

Libanicoccus massiliensis gen. nov., sp. nov., a new bacterium isolated from human stool

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Abstract

Strain Marseille-P3237 was isolated from a stool sample of a healthy 35-year-old Congolese pygmy female. This anaerobic, Gram-negative, non-spore-forming and non-motile coccus-shaped bacterium is a member of the order *Coriobacteriales*. It exhibits a 2 009 306-bp genome with a 65.46 mol% G+C content and is closely related to, but distinct from, members of the *Olsenella* genus. We propose the creation of the new genus *Libanicoccus* gen. nov. and of the new species *Libanicoccus massiliensis* sp. nov.

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Introduction

The human gut contains 10^{11} to 10^{12} bacteria per gram of stool. This complex microflora is known for its microbial diversity and role in health and diseases [1]. Deciphering the gut microbiota has become a challenge in the twenty-first century [2] and has been attempted using different tools yielding increasingly complex results [3]. To date, more than 2000 different bacterial species belonging to the human gut microbiota have been reported [4]. In our laboratory, we have developed a new technique named culturomics to isolate previously uncultured human gut bacteria [5,6]. Basically, stool samples are cultured under various conditions and all isolated colonies are identified using matrix-assisted laser desorption ionization-time of flight mass

spectrometry (MALDI-TOF MS). Among bacterial isolates that fail MALDI-TOF MS identification, those that show sufficient 16S rRNA gene sequence divergence with species with standing in nomenclature are further characterized using the taxonogenomics strategy, which combines phenotypic assays and genome sequencing and analysis [3,7]. In the present study, using the taxonogenomics approach, we describe the new genus *Libanicoccus* gen. nov. within the family *Coriobacteriaceae*. Strain Marseille-P3237^T (= CSUR P3237 = CCUG 71182) is the type strain of the new species *Libanicoccus massiliensis* gen. nov., sp. nov.

Material and methods

Ethics and sample collection

A stool sample from a healthy 35-year-old pygmy woman was collected in Congo and preserved at -80°C for further analysis at the URMITE Laboratory (Marseille, France). The sample donor gave a signed and informed consent. The study was approved by the ethics committee of the Institut Fédératif de Recherche IFR48 (Marseille, France) under number 09-022.

Strain isolation

The stool sample was diluted in PBS (Life Technologies, Carlsbad, CA, USA) and pre-incubated for 3 days in a blood culture vial (BD BACTEC[®], Plus Anaerobic/F Media, Le Pont de Claix, France) supplemented with 5 mL of sheep blood and 5 mL of filter-sterilized rumen at 37°C. Then, the culture suspension was inoculated on 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) and incubated at 37°C in anaerobic atmosphere.

MALDI-TOF MS and 16S rRNA gene sequencing identification

The individual identification of isolated colonies was first attempted using MALDI-TOF MS, as previously described [5,6]. The reference spectrum obtained for each colony was compared with the Bruker database using the MALDI BIOTYPER software version 3.0 (Bruker Daltonics, Bremen, Germany). Any score <1.9 was considered unreliable. In this case, colonies were subjected to 16S rRNA gene amplification and sequencing, using a GeneAmp 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA) and an ABI Prism 3130xl Genetic Analyzer capillary sequencer (Applied Biosystems) as previously described [8]. Each 16S rRNA nucleotide sequence was compared with the nr database of the National Center for Biotechnology Information using the BLAST software (<https://blast.ncbi.nlm.nih.gov/>). We used the 16S rRNA sequence similarity thresholds of 95% and 98.65% proposed by Kim et al. to consider bacterial isolates as putatively belonging to a new genus or a new species without performing DNA–DNA hybridization [9]. Finally, to determine the phylogenetic position of strain Marseille-P3237 with regard to species with standing in nomenclature, its 16S rRNA gene sequence was compared with the 16S rRNA database of the 'All-Species Living Tree' project of Silva (LTPs121) [10]. Sequence alignment was obtained using Muscle [11] and phylogenetic relationships were inferred with the maximum-likelihood method within the FastTree software [12].

Growth conditions

Culture of strain Marseille-P3237 was attempted using several conditions to determine its optimal growth requirements. First, strain Marseille P3237 was inoculated on 5% sheep blood-enriched Columbia agar (bioMérieux) and incubated in aerobic, micro-aerophilic and anaerobic conditions at 28, 37, 45 and 55°C. The GENbag anaer and GENbag microaer systems (bioMérieux) were used to evaluate the bacterial growth in anaerobic and microaerophilic atmospheres, respectively. In addition, optimal halophily and pH were estimated using 0, 5, 15 and 45% NaCl concentrations and pH values of 6, 6.5, 7 and 7.5, respectively.

Morphological and biochemical assays

The API 20A, API ZYM and API 50CH strips (bioMérieux) were used to biochemically characterize strain Marseille-P3237. Sporulation ability was tested after exposing a bacterial suspension to a thermic shock at 80°C for 10 min. Motility was evaluated using a DMI1000 photonic microscope (Leica Microsystems, Nanterre, France) with a 1000× magnification. Cell morphology was observed using electron microscopy and the following protocol. Bacteria were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 1 h at 4°C. Then, a drop of cell suspension was deposited for approximately 5 min on glow-discharged formvar carbon film on 400-mesh nickel grids (FCF400-Ni, EMS). The grids were dried on blotting paper and cells were negatively stained for 10 seconds with a solution of 1% ammonium molybdate in filtered water at room temperature. Electron micrographs were acquired with a Tecnai G20 Cryo (FEI) transmission electron microscope operated at 200 keV.

