

Massilicoli timonensis sp. nov., a new bacterium isolated from the human microbiota

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Abstract

Massilicoli timonensis sp. nov., strain Marseille-P3755^T (= CSUR P3755 = DSM 103513) is a new bacterial species from the phylum *Firmicutes* and the family *Clostridiales* which was isolated from the human gut microbiota.

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Introduction

Deciphering the bacterial diversity involved in normal and pathogenic functions appears fundamental [1]. In order to unveil the human gut microbial diversity, the culturomics approach, based on diversified culture conditions, was designed to isolate as yet uncultured species and to complement 16S ribosomal RNA (rRNA) metagenomics [2–4]. Furthermore, a new taxonomic strategy termed taxonogenomics was developed to include the analysis of complete genome sequences in combination with phenotypic characteristics [5]. Here we report a short description of strain Marseille-P3755^T that was isolated from the human gut microbiota.

Isolation and growth conditions

As part of a culturomics study, a stool sample was collected from an 85-year-old Frenchwoman admitted in the Timone

Hôpital Marseille in December 2016. A total of 0.3 g of faecal specimen was serially diluted in 900 µL of phosphate-buffered saline (Life Technologies, Carlsbad, CA, USA), and 50 µL of each dilution was seeded on 5% sheep's blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France). After 3 days of incubation at 37°C in an anaerobic atmosphere generated by AnaeroGen (bioMérieux), several colonies grew and were isolated. The purified isolate obtained after three sub-cultures from a single colony could not be identified by MALDI-TOF MS. The screening was performed on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany), as previously reported [6]. Spectra obtained (Fig. 1) were imported and analysed by Biotyper 3.0 software against the Bruker database, which is continuously updated with information from the Microbes Evolution Phylogeny and Infections (MEPHI) database [1].

This study was approved by the ethics committee of the Institut Fédératif de Recherche 48 under reference 2016-010. The patient provided written informed consent for participating in this study.

Phenotypic characteristics

The strain Marseille-P3755 colonies grown on Columbia agar plates after 3 days were circular and translucent, with a diameter of about 0.5 to 1 mm. Strain Marseille-P3755 is a strict anaerobic bacterium, has Gram-negative bacilli (0.3 µm × 2–3 µm), and is nonmotile and non-spore forming (Fig. 2). Strain

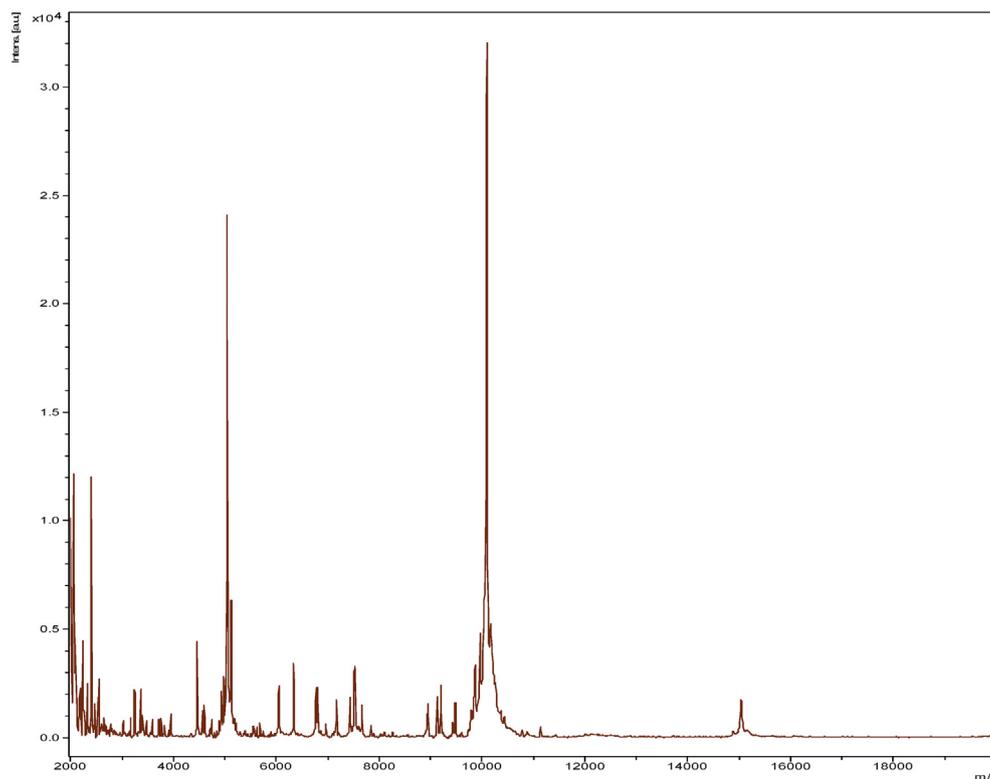


FIG. 1. MALDI-TOF MS reference spectrum of *Massilicoli timonensis* strain Marseille-P3755^T. Reference spectrum was generated by comparison of spectra from 12 individual colonies.

