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

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Abstract

Acidaminococcus provencensis strain Marseille-P4266T (= CSURP4266T) is a new species isolated from a fresh human stool specimen. © 2019 The Author(s). Published by Elsevier Ltd.

Keywords: Acidaminococcus provencensis, Culturomics, Microbiota, New species, Taxonogenomics, TM4000, Stool

Original Submission: 17 May 2019; Accepted: 5 June 2019

Article published online: 14 June 2019

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-P4266T 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...
(Eurogentec, Angers, France) and sequencing used the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary 3500xL sequencer (Thermo Fisher, Saint-Aubin, France), as previously described [8]. 16S rRNA nucleotide sequences were assembled and corrected using CODONCODE ALIGNER software (http://www.codoncode.com).

Strain Marseille-P4266T exhibited a 96.67% sequence identity with Acidaminococcus fermentans strain DSM-20731T (GenBank Accession no. NR_074928), the phylogenetically closest species with standing in nomenclature (Fig. 4). We consequently classify

**TABLE 1. Biochemical tests of Acidaminococcus provencensis**

| Test                   | Results (+/−) |
|------------------------|---------------|
| Control                | −             |
| Glycerol               | −             |
| Erythrob                | −             |
| D-arabinose             | −             |
| D-ribose                | −             |
| D-xylose                | −             |
| D-adonitol              | −             |
| Methyl-β-D-xylopyranoside | −           |
| D-glucose               | −             |
| D-fructose              | −             |
| D-mannose               | −             |
| L-sorbose               | −             |
| L-arabinose             | −             |
| D-maltose               | −             |
| D-melibiose             | −             |
| D-saccharose            | −             |
| D-trehalose             | −             |
| Inuline                 | −             |
| D-melezitose            | −             |
| D-rafinose              | −             |
| Amidon                  | −             |
| Glycogen                | −             |
| Xyitol                  | −             |
| Gentibiose              | −             |
| D-turanose              | −             |
| D-lycose                | −             |
| d-tagatose              | +             |
| i-fucose                | −             |
| i-arabitol              | −             |
| Methyl-α-D-mannopyranoside | −         |
| Methyl-α-D-glucopyranoside | −         |
| N-acetylglucosamine      | −             |
| Potassium glucosamine   | −             |
| Amygdaline              | −             |
| Potassium 2-cetogluconate | −        |
| Arbutine                | −             |
| Potassium 5-cetogluconate | +          |

| Test                   | Results (+/−) |
|------------------------|---------------|
| 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-galactosidase         | −             |
| β-glucuronidase         | −             |
| α-glicosidase           | −             |
| β-glucosidase           | −             |
| N-acetyl-β-glucosaminidase | −         |
| α-mannosidase           | −             |
| α-fucosidase            | −             |

**TABLE 2. Biochemical tests of Acidaminococcus provencensis**

| 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-galactosidase         | −             |
| β-glucuronidase         | −             |
| α-glicosidase           | −             |
| β-glucosidase           | −             |
| N-acetyl-β-glucosaminidase | −         |
| α-mannosidase           | −             |
| α-fucosidase            | −             |

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

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

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FIG. 3. Description of Acidaminococcus provencensis according to the digitalized protologue TA00942 on the www.imedea.uib.es/dprotologue website.
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 are removed.

**FIG. 4.** Phylogenetic tree showing the position of “*Acidaminococcus provencensis*” strain Marseille-P4266T 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.

**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-P4266T 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-P4266T 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 collection

Strain Marseille-P4266T was deposited in strain collection under number (= CSURP4266T).

Conflict of interest

None to declare.

Funding sources

The study was supported by the Méditerranée Infection Foundation, the National Research Agency under the programme Investissements d’avenir, reference ANR-10-IAHU-03, and by Région Provence Alpes Côte d’Azur and European funding FEDER PRIMI.

Acknowledgements

We sincerely thank Sakazume Taku, Takashi Irie, Kyoko Imai, Shigeki Matsubara, Akiko Hisada, Yusuke Ominami and all the Hitachi Team in Japan for the collaborative study we are conducting together between Hitachi High-Technologies and the Institut Hospitalo-Universitaire Méditerranée-Infection, and for the installation and services on the TM4000Plus microscope in our facility.

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