Varibaculum massiliense sp. nov., a new bacterium isolated from human urine with culturomics

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Abstract

Varibaculum massiliense sp. nov. strain Marseille-P2802^T (= CSUR P2802 = DSM 103074) is a new species within the genus *Varibaculum* in the phylum Actinobacteria that was isolated from the urine of a 59-year-old man treated with chronic haemodialysis for diabetic nephropathy.

Keywords: Bacteria, culturomics, human urine, taxono-genomics *Varibaculum massiliensesp.* nov
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Introduction

Bacteria constitute an important and highly diversified taxonomic group within the life tree of living organisms. Decoding the bacterial diversity underlying their normal and pathogenic functions is fundamental [1]. A high-throughput bacterial culture approach based on diversified culture conditions, known as culturomics, was designed to isolate as yet uncultured species to unveil human microbial diversity, and to complement 16S rRNA metagenomics [2–4]. Furthermore, a new taxonomic strategy named taxono-genomics was developed to include the analysis of complete genome sequences in combination with phenotypic characteristics [5]. Herein, we report a short description of strain Marseille-P2802, isolated from the urine of a man treated for diabetic nephropathy.

Isolation and growth conditions

We isolated from the urine of a 59-year-old man treated with chronic haemodialysis for diabetic nephropathy, a potential new bacterial strain that could not be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The screening 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 continually incremented with local MEPHI database. The strain was isolated from a human urine sample, after 7 days growth on 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) at 37°C in an anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France).

Phenotypic characteristics

Microcolonies with mean diameter 0.5 mm were entire edged, translucent, greyish and glistering. Cells were a Gram positive rod-shaped bacterium and were slightly curved, non-motile and non-spore-forming (Fig. 2). The Strain Marseille-P2802 exhibited neither catalase nor oxidase activities http://www.mediterranee-infection.com/article.php?larub=280&titre=umrs-database [7]. A comparative study of the biochemical characteristics of this strain with other closely related *Varibaculum* species is presented in Table 1. The biochemical characteristics of the Marseille-P2802^T strain obtained using the API ZYM and 20A strips (bioMérieux) are presented in Table 2. The major fatty acid found for this strain was hexadecanoic acid (47%).

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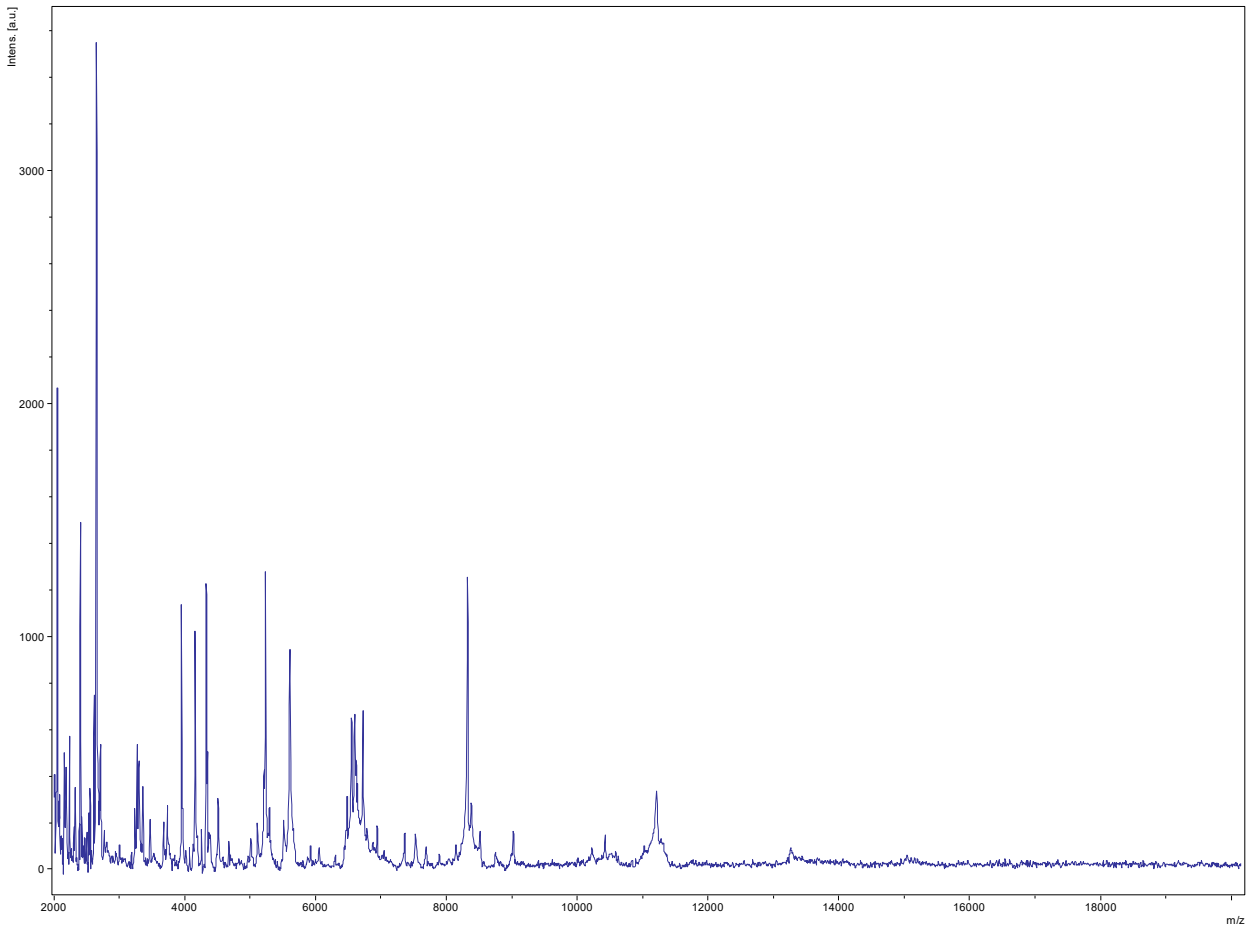

