Clostridium transplantifaecale sp. nov., a new bacterium isolated from patient with recurrent *Clostridium difficile* infection

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Abstract

Clostridium transplantifaecale strain Marseille-P8228^T (= CSURP8228) is a new species isolated from a patient with recurrent *Clostridium difficile* infection.

Keywords: *Clostridium transplantifaecale*, culturomics, new species, stool, taxono-genomics
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Introduction

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 [1–4]. Once it was isolated, we used a taxono-genomics approach including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description (Table 1) and genome sequencing to describe the isolate [5,6].

Isolation and growth conditions

In 2018, we isolated from the human stool an unidentified bacterial strain. The study was validated by the ethics committee of IHU Méditerranée Infection under number 2016-011. Screening was made by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectra (Fig. 1) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated with MEPHI database https://www.mediterranee-infection.com/urms-database/). Initial growth was obtained after 48 h of culture on Columbia agar with 5% sheep blood in anaerobic conditions at 37°C at pH 7.5.

Strain identification

The 16S rRNA gene was sequenced to classify this bacterium. Amplification was performed using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing used the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com). Strain *Clostridium transplantifaecale* exhibited a 96.46% sequence identity with *Clostridium symbiosum* strain ATCC 14940 (GenBank accession number NR_118730.1), the

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TABLE 1. Description of *Clostridium transplantifaecale* according to the digitalized protologue TA00973 on the www.imedea.uib.es/dprotologue website

Taxonumber	TA00973
Date of the entry	2019-06-22
First submission date	2019-06-22
Draft number / Date	003
Version	submitted
Type of description	new description
Species name	*Clostridium transplantifaecale*
Genus name	*Clostridium*
Specific epithet	*Clostridium transplantifaecale*
Species status	sp. nov.
Species etymology	from Late Latin *transplantare* 'plant again in a different place', from Latin *trans* 'across, beyond' (see trans-) + *plantare* 'to plant' (see plant (n.)). Extended to people (1550s) and then to organs or tissue (1786). Is the transfer of stool from a healthy donor into the gastrointestinal tract for the purpose of treating recurrent *C. difficile* colitis
Submitter	Kuete Yimagou Edmond
E-mail of the submitter	edmondkuete@yahoo.fr
Designation of the type strain	Marseille-P8228T
Strain collection numbers	CSURP8228.
16S rRNA gene accession number	LR031294
Genome accession number [RefSeq]	UYZY00000000
GENOME SIZE	5.65038
G+C mol%	48.1
Data on the origin of the sample from which the strain had been isolated	
Country of origin	France
Region of origin	Provence–Alpes–Côte d'Azure
Source of isolation	gut
Sampling date	2018-03-12
Geographic location	Marseille
Source of isolation of non-type strains	gut
Growth medium, incubation conditions (temperature, pH and further information] used for standard cultivation	Columbia agar with 5% sheep blood in anaerobic conditions
Gram stain	positive
Lowest temperature for growth	25°C
Highest temperature for growth	45°C
Temperature optimum	37°C
Oxidase	negative
Catalase	positive

