

'*Negativococcus massiliensis*', a new species identified from human stool from an obese patient after bariatric surgery

A. H. Togo¹, S. Khelaifia¹, R. Valero^{2,3}, F. Cadoret¹, D. Raoult^{1,4} and M. Million¹

1) Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, 2) Aix Marseille Université, NORT 'Nutrition, Obesity and Risk of Thrombosis', INSERM1062, INRA1260, 3) APHM, CHU Hôpital de la Conception, Service Nutrition, Maladies Métaboliques et Endocrinologie, Marseille, France and 4) Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

We report here the main characteristics of '*Negativococcus massiliensis*' strain AT7 (CSURP = P2082, DSM = 100853) isolated from a stool sample collected from a 47-year-old obese French man before bariatric surgery.

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Keywords: Culturomics, human gut microbiota, *Negativococcus massiliensis*, obesity, taxonomy

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Corresponding author: M. Million, 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: matthieumillion@gmail.com

In December 2011, we performed an exploratory study of gut microbiota from obese patients before and after bariatric surgery by culturomics, as described elsewhere [1]. Written consent was obtained from the patient at the Nutrition, Metabolic Disease and Endocrinology service at Timone Hospital, Marseille, France. The study and consent procedures were approved by the local IFR 48 ethics committee under consent number 09-022, 2010. The first growth was obtained after 30 days of culture in a blood culture bottle (BACTEC™ Lytic/10 Anaerobic/F Culture Vials; Le Pont de Claix, Isère, France) enriched with 4 mL of sheep blood and 4 mL of rumen under anaerobic atmosphere at 37°C medium. Agar-grown (Columbia agar + 5% sheep blood; bioMérieux, Marcy l'Étoile, France) colonies were circular, translucent and very small, with a mean diameter <0.5 mm after 72 h of incubation. A bacterium strain AT7 could not be identified by our systematic matrix-assisted laser desorption-ionization time-of-flight mass spectrometry

(MALDI-TOF MS) screening on Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [2]. The 16S rRNA gene sequencing was performed using rD1-rP2 primers with a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France) as previously described [3]. Strain AT7 exhibited a 98.6% 16S rRNA sequence similarity with *Negativococcus succinicivorans* strain ADV 07/08/06-B-1388 (= AIP 149.07 = CIP 109806 = DSM 21255 = CCUG 56017), the phylogenetically closest species with standing nomenclature (Fig. 1). This fact putatively classified strain AT7 as a member of the genus *Negativococcus* within the family *Veillonellaceae* in the phylum *Firmicutes*. However, this percentage remains lower than the 98.7% 16S rRNA gene sequence threshold recommended by Stackebrandt and Ebers to delineate a new species [4]. Strain AT7 exhibits a 16S rRNA gene sequence divergence of 1.4% with its phylogenetically closest species with standing in nomenclature [5]. Cells are Gram-negative staining, non-motile and coccoid; ranging in length from 0.2 to 0.5 µm. Strain AT7 was catalase-negative and oxidase-negative. On the basis of phenotypic and phylogenetic characteristics we propose the creation of this new species named '*Negativococcus massiliensis*' (ma.si.li.en'.sis. L. fem. adj. *massiliensis*, of Massilia, the Latin name of Marseille where *N. massiliensis* was first isolated). Strain AT7 is the type strain of the new species '*Negativococcus massiliensis*' sp. nov.

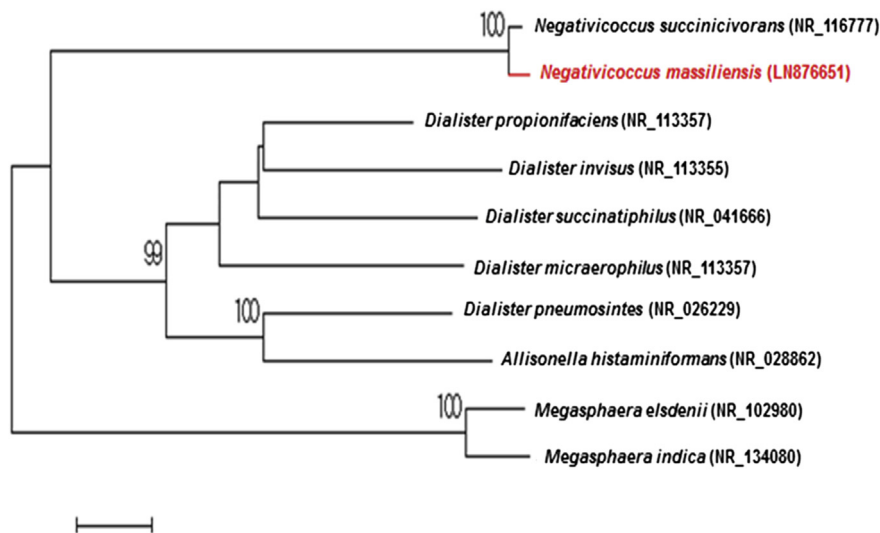


FIG. 1. Phylogenetic tree showing the position of 'Negativicoccus massiliensis' strain AT7 relative to other phylogenetically closest species. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using the maximum-likelihood method within the MEGA software. Numbers at the nodes depict bootstrap percentages values obtained by repeating the analysis 500 times to yield a consensus tree. Only bootstrap values >95% were retained. The scale bar indicates a 2% nucleotide sequence divergence.

MALDI-TOF MS Spectrum Accession Number

The MALDI-TOF-MS spectrum of this strain is available at <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 number LN876651.

Deposit in a Culture Collection

Strain AT7 was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR) under number P2082.

Conflict of Interest

The authors have no conflicts of interest to declare.

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