Fatty acid methyl ester analysis

Cellular fatty acid methyl ester analysis was performed by gas chromatography/mass spectrometry. Two samples were prepared with approximately 21 mg of bacterial biomass per tube, harvested from several culture plates. FAME analysis was carried out as previously described [5].

Antibiotic susceptibility testing

The antibiotic susceptibility of strain Marseille-P3237 was assessed using the E-test method for the following molecules: benzylpenicillin, amoxicillin, cefotaxime, ceftriaxone, imipenem, amikacin, erythromycin, daptomycin, rifampicin, minocycline, teicoplanin, vancomycin, colistin and metronidazole (bioMérieux).

Genomic DNA extraction and genome sequencing

Genomic DNA (gDNA) of strain Marseille-P3237 was extracted as previously described [5]. A final concentration of 67.8 ng μ L was measured with the Qubit assay and the high sensitivity kit (Life Technologies, Carlsbad, CA, USA). Afterwards, gDNA was sequenced on a MiSeq sequencer (Illumina, San Diego, CA, USA). Briefly, 1.5 μ g of gDNA was used for mate-pair library preparation using the Nextera mate pair Illumina guide (Illumina). After tagmentation and fragmentation of the gDNA with a mate-pair junction adapter, the fragmentation pattern was confirmed using an Agilent 2100 BioAnalyzer (Agilent Technologies Inc, Santa Clara, CA, USA) with a DNA 7500 labchip. The DNA fragments ranged in size from 1.5 kb up to 11 kb with an optimal size at 5.282 kb. No size selection was performed and 191.8 ng of tagmented fragments were circularized. Mechanical shearing of the circularized DNA was

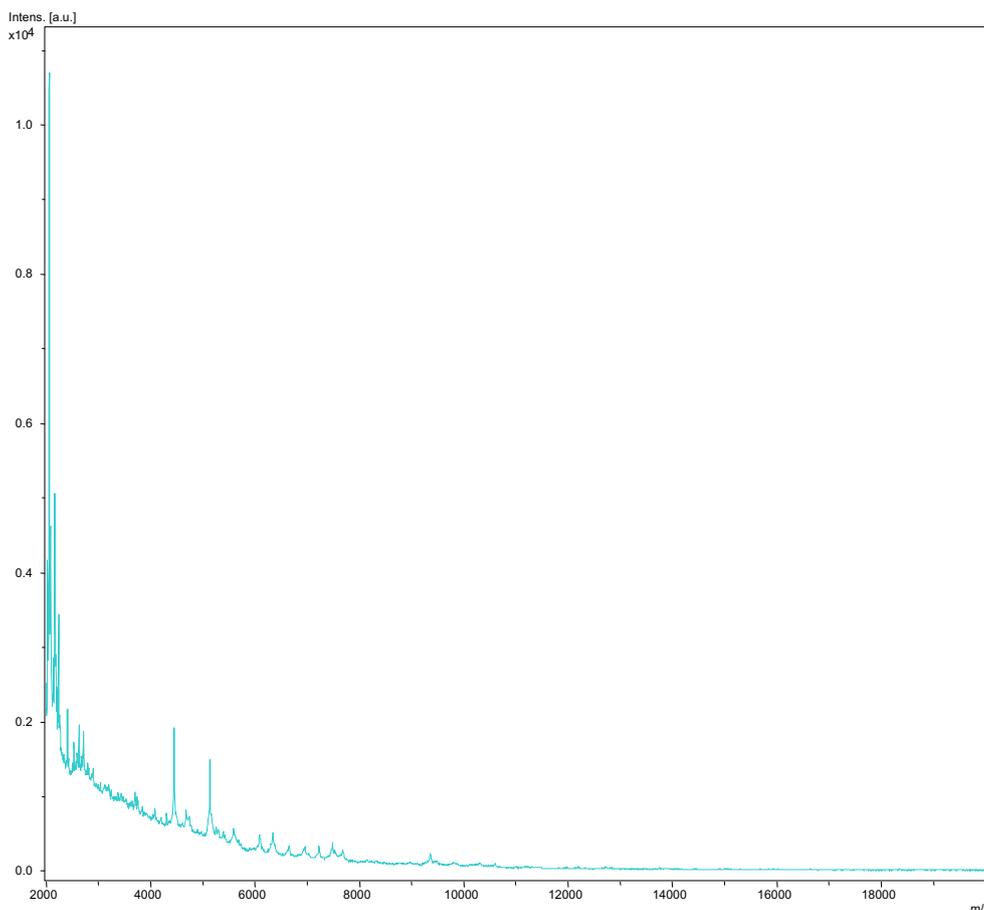


FIG. 1. MALDI-TOF MS-generated mass spectrum of *Libanicoccus massiliensis* gen. nov., sp. nov., strain Marseille-P3237^T.

performed with an optimal size of 1261 bp on the Covaris device S2 in T6 tubes (Covaris, Woburn, MA, USA). Library profile visualization was performed on a High Sensitivity Bio-analyzer LabChip (Agilent Technologies Inc) and the final concentration library was detected as 0.7626 nmol/L. Libraries were normalized to 2 nM, followed by a denaturation step and dilution to reach a 15 pM concentration. An automated cluster generation and sequencing run were performed in a single 39-h run in a 2 × 251-bp. Total information of 11.1 Gb was obtained from a 1332 K/mm² cluster density with a cluster passing quality control filters of 87.9% (21 937 000 passing filter paired reads). Within this run, the index representation for strain Marseille-P3237 was determined to be 3.05%. The 668 978 paired-reads were trimmed and then assembled.

