Genome sequence and description of *Traorella massiliensis* gen. nov., sp. nov., a new bacterial genus isolated from human left colon

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Abstract

A strictly anaerobic, motile, non–spore-forming, Gram-negative, rod-shaped bacterium designated Marseille-P3110^T was isolated from the left colon cleansing of a 76-year-old Frenchwoman. Its 16S ribosomal RNA (rRNA) gene showed a 93.2% similarity level with the 16S rRNA of *Dielma fastidiosa* strain JC13, the closest species with a validly published name. The genome of Marseille-P3110^T is 2 607 061 bp long with 35.99% G+C content. Of the 2642 predicted genes, 2582 were protein-coding genes and 60 were RNAs, including five 16S rRNA genes.

Keywords: Culturomics, genome, gut microbiota, taxonogenomics, *Traorella massiliensis*
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Introduction

Even though metagenomics has made it possible to explore the human microbiota, many bacteria remain to be cultivated. Culturomics is a culture-based approach that uses multiple culture conditions together with MALDI-TOF MS and 16S ribosomal RNA (rRNA) gene sequencing [1] for bacterial culture and identification. This approach was developed to improve the identification of uncultured bacteria by matching metagenomic unassigned sequences with identified and cultivated bacteria [1].

The project that allows the isolation of this new strain consists of the study the microbiota of different portions of the digestive tract. Its composition varies according to different factors such as pH, percentage of oxygen or health status [2].

During this project, we isolated a new bacterial genus, a member of the family *Erysipelotrichaceae.* This family was created in 2004 and actually regroups 11 genera [3]. Bacteria belonging to this family are all strictly anaerobic, except the genus *Erysipelothrix,* which contains facultative anaerobic or microaerophilic species. Most of these bacteria are Gram positive, non–spore forming and rod shaped. The bacteria belonging to this family seem to be highly immunogenic. The proportion of *Erysipelotrichaceae* is higher in patients with inflammatory bowel disease than in healthy individuals [4].

The new genus was described using the taxonogenomics approach. This approach combines next-generation sequencing, and phylogenetic and phenotypic techniques [5]. The MALDI-TOF MS protein profile plays also a role in the description of new bacterial species.

We describe *Traorella massiliensis* strain Marseille-P3110^T (= CSUR P3110 = DSM 103514), a new genus isolated from the left colon cleansing of a 76-year-old Frenchwoman.

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Materials and methods

Ethics and sample information

A sample from the left colon cleansing of a 76-year-old Frenchwoman who underwent a colonoscopy to check for colon polyps was collected at Hôpital Nord (Marseille, France) in May 2016. The study was authorized by the local ethics committee of the Institut Hospitalo-Universitaire Méditerranée-infection (Marseille, France) under agreement 2016-010, and the patient provided written informed consent. At the moment of the sample collection, she was treated with a proton pump inhibitor. The sample was transported in an antioxidant transport medium.

Strain identification by MALDI-TOF MS and 16S rRNA gene sequencing

The sample was seeded directly on Columbia medium supplemented with 5% sheep's blood (COS) (bioMérieux, Marcy l'Etoile, France), and incubated under anaerobic conditions at

