# Description of *Clostridium cagae* sp. nov., *Clostridium rectalis* sp. nov. and *Hathewaya massiliensis* sp. nov., new anaerobic bacteria isolated from human stool samples

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## Abstract

Using culturomics methods, three strains were isolated, identified and characterized following the taxonogenomics concept. *Clostridium cagae* strain Marseille-P4344$^T$ (=CSURP4344), *Clostridium rectalis* strain Marseille-P4200$^T$ (=CSURP4200) and *Hathewaya massiliensis* strain Marseille-P3545$^T$ (=CSURP3545) were isolated from human stool samples. The phylogenetic reconstruction, phenotypic criteria and genomic analyses were carried out and demonstrated that these three bacteria are different from previously known bacterial species with standing in nomenclature and were classified as new members of the *Clostridiaceae* family.


**Keywords:** *Clostridium cagae* sp. nov., *Clostridium rectalis* sp. nov., culturomics, *Hathewaya massiliensis sp*. nov., human immunodeficiency virus, malnutrition, taxonogenomics
**Original Submission:** 26 May 2020; **Accepted:** 19 June 2020
**Article published online:** 1 August 2020

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## Introduction

The taxonomy of the genus *Clostridium* has undergone many changes over the course of its history [1–3]. It was first described by Prazmowski in 1880 and classified in the phylum *Firmicutes* [4]. It is mainly composed of Gram-positive bacteria, strictly anaerobic and able to produce spores. Members of this genus are frequently encountered in various environments such as soils [5,6], commensal digestive flora of mammals [7] and algae [8]. Moreover, some species can fix nitrogen and play an important role in agriculture, such as *Clostridium butyricum* and *Clostridium pasteurianum* [9,10]. Several *Clostridium* species are also involved in human pathologies. For example, *Clostridium difficile* causes nosocomial infections [11,12]. Others, such as *Clostridium botulinum*, *Clostridium tetani* and *Clostridium perfringens* are responsible for neuroparalytic [13], tetanus [14] and gastrointestinal [15] diseases in humans or animals. The wide diversity of the genus *Clostridium* led to the creation of the genus *Hathewaya*, which regroups *Hathewaya histolytica*, *Hathewaya limosa* and *Hathewaya proteolytica* named in the past as *Clostridium histolytica*, *Clostridium limosa* and *Clostridium proteolytica*, respectively [16].

In order to identify most bacteria living in the human gut, even the most fastidious ones, our laboratory has developed a culturomic strategy based on diversified culture conditions (temperature, medium, atmosphere and pH), followed by rapid screening by matrix-assisted desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [17–19]. We describe here three new bacterial species using the taxonogenomic method that combines phenotypic characteristics and whole genome sequencing analysis as previously described [20,21]. In the present study, we aimed to compare strains Marseille-P4344,



https://doi.org/10.1016/j.nmni.2020.100719

Marseille-P4200 and Marseille-P3545 with their closely related phylogenetic neighbours, and propose, respectively, the creation of new species *Clostridium cagae* sp. nov., *Clostridium rectalis* sp. nov. and *Hathewaya massiliensis* sp. nov.

## Materials and methods

### Strain isolation

In 2016, as part of a culturomic study investigating the human microbiome, we isolated two bacterial strains from stool samples of individuals in Niger with marasmus. These were strains Marseille-P3545 and Marseille-P4200. Strain Marseille-P4334 was isolated from a stool sample from a human immunodeficiency virus-positive individual at La Timone Hospital in Marseille, France. All the participants gave an informed consent, and the study was approved by the ethics committee of the Institut Federatif de Recherche IFR48 under number 09-022.

Initial growth was obtained for strain Marseille-P3545 after 10 days in a blood-culture bottle at 37°C in an anaerobic atmosphere. Under anaerobic conditions at 37°C, strains Marseille-P4200 and Marseille-P4334 had formed colonies after 3 days of pre-incubation in an anaerobic blood-culture bottle (Thermo Scientific, Villebon sur Yvette, France) supplemented with 5 mL of 0.2-µm-filtered rumen fluid. For each strain, the enriched liquid medium in which it was initially incubated was then inoculated on 5% Columbia agar enriched with sheep blood (bioMérieux, Marcy l'Étoile, France) followed by a new incubation at 37°C in an anaerobic atmosphere (Thermo Scientific, Dardilly, France) for 24 hours.

### Strain identification

Strain identification was realized using MALDI-TOF MS according to the previously reported process [22,23]. A Microflex LT spectrometer (Bruker, Daltonics, Bremen, Germany) was used for this purpose. Spectra (Fig. 1) were output and analysed using the BIOTYPER 3.0 software against the Bruker database, which was regularly incremented with the local URMS database (https://www.mediterranee-infection.com/urms-data-base). If any identification was not provided, the 16S rRNA gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France), as

