

Description of *Gabonibacter massiliensis* gen. nov., sp. nov., a New Member of the Family *Porphyromonadaceae* Isolated from the Human Gut Microbiota

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Abstract The identification of human-associated bacteria is very important to control infectious diseases. In recent years, we diversified culture conditions in a strategy named culturomics, and isolated more than 100 new bacterial species and/or genera. Using this strategy, strain GM7, a strictly anaerobic gram-negative bacterium was recently isolated from a stool specimen of a healthy Gabonese patient. It is a motile coccobacillus without catalase and oxidase activities. The genome of *Gabonibacter massiliensis* is 3,397,022 bp long with 2880 ORFs and a G+C content of 42.09 %. Of the predicted genes, 2,819 are protein-coding genes, and 61 are RNAs. Strain GM7 differs from the closest genera within the family *Porphyromonadaceae* both genotypically and in shape and motility. Thus, we propose that strain GM7^T (=CSUR P2336 = DSM 101039) is the type strain of the new genus

Gabonibacter gen. nov. and the new species *G. massiliensis* gen. nov., sp. nov.

Keywords *Gabonibacter massiliensis* · Taxonogenomics · Culturomics · Gabon · Gut microbiota

Abbreviations

CSUR	Collection de Souches de l'Unité des Rickettsies
DSM	Deutsche Sammlung von Mikroorganismen
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
URMITE	Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes
FAME	Fatty acid methyl ester
GC/MS	gas chromatography/mass spectrometry
ORF	Open reading Frame
BMI	Body mass index

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Introduction

The human gut microbiota is abundant and diversified, and has a great influence on human health. Therefore, characterizing its various members is of great interest for understanding its role in health and diseases. It was previously demonstrated that patients infected with *Plasmodium falciparum* may present an increased risk of bacteremia caused by gut bacteria [35]. In Africa, studies of febrile patients have demonstrated that the proportion of cases without identified etiology remains elevated [2]. This may be due to emerging or as-yet unknown pathogens, or to the lack of adequate diagnostic tools as recently reported

in Gabon [23]. In this country, the prevalence of *Rickettsia felis* infections was demonstrated to be elevated in febrile patients [23], although many of them had been treated empirically for malaria [2]. As a consequence, it is important to search new bacteria using efficient tools such as culturomics [13].

The concept of culturomics recently developed in our laboratory is based on the use of diversified culture conditions (medium, atmosphere, and temperature). This strategy enabled us to isolate an important number of previously undetected or unknown bacteria in clinical samples [13]. Initially, culturomics was developed using more than 200 combinations of culture conditions [13]. Recently, our team demonstrated that the number of optimal culture conditions could be reduced to 18 [17]. Coupled to taxonogenomics, a new combination of genomic and phenotypic analyses for the taxonomic description of new bacteria [27], we proposed the creation of more than 50 new species and genera to date [7, 14–21, 24–26, 28]. Here, we report the biochemical features, complete genomic sequencing, and annotation of *Gabonibacter massiliensis* gen. nov., sp. nov., strain GM7^T (= CSUR P2336 = DSM 101039), a new bacterium isolated from the gut microbiota of a healthy Gabonese male.

Materials and Methods

Ethics and Sample Collection

A culturomics study of the human gut microflora aiming at exploring its composition was conducted in Gabon. The new species described here was isolated from a stool specimen of a healthy 16 year-old male (BMI = 19.03) from Lebamba, a city located in the Ngounié province of south Gabon. Written informed consent was obtained from the patient. This study was validated by the ethics committees of the institute fédératif de recherche 48 (Marseille, France) and the National Ethics Committee of Gabon under registration numbers 09–022 and 0023/2013/SG/CNE, respectively. The stool sample was collected in January 2015 and stored at –80 °C until being shipped to the URMITE laboratory in dry ice.

Strain Isolation and MALDI-TOF MS Identification

Strain isolation consisted of four main steps: (a) dilution of the stool sample in phosphate-buffered saline (PBS) (Life technologies, Paisley, UK); (b) preincubation of diluted stool in an anaerobic blood culture bottle (Becton–Dickinson, Pont de Claix, France); (c) subculture of the

inoculum on 5 % sheep blood-enriched Columbia agar (bioMérieux, Marcy l’Etoile, France) under anaerobic conditions at 37 °C [17]; and (d) identification of all different bacterial colonies using MALDI-TOF MS as previously reported [24–26, 33]. Reference spectra are available in our online database (<http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>). Protein profiles of the new bacterium were compared to those of the closest bacteria (Fig. 1). The gel view displays the raw spectra of loaded spectrum files arranged in a pseudo-gel-like look. The x-axis records the m/z value. The left y-axis displays the running spectrum number originating from subsequent spectra loading. The peak intensity is expressed by a Grayscale scheme code. The color bar and the right y-axis indicate the relation between the color a peak is displayed with and the peak intensity in arbitrary units. Displayed species are indicated on the left.

