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

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Abstract

Using the culturomics method, two strains were isolated, identified, and characterised following the taxonogenomics concept. *Bacillus marasmi* sp. nov. strain Marseille-P3556 (= CSURP3556) is isolated from a 13-month-old girl living in Niger. The phylogenetic tree, phenotypic criteria, and genomic analysis described here clearly show that this bacterium is different from previously known bacterial species withstanding in nomenclature and new members of *Bacillus* genus.

Keywords: *Bacillus marasmi* sp. nov., child, human stool, marasmus, taxonogenomics
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Introduction

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

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

Herein, we report the details of the isolation and taxonogenomic characterisation of strain Marseille-P3556^T, as a type strain of *Bacillus marasmi* sp. nov., for which its creation was previously announced [19].

Materials and methods

Strain isolation and identification

The human microbiome study was an opportunity for us to isolate bacterial strain from stool sample from a child from Niger with severe acute malnutrition. The child's parents provided an informed and signed consent. The study was validated by the ethics committee of the Institute Federatif de Recherche (Marseille, France) IFR48 under agreement number 09-022. The shipments of the sample, as well as its isolation were carried out as previously

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described [20]. Then, strain Marseille-P3556 was seeded in petri dishes containing 5% sheep blood agar (bioMérieux, Marcy l'Etoile, France) and incubated under anaerobic condition (Thermo Scientific, Dardilly, France) at 37°C. Identification by MALDI-TOF MS has failed with this strain despite numerous attempts because this reference spectrum is unknown in the MALDI-TOF database. The Microflex LT spectrometer (Bruker, Daltonics, Bremen, Germany) was used during the MALDI-TOF identification as previously described [21]. The obtained spectra were analysed with Biotyper 3.0 software and added in the local URMS database (https://www.mediterranee-infection.com/urms-data-base). The 16S rRNA gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and then sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France), as previously described [22]. The nucleotide sequences of the 16S rRNA gene were analysed by CodonCode Aligner software (http://www.codoncode.com). An obtained consensus sequence is compared to the NCBI nucleotide database (https://www.ncbi.nlm.nih.gov/nucleotide/).

Phenotypic and biochemical characterisation

Aerobic, microaerophilic, and anaerobic atmospheres (Thermo Scientific, Dardilly, France) were tested to assess the different growth conditions of this strain. Varied temperatures were tested (28, 37, 45, and 55 °C) to determine the optimal temperature of strain Marseille-P3556 on 5% sheep blood-enriched Columbia agar medium (bioMérieux, Marcy l'Etoile, France). API ZYM and API 50 CH strips (bioMérieux) were used to establish the biochemical characteristics of the strain in accordance with the manufacturer's recommendations. Further phenotypic tests, such as Gram staining, catalase, oxidase, and spore forming were performed as previously reported [23]. The morphological details were highlighted with a scanning electron microscope (Hitachi High-Technologies, Tokyo, Japan) as previously described [24].

The fatty acid methylester (FAME) analysis was performed by Gas Chromatography/ Mass Spectrometry (GC/MS). Samples were prepared with approximately 10 to 20 mg of bacterial biomass per tube harvested from several culture plates. FAMEs were prepared as described by Sasser [25] followed by GC/MS analyses [26]. Briefly, FAMEs were separated using an Elite 5-

