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

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Abstract

Brachybacterium timonense strain Marseille-P4339^T (=CSURP4339, =CECT9821) is a new species isolated from human sputum.

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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 (MEPHI) 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

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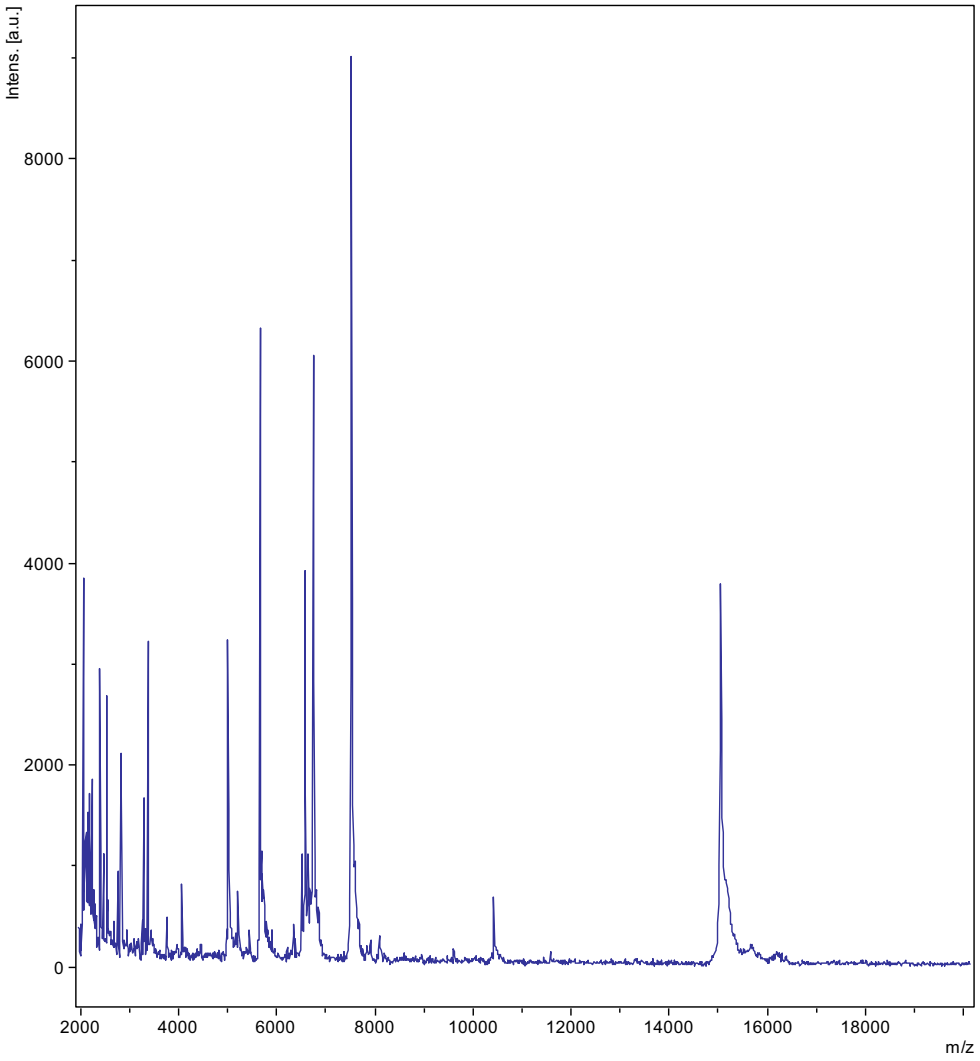

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and reference spectrum generated.

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-P4339^{T} 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 Marseile-P4339 has a similar phenotypic profile.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit, then sequenced using MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera XT Paired end (Illumina), as previously