16S rRNA Sequencing and Phylogeny

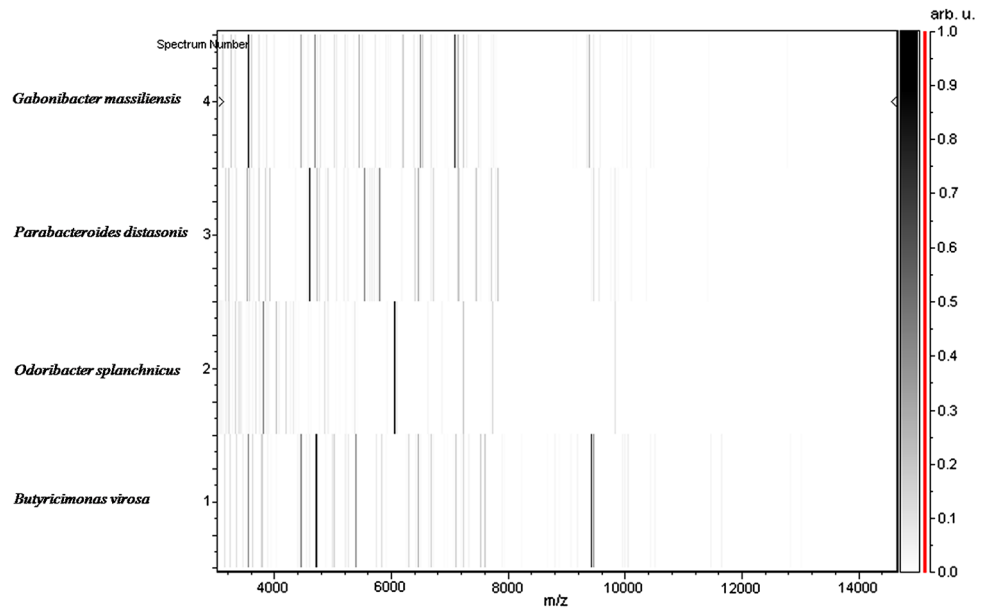
DNA from strain GM7 was extracted using the BioRobot EZ1 Advanced XL (Qiagen, Courtaboeuf, France). The 16S rRNA was then amplified using three sets of primers (fD1/800R, 536F/1050R, and 800F/rP2) [24–26]. Briefly, each PCR reaction was performed using the 2720 thermal cycler (Applied Biosystems, Bedford, MA, USA). Sequencing was performed using an ABI Prism 3130xl Genetic Analyzer sequencer (Applied Biosystems) as previously described [22]. The obtained 16S rRNA sequence was compared to those in GenBank using BLASTn (<http://blast.ncbi.nlm.nih.gov.gate1.inist.fr/Blast.cgi>) to determine the percentage of sequence similarity with the closest bacteria.

To construct a phylogenetic tree, 16S rRNA sequences were aligned using the CLUSTALW software, inferences were obtained using the neighbor joining and maximum-likelihood methods, and the MEGA software with 1000 bootstrap repetitions [24–26].

Growth Conditions

To test the range of growth temperatures of strain GM7, the bacterium was cultivated on 5 % sheep blood-enriched Columbia agar (BioMérieux) in anaerobic atmosphere at 28, 37, 45, and 55 °C. The pH tolerance was also tested from 6.0 to 9.0. The atmosphere preference was evaluated using 5 % CO₂, GENbag anaer and GENbag microaer systems (bioMérieux) at 37 °C to assess the ability of strain GM7 to grow in aerobic, anaerobic, or microaerophilic conditions. Additionally, the salinity test was performed [24–26].

Fig. 1 Gel view of MALDI-TOF MS spectra comparing *Gabonibacter massiliensis* strain GM7^T to other closely related bacteria. The gel view displays the raw spectra of loaded spectrum files arranged in a pseudo-gel-like look



Biochemical Properties and Motility Assays

Biochemical assays were performed using the API ZYM, API 20A, and API 50CH strips (bioMérieux). The catalase (bioMérieux) and oxidase (Becton, Dickinson and Company, Le Pont de Claix, France) activities were also tested. Fatty acid composition of outer membrane was performed using fatty acid methyl ester (FAME) analysis by GC/MS. Approximately, 40 mg of bacterial biomass was harvested from three different culture plates. Cellular FAMEs were prepared as previously described [32]. GC/MS analyses were carried out on a Clarus 500 gas chromatograph equipped with a SQ8S MS detector (Perkin Elmer, Courtaboeuf, France). Two μL of FAME extracts were volatilized at 250 °C (split 20 mL/min) in a Focus liner with wool and separated on an Elite-5MS column (30 m, 0.25 mm i.d., 0.25 mm film thickness) using a linear temperature gradient (70–290 °C at 6 °C/min) enabling the detection of C4–C24 FAMEs. Helium flowing at 1.2 mL/min was used as carrier gas. MS inlet line was set at 250 °C and EI source at 200 °C. Full-scan monitoring was performed from 45 to 500 m/z. All data were collected and processed using Turbomass 6.1 (Perkin Elmer). FAMEs were identified by comparison with the FAME mass spectral database (Wiley, Chichester, UK) and the MS search using MS 2.0 operated with the standard reference database 1A (NIST, Gaithersburg, USA). A 37-component FAME mix (Supelco, Sigma-Aldrich, Saint-Quentin Fallavier, France) was used for retention time correlations with estimated non-polar retention indexes from the NIST database; FAME identifications were confirmed using this index.

Antibiotic Susceptibility

Antibiotics used to test the susceptibility of strain GM7 included amoxicillin, amoxicillin/clavulanic acid, imipenem, doxycycline, rifampicin, nitrofurantoin, vancomycin, clindamycin, gentamicin, erythromycin, metronidazole, trimethoprim/sulfamethoxazole, ciprofloxacin, amikacin, and tobramycin (i2a, Montpellier, France). Inhibition diameters were measured using the Scan1200[®] scanner (Interscience, Saint-Nom-La Bretèche, France) [24–26].

