Noncontiguous finished genome sequences and description of *Bacteroides mediterraneensis* sp. nov., *Bacteroides ihuae* sp. nov., *Bacteroides togonis* sp. nov., *Bacteroides ndongoniae* sp. nov., *Bacteroides ilei* sp. nov. and *Bacteroides congonensis* sp. nov. identified by culturomics

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Abstract

Culturomics is a concept developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria. This enabled us to isolate six new species of the *Bacteroides* genus: *Bacteroides mediterraneensis* strain Marseille-P2644, *Bacteroides ihuae* strain Marseille-P2824, *Bacteroides togonis* strain Marseille-P3166, *Bacteroides ndongoniae* strain Marseille-P3108, *Bacteroides ilei* strain Marseille-P3208 and *Bacteroides congonensis* strain Marseille-P3132. Those bacteria are Gram-negative anaerobic bacilli. We describe here their phenotypic features, together with phylogenetic analysis, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry spectrum, fatty acid composition, and genome sequencing and annotation.

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Introduction

The *Bacteroides* genus was discovered in 1919 and defined as the type genus of the Bacteroidaceae family [1]. The first type species, *Bacteroides fragilis*, was discovered in 1898 and approved in 1980 [2]. This genus currently comprises 52 validated species (http://www.bacterio.net/) (Fig. 1) including *Bacteroides fragilis* and *Bacteroides thetaiotaomicron*, the oldest and most studied species. Bacteria of this genus are non-sporulating, anaerobic, Gram negative and rod shaped [3]. *Bacteroides* is one of the major lineages present in the human colon and is involved in ‘good’ processes (production of energy source, activation of immune response) as well as ‘bad’ processes leading to diseases (abscess, reservoir of antibiotic resistance determinants, bacteraemia) [3]. More recent studies have attempted to elucidate the implication of those bacteria in colorectal cancer [4,5].

Consequently, enlarging our knowledge of bacteria that colonize the human gut, but more generally of the human microbiota, is foundational to better understand the multiple functions in which they are involved and enable the treatment of diseases. This is the reason why we used the culturomics concept, consisting of the use of multiple growth conditions, in
order to identify new bacteria previously uncultivable with classic methods [6–9]. This enabled us to isolate six new species of the Bacteroides genus. We used a taxonomogenomics approach, including matrix-assisted desorption ionization—time of flight mass spectrometry (MALDI-TOF MS) spectrum, phylogenetic analysis, main phenotypic description and genome sequencing [10,11], to describe the following bacteria: Bacteroides mediterraneensis strain Marseille-P2644 (= CSUR P2644 = DSM 103033), Bacteroides ihuae strain Marseille-P2824 (= CSUR P2824 = CCUG 70550), Bacteroides togonis strain Marseille-P3166 (= CSUR P3166 = DSM 103637), Bacteroides ndongoniae strain Marseille-P3108 (= CSUR P3108 = DSM 103636), Bacteroides ilei strain Marseille-P3208 (= CSUR P3208 = CCUG 69964) and Bacteroides congonensis strain Marseille-P3132 (= CSUR P3132 = CCUG 70144).

Materials and methods

Strain identification by MALDI-TOF MS and 16S rRNA gene sequencing

According to the culturomics approach, we tested 18 conditions on samples to isolate these strains, as previously described [7,8]. The samples’ origins and conditions of isolation are summarized in Table 1.

Purified colonies were identified by MALDI-TOF MS using a Microflex LT spectrometer and an MSP 96 MALDI-TOF target plate (Bruker Daltonics, Bremen, Germany), as previously described [12,13]. The obtained spectra were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed by standard pattern matching (with default parameter settings) against the main spectra of the 7537 bacteria included in the databases (Bruker and constantly updated Microbes Evolution Phylogeny and Infections (MEPHI) databases). The resulting score enabled the identification (or not) of tested species: a score of ≥2 with a validly published species enabled identification at the species level, a score of ≥1.7 but <2 enabled identification at the genus level and a score of <1.7 did not enable any identification. No significant scores were obtained for the studied strains, suggesting that our isolates were not members of known species.

Consequently, sequencing of the 16S rRNA gene was performed in order to identify these strains. DNA was previously extracted by EZ1 DNA Tissue Kit using BioRobot EZ1 Advanced XL (Qiagen, Courtaboeuf, France). The amplification and purification of the 16S rRNA gene was performed as previously described [14,15] by use of the universal primers pair fD1 and rP2 (Eurogentec, Angers, France). Sequencing was then done using the Big Dye Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary sequencer (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA), as previously described [14,15]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com), and BLASTn searches were performed against the National Center for Biotechnology Information (NCBI) GenBank database (http://blast.ncbi.nlm.nih.gov/gate1/insti.fr/Blast.cgi) to determine the percentage of similarity with the closest bacteria. A similarity threshold of <98.65% enabled us to define a new species, whereas a threshold of <95% enabled us to define a new genus without performing DNA-DNA hybridization [16].

Phylogenetic analysis

A custom Python script was used to automatically retrieve all species from the same family as that of the new species, and we downloaded 16S sequences from NCBI by parsing NCBI eUtils
results and the NCBI taxonomy page. It only kept sequences from type strains. The script then divided 16S sequences into two groups: one containing the sequences of strains from the same genus (group A) and one containing the others (group B). Finally, it only kept the 49 closest strains from group A and one from group B (outgroup).

**Phenotypic, biochemical and antibiotic susceptibility tests**

Ideal growth conditions of the studied strains were determined by testing five growth temperatures (25, 30, 37, 42, 56°C) in an aerobic atmosphere with or without 5% CO2 and under anaerobic and microaerophilic conditions using the GENbag anaer and GENbag microaer systems, respectively (bioMérieux, Marcy l’Étoile, France). Different pH values (5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5) and NaCl concentrations (5, 10, 50, 75, 100, 150, 200 g/L) were also tested. Phenotypic characteristics such as Gram staining, motility, sporulation, and catalase and oxidase activities were tested as previously described [7].

