

Acidaminococcus provencensis sp. nov., a new bacterium isolated from a fresh human stool specimen

T. Takakura^{1,2}, H. Anani³, A. Fadlane², A. Fontanini², D. Raoult^{2,3} and J. Y. Bou Khalil²

1) Hitachi High-Technologies Corporation, Analytical & Medical Solution Business Group, Ibaraki-ken, Japan, 2) Institut Hospitalo-Universitaire Méditerranée-Infection, Marseille, France and 3) Aix-Marseille Université, Institut de Recherche pour le Développement (IRD), UMR Microbes Evolution Phylogeny and Infections (MEPHI), Marseille, France

Abstract

Acidaminococcus provencensis strain Marseille-P4266^T (= CSURP4266^T) is a new species isolated from a fresh human stool specimen.

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Corresponding author: J.Y. Bou Khalil, MEPHI, Institut Hospitalo-Universitaire Méditerranée Infection, 19–21 Boulevard Jean Moulin, 13005, Marseille Cedex 05, France.
E-mail: boukhaliljacques@gmail.com

Introduction

Culturomics is a concept that involves the development of different culture conditions to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. After isolation, we used a taxonogenomics approach including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description and genome sequencing to describe an isolate [5,6].

Isolation and growth conditions

In 2017, we isolated an unidentified bacterial strain from a fresh stool sample in the Hospital of Timone (Marseille). Screening was performed using MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The spectra obtained (Fig. 1) were

imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in two databases (Bruker and the constantly updated MEPHI databases: <https://www.mediterranee-infection.com/urms-data-base/>). The study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 09-022. Initial growth was obtained after 72 h of culture in Colombia agar enriched with 5% sheep's blood (bioMérieux, Marcy l'Etoile, France) in strict anaerobic conditions at 37°C.

Phenotypic characteristics

Colonies were circular and white. Bacterial cells were Gram-negative, coccus-shaped with a mean diameter of 0.8 µm (Fig. 2). Strain Marseille-P4266^T showed catalase-negative and oxidase-negative activities. API 50CH and API ZYM were performed under strict anaerobic conditions at 37°C; results are listed in Tables 1 and 2. Main characteristics of the strain are summarized in Fig. 3.

Strain identification

The 16S rRNA gene was sequenced to classify this bacterium. Amplification was done by using the primer pair fDI and rP2

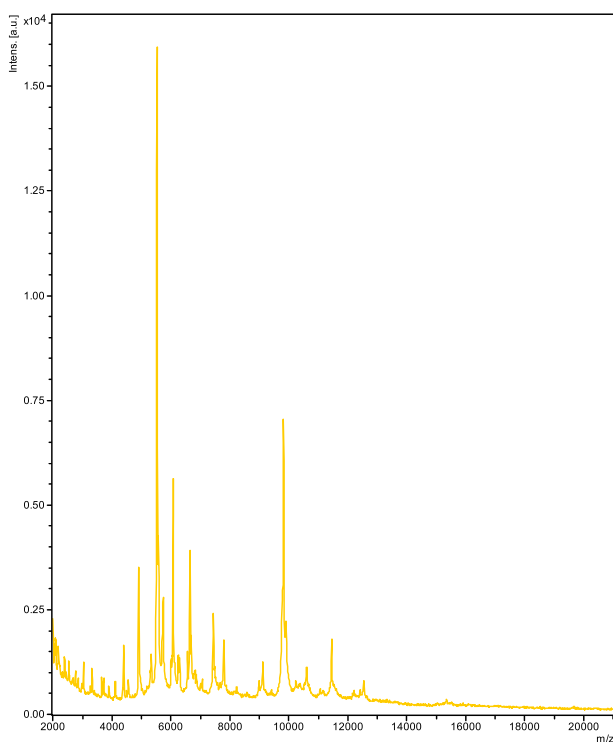


FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

(Eurogentec, Angers, France) and sequencing used the Big Dye[®] Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary 3500xL sequencer (ThermoFisher, Saint-Aubin, France), as previously described [8]. 16S rRNA nucleotide sequences were assembled and corrected using CODONCODE ALIGNER software (<http://www.codoncode.com>).

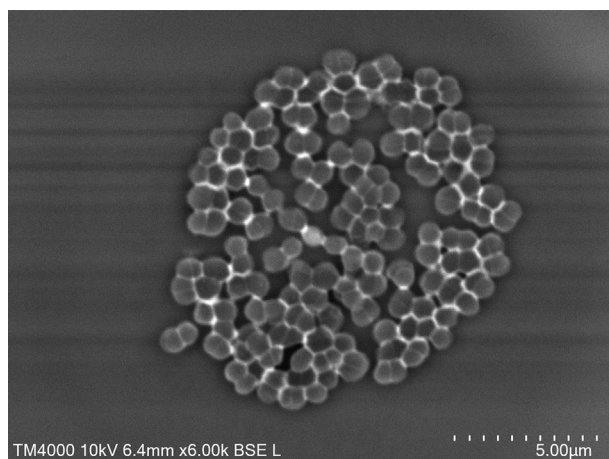


FIG. 2. Scanning electron micrograph of *Acidaminococcus provencensis* using TM4000Plus microscope from HITACHI. Scale bar and acquisition settings are shown on the original micrograph.

