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Research article

Peptostreptococcus faecalis sp. nov., new bacterial species isolated from healthy indigenous congolese volunteer

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ABSTRACT

The Microbial Culturomics Project aiming to discover several bacterial species made it possible to isolate the strain Marseille-P4308^T from a stool sample of a healthy indigenous Congolese volunteer. Strain Marseille-P4308^T is a Gram-positive coccus shaped bacterium that optimally grows at 37 °C. The 16S rRNA gene sequence of the strain has a 96.2% sequence similarity to *Peptostreptococcus anaerobius* strain NCTC 11460^T (GenBank accession number: NR_042847.1). In addition, the average nucleotide identity of strain Marseille-P4308^T with its closest related species was 71.1%, which was far below the recommended threshold (>95–96%). The genome of the strain Marseille-P4308^T has a length of 2.14 Mbp with G + C content of 30.4 mol%. Based on phenotypic, biochemical, genomic and phylogenetic analysis, strain Marseille-P4308^T (= CSUR P4308 = CECT 9960) clearly appears to be a new species for which the name *Peptostreptococcus faecalis* sp. nov., is proposed.

1. Introduction

Different genera of anaerobic cocci bacteria are involved in a broad range of infections, occurring in all parts of the human body [1]. In various environments, members of the *Peptostreptococcaceae* family belonging to the order *Clostridiales*, phylum *Firmicutes* can be found in the human body, manure, soil and sediment [2]. The closest phylogenetic neighbours to this family belong to the genera *Alkaliphilus*, *Natronincola*, and *Tindallia* [2]. The species of the genus *Peptostreptococcus* are commensal species that colonise almost every mucosal human tissue, forming a part of the intestinal, urinary, vaginal, oral tract microbiotas and also the skin. They are pathogenic under certain circumstances and can cause bacteraemia and abscesses in different organs [3].

The genus *Peptostreptococcus* is a group of Gram-positive, mostly anaerobic cocci species that are very diverse phenotypically and phylogenetically [4]. Cells measure between 0.3 and 2.0 μm and are arranged in chains, pairs, tetrads or masses. Some are aero-tolerant but do not form spores. Their ability to use carbohydrates varies considerably. The main source of energy would be the products of protein metabolism [1]. However, the pathogenic character of some members of the genus *Peptostreptococcus* is complex to determine, because some members are part of the normal microflora, while species such as *Peptostreptococcus anaerobius* and *Peptostreptococcus stomatis* have been frequently identified from clinical samples of diseased individuals [5, 6, 7]. Indeed, *Peptostreptococcus russellii* was isolated in environment as a storage pit [8].

Numerous previously uncultured members of the human microbiome have been isolated by the culturomics method [9]. Isolated species are then described by multiphasic approach including a MALDI-TOF MS identification, 16S rRNA gene sequencing and the phenotypic and biochemical analysis of the strain [10].We report here a taxonogenomic description of *Peptostreptococcus faecalis* sp. nov., strain Marseille-P4308^T isolated from human gut microbiota.

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2. Material and methods

2.1. Ethics and sample collection

The stool sample was collected from a healthy indigenous Congolese volunteer living in Bene Gain (1°59′24.7″S 15°52′18.1″E), in the Republic of the Congo, in August 2015 (supplementary Figure S1) [11]. Approval for this study was obtained from the Ministry for Health of the Republic of Congo (000208/MSP/CAB.15 du Ministère de la Santé et de la Population, 20 August 2015). The study was also approved by the ethics committee of the Institut Fédératif de Recherche IFR48, Faculty of Medicine, Marseille, France, under reference number 09–022. Prior to sampling, an informed consent form was obtained from each individual. In the presence of representatives from a local health centre and village elders, full information was given orally in the local language (Lingala) to ensure that the project was fully understood, as the participants were illiterate.

2.2. Strain isolation and growth conditions

One gram of the stool sample was pre-incubated in a blood culture bottle containing rumen and sheep blood, as described above for culturing human samples [12]. The culture medium was serially diluted and inoculated on Columbia agar with 5% sheep blood (BioMérieux, Marcy-L'Etoile, France). All pure colonies obtained by culture were identified by MALDI-TOF MS [13]. If, despite the good quality of the bacterial spectrum, the bacteria could not be identified, sequencing of the 16S rRNA gene of the bacterium was performed for identification.