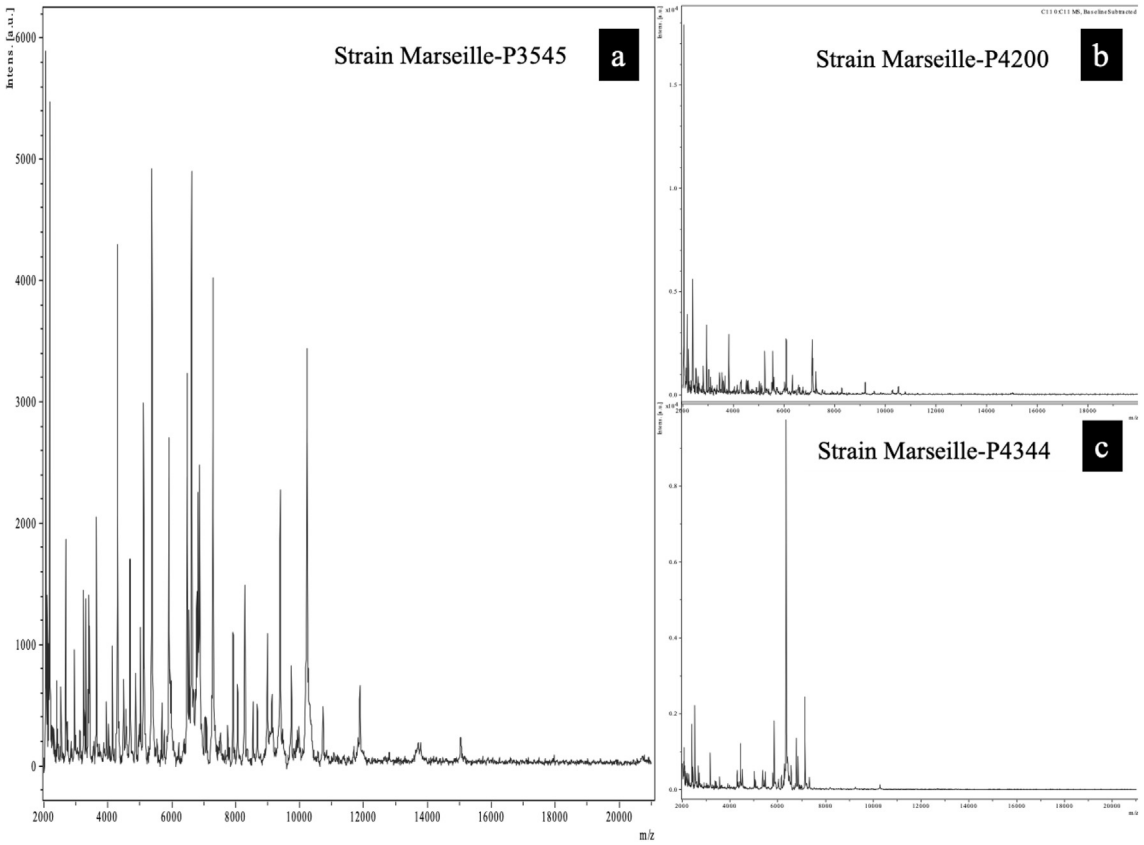


FIG. 1. MALDI-TOF MS reference spectrum of (a) *Hathewaya massiliensis* sp. nov., (b) *Clostridium rectalis* sp. nov. and (c) *Clostridium cagae* sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies.

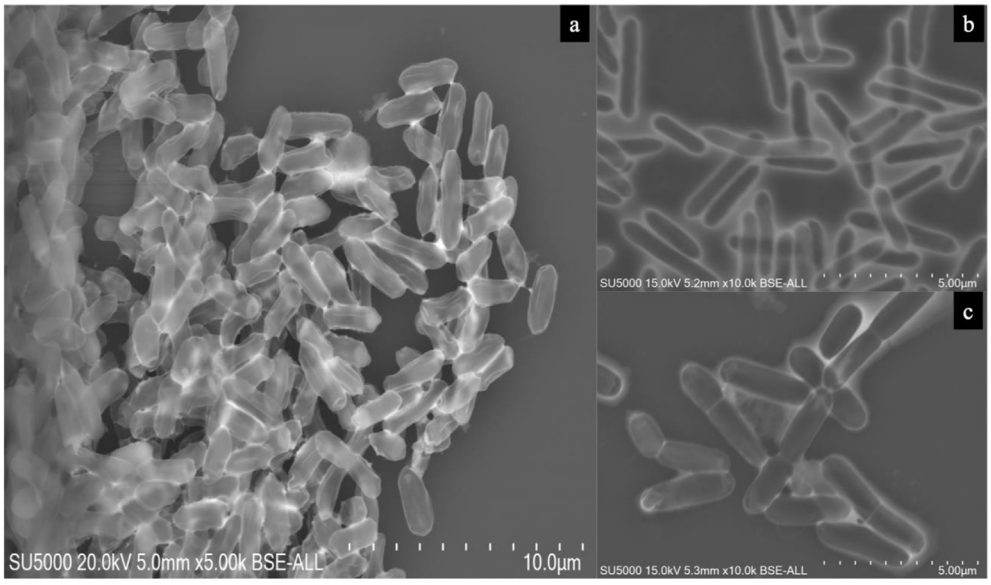


FIG. 2. Scanning electron microscopy (SEM) of stained (a) *Clostridium cagae* sp. nov., (b) *Hathewaya massiliensis* sp. nov. and (c) *Clostridium rectalis* sp. nov. A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution.

previously described [24]. All 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com/).

