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Maryam Tidjani Alou, Cheikh Sokhna, Fadi Bittar, Saber Khelaifia, Pierre-Edouard Fournier, et al.. ‘*Clostridium massiliodiemoense*’, a new species isolated from the human gut microbiota. *New Microbes and New Infections*, 2016, 13, pp.27 - 28. 10.1016/j.nmni.2016.05.014 . hal-01478544

HAL Id: hal-01478544

<https://amu.hal.science/hal-01478544v1>

Submitted on 26 Aug 2024

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## '*Clostridium massiliodiemoense*', a new species isolated from the human gut microbiota

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### Abstract

Herein, we report the main characteristics of '*Clostridium massiliodiemoense*' strain mt26 (= CSUR P2255), representative of a new species isolated from the gastrointestinal tract of a healthy 28-month-old Senegalese boy.

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**Keywords:** '*Clostridium massiliodiemoense*', culturomics, gut microbiota, taxono-genomics

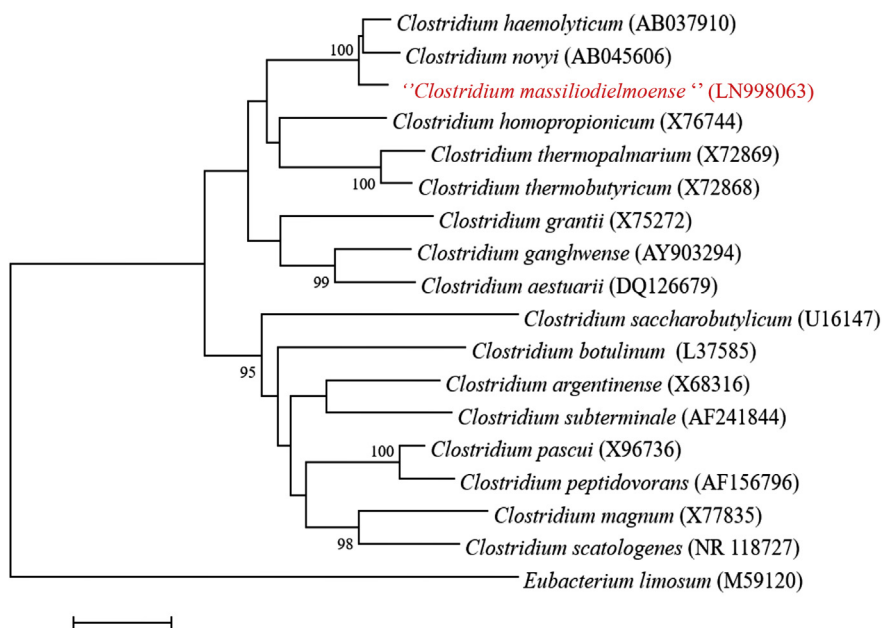
**Original Submission:** 11 May 2016; **Revised Submission:** 20 May 2016; **Accepted:** 23 May 2016

**Article published online:** 2 June 2016

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The culturomics approach is the method of choice to explore as exhaustively as possible the viable population of a microbial ecosystem [1,2]. We used culturomics to study the stool sample of a healthy 28-month-old Senegalese boy with a weight-for-height z-score of -0.99. Oral informed consent was given by the boy's parents and the study was approved by the ethics committee of the Institut Federatif de Recherche IFR48 under number 09-022. Primo-culture of strain mt26 was performed after a 7-day pre-incubation of the stool filtered on a 5-µm filter in an anaerobic blood-culture bottle supplemented with 5% sterile sheep blood. Subculture was performed on 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy-l'Etoile, France) at 37°C in anaerobic atmosphere. Strain mt26 formed a translucent biofilm covering the Petri dish. Cells were Gram-positive, rod-shaped and had mean diameter and length of 0.54 and 2.11 µm, respectively. Strain mt26 exhibited catalase and oxidase

activities. Our routine identification method, matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [3,4], failed to identify strain mt26 at the species level. Following 16S rRNA gene amplification and sequencing using the fD1-rP2 primers, as previously described [5], and using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France), strain mt26 was 98.4% similar to *Clostridium novyi* strain ATCC 17861 [6] (GenBank Accession number AB045606), the phylogenetically closest species with a validly published name (Fig. 1). *Clostridium novyi* strains have previously been isolated in soil, marine sediments and in animal and human wounds, and sometimes grow forming a biofilm covering the plate [7]. As the 16S rRNA gene similarity level was lower than the 98.65% threshold to define a new species [8,9], we propose the creation of a new species within the genus *Clostridium* that we named '*Clostridium massiliodiemoense*' (mas.si.li.o.di.el.mo.en'se, L. neutr. adj., a combination of *massilio*, of Massilia, the Roman name of Marseille, where strain mt26 was isolated, and *diemoense*, of Dielmo, the Senegalese village where the boy from whom the strain was cultivated lived). Strain mt26<sup>T</sup> is the type strain of '*C. massiliodiemoense*' sp. nov.



**FIG. 1.** Phylogenetic tree showing the position of 'Clostridium massiliodiemoense' strain mt26<sup>T</sup> relative to phylogenetically close species with standing in nomenclature. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using the maximum-likelihood method within the MEGA software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. Only bootstraps values >95% were displayed. *Eubacterium limosum* was used as an outgroup. The scale bar indicates a 2% nucleotide sequence.

## MALDI-TOF-MS Spectrum

The MALDI-TOF-MS spectrum of '*C. massiliodiemoens*' is available at <http://www.mediterranee-infection.com/article.php?leref=256&titre=urms-database>.

## Nucleotide Sequence Accession Number

The 16S rRNA gene sequence was deposited in GenBank under Accession number LN998063.

## Deposit in a Culture Collection

Strain mt26<sup>T</sup> was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number P2255.

## Conflicts of Interest

The authors certify that they have no conflict of interest in relation to this research.

## Funding

This work was funded by Méditerranée-Infection Foundation.

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