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Non-contiguous finished genome sequence and description of *Paenibacillus gorillae* sp. nov.

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Strain G¹T sp. nov. is the type strain of *Paenibacillus gorillae* a newly proposed species within the genus *Paenibacillus*. This strain, whose genome is described here, was isolated in France from the fecal sample of a wild western lowland gorilla from Cameroon. *P. gorillae* is a facultative anaerobic, Gram-negative, rod-shaped bacterium. Here we describe the features of this organism, together with the complete genome sequence and annotation. The 6,257,967 bp long genome (one chromosome but no plasmid) contains 5,856 protein-coding and 62 RNAs genes, including 60 tRNA genes.

**Introduction**

Strain G¹T (= CSUR P205 = DSM 26181) is the type strain of *Paenibacillus gorillae* sp. nov. This bacterium is a Gram-negative, flagellated, facultative anaerobic, indole-negative bacillus that has rounded-ends. It was isolated from the stool sample of *Gorilla gorilla* subsp. *gorilla* as part of a culturomics study aiming at cultivating bacterial species found within gorilla feces. By applying large-scale culture conditions, culturomics has previously facilitated the isolation of many new bacterial species from human stool samples [1-3]. The genus *Paenibacillus* was created by Ash *et al*. about 20 years ago [4,5]. To date, this genus comprises 145 validly published species [6] of Gram-positive, Gram-negative or variable, mostly motile and spore-forming bacteria. Members of the genus *Paenibacillus* are ubiquitous bacteria isolated from various environments including soil, water, rhizosphere, food, insect larvae and normal human flora [7]. Moreover, *Paenibacillus* species were also isolated from or involved in human infections including wound infections, bacteremia and endocarditis [8-13]. Currently, a polyphasic approach that combines proteomics by MALDI-TOF spectral analysis, genomic data and phenotypic characterization is being used as a new approach to describe bacterial species [7,14-25].

Here we present a summary classification and a set of features for *P. gorillae* sp. nov. strain G¹T together with the description of the complete genome sequence and annotation. These characteristics support the circumscription of the species *P. gorillae* [26].

**Classification and features**

In July 2011, a fecal sample was collected from a wild western lowland gorilla near Minton, a village in the south-central part of the DJA FAUNAL Park (Cameroon). The collection of the stool sample was approved by the Ministry of Scientific Research and Innovation of Cameroon. No experimentation was conducted on this gorilla. The fecal specimen was preserved at -80°C after collection and sent to Marseille. Strain G¹T (Table 1) was isolated in January 2012 by cultivation on Columbia agar with sheep blood 5% (BioMérieux, France). This strain exhibited a 98.28% 16S rRNA nucleotide sequence similarity with *Paenibacillus xinjiangensis*, the phylogenetically closest validly published *Paenibacillus* species (Figure 1). This value was lower than the 98.7% 16S rRNA gene sequence threshold recommended by Stackebrandt and Ebers to delineate a new species without carrying out DNA-DNA hybridization [42].
Table 1. Classification and general features of *Paenibacillus gorillae* strain G1\(^{T}\) according to the MIGS recommendations [27].

| MIGS ID   | Property                  | Term                        | Evidence code\(^{a}\) |
|-----------|---------------------------|-----------------------------|-----------------------|
|           | Domain *Bacteria*         | TAS [28]                    |                       |
|           | Phylum *Firmicutes*       | TAS [29-31]                 |                       |
|           | Class *Bacilli*           | TAS [32,33]                 |                       |
| Current classification | Order *Bacillales*        | TAS [34,35]                 |                       |
|           | Family *Paenibacillaceae* | TAS [33,36]                 |                       |
|           | Genus *Paenibacillus*     | TAS [4,5,37-39]             |                       |
|           | Species *Paenibacillus gorillae* | IDA                        |                       |
|           | Type strain G1\(^{T}\)    | IDA                         |                       |
|           | Gram stain                | negative                    | IDA                   |
|           | Cell shape                | rod-shaped                   | IDA                   |
|           | Motility                  | motile                      | IDA                   |
|           | Sporulation               | Sporulating                 | IDA                   |
|           | Temperature range         | mesophilic                  | IDA                   |
|           | Optimum temperature       | 25°C                        | IDA                   |
| MIGS-6.3  | Salinity                  | growth in BHI medium + 2% NaCl | IDA   |
| MIGS-22   | Oxygen requirement        | aerobic                     | IDA                   |
|           | Carbon source             | varied (see Table 2)        | IDA                   |
|           | Energy source             | chemoorganoheterotrophic    | IDA                   |
| MIGS-6    | Habitat                   | gorilla gut                 | IDA                   |
| MIGS-15   | Biotic relationship       | free living                 | IDA                   |
| MIGS-14   | Pathogenicity             | unknown                     | NAS                   |
|           | Biosafety level           | 2                           | NAS                   |
|           | Isolation                 | gorilla feces               | IDA                   |
| MIGS-4    | Geographic location       | Cameroon                    | IDA                   |
| MIGS-5    | Sample collection time    | July 2011                   | IDA                   |
| MIGS-4.1  | Latitude                  | 2.783938                    | IDA                   |
| MIGS-4.1  | Longitude                 | 13.030472                   | IDA                   |
| MIGS-4.3  | Depth                     | surface                     | IDA                   |
| MIGS-4.4  | Altitude                  | > 600 m above sea level     | IDA                   |

\(^{a}\)Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [40]. If the evidence is IDA, then the property was directly observed for a live isolate by one of the authors or an expert mentioned in the acknowledgements.
Figure 1. Phylogenetic tree highlighting the position of Paenibacillus gorillae strain G1T relative to other type strains within the Paenibacillus genus. GenBank accession numbers are indicated in parentheses. Sequences were aligned using CLUSTAL X (V2), and phylogenetic inferences obtained using the maximum-likelihood method within the MEGA 5 software [41]. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1,000 times to generate a majority consensus tree. Brevibacillus brevis was used as outgroup. The scale bar represents a 2% nucleotide sequence divergence.