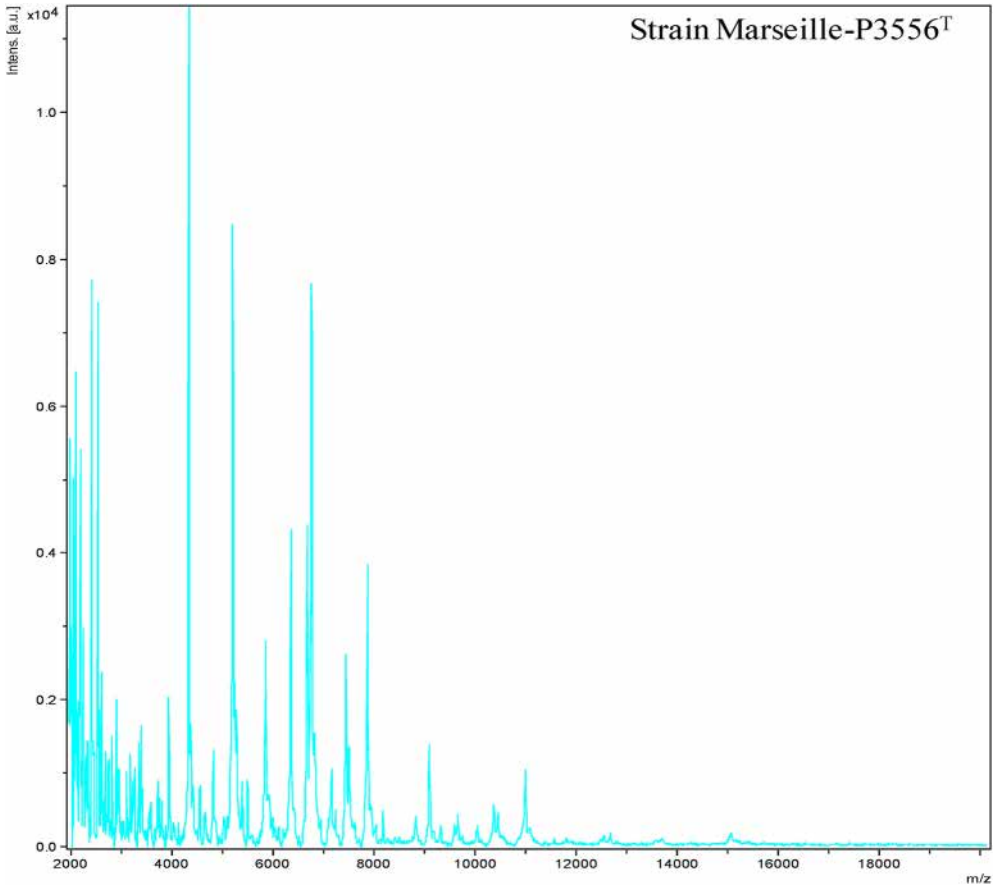

FIG. 1. Matrix-assisted laser desorption ionisation–time-of-flight mass spectrometry (MALDI-TOF MS) reference spectrum of *Bacillus marasmi* sp. nov. The reference spectrum was generated by comparison of spectra from 12 individual colonies of strain Marseille-P3556.

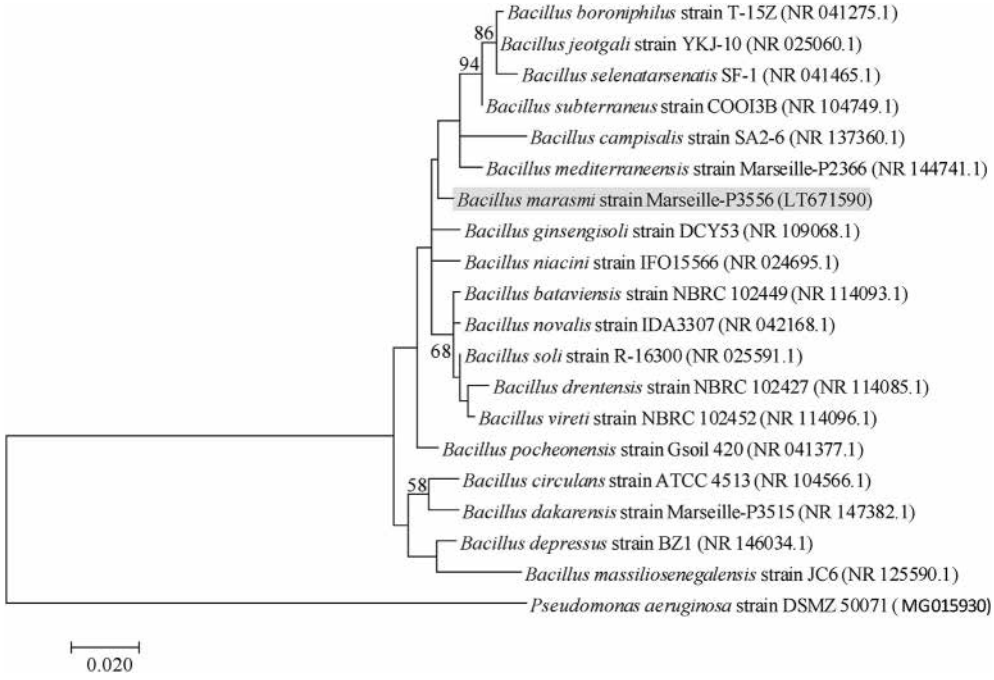

FIG. 2. The 16S rRNA phylogenetic tree displaying the position of *Bacillus marasmi* strain Marseille-P3556^T relative to its closest phylogenetically species. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequence alignment and phylogenetic inferences were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

MS column and monitored using mass spectrometry (Clarus 500 - SQ 8 S, PerkinElmer, Courtaboeuf, France). Spectral database search was performed using MS Search 2.0 operated with the Standard Reference Database1A (NIST, Gaithersburg, USA) and the FAMEs mass spectral database (Wiley, Chichester, UK).

