Brachybacterium timonense sp. nov., a new bacterium isolated from human sputum

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Abstract

Brachybacterium timonense strain Marseille-P4339T (=CSURP4339, =CECT9821) is a new species isolated from human sputum. © 2019 The Author(s). Published by Elsevier Ltd.

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Introduction

Culturomics is the concept of developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once a bacterium is isolated, a taxonogenomic approach is used, including MALDI-TOF MS, phylogenetic analysis, main phenotypic description and genome sequencing, to describe it [5,6].

Isolation and growth conditions

In 2017 we isolated from the human sputum an unidentified bacterial strain. The study was validated by the ethics committee of IHU Méditerranée Infection under number 2016-011. Screening was performed by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectra (Fig. 1) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated with Microbes Evolution Phylogeny and Infections (MEPIHI) database: http://www.mediterranee-infection.com/article.php?larub=280&titre=urms-database). The initial growth was obtained after 48 hours’ culture on Columbia agar with 5% sheep’s blood in anaerobic conditions at 37°C and pH 7.5.

Strain identification

The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification was done by using the primer pair fD1 and rP2 (Eurogentec, Angers, France), and sequencing by the Big Dye Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary sequencer (ThermoFisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com). Strain Brachybacterium timonense exhibited a 97.24% sequence identity with Brachybacterium faecium strain DSM 4810 (GenBank accession number NR_074655.2), the phylogenetically closest species with standing in...
nomenclature (Fig. 2). We consequently classified this strain as a member of a new species within the genus *Brachybacterium*, family *Dermabacteraceae*, phylum *Actinobacteria*.

**Phenotypic characteristics**

Colonies were cocci with a mean diameter of 0.8 μm. Bacterial cells were Gram positive and round (Fig. 3). Strain Marseille-P4339T showed catalase-positive and oxidase-negative activities. Characteristics of the strain are summarized in Table 1. API 50CH and API ZYM tests were performed at 37°C under anaerobic conditions (Table 2). By comparison with closely related species (*Brachybacterium faecium* Collins et al., 1988) [9], strain Marseille-P4339 has a similar phenotypic profile.

**Genome sequencing**

Genomic DNA was extracted using the EZ I biorobot (Qiagen, Courtaboeuf, France) with the EZI DNA tissue kit, then sequenced using MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera XT Paired end (Illumina), as previously
described [10]. The assembly was performed with a pipeline incorporating different software (Velvet [11], Spades [12] and Soap Denovo [13]) on trimmed (Trimmomatic [14]) or raw data. GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value lower than 25% of the mean depth were removed. The best assembly was selected by using different criteria (four scaffolds, nine contigs).

FIG. 2. Phylogenetic tree showing position of Brachybacterium timonense strain Marseille-P4339T relative to other phylogenetically close neighbours. Respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences were aligned using Muscle v3.8.31 with default parameters and phylogenetic inferences were obtained using maximum likelihood method within MEGA 7 software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 100 times to generate majority consensus tree. Scale bar indicates 5% nucleotide sequence divergence.

TABLE 1. Description of Brachybacterium timonense according to digitalized protologue TA00881 (www.imedea.uib.es/dprotologue)

| Characteristic                      | Value                          |
|------------------------------------|--------------------------------|
| Taxonumber                         | TA00881                        |
| Date of entry                      | 2019-04-19                     |
| Draft number/date                  | 001                            |
| Version                            | Submitted                      |
| Species name                       | Brachybacterium timonense      |
| Genus name                         | Brachybacterium                |
| Specific epithet                   | Brachybacterium timonense      |
| Species status                     | sp. nov.                       |
| Species etymology                  | tim.o.nen.e, N.L. masc. adj., timonense from Latin |
| Name of Hôpital de la Timone, hospital in Marseille, where strain Marseille-P4339 was isolated | Kuete Yimagou Edmond edmondkuete@yahoo.fr |
| E-mail of submitter                |                                |
| Designation of type strain         | Marseille-P4339                 |
| Strain collection numbers          | CSURP4339                      |
| 16S rRNA gene accession number     | LT962482                       |
| Genome accession number [EMBL]     | OIYW00000000                   |
| Data on origin of sample from which strain had been isolated | France |
| Country of origin                  | France                         |
| Region of origin                   | Paca                           |
| Source of isolation                | Sputum                         |
| Sampling date                      | 2016-08-14                     |
| Geographic location                | Marseille                      |
| Gram stain                         | Positive                       |
| Cell shape                         | Coccus                         |
| Motility                           | Nonmotile                      |
| Sporulation (resting cells)        | None                           |
| Lowest temperature for growth      | 25                             |
| Highest temperature for growth     | 45                             |
| Temperature optimum                | 37                             |
| Oxidase                            | Positive                       |
| Catalase                           | Negative                       |
| Habitat                            | Human                          |