Microscopy

Individual cells of strain GM7 were visualized using a Tecnai G20 electron microscope (FEI company, Limeil-Brevannes, France) [24–26]. The Color Gram 2 Kit (BioMérieux) was used for the gram staining observed at a 1000 \times magnification using the DM1000 photonic microscope (Leica Microsystems) [24–26].

Genome Sequencing and Assembly

The genome sequencing of strain GM7 was performed using the MiSeq sequencer (Illumina, San Diego, CA, USA) with the Mate Pair strategy [24–26]. To be mixed with 11 other bacterial DNAs using the Nextera Mate Pair sample prep kit (Illumina), the genomic DNA of strain GM7 was barcoded. Overall, all genome sequencing and assembly steps were performed as previously reported [26]. The reads were trimmed and assembled using the CLC genomics Workbench v4.7.2 software (CLC bio, Aarhus, Denmark) [24–26].

Genome Annotation and Comparison

Genome annotation and comparison were performed using the Multi-Agent Software System DAGOBAB [5] that includes Figenix [6]. The genomic features of strain GM7 were compared to those of *Butyricimonas virosa* strain MT12^T [31], *Butyricimonas synergistica* strain MT01^T [31], *Parabacteroides goldsteinii* strain WAL 12034^T [29], *Parabacteroides distasonis* strain JCM5825^T [29], *Parabacteroides merdae* strain ATCC 43184^T [29], and *Parabacteroides johnsonii* strain M-165^T [30]. Two parameters, dDDH which was highly correlated with DDH [1, 18], and AGIOS [27] which was designed to be independent from DDH, were determined in order to evaluate the genomic similarity among studied strains.

Results

MALDI-TOF and Phylogenic analysis

MALDI-TOF MS failed to identify strain GM7 (score <1.7). Therefore, its spectrum was added to our database to improve its content (Fig. 1). The 16S rRNA sequencing showed that strain GM7 was 90 % similar to *Butyricimonas virosa*, the closest phylogenetic neighbor with standing in nomenclature (Fig. 2). This percentage of similarity is lower than the 16S rRNA gene sequence threshold recommended to delineate a genus [12]. Therefore, we propose that strain GM7 be a member of a new genus in the family *Porphyromonadaceae* for which we propose the name *Gabonibacter* gen. nov. In addition, this strain exhibited a degree of 16S rRNA similarity of 97.85 % to “*Sanguibacteroides justesenii*” strain OUH 308042 that has no standing in nomenclature but may

belong to the same genus [9, 29, 34] (Table 1). The 16S rRNA sequence of strain GM7 was deposited in GenBank under accession number LN881588.

Phenotypic and Biochemical Characterization

Strain GM7 is a gram-negative coccobacillus. Cells are motile. Their mean length and diameter are 1.2 μm (range 0.92–1.38 μm) and 0.48 μm (range 0.4–0.56 μm), respectively (Fig. 3). Catalase and oxidase activities were negative (Table 2). Colonies of strain GM7 grown on 5 % sheep blood-enriched agar are white with a diameter of 2 mm. Strain GM7 was able to grow at 28 °C but its optimal growth temperature is 37 °C. No growth was observed at 45 and 55 °C. Strain GM7 is strictly anaerobic and unable to grow under microaerophilic or aerobic conditions. Its salinity tolerance varies from 0 to 5 g/L and pH tolerance ranges from 6.0 to 8.5. Results of API 50CH, API ZYM, and API 20A strips are detailed in Table 2 and in the species description below.

Among tested antibiotics, cells were susceptible to imipenem, metronidazole, and nitrofurantoin but resistant to amoxicillin, amoxicillin/clavulanic acid, doxycycline, rifampicin, vancomycin, clindamycin, gentamicin, erythromycin, trimethoprim/sulfamethoxazole, ciprofloxacin, amikacin, and tobramycin.

The predominant cellular fatty acids of strain GM7 and the closest species are summarized in Table 3.

Genome Properties

The genome of strain GM7 is 3,397,022 bp long with a G+C content of 42.09 % (Table 4). It is composed of seven scaffolds and eight contigs (Fig. 4). On the 2880 predicted genes, 2819 were protein-coding genes, and 61

Fig. 2 Neighbor Joining-based phylogenetic tree highlighting the position of *Gabonibacter massiliensis* strain GM7^T compared to other members of the family *Porphyromonadaceae*. The respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Numbers at the nodes are bootstrap values (>70 %) obtained by repeating the analysis 1000 times. The scale bar represents a 2 % nucleotide sequence divergence

