

'*Bittarella massiliensis*' gen. nov., sp. nov. isolated by culturomics from the gut of a healthy 28-year-old man

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Abstract

We report here the main features of the proposed new bacterial genus *Bittarella*. The type strain '*Bittarella massiliensis*' GD6^T (CSUR P2149) was isolated from a stool sample from a healthy French man.

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We isolated in April 2015, as part of the culturomics study of the Human Microbiome [1], an oxygen-intolerant species that could not be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) screening (score <1.7) using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [1–3]. The species was isolated from the faeces of a healthy, 28-year-old French man. The stool was inoculated without delay in different culture conditions used for culturomics [1]. The initial growth of the GD6 strain occurred after 48 h of anaerobic incubation in a 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France). The stool was pre-incubated for 10 days at 37°C in an anaerobic atmosphere in a culture bottle in the presence of 5% sheep blood and 5% rumen fluid filter-sterilized through a 0.2-µm-pore filter (Thermo Fisher Scientific, Villebon sur Yvette, France). The donor gave signed informed consent and the study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 09-022.

The colonies appeared to be 0.5 mm in size, homogeneous, translucent, smooth and non-haemolytic. Strain GD6 was a non-

motile, Gram-negative and rod-shaped bacteria with a mean diameter of 250 nm and a length of 1 µm without spore-forming activity. Catalase and oxidase were negative. The complete 16S rRNA gene of the bacterium was sequenced as previously described [4] and shared 89.5% of identity with *Anaerotruncus massiliensis* strain AT3 (GenBank Accession number LN866995) [5]. The phylogenetically closest species standing in nomenclature was *Hydrogenoanaerobacterium saccharovorans* with 89.2% of similarity (Fig. 1). The phylogenetic analysis confirms the bacterium as a member within the *Ruminococcaceae* family belonging to the phylum *Firmicutes* (Fig. 1). *Hydrogenoanaerobacterium saccharovorans* is an anaerobic, hydrogenogenic, rod-shaped bacterium isolated from a laboratory-scale H₂-producing up-flow anaerobic sludge blanket reactor [6].

Strain GD6 exhibits a 16S rRNA sequence divergence >5% with its phylogenetically closest species with a validly published name standing in nomenclature [7], so we propose the creation of the genus '*Bittarella*' whose type strain is '*Bittarella massiliensis*' GD6^T (Bit.ar. Masc. Adj., in honour of Dr Bittar, a French microbiologist, and mas.il.i.en'sis. L. gen. masc. n. *massiliensis*, of Massilia, the Latin name of Marseille where the strain GD6^T was first isolated).

MALDI-TOF-MS spectrum accession number. The MALDI-TOF-MS spectrum of '*Bittarella massiliensis*' is available at <http://mediterranee-infection.com/article.php?laref=256&titre=urms-database>. (Last accessed 28 November 2016).

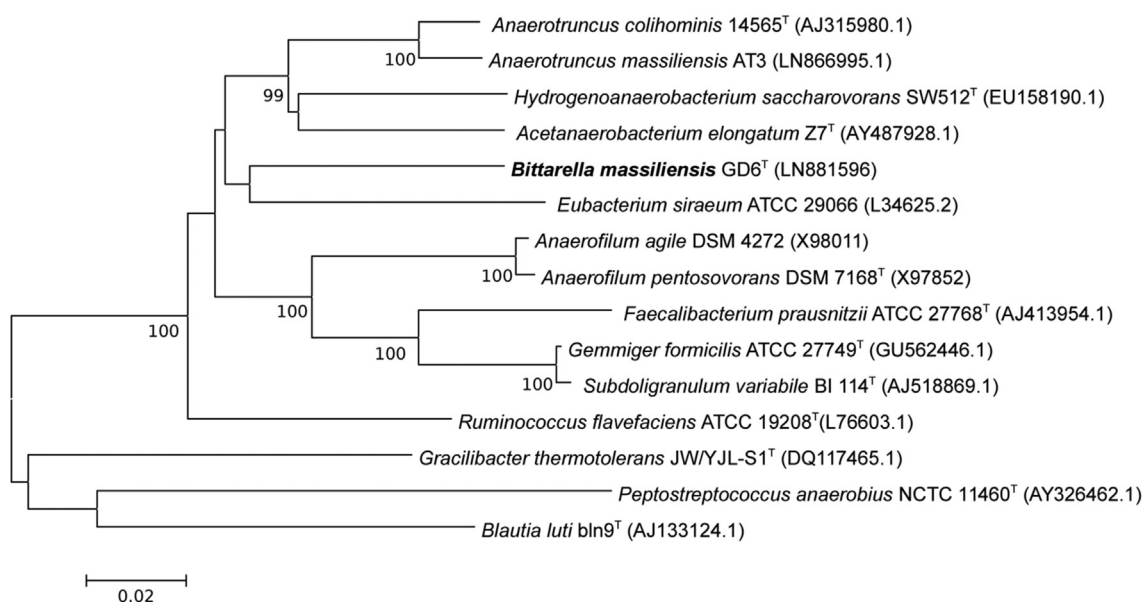


FIG. 1. Phylogenetic tree based on the 16S rRNA gene sequence showing the position of *Bittarella massiliensis* GD6^T (bold) with other closely related species among the *Ruminococcaceae*. The EMBL database accession numbers are indicated in parentheses. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained with a Kimura two-parameter model using the neighbour-joining method with 1000 bootstrap replicates, within MEGA6 software. The scale bar represents a 2% nucleotide sequence divergence.

Nucleotide sequence accession number. The 16S rRNA gene sequence was deposited in GenBank under Accession number LN881596.

Deposit in a culture collection. Strain GD6^T was deposited in the collection de Souches de l'Unités des Rickettsies (CSUR, WDCM 875) under number P2149.

Transparency declaration

No conflicts of interest are declared.

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