



**HAL**  
open science

## **Taxonogenomics description of *Bacillus marasmi* sp. nov., a new species isolated from the stool sample**

M. Sarr, F.S. Diouf, C.I. Lo, M. Tidjani Alou, S. Alibar, M. Million, C. Sokhna, F. Fenollar

### ► To cite this version:

M. Sarr, F.S. Diouf, C.I. Lo, M. Tidjani Alou, S. Alibar, et al.. Taxonogenomics description of *Bacillus marasmi* sp. nov., a new species isolated from the stool sample. *New Microbes and New Infections*, 2021, 42, pp.100906. 10.1016/j.nmni.2021.100906 . hal-03421343

**HAL Id: hal-03421343**

**<https://amu.hal.science/hal-03421343>**

Submitted on 16 Jun 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

**Taxonogenomics description of *Bacillus marasmi* sp. nov., a new species isolated from the stool sample.**

Marièma SARR<sup>1,2</sup>, Fatou Samba DIOUF<sup>1,2</sup>, Cheikh Ibrahima LO <sup>2,3</sup>, Maryam TIDJANI ALOU<sup>1,2</sup>, Stéphane ALIBAR<sup>1,2</sup>, Matthieu MILLION<sup>1,2</sup>, Cheikh SOKHNA<sup>2,4</sup> and Florence FENOLLAR<sup>2,3\*</sup>

<sup>1</sup>Aix Marseille Univ, IRD, AP-HM, MEPHI, Marseille, France

<sup>2</sup>IHU-Méditerranée Infection, Marseille, France

<sup>3</sup>Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France

<sup>4</sup>Campus Commun UCAD-IRD of Hann, Dakar, Senegal

\*Corresponding author : Florence FENOLLAR, Institut Hospitalo-Universitaire Méditerranée-Infection, 19-21 Boulevard Jean Moulin, 13005, Marseille cedex 05, France ; tel +33614876832 ; fax : +33614876832; e-mail: [florence.fenollar@univ-amu.fr](mailto:florence.fenollar@univ-amu.fr).

**Running title:** *Bacillus marasmi* sp. nov.

**Keywords:** *Bacillus marasmi* sp. nov.; Taxonogenomics; Human stool; Marasmus; Child.

1 **Abstract:**

2 Using culturomics method, two strains were isolated, identified and characterized following  
3 the taxono-genomics concept. *Bacillus marasmi* sp. nov. strain Marseille-P3556  
4 (=CSURP3556) is isolated from 13-month-old girl living in **Nigerien**. The phylogenic tree,  
5 phenotypic criteria and genomic analysis described here clearly show that this bacterium is  
6 different from previously known bacterial species with standing in nomenclature and new  
7 members of *Bacillus* genus.

8

## 9 **Introduction**

10 In 1872, Ferdinand Julius Cohn described a new genus belonging to the family *Bacillaceae*  
11 and named *Bacillus* [1]. Currently hundreds of new *Bacillus* species have been described with  
12 validly published names [2]. Most often members of the genus *Bacillus* are bacteria that live  
13 in various environments such as soil, food, fresh water and the sea [3-6]. *Bacillus species*  
14 *have varied lifestyles and can be saprophytes [7] or plant endophytes [8-9].* In addition, there  
15 are two species which constitute a burden for public health: one is associated with food  
16 poisoning (*Bacillus cereus*) [10] and the other is responsible for anthrax (*Bacillus anthracis*)  
17 [11].

18 An understanding of the origin of disease or the human health status depends in part on the  
19 study of bacteria involved in normal physiological functions [12]. Thus, the exploration of the  
20 human intestinal microbiota has been at the focus of scientific studies in recent years. The  
21 culturomics method was initiated in our laboratory to isolate bacteria under different culture  
22 conditions [13-14]. This method is associated with identification by MALDI-TOF MS and  
23 systematic sequencing of the 16S rRNA gene; which made it possible to better understand the  
24 microbial diversity of the human intestine [15-16]. New bacterial species have been isolated  
25 and described using these methods which combine phenotypic, morphological, biochemical  
26 and genotypic characteristics [17-18].

27 Herein, we report the details of the isolation and taxono-genomics characterization of strain  
28 Marseille-P3556<sup>T</sup>, as type strain of *Bacillus marasmi* sp. nov., *for which its creation was*  
29 *previously announced [19].*

## 30 **Materials and methods**

31 *Strains isolation and identification*

32 The human microbiome study was an opportunity for us to isolate bacterial strain from stool  
33 sample from a Nigerien child with severe acute malnutrition. The patient's guardian approved  
34 informed consent and then the study was validated by the ethics committee of the Institut  
35 Federatif de Recherche IFR48 under number 09-022. The shipments of the sample as well as  
36 its isolation were done as previously described [20]. Then, strain Marseille-P3556 was seeded  
37 in petri dishes containing 5% sheep blood agar (bioMérieux, Marcy l'Etoile, France) and  
38 incubated under anaerobic condition (Thermo Scientific, Dardilly, France) at 37°C.  
39 **Identification by MALDI-TOF mass spectrometry has failed with this strain despite numerous**  
40 **attempts because this reference spectrum is unknown in the MADI-TOF database.** The  
41 Microflex LT spectrometer (Bruker, Daltonics, Bremen, Germany) was used during the  
42 MALDI-TOF identification as previously described [21]. The obtained spectra were analyzed  
43 with Biotyper 3.0 software and added in the local URMS database  
44 (<https://www.mediterranee-infection.com/urms-data-base>). The 16S rRNA gene was  
45 amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and then sequenced  
46 using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer  
47 capillary sequencer (ThermoFisher, Saint-Aubin, France), as previously described [22]. The  
48 nucleotide sequences of the 16S rRNA gene were analyzed by CodonCode Aligner software  
49 (<http://www.codoncode.com>). An obtained consensus sequence is compared to the NCBI  
50 nucleotide database (<https://www.ncbi.nlm.nih.gov/nucleotide/>).