The genome of strain Marseille-P3237 was assembled, annotated and compared with other closely related species as previously described [5]. The genomic comparison included *Olsenella profusa* (GenBank accession number NZ_AWEZ00000000.1), *Olsenella uli* (NZ_JQCO00000000.1), *Slackia exigua* (ACUX00000000), *Atopobium vaginae* (NZ_ACGK00000000.2),

Atopobium parvulum (NC_013203.1), *Atopobium rimaie* (NZ_ACFE00000000.1), *Atopobium minutum* (NZ_AGXC-00000000.1), *Collinsella tanakaei* (NZ_ADLS00000000.1) and *Collinsella intestinalis* (NZ_ABXH00000000.2).

Results and discussion

Strain identification and phylogenetic analysis

The identification of strain Marseille-P3237 using MALDI-TOF MS failed. The generated reference mass spectrum (Fig. 1) was added to the URMS database (<http://www.mediterranee-infection.com/article.php?larub=280&titre=urms-database>).

Strain Marseille-P3237 exhibited a 93.05% sequence identity with *O. uli* (GenBank accession number AF292373), the phylogenetically closest species with a validly published name (Fig. 2). This 16S rRNA gene sequence divergence being greater than 5%, we investigated whether strain Marseille-P3237 could be the representative strain of a new species within a new genus (Table 1) [13]. The 16S rRNA gene sequence from strain

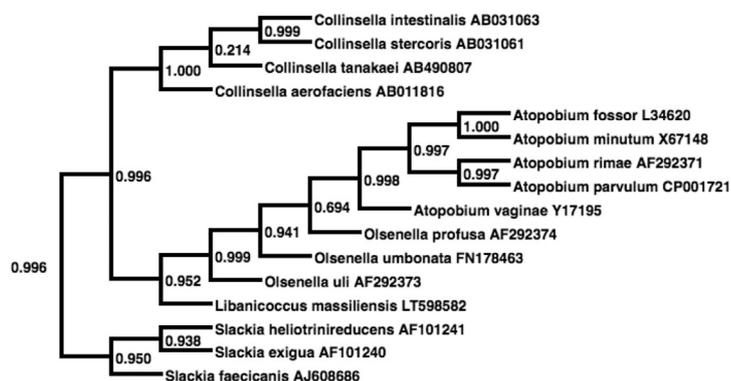


FIG. 2. Phylogenetic position of *Libanicoccus massiliensis* strain Marseille-P3237 relative to other closely related species. Phylogenetic relationships were inferred using the maximum-likelihood method. Numbers at the nodes are bootstrap values obtained from 1000 replicates with Shimodaira–Hasegawa test. The scale bar represents a 1% nucleotide sequence divergence.

TABLE 1. General features of *Libanicoccus massiliensis* gen. nov., sp. nov. strain Marseille-P3237^T

Properties	Term
Current classification	Domain: <i>Bacteria</i> Phylum: <i>Actinobacteria</i> Class: <i>Coriobacteria</i> Order: <i>Coriobacteriales</i> Family: <i>Coriobacteriaceae</i> Genus: <i>Libanicoccus</i> Species: <i>Libanicoccus massiliensis</i> Type Strain: Marseille-P3237 ^T
Gram stain	Negative
Cell shape	Coccus
Motility	Non-motile
Sporulation	Negative
Growth temperature range	30°C–42°C
Optimal growth temperature	37°C

Marseille-P3237 was submitted to EMBL-EBI under accession number LT598582. The MALDI-TOF MS spectrum of strain Marseille-P3237 was compared with those of other members of the *Coriobacteriaceae* family (Fig. 3).

Phenotypic and biochemical characterization

Strain Marseille-P3237 grew at an optimal temperature of 37°C under anaerobic conditions (Fig. 4). No growth was observed in aerobic or microaerophilic atmospheres. It also tolerated a pH range between 6 and 8.5 but could not sustain NaCl concentrations >5 g/L. In optimal culture conditions, bacterial colonies were dark white and rough, with a diameter of 0.8–1.2 mm. Cells of strain Marseille-P3237 were not motile, unable to sporulate, Gram-negative cocci. They were also catalase- and oxidase-negative. Using electron microscopy, bacterial cells had a mean diameter of 1.06 µm (Fig. 5).

Using an API 20A strip, strain Marseille-P3237 could hydrolyse esculin but could neither produce indole nor hydrolyse gelatine. In addition, it was urease negative and could not acidify D-glucose, D-saccharose, D-lactose, D-melezitose,

D-xylose, D-mannose, D-trehalose, L-rhamnose, L-arabinose, D-raffinose, D-mannitol, D-cellobiose, glycerol, salicin, D-maltose and D-sorbitol.

Using an API ZYM strip, strain Marseille-P3237 exhibited α-fucosidase, alkaline phosphatase, acid phosphatase, esterase lipase (C8), Valine arylamidase, Leucine arylamidase and naphthol-AS-BI phosphohydrolase activities but was negative for α-galactosidase, β-galactosidase, α-chymotrypsin, β-glucuronidase, α-glucosidase, β-glucosidase, esterase (C4), N-acetyl-β-glucosaminidase, lipase (C14), Cystine arylamidase, trypsin and α-mannosidase activities.

Using an API 50CH strip, the following sugars were fermented: D-ribose, L-arabinose, D-galactose, D-xylose, glycerol, D-fructose, D-glucose, D-mannose, D-sorbitol, methyl-α-D-mannopyranoside, N-acetylglucosamine, amygdaline, D-mannitol, salicin, arbutine, D-cellobiose, esculin, D-lactose, D-saccharose, D-melibiose, D-maltose, D-melezitose, D-trehalose, starch, xylitol, D-tagatose, D-raffinose, potassium gluconate and gentobiose. In contrast, strain Marseille-P3237 exhibited negative reactions for inositol, erythritol, D-lyxose, L-fucose, L-sorbose, D-arabitol, L-arabitol, L-rhamnose, inulin, methyl-α-D-glucosamin, potassium 5-ketogluconate, D-turanose, D-adonitol, glycogen, methyl-β-D-xylopyranoside, L-xylose, D-fucose, D-ulcitol and D-arabinose. By comparison with members of the *Olsenella* genus, strain Marseille-P3237 differed in acid production from D-mannose and D-glucose (Table 2).

