



'*Gracilibacillus phocaeensis*' sp. nov., '*Sediminibacillus massiliensis*' sp. nov. and '*Virgibacillus ndiopensis*' sp. nov., three halophilic species isolated from salty human stools by culturomics.

Bruno Senghor, Saber Khelaifia, Hubert Bassene, E. H. Seck, Pierre-Edouard Fournier, Cheikh Sokhna, Didier Raoult, J.-C. Lagier

► To cite this version:

Bruno Senghor, Saber Khelaifia, Hubert Bassene, E. H. Seck, Pierre-Edouard Fournier, et al.. '*Gracilibacillus phocaeensis*' sp. nov., '*Sediminibacillus massiliensis*' sp. nov. and '*Virgibacillus ndiopensis*' sp. nov., three halophilic species isolated from salty human stools by culturomics.. New Microbes and New Infections, 2017, 20, pp.51-54. 10.1016/j_nmni.2017.08.006 . hal-01788972

HAL Id: hal-01788972

<https://amu.hal.science/hal-01788972>

Submitted on 9 May 2018

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

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

'*Gracilibacillus phocaeensis*' sp. nov., '*Sediminibacillus massiliensis*' sp. nov. and '*Virgibacillus ndiopensis*' sp. nov., three halophilic species isolated from salty human stools by culturomics

B. Senghor¹, S. Khelaifia¹, H. Bassène², E. H. Seck¹, P.-E. Fournier¹, C. Sokhna², D. Raoult¹ and J.-C. Lagier¹

¹) URMITE, Aix Marseille Université, UM63, CNRS 7278, IRD 198, INSERM 1095, IHU—Méditerranée Infection, Marseille, France and 2) Aix-Marseille Université, URMITE, UMR63, CNRS 7278, IRD 198, INSERM 1095, Dakar, Senegal

Abstract

We report the isolation of three bacterial strains that could not be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry screening. '*Gracilibacillus phocaeensis*' sp. nov., '*Sediminibacillus massiliensis*' sp. nov. and '*Virgibacillus ndiopensis*' sp. nov. are halophilic species isolated from salty human stools by culturomics.

© 2017 The Authors. Published by Elsevier Ltd.

Keywords: Culturomics, human gut microbiota, MALDI-TOF, new bacterial species, taxono-genomics

Original Submission: 19 June 2017; **Revised Submission:** 28 August 2017; **Accepted:** 31 August 2017

Article published online: 6 September 2017

Corresponding author: J.-C. Lagier, URMITE, Aix Marseille Université, UM63, CNRS 7278, IRD 198, INSERM 1095, IHU—Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13005 Marseille, France

E-mail: jclagier@yahoo.fr

Culturomics is a new approach using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF MS) for bacteria identification and aiming to cultivate individually all bacterial species from the human gut and also from other human mucosa microbiota. Thus, this approach has allowed a considerable increase in the gut microbiota repertoire, with the description of more than 247 new species in the last few years [1]. Here we report the isolation of three bacterial strains that could not be identified by our MALDI-TOF MS screening on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [2,3]. These strains were isolated in 2017 from the salty stools (>1.7% NaCl) of healthy Senegalese individuals. The study was approved by the ethics committee of the Institut Hospitalo-Universitaire Méditerranée Infection under number 2016-011, and all patients provided signed informed consent.

The percentage of NaCl in the stool specimens was determined using a salinity refractometer (Thermo Scientific,

Villebon-sur-Yvette, France) by diluting 1 g in 10 mL of distilled water and centrifuging it for 10 minutes at 5000g. Then 100 µL of supernatant was deposited in the refractometer; the result was in a straight line, displayed on the screen in *per mille* and then reported in percentage of NaCl.

To cultivate the bacteria from stool samples, we used an aerobic blood culture bottle (Becton Dickinson, Le Pont-de-Claix, France) containing a halophilic medium prepared by modifying a Columbia broth medium (Sigma-Aldrich, Saint-Quentin-Fallavier, France), as detailed in our previous study [4]. The amount of solute per liter was determined by the following formula: concentration (in %, w/v) = 100 × [(mass solute in g)/(volume solution in mL)].

All strains were first isolated in a halophilic culture medium with 15% (w/v) NaCl.

