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. Boxberger[1,2], **H. Anani**[2,3] **and B. La Scola**[1,2]

1) *Aix Marseille University, IRD, AP-HM, MEΦI, 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-P3115^T (= 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.

Keywords: *Alterileibacterium massiliense*, Culturomics, Taxono-genomics
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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 fD1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic 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* ATCC700924^T (GenBank accession no. AB037875),

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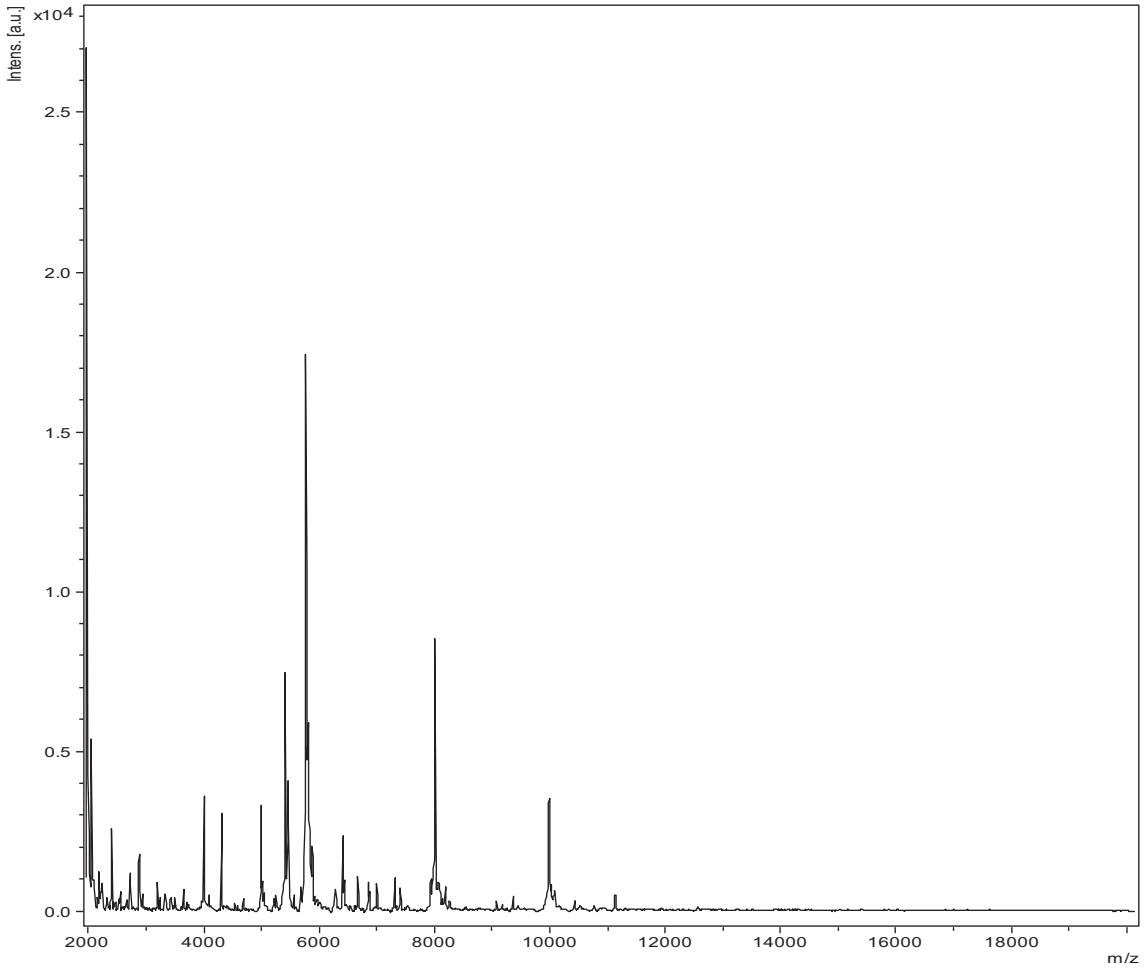