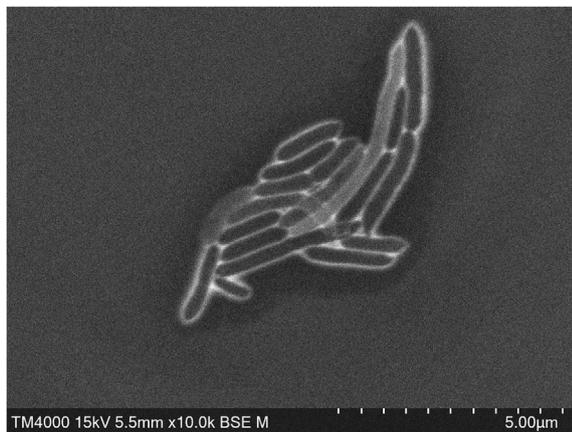


FIG. 2. Scanning electron microscopy (SEM) of stained *Massilicoli timonensis*, sp. nov. Colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then a drop of suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 minutes and treated with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

Marseille-P3755 was negative for catalase and oxidase activities. Biochemical characteristics were investigated using API ZYM, API 50CH and API 20NE strips (bioMérieux). In API ZYM, enzymatic activities were observed for phosphatase acid and naphthol-AS-BI-phosphohydrolase. A slightly positive reaction was observed for phosphatase alkaline, esterase (C4) and esterase lipase (C8); the results of the other tests were negative. Using API 50CH strips, positive reactions were observed with: L-arabinose, D-ribose, D-xylose, L-xylose D-adonitol, D-glucose, D-fructose, dulcitol, inositol, D-mannose methyl- α D-mannopyranoside, N-acetyl-glucosamine amygdalin, arbutin, salicin, Dmaltose, D-saccharose, inulin glycogen, xylitol, gentiobiose, Larabitol and potassium 5-keto-gluconate. Negative reactions were observed with: negative: glycerol, erythritol, D-arabinose, methyl- β D-xylopyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, Dsorbitol, methyl- α D-glucopyranoside, esculin ferric citrate, Dcellobiose, D-lactose, D-melibiose D-trehalose D-melezitose Draffinose, amidone, D-turanose, D-lyxose, D-tagatose D-fucose, Lfucose, D-arabitol, potassium gluconate and potassium 2-ketogluconate. In API 20NE, all test results were negative, including nitrate reduction, indole formation, arginine dihydrolase and hydrolysis of esculin and gelatin.

