Anaerosphaera massiliensis sp. nov., a new bacterium isolated from the stool of a 39-year-old Pygmy

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Abstract

Anaerosphaera massiliensis strain Marseille-P4592^T (= CSURP4592^T; = CCUG72452^T) is a new species isolated from the stool of a 39-year-old male Pygmy from the Democratic Republic of Congo.

Keywords: *Anaerosphaera massiliensis*, culturomics, new species, stool, taxono-genomics
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Introduction

Culturomics is the concept of developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once isolated, we used a taxono-genomics approach including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description and genome sequencing, to describe it [5,6].

Isolation and growth conditions

In 2017, we isolated from the stool sample of a healthy 39-year-old male Pygmy an unidentified bacterial strain. A screening was performed by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectrum (Fig. 1) was imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in two databases (Bruker and the constantly updated MEPHI databases; https://www.mediterranee-infection.com/urms-data-base/). The spectrum from strain Marseille-P4592 was compared with 71 other species from the genus of *Peptoniphilus* and one from the genus *Tissierella* (Fig. 2). The study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 2016-011. The growth was obtained 24 h after culture in a Colombia agar enriched with 5% sheep blood (bioMérieux, Marcy l'Etoile, France) under anaerobic conditions at 37°C.

Phenotypic characteristics

Colonies were white with a mean diameter of 1 mm. Bacterial cells were Gram-positive, round-shaped, with a diameter ranging from 662 to 763 nm (Fig. 3). Strain Marseille-P4592 showed negative catalase and oxidase activities. API 50 CH and API ZYM tests were performed at 37°C under anaerobic conditions. Using an API ZYM strip, a positive reaction was observed for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase and naphthol-AS-BI-phosphohydrolase but negative reactions were obtained for alkaline and acid phosphatases, lipase, trypsine, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase,

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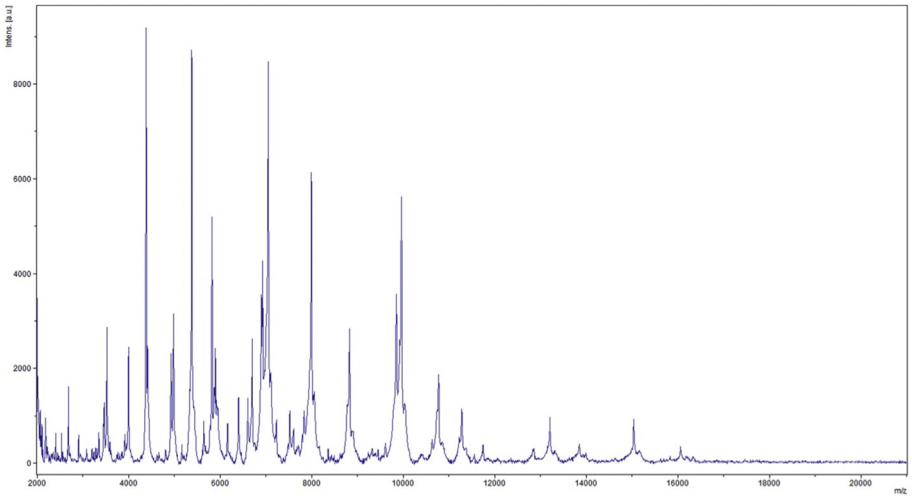

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

α-mannosidase and α-fucosidase enzymes. Using an API 50 CH strip, strain Marseille-P4592 was able to metabolize esculin, D-tagatose and potassium 5-ketogluconate. However, negative reactions were obtained for glycerol, D-glucose, D-galactose, D-maltose, D-fructose, D-mannose, methyl-αD-glucopyranoside, *N*-acetylglucosamine, D-lactose, D-saccharose, D-trehalose, D-turanose, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-βD-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-mannopyranoside, amygdalin, arbutin, salicin, D-