FIG. 1. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) reference mass spectrum for *Alterileibacterium massiliense* Marseille-P3115. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

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 Alterileibacterium, family 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 *Eubacterium* and *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/mm^2 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 *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 *A. massiliense* strain Marseille-P3115 and closely related species was estimated using the OrthoANI software [14]. Values

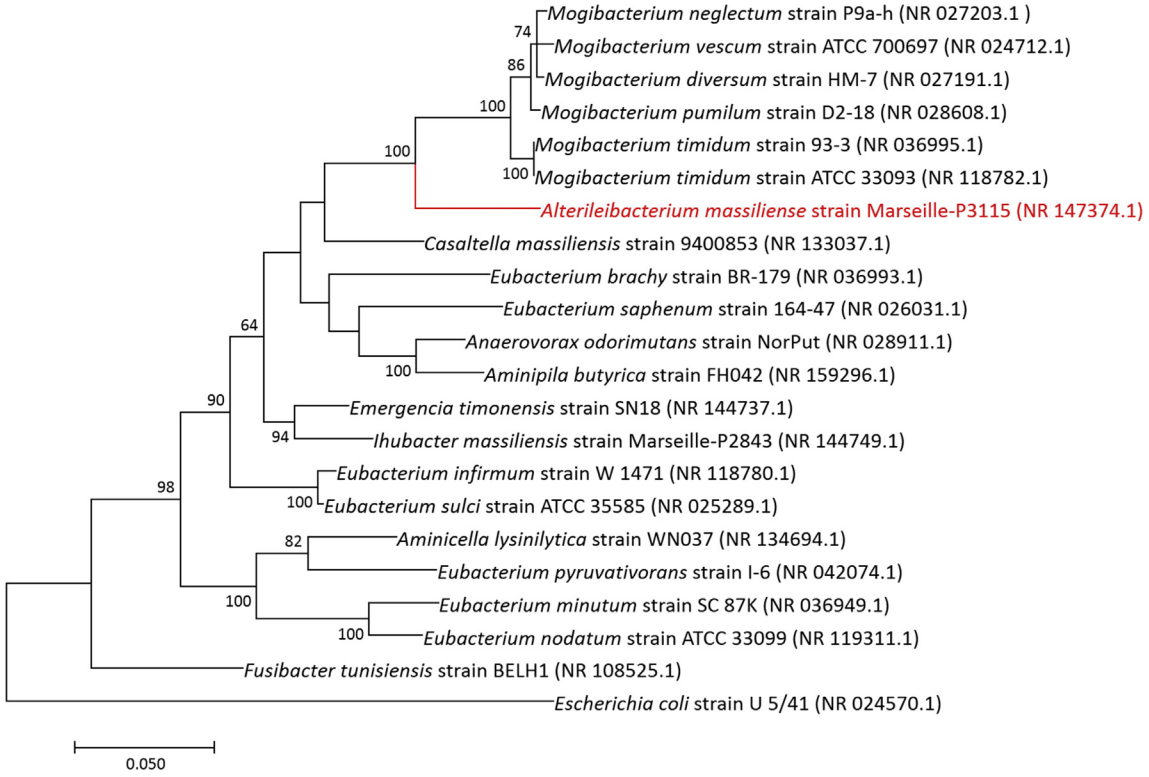

FIG. 2. Phylogenetic tree showing the position of Alterileibacterium massiliense strain Marseille-P3115 relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using Muscle v7.0.26 with default 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 1000 times to generate a majority consensus tree. Only bootstrap values > 70% were retained. The scale bar indicates a 5% nucleotide sequence divergence.

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* ATCC700923T (RefSeq NZ_CP027228.1) and *Mogibacterium pumilium* ATCC700696^T (RefSeq NZ_CP016199.1) When the isolate was compared to these closely related species, values ranged from 64.02% with *Eubacterium pyruvativorans* ATCCBAA574^T (GenBank FNBF01000001.1) to 68.04% with *Mogibacterium timidum* ATCC33093^T (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.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 '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 FNWE00000000, respectively.