### Phenotypic characterization

Growth of strains was tested under aerobic, microaerophilic and anaerobic atmospheres (Thermo Scientific, Dardilly, France). Several growth temperatures (28, 37, 45 and 55°C) were also studied to know the optimal temperature of growth on 5% sheep-blood-enriched Columbia agar medium (bioMérieux). In addition, API ZYM and API 50CH strips (bioMérieux) were used to evaluate the biochemical characteristics of each strain test following the manufacturer's recommendations. Using API 50CH and API ZYM strips, the three strains were incubated for 48 and 4 hours, respectively, under anaerobic conditions as indicated by the manufacturer. Gram staining as well as catalase and oxidase tests were performed. Spore formation tests were carried out on each strain as previously reported [25]. The morphology of these three new species was investigated using a scanning electron microscope

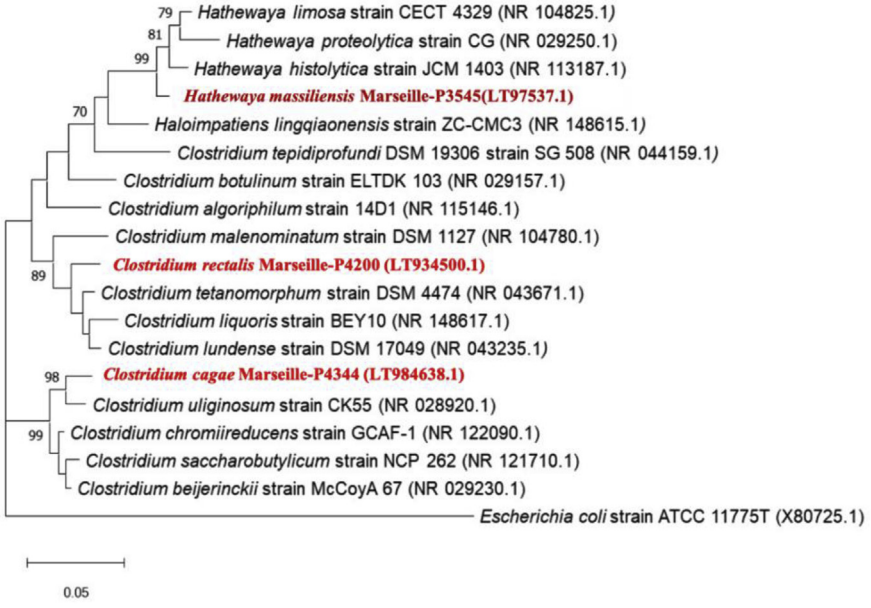


FIG. 3. Phylogenetic tree highlighting the position of *Clostridium rectalis* sp. nov., *Clostridium cagae* sp. nov. and *Hathewaya massiliensis* sp. nov., with regard to other closely related species. GenBank accession numbers of 16S rRNA are indicated in parentheses. Bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree are indicated at the nodes. The scale bar indicates a 5% nucleotide sequence divergence.

**TABLE 1. Different phenotypic characteristics between the studied strains and their closest relative species**

| Properties | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Cell diameter (µm) | 0.8–1.1 | 1.0 | 0.5–0.6 | 0.7–1.0 | 0.5–1.0 | 0.62 | 0.5–0.6 | 0.5–0.9 | 0.6–1.6 |
| Oxygen requirement | Strictly anaerobic | Anaerobic | Strictly anaerobic | Strictly anaerobic | Strictly anaerobic | Obligately anaerobic | Strictly anaerobic | Strictly anaerobic | Strictly anaerobic |
| Gram stain | + | — | + | + | + | + | + | + | + |
| Motility | — | + | — | + | — | + | + | + | + |
| Endospore formation | + | + | + | + | + | + | + | + | + |
| Alkaline phosphatase | — | NF | | NF | NF | + | + | + | NF |
| Catalase | + | NF | + | — | — | — | + | NF | NF |
| Oxidase | — | NF | — | NF | NF | — | — | NF | NF |
| β-Galactosidase | — | NF | — | NF | NF | + | — | NF | NF |
| *N*-acetyl-glucosamine | — | NF | — | NF | NF | + | — | NF | NF |
| Arabinose | — | — | — | — | NF | — | + | NF | NF |
| Esterase lipase (C8) | — | NF | — | + | NF | NF | — | NF | NF |
| Mannose | — | + | — | — | weakly + | + | + | NF | NF |
| Mannitol | + | + | — | NF | + | + | + | NF | NF |
| Sucrose | — | + | + | — | NF | + | + | NF | NF |
| D-Glucose | + | + | — | + | + | + | + | NF | + |
| D-Fructose | — | NF | — | + | NF | + | + | NF | NF |
| D-Maltose | — | — | — | + | + | + | + | NF | NF |
| G + C (mol%) | 27.3 | 28.0 | 28.1 | 27.5 | 34.4 | 34.0 | 29.5 | NF | 24.0 |
| Source | Stool | Acidic forest bog | Stool | Septic wounds | Old fermentation pit | Faecal flora | Stool | Soil, Human | Human, Animal |

**1**, *Clostridium cagae* strain Marseille-P4344; **2**, *Clostridium uliginosum*; **3**, *Clostridium rectalis* strain Marseille-P4200; **4**, *Clostridium tetanomorphum*; **5**, *Clostridium liquoris*; **6**, *Clostridium polynesiense*; **7**, *Hathewaya massiliensis* strain Marseille P-3545; **8**, *Hathewaya histolytica* and **9**, *Hathewaya limosa*.
NF, not found; +, positive test; –, negative test.

