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### ► To cite this version:

I. I. Ngom, I. Hasni, Bruno Senghor, C. I. Lo, N. Armstrong, et al.. Description of *Gracilibacillus phocaeensis* sp. nov., a new halophilic bacterium isolated from Senegalian human stool. *NEW MICROBES AND NEW INFECTIONS*, 2020, 38, 10.1016/j.nmni.2020.100799 . hal-03149259

**HAL Id: hal-03149259**

**<https://amu.hal.science/hal-03149259>**

Submitted on 15 Dec 2022

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Description of *Gracilibacillus phocaeensis* sp. nov., a new halophilic bacterium isolated from a Senegalian human stool.

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**Running title:** *Gracilibacillus phocaeensis* sp. nov.

**Keywords:** *Gracilibacillus phocaeensis* sp. nov. ; genome; taxonogenomics; culturomics; gut

**Abbreviations :**

**CSUR:** Collection de Souches de l'Unité des Rickettsies

**MALDI-TOF:** Matrix-Assisted Laser-Desorption/Ionization Time-Of-Flight

**FAME:** Fatty Acid Methyl Ester

**GC/MS:** Gaz Chromatography/Mass Spectrometry

**ASP:** Antibiotic susceptibility profile

1 **Abstract**

2 Using Taxonogenomics method, we described here *Gracilibacillus phocaeensis* strain  
3 Marseille-P380, a new species previously isolated from a salty stool of a 20-year-old man  
4 from N'Diop. It is Gram-positive, aerobic and motile bacilli. The major fatty acids are C<sub>15:0</sub>  
5 anteiso (59 %), C<sub>16:0</sub> (16 %) and C<sub>17:0</sub> anteiso (11 %). Strain Marseille-P3801 exhibits a 98.45%  
6 sequence similarity with *Gracilibacillus thailandensis* TP2-8, the phylogenetically closest  
7 species. Its genome is 4.66 Mb with 39.6 mol% G+C content.

8

## 9 **Introduction**

10 The genus *Gracilibacillus* has been described as moderately halophilic, motile endospore-  
11 forming bacteria [1]. Moderately halophilic bacteria have been found in a variety of  
12 fermented foods and one of the most important food preservation methods in history has been  
13 the use of salt (NaCl) [2]. Salt is the main source of sodium in our diet. Some gut bacteria,  
14 such as lactobacilli, are very sensitive to salt [3]. However, it has been demonstrated to favor  
15 the emergence and growth of others, mainly halophilic bacteria, including *Gracilibacillus* [4].  
16 Halophilic and halotolerant bacteria are most commonly isolated from the human gut  
17 microbiota [5,6]. The culturomics approach, based on the multiplication of culture conditions  
18 (variation of media, temperature, and atmosphere) with a more rapid bacterial identification  
19 by [Matrix-Assisted Laser-Desorption/Ionization Time-Of-Flight Mass Spectrometry](#)  
20 [\(MALDI-TOF MS\)](#) [7] was used to explore the human gut halophilic microbiota. Thanks to  
21 culturomics, we used high salt-containing culture media, which allowed us to isolate a new  
22 moderately halophilic bacterial strain Marseille-P3801<sup>T</sup>, which belongs to the genus  
23 *Gracilibacillus* [8]. The genus *Gracilibacillus* currently comprises 13 species validly  
24 standing in nomenclature [9]. *Gracilibacillus* species were isolated from diverse salty  
25 environmental samples, including sea water, salty lakes [4,10,11], soil [12,13] and/or food  
26 [1,14,15] and gut microbiota [6].

27 Various parameters, including phenotypic and genotypic characteristics, such as DNA-DNA  
28 hybridization, were used to define a new species, but they present certain limitations [16]. In  
29 our study, using a taxonogenomic approach that includes phenotypic characteristics,  
30 proteomic information obtained by MALDI-TOF MS and the analysis of the complete  
31 genome sequence, we were able to achieve a complete description of the new halophilic  
32 species called *G. phocaeensis*; the strain Marseille-P3801<sup>T</sup> (= CSUR P3801) is the type strain  
33 of *Gracilibacillus phocaeensis* sp. nov.