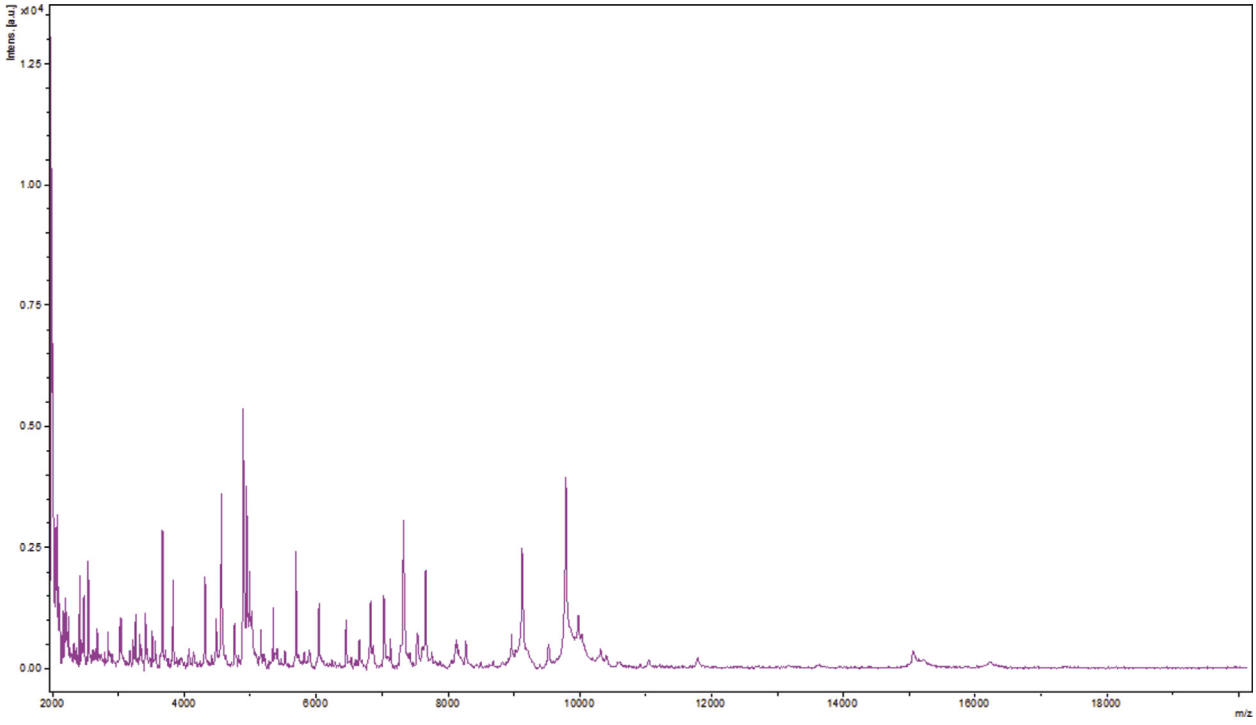

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

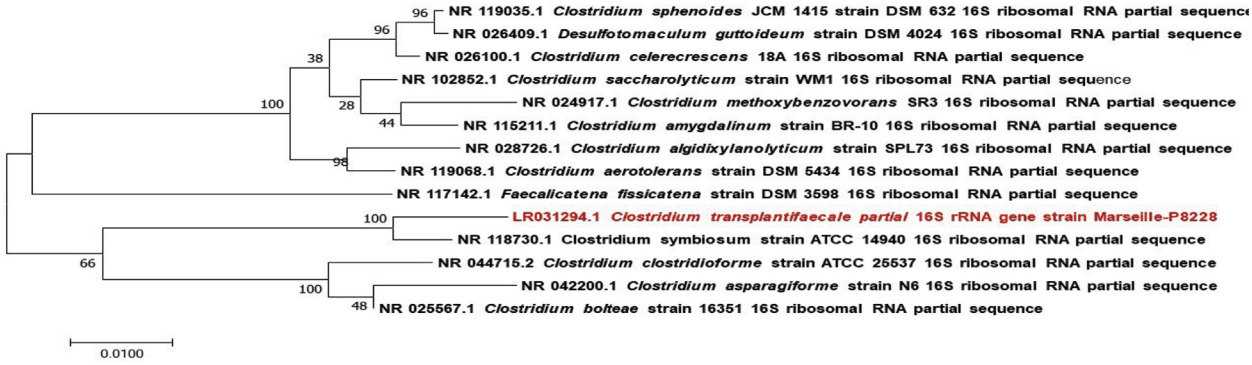

FIG. 2. Phylogenetic tree showing the position of *Clostridium transplantifaecale* strain Marseille-P8228^T relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using Muscle v3.8.31 with default parameters and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. The scale bar indicates a 5 % nucleotide sequence divergence.

phylogenetically closest species with standing in nomenclature (Fig. 2). We consequently classify this strain as a member of a new species within the genus *Clostridium*, family *Clostridiaceae*, phylum Firmicutes.