The biochemical analysis was carried out using API 50CH, API 20A, API ZYM strips (bioMérieux) in an anaerobic atmosphere, according to the manufacturer’s instructions. Cellular fatty acid methyl ester (FAME) analysis was performed by gas chromatography/mass spectrometry (GC/MS). Two samples were prepared with approximately 20 to 70 mg (according to bacteria) of bacterial biomass per tube collected from several culture plates. FAME were prepared as described by Sasser [17]. GC/MS analyses were carried out as previously described [18]. Briefly, FAME were separated using an Elite 5-MS column and monitored by mass spectrometry (Clarus 500–SQ 8 S; Perkin Elmer, Courtaboeuf, France). A spectral database search was performed using MS Search 2.0 operated with the Standard Reference Database 1A (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the FAMEs mass spectral database (Wiley, Chichester, UK). Antibiotic susceptibility was tested using the disc diffusion method [19] and according to European Committee on Antimicrobial Susceptibility Testing 2018 recommendations.

**Microscopy**

Negative staining was performed in order to observe the cells’ morphology. The cells were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 1 hour at 4°C. A drop of cell suspension was deposited for approximately 5 minutes on glow-discharged Formvar carbon film with 400 mesh nickel grids (FCF400-Ni; Electron Microscopy Sciences (EMS), Hatfield, PA, USA). The grids were negatively stained with 1% ammonium molybdate solution in filtered water at room temperature. Electron micrographs were acquired with a Tecnai G20 Cryo (FEI Company, Limeil-Brévannes, France) transmission electron microscope operated at 200 keV or with a Morgagni 268D (Philips, Amsterdam, The Netherlands) transmission electron microscope operated at 80 keV.

**DNA extraction and genome sequencing**

Genomic DNA (gDNA) of strains Marseille-P3132, Marseille-P3166 and Marseille-P3208 were first extracted by a mechanical treatment using acid-washed glass beads (G4649—500g; MilliporeSigma, St. Louis, MO, USA) and a FastPrep BIO 101 instrument (Qbiogene, Strasbourg, France) at maximum speed (6.5) for 3 × 30 seconds. Then for all the studied Bacteroides strains a 2-hour lysozyme incubation at 37°C was done and gDNA was extracted using the EZ1 biorobot (Qiagen) with the EZ1 DNA tissues kit. The elution volume was 50 μL. gDNA was quantified by a Qubit assay with the High Sensitivity kit (Life Technologies, Carlsbad, CA, USA) (Supplementary Table S1).

gDNA was sequenced on the MiSeq Technology (Illumina, San Diego, CA, USA) with the mate pair strategy. The gDNA was sequenced following the Nextera XT Library Preparation protocol of Illumina.
was barcoded in order to be mixed with 11 other projects with the Nextera Mate Pair sample prep kit (Illumina). The mate pair library was prepared with 1.5 μg of gDNA using the Nextera mate pair Illumina guide. The gDNA sample was simultaneously fragmented and tagged with a mate pair junction adapter. The pattern of fragmentation was validated on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) with a DNA 7500 labchip. The DNA fragments ranged in size from 1.5 to 11 kb (with an optimal size at 7.710, 6.047, 7.937, 7.840, 10.380 and 6.752 kb for strain Marseille-P2644, Marseille-P2824, Marseille-P3132, Marseille-P3108, Marseille-P3166 and Marseille-P3208, respectively). No size selection was performed, and 600 ng (610.4 ng for strain Marseille-P3132 and 369.6 ng for strain Marseille-P3108) of tagedgment fragments were circularized. The circularized DNA was mechanically sheared to small fragments (with an optimal size of 1209, 997, 1140 and 1167 bp for strain Marseille-P3132, Marseille-P3108, Marseille-P3166 and Marseille-P3208, with optima on a bimodal curve at 975 and 1514 bp for strain Marseille-P2824 and with optima on a trimodal curve at 675, 1252 and 2049 bp for strain Marseille-P2644) on the Covaris device S2 in T6 tubes (Covaris, Woburn, MA, USA). The library profile was visualized on a High Sensitivity Bioanalyzer LabChip (Agilent Technologies), and the final concentration library was measured (Supplementary Table S1).

The libraries were normalized at 2 nM and pooled. After a denaturation step and dilution at 15 pM, the pool of libraries was loaded onto the reagent cartridge and then onto the instrument along with the flow cell. Automated cluster generation and sequencing run were performed in a single 39-hour run in a 2 × 151 bp (2 × 251 bp for strains Marseille-P3108, Marseille-P3166 and Marseille-P3208). The paired reads were finally trimmed and assembled. Complementary information is available in Supplementary Table S1.

Genome assembly, annotation and comparison

The genomes’ assembly was performed with a pipeline that enabled us to create an assembly with different software packages (Velvet [20], Spades [21] and Soap Denovo [22]), on trimmed (MiSeq and Trimmomatic [23] software) or untrimmed data (only MiSeq software). For each of the six assemblies performed, GapCloser [22] was used to reduce gaps. Then contamination with Phage Phix was identified (BLASTn against Phage Phix174 DNA sequence) and eliminated. Finally, scaffolds under 800 bp in size were removed and scaffolds with a depth value lower than 25% of the mean depth were removed (identified as possible contaminants). The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). For all studied strains, Spades gave the best assembly (with a depth coverage of 153, 44, 647, 99 and 94, respectively, for strains Marseille-P2824, Marseille-P3132, Marseille-P3108, Marseille-P3166 and Marseille-P3208), except for strain Marseille-P2644, which obtained the best assembly with Velvet (with a depth coverage of 174).