TABLE 1. Biochemical tests of *Acidaminococcus provencensis* (API 50 CH)

Bacteria: <i>Acidaminococcus provencensis</i>			
API 50 CH			
Test	Results (+/-)	Test	Results (+/-)
Control	–	Esculine	–
Glycerol	–	Salicine	–
Erythrol	–	D-cellobiose	–
D-arabinose	–	D-maltose	–
L-arabinose	–	D-lactose	–
D-ribose	–	D-melibiose	–
D-xylose	–	D-saccharose	–
L-xylose	–	D-trehalose	–
D-adonitol	–	Inuline	–
Methyl-β-D-xylopyranoside	–	D-melezitose	–
D-galactose	–	D-raffinose	–
D-glucose	–	Amidon	–
D-fructose	–	Glycogene	–
D-mannose	–	Xylitol	–
L-sorbose	–	Gentibiose	–
L-rhamnose	–	D-turanose	–
Dulcitol	–	D-lyxose	–
Inositol	–	D-tagatose	+
D-mannitol	–	D-fucose	–
D-sorbitol	–	L-fucose	–
Methyl-α-D-mannopyranoside	–	D-arabitol	–
Methyl-α-D-glucopyranoside	–	L-arabitol	–
N-acetylglucosamine	–	Potassium gluconate	–
Amygdaline	–	Potassium 2-cetogluconate	–
Arbutine	–	Potassium 5-cetogluconate	+

Strain Marseille-P4266^T exhibited a 96.67% sequence identity with *Acidaminococcus fermentans* strain DSM-20731^T (GenBank Accession no. NR_074928), the phylogenetically closest species with standing in nomenclature (Fig. 4). We consequently classify

TABLE 2. Biochemical tests of *Acidaminococcus provencensis* (API ZYM)

Bacteria: <i>Acidaminococcus provencensis</i>	
API ZYM	
Test	Results (+/-)
Control	–
Alkaline phosphatase	–
Esterase (C 4)	+
Esterase Lipase (C 8)	–
Lipase (C 14)	–
Leucine arylamidase	+
Valine arylamidase	–
Cystine arylamidase	–
Trypsin	–
α-chymotrypsine	–
Acid phosphatase	+
Naphthalo-AS-BI-phosphohydrolase	+
α-galactosidase	–
β-galactosodase	–
β-glucuronidase	–
α-glucosidase	–
β-glucosidase	–
N-acetyl-β-glucosaminidase	–
α-mannosidase	–
α-fucosidase	–

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SPNA Acidaminococcus Provencensis
GENA Acidaminococcus
SPEP Provencensis
SPST sp. nov.
SPTY pro.ven.cen' sis, N.L. adj. neut., from Provence,
the region in France, where the strain was
isolated

SUBM TATSUKI TAKAKURA
EMSU ttttkkr@gmail.com
TYPE strain-marseille P4266
COLN CSUR P4266
16SR LT969383
GARE OLMM00000000
GSTA draft
GSIZ 2605.487 kbp
GGCM 53.2
COUN France
REGI Bouches de Rhone
SOUR human gut
DATS 2017-12-15
GRAM NEGATIVE
CSHA coccus
TEMO 37
OREL anaerobe
OXID negative
CATA negative

FIG. 3. Description of *Acidaminococcus provencensis* according to the digitalized protologue TA00942 on the www.imedeauib.es/dprotologue website.