Different growth temperatures (25, 30, 37, 45°C) were tested. Growth occurred for the temperatures (25°C-37°C), but the optimal growth was observed at 25°C. Colonies were 2-8 mm in diameter on Columbia agar, appear whitish in color at 25°C and produce a clear liquid. Growth of the strain was tested under anaerobic and microaerophilic conditions using the GENbag anaer and GENbag microaer systems, respectively (BioMérieux), and in aerobic conditions, with or without 5% CO₂. Growth was achieved under aerobic (with and without CO₂), microaerophilic and anaerobic conditions. Gram staining showed Gram-negative bacilli (Figure 2). A motility test was positive. Cells grown on agar sporulate and the rods have a length ranging from 2.5 to 3.97 µm (mean 3.2 µm) and a diameter ranging from 0.76 to 0.83 µm (mean 0.79 µm) as determined by negative staining transmission electron microscopy (Figure 3).
Figure 2. Gram stain of *P. gorillae* strain G1T

Figure 3. Transmission electron micrograph of *P. gorillae* strain G1T, taken using a Morgani 268D (Philips) at an operating voltage of 60kV. The scale bar represents 2 μm.
Strain G1\textsuperscript{T} exhibited oxidase activity but not catalase activity. Using the API 50CH system (BioMerieux), a positive reaction was observed for D-mannose, amygdalin, L-arabinose, cellobiose, lactose, D-xylene, D-glucose, mannitol, arabinose, xylose, glycerol, D-galactose, N-acetylglucosamine, arbutin, aesculin, D-sorbitol, D-maltose, D-saccharose, D-trehalose, D-tagatose, L-rhamnose, salicin, adonitol, D-melibiose, D-raffinose, D-ribose, D-fructose and hydrolysis of starch. Negative reactions were observed for potassium gluconate, potassium 2-cetogluconate, inulin, D-melezitose, Glycogen, β-gentiobiose, D-turanose, methyl-αD-mannopyranoside and methyl-αD-glucopyranoside. Using the API ZYM system, negative reactions were observed for lipase (C14), a-chymotrypsin, esterase (C4), esterase lipase (C8), naphthyl-AS-BI-phosphohydrolase, phenylalanine arylamidase, leucine arylamidase, cystine arylamidase, valine arylamidase, glycine arylamidase, arginine arylamidase and β-glucosidase. Using the API Coryne system, positive reactions were observed for β-glucuronidase, alkaline phosphatase, α-glucosidase, α-galactosidase and N-acetyl-β-glucosaminidase activities. The urease reaction, nitrate reduction and indole production were negative. *P. gorillae* is susceptible to imipenem, rifampicin, gentamycin, nitrofurantoin and vancomycin, but resistant to metronidazole, trimethoprim/sulfamethoxazole, ceftriaxone, ciprofloxacin and amoxicillin.

When compared to other *Paenibacillus* species [43-46] and *Brevibacillus brevis* [47], *P. gorillae* sp. nov. strain G1\textsuperscript{T} exhibited the phenotypic differences detailed in Table 2.

Matrix-assisted laser-desorption/ionization time-of-flight (MALDI-TOF) MS protein analysis was carried out as previously described [14] using a Microflex spectrometer (Bruker Daltonics, Leipzig, Germany). Twelve distinct deposits were made for strain G1\textsuperscript{T} from 12 isolated colonies. The 12 G1\textsuperscript{T} spectra were imported into the MALDI BioTyper software (version 2.0, Bruker) and analyzed by standard pattern matching (with default parameter settings) against 6,252 bacterial spectra including 123 spectra from 67 *Paenibacillus* species, used as reference data, in the BioTyper database. Interpretation of scores was as follows: a score ≥ 2 to a validly published species enabled the identification at the species level, a score ≥ 1.7 but < 2 enabled the identification at the genus level; and a score < 1.7 did not enable any identification. For strain G1\textsuperscript{T}, the obtained scores ranged from 1.177 to 1.343, thus suggesting that our isolate was not a member of a known species. We incremented our database with the spectrum from strain G1\textsuperscript{T} (Figure 4). Spectrum differences with other of *Paenibacillus* species are shown in Figure 5.

![Figure 4. Reference mass spectrum from *P. gorillae* strain G1\textsuperscript{T}. Spectra from 12 individual colonies were compared and a reference spectrum was generated.](http://standardsingenomics.org)
Table 2. Differential phenotypic characteristics between *P. gorillae* sp. nov. strain G1T and phylogenetically close *Paenibacillus* species