FIG. 1. MALDI-TOF MS reference spectrum of *Varibaculum massiliense* sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

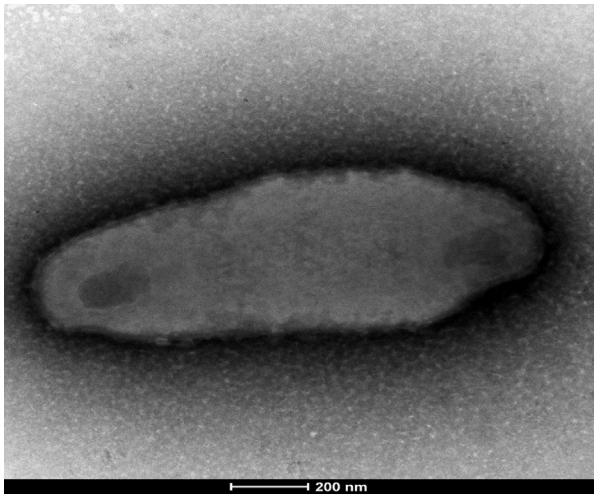

FIG. 2. Scanning electron microscopy (SEM) of stained *Varibaculum massiliense* sp. nov. A colony was collected from agar and immersed in a 2.5% glutaraldehyde fixative solution. Then a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 minutes and treated 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 (Tecnai G20). Scales and acquisition settings are shown in the figure.

TABLE 1. Differential characteristics of *Varibaculum massiliense* sp. nov., *Varibaculum timonense* [15], *Varibaculum cambriensis* [16] and *Varibaculum anthropi* [17]

Properties	*V. massiliense*	*V. timonense*	*V. cambriensis*	*V. anthropi*
Cell diameter (μm)	0.5–0.6	0.4–0.5	NA	NA
Oxygen requirement	anaerobic	anaerobic	anaerobic	anaerobic
Gram stain	+	–	+	+
Salt requirement	–	–	–	–
Motility	–	–	–	–
Endospore formation	–	–	–	–
Alkaline phosphatase	+	+	–	–
Catalase	–	–	–	–
Indole	–	+	–	–
Urease	+	+	–	+
β-galactosidase	–	–	–	–
N-acetyl-glucosamine	–	–	–	–
Arabinose	–	–	–	–
Lipase (C8)	+	+	+	NA
Trypsin	+	+	–	NA
Mannose	–	–	–	–
Mannitol	–	+	–	–
Glucose	–	–	+	+
Maltose	+	+	+	+
Source	Urine sample	Stool sample	Human sources	Clinical sample

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

The 18 carbons, mostly unsaturated structures, were also abundant and represented almost 50% of the total composition: 18:1n9 (22%), 18:2n6 (12%), 18:0 (12%) and 18:1n7 (4%) (Table 3).