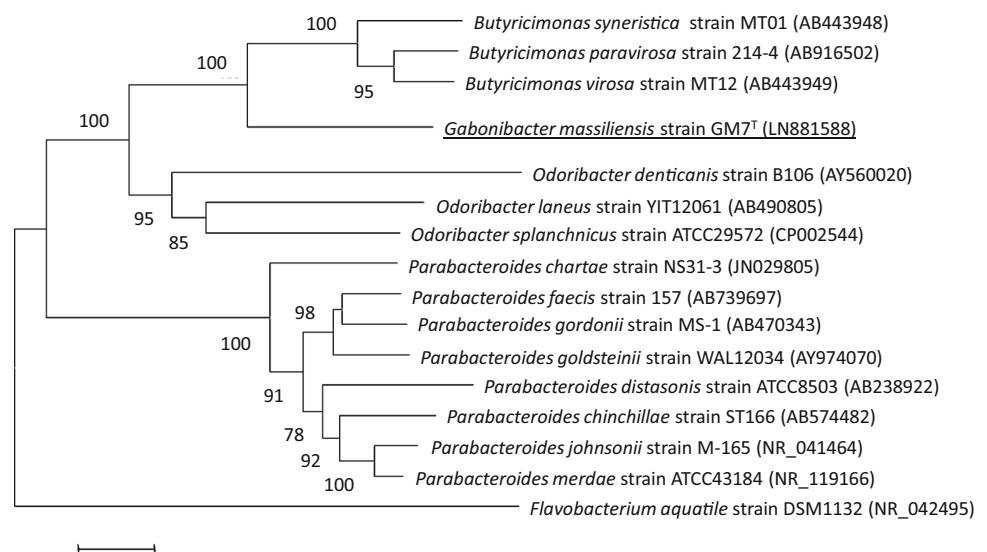


Table 1 Classification and general features of *Gabonibacter massiliensis* strain GM7^T

Property	Term	References
Current classification	Domain: <i>Bacteria</i>	[36]
	Phylum: <i>Bacteroidetes</i>	[3, 11]
	Class: <i>Bacteroidia</i>	[3, 8]
	Order: <i>Bacteroidales</i>	[3, 10]
	Family: <i>Porphyromonadaceae</i>	[9]
	Genus: <i>Gabonibacter</i>	IDA
	Species: <i>Gabonibacter massiliensis</i>	IDA
	Type strain: GM7	IDA
	Gram stain	Negative
Cell shape	Coccobacillus	IDA
Motility	Yes	IDA
Sporulation	Yes	IDA
Temperature range	Mesophilic	IDA
Optimum temperature	37 °C	IDA
Atmosphere	Anaerobic	IDA
Salinity	0–5 g.L ⁻¹	IDA
GPS and altitude data from Lebamba	2°12'36''S	IDA
	11°28'32''E	

Evidence codes *IDA* inferred from direct assay, *TAS* traceable author statement (i.e., a direct report exists in the literature)

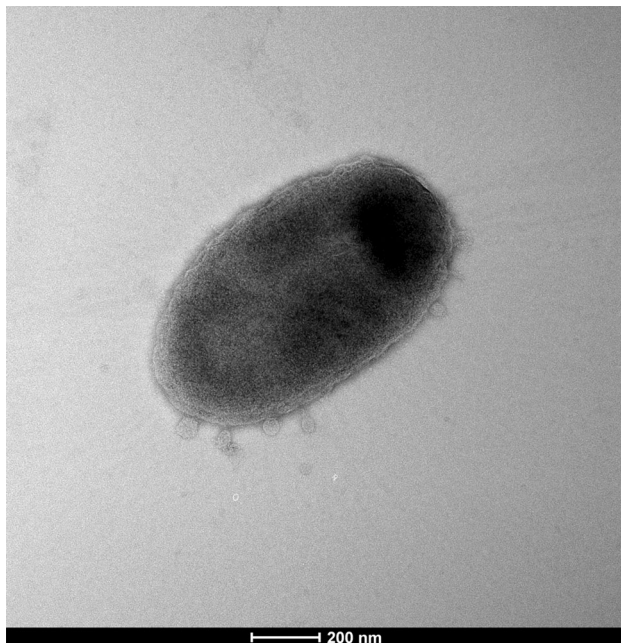


Fig. 3 Electron microscopy of *Gabonibacter massiliensis* strain GM7^T. Transmission electron microscopy of *Gabonibacter massiliensis* strain GM7^T, using a Morgagni 268D (Philips) at an operating voltage of 60 kV. The scale bar represents 200 nm

were RNAs (1 is 16S rRNA, 2 23S rRNA, 4 5S rRNA, 54 tRNAs). A total of 1858 genes (65.91 %) were assigned a putative function (by comparison to the COG or NR databases). Another 777 genes (27.56 %) were annotated

as hypothetical proteins and 124 genes were identified as ORFans (4.40 %). Table 3 details the properties and statistics of the genome. The distribution of genes into COG functional categories is summarized in Table 5. The genome from strain GM7 was deposited in Genbank under accession number FAVK00000000.

Genome Comparison

The genome size of strain GM7 (3.39 Mb) is smaller than those of all compared species (*B. virosa* [4.72 Mb], *B. synergistica* [4.77 Mb], *P. goldsteinii* [7.09], *P. distasonis* [4.81 Mb], *P. merdae* [4.43], and *P. johnsonii* [4.63]) (Table 5). The G+C content of strain GM7 (42.09 %) is lower than those of *B. virosa* (42.3 %), *B. synergistica* (44.8 %), *P. goldsteinii* (43.5 %), *P. distasonis* (45.1 %), *P. merdae* (45.3 %), and *P. johnsonii* (45.1 %). The total number of genes of strain GM7 (2880) is smaller than those of *B. virosa* (3895), *P. distasonis* (3980), *B. synergistica* (3851), *P. goldsteinii* (5593), *P. merdae* (3653), and *P. johnsonii* (3731). The number of protein-coding genes of strain GM7 (2819) is smaller than those of *B. virosa* (3739), *P. distasonis* (3829), *B. synergistica* (3737), *P. goldsteinii* (5386), *P. merdae* (3414), and *P. johnsonii* (3599). The degrees of 16S rRNA similarity and orthologous protein content between these bacteria are summarized in Table 6. The AGIOS value ranged from 62.91 % between *B. virosa* and *P. merdae* to 90.95 % between *P. merdae* and *P. johnsonii* among compared species except

