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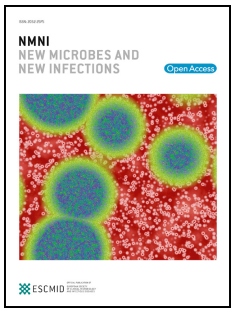

"*Khelaifiella massiliensis*", "*Niameybacter massiliensis*" "*Brachybacterium massiliense*" "*Enterobacter timonensis*", "*Massilibacillus massiliensis*", new bacterial species and genera isolated from the gut microbiota of healthy infants

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"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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Running title: New bacteria from the gut of healthy infants.

Keywords: new species; gut microbiota; culturomics; taxono-genomic

Abstract

Here are presented the main characteristics of "*Khelaifiella massiliensis*" strain Mt13^{T} (= CSUR P1935, = DSM100591), "*Niameybacter massiliensis*" strain Mt14^{T} (= CSUR P1909, = DSM100592), "*Brachybacterium massiliense*" strain mt5^{T} (= CSUR P2240 = DSM101766), "*Enterobacter timonensis*" strain mt20^{T} (= CSUR P2201 = DSM 101775) and "*Massilibacillus massiliensis*" strain Marseille-P2411^{T} (= CSUR P2411 = DSM102838), new species isolated from the gut of healthy African infants.

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 (WHZ) of 0.15 from Niger and a 38-month-old healthy girl with a WHZ 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 child's parents and the study received authorization from the local ethics committee of the Institut Federatif de Recherche IFR48 under number 09-022. As a part of this study, strains Mt13^T and Mt14^T were isolated in the sample from Niger while strains mt20^T, mt5^T and Marseille-P2411^T were isolated in the sample from Senegal.

Growth conditions and strains phenotypic characteristics

Strain Mt13^T was first isolated after a 3-day preincubation 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 anaerobic atmosphere. Large greyish and irregular colonies 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 Mt14^T was first isolated after a 7-day preincubation 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 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 MT5^T and strain mt20^T were first isolated after a 24-hour and 3-day aerobic preincubation 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 condition. Strain mt20^T 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 mt20^T exhibited oxidase and catalase activities. As for strain mt21^T, it formed white full, circular colonies with a mean diameter of 3 mm which were formed with Gram-stain-positive cooci. Cells had a mean diameter of 0.57 µm and exhibited catalase activity and no oxidase activity.

After a 7-day preincubation in an anaerobic blood culture bottle supplemented with sheep blood and rumen and seeding on 5% sheep blood enriched Colombia agar in anaerobic atmosphere, strain Marseille-P2411^T was isolated. Small translucent colonies with a white center 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-P2411^T.

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 mt20^T [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 Mt13^T (accession number LN850733) showed a 94.23% similarity level with *Hathewaya histolytica* strain ATCC 19401^T (accession number AB566416) [8]. This classifies strain Mt13^T as a putative new genus within the family *Clostridiaceae* (Figure 1) for which we suggest the name *Khelaifiella* (Khe.lai.fi.el'la N.L. fem. n. composed of *Khelaifia*, in honor of the microbiologist Saber Khelaifia and *bacterium* mean rod) with the type species being "*Khelaifiella massiliensis*" (mas.si.li.en'sis; L. fem.

adj. *massiliensis* for Massilia, the Roman name of Marseille, where strain Mt13^T was first isolated). Strain Mt13^T is the type strain of the species "*Khelaifiella massiliensis*".

The 16S rRNA gene sequences of strain Mt14^T (accession number LN850735) revealed a 92.18% similarity level with *Cellulosilyticum lentocellum* strain RHM5^T (accession number X71851) [9] thus confirming strain Mt14^T as a new genus within the family *Lachnospiraceae* (Figure 2). The name *Niameybacter* (Nia.mey.bac'ter for Niamey, the capital of Niger where the stool sample was collected and *bacter* for bacterium) is proposed for this new genus as well the type species "*Niameybacter massiliensis"* (mas.si.li.en'sis; L. masc. adj. *massiliensis* for Massilia, the Roman name of Marseille, where strain Mt14^T was first isolated). Strain Mt14^T is the type strain of the species "*Niameybacter massiliensis*".

