Luxibacter massiliensis gen. nov., sp. nov., a new bacterium isolated from the human gut microbiota

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Abstract

An anaerobic facultative Gram-stain positive bacterium was isolated from human gut microbiota. Strain Marseille-P5551^T was considered to be a new genus within the phylum *Firmicutes*, as it exhibits a 91.87% similarity level with *Faecalicatena orotica* (NR_117129.1), the phylogenetically closest related species. The draft genome size of strain Marseille-P5551^T is 4 142 938 bp with 44.4% of G + C content. We hereby suggest the creation of *Luxibacter massiliensis* gen. nov., sp. nov., as a new bacterial genus.

Keywords: culturomics, genome, human gut, *Luxibacter massiliensis* gen. nov., sp. nov., taxonogenomics
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Introduction

The human intestinal microbiota continues to reveal its secrets through numerous studies seeking characterization [1]. However, to understand the relationship between the components of the intestinal microbiota and the interactions between the host and the microbe, it is imperative to perform complete phenotypic and genomic characterization [2]. Indeed, it is crucial in order to better understand its potential roles that bacterial diversity can play in physiology and human diseases [3]. In order to explore the bacterial diversity of the human gut, the culturomics approach, which is based on various culture conditions, was chosen to isolate species not previously cultivated; in addition, this modality was chosen because of its complementarity with 16S rRNA amplicon sequencing [4–6].

Using the taxonogenomics approach [7], which consists of characterizing new bacterial species by using phenotypic and genomic characteristics as well as proteomic characteristics from matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis [8,9], we provide here a brief description of a new bacterial genus isolated from the human gut belonging to the family *Lachnospiraceae* which consists of 57 genera according to the List of Prokaryotic Names With Standing in Nomenclature (LPSN; https://www.bacterio.net/).

Isolation and growth conditions

As part of the 'RHU Torino-Lumiere' project, samples from non–small-cell lung cancer patients were collected with the end goal of developing new diagnostic tools and therapeutic options. In 2017, the stool sample of a 69-year-old patient with non–small-cell lung cancer was collected at Institut Gustave Roussy, stored at −80°C and transported to the Institut Hospitalo-Universitaire Méditerranée Infection in Marseille for cultivation using the 18 culture conditions of standardized culturomics [6]. Written informed consent was obtained from the patient for faeces collection, which was part of the oncobiotics study. The study was validated by the B2M ethics committee (protocol

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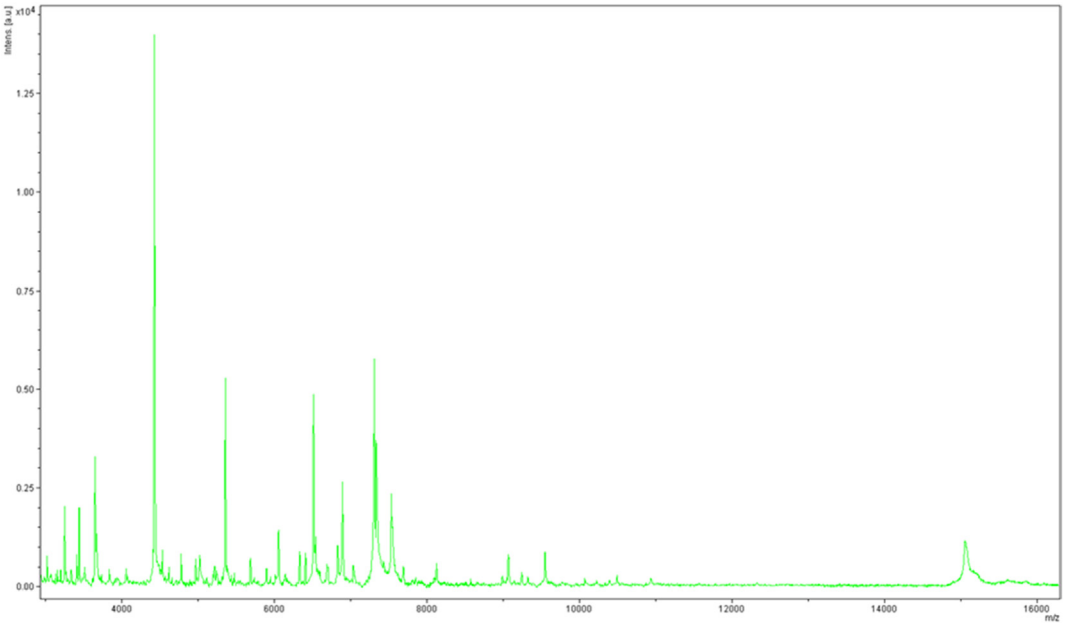