FIG. 3. Electron micrograph of Brachybacterium timonense strain Marseille-P4339T was acquired with Hitachi TM4000Plus tabletop scanning electron microscope. Scale bar and acquisition settings are detailed on micrograph.
The genome of strain Marseille-P4339 is 3,092,417 bp long with a 67.3 mol% G+C content and contains 2,727 predicted genes. The degree of genomic similarity of strain Marseille-P4339 with closely related species was estimated by OrthoANI software [14]. Values among closely related species (Fig. 4) ranged from 73.98% between Brachybacterium nesterenkovii and strain Marseille-P4339 to 83.79% between Brachybacterium faecium.

### TABLE 2. Phenotypic characterization of Brachybacterium timonense based on biochemical tests

| Test        | Result |
|-------------|--------|
| **API 50 CH** |        |
| Control     | —      |
| Glycerol    | +      |
| Erythrol    | +      |
| α-Arabinose | +      |
| L-Arabinose | +      |
| α-Ribose    | +      |
| α-Xylose    | +      |
| L-Xylose    | +      |
| α-Adonitol  | +      |
| Methyl-β-D-xylopyranoside | + |
| α-Galactose | +      |
| α-Glucose   | +      |
| α-Fructose  | +      |
| α-Manose    | +      |
| L-Sorbose   | +      |
| α-Rhamnose  | +      |
| Dulcitol    | +      |
| Inositol    | +      |
| α-Mannitol  | +      |
| α-Sorbitol  | +      |
| Methyl-α-D-mannopyranoside | + |
| Methyl-α-D-glucopyranoside | + |
| N-Acetylglucosamine | + |
| Amygdaline  | +      |
| Arbutin     | +      |
| Esculine    | +      |
| Salicine    | +      |
| α-Galactoside | + |
| α-Maltose   | +      |
| α-Lactose   | +      |
| α-Melibiose | +      |
| α-Saccharose| +      |
| α-Trehalose | +      |
| Inuline     | +      |
| α-Melezitose| +      |
| α-Raffinose | +      |
| Amidon      | +      |
| Glycogen    | +      |
| Xyitol      | +      |
| Gentibiose  | +      |
| α-Turanose  | +      |
| α-Lyxose    | +      |
| α-Tartarate | +      |
| α-Fucose    | +      |
| L-Fucose    | +      |
| α-Arabinol  | +      |
| L-Arabinol  | +      |
| Potassium gluconate | + |
| Potassium 3-cetoglucurate | — |
| Potassium 5-cetoglucurate | + |
| **API ZYM** |        |
| Control     | —      |
| Alkaline phosphatase | + |
| Esterase (C4) | + |
| Esterase lipase (C8) | + |
| Lipase (C14) | — |
| Leucine aryiamidase | + |
| Valine aryiamidase | — |
| Cystine aryiamidase | + |
| Trypsine    | +      |
| α-Chymotrypsine | — |
| Acid phosphatase | — |
| Naphtal-AS-Bi-phosphohydrolase | — |
| α-Galactosidase | + |
| β-Galactosidase | + |
| α-Glucuronidase | — |
| α-Glucosidase | + |
| β-Glucosidase | + |
| N-Acetyl-β-glucosaminidase | + |
| α-Mannosidase | + |
| α-Fucosidase | — |

+ = positive result; − = negative result.
and Brachybacterium saurashtrense. When the isolate was compared to these closely species, values ranged from 73.98% with Brachybacterium nesterenkovi to 75.53% with Brachybacterium muris.

**Conclusion**

Strain *Brachybacterium timonense* exhibited a 16S rRNA sequence divergence of <98.65% and an OrthoANI value < 95% with its phylogenetically closest species with standing in nomenclature, together with unique phenotypic features. It is consequently proposed as the type strain of the new species *Brachybacterium timonense* sp. nov.

**Nucleotide sequence accession number**

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT962482 and OIWW00000000 respectively.

**Deposit in culture collections**

Strain Maseille-P4339 was deposited in two different strain collections under numbers CSURP4339 and CECT9821.

**Conflict of interest**

None declared.
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