Open reading frames (ORFs) were predicted using Prodigal [24] with default parameters, but the predicted ORFs were excluded if they spanned a sequencing gap region (contained N). The predicted bacterial protein sequences were searched against the Clusters of Orthologous Groups (COGs) database using BLASTP (E value of 1e-03, coverage 0.7 and identity percentage 30%). If no hit was found, we searched against the NR database using BLASTP (E value of 1e-03, coverage 0.7 and identity percent of 30%). If the sequence length was smaller than 80 aa, we used an E value of 1e-05. The tRNAscanSE [25] tool was used to find transfer RNA genes, whereas ribosomal RNA genes were found by using Rfam [26]. Lipoprotein signal peptides and the number of transmembrane helices were predicted using Phobius [27]. ORFans were identified if all the BLASTP searches we performed failed to provide positive results (E value smaller than 1e-03 for ORFs with sequence size superior to 80 aa or E value smaller than 1e-05 for ORFs with sequence length smaller than 80 aa). Such parameter thresholds have already been used in previous works to define ORFans. Pfam-conserved domains (Pfam-A and Pfam-B domains) were searched on each protein with an HMMScan of the HMMER3 suite [28]. Number of genes associated to polyketide synthase (PKS) or nonribosomal peptide synthase (NRPS) and antibiotic resistance genes were found using the BLAST program against the Clusters of Orthologous Groups of proteins (with same domain) and the Homemade database [30], respectively. The annotation process was performed in the multi-agent software system DAGOBABH [31], which includes Figenix [32] libraries, which provided the pipeline analysis.

Species that must be compared were automatically retrieved from the 16S RNA tree using PhyloPattern. For each selected species, the complete genome sequence, proteome sequence and ORFeome sequence were retrieved from the NCBI’s FTP site. If one specific strain did not have a complete and available genome, a complete genome of the same species was used. If ORFeome and proteome were not predicted, Prodigal was used with default parameters to predict them. All proteomes were analysed with proteinOrtho [33]. Then, for each couple of genomes, a similarity score was computed. This score is the mean value of nucleotide similarity between all couples of orthologous genes between the two genomes studied (average genomic identity of orthologous gene sequences (AGIOS) tool). An annotation of all proteomes was also realized to define the distribution of functional classes of predicted genes according to the clusters of orthologous groups of proteins (with same method as that used for genome annotation). The comparison
The studied strains could not be identified using MALDI-TOF MS, and therefore their 16S rRNA gene was sequenced. Strain Marseille-P3132 (accession no. LT598566) revealed 96.74% sequence similarity with the 16S rRNA of Bacteroides thetaiotaomicron strain VPI-5482, the closest species with a validly published name. We consequently propose that our strain is a representative strain of a new species within the genus Bacteroides for which we suggest the name ‘Bacteroides congonensis’ strain Marseille-P3132. Strain Marseille-P2824 (accession no. LT576386) revealed 95.62% sequence similarity with the 16S rRNA of Bacteroides graminisolvens strain JCM15093, the closest species with a validly published name. We consequently propose that our strain is a representative strain of a new species within the genus Bacteroides for which we suggest the name ‘Bacteroides ilei’ strain Marseille-P2824. Strain Marseille-P3208 (accession no. LT623890) revealed 94.29% sequence similarity with the 16S rRNA of Bacteroides coprophilus strain CB42, the closest species with a validly published name. Although this value is under the threshold of 95%, we proposed this strain as a new species and not a new genus according to the low percentage of similarity already existing between two validly published Bacteroides species (74.8–98.7%) [35]. We consequently propose that our strain is a representative strain of a new species within the genus Bacteroides for which we suggest the name ‘Bacteroides ilei’ strain Marseille-P3208. Strain Marseille-P3108 (accession no. LT615364) revealed 93.91% sequence similarity with the 16S rRNA of Bacteroides egerthii strain JCM12986, the closest species with a validly published name. We consequently propose that our strain is a representative strain of a new species within the genus Bacteroides for which we suggest the name ‘Bacteroides ilei’ strain Marseille-P3108. Strain Marseille-P3166 (accession no. LT631521) revealed 93.14% sequence similarity with the 16S rRNA of Bacteroides egerthii strain JCM12986, the closest species with a validly published name. We consequently propose that our strain is a representative strain of a new species within the genus Bacteroides for which we suggest the name ‘Bacteroides ilei’ strain Marseille-P3166. Strain Marseille-P2644 (accession no. LT558804) revealed a 94.61% sequence similarity with the 16S rRNA of Bacteroides coprocola strain M16, the closest species with a validly published name. We consequently propose that our strain is a representative strain of a new species within the genus Bacteroides for which we suggest the name ‘Bacteroides ilei’ strain Marseille-P2644.

A phylogenetic tree showing the position of the studied strains in the Bacteroides genus is shown in Fig. 2. We observed that B. ilei and B. mediterraneensis, which are closely related, exhibited a 95.31% similarity, which confirms that they belong to two distinct species. The same observation was made for B. ndongonae and B. togoensis, which exhibited a 93.05% 16S rRNA similarity. All spectra (Fig. 3(A)) were added to MEGH database (http://www.mediterranee-infection.com/article.php?arub=280&dore=urms-database). A gel view was made in order to compare proteomic profiles between the studied strains and their closest species (Fig. 3(B)). The analysis revealed a common general profile between Bacteroides strains, including the studied strains, whereas the outsider species Parabacteroides goldsteinii profile shows several unique differences.

Phenotypic features

The main phenotypic results of each studied strain compared to published data of close species are presented in Table 2 [36–38]. The results show that the morphologic description, notably Gram staining, cell shape, spore formation and atmosphere, supports the fact that these six new species are members of the Bacteroides genus. These observations were confirmed by electronic microscopy (Fig. 4), which revealed a similar morphology. Regarding the biochemical characteristics, nearly all compared strains were negative for oxidase and urease activities and mannitol metabolism, whereas they were positive for β-galactosidase and alkaline phosphatase activities (Table 2). A more detailed analysis of biochemical features of the studied strains is available in Supplementary Tables S2, S3 and S4, corresponding to API strip results. Finally, we observed that the main fatty acid is C15:0 anteiso for nearly all compared strains (Table 2).

The composition in cellular fatty acids of the studied strains is presented in detail in Table 3; results of antibiotic susceptibility testing are presented in Table 4. We observed that the major cellular fatty acid of the studied Bacteroides strains is 12-methyl-tetradecanoic acid except for the Marseille-P3166 and Marseille-P3108 strains (Table 3). Table 4 shows that the strains often have the same antibiotic susceptibility profile, except strain Marseille-P3132. Nevertheless, all strains are susceptible to imipenem, amoxicillin, amoxicillin/clavulanic acid, metronidazole and rifampicin and resistant to gentamicin.