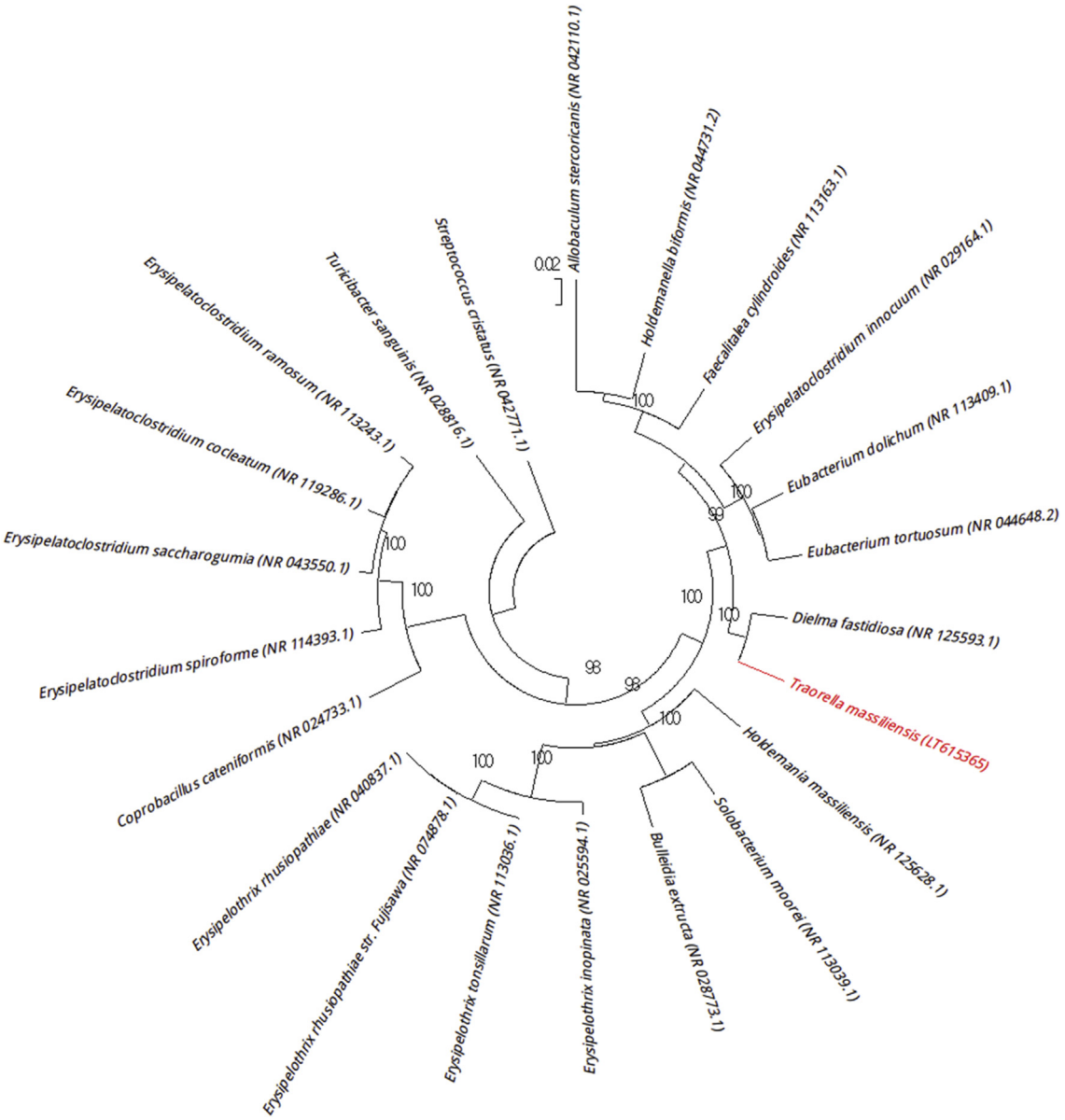


FIG. 1. Phylogenetic tree highlighting position of *Traorella massiliensis* strain Marseille-P3110^T relative to other close strains. GenBank accession numbers of each 16S rRNA are noted in parentheses. Sequences were aligned using Muscle version 3.8.31 with default parameters, and phylogenetic inferences were obtained using neighbour-joining method with 500 bootstrap replicates within MEGA6 software. Only bootstraps >95% are shown. Scale bar represents 0.02% nucleotide sequence divergence.

37°C during 3 days. Colonies were purified through subculture and identified by MALDI-TOF MS using a Microflex spectrometer and a MTP 96 MALDI-TOF target plate (Bruker Daltonics, Leipzig, Germany), as described previously [6,7]. The spectra obtained for each colony were matched against the MALDI Biotyper software version 3.0 (Bruker) and Unité des Maladies Infectieuses et Tropicales Emergentes (URMITE) databases using standard pattern matching (with default parameter settings). The identification scores used were as follows: a score over 1.9 allowed identification at the species level, while a score under 1.7 did not allow any identification. In the latter case, the colony was identified by sequencing its 16S rRNA gene as previously described [8]. According to Stackebrandt and Ebers [9], a similarity threshold of 98.65% was used to define a new species, whereas a threshold of 95% was used to define a new genus without performing DNA-DNA hybridization. Upon identification, a reference spectrum for strain Marseille-P3110^T was incremented in the URMITE database.

16S rRNA phylogenetic tree

A custom Python script was used to automatically retrieve all species from the same order as the new genus and download 16S sequences from National Center for Biotechnology Information (NCBI) by parsing the NCBI eUtils results and NCBI taxonomy page. It only keeps sequences from type strains. In case of multiple sequences for one type strain, it selects the sequence obtaining the best identity rate from the BLASTn alignment with our sequence. The script then separates 16S sequences in two groups: one containing the sequences of strains from the same family (group a) and one containing the others (group b). It finally only keeps the 15 strains closest to the group and the closest to group b. If it is impossible to get 15 sequences from group a, the script selects more sequences from group b to get at least nine strains from both groups.

TABLE 1. Classification and general features of *Traorella massiliensis* strain Marseille-P3110^T

Property	Term
Current classification	Domain: *Bacteria* Phylum: *Firmicutes* Class: *Erysipelotrichia* Order: *Erysipelotrichales* Family: *Erysipelotrichaceae* Genus: *Traorella* Species: *Traorella massiliensis* Type strain: Marseille-P3110^T
Gram stain	Negative
Cell shape	Rod
Motility	Motile
Sporulation	Nonsporulating
Temperature range	Mesophilic
Optimum temperature	37°C

Growth conditions

Ideal growth conditions for strain Marseille-P3110^T were determined by testing five growth temperatures (25, 28, 37, 45 and 56°C) in an aerobic atmosphere with or without 5% CO_2, and under anaerobic and microaerophilic conditions using the GENbag anaer and GENbag microaer systems, respectively (bioMérieux). Different pH values (5, 5.5, 6.5, 7, 7.5 and 8) and NaCl concentrations (10, 15 and 20%) were also tested.

Morphologic, biochemical and antibiotic susceptibility tests

Phenotypic characteristics such as Gram staining, motility, sporulation, and catalase and oxidase activities were tested as previously described [3,10]. Biochemical analysis of strain Marseille-P3110^T was carried out using API 50 CH, API 20A and API ZYM strips (bioMérieux) in an anaerobic atmosphere. Antibiotic susceptibility was tested using the disc diffusion method (i2a, Montpellier, France) [11] and according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2015 recommendations.

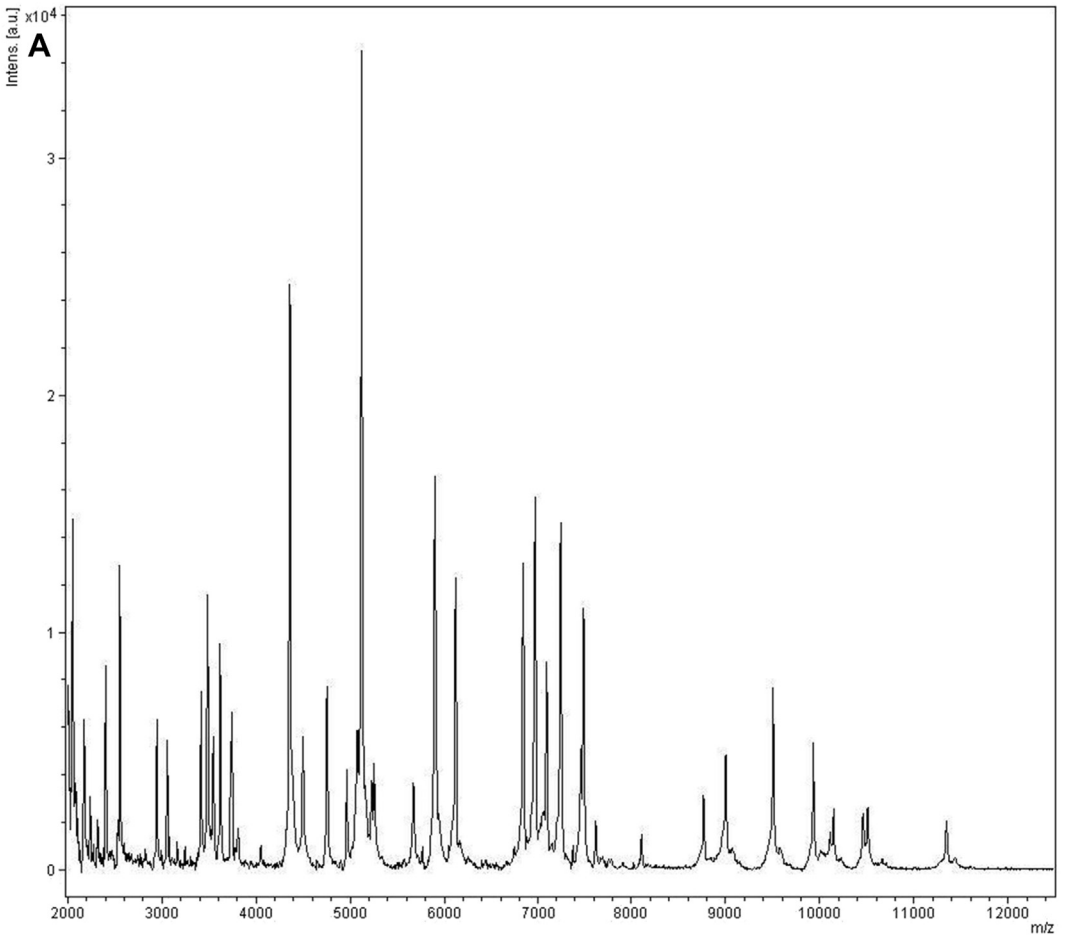
In order to observe their morphology, the cells were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 1 hour at 4°C. A drop of cell suspension was deposited for approximately 5 minutes on glow-discharged formvar carbon film on 400 mesh nickel grids (FCF400-Ni, Electron Microscopy Sciences (EMS), Hatfield, PA, USA). The grids were dried on blotting paper, and cells were negatively stained for 10 seconds with 1% ammonium molybdate solution in filtered water at room temperature. Electron micrographs were acquired with a Morgagni 268D (Philips, Amsterdam, The Netherlands) transmission electron microscope operated at 80 keV.