## 34 **Materials and Methods**

### 35 **Bacterial strains and growth conditions**

36 Strain Marseille-P3801<sup>T</sup> was isolated from stool samples of a 20-year-old man from N'Diop.  
37 The study was approved by the ethics committee of the Institut Hospitalo-Universitaire  
38 Méditerranée Infection under number 2016-011, and the patient provided a signed informed  
39 consent. The percentage of NaCl in the stool sample was determined using a salinity  
40 refractometer (Thermo Scientific, Villebon-sur-Yvette, France) by diluting 1 g in 10 mL of  
41 distilled water and centrifuging it for 10 minutes at 5000g. In a second time, 100 µL of  
42 supernatant was deposited in the refractometer; the result was in a straight line, displayed on  
43 the screen in per mille and then reported in percentage of NaCl. To culture the bacteria from  
44 stool samples, we used an aerobic blood culture bottle (Becton Dickinson, Le Pont-de-Claix,  
45 France) containing a halophilic medium prepared in a [modified](#) Columbia broth (Sigma-  
46 Aldrich, Saint-Quentin-Fallavier, France), by adding (per litre: 1% (w/v) MgSO<sub>4</sub>, 0.1% (w/v)  
47 MgCl<sub>2</sub>, 0.4% (w/v) KCl, 0.1% (w/v) CaCl<sub>2</sub>, 0.05% (w/v) NaHCO<sub>3</sub>, 0.2% (w/v) of glucose,  
48 0.5% (w/v) of yeast extract (Becton Dickinson), and from 10 to 15% (w/v) NaCl according to  
49 the required salinity with a pH adjusted to 7.5 and incubated for 3 days in an aerobic  
50 atmosphere at 37°C [8]. All strains were first isolated in a halophilic culture medium with  
51 15% (w/v) NaCl. [The initial growth of colonies on agar were obtained after 24 hours of](#)  
52 [incubation at 37°C under aerobic conditions. The oxygen requirement was evaluated by](#)  
53 [incubating strain Marseille-P3801<sup>T</sup> under aerobic, microaerophilic, and anaerobic conditions](#)  
54 [using AnaeroGen<sup>TM</sup> \(Atmosphere Generation Systems, Dardilly, France\) at 37°C.](#) The  
55 colonies isolated were identified using [MALDI-TOF MS](#), as previously described [17]. As for  
56 unidentified colonies, the 16S rRNA gene was sequenced and the obtained sequence was  
57 matched against the NCBI database using the BLAST algorithm [18].

### 58 **16S rRNA gene sequencing and phylogenetic analysis**

59 The 16S rRNA gene sequence of the strain was determined for subsequent phylogenetic  
60 analysis. The genomic DNA of the strain was amplified by PCR using the primers pair fD1  
61 and rP2 (Eurogentec, Angers, France) [19] and sequenced with the MiSeq Technology  
62 (Illumina Inc, San Diego, CA, USA) as previously described [20]. The 16S rRNA nucleotide  
63 sequences were assembled and corrected using CodonCode Aligner software  
64 (<http://www.codoncode.com> ). A BLAST research (Basic Local Alignment Search Tool) was  
65 further performed against the GenBank nucleotide collection  
66 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). If the 16S rRNA sequence similarity value was lower  
67 than 98.65% with the most closely related species with standing in nomenclature, as proposed  
68 by Stackebrandt [21], the strain was proposed as belonging to a new species [22].

#### 69 **Phenotypic and biochemical characteristics**

70 The morphology of strain Marseille-P3801<sup>T</sup> was revealed by a negative staining observed on  
71 a Hitachi SU5000 scanning electron microscope (Hitachi Group, Krefeld, Germany) and  
72 Gram staining observed on photonic microscope Leica DM2500 (Leica Microsystems,  
73 Nanterre, France) with a 100X oil-immersion objective. Sporulation, motility catalase and  
74 oxidase were tested, as previously reported [23,24]. To determine the optimal growth  
75 conditions, the strain Marseille-P3801 was cultivated in Mueller Hinton agar (Sigma-Aldrich)  
76 by varying the NaCl concentrations from 5 to 20% (w/v) as well as the pH values (5, 5.5, 6,  
77 6.5, 7, 7.5 and 8). It was also seeded on 5% sheep blood-enriched Columbia agar  
78 (bioMérieux, Marcy l'Etoile, France) and incubated under different temperatures (25, 28, 37,  
79 45 and 55°C). API 50CH, ZYM and 20 NE test strips (bioMérieux, Marcy-l'Etoile, France)  
80 were used to study the carbohydrate metabolism, enzymes activities and biochemical criteria  
81 of strain Marseille-P3801, according to the manufacturer's instructions.  
82 Cellular fatty acid methyl ester (FAME) analysis was performed by GC/MS. Two samples  
83 were prepared with approximately 90 mg of bacterial biomass per tube, harvested from

84 several culture plates. Fatty acid methyl esters were prepared as described by Sasser [25].  
85 GC/MS analyses were carried out as previously described [26]. Briefly, fatty acid methyl  
86 esters were separated using an Elite 5-MS column and monitored by mass spectrometry  
87 (Clarus 500 - SQ 8 S, Perkin Elmer, Courtaboeuf, France). Spectral database search was  
88 performed using MS Search 2.0 operated with the Standard Reference Database 1A (NIST,  
89 Gaithersburg, USA) and the FAMES mass spectral database (Wiley, Chichester, UK).