Genome description and comparison

The genomes of the studied strains are represented in a map in Supplementary Fig. 1. The detail of the genomes’ content and statistics are summarized in Table 5, and the distribution of
predicted genes in COGs categories is shown in Table 6. We observed that the gene distribution in the 25 general COGs functional categories of the six new Bacteroides species is similar. The genomic characteristics of the studied strains were compared to the available genomes of closely related species in Table 7. This enabled us to observe that for all studied strains, the genome’s size, percentage of G+C content and number of total genes are in the same range as those of other Bacteroides species.

Furthermore, digital DNA-DNA hybridization values (Table 8) between compared species except for the studied strains ranged from 17.90% to 34.50%. When the studied strains were compared to other Bacteroides species, the values were approximately in the same range: 19.70–29.80% for strain...
FIG. 3. (A) Reference mass spectra. Spectra from 12 individual colonies were compared and each reference spectrum generated. (B) Gel view comparing studied strains to other species within Bacteroides genus. Gel view displays raw spectra of loaded spectrum files arranged in a pseudo-gel-like look. X-axis records m/z value. Left y-axis displays running spectrum number originating from subsequent spectra loading, peak intensity is expressed by greyscale scheme code. Colour bar and right y-axis indicate relation between colour and peak intensity in arbitrary units. Displayed species are indicated at left.
### TABLE 2. Comparison of phenotypic characteristics between nine Bacteroides species [36–38]

| Property                        | B. ilei            | B. mediterraneensis | B. coprocola | B. ndongoniae | B. togonis   | B. finegoldii | B. congensis | B. ihuae | B. graminisolvens |
|--------------------------------|--------------------|---------------------|--------------|---------------|--------------|--------------|--------------|----------|------------------|
| Strain                          | Marseille-P3208    | Marseille-P2644     | M16<sup>7</sup> | Marseille-P3108 | Marseille-P3166 | 199<sup>2</sup> | Marseille-P3132 | Marseille-P2824 | XDT-1<sup>7</sup> |
| Optimal temperature             | 37°C               | 37°C                | 37°C         | 37°C          | 37°C         | 37°C         | 37°C         | 30°C     | 30–35°C          |
| Atmosphere                      | Anaerobic          | Anaerobic           | Anaerobic    | Anaerobic     | Anaerobic    | Anaerobic    | Anaerobic    | Anaerobic | Anaerobic        |
| pH range                        | 5–7.5              | 5–8                 | NA           | 7–7.5         | 7–7.5        | NA           | Anaerobic    | 6–8.5    | Anaerobic        |
| Colony aspect                   | Circular, translucent | Circular, grey, translucent, shiny | Disc shaped and greyish-white | Circular, grey, translucent, small | Circular, small, circular and white | Circular, translucent-wisitsh, raised, convex | Circular, smooth | Mucous | Thic, smooth-surfaced, with pearl-like, iridescent lustre |
| Cell shape                      | Fusiform           | Rod                 | Rod          | Rod           | Rod          | Rod          | Rod          | Rod      | Rod              |
| Cell length (μm)                | 3.9–5              | 0.9–2.3             | 1–4          | 2.2–3.1       | 1.4–3.2      | 1.5–4.5      | 2–2.1       | 1.2–1.8  | 1.2–4.5          |
| Cell width (μm)                 | 0.5–0.6            | 0.6–0.7             | 0.8          | 0.6–1.1       | 0.6–0.7      | 0.8          | 2–2.1       | 0.5–0.6  | 0.4–0.6          |
| Gram staining                   | Negative           | Negative            | Negative     | Negative      | Negative     | Negative     | Negative     | Negative | Negative         |
| Salt tolerance (g·L<sup>−1</sup>)| 0                  | 0                   | NA           | 0             | 0            | NA           | 50          | 5        | 0–40             |
| Motility                        | −                  | +                   | −            | −             | −            | +            | −            | −        | −                |
| Endospore formation             | −                  | +                   | −            | −             | −            | +            | −            | −        | −                |
| Major cellular fatty acid       | 15:0 anteiso       | 15:0 anteiso        | 15:0 anteiso | 15:0 iso      | 15:0 iso     | 15:0 iso     | 15:0 iso     | 15:0 iso  | 15:0 iso         |
| Production of:                  |                    |                     |              |               |              |              |              |          |                  |
| Alkaline phosphatase            | +                  | +                   | +            | +             | +            | +            | +           | −        | −                |
| Catalase                        | −                  | +                   | −            | +             | −            | +            | +           | +        | +                |
| Oxidase                         | −                  | −                   | NA           | −             | −            | +            | +           | −        | −                |
| Urease                          | −                  | −                   | −            | −             | −            | −            | −           | −        | −                |
| β-Galactosidase                 | +                  | +                   | +            | +             | +            | +            | +           | +        | +                |
| N-Acetyl-glucosamine            | −                  | +                   | NA           | −             | +            | NA           | +           | +        | −                |
| Acid from:                      |                    |                     |              |               |              |              |              |          |                  |
| L-Arabinose                     | −                  | +                   | −            | −             | −            | +            | +           | +        | +                |
| Ribose                          | −                  | −                   | NA           | −             | −            | −            | −           | −        | −                |
| Mannose                         | −                  | −                   | −            | −             | −            | +            | +           | −        | −                |
| Mannitol                        | −                  | −                   | −            | −             | −            | −            | −           | −        | −                |
| α-Saccharose                    | −                  | +                   | −            | −             | −            | +            | +           | +        | +                |
| β-Glucose                      | −                  | +                   | +            | +             | +            | +            | +           | +        | +                |
| α-Fructose                      | −                  | −                   | NA           | −             | −            | +            | +           | +        | +                |
| α-Maltose                       | −                  | +                   | −            | −             | −            | +            | +           | +        | +                |
| α-Lactose                       | −                  | −                   | −            | −             | −            | −            | −           | −        | −                |
| Habitat                         | Human ileum        | Human ileum         | Human stool  | Human right colon | Human right colon | Human faeces | Human stool  | Human sputum | Rice-straw residue |