(SEM; Hitachi High-Technologies, Tokyo, Japan) (Fig. 2). A bacterial colony from each strain was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then, a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 minutes and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 minutes to increase SEM image contrast. The slide was gently washed in water, air-dried and examined in a tabletop TM4000 SEM.

### Genome characteristics

An EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) was used for genomic DNA extraction. Then, sequencing was performed with a MiSeq sequencer (Illumina Inc., San Diego, CA, USA) using the Nextera Mate Pair sample prep kit and Nextera XT Paired End (Illumina), as previously reported [24]. The assembly was carried out using a set of software such as Velvet [26], Spades [27] and Soap Denovo [28]. Sequences are trimmed with MiSeq and Trimmomatic [29] software, whereas untrimmed data were processed using only MiSeq software. To decrease assembly gaps, we used GapCloser software [30]. Scaffolds that have a nucleotide number <800 bp and scaffolds that have a depth value < 25% of the mean depths were removed. The best assembly was selected using different criteria (number of scaffolds, N50, number of N).

Genomes of these three species were annotated as previously described [31]. Furthermore, the Genome-to-Genome Distance Calculator web server (available at http://ggdc.dsmz.de) was used to evaluate the overall similarity among the compared genomes and to replace the wet-laboratory DNA–DNA hybridization (DDH) with a digital DDH (dDDH) [32]. Average nucleotide identity analysis was also estimated using OAT software [33]. Finally, the Clustage software [34] was employed to group the genomes of these three strains to study the distribution of accessory elements within each strain.

## Results

### Strain identification and phylogenetic analysis

Mass spectrometric identification of a single colony from each strain first cultivated on blood agar in an anaerobic atmosphere

**TABLE 2. Statistical details from genomes of *Clostridium cagae* strain Marseille-P4344, *Clostridium rectalis* strain Marseille-P4200 and *Hathewaya strain massiliensis* Marseille-P3545**

| Characteristics | Strain Marseille-P4200 | Strain Marseille-P3545 | Strain Marseille-P4344 |
|---|---|---|---|
| GenBank accession numbers | UYZZ00000000 | CABFVD000000000 | OKRA00000000 |
| Size (bp) | 3 279 426 | 3058 214 | 3 738 409 |
| RNAs | 90 | 112 | 107 |
| Protein-coding genes | 3083 | 2738 | 3299 |
| Genes | 3233 | 2911 | 3480 |
| G + C content (%) | 36.6 | 29.4 | 36.6 |

at 37°C failed. This suggested that these isolates were not referenced in the database and may be unknown species. Their MALDI-TOF MS spectra were added to our database to expand its content. 16S rDNA-based similarity analysis of strain Marseille-P4344$^T$, strain Marseille-P4200$^T$ and strain Marseille-P3545$^T$ against GenBank yielded highest nucleotide sequence similarities of 97.21% sequence identity with *Clostridium uliginosum* strain CK55 (GenBank accession no. NR_028920.1), 97.02% sequence identity with *Clostridium tetanomorphum* strain DSM 4474 (GenBank accession no. NR_043671.1) and 97.63% sequence identity with *H. histolytica* strain JCM 1403 (GenBank accession no. NR_113187.1), respectively. As these similarity values were under the threshold of 98.65%, established to delineate new bacterial species [32,35], strain Marseille-P4344$^T$, strain Marseille-P4200$^T$ and strain Marseille-P3545$^T$ were considered as potentially new species within the family *Clostridiaceae*. The phylogenetic tree highlighting the position of these three strains relative to other closely related species with a validly published name is shown in Fig. 3.

## Biochemical properties of the strains

For all three strains, growth occurred only in an anaerobic atmosphere at temperatures ranging from 28 to 55°C, with optimal growth observed at 37°C. Cells from strain Marseille-P4344$^T$, strain Marseille-P4200$^T$ and strain Marseille-P3545$^T$ were strictly anaerobic, Gram-positive, rod-shaped bacilli with mean diameters of 0.95, 0.63 and 0.60 µm, respectively. The colonies of strains Marseille-P4344 and Marseille-P4200 had a similar appearance on blood agar after 24 hours of anaerobic growth. They were fine, translucent and non-haemolytic with a mean diameter of 1–2 mm. Colonies of strain Marseille-P3545 were circular, white and smooth with regular boundaries and a diameter ranging from 2 to 5 mm. They all exhibited catalase-positive and oxidase-negative activities. A biochemical