2.3. 16S rRNA sequencing and phylogenetic analysis

The DNA of the bacterial strain was extracted using the EZ1 (Qiagen, Venlo, The Netherlands) DNA tissue kit on the EZ1 (Qiagen) automate. The universal primers fD1 and rP2 (Eurogentec, Angers, France) were used to amplify the16S rRNA gene sequence. Sequencing was performed using the BigDye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary sequencer (Thermo Fisher, Saint-Aubin, France), as previously described [14]. Using Codon Code Aligner software (http://www.codoncode.com), the 16S rRNA nucleotide sequences were assembled and corrected. Consensus sequence from 16S rRNA gene sequencing was compared by BLASTn within the NCBI 16S rRNA database (https://blast.ncbi.nlm.nih.gov/). In order to create a robust phylogenetic tree, the 16S rRNA sequences of species with a validly published name were downloaded from the LPSN website (https://lpsn.dsmz.de/). Using MEGA X software [15], the sequences were aligned and a phylogenetic tree was constructed with 1,000 bootstrap replicates.

2.4. Genome annotation and comparison

Genomes of closely related species were downloaded from the GenBank database and annotated with Prokka software v1.14.6 [16]. Coding sequences were predicted using Prodigal software 2.6 [17], then the predicted bacterial protein sequences were searched against the GenBank (https://www.ncbi.nlm.nih.gov/genbank/) database using BLASTp. ARAGORN software 1.2 [18] was used to find tRNA and mRNA genes, whereas rRNA genes were predicted with Barrnap software 0.4.

The genome of strain Marseille-P4308^T was compared to the genomes of the following closely related species, including *Peptostreptococcus anaerobius* NCTC11460^T (GenBank accession: UGTB00000000), *Peptostreptococcus russellii* RT-10B^T (JYGE00000000), *Peptostreptococcus stomatis* DSM 17678^T (ADGQ00000000), *Peptostreptococcus canis* DSM 27025^T (JABGBW000000000), *Asaccharospora irregularis* DSM 2635^T (FQWX00000000), *Clostridioides difficile* ATCC 9689^T (AUOX00000000), *Clostridioides mangenotii* LM2 (JIAA00000000) and *Clostridium hiranonis* DSM 13275^T (CP036523). The DNA-DNA hybridisation (dDDH) was calculated to assess similarity between studied genome sequences using the online server of Genome-to-Genome Distance Calculator (GGDC 2.1) (http://ggdc.dsmz.de/), taking into account the method and formula two, as suggested [19, 20]. Furthermore, the genomic average nucleotide identity (ANI) was determined using OrthoANI software v0.93.1 [21]. Clusters of Orthologous genes (COGs) were detected by performing BLASTp of genomes against the COG database [22].

2.5. Conditions of growth

The strain was grown under different conditions. In terms of temperature, the growth of the strain was evaluated at room temperature, 28 °C, 37 °C, 45 °C and 55 °C and incubated under aerobic, anaerobic and microaerophilic conditions on Colombia agar enriched with 5% sheep blood (BioMérieux), using the GENbag anaer and GENbag microaer systems (ThermoFisher Scientific, Basingstoke), respectively. To determine the tolerated salt concentration and the optimum pH for growth, the strain was cultured in different media with varied pH (6, 6.5, 7 and 8.5) and at different NaCl concentrations (5, 10, 50, 75 and 100 g/L NaCl) at 37 °C under anaerobic conditions.

2.6. Phenotypic characteristics

Motility test and Gram staining of the strain were verified using a DM1000 photomicroscope (Leica Microsystems, Nanterre, France) under a 100× objective. In addition, a bacterial suspension was fixed with a 2.5% glutaraldehyde solution at 0.1 mol/L with the aim of observing the morphology of cells using a Hitachi TM4000 electron microscope (Hitachi Group, Krefeld, Germany). Spore-forming was investigated by exposing a bacterial suspension for 10 min under thermal shock at 80 °C. Multiple biochemical criteria from strain Marseille-P4308^T were revealed using API tests (50CH, ZYM and 20A; bioMérieux). Oxidase and catalase reactions were determined using a BD BBL™ DrySlide (Becton Dickinson, Le Pont-de-Claix, France).