*+, positive result; −, negative result; NA, data not available.*
FIG. 4. Gram staining and electron micrographs of Bacteroides new species: B. ihuae (A, B), B. togonis (C, D), B. ndongoniae (E, F), B. mediterraneensis (G, H), B. ilei (I, J) and B. congonensis (K, L), respectively.
TABLE 4. Antibiotic resistance test results of six Bacteroides species

| Antibiotic         | Disc load (μg/mL) | B. ilei Marseille-P3208 | B. mediterraneensis Marseille-P32644 | B. ndongoniae Marseille-P3108 | B. tognosis Marseille-P3166 | B. congonensis Marseille-P3132 | B. ihuae Marseille-P3248 |
|--------------------|-------------------|-------------------------|--------------------------------------|-------------------------------|-----------------------------|--------------------------------|--------------------------|
| Amoxicillin        | 25                | S                       | S                                    | S                             | S                           | S                               | S                        |
| Clindamycin        | 100               | S                       | R                                    | R                             | R                           | R                               | S                        |
| Gentamicin         | 25                | S                       | S                                    | S                             | S                           | S                               | S                        |
| Metronidazole      | 25                | S                       | S                                    | S                             | S                           | S                               | S                        |
| Oxacillin          | 5                 | S                       | R                                    | R                             | R                           | R                               | R                        |
| Tobramycin         | 10                | S                       | S                                    | S                             | S                           | S                               | S                        |
| Rifampicin         | 25                | S                       | S                                    | S                             | S                           | S                               | S                        |
| Doxycycline        | 25                | S                       | S                                    | S                             | S                           | S                               | S                        |
| Erythromycin       | 15                | S                       | R                                    | R                             | R                           | R                               | S                        |

NA, not available; R, resistant; S, susceptible.

Marseille-P2644, 18.50–20.40% for strain Marseille-P2824, 18.60–25.60% for strain Marseille-P3132, 19.40–50.00% for strain Marseille-P3108, 17.90–25.70% for strain Marseille-P3166 and 19.40–50.00% for strain Marseille-P3208. This confirms their new Bacteroides species status. These results are supported by values lower than 70%.

Conclusion

According to their phylogenetic, phenotypic and biochemical features and genomic comparisons, we propose the creation of six new species as members of the Bacteroides genus: Bacteroides mediterraneensis strain Marseille-P2644, Bacteroides ihuae strain Marseille-P2824, Bacteroides tognosis strain Marseille-P3166, Bacteroides ndongoniae strain Marseille-P3108, Bacteroides ilei strain Marseille-P3208 and Bacteroides congonensis strain Marseille-P3132.

Description of Bacteroides ilei sp. nov.

Bacteroides ilei (‘ilei’, L. gen. n., ilei, ‘of the ileum,’ the part of the digestive tract from which the bacterium was collected).

Cells are Gram-negative bacilli and have a fusiform shape with a length of 4 to 5 μm and a width of 0.5 to 0.6 μm. The
type strain did not exhibit catalase or oxidase activities. *Bacteroides ilei* is non-spore forming and nonmotile. Colonies are circular, small and translucent with a diameter of 0.8 to 1 mm. Optimum growth occurred in an anaerobic atmosphere at 37°C and pH 7 on Columbia agar enriched with 5% sheep’s blood after 7 days of growth. Growth was observed at pH values between 5 to 8 and without NaCl.

Results of the API ZYM strip show that the strain possesses an activity for the following enzymes: alkaline phosphatase, esterase (C4), α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and α-fucosidase. The other enzymes of the strip have no activities (Supplementary Table S2). Results of the API 50CH strip show that the strain is able to metabolize the following substrates: amygdalin, esculin ferric citrate, glycogen and potassium 5-ketogluconate. The others are negative (Supplementary Table S3). Finally, the results of the API 20A strip show a positive reaction only for β-gluco-sidase (Supplementary Table S4).

*Bacteroides ilei* is resistant to gentamicin and susceptible to other tested antibiotics (Table 4).

The major fatty acid is 12-methyl-tetradecanoic acid (47%).

The genome of strain Marseille-P3208 is 3,974,619 bp long with a 45.02 mol% G+C content. In the European Molecular Biology Laboratory–European Bioinformatics Institute (EMBL-EBI) database, the 16S rRNA gene and genome sequences are available under accession numbers LT623890 and FQSC00000000, respectively. The strain Marseille-P3208T is the type strain of the species *Bacteroides ilei* (= CSUR P3208 = CCUG 69964) and was isolated from the ileum of a 76-year-old woman with oesophagitis in Marseille, France. This bacteria was also found in the left colon of the same patient.

**Description of Bacteroides mediterraneensis sp. nov.**

*Bacteroides mediterraneensis* (me ди trа ra ne es'nis, L. masc. adj., mediterraneensis, ‘of Mediterranea,’ the sea bordering Marseille, where the strain was isolated).

Cells are Gram-negative bacilli and are rod shaped with a length of 0.9 to 2.3 μm and a width of 0.6 to 0.7 μm. The type strain exhibited a catalase activity but no oxidase activity. *Bacteroides mediterraneensis* is non-spore forming but motile. Colonies are circular, translucent, grey and shiny, with a diameter of 0.6 mm. Optimum growth occurred in an anaerobic atmosphere at 37°C and pH 7 on Columbia agar enriched with 5% sheep’s blood after 1 day of growth. Growth was observed at pH values between 5 to 8 and without NaCl.

Results of the API ZYM strip show that the strain possesses an activity for the following enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and α-fucosidase. The other enzymes of the strip have no activities (Supplementary Table S2). Results of the API 50CH strip show that the strain is able to metabolize the following substrates: amygdalin, esculin ferric citrate, glycogen and potassium 5-ketogluconate. The others are negative (Supplementary Table S3). Finally, the results of the API 20A strip show a positive reaction only for β-glucosidase (Supplementary Table S4).

*Bacteroides ilei* is resistant to gentamicin and susceptible to other tested antibiotics (Table 4).

The major fatty acid is 12-methyl-tetradecanoic acid (47%).