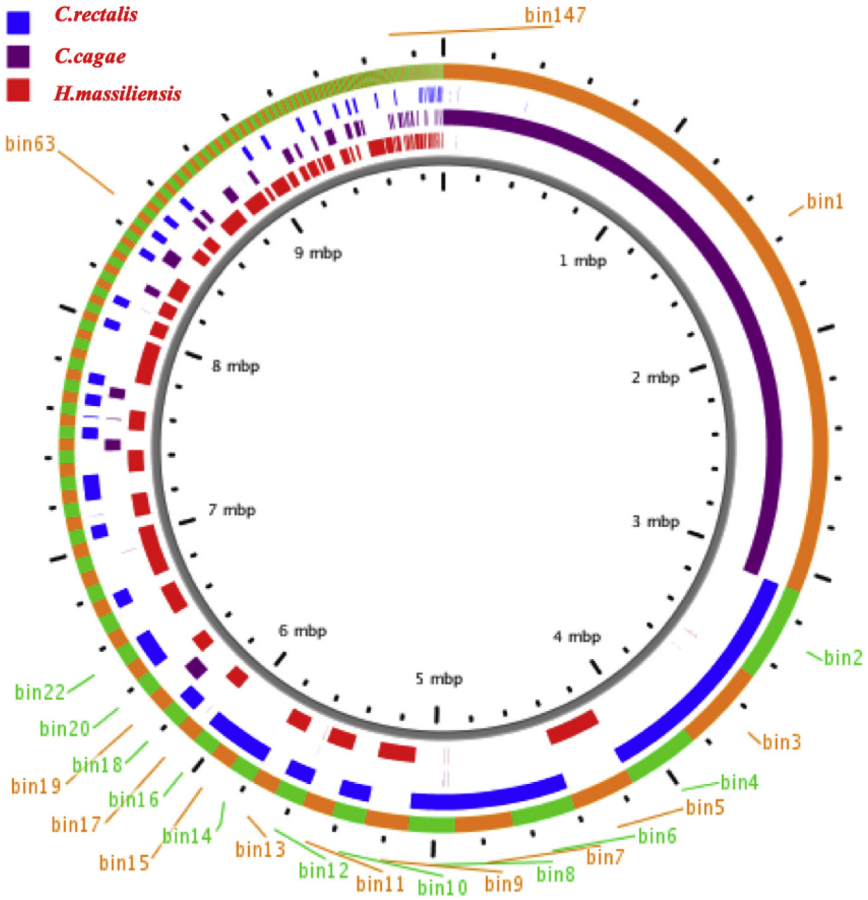


FIG. 4. Pan accessory genome distribution among *Clostridium rectalis* sp. nov., *Clostridium cagae* sp. nov. and *Hathewaya massiliensis* sp. nov. The outer ring shows the ClustAGE trays, classified from the largest to the smallest, with alternating orange and green colours indicating the boundaries of the trays. The concentric inner rings show the distribution of accessory elements within each strain. The ruler in the centre of the figure shows the cumulative size of the representatives of the accessory trays of the genome.

comparison was performed between these three new strains and other closest species (Table 1).

Using API ZYM and API 50 CH (bioMérieux), the three strains tested were positive for acid phosphatase, naphthol-AS-BI-phosphohydrolase, arbutin, D-melezitose, D-turanose, D-lyxose, D-tagalose, D-fucose, L-fucose, D-arabitol, L-arabitol and potassium 5-ketogluconate. In addition, both strains Marseille-P4200$^T$ and Marseille-P3545$^T$ were also positive for methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetyl-glucosamine, amygdalin, salicin, D-cellobiose, D-melibiose, sucrose, D-trehalose, starch and glycogen while D-glucose, D-mannitol and inulin were fermented by both strains Marseille-P4344 and Marseille-P3545$^T$. The remaining negative tests were reported in the Supplementary material (Tables S1 and S2).

## Genomic analysis

The genomes of strains Marseille-P4344 and Marseille-P4200 were 3 738 409 and 3 279 426 bp long with 36.6 and 36.6 mol% G + C content, respectively. The assembly was carried out into ten scaffolds for Marseille-P4344 and into 30 scaffolds for Marseille-P4200. They had 3480 and 3233 predicted genes, respectively. Their genomes contained 3299 and 3083 protein-coding genes, and 107 and 90 RNA genes (26 and 23 rRNAs, 77 and 63 tRNAs, 4 and 4 ncRNAs) and 74 and 60 pseudo genes, respectively. Genes with putative function (by Clusters of Orthologous Groups) were 2618 for strain Marseille-P4344 (72%) and 2592 for strain Marseille-P4200 (74%). Finally, 993 and 916 genes (28% and 26%) were annotated as hypothetical proteins for strain Marseille-P4344 and strain Marseille-P4200, respectively. In contrast, the genome size of *H. massiliensis* strain Marseille-P3545 was 3 058 214 bp with 29.4 mol% G + C content. The genome assembly of this strain was achieved on ten contigs. Of the 2911 predicted genes, 2738 were protein-coding genes and 112 were RNAs (9 5S rRNA, 7 16S rRNA, 7 23S rRNA, 85 tRNA and 4 ncRNA genes) and 61 were pseudo genes. More statistical data from the genomes are listed in Table 2. The properties and distribution of accessory genes into the pan-genome of these strains showed that both strain Marseille-P4344 and strain Marseille-P4200 shared several genes, whereas *H. massiliensis* displayed a narrower distribution than that of *Clostridium* species (Fig. 4).

**TABLE 3. Number of genes associated with the 25 general clusters of orthologous group (COG) functional categories of *Clostridium cagae* strain Marseille-P4344, *Clostridium rectalis* strain Marseille-P4200 and *Hathewaya massiliensis* strain Marseille-P3545**