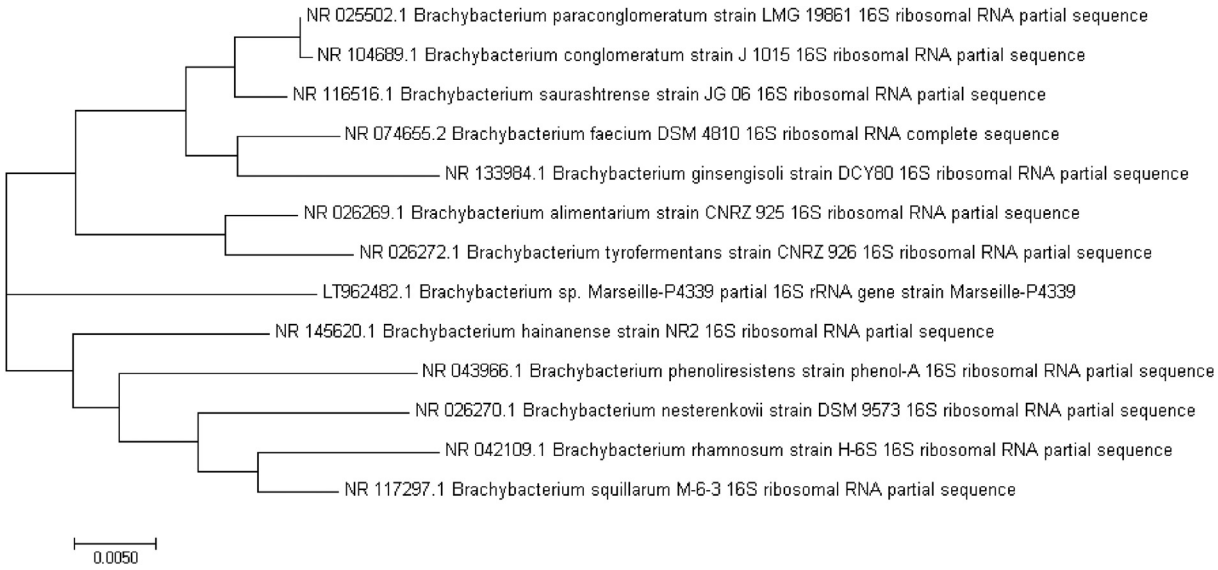

FIG. 2. Phylogenetic tree showing position of *Brachybacterium timonense* strain Marseille-P4339^T relative to other phylogenetically close neighbours. Respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences were aligned using Muscle v3.8.31 with default parameters 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.

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).

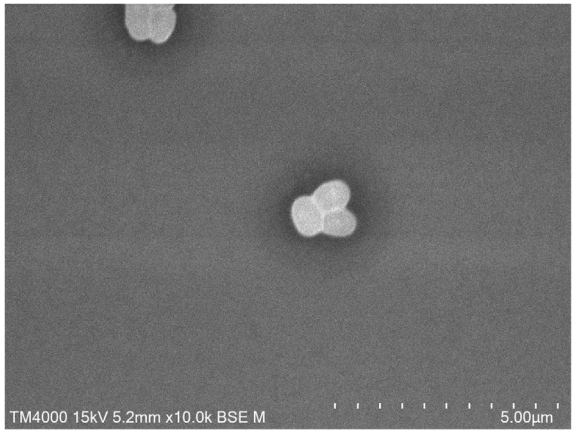

FIG. 3. Electron micrograph of *Brachybacterium timonense* strain Marseille-P4339^T was acquired with Hitachi TM4000Plus tabletop scanning electron microscope. Scale bar and acquisition settings are detailed on micrograph.

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	ti.mo.nen'se, N.L. masc. adj., *timonense* from Latin name of Hôpital de la Timone, hospital in Marseille, where strain Marseille-P4339 was isolated
Submitter	Kuete Yimagou Edmond
E-mail of submitter	edmondkuete@yahoo.fr
Designation of type strain	Marseille-P4339^T
Strain collection numbers	CSURP4339
16S rRNA gene accession number	LT962482
Genome accession number [EMBL]	OIWY00000000
Data on origin of sample from which strain had been isolated	
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

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

Test	Result
API 50 CH	
Control	—
Glycerol	+
Erythrol	+
D-Arabinose	+
L-Arabinose	+
D-Ribose	+
D-Xylose	+
L-Xylose	+
D-Adonitol	+
Methyl-βD-xylopyranoside	+
D-Galactose	+
D-Glucose	+
D-Fructose	+
D-Mannose	+
L-Sorbose	+
L-Rhammose	+
Dulcitol	+
Inositol	+
D-Mannitol	+
D-Sorbitol	+
Methyl-αD-mannopyranoside	+
Methyl-αD-glucopyranoside	+
N-Acetylglucosamine	+
Amygdaline	+
Arbutine	+
Esculine	+
Salicine	+
D-Cellobiose	+
D-Maltose	+
D-Lactose	+
D-Melibiose	+
D-Saccharose	+
D-Trehalose	+
Inuline	+
D-Melezitose	+
D-Raffinose	+
Amidon	+
Glycogene	+
Xylitol	+
Gentibiose	+
D-Turanose	+
D-Lyxose	+
D-Tagatose	+
D-Fucose	+
L-Fucose	+
D-Arabitol	+
L-Arabitol	+
Potassium gluconate	+
Potassium 2-cetogluconate	—
Potassium 5-cetogluconate	+
API ZYM	
Control	—
Alkaline phosphatase	+
Esterase (C4)	+
Esterase lipase (C8)	+
Lipase (C14)	—
Leucine arylamidase	+
Valine arylamidase	—
Cystine arylamidase	+
Trypsine	+
α-Chymotrypsine	—
Acid phosphatase	—
Naphtalo-AS-BI-phosphohydrolase	+
α-Galactosidase	+
β-Galactosodase	+
β-Glucuronidase	—
α-Glucosidase	+
β-Glucosidase	+
N-Acetyl-β-glucosaminidase	+
α-Mannosidase	+
α-Fucosidase	—

+, positive result; −, negative result.