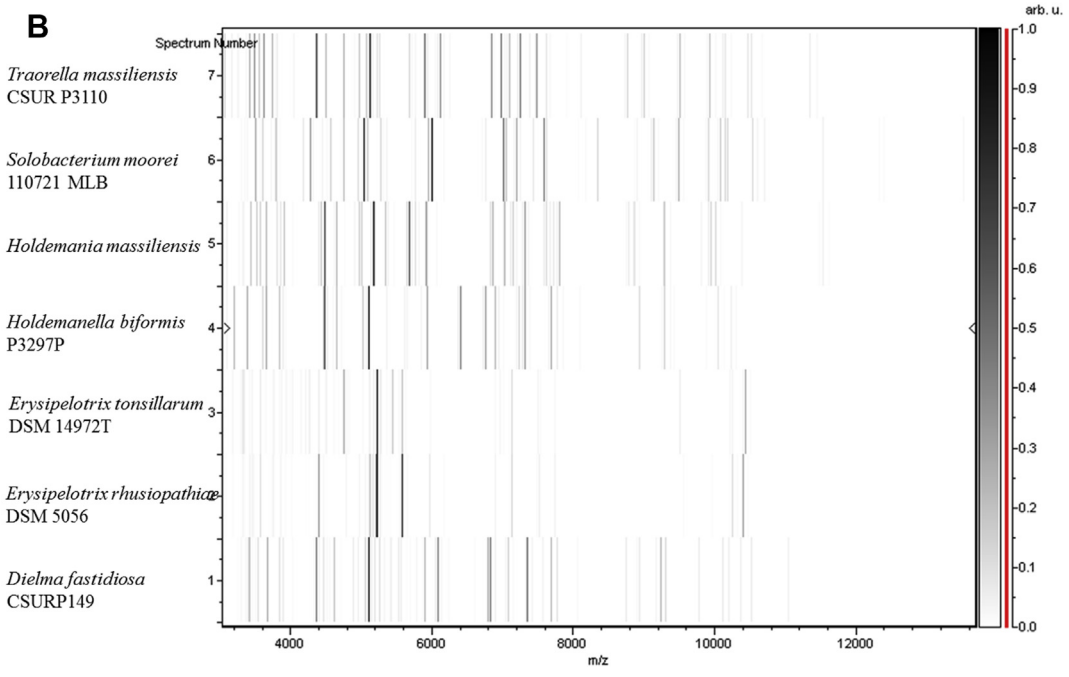
Fatty acid methyl ester analysis by gas chromatography/mass spectrometry

Two samples were prepared with approximately 30 mg of bacterial biomass per tube collected from several culture plates. Fatty acid methyl esters (FAME) were prepared as described by Sasser [12]. Gas chromatography/mass spectrometry analyses were carried out as described previously [13]. Briefly, FAME were separated using an Elite 5-MS column and monitored by mass spectrometry (Clarus 500–SQ 8 S; PerkinElmer, Courtaboeuf, France). Spectral database search was performed using MS Search 2.0 operated with the Standard Reference Database 1A (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the FAMEs mass spectral database (Wiley, Chichester, UK).

Genomic DNA extraction and genome sequencing

After a lysozyme pretreatment and incubation at 37°C for 2 hours, DNA was extracted on the EZ1 biorobot (Qiagen,





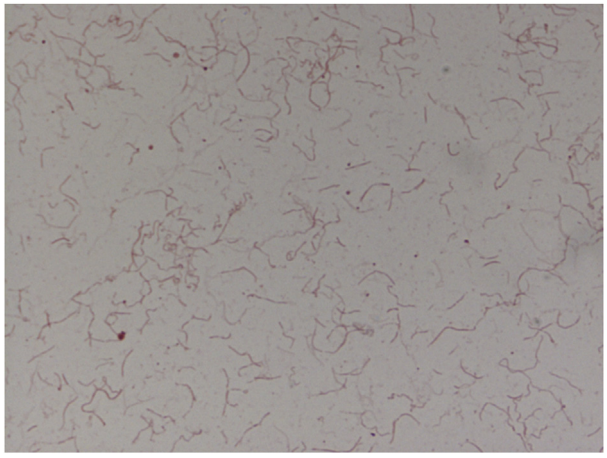


FIG. 3. Gram staining of *Traorella massiliensis* strain Marseille-P3110^T.

