1 INTRODUCTION

The gut microbiota is well-known for its microbial diversity and its role in health as well as in diseases. Even though scientific technologies have been greatly developed over the past years and have drastically facilitated the description of the gut microbiota, it still remains a challenging task (Turnbaugh et al., 2007) as the massive data generated over the last decade do not yet allow the clear depiction of the gut microbiota composition (Lagier et al., 2012).

Nevertheless, the fact that 1 g of human stool might contain up to $10^{12}$ bacteria drives us to pursue our efforts in describing the human gut microbiota for which only around 2,776 species have been reported (Bilen et al., 2018; Hugon et al., 2015). Consequently, our laboratory has developed a new approach called culturomics which aims to isolate previously uncultured bacteria using sophisticated culture methods (Lagier et al., 2012). In doing so, culturomics has expanded our capabilities in human gut microbiota description and therefore lead to the isolation of a significant number of new species, such as Parabacteroides timonensis.
genera and species (Lagier et al., 2016). The process begins with the
cultivation of stool samples under varying conditions and bacteri-
ial growth is assessed over 30 days. At this point, Matrix-assisted
laser desorption/ionization time-of-flight mass spectrometry
(MALDI-TOF MS) is primarily used for colony identification and 16S
rRNA sequencing is adapted in case of MALDI-TOF MS’s identifi-
cation failure. Subsequently, unidentified species are subjected to
taxonomics description (Fournier & Drancourt, 2015). The ge-
nome of the concerned species is then sequenced for a genomic
description, followed by a phenotypic and biochemical analysis
(Fournier & Drancourt, 2015; Fournier, Lagier, Dubourg, & Raoult,
2015; Lagier et al., 2012). By adapting this procedure, we isolated
a new species known as Parabacteroides timonensis (P. timonensis), a
member of the Parabacteroides genus known to be gram-negative,
obligate anaerobic, nonmotile, rod-shaped, and nonspore-forming
(Sakamoto & Benno, 2006). To date, eight Parabacteroides species
have been isolated, out of which six were isolated from the human
gut (www.bacterio.net). We demonstrate here the description of P.
timonensis strain Marseille-P3236T (=Culture Collection University
Gothenburg (CCUG) 71183, =Collection de Souches de l’Unité des
Rickettsies (CSUR) P3236) using the taxononomic approach.

2 | MATERIALS AND METHODS

2.1 | Ethics and sample collection

Before stool sample collection in Congo, the sample’s donor has
signed an informed consent. The donor is a healthy 39-year-old
pygmy male and the collected stool samples were stored at −80°C
for further analysis. In addition, an approval from the ethic com-
mittee of the Institut Fédératif de Recherche IFR48 (Marseille, France)
carrying the number 09-022 was obtained before launching the
study.

2.2 | Strain isolation

A loop of stool sample was diluted in phosphate-buffered saline (Life
Technologies, Carlsbad, CA, USA) prior to incubation in a blood culture
bottle (BD BACTEC®, Plus Anaerobic/F Media, Le Pont de Claix, France),
supplemented with 5% sheep blood and 5% filtered rumen, at 37°C
under anaerobic conditions. Bacterial growth and isolation was done
by subculturing samples after 5 days on 5% sheep’s blood–enriched
Columbia agar solid medium (bioMérieux, Marcy l’Etoile, France).

2.3 | Colony identification

Isolated bacterial colonies identification trials were done first by
using MALDI-TOF MS analysis as previously described (Elsawi et al.,
2017). In case of MALDI-TOF MS identification failure, complete
16S rRNA sequencing was performed for further analysis with the
same protocol used in our previous studies (Elsawi et al., 2017).
Complete 16S rRNA nucleotide sequence are assembled and ma-
nipulated using CodonCode Aligner software (http://www.co-
doncode.com) and blasted in the online PubMed National Center
for Biotechnology Information (NCBI) database for phylogenetic
analysis. According to Kim, Oh, Park, & Chun (2014), a threshold of
98.65% 16S rRNA gene sequence similarity was used to classify a
new species, whereas a threshold of 95% 16S rRNA gene sequence
was used for new genus classification. Generated mass spectrum
of the concerned species was added to our custom database and
its 16S rRNA gene sequence was submitted to EMBL-EBI database.

