Genome sequence and description of Alterileibacterium massiliense gen. nov., sp. nov., a new bacterium isolated from human ileum of a patient with Crohn’s disease

M. Boxberger1,2, H. Anani2,3 and B. La Scola1,2
1) Aix Marseille University, IRD, AP-HM, ME01, Marseille, France, 2) IHU-Méditerranée Infection, Marseille, France and 3) Aix Marseille University, IRD, AP-HM, SSA, VITROME, Marseille, France

Abstract

Alterileibacterium massiliense gen. nov. sp. nov. strain Marseille-P3115T (= CSURP-3115; DSM 103486), formerly proposed as Ileibacterium massiliense, is a new genus of bacteria isolated from the ileum of a human patient with Crohn’s disease. © 2019 The Author(s). Published by Elsevier Ltd.

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Corresponding author: B. La Scola, Aix Marseille University, Marseille, France. E-mail: bernard.la-scola@univ-amu.fr

Introduction

Alterileibacterium massiliense was isolated using the culturomics approach, an approach based on the use of a large panel of culture conditions in order to describe the microbial composition of a sample by high-throughput culture [1–4]. A taxono-genomics approach—including matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description and genome sequencing—was used to describe this species [5,6].

Isolation and growth conditions

In April 2016, an unidentified bacterial strain was isolated from the human ileum of a patient with Crohn’s disease and provisionally named Ileibacterium massiliense [7]. Tentative identification was done using MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [8]. 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 two databases (Bruker Daltonics) and constantly updated MEPHI databases (http://www.mediterranee-infection.com/article.php?larub=280&titre=urms-database). This strain was cultured routinely on Columbia sheep-blood agar (Biomerieux, Marcy l’Etoile, France) at 37°C under anaerobic conditions.

Strain identification

To identify this bacterium, the 16S rRNA gene was amplified using the primer pair ID1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xL Genetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France) as previously described [9]. The 16S rRNA nucleotide sequence was assembled and corrected using CodonCode Aligner software (http://www.codoncode.com).

The 16s rDNA gene sequence of strain Marseille-P3115 exhibited a 90.7% sequence similarity with Mogibacterium neglectum ATCC700924T (GenBank accession no. AB037875),...
the phylogenetically closest species with a standing in nomenclature (Fig. 2). We consequently classify this strain as a member of a new species within the new genus \textit{Alterileibacterium}, family \textit{Clostridiales} XIII. Incertae Sedis, phylum Firmicutes.

**Phenotypic characteristics**

Microcolonies are white and circular with a mean diameter of 0.08 mm. Bacterial cells of this gram-positive bacterium are easily discoloured and appear rather as Gram-negative bacilli that differ from their neighbouring genera \textit{Eubacterium} and \textit{Mogibacterium}. The cells have a mean diameter of 0.3 μm and a mean length of 1 μm (Fig. 3). Strain Marseille-P3115 showed catalase-negative and oxidase-negative activities (Table 1).

**Genomics**

Genomic DNA of the bacterium was sequenced using the MiSeq Technology (Illumina Inc, San Diego, CA, USA) with the mate-pair strategy as previously described [10]. Total information of 5.1 Gb was obtained from a 544K/mm² cluster density with a cluster passing quality control filters of 96.8% (10 139 000 passing filter paired reads). Within this run, the index representation for \textit{A. massiliense} was determined to 4.43%. The 449 618 paired reads were trimmed using the Trimmomatic software [11]; GapCloser [12] was used to reduce gaps, then assembly was carried out with the Spades software [13] in two scaffolds.

The genome of strain Marseille-P3115 is 1 450 823 bp long with a 35.9 mol% G+C content. The degree of genomic similarity between \textit{A. massiliense} strain Marseille-P3115 and closely related species was estimated using the OrthoANI software [14]. Values

![FIG. 1. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) reference mass spectrum for \textit{Alterileibacterium massiliense} Marseille-P3115. Spectra from 12 individual colonies were compared and a reference spectrum was generated.](image)
among closely related species (Fig. 4) ranged from 63.39% between Eubacterium saphenum strain ATCC49989 T (GenBank ACON01000001) and Eubacterium pyruvativorans ATCC BAA-574 T (GenBank FNBF01000001.1) to 83.43% between Mogibacterium diversum strain ATCC700923T (RefSeq NZ_CP027228.1) and Mogibacterium timidum strain ATCC33093 (NR 036993.1). When the isolate was compared to these closely related species, values ranged from 64.02% with Eubacterium pyruvativorans ATCC BAA-574 T (GenBank FNBF01000001.1) to 68.04% with Mogibacterium timidum strain ATCC33093 (GenBank JALU01000001.1).