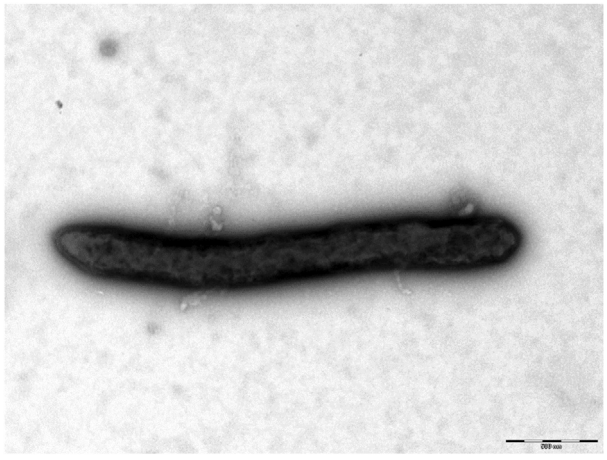


FIG. 4. Transmission electron microscopy of *Traorella massiliensis* strain Marseille-P3110^T using Morgagni 268D (Philips) transmission electron microscope operated at 80 keV. Scale bar represents 500 nm.

Germantown, MD, USA) with EZ1 DNA tissues kit. The elution volume was 50 µL. Genomic DNA (gDNA) was quantified by a Qubit assay with a high-sensitivity kit (Life Technologies, Carlsbad, CA, USA) to 25.5 ng/µL.

gDNA of strain Marseille-P3110^T was sequenced on the MiSeq Technology (Illumina, San Diego, CA, USA) with the mate-pair strategy. The gDNA was barcoded in order to be mixed with 11 other projects with the Nextera Mate Pair sample prep kit (Illumina).

The mate-pair library was prepared with 1.5 µg of gDNA using the Nextera mate-pair Illumina guide. The gDNA sample was simultaneously fragmented and tagged with a mate-pair junction adapter. The pattern of the fragmentation was validated on an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) with a DNA 7500 labchip. The DNA fragments ranged in size from 1.5 to 11 kb, with an optimal size at 5.176 kb. No size selection was performed, and 378.6 ng of tagmented fragments were circularized. The circularized DNA was mechanically sheared to small fragments with an optimal at 1054 bp on the Covaris device S2 in T6 tubes (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent), and the final concentration library was measured at 31.57 nmol/L.

The libraries were normalized at 2 nM and pooled. After a denaturation step and dilution at 15 pM, the pool of libraries was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. Automated cluster generation and sequencing run were performed in a single 39-hour run at a 2 × 251 bp read length.

Total information of 5.1 Gb was obtained from a 544K/mm^2 cluster density with a cluster passing quality control filters of 96.8% (10 139 000 passing filter paired reads). Within this run, the index representation for strain Marseille-P3110^T was determined to be of 8.93%. The 905 502 paired reads were trimmed then assembled in nine scaffolds.

Genome assembly

The genome assembly was performed with a pipeline that enabled creation of an assembly with different software (Velvet [14], Spades [15] and Soap Denovo [16]) on trimmed (MiSeq and Trimmomatic [17] software) or untrimmed data (only MiSeq software). For each of the six assemblies performed, GapCloser [16] was used to reduce gaps. Then contamination with Phage Phix was identified (BLASTn against Phage Phix174 DNA sequence) and eliminated. Finally, scaffolds under 800 bp in size were removed, and scaffolds with a depth value lower than 25% of the mean depth were removed (identified as possible contaminants). The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). For the studied strain, Spades gave the best assembly, with a depth coverage of 128×.

FIG. 2. (A) Reference mass spectrum from *Traorella massiliensis* strain Marseille-P3110^T. (B) Gel view comparing *Traorella massiliensis* strain Marseille-P3110^T to other species within genera *Erysipelotrix, Dielma, Holdemanella, Holdemania* and *Solobacterium*. Gel view displays raw spectra of loaded spectrum files arranged in pseudo–gel-like look. *X*-axis records *m/z* value; left *y*-axis displays running spectrum number originating from subsequent spectra loading. Peak intensity is expressed by greyscale scheme code. Right *y*-axis indicates relation between colour of peak and its intensity in arbitrary units. Displayed species are indicated at left.

TABLE 2. Differential characteristics of *Traorella massiliensis* strain Marseille-P3110^T, *Dielma fastidiosa* strain JC13^T [3], *Eubacterium dolichum* strain JCM 10413^T DSM 3991 [32], *Faecalitalea cylindroides* JCM 10261^T DSM 3983 [33], *Holdemanella biformis* strain DSM 3989 [33], *Allobaculum stercoricanis* strain 2CLOS2^T DSM 13633 [34], *Holdemania massiliensis* strain AP2^T DSM 26143 [35], *Solobacterium moorei* strain RCA59–74^T DSM 22971 [36] and *Bulleidia extructa* strain W 1219^T DSM 13220 [37]

Property	*Traorella massiliensis*	*Dielma fastidiosa*	*Eubacterium dolichum*	*Faecalitalea cylindroides*	*Holdemanella biformis*	*Allobaculum stercoricanis*	*Holdemania massiliensis*	*Solobacterium moorei*	*Bulleidia extructa*
Cell diameter (µm)	0.28–0.37	0.6	0.4–0.6	NA	NA	0.75–0.9	0.57	0.2	0.5
Gram stain	–	–	+	+	+	+	+	+	+
Salt tolerance	–	NA	NA	NA	NA	NA	NA	NA	NA
Motility	+	+	–	–	–	–	–	–	–
Endospore formation	–	–	–	–	–	–	–	–	–
Major cellular fatty acid	18:1n9	NA	NA	C 16 : 0	C 16 : 0	NA	NA	NA	NA
Acid from:									
D-Xylose	–	–	–	–	+	–	NA	–	–
Ribose	+	NA	–	NA	NA	–	+	+	NA
Mannose	–	–	–	+	+	–	+	–	–
Mannitol	–	NA	–	–	+	–	+	–	–
Sucrose	–	NA	–	+	–	+	+	–	–
D-Glucose	–	–	+	+	+	+	+	+	+
D-Fructose	–	NA	+	NA	NA	+	+	+	+
D-Maltose	–	NA	+	–	–	+	+	+	+
Habitat	Human gut	Human gut	Human gut	Human gut	Human gut	Human Gut	Human gut	Human gut	Human oral cavity

+, positive result; –, negative result; NA, data not available.