2.4 | Growth conditions

In order to determine the optimal growth environment, strain
Marseille-P3236T was cultured using different conditions such
as temperature, pH, atmosphere, and salinity. To begin with, this
strain was cultured under anaerobic, aerobic, and microaerophilic

FIGURE 1 Reference mass spectrum representing Parabacteroides timonensis
strain Marseille-P3236T
conditions on 5% sheep’s blood–enriched Colombia agar (bioMérieux) at 28, 45, 37, and 55°C. GENbag anaer and GENbag microaer systems (bioMérieux) were used for anaerobic and microaerophilic environment establishment, respectively. Furthermore, salt and acidity tolerance were evaluated using concentration of 0%, 5%, 15%, and 45% NaCl and pH values of 6, 6.5, 7, and 8.5.

2.5 | Morphological and biochemical assays

Biochemical characteristics of Marseille-P3236T strain were determined using different API galleries (20A, ZYM, and 50CH, bioMérieux) according to the manufacturer’s instructions. Not to mention, sporulation ability was tested by culturing this strain after exposing a bacterial suspension to a thermal shock of 80°C for 10 min. Strain Marseille-P3236T morphology was determined as previously described (Elsawi et al., 2017). Additionally, DM1000 photonic microscope (Leica Microsystems, Nanterre, France) was used to observe the motility of strain Marseille-P3236T from a fresh culture with a 100× objective lens. Cellular fatty acid methyl ester (FAME) analysis was performed using around 43 mg of bacterial biomass per tube as formerly described (Elsawi et al., 2017).

2.6 | Antibiotic susceptibility

Antibiotics susceptibility testing (AST) of Marseille-P-3236T strain was evaluated by performing minimum inhibitory concentrations (MICs) using the E-test strip method (Biomérieux) on Columbia agar + 5% sheep blood (bioMérieux) with the following agents: rifampicin, imipenem, amikacin, cefotaxime, benzylpenicillin, vancomycin, erythromycin, amoxicillin, ceftriaxone, minocycline, metronidazole, teicoplanin, and daptomycin.

2.7 | Genome sequencing and analysis

Genomic DNA (gDNA) of Marseille-P3236T strain was extracted as previously described with 50 μL being eluted (Elsawi et al., 2017). Quantification was done using the Qubit assay with the high

![FIGURE 2](image2.png)

Phylogenetic subtree highlighting the position of Parabacteroides timonensis strain Marseille-P3236T relative to other close species

![FIGURE 3](image3.png)

Gel view comparing mass the mass spectrum of Parabacteroides timonensis strain Marseille-P3236T to other species. The gel view displays the raw spectra of loaded spectrum files arranged in a pseudo-gel like look. The x-axis records the m/z value. The left y-axis displays the running spectrum number originating from subsequent spectra loading. The peak intensity is expressed by a Gray scale scheme code. The right y-axis indicates the relation between the color of a peak and its intensity, in arbitrary units. Displayed species are indicated on the left
sensitivity kit (Life technologies, Carlsbad, CA, USA) and determined to be 134 ng/μl.

gDNA sequencing, library preparation, fragmentation, and tagmentation were performed as previously described with an optimal DNA fragment size of 8.675 kb (Lagier et al., 2016). Circulization was done using 511.4 ng of tagmented fragments with no size selection. The circulized DNA was mechanically sheared to small fragments with optima at 1059 bp on the Covaris device S2 in T6 tubes (Covaris, Woburn, MA, USA). Library profile visualization was done on a High Sensitivity Bioanalyzer LabChip (Agilent Technologies Inc, Santa Clara, CA, USA) and the final library concentration was determined at 27.8 nmol/L. After that, libraries were normalized at 2 nM and pooled. Dilution at 15 pM was done after a denaturation step and pooled libraries were loaded in the sequencer for automated cluster generation and a single sequencing run of 39 h in a 2 × 151-bp was performed. Total information of 5.1 Gb were generated from a 542 K/mm² cluster density with a quality control filters threshold of 95.7% (10,171,000 passing filter paired reads). Within that, libraries were normalized at 2 nM and pooled. Dilution at 15 pM was done after a denaturation step and pooled libraries were loaded in the sequencer for automated cluster generation and a single sequencing run of 39 h in a 2 × 151-bp was performed.