#### 51 *Phenotypic and biochemical characterization*

52 Aerobic, microaerophilic and anaerobic atmospheres (Thermo Scientific, Dardilly, France)  
53 were tested to assess the different growth conditions of this strain. Varied temperatures were  
54 tested (28, 37, 45 and 55 °C) to determine the optimal temperature of strain Marseille-P3556  
55 on 5% sheep blood-enriched Columbia agar medium (bioMérieux, Marcy l'Etoile, France).  
56 API ZYM and API 50 CH strips (bioMérieux) were used to establish the biochemical

57 characteristics of the strain in accordance with the manufacturer's recommendations. Further  
58 phenotypic tests, such as Gram-staining, catalase, oxidase and spore-forming were performed  
59 as previously reported [23]. The morphological details were highlighted with a scanning  
60 electron microscope (Hitachi High-Technologies, Tokyo, Japan) as previously described [24].  
61 The fatty acid methyl ester (FAME) analysis was performed by GC/MS. Samples were  
62 prepared with approximately 10 to 20 mg of bacterial biomass per tube harvested from several  
63 culture plates. FAMEs were prepared as described by Sasser [25] followed by GC/MS  
64 analyses [26]. Briefly, FAMEs were separated using an Elite 5-MS column and monitored  
65 using mass spectrometry (Clarus 500 - SQ 8 S, Perkin Elmer, Courtaboeuf, France). Spectral  
66 database search was performed using MS Search 2.0 operated with the Standard Reference  
67 Database1A (NIST, Gaithersburg, USA) and the FAMEs mass spectral database (Wiley,  
68 Chichester, UK).

#### 69 *Genome characteristics*

70 Extraction of the genomic DNA (gDNA) was carried out on EZ1 biorobot using the EZ1  
71 DNA tissue kit (Qiagen, Hilden, Germany). Then, it's sequenced on the MiSeq instrument  
72 (Illumina Inc, San Diego, CA, USA) using the Nextera Mate Pair and Nextera XT Paired End  
73 (Illumina) sample preparation kit, as previously reported [17]. The genomic assembly was  
74 performed with three softwares such as Velvet [27], Spades [28] and Soap Denovo [29].  
75 Sequences were trimmed or untrimmed using MiSeq and Trimmomatic [30] softwares. Best  
76 assembly was done using different criteria as previously described [20]. Annotation of  
77 Genome of Marseille-P3556 was performed as reported elsewhere [20]. The Genome-to-  
78 Genome Distance Calculator (GGDC) web server available online (<http://ggdc.dsmz.de>) was  
79 used to calculate similarity between the related closed genomes. Thus the DNA–DNA  
80 hybridization (DDH) was also determined [31]. Average nucleotide identity analysis was  
81 assessed using the OAT software [32].

## 82 **Results**

### 83 *Strain identification and phylogenetic analysis*

84 Colonies of strain Marseille-P3556 was not identified by mass spectrometry. **In other words**  
85 **the spectral reference of the strain is absent in the MALDI-TOF database.** Therefore, its  
86 generated reference spectrum was incremented in our local database (**Figure 1**). A similarity  
87 analysis of the 16S rDNA gene of strain Marseille-P3556 revealed a nucleotide sequence  
88 identity of 97.29% with *Bacillus subterraneus* strain COOI3B (accession number  
89 NR\_104749.1), the phylogenetically closest species. This similarity value was lower than the  
90 recommended cut-off value (98.65%) to delimit the species barrier in bacteria [33]. Therefore,  
91 strain Marseille-P3556 was potentially new species within the family *Bacillaceae*. The 16S  
92 rRNA-based phylogenetic tree of *Bacillus* species (Figure 2) highlighted the position of strain  
93 Marseille-P3556 among its closely related species with a validly published name. In addition,  
94 the shape of bacterial cell was visualized using the Hitachi SU5000 instrument (Figure 3).

### 95 *Biochemical and phenotypic properties of the strain*

96 Strain Marseille-P3556 grows under aerobic conditions with an optimal temperature at 37°C.  
97 It is Gram-positive aerobic rod-shaped bacterium with a mean cell diameter of 0.8 µm.  
98 Colonies of the strain Marseille-P3556 were flat, smooth, small, circular and pale grey with a  
99 mean diameter of 0.5 to 2 mm. It presents catalase-negative and oxidase-negative activities.

100 Using the API ZYM strip, **esterase lipase (C 8)** and naphthol-AS-BI-phosphohydrolase were  
101 positive for strain Marseille-P3556, while alkaline phosphatase, valine arylamidase, trypsin, α  
102 -chymotrypsin, acid phosphatase, **lipase (C14)**, **esterase (C4)** β-galactosidase, β-  
103 glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase  
104 were negative. In addition, using 50 CH strip, *Bacillus marasmi* strain Marseille-P3556 was  
105 positive for glycerol, ribose, D-turanose, adonitol, galactose, glucose, mannose, D-fructose,

106 inositol, sorbitol, methyl  $\alpha$ D-glucopyranoside, N-acetyl-glucosamine, amygdalin, arbutin,  
107 salicin, D-maltose, D-lactose, D-melibiose, starch, sucrose, inulin, D-melezitose, D-raffinose,  
108 glycogen, xylitol, gentiobiose, D-cellobiose, D-trehalose, D-lyxose, and potassium 5-  
109 ketogluconate. But negative reactions were observed for erythritol, arabinose, xylose, D-  
110 tagalose, fucose and potassium 2-ketogluconate. Phenotypic criteria of strain Marseille-P3556  
111 were compared with closely related species in Table 1. The major fatty acids found for  
112 Marseille-P3556 were C<sub>16:0</sub> (31.4%), C<sub>14:0iso</sub> (20%) and C<sub>16:1n7</sub> (14.8%). Minor amounts of  
113 saturated fatty acids were also found with strain Marseille-P3556.

