

'*Caecibacter massiliensis*' gen. nov., sp. nov., isolated from human right colon

D. Ricaboni^{1,3}, M. Mailhe¹, A. Benezech², F. Cadoret¹, P.-E. Fournier¹ and D. Raoult¹

1) Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, 2) Service de Gastroenterologie, Hôpital Nord, Assistance Publique-Hôpitaux de Marseille, Marseille, France and 3) Department of Biomedical and Clinical Sciences, 3rd Division of Clinical Infectious Disease, University of Milan, Luigi Sacco Hospital, Milan, Italy

Abstract

We report here the main characteristics of '*Caecibacter massiliensis*' strain Marseille-P-2974^T (CSUR P2974), which was isolated from a human right colon sample.

© 2016 The Author(s). Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Keywords: '*Caecibacter massiliensis*', culturomics, human, gut, microbiota, taxonogenomics

Original Submission: 1 November 2016; **Revised Submission:** 14 November 2016; **Accepted:** 18 November 2016

Article published online: 24 November 2016

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 the context of an ongoing culturomics [1] and metagenomic study which targets the modifications of the human microbiome in the different gastrointestinal tracts, we isolated from a right colon lavage sample of a 27-year-old obese patient a bacterial strain that escaped identification by our systematic matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [2]. The patient provided signed informed consent, and the study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 2016-010.

Strain Marseille-P2974^T first growth was obtained on 5% sheep's blood–enriched Columbia agar medium (bioMérieux, Marcy l'Etoile, France) under anaerobic atmosphere (anaeroGEN, Oxoid, Dardilly, France) after a 7-day enrichment of the fresh right colon lavage sample in an anaerobic hemoculture bottle (Becton Dickinson, Pont de Claix, France) added with 5 mL of sterile sheep's blood (bioMérieux) and 5 mL of 0.2 µm filtered (Thermo Fisher Scientific, Villebon-sur-Yvette, France) rumen at 37°C.

After 96 hours of anaerobic incubation on 5% sheep's blood–enriched agar (bioMérieux) at 37°C, colonies were circular and convex, with entire edges and whitish in colour. Mean diameter was 0.2 to 0.8 mm. Neither haemolysis nor pigmentation was observed.

Bacterial cells were Gram-negative, nonmotile cocci with a mean diameter ranging from 0.8 to 1.2 µm. Strain Marseille-P2974^T tested catalase and oxidase negative. Different temperatures (20, 28, 37, 45 and 55°C) and atmospheres (anaerobic, microaerophilic (CampyGEN, Oxoid) and aerobic conditions) were tested on 5% sheep's blood–enriched Columbia agar (bioMérieux). Growth was achieved only under anaerobic atmosphere at 37°C. Sporulation test (20 minutes at 80°C) was negative.

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-P2974^T exhibited a 94.24% sequence identity with *Megasphaera cerevisiae* strain JCM 6130^T (GenBank accession no. LC037207), the phylogenetically closest species with standing in nomenclature (Fig. 1), which putatively classifies it as a member of a new genus within the family Veillonellaceae in the phylum Firmicutes.

The family Veillonellaceae comprises six genera with validated and published names (*Allisonella*, *Anaeroglobus*, *Dialister*, *Megasphaera*, *Veillonella* and *Negativicoccus*). Members of this family are