The 16S rRNA sequence of strain MT5^T (accession number LN906631) presented a 98.18% similarity level with *Brachybacterium saurashtrense* strain JG 06^T (accession number EU937750) [10]. Strain MT5^T was thus classified as a new species within the genus *Brachybacterium* (Figure 3) for which we suggest the name "*Brachybacterium massiliense*" (mas.si.li.en'se; L. masc. neut. adj. *massiliense* for Massilia, the Roman name of Marseille, where strain MT5^T was first isolated). Strain MT5^T is the type strain of the species "*Brachybacterium massiliense*".

The 16S rRNA gene sequence of strain mt20^T (accession number LN906632) showed a 98.4% similarity level with *Enterobacter cloacae* strain ATCC 13047^T (accession number NR_102794) [7,8] thus classifying strain mt20^T as a new species within the genus *Enterobacter* (Figure 4). The rpoB gene sequence (accession number LN906633) showed a 95.12% similarity level with the rpoB gene of *Enterobacter cloacae* strain ATCC 13047^T (accession number AJ543726), thus confirming the status of strain mt20^T as a putative new species [6]. Strain mt20^T 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 mt20^T was first isolated).

The 16S rRNA gene sequence of strain Marseille-P2411 (accession number LT161896) presented a similarity level of 94.2% with *Anaerosinus glycerini* strain DSM 5192 (GenBank accession number NR_025297) [11], which is the phylogenetically closest species with a validly published name, was obtained (Figure 5). A new genus was thus created within the family *Veillonellaceae* named "*Massilibacillus*" (mas.si'li, L., masc. adj., massili for Massilia, the old Roman name for Marseille where the genus was first isolated; bacillus as a reference to the rod shape of the cell). Strain Marseille-P2411^T is the type species of "*Massilibacillus massiliensis*" (mas.si.li.en'sis, L., masc. adj., massiliensis for Massilia, the old Roman name for Marseille where strain Marseille-P2411^T was first isolated).

MALDI-TOF MS spectra

The MALDI-TOF MS spectrum of "*Khelaifiella massiliensis*", "*Niameybacter massiliensis*", "*Enterobacter massiliensis*", "*Brachybacterium massiliense*" and "*Massilibacillus massiliensis*" are available at http://www.mediterraneeinfection.com/article.php?laref=256&titre=urms-database.

Nucleotide sequence accession number

The 16S rRNA sequences of strains Mt13^T, Mt14^T, MT5^T and Marseille-P2411^T are deposited in the Genbank database under accession numbers LN850733, LN850735, LN906631 and LT161896 respectively.

The 16S rRNA and rpoB gene sequences for strain mt20^T are also deposited in the Genbank database under accession number LN906632 and LN906633 respectively.

Deposit in a culture collection

Strains Mt13^T, Mt14^T, MT5^T, mt20^T and Marseille-P2411^T were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under numbers P1935, P1909,

P2240, P2201 and P2411 respectively. Strains Mt14^T, MT5^T, mt20^T and Marseille-P2411^T 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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Conflict of Interest

None to declare.

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Figure legends

Figure 1. Phylogenetic tree showing position of "*Khelaifiella massiliensis*" strain Mt13^T relative to other phylogenetically close species with validly published name. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum likelihood method within MEGA software [12]. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. *Selenomonas sputigena* was used as outgroup. Scale bar indicates 2% nucleotide sequence divergence.

Figure 2. Phylogenetic tree showing position of "*Niameybacter massiliensis*" strain Mt14^T relative to other phylogenetically close species with validly published name. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum likelihood method within MEGA software [12]. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. *Veillonella parvula* was used as outgroup. Scale bar indicates 2% nucleotide sequence divergence.

Figure 3. Phylogenetic tree showing position of "*Brachybacterium massiliensis*" strain MT5^T relative to other phylogenetically close species with validly published name. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum likelihood method within MEGA software [12]. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. *Dermabacter hominis* was used as outgroup. Scale bar indicates 0.5 % nucleotide sequence divergence.

Figure 4. Phylogenetic tree showing position of "*Enterobacter timonensis*" strain mt20^T relative to other phylogenetically close species with validly published name. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum

likelihood method within MEGA software [12]. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generatemajority consensus tree. *Rosenbergiella nectarea* was used as outgroup. Scale bar indicates 0.5% nucleotide sequence divergence.

Figure 5. Phylogenetic tree showing position of "*Massilibacillus massiliensis*" strain Marseille-P2411^{T} relative to other phylogenetically close species with validly published name. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum likelihood method within MEGA software [12]. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. *Thermosinus carboxydivorans* was used as outgroup. Scale bar indicates 1% nucleotide sequence divergence.