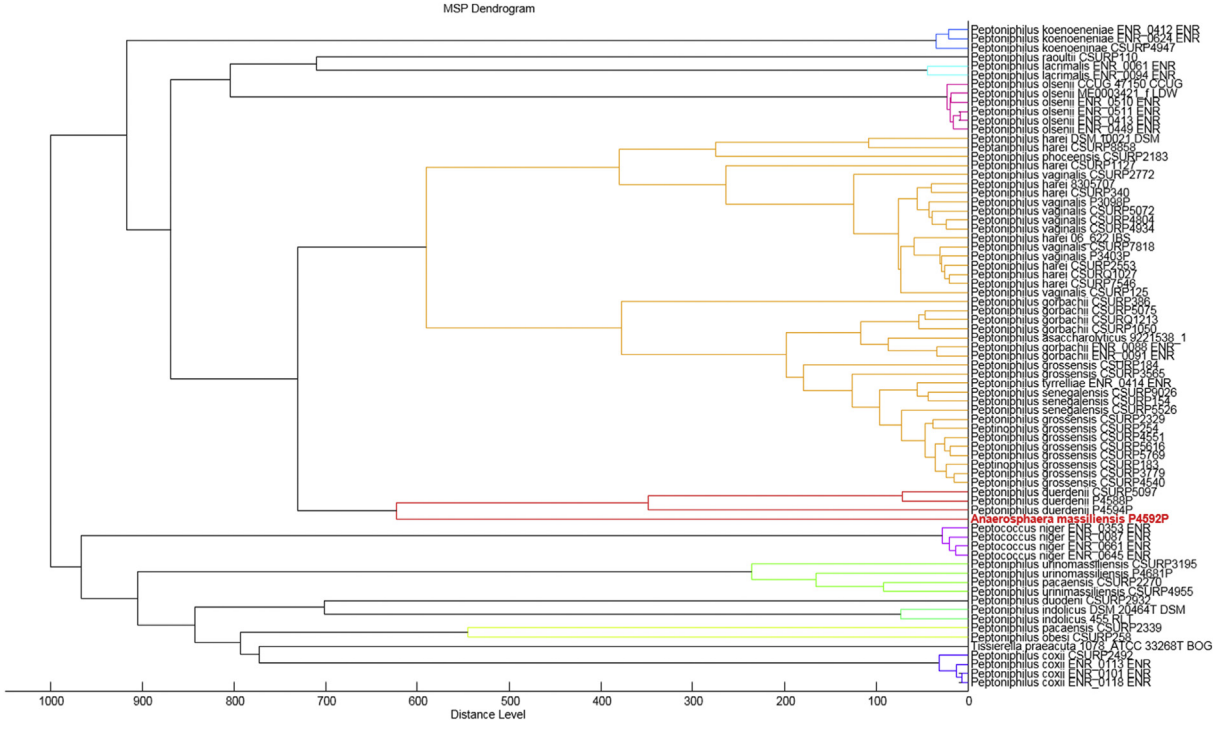

FIG. 2. Dendrogram based on the comparison of the *Anaerosphaera massiliensis* strain Marseille-P4592^T MALDI-TOF reference spectrum (in red), 71 other species of the genus of *Peptoniphilus* and one from the genus *Tissierella*.

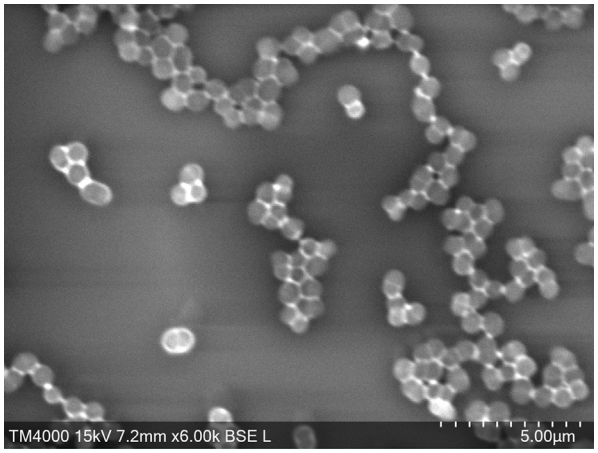

FIG. 3. Scanning electron micrograph of *Anaerosphaera massiliensis* sp. nov. strain Marseille-P4592^T using the Tabletop Microscope TM4000Plus from Hitachi. Scale bar and acquisition settings are shown on the original micrograph.

cellobiose, D-melibiose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 2-ketogluconate. The main biochemical characteristics of strain Marseille-P4592 and its closest species with standing in nomenclature are detailed in Table 1.

