Virgibacillus ndiopensis sp. nov., a new halophilic bacterium isolated from the stool of a healthy 11-year-old boy

R. Francis[1,2], H. Anani[1,3], A. Fontanini[1], P. E. Fournier[1,3], B. L. A. Scola[1,2], D. Raoult[1,2,4] and J. Y. Bou Khalil[1]

1) *Institut Hospitalo-Universitaire Méditerranée-Infection,* 2) *Aix-Marseille Université, Institut de Recherche pour le Développement (IRD), UMR Microbes Evolution Phylogeny and Infections (MEPHI),* 3) *Aix-Marseille Université, Institut de Recherche pour le Développement (IRD), Service de Santé des Armées, AP-HM, UMR Vecteurs Infections Tropicales et Méditeranéennes (VITROME), Marseille, France and* 4) *Special Infectious Agents Unit, King Fahed Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*

Abstract

Virgibacillus ndiopensis strain Marseille-P3835^T (= CSURP3835^T; = CCUG70388^T) is a new specie isolated from the stool of a healthy 11-year-old boy from N'Diop, Senegal.

Keywords: Culturomics, taxonogenomics, *Virgibacillus ndiopensis*
Original Submission: 4 February 2019; **Revised Submission:** 25 March 2019; **Accepted:** 28 March 2019
Article published online: 10 April 2019

Corresponding author: J. Y. Bou Khalil, Aix-Marseille Université, IRD, APHM, MEPHI, IHU-Méditerranée Infection, 19-21 boulevard Jean Moulin, 13005 Marseille, France.
E-mail: boukhaliljacques@gmail.com

Introduction

Culturomics is the concept of developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once a bacterium was isolated, we used a taxonogenomics approach including MALDI-TOF MS, phylogenetic analysis, main phenotypic description and genome sequencing to describe it [5,6].

Isolation and Growth Conditions

In 2017, we isolated from the salty stool sample of a healthy 11-year-old boy an unidentified bacterial strain [7]. A screening was performed by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [8]. The obtained spectra (Fig. 1) was imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in two databases (Bruker and constantly updated MEPHI databases; http://www.mediterranee-infection.com/article.php?larub=280&titre=urms-database). The study was validated by the ethics committee of Institut Fédératif de Recherche IFR48 under number 2016-011. The initial growth was obtained 24 hours after culture in a halophilic modified Colombia broth medium (Sigma-Aldrich, Saint-Quentin-Fallavier, France) with 15% (w/v) NaCl under aerobic conditions at 37°C.

Phenotypic Characteristics

Colonies were pink in colour and circular in shape, with a mean diameter of 1 mm. Bacterial cells were Gram positive and rod-shaped, ranging from 1.54 to 3.04 µm in length and from 0.35 to 0.48 µm in width (Fig. 2). Strain Marseille-P3835^T showed catalase-positive and oxidase-positive activities. API 50 CH and API ZYM tests were performed at 37°C under aerobic conditions (Table 1). Table 2 compares the main biochemical characteristics of the closest *Virgibacillus* species with standing in nomenclature. The main characteristics of this strain are summarized in Fig. 3.