TABLE 1 Classification and general features of Parabacteroides timonensis strain Marseille-P3236†

| Property               | Term                                      |
|------------------------|-------------------------------------------|
| Current classification | Domain: Bacteria                          |
|                        | Phylum: Bacteroidetes                     |
|                        | Class: Bacteroidia                        |
|                        | Order: Bacteroidales                      |
|                        | Family: Porphyromonadaceae                |
|                        | Genus: Parabacteroides                    |
|                        | Species: Parabacteroides timonensis       |
|                        | Type strain: P3236                        |
| Gram strain            | Negative                                  |
| Cell shape             | Rod                                       |
| Motility               | Motile                                    |
| Sporulation            | Negative                                  |
| Temperature range      | 30–42°C                                   |
| Optimum temperature    | 37°C                                      |

Strain Marseille-P3236† exhibited a 97.05% sequence similarity with Parabacteroides gordonii strain MS-1 (NR_112835.1), the phylogenetically closest species with standing nomenclature (Figure 2). Accordingly, with more than 1.3% sequence divergence from its phylogenetically closest species with standing in nomenclature, we propose the classification of Marseille-P3236† strain as a new species within the Parabacteroides genus. The protein spectrum of Marseille-P3236† strain was compared with those of other close species under the same family. This comparison revealed its uniqueness with specific and different peaks compared to others (Figure 3).

3.2 | Phenotypic and biochemical characterization

Strain Marseille-P3236† is a gram-negative rod, motile, unable to sporulate, and grows anaerobically between 25 and 42°C but optimally at 37°C (Table 1). It is able to endure a range of pH between 6 and 8.5 and can sustain only a 5% salinity concentration. Strain Marseille-P3236† is catalase positive, oxidase negative, and can be seen as smooth colonies with a diameter of 0.9–1 mm. Under electron microscopy, each bacterial cell has an average length of 1.4–2.7 μm and an average diameter of 0.5 μm (Figure 4).

MICs of strain Marseille-P3236† for various antimicrobial agents were as follows: rifampicin (8 μg/ml), imipenem (8 μg/ml), amikacin (>256 μg/ml), cefotaxime (6 μg/ml), benzylpenicillin (16 μg/ml), vancomycin (4 μg/ml), erythromycin (0.016 μg/ml), amoxicillin (1.5 μg/ml), ceftriaxone (4 μg/ml), minocycline (0.25 μg/ml), metronidazole (>256 μg/ml), teicoplanin (2 μg/ml), and daptomycin (12 μg/ml).
The main biochemical features obtained by API tests are summarized in Table 2. Additionally, the general features of strain Marseille-P3236T have been listed in Table 3. The major fatty acid found in this strain was 12-methyl-tetradecanoic acid (46%). Several specific 3-hydroxy branched structures were described. Minor amounts of unsaturated, branched, and other saturated fatty acids were also detected (Table 4).

### Table 2: Main biochemical features of strain Parabacteroides timonensis strain Marseille-P3236T obtained by API tests (20A, 50CH, and ZYM)