Strain identification

The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification and sequencing were performed using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary 3500xL Genetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France), respectively, as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com). Strain Marseille-P4592 exhibited a 95.39% sequence identity with *Anaerosphaera aminiphila* strain WN036 (GenBank accession number NR041586), the phylogenetically closest species with standing in nomenclature (Fig. 4). Consequently, strain Marseille-P4592 was classified as a new member of the genus *Anaerosphaera*, family *Peptoniphilaceae*, phylum *Firmicutes*, with the stain Marseille P4592^T as the type strain of the new species *Anaerosphaera massiliensis*.

TABLE 1. **Comparative biochemical characteristics of (1) *Anaerosphaera massiliensis*, (2) *Anaerosphaera aminiphila*, (3) *Peptoniphilus asaccharolyticus*, (4) *Peptoniphilus indolicus*, (5) *Peptoniphilus coxii*, (6) *Peptoniphilus duerdenii*, (7) *Tepidimicrobium xylanilyticum*, and (8) *Tissierella creatinini* [19–24]**

Characteristic	1	2	3	4	5	6	7	8
Indole	–	–	w	+	–	–	+	–
Urease	–	–	–	–	–	–	–	–
Alkaline phosphatase	–	–	–	+	–	–	–	–
Coagulase	–	–	–	+	–	–	–	–
Glucose	–	–	–	–	–	–	+	–
Lactose	–	–	–	–	–	–	–	–
Raffinose	–	–	–	–	–	–	–	–
Mannose	–	–	–	–	–	–	–	–
α-galactosidase	–	–	–	–	–	–	–	–
β-galactosodase	–	–	–	–	–	–	–	–
α-glucosidase	–	–	–	–	–	–	–	–
β-glucosidase	–	–	–	–	–	–	–	–
Arginine arylamidase	–	–	+	+	–	–	–	–
Proline arylamidase	–	–	–	–	+	–	–	–
Phenylalanine arylamidase	–	–	–	+	–		–	–
Leucine arylamidase	+	–	w	+	–	+	–	–
Pyroglutamyl arylamidase	–	–	–	–	–	–	–	–
Histidine arylamidase	–	–	w	+	–	–	–	–
β–glucuronidase	–	–	–	–	–	–	–	–
Tyrosine aramylidase	–	–	–	+	–	–	–	–

