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

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Abstract

Using the strategy of taxonogenomics, we described *Clostridium pacaense* sp. nov. strain Marseille-P3100^T, 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.

Keywords: *Clostridium pacaense*, culturomics, taxonogenomics
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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 *Clostridia* and has traditionally been described by anaerobic growth and spore formation [3,6]. *Clostridia* 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-P3100^T (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

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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'Etoile, 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).

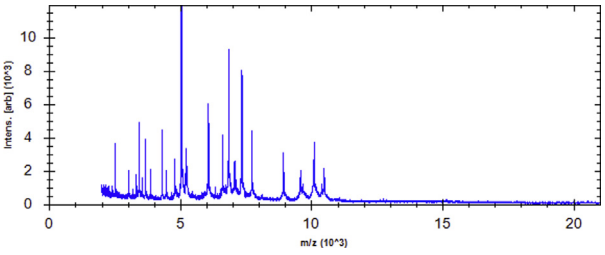

FIG. 1. Reference mass spectrum (via MALDI-TOF MS) from Clostridium pacaense strain Marseille-P3100.

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 $1e^{-03}$, 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

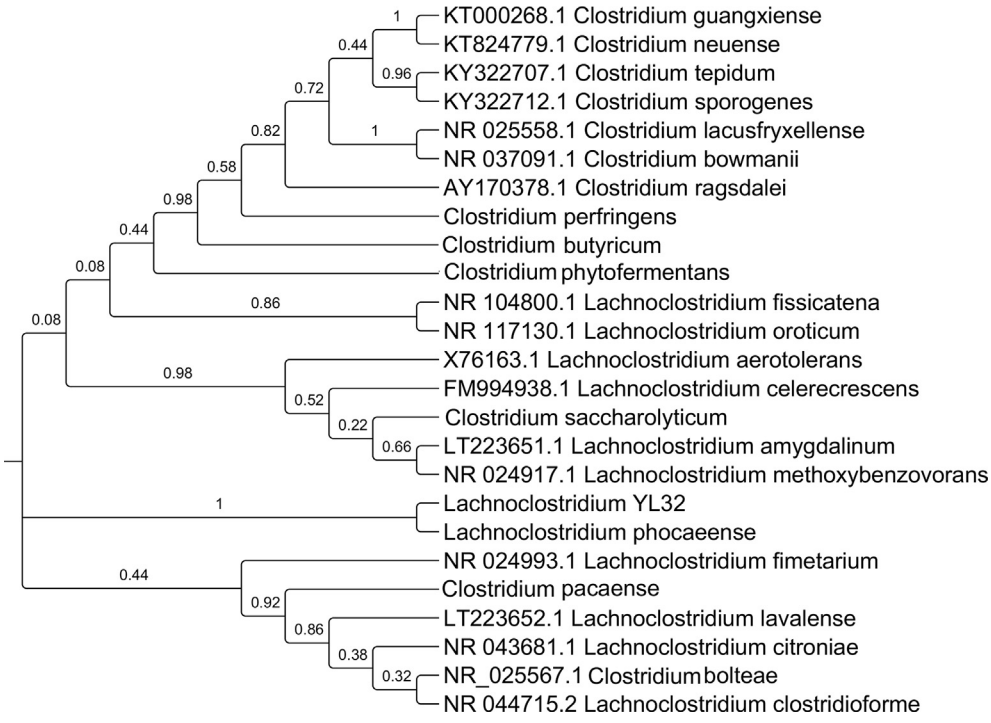

FIG. 2. Phylogenetic tree analysis based on 16S ribosomal RNA (rRNA) gene sequences. The 16S rRNA genes were aligned using CLUSTALW, and phylogenetic tree was generated using MEGA 7 software [19].