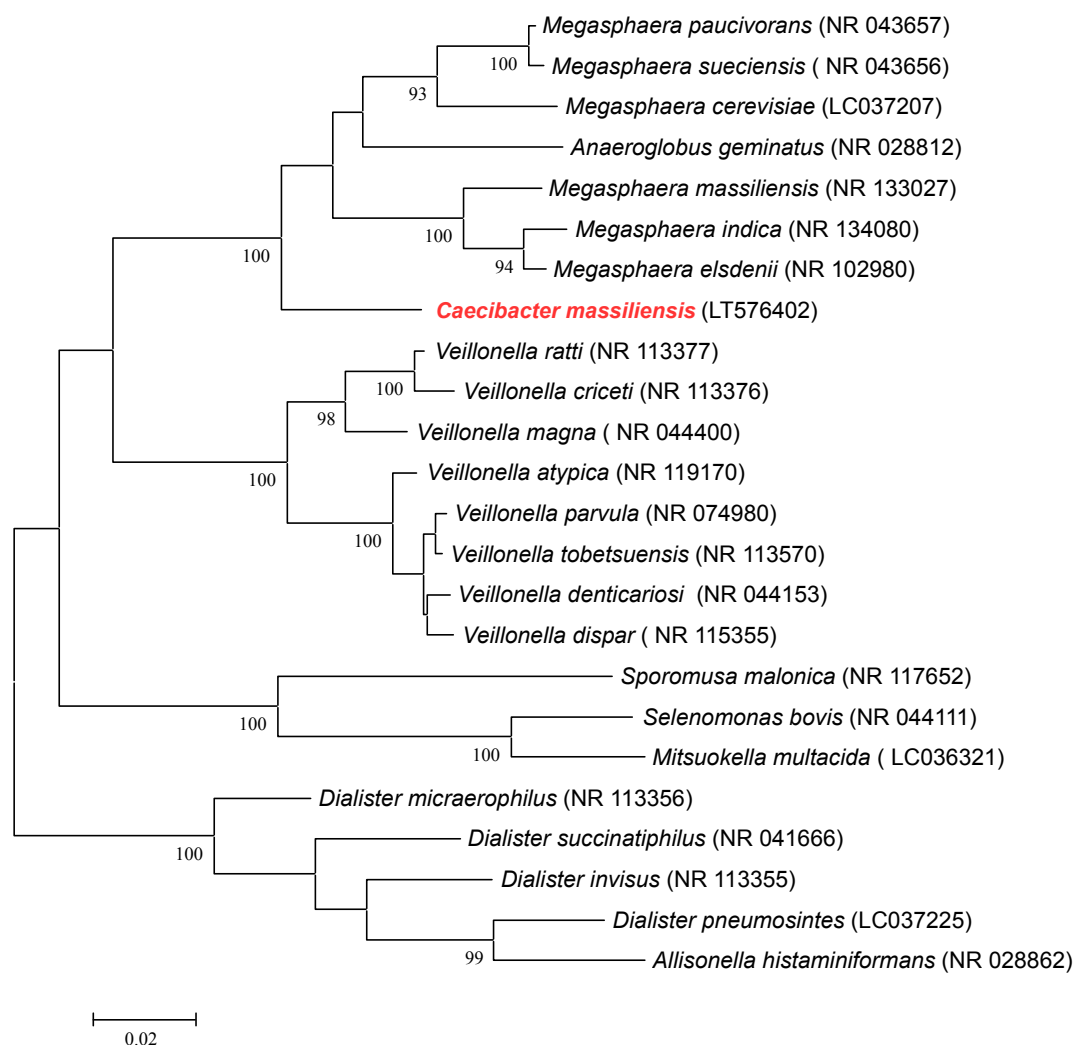


FIG. 1. Phylogenetic tree showing position of 'Caecibacter massiliensis' strain Marseille-P2974^T relative to other phylogenetically close neighbors. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Only bootstrap scores of at least 90 were retained.

characterized by being Gram-negative cocci or coccobacilli with a microaerophilic or anaerobic metabolism [4]. The closest genus to strain Marseille-P2974^T, *Megasphaera*, was created in 1971, and all its six recognized species possess a strictly anaerobic metabolism [5]. Members of this genus are commonly found in rumen, human and pig intestine (*Megasphaera elsdenii*), brewery-associated environments (*Megasphaera cerevisiae*, *Megasphaera paucivorans*, *Megasphaera sueciensis*), while *Megasphaera micro-nuciformis* was isolated from a human liver abscess [6].

On the basis of the 16S rRNA sequence divergence of strain Marseille-P2974^T with the phylogenetically closest species with standing in nomenclature [7] we propose here the creation of the new genus 'Caecibacter' (Cae.ci.bac.ter, L.

neut. n. caecum, 'the caecum of the large intestine'; N.L. masc. n. bacter, 'a rod'; N.L. masc. n. Caecibacter, 'a rod from the large intestine') for which the strain Marseille-P2974^T (= CSUR P2974) is the type strain. Strain Marseille-P2974^T is the type strain of 'Caecibacter 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 'Caecibacter massiliensis' strain Marseille-P2974^T is available online (<http://www>.

mediterranean-infection.com/article.php?leref=256&titre=urms-database).

Nucleotide sequence accession number

The 16S r RNA gene sequence was deposited in GenBank under accession number LT576402.

Deposit in a culture collection

Strain Marseille-P2974^T was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number P2974.

Acknowledgement

This study was funded by the Fondation Méditerranée Infection.

Conflict of Interest

None declared.

References

- [1] Lagier JC, Hugon P, Khelaifa S, Fournier PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. *Clin Microbiol Rev* 2015;28:237–64.
- [2] Seng P, Abat C, Rolain JM, Colson P, Lagier JC, Gouriet F, et al. Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol* 2013;51:2182–94.
- [3] Drancourt M, Bollet C, Carlioz A, Martelin R, Gayral JP, Raoult D. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J Clin Microbiol* 2000;38:3623–30.
- [4] Rogosa M. Transfer of *Peptostreptococcus elsdenii* Gutierrez et al. to a new genus, *Megasphaera* [*M. elsdenii* (Gutierrez et al.) comb. nov.]. *Int J Syst Evol Microbiol* 1971;21:187–9.
- [5] Marchandin H, Teyssier C, Campos J, Jean-Pierre H, Roger F, Gay B, et al. *Negativicoccus succinicivorans* gen. nov., sp. nov., isolated from human clinical samples, emended description of the family *Veillonellaceae* and description of *Negativicutes classis* nov., *Selenomonadales* ord. nov. and *Acidaminococcaceae* fam. nov. in the bacterial phylum *Firmicutes*. *Int J Syst Evol Microbiol* 2010;60:1271–9.
- [6] Lanjekar VB, Marathe NP, Ramana VV, Shouche YS, Ranade DR. *Megasphaera indica* sp. nov., an obligate anaerobic bacteria isolated from human faeces. *Int J Syst Evol Microbiol* 2014;64:2250–6.
- [7] Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346–51.