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

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Abstract

Massilimicrobiota timonensis gen. nov., sp. nov. strain Marseille-P2264 is a new species from *Firmicutes* phylum isolated from the human gut. Its genome was 2,849,574 bp-long with a 31.8% G+C content. The closest species based on 16S rRNA sequence was *Longibaculum muris* with 95.6% sequence similarity. Considering phenotypic features, 16S rRNA sequence and comparative genome studies, we proposed Marseille-P2264 as the type strain of *Massilimicrobiota timonensis* gen. nov., sp. nov.

Keywords: Culturomics, Gut microbiota, *Massilimicrobiota timonensis* gen. nov., sp. nov.
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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 human gut microbiota diversity, 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 taxono-genomics, 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 SN16^T which has been isolated from the human intestinal microbiota.

Isolation and growth conditions

The SN16 strain was isolated from the stool of an 87-year-old patient admitted to Timone Hospital in Marseille in September 2015. The patient had a cognitive impairment that was accompanied by a loss of weight. The isolated bacterial strain 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]. 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 [1]. The stool sample was pre-incubated for 5 days in an anaerobic blood culture vial (Becton-Dickinson, Pont de Claix, France) enriched of 5% sheep's blood and filter-sterilized rumen at 37°C. Colonies of the strain SN16 were obtained after subculture on Columbia agar enriched of 5% sheep's blood (bioMérieux, Marcy l'Etoile, France) following 3 days of incubation at 37°C under anaerobic conditions generated by AnaeroGen (bioMérieux).

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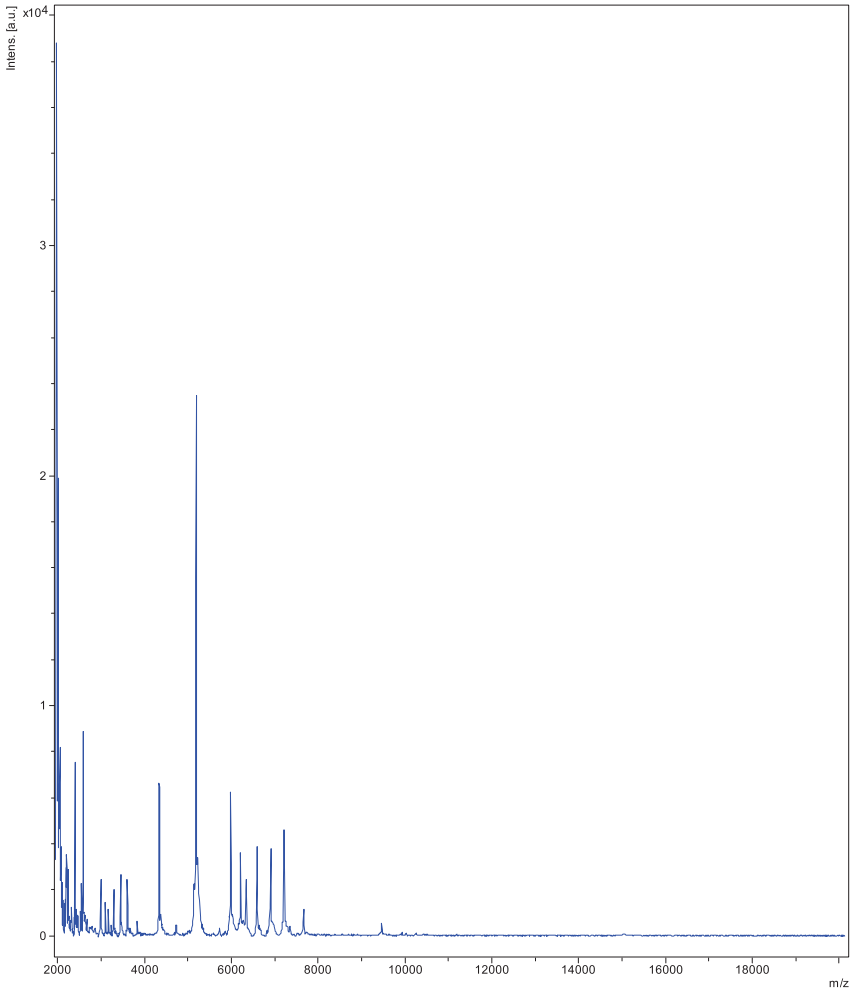

FIG. 1. MALDI-TOF MS reference spectrum of *Massilimicrobiota timonensis* gen. nov., sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