| Characteristic                  | 1       | 2       | 3       | 4       | 5       | 6       |
|--------------------------------|---------|---------|---------|---------|---------|---------|
| **Gram stain**                 | -       | -       | var     | var     | +       | +/var   |
| **Production of**              |         |         |         |         |         |         |
| Catalase                       | -       | +       | +       | +       | +       | +       |
| Oxidase                        | +       | -       | -       | +       | -       | +       |
| Nitrate reductase              | -       | +       | +       | -       | +       | +       |
| Urease                         | -       | +       | +       | na      | na      | -       |
| Indole                         | -       | -       | +       | +       | na      | -       |
| **Utilization of**             |         |         |         |         |         |         |
| D-mannose                      | +       | +       | +       | -       | +       | -       |
| Amygdalin                      | +       | -       | -       | -       | +       | -       |
| L-Arabinose                    | +       | w       | -       | -       | +       | -       |
| Cellobiose                     | +       | +       | +       | -       | +       | +       |
| D-lactose                      | +       | +       | +       | na      | -       | +       |
| D-xylene                      | +       | +       | var     | -       | -       | -       |
| D-glucose                      | +       | +       | +       | +       | +       | -       |
| D-Mannitol                     | +       | -       | +       | -       | +       | -       |
| D-Arabinose                    | +       | -       | na      | na      | -       | -       |
| Glycerol                       | +       | -       | var     | -       | +       | -       |
| D-Galactose                    | +       | -       | +       | na      | +       | +       |
| Starch                         | +       | +       | +       | +       | -       | -       |
| N-acetylglucosamine            | +       | +       | +       | na      | -       | +       |
| Arbutin                        | +       | -       | na      | +       | +       | na      |
| Aesculin                       | +       | +       | +       | na      | +       | +       |
| D-sorbitol                     | +       | -       | na      | na      | na      | -       |
| D-maltose                      | +       | +       | +       | na      | +       | +       |
| D-saccharose                   | +       | +       | na      | -       | +       | -       |
| D-trehalose                    | +       | +       | +       | -       | +       | -       |
| D-tagatose                     | +       | -       | na      | na      | na      | -       |
| Potassium gluconate            | -       | -       | +       | -       | -       | w       |
| L-rhamnose                     | +       | -       | na      | na      | na      | -       |
| Salicin                        | +       | +       | -       | -       | +       | -       |
| Adonitol                       | +       | -       | na      | +       | -       | -       |
| D-melibiose                    | +       | +       | na      | +       | -       | -       |
| D-raffinose                    | +       | +       | na      | +       | -       | -       |
| D-ribose                       | +       | -       | +       | na      | na      | -       |
| D-fructose                     | +       | +       | w       | na      | -       | -       |
| **Habitat**                    | Gorilla gut | Gorilla gut | Roots of *Perilla frutescens* | Honeybee larvae | Human blood culture | Soil |

*Strains shown: 1, *Paenibacillus gorillae* G1T; 2, “*Gorillibacterium massiliense*” G5T; 3, *Paenibacillus elgii* SD1T; 4, *Paenibacillus alvei* BCRC 11220T; 5, *Paenibacillus massiliensis* 2301065T; 6, *Brevibacillus brevis* NBRC 15304T. Symbols: var: variable, +: positive result, -: negative result, na: data not available, w: weak positive result.
Figure 5. Gel view comparing *Paenibacillus gorillae* G1\(^T\) spectra with other members of the *Paenibacillus* genus (*P. massiliensis*, *P. kobensis*, and *P. alvei*) and with *Brevibacillus brevis* and “*Gorillibacterium massiliense*” G5\(^T\). The Gel View displays the raw spectra of all loaded spectrum files arranged in a pseudo-gel like look. The x-axis records the m/z value. The left y-axis displays the running spectrum number originating from subsequent spectra loading. The peak intensity is expressed by a Gray scale scheme code. The color bar and the right y-axis indicate the relation between the color a peak is displayed with and the peak intensity in arbitrary units.

**Genome sequencing information**

**Genome project history**
The organism was selected for sequencing on the basis of its phylogenetic position and 16S rRNA similarity to other members of the genus *Paenibacillus*, and is part of a “culturomics” study of the gorilla flora which aims to isolate all bacterial species within gorilla feces. It is the 44th genome of a *Paenibacillus* species sequenced and the first genome of *Paenibacillus gorillae* sp. nov. sequenced. A summary of the project information is shown in Table 3. The Genbank accession number is CBVJ000000000 and consists of 167 contigs (150 large contigs). Table 3 shows the project information and its association with MIGS version 2.0 compliance [48].

| MIGS ID | Property                      | Term                                      |
|---------|-------------------------------|-------------------------------------------|
| MIGS-31 | Finishing quality             | High-quality draft                        |
| MIGS-28 | Libraries used                | 454 paired-end 3-kb libraries             |
| MIGS-29 | Sequencing platform           | 454 GS FLX Titanium                       |
| MIGS-31.2| Sequencing coverage          | 17.2×                                     |
| MIGS-30 | Assemblers                    | Newbler version 2.5.3                     |
| MIGS-32 | Gene calling method           | Prodigal                                  |
| EMBL Date of Release   |                               | November 26, 2013                         |
| EMBL ID             |                               | CBVJ000000000                             |
| MIGS-13 | Project relevance             | Study of the gorilla gut microbiome       |
Growth conditions and DNA isolation

*P. gorillae* sp. nov. strain G1T, CSUR P205, DSM 26181, was grown aerobically on 5% sheep blood-enriched Columbia agar at 25°C. Four Petri dishes were spread and resuspended in 3×500µl of TE buffer and stored at 80°C. Then, 500µl of this suspension were thawed, centrifuged 3 minutes at 10,000 rpm and resuspended in 3×100µL of G2 buffer (EZ1 DNA Tissue kit, Qiagen). A first mechanical lysis was performed by glass powder on the Fastprep-24 device (Sample Preparation system, MP Biomedicals, USA) using 2×20 seconds cycles. DNA was then treated with 2.5µg/µL lysozyme (30 minutes at 37°C) and extracted using the BioRobot EZ1 Advanced XL (Qiagen). The DNA was then concentrated and purified using the Qiamp kit (Qiagen). The yield and the concentration was measured by the Quant-it Picogreen kit (Invitrogen) on the Genios Tecan fluorometer at 50ng/µl.