#### 90 **Genome: extraction, sequencing and assembly**

91 Genomic DNA (gDNA) of *Gracilibacillus phocaeensis* was extracted in two steps : a  
92 mechanical treatment was first performed by glass beads acid washed (G4649-500g Sigma)  
93 using a FastPrep BIO 101 instrument (Qbiogene, Strasbourg, France) at maximum speed  
94 (6.5m/s) for 90 seconds. After 30 minutes incubation of the lysozyme at 37°C, the DNA was  
95 extracted on the EZ1 biorobot (Qiagen) with the EZ1 DNA tissue kit. The elution volume is  
96 50µL. Genomic DNA was evaluated with a Qubit test (Life technologies, Carlsbad, CA,  
97 USA). The mate pair library was prepared with 1.5 µg of genomic DNA using the Nextera  
98 mate pair Illumina guide. The DNA sample was simultaneously fragmented and tagged with a  
99 mate pair junction adapter. The pattern of fragmentation was validated on an Agilent 2100  
100 BioAnalyzer (Agilent Technologies Inc, Santa Clara, CA, USA) with a DNA 7500 labchip  
101 kit. The DNA fragments size ranged from 1.5 kb up to 11 kb, with an optimal size at 8.10 kb.  
102 No size selection was performed and 600 ng of tagmented fragments were circularized. The  
103 circularized DNA was mechanically sheared to small fragments with an optimal at 1086 bp on  
104 the Covaris device S2 in T6 tubes (Covaris, Woburn, MA, USA). The library profile was  
105 visualized on a High Sensitivity Bioanalyzer LabChip kit (Agilent Technologies Inc) and the  
106 final concentration library was measured at 31.31 nmol/l. The libraries were normalized at  
107 2nM and pooled. After a denaturation step and dilution at 15 pM, the pool of libraries was  
108 loaded onto the reagent cartridge and then onto the instrument along with the flow cell.

109 Automated cluster generation and sequencing run were performed in a single 39-hour run in a  
110 2x251-bp. Total information of 8.2 Gb was obtained from a 932 K/mm<sup>2</sup> cluster density with a  
111 cluster passing quality control filters of 91 %. Within this run, the index representation for  
112 *Gracilibacillus phocaeensis* was determined at 13.20 %. The 2 141 870 paired end reads were  
113 filtered according to the read qualities.

114 The assembly was performed with a pipeline incorporating different softwares, including  
115 Velvet[27], Spades [28] and Soap Denovo [29] on trimmed (Trimmomatic) [30] or raw data.  
116 To reduce assembly gap, GapCloser software was used. The scaffolds inferior to 800 bp and  
117 scaffolds with a depth value < 25% of the mean depth were discarded. The best assembly was  
118 selected using different criteria (number of scaffolds, N50, number of N).

## 119 **Genome annotation and comparisons**

120 Open reading frames (ORFs) were predicted using the Prodigal tool (<http://prodigal.ornl.gov>)  
121 with defaults parameters. tRNAs and rRNAs were detected using tRNAscan-SE v.1.2129 and  
122 RNAmmer v.1.230, respectively[31, 32]. The protein sequence annotation was performed on  
123 NCBI GenBank non-redundant protein sequence database (nr) using BLAST protein with an  
124 e-value of 1e-03 as significance thresholds [33]. Then, we obtained the functional  
125 classification of gene families (COG ID and letters) using EggNOG against the COG database  
126 [34]. The genome of *Gracilibacillus phocaeensis* strain Marseille-P3801<sup>T</sup> (EMBL EBI  
127 accession number UZBG000000000) was compared with that of *Gracilibacillus*  
128 *boracitolerans* strain JCM 21714 (BAVS000000000), *Gracilibacillus massiliensis* strain Awa-  
129 1<sup>T</sup> (CZRP000000000), *Gracilibacillus lacisalsi* DSM 19029 (ARIY000000000), *Gracilibacillus*  
130 *ureilyticus* CGMCC (FOGL000000000), *Gracilibacillus dipsosauri* (QGTD000000000) and  
131 *Halobacillus karajensis* DSM 14948 (FNWW000000000) using OrthoANI software [35].

## 132 **Results**

### 133 **Strain identification and phylogenetic analysis**



134 Strain Marseille-P3801<sup>T</sup> formed yellow colonies after 1 day of culture on agar with horse  
135 blood ranging from 2 to 20% (w/v) NaCl (optimum at 7.5 (w/v) NaCl) at 37°C. The spectrum  
136 resulting from the 8 pure colonies of strain Marseille-P3801 deposited on MALDI-TOF target  
137 plate did not allow the identification of this bacterium because there was no spectrum match  
138 with those in the Bruker database (**Supplementary Fig. S1**). Using 16S rRNA sequence of *G.*  
139 *phocaeensis* (LT934503.1), the phylogenetic analysis revealed that strain Marseille-P3801  
140 exhibited a sequence similarity of 98.39% with *Gracilibacillus thailandensis* strain TP2-8  
141 (GenBank accession no. NR\_116568.1), the phylogenetically closest species with standing in  
142 nomenclature (**Fig. 1**). Therefore, we classified this strain as a member of a new species  
143 within the genus *Gracilibacillus*, family *Bacillaceae* and phylum *Firmicutes*. This value was  
144 lower than the 98.7% 16S rRNA gene sequence, threshold advised by Meier-Kolthoff *et al.*  
145 [36] to delineate a new species without carrying out DNA-DNA hybridization. Classification  
146 and general features of strain Marseille-P3801 are summarized in **Table S1**.

