NEW SPECIES

‘Khelaifiella massiliensis’, ‘Niameybacter massiliensis’, ‘Brachybacterium massiliense’, ‘Enterobacter timonensis’, ‘Massilibacillus massiliensis’, new bacterial species and genera isolated from the gut microbiota of healthy infants

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Abstract

The main characteristics of ‘Khelaifiella massiliensis’ strain Mt13T (= CSUR P1935, = DSM100591), ‘Niameybacter massiliensis’ strain Mt14T (= CSUR P1909, = DSM100592), ‘Brachybacterium massiliense’ strain MT5T (= CSUR P2240, = DSM101766), ‘Enterobacter timonensis’ strain mt20T (= CSUR P2201, = DSM 101775) and ‘Massilibacillus massiliensis’ strain Marseille-P2411T (= CSUR P2411, = DSM102838), new species isolated from the gut of healthy African infants, are presented.

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Extensive knowledge of the gut microbiota composition has become essential for the understanding of many aspects of health and disease. For that purpose, stool samples were collected from a 7-month-old healthy girl with a weight-for-height z-score of 0.15 from Niger and a 38-month-old healthy girl with a weight-for-height z-score of – 0.12 from Senegal. The diversity of these samples was explored using the culturomics concept [1,2]. Oral consent for this study was obtained from the children’s parents and the study received authorization from the local ethics committee of the Institut Federatif de Recherche IFR48 under number 09-022. As part of this study, strains Mt13T and Mt14T were isolated in the sample from Niger whereas strains mt20T, MT5T and Marseille-P2411T were isolated in the sample from Senegal.

Growth conditions and strains phenotypic characteristics

Strain Mt13T was first isolated after a 3-day pre-incubation in an anaerobic blood culture bottle supplemented with sheep blood and seeding on 5% sheep-blood-enriched Columbia agar (bioMérieux, Marcy l’Etoile, France) at 37°C in an anaerobic atmosphere. Large greyish and irregular colonies were obtained with a mean diameter of 8 mm. Cells were Gram-stain-positive bacilli with a mean diameter of 0.67 μm and a mean length of 2.99 μm. Catalase and oxidase activities were absent.

Strain Mt14T was first isolated after a 7-day pre-incubation in an anaerobic blood culture bottle supplemented with rumen and sheep blood and seeding on 5% sheep-blood-enriched Columbia agar at 37°C in an anaerobic
atmosphere. Small white colonies were obtained with a mean diameter of 3 mm. These colonies were formed with Gram-stain-positive bacilli with a mean diameter of 0.61 μm and a mean length of 4.47 μm.

Strain MT5T and strain mt20T were first isolated after a 24-h and 3-day aerobic pre-incubation, respectively, in a liquid medium containing 37 g of Difco Marine Broth (Becton Dickinson, Le Pont de Claix, France) per litre of sterile water at 37°C and on 5% sheep-blood-enriched Columbia agar in aerobic conditions. Strain mt20T formed large brown colonies with a mean diameter of 8 mm. Cells were Gram-stain-negative bacilli with a mean diameter of 0.8 μm and a mean length of 1.3 μm. Strain mt20T exhibited oxidase activity and catalase activities. As for strain mt21T, it formed white full, circular colonies with a mean diameter of 3 mm, which were formed with Gram-stain-positive cocci. Cells had a mean diameter of 0.57 μm and exhibited catalase activity and no oxidase activity.

After a 7-day pre-incubation in an anaerobic blood culture bottle supplemented with sheep blood and rumen and seeding on 5% sheep-blood-enriched Columbia agar in an anaerobic atmosphere, strain Marseille-P2411T was isolated. Small translucent colonies with a white centre measuring a mean diameter of 2 mm were obtained. These Gram-stain-positive bacilli had a mean diameter of 0.6 μm and a mean length of 3.65 μm. Catalase and oxidase activities were not exhibited by strain Marseille-P2411T.

FIG 1. Phylogenetic tree showing position of ‘Khelaifella massiliensis’ strain Mt13T relative to other phylogenetically close species with validly published name. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using a maximum likelihood method within MEGA software [12]. Numbers at nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. Selenomonas sputigena was used as outgroup. Scale bar indicates 2% nucleotide sequence divergence.
FIG 2. Phylogenetic tree showing position of ‘Nameybac ter massiliensis’ strain Mt14T relative to other phylogenetically close species with validly published name. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using a maximum likelihood method within MEGA software [12]. Numbers at nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. Veillonella parvula was used as outgroup. Scale bar indicates 2% nucleotide sequence divergence.
Strain identification

After a failed identification of all five strains using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [3,4], the 16S rRNA gene was sequenced using fD1 and rP2 primers [5] as well as the rpoB gene for strain mt20T [6]. Kim et al. suggested a 98.65% and a 95% similarity level threshold to define a new species and a new genus, respectively, without performing DNA–DNA hybridization [7] using the 16S rRNA gene while a 97.7% threshold was set for identification using the rpoB gene [6].