| Test                                | Results | Test                               | Results | Test                               | Results |
|--------------------------------------|---------|------------------------------------|---------|------------------------------------|---------|
| Alkaline phosphatase                 | +       | Fermentation (d-glucose)           | +       | Fermentation (d-turanose)          | +       |
| Esterase (C4)                        | +       | Fermentation (d-fructose)          | +       | Fermentation (d-lyxose)            | −       |
| Esterase Lipase (C8)                 | +       | Fermentation (d-mannose)           | +       | Fermentation (d-tagatose)          | +       |
| Lipase (C14)                         | −       | Fermentation (l-sorbose)           | −       | Fermentation (d-fucose)            | −       |
| Leucine arylamidase                  | +       | Fermentation (l-rhamnose)          | −       | Fermentation (l-fucoside)          | −       |
| Valine arylamidase                   | +       | Fermentation (o-ucilol)            | −       | Fermentation (o-arabitol)          | −       |
| Cystine arylamidase                  | +       | Fermentation (Inositol)            | −       | Fermentation (l-arabitol)          | −       |
| Trypsin                              | −       | Fermentation (o-mannitrol)         | +       | Fermentation (potassium gluconate) | +       |
| α-chymotrypsin                       | −       | Fermentation (o-sorbitol)          | +       | Fermentation (potassium 2-Ketogluconate) | −       |
| Acid phosphatase                     | +       | Fermentation (Methyl-αd-mannopyranoside) | +   | Fermentation (potassium 5-Ketogluconate) | −       |
| Naphthol-AS-BI-phosphohydrolase      | +       | (Fermentation (Methyl-αd-glucosamine)) | +   | Indole formation                   | −       |
| α-galactosidase                      | +       | Fermentation (N-acetylglucosamine) | +       | Urease                             | −       |
| β-galactosidase                      | +       | Fermentation (Amygdaline)          | +       | Acidification (Glucose)            | +       |
| β-glucuronidase                      | +       | Fermentation (Arbutin)             | +       | Acidification (Mannitol)           | −       |
| α-glucosidase                        | +       | Fermentation (Esculin ferric citrate) | +  | Acidification (Lactose)            | +       |
| β-glucosidase                        | +       | Fermentation (Salicin)             | +       | Acidification (Saccharose)         | +       |
| N-acetyl-β-glucosaminidase           | +       | Fermentation (o-cellobiose)        | +       | Acidification (Maltose)            | +       |
| α-mannosidase                        | −       | Fermentation (o-Maltose)           | +       | Acidification (Salicin)            | −       |
| α-fucosidase                         | −       | Fermentation (o-lactose)           | +       | Acidification (Xylose)             | +       |
| Fermentation (Glycerol)              | −       | Fermentation (o-melibiose)         | +       | Acidification (Arabinose)          | +       |
| Fermentation (Erythritol)            | +       | Fermentation (o-saccharose/sucrose) | +   | Hydrolysis (protease) (Gelatin)    | −       |
| Fermentation (o-arabinose)           | +       | Fermentation (o-trelahose)         | +       | Hydrolysis β-glucosidase (Esculin) | −       |
| Fermentation (l-arabinose)           | +       | Fermentation (Inuline)             | +       | Acidification (Glycerol)           | −       |
| Fermentation (p-ribose)              | +       | Fermentation (p-melezitose)        | +       | Acidification (Cellulbiose)        | −       |
| Fermentation (o-xylose)              | +       | Fermentation (o-raffinose)         | +       | Acidification (Mannose)            | +       |
| Fermentation (l-xylose)              | −       | Fermentation (Starch)              | +       | Acidification (Melezitose)         | −       |
| Fermentation (p-adonitol)            | −       | Fermentation (Glycogen)            | −       | Acidification (Raffinose)          | +       |
| Fermentation (Methyl-p-α-xylopyranoside) | −   | Fermentation (xylitol)             | −       | Acidification (Sorbitol)           | −       |
| Fermentation (o-galactose)           | +       | Fermentation (Gentiobiose)         | +       | Acidification (Rhamnose)           | +       |
|                                      |         |                                    |         |                                    |         |
|                                      |         |                                    |         |                                    |         |

#### 3.3 | Genome properties

Strain Marseille-P3236T had a genome of 6,483,434 bp long with 43.41 mol% of G+C content. It is composed of eight scaffolds (composed of 13 contigs). Of the 5,130 predicted genes, 5,046 were protein-coding genes and 84 were RNAs (6 genes are 5S rRNA, 1 gene is 16S rRNA, 5 genes are 23S rRNA, and 72 genes are tRNA
genes). A total of 3,253 genes (64.47%) were assigned as putative function (by cogs or by NR blast) and 202 genes were identified as ORFans (4%). The remaining genes were annotated as hypothetical proteins (1,483 genes; 29.39%) (Table 5). The representations of strain Marseille-P3236T genome and its genes repartition into COG functional categories were done in Figure 5 and Table 6, respectively.

### 3.4 Comparison of genome properties

The draft genome sequence of Marseille-P3236T strain was compared to those of *Parabacteroides gordonii* (P. gordonii) (AUAE00000000), *Parabacteroides distasonis* (P. distasonis) (JNHP00000000), *Porphyromonas canoris* (P. canoris) (JQZV00000000), *Parabacteroides johnsonii* (P. johnsonii) (ABYH00000000), *Parabacteroides merdae* (P. merdae) (AJPU00000000), and *Tannerella forsythia* (T. forsythia) (CP003191).

Genome of Marseille-P3236T strain was smaller than those of *P. gordonii* (6,483 and 6,677 MB, respectively), but was nevertheless larger than those of *P. distasonis*, *P. canoris*, *P. johnsonii*, *P. merdae*, and *T. forsythia* (5,316, 2,203, 4,787, 4,459, and 3,282 MB, respectively). The G+C content of Marseille-P3236T strain was smaller than those of *P. gordonii* (43.41%, 44.85%, 44.75%, 45.2%, 45.25%, 47.14%, and 44.43%, respectively). The gene content of Marseille-P3236T strain was smaller than those of *P. gordonii* (5,046 and 5,326, respectively), but was larger than those of *P. distasonis*, *P. canoris*, *P. johnsonii*, *P. merdae*, and *T. forsythia* (4,800, 1,612, 4,515, 3,703, and 2,492, respectively).