Phenotypic characteristics

On Columbia agar, the colonies of strain SN16 were pale grey, haemolytic, circular and non-uniform border, raised, convex and measuring 1–2 mm of diameter after 3 days of incubation. Strictly anaerobic, strain SN16 was able to grow at 42°C with an optimum at 37°C. It is Gram-negative and bacterial cells are in the shape of rods in chains, no-motile and no-spore-forming. They were about 0.4–0.7 µm in diameter and 1.8–3.0 µm in length on electron micrographs (Fig. 2). Catalase and oxidase activity were not detected. The biochemical characteristics were tested using API 50CH, API ZYM and API 20NE strips (bioMérieux). Using API 50CH strip; positive reactions were found for erythritol, L-xylose, D-adonitol, D-galactose, D-glucose, D-fructose, D-mannose, dulcitol, methyl-α-D-mannopyranoside, amygdalin, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, amidon, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, potassium gluconate and potassium 5-ketogluconate. Negative reactions were obtained for glycerol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-sorbose, L-rhamnose, inositol, D-mannitol, D-sorbitol, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, D-arabitol and potassium 2-ketogluconate. An API ZYM, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase were positive. All other enzymatic activities, including alkaline phosphatase, α-galactosidase, lipase (C14), cystine arylamidase and α-mannosidase, were negative. An API 20NE, glucose fermentation, arginine dihydrolase and hydrolysis of esculin and gelatin were positive. All other tests were negative including nitrates reduction and indole formation.

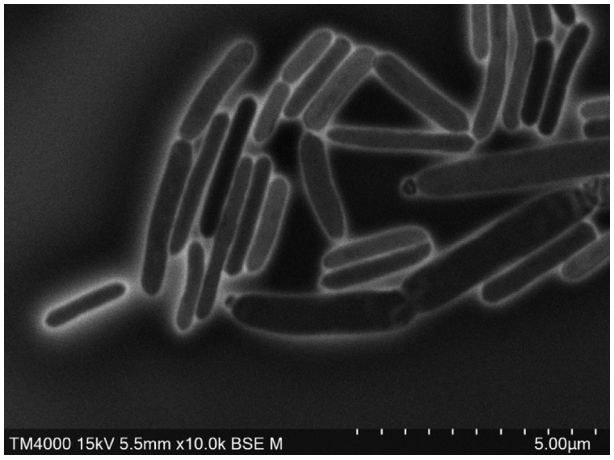

FIG. 2. Scanning electron microscopy (SEM) of stained *Massilimicrobiota timonensis* gen. nov., sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then, a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 minutes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

Fatty acid methyl ester (FAME) analysis by Gas Chromatography/ Mass Spectrometry (GC/MS)

Cellular fatty acid methyl ester (FAME) analysis was performed by GC/MS as described by Sasser [7]. GC/MS analyses were carried out as described previously [8]. Spectral database search was performed using MS Search 2.0 operated with the Standard Reference Database 1A (NIST, Gaithersburg, USA) and the FAMEs mass spectral database (Wiley, Chichester, UK). The major fatty acid was hexadecanoic acid (41%). The most abundant fatty acids were saturated (65%). Minor amounts of unsaturated, branched and other saturated fatty acids were also described (Table 1).

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 [9]. The 16S rRNA nucleotide sequence was assembled and corrected using the CodonCode Aligner software (http://www.codoncode.com).

TABLE 1. Cellular fatty acid composition (%)

Fatty acids	Name	Mean relative % [a]
16:0	Hexadecanoic acid	41.4 ± 1.2
18:0	Octadecanoic acid	20.6 ± 1.7
18:1n9	9-octadecanoic acid	19.4 ± 0.3
18:2n6	9,12-octadecadienoic acid	8.0 ± 0.1
14:0	Tetradecanoic acid	4.0 ± 0.5
18:1n7	11-octadecanoic acid	3.2 ± 0.1
16:1n7	9-hexadecanoic acid	1.0 ± 0.2
17:0 anteiso	14-methyl-hexadecanoic acid	TR
17:0	Heptadecanoic acid	TR
15:0	Pentadecanoic acid	TR
16:1n9	7-hexadecanoic acid	TR
16:0 9,10-methylene	2-hexyl-cyclopropaneoctanoic acid	TR
20:4n6	5,8,11,14-eicosatetraenoic acid	TR
15:0 anteiso	12-methyl-tetradecanoic acid	TR
15:0 iso	13-methyl-tetradecanoic acid	TR
18:1n6	12-octadecanoic acid	TR

TR = trace amounts <1%.
[a]Mean peak area percentage.