Genome sequencing and assembly

A shotgun and a 3 kb paired end library were pyrosequenced on the 454 Roche Titanium. This project was loaded on a 1/4 region for each application on PTP Picotiterplates. The shotgun library was constructed with 50ng of DNA as described by the manufacturer Roche with the Rapid library Preparation kit for XL+. The concentration of the shotgun library was measured with a TBS fluorometer and determined to be 2.89E+09 molecules/µL. The paired-end library was prepared with 5 µg of bacterial DNA using the DNA fragmentation on a Covaris S-Series (S2) instrument (Woburn, Massachusetts, USA) with an enrichment size at 3.2kb. The DNA fragmentation was visualized with an Agilent 2100 BioAnalyzer on a DNA labchip 7500. The library was constructed according to the 454 GS FLX Titanium paired-end protocol (Roche). Circularization and nebulization were performed and generated a pattern with an optimum at 591bp. After PCR amplification through 17 cycles followed by double size selection, the single stranded paired-end library was quantified using the Quant-it Picogreen kit (Invitrogen) on a Genios Tecan fluorometer at 691 pg/µL. The library concentration equivalence was calculated as 1.07E+10 molecules/µL. The library was stored at -20°C until further use.

The shotgun XL+ library was clonally amplified with 6 cpb in 2 emPCR reactions. The paired-end library was clonally amplified with 0.5 cpb in 3 emPCR reactions with the GS Titanium SV emPCR Kit (Lib-L) v2 (Roche). The yields of the emPCR were 16.9% and 8.61% respectively, and within the expected yield range of 5 to 20%, as recommended by the Roche procedure.

A total of 790,000 beads for each ¼ region per application were loaded on the GS Titanium PicoTiterPlate PTP Kit 70×75 and sequenced with the GS FLX Titanium Sequencing Kit XL/R70 (Roche). The run was performed overnight and then analyzed on the cluster through the gsRunBrowser and Newbler assembler (Roche). A total of 339,189 passed filter wells were obtained and generated 108.46 Mb of sequences with a length average of 330 bp. The passed filter sequences were assembled using Newbler with 90% identity and 40-bp as overlap. The final assembly identified 11 scaffolds with 150 large contigs (>1.5kb) generating a genome size of 6.22 Mb corresponding to a genome coverage of 17.2×.

Genome annotation

Open Reading Frames (ORFs) were predicted using Prodigal [49] with default parameters but the predicted ORFs were excluded if they spanned a sequencing gap region. The predicted bacterial protein sequences were searched against the GenBank database [50] and the Clusters of Orthologous Groups (COG) databases using BLASTP. The tRNAscanSE tool [51] was used to find tRNA genes, whereas ribosomal RNAs were found by using RNAmmer [52] and BLASTn against the GenBank database. ORFans were identified if their BLASTP E-value was lower than 1e-03 for alignment length greater than 80 amino acids. If alignment lengths were smaller than 80 amino acids, we used an E-value of 1e-05.

To estimate the mean level of nucleotide sequence similarity at the genome level between *P. gorillae* sp nov. strain G1T and other *Paenibacillaceae* species, we use the Average Genomic Identity of orthologous gene Sequences (AGIOS) program. Briefly, this software combines the Proteinortho software [53] to detect orthologous proteins between genomes compared on a pair-wise basis, then retrieves the corresponding genes and determines the mean percentage of nucleotide sequence identity among orthologous ORFs using the Needleman-Wunsch global alignment algorithm.

Genome properties

The genome 6,257,967 bp long (1 chromosome, but no plasmid) with a 48,80% G+C content (Fig-
It is composed of 167 contigs (150 large contigs, 11 scaffolds). Of the 5,918 predicted genes, 5,856 were protein-coding genes and 62 were RNAs (1 gene is 16S rRNA, 1 gene is 23S rRNA and 60 are tRNA genes). A total of 4,296 genes (73.36%) were assigned a putative function (by COGS or by NR blast) and 304 genes were identified as ORFans (5.19%). The remaining genes were annotated as hypothetical proteins (917 genes, 15.66%). The distribution of genes into COGs functional categories is presented in Table 5. The properties and statistics of the genome are summarized in Tables 4 and 5.

Figure 6. Graphical circular map of the chromosome. From outside to the center: Genes on the forward strand colored by COG categories (only genes assigned to COG), genes on the reverse strand colored by COG categories (only gene assigned to COG), RNA genes (tRNAs green, rRNAs red), G+C content and GC skew. Purple and olive indicating negative and positive values, respectively.
Paenibacillus gorillae G1T

Table 4. Nucleotide content and gene count levels of the genome

| Attribute                        | Value     | % of totala |
|----------------------------------|-----------|-------------|
| Genome size (bp)                 | 6,257,967 | 100         |
| DNA G+C content (bp)             | 3,053,870 | 48.80       |
| DNA coding region (bp)           | 5,416,322 | 86.55       |
| Total genes                      | 5,918     | 100         |
| RNA genes                        | 62        | 1.05        |
| Protein-coding genes             | 5,856     | 98.95       |
| Genes with function prediction   | 4,296     | 73.36       |
| Genes assigned to COGs           | 4,305     | 73.51       |
| Genes with peptide signals       | 809       | 13.81       |
| Genes with transmembrane helices | 1,440     | 24.59       |