#### 147 **Physiological and biochemical characteristics**

148 *G. phocaeensis* sp. nov. strain Marseille-P3801<sup>T</sup> (= CSUR P3801 ) is Gram positive. Colonies  
149 are circular, yellow with a mean diameter of 2 mm after 2-3 days of growth on 5% sheep  
150 blood-enriched Columbia agar medium (BioMérieux, Marcy l'Etoile, France). Bacterial cells  
151 of strain Marseille-P3801 were motile, rod shaped and polymorphic (**Fig. 2**).

152 The major fatty acids were saturated structures mainly branched: 12-methyl-tetradecanoic  
153 acid (59 %), hexadecanoic acid (16 %) and 14-methyl-hexadecanoic acid (11 %). Other  
154 saturated and branched fatty acids were also described. 7-Hexadecenoic acid was the only one  
155 unsaturated structure detected (**Supplementary Table S2**). Catalase and oxidase were  
156 positive. Using API ZYM trips, positive reaction were detected for lipases (C4, C8 and C14),  
157 leucine arylamidase, acid phosphatase, naphthol-as-bi-phosphohydrolase, β-galactosidase, α-  
158 glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase, but no reaction was observed for

159 alkaline phosphatase, valine and cysteine arylamidase,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  
160 trypsin,  $\beta$ -glucuronidase,  $\alpha$ -mannosidase and  $\alpha$ -fucosidase. API 20NE strip exhibited positive  
161 reactions of fermentation of glucose, urease activity, and metabolism of L-arginine and  
162 esculin. In contrast, negative reactions were observed for nitrate and indole production as well  
163 as metabolism of D-glucose, L-arabinose, D-mannose, D-maltose, D-mannitol,  
164 N-acetyl-glucosamine, potassium gluconate, capric acid, malic acid and phenylacetic acid.  
165 Using API 50CH strip, strain Marseille-P3801<sup>T</sup> exhibited esculin hydrolysis and negative  
166 reactions for D-galactose, D-lactose, D-maltose, D-ribose, D-saccharose, D-lyxose,  
167 D-mannose, L-sorbose, D-tagatose, D-turanose, D-xylose, L-xylose, D-arabinose,  
168 L-arabinose, D-sorbitol, D-cellobiose, D-melezitose, D-melibiose, D-trehalose, D-raffinose,  
169 D-arabitol, L-arabitol, D-glucose, D-fructose, D-fucose, L-rhamnose, D-adonitol, D-mannitol,  
170 L-fucose, amygdalin, arbutin, erythritol, dulcitol, gentiobiose, glycerol, glycogen, inositol,  
171 inulin, salicin, starch, xylitol,  $\alpha$ D-glucopyranoside, methyl- $\beta$ D-xylopyranoside,  
172 methyl- $\alpha$ D-mannopyranoside, potassium gluconate and N-acetylglucosamine. The differential  
173 characteristics of *Gracilibacillus phocaeensis* with respect to other bacteria related to the  
174 genus *Gracilibacillus* are outlined in **Table 1**. Phenotypic characterization of *Gracilibacillus*  
175 *phocaeensis* sp. nov., based on analytical profile index (API) tests was summarized on **Table**  
176 **2**.

## 177 **Genome properties**

178 The genome length of *Gracilibacillus phocaeensis* strain Marseille-P3801<sup>T</sup> is 4.66 megabases  
179 encompassing 12 scaffolds (11 contigs). The guanine-cytosine (GC) content is 39.6 mol%.  
180 Among the 4,390 predicted genes, 4,255 were protein-coding genes, 67 were RNAs (11  
181 rRNA, 52 tRNAs and 4 non-coding RNAs) and 68 pseudogenes. The BLASTp annotation of  
182 *G. phocaeensis* strain Marseille-P3801 had assigned a putative function to 3,606 genes and  
183 649 genes annotated as hypothetical proteins. To further insight on the gene function, a

184 comparison of *G. phocaeensis* protein sequences with COG database was realized. Out of  
185 4,390 protein encoding genes, 3,774 were assigned to a COG function (86%) distributed in 20  
186 COG categories (Supplementary Fig. S2).

### 187 **Genome comparison**

188 To explore the genomic similarity of *G. phoceeanensis* with closely related bacteria, we  
189 performed an OrthoANI analysis. Among closely related species, we found OrthoANI values  
190 ranging from 66.64% between *Gracilibacillus phocaeensis* strain Marseille-P3801 and  
191 *Halobacillus karajensis* DSM 14948, to 78.39 % between *Gracilibacillus boraciitolerans*  
192 strain JCM 21714 and *Gracilibacillus massiliensis* strain Awa-1. When *Gracilibacillus*  
193 *phocaeensis* strain Marseille-P3801 was compared with these closely related species, we  
194 found values ranging from 66.64% with *Halobacillus karajensis* DSM 14948, to 72.42% with  
195 *Gracilibacillus lacisalsi* strain DSM 19029 (Fig. 3). The representations of strain Marseille-  
196 P3801 genome and its genes repartition into functional categories are illustrated in Fig. 4.