**Conclusion**

Strain Marseille-P3115 T, exhibiting a 16S rRNA sequence divergence >5% with its phylogenetically closest species with standing in nomenclature, is consequently proposed as the type strain of the new genus and species Alterileibacterium massiliense gen. nov., sp. nov (Alter.i.lei.bac.te’ri.um, Gr. adj. Alter ‘other’, Gr. n. ilei, ‘ileum’; Gr. n. bakterion, ‘bacterium’; N.L. neut. n. ileibacterium, ‘bacterium isolated from the human ileum sample ’mas.si.li. en’se, L. neut. adj., massiliense for Massilia, the Latin name of Marseille, where the strain was first isolated). This strain was previously provisionally named Ileibacterium massiliense [7]. However, at the same time the genus name Ileibacterium gen. nov. was used to name an isolate of the family Erysipelotrichaceae [15]. This is the reason we modified the name of our isolate from Ileibacterium massiliense gen. nov. sp. nov. to Alterileibacterium massiliense gen. nov. sp. nov.

**Nucleotide sequence accession number**

The 16S rRNA gene and genome sequences were deposited in GenBank under accession number LT598557 and FNWE0000000, respectively.
FIG. 3. Micrograph electron microscopy of strain Alterileibacterium mas-

siliense gen. nov., sp. nov. A colony was collected from agar and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 1 h at 4°

C. A drop of cell suspension was deposited for approximately 5 min on glow-discharged formvar carbon film with 400 mesh nickel grids (FCF4O0-

Ni, EMS). The grids were dried on blotting paper and the cells were negatively stained for 10 s with 1% ammonium molybdate solution in

filtered water at room temperature. Electron micrographs were acquired with a Morgagni 268D (Philips) transmission electron microscope operated at 80 keV. Scale bar 200 nm.

TABLE 1. Description of Alterileibacterium massiliense according to the digitalized protologue under the number TA00884 on the

website www.imedea.uib.es/dprotologue

| Taxonumber | TA00884 |
|------------|---------|
| Date of the entry | 2019-01-29 |
| Version | Draft |
| Species name | Alterileibacterium massiliense |
| Genus name | Alterileibacterium |
| Specific epithet | massiliense |
| Species status | gen. nov.; sp. nov. |
| Species etymology | Alterileibacterium massiliense gen. nov., sp. nov. (Alter.il.ei.bac.te.ri.um, Gr. adj. Alter ‘other’, Gr. n. ilei, ‘ileum’; Gr. n. bakterion, ‘bacterium’, N.L. neut. n. Ileibacterium, ‘bacterium isolated from the human ileum sample’; massa.i.l. en.se, L. neut. adj. massiliense for Massilia, the Latin name of Marseille, where the strain was first isolated.) |
| Submitter | BOXBERGER Manon |
| E-mail of the submitter | manon.boxberger@hotmail.fr |
| Designation of the type strain | Marseille-P3115 |
| Strain collection numbers | CSURP3115 = DSM103486 LT59857 |
| Genome accession number [EMBL] | FNWE00000000 |
| Genome status | Draft |
| Genome size | 1 450 823 bp |
| GC mol% | 35.9 |
| Data on the origin of the sample from which the strain had been isolated | France |
| Country of origin | Marseille-P3115 |
| Region of origin | 2016-01-01 |
| Date of isolation | Human ileum |
| Source of isolation | 2019-01-01 |
| Sampling date | Columbia agar supplemented with 5% sheep blood, 37°C for 48 h of incubation |
| Growth medium, incubation conditions (temperature, pH, and further information) used for standard cultivation | |
| Gram stain | Negative |
| Cell shape | Bacilli |
| Cell size (mean length; mean diameter) | 1: 0.3 (μm) |
| Colony morphology | White, circular |
| Motility | Non-motile |
| Sporulation | No sporulation |
| Temperature range | 37°C |
| Temperature optimum | 37°C |
| Lowest pH for growth | 7 |
| Highest pH for growth | 7.5 |
| Relationship to O2 | Strictly anaerobic |
| O2 conditions for strain testing | Aerobiosis, anaerobiosis, microaerophilic |
| Oxidase | Negative |
| Catalase | Negative |
Deposit in culture collections

Strain Marseille-P3115T was deposited in two different strain collections under numbers CSURP3115 and DSM103486.

Transparency declaration

The authors declare no conflicts of interest. This work was supported by the French Government under the ‘Investissements d’avenir’ programme managed by the Agence Nationale de la Recherche (ANR) [reference: Méditerranée-Infection 10-IHU-03], by Région Provence-Alpes-Côte d’Azur and European funding FEDER, PRIMI.

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