Olsenella timonensis sp. nov., a new bacteria species isolated from the human gut microbiota

S. Ndongo[1], M. L. Tall[1], I. I. Ngom[1], J. Delerce[1], A. Levasseur[1], D. Raoult[1,2], P.-E. Fournier[3] and S. Khelaifia[1]

1) *Aix-Marseille Univ, IRD, APHM, MEPHI,* 2) *Institut Hospitalo-Universitaire Méditerranée Infection and* 3) *UMR VITROME, Aix Marseille Université, IRD, SSA, AP-HM, Marseille, France*

Abstract

Olsenella timonensis sp. nov., strain Marseille-P2300^T (= CSUR P2300; =DSM102072), is a new bacterial species from the phylum *Firmicutes* in the family *Atopobiaceae.*This bacteria species was isolated from the human gut microbiota.
© 2019 The Authors. Published by Elsevier Ltd.

Keywords: Anaerobic culture, Culturomics, Gut microbiota, *Olsenella timonensis* sp. nov., Taxonogenomics
Original Submission: 20 May 2019; **Revised Submission:** 30 September 2019; **Accepted:** 1 October 2019
Article published online: 10 October 2019

Corresponding author: S. Khelaifia, Institut Hospitalo-Universitaire Méditerranée Infection, 19–21 Boulevard Jean Moulin, 13385, Marseille cedex 5, France.
E-mail: khelaifia_saber@yahoo.fr

Introduction

Decoding the bacterial diversity involved in normal and pathogenic functions is fundamental [1]. To unveil the diversity of the human gut microbiota, the culturomics approach, based on diversified culture conditions, has been implemented to isolate uncultured species 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-P2300^T that has been isolated from the human gut microbiota.

Isolation and growth conditions

The strain Marseille-P2300^T was isolated from the stool of a 73-year-old man on haemodialysis who was hospitalized in October 2015 in the intensive care unit of the Timone Hospital in Marseille, France. The patient had an inflammatory syndrome with a previous digestive history (diverticulum, colonic polyposis) and dyslipidaemic hypertension. The isolate was obtained after 5 days of pre-incubation at 37°C, in an anaerobic blood-culture bottle enriched with 3 mL of filter sterilized rumen and 3 mL of sheep blood (bioMérieux, Marcy l'Etoile, France). A 100-µL sample was taken from the blood-culture bottle and, after ten serial dilutions, 50 µL of each dilution was seeded into 5% sheep-blood-enriched Columbia agar (bioMérieux). The emerging colonies were observed after 3 days of incubation at 37°C under anaerobic conditions generated by AnaeroGen (bioMérieux), then sub-cultured in the same medium and purified for better identification. The pure isolated colonies of the strain could not be identified by proteomic analysis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as previously described [6] using a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany). The spectra obtained from this strain (Fig. 1) were imported and compared with those of the Bruker database, which is routinely supplemented with the internet database of MEPHI [1].

Phenotypic characteristics

The strain Marseille-P2300 is an obligate anaerobic bacterium, non-motile and non-spore-forming. Cells are Gram-positive with short rods presented singly or in chains

© 2019 The Authors. Published by Elsevier Ltd
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
https://doi.org/10.1016/j.nmni.2019.100610