The major fatty acid found for this strain was 9-octadecenoic acid (18:1n9, 38%). The other most abundant fatty acids were the saturated structures hexadecanoic acid (16:0, 28 %) and octadecanoic acid (18:0, 11 %) (Table 3).

Strain Marseille-P3237 MICs (mg/L) of 0.38, 0.75, 1, 1, 0.047, >256, 0.016, 1.5, 0.19, 4, 0.5, 32, >256, 0.125 and for benzylpenicillin, amoxicillin, cefotaxime, ceftriaxone, imipenem, amikacin, erythromycin, daptomycin, rifampicin, minocycline,

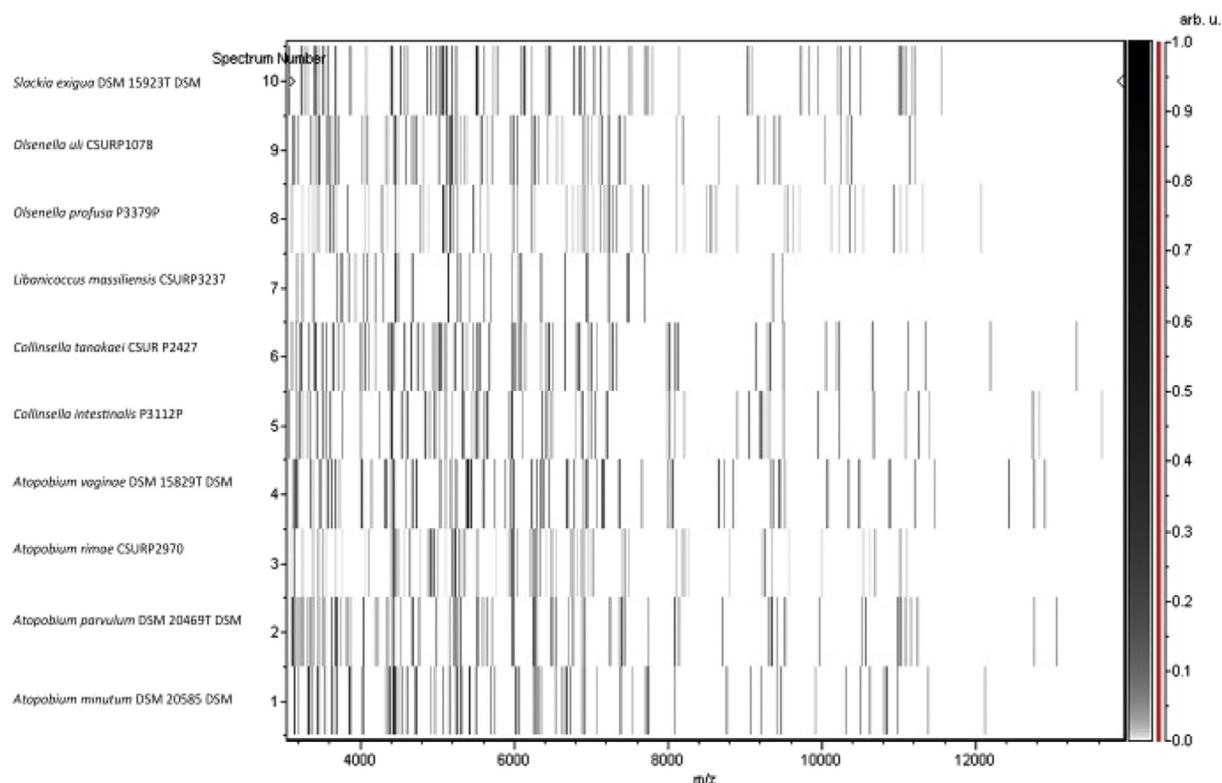


FIG. 3. Gel view comparing *Libanicoccus massiliensis* gen. nov., sp. nov. strain Marseille-P3237^T with other closely related species. The gel view displays the raw spectra of loaded spectrum files arranged in a pseudo-gel like look. M/z values are shown on the x axis and the left y-axis represents the running spectrum number originating from subsequent spectra loading. The peak intensity is expressed by a Grey scale scheme code. The right y-axis indicates the relation between the colour of a peak and its intensity, in arbitrary units.

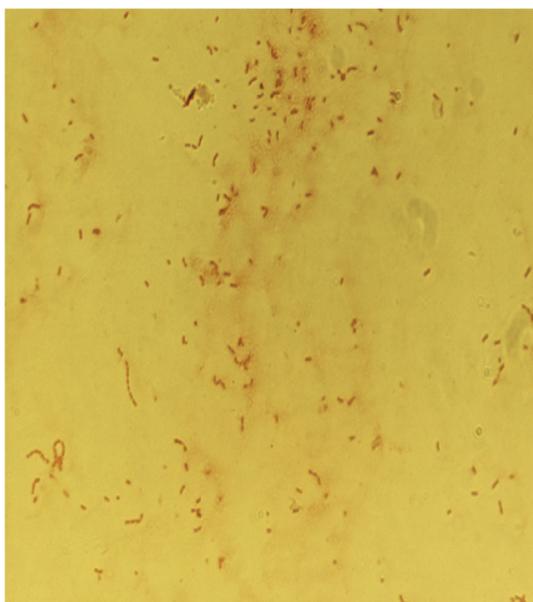


FIG. 4. Gram staining of *Libanicoccus massiliensis* gen. nov., sp. nov. strain Marseille-P3237^T.

teicoplanin, vancomycin, colistin and metronidazole, respectively.

