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

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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.

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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-P3756^T 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'Etoile, 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

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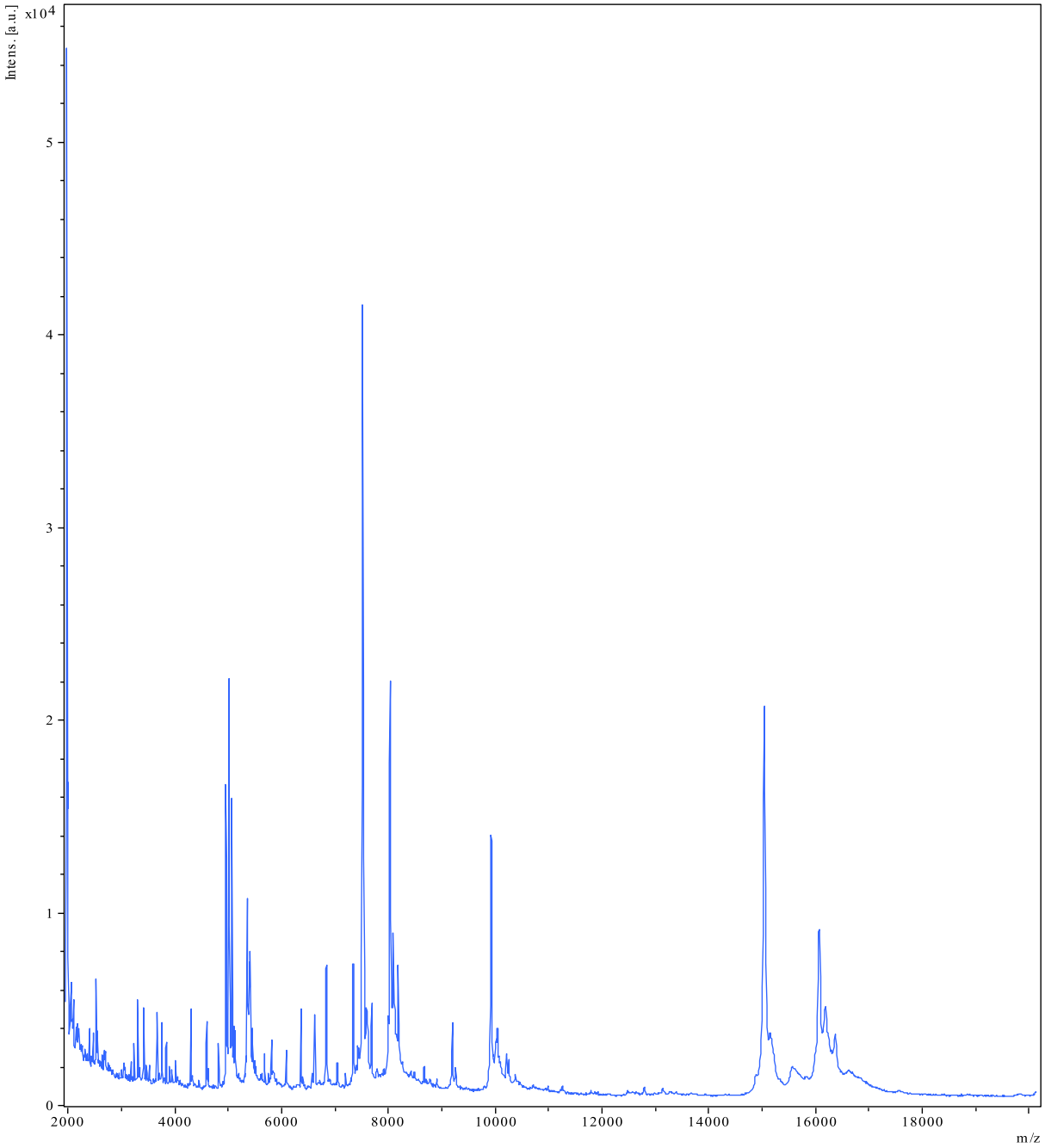

FIG. 1. Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) reference spectrum of *Massilistercora timonensis* gen. nov., sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