Phenotypic characteristics

Colonies were beige in colour and circular in shape with a mean diameter of 1 mm. Bacterial cells were Gram-positive, rod-shaped, ranging in length from 2 to 3 µm and in width from 0.5 to 0.7 µm and were non-motile (Fig. 3). Strain Marseille-P8228^T showed catalase-positive and oxidase-negative activities. Characteristics of the strain are summarized in Table 1. API 50CH and API ZYM tests were performed at 37°C under anaerobic conditions and the results are summarized in Table 2.

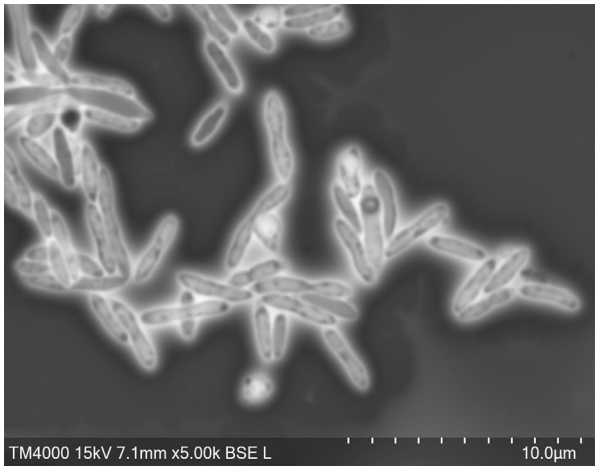

FIG. 3. Electron micrograph of *Clostridium transplantifaecale* strain Marseille-P8228^{T}was acquired with a Hitachi TM 4000 Plus tabletop scanning electron microscope.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit and then sequenced using MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera XT Paired end (Illumina), as previously described [9]. The assembly was performed with a pipeline incorporating different softwares (Velvet [10], Spades [11] and Soap Denovo [12]) on trimmed (Trimmomatic [13]) or raw data. GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value <25% of the mean depth were removed. The best assembly was selected using different criteria (17 scaffolds, 19 contigs). The genome of strain Marseille-P8228^T is 5.65038 bp long with a 48.1 mol% G+C content and contains 4705 predicted genes. The degree of genomic similarity of strain Marseille-P8228^T with closely related species was estimated using the OrthoANI software [14]. Values among closely related species (Fig. 4) ranged from 68.25% between *Clostridium asparagiforme* and *Clostridium amygdalinum* to 91.62% between *Clostridium celerecrescens* and *Clostridium sphenoides*. When the isolate was compared with these closely related species, values ranged from 68.57% with *Clostridium amygdalinum* to 79.75% with *Clostridium symbiosum*.

TABLE 2. Phenotypic characterization of *Clostridium transplantifaecale* sp. nov. based on the biochemical tests API 50 CH, and API ZYM

Bacteria: *Clostridium transplantifaecale*

Test	Results (+/–)	Test	Results (+/–)
API 50 CH			
Control	–	Esculine	–
Glycerol	+	Salicine	+
Erythrol	+	d-cellobiose	–
D-arabinose	+	d-maltose	+
L-arabinose	+	d-lactose	+
D-ribose	+	d-melibiose	+
D-xylose	+	d-saccharose	+
L-xylose	+	d-trehalose	+
D-adonitol	+	Inuline	+
Methyl-βD-xylopyranoside	+	d-melezitose	+
D-galactose	+	d-raffinose	+
D-glucose	+	Amidon	+
D-fructose	+	Glycogene	+
D-mannose	+	Xylitol	+
L-sorbose	+	Gentibiose	+
L-rhammose	+	d-turanose	+
Dulcitol	+	d-lyxose	+
Inositol	+	d-tagatose	+
D-mannitol	+	d-fucose	+
D-sorbitol	+	l-fucose	+
Methyl-αD-mannopyranoside	+	d-arabitol	+
Methyl-αD-glucopyranoside	+	l-arabitol	+
N-acetylglucosamine	+	Potassium gluconate	+
Amygdaline	+	Potassium 2-cetogluconate	–
Arbutine	+	Potassium 5-cetogluconate	+
API ZYM			
Control	–		
Alkaline phosphatase	+		
Esterase (C 4)	+		
Esterase lipase (C 8)	+		
Lipase (C 14)	–		
Leucine arylamidase	+		
Valine arylamidase	–		
Cystine arylamidase	–		
Trypsine	–		
α-chymotrypsine	–		
Acid phosphatase	+		
Naphthalo-AS-BI-phosphohydrolase	+		
α-galactosidase	+		
β-galactosodase	+		
β-glucuronidase	–		
α-glucosidase	+		
β-glucosidase	–		
N-acetyl-β-glucosaminidase	+		
α-mannosidase	–		