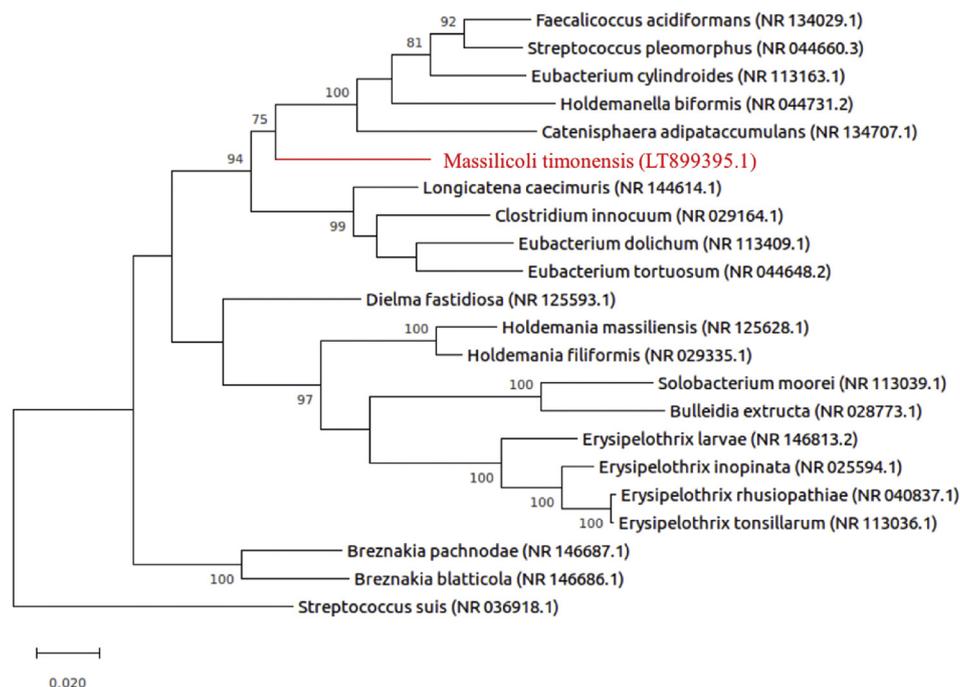


FIG. 3. Phylogenetic tree highlighting position of *Massilicoli timonensis* sp. nov. with regard to other closely related species. GenBank accession numbers of 16S ribosomal RNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters; phylogenetic inferences were obtained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

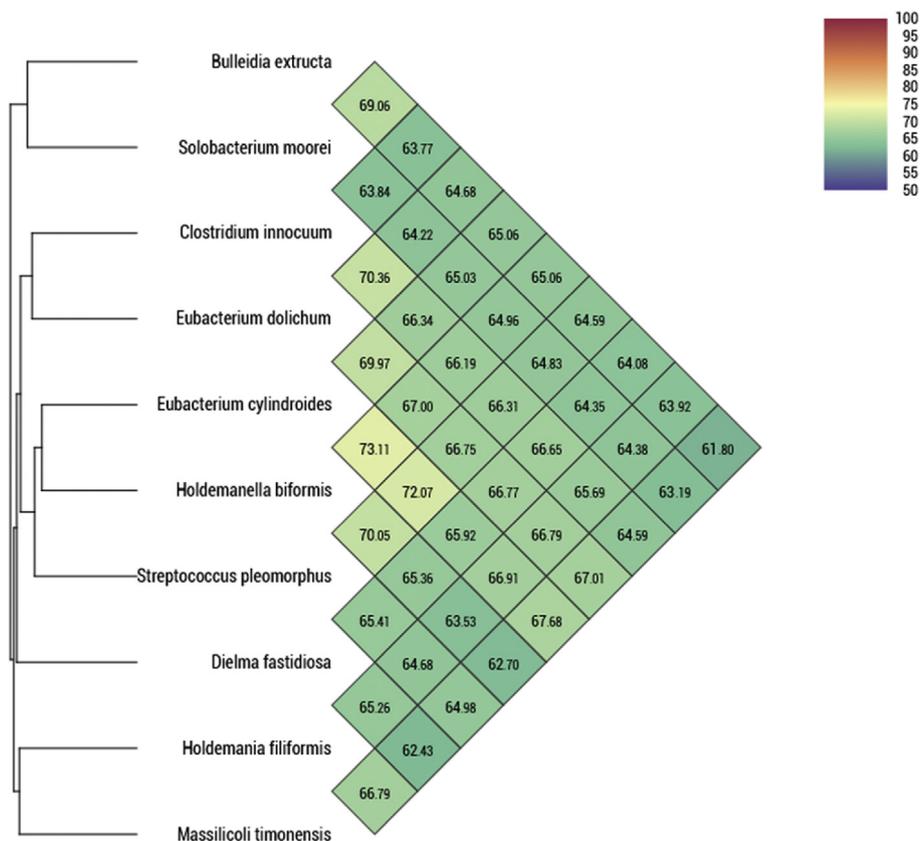


FIG. 4. Heat map generated with OrthoANI values calculated using OAT software between *Massilicoli timonensis* sp. nov. and other closely related species with standing in nomenclature.

TABLE I. Description of *Massilicoli timonensis* sp. nov.