W, weak

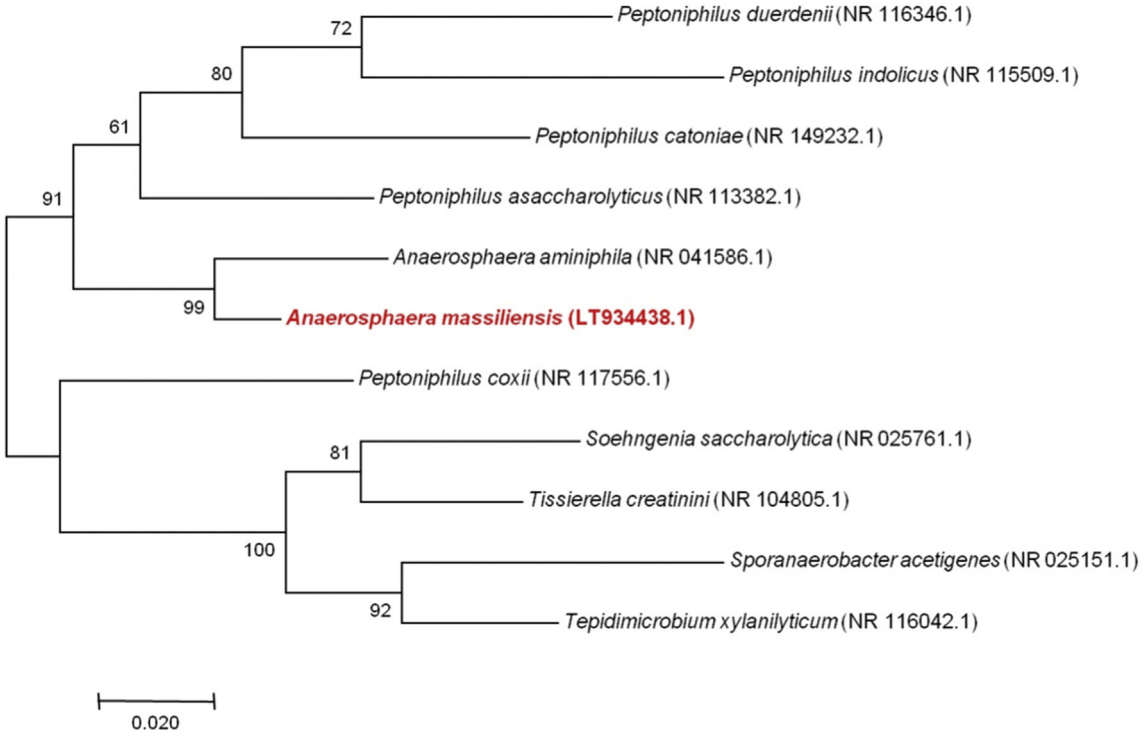

FIG. 4. Phylogenetic tree showing the position of *Anaerosphaera massiliensis* sp. nov. strain Marseille P4592T relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequence alignments and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit, then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit (Illumina), as previously described [9–12]. Genome assembly was performed with a pipeline incorporating different softwares (SPADES [13]), on trimmed (TRIMMOMATIC [14]) or raw data. GAPCLOSER was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value < 25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds: 56, number of contigs: 56). The genome of strain Marseille-P4592^T is 2 064 271 bp long with a 28.1 mol% G + C content. Genomic characteristics of strain Marseille-P4592 and its closest species with standing in nomenclature are summarized in Table 2. The degree of genomic similarity of strain Marseille-P4592^T with closely related species was estimated using the ORTHOANI software

TABLE 2. **Genomic characteristics of *Anaerosphaera massiliensis* sp. nov. and the seven most closely related bacterial taxa for which genome sequences are available**

Type strains	Accession number	Size (Mb)	GC %	Gene content
Anaerosphaera aminiphila	FQXI00000000	2.02	31.5	1960
Anaerosphaera massiliensis	CABHLS01000001 –CABHLS01000061	2.06	28.1	2041
Peptoniphilus asaccharolyticus	FWWR00000000	2.23	32.3	2268
Peptoniphilus coxii	LSDG00000000	1.83	44.6	1783
Peptoniphilus duerdenii	AEEH00000000	2.08	34.2	2018
Peptoniphilus indolicus	AGBB00000000	2.24	31.6	2145
Tepidimicrobium xylanilyticum	FNNG00000000	3.00	33.2	3094
Tissierella creatinini	SUSS00000000	2.61	35.7	2581

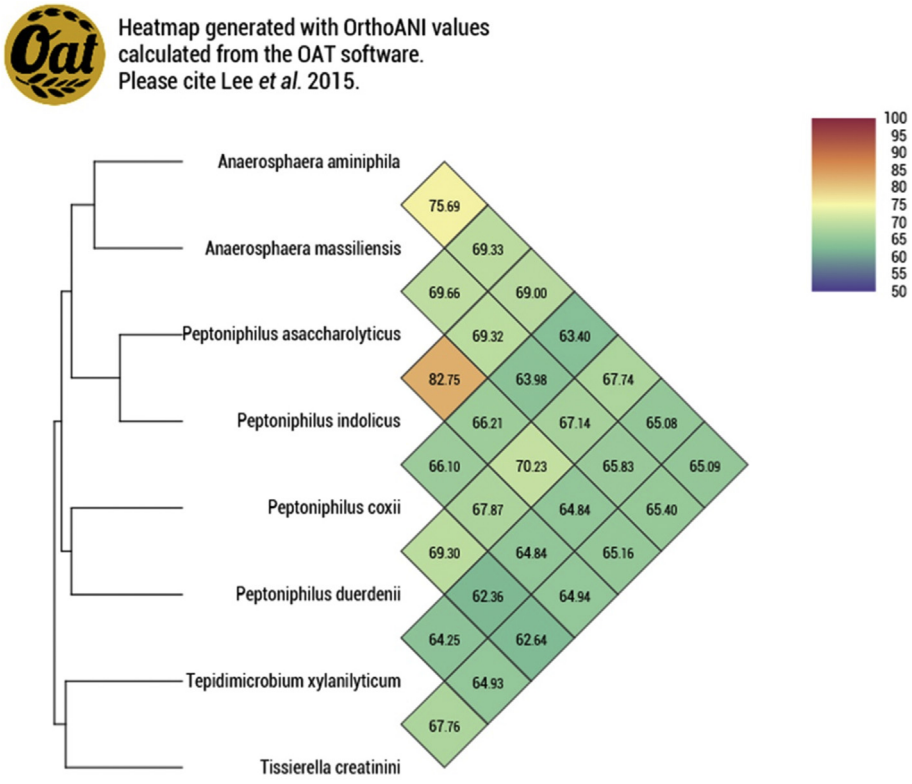

FIG. 5. Heatmap generated with OrthoANI values calculated using the OAT software between *Anaerosphaera massiliensis* sp. nov. and other closely related species with standing in nomenclature.