| Code | Strain Marseille-P4344 | Strain Marseille-P3545 | Strain Marseille-P4200 | Description |
|---|---|---|---|---|
| [J] | 224 | 218 | 209 | Translation, ribosomal structure and biogenesis |
| [A] | 1 | 0 | 0 | RNA processing and modification |
| [K] | 245 | 175 | 220 | Transcription |
| [L] | 118 | 128 | 130 | Replication, recombination and repair |
| [B] | 1 | 1 | 1 | Chromatin structure and dynamics |
| [D] | 44 | 40 | 47 | Cell cycle control, cell division, chromosome partitioning |
| [Y] | 0 | 0 | 0 | Nuclear structure |
| [V] | 72 | 88 | 94 | Defense mechanisms |
| [T] | 202 | 146 | 187 | Signal transduction mechanisms |
| [M] | 162 | 143 | 162 | Cell wall/membrane/envelope biogenesis |
| [N] | 79 | 60 | 74 | Cell motility |
| [Z] | 0 | 0 | 2 | Cytoskeleton |
| [W] | 9 | 4 | 10 | Extracellular structures |
| [U] | 33 | 25 | 23 | Intracellular trafficking, secretion and vesicular transport |
| [O] | 106 | 111 | 97 | Post-translational modification, protein turnover, chaperones |
| [X] | 32 | 88 | 35 | Mobilome: prophages, transposons |
| [C] | 164 | 114 | 144 | Energy production and conversion |
| [G] | 223 | 94 | 147 | Carbohydrate transport and metabolism |
| [E] | 194 | 191 | 195 | Amino acid transport and metabolism |
| [F] | 102 | 76 | 83 | Nucleotide transport and metabolism |
| [H] | 135 | 138 | 105 | Coenzyme transport and metabolism |
| [I] | 92 | 65 | 78 | Lipid transport and metabolism |
| [P] | 135 | 117 | 131 | Inorganic ion transport and metabolism |
| [Q] | 44 | 25 | 26 | Secondary metabolites biosynthesis, transport and catabolism |
| [R] | 261 | 218 | 243 | General function prediction only |
| [S] | 167 | 121 | 149 | Function unknown |
| — | 993 | 786 | 916 | Not in COG |

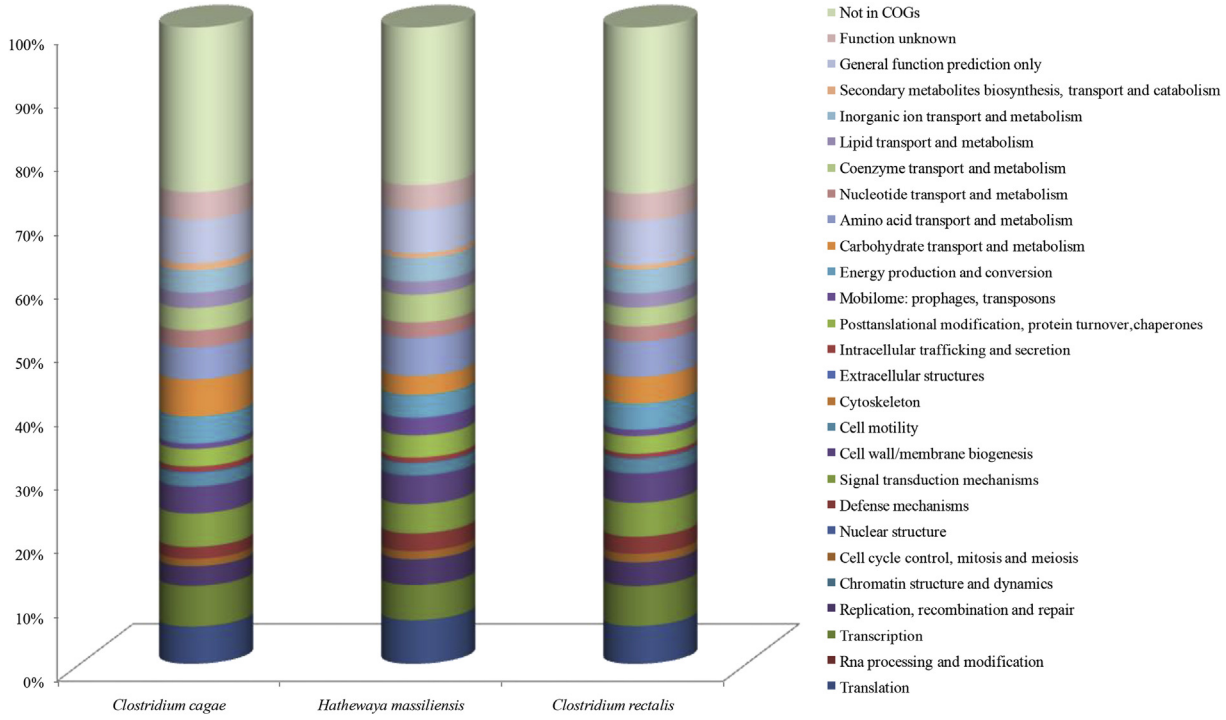


**FIG. 5.** Distribution of functional classes of the predicted genes in *Clostridium cagae*, *Clostridium rectalis* and *Hathewaya massiliensis* chromosomes according to the clusters of orthologous groups of proteins.

Analysis of the Clusters of Orthologous Groups categories showed that the mobile elements of the *H. massiliensis* genome appeared to be more numerous than those of the genomes of other *Clostridium* species (32, 35 and 88 in category [X], respectively). In contrast, Marseille-P4344 and Marseille-P4200 strains exhibited a greater ability to transport and metabolize carbohydrates than Marseille-P3545, with 223, 147 and 97 genes associated to category [G] (Table 3, Fig. 5).

