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

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Running title: *Alterileibacterium massiliense* gen. nov., sp. nov.

Keywords: Culturomics, taxono-genomics, *Alterileibacterium massiliense*

1 **Abstract:**

2 *Alterileibacterium massiliense* gen. nov. sp. nov. strain Marseille-P3115^T (= CSURP-3115;
3 DSM 103486), formerly proposed as *Ileibacterium massiliense*, is a new genus isolated from
4 human ileum of a patient with Crohn disease.

5

6 **Introduction**

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

13 **Isolation and growth conditions**

14 In April 2016, an unidentified bacterial strain was isolated from the human ileum of a
15 patient with Crohn disease and provisionally named *Ileibacterium massiliense* [7]. Tentative
16 identification was done by MALDI-TOF MS on a Microflex LT spectrometer (Bruker
17 Daltonics, Bremen, Germany) as previously described [8]. The obtained spectra (Figure 1)
18 was imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analyzed against the
19 main spectra of the bacteria included in two databases (Bruker Daltonics and constantly
20 updated MEPHI databases ([http://www.mediterranee-](http://www.mediterranee-infection.com/article.php?larub=280&titre=urms-database)
21 [infection.com/article.php?larub=280&titre=urms-database](http://www.mediterranee-infection.com/article.php?larub=280&titre=urms-database)). This strain was cultured routinely
22 on columbia sheep blood agar (Biomerieux, Marcy l'Etoile, France) at 37°C under anaerobic
23 conditions.

24 **Strain identification**

25 To identify this bacterium, the 16S rRNA gene was amplified using the primer pair
26 fD1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator
27 v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer capillary sequencer (Thermofisher,
28 Saint-Aubin, France) as previously described [9]. The 16S rRNA nucleotide sequence was
29 assembled and corrected using CodonCode Aligner software (<http://www.codoncode.com>).

30 The 16s rDNA gene sequence strain Marseille-P3115 exhibited a 90.7 % sequence similarity
31 with *Mogibacterium neglectum* ATCC700924^T (GenBank accession no. AB037875) the
32 phylogenetically closest species with standing in nomenclature (Figure 3). We consequently
33 classify this strain as a member of a new species within the new genus *Alterileibacterium*,
34 family *Clostridiales XIII. Incertae Sedis*, phylum *Firmicutes*.

35

36 **Phenotypic characteristics (Table 1)**

37 Microcolonies are white and circular with a mean diameter of 0.08 mm. Bacterial cells
38 of this gram-positive bacterium are easily discolored and appear rather as Gram-negative
39 bacilli that differs with their neighbor's genus *Eubacterium* and *Mogibacterium*. They present
40 a mean diameter of 0.3 μm and a mean length of 1 μm . (Figure 2) Strain Marseille-P3115
41 showed catalase-negative and oxidase-negative activities.

42 **Genomics**

43 Genomic DNA of the bacterium was sequenced using the MiSeq Technology
44 (Illumina Inc, San Diego, CA, USA) with the mate pair strategy as previously described [10].
45 Total information of 5.1 Gb was obtained from a 544K/mm² cluster density with a cluster
46 passing quality control filters of 96.8 % (10.139.000 passing filter paired reads). Within this
47 run, the index representation for *A. massiliense* was determined to 4.43%. The 449.618 paired
48 reads were trimmed using the Trimmomatic software [11], GapCloser [12] was used to reduce
49 gaps, then assembled with the Spades software [13] in 2 scaffolds.

50 The genome of strain Marseille-P3115 is 1.450.823 bp long with a 35.9 mol% G+C
51 content. The degree of genomic similarity of *A. massiliense* strain Marseille-P3115 with
52 closely related species was estimated using the OrthoANI software [14]. Values among
53 closely related species (Figure 4) ranged from 63.39% between *Eubacterium brachy* ATCC

54 33089^T (GenBank: AXUD01000001.1) and *Eubacterium pyruvativorans* ATCC BAA-574^T
55 (GenBank FNBF01000001.1) to 83.43% between *Mogibacterium diversum* ATCC700923^T
56 (RefSeq NZ_CP027228.1) and *Mogibacterium pumilium* ATCC700696^T (RefSeq
57 NZ_CP016199.1). When the isolate was compared to these closely species, values ranged
58 from 64.02% with *Eubacterium pyruvativorans* ATCCBAA574^T (GenBank
59 FNBF01000001.1) to 68.04% with *Mogibacterium timidum* ATCC33093^T (GenBank
60 JALU01000001.1).