### 197 **Description of *Gracilibacillus phocaeensis***

198 *Gracilibacillus phocaeensis* (pho.ca.een'sis, N.L. masc. adj., from *phocaeensis*, related to the  
199 Phocaeans, the founders of Marseille, France where the type strain was isolated and  
200 characterized like many others species). It is Gram positive, motile and aerobic bacterium.  
201 The colonies are yellow, circular with a mean diameter of 2 mm. Bacterial cells were rod-  
202 shaped and polymorphic. Strain Marseille-P3801 grows at an optimal temperature of 37°C,  
203 pH 7 with 7.5% (w/v) of NaCl. It is catalase and oxidase positive. Positive reactions were  
204 observed for esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-as-bi-  
205 phosphohydrolase, leucine arylamidase,  $\beta$ -galactosidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase and N-  
206 acetyl- $\beta$ -glucosaminidase. *G. phocaeensis* strain Marseille-P3801<sup>T</sup> was isolated from a stool  
207 sample of a 20-year-old man from N'Diop, Senegal. This strain exhibited a G+C content of  
208 39.6 mol%. Its 16S rRNA sequence was deposited in GenBank under accession number

209 LT934503, and the whole genome shotgun sequence was deposited in GenBank under  
210 accession number UZBG00000000.

## 211 **Discussion and conclusion**

212 The concept of “microbial culturomics”, based on the variation of physicochemical  
213 parameters of the culture conditions, allows the exploration of microbial diversity of different  
214 ecosystems such as gut microbiota [7]. Microbial culturomics provides culture conditions that  
215 simulate, reproduce or mimic all the selective constraints that have shaped the natural  
216 microbiota for millions of years. Many new bacterial species have been discovered,  
217 particularly those belonging to the *Bacillales* order, which is one of the most represented  
218 bacterial orders [37]. To explore the halophilic microbiota of the human gut, the use of culture  
219 media with a high salt content, allowed us to isolate a new moderately halophilic bacterial  
220 strain, Marseille-P3801<sup>T</sup> which belongs to the genus *Gracilibacillus* [8]. To the best of our  
221 knowledge, this is the second *Gracilibacillus* species described in the human gut. Based on its  
222 phenotypic, phylogenetic and genomic characteristics, this strain is proposed to represent a  
223 novel species in the genus *Gracilibacillus*, for which the name *Gracilibacillus phocaeensis*  
224 sp. nov. is proposed, with Marseille-P3801<sup>T</sup> as the type strain.