Axonumber	TA00843
Species name	<i>Massilicoli timonensis</i>
Genus name	<i>Massilicoli</i>
Specific epithet	<i>timonensis</i>
Species status	sp. nov.
Species etymology	<i>Massilicoli</i> (mas.si.li.co.li, N.L. masc. n., association of Massilia, the Latin name of Marseille, France, and colon, from which the type strain was isolated) <i>Massilicoli timonensis</i> (ti.mo.nen'sis, L. masc. adj., <i>timonensis</i> from Timone, the name of university hospital in Marseille, France, where the strain type was isolated)
Designation of type strain	Strain Marseille-P3755
Strain collection numbers	(= CSUR P3755 = DSM 103513).
16S rRNA gene accession number	LT899395
Genome accession number (EMBL)	OEMR00000000
Genome status	Draft
Genome size	3 118 584 bp
GC mol%	53.0
Data on origin of sample from which strain had been isolated	
Country of origin	France
Region of origin	Marseille
Source of isolation	Human stool
Gram stain	Negative
Cell shape	Rod
Motility	Motile
Colony morphology	On Columbia agar plates, colonies are circular and translucent, with diameter about 0.5 to 1 mm after 3 days of incubation at 37°C
Temperature optimum	37°C
pH optimum	7
Oxidase	Negative
Catalase	Negative

Data shown according to protologue TA00843 at Digital Protologue website (<http://imedea.uib-csic.es/dprotologue/>).

Strain identification

In order to classify this bacterium, the 16S recombinant DNA (rDNA) gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequenced with the Big Dye Terminator v1.1 Cycle Sequencing Kit and the 3500xL Genetic Analyzer capillary sequencer (Thermo Fisher Scientific, Waltham, MA, USA) as previously described [7]. The 16S rDNA nucleotide sequence was assembled and corrected using CodonCode Aligner software (<https://www.codoncode.com/>).

Strain Marseille-P3755^T exhibited a 95.0% 16S rDNA similarity with *Dielma fastidiosa* strain JC13 (GenBank accession no. NR_125593.1), the phylogenetically closest species with standing in nomenclature (Fig. 3). We consequently proposed to classify this strain as a new genus named *Massilicoli* within the *Firmicutes* phylum. *Massilicoli timonensis* strain Marseille-P3755^T is the species type.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany), then sequenced on a MiSeq sequencer (Illumina, 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 (MiSeq and Trimmomatic [12] 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-P3755^T was 3 118 584 bp long with a 53.0 mol% G + C content. The degree of genomic similarity of strain Marseille-P3755^T with closely related species was estimated with OrthoANI software [13]. OrthoANI values among closely related species (Fig. 4) ranged from 61.80% between *Bulleidia extracta* and *Massilicoli timonensis* to 70.36% between *Clostridium innocuum* and *Eubacterium cylindroides*. When *M. timonensis* was compared to these closely species, values ranged from 62.43% with *Dielma fastidia* to 70.36% with *Clostridium innocuum*.

Conclusion

On the basis of unique phenotypic features, including MALDI-TOF MS spectrum, a 16S rRNA sequence divergence greater than >1.3% and an OrthoANI value < 95% with the phylogenetically closest species with standing in nomenclature, we formally propose the creation of the new genus '*Massilicoli*' gen. nov., and *Massilicoli timonensis* sp. nov., strain Marseille-P3755^T is the type strain.

Description of *Massilicoli* gen. nov.

Massilicoli (mas.si.li.co.li, N.L. masc. n., association of Massilia, the Latin name of Marseille, France, and colon, from which the type strain was isolated).

Description of *Massilicoli timonensis* strain Marseille-P3755^T gen. nov., sp. nov.

Massilicoli timonensis (ti.mo.nen'sis, L. masc. adj., *timonensis* from Timone, the name of the university hospital in Marseille, France, where the strain type was isolated).

The characteristics of the species are listed in Table I. The type strain is Marseille-P3755^T (= CSUR P3755 = DSM 103513).

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT899395 and OEMR00000000 respectively.

Deposit in a culture collection

Strain Marseille-P3755^T was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR) under P3755 and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) under DSM 103513.

MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of '*Massilicoli timonensis*' Marseille-P3755^T is available online (<http://backup.mediterranee-infection.com/article.php?larub=280&titre=urms-database>).

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Conflict of interest

None declared.

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 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, Khelaifia 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, 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 JC, 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] 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] 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.
- [8] Ndongo S, Bittar F, Beye M, Robert C, Di Pinto F, Fournier PE, et al. '*Cellulomonas timonensis*' sp. nov., a taxonogenomics description of the new bacterial species isolated from the human gut. *New Microbe New Infect* 2018;23:7–16.
- [9] Zerbino DR, Birney E. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 2008;18:821–9.
- [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. SOAPdenov02: 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 SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100–3.