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

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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.
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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 5000*g*. 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-

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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 in 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,

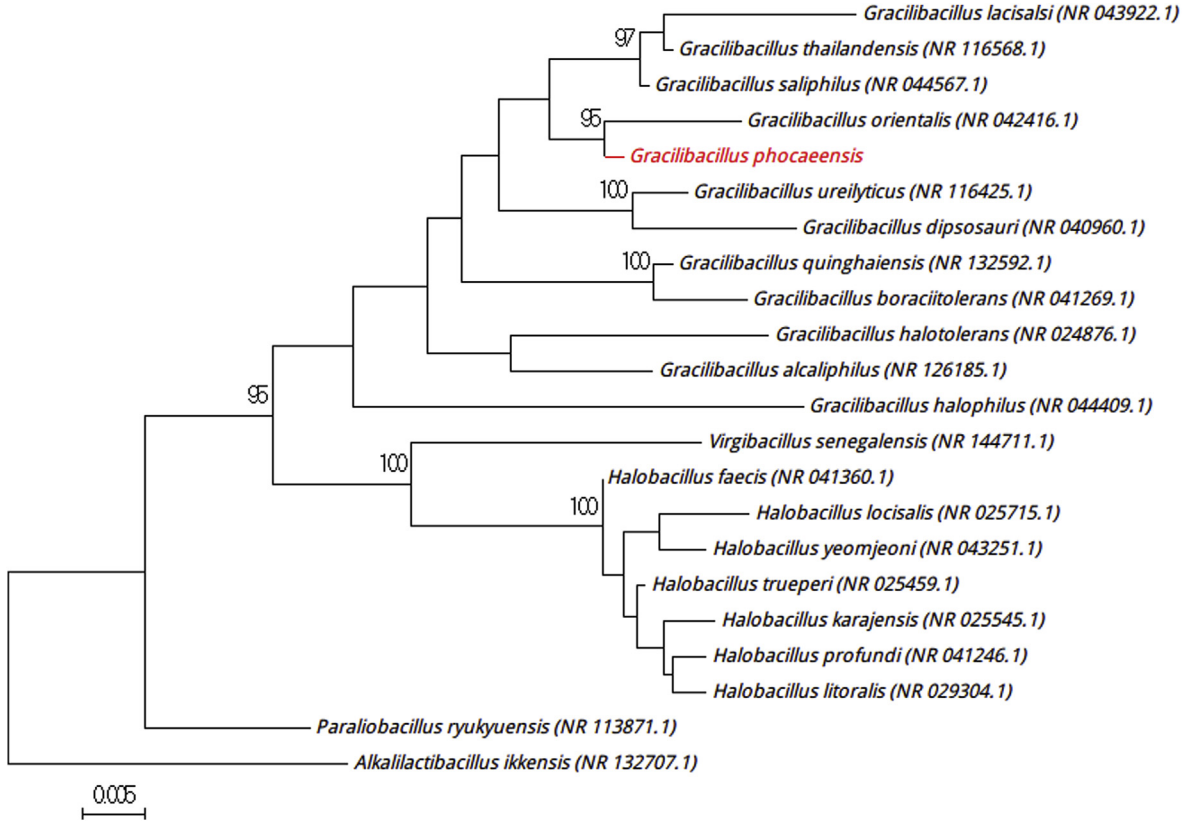

FIG. 1. Phylogenetic tree showing position of '*Gracilibacillus phocaeensis*' Marseille-P3801^T relative to other phylogenetically close neighbours. 16S rRNA gene sequences were aligned using ClustalW, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers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