Genome properties

The genome from strain Marseille-P3237 is 2 009 306-bp long with a 65.46 mol% G+C content (Table 4). It is composed of one scaffold (two contigs). Of the 1805 predicted genes, 1747 are protein-coding genes and 58 are RNAs (two complete rRNA operons and an additional 5S rRNA and 51 tRNA genes). A total of 1375 genes (78.71%) are assigned a putative function (by BLAST against COGs or nr). A total of 108 genes are identified as ORFans (6.18%). The remaining 216 genes are annotated as hypothetical proteins (12.36%, Table 4). A graphical representation of the genome is depicted in Fig. 6. The distribution of genes into COG functional categories is presented in Table 5.

Genomic comparison

The compared distribution of functional classes of predicted genes from strain Marseille-P3237 and closely related species, according to the COGs database, is represented in Fig. 7. The

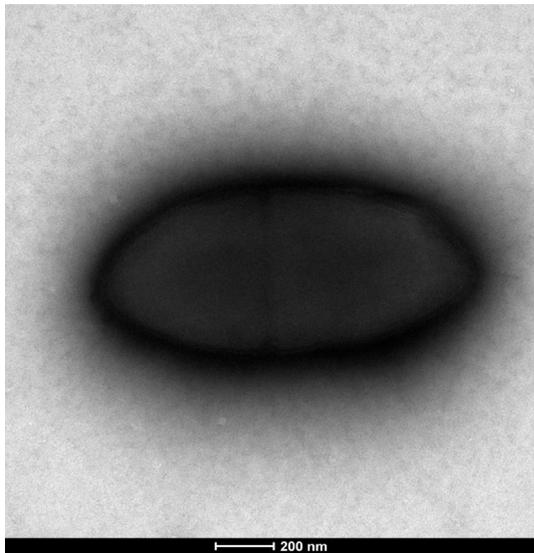


FIG. 5. Transmission electron microscopy of *Libanicoccus massiliensis* gen. nov., sp. nov. strain Marseille-P3237^T.

TABLE 2. Differential characteristics of *Libanicoccus massiliensis* gen. nov., sp. nov. strain Marseille-P3237^T (present study), *Olsenella scatoligenes* strain SK9K4^T [14], *Olsenella uli* strain VPI D76D-27C^T (15) and *Olsenella profusa* strain D315A-29^T [15]

Properties	<i>L. massiliensis</i>	<i>O. scatoligenes</i>	<i>O. uli</i>	<i>O. profusa</i>
Cell length (µm)	1.6	1–2	NA	0.8–2
Oxygen requirement	Strictly anaerobic	Strictly anaerobic	Strictly anaerobic	Strictly anaerobic
Gram stain	Negative	Positive	Positive	Positive
Salt requirement	—	NA	+	+
Motility	—	—	—	—
Endospore formation	—	—	NA	—
Indole production	—	—	—	—
Production of:				
Alkaline phosphatase	+	+	NA	NA
Catalase	—	—	—	—
Oxidase	—	NA	NA	NA
Urease	—	—	NA	NA
β-galactosidase	—	+	NA	NA
N-acetyl-glucosamine	+	NA	NA	NA
Acid production from:				
L-arabinose	—	—	—	+
D-mannose	—	+	+	+
D-mannitol	—	+	—	+
D-glucose	—	+	+	+
D-maltose	—	+	—	+
D-lactose	—	+	—	+
G+C content (%)	65.46%	62.1	64	64
Habitat	Human gut	Pig gut	Human gingival crevices	Human subgingival plaque

NA, data not available.

genome from strain Marseille-P3237 was smaller than those of *O. profusa*, *O. uli*, *S. exigua* and *C. tanakaei* (2009, 2725, 2057, 2096 and 2492 Mb, respectively), but larger than those of *A. vaginae*, *A. parvulum*, *A. rimae*, *A. minutum* and *C. intestinalis* (1419, 1544, 1626, 1707 and 1809 Mb, respectively). The G+C content of strain Marseille-P3237 was larger than those of

TABLE 3. Cellular fatty acid composition of *Libanicoccus massiliensis* gen. nov., sp. nov. strain Marseille-P3237^T

Fatty acids	Name	Mean relative % ^a
18:1n9	9-Octadecenoic acid	37.8 ± 1.8
16:0	Hexadecanoic acid	28.4 ± 1.4
18:0	Octadecanoic acid	10.6 ± 0.5
14:0	Tetradecanoic acid	10.4 ± 0.5
18:1n5	13-Octadecenoic acid	7.2 ± 1.0
10:0	Decanoic acid	2.4 ± 0.4
18:2n6	9,12-Octadecadienoic acid	1.8 ± 0.7
15:0	Pentadecanoic acid	TR
12:0	Dodecanoic acid	TR
15:0 anteiso	12-methyl-tetradecanoic acid	TR

^aMean peak area percentage; TR = trace amounts <1%.