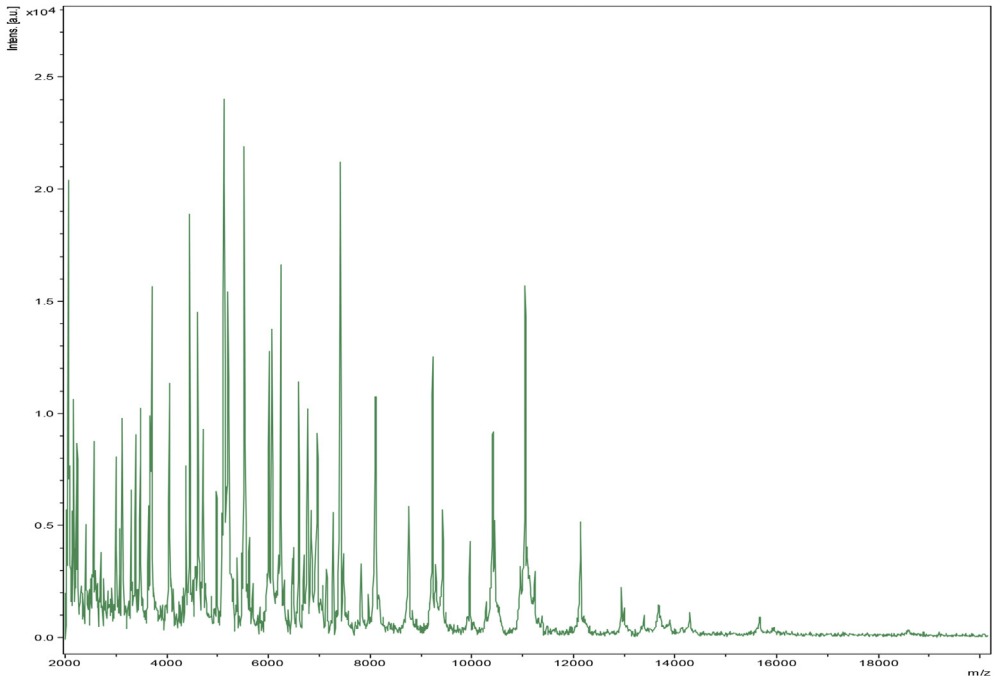

FIG. 1. MALDI-TOF MS reference spectrum of *Olsenella timonensis* sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

(0.4 × 1.0–1.5 µm) (Fig. 2). Colonies were pale grey, measuring up to 2 mm in diameter, with a maximum recorded at 37°C for 72 h. It exhibited no catalase or oxidase activity (Table 1).

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 (Thermofisher, Saint-Aubin, France) as previously described [7]. The 16S rRNA nucleotide sequence was Strain Marseille-P2300^T and exhibited a 96.9% 16S rRNA similarity with *Olsenella umbonata* strain lac31 (GenBank accession number NR_116936.1), the phylogenetically closest species with standing in nomenclature (Fig. 3). We consequently proposed to classify strain Marseille-P2300^T as a new species within the genus *Olsenella* in the phylum Firmicutes.

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 [8]. The assembly was performed using a pipeline containing several softwares (Velvet [9], Spades [10] and Soap Denovo [11], on trimmed data (MiSeq and Trimmomatic [12] softwares) or untrimmed data (only MiSeq

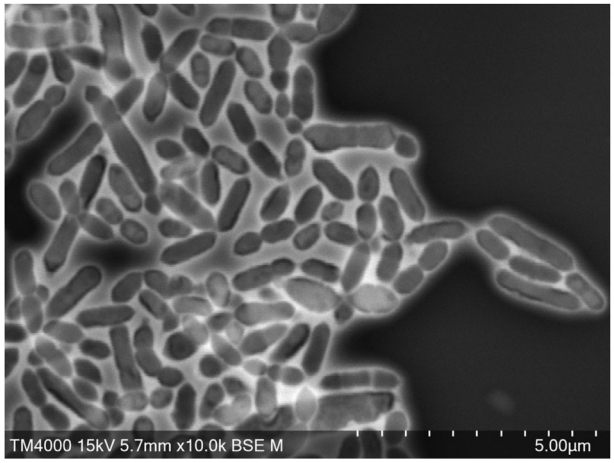

FIG. 2. Scanning electron microscopy (SEM) of stained *Olsenella timonensis* sp. nov. A colony was collected from agar and immersed in a 2.5% glutaraldehyde fixative solution. Then, a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 min and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 min to increase SEM image contrast. The slide was gently washed in water, air-dried and examined in a tabletop SEM (Hitachi TM4000) approximately 60 cm in height and 33 cm in width to evaluate bacteria structure. Scales and acquisition settings are shown in the figure.

© 2019 The Authors. Published by Elsevier Ltd, *NMNI*, **32**, 100610
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

TABLE 1. Description of *Olsenella timonensis* sp. nov., according to the digitalized protologue TA00827 at the www.imedea.uib.es/dprotologue website

Taxonumber	TA00827
Type of description	new description
Species name	*Olsenella timonensis*
Genus name	*Olsenella*
Specific epithet	*timonensis*
Species status	sp. nov.
Species etymology	tim.o.nen'sis. L. masc. adj. *timonensis*, of Timone, the name of the hospital where strain was isolated
Designation of the type strain	strain Marseille-P2300
Strain collection numbers	DSM102072 = CSUR P2300
16S rRNA gene accession number	LT635455
Genome accession number (EMBL)	NZ_LT635455
Genome size	2,176,737 bp
GC mol %	65.4
Source of isolation	human stool
Sampling date	2015-10-28
Gram stain	positive
Cell shape	rod
Cell size (length or diameter)	0.4 × 1.0–1.5 µm
Motility	non-motile
Sporulation (resting cells)	none
Colony morphology	pale grey, measuring up to 2 mm in diameter
Temperature optimum	37°C
pH optimum	7
Relationship to O_2	anaerobe
Oxidase	negative
Catalase	negative

