'*Collinsella phocaeensis*' sp. nov., '*Clostridium merdae*' sp. nov., '*Sutterella massiliensis*' sp. nov., '*Sutturella 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

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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., *'Sutturella 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.

Keywords: Culturomics, human gut microbiota, new bacteria, new species, taxonogenomics
Original Submission: 16 January 2017; **Revised Submission:** 20 February 2017; **Accepted:** 27 February 2017
Article published online: 4 March 2017

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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

http://dx.doi.org/10.1016/j.nmni.2017.02.005

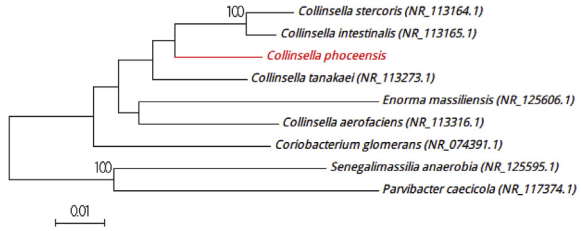

FIG. 1. Phylogenetic tree showing position of *'Collinsella phocaeensis'* strain Marseille-P3245^T (red) relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences obtained with Kimura-2 parameter models using maximum-likelihood method within 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 phocaeensis'* (pho.cae.en′sis, N.L. adj. fem., from *Phocaea,* the antic name of Phocea, the Greek city which founded Marseille, France, where the strain was isolated). Marseille-P3245^T is the type strain of the species *'Collinsella phocaeensis.'*

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, nonhaemolytic 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