**Parabacteroides pacaensis** sp. nov. and **Parabacteroides provencensis** sp. nov., two new species identified from human gut microbiota

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**Abstract**

Strains Marseille-P4001 and Marseille-P3668 are new species from the order Bacteroidales isolated from healthy French volunteers. They are anaerobic Gram-negative rod-shaped bacteria. They exhibited 92.68% and 96.68% 16S rRNA sequence identities with Parabacteroides gordonii strain MS-1 and Parabacteroides chinchillae JCM 17104, respectively, the phylogenetically closest species. Their respective draft genomes measured 5.23 Mb and 3.73 Mb with 39.2 mol% and 40.8 mol% of G + C content. Using a taxonogenomics approach, we propose here a brief description of **Parabacteroides pacaensis** sp. nov., strain Marseille-P4001T and **Parabacteroides provencensis** sp. nov., strain Marseille-P3668T as new bacterial species.

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Here we describe **Parabacteroides pacaensis** sp. nov., strain Marseille-P4001T (= CSUR P4001), and **Parabacteroides provencensis** sp. nov., strain Marseille-P3668T (= CSUR P3668), according this taxonogenomics concept.

**Isolation and growth conditions**

We isolated two unidentified bacterial strains from the fresh stools of two volunteers living in France. A screening was made by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [8]. The obtained spectra (Fig. 1) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in two databases (Bruker and constantly updated URMS databases). The study was validated by the ethics committee of Institut Fédératif de Recherche IFR48 under number 2016-010. Strains Marseille-P4001T and Marseille-P3668T were first isolated after 7 days of pre-incubation in an anaerobic blood culture bottle (Becton-
Dickinson Diagnostics, Le Pont-de-Clairaix, France) supplemented with 5% sheep blood at 37°C.

**Phenotypic characteristics**

After the isolation step, the strain Marseille-P4001T and strain Marseille-P3668T were cultured with the aim to get pure and isolated colonies on blood agar. The colonies of Marseille-P4001 and Marseille-P3668 had almost the same morphological aspect, namely beige, small and smooth. Bacterial cells were Gram-negative for both strains. The sporulation test (10 min at 80°C) was negative. Different growth temperatures (20, 28, 32, 37, 45 and 56°C), pH (5, 6, 7, 7.5, 8 and 8.5), and atmospheres (aerobic, anaerobic and microaerophilic (CampyGEN, Oxoid, Basingstoke, UK)) were tested on 5% sheep-blood-enriched Columbia agar. Strain Marseille-P4001T grows at 28 and 37°C in anaerobic conditions at pH 7. Strain Marseille-P3668T grows from 28 to 45°C (optimally at 37°C) at pH ranging from 6 to 8.5 (optimally at pH 7) in anaerobic conditions. API ZYM (bioMérieux, Marcy l’Étoile, France) was performed to determine specific enzymatic properties for both strains. The results are tabulated in Table 1. Using API 50 CH strips (bioMérieux) the carbohydrate metabolism of both strains was evaluated according to the manufacturer’s instructions (Table 2). For strain Marseille-P4001T the following positive reactions were noted: esterase (C4), leucine arylamidase, α-galactosidase, β-galactosidase, N-acetyl-β-galosaminidase, alkaline phosphatase, esculin ferric citrate, d-melezitose, d-saccharose, d-mannitol, methyl-α-D-glucopyranose and glycogen. All the other reactions tested were negative. Strain Marseille-P3668T had positive reactions for alkaline phosphatase, leucine arylamidase, α-galactosidase, β-galactosidase, naphthol-AS-BI-phosphohydrolase, phosphatase acid, N-acetyl-

| Tests                  | Characteristics | P4001T | P3668T |
|------------------------|-----------------|--------|--------|
| Alkaline phosphatase   | +               | +      |        |
| Esterase (C4)          | +               | +      |        |
| Esterase lipase (C8)   |                |        |        |
| Lipase (C14)           |                |        |        |
| Leucine arylamidase    | +               | +      |        |
| Valine arylamidase     |                |        |        |
| Cystine arylamidase    |                |        |        |
| Trypsin                |                |        |        |
| α-chymotrypsin         |                |        |        |
| Acid phosphatase       |                |        |        |
| Naphthol-AS-BI-phosphohydrolase |          | +      | +      |
| α-galactosidase        |                |        |        |
| β-galactosidase        |                |        |        |
| β-glucuronidase        |                |        |        |
| α-glucosidase          |                |        |        |
| β-glucosidase          |                |        |        |
| N-acetyl-β-glucosaminidase |            | +      | +      |
| α-mannosidase          |                |        |        |
| α-fucosidase           |                |        |        |
| Glycerol               |                |        |        |