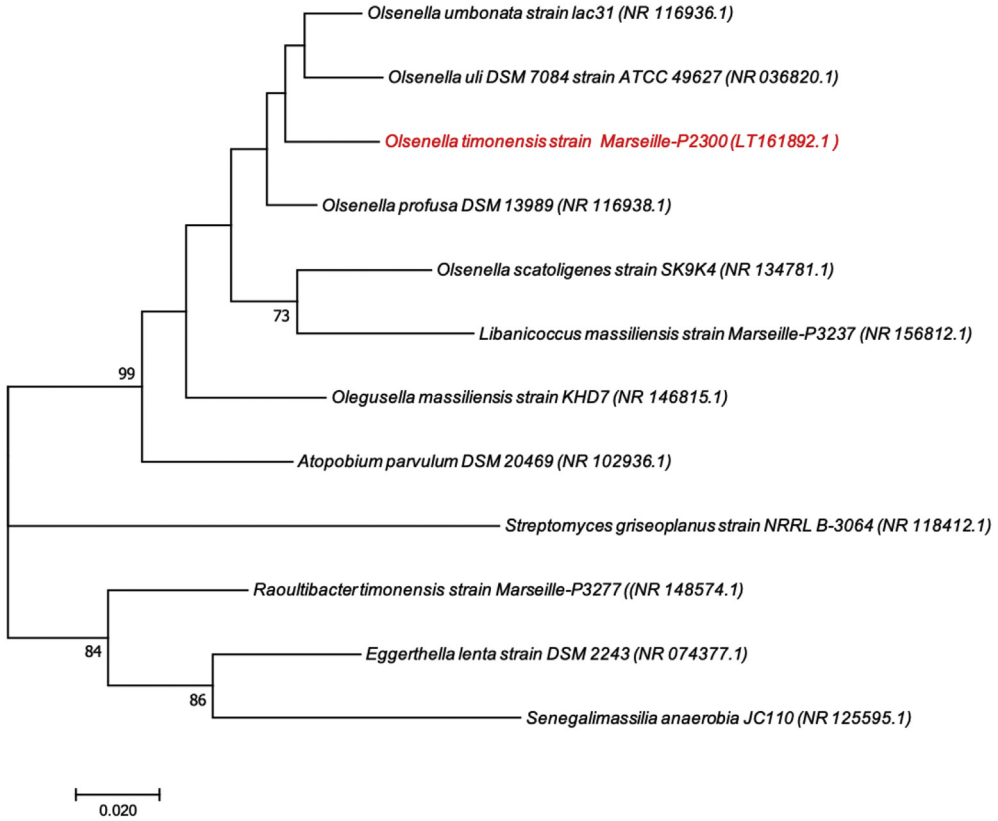

FIG. 3. Phylogenetic tree highlighting the position of *Olsenella timonensis* sp. nov. with regard to other closely related species. GenBank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inference was obtained using 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.

© 2019 The Authors. Published by Elsevier Ltd, *NMNI*, **32**, 100610
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

