

Description of '*Blautia phocaeensis*' sp. nov. and '*Lachnoclostridium edouardi*' sp. nov., isolated from healthy fresh stools of Saudi Arabia Bedouins by culturomics

S. I. Traore¹, E. I. Azhar^{2,3}, M. Yasir², F. Bibi², P.-E. Fournier¹, A. A. Jiman-Fatani⁴, J. Delerce¹, F. Cadoret¹, J.-C. Lagier¹ and D. Raoult^{1,2}

1) Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, Marseille, France, 2) Special Infectious Agents Unit, King Fahd Medical Research Centre, 3) Medical Laboratory Technology Department, Faculty of Applied Medical Sciences and 4) Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

We report here the main characteristics of '*Blautia phocaeensis*' strain Marseille-P3441 sp. nov. and '*Lachnoclostridium edouardi*' strain Marseille-P3397 sp. nov., that were isolated from a faecal specimen of a 42-year-old female Saudi Bedouin. We used a bacterial culturomics approach combined with taxono-genomics.

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Keywords: '*Blautia phocaeensis*', gut microbiota, '*Lachnoclostridium edouardi*', new species, taxono-genomics

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Corresponding author: D. Raoult, Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, 27 Boulevard Jean Moulin, 13385, Marseille cedex 05, France
E-mail: didier.raoult@gmail.com

In 2016 we isolated two bacteria, using a bacterial culturomics approach in the human gut microbiota, that could not be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [1,2]. These species were isolated from the stool of a Saudi Arabia Bedouin woman. The donor of the stool has signed informed consent and the study has been validated by the Ethics Committee of the IFR48 Federative Research Institute under number 09-022. Both strains failed to be identified by MALDI-TOF MS and their 16S rRNA genes were sequenced using fD1-rP2 primers as described previously using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France) [3].

The stool sample was pre-incubated for 1 day at 37°C in a blood culture bottle (BD Diagnostics, Le Pont-de-Claix, France) supplemented with 2 mL of rumen fluid filter-sterilized through a 0.2-µm pore filter (Thermo Fisher Scientific, Villebon sur Yvette, France) and 2 mL of sheep blood (bioMérieux, Marcy l'Etoile, France).

Strain Marseille-P3441 was isolated after initial growth of 2 days on Columbia agar supplemented with 5% sheep blood at 37°C under strict anaerobic conditions. The colonies appeared ochre, non-haemolytic, motile, spore forming and were 1 mm in size. The cells were Gram-positive, cocci-shaped with a 1-µm diameter. The strain did not show catalase or oxidase activity. The strain Marseille-P3441 had a 16S rRNA gene sequence identity of 95.53% with *Blautia glucersea* strain JCM 17039 (NR_113231), the phylogenetically closest species with standing in nomenclature (Fig. 1). Strain HFTH-1^T isolated from dog faeces, was Gram-positive, anaerobic, oval spore forming, rod-shaped, lecithinase-negative and lipase-negative [4]. The similarity was <98.65%, which led us to putatively classify Marseille-P3441^T as a new member in the *Lachnospiraceae* family of the *Clostridiales* order of the phylum *Firmicutes* [5]. Therefore we



FIG. 1. Phylogenetic tree showing the position of '*Blautia phocaensis*' strain Marseille-P3441^T relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained with Kimura two-parameter models using the maximum-likelihood method within the MEGA software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 0.5% nucleotide sequence divergence.

