**Clostridium pacaense: a new species within the genus Clostridium**

M. Hosny1, R. Abou Abdallah2, J. Bou Khalil1, A. Fontanini1, E. Baptiste1, N. Armstrong1 and B. La Scola1

1) Aix-Marseille Université UM63, Institut de Recherche pour le Développement IRD 198, Assistance Publique—Hôpitaux de Marseille (AP-HM), Microbes, Evolution, Phylogeny and Infection (MEΦI), Institut Hospitalo-Universitaire (IHU)-Méditerranée Infection and 2) Aix-Marseille Université UM63, Institut de Recherche pour le Développement IRD 198, Assistance Publique—Hôpitaux de Marseille (AP-HM), Vecteurs—Infections Tropicales et Méditerranéennes (VITROME), Service de Santé des Armées, IHU-Méditerranée Infection, Marseille, France

**Abstract**

Using the strategy of taxonogenomics, we described Clostridium pacaense sp. nov. strain Marseille-P3100T, a Gram-variable, nonmotile, spore-forming anaerobic bacillus. This strain was isolated from a 3.3-month-old Senegalese girl with clinical aspects of marasmus. The closest species based on 16S ribosomal RNA was Clostridium aldenense, with a similarity of 98.4%. The genome length was 2 672 129 bp, with a 50% GC content; 2360 proteins were predicted. Finally, predominant fatty acids were hexadecanoic acid, tetradecanoic acid and 9-hexadecenoic acid.

© 2019 The Authors. Published by Elsevier Ltd.

**Keywords:** Clostridium pacaense, culturomics, taxonogenomics

**Original Submission:** 16 October 2018; **Revised Submission:** 21 December 2018; **Accepted:** 21 December 2018

**Article published online:** 31 December 2018

**Introduction**

Human intestinal flora is incorporated mainly in the terminal part of small intestine and colon. It consists of about 100 000 billion bacteria grouped into 500 species, including 90% anaerobic bacteria [1,2]. Oxygen-tolerant species such as lactobacilli, and thus aerobic organisms such as *Escherichia coli* and enterococci, represent a minority of intestinal microbiota [2]. It appears that each adult has a unique signature of microbial community, which is increasingly understood to influence human health [3–5]. *Clostridiaceae* is a family of *Clostridium* and has traditionally been described by anaerobic growth and spore formation [3,6]. *Clostridium* comprises the major composition of mammalian gastrointestinal tract microbiomes [7]. Culturomics combined with taxonogenomics is an important tool for the isolation and characterization of new bacterial species. These techniques permit the study of their phenotypes, and thus of their antibiotic resistance and biochemical features; analyses of characteristics of the genome may thus potentially have an impact on human health [8,9].

Here we propose *Clostridium pacaense* sp. nov. strain Marseille-P3100T (CSUR P3100) as a new species within the *Clostridium* genus. This strain was isolated from a 3.3-month-old Senegalese girl with clinical aspects of marasmus [10].

**Materials and methods**

**Phenotypic, biochemical and antibiotics susceptibility**

Gram staining, motility, and catalase and oxidase were determined as described by Lagier et al. [11]. Sporulation was tested using a thermal shock on bacterial colonies (diluted in phosphate-buffered saline) for 20 minutes at 80°C. For electronic microscopy, a colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. The slide was gently washed in water and air dried; then the colony, approximately 60 cm in height and 33 cm in width, was
examined to evaluate the bacteria’s structure on a TM4000 microscope (Hitachi, Yokohama, Japan). Mass spectra were obtained from C. pacaense colonies using MALDI-TOF MS (Fig. 1). Biochemical characteristics were tested using API 50CH, API ZYM and API 20A strips (bioMérieux, Marcy l’Étoile, France). Antibiotic susceptibility referred to European Committee on Antimicrobial Susceptibility Testing 2018 recommendations.

**Fatty acid methyl ester analysis**

Cellular fatty acid methyl ester analysis was performed by GC/MS. Two samples were prepared with approximately 35 mg of bacterial biomass per tube collected from several culture plates. Fatty acid methyl esters were prepared as described previously [12]. GC/MS analyses were carried out as described previously [13]. Briefly, fatty acid methyl esters were separated using an Elite 5-MS column and monitored by mass spectrometry (Clarus 500-SQ 8 S; PerkinElmer, 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 fatty acid methyl ester mass spectral database (Wiley, Chichester, UK).