The genome of strain Marseille-P4339 is 3<thinsp>092<thinsp>417 bp long with a 67.3 mol% G+C content and contains 2727 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*

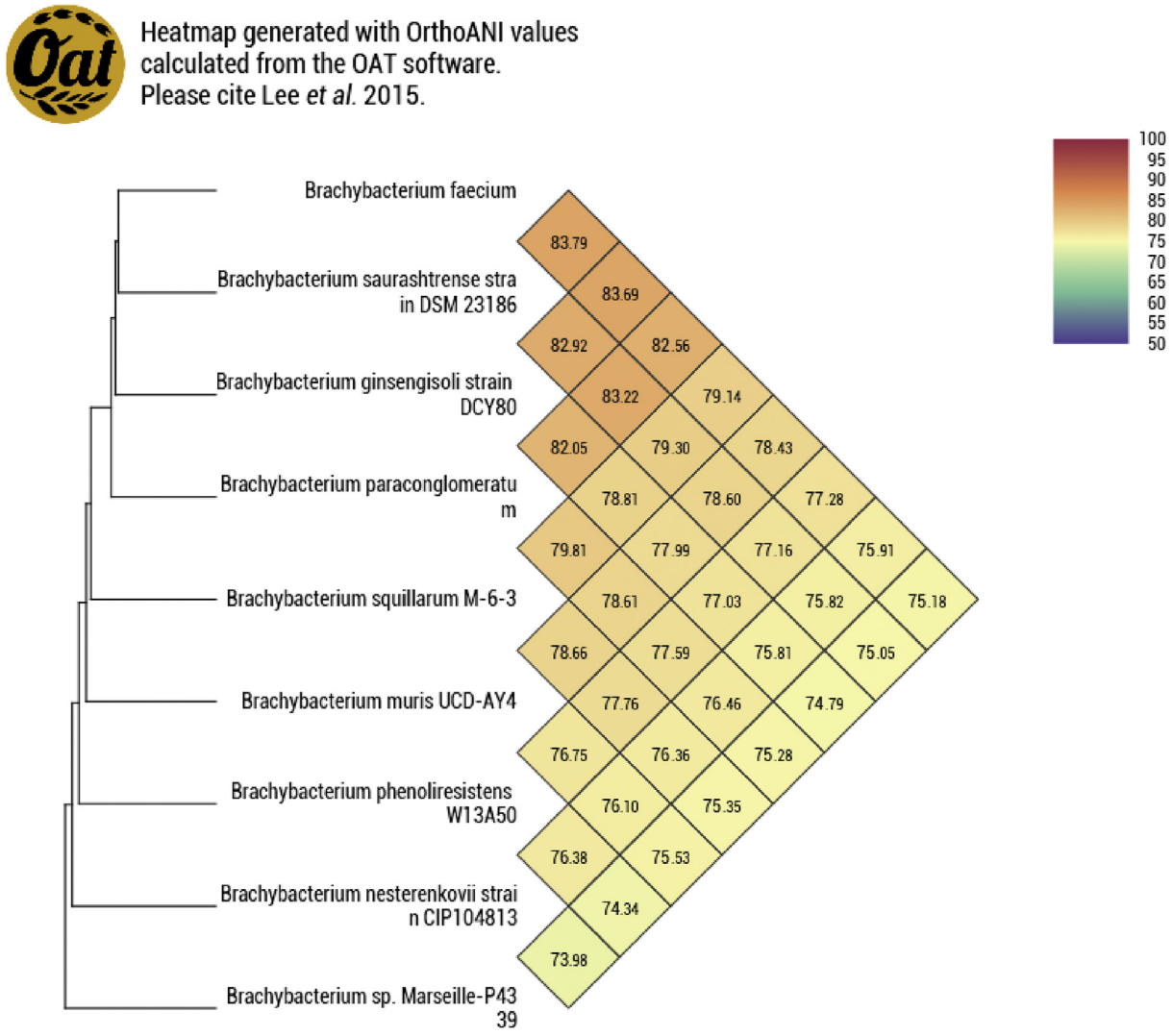

FIG. 4. Heat map generated with OrthoANI values calculated using OAT software between genus and species and other closely related species with standing in nomenclature.

and *Brachybacterium saurashtrense*. When the isolate was compared to these closely species, values ranged from 73.98% with *Brachybacterium nesterenkovii* 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 OIWY00000000 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.

Acknowledgements

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