

'*Flaviflexus massiliensis*', a new bacterial species isolated from the human gut

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Abstract

Herein, we report the main characteristics of '*Flaviflexus massiliensis*' strain SIT4^T (CSURP1300), a new bacterium that was isolated from the stool specimen of a 2-year-old girl from Niger suffering from kwashiorkor.

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Keywords: Culturomics, emerging bacterium, gut microbiota, *Flaviflexus massiliensis*, taxonomy

Original Submission: 10 June 2016; **Revised Submission:** 5 July 2016; **Accepted:** 25 July 2016

Article published online: 29 July 2016

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In 2014, as part of the exploration of the human microbiota by culturomics [1,2], we isolated the new bacterial strain SIT4, from the stool of a 2-year-old girl from Niger suffering from kwashiorkor. This strain could not be identified by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [3]. After obtaining the informed consent of the girl's parents and the approval of the National Ethics Committee of Niger and the ethics committee of the Institut Fédératif de Recherche 48 (Marseille, France) under number 09-022, the stool specimen was collected in Niger in 2014 and shipped to Marseille, France, where it was stored at -80°C until use for analysis. The stool specimen was pre-incubated for 15 days in an aerobic blood culture bottle (Becton Dickinson, Le Pont de Claix, France) enriched with 37 g/L of Difco Marine Broth (Becton Dickinson). It was then subcultured on 5% sheep-blood-enriched Columbia agar (bioMérieux, Marcy L'Etoile, France) at 37°C in an aerobic atmosphere. After 24 h of incubation, smooth white colonies with a diameter ranging from 0.2 to 0.5 mm were observed. Bacterial cells were Gram-positive cocco-bacilli with

diameter and length ranging from 0.3 to 0.4 μm and 0.5 to 0.8 μm , respectively. Strain SIT4 was catalase-positive but oxidase-negative. The 16S rRNA gene was sequenced using the fD1-rP2 primers as previously described [4], using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Strain SIT4 exhibited a 96.7% sequence identity with *Flaviflexus huanghaiensis* strain H5 (GenBank Accession number JN815236.1), the phylogenetically closest species with standing in nomenclature (Fig. 1). *Flaviflexus huanghaiensis* was first isolated from a sediment sample and described in 2013. It is a facultatively anaerobic, Gram-positive, non-motile and straight to slightly curved rod-shaped bacillus [5].

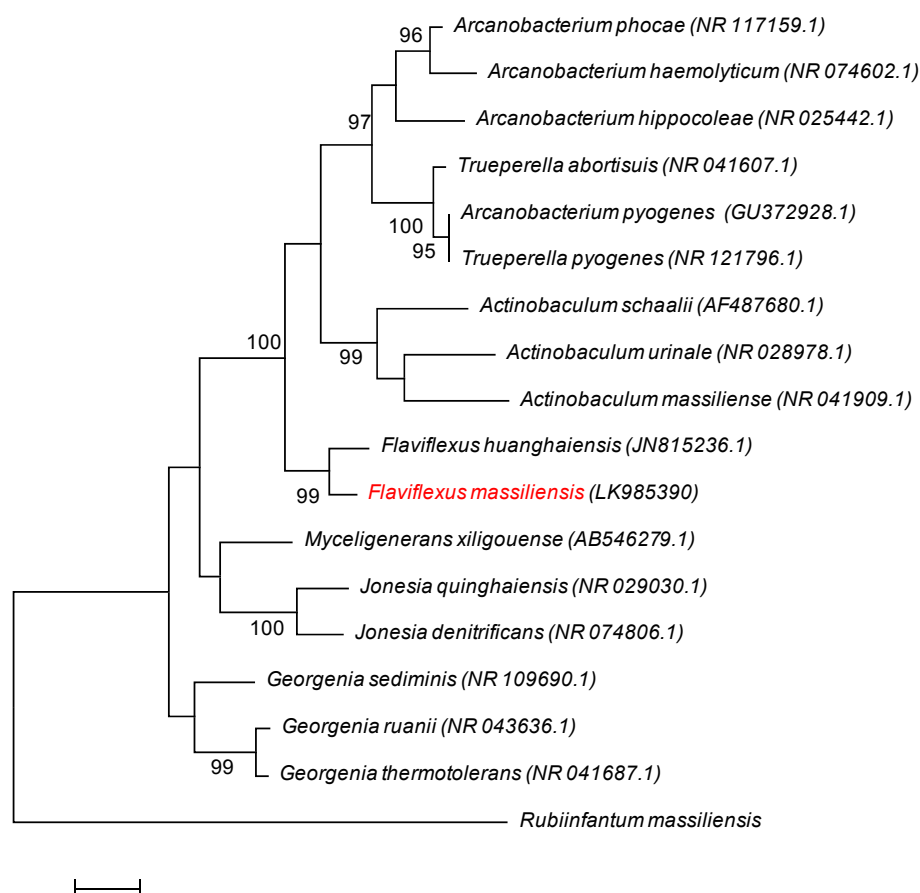
Strain SIT4^T exhibited a 16S rRNA sequence divergence $>1.3\%$ with its phylogenetically closest species with standing in nomenclature [6]; for this reason, we propose that it is the type strain of a new species within the genus *Flaviflexus* for which we propose the name '*Flaviflexus massiliensis*' (L., masc. adj., *massiliensis* for Massilia, the Roman name of Marseille, where strain SIT4^T was isolated).

MALDI-TOF-MS Spectrum. The MALDI-TOF-MS spectrum of '*F. massiliensis*' 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 Accession number LK985390.

Deposit in a culture collection. Strain SIT4 was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number P1300.

FIG. 1. Phylogenetic tree showing the position of '*Flaviflexus massiliensis*' strain SIT4^T relative to other phylogenetically close species with validly published names. Sequences were aligned using CLUSTALW, and phylogenetic inferences obtained using the maximum likelihood method within the MEGA software. Accession numbers are indicated in parentheses. Numbers at the nodes are percentages of bootstrap values ($\geq 95\%$) obtained by repeating the analysis 500 times to generate a majority consensus tree. The scale bar indicates a 2% nucleotide sequence divergence.



Conflict of interest

The authors certify that they do not have any conflict of interest in relation to this research.

Funding

This work was funded by the Méditerranée-Infection Foundation.

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