2.7. Antibiotic susceptibility

The E test method was used to obtain an antimicrobial sensitivity profile and minimum inhibitory concentration (µg/mL) of the strain Marseille-P4308^T [23]. Antimicrobial discs placed on a blood agar Petri dish were amikacin, amoxicillin, benzylpenicillin, ceftazidime, ceftriaxone, ciprofloxacin, clindamycin, daptomycin, erythromycin, imipenem, linezolid, metronidazole, minocycline, rifampicin, teicoplanin, tigecycline, trimethoprim/sulfamethoxazole, tobramycin and vancomycin.

3. Results

3.1. Identification and classification

Analysis of the 16S rRNA gene sequence on NCBI Blast showed that the Marseille-P4308^T strain has the highest similarity score of 96.2% to *Peptostreptococcus anaerobius* strain NCTC 11460^T (GenBank accession number: NR_042847.1). This value obtained is below the threshold value recommended for delimiting a new species of prokaryote [24]. Therefore, the strain Marseille-P4308^T is presumed to be a potential new bacterial species belonging to the genus *Peptostreptococcus* within the *Peptostreptococcaceae* family and phylum *Firmicutes*. The phylogenetic tree constructed on the basis of the sequences of the 16S rRNA gene shows the strain Marseille-P4308^T anchored in the *Peptostreptococcus* sp group with closely related species (Figure 1). The position is confirmed with the phylogenomic tree basing on the genomic sequences of the closely related species (Figure 2).

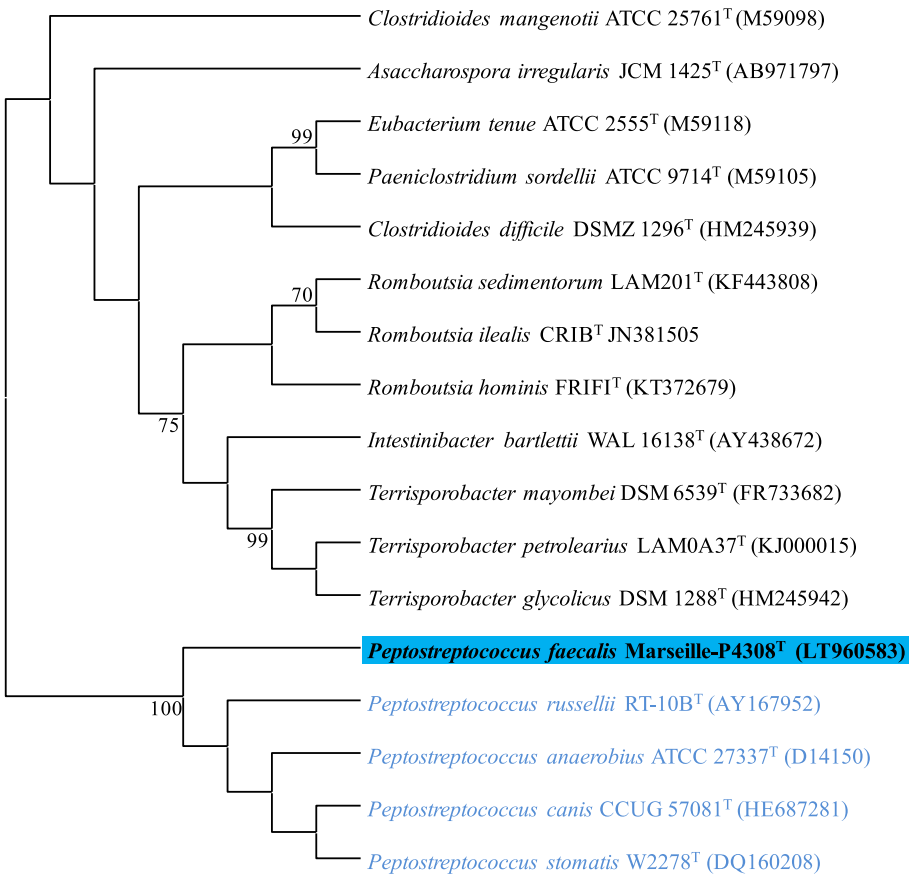

Figure 1. Phylogenetic tree with the 16S rRNA gene sequences indicating the position of *Peptostreptococcus faecalis* sp. nov. Marseille-P4308^T among other closely related species. Sequences were aligned using MUSCLE, and phylogenetic inferences were obtained using the Neighbor-Joining method [29] within the MEGA X software [15]. Accession numbers are indicated in parenthesis.

