**Abstract**

*Massilistercora timonensis* gen. nov., sp. nov. strain Marseille-P3756T is a new species of the phylum *Firmicutes*; it was isolated from the human gut microbiota and has a genome of 2 769 591 bp (51.2% G + C). The closest species based on 16S rRNA sequence was *Merdimonas faecis* strain BR31 with 95.2% sequence similarity. Considering phenotypic features and comparative genome studies, we proposed the strain Marseille-P3756T as the type strain of *Massilistercora timonensis* sp. nov., a new species within the genus Massilistercora.

© 2020 Published by Elsevier Ltd.

**Keywords:** Culturomics, Gut microbiota, *Massilistercora timonensis* gen. nov., sp. nov, taxonogenomics

**Original Submission:** 16 December 2019; **Accepted:** 5 March 2020

**Article published online:** 12 March 2020

**Corresponding author:** A. Levasseur, AMU, MEPHI (Microbes, Evolution, Phylogeny and Infections), UM63, IRD, IHU-Méditerranée Infection, 19–21 Boulevard Jean Moulin, 13385, Marseille cedex 05, France.

E-mail: anthony.levasseur@univ-amu.fr

**Introduction**

Deciphering the pathogenic functions associated with bacterial diversity is a challenge in medical microbiology [1]. In order to unveil the diversity of the human gut microbiota, the culturomics approach, based on diversified culture conditions, has been designed to isolate species not yet cultured and to complement 16S rRNA metagenomics [2–4]. Furthermore, a new taxonomic strategy, named taxonogenomics, has been developed to include the analysis of complete genome sequences in combination with phenotypic characteristics [5]. Herein, we report a short description of strain Marseille-P3756T which has been isolated from the human gut microbiota.

**Isolation and growth conditions**

In December 2016 we isolated a bacterial strain from the stool sample of an 85-year-old French woman hospitalized at Timone Hospital in Marseille. This isolate 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 reported [6]. The spectra obtained (Fig. 1) were imported and analysed using the Biotyper 3.0 software against the Bruker database that was continually incremented with the MEPHI database. The strain was isolated on 5% sheep-blood-enriched Columbia agar (COS) (bioMérieux, Marcy l’Étoile, France) after a 3-day incubation at 37°C in an anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France).

**Phenotypic characteristics**

Colonies were beige, haemolytic, and circular with a mean diameter of 0.5–1 mm. Bacterial cells were gram-negative bacilli, motile, with a mean diameter of 0.3 μm and length...
ranging from 0.8 to 1.2 μm (Fig. 2). It is a strict anaerobic bacterium and is not spore-forming. Catalase and oxidase activities were negative. Biochemical characteristics were analysed using API ZYM, API 50CH and API 20NE strips (bioMérieux). In API ZYM, positive enzymatic reactions for naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and β-glucosidase were observed. Alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase, leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase were negative. In API 50CH strips, glycerol, D-glucose, D-fructose, N-acetyl-glucosamine and potassium 5-ketogluconate were positive. Erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-

**FIG. 1.** Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) reference spectrum of Massilister cora timonensis gen. nov., sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.
adonitol, methyl-β-D-xylopyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-glucopyranoside, methyl-α-D-glucopyranoside, D-saccharose, D-trehalose, amidon, glycogen, xylitol, gentiobiose and L-arabitol were slightly positive. Negative reactions were obtained for amygdalin, arbutin, aesculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, inulin, D-melezitose, D-raffinose D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, potassium gluconate and potassium 2-ketogluconate. Using API 20NE, nitrates

FIG. 2. Scanning electron microscopy of stained Massilistercora timonensis gen. nov., sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. A drop of the suspension was then deposited directly on a poly-L-lysine-coated microscope slide for 5 min and treated with a 1% phosphotungstic acid (PTA) aqueous solution (pH 2.0) for 2 min to increase the 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. The scales and acquisition settings are shown in figure.

FIG. 3. Phylogenetic tree highlighting the position of Massilistercora timonensis gen. nov., 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 1% nucleotide sequence divergence.
were reduced to nitrites and arginine dihydrolase was positive. All other reactions were negative, including indole formation, urease, gelatine and aesculin hydrolysis.