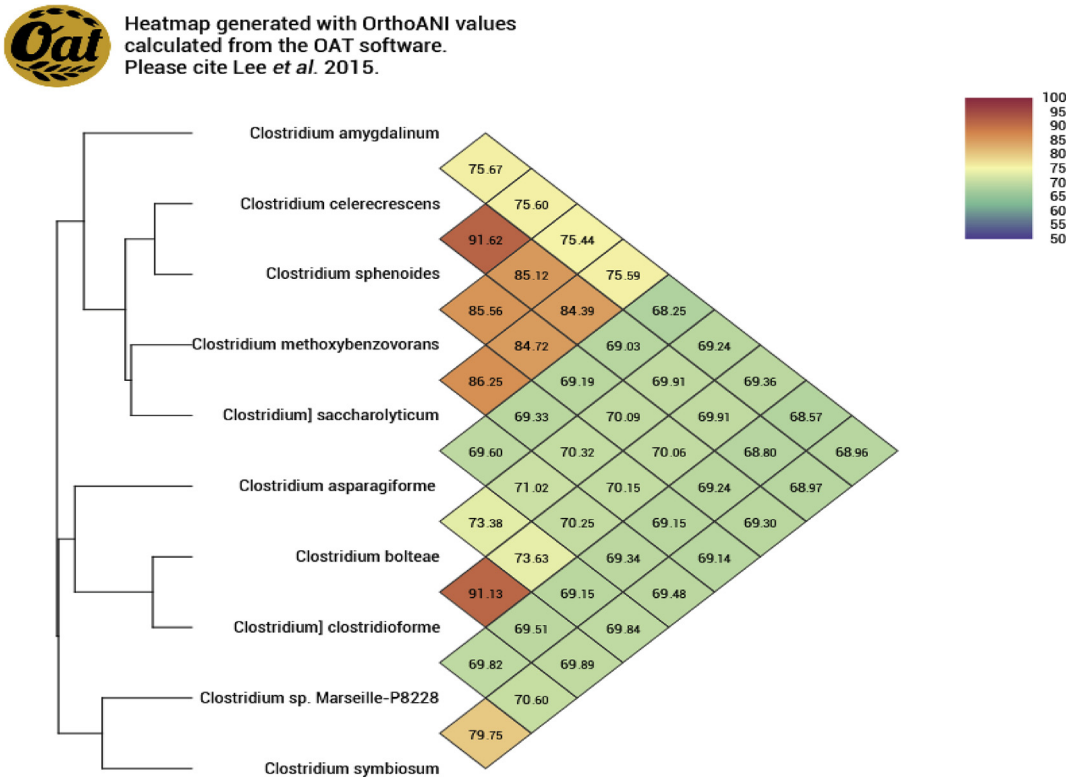

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between Genus species and other closely related species with standing in nomenclature.

Conclusion

Strain Marseille-P8228T exhibits a 16S rRNA sequence divergence <98.65% and an OrthoANI value <95% with its phylogenetically closest species with standing in nomenclature, together with unique phenotypic features. It is consequently proposed as the type strain of the new species: *Clostridium transplantifaecale* sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LR031294 and UYZY 00000000, respectively.

Deposit in culture collections

Strain Marseille-P8228^T was deposited in the collections under number CSURP8228.

Acknowledgements

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Conflict of interest

None to declare.

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