3.2. Phenotypic characteristics

Strain Marseille-P4308^T grows under anaerobic and micro-aerobic conditions between 25 °C and 42 °C, with an optimal growth at 37 °C. It is a Gram-positive coccus-shaped bacterium, non-motile, non-spore-forming, oxidase-negative and catalase-negative. Using a Hitachi electron microscope, the strain's morphology is highlighted; cells had a mean length of 0.84 µm and a mean diameter of 0.7 µm (supplementary Figure S2). Furthermore, this strain was able to grow on media at a pH between 6 and 8.5 and tolerated NaCl concentration up to 50 g/L. Colonies were smooth, convex and regular in appearance, with a mean diameter of 0.5 mm on Columbia Agar with 5% Sheep Blood (bioMérieux).

Using API 50CH, the following carbohydrates are fermented: glycerol, erythritol, D-arabinose, D-ribose, L-xylose, D-adonitol, methyl ß-D-xylo-pyranoside, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, D-cellobiose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-tagatose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 5-ketogluconate. Negative reactions were observed for D-

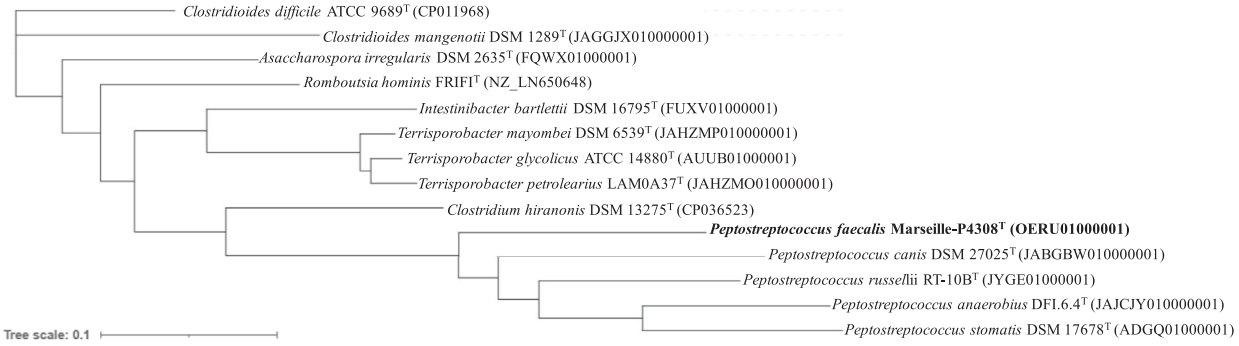

Figure 2. Phylogenomic tree basing on genomic sequences of *Peptostreptococcus faecalis* sp. nov., Marseille-P4308^T and other its related species. Sequences were concatenated with union EMBOSS (https://www.bioinformatics.nl/cgi-bin/emboss/union) and aligned by Mugsy software [30]. The tree was built using iTOL an online bioinformatic tool [31].

Table 1. Differential characteristics of Marseille-P4308^T, *Peptostreptococcus stomatis* W2278^T [5], *Peptostreptococcus canis* CCUG 57081^T [27], and *Peptostreptococcus anaerobius* ATCC 27337^T [28].

Characteristics	Marseille-P4308^T	W2278^T	CCUG 57081^T	ATCC 27337^T
Oxygen requirement	Strictly anaerobic	Strictly anaerobic	Facultative anaerobic	Strictly anaerobic
Acid production from:				
D-Cellobiose	+	−	−	+
α-Galactosidase	+	+	−	+
D-Glucose	−	+	−	+
α-Glucosidase	+	+	NP	+
D-Lactose	−	−	−	+
D-Mannose	+	−	+	NP
D-Raffinose	+	−	−	NP
Sucrose	−	−	−	+
G + C content (mol%)	30.4	36	30.8	34–36
Habitat	Human	Human	Dog	Cat

NP, not performed.

glucose, D-lactose, D-maltose, D-mannitol, D-turanose, D-xylose, salicin and sucrose. Indeed, the use of API ZYM strips revealed positive reactions for esterase, leucine arylamidase, cystine arylamidase, α-glucosidase and α-galactosidase, while the following tests, such as alkaline phosphatase, esterase lipase, lipase (C14), valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase were negative. Finally, the use of API 20A strips shows that strain Marseille-P4308^T was positive for gelatin and esculin ferric citrate, while it was negative for tryptophan, urea, D-glucose, D-mannitol, D-lactose, D-sucrose, D-maltose, salicin, and D-xylose. The comparison of the main phenotypic and chemical differences between strain Marseille-P4308^T and its phylogenetically related species is given in Table 1.