TABLE 4. Nucleotide content of strain Marseille-P3237^T and gene count levels of the genome

	Number	%
Size (bp)	2 009 306	100
Number of G+C nucleotides	1 313 861	65.46
Total number of genes	1805	100
Number of protein-coding genes	1747	96.79
Total number of RNA genes	58	3.21
Number of tRNA genes	51	2.82
Number of rRNA (5S, 16S, 23S) genes	7	0.39
Coding sequence size	1 782 220	88.7
Protein-coding gene sequence size	1 768 986	88.04
tRNA gene sequence size	3993	0.2
rRNA gene (5S, 16S, 23S) sequence size	9241	0.46
Number of proteins associated to COGs	1223	70.00
Number of proteins classified as orfans	108	6.18
Number of proteins with signal peptides	152	8.70
Number of genes associated with antibiotic resistance	1	0.06
Number of genes associated with PKS or NRPS	1	0.06
Number of genes associated with virulence	339	19.40

O. profusa, *O. uli*, *A. vaginae*, *A. parvulum*, *A. rimae*, *A. minutum*, *C. intestinalis*, *C. tanakaei* and *S. exigua* (65.46, 64.17, 64.69, 42.66, 45.69, 49.26, 48.94, 62.47, 60.24 and 62.15 mol% respectively). The gene content of strain Marseille-P3237 is smaller than those of *O. profusa*, *C. intestinalis*, *O. uli*, *C. tanakaei* and *S. exigua* (1747, 2650, 1784, 1770, 2212 and 2029, respectively), but larger than those of *A. vaginae*, *A. parvulum*, *A. rimae* and *A. minutum* (1179, 1353, 1548 and 1539, respectively). Moreover, strain Marseille-P3237 exhibited the highest Average Genomic Identity of Orthologous gene Sequences values (Table 6) with *O. profusa* and *O. uli* (61.51 and 62.78%, respectively) and dDDH values (Table 7) with these species (20.2% (18–22.6) and 19.9% (17.7–22.3), respectively) that were lower than that obtained within the *Olsenella* genus (22.4%; 20.1–24.8).

Conclusion

On the basis of phenotypic, biochemical, genomic and phylogenetic results, we formally propose the creation of the new genus *Libanicoccus* gen. nov., with *Libanicoccus massiliensis*

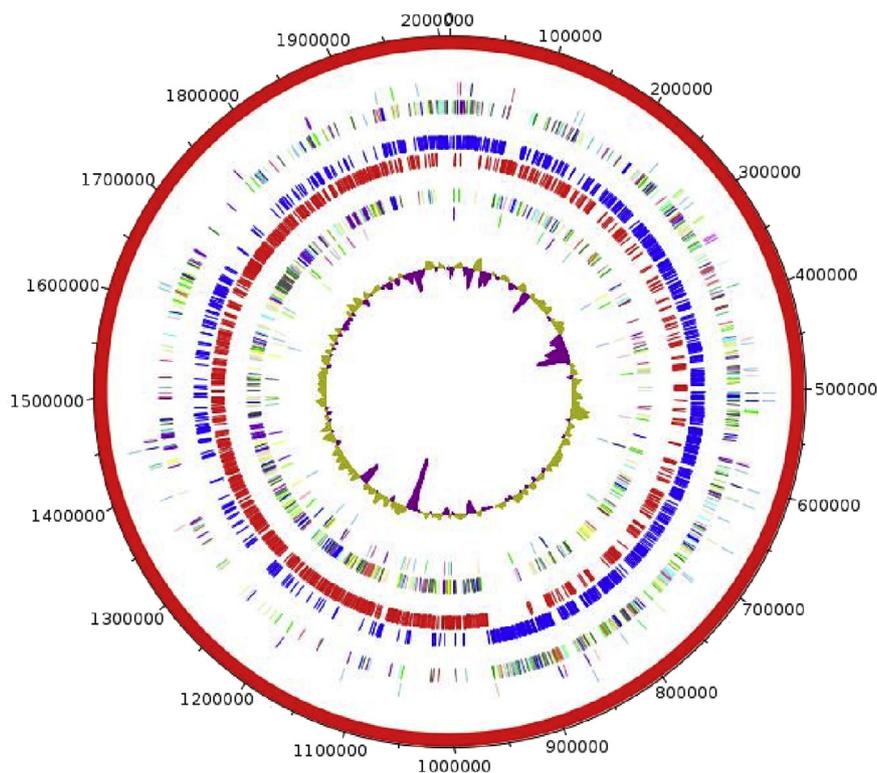


FIG. 6. Graphical circular map of the genome of *Libanicoccus massiliensis* strain Marseille-P3237^T. From outside in: contigs (red/grey), COG categories of genes on the forward strand (three circles), genes on the forward strand (blue circle), genes on the reverse strand (red circle), COG categories on the reverse strand (three circles), G+C content.

TABLE 5. Numbers of genes associated with the 25 general COG functional categories

Code	Value	% of total ^a	Description
[J]	160	9.16	Translation
[A]	0	0	RNA processing and modification
[K]	85	4.86	Transcription
[L]	71	4.06	Replication, recombination and repair
[B]	0	0	Chromatin structure and dynamics
[D]	20	1.14	Cell cycle control, mitosis and meiosis
[Y]	0	0	Nuclear structure
[V]	37	2.12	Defence mechanisms
[T]	51	2.92	Signal transduction mechanisms
[M]	65	3.72	Cell wall/membrane biogenesis
[N]	7	0.40	Cell motility
[Z]	0	0	Cytoskeleton
[W]	4	0.23	Extracellular structures
[U]	18	1.03	Intracellular trafficking and secretion
[O]	43	2.46	Post-translational modification, protein turnover, chaperones
[X]	19	1.09	Mobilome: prophages, transposons
[C]	69	3.95	Energy production and conversion
[G]	142	8.13	Carbohydrate transport and metabolism
[E]	159	9.10	Amino acid transport and metabolism
[F]	56	3.20	Nucleotide transport and metabolism
[H]	63	3.61	Coenzyme transport and metabolism
[I]	48	2.75	Lipid transport and metabolism
[P]	64	3.66	Inorganic ion transport and metabolism
[Q]	26	1.49	Secondary metabolites biosynthesis, transport and catabolism
[R]	119	6.81	General function prediction only
[S]	48	2.75	Function unknown
-	524	29.99	Not in COGs

^aThe total is based on the total number of protein-coding genes in the annotated genome.

sp. nov. being the type species. Strain Marseille-P3237^T, isolated from the gut microbiota of a healthy 35-year-old Congolese pigmy female, is the type strain of *L. massiliensis* gen. nov., sp. nov.