#### 114 *Genomic analysis*

115 The size of the genome of strains Marseille-P3556 was 4,638,282 bp long with 38.2 mol%  
116 G+C content (Figure 4). The genomic assembly was carried out into 47 contigs and into 46  
117 scaffolds. Strain Marseille-P3556 has 4,586 assigned as predicted genes. Furthermore, 4,415  
118 protein-coding genes and 116 RNAs genes (28 rRNAs, 83 tRNAs and 5 ncRNAs) were  
119 detected inside genome of Marseille-P3556. In Table 2, the composition of the genome of *B.*  
120 *marasmi* is contrasted against that of the genomes of phylogenetically close species.

121 DDH analysis showed values ranged from 19.1% between *B. drentensis* and *B. subterraneus*  
122 to 31.8% between *B. circulans* and *B. subterraneus*. Obtained values are below to the 70%  
123 recommended threshold to delineate new prokaryotic species, thus confirming that this strain  
124 is new species. The DDH values comparison of species in the study here is detailed in Table  
125 3. Moreover, OrthoANI analysis among closely related species (Figure 5) showed that  
126 *Bacillus* species had higher value of percentage of identity of 77.22% shared between *B.*  
127 *drentensis* and *B. bataviensis*. On the other hand, 67.53% was lowest value of similarity  
128 obtained between *B. subterraneus* and *B. circulans*. These OrthoANI values are lower than  
129 the recommended threshold (<95%), which suggest strain Marseille-P3556 is new member of  
130 *Bacillus* genus.



131 **Conclusion**

132 Considering the phenotypic tests, biochemical criteria and genomic analysis performed on  
133 strain Marseille-P3556, we proposed it as new bacterial species. Therefore, in this study, the  
134 genomic findings obtained such as the sequence identity of the 16S rRNA gene below the  
135 threshold value of 98.65%, the OrthoANI values (<95%) and DDH averages allowed us to  
136 formally report that *Bacillus marasmi* sp. nov., is new species within the family *Bacillaceae*  
137 in the phylum *Firmicutes*.

138 **Description of *Bacillus marasmi* sp. nov.**

139 *Bacillus marasmi* sp. nov. (ma.ras.mi, L. adj. fem, 'marasmus,' the disease meaning a form of  
140 severe malnutrition in children). It is Gram-positive aerobic bacterium and rod-shaped. Cells  
141 have diameter varying between 0.6 to 1 µm. Catalase and oxidase activities are negative.  
142 Colonies are small, circular and pale grey with a mean diameter of 1.25 mm on blood agar.  
143 The strain Marseille-P3556 was positive for glycerol, ribose, D-turanose, adonitol, galactose,  
144 glucose, mannose, D-fructose, inositol, sorbitol, methyl αD-glucopyranoside, N-acetyl-  
145 glucosamine, amygdalin, arbutin, salicin, D-maltose, D-lactose, D-melibiose, starch, sucrose,  
146 inulin, D-melezitose, D-raffinose, glycogen, xylitol, gentiobiose, D-lyxose, and potassium 5-  
147 ketogluconate. C<sub>16:0</sub> (31.4%), C<sub>14:0iso</sub> (20%) and C<sub>16:1n7</sub> (14.8%). are the major fatty acids  
148 detected in cells of *Bacillus marasmi* sp. nov. The genome of strain Marseille-P3556 was 4.64  
149 Mbp with 38.2 mol% of G+C content. The 16S rRNA and draft genome sequences are  
150 deposited in the Genbank database under Accession numbers LT671590 and  
151 CABHPS0000000000, respectively. The type strain of *Bacillus marasmi* sp. nov., strain  
152 Marseille-P3556 was isolated from a Nigerian child with clinical aspects of marasmus.

153 **Conflict of interest:**

154 Authors declare that there are not conflicts of interest.

155 **Acknowledgments:**

156 The authors thank Amael Fadlane for culturing the strains and Aurelia Caputo for submitting  
157 the genomic sequence to GenBank.

158 **Funding sources:**

159 This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée  
160 Infection, the National Research Agency under the program « Investissements d'avenir »,  
161 reference ANR-10-IAHU-03, the Région Provence Alpes Côte d'Azur and European funding  
162 FEDER PRIMI.