Using dDDH analysis, values ranged from 18% between *Clostridium tepidiprofundi* and *Clostridium lundense*, to 27.6% between *C. lundense* and *Clostridium liquoris* (Table 4). These values are lower than the 70% threshold used for delineating prokaryotic species, confirming that these three strains represent new species. Finally, OrthoANI analysis among closely related species (Fig. 6) showed that the higher percentage value was 83.56% shared between *C. liquoris* and *C. lundense*, whereas the lowest was 67.73% between *H. histolytica* and *Clostridium chromiireducens*. In addition, when *C. rectalis* was compared with other species, the values ranged from 69.09% with *Clostridium cagae* to 75.56% with *C. tetanomorphum*. For *C. cagae*, values ranged from 68.46% with *H. histolytica* to 79.45% with *C. uliginosum* and for *H. massiliensis* they ranged from 67.86% with *C. chromiireducens* to 78.27% with *H. histolytica*.

**TABLE 4. Numerical DNA–DNA hybridization values (%) obtained by comparison between *Clostridium rectalis* strain Marseille-P4200, *Hathewaya massiliensis* strain Marseille-P3545, *Clostridium cagae* strain Marseille-P4344 and other closely related species using GGDC formula 2 software (DDH estimates based on HSP identities/length) (https://ggdc.dsmz.de/ggdc.php#), top right**

| | ***Hh*** | ***Cu*** | ***Ct*** | ***Cte*** | ***Cre*** | ***Clu*** | ***Clq*** | ***Hm*** | ***Cch*** | ***Cca*** |
|---|---|---|---|---|---|---|---|---|---|---|
| *Hh* | 100 | 23.1 | 21.0 | 22.5 | 21.3 | 20.1 | 22.7 | 20.7 | 23.3 | 23.0 |
| *Cu* | | 100 | 23.5 | 20.1 | 19.8 | 21.5 | 21.4 | 24.3 | 23.6 | 24.9 |
| *Ct* | | | 100 | 19.8 | 19.7 | 27.5 | 24.2 | 19.4 | 21.9 | 19.2 |
| *Cte* | | | | 100 | 19.0 | 18.0 | 21.7 | 21.9 | 22.2 | 21.4 |
| ***Cre*** | | | | | 100 | 19.9 | 19.3 | 21.1 | 21.2 | 19.5 |
| *Clu* | | | | | | 100 | 27.6 | 21.8 | 21.3 | 19.3 |
| *Clq* | | | | | | | 100 | 23.2 | 22.2 | 19.2 |
| ***Hm*** | | | | | | | | 100 | 23.3 | 20.3 |
| *Cch* | | | | | | | | | 100 | 22.0 |
| ***Cca*** | | | | | | | | | | 100 |

*Hh*, *Hathewaya histolytica*; *Cu*, *Clostridium uliginosum*; *Ct*, *Clostridium tetanomorphum*; Abbreviations: *Cte*, *Clostridium tepidiprofundi*; *Cre*, *Clostridium rectalis*; **Clu,** *Clostridium lundense*; *Clq*, *Clostridium liquoris*; *Hm*, *Hathewaya massiliensis*; Cch, *Clostridium chromiireducens*; Cca, *Clostridium cagae*.

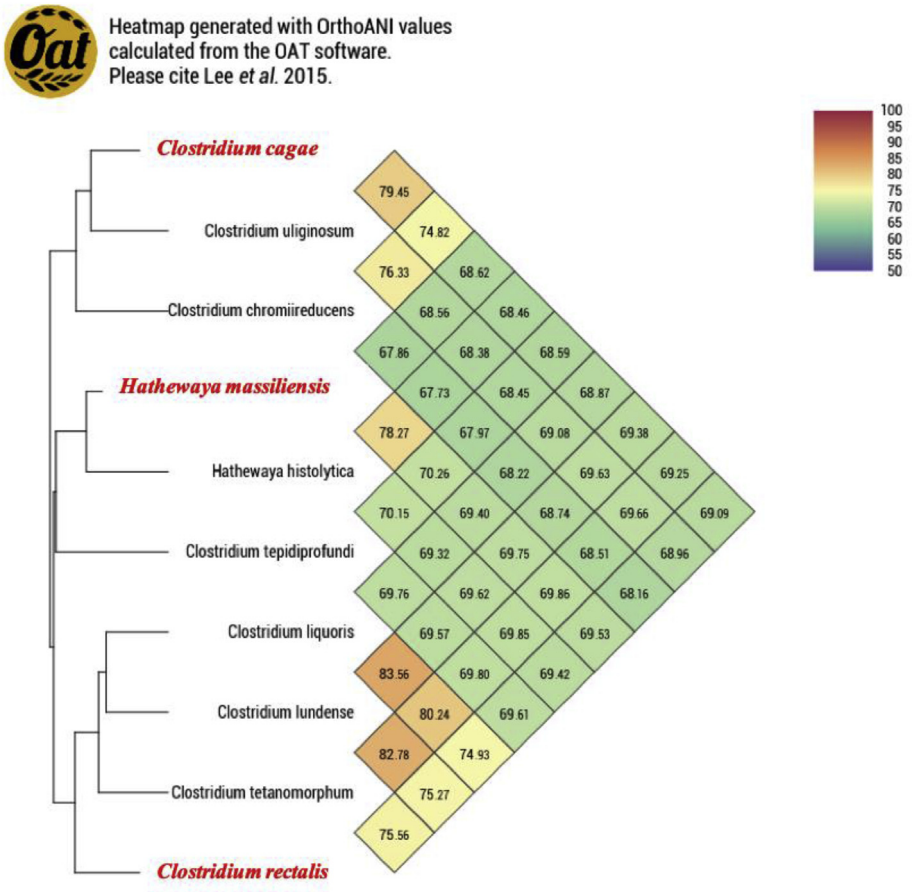


FIG. 6. Heatmap generated with OrthoANI values calculated using the OAT software between *Clostridium cagae* sp. nov., *Clostridium rectalis* sp. nov. and *Hathewaya massiliensis* with other closely related species validly described.