FIG. 1. Matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) reference spectrum of *Luxibacter massiliensis* gen. nov., sp. nov. Reference spectrum was generated by comparison of spectra from 12 individual colonies.

PP:15-013) and the ethics committee of 'Institut Fédératif de Recherche IFR48' (agreement 09-022).

A thermic shock was delivered by heating the stool sample to 80°C during 20 minutes; the sample was then preincubated for 10 days in anaerobic conditions at 37°C in an anaerobic blood culture bottle (bioMérieux, Marcy l'Etoile, France). Then colonies were obtained by subculture on 5% sheep's blood–enriched Columbia agar (bioMérieux) at 37°C and pH 7.5 in anaerobic atmosphere generated using AnaeroGen (bioMérieux) after 48 hours. The identification of strain Marseille-P5551^T by MALDI-TOF MS using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) was unsuccessful. The obtained spectra (Fig. 1) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated with MEPHI database) (https://www.mediterranee-infection.com/urms-data-base). Specific data were collected for this strain and are presented in Table 1.

TABLE 1. General information concerning *Luxibacter massiliensis* gen. nov., sp. nov., strain Marseille-P5551

Property	Term
Taxonumber	GA00118
Species name	*Luxibacter massiliensis*
Genus name	*Luxibacter*
Specific epithet	*massiliensis*
Species status	gen. nov., sp. nov.
Designation of the type strain	Strain Marseille-P5551
Strain collection numbers	CSUR P5551
16S rRNA gene accession number	LS488978
Genome accession number	UWOE00000000
Genome size (bp)	4 142 938
G + C (mol%)	44.4
Origin	Marseille, France
Date of isolation	10 January 2017
Source of isolation	Human stool
Sampling date	9 January 2017
Conditions used for standard cultivation	COS for 48 hours of incubation
Gram stain	Positive
Cell shape	Rod shaped
Cell size (length × diameter) (µm)	2.05 × 0.5
Motility	Nonmotile
Colony colour	Cream coloured
Temperature optimum	37°C
pH optimum	7.5
Relationship to O2	Facultative anaerobe
Oxidase	Positive
Catalase	Negative

Phenotypic characteristics

Colonies of strain Marseille-P5551^T, grown on 5% sheep blood–enriched Columbia agar (bioMérieux), were cream coloured with a mean diameter of 0.75 mm. Bacterial cells were Gram-stain positive and rod shaped, with a mean length of 2.05 ± 0.41 µm and a mean width of 0.5 ± 0.04 µm (Fig. 2).