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-

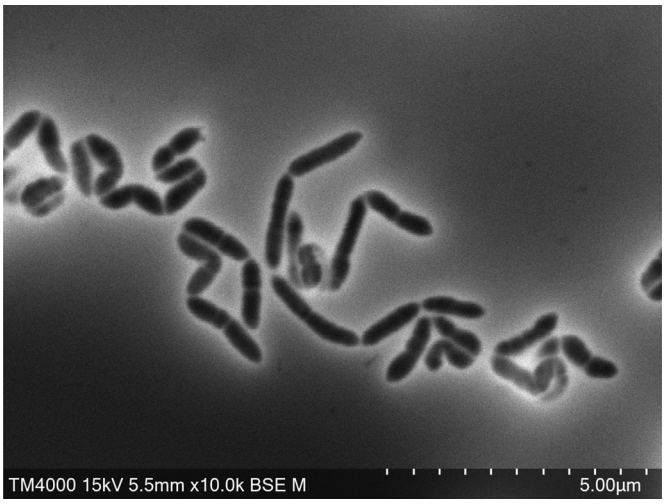

FIG. 2. Scanning electron microscopy of stained *Massilistercora timonensis* gen. nov., sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. A drop of the suspension was then deposited directly on a poly-L-lysine-coated microscope slide for 5 min and treated 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.

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

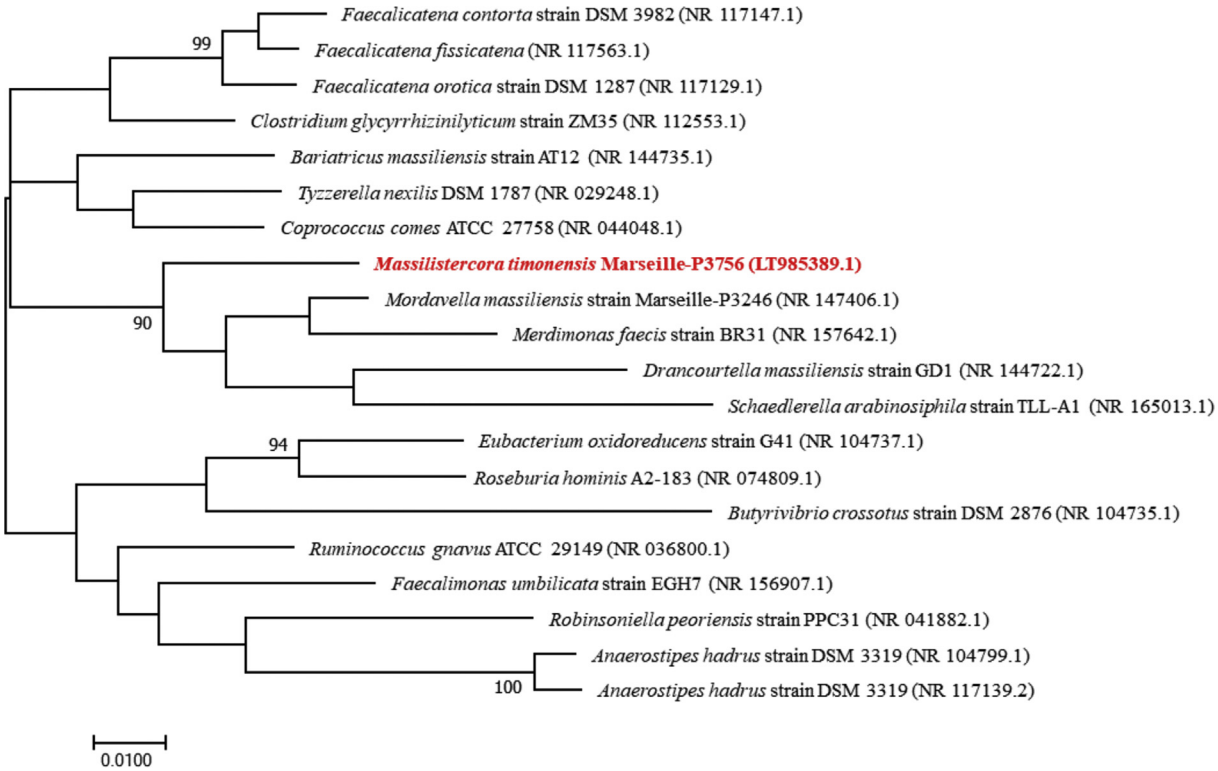

FIG. 3. Phylogenetic tree highlighting the position of *Massilistercora timonensis* gen. nov., sp. nov. with regard to other closely related species. Genbank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inferences 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 3500xLGenetic Analyzer capillary sequencer (Thermofisher, 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-P3756^T 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-P3756^T 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).