Genome characteristics

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

Results

Strain identification and phylogenetic analysis

Colonies of strain Marseille-P3556 was not identified by mass spectrometry. In other words, the spectral reference of the strain is absent in the MALDI-TOF database. Therefore, its generated reference spectrum was incremented in our local database (Fig. 1). A similarity analysis of the 16S rDNA gene

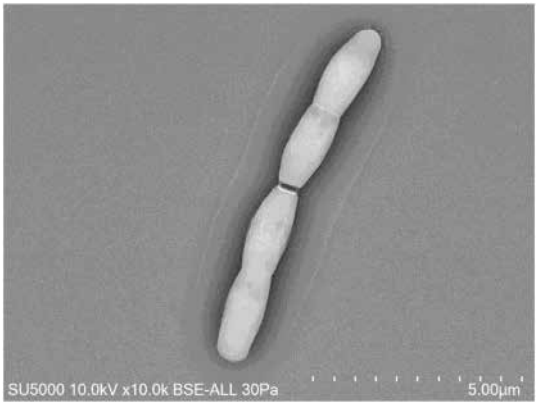

FIG. 3. Scanning electron micrograph of *Bacillus marasmi* strain Marseille-P3556T using the Scanning Electron Microscope SU5000 from Hitachi. Scale bar and acquisition settings are presented on the pictures.

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

Properties	1	2	3	4	5	6
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	+	+	+	—	+	+
Production of:						
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	+	—	—	—	+	+
Utilisation of:						
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