Distribution of functional classes of predicted genes according to the COGs database regarding strain Marseille-P3236T is presented in Figure 6. The distribution was similar in all the studied genomes. Strain Marseille-P3236T shared the highest number of orthologous proteins with *P. gordonii* (2,614 with 84.62% similarity at the nucleotide level, Table 7).

As for digital DNA-DNA hybridization (dDDH) values between strain Marseille-P3236T and its phylogenetic closest species, it was

| Properties | Parabacteroides timonensis |
|------------|-----------------------------|
| Cell length (μm) | 1.4-2.7 |
| Oxygen requirement | Strictly anaerobic |
| Gram stain | Negative |
| Salt requirement | – |
| Motility | + |
| Endospore formation | – |
| Indole | – |
| Production of | + |
| Alkaline phosphatase | + |
| Catalase | + |
| Oxidase | – |
| Urease | – |
| β-galactosidase | + |
| N-acetyl-glucosamine | + |
| Acid from | + |
| D-Arabinose | + |
| D-Ribose | + |
| D-Mannose | + |
| D-Mannitol | + |
| D-glucose | + |
| D-fructose | + |
| D-maltose | + |
| D-lactose | + |
| G+C content (mol%) | 43.41 |
| Habitat | Human gut |

### Table 3 General characteristics of *Parabacteroides timonensis* strain Marseille-P3236T

| Properties       | Parabacteroides timonensis |
|------------------|-----------------------------|
| Cell length (μm) | 1.4-2.7 |
| Oxygen requirement | Strictly anaerobic |
| Gram stain | Negative |
| Salt requirement | – |
| Motility | + |
| Endospore formation | – |
| Indole | – |
| Production of | + |
| Alkaline phosphatase | + |
| Catalase | + |
| Oxidase | – |
| Urease | – |
| β-galactosidase | + |
| N-acetyl-glucosamine | + |
| Acid from | + |
| D-Arabinose | + |
| D-Ribose | + |
| D-Mannose | + |
| D-Mannitol | + |
| D-glucose | + |
| D-fructose | + |
| D-maltose | + |
| D-lactose | + |
| G+C content (mol%) | 43.41 |
| Habitat | Human gut |

### Table 4 Cellular fatty acids composition of *Parabacteroides timonensis* strain Marseille-P3236T

| Fatty acids | Name                              | Mean relative % (a) |
|-------------|-----------------------------------|---------------------|
| 15:0 anteiso | 12-methyl-tetradecanoic acid      | 46.0 ± 0.5          |
| 15:0         | Pentadecanoic acid                | 8.6 ± 0.4           |
| 18:1n9       | 9-Octadecenoic acid               | 8.4 ± 0.4           |
| 17:0 3-OH iso| 3-hydroxy-15-methyl-Hexadecanoic acid | 7.6 ± 0.4 |
| 16:0         | Hexadecanoic acid                 | 6.1 ± 0.1           |
| 18:2n6       | 9,12-Octadecadienoic acid         | 5.8 ± 0.5           |
| 15:0 iso     | 13-methyl-tetradecanoic acid      | 4.7 ± 0.1           |
| 16:0 3-OH    | 3-hydroxy-Hexadecanoic acid       | 3.9 ± 0.3           |
| 17:0 3-OH anteiso | 3-hydroxy-14-methyl-Hexadecanoic acid | 1.4 ± 0.6 |
| 14:0 iso     | 12-methyl-Tridecanoic acid        | 1.2 ± 0.1           |
| 5:0 iso      | 3-methyl-butanoic acid            | 1.0 ± 0.1           |
| 14:0         | Tetradecanoic acid                | 1.0 ± 0.1           |
| 18:0         | Octadecanoic acid                 | TR                  |
| 16:1n7       | 9-Hexadecenoic acid               | TR                  |
| 15:0 3-OH    | 3-hydroxy-Pentadecanoic acid      | TR                  |
| 17:0 3-OH    | 3-hydroxy-Heptadecanoic acid      | TR                  |
| 17:1n7       | 10-Heptadecanoic acid             | TR                  |
| 20:4n6       | 5,8,11,14-Eicosatetraenoic acid   | TR                  |
| 13:0 iso     | 11-methyl-Dodecanoic acid         | TR                  |
| 18:1n7       | 11-Octadecanoic acid              | TR                  |
| 16:0 3-OH iso| 3-hydroxy-14-methyl-Pentadecanoic acid | TR  |
| 16:0         | 9,10-methylene-2-hexyl-Cyclopropaneoctanoic acid | TR  |
| 13:0 anteiso | 10-methyl-Dodecanoic acid         | TR                  |
| 17:0         | Heptadecanoic acid                | TR                  |
| 13:0         | Tridecanoic acid                  | TR                  |

(a) Mean peak area percentage; TR = trace amounts <1%.