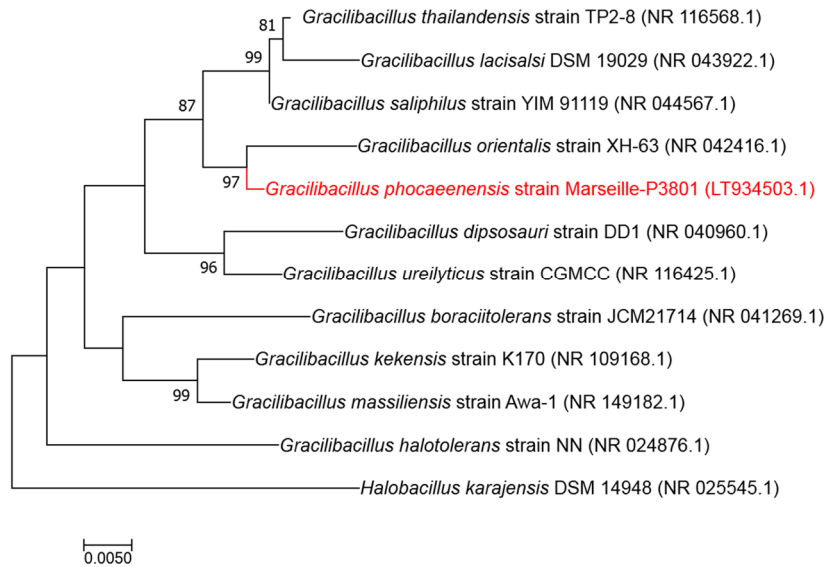
225

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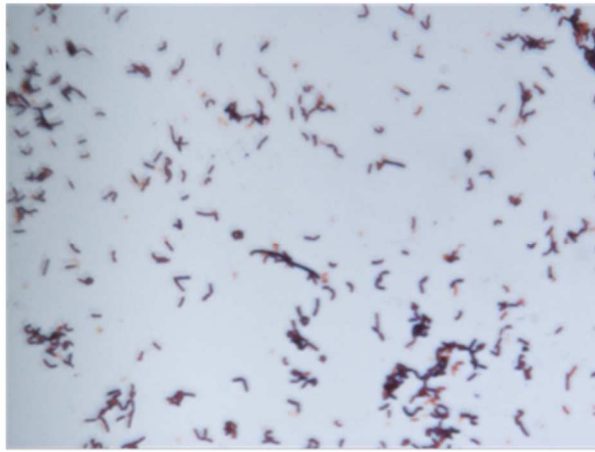
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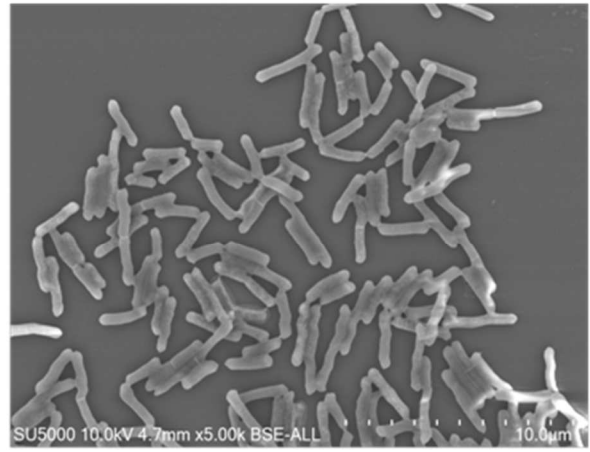


**Fig. 1:** Phylogenetic tree highlighting the phylogenetic position of *Gracilibacillus phocaeensis* strain Marseille-P3801T relative to other phylogenetically close members of the family *Bacillaceae*. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using the maximum likelihood method within the MEGA7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree.



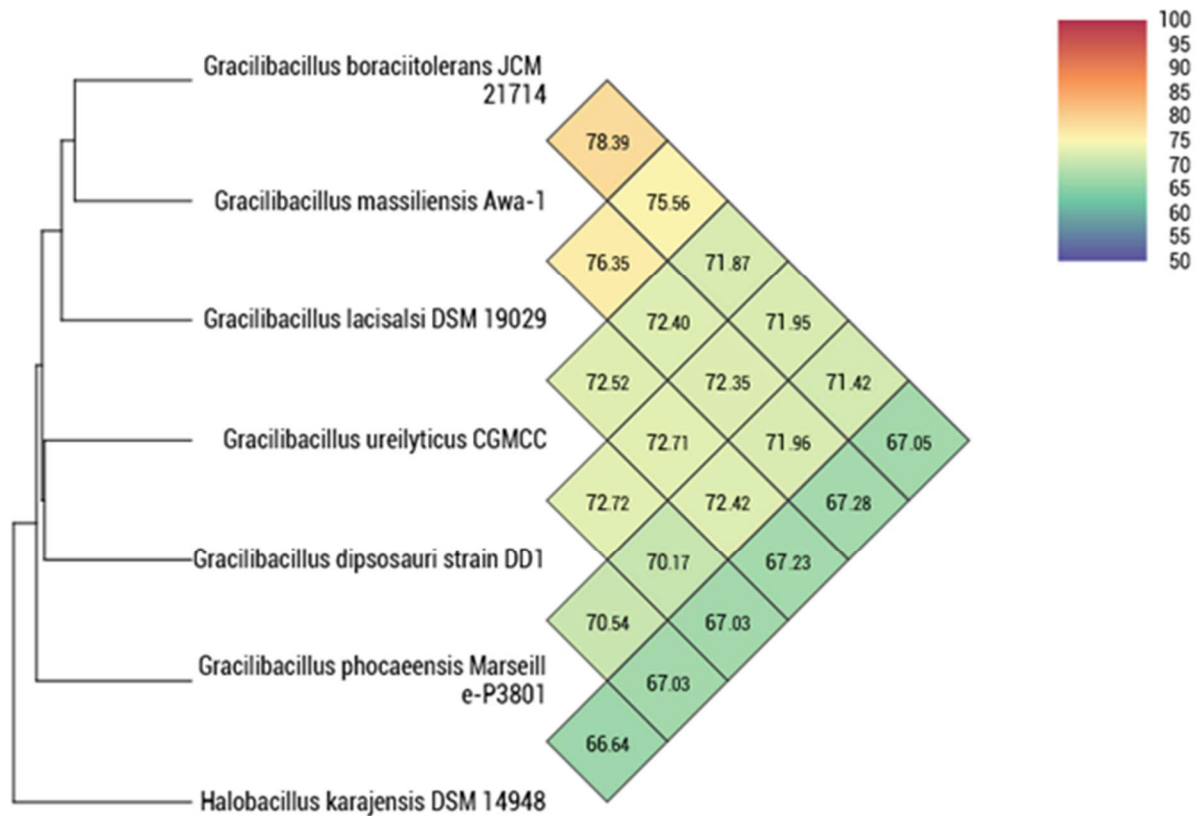


(a)

