

# “*Negativibacillus massiliensis*” gen. nov., sp. nov., isolated from human left colon

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## Abstract

We report here the main characteristics of “*Negativibacillus massiliensis*” strain Marseille-P3213<sup>T</sup>, isolated from a human left-colon wash sample.

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**Keywords:** Culturomics, human gut, microbiota, “*Negativibacillus massiliensis*”, taxonogenomics

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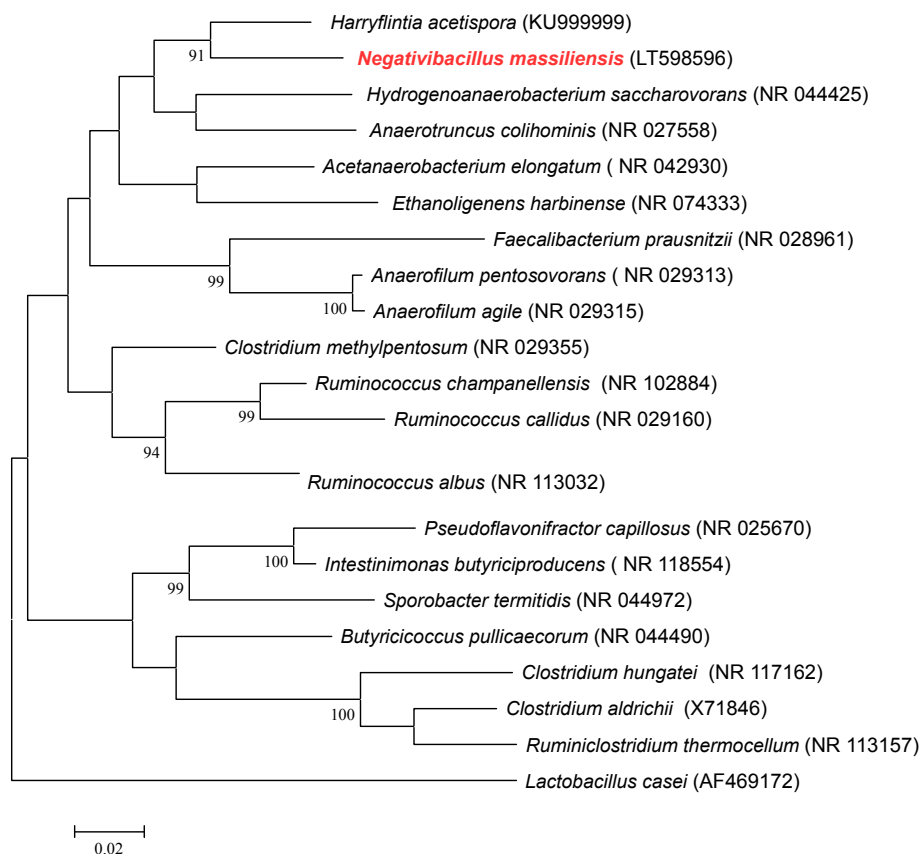
In 2016, as a part of a culturomics study [1] focused on the modifications of the human gut microbiome along the whole gastrointestinal tract, we isolated from the left colon of a 76-year-old patient a bacterial strain that escaped our systematic matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) screening on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [2]. The patient, who underwent simultaneous upper and lower endoscopy for medical reasons, provided signed informed consent, and the study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 09-022.

Strain Marseille-P3213<sup>T</sup> growth was obtained on 5% sheep’s blood–Columbia agar medium (bioMérieux, Marcy l’Étoile, France) under anaerobic atmosphere (anaeroGEN, Oxoid, Dardilly, France) after a 14-day enrichment of the fresh left-colon sample in an anaerobic blood culture bottle

(Becton Dickinson, Pont de Claix, France) added with 5 mL sheep’s blood (bioMérieux) and 5 mL 0.2 µm filtered (Thermo Fisher Scientific, Villebon-sur-Yvette, France) rumen at 37°C. After a 5-day anaerobic incubation on 5% sheep’s blood–enriched Columbia agar (bioMérieux), colonies were approximately circular, raised with undulated edges, whitish and not haemolytic. The mean diameter was 0.5 to 3 mm.

Bacterial cells were Gram-negative, nonmotile rods 0.5 to 0.8 µm wide by 3.0 to 4.5 µm long. Strain Marseille-P3213<sup>T</sup> tested catalase and oxidase negative. Sporulation was obtained in 20 minutes at 80°C, while no growth was observed under aerobic or microaerophilic (campyGen, Oxoid) conditions. Growth was obtained on blood-enriched agar under an anaerobic atmosphere at 37°C and 45°C (no growth at 20°C, 28°C and 55°C).

The 16S rRNA gene was sequenced using fD1-rP2 primers as previously described [3], using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Strain Marseille-P3213<sup>T</sup> exhibited a 93.52% sequence identity with *Harrifyntia acetispora* strain V20-281a<sup>T</sup> (GenBank accession no. KU999999), the phylogenetically closest species with standing in nomenclature (Fig. 1), which putatively classifies



**FIG. 1.** Phylogenetic tree showing position of “*Negativibacillus massiliensis*” strain Marseille-P3213<sup>T</sup> relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW and phylogenetic inferences obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstrap scores of at least 90 were retained.

strain Marseille-P3213<sup>T</sup> as a member of a new genus within the *Clostridiales* cluster IV in the phylum *Firmicutes*.

*Clostridiales* cluster IV was created in 1994 and groups microorganisms that exhibit *Clostridium* and non-*Clostridium*-like characteristics [4], including Gram-negative microorganisms [5]. *Harryflintia acetispora*, the phylogenetically closest species to strain Marseille-P3213<sup>T</sup>, is a Gram-negative, endospore-forming rod isolated from chicken gut. Unlike strain Marseille-P3213<sup>T</sup>, it is catalase positive [6].

On the basis of the phenotypic (catalase activity) and 16S rRNA gene sequence divergence of strain Marseille-P3213<sup>T</sup> with the phylogenetically closest species with standing in nomenclature [7], we propose here the creation of the new genus “*Negativibacillus*” (Ne.ga.ti.vi.ba.cil’lus, L. adj. *negativus*, ‘negative’; L. masc. n. *bacillus*, ‘a small staff’; N.L. masc. n. *Negativibacillus*, bacillus with a Gram-negative cell wall structure). Strain Marseille-P3213<sup>T</sup> (= CSURP3213 = DSM103594) is the type strain of “*Negativibacillus massiliensis*” gen. nov., sp. nov., (mas.si.li.en’sis, L. masc. adj. *massiliensis* from Massilia, the Roman name of Marseille).

## MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of “*Negativibacillus massiliensis*” strain Marseille-P3213<sup>T</sup> is available online (<http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>).

## Nucleotide sequence accession number

The 16S rRNA gene sequence was deposited in GenBank under accession number LT598596.

## Deposit in a culture collection

Strain Marseille-P3213<sup>T</sup> was deposited in the Collection de Souches de l’Unité des Rickettsies (CSUR, WDCM 875) under number P3213 and in the Deutsche Sammlung von Mikroorganismen und Zellkulturen under number DSM 103594.

## Acknowledgement

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## Conflict of Interest

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None declared.

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