

Varibaculum timonense sp. nov., a new bacterial species isolated from human stool sample

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Abstract

Varibaculum timonense sp. nov. strain Marseille-P3369^T (= CSURP3369) is a new species from the order *Actinomycetales* that has been isolated from a fresh stool sample of a healthy French woman.

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Introduction

Currently, the implications of bacterial diversity for normal physiological functions and for disease must be understood [1]. To explore the diversity of human intestinal bacteria, the culturomics approach, based on diversified culture conditions, was designed to isolate species never cultivated before and also to complete the metagenomics of 16S rRNAs [2–4]. A new taxonomic method called taxonogenomics has been developed for a description associating the analysis of complete sequences of the genome and the phenotypic characteristics of novel bacterial species [5]. By integrating this new approach, we give here a brief description of a new species within the genus *Varibaculum*, isolated from a fresh stool sample of a healthy French woman.

Isolation and growth conditions

In September 2016, we isolated, from a fresh stool sample of a 26-year-old healthy French woman, a bacterial strain (Marseille-

P3369^T). This strain was not identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The analysis was performed on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany), as previously described [6]. Spectra obtained (Fig. 1) were imported and analysed using the BIOTYPER 3.0 software against the Bruker database, which was constantly updated with the MEPHI database [1]. Growth of colonies of the strain Marseille-P3369^T was observed after 48 hours of incubation at 37°C on 5% sheep's blood agar (bioMérieux, Marcy l'Etoile, France) under strict anaerobic conditions generated by anaeroGEN (Oxoid, Dardilly, France) [7].

Phenotypic characteristics

The bacterial strain Marseille-P3369^T is not motile and not spore-forming. Its colonies are circular and white with a mean diameter of 0.5 mm. Cells were Gram-positive, small rod-shaped, and slightly curved, ranging in diameter from 0.35 to 0.4 μm (Fig. 2). Catalase and oxidase activities were not observed for strain Marseille-P3369^T. The optimal growth of this strain was observed at 37°C and pH 7.5 under strict anaerobic conditions. Results issued from API ZYM and API 20A tests are shown in Table 1. The main biochemical characteristics of the closest *Varibaculum* species with standing in nomenclature are compared in Table 2. The major fatty acid

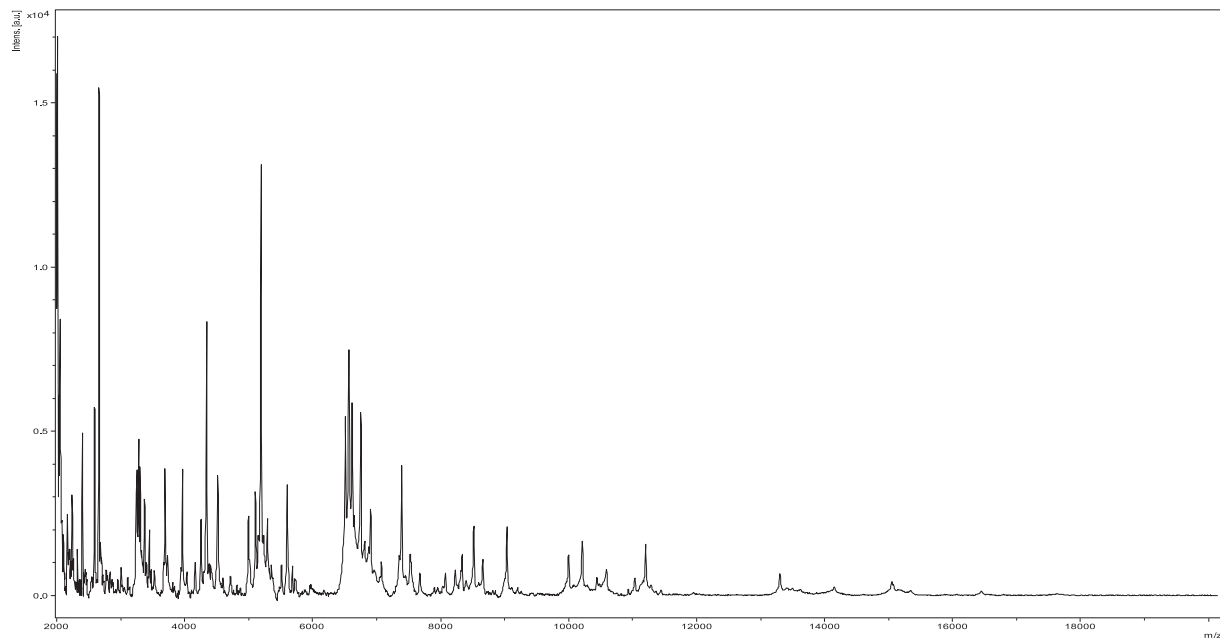


FIG. 1. MALDI-TOF MS reference spectrum of *Varibaculum timonense* sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