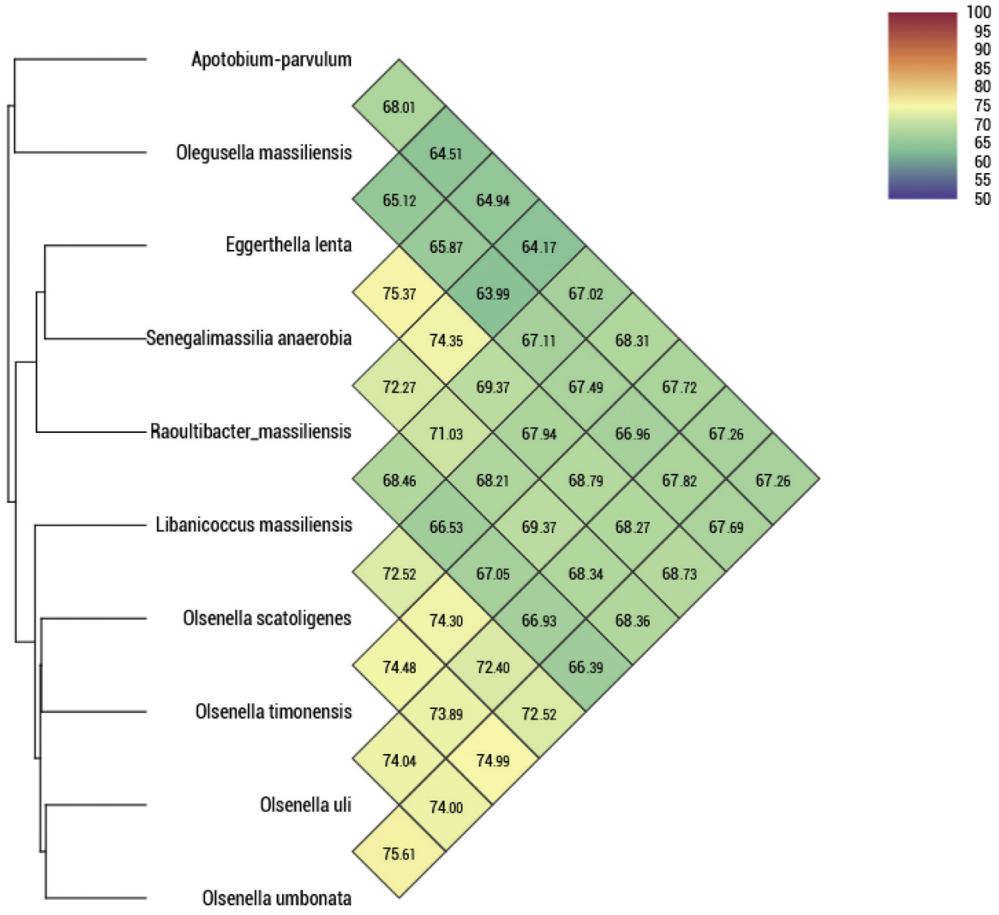

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Olsenella timonensis* sp. nov and other closely related species with standing in nomenclature.

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 using different criteria (number of scaffolds, N50, number of N). The genome size of strain Marseille-P2300^T was 2 176 737 bp with a 65.4 mol% G + C content. The degree of genomic similarity of strain Marseille-P2300^T with closely related species was estimated using the OrthoANI software [13].

OrthoANI values among closely related species (Fig. 4) ranged from 63.99% between *Olegusella massiliensis* and *Raoultibacter massiliensis*, to 75.61% between *Olsenalla uli* and *Olsenella umbonata*. When *Olsenella timonensis* was compared with these closely related species, values ranged from 66.96% with *Olegusella massiliensis* to 74.48% with *Olsenella scatoligenes*.

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-P2300^T as the type strain of *Olsenella timonensis* sp. nov., a new bacterial species within the genus *Olsenella*.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences of strain Marseille-P2300^T were deposited in GenBank under accession number LT161892 and NZ_LT635455.

Description of *Olsenella timonensis* sp. nov.

Olsenella timonensis (tim.o.nen'sis. L. masc. adj. *timonensis*, of Timone, the name of the hospital where the strain was isolated).

© 2019 The Authors. Published by Elsevier Ltd, *NMNI*, **32**, 100610
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Conflicts of interest

None to declare.

Funding sources

The research was funded by the Mediterranee-Infection foundation and the French National Research Agency under the programme *Investissements d'Avenir*, reference ANR-10-IAHU-03.

Ethics and consent

The study was approved by the ethics committee of the Institut Federatif de Recherche 48 under reference 2016-010. The patient gave, approved and signed consent for participating in the study.

Acknowledgements

The authors thank Catherine Robert for sequencing the genome, Aurelia Caputo for submitting the genomic sequence to GenBank and Magdalen Lardière for English reviewing. We also thank Takashi Irie, Kyoko Imai, Shigeki Matsubara, Taku Sakazume, Yusuke Ominami, Hishada Akiko and the Hitachi team of Japan (Hitachi High-Technologies Corporation, Tokyo, Japan) for the collaborative study conducted together with the IHU Méditerranée Infection, and for the installation of a TM4000 microscope at the IHU Méditerranée Infection facility.

References

[1] Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007;449:804–10.
[2] Lagier J-C, 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 J-C, Hugon P, Khelaifia S, Fournier P-E, 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 J-C, Khelaifia 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 J-C, Padhmanabhan 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] Ndongo S, Bittar F, Beye M, Robert C, Di Pinto F, Fournier P-E, et al. '*Cellulomonas timonensis*' sp. nov., taxonogenomics description of a new bacterial species isolated from human gut. New Microbe New Infect 2018;23:7–16.
[7] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, 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.
[8] Morel A-S, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta J-P, 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.
[9] Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18:821–9.
[10] 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.
[11] Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience 2012;1:18.
[12] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
[13] Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.

© 2019 The Authors. Published by Elsevier Ltd, *NMNI*, **32**, 100610
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).