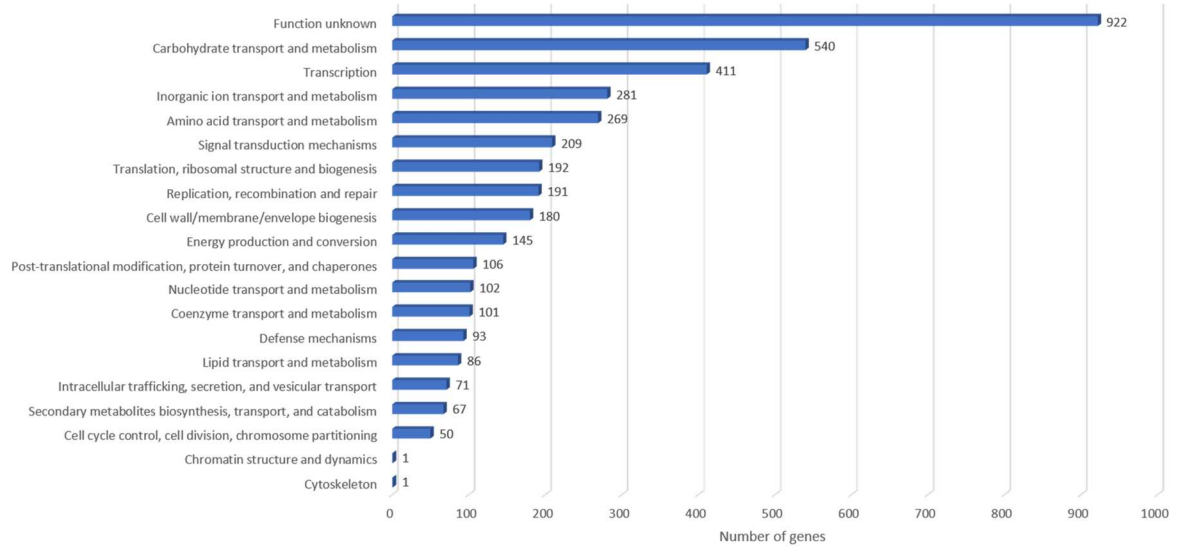


(b)

**Fig. 2:** (a) Gram staining of *Gracilibacillus phocaeensis* strain Marseille-P3801T. (b) Morphological structure of *G. phocaeensis* strain Marseille-P3801T obtained with Hitachi SU5000 scanning electron microscope. Scale bar and acquisition settings are shown of figure.



1  
2 **Fig. 3:** Heatmap generated with OrthoANI values calculated using the OAT software between  
3 *Gracilibacillus phocaeensis* *sp. nov.* strain Marseille-P3801. *nov.* and other closely related species  
4 with standing in nomenclature.  
5



6  
7 **Fig. 4:** Distribution of functional classes of predicted genes according to the clusters of orthologous  
8 groups of proteins of *Gracilibacillus phocaeensis* strain Marseille-P3801

1 **Table 1:** Differential characteristics of **1**, *Gracilibacillus phocaeensis* strain Marseille-P3801 compared to other close bacteria of the genus *Gracilibacillus*: **2**,  
2 *G. timonensis* strain Marseille-P2481[6]; **3**, *G. massiliensis* strain Marseille-P1441[15]; **4**, *G. alcaliphilus* strain SG103 [38]; **5**, *G. saliphilus* strain YIM 91119  
3 [39]; **6**, *G. orientalis* strain XH-63 [40] and **7**, *G. halophilus* strain YIM-C55.5 [13].

| <b>Properties</b>      | <b>1</b> | <b>2</b>      | <b>3</b> | <b>4</b>     | <b>5</b>     | <b>6</b> | <b>7</b> |
|------------------------|----------|---------------|----------|--------------|--------------|----------|----------|
| Cell diameter (µm)     | 0.3-0.6  | 0.5-0.8       | 0.3-1.8  | 0.5-0.7      | 0.7-0.9      | 0.7-0.9  | 0.3-0.5  |
| Pigmentation           | Yellow   | Creamy orange | White    | Creamy white | Creamy white | Creamy   | White    |
| Oxygen requirement     | Aerobic  | Aerobic       | Aerobic  | Aerobic      | Aerobic      | Aerobic  | Aerobic  |
| Gram stain             | +        | +             | +        | +            | +            | +        | +        |
| Salt requirement       | +        | +             | +        | +            | +            | +        | +        |
| Motility               | +        | +             | +        | +            | +            | +        | +        |
| Sporulation            | +        | +             | -        | +            | +            | +        | +        |
| Indole                 | -        | -             | -        | -            | -            | -        | -        |
| <b>Production of :</b> |          |               |          |              |              |          |          |
| Alkaline phosphate     | -        | -             | -        | -            | +            | NA       | +        |
| Catalase               | +        | +             | +        | +            | +            | +        | +        |
| Oxidase                | +        | -             | -        | -            | +            | -        | +        |
| Nitrate reductase      | -        | -             | -        | +            | +            | -        | +        |
| Urease                 | +        | +             | +        | +            | +            | -        | -        |
| β-galactosidase        | +        | +             | -        | NA           | +            | NA       | +        |
| α-galactosidase        | -        | -             | +        | -            | -            | NA       | -        |
| N-acetyl-glucosamine   | -        | -             | -        | +            | +            | NA       | -        |
| <b>Acid from</b>       |          |               |          |              |              |          |          |
| L-Arabinose            | -        | -             | -        | +            | +            | +        | -        |
| D-mannose              | -        | -             | -        | -            | +            | -        | -        |
| D-mannitol             | -        | -             | -        | +            | +            | +        | +        |
| D-glucose              | -        | -             | -        | +            | +            | +        | +        |
| D-fructose             | -        | -             | -        | +            | +            | +        | +        |
| D-maltose              | -        | -             | -        | +            | +            | +        | -        |
| D-lactose              | -        | -             | -        | +            | +            | +        | -        |