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

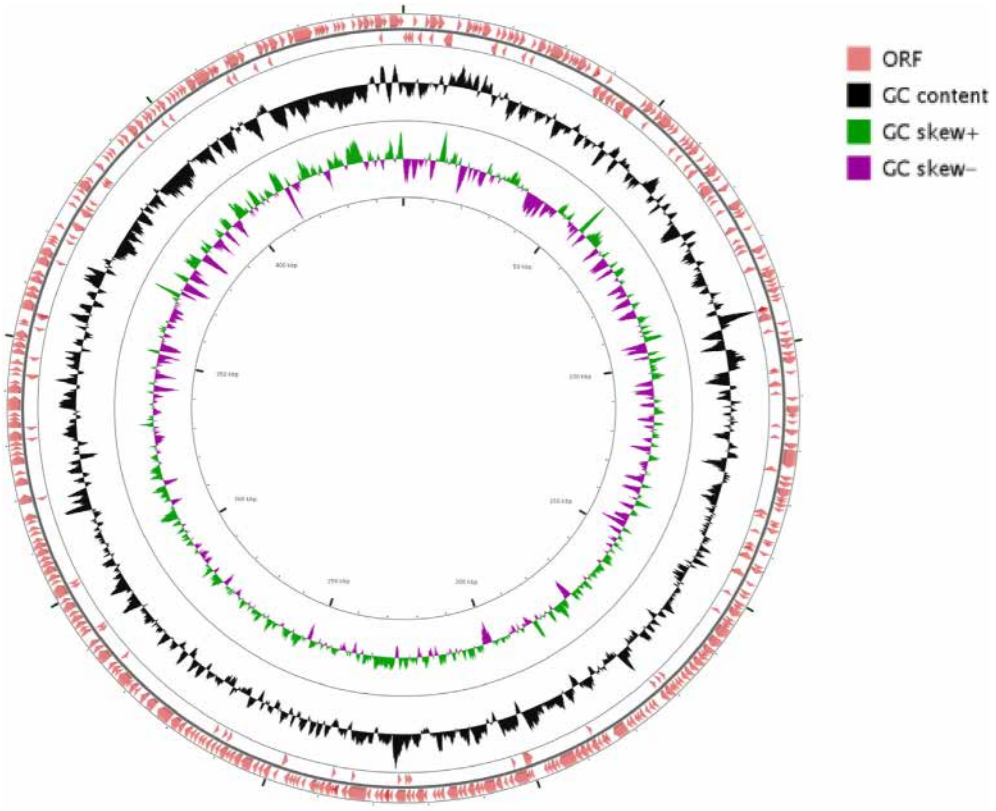

FIG. 4. Circular map of the genome *Bacillus marasmi* strain Marseille-P3556 (4,638,282 bp). CGView Server [34] was used to perform this genome cartography. From outside to the centre: region coding genes and RNA genes from the forward and reverse strands, respectively, GC content (black) and GC skew (green/mauve).

TABLE 2. **Genome comparison of closely related species to *B. marasmi* strain Marseille-P3556^T**

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

TABLE 3. **Genomic comparison of *Bacillus marasmi* strain Marseille-P3556 between its closely related species using GGDC and formula 2 (dDDH 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%

BMA, *Bacillus marasmi* Marseille-P3556 (CABHPS000000000); **BDA**, *Bacillus dakarensis* Marseille-P3515 (FTOZ00000000); **BMG**, *Bacillus massiliogabonensis* Marseille-P2639 (FZRJ00000000); **BCI**, *Bacillus circulans* NBRC 13626 (NZ_CP026033.1); **BSU**, *Bacillus subterraneus* DSM 13966 (RSFW00000000); **BDR**, *Bacillus drentensis* NBRC 102427 (BCUX00000000); **BBA**, *Bacillus bataviensis* LMG 21833 (AJLS00000000); **BME**, *Bacillus mediterraneensis* Marseille-P2366 (FOJL00000000); GGDC, Genome-to-Genome Distance Calculator; DDH, DNA-DNA hybridisation.

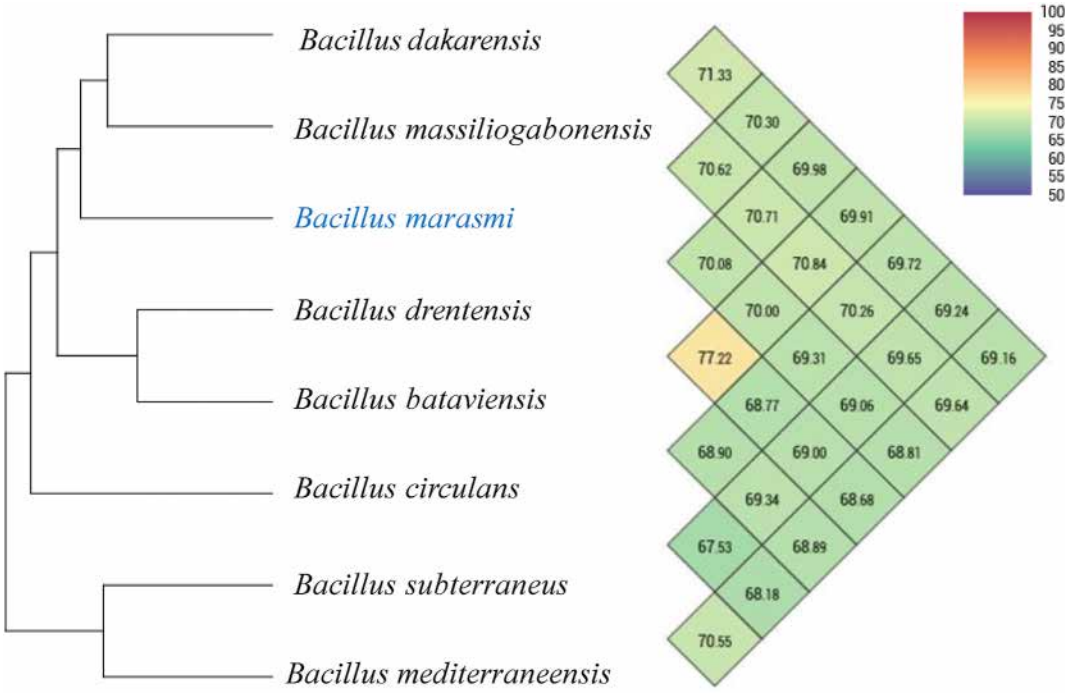

FIG. 5. Heatmap generated with OrthoANI values calculated using the OAT software for *Bacillus marasmi* sp. nov., strain Marseille-P3556 with its respective closely related species with standing in nomenclature.