The genome of strain Marseille-P3208 is 3,974,619 bp long with a 45.02 mol% G+C content. In the European Molecular Biology Laboratory–European Bioinformatics Institute (EMBL-EBI) database, the 16S rRNA gene and genome sequences are available under accession numbers LT623890 and FQSC00000000, respectively. The strain Marseille-P3208T is the type strain of the species *Bacteroides ilei* (= CSUR P3208 = CCUG 69964) and was isolated from the ileum of a 76-year-old woman with oesophagitis in Marseille, France. This bacteria was also found in the left colon of the same patient.

**Description of Bacteroides mediterraneensis sp. nov.**

*Bacteroides mediterraneensis* (me ди trа ra ne es’nis, L. masc. adj., mediterraneensis, ‘of Mediterranea,’ the sea bordering Marseille, where the strain was isolated).

Cells are Gram-negative bacilli and are rod shaped with a length of 0.9 to 2.3 μm and a width of 0.6 to 0.7 μm. The type strain exhibited a catalase activity but no oxidase activity. *Bacteroides mediterraneensis* is non-spore forming but motile. Colonies are circular, translucent, grey and shiny, with a diameter of 0.6 mm. Optimum growth occurred in an anaerobic atmosphere at 37°C and pH 7 on Columbia agar enriched with 5% sheep’s blood after 1 day of growth. Growth was observed at pH values between 5 to 8 and without NaCl.

Results of the API ZYM strip show that the strain possesses an activity for the following enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-
TABLE 6. Number of genes associated with 25 general COGs functional categories for six Bacteroides species.

| Code | Description | Value | % of total | Value | % of total | Value | % of total | Value | % of total | Value | % of total | Value | % of total |
|------|-------------|--------|------------|--------|------------|--------|------------|--------|------------|--------|------------|--------|------------|
| J    | Translation | 180    | 5.25       | 178    | 5.20       | 182    | 4.32       | 179    | 5.23       | 194    | 3.88       | 190    | 5.70       |
| A    | RNA processing and modification | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| K    | Transcription | 76 | 2.21 | 93 | 2.72 | 106 | 2.44 | 107 | 3.12 | 162 | 3.24 | 115 | 3.45 |
| L    | Replication, recombination and repair | 108 | 3.15 | 112 | 3.27 | 135 | 3.21 | 111 | 3.27 | 131 | 2.62 | 92 | 2.76 |
| B    | Chromatin structure and dynamics | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D    | Cell cycle control, mitosis and meiosis | 26 | 0.75 | 23 | 0.67 | 29 | 0.68 | 27 | 0.78 | 25 | 0.50 | 25 | 0.75 |
| N    | Energy production and conversion | 112 | 3.27 | 112 | 3.27 | 105 | 2.49 | 99 | 2.89 | 125 | 3.05 | 114 | 3.42 |
| E    | Amino acid transport and metabolism | 134 | 3.91 | 132 | 3.86 | 128 | 3.04 | 118 | 3.45 | 164 | 3.28 | 149 | 4.47 |
| C    | Coenzyme transport and metabolism | 109 | 3.18 | 121 | 3.53 | 103 | 2.44 | 97 | 2.83 | 114 | 2.28 | 116 | 3.48 |
| H    | Secondary metabolites biosynthesis, transport and catabolism | 25 | 0.72 | 33 | 0.96 | 37 | 0.87 | 29 | 0.84 | 34 | 0.68 | 23 | 0.69 |
| X    | Post-translational modification, protein turnover, chaperones | 75 | 2.18 | 80 | 2.33 | 76 | 1.80 | 70 | 2.04 | 81 | 1.62 | 77 | 2.31 |
| A    | Protein turnover, chaperones | 47 | 1.37 | 14 | 0.40 | 35 | 0.83 | 21 | 0.61 | 51 | 1.02 | 16 | 0.48 |
| C    | Energy production and conversion | 112 | 3.27 | 112 | 3.27 | 105 | 2.49 | 99 | 2.89 | 125 | 3.05 | 114 | 3.42 |
| D    | Carbohydrate transport and metabolism | 162 | 4.72 | 156 | 4.35 | 176 | 4.18 | 154 | 4.50 | 257 | 5.15 | 190 | 5.70 |
| E    | Amino acid transport and metabolism | 134 | 3.91 | 132 | 3.86 | 128 | 3.04 | 118 | 3.45 | 164 | 3.28 | 149 | 4.47 |
| F    | Lipid transport and metabolism | 70 | 2.04 | 64 | 1.87 | 63 | 1.49 | 64 | 1.87 | 69 | 1.38 | 71 | 2.13 |
| G    | Nuclear structure | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| R    | Defense mechanisms | 70 | 2.04 | 83 | 2.42 | 101 | 2.40 | 86 | 2.51 | 88 | 1.76 | 83 | 2.49 |
| S    | Inorganic ion transport and metabolism | 102 | 2.97 | 111 | 3.24 | 110 | 2.61 | 11 | 0.28 | 20 | 0.40 | 14 | 0.42 |
| U    | Not in COGs | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

COGs. Clusters of Orthologous Groups database.

TABLE 7. Genome comparison of closely related Bacteroides species.

| Organism | Strain | INSDC | Size (Mb) | G+C (mol%) | Total genes |
|----------|--------|-------|-----------|------------|-------------|
| B. ilei | Marseille-P3208 | FQSC00000000 | 3.97 | 45.02 | 3425 |
| B. mediterraneensis | Marseille-P2444 | FQRZ00000000 | 4.07 | 47.47 | 3419 |
| B. capillosa | DSM 18228 | ACB70000000 | 3.87 | 46.71 | 3393 |
| B. coprocola | DSM 17136 | ABIY00000000 | 4.30 | 41.86 | 4291 |
| B. ndongonae | Marseille-P3108 | FVNN00000000 | 3.97 | 47.47 | 4205 |
| B. tagonae | Marseille-P3165 | FOX90000000 | 4.06 | 39.71 | 3332 |
| B. congoniae | Marseille-P3132 | FVNX00000000 | 4.06 | 39.71 | 3332 |
| B. coprophilus | Marseille-P2824 | FVNY00000000 | 4.06 | 39.71 | 3332 |
| B. graminiphilus | JCM15093 | BAJ50000000 | 3.68 | 41.5 | 3403 |
| B. raffinoides | JCM12986 | ABWV00000000 | 4.19 | 44.6 | 3488 |
| B. distasonis | VPI5482 | PUCO00000000 | 6.29 | 48.06 | 4823 |

INSDC, International Nucleotide Sequence Database Collaboration.