163 **References**

- 164 1. Cohn F. Untersuchungen über Bakterien. Beitrage zur Biologie der Pflanzen Heft.  
165 1872; 1:127-224.
- 166 2. LPSN *Bacillus*. In: LPSN. <https://lpsn.dsmz.de/genus/bacillus>. Accessed 14 Sept  
167 2020.
- 168 3. Díez-Méndez A, Rivas R, Mateos PF, et al. *Bacillus terrae* sp. nov. isolated from  
169 Cistus ladanifer rhizosphere soil. Int J Syst Evol Microbiol. 2017;67(5):1478-1481.  
170 doi:10.1099/ijsem.0.001742.
- 171 4. Abril AG, Rama JLR, Feijoo-Siota L, et al. *Bacillus safensis* subsp. *osmophilus* subsp.  
172 nov., isolated from condensed milk, and description of *Bacillus safensis* subsp.  
173 *safensis* subsp. nov. Int J Syst Evol Microbiol. 2019; 69(1):189-195.  
174 doi:10.1099/ijsem.0.003126.
- 175 5. Baik KS, Lim CH, Park SC, Kim EM, Rhee MS, Seong CN. *Bacillus rigui* sp. nov.,  
176 isolated from wetland fresh water. Int J Syst Evol Microbiol. 2010; 60(Pt 9):2204-  
177 2209. doi:10.1099/ijse.0.018184-0.
- 178 6. Zhu D, Zhang P, Niu L, et al. *Bacillus ectoiniformans* sp. nov., a halotolerant  
179 bacterium isolated from deep-sea sediments. Int J Syst Evol Microbiol. 2016;  
180 66(2):616-622. doi:10.1099/ijsem.0.000763.
- 181 7. Debois D, Fernandez O, Franzil L, Jourdan E, de Brogniez A, Willems L et al. Plant  
182 polysaccharides initiate underground crosstalk with bacilli by inducing synthesis of  
183 the immunogenic lipopeptide surfactin. Environ Microbiol Rep. 2015;7(3):570-582.  
184 doi:10.1111/1758-2229.12286
- 185 8. de Los Santos Villalobos S, Robles RI, Parra Cota FI, Larsen J, Lozano P, Tiedje JM.  
186 *Bacillus cabrialesii* sp. nov., an endophytic plant growth promoting bacterium isolated  
187 from wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley, Mexico. Int J Syst  
188 Evol Microbiol. 2019; 69(12):3939-3945. doi:10.1099/ijsem.0.003711.
- 189 9. Ma L, Xi JQ, Cao YH, Wang XY, Zheng SC, Yang CG et al. *Bacillus*  
190 *endozanthoxylicus* sp. nov., an endophytic bacterium isolated from *Zanthoxylum*  
191 *bungeanum* Maxim leaves. Int J Syst Evol Microbiol. 2017; 67(10):3699-3705.  
192 doi:10.1099/ijsem.0.002138
- 193 10. Bottone EJ. *Bacillus cereus*, a volatile human pathogen. Clin Microbiol Rev 2010;  
194 23:382-98. doi.org/10.1128/CMR.00073-09.
- 195 11. Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M *et al.*  
196 Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United  
197 States. Emerg Infect Dis 2001; 7:933-44. doi.org/10.3201/eid0706.010604.
- 198 12. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R and Gordon JI. The  
199 human Microbiome Project. Nature 2007; 449: 804-810.  
200 <https://www.nature.com/articles/nature06244>.
- 201 13. Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial  
202 culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect  
203 2012; 18:1185-93. doi:10.1111/1469-0691.12023.

- 204 14. Lagier JC, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of  
205 previously uncultured members of the human gut microbiota by culturomics. *Nat*  
206 *Microbiol* 2016; 1:16203. doi:10.1038/nmicrobiol.2016.203.
- 207 15. Ramasamy D, Mishra AK, Lagier JC, Padhmanabhan R, Rossi M, Sentausa E, et al. A  
208 polyphasic strategy incorporating genomic data for the taxonomic description of novel  
209 bacterial species. *Int J Syst Evol Microbiol* 2014; 64:384–91.  
210 doi:10.1099/ijs.0.057091-0.
- 211 16. Lagier JC, Hugon P, Khelaifia S, Fournier PE, La Scola B, Raoult D. The rebirth of  
212 culture in microbiology through the example of culturomics to study human gut  
213 microbiota. *Clin Microbiol Rev* 2015; 28:237–64. doi:10.1128/CMR.00014-14.
- 214 17. Lo CI, Padhmanabhan R, Mediannikov O, Terras J, Robert C, Faye N, et al. High-  
215 quality genome sequence and description of *Bacillus dielmoensis* strain FF4(T) sp.  
216 nov. *Stand Genomic Sci.* 2015; 10:41. doi:10.1186/s40793-015-0019-8
- 217 18. Sarr M, Lo CI, Tall ML, Fadlane A, Senghor B, Sokhna C, Raoult D et al.  
218 Taxonogenomics description of *Bacillus dakarensis* sp. nov., *Bacillus sinesaloumensis*  
219 sp. nov. and *Bacillus massiliogabonensis* sp. nov., three new species isolated from  
220 human stools. *New Microbes New Infect* 2020; 37: 100718.
- 221 19. Pham TP, Cadoret F, Alou MT, Brah S, Diallo BA, Diallo A, Sokhna C, Delerce J,  
222 Fournier PE, Million M, Raoult D. '*Urmitella timonensis*' gen. nov., sp. nov., '*Blautia*  
223 *marasmi*' sp. nov., '*Lachnoclostridium pacaense*' sp. nov., '*Bacillus marasmi*' sp. nov.  
224 and '*Anaerotruncus rubiinfantis*' sp. nov., isolated from stool samples of  
225 undernourished African children. *New Microbes New Infect.* 2017 Mar 3;17:84-88.  
226 doi: 10.1016/j.nmni.2017.02.004.
- 227 20. Bilen M, Mbogning Fonkou MD, Nguyen TT, Richez M, Daoud Z, Fournier PE, et al.  
228 *Miniphocibacter massiliensis* gen. nov., sp. nov., a new species isolated from the  
229 human gut and its taxono-genomics description. *Microbiologyopen.* 2019;  
230 8(5):e00735. doi:10.1002/mbo3.735.
- 231 21. Lo CI, Fall B, Sambe-Ba B, Diawara S, Gueye MW, Mediannikov O, Sokhna C et al.  
232 MALDI-TOF Mass Spectrometry: A Powerful Tool for Clinical Microbiology at  
233 Hôpital Principal de Dakar, Senegal (West Africa). *PLoS One* 2015; 10(12):e0145889.  
234 doi: 10.1371/journal.pone.0145889.
- 235 22. Morel AS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta JP, et al.  
236 Complementarity between targeted real-time specific PCR and conventional broad-  
237 range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. *Eur J*  
238 *Clin Microbiol Infect Dis* 2015; 34:561-70. doi:10.1007/s10096-014-2263-z.
- 239 23. Gary P. Wormser, Charles Stratton, *Manual of Clinical Microbiology*, 9th Edition  
240 Edited by Patrick R. Murray, Ellen Jo Baron, James H. Jorgensen, Marie Louise  
241 Landry, and Michael A. Pfaller Washington, DC: ASM Press, 2007 2488 pp.,  
242 illustrated. \$209.95 (hardcover), *Clinical Infectious Diseases*, Volume 46, Issue 1, 1  
243 January 2008, Page 153, doi.org/10.1086/524076 18.
- 244 24. Belkacemi S, Bou Khalil J, Ominami Y, Hisada A, Fontanini A, Caputo A et al.  
245 Passive Filtration, Rapid Scanning Electron Microscopy, and Matrix-Assisted Laser  
246 Desorption Ionization-Time of Flight Mass Spectrometry for *Treponema* Culture and