As for digital DNA-DNA hybridization (dDDH) values between strain Marseille-P3236T and its phylogenetic closest species, it was

those of *P. distasonis*, *P. canoris*, *P. johnsonii*, *P. merdae*, *T. forsythia*, and *P. gordonii* (43.41%, 44.85%, 44.75%, 45.2%, 45.25%, 47.14%, and 44.43%, respectively). The gene content of Marseille-P3236T strain was smaller than those of *P. gordonii* (5,046 and 5,326, respectively), but was nevertheless larger than those of *P. distasonis*, *P. canoris*, *P. johnsonii*, *P. merdae*, and *T. forsythia* (4,800, 1,612, 4,515, 3,703, and 2,492, respectively).

Distribution of functional classes of predicted genes according to the COGs database regarding strain Marseille-P3236T is presented in Figure 6. The distribution was similar in all the studied genomes. Strain Marseille-P3236T shared the highest number of orthologous proteins with *P. gordonii* (2,614 with 84.62% similarity at the nucleotide level, Table 7).

As for digital DNA-DNA hybridization (dDDH) values between strain Marseille-P3236T and its phylogenetic closest species, it was
TABLE 5 General genomic characteristics of strain Marseille-P3236<sup>T</sup>

| Characteristic                        | Number     | Percent<sup>a</sup> |
|--------------------------------------|------------|----------------------|
| Size (bp)                            | 6,483,434  | 100                  |
| Number of G+C                        | 2,813,492  | 43.41                |
| Number total of genes                | 5,130      | 100                  |
| Number total of protein genes        | 5,046      | 98.36                |
| Number total of RNA genes            | 84         | 1.64                 |
| Number total of tRNA genes           | 72         | 1.40                 |
| Number total of RNA (5S, 16S, 23S)   | 12         | 0.23                 |
| Coding sequence size                 | 5,884,794  | 90.77                |
| Coding sequence gene protein size    | 5,858,682  | 90.36                |
| Coding sequence tRNA gene size       | 5,555      | 0.09                 |
| Coding sequence (5S, 16S, 23S) gene size | 20,557    | 0.32                 |
| Number of protein-coding gene        | 5,046      | 100                  |
| Number of protein associated to COGs | 2,513      | 49.80                |
| Number of protein associated to orphan | 202      | 4.00                 |
| Number of protein with peptide sigNAl | 1,641      | 32.52                |
| Number of gene associated to resistance genes | 0 | 0                   |
| Number of gene associated to PKS or NRPS | 11 | 0.22                |
| Number of genes associated to virulence | 705 | 13.97                |

<sup>a</sup>The total is based on either the size of the genome in base pairs or the total number of protein-coding genes in the annotated genome.

21.6 [19.3–24], 31 [28.6–33.5], 21.5 [19.2–23.9], 28 [25.7–30.5], and 23.5 [21.2–25.9] with P. johnsonii, P. canoris, P. distasonis, P. gordonii, and T. forsythia, respectively. In fact, 70% dDDH value is considered as a threshold to define a new bacterial species (Tindall, Rosselló-Móra, Busse, Ludwig, & Kämpfer, 2010; Wayne et al., 1987). According to our results, strain Marseille-P3236<sup>T</sup> shared with all its phylogenetically closest species with standing in nomenclature dDDH value of less than 70% and thus confirming it as a new species (Table 8).

FIGURE 5 Graphical circular map of the genome of Parabacteroides timonensis strain Marseille-P3236<sup>T</sup>. From outside to the center: Contigs (red/gray), COG category of genes on the forward strand (three circles), genes on forward strand (blue circle), genes on the reverse strand (red circle), COG category on the reverse strand (three circles), G+C content.
DISCUSSION

