'Acidaminococcus timonensis' sp. nov. and *'Acidaminococcus massiliensis'* sp. nov. isolated from human gut

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Abstract

We report here the main characteristics of *'Acidaminococcus timonensis'* strain Marseille-P2764^T, isolated from human right colon, and *'Acidaminococcus massiliensis'* strain Marseille-P2828^T, isolated from human duodenum.

Keywords: *'Acidaminococcus massiliensis'*, *'Acidaminococcus timonensis'*, culturomics, human gut, microbiota
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In the context of ongoing work centred on the study of the whole gut human microbiome by culturomics [1] and metagenomics, two bacterial strains that could not be identified by our matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; MicroFlex, Bruker Daltonics, Wissembourg, France) [2] were isolated in April 2016. Both strains were isolated from the same 60-year-old patient who underwent an upper and lower digestive tract endoscopy to investigate severe anaemia. Strain Marseille-P2764^T was isolated from a right colon liquid sample, while strain Marseille-P2828^T was isolated from a duodenal liquid sample. The patient received clear information and provided signed informed consent. The study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 09-022.

The initial growth of strain Marseille-P2764^T was achieved under anaerobic conditions (AnaeroGen Compact; Oxoid, Thermo Scientific, Dardilly, France) on 5% sheep's blood–enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) at 37°C after 7-day liquid enrichment of the right colon sample in a blood culture bottle (Plus Anaerobic/F Media, BD BACTEC, Le Pont de Claix, France) previously supplemented with 5 mL of sheep's blood (bioMérieux) and 5 mL of 0.2 µm filtered (Thermo Fisher Scientific, Villebon-sur-Yvette, France) rumen. The first growth of strain Marseille-P2828^T was obtained on 5% sheep's blood–enriched Columbia agar (bioMérieux) under anaerobic atmosphere (AnaeroGen Compact) at 37°C after 21-day liquid enrichment in a blood culture bottle (BD BACTEC, Plus Anaerobic/F Media) previously supplemented with 5 mL sheep's blood (bioMérieux) and 5 mL of 0.2 µm filtered (Thermo Fisher Scientific) rumen.

The colony morphology of both strains after 72 hours of anaerobic incubation on blood-enriched Columbia agar was similar. They were circular with entire edges, convex and translucent/whiteish, with a mean diameter of 0.3 mm. No pigment production or haemolysis were registered. Both strains were Gram-negative cocci, with a mean diameter ranging from 0.6 to 0.8 µm. Both strains were nonmotile, non–spore forming and catalase and oxidase negative.

The 16S rRNA gene was sequenced for both strains using fD1-rP2 primers as previously described [3] with a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Strain Marseille-P2764^T and strain Marseille-P2828^T, respectively, exhibited 97.30% and 98.09% sequence identity with