- 247 Identification from the Oral Cavity. J Clin Microbiol. 2019; 57(10):e00517-19.  
248 doi:10.1128/JCM.00517-19.
- 249 25. Myron S, Bacterial Identification by Gas Chromatographic Analysis of Fatty Acids  
250 Methyl Esters (GC-FAME). MIDI. 2006;(Technical Note #101).
- 251 26. N. Dione, S.A. Sankar, J.C. Lagier, S. Khelaifia, C. Michele, N. Armstrong, M.  
252 Richez, J. Abrahao, D. Raoult and P.E. Fournier Genome sequence and description of  
253 *Anaerosalibacter massiliensis* sp. nov. New Microbes and New Infections.  
254 2016;10:66-76.
- 255 27. Zerbino DR, Birney E. Velvet: Algorithms for de novo short read assembly using de  
256 Bruijn graphs. Genome Res 2008; 18:821–9. doi:10.1101/gr.074492.107.
- 257 28. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al.  
258 SPAdes: a new genome assembly algorithm and its applications to single-cell  
259 sequencing. J Comput Biol 2012; 19:455–77. doi:10.1089/cmb.2012.0021.
- 260 29. Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically  
261 improved memory-efficient short-read de novo assembler. Gigascience. 2012; 1:18.  
262 doi:10.1186/2047-217X-1-18.
- 263 30. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina  
264 sequence data. Bioinformatics 2014; 30:2114–20. doi:10.1093/bioinformatics/btu170
- 265 31. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species  
266 delimitation with confidence intervals and improved distance functions. BMC  
267 Bioinformatics. 2013; 14:60. doi:10.1186/1471-2105-14-60.
- 268 32. Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: An improved algorithm and software  
269 for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016; 66:1100–3.  
270 doi:10.1099/ijsem.0.000760.
- 271 33. Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average  
272 nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of  
273 prokaryotes [published correction appears in Int J Syst Evol Microbiol. 2014  
274 May;64(Pt 5):1825]. Int J Syst Evol Microbiol. 2014; 64(Pt 2):346–351.  
275 doi:10.1099/ijss.0.059774-0
- 276 34. Stothard P, Grant JR, Van Domselaar G. Visualizing and comparing circular genomes  
277 using the CGView family of tools. Brief Bioinform 2017 Jul 26. doi:  
278 10.1093/bib/bbx081.
- 279 35. Kanso S, Greene AC, Patel BKC. *Bacillus subterraneus* sp. nov., an iron- and  
280 manganese-reducing bacterium from a deep subsurface Australian thermal aquifer. Int  
281 J Syst Evol Microbiol. 2002 May;52(Pt 3):869-874. doi: 10.1099/00207713-52-3-869.
- 282 36. Heyrman J, Vanparys B, Logan NA, Balcaen A, Rodríguez-Díaz M, Felske A, De Vos  
283 P. *Bacillus novalis* sp. nov., *Bacillus vireti* sp. nov., *Bacillus soli* sp. nov., *Bacillus*  
284 *bataviensis* sp. nov. and *Bacillus drentensis* sp. nov., from the Drentse A grasslands.  
285 Int J Syst Evol Microbiol. 2004 Jan;54(Pt 1):47-57. doi: 10.1099/ijss.0.02723-0.

286 **Table 1:** Different characteristics of **1**, *Bacillus marasmi* sp. nov., strain Marseille-P3556; **2**, *Bacillus dakarensis* strain Marseille-P3515 [18]; **3**,  
 287 *Bacillus massiliogabonensis* strain Marseille P2639 [18]; **4**, *Bacillus subterraneus* strain DSM 13966 [35]; **5**, *Bacillus drentensis* strain DSM  
 288 15600 [36]; **6**, *Bacillus bataviensis* strain DSM 15601 [36].

<b>Properties</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
Cell diameter (µm)	0.6-1	0.5-1	0.7-1	0.5–0.8	0.6-1.2	0.7-1.2
Oxygen requirement	Aerobic	Aerobic	Aerobic	FA	FA	FA
Gram stain	+	+	–	–	+	+
Motility		+	–	+	+	+
Endospore formation	+	+	+	–	+	+
<b>Production of:</b>						
Alkaline phosphatase	–	–	+	na	na	na
Catalase	–	+	+	+	na	na
Oxidase	–	+	–	–	na	na
β-Galactosidase	–	–	–	–	+	+
α-Glucosidase	–	–	–	+	na	na
Naphthol-AS-BI-phosphohydrolase	+	–	–	na	na	na
N-acetyl-β-glucosaminidase	+	–	–	–	+	+
<b>Utilization of:</b>						
Potassium 5-ketogluconate	+	–	–	na	–	–
D-Xylose	–	+	–	+	w	–
D-Fructose	+	–	–	+	+	+
D-Glucose	+	–	–	+	+	+
D-Mannose	+	–	–	–	w	+
G+C content (mol%)	38.2	38.6	37.9	43.1	39.4	39.6
Habitat	Human stool	Human stool	Stool sample	Thermal waters	Soil	Soil

289 FA, facultative anaerobic; +, positive reaction; -, reaction; na, not available data.

290

291 **Table 2:** Genome comparison of closely related species to *B. marasmi* strain Marseille-P3556<sup>T</sup>