Table 2 Differential characteristics of *Gabonibacter massiliensis* strain GM7^T (data from this study), *Butyricimonas synergistica* [31], *Butyricimonas virosa* [31], *Parabacteroides merdae* [29], *Parabacteroides distasonis* [29], *Parabacteroides goldsteinii* [29], and *Parabacteroides johnsonii* [30]

Properties	<i>G. massiliensis</i>	<i>B. synergistica</i>	<i>P. johnsonii</i>	<i>P. distasonis</i>	<i>B. virosa</i>	<i>P. merdae</i>	<i>P. goldsteinii</i>
Cell diameter	0.4–0.56 µm	1 µm	0.8 µm	0.8–1.6 µm	1 µm	0.8–1.6 µm	0.8–1.6 µm
Oxygen requirement	–	–	–	–	–	–	–
Shape	Coccobacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus	Bacillus
Gram stain	–	–	–	–	–	–	–
Motility	+	–	–	–	–	–	–
Indole	+	+	–	–	+	–	–
Production of							
Alkaline phosphatase	–	+	+	+	+	+	+
Catalase	–	–	+	+	+	–	±
Oxidase	–	Na	Na	Na	Na	Na	Na
Nitrate reductase	–	Na	–	–	Na	–	–
Urease	–	–	–	–	–	–	–
β-galactosidase	–	–	+	+	+	+	+
N-acetyl-glucosaminidase	–	+	+	+	+	+	+
Acid from							
L-arabinose	–	–	+	–	–	–	–
Ribose	+	–	Na	Na	–	Na	Na
Mannose	–	+	+	+	–	+	+
Mannitol	+	–	–	–	–	–	–
Sucrose	–	–	+	+	–	+	+
D-glucose	+	+	+	+	+	+	+
D-fructose	–	–	–	–	–	–	–
D-maltose	–	–	+	–	–	+	–
D-lactose	–	–	+	+	–	+	–
G + C content (%)	42.09	44.8	45.1	45.1	42.3	45.3	43.5
Habitat	Human	Rat	Human	Haman	Rat	Human	Human

Na not available

Table 3 Differential main membrane fatty acids compositions of *Gabonibacter massiliensis* strain GM7^T (data from this study), *Butyricimonas synergistica* [31], *Butyricimonas virosa* [31], *Parabacteroides merdae* [29], *Parabacteroides distasonis* [29], *Parabacteroides goldsteinii* [29], and *Parabacteroides johnsonii* [30] in %

Fatty acids	<i>G. massiliensis</i>	<i>B. synergistica</i>	<i>P. johnsonii</i>	<i>P. distasonis</i>	<i>B. virosa</i>	<i>P. merdae</i>	<i>P. goldsteinii</i>
Iso-C _{15:0}	75.6	66.1	8.1	7.2	74.1	8	4.3
C _{16:0}	4.3	1.3	5.4	5.3	1.9	5.9	7.2
C _{18:1 n9}	3.1	14.1	9	14	4.4	14	11.7
Iso-C _{5:0}	2.8	–	–	–	–	–	–
C _{17:0} 3-OH	2.8	–	2	1.2	–	1.2	1.2
C _{16:0} 3-OH	2.7	2.4	3	4.9	4	5.7	3.6
Anteiso-C _{15:0}	2.1	–	32.2	28	–	25.3	26.5
C _{14:0}	1.9	–	1.1	1.3	1.4	1.4	1.1
C _{18:2 n6}	1.4	–	2.4	1.3	–	1.3	2.2
Iso-C _{17:0} 3-OH	–	10.1	21.3	24.7	6.8	26	26.3

Na not available

Table 4 Nucleotide content and gene count levels of the genome

Attribute	Genome (total)	
	Value	% of total ^a
Size (bp)	3,397,022	100
G + C content (%)	1,429,591	42.08
Coding region (bp)	3,062,697	90.15
Total genes	2880	100
RNA genes	61	2.11
Protein-coding genes	2819	97.88
Genes with function prediction	1262	43.81
Genes assigned to COGs	1557	54.06
Genes with peptide signals	717	24.89
CRISPR repeats	1	0.03
ORFan genes	124	4.30
Genes with transmembrane helices	645	22.39
Genes associated with PKS or NRPS	07	0.24
No. of antibiotic resistance genes	0	0
No. of genes associated to virulence	410	14.23

^a The total is based on either the size of the genome in base pairs or the total number of protein-coding genes in the annotated genome

strain GM7. When strain GM7 was compared to other species, AGIOS values ranged from 62.71 (*P. merdae* and *P. johnsonii*) to 72.04 (*B. virosa*) (Table 7). The dDDH value ranged from 12.6 ± 0.99 to 49.4 ± 0.31 among compared species except strain GM7 (Table 8). When strain GM7 was compared to other species, dDDH values ranged from 12.5 ± 0.99 (*P. merdae*, *P. johnsonii*, and *P. distasonis*) to 13.2 ± 0.96 (*B. synergistica*) (Table 8). The distribution of functional classes of predicted genes into COG categories was similar in all compared genomes (Fig. 5).