*The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

Table 5. Number of genes associated with the 25 general COG functional categories

| Code | Value | %age | Description                                                                 |
|------|-------|------|-----------------------------------------------------------------------------|
| J    | 195   | 3.33 | Translation                                                                 |
| A    | 0     | 0    | RNA processing and modification                                              |
| K    | 570   | 9.73 | Transcription                                                               |
| L    | 186   | 3.18 | Replication, recombination and repair                                        |
| B    | 1     | 0.02 | Chromatin structure and dynamics                                             |
| D    | 31    | 0.53 | Cell cycle control, mitosis and meiosis                                      |
| Y    | 0     | 0    | Nuclear structure                                                           |
| V    | 101   | 1.72 | Defense mechanisms                                                          |
| T    | 354   | 6.05 | Signal transduction mechanisms                                              |
| M    | 247   | 4.22 | Cell wall/membrane biogenesis                                               |
| N    | 76    | 1.30 | Cell motility                                                               |
| Z    | 2     | 0.03 | Cytoskeleton                                                                |
| W    | 1     | 0.02 | Extracellular structures                                                    |
| U    | 59    | 1.01 | Intracellular trafficking and secretion                                     |
| O    | 127   | 2.17 | Posttranslational modification, protein turnover, chaperones                |
| C    | 189   | 3.23 | Energy production and conversion                                            |
| G    | 591   | 10.09| Carbohydrate transport and metabolism                                        |
| E    | 414   | 7.07 | Amino acid transport and metabolism                                          |
| F    | 96    | 1.64 | Nucleotide transport and metabolism                                         |
| H    | 125   | 2.13 | Coenzyme transport and metabolism                                           |
| I    | 144   | 2.46 | Lipid transport and metabolism                                              |
| P    | 365   | 6.23 | Inorganic ion transport and metabolism                                       |
| Q    | 147   | 2.51 | Secondary metabolites biosynthesis, transport and catabolism                |
| R    | 735   | 12.55| General function prediction only                                            |
| S    | 339   | 5.79 | Function unknown                                                            |
| -    | 1,551 | 26.49| Not in COGs                                                                 |

*The total is based on the total number of protein coding genes in the annotated genome.
Genomic comparison of *P. gorillae* and other members of the family **Paenibacillaceae**

Here, we compared the genome of *P. gorillae* strain G1\(^\text{T}\) with those of “*G. massiliense*” strain G5\(^\text{T}\), *P. elgii* strain B69, *P. alvei* strain DSM 29, *P. massiliensis* strain DSM 16942 and *B. brevis* strain NBRC 100599 (Table 6). The draft genome of *P. gorillae* is larger in size than that of “*G. massiliense*” (6.25 vs 5.54 Mb) and smaller in size than those of *P. elgii*, *P. alvei*, *P. massiliensis* and *B. brevis* (6.25 vs 7.96, 6.83, 6.39 and 6.3 Mb respectively). *P. gorillae* has a lower G+C content than those of “*G. massiliense*” and *P. elgii* (48.8% vs 50.39% and 52.6% respectively) but higher than those of *P. alvei* and *B. brevis* (48.8% vs 45.9% and 47.3% respectively) and slightly higher than *P. massiliensis* (48.8% vs 48.5%). The protein content of *P. gorillae* is lower than that of *P. elgii*, *P. alvei* and *B. brevis* (5,856 vs 7,597, 6,823 and 5,946 respectively) but higher than that of “*G. massiliense*” and *P. massiliensis* (5,856 vs 5,146 and 5,496 respectively) (Table 6). In addition, *P. gorillae* shares 1,987, 2,380, 2,055, 2,121 and 1,935 orthologous genes with “*G. massiliense*”, *P. elgii*, *P. alvei*, *P. massiliensis* and *B. brevis*, respectively (Table 7). The nucleotide sequence identity of orthologous genes ranges from 66.3 to 68.7% among previously published genomes, and from 65.7 to 68.6% between *P. gorillae* and the other studied genomes (Table 7), thus confirming its status as a new species. Table 7 summarizes the number of orthologous genes and the average percentage of nucleotide sequence identity between the different genomes studied.

### Table 6. Genomic comparison of *P. gorillae* sp. nov., strain G1\(^\text{T}\) with four other members of the family *Paenibacillaceae*

| Species                      | Strain  | NCBI accession number | Genome size (Mb) | G+C content |
|------------------------------|---------|-----------------------|------------------|-------------|
| *Paenibacillus gorillae*     | G1\(^\text{T}\) | CBVJ0000000000       | 6.25             | 48.8        |
| “*Gorillibacterium massiliense*” | G5\(^\text{T}\) | CBQR0000000000       | 5.54             | 50.39       |
| *Paenibacillus elgii*        | B69     | AFHW0000000000       | 7.96             | 52.6        |
| *Paenibacillus alvei*        | DSM 29  | AMBZ0000000000       | 6.83             | 45.9        |
| *Paenibacillus massiliensis* | DSM 16942 | ARIL0000000000   | 6.39             | 48.5        |
| *Brevibacillus brevis*       | NBRC 100599 | AP008955             | 6.3              | 47.3        |

Species and strain names, GenBank genome accession numbers, sizes and G+C contents

### Table 7. Genomic comparison of *P. gorillae* sp. nov., strain G1\(^\text{T}\) with four other members of the family *Paenibacillaceae*

|                      | *P. gorillae* | “*G. massiliense*” | *P. elgii* | *P. alvei* | *P. massiliensis* | *B. brevis* |
|----------------------|--------------|--------------------|------------|------------|------------------|------------|
| *P. gorillae*        | 5,856        | 67.8               | 68.6       | 68         | 67.8             | 65.7       |
| “*G. massiliense*”   | 1,987        | 5,146              | 68.7       | 66.7       | 66.9             | 65.3       |
| *P. elgii*           | 2,380        | 2,122              | 7,597      | 67.6       | 67               | 66.4       |
| *P. alvei*           | 2,055        | 1,846              | 2,336      | 6,823      | 67.9             | 66         |
| *P. massiliensis*    | 2,121        | 1,902              | 2,296      | 1,994      | 5,496            | 65.3       |
| *B. brevis*          | 1,935        | 1,716              | 2,278      | 1,936      | 1,872            | 5,946      |

Numbers of orthologous proteins shared between genomes (lower left triangle), average percentage similarity of nucleotides corresponding to orthologous proteins shared between genomes (upper right triangle). Bold numbers indicate numbers of proteins per genome.