Table 2. Phenotypic characterization of *Parabacteroides pacoensis* strain Marseille-P4001T sp. nov. and *Parabacteroides provencensis* sp. nov. strain Marseille-P3668T, based on API 50 CH test

| Tests            | Characteristics | P4001T | P3668T |
|------------------|-----------------|--------|--------|
| 50 CH             |                 |        |        |
| Erythritol       | −               | −      | −      |
| α-arabinose      | −               | −      | −      |
| β-arabinose      | −               | −      | −      |
| α-xylose         | −               | −      | −      |
| β-xylose         | −               | −      | −      |
| α-Adonitol       | −               | −      | −      |
| Methyl β-oxylypyranoside | − | −      | −      |
| α-glucose        | −               | −      | −      |
| α-fructose       | −               | −      | −      |
| α-mannose        | −               | −      | −      |
| α-sorbose        | −               | −      | −      |
| α-rhamnose       | −               | −      | −      |
| Dulcitol         | +               | +      | +      |
| Inositol         | −               | −      | −      |
| α-mannitol       | +               | +      | +      |
| α-sorbitol       | −               | −      | −      |
| Methyl α-αmannopyranoside | − | −      | −      |
| Methyl β-αglucopyranoside | + | +      | +      |
| N-acetyl-glucosamine | − | −      | −      |
| Amygdalin        | −               | −      | −      |
| Arbutin          | −               | −      | −      |
| Esculin ferric citrate | +          | +      | +      |
| Salicin          | −               | −      | −      |
| α-cellobiose     | −               | −      | −      |
| α-maltose        | −               | −      | −      |
| α-lactose        | −               | −      | −      |
| α-malbiose       | −               | −      | −      |
| α-saccharose     | +               | +      | +      |
| α-trehalose      | −               | −      | −      |
| Inulin           | −               | −      | −      |
| α-melezitose     | +               | +      | +      |
| α-raffinose      | −               | −      | −      |
| Amidon           | −               | −      | −      |
| Glycogen         | −               | −      | −      |
| Xylose           | −               | −      | −      |
| Gentobiose       | −               | −      | −      |
| α-turanose       | −               | −      | −      |
| α-xylose         | −               | −      | −      |
| α-saprose        | −               | −      | −      |
| α-fucose         | −               | −      | −      |
| α-l-fucose       | −               | −      | −      |
| α-arabitol       | −               | −      | −      |
| α-arabinol       | −               | −      | −      |
| Potassium glutonate | −            | −      | −      |
| Potassium 2-ketogluconate | − | −      | −      |
| Potassium 5-ketogluconate | − | −      | −      |

Strain identification

The 16S rRNA gene was sequenced to classify the bacteria. Amplification was performed by using the primer pair F1 and R2 (Eurogentec, Angers, France) and sequencing used the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xL Genetic Analyzer capillary3500xL sequencers (Thermo Fisher, Saint-Aubin, France), as previously described [9]. The 16S rRNA nucleotide sequences were assembled and corrected using CODONCODE ALIGNER software (http://www.codoncode.com). Strain Marseille-P4001T exhibited a 92.68% sequence identity with *Parabacteroides gordonii* strain MS-I (GenBank accession number NR128351.1) and strain Marseille-P3668T exhibited a 96.68% sequence identity with *Parabacteroides chinchillae* JCM 17104 (GenBank accession number NR113208.1), the phylogenetically closest species with standing in nomenclature (Fig. 3). Considering these phylogenetic values lower than the thresholds fixed to delineate new bacterial taxa [10,11], we consequently classify these strains as members within the genus *Parabacteroides* belonging to family Tannellaceae.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue Kit and then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera

Table 3. Comparison of differential characteristics of *Parabacteroides pacoensis* sp. nov., *Parabacteroides provencensis* sp. nov., *Parabacteroides timonensis* and *Parabacteroides chartae*

| Property                  | P. pacoensis | P. provencensis | P. timonensis | P. chartae |
|---------------------------|--------------|-----------------|---------------|------------|
| Cell diameter (μm)        | 0.5          | 0.7             | 0.5           | 0.7 – 1    |
| Oxygen requirement        | −            | −               | −             | −          |
| Gram stain                | −            | −               | −             | −          |
| Salt requirement          | −            | −               | −             | −          |
| Motility                  | −            | −               | −             | −          |
| Endospore formation       | −            | −               | −             | −          |
| Alkaline phosphatase      | +            | +               | +             | +          |
| Catalase                  | +            | +               | +             | −          |
| Oxidase                   | −            | −               | −             | NA         |
| Urease                    | −            | −               | −             | −          |
| β-Galactosidase           | +            | +               | +             | +          |
| N-acetyl-glucosamine      | +            | +               | +             | +          |
| Arabinose                 | +            | +               | +             | +          |
| Lipase (C8)               | +            | +               | +             | +          |
| Mannose                   | −            | −               | −             | −          |
| Mannitol                  | −            | −               | −             | −          |
| Sucrose                   | +            | +               | +             | +          |
| d-Glucose                 | −            | −               | −             | −          |
| d-Fructose                | −            | −               | −             | −          |
| d-Maltose                 | −            | −               | −             | −          |
| Source                    | Human        | Human           | Human         | Environment |