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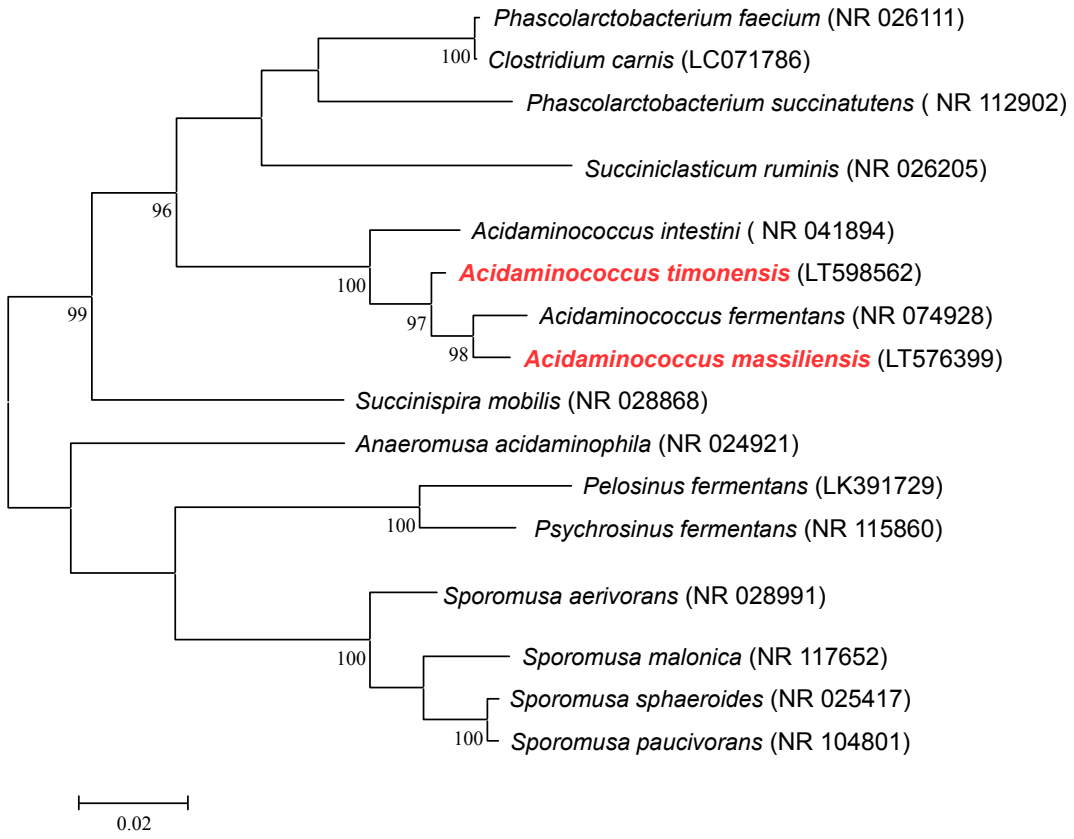

FIG. 1. Phylogenetic tree showing position of *'Acidaminococcus timonensis'* strain Marseille-P2764^T and *'Acidaminococcus massiliensis'* strain Marseille-P2828^T relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using 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 95% were retained. Scale bar indicates 2% nucleotide sequence divergence.

Acidaminococcus fermentans strain DSM 20731 (NR_074928), the phylogenetically closest species with standing in nomenclature (Fig. 1). Strain Marseille-P2764^T and strain Marseille-P2828^T showed 97.50% sequence identity, which putatively classifies them as new members of the genus *Acidaminococcus* in the family *Acidaminococcaceae* within the phylum *Firmicutes* [4].

The *Acidaminococcus* genus was created in 1969 and actually counts as two species with standing in nomenclature: *A. fermentans* and *A. intestini*. Cells belonging to this genus are Gram-negative, anaerobic cocci, generally isolated from the digestive tract of mammals [5,6].

On the basis of 16S rRNA gene sequence, we found a divergence of >1.3% for strains Marseille-P2764^T and Marseille-P2828^T from the phylogenetically closest species with standing in nomenclature. Within them we propose here the creation of the new species *'Acidaminococcus timonensis'* sp. nov. (ti.m-on.en′sis, L. masc. adj. *timonensis*, originating from La Timone, the hospital where the specimen from which the type strain was cultivated was collected) and *'Acidaminococcus massiliensis'* sp. nov. (mas.si.li.en′sis, L. masc. adj. *massiliensis* from Massilia, the Roman name of Marseille, where the type strain was isolated). Strain Marseille-P2764^T is the type strain of the new species *'A. timonensis'* while strain Marseille-P2828^T is the type strain of the new species *'A. massiliensis.'*

MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of *'A. timonensis'* strain Marseille-P2764^T and *'A. massiliensis'* strain Marseille-P2828^T are available online (http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database).

Nucleotide sequence accession number

The 16S rRNA gene sequence for *'A. timonensis'* strain Marseille-P2764^T and *'A. massiliensis'* strain Marseille-P2828^T were

deposited in GenBank under accession numbers LT598562 and LT576399, respectively.

Deposit in a culture collection

'A. timonensis' strain Marseille-P2764^T was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number P2764, while *'A. massiliensis'* strain Marseille-P2828^T was deposited in CSUR under number P2828 and in the Deutsche Sammlung von Mikroorganismen und Zellkulturen under number DSM 103158.

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Conflict of Interest

None declared.

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