**Genome sequencing, assembly and annotation**

Genomic DNA was sequenced on MiSeq sequencer (Illumina, San Diego, CA, USA) using the paired-end strategy, as described previously [6]. SPAdes software was used for genome assembly [14]. Contaminations were eliminated after performing BLASTn. Open reading frames were predicted and annotated using Prokka software [15]. The C. pacaense genome was used for protein functions against the Clusters of Orthologous Groups (COGs) database using BLASTP (E value of $10^{-03}$, coverage 0.7, identity percentage 30%). The genome is available on the European Molecular Biology Laboratory–European Bioinformatics Institute (EMBL-EBI) scaffolds under accession numbers LS999944 to LS999965.

**Comparative genomics**

Species to be compared were those with higher similarity based on 16S RNA (Fig. 2), provided the genome is available. The

---

**FIG. 1.** Reference mass spectrum (via MALDI-TOF MS) from Clostridium pacaense strain Marseille-P3100.

**FIG. 2.** Phylogenetic tree analysis based on 16S ribosomal RNA (rRNA) gene sequences. The 16S rRNA genes were aligned using CLUSTALW, and phylogenetic tree was generated using MEGA 7 software [19].
following bacterial species were used in this analysis (their genomics features are summarized in Supplementary Table S1): Clostridium bolteae (GCA_002234575.2), Clostridium lavalense (GCA_003024655.1), Clostridium saccharolyticum (GCA_00144625.1), Clostridium aldenense (GCA_003434055.1), Lachnoclostridium citroniae (GCA_000233455.1), Clostridium amygdalinum (GCA_900205965.1) and Clostridium celerecrescens (GCA_000732605.1). Amino acids and open reading frame sequences were predicted using Prodigal software [16] to obtain optimized prediction within all genomes. Then, for each couple of genomes, a similarity percentage was computed using OrthoANI software [17].

**Results**

**Phenotypic and biochemical characterization**

C. pacaense is a Gram-variable, spore-forming, nonmotile, anaerobic bacillus, with no catalase and oxidase activities. Electron microscopy revealed that its was 3.5 μm long and 0.5 μm in diameter (Fig. 3). C. pacaense produced α-glucosidase and naphthol-AS-Bl-phosphohydrolase. General features and biochemical characteristics are summarized in Table I. Antibiotic susceptibility testing revealed that C. pacaense was

---

**TABLE I. General feature and biochemical tests of Lachnoclostridium pacaense**

| Characteristic             | Value          |
|----------------------------|----------------|
| Current classification     |                |
| Domain                     | Bacteria       |
| Phylum                     | Firmicutes     |
| Class                      | Clostridum     |
| Order                      | Clostridales   |
| Family                     | Clostridiaceae |
| Genus                      | Clostridium    |
| Species                    | Lachnoclostridium pacaense |
| Type strain                | Marseille-P3100T |
| Gram staining              | Variable       |
| Cell shape                 | Bacillus       |
| Diameter                   | 0.5 μm         |
| Cell length                | 3.5 μm         |
| Motility                   | No             |
| Sporulation                | Yes            |
| Indole                     | No             |
| Production of:             |                |
| Alkaline phosphatase       | No             |
| Catalase                   | No             |
| Oxidase                    | No             |
| Nitrate reductase          | No             |
| Urease                     | No             |
| β-Galactosidase            | No             |
| α-Glucosidase              | Yes            |
| N-Acetyl-glucosamine       | No             |
| Esterase                   | No             |
| Acid from:                 |                |
| L-Arabinose                | No             |
| Ribose                     | No             |
| Mannose                    | No             |
| Mannitol                   | No             |
| Sucrose                    | No             |
| α-Glucose                  | No             |
| α-Fructose                 | No             |
| α-Maltose                  | No             |
| α-Lactose                  | No             |

---

**FIG. 3.** Electron microscopy of Clostridium pacaense.
susceptible to amoxicillin, amoxicillin–clavulanic acid, ceftriaxone, ceftazidime, cefepime, ertapenem, metronidazole and vancomycin.

Predominant fatty acids

The major fatty acids were hexadecanoic acid (59%), tetradecanoic acid (20%) and 9-hexadecenoic acid (9%). No branched structures were detected (Table 2).

