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-P4308T from a stool sample of a healthy indigenous Congolese volunteer. Strain Marseille-P4308T 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 11460T (GenBank accession number: NR_042847.1). In addition, the average nucleotide identity of strain Marseille-P4308T with its closest related species was 71.1%, which was far below the recommended threshold (>95–96%). The genome of the strain Marseille-P4308T 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-P4308T (¼ CSUR P4308 ¼ 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, mucure, 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 microbiota 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 russelli* 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 taxononomic description of *Peptostreptococcus faecalis* sp. nov., strain Marseille-P4308T isolated from human gut microbiota.
2. Material and methods

2.1. Ethics and sample collection

The stool sample was collected from a healthy indigenous Congolese volunteer living in Beni 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 de la Population, 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 the 16S 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://www.lpsn.org/). 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 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-P4308T was compared to the genomes of the following closely related species, including Peptostreptococcus anaerobius NCTC11460T (GenBank accession: UGTB0000000000), Peptostreptococcus russelli RT-10B1 (JYGE000000000), Peptostreptococcus stomatis DSM 17678T (ADGG0000000000), Peptostreptococcus canis DSM 27025T (JABGW0000000000), Asaccharospora irregulans DSM 2635T (FQWX0000000000), Clostridoides difficile ATCC 9689T (AUOX0000000000), Clostridoides mangenotii LM2 (JIAA000000000) and Clostridium hiranonis DSM 13275T (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 Columbia agar enriched with 5% sheep blood (BioMérieux), using the GENbag anaer and GENbag micro aer 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). Spawn-forming was investigated by exposing a bacterial suspension for 10 min under thermal shock at 80 °C. Multiple biochemical criteria from strain Marseille-P4308T were revealed using API tests (50CH, 2YM and 20A; bioMérieux). Oxidase and catalase reactions were determined using a BD BBL™ DrySlide (Becton Dickinson, Le Pont-de-Clair, 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-P4308T [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-P4308T strain has the highest similarity score of 96.2% to Peptostreptococcus anaerobius strain NCTC 11460T (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-P4308T 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-P4308T 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).
3.2. Phenotypic characteristics

Strain Marseille-P4308T 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-xylene, D-adonitol, methyl β-D-xylopyranoside, 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-cellulose, D-melibiose, D-saccharose, D-trehalose, starch, glycogen, xyitol, gentiobiose, D-lyxose, D-tagatose, L-fucose, D-arabitol, potassium gluconate and potassium 5-ketogluconate. Negative reactions were observed for D-
Table 1. Differential characteristics of Marseille-P4308T, *Peptostreptococcus stomatis* W2278T [5], *Peptostreptococcus canis* CCUG 57081T [27], and *Peptostreptococcus anaerobius* ATCC 27337T [28].

| Characteristics | Marseille-P4308T | W2278T | CCUG 57081T | ATCC 27337T |
|-----------------|-----------------|--------|-------------|------------|
| Oxygen requirement | Strikely anaerobic | Strictly anaerobic | Facultative anaerobic | Strictly anaerobic |
| Acid production from: | D-Cellulose | + | - | - |
| | a-Galactosidase | + | + | + |
| | D-Glucose | - | + | - |
| | D-Acetolactate | + | + | NP |
| | D-Lactate | - | - | + |
| | D-Mannose | + | + | NP |
| | D-Raffinose | + | - | NP |
| | Sucrose | - | - | - |
| G + C content (mol%) | 30.4 | 36 | 30.8 | 34-36 |
| Habitat | Human | Human | Dog | Cat |

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-P4308T with the other genomes of related species.

| Species | Strain Number | Genbank accession | Size (bp) | G + C mol% | Nb of proteins |
|---------|---------------|--------------------|-----------|-------------|---------------|
| *Peptostreptococcus faecalis* | Marseille-P4308T | OERU00000000 | 2,145,294 | 30.4 | 2,031 |
| *Peptostreptococcus anaerobius* | ATCC 27337T | UGTB00000000 | 2,256,756 | 35.7 | 2,070 |
| *Peptostreptococcus canis* | CCUG 57081T | JABGW00000000 | 2,064,240 | 30.2 | 1,834 |
| *Peptostreptococcus russelli* | RT-10B | JYGE00000000 | 2,082,949 | 30.9 | 1,756 |
| *Peptostreptococcus stomatis* | W2278T | 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-P4308T; 2, *Peptostreptococcus anaerobius* ATCC 27337T; 3, *Peptostreptococcus canis* CCUG 57081T; 4, *Peptostreptococcus russelli* RT-10B; 5, *Peptostreptococcus stomatis* W2278T; 6, *Asaccharospora irregulraris* DSM 2635T; 7, *Clostridoides difficile* DSM 1296; 8, *Clostridoides mangenotii* DSM 1289; 9, *Clostridium hiranonis* DSM 13275T. OrthoANI values are shown on right bottom (in bold) 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-P4308T | ATCC 27337T | CCUG 57081T | RT-10B | W2278T | DSM 2635T | DSM 1296T | DSM 1289T | DSM 13275T |
|---------|-----------------|-------------|-------------|--------|--------|-----------|-----------|-----------|-----------|
| Marseille-P4308T | 22.6% | 19.5% | 21.2% | 20.7% | 18.9% | 19.3% | 23.7% | 26.0% |
| ATCC 27337T | 71.16% | 71.68% | 21.3% | 25.1% | 18.9% | 18.8% | 25.8% | 25.7% |
| CCUG 57081T | 71.17% | 71.68% | 23.4% | 27.5% | 20.4% | 21.3% | 28.3% | 26.0% |
| RT-10B | 73.25% | 72.26% | 73.83% | 23.3% | 25.7% | 19.4% | 21.2% | 28.4% | 27.8% |
| W2278T | 71.16% | 73.83% | 71.68% | 72.29% | 19.4% | 21.2% | 28.4% | 27.8% |
| DSM 2635T | 69.14% | 68.03% | 68.85% | 70.06% | 67.83% | 21.1% | 20.3% | 20.7% |
| DSM 1296T | 69.16% | 68.00% | 69.31% | 69.40% | 67.60% | 75.83% | 19.8% | 21.6% |
| DSM 1289T | 68.82% | 68.28% | 68.58% | 68.80% | 67.50% | 72.90% | 73.53% | 26.1% |
| DSM 13275T | 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-P4308T 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-P4308T 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-P4308T, is formally considered as a new species within the genus Peptostreptococcus. The type strain of this new species is Marseille-P4803T.

4.1. Description of Peptostreptococcus faecalis sp. nov.

Peptostreptococcus faecalis (fae.ca'lis. L.N. fem. adj. faecalis of faeces). Bacterial colonies of strain Marseille-P4308T 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 glutonate, D-ribose, L-xylitol, D-adonitol, methyl β-D-xylulopyranoside, D-galactose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglycosamine, amygdalin, arbutin, esculin ferric chloride, 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, dulcitol, inositol, D-sorbitol, methyl fi-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-P4308T is about 1.24 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-P4308T (=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 Mediniakov, Jean Akiana and Geor Mongo Ndombe: Performed the experiments.
Rita Zghelbi: 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

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