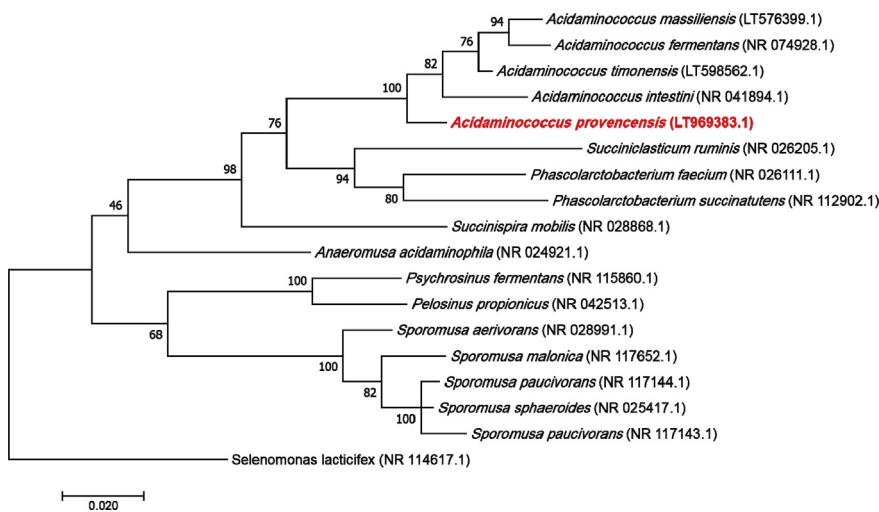


FIG. 4. Phylogenetic tree showing the position of “*Acidaminococcus provencensis*” strain Marseille-P4266^T relative to other phylogenetically close neighbours. The respective GenBank Accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using MUSCLE v3.8.31 with default parameters and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 2% nucleotide sequence divergence.

this strain as a member of a new species within the genus *Acidaminococcus*, family *Acidaminococcaceae*, phylum *Firmicutes*.

Genome sequencing

Genomic DNA was extracted with an EZI biorobot (Qiagen, Courtaboeuf, France) with the EZI DNA tissue kit and was

sequenced using MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit (Illumina), as previously described [9]. The assembly was performed with a pipeline incorporating different softwares (VELVET [10], SPADIS [11] and SOAP DENOVO [12]), on trimmed (MiSeq and TRIMMOMATIC [13] software) or untrimmed (only MiSeq software) data. GAPCLOSER was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value < 25% of the mean



Heatmap generated with OrthoANI values calculated from the OAT software. Please cite Lee et al. 2015.

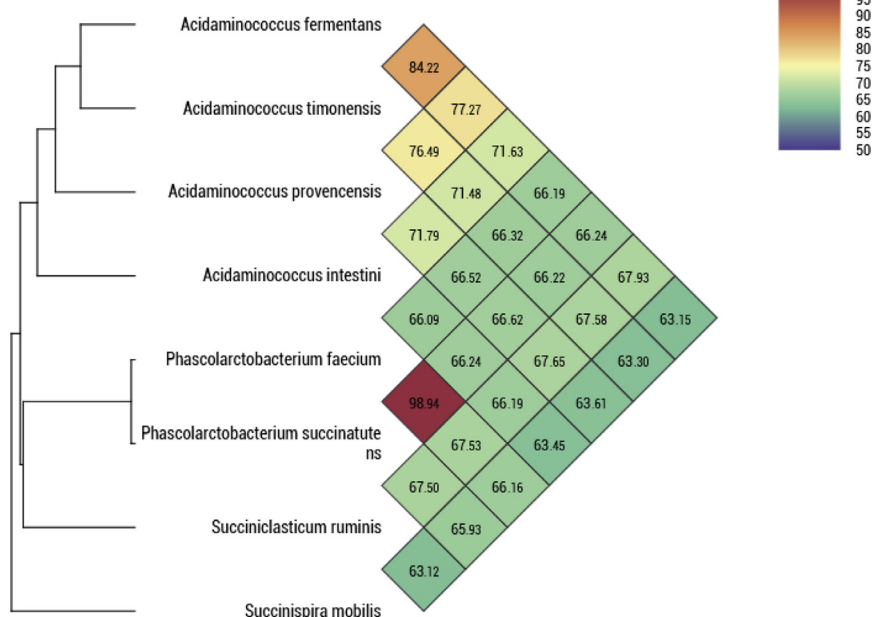


FIG. 5. Heatmap generated with ORTHOANI values calculated using the OAT software between *Acidaminococcus provencensis* and other closely related species with standing in nomenclature.

depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). The genome of strain Marseille-P4266^T is 2 605 487 bp long with a 53.2 mol% G+C content and 23 contigs. The degree of genomic similarity of Marseille-P4266^T with closely related species was estimated using ORTHOANI software [14]. Values among closely related species (Fig. 5) ranged from 63.12% between *Succiniclasticum ruminis* and *Succinispira mobilis* to 98.94% between *Phascolarctobacterium faecium* and *Phascolarctobacterium succinatutens*. When the isolate was compared with these closely related species, values ranged from 63.61% with *Succinispira mobilis* to 77.27% with *Acidaminococcus fermentans*.

Conclusion

Strain Marseille-P4266^T exhibited a 16S rRNA sequence divergence <98.65% with its phylogenetically closest species with standing in nomenclature, and is consequently proposed as the type strain of the new species *Acidaminococcus provencensis* sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under Accession numbers LT969383 and OLMM00000000, respectively.

Deposit in culture collections

Strain Marseille-P4266^T was deposited in strain collection under number (= CSURP4266^T).

Conflict of interest

None to declare.

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