https://doi.org/10.1016/j.nmni.2019.100541

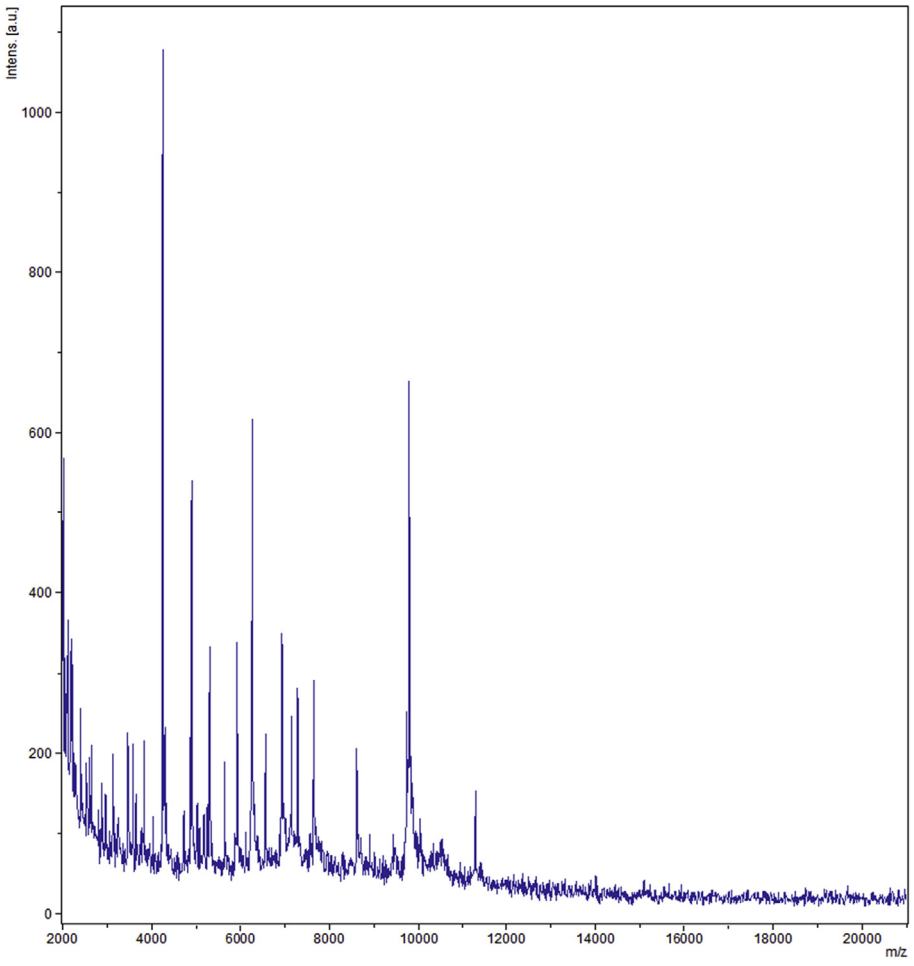

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and reference spectrum was generated.

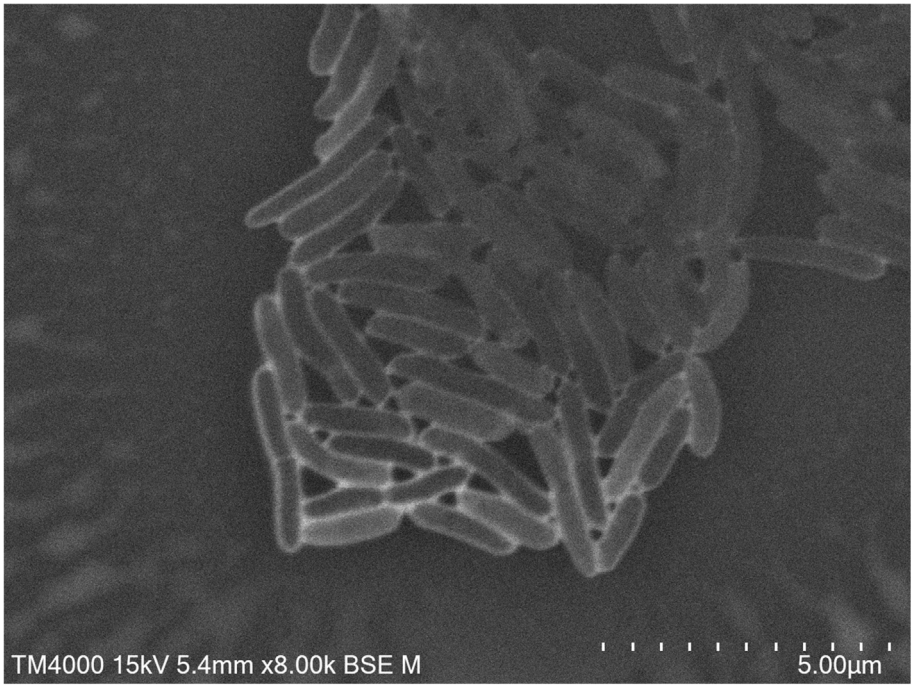

FIG. 2. Scanning electron micrograph of *Virgibacillus ndiopensis* strain Marseille P3835^{T} using Hitachi TM4000 microscope. Scale bar and acquisition settings are shown on original micrograph.