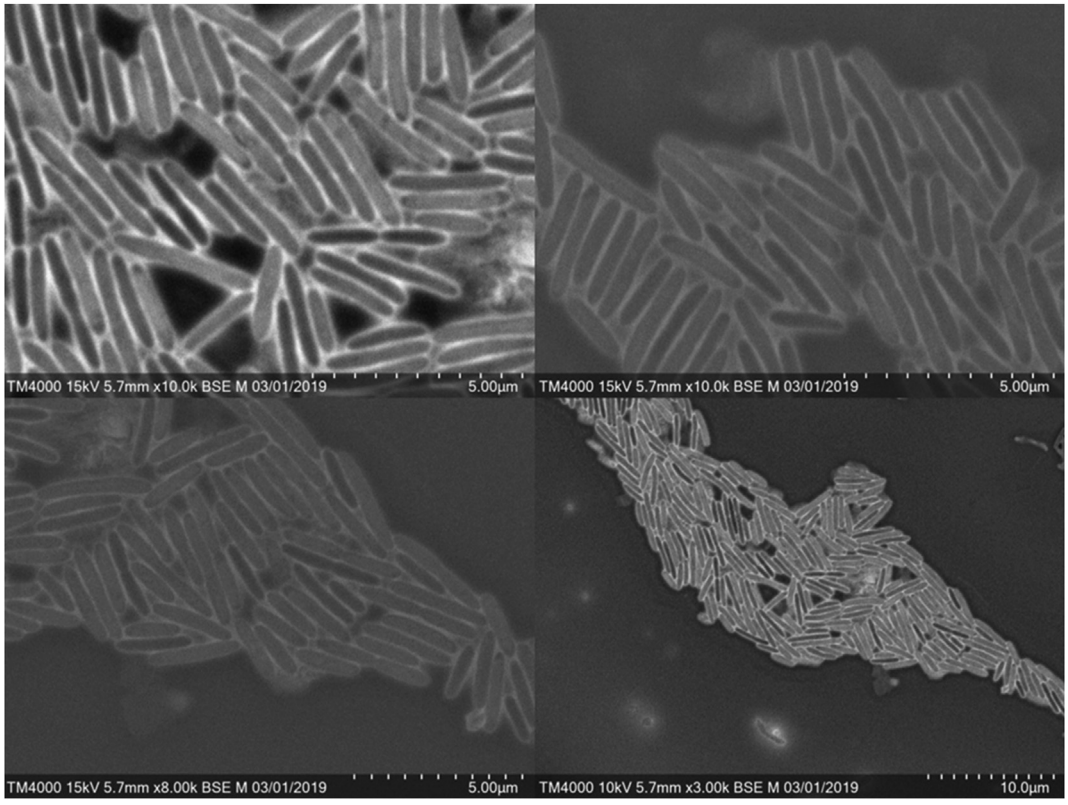

FIG. 2. Scanning electron microscopy (SEM) of stained *Luxibacter massiliensis* gen. nov., sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then a drop of suspension was directly deposited on a poly-L-lysine–coated microscope slide for 5 minutes and treated with 1% phosphotungstic acid (PTA) aqueous solution (pH 2.0) for 2 minutes to increase SEM image contrast. The slide was gently washed in water, air dried and examined with a Hitachi TM4000Plus tabletop microscope (Hitachi High-Tech, Tokyo, Japan). Scales and acquisition settings are shown.

Strain Marseille-P5551^T was catalase negative, oxidase positive and no nhaemolytic. Further biochemical characteristics of strain Marseille-P5551^T were determined using API 50CH and 20A strips (bioMérieux), the results of which are displayed in Table 2. A comparison of the different characteristics of this strain with other closely related species is presented in Table 3.

Cellular fatty acid methyl ester analysis was performed by gas chromatography/mass spectrometry (GC/MS). For this, two samples were prepared with approximately 7 mg of bacterial biomass per tube collected from several culture plates. Fatty acid methyl esters were prepared as previously described [10], and GC/MS analyses were carried out as previously described [11]. Finally, we showed that the most abundant fatty acid by far was hexadecanoic acid (63%), followed by octadecanoic acid (13%) and 9-octadecenoic acid (8%) (Table 4). Minor amounts of other unsaturated, saturated and branched fatty acids were also described (Table 4).