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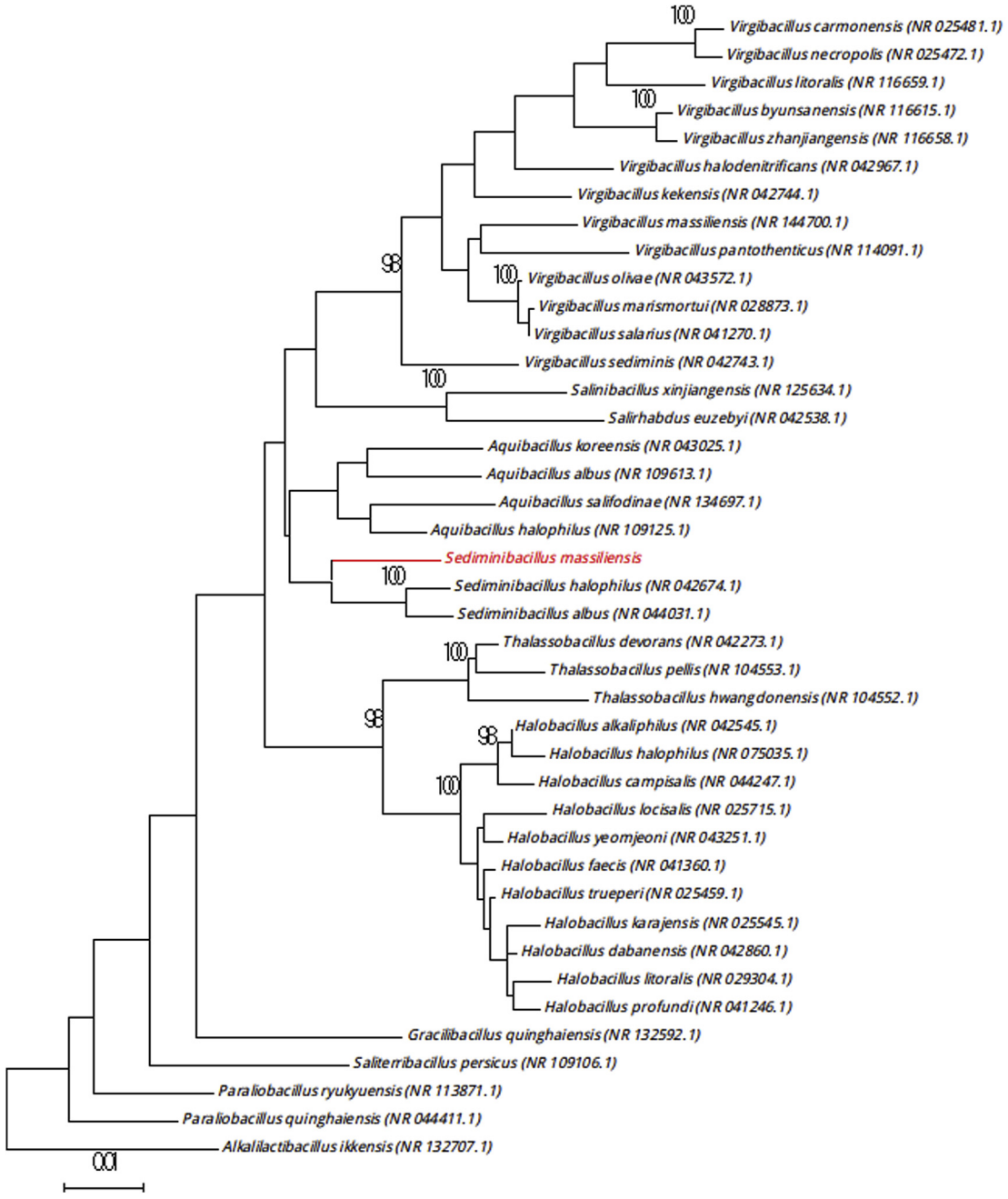

FIG. 2. Phylogenetic tree showing position of '*Sediminibacillus massiliensis*' Marseille-P3518^T relative to other phylogenetically close neighbours. Sequences alignment and phylogenetic inferences were realized as explained in Fig. 1. Scale bar represents 0.01 nucleotide sequence divergence.

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).

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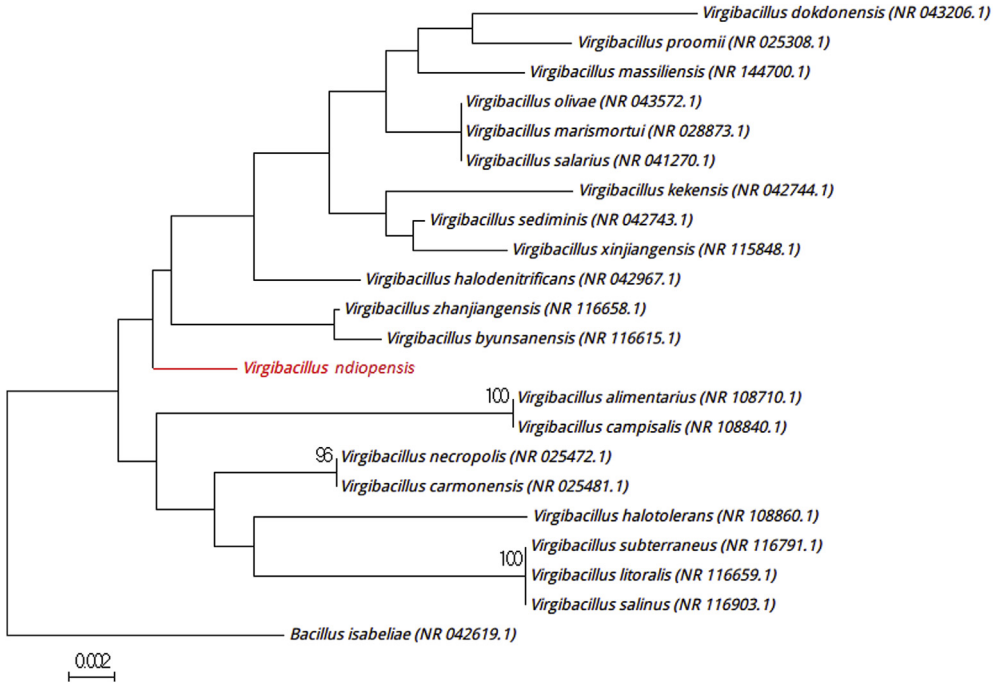

FIG. 3. Phylogenetic tree showing position of '*Virgibacillus ndiopensis*' Marseille-P3835^T relative to other phylogenetically close neighbours. Sequences alignment and phylogenetic inferences were realized as explained for Fig. 1. Scale bar represents 0.002 nucleotide sequence divergence.

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: '*Gracilibacillus phocaeensis*' Marseille-P3801^T (P3801), '*Sediminibacillus massiliensis*' Marseille-P3518^T (3518) and '*Virgibacillus ndiopensis*' Marseille-P3835^T (P3835).

Conflict of interest

None declared.

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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, Threenier PE, et al. Description of *'Bacillus dakarensis'* sp. nov., *'Bacillus sinesaloumensis'* sp. nov., *'Gracilibacillus 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, Padhmanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 2014;64:384–91.

[7] Chamroensaksri N, Tanasupawat S, Akaracharanya A, Visessanguan W, Kudo T, et al. *Gracilibacillus 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.

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