61

62 **Conclusion**

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

74 **Nucleotide sequence accession number.** The 16S rRNA gene and genome sequences were
75 deposited in GenBank under accession number LT598557 and FNWE00000000, respectively.

76 **Deposit in culture collections.** Strain Marseille-P3115^T was deposited in two different strain
77 collections under number CSURP3115 and DSM103486.

78

79 **Conflict of interest:**

80 None to declare.

81 **Acknowledgements**

82 The authors are indebted to Catherine Robert for sequencing the genome, Aurelia Caputo for
83 submitting the genome sequence to GenBank and the platform of electron microscopy of IHU
84 for the electron micrographs.

85

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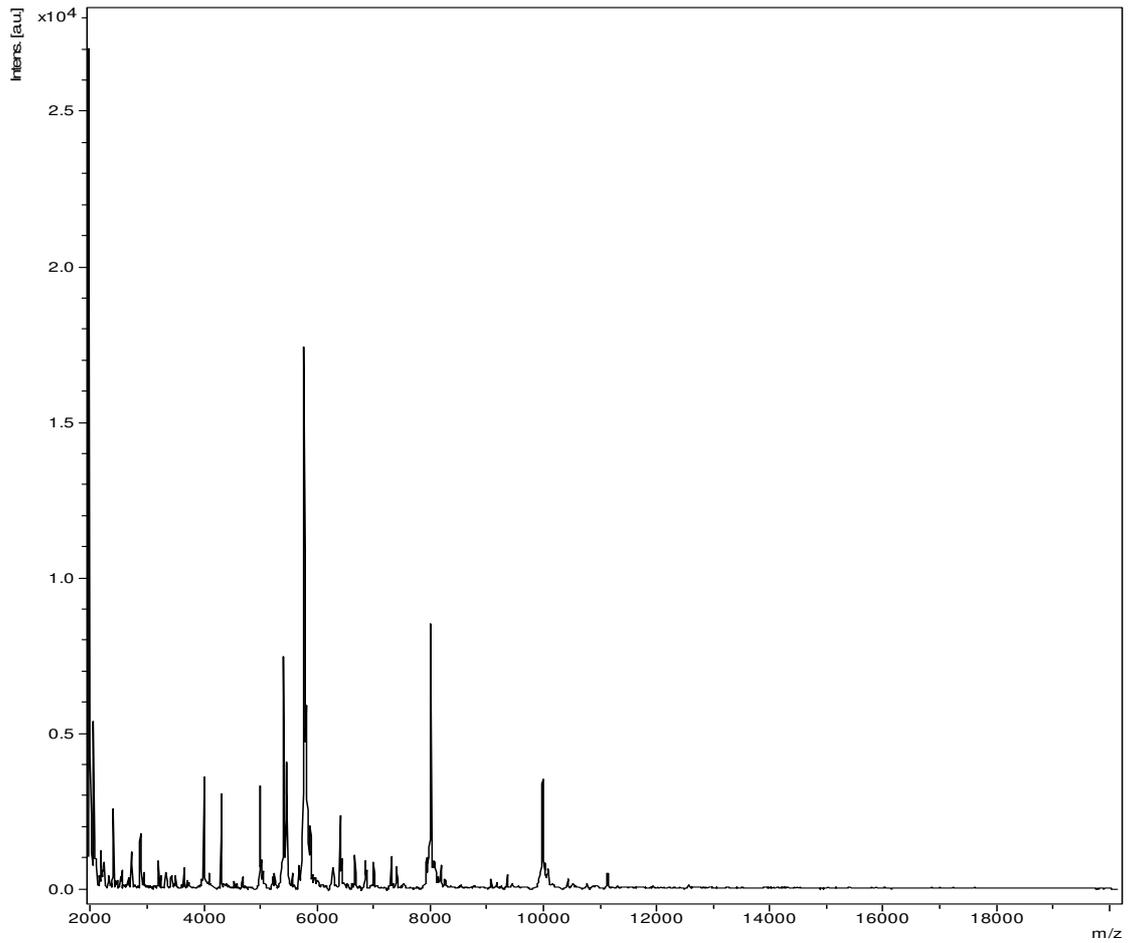
87 This work was supported by the French Government under the “*Investissements d’avenir*”
88 program managed by the *Agence Nationale de la Recherche (ANR)*, [reference: *Méditerranée-*
89 *Infection* 10-IAHU-03], by Région Provence-Alpes-Côte d’Azur and European funding
90 FEDER PRIMI

91 **Table 1:** Description of *Alterileibacterium massiliense* according to the digitalized protologue
 92 under the number TA00884 on the www.imedeauib.es/dprotologue website.