TABLE 2. Phenotypic characterization of *Luxibacter massiliensis* gen. nov., sp. nov., based on analytical profile index (API) test results

Strip	Test	Result
API 50 CH	Control	–
	Glycerol	–
	Erythrol	–
	D-Arabinose	–
	L-Arabinose	+
	D-Ribose	+
	D-Xylose	+
	L-Xylose	–
	D-Adonitol	–
	Methyl-βD-xylopyranoside	+
	D-Galactose	+
	D-Glucose	+
	D-Fructose	+
	D-Mannose	+
	L-Sorbose	–
	L-Rhamnose	+
	Dulcitol	–
	Inositol	–
	D-Mannitol	+
	D-Sorbitol	+
	Methyl-αD-mannopyranoside	–
	Methyl-αD-glucopyranoside	–

Continued

TABLE 2. Continued

Strip	Test	Result
	N-Acetylglucosamine	−
	Amygdalin	+
	Arbutin	+
	Esculin	+
	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	−
	D-Arabitol	−
	L-Arabitol	−
	Potassium gluconate	−
	Potassium 2-ketogluconate	+
	Potassium 5-ketogluconate	+
API 20 A	L-Tryptophane	+
	Urea	+
	D-Glucose	+
	D-Mannitol	+
	D-Lactose (bovine origin)	+
	D-Saccharose (sucrose)	+
	D-Maltose	+
	Salicin	+
	D-Xylose	+
	L-Arabinose	+
	Gelatin (bovine origin)	+
	Esculin ferric citrate	+
	Glycerol	−
	D-Cellobiose	+
	D-Mannose	+
	D-Melezitose	−
	D-Raffinose	−
	D-Sorbitol	−
	L-Rhamnose	+
	D-Trehalose	+

TABLE 4. Cellular fatty acid composition (%)

Fatty acid	Name	Mean relative %[a]
16:0	Hexadecanoic acid	63.1 ± 0.6
18:0	Octadecanoic acid	12.9 ± 0.4
18:1n9	9-Octadecenoic acid	8.0 ± 0.3
14:0	Tetradecanoic acid	7.2 ± 0.4
18:2n6	9,12-Octadecadienoic acid	3.0 ± 0.1
17:0	Heptadecanoic acid	1.7 ± 0.3
18:1n7	11-Octadecenoic acid	1.4 ± 0.1
15:0	Pentadecanoic acid	1.2 ± 0.1
16:1n7	9-Hexadecenoic acid	TR
12:0	Dodecanoic acid	TR
15:0 anteiso	12-Methyl-tetradecanoic acid	TR
15:0 iso	13-Methyl-tetradecanoic acid	TR
13:0	Tridecanoic acid	TR

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

Strain identification

The 16S rRNA gene was sequenced in order to identify this strain. Amplification was performed using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing using the Big Dye Terminator v1.1 Cycle Sequencing Kit as previously described [12]. The 16S rRNA nucleotide sequences were assembled and corrected by CodonCode Aligner software (https://www.codoncode.com/). Strain Marseille-P5551^T exhibited a 91.87% sequence identity with *Faecalicatena orotica* strain DSM 1287 (GenBank accession no. NR_117129.1) (Fig. 3). We consequently classify this strain as a new genus within the *Lachnospiraceae* family and the *Firmicutes* phylum.

TABLE 3. Differential characteristics of , *Luxibacter massiliensis* gen. nov., sp. nov.; 2, *Clostridium scindens* strain ATCC 35704 [19]; 3, *Blautia marasmi* strain Marseille-P2377^T [20]; 4, *Faecalicatena fissicatena* strain JCM 31501 [21]; and 5, *Robinsoniella peoriensis* strain CCUG 52336 [22]