### Conclusion

On the basis of phenotypic (Table 2), phylogenetic and genomic analyses (taxonogenomics) (Table 7), we formally propose the creation of *Paenibacillus gorillae* sp. nov. that contains the strain G1\(^\text{T}\). This strain has been found in gorilla stool sample collected from Cameroon.
**Description of *Paenibacillus gorillae* sp. nov.**

*Paenibacillus gorillae* (gor.ill.ae, N.L. gen fem. of the gorilla from which the stool sample was obtained).

*P. gorillae* is a facultative aerobic Gram-negative. Optimal growth is achieved aerobically. A weak growth is observed in microaerophilic or anaerobic conditions. Growth occurs between 25 and 37°C, with optimal growth observed at 25°C. Cells stain Gram-negative, are rod-shaped, endospore-forming and motile with a mean diameter of 0.79 μm (range 0.76 to 0.83 μm) and a mean length of 3.2 μm (range 2.5 to 3.97 μm). Colonies are white and 2-8 mm in diameter on blood-enriched Columbia agar. Catalase negative, oxidase positive. Using the API 50CH system (BioMérieux), a positive reaction is obtained for D-mannose, amygdalin, L-arabinose, cellobiose, lactose, D-xylene, D-glucose, mannitol, arabinose, xylose, glycerol, D-galactose, N-acetylgalactosamine, arbutin, aesculin, D-sorbitol, D-maltose, D-saccharose, D-tagatose, L-rhamnose, salicin, adonitol, D-melibiose, D-raffinose, D-ribose, D-fructose and hydrolysis of starch. Negative reactions are obtained for potassium gluconate, potassium 2-cetogluconate, inulin, D-melezitose, Glycogen, β-glucosaminidase activities. The API ZYM system, negative reactions are obtained for lipase (C14), a-chymotrypsin, esterase (C4), esterase lipase (C8), naphthyl-AS-BI-phosphohydrolase, phenylalanine arylamidase, leucine arylamidase, cystine arylamidase, valine arylamidase, glycine arylamidase, arginine arylamidase and β-glucosidase. Using the API Coryne system, positive reactions are observed for β-glucuronidase, alkaline phosphatase, α-glucosidase, α-galactosidase and N-acetyl-β-glucosaminidase activities. The urease reaction, nitrate reduction and indole production were negative. *P. gorillae* is susceptible to imipenem, rifampicin, gentamycin, nitrofurantoin and vancomycin, but resistant to metronidazole, trimethoprim/sulfamethoxazole, ceftriaxone, ciprofloxacin and amoxicillin.

The G+C content of the genome is 48.8%. The 16S rRNA and genome sequences are deposited in GenBank under accession numbers JX650054 and CBVJ000000000, respectively. The type strain G1T (= CSUR P205 = DSM 26181) was isolated from the fecal flora of a *Gorilla gorilla gorilla* from Cameroon.

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**References**

1. Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, Bittar F, Fournous G, Gimenez G, Maraninchi M, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* 2012; 18:1185-1193. PubMed

2. Dubourg G, Lagier JC, Armougom F, Robert C, Hamad I, Brouqui P, Raoult D. The gut microbiota of a patient with resistant tuberculosis is more comprehensively studied by culturomics than by metagenomics. *Eur J Clin Microbiol Infect Dis* 2013; 32:637-645. PubMed http://dx.doi.org/10.1007/s10096-012-1787-3

3. Pfleiderer A, Lagier JC, Armougom F, Robert C, Vialettes B, Raoult D. Culturomics identified 11 new bacterial species from a single anorexia nervosa stool sample. *Eur J Clin Microbiol Infect Dis* 2013; 32:1471-1481. PubMed http://dx.doi.org/10.1007/s10096-013-1900-2

4. Ash C, Priest FG, Collins MD. Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek* 1993; 64:253-260. PubMed http://dx.doi.org/10.1007/BF00873085

5. Judicial Commission of the International Committee on Systematics of Prokaryotes. The type species of the genus *Paenibacillus* Ash et al. 1994 is *Paenibacillus polymyxa*. Opinion 77. *Int J Syst Evol Microbiol* 2005; 55:513. PubMed http://dx.doi.org/10.1099/ijs.0.63546-0

6. Abstract for the genus *Paenibacillus*. NamesforLife, LLC. Retrieved November 1, 2013.

7. Mishra AK, Lagier JC, Rivet R, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Paenibacillus senegalensis* sp. nov. *Stand Genomic Sci* 2012; 7:70-81. PubMed http://dx.doi.org/10.4056/sigs.3056450

8. Glaeser SP, Falsen E, Busse HJ, Kämpfer P. *Paenibacillus vulneris* sp. nov., isolated from a necrotic wound. *Int J Syst Evol Microbiol* 2013;
9. Anikphe YF, Keller P, Bloemberg GV, Grünenfelder J, Zinkernagel AS. Spacecraft bacterium, *Paenibacillus pasadenensis*, causing wound infection in humans. *BMJ Case Rep* 2010.