## Conclusion

Based on all the phenotypic, biochemical and genomic tests performed on these bacterial strains, we consequently considered that strains Marseille-P4200, Marseille-P3545 and Marseille-P4344 are new species. Indeed, the taxonogenomic evidence from this study, such as the sequence similarity of the 16S rRNA gene below the threshold value of 98.65%, OrthoANI values also <95%, as well as the several phenotypic divergences obtained, have allowed us to formally propose *Clostrium cagae* sp. nov., *Clostridium rectalis* sp. nov. and *Hathewaya massiliensis* sp. nov. as new species within the phylum *Firmicutes*.

### Description of *Clostridium cagae* sp. nov

Strain Marseille-P4344$^T$ is the type strain of *Clostridium cagae* sp. nov. (ca.ga.e, Gr.n. pronounced as kakke, referring to 'faeces' the clinical sample from which this bacterium was isolated). *Clostridium cagae* is a strictly anaerobic, spore-forming and Gram-positive rod bacterium with a mean diameter of 0.95 µm. It exhibited catalase-positive and oxidase-negative activities. Strain Marseille-P4344$^T$ grows under anaerobic conditions at temperatures ranging between 28 and 55°C, with an optimal temperature of 37°C. Colonies of strain Marseille-P4344 are fine, translucent and non-haemolytic with a mean diameter of 1–2 mm. The genome of strain Marseille-P4344$^T$ was 3 738 409 bp with 36.6 mol% of G + C content. Strain Marseille-P4344 is able to ferment D-arabitol, D-melezitose, D-lyxose, L-arabitol, D-turanose, D-tagalose, L-fucose, D-fucose and potassium 5-ketogluconate. The type strain of *Clostridium cagae* sp. nov., strain Marseille-P4344, was isolated from the stool sample of an individual with human immunodeficiency virus infection.

### Description of *Clostridium rectalis* sp. nov

Strain Marseille-P4200$^T$ is the type strain of *Clostridium rectalis* sp. nov. (rec.ta.lis, N.L. masc. adj. rectalis to rectal referring to rectum the straight bowel from which this bacterium was isolated). *Clostridium rectalis* is an anaerobic bacterium. It can

form spores and is a Gram-positive bacilli with a mean diameter of 0.63 μm. It exhibited catalase-positive and oxidase-negative activities. Strain Marseille-P4200$^T$ grows under anaerobic conditions at 37°C. Strain Marseille-P4200 presents translucent, fine and non-haemolytic colonies with a mean diameter of 2 mm. The genome of strain Marseille-P4200$^T$ was 3 279 426 bp with 36.6 mol% of G + C content. Strain Marseille-P4200 is positive for acid phosphatase, naphthol-AS-BI-phosphohydrolase, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetyl-glucosamine, amygdalin, salicin, D-cellobiose, D-melibiose, sucrose, D-trehalose and glycogen. The type strain of *Clostridium rectalis* sp. nov., strain Marseille-P4200, was isolated from the stool sample of a child with marasmus.

**Description of *Hathewaya massiliensis* sp. nov.**

*Hathewaya massiliensis* sp. nov (mas.si.li.en'sis N.L. fem. adj. *massiliensis*, to Massilia, the Latin name of Marseille where the type strain was first isolated and characterized). is classified as a member of the family *Clostridiaceae* in the phylum *Firmicutes*. Strain Marseille-P3545$^T$ is the type strain of the new species *Hathewaya massiliensis* sp. nov. It is an anaerobic, Gram-positive, rod-shaped bacterium, spore-forming and motile. Colonies of strain Marseille-P3545$^T$ observed on blood agar medium are circular, white and smooth with regular edges and a diameter of 3 mm. This bacterial strain possesses catalase-positive and oxidase-negative activities. The genome size of *Hathewaya massiliensis* strain Marseille-P3545$^T$ is 3 058 214 bp with 29.4 mol% G + C content. The GenBank accession number for the 16S rRNA gene sequence of strain Marseille-P3545$^T$ is LT797537 and for the whole genome shotgun project is CABFVD000000000. It was isolated from a stool sample from a young individual with malnutrition.

## Conflicts of interest

None to declare.

## Funding sources

This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the programme *Investissements d'avenir*, reference ANR-10-IAHU-03, the Région Provence-Alpes-Côte d'Azur and European funding FEDER PRIMI. This research was supported by a grant from the Institut Universitaire de France (IUF, Paris, France) to Professor Anthony Levasseur.

## Ethics and consent

The study was approved and authorized by the ethics committee of the Institut Hospitalo-Universitaire Méditerranée Infection (IHU-MI) under reference 2016-010.

## Acknowledgements

The authors thank Aurelia Caputo for submitting the genomic sequence to GenBank and Amael Fadlane for subculturing the strains.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2020.100719.

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