Taxonumber	TA00884
Date of the entry	2019-01-29
Version	Draft
Species name	<i>Alterileibacterium massiliense</i>
Genus name	<i>Alterileibacterium</i>
Specific epithet	<i>massiliense</i>
Species status	gen. nov ; sp. nov.
Species etymology	<i>Alterileibacterium massiliense</i> gen. nov., sp. nov. (<i>Alter.il.ei.bac.te'ri.um</i> , Gr. adj. <i>Alter</i> 'other', Gr. n. <i>ilei</i> , 'ileum'; Gr. n. <i>bakterion</i> , 'bacterium'; N.L. neut. n. <i>Ileibacterium</i> , 'bacterium isolated from the human ileum sample'; 'mas.si.li. en'se, L. neut. adj., <i>massiliense</i> for <i>Massilia</i> , the Latin name of <i>Marseille</i> , 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 48h 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 O₂	Strictly anaerobe
O₂ conditions for strain testing	Aerobiosis, Anaerobiosis, Microaerophilic
Oxidase	Negative
Catalase	Negative

93

94



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96

97 **Figure 1:** MALDI-TOF MS Reference mass spectrum for *Alterileibacterium massiliense*

98 Marseille-P3115. Spectra from 12 individual colonies were compared and a reference

99 spectrum was generated.