The initial agar-grown colonies were obtained after 24 hours of incubation at 37°C in aerobic conditions. The 16S rRNA genes were sequenced using the universal primer pair fD1-rP2 as previously described [5] using a 3130-XL sequencer (Applied Biosciences, Saint-Aubin, France). Because all the strains exhibited a 16S rRNA sequence homology of <98.7% with their phylogenetically closest species, we thus propose the creation of these three new species according to the nomenclature [6].

Strain Marseille-P3801^T was isolated from stool samples (2% NaCl) of a 20-year-old man from N'Diop. Strain Marseille-

P3801^T can grow in media ranging from 2 to 20% (w/v) NaCl (optimum at 7.5 (w/v) NaCl). The growing colonies are yellow and circular with a mean diameter of 2 mm. Bacterial cells were motile by using peritrichous flagella under electron microscopy, and were Gram positive, rod shaped and polymorphic, and catalase and oxidase positive. Strain Marseille-P3801^T exhibited a 98.45% sequence identity with *Gracilibacillus thailandensis* strain TP2-8 (GenBank accession no. NR_116568.1) (Fig. 1) [7], which allowed us to classify it as a member of the genus *Gracilibacillus* within the family *Bacillaceae* in the phylum *Firmicutes*. Strain Marseille-P3801^T is the type strain of the new species '*Gracilibacillus phocaeensis*' (pho.ca.een'sis, N.L. masc. adj., from *phocaeensis*, related to the Phocaeans, the founders of Marseille).

Strain Marseille-P3518^T was isolated from stool samples (2% NaCl) of a 15-year-old boy from Dielmo. Agar-grown colonies were beige, circular and shiny with a mean diameter of 2 mm. Bacterial cells were Gram positive, rod shaped and polymorphic, and had positive catalase and oxidase reaction. Strain Marseille-P3518^T exhibited a 97.4% sequence identity with *Sediminibacillus albus* strain NHBX5 (GenBank accession no.

NR_044031.1) [8], the phylogenetically closest species with standing in nomenclature (Fig. 2), which putatively classifies it as a member of the genus *Sediminibacillus* within the family *Bacillaceae* in the phylum *Firmicutes*. Strain Marseille-P3518^T is the type strain of the new species *Sediminibacillus massiliensis* (ma.si.lien'sis, L. masc. adj., from *massiliensis*, related to the university hospital in Marseille, France, where the strain was isolated).

Strain Marseille-P3835^T was isolated from stool samples (3.7% NaCl) of a 11-year-old boy from N'Diop. Strain Marseille-P3835^T is Gram positive, and catalase and oxidase positive. The strain was able to grow in 0.5 to 15% (w/v) NaCl, with an optimum growth at 5% (w/v) NaCl. The agar colonies are pink and circular, with a mean diameter of 2 mm. Strain Marseille-P3835^T exhibited a 16S rRNA sequence similarity of 98.6% with *Virgibacillus zhanjiangensis* strain JSM 079157 (GenBank accession no. NR_116658.1) (Fig. 3) [9]. On the basis of this result, we propose to classify '*Virgibacillus ndiopensis*' as a new representative of the *Virgibacillus* genus belonging to the family *Bacillaceae*, of the phylum *Firmicutes*. Strain Marseille-P3835^T is the type strain of '*Virgibacillus ndiopensis*' (ndiop.en'sis,