|   |                        |           |           |              |                     |           |           |           |       |
|---|------------------------|-----------|-----------|--------------|---------------------|-----------|-----------|-----------|-------|
|   | DNA G+C content (mol%) | 39.6      | 39.8      | 36.05        | 41.3                | 40.1      | 37.1      | 42.3      |       |
|   | Habitat                | Human gut | Human gut | Cooking salt | Fermentation liquor | Salt lake | Salt lake | Salt soil |       |
| 4 | +, positive            | reaction; | -,        | negative     | reaction;           | NA,       | Not       | available | data. |

1 **Table 2:** Phenotypic characterization of *Gracilibacillus phocaeensis* sp. nov., strain Marseille-P3801  
 2 based on analytical profile index (API) tests.

| Tests     | Characteristics                    | Results | Tests                             | Characteristics                 | Results |
|-----------|------------------------------------|---------|-----------------------------------|---------------------------------|---------|
| API ZYM   | Alkaline phosphatase               | -       | API 50 CH                         | Glycerol                        | -       |
|           | Esterase (C4)                      | +       |                                   | Erythritol                      | -       |
|           | Esterase Lipase (C8)               | +       |                                   | D-arabinose                     | -       |
|           | Lipase (C14)                       | +       |                                   | L-arabinose                     | -       |
|           | Leucine arylamidase                | +       |                                   | D-ribose                        | -       |
|           | Valine arylamidase                 | -       |                                   | D-xylose                        | -       |
|           | Cystine arylamidase                | -       |                                   | L-xylose                        | -       |
|           | Trypsin                            | -       |                                   | D-Adonitol                      | -       |
|           | $\alpha$ -chymotrypsin             | -       |                                   | Methyl $\beta$ D-xylopyranoside | -       |
|           | Acid phosphatase                   | +       |                                   | D-galactose                     | -       |
|           | Naphthol-AS-BI-phosphohydrolase    | +       |                                   | D-glucose                       | -       |
|           | $\alpha$ -galactosidase            | -       |                                   | D-fructose                      | -       |
|           | $\beta$ -galactosidase             | +       |                                   | D-mannose                       | -       |
|           | $\beta$ -glucuronidase             | -       |                                   | L-sorbose                       | -       |
|           | $\alpha$ -glucosidase              | +       |                                   | L-rhamnose                      | -       |
|           | $\beta$ -glucosidase               | +       |                                   | Dulcitol                        | -       |
|           | N-acetyl- $\beta$ -glucosaminidase | +       |                                   | Inositol                        | -       |
|           | $\alpha$ -mannosidase              | -       |                                   | D-mannitol                      | -       |
|           | $\alpha$ -fucosidase               | -       |                                   | D-sorbitol                      | -       |
| API 20 NE | Nitrates to nitrites               | -       | Methyl $\alpha$ D-mannopyranoside | -                               |         |
|           | Indole                             | -       | Methyl $\alpha$ D-glucopyranoside | -                               |         |
|           | Glucose fermentation               | +       | N-acetyl-glucosamine              | -                               |         |
|           | Arginine dihydrolase               | -       | Amygdalin                         | -                               |         |
|           | Urease                             | +       | Arbutin                           | -                               |         |
|           | $\beta$ -glucosidase               | -       | Esculin ferric citrate            | +                               |         |
|           | Protease                           | -       | Salicin                           | -                               |         |
|           | $\beta$ -galactosidase             | -       | D-cellobiose                      | -                               |         |
|           | Glucose assimilation               | -       | D-maltose                         | -                               |         |
|           | Arabinose                          | -       | D-lactose                         | -                               |         |
|           | Mannose                            | -       | D-melibiose                       | -                               |         |
|           | Mannitol                           | -       | Sucrose                           | -                               |         |
|           | N-acetyl-glucosamine               | -       | D-trehalose                       | -                               |         |
|           | Maltose                            | -       | Inulin                            | -                               |         |
|           | Potassium gluconate                | -       | D-melezitose                      | -                               |         |
|           | Capric acid                        | -       | D-raffinose                       | -                               |         |
|           | Adipic acid                        | -       | Starch                            | -                               |         |
|           | Malate                             | -       | Glycogen                          | -                               |         |
|           | Trisodium Citrate                  | -       | Xylitol                           | -                               |         |

Phenylacetic acid -

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Gentiobiose -  
D-turanose -  
D-lyxose -  
D-tagalose -  
D-fucose -  
L-fucose -  
D-arabitol -  
L-arabitol -  
Potassium gluconate -  
Potassium 2-ketogluconate -  
Potassium 5-ketogluconate -

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3 +, positive reaction; -, negative reaction.