Strain Marseille-P2264^T exhibited a 95.6% 16S rRNA similarity with *Longibaculum muris* strain MT10-315-CC-1.2-2 (GenBank Accession number NR_144615.1), the phylogenetically closest species with standing in nomenclature (Fig. 3). We consequently proposed to classify this strain as new genus called *Massilimicrobiota* within the Firmicutes phylum and *Massilimicrobiota timonensis* SN16^T is the type species.

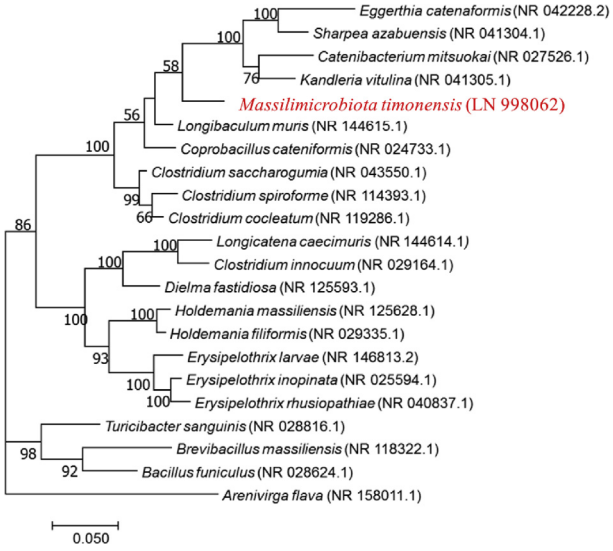

FIG. 3. Phylogenetic tree highlighting the position of *Massilimicrobiota timonensis* gen. nov., sp. nov. with regard to other closely related species. GenBank Accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inference was obtained using the Maximum Likelihood method and the MEGA 7 software. Bootstrap values obtained by repeating the analysis 1,000 times to generate a majority consensus tree are indicated at the nodes. The scale bar indicates a 5% nucleotide sequence divergence.

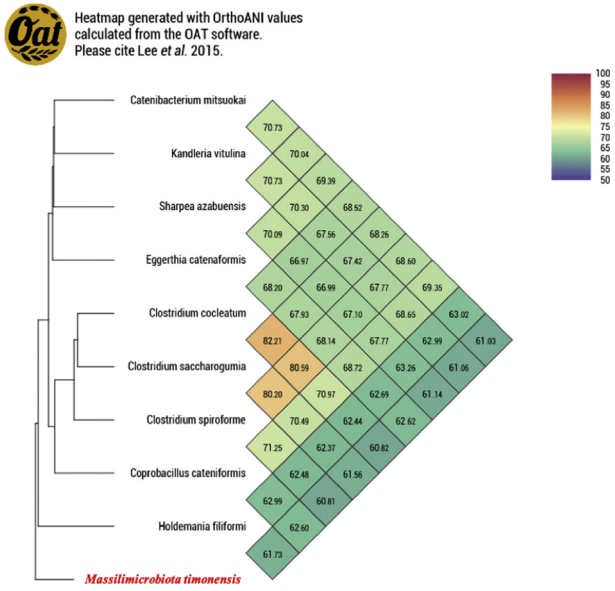

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Massilimicrobiota timonensis* gen. nov., sp. nov. and other closely related species with standing in nomenclature.

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 [10], Spades [5,11] and Soap Denovo [12], on trimmed (MiSeq and Trimmomatic [13] software) or 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). The genome of strain Marseille-P2264^T was 2,849,574 bp-long with a 31.8% G+C content. The degree of genomic similarity of strain SN16^T with closely related species was estimated using the OrthoANI software [14]. OrthoANI values among closely related species (Fig. 4) ranged from 60.81% between *Clostridium spiriforme* and *Massilimicrobiota timonensis* to 82.21 % between *Clostridium saccharogumia* and *Clostridium cocleatum*. When *Massilimicrobiota timonensis* was compared to these closely related species, values ranged from 60.81% with *Clostridium spiriforme* to 62.62% with *Eggerthia catenaformis*.

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 the creation of the new genus "*Massilimicrobiota*" gen. nov and the species type is "*Massilimicrobiota timonensis*" gen. nov., sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under Accession number LN998062 and NZ_UYXN00000000.1, respectively.

Description of Massilimicrobiota gen. nov

Massilimicrobiota (mas.si.li.mi.cro.bio'ta N.L. fem. n., combination of *Massilia*, the Latin name of Marseille, and microbiota, in reference to the human intestinal flora from which the type strain was isolated).

Description of Massilimicrobiota timonensis strain SN16^T gen. nov., sp. nov.

Massilimicrobiota 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 detailed in Table 1. The type strain is SN16^T (= CSUR P2264 = DSM101840).

Conflicts of interest

None to declare.

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Ethics and consent

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

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