Genome properties and comparison

The C. pacaense draft genome consisted of 22 scaffolds. Genome length was 2 672 129 bp, with a 50% of GC content. A total of 2360 proteins were predicted. The draft genome sequence of C. pacaense owned the smallest genome. Its GC content was same as C. aldenense, but smaller than C. lavalense and greater than others. Additionally, C. pacaense owned the smallest number of predicted genes. Carbohydrate transport and metabolism (and thus secondary metabolite biosynthesis, transport and catabolism) were the predominant COGs categories identified within C. pacaense (Table 3). On the basis of 16S RNA similarity, the closest species was C. aldenense (Table 4). This was in agreement with genome data, as C. aldenense was also the closest species, with an OrthoANI value of 89.9744% (C. aldenense) but below the 95% cutoff for defining a species (Fig. 4).

Description of Clostridium pacaense sp. nov

Clostridium pacaense (pa.ca.en’se, L. masc. adj. pacaense, ‘of PACA,’ the abbreviation of Provence Alpes Cote d’Azur, the French area where the strain was isolated). In addition to the characteristics in the genus description, cells are Gram variable

| Fatty acid | Name               | Mean relative %a   |
|------------|--------------------|--------------------|
| 16:0       | Hexadecanoic acid  | 58.5 ± 0.5         |
| 14:0       | Tetradecanoic acid | 19.7 ± 0.3         |
| 16:1n7     | 9-Hexadecenoic acid| 8.9 ± 0.2          |
| 18:1n9     | 9-Octadecenoic acid| 5.5 ± 0.2          |
| 18:1n7     | 11-Octadecenoic acid| 4.4 ± 0.3          |
| 18:0       | Octadecanoic acid  | 1.0 ± 0.1          |
| 15:0       | Pentadecanoic acid | TR                 |
| 16:1n9     | 7-Hexadecenoic acid| TR                 |
| 12:0       | Dodecanoic acid    | TR                 |

TR, trace amounts <1%.

*Mean peak area percentage.

TABLE 2. Cellular fatty acids of Clostridium pacaense

| COGs category | COGs description                          | Total |
|---------------|------------------------------------------|-------|
| C             | Chromatin structure and dynamics          | 119   |
| D             | Cell cycle control, mitosis and meiosis   | 17    |
| E             | Amino acid transport and metabolism       | 110   |
| F             | Nucleotide transport and metabolism       | 48    |
| G             | Carbohydrate transport and metabolism     | 280   |
| H             | Coenzyme transport and metabolism         | 44    |
| I             | Lipid transport and metabolism            | 31    |
| J             | Translation                               | 41    |
| K             | Replication, recombination and repair     | 73    |
| M             | Cell wall/membrane biogenesis             | 73    |
| N             | Cell motility                             | 18    |
| O             | Posttranslational modification, protein turnover, chaperones | 28 |
| P             | Inorganic ion transport and metabolism    | 76    |
| Q             | Secondary metabolites biosynthesis, transport and catabolism | 7 |
| R             | General function prediction only          | 222   |
| S             | Function unknown                          | 98    |
| T             | Signal transduction mechanisms            | 93    |
| U             | Intracellular trafficking and secretion   | 4     |
| V             | Defense mechanisms                        | 55    |

COGs, Clusters of Orthologous Groups database.

TABLE 3. Clostridium pacaense number of genes associated with COGs categories

| COGs matrix of similarity based on 16S rRNA gene |
|-----------------------------------------------|

| C. pacaense | C. lavalense | C. citroniae | C. celerecrescens | C. bolteae | C. amygdalinum | C. aldenense | C. saccharolyticum |
|------------|--------------|--------------|-------------------|-----------|----------------|--------------|-------------------|
| C. pacaense | —            | —            | —                 | —         | —              | —            | —                 |
| C. lavalense| 96.3         | —            | —                 | —         | —              | —            | —                 |
| C. citroniae| 96.7         | 96.1         | —                 | —         | —              | —            | —                 |
| C. celerecrescens| 93.7       | 92.9         | 93.5              | —         | —              | —            | —                 |
| C. bolteae | 95.7         | 97           | 96.8              | 94.1      | —              | —            | —                 |
| C. amygdalinum| 94.2        | 93.2         | 93.7              | 97.9      | 94.3           | —            | —                 |
| C. aldenense| 98.4         | 95.9         | 96.7              | 93.9      | 95.8           | 94.1         | 94                 |
| C. saccharolyticum| 94.2  | 93.2         | 93.6              | 98.5      | 94.1           | 98.8         | 94                 |