TABLE 3. Cellular fatty acid composition (%)

Fatty acids	Name	Mean relative %[a]
18:1n9	9-Octadecenoic acid	34.1 ± 0.6
16:0	Hexadecanoic acid	30.1 ± 2.2
18:0	Octadecanoic acid	15.9 ± 2.0
18:2n6	9,12-Octadecadienoic acid	13.2 ± 2.8
14:0	Tetradecanoic acid	1.9 ± 0.2
18:1n7	11-Octadecenoic acid	1.8 ± 0.3
18:1n6	12-Octadecenoic acid	1.2 ± 0.1
17:0	Heptadecanoic acid	TR
16:1n7	9-Hexadecenoic acid	TR
15:0	Pentadecanoic acid	TR
17:0 anteiso	14-methyl-Hexadecanoic acid	TR
16:0 9,10-methylene	2-hexyl-Cyclopropaneoctanoic acid	TR
17:0 iso	15-methyl-Hexadecanoic acid	TR

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

Genome annotation and comparison

Open reading frames (ORFs) were predicted using Prodigal [18] with default parameters, but the predicted ORFs were excluded if they spanned a sequencing gap region (contained N). The predicted bacterial protein sequences were searched against the Clusters of Orthologous Groups (COGs) database using BLASTP (*E* value 1e-03, coverage 0.7 and identity percentage 30%). If no hit was found, then the predicted bacterial protein sequences were searched against the NR database using BLASTP with an *E* value of 1e-03, coverage of 0.7 and identity percentage of 30%, and if the sequence length was smaller than 80 aa, we used an *E* value of 1e-05. The tRNAScanSE tool [19] was used to find transfer RNA genes, while ribosomal RNAs were found using RNAmmer [20]. Lipoprotein signal peptides and the number of transmembrane helices were predicted using Phobius [21]. ORFans were identified if all the BLASTP performed did not give positive results (*E* value smaller than 1e-03 for ORFs with sequence size larger than 80 aa or *E* value smaller than 1e-05 for ORFs with sequence length smaller 80 aa). Such parameter thresholds have already been used in previous studies to define ORFans. Pfam-conserved domains (Pfam-A and Pfam-B domains) were search on each protein with the HMMscan of the HMMER3 suite [6]. PKS and NRPS were searched against the ClusterMine360 [17] database. Resistome was analysed by using the ARG-ANNOT database [22].

Species used for genomic comparison were identified in the 16S rRNA tree using PhyloPattern software [23]. For each selected species, the complete genome sequence, proteome sequence and ORFeome sequence were retrieved from the NCBI's FTP site. If one specific strain did not have a complete and available genome, a complete genome of the same species was used. If ORFeome and proteome were not predicted, Prodigal was used with default parameters to predict them. All proteome were analysed with proteinOrtho [24]. Then, for each couple of genomes, a similarity score was computed. This score is the mean value of nucleotide similarity between all couples of orthologous genes between the two genomes studied (average genomic identity of orthologous gene sequences (AGIOS) tool). An annotation of the entire proteome was performed to define the distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins (using the same method as for the genome annotation). Annotation and comparison processes were performed in the multiagent software system DAGOBAH [25], which includes Figenix [26] libraries that provide pipeline analysis, and by using PhyloPattern [23] for tree manipulation. To evaluate the genomic similarity between studied genomes,

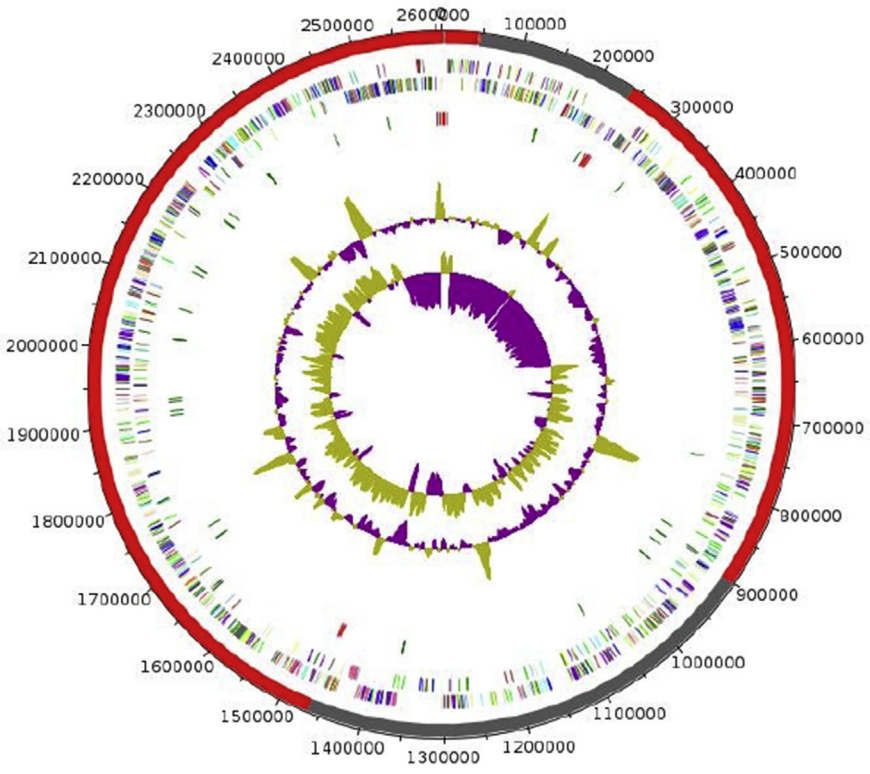


FIG. 5. Graphical circular map of chromosome. From outside to centre, genes on forward strain coloured by COGs categories (only gene assigned to COGs), RNA genes (tRNAs green, rRNAs red), G+C content and G+C skew. COGs, Clusters of Orthologous Groups database; rRNA, ribosomal RNA; tRNA, transfer RNA.

TABLE 4. Nucleotide content and gene count levels of genome

	Genome (total)	
Attribute	**Value**	**% of total[a]**
Size (bp)	2 607 061	100
G+C content (%)	937 722	35.99
Coding region (bp)	2 346 961	90.02
Total genes	2642	100
RNA genes	60	2.27
Protein-coding genes	2582	100
Genes with function prediction	1757	68.05
Genes assigned to COGs	1468	56.86
Genes with peptide signals	296	11.46
ORFans genes	279	10.81
Genes associated with PKS or NRPS	1	0.039
No. of antibiotic resistance genes	1	0.039
No. of genes associated with Pfam-A domains	2308	87

COGs, Clusters of Orthologous Groups database; NRPS, nonribosomal peptide synthase; PKS, polyketide synthase.
[a]Total is based on either size of genome in base pairs or total number of protein-coding genes in annotated genome.

we determined two parameters, digital DNA-DNA hybridization (dDDH), which exhibits a high correlation with DNA-DNA hybridization (DDH) [27,28], and AGIOS [29], which was designed to be independent from DDH [31]. Genome-to-Genome Distance Calculator (GGDC) analysis was performed using the GGDC web server as previously reported [28].