[15–17]. Values among closely related species (Fig. 5) ranged from 62.36% between *Peptoniphilus coxii* and *Tepidimicrobium xylanilyticum* to 82.75% between *Peptoniphilus asaccharolyticus* and *Peptoniphilus indolicus*. When the isolate was compared with these closely related species, values ranged from 63.98% with *P. coxii* to 75.69% with *Anaerosphaera aminiphila*. An average nucleotide identity value < 95% suggesting that two strains belong to distinct species. The degree of genomic similarity of strain Marseille-P4592 with closely related species was calculated also using the digital DNA–DNA hybridization software [18]. Values among closely related species (Table 3) ranged from 17.7% between strain Marseille-P4592 and *P. asaccharolyticus* to 41.8% between *P. coxii* and *P. indolicus* (Table 3). When strain Marseille-P4592 was compared with these closely related species, values ranged from 17.7% with *P. asaccharolyticus* to 27.3% with *Tissierella creatinini* (Table 3). These values are lower than the 70% threshold used for delineating prokaryotic species, which confirmed that this strain represents a new species. Core-genome-based phylogenetic relationships of strain Marseille-P4592^T and the closest species with standing in nomenclature confirmed the phylogenetic position of strain Marseille-P4592^T within *Anaerosphaera* cluster (Fig. 6). Therefore, the genomic analysis suggested that strain Marseille-P4592^T was classified as a

TABLE 3. Digital DNA–DNA hybridization values (%) obtained by comparison of all studied genomess

	1	2	3	4	5	6	7	8
1- *Anaerosphaera aminiphila*	100	19.2	19.2	32.3	33.2	18.9	22.6	30.7
2- *Anaerosphaera massiliensis*		100	17.7	25.5	20.6	18.1	24.8	27.3
3- *Peptoniphilus asaccharolyticus*			100	35.4	32.9	26.4	20.2	33.1
4- *Peptoniphilus coxii*				100	38.3	41.8	25.4	17.5
5- *Peptoniphilus duerdenii*					100	25.4	25.9	35.7
6- *Peptoniphilus indolicus*						100	20.2	31.1
7- *Tepidimicrobium xylanilyticum*							100	18.8
8- *Tissierella creatinini*								100