Phenotypic Comparison

All compared species (*G. massiliensis*, *B. synergistica*, *B. virosa*, *P. merdae*, *P. distasonis*, *P. goldsteinii*, and *P. johnsonii*) are gram-negative, strictly anaerobic, without urease activity. However, only *G. massiliensis* is motile. Indole is produced only by strain GM7, *B. virosa*, and *B. synergistica*. Except for *G. massiliensis*, other compared species exhibit alkaline phosphatase activity. Catalase activity varies among studied species (Table 2). The most common cellular fatty acids shared by *G. massiliensis*, *B.*

Fig. 4 Graphical circular map of the genome of *Gabonibacter massiliensis* strain GM7^T. From outside to the center: Contigs (red/gray), COG category of genes on the forward strand (three circles), genes on forward strand (blue circle), genes on reverse strand (red circle), COG category on the reverse strand (three circles), GC content

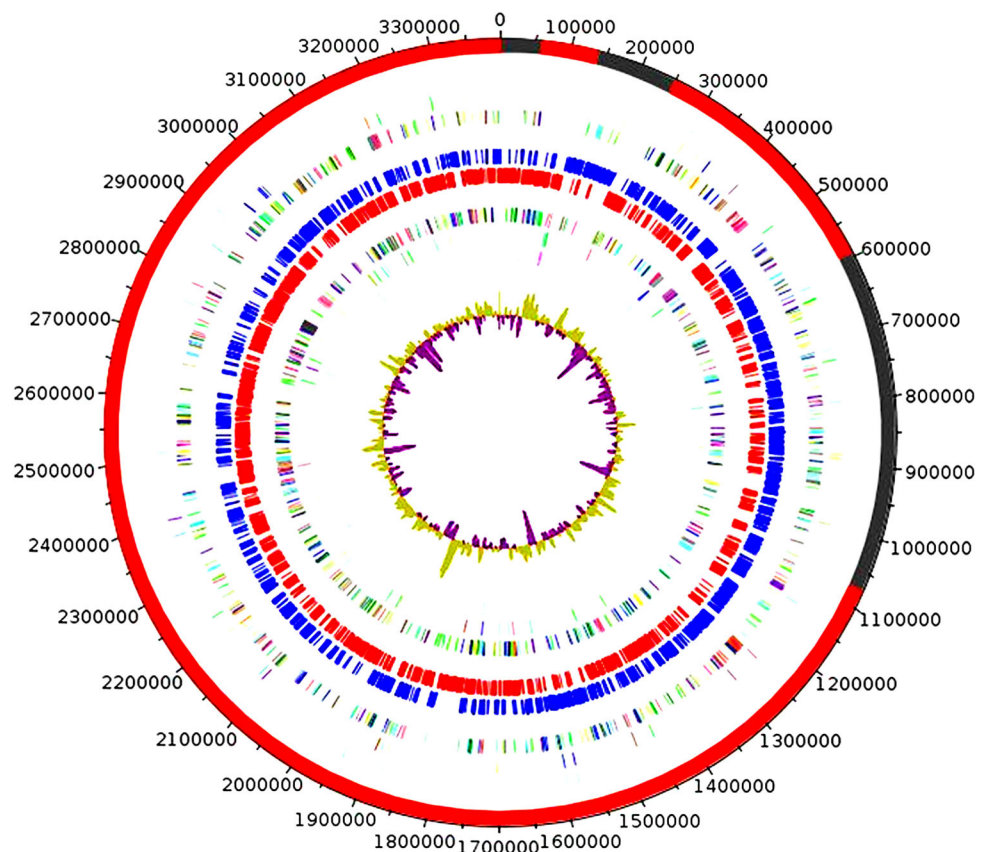


Table 5 Number of genes associated with the 25 general COG functional categories

Code	Value	% of total ^a	Description
J	148	5.25	Translation
A	0	0.0	RNA processing and modification
K	145	5.14	Transcription
L	111	3.94	Replication, recombination and repair
B	0	0.0	Chromatin structure and dynamics
D	23	0.82	Cell cycle control, mitosis and meiosis
Y	0	0.0	Nuclear structure
V	63	2.23	Defense mechanisms
T	121	4.29	Signal transduction mechanisms
M	194	6.88	Cell wall/membrane biogenesis
N	9	0.32	Cell motility
Z	0	0.0	Cytoskeleton
W	0	0.0	Extracellular structures
U	41	1.45	Intracellular trafficking and secretion
O	82	2.91	Post-translational modification, protein turnover, chaperones
C	148	5.25	Energy production and conversion
G	86	3.05	Carbohydrate transport and metabolism
E	147	5.21	Amino acid transport and metabolism
F	61	2.16	Nucleotide transport and metabolism
H	105	3.72	Coenzyme transport and metabolism
I	51	1.81	Lipid transport and metabolism
P	135	4.79	Inorganic ion transport and metabolism
Q	22	0.78	Secondary metabolites biosynthesis, transport and catabolism
R	278	9.86	General function prediction only
S	111	3.94	Function unknown
–	295	10.25	Not in COGs

