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

S. Ndongo1, M. L. Tall1, I. I. Ngom1, J. Delerce1, A. Levasseur1, D. Raoult1,2, P.-E. Fournier3 and S. Khelafia1
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-P2300T (= CSUR P2300; =DSM102072), is a new bacterial species from the phylum Firmicutes in the family Atopobiacae. 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

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 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-P2300T that has been isolated from the human gut microbiota.

Isolation and growth conditions

The strain Marseille-P2300T 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 hypertenion. 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...
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 3500xLGentic Analyzer capillary sequencer (Thermo-fisher, Saint-Aubin, France) as previously described [7]. The 16S rRNA nucleotide sequence was Strain Marseille-P2300T 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-P2300T 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).
### 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 O2 | anaerobe |
| Oxidase | negative |
| Catalase | negative |

![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.](image)
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-P2300T was 2,176,737 bp with a 65.4 mol% G + C content. The degree of genomic similarity of strain Marseille-P2300T with closely related species was estimated using the ORTHOANI software [13].

ORTHOANI values among closely related species (Fig. 4) ranged from 63.99% between Olegussella massiliensis and Raoulbacter massiliensis, to 75.61% between Olsenella uli and Olsenella umbonata. When Olsenella timonensis was compared with these closely related species, values ranged from 66.96% with Olegussella 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-P2300T 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-P2300T 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).
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, Khelaifi 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, Khelaifi 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, Padmanabhan 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] Bankier A, Nurk S, Antoś 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. Trimmmomatic: 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.