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XT Paired end (Illumina), as previously described [12]. The assembly was performed with a pipeline incorporating different software (VELVET [13], SPades [14] and SOAP DENOVO [15]) and trimmed data (MiSeq and TRIMMOMATIC [16] softwares) or untrimmed data (only MiSeq software). GAPCLOSER software [17] 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, N50, number of N). The genome of Parabacteroides pacensis strain Marseille-P4001^T is 5 238 628 bp long with a 39.21 mol% G + C content. Hence, the genome of Parabacteroides provencensis strain Marseille-P3668^T is 3 732 078 bp long with a 40.8 mol% G + C content. The degree of genomic similarity of strain Marseille-P4001^T and Marseille-P3668^T with closest species was estimated using the ORTHOANI software [18]. Values among closely related species (Fig. 4) ranged from 78.31% between Parabacteroides chinchillae and Parabacteroides provencensis to 82.18% between Parabacteroides goldsteinii and Parabacteroides gordonii; 71.26% of similarity is shared between P. provencensis and P. pacensis.
Conclusion

Based on the results from unique phenotypic characteristics, including API gallery tests, MALDI-TOF spectrum, and phylogenetic and genomic analysis such as 16S rRNA sequence similarity <95% and ORTHOANI value <95% with the phylogenetically closest species with standing in nomenclature, we formally propose strain Marseille-P4001T and strain Marseille-P3668T as type strains of Parabacteroides pacaensis sp. nov and Parabacteroides provencensis sp. nov., and other closely related species with standing in nomenclature.

Description of Parabacteroides pacaensis sp. nov.

Parabacteroides pacaensis (pa.ca.en.sis N.L. masc. adj. pacaensis, derived from the abbreviation PACA, for the region of Provence Alpes Côte d’Azur, where the strain was first isolated). The strain grows in varied conditions. Optimum growth of colonies was obtained at 37°C on 5% sheep-blood-enriched Columbia agar after 3 days in an anaerobic atmosphere. They appear smooth and small. Parabacteroides pacaensis is a Gram-negative rod-shaped bacterium with a mean length of 1.4 μm and a mean diameter of 0.5 μm. Strain Marseille-P4001T produced esterase (C4), leucine arylamidase, α- and β-galactosidase, N-acetyl-β-glycosaminidase and alkaline phosphatase, and metabolized esculin ferric citrate, D-melezitose, D-saccharose, D-mannitol, methyl-α-D-glucopyranoside and glycogen. No activities were observed with trypsin, α-glucosidase, glycerol, α-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-lactose, α-fucoside and D-arabitol. Strain Marseille-P4001T is catalase-positive and oxidase-negative. The genome size of Parabacteroides pacaensis sp. nov. is about 5.24 Mb long with 39.2 mol% G + C content. The GenBank accession number for the 16S rRNA gene sequence of strain Marseille-P4001T is LT985457 and for the whole genome shotgun project is OLMS01000001-OLMS01000014. This strain was isolated from the fresh stool of a healthy French volunteer.

Description of Parabacteroides provencensis sp. nov.

Parabacteroides provencensis (pro.ven.ce.nsis, N.L. fem. adj. provencensis, pertaining to Provence, the region of France where the type strain was isolated). The strain grows in varied conditions. Optimum growth of colonies was obtained at 37°C on 5% sheep-blood-enriched Columbia agar after 3 days in anaerobic conditions. They appear smooth and small. Parabacteroides provencensis is a Gram-negative rod-shaped bacterium with a mean length of 2 μm and a mean diameter of 0.7 μm. Strain Marseille-P3668T produced alkaline phosphatase, leucine arylamidase, α- and β-galactosidase, naphthol-AS-BI-phosphohydrolase, acid phosphatase, N-acetyl-β-glycosaminidase, and α-fucosidase and metabolize only esculin ferric citrate and Dulcitol. But any activities were observed with...
trypsin, α-glucosidase, glycerol, d-arabinose, d-ribose, d-xylitol, d-glucose, d-fructose, d-mannose, l-rhamnose, d-lactose, d-fucose and d-arabitol. Strain Marseille-P3668T is catalase-positive and oxidase-negative. The genome size of *P. provencensis* strain Marseille-P3668T is about 3.73 Mb long with 40.8 mol% G + C content. The GenBank accession number for the 16S rRNA gene sequence of strain Marseille-P3668T is LT722681 and for the whole genome shotgun project number for the 16S rRNA gene sequence of strain Marseille-P4001T is FYCK01000001-FYCK01000021. This strain was isolated from the fresh stool of a healthy French volunteer.

**Nucleotide sequence accession number**

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT985457 and OLMS01000001-OLMS01000014, respectively, for Strain Marseille-P4001T and under accession numbers LT722681 and FYCK01000001-FYCK01000021, respectively, for Strain Marseille-P3668T.

**Deposit in culture collections**

Strain Marseille-P4001T was deposited in our strain collections under number (= CSUR P4001) and Strain Marseille-P3668T under number (= CSUR P3668).

**Conflict of interest**

None to declare.

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