Phosphorylase, alpha-B-galactosidase, beta-galactosidase, alphagalactosidase, alpha-glucosidase, beta-glucosidase, N-acetylbeta-D-glucosaminidase and alpha-fucosidase. The other enzymes of the strip have no activity (Supplementary Table S2). Results of the API 50CH strip show that the strain is able to metabolize the following substrates: D-arabinose, L-arabinose, D-xylose, D-glucose, methyl-D-glucopyranoside, N-acetylgalactosamine, esculin ferric citrate, salicin, D-cellulose, D-maltose, D-melibiose, D-saccharose, inulin, D-}

raffinose, amylopectin and potassium 5-ketogluconate. The others are negative (Supplementary Table S3). Finally, the results of the API 20A strip show positive reactions for the following: glucose, lactose, saccharose, maltose, salicin, xylose, arabinose, cellobiose, mannose, raffinose and beta-glucosidase (Supplementary Table S4).

Bacteroides mediterraneensis was resistant to gentamicin, trimethoprim/sulfamethoxazole, oxacinil and erythromycin and susceptible to other tested antibiotics (Table 4). The major fatty acid is 12-methyl-tetradecanoic acid (39%).

The genome of strain Marseille-P2644 is 4 075 657 bp long with a 47.47 mol% G+C content. In the EMBL-EBI database, the 16S rRNA gene and genome sequences are available under accession numbers LT558804 and FQR020000000, respectively. The strain Marseille-P2644 T is the type strain of the species Bacteroides mediterraneensis (= CSUR P2644 = DSM 103033) and was isolated from the ileum of a 58-year-old woman undergoing colorectal cancer screening in Marseille, France. This bacterium was also found in the stomach and the right and left colon of the same patient.

Description of Bacteroides ndongonae sp. nov.

Bacteroides ndongonae (ndon.go’ni ae, N.L. fem. gen. n., ndon-gonae, ‘of Ndongo,’ the family name of Sokhna Ndongo, a Senegalese microbiologist involved in culturomics and in the description of new bacterial species).
Cells are Gram-negative bacilli and are rod shaped with a length of 2.2 to 3.1 μm and a width of 0.6 to 1.1 μm. The type strain exhibits a catalase activity but no oxidase activity. Bacteroides ndongoniae is non–spore forming and also nonmotile. Colonies are circular, translucent, small and grey, with a diameter of 1 to 1.2 mm. Optimum growth occurred in an anaerobic atmosphere at 37°C and pH 7 on Columbia agar enriched with 5% sheep’s blood after 7 days of growth. Growth was observed at pH values between 7 to 7.5 and without NaCl.

Results of the API ZYM strip show that the strain possesses an activity for the following enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-fucosidase. The other enzymes of the strip have no activity (Supplementary Table S2). Results of the API 50CH strip show that the strain is able to metabolize the following substrates: esculin ferric citrate, α-turanose, potassium 5-ketoglucuronate. The others are negative (Supplementary Table S3). Finally, the results of the API 20A strip show positive reactions for the following: glucose, lactose, saccharose, maltose, xylose, arabinose, cellobiose, mannose, raffinose, rhamnose and β-glucosidase (Supplementary Table S4).

Bacteroides ndongoniae was resistant to clindamycin, gentamicin, trimethoprim/sulfamethoxazole, oxacillin and erythromycin and susceptible to other tested antibiotics (Table 4). The major fatty acid is hexadecanoic acid (26%).

The genome of strain Marseille-P3108 is 4 994 270 bp long with a 47.67 mol% G+C content. In the EMBL-EBI database, the 16S rRNA gene and genome sequences are available under accession numbers LT615364 and FNV00000000, respectively. The strain Marseille-P3108 is the type strain of the new species Bacteroides ndongoniae (= CSUR P3108 = DSM 103636) and was isolated from the right colon of a 76-year-old woman with oesophagitis in Marseille, France. This bacterium was also found in the left colon of the same patient.

**Description of Bacteroides togonis sp. nov.**

Bacteroides togonis (to.go.nis, N. L. masc. gen. n., togonis, named in honor of Amadou Togo, a Malian microbiologist involved in culturomics and in the description of new bacterial species).

Cells are Gram-negative bacilli and are rod shaped with a length of 1.4 to 3.2 μm and a width of 0.6 to 0.7 μm. The type strain did not exhibit activities for catalase and oxidase. Bacteroides togonis is non–spore forming but motile. Colonies are small, circular and white, with a diameter of 0.6 to 0.8 mm.

Optimum growth occurred in an anaerobic atmosphere at 37°C and pH 7 on Columbia agar enriched with 5% sheep’s blood after 3 days of growth. Growth was observed at pH values between 7 to 7.5 and without NaCl.

Results of the API ZYM strip show that the strain possesses activity for the following enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-fucosidase. The other enzymes of the strip had no activity (Supplementary Table S2).

Results of the API 50CH strip show that the strain is able to metabolize the following substrates: esculin ferric citrate, α-turanose, potassium 5-ketoglucuronate. The others are negative (Supplementary Table S3). Finally, the results of the API 20A strip show positive reactions for the following: glucose, lactose, saccharose, maltose, xylose, arabinose, mannose, raffinose, rhamnose and β-glucosidase (Supplementary Table S4).

| TABLE 8. Pairwise comparison of Bacteroides species using GGDC, formula 2 (DDH estimates based on identities/HSP length), a upper right |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1               | 2              | 3              | 4              | 5              | 6              | 7              | 8              | 9              | 10             | 11             |
| 100%            | 25.60% ± 2.3   | 29.80% ± 2.4   | 50.00% ± 2.6   | 24.60% ± 2.4   | 20.20% ± 2.3   | 25.00% ± 2.4   | 24.60% ± 2.4   | 23.40% ± 2.3   | 22.20% ± 2.3   | 19.70% ± 2.3   | 22.80% ± 2.3   |
| 100%            | 100%           | 100%           | 100%           | 100%           | 100%           | 100%           | 100%           | 100%           | 100%           | 100%           | 100%           |

Bold indicates comparison between strain and itself.