10. Rieg S, Martin Bauer T, Peyerl-Hoffmann G, Held J, Ritter W, Wagner D, Kern WV, Serr A. *Paenibacillus larvae* bacteremia in injection drug users. *Emerg Infect Dis* 2010; **16**:487-489. [PubMed](http://dx.doi.org/10.3201/eid1603.091457)

11. Ouyang J, Pei Z, Lutwick L, Dalal S, Yang L, Cassai N, Sandhu K, Hanna B, Wieczorek RL, Bluth M, Pincus MR. Case report: *Paenibacillus thiaminolyticus*: a new cause of human infection, inducing bacteremia in a patient on hemodialysis. *Ann Clin Lab Sci* 2008; **38**:393-400. [PubMed](http://dx.doi.org/10.1089/cll.2007.0068)

12. Teng JL, Woo PC, Leung KW, Lau SK, Wong MK, Yuen KY. Pseudobacteremia in a patient with neutropenic fever caused by a novel *paenibacillus* species: *Paenibacillus hongkongensis* sp. nov. *Mol Pathol* 2003; **56**:29-35. [PubMed](http://dx.doi.org/10.1111/mpe.12097)

13. Ferrand J, Hadou T, Selton-Suty C, Goehringer F, Sadoul N, Alauzet C, Lozniewski A. Cardiac Device-Related Endocarditis Caused by *Paenibacillus glucanolyticus*. *J Clin Microbiol* 2013; **51**:3439-3442. [PubMed](http://dx.doi.org/10.1128/JCM.00864-13)

14. Keita MB, Diene S, Robert C, Raoult D, Fournier PE, Bittar F. Non-contiguous finished genome sequence and description of *Bacillus massiliogorillae* sp. nov. *Stand Genomic Sci* 2013; **9**:93-105. [PubMed](http://dx.doi.org/10.4056/sigs.4388124)

15. Mishra AK, Lagier JC, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Clostridium senegalense* sp. nov. *Stand Genomic Sci* 2012; **6**:386-395. [PubMed](http://dx.doi.org/10.4056/sigs.2685971)

16. Lagier JC, Armougom F, Mishra AK, Nguyen TT, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Alistipes timonensis* sp. nov. *Stand Genomic Sci* 2012; **6**:315-324. [PubMed](http://dx.doi.org/10.4056/sigs.2415480)

17. Lagier JC, El Karkouri K, Nguyen TT, Armougom F, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Anaerococcus senegalensis* sp. nov. *Stand Genomic Sci* 2012; **6**:116-125. [PubMed](http://dx.doi.org/10.4056/sigs.2415480)

18. Roux V, El Karkouri K, Lagier JC, Robert C, Raoult D. Non-contiguous finished genome sequence and description of *Kurthia massiliensis* sp. nov. *Stand Genomic Sci* 2012; **7**:221-232. [PubMed](http://dx.doi.org/10.4056/sigs.3206554)

19. Mishra AK, Lagier JC, Robert C, Raoult D, Fournier PE. Non-contiguous finished genome sequence and description of *Peptoniphilus timonensis* sp. nov. *Stand Genomic Sci* 2012; **7**:1-11. [PubMed](http://dx.doi.org/10.4056/sigs.2956294)

20. Hugon P, Ramasamy D, Lagier JC, Rivet R, Couderc C, Raoult D, Fournier PE. Non contiguous-finished genome sequence and description of *Alistipes obesi* sp. nov. *Stand Genomic Sci* 2013; **7**:427-439. [PubMed](http://dx.doi.org/10.4056/sigs.3336746)

21. Ramasamy D, Lagier JC, Nguyen TT, Raoult D, Fournier PE. Non contiguous-finished genome sequence and description of *Timonella senegalensis* gen. nov., sp. nov., a new member of the Family *Erysipelotrichaceae*. *Stand Genomic Sci* 2013; **8**:336-351. [PubMed](http://dx.doi.org/10.4056/sigs.3567059)

22. Mishra AK, Lagier JC, Robert C, Raoult D, Fournier PE. Genome sequence and description of *Enorma massiliensis* gen. nov., sp. nov., a new member of the suborder *Micrococcineae*. *Stand Genomic Sci* 2013; **8**:316-335. [PubMed](http://dx.doi.org/10.4056/sigs.3476977)

23. Mishra AK, Hugon P, Lagier JC, Nguyen TT, Couderc C, Raoult D, Fournier PE. Non contiguous-finished genome sequence and description of *Enorma massiliensis* gen. nov., sp. nov., a new member of the Family *Coriobacteriaceae*. *Stand Genomic Sci* 2013; **8**:290-305. [PubMed](http://dx.doi.org/10.4056/sigs.3426906)

24. Ramasamy D, Lagier JC, Gorlas A, Raoult D, Fournier PE. Non contiguous-finished genome sequence and description of *Bacillus massiliogorillae* sp. nov. *Stand Genomic Sci* 2013; **8**:264-278. [PubMed](http://dx.doi.org/10.4056/sigs.3496989)

25. Hugon P, Mishra AK, Lagier JC, Nguyen TT, Couderc C, Raoult D, Fournier PE. Non contiguous-finished genome sequence and description of *Brevibacillus massiliosenegalensis* sp. nov. *Stand Genomic Sci* 2013; **8**:1-14. [PubMed](http://dx.doi.org/10.4056/sigs.3466975)

26. Sentausa E, Fournier PE. Advantages and limitations of genomics in prokaryotic taxonomy. *Clin Microbiol Infect* 2013. [PubMed](http://dx.doi.org/10.1111/1469-0691.12181)

27. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angiuoli SV, et al. The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* 2008; **26**:541-547. [PubMed](http://dx.doi.org/10.1038/nbt1360)
28. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains *Archae, Bacteria, and Eukarya*. *Proc Natl Acad Sci USA* 1990; 87:4576-4579. PubMed http://dx.doi.org/10.1073/pnas.87.12.4576

29. Gibbons NE, Murray RGE. Proposals concerning the higher taxa of *Bacteria*. *Int J Syst Bacteriol* 1978; 28:1-6. http://dx.doi.org/10.1099/00207713-28-1-1

30. Garrity GM, Holt JG. The Road Map to the Manual. In: Garrity GM, Boone DR, Castenholz RW (eds), Bergey’s Manual of Systematic Bacteriology, Second Edition, Volume 1, The Williams and Wilkins Co., Baltimore, 1984, p. 31-34.

