

'*Lactobacillus timonensis*' sp. nov., a new bacterial species isolated from the human gut

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Abstract

Here, we describe the main characteristics of '*Lactobacillus timonensis*' sp. nov., strain Marseille-P3825^T (CSUR=P3825), isolated from a stool sample of a healthy Beninese woman.

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Keywords: Culturomics, disinfection with alcohol, human gut, '*Lactobacillus timonensis*' sp. nov., new species

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In February 2017, as part of a culturomics [1] study to map the microbial diversity of the human gut, a new bacterial species was isolated from a sample stool of a healthy 26-year-old woman of Beninese nationality, living in France. We obtained consent and the study was approved by the Institut Fédératif de Recherche 48 (Faculty of Medicine, Marseille, France), under agreement number 09-022. Stools were decontaminated with 70% alcohol (volume/volume) to eliminate vegetative forms, as previously described [2]. Strain Marseille-P3825^T was first isolated on 5% sheep blood-enriched Columbia agar (COS) (bio-Mérieux, Marcy l'Etoile, France) at 37°C in anaerobic atmosphere, after a 3-day pre-incubation in an anaerobic bottle of blood culture containing 5% sheep blood supplemented with 5% rumen fluid previously filter sterilized through a 0.2-µm pore filter (Thermo Fisher Scientific, Villebon sur Yvette, France). Colonies were greyish with a diameter ranging from 0.5 to 1.5 mm. Bacterial cells were non-motile Gram-positive rod-shaped bacilli of 0.7 µm wide by 3.5 µm long. Strain Marseille-P3825^T grew correctly after 48 hours of incubation in anaerobic conditions at a temperature ranging from 28°C to 45°C, but optimal growth was observed at 37°C. Growth was

also possible in microaerophilic conditions. Bacterial cells tolerate a pH of 6.5 to 8.5 and an amount of NaCl of 0–5 g/L. After 20 min of thermal shock at 80°C, this bacterium still grew at 37°C on 5% sheep blood-enriched Columbia agar but the result of a search for spores by electron microscopy was negative. The catalase and oxidase tests for strain Marseille-P3825^T were negative.

After three failed identifications by our systematic matrix-assisted desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) screening on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [3], the 16S rRNA gene was sequenced, using universal primers FDI and RP2 (Eurogentec, Angers, France) as previously described [4], using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Strain Marseille-P3825^T exhibited a 96.13% sequence identity with the type strain *Lactobacillus frumenti* DSM 13145 (GenBank Accession no. AJ250074), the phylogenetically closest species with standing in nomenclature (Fig. 1). Therefore, strain Marseille-P3825^T was classified as a member of the genus *Lactobacillus* in the order of *Lactobacillales* within the Firmicutes phylum. As the sequence identity with the phylogenetically closest validated species was <98.65%, which is the threshold recommended to define a species [5], we suggest the creation of a new species named '*Lactobacillus timonensis*' sp. nov. strain Marseille-P3825^T (*ti.mo.nen'sis*, L adj. fem., 'Timone,' the name of the main hospital of Marseille, France, where the strain was first isolated).

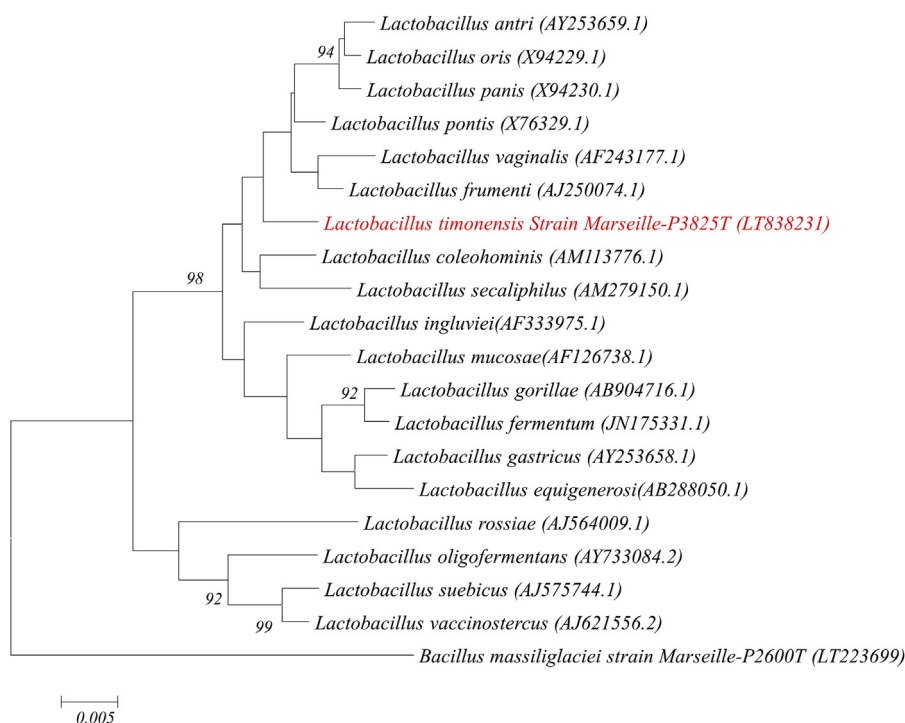


FIG. 1. Phylogenetic tree showing position of '*Lactobacillus timonensis*' strain Marseille-P3825^T relative to other phylogenetically close species with standing in nomenclature. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. Only bootstrap scores of at least 90 were retained. The scale bar indicates a 5‰ nucleotide sequence divergence.

Deposit in a culture collection

Strain Marseille-P3825^T was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number P3825.

MALDI-TOF-MS spectrum

The MALDI-TOF-MS spectrum of '*Lactobacillus timonensis*' Marseille-P3825^T 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 LT838231.

Transparency declaration

The authors have no conflicts of interest to declare.

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