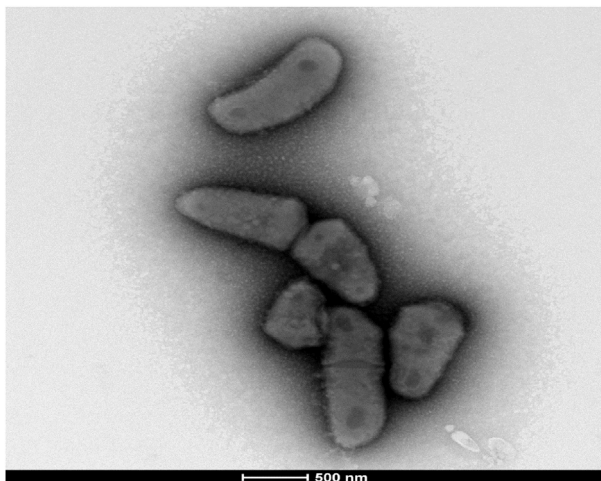


FIG. 2. Scanning electron microscopy (SEM) of stained *Varibaculum timonense* sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 minutes and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 minutes to increase SEM image contrast. The slide was gently washed in water; air-dried and examined in a tabletop SEM (Hitachi TM4000). The scale is shown on the figure.

TABLE 1. Phenotypic characterization of *Varibaculum timonense* sp. nov., based on analytical profile index (API) tests

Biochemical characteristics	Results
Alkaline phosphatase	+
Esterase (C-4)	+
Esterase lipase (C-8)	+
Lipase (C-14)	+
Leucine arylamidase	+
Valine arylamidase	-
Cystine arylamidase	-
Trypsine	+
α -chymotrypsine	-
Acid phosphatase	+
Naphthalo-AS-BI-phosphohydrolase	+
α -galactosidase	-
β -galactosidase	-
β -glucuronidase	-
α -glucosidase	+
β -glucosidase	-
N-acetyl- β -glucosaminidase	-
α -mannosidase	+
α -fucosidase	-
Indole production	-
Urease	+
Glucose	-
Mannitol	+
Lactose	+
Sucrose	+
Maltose	+
Salicin	+
Xylose	-
Arabinose	-
Gelatin	-
Esculin	+
Glycerol	-
Cellulose	+
Mannose	-
Melezitose	+
Raffinose	-
Sorbitol	-
Rhamnose	-
Trehalose	-

TABLE 2. Differential characteristics of *Varibaculum timonensis* sp. nov., *Varibaculum cambriensis* and *Varibaculum anthropi*

Properties	<i>V. timonensis</i> sp. nov.	<i>V. cambriensis</i>	<i>V. anthropi</i>
Cell diameter (µm)	0.4-0.5	NA	NA
Oxygen requirement	-	-	-
Gram stain	+	+	+
Salt requirement	-	-	-
Motility	-	-	-
Endospore formation	-	-	-
Alkaline phosphatase	+	-	-
Catalase	-	-	-
Indole	+	-	-
Nitrate reductase	-	+	-
Urease	+	-	+
β-galactosidase	-	-	-
N-acetyl-glucosamine	-	-	-
Arabinose	-	-	-
Lipase (C8)	+	+	NA
Trypsine	+	-	NA
Mannose	-	-	-
Mannitol	+	-	-
D-Glucose	-	+	+
D-Maltose	+	+	+
Source	Stool samples	Human samples	Human samples

+, positive result; -, negative result; NA, data not available.

found for this strain was hexadecanoic acid (52%) followed by 9-octadecenoic acid (22%), 9,12-octadecadienoic acid (12%) and octadecanoic acid (9%). Minor amounts of unsaturated, branched and other saturated fatty acids were also detected (Table 3).