^a The total is based on the total number of protein-coding genes in the annotated genome

Table 6 Genome comparison of closely related species to *Gabonibacter massiliensis* strain GM7

Name of organisms	16S rRNA similarity (%)	Size (Mb)	G + C (%)	Protein-coding genes	Total Genes
<i>Gabonibacter massiliensis</i> strain GM7 ^T	100	3.39	42.0	2819	2880
<i>Butyricimonas virosa</i> strain MT12 ^T	90	4.72	42.3	3739	3895
<i>Butyricimonas synergistica</i> strain MT01 ^T	90	4.77	44.8	3737	3851
<i>Parabacteroides distasonis</i> strain JCM5825 ^T	83.9	4.81	45.1	3829	3980
<i>Parabacteroides goldsteinii</i> strain WAL 12034 ^T	82	7.09	43.5	5386	5593
<i>Parabacteroides johnsonii</i> strain M-165 ^T	82.1	4.63	45.1	3599	3731
<i>Parabacteroides merdae</i> strain ATCC 43184 ^T	82	4.43	45.3	3414	3653

synergistica, *B. virosa*, *P. merdae*, *P. distasonis*, *P. goldsteinii*, and *P. johnsonii* are summarized in Table 3.

Discussion

Assessment of the human gut microbiota is of great interest in human health. Indeed, it was reported that the human gut microbiota composition (relative abundance of the distinct

phyla) is linked to various diseases including, among others, obesity, type II diabetes, kwashiorkor, and marasmus [13, 17, 37]. However, the exhaustive assessment of complex microfloras is difficult. Recent efforts such as metagenomics and culturomics have permitted to increase the known repertory of bacteria thanks to the discovery of new families, genera, and species [4, 13, 17]. In our laboratory, over the past 5 years, we isolated representative strains from 124 putative new bacterial species including

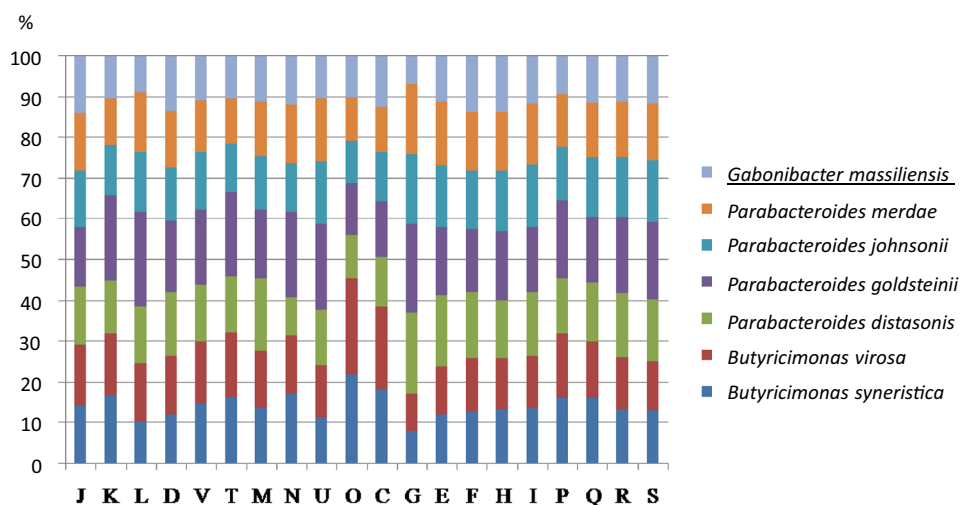
Table 7 Numbers of orthologous protein (upper right)* and AGIOS values (lower left) observed between genomes

	<i>B. synergistic</i>	<i>B. virosa</i>	<i>P. distasonis</i>	<i>P. goldsteinii</i>	<i>P. johnsonii</i>	<i>P. merdae</i>	<i>G. massiliensis</i>
<i>B. synergistica</i>	3874	1767	1207	1264	1191	1159	1755
<i>B. virosa</i>	77.53	3915	1236	1284	1208	1173	1735
<i>P. distasonis</i>	63.04	63.04	4003	2069	2056	1991	1199
<i>P. goldsteinii</i>	62.91	63.01	73.50	5648	2210	2133	1222
<i>P. johnsonii</i>	62.87	62.93	73.58	75.70	3761	2162	1170
<i>P. merdae</i>	63.00	62.91	73.81	76.03	90.95	3743	1135
<i>G. massiliensis</i>	71.65	72.04	62.82	62.89	62.71	62.71	295

Table 8 Pairwise comparison of *Gabonibacter massiliensis* with other species using GGDC, formula 2 (dDDH estimates based on identities/HSP length)* upper right

	<i>B. synergistic</i>	<i>B. virosa</i>	<i>P. distasonis</i>	<i>P. goldsteinii</i>	<i>P. johnsonii</i>	<i>P. merdae</i>	<i>G. massiliensis</i>
<i>B. synergistica</i>	100 % ± 00	18.5 ± 0.75	12.6 ± 0.99	12.6 ± 0.99	12.6 ± 0.99	12.6 ± 0.99	13.2 ± 0.96
<i>B. virosa</i>		100 % ± 00	12.6 ± 0.99	12.6 ± 0.99	12.6 ± 0.99	12.6 ± 0.99	12.9 ± 0.97
<i>P. distasonis</i>			100 % ± 00	14.6 ± 0.89	14.8 ± 0.88	15.3 ± 0.86	±12.5 ± 0.99
<i>P. goldsteinii</i>				100 % ± 00	15.9 ± 0.84	16.2 ± 0.83	12.6 ± 0.99
<i>P. johnsonii</i>					100 % ± 00	49.4 ± 0.31	12.5 ± 0.99
<i>P. merdae</i>						100 % ± 00	12.5 ± 0.99
<i>G. massiliensis</i>							100 % ± 00