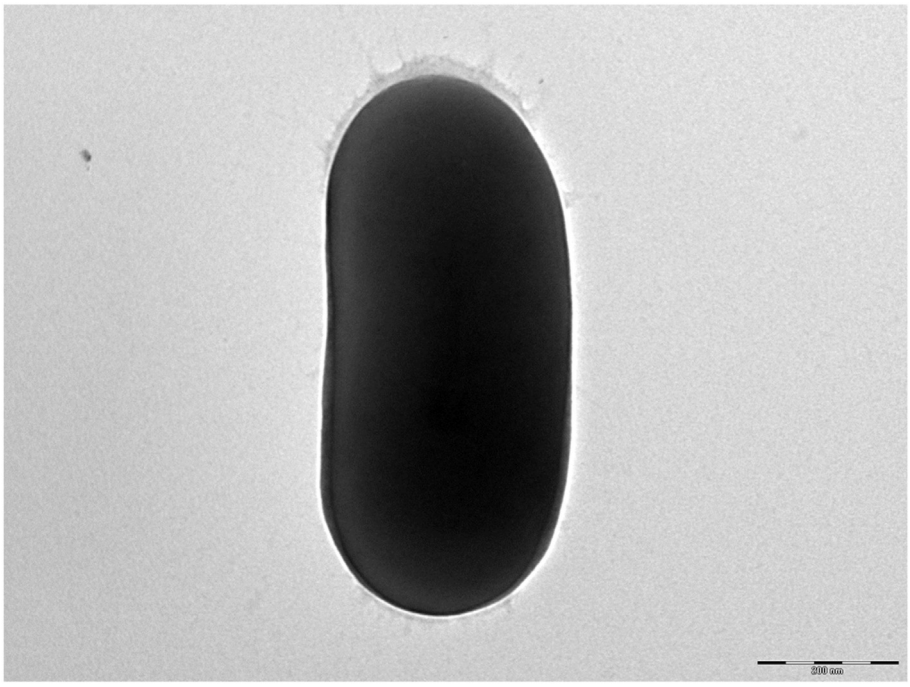

FIG. 3. Micrograph electron microscopy of strain *Alterileibacterium massiliense* gen. nov., sp. nov. A colony was collected from agar and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least 1 h at 4° C. A drop of cell suspension was deposited for approximately 5 min on glow-discharged formvar carbon film with 400 mesh nickel grids (FCF400-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';'mas.si.li. 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
16S rRNA gene accession number	LT598557
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	
Country of origin	France
Region of origin	Marseille
Date of isolation	2016-01-01
Source of isolation	Human ileum
Sampling date	2019-01-01
Growth medium, incubation conditions (temperature, pH, and further information) used for standard cultivation	Columbia agar supplemented with 5% sheep blood, 37°C for 48 h of incubation
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 O_2	Strictly anaerobic
O_2 conditions for strain testing	Aerobiosis, anaerobiosis, microaerophilic
Oxidase	Negative
Catalase	Negative