Antimicrobial susceptibility testing of strain Marseille-P4308^T showed the following minimal inhibitory concentrations (in parenthesis) for the following antibiotics: ceftazidime (0.75 μg/mL), trimethoprim (0.25 μg/mL), daptomycin (0.016 μg/mL), tobramycin (8 μg/mL), benzylpenicillin (5 μg/mL), ciprofloxacin (1 μg/mL), ceftriaxone (0.5 μg/mL), linezolid (0.094 μg/mL), ciprofloxacin (0.75 μg/mL), clindamycin (0.064 μg/mL) and amikacin (16 μg/mL).

3.3. Genome properties and comparison

Strain Marseille-P4308^T has a genome size of 2,145,294 bp, assembled into 29 contigs with 30.4 mol% G + C content. Its genome possesses 2,095 predicted genes, of which 2,031 are protein-coding genes and 64 code for RNAs (9 rRNA genes, 53 tRNA genes and 2 tmRNA gene). Of the 2,031 protein-coding genes, 1,442 were assigned to COG functional categories. The genomic structure of the strain is represented by a circular map showing the coding regions of the genome (supplementary Figure S3). The genome of strain Marseille-P4308^T was compared with eight other genomes belonging to closely-related species in the *Peptostreptococcaceae* family (Table 2). Among the *Peptostreptococcus* species, strain Marseille-P4308^T has the second largest genome (2.14 Mbp), shorter only than the genome of *Peptostreptococcus anaerobius* ATCC 27337^T (2.25 Mbp). The distribution of genes among the different COG categories is almost the same proportion in all the genomes of the compared *Peptostreptococcus* species (supplementary Figure S4).

The DDH values obtained after genomic analysis vary for strain Marseille-P4308^T from 18.9% with *Asaccharospora irregularis* DSM 2635^T to 23.7% with *Clostridioides mangenotii* DSM 1289^T. Among species within *Peptostreptococcus* genus, DDH values ranged from 19.5%, between *P. faecalis* Marseille-P4308^T and *P. canis* CCUG 57081^T, to 27.9% between *P. stomatis* W2278^T and *P. anaerobius* ATCC 27337^T (Table 3). These values are therefore lower than the recommended threshold value of 70% for predicting a new prokaryote species [19, 24]. However, on this basis, strain Marseille-P4308^T is considered to be a new species in the genus *Peptostreptococcus*. In addition, among the *Peptostreptococcus* species studied, the lowest OrthoANI value was 71.16% (between *P. faecalis* Marseille-P4308^T and *P. anaerobius* ATCC 27337^T or *P. stomatis* W2278^T) while 73.25% (between *P. canis* CCUG 57081^T and *P. russellii* RT-10B^T) was the highest OrthoANI value obtained in this analysis (Table 3). The

Table 2. Comparison of the size, the content of G + C mol% and the number of proteins of the genome of *Peptostreptococcus faecalis* sp. nov., strain Marseille-P4308^T with the other genomes of related species.

Species	Strain Number	Genbank accession	Size (bp)	G + C mol%	Nb of proteins
Peptostreptococcus faecalis	Marseille-P4308^T	OERU00000000	2,145,294	30.4	2,031
Peptostreptococcus anaerobius	ATCC 27337^T	UGTB00000000	2,256,756	35.7	2,070
Peptostreptococcus canis	CCUG 57081^T	JABGBW000000000	2,064,240	30.2	1,834
Peptostreptococcus russellii	RT-10B^T	JYGE00000000	2,082,949	30.9	1,756
Peptostreptococcus stomatis	W2278^T	ADGQ00000000	1,988,044	36.6	1,799

Table 3. Average nucleotide identity (ANI) and dDDH values (%) obtained by pairwise comparison of the nine studied genomes. Compared genomes: 1, *Peptostreptococcus faecalis* Marseille-P4308^T; 2, *Peptostreptococcus anaerobius* ATCC 27337^T; 3, *Peptostreptococcus canis* CCUG 57081^T; 4, *Peptostreptococcus russellii* RT-10B^T; 5, *Peptostreptococcus stomatis* W2278^T; 6, *Asaccharospora irregularis* DSM 2635^T; 7, *Clostridioides difficile* DSM 1296^T; 8, *Clostridioides mangenotii* DSM 1289^T; 9, *Clostridium hiranonis* DSM 13275^T. OrthoANI values are shown on right bottom (in bolt) and dDDH values calculated using GGDC formula 2 software (DDH estimates based on HSP identities/length) shown on upper left. The empty boxes between them are 100%.