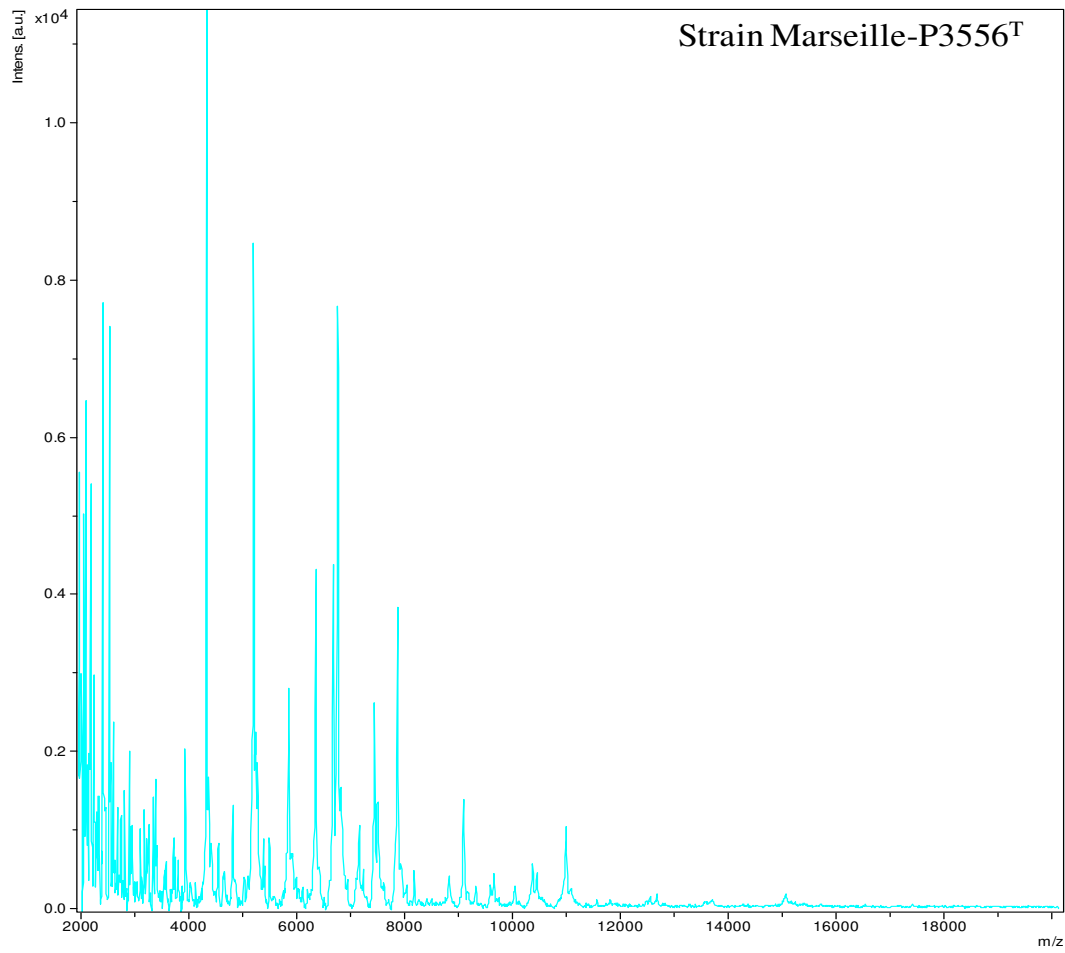
Species	Size (Mb)	G+C (mol%)	Proteins	RNAs	Genes	Pseudogenes
<i>Bacillus marasmi</i>	4.64	38.2	4,415	116	4,585	54
<i>Bacillus subterraneus</i>	4.57	43.9	4,526	93	4,691	72
<i>Bacillus circulans</i>	5.10	35.6	4,836	79	5,008	93
<i>Bacillus massiliogabonensis</i>	5.23	38.1	4,917	193	5,153	48
<i>Bacillus drementensis</i>	5.31	38.9	4,946	185	5,213	82
<i>Bacillus bataviensis</i>	5.37	39.6	5,126	36	5,277	115
<i>Bacillus mediterraneensis</i>	3.34	42.3	3,253	108	3,467	106
<i>Bacillus niacini</i>	6.18	38.2	5,793	46	5,920	81

292

293 **Table 3:** Genomic comparison of *Bacillus marasmi* strain Marseille-P3556 between its closely related species using GGDC and formula 2 (dDDH  
294 estimates based on identities over HSP length).

	BMA	BDA	BMG	BCI	BSU	BDR	BBA	BME
BMA	100%	21.90 ±4.7%	22.60 ±4.8%	28.80 ±4.9%	22.40 ±4.7%	20.60 ±4.7%	20.60 ±4.7%	21.40 ±4.6%
BDA		100%	24.30 ±4.8%	28.50 ±4.9%	21.60 ±4.7%	21.30 ±4.7%	22.40 ±4.7%	23.00 ±4.7%
BMG			100%	27.40 ±4.8%	26.20 ±4.9%	23.80 ±4.8%	23.10 ±4.7%	27.10 ±4.8%
BCI				100%	31.80 ±4.9%	30.60 ±4.9%	25.40 ±4.8%	28.60 ±4.9%
BSU					100%	19.10 ±4.5%	19.60 ±4.6%	19.70 ±4.6%
BDR						100%	21.00 ±4.7%	20.50 ±4.6%
BBA							100%	21.60 ±4.7%
BME								100%