of strain Marseille-P3556 revealed a nucleotide sequence identity of 97.29% with *Bacillus subterraneus* strain COOI3B (accession number NR_104749.1), the phylogenetically closest species. This similarity value was lower than the recommended cut-off value (98.65%) to delimit the species barrier in bacteria [33]. Therefore, strain Marseille-P3556 was potentially new species within the family *Bacillaceae*. The 16S rRNA-based phylogenetic tree of *Bacillus* species (Fig. 2) highlighted the position of strain Marseille-P3556 among its closely related species with a validly published name. In addition, the shape of bacterial cell was visualised using the Hitachi SU5000 instrument (Fig. 3).

Biochemical and phenotypic properties of the strain

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

Using the API ZYM strip, esterase lipase (C 8) and naphthol-AS-BI-phosphohydrolase were positive for strain Marseille-P3556, whereas alkaline phosphatase, valine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, lipase (C14), esterase (C4) ß-galactosidase, ß-glucuronidase, α-glucosidase, N-acetyl-ß-glucosaminidase, α-mannosidase, and α-fucosidase were negative. In addition, using 50 CH strip, *Bacillus marasmi* strain Marseille-P3556 was positive for glycerol, ribose, D-turanose, adonitol, galactose, glucose, mannose, D-fructose, inositol, sorbitol, methyl αD-glucopyranoside, N-acetyl-glucosamine, amygdalin, arbutin, salicin, D-maltose, D-lactose, D-melibiose, starch, sucrose, inulin, D-melezitose, D-raffinose, glycogen, xylitol, gentiobiose, D-cellobiose, D-trehalose, D-lyxose, and potassium 5-ketogluconate. But negative reactions were observed for erythritol, arabinose, xylose, D-tagalose, fucose, and potassium 2-ketogluconate. Phenotypic criteria of strain Marseille-P3556 were compared with closely related species in Table 1. The major fatty acids found for Marseille-P3556 were $C_{16:0}$ (31.4%), $C_{14:0iso}$ (20%), and $C_{16:1n7}$ (14.8%). Minor amounts of saturated fatty acids were also found with strain Marseille-P3556.

Genomic analysis

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

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

Conclusion

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

Description of *Bacillus marasmi* sp. nov

Bacillus marasmi sp. nov. (ma.ras.mi, L. adj. fem, 'marasmus,' the disease meaning a form of severe malnutrition in children). It is a Gram-positive aerobic bacterium and is rod-shaped. Cells have a diameter varying between 0.6 and 1 µm. Catalase and oxidase activities are negative. Colonies are small, circular, and pale grey with a mean diameter of 1.25 mm on blood agar. The strain Marseille-P3556 was positive for glycerol, ribose, D-turanose, adonitol, galactose, glucose, mannose, D-fructose, inositol, sorbitol, methyl αD-glucopyranoside, N-acetyl-glucosamine, amygdalin, arbutin, salicin, D-maltose, D-lactose, D-melibiose, starch, sucrose, inulin, D-melezitose, D-raffinose, glycogen, xylitol, gentiobiose, D-lyxose, and potassium 5-ketogluconate. $C_{16:0}$ (31.4%), $C_{14:0iso}$ (20%), and $C_{16:1n7}$

(14.8%) are the major fatty acids detected in cells of *Bacillus marasmi* sp. nov. The genome of strain Marseille-P3556 was 4.64 Mbp with 38.2 mol% of G + C content. The 16S rRNA and draft genome sequences are deposited in the Genbank database under Accession numbers LT671590 and CABHPS000000000, respectively. The type strain of *Bacillus marasmi* sp. nov., strain Marseille-P3556 was isolated from a Nigerien child presenting clinical aspects of marasmus.

Transparency declaration

The authors declare that there are no conflicts of interest. This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the program « Investissements d'avenir », reference ANR-10-IAHU-03, the Région Provence Alpes Côte d'Azur, and European funding FEDER PRIMI.