Description of *Libanicoccus* gen. nov.

Libanicoccus gen. nov. (li. ba.ni.coc'cus N.L. masc. n, *Libanicoccus*, composed of *Liban*, the country of origin of the microbiologist who first cultivated strain Marseille-P3237^T, and *coccus* for the shape of bacterial cells).

Gram-negative cocci. Strictly anaerobic. Mesophilic. Not motile. Unable to sporulate. Absent catalase, oxidase and indole productions. Hydrolyses esculin. Positive for α-fucosidase, alkaline phosphatase, acid phosphatase, esterase lipase (C8), valine arylamidase, leucine arylamidase and naphthol-AS-BI phosphohydrolase activities. Ferments D-ribose, L-arabinose, D-galactose, D-xylose, glycerol, D-fructose, D-glucose, D-mannose, D-sorbitol, methyl-αD-mannopyranoside, N-acetylglucosamine, amygdaline, D-mannitol, salicin, arbutine, D-cellobiose, esculin, D-lactose, D-saccharose, D-melibiose, D-maltose, D-melezitose, D-trehalose, starch, xylitol, D-tagatose, D-raffinose, potassium gluconate and gentobiose. Habitat: human digestive tract. Type species: *Libanicoccus massiliensis*. Type strain *Libanicoccus massiliensis* strain Marseille-P3237^T (= CSUR P3237 = CCUG 71182).

Description of *Libanicoccus massiliensis* sp. nov.

Libanicoccus massiliensis, sp. nov. (ma.ssi.li.en'sis. L. masc. adj. *massiliensis* pertaining to Massilia, the Roman name of Marseille, where strain Marseille-P3237^T was first isolated).

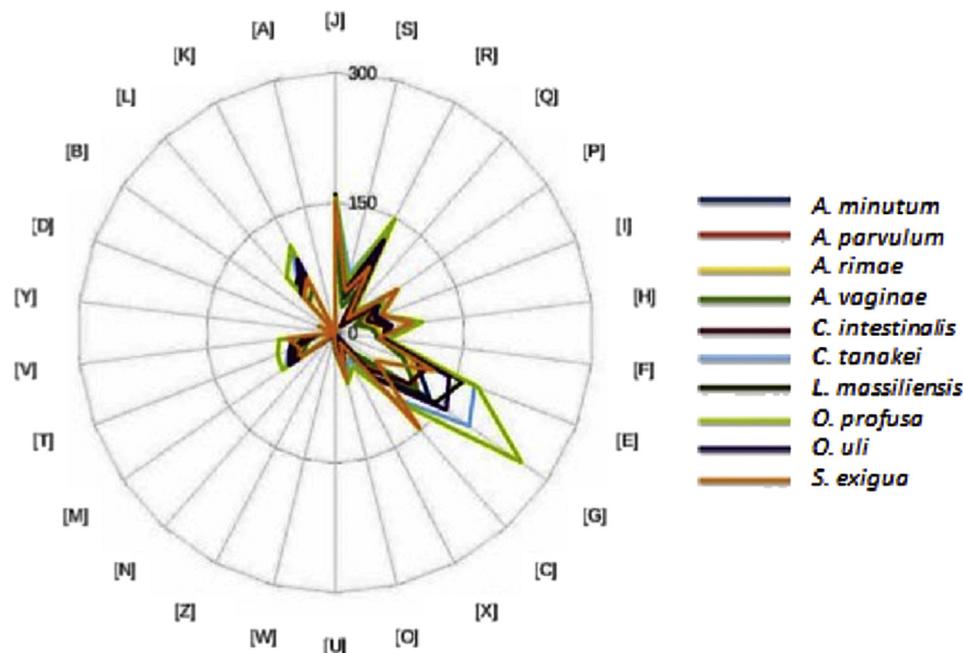


FIG. 7. Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins.

TABLE 6. Numbers of orthologous proteins between strain Marseille-P3237^T and other closely related species (upper right), numbers of proteins per genome (bold numbers), and Average Genomic Identity of Orthologous gene Sequences values (%), lower left)

	AV	OP	AR	SE	OU	LM	AM	CI	CT	AP
AV	1179	676	645	482	695	629	680	601	613	658
OP	51.87	2650	808	624	920	865	799	759	875	818
AR	54.15	59.38	1548	560	818	734	743	669	714	904
SE	49.53	60.25	57.01	2029	624	591	544	577	625	560
OU	53.06	62.94	56.53	60.81	1770	814	820	747	826	809
LM	58.27	61.51	53.92	56.9	62.78	1747	738	796	870	751
AM	57.22	55.35	57.54	54.14	58.02	56.91	1539	704	768	767
CI	51.9	61.42	56.84	60.42	62.31	60.82	55.67	1784	928	691
CT	53.82	59.71	56.27	58.88	59.6	60.92	55.29	63.12	2212	738
AP	56.19	57.91	63.66	54.48	54.38	54.37	57.78	54.92	55.34	1353

Abbreviations: AV, *Atopobium vaginae* strain DSM 15829; OP, *Olsenella profusa* strain DSM 13989; AR, *Atopobium rimae* strain ATCC 49626; SE, *Slackia exigua* strain ATCC 700122; OU, *Olsenella uli* strain DSM 7084; LM, *Libanicoccus massiliensis* strain Marseille-P3237^T; AM, *Atopobium minutum* strain NCFB 2751; CI, *Collinsella intestinalis* strain DSM 13280; CT, *Collinsella tanakai* strain YIT 12063; AP, *Atopobium parvulum* strain DSM 20469.