100

101

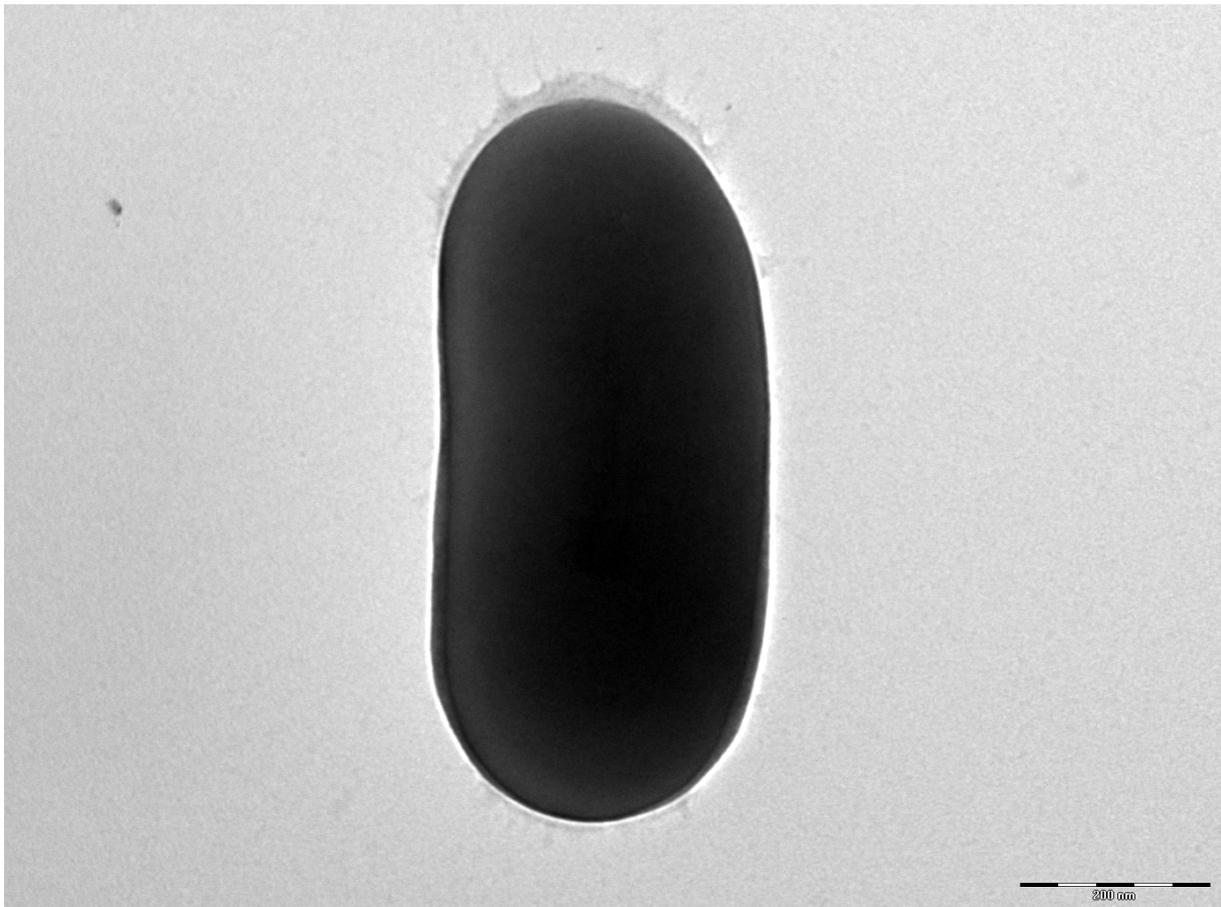
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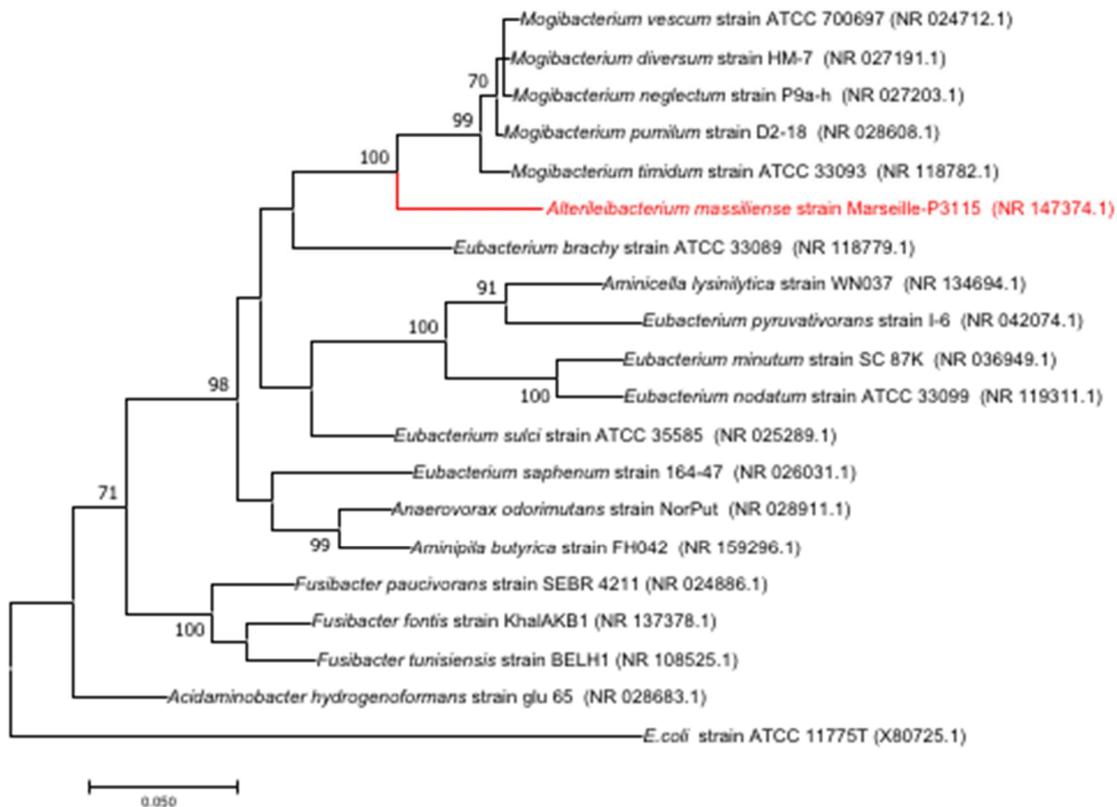
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108

109 **Figure 2.** Micrograph electron microscopy of strain *Alterileibacterium massiliense* gen. nov.,
110 sp. nov. A colony was collected from agar and fixed with 2.5 % glutaraldehyde in 0.1M
111 cacodylate buffer for at least 1h at 4°C. A drop of cell suspension was deposited for
112 approximately 5 minutes on glow-discharged formvar carbon film with 400 mesh nickel grids
113 (FCF400-Ni, EMS). The grids were dried on blotting paper and the cells were negatively
114 stained for 10 seconds with 1% ammonium molybdate solution in filtered water at RT.
115 Electron micrographs were acquired with a Morgagni 268D (Philips) transmission electron
116 microscope operated at 80 keV. Scale is shown of figures.