Property	1	2	3	4	5
Cell diameter (µm)	0.5–0.6	0.5–0.7	NA	0.3–0.5	NA
Oxygen requirement	Anaerobic	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Gram stain	+	+	+	+	+
Motility	Nonmotile	Nonmotile	Nonmotile	Nonmotile	Nonmotile
Endospore formation	+	+	−	−	+
Catalase	−	−	−	−	v
Oxidase	+	NA	−	NA	+
Amygdalin	+	−	NA	NA	+
Maltose	+	−	NA	+	+
D-xylose	−	+	NA	+	+
D-ribose	+	+	NA	+	+
Arabinose	−	−	NA	+	+
Rhamnose	+	−	NA	+	+
Melibiose	+	−	NA	NA	+
Mannitol	+	−	NA	−	−
D-Fructose	+	+	NA	+	+
D-Glucose	+	+	+	+	+
Sucrose	−	−	NA	+	+
Source	Human faeces	Human faeces	Human faeces	Human faeces	Human wound

+, positive result; −, negative result; v, variable result; w, weakly positive result; NA, data not available.

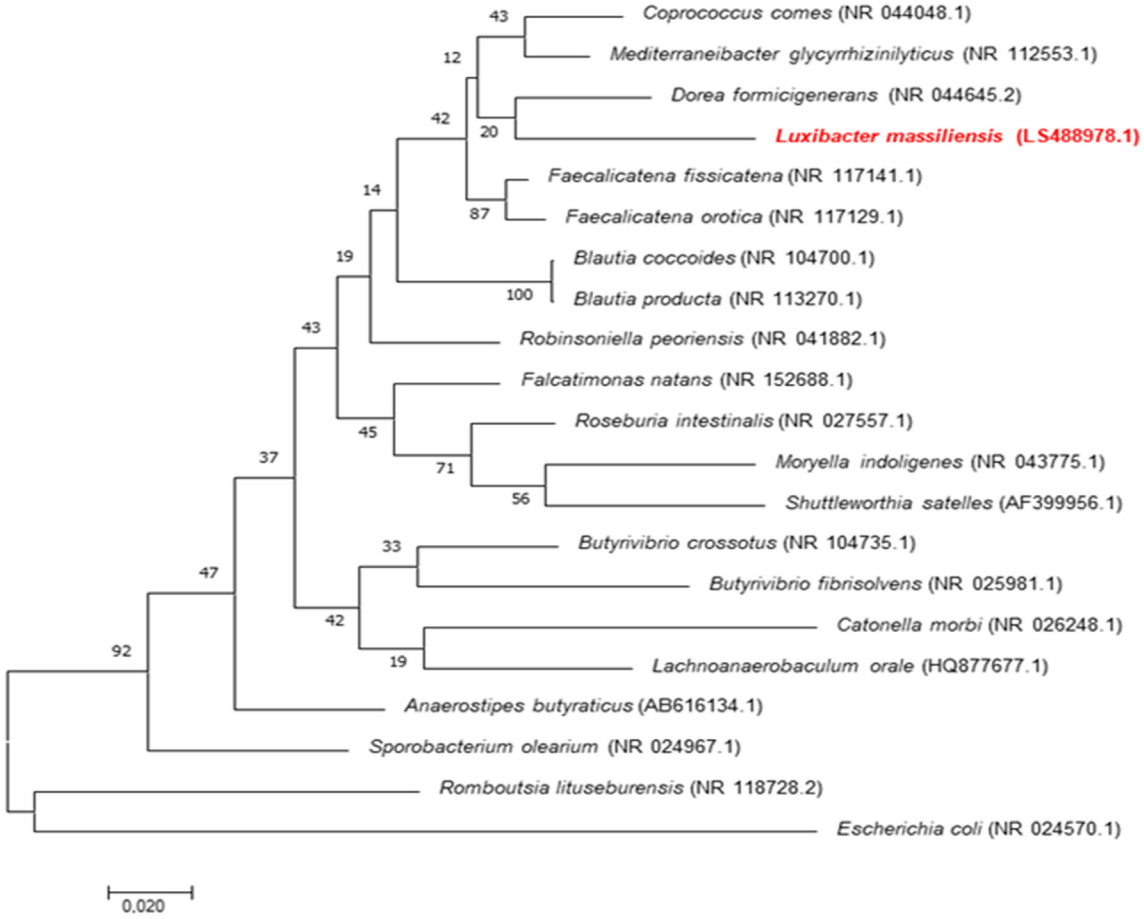

FIG. 3. Phylogenetic tree showing position of *Luxibacter massiliensis* gen. nov., sp. nov., strain Marseille-P5551^T, relative to other phylogenetically close neighbours. Respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences were aligned by Muscle v3.8.31 with default parameters, and phylogenetic inferences were obtained by maximum likelihood method within MEGA 7 software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Scale bar indicates 1% nucleotide sequence divergence.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit, then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT Paired end (Illumina), as previously described [13]. The assembly was performed with a pipeline incorporating different software packages (Velvet [14], Spades [15] and Soap Denovo [16]) and trimmed (MiSeq and Trimmomatic [17] software) or untrimmed data (only MiSeq software). GapCloser was used to decrease assembly gaps. Scaffolds with <800 bp and those with a depth value of <25% at mean depth were removed. Therefore, the best assembly was chosen by using different criteria (number of scaffolds, N50, number of N).

The genome of strain Marseille-P5551^T is 4.14 Mb long with a 44.4 mol% G + C content and contains 3940 predicted genes. The degree of genomic similarity of strain Marseille-P5551^T with closely related species was estimated by OrthoANI software [18]. OrthoANI values among closely related species ranged from 67.56% between *Robinsoniella peoriensis* and strain Marseille-P5551^T to 84.42% between *Blautia marasmi* and *Blautia producta* (Fig. 4). When strain Marseille-P5551^T was compared to these closely related species, values ranged from 67.56% with *R. peoriensis* to 74.92% with *Faecalicatena contorta* (Fig. 4).