The human gut microbiota has been extensively studied by the scientific community and it has already been correlated with several health conditions such as obesity (Million et al., 2016), gastrointestinal diseases (Guinane & Cotter, 2013), or nonalcoholic fatty acid liver disease (Abu-Shanab & Quigley, 2010). Profiling the bacterial content and its ratio in the human gut have led to the development of several therapeutic strategies such as probiotics, and also therapeutic improvements such as the case of CTLA-4-based cancer immunotherapy (Vétizou et al., 2015). Hence, describing the human gut microbiota without neglecting a group of its population is essential. Culturomics was developed for the purpose of isolating previously uncultured organisms along with attempting to correlate its sequences to operational taxonomic units (Lagier et al., 2012, 2016). This work adds on the previously performed descriptive studies on the human gut microbiota via culturomics (Lagier et al., 2016) by isolating a new bacterial species belonging to the Parabacteroides genus (Sakamoto & Benno, 2006). However, Parabacteroides has been previously isolated from clinical cases (Kierzkowska et al., 2017) and was the causative agent of bacteremia (Awadel-Kariem, Patel, Kapoor, Brazier, & Goldstein, 2010). This fact renders the isolation and description of a new Parbacteroides species important as it can now be more easily identified if isolated in the future during a clinical case and thus facilitating medical diagnoses.

CONCLUSION

In conclusion, describing the human microbiota remains a challenging task requiring intensive efforts. Even though sequencing approaches proved to be efficient in this field, culturomics reemphasized the importance of culture in deciphering the dark matter of the human microbiome by shedding the light on previously unidentified and uncultured species (Lagier et al., 2016). Herein, we report the isolation of a new bacterial species from the human gut, Parabacteroides timonensis strain P3236T which represents the ninth Parabacteroides species and the seventh found in the human gut.

5.1 Description of Parabacteroides timonensis sp. nov

Parabacteroides timonensis (ti.mo.nen’sis. N.L. masc. adj. timonensis pertaining to La Timone, Marseilles’ hospital name, France, where the strain was isolated).

P. timonensis is motile, gram-negative rod, catalase positive, oxidase negative, nonspore-forming, strictly anaerobic, and has a cell length ranging between 1.4 and 2.7 μm. Colonies of P. timonensis are smooth and exhibit a diameter of 0.8–1 mm. The optimal growth temperature of P. timonensis is 37°C.

Positive reactions are observed with alkaline phosphatase, α-glucosidase, esterase lipase C8, valine arylamidase, acid phosphatase, cystine arylamidase, α-galactosidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, leucine arylamidase, β-glucuronidase, β-glucosidase, esterase C4, N-acetyl-β-glucosaminidase but negative with lipase (C14), trypsin,

| Code | Value | % of total | Description |
|------|-------|------------|-------------|
| [J]  | 194   | 3.8446293  | Translation |
| [A]  | 0     | 0          | RNA processing and modification |
| [K]  | 194   | 3.8446293  | Transcription |
| [L]  | 165   | 3.2699168  | Replication, recombination and repair |
| [B]  | 0     | 0          | Chromatin structure and dynamics |
| [D]  | 25    | 0.49544194 | Cell cycle control, mitosis, and meiosis |
| [Y]  | 0     | 0          | Nuclear structure |
| [V]  | 128   | 2.5366626  | Defense mechanisms |
| [T]  | 183   | 3.6266348  | Signal transduction mechanisms |
| [M]  | 233   | 4.617519   | Cell wall/membrane biogenesis |
| [N]  | 22    | 0.4399887  | Cell motility |
| [Z]  | 1     | 0.01981767 | Cytoskeleton |
| [W]  | 0     | 0          | Extracellular structures |
| [U]  | 96    | 1.902497   | Intracellular trafficking and secretion |
| [O]  | 183   | 3.2699168  | Signal transduction mechanisms |
| [C]  | 183   | 3.6266348  | Cell cycle control, mitosis, and meiosis |
| [G]  | 248   | 4.914784   | Carbohydrate transport and metabolism |
| [E]  | 180   | 3.5671818  | Amino acid transport and metabolism |
| [F]  | 128   | 2.5366626  | Defense mechanisms |
| [H]  | 128   | 2.5366626  | Defense mechanisms |
| [I]  | 128   | 2.5366626  | Defense mechanisms |
| [P]  | 297   | 5.8858504  | Inorganic ion transport and metabolism |
| [Q]  | 36    | 0.7134364  | Secondary metabolites biosynthesis, transport and catabolism |
| [R]  | 297   | 5.8858504  | Inorganic ion transport and metabolism |
| [S]  | 108   | 2.140309   | Function unknown |
|      | 2,533 | 50.19818   | Not in COGs |
FIGURE 6  Distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins for Parabacteroides timonensis strain Marseille-P3236T