TABLE 5. Number of genes associated with 25 general COGs functional categories

Code	Value	% of total[a]	Description
[J]	191	7.40	Translation
[A]	0	0	RNA processing and modification
[K]	125	4.84	Transcription
[L]	88	3.41	Replication, recombination and repair
[B]	0	0	Chromatin structure and dynamics
[D]	32	1.24	Cell cycle control, mitosis and meiosis
[Y]	0	0	Nuclear structure
[V]	73	2.83	Defense mechanisms
[T]	64	2.48	Signal transduction mechanisms
[M]	96	3.72	Cell wall/membrane biogenesis
[N]	7	0.27	Cell motility
[Z]	0	0	Cytoskeleton
[W]	1	0.04	Extracellular structures
[U]	18	0.70	Intracellular trafficking and secretion
[O]	53	2.05	Posttranslational modification, protein turnover, chaperones
[X]	58	2.25	Mobilome: prophages, transposons
[C]	73	2.83	Energy production and conversion
[G]	155	6.00	Carbohydrate transport and metabolism
[E]	116	4.49	Amino acid transport and metabolism
[F]	69	2.67	Nucleotide transport and metabolism
[H]	47	1.82	Coenzyme transport and metabolism
[I]	42	1.63	Lipid transport and metabolism
[P]	68	2.63	Inorganic ion transport and metabolism
[Q]	21	0.81	Secondary metabolites biosynthesis, transport and catabolism
[R]	152	5.89	General function prediction only
[S]	74	2.87	Function unknown
–	1114	43.14	Not in COGs

COGs, Clusters of Orthologous Groups database.
[a]Total is based on total number of protein-coding genes in annotated genome.

Results

Strain identification and phylogenetic analyses

Strain Marseille-P3110^T was first cultivated on COS under anaerobic atmosphere at 37°C. The sample containing this bacterium was transported in an antioxidant transport medium and seeded directly onto COS. Strain Marseille-P3110^T could not be identified using MALDI-TOF MS, and therefore the 16S rRNA was sequenced. The resulting sequence (accession no. LT615365) showed a 93.2% similarity level with the 16S rRNA gene of *Dielma fastidiosa* strain JC13, the closest species with a validly published name [30] (Fig. 1). Because this 16S rRNA nucleotide sequence similarity was lower than 95%, strain Marseille-P3110^T is considered to be a new genus according to the threshold described by Stackebrandt and Ebers [9]. This new genus belongs to the family *Erysipelotrichaceae* for which we suggest the name *Traorella,* with *Traorella massiliensis* as type species and Marseille-P3110^T (= CSUR P3110 = DSM 103514) as type strain (Table 1). Consequently, the reference protein spectra for *Traorella massiliensis* (Fig. 2(A)) were incremented in the URMITE database (http://www.mediterranee infection.com/article.php?laref=256&titre=urms-database). A gel view was also performed to observe the spectra's comparison with the closest bacteria (Fig. 2(B)).

Phenotypic description

The bacterium was cultivated at 37°C in anaerobic conditions. No growth was noted in aerobic and microaerophilic conditions. The optimal growth condition was observed after 72 hours in anaerobic conditions. Growth was observed at pH 7, 7.5 and 8 but not at pH 5, 5.5 and 6.5. No growth was observed for the salinities tested (10, 15 and 20%). Colonies of the strain Marseille-P3110^T were motile, non–spore forming, Gram negative and rod shaped (Fig. 3). The strain Marseille-P3110^T forms regular white colonies with a mean diameter of 1 mm. Individual cells have a width ranging from 0.28 to 0.37 µm and a length ranging from 2.5 to 3.1 µm (Fig. 4). No catalase and oxidase activities were observed. Using an API ZYM strip, an API 20A

TABLE 6. Genome comparison of closely related species to *Traorella massiliensis* strain Marseille-P3110^T

Organism	INSDC	Size (Mb)	G+C (%)	Protein-coding genes	Total genes
Traorella massiliensis strain Marseille-P3110^T	FNLJ00000000	2.61	35.99	2582	2582
Faecalitalea cylindroides strain ATCC 27803	AWVI00000000	1.95	34.68	1841	2057
Erysipelothrix rhusiopathiae strain ATCC 19414	ACLK00000000	1.75	36.47	1613	1645
Solobacterium moorei strain F0204	AUKY00000000	2.01	36.78	2181	2035
Eubacterium dolichum strain CAG:375	ABAW00000000	2.03	37.40	2076	1927
Erysipelothrix tonsillarum strain DSM 14972	AREO00000000	1.93	36.83	1750	1792
Erysipelatoclostridium ramosum strain DSM 1402	ABFX00000000	3.24	31.39	2941	3169
Holdemanella biformis strain DSM 3989	ABYT00000000	2.52	33.79	2248	2529
Dielma fastidiosa strain DSM 26099	CAEN00000000	3.62	39.97	3321	3496

INSDC, International Nucleotide Sequence Database Collaboration.

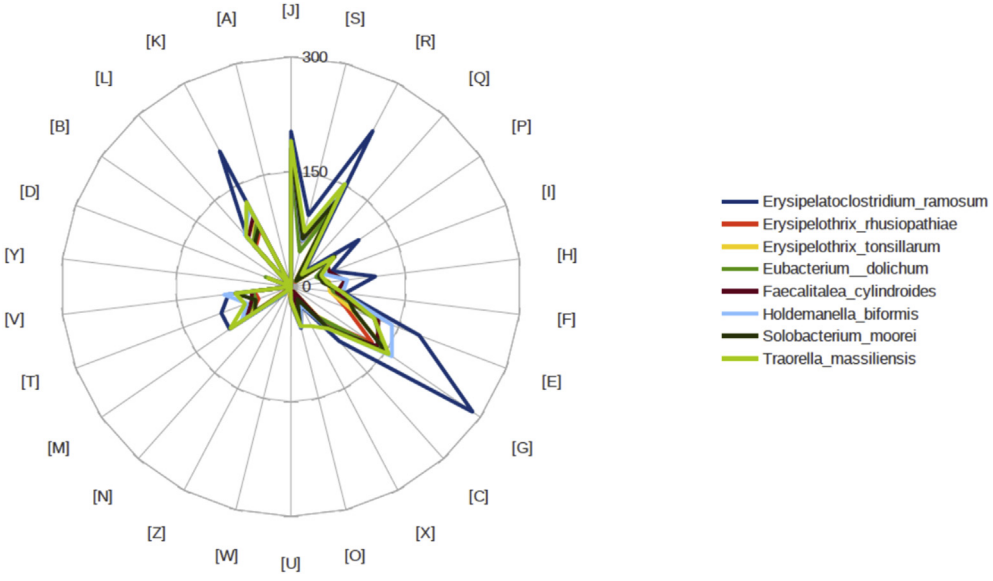


FIG. 6. Distribution of functional classes of predicted genes according to clusters of orthologous groups of proteins.