The 16S rRNA gene of strain Mt13T (Accession number LN850733) showed a 94.23% similarity level with Hathewaya histolytica strain ATCC 19401T (Accession number AB566416) [8]. This classifies strain Mt13T as a putative new genus within the family Clostridiaceae (Fig. 1), for which we suggest the name Khelaiella (Khe.lai.e.lai.N.L. fem. n. composed of Khelaifa, in honour of the microbiologist Saber Khelaifa, and bacterium, meaning rod) with the type species being ‘Khelaiella massiliensis’ (mas.si.li.en’sis; L. masc. adj. massiliensis for Massilia, the Roman name of Marseille, where strain Mt13T was first isolated). Strain Mt13T is the type strain of the species ‘Khelaiella massiliensis’.

The 16S rRNA sequences of strain Mt14T (Accession number LN906631) presented a 98.18% similarity level with Brachybacterium saurashtraense strain JG 06T (Accession number EU937750) [9]. Strain Mt14T was therefore classified as a new species within the genus Brachybacterium (Fig. 3) for which we suggest the name ‘Brachybacterium massiliense’ (mas.si.li.en’sis; L. masc. neut. adj. massiliense for Massilia, the Roman name of Marseille, where strain Mt14T was first isolated). Strain Mt14T is the type strain of the species ‘Brachybacterium massiliense’.

The 16S rRNA sequence of strain MT5T (Accession number LN906631) presented a 98.18% similarity level with Brachybacterium saurashtraense strain JG 06T (Accession number EU937750) [10]. Strain MT5T was therefore classified as a new species within the genus Brachybacterium (Fig. 3) for which we suggest the name ‘Brachybacterium massiliense’ (mas.si.li.en’sis; L. masc. neut. adj. massiliense for Massilia, the Roman name of Marseille, where strain Mt14T was first isolated). Strain MT5T is the type strain of the species ‘Brachybacterium massiliense’.

FIG 3. Phylogenetic tree showing position of ‘Brachybacterium massiliense’ strain MT5T relative to other phylogenetically close species with validly published name. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using a maximum likelihood method within MEGA software [12]. Numbers at nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. Dermabacter hominis was used as outgroup. Scale bar indicates 0.5% nucleotide sequence divergence.
The 16S rRNA gene sequence of strain mt20T (accession number LN906632) showed a 98.4% similarity level with *Enterobacter cloacae* strain ATCC 13047T (Accession number NR_102794) [7,8] so classifying strain mt20T as a new species within the genus *Enterobacter* (Fig. 4). The rpoB gene sequence (Accession number LN906633) showed a 95.12% similarity level with the rpoB gene of *Enterobacter cloacae* strain ATCC 13047T (Accession number AJ543726), confirming the status of strain mt20T as a putative new species [6]. Strain mt20T is the type strain of *Enterobacter timonensis* sp. nov. (ti.mo.nen'sis, L. gen. masc. timonensis, of Timone, the name of the hospital where strain mt20T was first isolated).

The 16S rRNA gene sequence of strain Marseille-P2411T (accession number LN906632) showed a 98.4% similarity level with *Enterobacter cloacae* strain ATCC 13047T (Accession number NR_102794) [7,8] so classifying strain mt20T as a new species within the genus *Enterobacter* (Fig. 4). The rpoB gene sequence (Accession number LN906633) showed a 95.12% similarity level with the rpoB gene of *Enterobacter cloacae* strain ATCC 13047T (Accession number AJ543726), confirming the status of strain mt20T as a putative new species [6]. Strain mt20T is the type strain of *Enterobacter timonensis* sp. nov. (ti.mo.nen'sis, L. gen. masc. timonensis, of Timone, the name of the hospital where strain mt20T was first isolated).

The MALDI-TOF MS spectra of *Khelaifiella massiliensis*, *Nia-meybacter massiliensis*, *Enterobacter massiliensis*, *Brachybacterium massiliense* and *Massilibacillus massiliensis* are available at http://www.mediterraneoinfection.com/article.php?laref=256&titre=urms-database.
The 16S rRNA sequences of strains Mt13T, Mt14T, MT5T and Marseille-P2411T are deposited in the GenBank database under accession numbers LN850733, LN850735, LN906631 and LT161896, respectively.

The 16S rRNA and \textit{rpoB} gene sequences for strain mt20T are also deposited in the GenBank database under accession numbers LN906632 and LN906633, respectively.

**Deposit in a culture collection**

Strains Mt13T, Mt14T, MT5T, mt20T and Marseille-P2411T were deposited in the Collection de Souches de l'Unité des Rick-ettsies (CSUR, WDCM 875) under numbers P1935, P1909, P2240, P2201 and P2411, respectively. Strains Mt14T, MT5T, mt20T and Marseille-P2411T were also deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) under numbers DSM100592, DSM101766, DSM101775 and DSM102838, respectively.

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