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

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Abstract

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

Keywords: *Acidaminococcus provencensis*, Culturomics, Microbiota, New species, Taxonogenomics, TM4000, Stool
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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 fD1 and rP2

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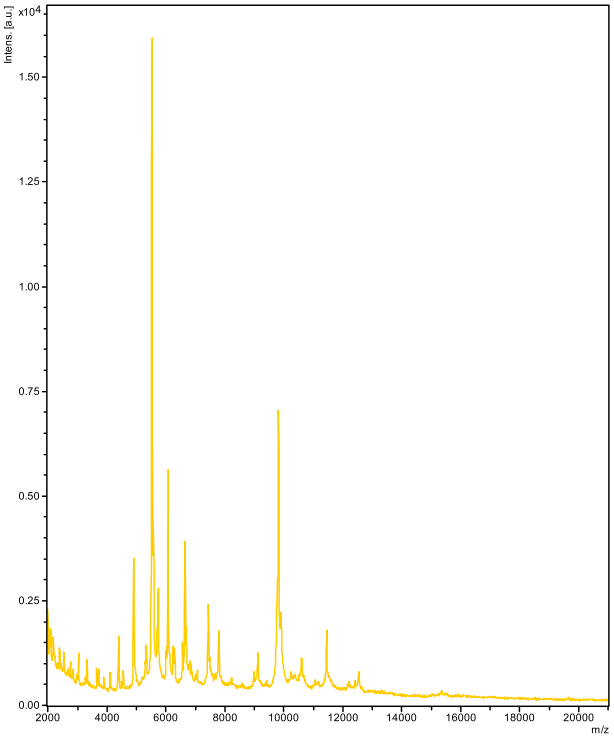


FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

(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).

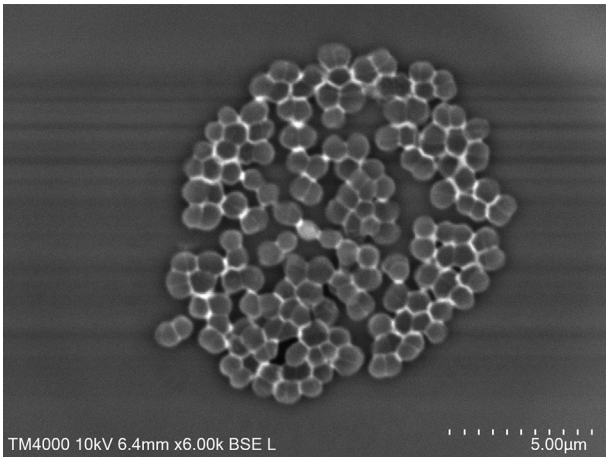


FIG. 2. Scanning electron micrograph of *Acidaminococcus provencensis* using TM4000Plus microscope from HITACHI. Scale bar and acquisition settings are shown on the original micrograph.

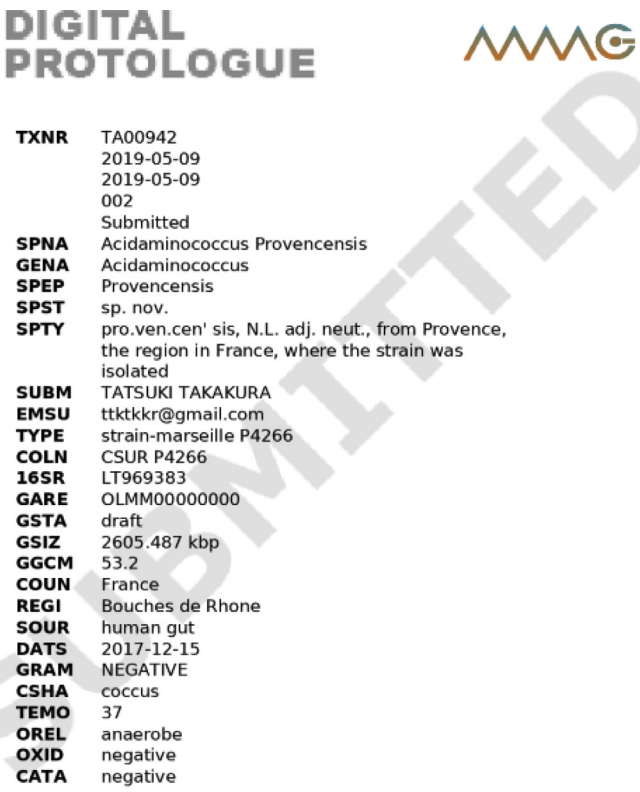
TABLE 1. Biochemical tests of *Acidaminococcus provencensis* (API 50 CH)

Bacteria: *Acidaminococcus provencensis*			
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-βD-xylopyranoside	−	D-melezitose	−
D-galactose	−	D-raffinose	−
D-glucose	−	Amidon	−
D-fructose	−	Glycogene	−
D-mannose	−	Xylitol	−
L-sorbose	−	Gentibiose	−
L-rhammose	−	D-turanose	−
Dulcitol	−	D-lyxose	−
Inositol	−	D-tagatose	+
D-mannitol	−	D-fucose	−
D-sorbitol	−	L-fucose	−
Methyl-αD-mannopyranoside	−	D-arabitol	−
Methyl-αD-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: *Acidaminococcus provencensis*	
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	−



DIGITAL PROTOLOGUE

TXNR	TA00942
	2019-05-09
	2019-05-09
	002
	Submitted
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	ttktkkr@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.imedea.uib.es/dprotologue website.