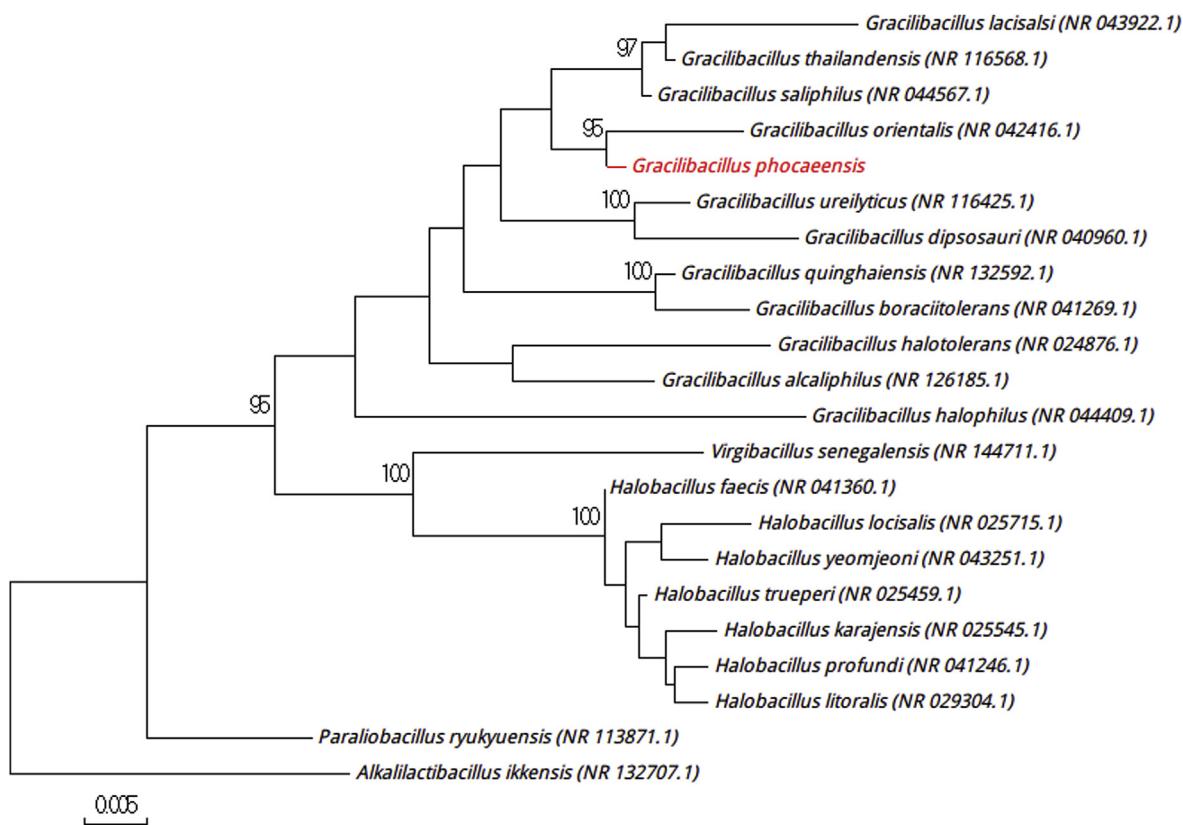


FIG. 1. Phylogenetic tree showing position of '*Gracilibacillus phocaeensis*' Marseille-P3801^T relative to other phylogenetically close neighbours. 16S rRNA gene sequences were aligned using ClustalW, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstrap scores of ≥ 75 were retained. Scale bar indicates 0.005 nucleotide sequence divergence.