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_000144625.1), *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 the 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. pacanese produced α-glucosidase and naphthol-AS-BI-phosphohydrolase. General features and biochemical characteristics are summarized in Table 1. Antibiotic susceptibility testing revealed that *C. pacaense* was

TABLE 1. General feature and biochemical tests of Lachnoclostridium pacaense

Characteristic	Value
Current classification	
Domain	*Bacteria*
Phylum	*Firmicutes*
Class	*Clostridia*
Order	*Clostridiales*
Family	*Clostridiaceae*
Genus	*Clostridium*
Species	Clostridium 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
D-Glucose	No
D-Fructose	No
D-Maltose	No
D-Lactose	No

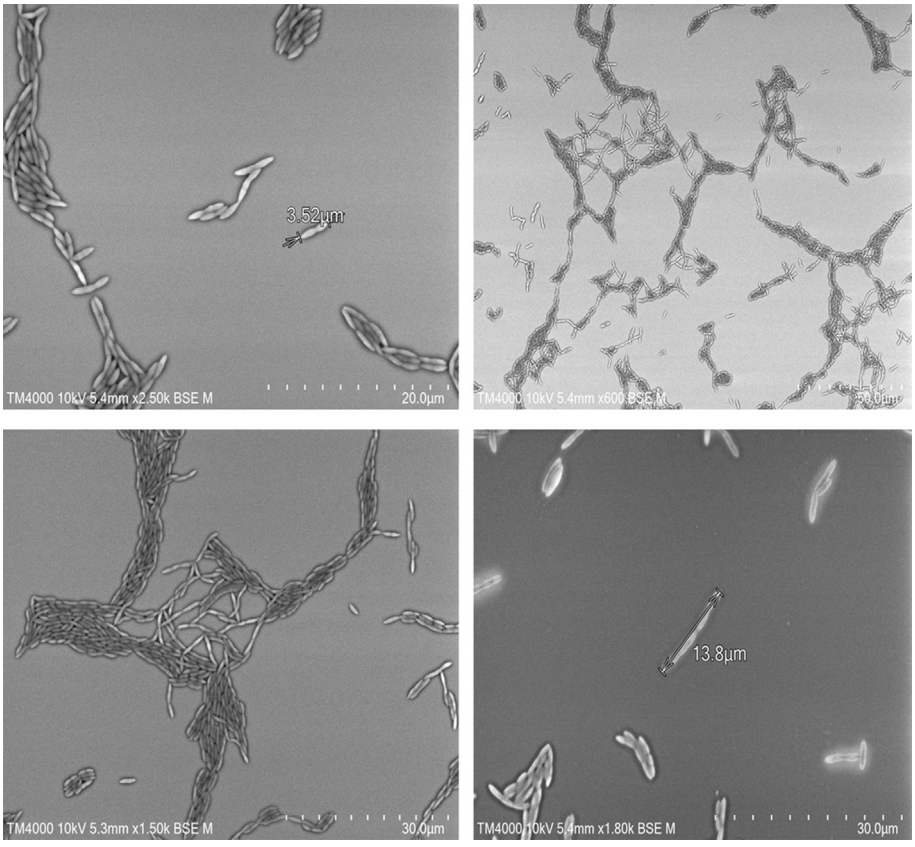

FIG. 3. Electron microscopy of *Clostridium pacaense*.

TABLE 2. Cellular fatty acids of Clostridium pacaense

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%.
[a]Mean peak area percentage.

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

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	Transcription	169
L	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.

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).

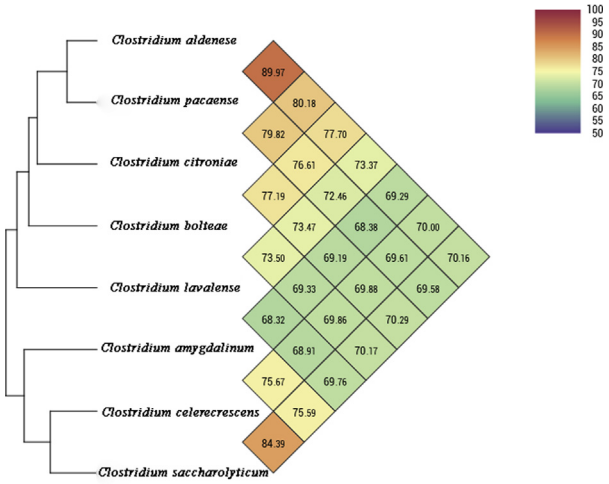

FIG. 4. OrthoANI heat map of implicated genomes.

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

TABLE 4. Clostridium pacaense 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	—	
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 $C_{16}H_{32}O_2$, $C_{14}H_{28}O_2$ and $C_{16}H_{30}O_2$. The type strain Marseille-P3100^T 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.

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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.

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