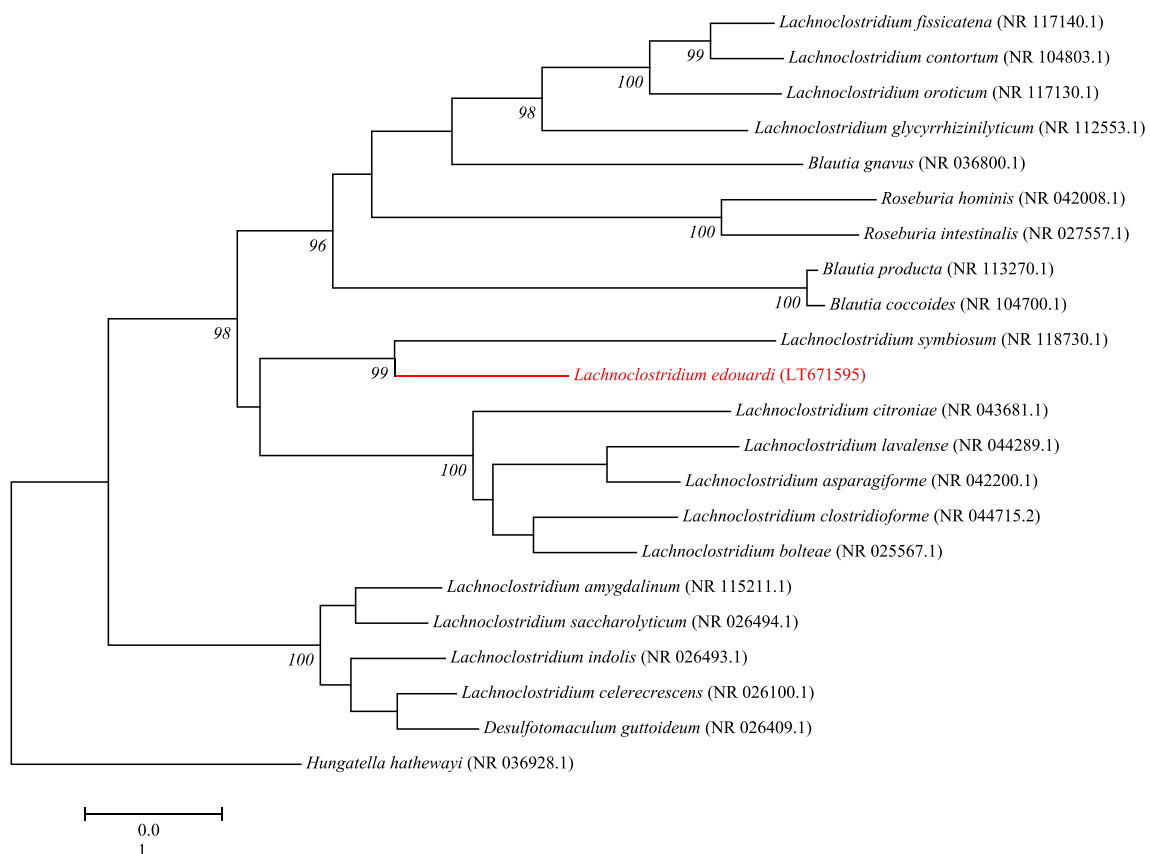


FIG. 2. Phylogenetic tree showing the position of '*Lachnospirillum edouardi*' strain Marseille-P3397^T relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained with Kimura two-parameter models using the maximum-likelihood method within the MEGA software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 1% nucleotide sequence divergence.

propose the creation of the new species '*Blautia phocaeensis*' (pho.ca.een'sis NL adj fem, to refer to Phocaea, the Latin name of Phocea, the city from whence came the founders of Marseille, France, where the strain was isolated). Marseille-P3441^T is the type strain of the species '*Blautia phocaeensis*'.

Strain Marseille-P3397 was isolated after an initial growth of 3 days on Columbia agar supplemented with 5% sheep blood at 37°C under strict anaerobic conditions. The colonies appeared beige, non-haemolytic, motile, non-spore forming and were 2 mm in size. The cells were Gram-positive, rod-shaped and ranged from 5 µm and 0.7 µm. The strain did not show catalase or oxidase activity. The strain Marseille-P3397 had a 16S rRNA gene sequence identity of 94.26% with *Clostridium symbiosum* strain ATCC 14940 (NR_118730), the phylogenetically closest species with standing in nomenclature (Fig. 2). This similarity of ≈ 95% leads us to putatively classify Marseille-P3397 as a new member in the *Clostridiaceae* family of the *Clostridiales* order of the *Firmicutes* phylum [5]. Therefore we propose the creation of the new species '*Lachnoclostridium edouardi*'. Marseille-P3397^T is the type strain of the species '*Lachnoclostridium edouardi*' (e.dou.ar'di L. adj. neut. Edouardi in honour of the microbiologist Sophie Edouard, Marseille, France).

MALDI-TOF MS spectra accession numbers

The MALDI-TOF MS spectra of these species are available at <http://mediterranee-infection.com/article.php?laref=256&titre=urms-database>.

Nucleotide sequence accession numbers

The 16S r RNA gene sequence was deposited in GenBank under accession numbers: '*Blautia phocaeensis*' strain Marseille-P3441^T (LT631543) and '*Lachnoclostridium edouardi*' strain Marseille-P3397^T (LT671595).

Deposit in a culture collection

The strains were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under numbers P3441 ('*Blautia phocaeensis*' strain Marseille-P3441^T) and P3397 ('*Lachnoclostridium edouardi*' strain Marseille-P3397^T).

Transparency declaration

None to declare.

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