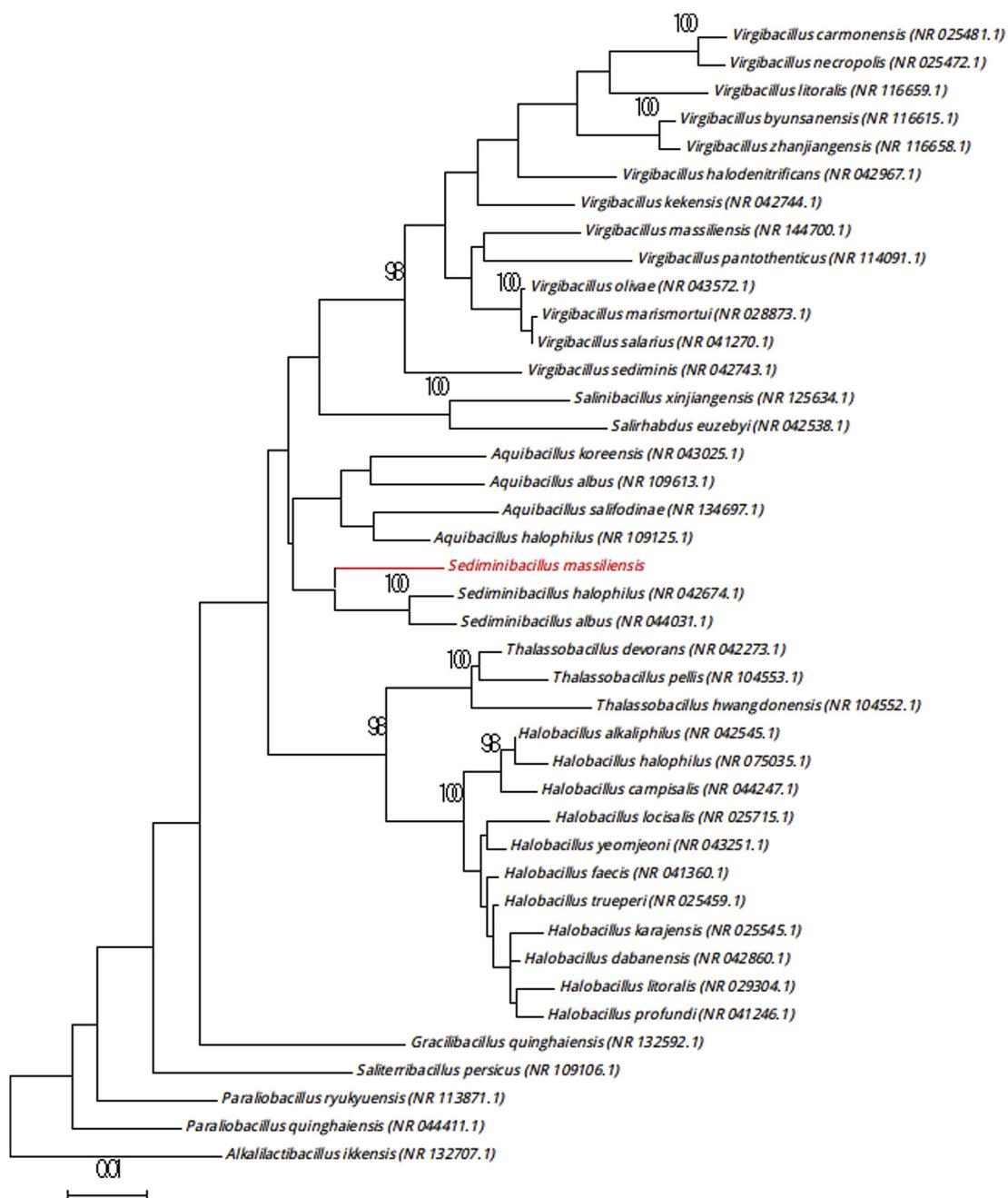


FIG. 2. Phylogenetic tree showing position of '*Sediminibacillus massiliensis*' Marseille-P3518^T relative to other phylogenetically close neighbours. Sequences alignment and phylogenetic inferences were realized as explained in Fig. 1. Scale bar represents 0.01 nucleotide sequence divergence.

L. masc. adj., from *ndiopensis*, related to N'Diop, a Senegalese village from which stool samples were collected).

MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of strains is available online (<http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>).

Nucleotide sequence accession number

The 16S rRNA gene sequences were deposited in GenBank under accession numbers '*Gracilibacillus phocaeensis*' Marseille-P3801^T (LT934503), '*Sediminibacillus massiliensis*' Marseille-P3518^T (LT671588) and '*Virgibacillus ndiopensis*' Marseille-P3835^T (LT883149).