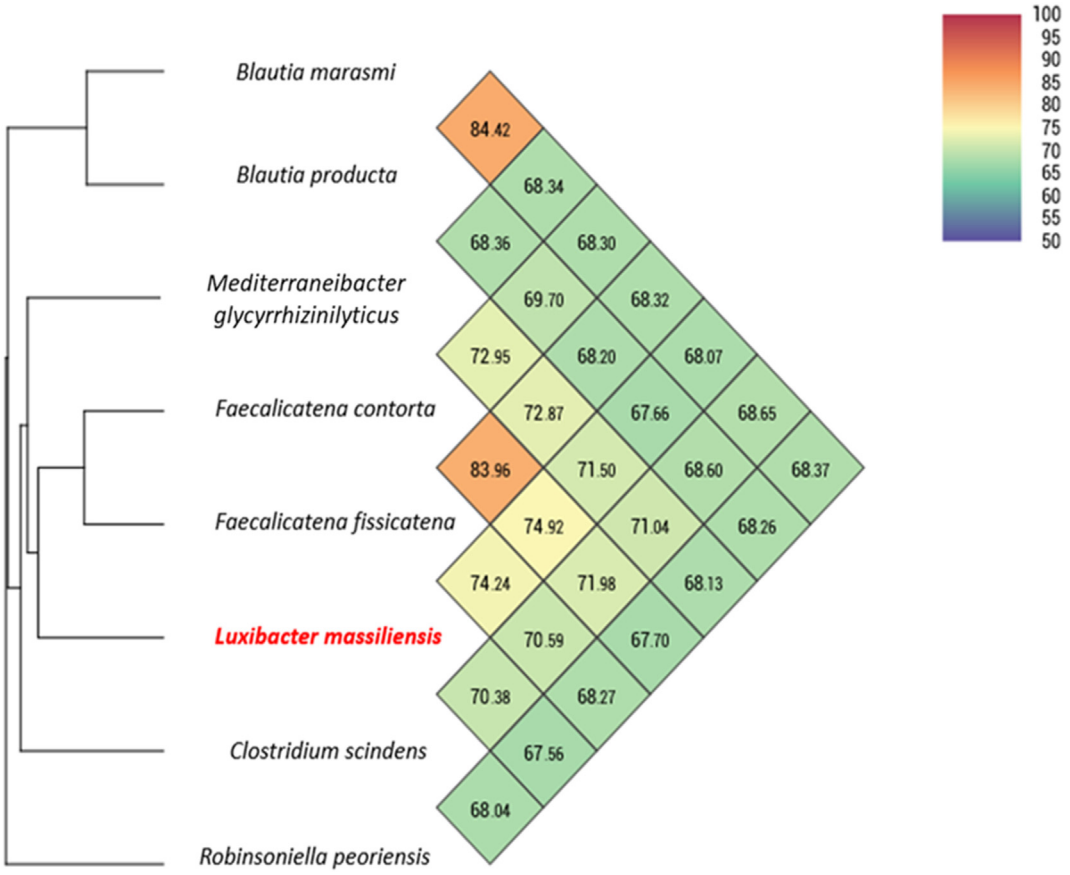

FIG. 4. Heat map generated using OrthoANI values calculated by OAT software between *Luxibacter massiliensis* gen. nov., sp. nov., strain Marseille-P5551^T, and other closely related species with standing in nomenclature.

Conclusion

The taxonogenomics concept based on phenotypic and genotypic features has been used to describe this new bacterium. Indeed, the unmatched MALDI-TOF spectrum, an OrthoANI value under 95% and a 16S rRNA sequence similarity under 94% with the phylogenetically closest species with standing in nomenclature lead us to conclude that this bacterial strain was previously unknown. Therefore, we formally propose the creation of *Luxibacter massiliensis* gen. nov., sp. nov., a new genus of bacteria in the Lachnospiraceae family within the Firmicutes phylum. Strain Marseille-P5551^T (CSURP5551), which was isolated from the human gut, is the type strain of L. massiliensis sp. nov.

Description of *Luxibacter* gen. nov.

Luxibacter (lu.xi.bac'ter, L. masc. n. *Luxibacter*, combination of *lux*, 'light', and *bacter*, 'rod'; *Luxibacter*, 'bacterium from light', in reference to the LUMIERE project in France). Cells are Gram-positive, motile, anaerobic bacilli. The growth of colonies was obtained after an anaerobic condition at 37°C during 48 hours. The DNA G + C content is about 44.4%. The type species of the genus is *Luxibacter massiliensis*.

Description of *Luxibacter massiliensis* sp. nov.

Luxibacter massiliensis gen. nov., sp. nov. (mas.si.li.en'sis, N.L. fem. adj. *massiliensis*, 'to Massilia', the Latin name of Marseille, where the type strain was first isolated and characterized) is classified as a member of the family *Lachnospiraceae* in the phylum *Firmicutes*. Strain Marseille-P5551^T is the type strain of the new species '*Luxibacter massiliensis*' gen. nov., sp. nov. It is an anaerobic Gram-positive bacterium and is motile. Colonies of strain Marseille-P5551^T observed on blood agar medium are cream coloured with a mean diameter of 0.75 mm and are catalase negative and oxidase positive. The genome size of *Luxibacter massiliensis* strain Marseille-P5551^T is 4 142 938 bp with 44.4 mol% G + C content. The GenBank accession

number for the 16S rRNA gene sequence of strain Marseille-P5551^T is LS488978 and for the whole genome shotgun project is UWOE00000000. *L. massiliensis* strain Marseille-P5551^T was isolated from the gut microbiota of a 69-year-old non–small-cell lung cancer patient.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LS488978 and UWOE00 000000 respectively.

Deposit in culture collections

Strain Marseille-P5551^T was deposited in the Collection de Souches de l'Unité des Rickettsies under accession number CSURP5551 and in the Spanish Type Culture Collection under accession number CECT 30111.

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Conflict of interest

None declared.

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