TABLE 7. Digital DNA–DNA hybridization values (%) obtained by pairwise genomic comparison of strain Marseille-P3237^T with other closely related species using the GGDC formula 2 software. The inherent uncertainty in approximating dDDH values from intergenomic distances established on models derived from empirical test data sets are represented in confidence intervals.

	LM	AV	OP	AR	SE	OU	AM	CI	CT	AP
LM	100	17.2 [15.1–19.5]	20.2 [18–22.6]	20.3 [18.1–22.7]	19.8 [17.6–22.2]	19.9 [17.7–22.3]	23.4 [21.1–25.8]	21.4 [19.2–23.9]	21.2 [19–23.7]	21.9 [19.6–24.3]
AV		100	19 [16.8–21.4]	23.3 [21.1–25.8]	24.5 [22.1–26.9]	20.3 [18.1–22.7]	20.7 [18.5–23.1]	24.5 [22.2–27]	26.9 [24.5–29.4]	20.3 [18.1–22.8]
OP			100	21.6 [19.3–24]	20.4 [18.2–22.8]	22.4 [20.1–24.8]	20.7 [18.5–23.2]	19.5 [17.3–21.9]	20 [17.8–22.5]	24 [21.7–26.5]
AR				100	21.8 [19.5–24.2]	25.2 [22.9–27.7]	25.5 [23.2–28]	24.7 [22.4–27.2]	22 [19.7–24.4]	23.9 [21.6–26.4]
SE					100	32.6 [30.2–35.1]	20.6 [18.3–23]	18.9 [16.7–21.2]	20.5 [18.3–22.9]	22.1 [19.8–24.5]
OU						100	21.4 [19.1–23.8]	20.4 [18.2–22.8]	19.5 [17.3–21.9]	25.3 [23–27.8]
AM							100	22.5 [20.2–25]	22.2 [20–24.7]	20.2 [18–22.6]
CI								100	24.7 [22.3–27.1]	22 [19.7–24.4]
CT									100	20.8 [18.6–23.3]
AP										100

Abbreviations: AV, *Atopobium vaginae* strain DSM 15829; OP, *Olsenella profusa* strain DSM 13989; AR, *Atopobium rimae* strain ATCC 49626; SE, *Slackia exigua* strain ATCC 700122; OU, *Olsenella uli* strain DSM 7084; LM, *Libanicoccus massiliensis* strain Marseille-P3237^T; AM, *Atopobium minutum* strain NCFB 2751; CI, *Collinsella intestinalis* strain DSM 13280; CT, *Collinsella tanakai* strain YIT 12063; AP, *Atopobium parvulum* strain DSM 20469.

Colonies are dark white and rough with a diameter of 0.8–1.2 mm on blood-enriched Columbia agar. Strictly anaerobic. Mesophilic. Can grow at a pH range of 6.0 to 8.5 but cannot grow at NaCl concentrations >5 g/L. Cells are Gram-negative cocci with a mean diameter of 1.06 µm. Not motile and non-sporulating. Catalase, oxidase and indole negative.

Using an API 20A strip, strain Marseille-P3237 could hydrolyse esculin but not gelatin. In addition, it was urease negative and could not acidify D-glucose, D-saccharose, D-lactose, D-melezitose, D-xylose, D-mannose, D-trehalose, L-rhamnose, L-arabinose, D-raffinose, D-mannitol, D-cellobiose, glycerol, salicin, D-maltose and D-sorbitol. Using an API ZYM strip, strain Marseille-P3237 exhibited α-fucosidase, alkaline phosphatase, acid phosphatase, esterase lipase (C8), valine arylamidase, leucine arylamidase and naphthol-AS-BI phosphohydrolase activities but was negative for α-galactosidase, β-galactosidase, α-chymotrypsin, β-glucuronidase, α-glucosidase, β-glucosidase, esterase (C4), N-acetyl-β-glucosaminidase, lipase (C14), cystine arylamidase, trypsin, and α-mannosidase activities. Using an API 50CH strip, D-ribose, L-arabinose, D-galactose, D-xylose, glycerol, D-fructose, D-glucose, D-mannose, D-sorbitol, methyl-α-D-mannopyranoside, N-acetylglucosamine, amygdaline, D-mannitol, salicin, arbutine, D-cellobiose, esculin, D-lactose, D-saccharose, D-melibiose, D-maltose, D-melezitose, D-trehalose, starch, xylitol, D-tagatose, D-raffinose, potassium gluconate and gentiobiose were fermented. Inositol, erythritol, D-lyxose, L-fucose, L-sorbose, D-arabitol, L-arabitol, L-rhamnose, inulin, methyl-α-D-glucosamin, potassium 5-ketogluconate, D-turanose, D-adonitol, glycogen, methyl-β-D-xylopyranoside, L-xylose, D-fucose, D-ulcitol and D-arabinose were not fermented.

The major fatty acid is 9-octadecenoic acid (18:1n9, 38 %), followed by hexadecanoic acid (16:0, 28 %) and octadecanoic acid (18:0, 11 %).

The 16S rRNA gene and genome sequences are deposited in the EBI/EMBL database under accession numbers LT598582 and LT671675, respectively. The G+C content of the genome is 65.46%. Habitat: human digestive tract. Type strain *Libanicoccus massiliensis* strain Marseille-P3237^T (= CSUR P3237 = CCUG 71182).

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Conflict of interest

The authors declare no conflict of interest.

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