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References

[1] Cohn F. Untersuchungen über Bakterien. Beitrage zur Biologie der Pflanzen Heft 1872;1:127–224.
[2] LPSN *Bacillus*. In: LPSN. https://lpsn.dsmz.de/genus/bacillus. [Accessed 14 September 2020].
[3] Díez-Méndez A, Rivas R, Mateos PF, et al. *Bacillus terrae* sp. nov. isolated from Cistus ladanifer rhizosphere soil. Int J Syst Evol Microbiol 2017;67(5):1478–81. https://doi.org/10.1099/ijsem.0.001742.
[4] Abril AG, Rama JLR, Feijoo-Siota L, et al. *Bacillus safensis* subsp. *osmophilus* subsp. nov., isolated from condensed milk, and description of *Bacillus safensis* subsp. *safensis* subsp. nov. Int J Syst Evol Microbiol 2019;69(1):189–95. https://doi.org/10.1099/ijsem.0.003126.
[5] Baik KS, Lim CH, Park SC, Kim EM, Rhee MS, Seong CN. *Bacillus rigui* sp. nov., isolated from wetland fresh water. Int J Syst Evol Microbiol 2010;60(Pt 9):2204–9. https://doi.org/10.1099/ijs.0.018184-0.
[6] Zhu D, Zhang P, Niu L, et al. *Bacillus ectoiniformans* sp. nov., a halotolerant bacterium isolated from deep-sea sediments. Int J Syst Evol Microbiol 2016;66(2):616–22. https://doi.org/10.1099/ijsem.0.000763.
[7] Debois D, Fernandez O, Franzil L, Jourdan E, de Brogniez A, Willems L, et al. Plant polysaccharides initiate underground crosstalk with bacilli by inducing synthesis of the immunogenic lipopeptide surfactin. Environ Microbiol Rep 2015;7(3):570–82. https://doi.org/10.1111/1758-2229.12286.
[8] de Los Santos Villalobos S, Robles RI, Parra Cota FI, Larsen J, Lozano P, Tiedje JM. *Bacillus cabrialesii* sp. nov., an endophytic plant growth promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) in the Yaqui Valley, Mexico. Int J Syst Evol Microbiol 2019;69(12):3939–45. https://doi.org/10.1099/ijsem.0.003711.
[9] Ma L, Xi JQ, Cao YH, Wang XY, Zheng SC, Yang CG, et al. *Bacillus endozanthoxylicus* sp. nov., an endophytic bacterium isolated from *Zanthoxylum bungeanum* Maxim leaves. Int J Syst Evol Microbiol 2017;67(10):3699–705. https://doi.org/10.1099/ijsem.0.002138.
[10] Bottone EJ. *Bacillus cereus*, a volatile human pathogen. Clin Microbiol Rev 2010;23:382–98. https://doi.org/10.1128/CMR.00073-09.
[11] Jernigan JA, Stephens DS, Ashford DA, Omenaca C, Topiel MS, Galbraith M, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. Emerg Infect Dis 2001;7:933–44. https://doi.org/10.3201/eid0706.010604.
[12] Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007;449:804–10. https://www.nature.com/articles/nature06244.
[13] Lagier JC, 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. https://doi.org/10.1111/1469-0691.12023.
[14] Lagier JC, 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. https://doi.org/10.1038/nmicrobiol.2016.203.
[15] Ramasamy D, Mishra AK, Lagier JC, Padhmanabhan 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. https://doi.org/10.1099/ijs.0.057091-0.
[16] Lagier JC, Hugon P, Khelaifia S, Fournier PE, 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. https://doi.org/10.1128/CMR.00014-14.
[17] Lo CI, Padhmanabhan R, Mediannikov O, Terras J, Robert C, Faye N, et al. High-quality genome sequence and description of *Bacillus dielmoensis* strain FF4(T) sp. nov. Stand Genomic Sci 2015;10:41. https://doi.org/10.1186/s40793-015-0019-8.
[18] Sarr M, Lo CI, Tall ML, Fadlane A, Senghor B, Sokhna C, Raoult D, et al. Taxonogenomics description of *Bacillus dakarensis* sp. nov., *Bacillus sinesaloumensis* sp. nov. and *Bacillus massiliogabonensis* sp. nov., three new species isolated from human stools. New Microbe. New Infect 2020;37:100718.
[19] Pham TP, Cadoret F, Alou MT, Brah S, Diallo BA, Diallo A, Sokhna C, Delerce J, Fournier PE, Million M, Raoult D. *'Urmitella timonensis'* gen. *nov., sp. nov.*, '*Blautia marasmi*' *sp. nov.*, '*Lachnoclostridium* pacaense' *sp. nov.*, *'Bacillus marasmi'* sp. nov. and *'Anaerotruncus rubiinfantis'* sp. nov., isolated from stool samples of undernourished African children. New Microbe. New Infect 2017 Mar 3;17:84–8. https://doi.org/10.1016/j.nmni.2017.02.004.
[20] Bilen M, Mbogning Fonkou MD, Nguyen TT, Richez M, Daoud Z, Fournier PE, et al. *Miniphocibacter massiliensis* gen. nov., sp. nov., a new species isolated from the human gut and its taxono-genomics description. Microbiologyopen 2019;8(5):e00735. https://doi.org/10.1002/mbo3.735.
[21] Lo CI, Fall B, Sambe-Ba B, Diawara S, Gueye MW, Mediannikov O, Sokhna C, et al. MALDI-TOF mass spectrometry: a powerful tool for clinical microbiology at hôpital principal de Dakar, Senegal (West africa). PLoS One 2015;10(12):e0145889. https://doi.org/10.1371/journal.pone.0145889.
[22] Morel AS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta JP, 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 Microbiol Infect Dis 2015;34:561–70. https://doi.org/10.1007/s10096-014-2263-z.
[23] Wormser Gary P, Charles Stratton. Manual of clinical microbiology. 2007 2488 pp., illustrated. $209.95 (hardcover). In: Murray Patrick R,