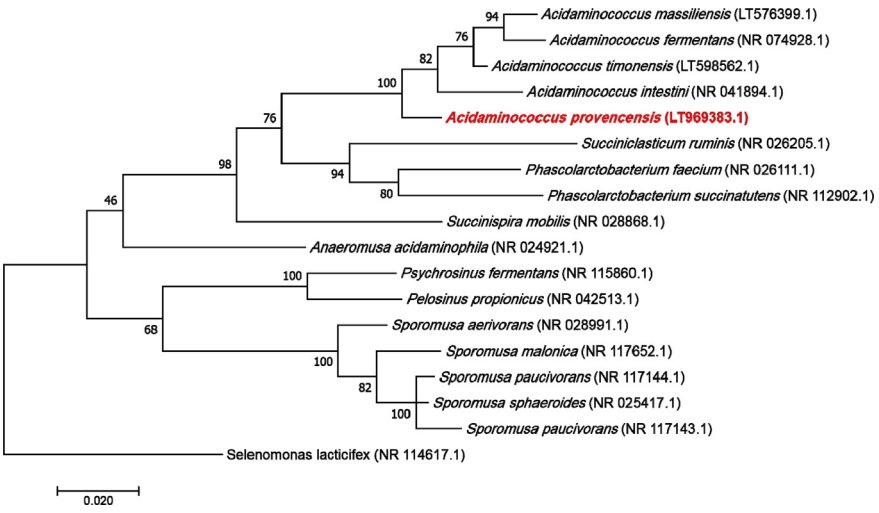


FIG. 4. Phylogenetic tree showing the position of "*Acidaminococcus provencensis*" strain Marseille-P4266^T relative to other phylogenetically close neighbours. The respective GenBank Accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using MUSCLE v3.8.31 with default 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 EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 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], SPADES [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

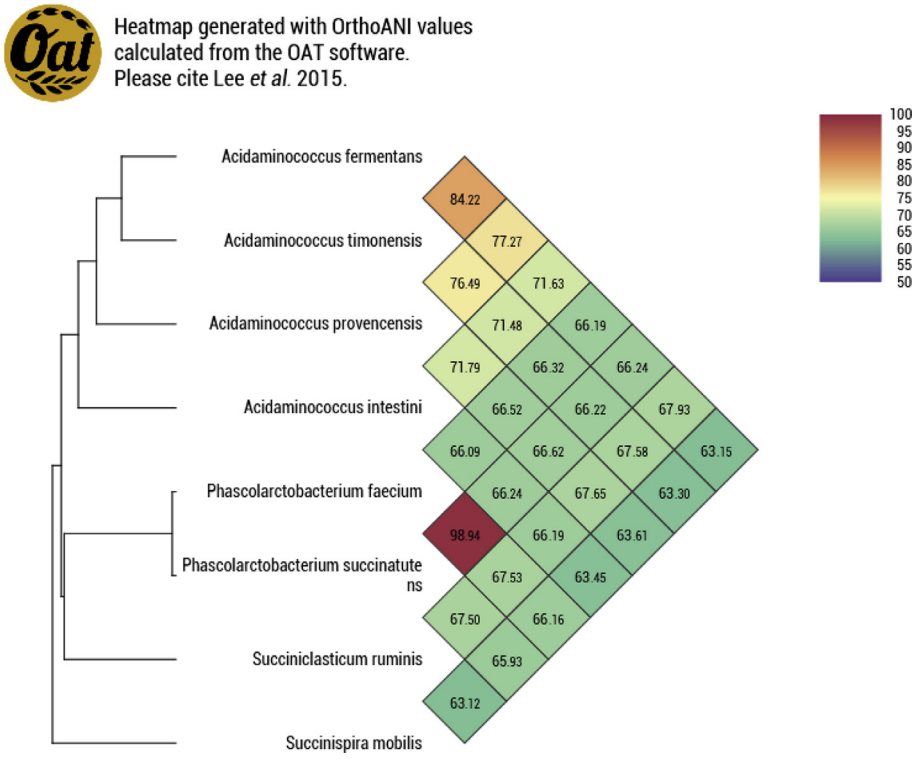


FIG. 5. Heatmap generated with ORTHOANI values calculated using the OAT software between *Acidaminococcus provencensis* and other closely related species with standing in nomenclature.

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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