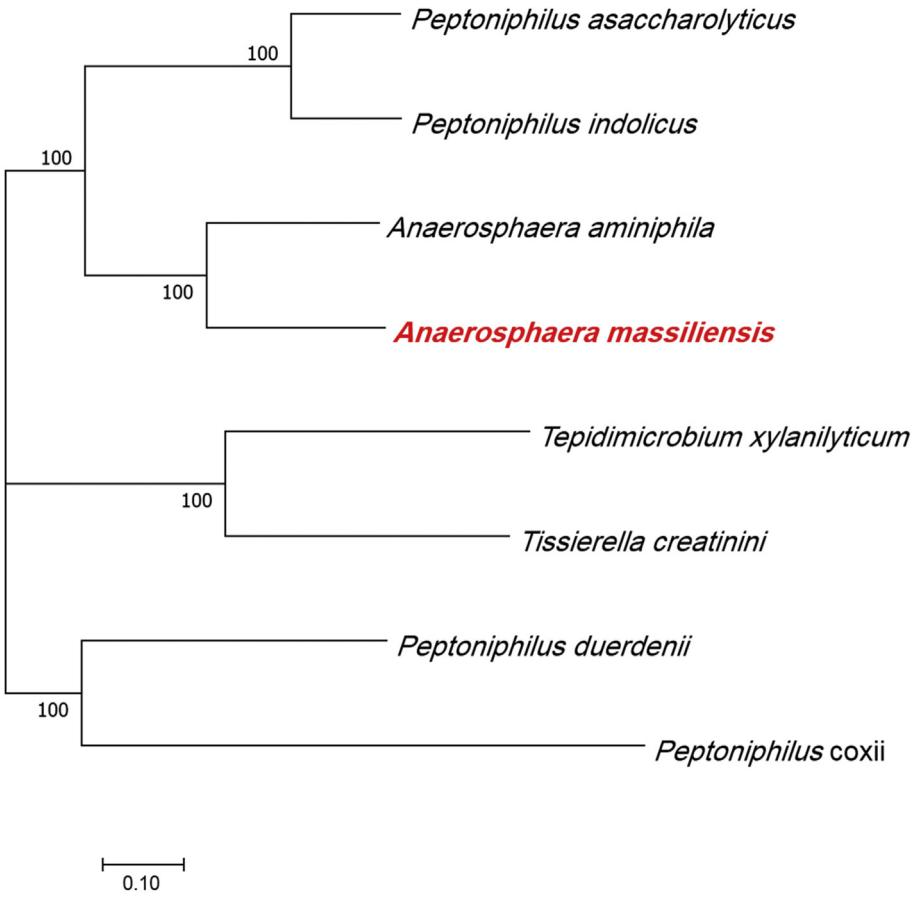

FIG. 6. Phylogenetic tree showing the core genome-based phylogeny between *Anaerosphaera massiliensis* sp. nov. strain Marseille-P4592 (in red) and other closely related species with standing in nomenclature. The genomes were annotated with PROKKA and these annotations were used by ROARY with identity of 50% to identify the pangenome of strains, including 337 core genes, as previously described [16]. Phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree.

member of a new species within the genus *Anaerosphaera*, family *Peptoniphilaceae*, phylum *Firmicutes*.

Conclusion

Strain Marseille-P4592^T exhibiting a 16S rRNA sequence identity <98.65%, an average nucleotide identity value < 95% and a digital DNA–DNA hybridization value < 70% with its phylogenetically closest species with standing in nomenclature, is consequently proposed as the type strain of the new species *Anaerosphaera massiliensis* sp. nov.

Description of *Anaerosphaera massiliensis* sp. nov

Anaerosphaera massiliensis (mas.si.li.en'sis; L. masc. adj. massiliensis for Massilia, the Roman name of Marseille, where strain Marseille-P4592 was first isolated). Cells are anaerobic, Gram-positive, with a mean diameter of 1 mm. Catalase and oxidase activities are negative. Cells are round-shaped with a diameter ranging from 662 to 763 nm. Colonies grown on 5% sheep blood-enriched Columbia agar (bioMérieux) are white after 24 hours of incubation in a strict anaerobic atmosphere. Growth occurs at 37°C. Cells grow anaerobically only. Using an API ZYM strip, a positive reaction was observed for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase and naphthol-AS-BI-phosphohydrolase but negative reactions were obtained for alkaline and acid phosphatases, lipase, trypsine, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *α*-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase enzymes. Using an API 50 CH strip, strain Marseille-P4592^T was able to metabolize esculin, D-tagatose and potassium 5-ketogluconate. However, negative reactions were obtained for glycerol, D-glucose, D-galactose, D-maltose, D-fructose, D-mannose, methyl-αD-glucopyranoside, *N*-acetylglucosamine, D-lactose, D-saccharose, D-trehalose, D-turanose erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-βD-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-mannopyranoside, amygdalin, arbutin, salicin, D-cellobiose, D-melibiose, inulin, D-

melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 2-ketogluconate. The genome is 2 064 271 bp long and its G + C content is 28.1%.

The type strain, Marseille-P4592^T, isolated from a the stool sample of a healthy 39-year-old male Pygmy, was deposited in the CSUR and CCUG collections under accession numbers CSUR P4592 and CCUG 72 452, respectively. The 16S rRNA and genome sequences are available in GenBank under accession numbers LT934438 and CABHLS010000001–CABHLS010000061, respectively.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT934438 and CABHLS010000001–CABHLS010000061, respectively.

Deposit in culture collections

Strain Marseille-P4592^T was deposited in two different strain collections under numbers (= CSURP4592^T; = CCUG72452^T).

Conflict of interest

We have no conflict of interest to declare.

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