Strain identification

To classify this bacterium, the 16S rRNA gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xL Genetic Analyzer capillary sequencer (ThermoFisher, Saint-Aubin, France), as previously reported [8]. The 16S rRNA nucleotide sequences were

assembled and corrected using CODONCODE ALIGNER software (<http://www.codoncode.com>). Strain Marseille-P3369^T exhibited 98.32% similarity with *Varibaculum cambriense* strain CCUG 44998 (GenBank Accession no. NR_114873.1), its phylogenetically closest species with standing in nomenclature (Fig. 3). We consequently proposed classifying strain Marseille-P3369^T as a new species within the genus *Varibaculum* belonging to the phylum *Actinobacteria*.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) and then sequenced on a MiSeq sequencer (Illumina Inc., San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT Paired End (Illumina), as previously described [9]. The assembly was performed using a pipeline containing several softwares (VELVET [10], SPADES [11] and SOAP DENOVO [12]), and trimmed data (MiSEQ and TRIMMOMATIC [13] softwares) or untrimmed data (only MiSEQ software). GAPCLOSER was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value < 25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). The genome of Strain Marseille-P3369^T was 2.73 Mb with 33.2% G + C content. The degree of genomic similarity of the strain with closely related species was calculated using ORTHOANI software [14]. OrthoANI values among closely related species (Fig. 4) ranged from 58.83% between *Actinomyces neuii* and *Varibaculum timonense* to 77.54% between *Actinomyces odontolyticus* and *Actinomyces georgiae*. When *Varibaculum timonense* was compared with these closely related species values ranged from 58.83% with *Actinomyces neuii* to 66% with *Actinomyces georgiae*.

TABLE 3. Fatty acid profiles (%) of *Varibaculum timonensis* strain Marseille-P3369

Fatty acids	Name	Mean relative % ^a
16:0	hexadecanoic acid	52.2 ± 1.0
18:1n9	9-octadecenoic acid	21.5 ± 0.2
18:2n6	9,12-octadecadienoic acid	11.5 ± 0.5
18:0	octadecanoic acid	8.6 ± 0.3
14:0	tetradecanoic acid	1.6 ± 0.1
18:1n5	13-octadecenoic acid	1.5 ± 0.1
18:1n7	11-octadecenoic acid	TR
15:0	pentadecanoic acid	TR
17:0 anteiso	14-methyl-hexadecanoic acid	TR
17:0	heptadecanoic acid	TR
16:1n7	9-hexadecenoic acid	TR
15:0 iso	13-methyl-tetradecanoic acid	TR
17:0 iso	15-methyl-hexadecanoic acid	TR

TR = trace amounts <1%.
^aMean peak area percentage.

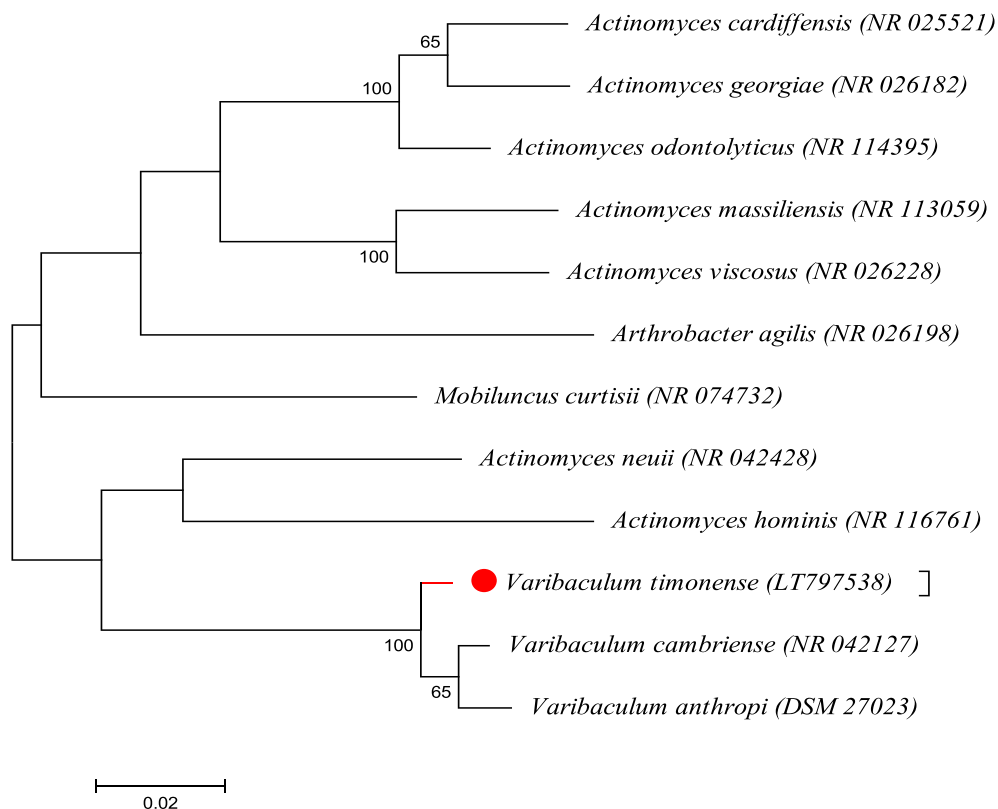


FIG. 3. Phylogenetic tree highlighting the position of *Varibaculum timonense* sp. nov., relative to the most closely related type strains within the genus *Varibaculum*. GenBank Accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inference were obtained using the maximum likelihood method and 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.

