**NEW SPECIES**

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

E. H. A. Niang1,2, C. I. Lo2,3, S. Brahimi1,2, N. Armstrong1,2, D. Raoult2,3, P.-E. Fournier1,2 and F. Fenollar1,2

1) Aix Marseille Univ, IRD, AP-HM, MEV, 2) IHU-Méditerranée Infection and 3) Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France

Abstract

*Varibaculum massiliense* sp. nov. strain Marseille-P2802T (= 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. © 2019 The Authors. Published by Elsevier Ltd.

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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 taxonogenomics 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 [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-P2802T 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%).
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.
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).

**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 (Thermo Fisher, 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.

| 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                  | +             | −            | +             | +           |
| Motility                    | −             | −            | −             | −           |
| Endospore formation         | −             | −            | −             | −           |
| Alkaline phosphatase        | +             | +            | −             | −           |
| Catalase                    | −             | −            | −             | −           |
| Indole                      | −             | −            | −             | −           |
| Urease                      | +             | +            | +             | +           |
| β-galactosidase             | −             | −            | −             | −           |
| N-acetylglucosamine         | −             | −            | −             | −           |
| Arabinose                   | −             | −            | −             | −           |
| Lipase (C8)                 | −             | −            | −             | −           |
| Tryptoph                      | +             | +            | −             | +           |
| Mannose                      | −             | −            | −             | −           |
| Mannitol                     | −             | −            | −             | −           |
| Glucose                      | −             | −            | −             | −           |
| Maltose                      | +             | +            | +             | +           |
| Source                       | Urine sample  | stool sample | Human sources | Clinical sample |

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

**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 | −       |
|                  | Cysteine arylamidase | −       |
|                  | Tryptoph | +       |
|                  | a-chymotrypsin    | −       |
|                  | Acid phosphatase  | +       |
|                  | Naphthol-AS-BI-phosphohydrolase | +       |
|                  | α-galactosidase   | −       |
|                  | β-galactosidase   | −       |
|                  | β-glucuronidase   | −       |
|                  | α-glucosidase     | +       |
|                  | β-glucosidase     | −       |
|                  | N-acetyl-β-glucosaminidase | −       |
|                  | α-mannosidase     | −       |
|                  | α-fucosidase      | −       |
| API 20A          | Indole production | −       |
|                  | Urease            | +       |
|                  | Glucose           | +       |
|                  | Mannitol          | +       |
|                  | Lactose           | +       |
|                  | Sucrose           | +       |
|                  | Maltose           | +       |
|                  | Salicin           | −       |
|                  | Xylose            | −       |
|                  | Arabinose         | −       |
|                  | Gelatin           | −       |
|                  | Esculin           | −       |
|                  | Glycerol          | +       |
|                  | Cellulose         | −       |
|                  | Mannose           | −       |
|                  | Melezitose        | +       |
|                  | Raffinose         | −       |
|                  | Sorbitol          | −       |
|                  | Rhamnose          | −       |
|                  | Trehalose         | −       |

| Fatty acids | Name                  | Mean relative % |
|-------------|-----------------------|-----------------|
| 16:0        | Hexadecanoic acid      | 46.5 ± 0.6      |
| 18:1n9      | 9-octadecenoic acid    | 21.9 ± 0.4      |
| 18:2n6      | 9,12-octadecadienoic acid | 11.4 ± 0.4  |
| 18:0        | Octadecanoic acid      | 11.6 ± 0.2      |
| 18:1n7      | 11-octadecenoic acid   | 3.6 ± 0.2       |
| 14:0        | Tetradecanoic acid     | 1.3 ± 0.2       |
| 17:0        | Heptadecanoic acid     | 1.2 ± 0.3       |
| 15:0        | Pentadecenoic acid     | TR              |
| 17:0        | 14-methyl-hexadecanoic acid | TR                |
| 17:0        | 15-methyl-hexadecanoic acid | TR                |
| 16:1n7      | 9-hexadecenoic acid    | TR              |
| 17:1n7      | 10-heptadecanoic acid  | TR              |

*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.

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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 (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 was estimated using the ORTHOANI software [14]. ORTHOANI values among closely related species was estimated using the ORTHOANI software [14]. ORTHOANI values among closely related species (Fig. 4) ranged from 85.95% between Vanibaculum 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 Vanibaculum massiliense sp. nov., a new species within the genus Vanibaculum.

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 FNW100000000, respectively.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession number LT576396 and FNW100000000, 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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References

[1] The Human Microbiome Project | Nature n.d. Available at: https://www.nature.com/articles/nature06244 (accessed 4 May 2018).
[2] Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185–93.
[3] Lagier JC, Hugon P, Khelaifa S, Fournier PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 2015;28:237–64.
[4] Lagier JC, Khelaifa S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016;1:16203.
[5] Ramasamy D, Mishra AK, Lagier JC, Padvamanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 2014;64:384–91.
[6] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51.
[7] Brahimi SE, Khelaifa S, Raoult D, Moal V. Vanibaculum massiliense sp. nov., a new bacterial species isolated from human urine. New Microbe New Infect 2016;13:75–6.

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Morel AS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta JP, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis 2015;34:561–70.

Diop A, Khelafia S, Armstrong N, Labas N, Fournier PE, Raoult D, et al. Microbial culturomics unravels the halophilic microbiota repertoire of table salt: description of Gracilibacillus massiliensis sp. nov. Microb Ecol Health Dis 2016;27.

Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18:821–9.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.

Luo R, Liu B, Xia Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience 2012;1:18.

Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.

Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.

Guilhot E, Lagier JC, Raoult D, Khelafia S. ‘Prevotella ihumii’ sp. nov. and ‘Varibaculum timonense’ sp. nov., two new bacterial species isolated from a fresh human stool specimen. New Microbe New Infect 2017;18:3–5.

Hall V, Collins MD, Lawson PA, Hutson RA, Falsen E, Ingnas E, et al. Characterization of some actinomyces-like isolates from human clinical sources: description of Varibaculum cambriensis gen nov, sp nov. J Clin Microbiol 2003;41:640–4.

Glaeser SP, Doijad S, Hijazin M, Chakraborty T, Falsen E, Hall V, et al. Varibaculum anthropi sp. nov. represented by three genetically different genomovars isolated from clinical material and emended description of the genus Varibaculum. Syst Appl Microbiol 2016;39:546–52.