117

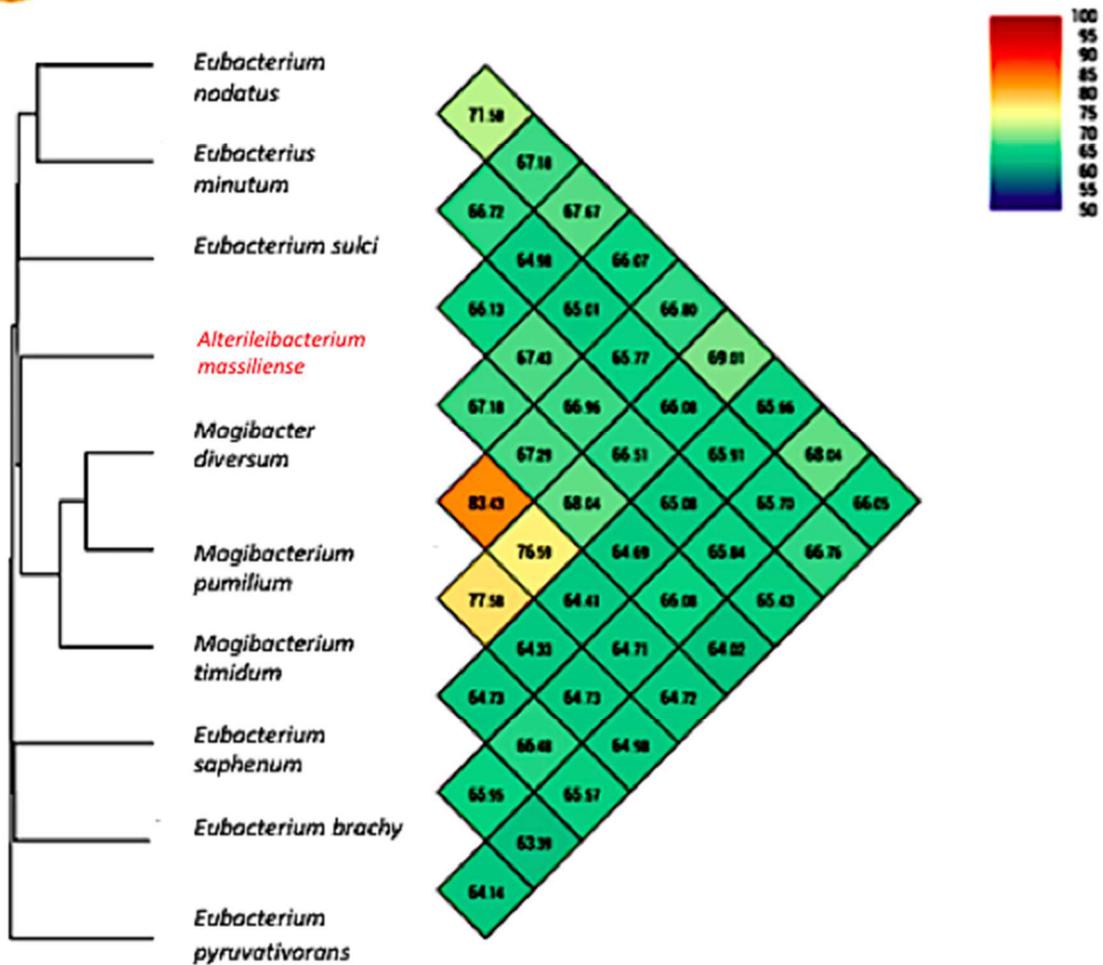


119

120 **Figure 3:** Phylogenetic tree showing the position of *Alterileibacterium massiliense* strain
 121 Marseille-P3115 relative to other phylogenetically-close neighbors. The respective GenBank
 122 accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned
 123 using Muscle v7.0.26 with default parameters and phylogenetic inferences were obtained
 124 using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are
 125 percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a
 126 majority consensus tree. Only bootstrap values > 70 % were retained. The scale bar indicates
 127 a 5 % nucleotide sequence divergence.



Heatmap generated with OrthoANI values calculated from the OAT software. Please cite Lee et al. 2015.



128

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

137 (AXUD01000001.1); *Eubacterium pyruvativorans* ATCCBAA574^T (GenBank

138 FNBF01000001.1)

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