strain Marseille-P2435^T (LT223579), 'Sutterella timonensis' Marseille-P3282^T (LT623892), 'Enorma phocaeensis' Marseille-P3242^T (LT598579), 'Mailhella massiliensis' strain Marseille-P3199^T (LT598584), 'Mordavella massiliensis' strain Marseille-P3246^T (LT615363) and 'Massiliprevotella massiliensis' strain Marseille-P2439^T (LT223577).

Deposit in a culture collection

The strains were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under numbers P3245 ('*Collinsella phocaeensis*' strain Marseille-P3245^T), P2953 (*Clostridium merdae*' strain Marseille-P2953), P2435 ('*Sutterella massiliensis*' strain Marseille-P2435^T), P3282 ('*Sutterella timonensis*' Marseille-P3282^T), P3242 ('*Enorma phocaeensis*' Marseille-P3242^T), P3246 '*Mordavella massiliensis*' strain Marseille-P3246^T), P3199 ('*Mailhella massiliensis*' strain Marseille-P3199^T) and P2439 ('*Massiliprevotella massiliensis*' strain Marseille-P2439^T).

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Conflict of Interest

None declared.

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