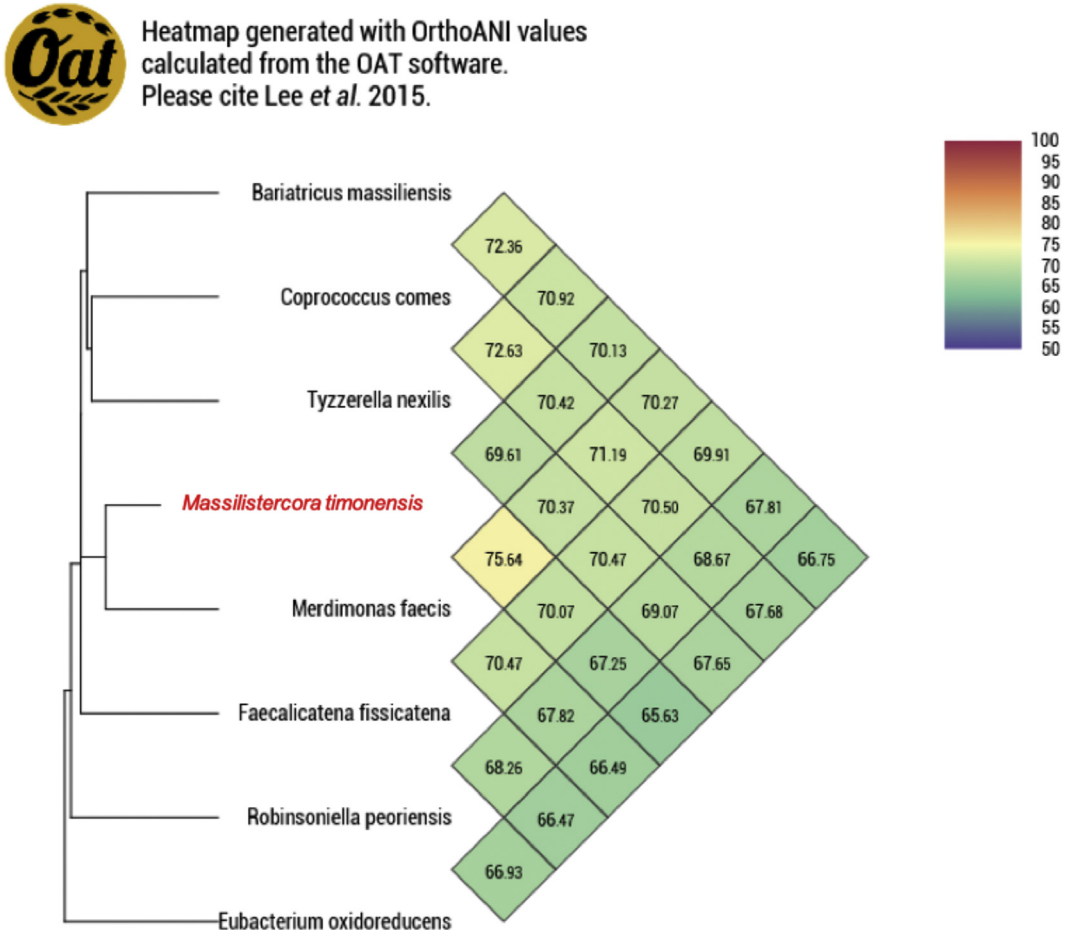

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Massilistercora timonensis* gen. nov., sp. nov. and other closely related species with standing in nomenclature.

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

TYPE OF DESCRIPTION	New Description
SPECIES NAME	*Massilistercora timonensis*
GENUS NAME	*Massilistercora*
SPECIFIC EPITHET	*timonensis*
SPECIES STATUS	sp. nov.
SPECIES ETYMOLOGY	Massilistercora (mas.si.lis.ter.co'ra. N.L. fem. n., association of Massilia, the Latin name of Marseille, France) 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)
STRAIN COLLECTION NUMBERS	CSUR P3756
16S rRNA GENE ACCESSION NUMBER	LT985389
GENOME ACCESSION NUMBER [EMBL]	NZ_LT990039
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
GRAM STAIN	Negative
CELL SHAPE	Rod
CELL SIZE (length or diameter)	0.3 × 0.8–1.2 µm
MOTILITY	Motile
SPORULATION (resting cells)	None
COLONY MORPHOLOGY	Beige, haemolytic, circular form with a mean diameter of 0.5–1 mm after 3 days' incubation
TEMPERATURE OPTIMUM	37°C
pH OPTIMUM	7
OXIDASE	Negative
CATALASE	Negative

The genome of strain Marseille-P3756^T was 2 769 591 bp long with a 51.2% G + C content. The degree of genomic similarity of strain Marseille-P3756^T with closely related species was estimated using the OrthoANI software [13]. OrthoANI values, among closely related species (Fig. 4) ranged from 66.47% between *Faeacalitena fissicatena* 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-P3756^T 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.lis.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-P3756^T 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-P3756^T (=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.

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