rRNA, ribosomal RNA. The 16S rRNA sequences were aligned, and similarity matrix was calculated by Bioedit software [18].
with a length of 3.5 μm and a width of 0.5 μm. It produces α-glucosidase and napthol-AS-BI-phosphohydrolase. The major fatty acids are C₁₆H₃₂O₂, C₁₄H₂₈O₂ and C₁₆H₃₀O₂. The type strain Marseille-P3100T has been deposited in the CSUR and CCUG culture collections under accession numbers CSUR P3100 and CCUG 71489, respectively. The type strain was isolated from a stool sample from a Senegalese girl with marasmus. The draft genome of the type strain is 2,672,129 bp long with a DNA G+C content of 50%, and is available on the EMBL-EBI scaffolds under accession numbers LS999944 to LS999965.

Acknowledgements

We thank the genomics and biobank platforms of the Institut Hospitalo-Universitaire (IHU)-Méditerranée Infection for technical assistance. Supported in part by a grant from the French state managed by the National Research Agency under the ‘Investissements d’avenir (Investments for the Future)’ programme (ANR-10-IAHU-03 Méditerranée Infection), and by Région Provence Alpes Côte d’Azur and European funding (‘Fond Européen de Développement Régional (FEDER)-PRIMI’).

Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2018.12.003.

References

[1] Eckburg PB, Bik EM, Bernstein CN, Purdom E, Deethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005;308:1635–8.
[2] Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. PLoS Biol 2007;5:1556–73.
[3] Hosny M, Cassir N, La Scola B. Updating on gut microbiota and its relationship with the occurrence of necrotizing enterocolitis. Hum Microbiome J 2017:4:14–9.
[4] Cassir N, Simeoni U, La Scola B. Gut microbiota and the pathogenesis of necrotizing enterocolitis in preterm neonates. Future Microbiol 2016;11:273–92.
[5] Lagier JC, Million M, Hugon P, Armougom F, Raoult D. Human gut microbiota: repertoire and variations. Front Cell Infect Microbiol 2012;2:136.
[6] Hosny M, Benamar S, Durand G, Armstrong N, Michelle C, Cadoret F, et al. Description of Clostridium phoceensis sp. nov., a new species within the genus Clostridium. New Microbe New Infect 2016;14:85–92.
[7] Lagier JC, Hugon P, Khelfaïa S, Fournier PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 2015;28:237–64.
[8] Abdallah RA, Beye M, Diop A, Bakour S, Raoult D, Fournier PE. The impact of culturomics on taxonomy in clinical microbiology. Antonie Van Leeuwenhoek 2017;10:1237–37.
[9] Fournier PE, Lagier JC, Dubourg G, Raoult D. Anaerobes in the microbiome from culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. Anaerobe 2015;36:73–8.
[10] Pharm TPT, Cadoret F, Alou MT, Brah S, Diallo BA, Diallo A, et al. ‘Umitella timonensis’ gen. nov., sp. nov., ‘Blautia marasmi’ sp. nov., ‘Lachnoclostridium pacaense’ sp. nov., ‘Boidulis marasmi’ sp. nov. and ‘Anserotrunctus rubifantis’ sp. nov., isolated from stool samples of undernourished African children. New Microbe New Infect 2017;17:84–8.
[11] Lagier JC, Edouard S, Pagnier I, Medianannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev 2015;28:208–36.
[12] Sasser M. Bacterial identification by gas chromatographic analysis of fatty acids methyl esters (GC-FAME). Newark, NY: Microbial ID; 2006.
[13] Dione N, Sankar SA, Lagier JC, Khelifaïa S, Micheïle C, Armstrong N, et al. Genome sequence and description of Anaerobisphaera massiliensis sp. nov. New Microbe New Infect 2016;10:66–76.
[14] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.
[15] Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics 2014;30:2068–9.
[16] Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinform 2010;11:119.
[17] Lee I, Kim YO, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.
[18] Hall T. BioEdit: an important software for molecular biology. GERF Bull Biosci 2011;2:60–1.
[19] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016;33:1870–4.