DDH: DNA-DNA hybridization; GGDC: Genome-to-Genome Distance Calculator; HSP, high-scoring segment pairs.

1. B. mediterraneensis; 2. B. ilei; 3. B. ndongoniae; 4. B. coganensis; 5. B. ihuae; 6. B. togonis; 7. B. coprocola; 8. B. coprophilus; 9. B. thetaiotaomicron; 10. B. graminisens; 11. B. eggerthii.

Confidence intervals indicate inherent uncertainty in estimating DDH values from intergenic distances based on models derived from empirical test data sets (which are always limited in size).
Bacteroides togonis was resistant to amoxicillin, clindamycin, gentamicin, trimethoprim/sulfamethoxazole, oxacillin and erythromycin and susceptible to other tested antibiotics (Table 4). The major fatty acid is 13-methyl-tetradecanoic acid (26%).

The genome of strain Marseille-P3166 is 3 977 096 bp long with a 48.26 mol% G+C content. In the EMBL-EBI database, the 16S rRNA gene and genome sequences are available under accession numbers LT631521 and FQXX00000000, respectively. The strain Marseille-P3166\textsuperscript{T} is the type strain of the species Bacteroides togonis (= CSUR P3166 = DSM 103637) and was isolated from the right colon of a 76-year-old woman with oesophagitis in Marseille, France.

**Description of Bacteroides togonis sp. nov.**

Bacteroides togonis (con.go.ne.nis, L. masc. adj., congonenis, ‘from Congo,’ the country where the sample was collected).

Cells are Gram-negative bacilli and are rod shaped with a length of 1 to 1.2 μm and a width of 0.6 to 0.7 μm. The type strain exhibits catalase activity but no oxidase activity. Bacteroides togonis is non–spore forming and motile. Colonies are smooth, with a diameter of 0.8 to 1 mm. Optimum growth occurred in an anaerobic atmosphere at 37°C and pH 7 on Columbia agar enriched with 5% sheep’s blood after 10 days of growth. Growth was observed at pH values between 6 to 8.5 and at a concentration of 5 g/L of NaCl.

Results of the API ZYM strip show that the strain possesses an activity for the following enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphtho-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β--glucosaminidase and α-fucosidase. The other enzymes of the strip had no activity (Supplementary Table S2). Results of the API 50CH strip showed that the strain is able to metabolize most of the substrates except: erythritol, L-arabinose, L-xylose, D-adenitol, methyl-β-D-xylopyranoside, L-sorbose, dulcitol, inositol, methyl-α-D-mannopyranoside, methyl-α-D-glucosepyranoside, arbutin, salicin, D-α-trehalose, inulin, D-melezitose, D-α-turanose, D-lyxose, D-α-tagatose, D-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 2-ketogluconate (Supplementary Table S3). Finally, the results of the API 20A strip show positive reactions for all studied reactions except for the formation of indole, urease, hydrolysis of gelatin and esculin (Supplementary Table S4).

Bacteroides togonis was susceptible to rifampicin, amoxicillin, imipenem, amoxicillin/clavulanic acid, meronidazole and trimethoprim/sulfamethoxazole and resistant to other tested antibiotics (Table 4). The major fatty acid is 12-methyl-tetradecanoic acid (60%).

The genome of strain Marseille-P3132 is 6 373 337 bp long with a 42.96 mol% G+C content. In the EMBL-EBI database, the 16S rRNA gene and genome sequences are available under accession numbers LT598566 and FOXY00000000, respectively. The strain Marseille-P3132\textsuperscript{T} is the type strain of the species Bacteroides congonensis (= CSUR P3132 = CCUG 70144) and was isolated from the stool of a 35-year-old healthy pygmy woman in Congo.

**Description of Bacteroides congonensis sp. nov.**

Bacteroides congonensis (con.go.ne.nis, L. masc. adj., congonensis, ‘from Congo,’ the country where the sample was collected).

Cells are Gram-negative bacilli and are rod shaped with a length of 1 to 1.6 μm and a width of 0.5 to 0.6 μm. The type strain does not exhibit activities for catalase or oxidase. B. congonensis is non–spore forming and motile. Colonies are mucous, with a diameter of 1 to 3 mm. Optimum growth occurred in an anaerobic atmosphere at 30°C and pH 7 on Columbia agar enriched with 5% sheep’s blood after 10 days of growth. Growth was observed at pH values between 6 to 8.5 and at a concentration of 5 g/L of NaCl.

Results of the API ZYM strip show that the strain possesses an activity for the following enzymes: alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphtho–AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase. The other enzymes of the strip had no activity (Supplementary Table S2). Results of the API 50CH strip show that the strain is able to metabolize most of the substrates except: erythritol, D-arabinose, L-xylose, D-adenitol, methyl-β-D-xylopyranoside, L-sorbose, dulcitol, inositol, methyl-α-D-mannopyranoside, D-melezitose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate (Supplementary Table S3). Finally, the results of the API 20A strip show positive reactions for all studied reactions except for the formation of indole, urease, hydrolysis of gelatin, glycerol, melezitose, sorbitol and rhamnose (Supplementary Table S4).

B. congonensis was resistant to gentamicin, oxacillin, fosfomycin and ceftriaxone and susceptible to others tested antibiotics (Table 4). The major fatty acid is 12-methyl-tetradecanoic acid (47%).

The genome of strain Marseille-P2824 is 4 063 701 bp long with a 39.71 mol% G+C content. In the EMBL-EBI database, the 16S rRNA gene and genome sequences are available under accession numbers LT756386 and FNVX00000000, respectively. The strain Marseille-P2824\textsuperscript{T} is the type strain of the species Bacteroides ihuae (= CSUR P2824 = CCUG 70550) was isolated from the sputum of a healthy 27-year-old woman in Marseille, France.
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Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nmni.2018.06.006.

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