* The confidence intervals indicate the inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test datasets (which are always limited in size). These results are in accordance with the phylogenetic analysis (Fig. 2) as well as the GGDC results

Fig. 5 Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins

39 strict anaerobes [4]. Of these, the names of 17 species have officially been validated [4, 7, 16, 19, 28]. The new bacterium described in this article, *G. massiliensis* gen. nov., sp. nov., strain GM7 was isolated from the same stool specimen as another two recently described new species,

Gabonia massiliensis gen. nov., sp. nov., strain GM3 and *Kallipyga gabonensis* sp. nov., strain GM4 [24, 25].

As a large fraction of febrile infections remain undiagnosed in Gabon [2], due to the lack of adequate diagnostic tools [23] and/or the existence of as-yet undetected agents,

our study participates in a better knowledge of human-associated microorganisms.

Conclusion

On the basis, the observed phenotypic and genomic differences with phylogenetically close species, we formally propose the creation of *Gabonibacter massiliensis* gen. nov., sp. nov. that contains strain GM7^T. Strain GM7^T was isolated from a stool specimen from a healthy Gabonese male and is the type strain of *G. massiliensis* gen. nov., sp. nov.

Taxonomic and Nomenclatural Proposals

Description of *Gabonibacter* gen. nov

Gabonibacter (Ga.bo.ni.bac.ter N. L. masc. n. Combination of *Gaboni*, from Gabon, the Sub-saharan African Country where stool specimen was collected and *bacter*, of bacteria the domain to which this microorganism belongs) cells are gram-negative and motile coccobacilli exhibiting a mean length and diameter of 1.2 µm (range 0.92–1.38 µm) and 0.48 µm (range 0.4–0.56 µm), respectively. *Gabonibacter* bacteria are strictly anaerobic and their optimal growth is obtained at 37 °C and their pH tolerance ranges from 6 to 8.5. Colonies grown on 5 % sheep blood-enriched Columbia agar are white with a diameter of 2 mm. Cells do not produce catalase and oxidase. Based upon 16S rRNA gene sequence analysis, *Gabonibacter* is a member of the family *Porphyromonadaceae*. *Gabonibacter massiliensis* sp. nov. and strain GM7^T are the type species and type strain, respectively, of *Gabonibacter* gen. nov.

Description of *Gabonibacter massiliensis* gen nov., sp nov

Gabonibacter massiliensis strain GM7^T (ma.si.li.e'n.sis. L. adj. masc. *massiliensis*, of *Massilia*, the Latin name of Marseille, where strain GM7^T was isolated) is a gram-negative and motile coccobacillus whose individual cells measure 0.86 µm in length and 0.5 µm in diameter. It is strictly anaerobic and optimal growth is obtained at 37 °C. Colonies grown on 5 % sheep blood-enriched Columbia agar (bioMérieux) are white and exhibit a diameter of 2 mm. Strain GM7^T produces indole but has no catalase and oxidase activities. Using an API 50 CH strip, cells use D-ribose, D-glucose, D-trehalose, D-tagatose, and potassium 5-ketogluconate. Using API ZYM, strain GM7 exhibits esterase (C4), esterase lipase (C8), acid phosphatase, and naphthol-AS-BI-phosphohydrolase activities. Using API 20A, positive reactions are only observed for D-mannitol,

salicin, gelatin, and D-trehalose assays. Strain GM7 exhibits no alkaline phosphatase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, α and β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase and α-fucosidase activities. Additionally, strain GM7 does not metabolize L-tryptophane, urea, D-glucose, D-lactose, sucrose, D-maltose, D-xylose, L-arabinose, esculin ferric citrate, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, and L-rhamnose. The bacterium is susceptible to imipenem, metronidazole, and nitrofurantoin. Its predominant cellular fatty acids are 13-methyl-tetradecanoic acid (15:0 iso), hexadecanoic acid (16:0), 9-octadecenoic acid (18:1 n9), 3-methyl-butanoic acid (5:0 iso), 3-hydroxy-heptadecanoic acid (17:0 3-OH), 3-hydroxy-hexadecanoic acid (16:0 3-OH), 12-methyl-tetradecanoic acid (15:0 anteiso), tetradecanoic acid (14:0), and 9,12-octadecadienoic acid (18:2 n6). The genome of *G. massiliensis* is 3,397,022 bp long with a G+C content of 42.09 %. The genome and 16S rRNA sequences from strain GM7^T were deposited in Genbank under accession numbers FAVK00000000 and LN881588, respectively. The type strain GM7^T, deposited in the CSUR and DSMZ collections under numbers CSUR P2336 and DSM 101039, respectively, was cultivated from the feces of a healthy 16-year-old Gabonese male.

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Compliance with Ethical Standards

Conflicts of interest The authors declare no conflict of interest.

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