**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 3500xL Genetic Analyzer capillary sequencer (Thermo Fisher, Saint-Aubin, France) as previously described [7]. The 16S rRNA nucleotide sequence was assembled and corrected using the CodonCode Aligner software (http://www.codoncode.com). Strain Marseille-P3756T exhibited a 95.2% 16S rRNA similarity with *Merdimonas faecis* strain BR31 (Genbank accession number NR_157642), a phylogenetically close species with standing in nomenclature (Fig. 3). We consequently proposed to classify strain Marseille-P3756T as a new species within the genus *Massilistercora* 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 software (Velvet [9], Spades [10] and Soap Denovo [11]), on trimmed data (MiSeq and Trimmomatic [12] software) or on 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 using different criteria (number of scaffolds, N50, number of N).

**FIG. 4.** Heatmap generated with OrthoANI values calculated using the OAT software between *Massilistercora timonensis* gen. nov., sp. nov. and other closely related species with standing in nomenclature. © 2020 Published by Elsevier Ltd, NMNI, 35, 100664 This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
The genome of strain Marseille-P3756T was 2,769,591 bp long with a 51.2% G + C content. The degree of genomic similarity of strain Marseille-P3756T with closely related species was estimated using the OrthoANI software [13]. OrthoANI values, among closely related species (Fig. 4) ranged from 66.47% between Faecalitena fiscicatena and Eubacterium oxidoreducens to 75.64% between Massilistercora timonensis and Merdimonas faecis. When Massilistercora timonensis was compared to these closely related species, values ranged from 65.63% with Eubacterium oxidoreducens to 75.64% with Merdimonas faecis.

**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-P3756T as a type strain of Massilistercora timonensis gen. nov., sp. nov. a new species within the genus Massilistercora.

**Nucleotide sequence accession number**

The 16S rRNA gene and genome sequences were deposited in Genbank under accession numbers LT985389 and NZ_LT990039, respectively.

**Description of Massilistercora gen. nov**

Massilistercora (mas.si.li.ter.co’ra. N.L. fem. n., association of Massilia, the Latin name of Marseille, France, and stercora, stool, from which the type strain was isolated).

**Description of Massilistercora timonensis strains Marseille-P3756T gen. nov., sp. nov**

Massilistercora timonensis (ti.mo.nen’sis. L. masc. adj., timonensis from Timone, the name of university hospital in Marseille, France, where the strain type was isolated). The characteristics of the species are given in Table 1. The type strain is Marseille-P3756T (=CSUR P3756).

**Conflict of interest**

The authors have no conflicts of interest to declare.

**Ethics and consent**

The study was approved by the ethics committee of the Institut Fédératif de Recherche 48 under reference 2016-010. The patient provided an approved and signed consent to participate in this study.

---

**Table 1. Description of Massilistercora timonensis gen. nov., sp. nov. strain Marseille-P3756T**

| TYPE OF DESCRIPTION | NEW DESCRIPTION |
|---------------------|-----------------|
| SPECIES NAME        | Massilistercora |
| GENUS NAME          | timonensis      |
| SPECIFIC EPITHET    | sp. nov.        |
| SPECIES STATUS      | Massilistercora |
| SPECIES ETYMOLOGY   | timonensis (ti.mo.nen’sis. L. masc. adj., timonensis from Timone, the name of university hospital in Marseille, France where the strain type was isolated) |
| GENUS NAME          | Massilistercora |
| SPECIFIC EPITHET    | timonensis      |
| SPECIES STATUS      | sp. nov.        |
| SPECIES ETYMOLOGY   | Massilistercora |

| STRAIN COLLECTION NUMBERS | NEW DESCRIPTION |
|---------------------------|-----------------|
| 16S rRNA GENE ACCESSION NUMBER | Massilistercora timonensis, strain Marseille-P3756T (=CSUR P3756) |
| GENOME ACCESSION NUMBER [EMBL] | Massilistercora timonensis, strain Marseille-P3756T (=CSUR P3756) |
| GENOME SIZE | 2,769,591 bp |
| GC mol % | 51.2 |
| COUNTRY OF ORIGIN | France |
| REGION OF ORIGIN | Marseille |
| SOURCE OF ISOLATION | Human stool |
| GROWTH MEDIUM, INCUBATION CONDITIONS (temperature, pH, and further information) USED FOR STANDARD CULTIVATION | 5% sheep-blood-enriched Columbia agar (bioMérieux) for 3 days' incubation at 37°C in anaerobic atmosphere |
| PH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |
| pH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |
| COUNTRY OF ORIGIN | France |
| REGION OF ORIGIN | Marseille |
| SOURCE OF ISOLATION | Human stool |
| GROWTH MEDIUM, INCUBATION CONDITIONS (temperature, pH, and further information) USED FOR STANDARD CULTIVATION | 5% sheep-blood-enriched Columbia agar (bioMérieux) for 3 days' incubation at 37°C in anaerobic atmosphere |
| PH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |
| pH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |

| STRAIN COLLECTION NUMBERS | NEW DESCRIPTION |
|---------------------------|-----------------|
| 16S rRNA GENE ACCESSION NUMBER | Massilistercora timonensis, strain Marseille-P3756T (=CSUR P3756) |
| GENOME ACCESSION NUMBER [EMBL] | Massilistercora timonensis, strain Marseille-P3756T (=CSUR P3756) |
| GENOME SIZE | 2,769,591 bp |
| GC mol % | 51.2 |
| COUNTRY OF ORIGIN | France |
| REGION OF ORIGIN | Marseille |
| SOURCE OF ISOLATION | Human stool |
| GROWTH MEDIUM, INCUBATION CONDITIONS (temperature, pH, and further information) USED FOR STANDARD CULTIVATION | 5% sheep-blood-enriched Columbia agar (bioMérieux) for 3 days' incubation at 37°C in anaerobic atmosphere |
| PH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |
| pH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |

| STRAIN COLLECTION NUMBERS | NEW DESCRIPTION |
|---------------------------|-----------------|
| 16S rRNA GENE ACCESSION NUMBER | Massilistercora timonensis, strain Marseille-P3756T (=CSUR P3756) |
| GENOME ACCESSION NUMBER [EMBL] | Massilistercora timonensis, strain Marseille-P3756T (=CSUR P3756) |
| GENOME SIZE | 2,769,591 bp |
| GC mol % | 51.2 |
| COUNTRY OF ORIGIN | France |
| REGION OF ORIGIN | Marseille |
| SOURCE OF ISOLATION | Human stool |
| GROWTH MEDIUM, INCUBATION CONDITIONS (temperature, pH, and further information) USED FOR STANDARD CULTIVATION | 5% sheep-blood-enriched Columbia agar (bioMérieux) for 3 days' incubation at 37°C in anaerobic atmosphere |
| PH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |
| pH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |

| STRAIN COLLECTION NUMBERS | NEW DESCRIPTION |
|---------------------------|-----------------|
| 16S rRNA GENE ACCESSION NUMBER | Massilistercora timonensis, strain Marseille-P3756T (=CSUR P3756) |
| GENOME ACCESSION NUMBER [EMBL] | Massilistercora timonensis, strain Marseille-P3756T (=CSUR P3756) |
| GENOME SIZE | 2,769,591 bp |
| GC mol % | 51.2 |
| COUNTRY OF ORIGIN | France |
| REGION OF ORIGIN | Marseille |
| SOURCE OF ISOLATION | Human stool |
| GROWTH MEDIUM, INCUBATION CONDITIONS (temperature, pH, and further information) USED FOR STANDARD CULTIVATION | 5% sheep-blood-enriched Columbia agar (bioMérieux) for 3 days' incubation at 37°C in anaerobic atmosphere |
| PH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |
| pH OPTIMUM | 7 |
| TEMPERATURE OPTIMUM | 37°C |
Acknowledgements

The authors thank Aurelia Caputo for submitting the genomic sequence to GenBank. 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, Science & Medical Systems Business Group 24-14, Nishi-shimbashi I-chome, Minato-ku, Tokyo 105-8717 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. The research was funded by the Meditérranée-Infection foundation and the French National Research Agency under the program ‘Investissements d’Avenir’, reference ANR-10-IAHU-03. This research was also supported by a grant from the Institut Universitaire de France (IUF, Paris, France) allocated to Anthony Levasseur.

References

[1] Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JL. 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(12):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(1):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, 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(2):384–91.
[6] 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(4):543–51.
[7] Morel A-S, Dubourg G, Prudent E, Edourard 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(3):561–70.
[8] Ndongo S, Bittar F, Beye M, Robert C, Di Pinto F, Fournier P-E, et al. ‘Cellulomonas timonensis sp. nov.’, a taxonogenomics description of the new bacterial species isolated from the human gut. New Microbe New Infections 2018;23:7–16.
[9] Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18(5):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(5):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 trimer for Illumina sequence data. Bioinformatics 2014;30(15):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(2):1100–3.