TABLE 2. Phenotypic characterization of *Varibaculum massiliense* sp. nov., based on analytical profile index (API) tests

Tests	Characteristics	Results
API ZYM	Alkaline phosphatase	+
	Esterase (C4)	–
	Esterase lipase (C8)	+
	Lipase (C14)	–
	Leucine arylamidase	+
	Valine arylamidase	–
	Cystine arylamidase	–
	Trypsin	+
	α-chymotrypsin	–
	Acid phosphatase	+
	Naphthalo-AS-BI-phosphohydrolase	+
	α-galactosidase	–
	β-galactosidase	–
	β-glucuronidase	–
	α-glucosidase	+
	β-glucosidase	–
	N-acetyl-β-glucosaminidase	–
	α-mannosidase	–
	α-fucosidase	–
API 20A	Indole producttion	–
	Urease	+
	Glucose	–
	Mannitol	–
	Lactose	+
	Sucrose	+
	Maltose	+
	Salicin	–
	Xylose	+
	Arabinose	–
	Gelatin	–
	Esculin	–
	Glycerol	+
	Cellobiose	–
	Mannose	–
	Melezitose	+
	Raffinose	+
	Sorbitol	–
	Rhamnose	–
	Trehalose	–

Strain identification

In order 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 (Thermofisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequence was assembled and corrected using CodonCode Aligner software (http://www.codoncode.com). Strain Marseille-P2802 exhibited 98.6% 16S rRNA similarity with *Varibaculum cambriense* strain CCUG 44998 (GenBank accession number NR_042127), the phylogenetically closest species with standing in nomenclature (Fig. 3). We consequently proposed to classify strain Marseille-P2802 as a new species within the genus *Varibaculum* belonging to the family *Actinomycetaceae* within the phylum Actinobacteria.

TABLE 3. Cellular fatty acid composition (%)

Fatty acids	Name	Mean relative % [a]
16:00	hexadecanoic acid	46.5 ± 0.6
18:1n9	9-octadecenoic acid	21.9 ± 0.5
18:2n6	9,12-octadecadienoic acid	11.8 ± 0.4
18:00	octadecanoic acid	11.6 ± 0.2
18:1n7	11-octadecenoic acid	3.6 ± 0.2
14:00	tetradecanoic acid	1.3 ± 0.2
17:00	heptadecanoic acid	1.2 ± 0.3
15:00	pentadecanoic acid	TR
17:00	14-methyl-hexadecanoic acid	TR
17:00	15-methyl-hexadecanoic acid	TR
16:1n7	9-hexadecenoic acid	TR
17:1n7	10-heptadecenoic acid	TR

[a]Mean peak area percentage; TR, trace amounts <1%.