Conclusion

On the basis of unique phenotypic features, including MALDI-TOF spectrum, a 16S rRNA sequence divergence >1.3%, and an OrthoANI value < 95% with the phylogenetically closest species with standing in nomenclature, we formally proposed strain Marseille-P3369^T as the type strain of *Varibaculum timonense* sp. nov., which is a new species in the genus *Varibaculum*.

Description of *Varibaculum timonense* strain Marseille-P3369^T sp. nov.

Strain Marseille-P3369^T is the type strain of *Varibaculum timonense* sp. nov. (ti.mo.nen'se, N.L. neut. adj. *timonense*, related to Timone, the name of the main university hospital in Marseille, France, from where the strain was isolated). *Varibaculum*

timonense is a strict anaerobic, non-motile and non-sporulating Gram-stain-positive rod bacterium. Strain Marseille-P3369^T grows under anaerobic conditions at temperatures ranging between 37°C and 45°C, with an optimal temperature of 37°C. It exhibits neither catalase nor oxidase activities. The genome of Strain Marseille-P3369^T was 2.73 Mb with 33.2% G + C content. The potential pathogenicity of the type strain Marseille-P3369^T (= CSURP3369) is unknown. It was isolated from the fresh stool sample of a 26-year-old French healthy woman.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under Accession numbers LT797538 and FWWDK00000000, respectively.

Deposit in culture collections

Strain Marseille-P3369^T was deposited in two different strain collections under the following number (= CSURP3369).

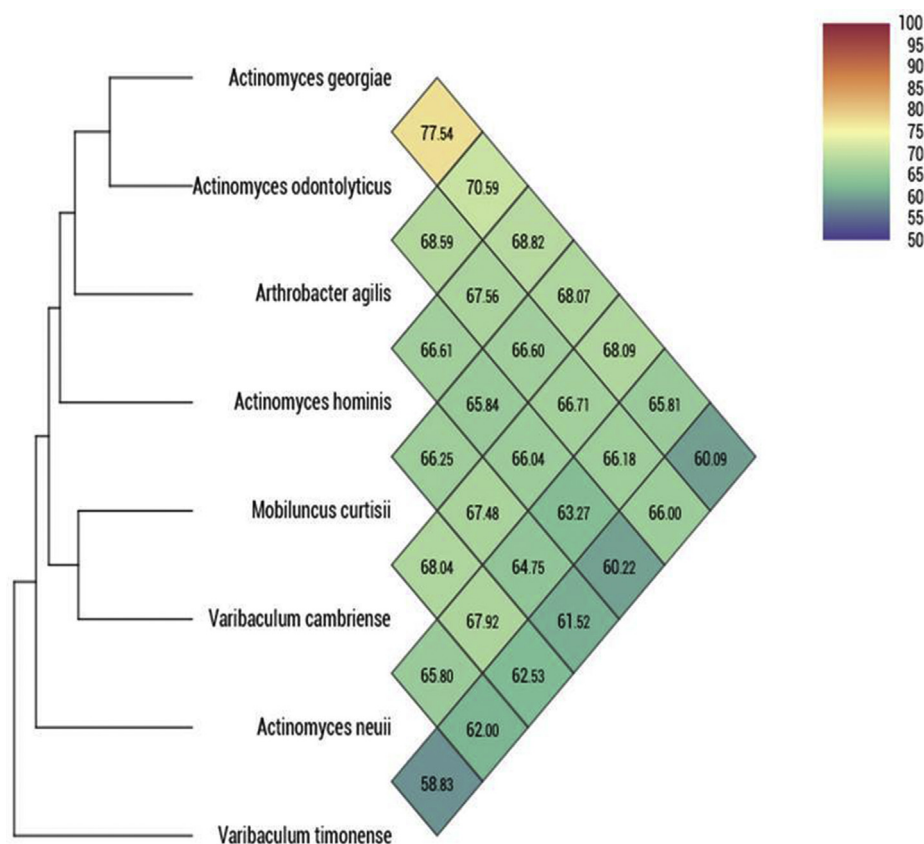


FIG. 4. Heatmap generated with ORTHOANI values calculated using the OAT software between *Varibaculum timonense* sp. nov. and other closely related species with standing in nomenclature.

Conflicts of interest

None to declare.

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Ethics and consent

The study was approved by the ethics committee of the Institut Federatif de Recherche 48 under reference 2016-010. The woman provided written consent.

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