**NEW SPECIES**

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

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**Abstract**

*Clostridium transplantifaecale* strain Marseille-P8228T (≡ CSURP8228) is a new species isolated from a patient with recurrent *Clostridium difficile* infection. © 2019 The Author(s). Published by Elsevier Ltd.

**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 6F1 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
TABLE 1. Description of Clostridium transplantifaciale according to the digitalized protologue TA00973 on the www.imedea.uib.es/dprotologue website

| Parameter                                      | Value                                      |
|------------------------------------------------|--------------------------------------------|
| Taxonumber                                     | TA00973                                   |
| Date of the entry                              | 2019-06-22                                |
| First submission date                          | 2019-06-22                                |
| Draft number / Date                            | 003 submitted                             |
| Type of description                            | new description                           |
| Species name                                   | Clostridium transplantifaciale             |
| Genus name                                     | Clostridium                               |
| Specific epithet                               | Clostridium transplantifaciale sp. nov.   |
| Species status                                 | from Late Latin transplantare 'plant again in a different place', from Latin trans 'across, beyond' (see trans-) + plante (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 | France
| Country of origin                              | Provence–Alpes–Côte d’Azur                |
| Region of origin                               | gut                                       |
| Sampling date                                  | 2018-03-12                                |
| Geographic location                            | Marseille                                  |
| Source of isolation of non-type strains        | gut                                       |
| Source of isolation                            | Columbia agar with 5% sheep blood in anaerobic conditions |
| Growth medium, incubation conditions (temperature, pH and further information) used for standard cultivation | Gram stain positive
| 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.
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<sup>T</sup> 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.

**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<sup>T</sup> is 5,650,38 bp long with a 48.1 mol% G+C content and contains 4705 predicted genes. The degree of genomic similarity of strain Marseille-P8228<sup>T</sup> 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 sphenoide*. When the isolate was compared with these closely related species, values ranged from 68.57% with *Clostridium amygdalinum* to 79.75% with *Clostridium symbiosum*. 

FIG. 2. Phylogenetic tree showing the position of *Clostridium transplantifaecale* strain Marseille-P8228<sup>T</sup> 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.

FIG. 3. Electron micrograph of *Clostridium transplantifaecale* strain Marseille-P8228<sup>T</sup> was acquired with a Hitachi TM 4000 Plus tabletop scanning electron microscope.
## 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-xylene                                | +    | d-saccharose   | +    |               |
| l-xylene                                | +    | d-trehalose    | +    |               |
| d-arabinitol                             | +    | Inuline        | +    |               |
| Methyl-β-d-xlylopyranoside               | +    | d-melezitose   | +    |               |
| d-galactose                              | +    | d-raffinose    | +    |               |
| d-glucose                               | +    | Amidon         | +    |               |
| d-fructose                              | +    | Glycogene      | +    |               |
| l-sorbose                                | +    | Xylitol        | +    |               |
| l-rhamnose                               | +    | d-turanose     | +    |               |
| Dulcitol                                 | +    | d-lyxose       | +    |               |
| m-sorbitol                               | +    | d-fucrose      | +    |               |
| Methyl-α-d-mannopyranoside               | +    | d-arabitol     | +    |               |
| Methyl-α-d-glucopyranoside               | +    | i-arabitol     | +    |               |
| N-acetylglucosamine                      | +    | Potassium gluconate | + |               |
| Amygdaline                               | +    | Potassium 2-cetogluconate | – |               |
| Arbutine                                 | +    | Potassium 5-cetoglucuronate | + |               |
| **API ZYM**                               |      |               |      |               |
| Control                                  | –    |               | –    |               |
| Alkaline phosphatase                     | +    |               | +    |               |
| Esterase (C 4)                           | +    |               | +    |               |
| Esterase lipase (C 8)                    | +    |               | +    |               |
| Lipase (C 14)                            | –    |               | –    |               |
| Leucine arylamidase                      | +    |               | +    |               |
| Valine arylamidase                       | –    |               | –    |               |
| Cystine arylamidase                      | –    |               | –    |               |
| Trypsin                                  | –    |               | –    |               |
| α-chymotrypsine                          | –    |               | –    |               |
| Acid phosphatase                         | +    |               | +    |               |
| Naphthol-A5-Bi-phosphohydrolase          | +    |               | +    |               |
| d-galactosidase                          | +    |               | +    |               |
| β-glucosidase                           | +    |               | +    |               |
| β-glucuronidase                          | –    |               | –    |               |
| d-glucosidase                           | +    |               | +    |               |
| β-glucosidase                           | –    |               | –    |               |
| N-acetyl-β-glucosaminidase               | +    |               | +    |               |
| α-mannosidase                           | –    |               | –    |               |
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 UYZY00000000, respectively.

Deposit in culture collections

Strain Marseille-P8228T was deposited in the collections under number CSURP8228.

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Conflict of interest

None to declare.

References

[1] Lagier J-C, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185–93.

[2] Lagier J-C, Hugon P, Khelafi S, Fournier P-E, 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.

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between Genus species and other closely related species with standing in nomenclature.
[3] Lagier J-C, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016;1:16203.

[4] Lagier JC, Edouard S, Pagnier I, Medianikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev 2015;28:208–36.

[5] Fournier PE, Lagier JC, Dubourg G, Raoult D. From culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. Anaerobe 2015;36:73–8.

[6] Ramasamy D, Mishra AK, Lagier J-C, Padmanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 2014;64:384–91.

[7] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51.

[8] Morel A-S, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta J-P, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbial Infect Dis 2015;34:561–70.

[9] Diop A, Khelaifia S, Armstrong N, Labas N, Fournier P-E, Raoult D, et al. Microbial culturomics unravels the halophilic microbiota repertoire of table salt: description of Gracilibacillus massiliensis sp. nov. Microb Ecol Health Dis 2016;27.

[10] Zerbino DR, Birney E. Velvett algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18:821–9.

[11] 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.

[12] Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience 2012;1:18.

[13] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.

[14] Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.