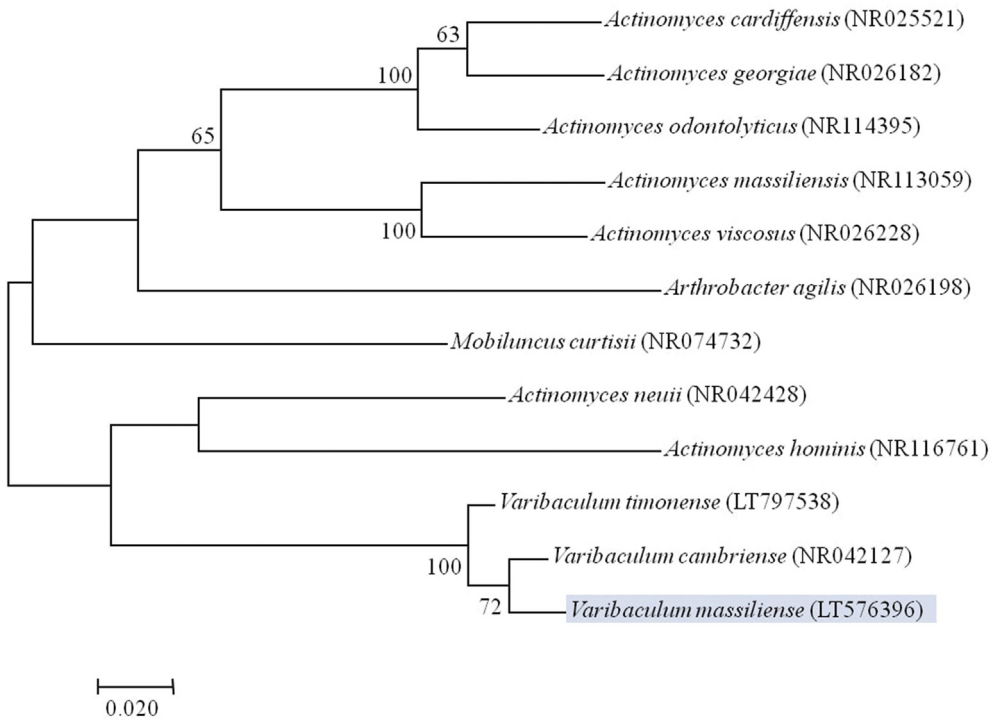

FIG. 3. Phylogenetic tree highlighting the position of *Varibaculum massiliense* sp. nov. with regard to other closely related species. GenBank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inferences were obtained using the maximum likelihood method and the MEGA 7 software. Bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree are indicated at the nodes. The scale bar indicates a 2% nucleotide sequence divergence.

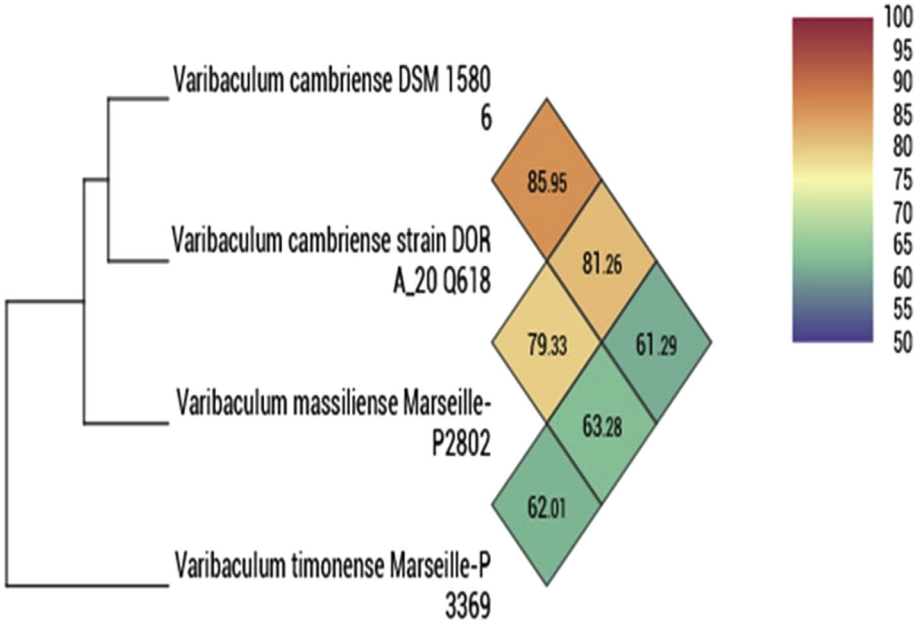

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Varibaculum massiliense* sp. nov. and other closely related species with standing in nomenclature.

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 lower than 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-P2802 was 2.14 Mb long with a 52.3 mol% of G+C content. The degree of genomic similarity of strain Marseille-P2802 with closely related species was estimated using the OrthoANI software [14]. OrthoANI values among closely related species (Fig. 4) ranged from 85.95% between *Varibaculum cambriense* strain DSM 15806 and *V. cambriense* strain DORA_20Q618 to 61.29% between *V. cambriense* strain DSM 15806 and *Varibaculum timonense* Marseille-P3369. When *Varibaculum massiliense* was compared with these closely related species, values ranged from 62.01% with *V. timonensis* to 81.26% with *V. cambriense* strain DORA_20Q618.

Conclusion

On the basis of unique phenotypic features, including the 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-P2802 as the type strain of *Varibaculum massiliense* sp. nov., a new species within the genus *Varibaculum*.

Description of *Varibaculum massiliense* strain Marseille-P2802 sp. nov.

Marseille-P2802 is the type strain of *Varibaculum massiliense* sp. nov. (mas.si.li.en'sis, L. fem. adj., from *Massilia*, the Latin name of Marseille, where the strain was first cultivated). The strain grows strictly under anaerobic conditions at 37°C. The potential pathogenicity of the type strain Marseille-P2802 (= CSUR P2802 = DSM 103074) is unknown. This strain has a genome length of 2.14 Mb with a 52.3% G+C content. The 16S rRNA gene sequence and whole-genome shotgun sequence of Marseille-P2802 were deposited in GenBank under accession numbers LT576396 and FNWI00000000, respectively.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession number LT576396 and FNWI00000000, respectively.

Deposit in culture collections

Strain Marseille-P2802 was deposited in two different strain collections under number (= CSURP2802 = DSM 103074).

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Conflict of interest

None declared.

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