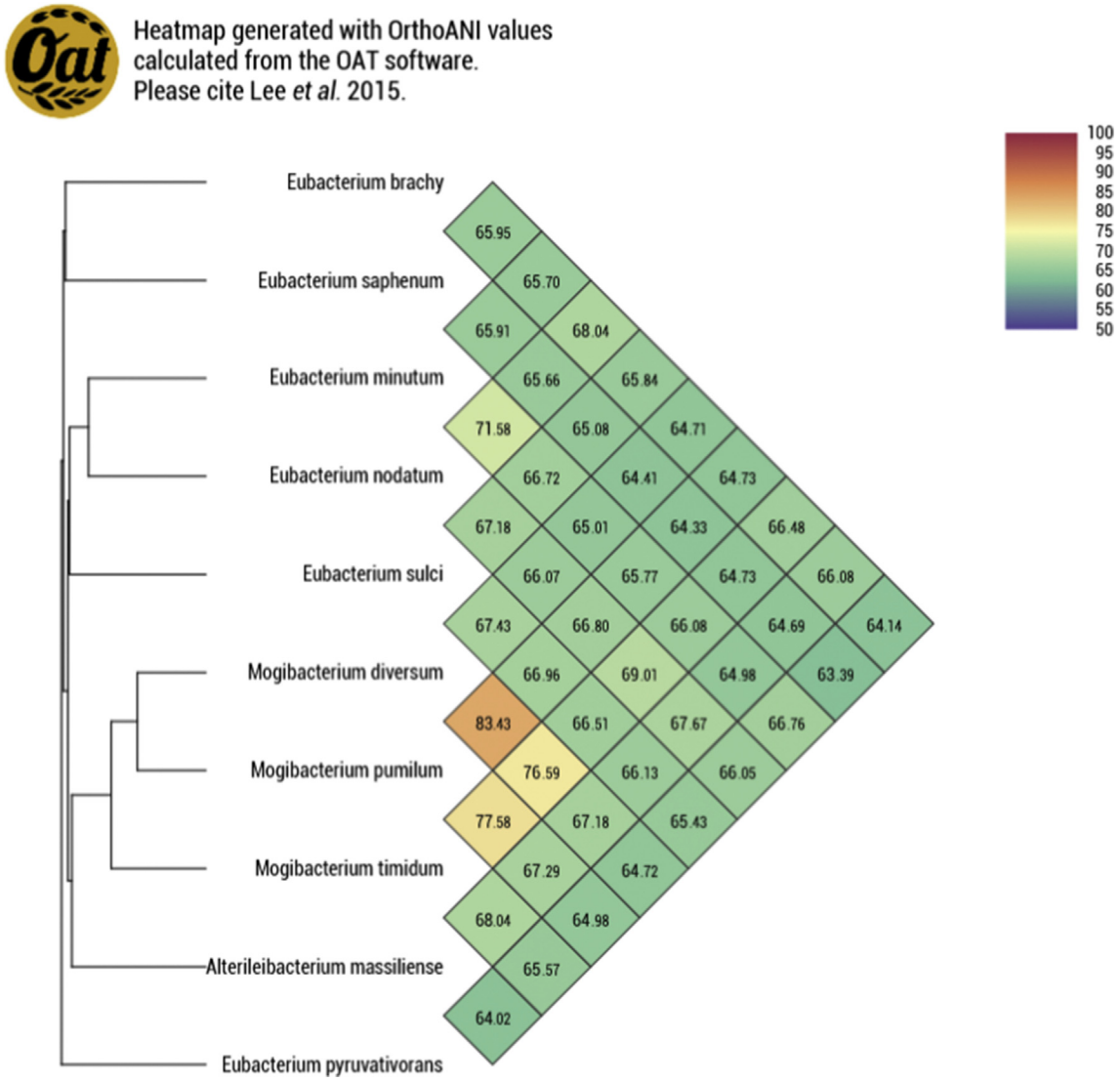

FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Alterileibacterium massiliense* and other closely related species with standing in nomenclature: *Eubacterium nodatum* ATCC33099^T (GenBank AZKM01000001); *Eubacterium minutum* ATCC 700079^T (RefSeq: NZ_CP016202.1); *Eubacterium sulci* ATCC35585^T (NZ_CP012068.1); *Mogibacterium diversum* ATCC700923^T (RefSeq NZ_CP027228.1); *Mogibacterium pumilium* ATCC700696^T (RefSeq NZ_CP016199.1); *Mogibacterium timidum* ATCC33093^T (GenBank JALU01000001.1); *Eubacterium saphenum* ATCC49989^T (GenBank ACON01000001.); *Eubacterium brachy* ATCC33089^T (AXUD01000001.1); and *Eubacterium pyruvativorans* ATCCBAA574^T (GenBank FNBF01000001.1).

Deposit in culture collections

Strain Marseille-P3115^T 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-IAHU-03], by Région Provence-Alpes-Côte d'Azur and European funding FEDER, PRIMI.

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