strip and an API 50 CH strip, positive enzymatic activities included esterase C4, leucine arylamidase, *N*-acetyl-β-glucosaminidase, α-fucosidase, α-glucosidase, acid phosphatase and naphtol-AS-BI-phosphohydrolase. No activity was found for the following enzymes: valine arylamidase, β-galactosidase, esterase lipase C8, protease, urease, alkaline phosphatase, lipase C14, cystine arylamidase, trypsin, β-glucuronidase, α-chymotrypsin, α-galactosidase, β-glucosidase and α-mannosidase. No acid production was observed from D-glucose, D-lactose, D-sucrose, D-maltose, salicin, D-cellobiose, D-mannose, D-raffinose, D-sorbitol, D-trehalose, D-mannitol, D-xylose, L-arabinose, glycerol, D-melezitose and L-rhamnose. Only a few carbohydrates were metabolized: D-ribose, D-tagatose and potassium 5-ketogluconate as revealed by an API 50 CH strip. The other tested carbohydrates (D-melibiose, glycerol, glycogen, D-arabinose, L-arabinose, erythritol, D-xylose, L-xylose, D-galactose, D-adonitol, methyl-βD-xylopyranoside, D-glucose, D-fructose, D-mannose, L-sorbose, dulcitol, L-rhamnose, inositol, D-mannitol, D-sorbitol, methyl-αD-glucopyranoside, methyl-αD-mannopyranoside, *N*-acetylglucosamine, esculin ferric citrate, amygdalin, D-cellobiose, arbutin, salicin, D-maltose, D-sucrose, D-lactose, D-raffinose, D-trehalose, inulin, D-melezitose, starch, xylitol, gentiobiose, D-arabitol, L-arabitol, D-lyxose, D-turanose, D-fucose, L-fucose, potassium gluconate and potassium 2-ketogluconate) were not utilized.

Strain Marseille-P3110^T was susceptible to amoxicillin/clavulanic acid, clindamycin, metronidazole, imipenem, tobramycin, fosfomycin, erythromycin and amoxicillin but was resistant to rifampicin, gentamicin, vancomycin, colistin, trimethoprim/sulfamethoxazole, oxacillin and doxycycline. Differential characteristics between strain Marseille-P3110^T and close relatives are presented in Table 2. Strain Marseille-P3110^T differed from other compared species for several phenotypic characteristics including Gram staining and motility. All species were non−spore forming.

The most abundant cellular fatty acids were 9-octadecenoic acid (34%), hexadecanoic acid (30%), octadecanoic acid (16%) and 9,12-octadecadienoic acid (13%). Other fatty acids were present in lower amounts (Table 3).

Genome properties

The genome of Marseille-P3110^T is 2 607 061 bp long with 35.99% G+C content (Fig. 5 and Table 4). It is composed of nine scaffolds (composed of 14 contigs). Of the 2642 predicted genes, 2582 were protein-coding genes and 60 were RNAs (five genes are 5S rRNA, five genes are 16S rRNA, five genes are 23S rRNA, 45 genes are transfer RNA genes). A total of 1757 genes (68.05%) were assigned as putative function (by COGs or by NR BLAST). A total of 279 genes were identified as ORFans (10.81%). The remaining genes were annotated as hypothetical proteins (472 genes, 18.28%). The distribution of genes into COGs functional categories is presented in Table 5.

Genome comparison

Genomic characteristics of strain Marseille-P3110^T were compared to those of closely related species with an available genome: *Dielma fastidiosa* (DSM 26099; CAEN00000000), *Erysipelatoclostridium ramosum* (DSM 1402; ABFX00000000), *Faecalitalea cylindroides* (ATCC 27803; AWVI00000000), *Erysipelothrix rhusiopathiae* (ATCC 19414; ACLK00000000), *Solobacterium moorei* (F0204; AUKY00000000), *Eubacterium dolichum* (CAG:375; ABAW00000000), *Erysipelothrix tonsillarum* (DSM 14972; AREO00000000) and *Holdemanella biformis* (DSM 3989; ABYT00000000) (Table 6). The draft genome sequence of strain Marseille-P3110^T is smaller than that of *Dielma fastidiosa* and *E. ramosum* (3615, 2607 and 3235 MB respectively), but larger than that of *F. cylindroides*, *E. rhusiopathiae*, *S. moorei*, *E. dolichum*, *E. tonsillarum* and *H. biformis* (1946, 1746, 2008, 2025, 1931 and 2518 MB respectively). The G+C content of strain Marseille-P3110^T is smaller than that of *Solobacterium moorei*, *Eubacterium dolichum*, *Dielma fastidiosa*, *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* (35.99, 36.78, 37.4, 39.97, 36.47 and 36.83% respectively), but larger than that of *F. cylindroides*, *E. ramosum* and *H. biformis* (34.68, 31.39 and 33.79% respectively) (Table 6).