|       | TF | PM | PC | PT | PJ | PG | PD |
|-------|----|----|----|----|----|----|----|
| TF    | 2,492 | 1,287 | 822 | 1,388 | 1,207 | 1,379 | 1,248 |
| PM    | 59.79 | 3,703 | 905 | 2,279 | 2,147 | 2,224 | 1,991 |
| PC    | 56.21 | 55.97 | 1,612 | 935 | 860 | 922 | 871 |
| PT    | 58.35 | 61.94 | 56.9 | 5,046 | 2,222 | 2,614 | 2,237 |
| PJ    | 59.7 | 76.79 | 56.11 | 61.54 | 4,515 | 2,176 | 1,943 |
| PG    | 58.45 | 64.48 | 55.61 | 61.98 | 63.46 | 62 | 4,800 |
| PD    | 59.15 | 64.48 | 55.61 | 61.98 | 63.46 | 62 | 4,800 |

Note. TF, Tannerella forsythia; PM, Parabacteroides merdae; PC, Porphyromonas canoris; PT, Parabacteroides timonensis; PJ, Parabacteroides johnsonii; PG, Parabacteroides gordonii; PD, Parabacteroides distasonis.

TABLE 7  The numbers of orthologous proteins shared between strain Marseille-P3236T genomes and others closely related species genomes (upper right), average percentage similarity of nucleotides of shared orthologous proteins between genomes (lower left) and numbers of proteins per genome (bold)

TABLE 8  Pairwise comparison of Parabacteroides timonensis strain Marseille-P3236T with other species using GGDC, formula 2 (DDH estimates based on identities/HSP length) upper right

|       | TF | PM | PC | PT | PJ | PG | PD |
|-------|----|----|----|----|----|----|----|
| PT    | 100% | 21.6 [19.3–24%] | 31 [28.6–33.5%] | 21.5 [19.2–23.9%] | 28 [25.7–30.5%] | 23.5 [21.2–25.9%] |
| PJ    | 100% | 30.8 [28.4–33.3%] | 22.5 [20.2–24.9%] | 21.3 [19.1–23.8%] | 28.8 [26.4–31.3%] |
| PC    | 100% | 28 [25.7–30.5%] | 33.3 [25.7–30.5%] | 32.9 [25.7–30.5%] |
| PD    | 100% | 20.5 [18.3–23%] | 23.2 [20.9–25.6%] |
| PG    | 100% | 17.1 [15–19.5%] |
| TF    | 100% |

Note. TF, Tannerella forsythia; PM, Parabacteroides merdae; PC, Porphyromonas canoris; PT, Parabacteroides timonensis; PJ, Parabacteroides johnsonii; PG, Parabacteroides gordonii; PD, Parabacteroides distasonis.

*aThe confidence intervals indicate the inherent uncertainty in estimating DDH values from intergenomic distances based on models derived from empirical test data sets.
α-chymotrypsin, α-mannosidase, and α-fucosidase. Acidification reactions are positive for saccharose, glucose, arabinose, lactose, maltose, raffinose, xylose, trehalose, and rhamnose but negative for glycerol, cellobiose, melezitose, sorbitol, mannitol, and salicin. This strain fails to form indole, is urease negative, and cannot hydrolyze gelatin or esculin. Strain Marseille-P3236T is able to ferment d-saccharose, d-arabinose, d-xylose, d-glucose, d-fructose, d-ribose, d-mannose, d-glucose, l-rhamnose, erythritol, salicin, methyl-α-d-mannopyranoside, l-arabinose, d-cellobiose, d-fructose, d-galactose, d-raffinose, d-mannitol, d-tagatose, d-sorbitol, d-maltose, arbutin, d-lactose, methyl-α-d-glucosamine, amygdaline, N-acetylglucosamine, esculin ferric citrate, d-trehalose, d-melibiose, inulin, d-melezitose, d-turanose, starch, and potassium gluconate but not xylitol, potassium 5-ketogluconate, potassium 2-ketogluconate, methyl-β-d-xylopyranoside, l-xylose, l-sorbose, l-fucose, l-arabitol, inositol, glycerol, glycogen, d-ucilcit, d-lyxose, d-fucose, d-arabitol, and d-adonitol. Its major fatty acid is 12-methyl-tetradecanoic acid (46%).

Strain Marseille-P3236T genome is 6,483,434 bp long with 43.41 mol% of G+C content. The 16S rRNA and genome sequences from the stool sample of a healthy 39-year-old pygmy male from Congo.

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