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

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**Abstract**

*Varibaculum timonense* sp. nov. strain Marseille-P3369T (= 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-P3369T). 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-P3369T 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-P3369T 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-P3369T. 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           | +       |
| Naphthol-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                  | −       |
| Galactin                   | −       |
| Esculin                    | +       |
| Glycerol                   | −       |
| Cellulobiose               | +       |
| Mannose                    | −       |
| Melezitose                 | −       |
| Raffinose                  | −       |
| Sorbitol                   | −       |
| Rh symbol                  | −       |
| Trehalose                  | −       |
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 3500xLGenetic Analyzer capillary sequencer (Thermo Fisher, 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-P3369T 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-P3369T 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-P3369T 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          |   |
| 18:1n7      | 13-octadecanoic acid        | 1.5 ± 0.1          |   |
| 18:1t17     | 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%.

Mean peak area percentage.
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-P3369T as the type strain of *Varibaculum timonense* sp. nov., which is a new species in the genus *Varibaculum*.

Description of *Varibaculum timonense* strain Marseille-P3369T sp. nov.

Strain Marseille-P3369T 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-P3369T 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-P3369T was 2.73 Mb with 33.2% G + C content. The potential pathogenicity of the type strain Marseille-P3369T (= 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 FWDK00000000, respectively.

Deposit in culture collections
Strain Marseille-P3369T was deposited in two different strain collections under the following number (= CSURP3369).
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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