The gene content of strain Marseille-P3110^T is smaller than that of *Dielma fastidiosa* and *E. ramosum* (3319, 2582 and

TABLE 7. Numbers of orthologous proteins shared between genomes (upper right) and AGIOS values obtained (lower left)

	Tm	Fc	Ed	Er	Et	Hb	Sm	Era	Df
Tm	**2582**	769	696	605	614	757	663	768	940
Fc	57.72	**2057**	696	534	541	851	606	723	797
Ed	59.24	59.86	**1927**	484	501	691	529	632	785
Er	58.60	57.06	57.12	**1645**	769	527	567	579	647
Et	62.91	56.24	56.25	70.60	**1792**	544	580	590	654
Hb	55.70	60.78	57.01	56.11	54.85	**2529**	614	722	800
Sm	58.87	57.70	58.21	57.81	58.48	56.99	**2035**	595	708
Era	59.80	56.40	56.80	57.56	58.29	57.36	56.95	**3169**	836
Df	59.29	56.88	58.13	56.47	56.39	55.88	56.07	56.88	**3319**

Number of proteins per genome is indicated in bold.
Df, *Diema fastidiosa* DSM 26099; Ed, *Eubacterium dolichum* CAG, 375; Er, *Erysipelothrix rhusiopathiae* ATCC 19414; Era, *Erysipelatoclostridium ramosum* DSM 1402; Et, *Erysipelothrix tonsillarum* DSM 14972; Fc, *Faecalitalea cylindroides* ATCC 27803; Hb, *Holdemanella biformis* DSM 3989; Sm, *Solobacterium moorei* F0204; Tm, *Traorella massiliensis* Marseille-P3110^T.

TABLE 8. Pairwise comparison of *Traorella massiliensis* strain Marseille-P3110^T with other species using GGDC, formula 2 (DDH estimates based on identities/HSP length)[a]

	Traorella massiliensis	*Faecalitalea cylindroides*	*Erysipelothrix rhusiopathiae*	*Solobacterium moorei*	*Eubacterium dolichum*	*Erysipelothrix tonsillarum*	*Erysipelatoclostridium ramosum*	*Holdemanella biformis*	*Dielma fastidiosa*
Traorella massiliensis	100% ± 00	24% ± 2.40	27.8% ± 2.45	23.3% ± 2.35	25.5% ± 2.40	20.2% ± 2.35	23.1% ± 2.35	23.9% ± 2.40	22.0% ± 2.35
Faecalitalea cylindroides		100% ± 00	16.50% ± 2.20	15.10% ± 2.15	23.4% ± 2.40	44.10% ± 2.55	17.9% ± 2.25	23.4% ± 2.40	17.5% ± 2.20
Erysipelothrix rhusiopathiae			100% ± 00	27.89 ± 2.40	28.4% ± 2.45	22.80% ± 2.40	24.6% ± 2.35	26.5% ± 2.40	24.2% ± 2.35
Solobacterium moorei				100% ± 00	23.5% ± 2.35	37.30% ± 2.50	21.7% ± 2.35	16.20% ± 2.20	28.1% ± 2.45
Eubacterium dolichum					100% ± 00	28% ± 2.40	22.5% ± 2.40	30.1% ± 2.45	22.20% ± 2.4
Erysipelothrix tonsillarum						100% ± 00	25.9% ± 2.40	29.0% ± 2.40	18.4% ± 2.3
Erysipelatoclostridium ramosum							100% ± 00	19.4% ± 2.25	27.3% ± 2.4
Holdemanella biformis								100% ± 00	21.2% ± 2.3
Dielma fastidiosa									100% ± 00

DDH, DNA-DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; HSP, high-scoring segment pairs.
[a]Confidence intervals indicate inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test data sets (which are always limited in size).

3169 respectively), but larger than that of *F. cylindroides, E. rhusiopathiae, S. moorei, E. dolichum, E. tonsillarum* and *H. biformis* (2057, 1645, 2035, 1927, 1792 and 2529 respectively). Distribution of genes into COGs categories was similar among all compared genomes (Fig. 6).

Among species with standing in nomenclature, AGIOS values ranged from 54.85 between *H. biformis* and *E. tonsillarum* to 70.60 between *E. tonsillarum* and *E. rhusiopathiae*. When compared to strain Marseille-P3110^T, AGIOS values ranged from 55.70 with *H. biformis* to 62.91 with *E. tonsillarum* (Table 7). Among species with standing in nomenclature, dDDH values ranged from 15.10% between *F. cylindroides* and *S. moorei* to 44.10% between *F. cylindroides* and *E. tonsillarum*. dDDH values between strain Marseille-P3110^T and compared species ranged from 20.20% with *E. tonsillarum* to 27.80% with *E. rhusiopathiae* (Table 8).

Conclusion

Considering the specific phenotypic properties of strain Marseille-P3110^T, including its low matching MALDI-TOF MS score, the 93.2% 16S rRNA similarity level with *Dielma fastidiosa,* and its genomic analysis, we propose the creation of a new genus within the family *Erysipelotrichaceae,* named *Traorella,* with *Traorella massiliensis* as type species and strain Marseille-P3110^T as type strain.

Description of *Traorella* gen. nov.

Traorella (tra.o.rel'la, N.L. fem. gen. n., *Traorella,* 'of Traore,' the family name of Sory Ibrahima Traore, a Malian microbiologist, for his contribution to the description of the human gut microbiota). Bacteria belonging to this genus are strictly anaerobic, non−spore forming, motile, Gram negative and rod shaped. The type species is *Traorella massiliensis.*

Description of *Traorella massiliensis* sp. nov.

Traorella massiliensis (mas.si.li.en'sis, L. masc. adj., *massiliensis,* 'of Massilia,' the Latin name of Marseille, where strain Marseille-P3110^T was isolated) is an anaerobic Gram-negative and motile bacilli with a mean length of 2.8 µm and a mean diameter of 0.33 µm. Strain Marseille-P3110^T forms regular white colonies with a mean diameter of 1 mm. This bacterium is catalase and oxidase negative, and non−spore forming. Optimal growth was observed after 72 hours of incubation in anaerobic conditions at 37°C. Strain Marseille-P3110^T was susceptible to amoxicillin/

clavulanic acid, clindamycin, metronidazole, imipenem, tobramycin, fosfomycin, erythromycin and amoxicillin but was resistant to rifampicin, gentamicin, vancomycin, colistin, trimethoprim/sulfamethoxazole, oxacillin and doxycycline.

The major cellular fatty acid was 9-octadecenoic acid. The genome of strain Marseille-P3110^T is 2 607 061 bp long with 35.99% G+C content. The 16S rRNA and genome sequences are available in the European Bioinformatics Institute/European Molecular Biology Laboratory (EBI/EMBL) database under accession numbers LT615365 and FNLJ00000000, respectively. The type strain Marseille-P3110^T (= CSUR P3110 = DSM 103514) was isolated from the left colon cleansing of a 76-year-old Frenchwoman.

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Conflict of interest

None declared.

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