31. Murray RGE. The Higher Taxa, or, a Place for Everything...? In: Holt JG (ed), Bergey’s Manual of Systematic Bacteriology, First Edition, Volume 1,-Revised Edition, Masson et Cie, Paris, 1953, p. 1-692

32. Ludwig W, Schleifer KH, Whitman WB. Class I. *Bacilli* class nov. In: De Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman WB (eds), Bergey’s Manual of Systematic Bacteriology, Second Edition, Volume 3, Springer-Verlag, New York, 2009, p. 19-20.

33. List of new names and new combinations previously effectively published, not validly published. List no. 132. *Int J Syst Evol Microbiol* 2010; 60:469-472. http://dx.doi.org/10.1099/ijs.0.022855-0

34. Skerman VBD, McGowan V, Sneath PHA. Approved Lists of Bacterial Names. *Int J Syst Bacteriol* 1980; 30:225-420. http://dx.doi.org/10.1099/00207713-30-1-225

35. Prévot AR. In: Hauderoy P, Ehringer G, Guillot G, Magrou. J., Prévot AR, Rosset D, Urbain A (eds), Dictionnaire des Bactéries Pathogènes, Second Edition, Masson et Cie, Paris, 1953, p. 1-692

36. De Vos P, Ludwig W, Schleifer KH, Whitman WB. Family IV. *Paenibacillaceae* fam. nov. In Bergey’s Manual of Systematic Bacteriology, 2nd Edition, vol 3 (The *Firmicutes*), Springer; New York, 2009, p. 269.

37. Validation List no. 51. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. *Int J Syst Bacteriol* 1994; 44:852. http://dx.doi.org/10.1099/00207713-44-4-852

38. Shida O, Takagi H, Kadowaki K, Nakamura LK, Komagata K. Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdianolycyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* 1997; 47:289-298. PubMed http://dx.doi.org/10.1099/00207713-34-2-1

39. Behrendt U, Schumann P, Steiglmeier M, Pukall R, Augustin J, Spröer C, Schwendner P, Moissl-Eichinger C, Ulrich A. Characterization of heterotrophic nitrifying bacteria with respiratory ammonification and denitrification activity – Description of *Paenibacillus uliginis* sp. nov., an inhabitant of fen peat soil and *Paenibacillus purispathii* sp. nov., isolated from a spacecraft assembly clean room. *Syst Appl Microbiol* 2010; 33:328-336. PubMed http://dx.doi.org/10.1016/j.syapm.2010.07.004

40. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; 25:29-25. PubMed http://dx.doi.org/10.1038/75556

41. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol Biol Evol* 2011; 28:2731-2739. PubMed http://dx.doi.org/10.1093/molbev/msr121

42. Stackebrandt E, Ebers J. Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 2006; 33:152-155.

43. Kim DS, Bae CY, Jeon JJ, Chun SJ, Oh HW, Hong SG, Baek KS, Moon EY, Bae KS. *Paenibacillus elgii* sp. nov., with broad antimicrobial activity. *Int J Syst Evol Microbiol* 2004; 54:2031-2035. PubMed http://dx.doi.org/10.1099/ijs.0.02414-0

44. Ueda J, Yamamoto S, Kurosawa N. *Paenibacillus thermoaerophilus* sp. nov., a moderately thermophilic bacterium isolated from compost. *Int J Syst Evol Microbiol* 2013; 63:3330-3335. PubMed http://dx.doi.org/10.1099/ijs.0.048090-0

45. Lee FL, Kuo HP, Tai CJ, Yokota A, Lo CC. *Paenibacillus taiwanensis* sp. nov., isolated from soil in Taiwan. *Int J Syst Evol Microbiol* 2007; 57:1351-1354. PubMed http://dx.doi.org/10.1099/ijs.0.06764-0

46. Roux V, Raoult D. *Paenibacillus massiliensis* sp. nov., *Paenibacillus sanguinis* sp. nov., and *Paenibacillus timonensis* sp. nov., isolated from blood cultures. *Int J Syst Evol Microbiol* 2004; 54:1049-1054. PubMed http://dx.doi.org/10.1099/ijs.0.02954-0

47. Takebe F, Hirota K, Noda-saka Y, Yumoto I. *Brevibacillus nitrificans* sp. nov., a nitrifying bacterium isolated from a microbiological agent for enhancing microbial digestion in sewage treatment tanks. *Int J Syst Evol Microbiol* 2012; 62:2121-2126. PubMed http://dx.doi.org/10.1099/ijs.0.032342-0
48. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angiuoli SV, et al. The minimum information about a genome sequence (MIGS) specification. Nat Biotechnol 2008; 26:541-547. PubMed http://dx.doi.org/10.1038/nbt1360

49. Prodigal. http://prodigal.ornl.gov

50. GenBank database. http://www.ncbi.nlm.nih.gov/genbank

51. Lowe TM, Eddy SR. t-RNAscan-SE: a program for improved detection of transfer RNA gene in genomic sequence. Nucleic Acids Res 1997;

52. Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 2007; 35:3100-3108. PubMed http://dx.doi.org/10.1093/nar/gkm160

53. Lechner M, Findeib S, Steiner L, Marz M, Stadler PF, Prohaska SJ. Proteinortho: Detection of (Co-)orthologs in large-scale analysis. BMC Bioinformatics 2011; 12:124. PubMed http://dx.doi.org/10.1186/1471-2105-12-124