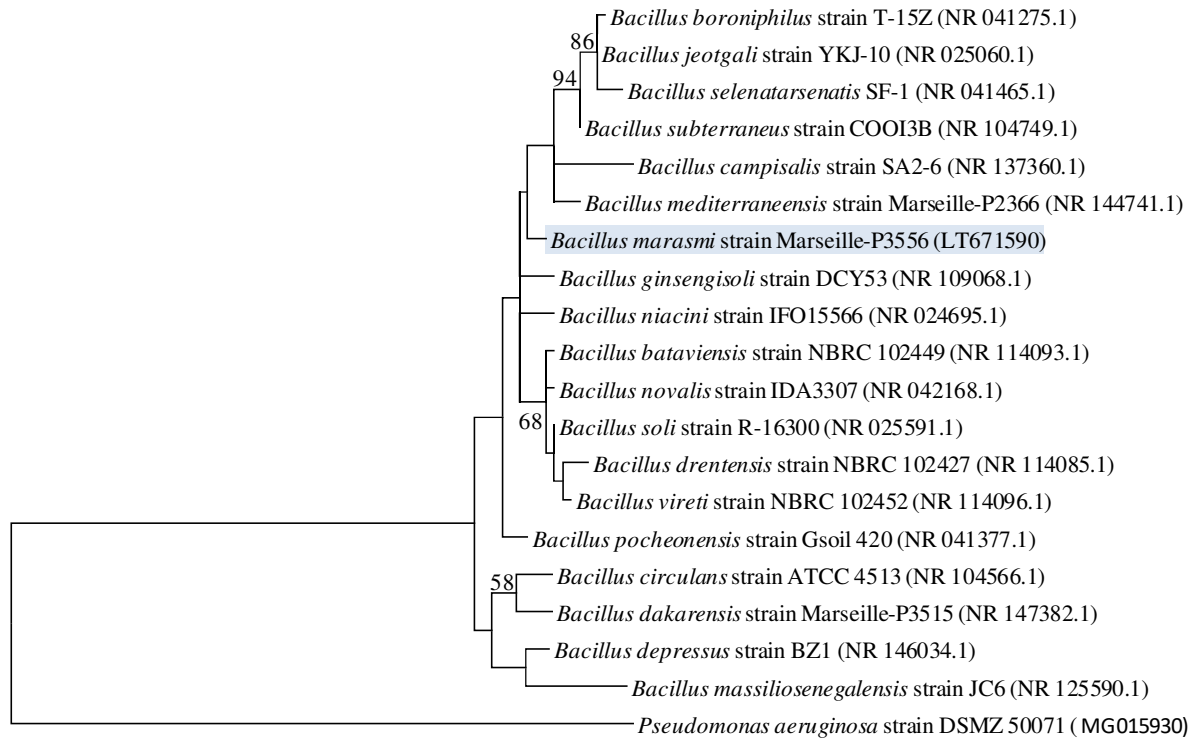
295 **BMA**, *Bacillus marasmi* Marseille-P3556 (CABHPS000000000); **BDA**, *Bacillus dakarensis* Marseille-P3515 (FTOZ000000000); **BMG**, *Bacillus*  
296 *massiliogabonensis* Marseille-P2639 (FZRJ000000000); **BCI**, *Bacillus circulans* NBRC 13626 (NZ\_CP026033.1); **BSU**, *Bacillus subterraneus* DSM 13966  
297 (RSFW000000000); **BDR**, *Bacillus drementensis* NBRC 102427 (BCUX000000000); **BBA**, *Bacillus bataviensis* LMG 21833 (AJLS000000000); **BME**, *Bacillus*  
298 *mediterraneensis* Marseille-P2366 (FOJL000000000).



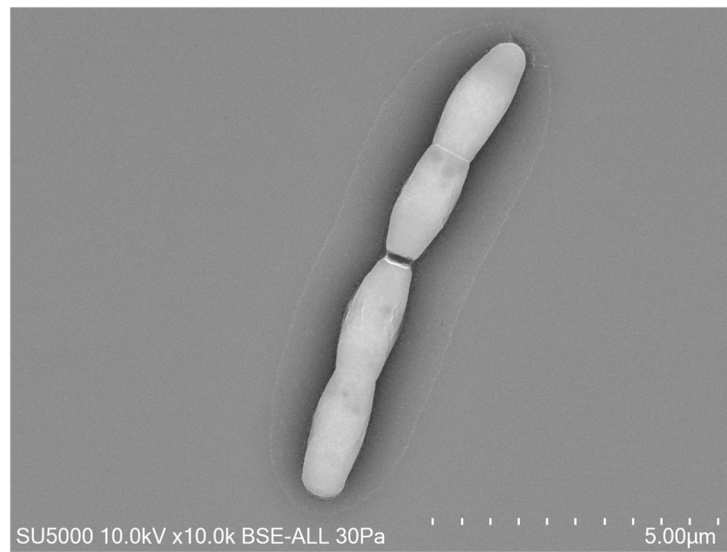
300

301 **Figure 1:** Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry  
302 (MALDI-TOF MS) reference spectrum of *Bacillus marasmi* sp. nov. The reference spectrum  
303 was generated by comparison of spectra from 12 individual colonies of strain Marseille-  
304 P3556.