Strains	Marseille-P4308^T	ATCC 27337^T	CCUG 57081^T	RT-10B^T	W2278^T	DSM 2635^T	DSM 1296^T	DSM 1289^T	DSM 13275^T
Marseille-P4308^T		22.6%	19.5%	21.2%	20.7%	18.9%	19.3%	23.7%	23.1%
ATCC 27337^T	**71.16%**		24.0%	22.5%	27.9%	20.4%	21.3%	28.3%	26.0%
CCUG 57081^T	**71.17%**	**71.68%**		21.3%	25.1%	18.9%	18.8%	25.8%	25.7%
RT-10B^T	**73.25%**	**72.26%**	**73.83%**		23.3%	25.7%	19.4%	27.1%	22.6%
W2278^T	**71.16%**	**73.83%**	**71.63%**	**72.29%**		19.4%	21.2%	28.4%	27.8%
DSM 2635^T	**69.14%**	**68.03%**	**68.85%**	**70.06%**	**67.83%**		21.1%	20.3%	20.7%
DSM 1296^T	**69.16%**	**68.00%**	**69.31%**	**69.40%**	**67.60%**	**75.83%**		19.8%	21.6%
DSM 1289^T	**68.82%**	**68.28%**	**68.58%**	**68.80%**	**67.50%**	**72.90%**	**73.53%**		26.1%
DSM 13275^T	**69.71%**	**69.54%**	**69.13%**	**69.66%**	**68.38%**	**72.03%**	**71.95%**	**71.65%**	

mean nucleotide identity (ANI) analysis based on the genomes of species close to the Marseille-P4308^T strain revealed genomic sequence similarities of less than 80%. Indeed, the cut-off value of 95–96% was recommended to delineate the species barrier in prokaryotes [25, 26]. Therefore, our strain Marseille-P4308^T sharing with its all other related species is considered as a new bacterial species.

4. Conclusion

Based on the results of phylogenetic, phenotypic and biochemical analyses, it appeared that *Peptostreptococcus faecalis* sp. nov., strain Marseille-P4308^T, is formally considered as a new species within the genus *Peptostreptococcus*. The type strain of this new species is Marseille-P4803^T.

4.1. Description of Peptostreptococcus faecalis sp. nov.

Peptostreptococcus faecalis (fae.ca'lis. N.L. fem. adj. *faecalis* of faeces). Bacterial colonies of strain Marseille-P4308^T are convex, smooth and regular with a mean diameter of 0.7 mm on blood agar. Cells are non-motile and non-spore-forming. They are Gram-positive cocci bacteria and are oxidase and catalase negative. Glycerol, erythritol, D-arabinose, potassium gluconate, D-ribose, L-xylose, D-adonitol, methyl ß-D-xylo-pyranoside, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, D-cellobiose, D-melibiose, D-saccharose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-tagatose, L-fucose, D-arabitol, L-arabitol, potassium 5-ketogluconate, esterase, leucine arylamidase, cystine arylamidase, α-galactosidase, α-glucosidase and gelatin are fermented. The genome size of strain Marseille-P4308^T is about 2.14 Mbp with a 30.4 mol% of G + C content. The 16S rRNA and genome sequences are deposited in the GenBank database under Accession numbers LT960583 and OERU00000000, respectively.

The type strain Marseille-P4308^T (=CSUR P4308 = CECT 9960) was isolated from stool sample from a healthy indigenous Congolese volunteer.

Declarations

Author contribution statement

Pierre-Edouard Fournier: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Florence Fenollar: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Didier Raoult: Conceived and designed the experiments.

Oleg Mediannikov, Jean Akiana and Geor Mongo Ndombe: Performed the experiments.

Rita Zgheib: Analyzed and interpreted the data.

Fatima Mekhalif: Analyzed and interpreted the data; Wrote the paper.

Cheikh Ibrahima Lo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Melhem Bilen and Stéphane Alibar: Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data associated with this study has been deposited at CSUR collection under the accession number CSUR P4308, CECT collection under the accession number CECT 9960 and GenBank database under the accession numbers LT960583 and OERU00000000.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e09102.

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