Baron Ellen Jo, Jorgensen James H, Landry Marie Louise, Pfaller Michael A, editors. Clinical infectious diseases. 9th ed., vol. 46. Washington, DC: ASM Press; 1 January 2008. p. 153. https://doi.org/10.1086/52407618. Issue 1.

[24] Belkacemi S, Bou Khalil J, Ominami Y, Hisada A, Fontanini A, Caputo A, et al. Passive filtration, rapid scanning electron microscopy, and matrix-assisted laser desorption ionization-time of flight mass spectrometry for *Treponema* culture and identification from the oral cavity. J Clin Microbiol 2019;57(10):e00517–9. https://doi.org/10.1128/JCM.00517-19.

[25] Myron S. Bacterial Identification by gas chromatographic Analysis of fatty acids methyl esters (GC-FAME). MIDI 2006 *(Technical Note #101)*.

[26] Dione N, Sankar SA, Lagier JC, Khelaifia S, Michele C, Armstrong N, Richez M, Abrahao J, Raoult D, Fournier PE. Genome sequence and description of *Anaerosalibacter massiliensis* sp. nov. New Microbe New Infections 2016;10:66–76.

[27] Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18:821–9. https://doi.org/10.1101/gr.074492.107.

[28] 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. https://doi.org/10.1089/cmb.2012.0021.

[29] 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. https://doi.org/10.1186/2047-217X-1-18.

[30] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20. https://doi.org/10.1093/bioinformatics/btu170.

[31] Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 2013;14:60. https://doi.org/10.1186/1471-2105-14-60.

[32] Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3. https://doi.org/10.1099/ijsem.0.000760.

[33] Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes [published correction appears in Int J Syst Evol Microbiol. Int J Syst Evol Microbiol 2014;64(Pt 2):346–51. https://doi.org/10.1099/ijs.0.059774-0.

[34] Stothard P, Grant JR, Van Domselaar G. Visualizing and comparing circular genomes using the CGView family of tools. Brief Bioinform 2017 Jul 26. https://doi.org/10.1093/bib/bbx081.

[35] Kanso S, Greene AC, Patel BKC. *Bacillus subterraneus* sp. nov., an iron- and manganese-reducing bacterium from a deep subsurface Australian thermal aquifer. Int J Syst Evol Microbiol 2002 May;52(Pt 3):869–74. https://doi.org/10.1099/00207713-52-3-869.

[36] Heyrman J, Vanparys B, Logan NA, Balcaen A, Rodríguez-Díaz M, Felske A, De Vos P. *Bacillus novalis* sp. nov., *Bacillus vireti* sp. nov., *Bacillus soli* sp. nov., *Bacillus bataviensis* sp. nov. and *Bacillus drentensis* sp. nov., from the Drentse A grasslands. Int J Syst Evol Microbiol 2004 Jan;54(Pt 1):47–57. https://doi.org/10.1099/ijs.0.02723-0.