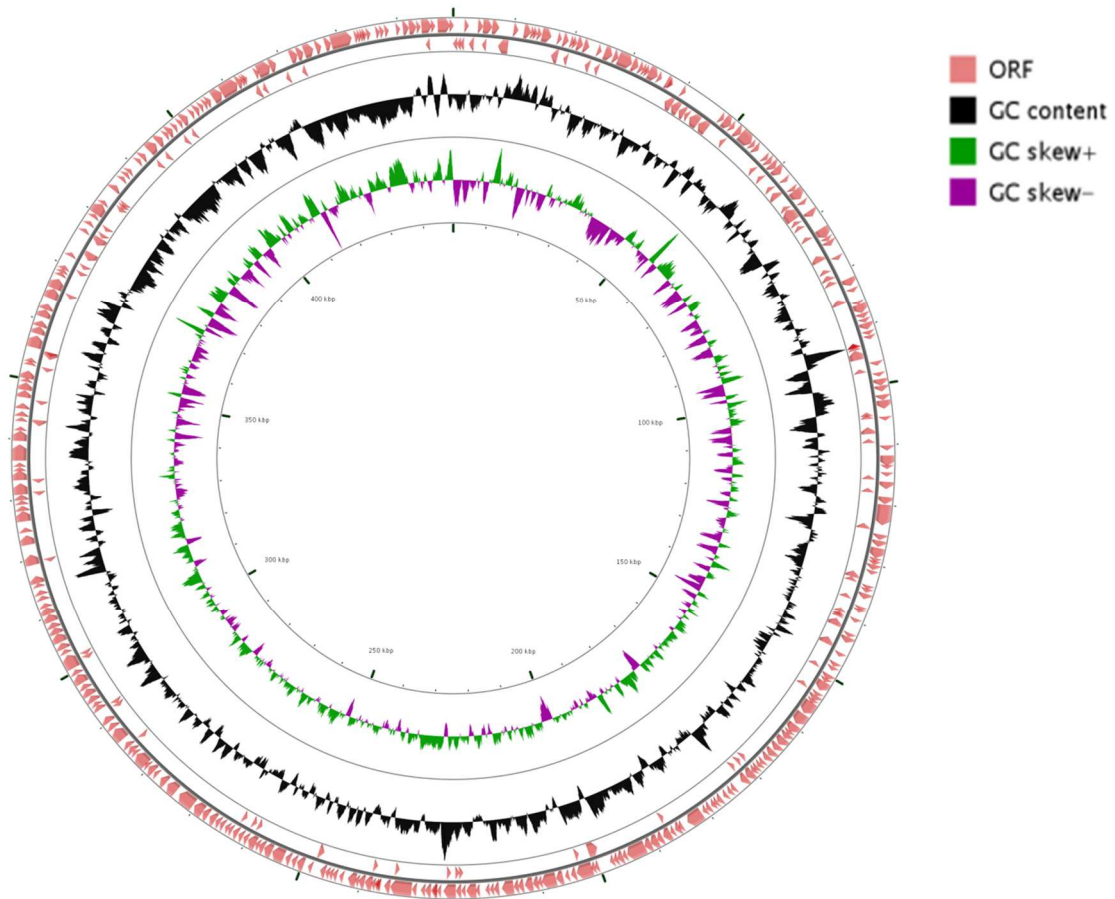




305  
 306 **Figure 2:** The 16S rRNA phylogenetic tree displaying the position of *Bacillus marasmi* strain  
 307 Marseille-P3556<sup>T</sup> relative to its closest phylogenetically species. The respective GenBank  
 308 accession numbers for 16S rRNA genes are indicated in parenthesis. Sequence alignment and  
 309 phylogenetic inferences were obtained using the maximum likelihood method within MEGA  
 310 7 software. The numbers at the nodes are percentages of bootstrap values obtained by  
 311 repeating the analysis 1000 times to generate a majority consensus tree.



313 **Figure 3:** Scanning electron micrograph of *Bacillus marasmi* strain Marseille-P3556T using  
314 the Scanning Electron Microscope SU5000 from Hitachi. Scale bar and acquisition settings  
315 are presented on the pictures.



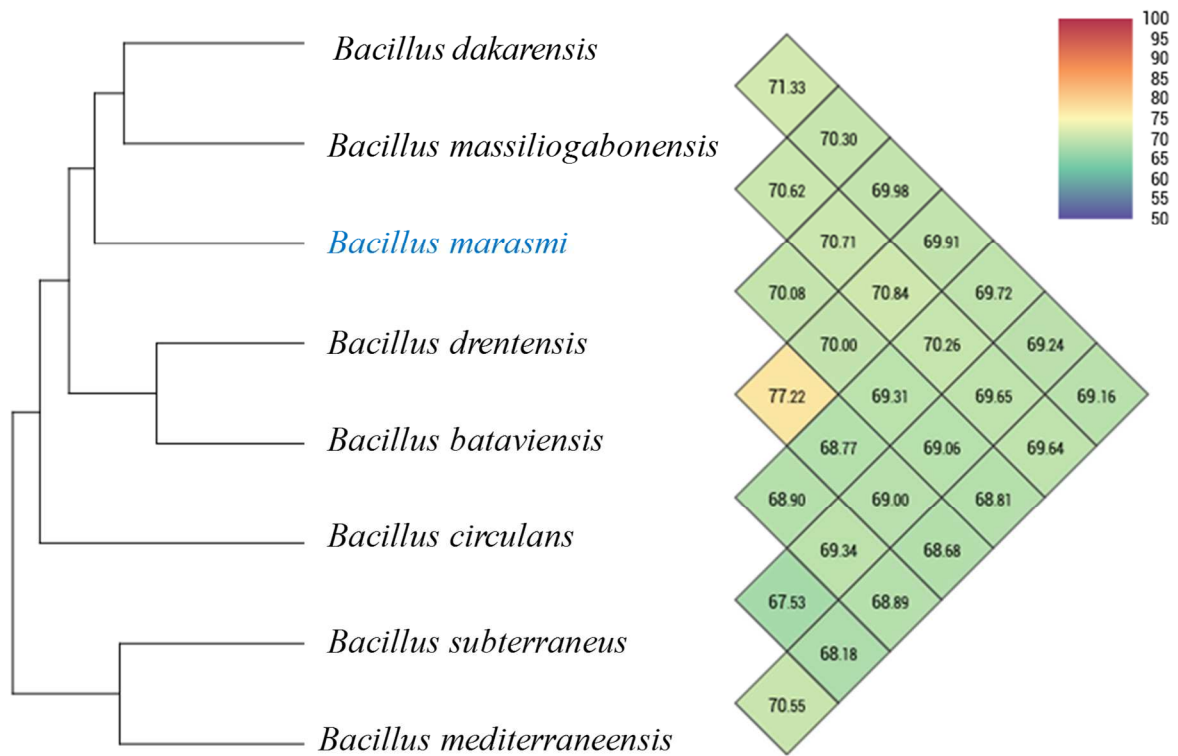
316

317 **Figure 4:** Circular map of the genome *Bacillus marasmi* strain Marseille-P3556 (4,638,282 bp).

318 CGView Server [34] was used to perform this genome cartography. From outside to the center: region

319 coding genes and RNA genes from the forward and reverse strands, respectively, GC content (black)

320 and GC skew (green/mauve).



322

323 **Figure 5:** Heatmap generated with OrthoANI values calculated using the OAT software for  
 324 *Bacillus marasmi* sp. nov., strain Marseille-P3556 with its respective closely related species  
 325 with standing in nomenclature.