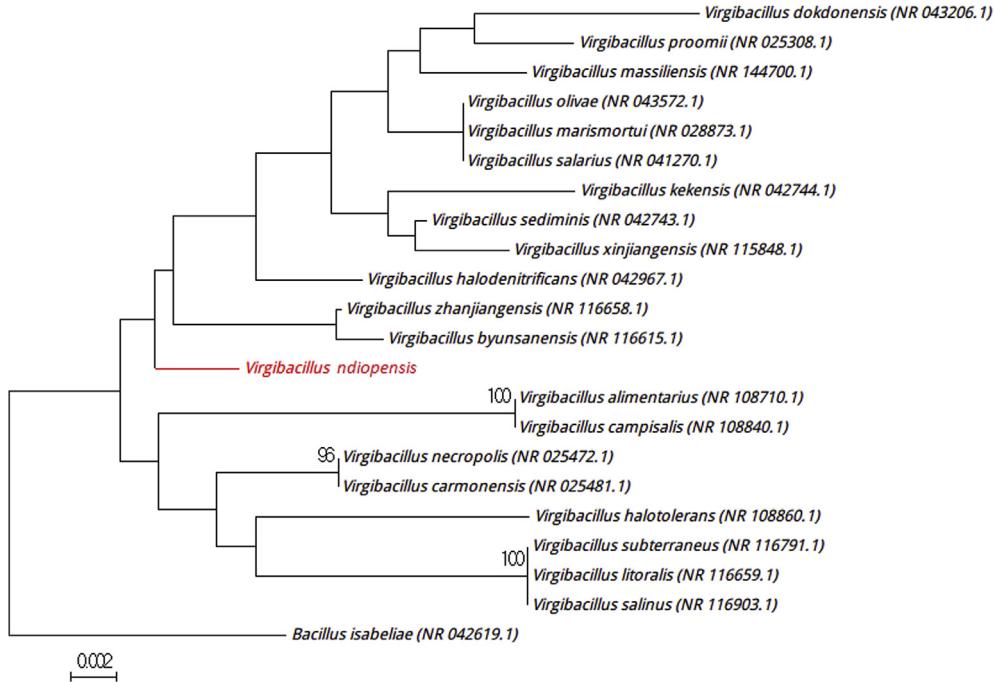


FIG. 3. Phylogenetic tree showing position of '*Virgibacillus ndiopensis*' Marseille-P3835^T relative to other phylogenetically close neighbours. Sequences alignment and phylogenetic inferences were realized as explained for Fig. 1. Scale bar represents 0.002 nucleotide sequence divergence.

Deposit in a culture collection

The strains were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under the following accession numbers: '*Gracilicoccus phocaeensis*' Marseille-P3801^T (P3801), '*Sediminibacillus massiliensis*' Marseille-P3518^T (3518) and '*Virgibacillus ndiopensis*' Marseille-P3835^T (P3835).

Conflict of interest

None declared.

Acknowledgement

This work has benefited from the French State support, managed by the 'Agence Nationale pour la Recherche' including the "Programme d'Investissement d'avenir" under the reference Méditerranée Infection 10-IAHU-03.

References

- [1] 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.
- [2] Seng P, Abat C, Rolain JM, Colson P, Lagier JC, Gouriet F, et al. Identification of rare pathogenic bacteria in a clinical microbiology michel laboratory: impact of matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2013;51: 2182–94.
- [3] Lo CI, Fall B, Sambe-Ba B, Diawara S, Gueye MW, Mediannikov O, et al. MALDI-TOF mass spectrometry: a powerful tool for clinical microbiology at Hôpital Principal de Dakar, Senegal (West Africa). *PLoS One* 2015;10:e0145889.
- [4] Senghor B, Seck EH, Khelaifia S, Bassène H, Sokhna C, Threener PE, et al. Description of '*Bacillus dakarensis*' sp. nov., '*Bacillus sinesalemensis*' sp. nov., '*Gracilicoccus timonensis*' sp. nov., '*Halobacillus massiliensis*' sp. nov., '*Lentibacillus massiliensis*' sp. nov., '*Oceanobacillus senegalensis*' sp. nov., '*Oceanobacillus timonensis*' sp. nov., '*Virgibacillus dakarensis*' sp. nov. and '*Virgibacillus marseillensis*' sp. nov., nine halophilic new species isolated from human stool. *New Microbes New Infect* 2017;17:45–51.
- [5] Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *Bacteriology* 1991;173:697–703.
- [6] Ramasamy D, Mishra AK, Lagier JC, Padmanabhan 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.
- [7] Chamroensaksri N, Tanasupawat S, Akaracharanya A, Visessanguan W, Kudo T, et al. *Gracilicoccus thailandensis* sp. nov., from fermented fish (pla-ra). *Int J Syst Evol Microbiol* 2010;60:944–8.
- [8] Wang X, Xue Y, Ma Y. *Sediminibacillus albus* sp. nov., a moderately halophilic, Gram-positive bacterium isolated from a hypersaline lake, and emended description of the genus *Sediminibacillus*. Carrasco et al. 2008. *Int J Syst Evol Microbiol* 2009;59(pt. 7):1640–4.
- [9] Peng QZ, Chen J, Zhang YQ, Chen QH, Peng DJ, Cui XL, et al. *Virgibacillus zhanjiangensis* sp. nov., a marine bacterium isolated from sea water. *Antonie Van Leeuwenhoek* 2009;96:645–52.