TABLE 1. Phenotypic characterization of *Virgibacillus ndiopensis* based on analytical profile index (API)

Test	Characteristic	Result
API 50 CH	Control	—
	Glycerol	—
	Erythrol	—
	D-Arabinose	—
	L-Arabinose	—
	D-Ribose	—
	D-Xylose	—
	L-Xylose	—
	D-Adonitol	—
	Methyl-βD-xylopyranoside	—
	D-Galactose	—
	D-Glucose	—
	D-Fructose	+
	D-Mannose	—
	L-Sorbose	—
	L-Rhammose	—
	Dulcitol	—
	Inositol	—
	D-Mannitol	—
	D-Sorbitol	—
	Methyl-αD-mannopyranoside	—
	Methyl-αD-glucopyranoside	—
	N-acetylglucosamine	—
	Amygdaline	—
	Arbutine	—
	Esculine	+
	Salicine	—
	D-Cellobiose	—
	D-Maltose	—
	D-Lactose	—
	D-Melibiose	—
	D-Saccharose	—
	D-Trehalose	—
	Inuline	—
	D-Melezitose	—
	D-Raffinose	—
	Amidon	—
	Glycogene	—
	Xylitol	—
	Gentibiose	—
	D-Turanose	—
	D-Lyxose	—
	D-Tagatose	—
	D-Fucose	—
	L-Fucose	—
	D-Arabitol	—
	L-Arabitol	—
	Potassium gluconate	—
	Potassium 2-cetogluconate	—
	Potassium 5-cetogluconate	+
API ZYM	Control	—
	Alkaline phosphatase	+
	Esterase (C 4)	+
	Esterase Lipase (C 8)	+
	Lipase (C 14)	—
	Leucine arylamidase	—
	Valine arylamidase	—
	Cystine arylamidase	—
	Trypsine	—
	α-Chymotrypsine	—
	Acid phosphatase	+
	Naphtalo-AS-BI-phosphohydrolase	+
	α-galactosidase	—
	β-Galactosodase	—
	β-Glucuronidase	—
	α-Glucosidase	—
	β-Glucosidase	—
	N-acetyl-β-glucosaminidase	—
	α-Mannosidase	—
	α-Fucosidase	—

Strain Identification

The 16S ribosomal RNA (rRNA) gene was sequenced in order to classify this bacterium. Amplification and sequencing were performed using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and the Big Dye Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary 3500xL Genetic Analyzer capillary sequencer (Thermo Fisher, Saint-Aubin, France), respectively, as previously described [9]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com). Strain Marseille-P3835^T exhibited a 97.63% sequence identity with *Virgibacillus necropolis* strain LMG 19488 (GenBank accession no. NR025472), the phylogenetically closest species with standing in nomenclature (Fig. 4). Consequently, *Virgibacillus ndiopensis* was classified as a new member

TABLE 2. Biochemical characteristics of *Virgibacillus* species

Characteristic	1	2	3	4	5	6	7	8
D-Galactose	—	+	—	+	+	+	—	—
D-Glucose	—	+	—	+	+	+	+	w
D-Fructose	+	+	—	+	+	+	+	—
D-Mannose	—	+	—	+	+	NA	w	—
D-Melibiose	—	—	—	—	—	NA	—	NA
D-Trehalose	—	—	+	+	+	NA	w	—
D-Mannitol	—	—	—	—	v	+	—	—
L-Rhamnose	—	—	+	v	—	+	—	NA
D-Turanose	—	NA	+	—	NA	NA	—	NA
D-Arabinose	—	NA	+	—	NA	NA	—	NA
L-Fucose	—	NA	+	—	NA	NA	—	NA
N-acetylglucosamine	—	NA	+	+	NA	NA	w	—
5-Keto-D-gluconate	+	NA	—	—	NA	NA	w	NA
Amygdaline	—	NA	+	—	NA	NA	—	NA
Glycerol	—	NA	+	—	NA	NA	w	NA
Glycogen	—	NA	—	v	NA	NA	—	NA
Inositol	—	NA	—	+	NA	NA	—	NA

(1) *Virgibacillus ndiopensis*, (2) *Virgibacillus dokdonensis*, (3) *Virgibacillus pantothenticus*, (4) *Virgibacillus proomii*, (5) *Virgibacillus halodentrificans*, (6) *Virgibacillus salinus*, (7) *Virgibacillus necropolis*, (8) *Virgibacillus soli*.
+, positive result; −, negative result; v, variable result; w, weakly positive result; NA, data not available.

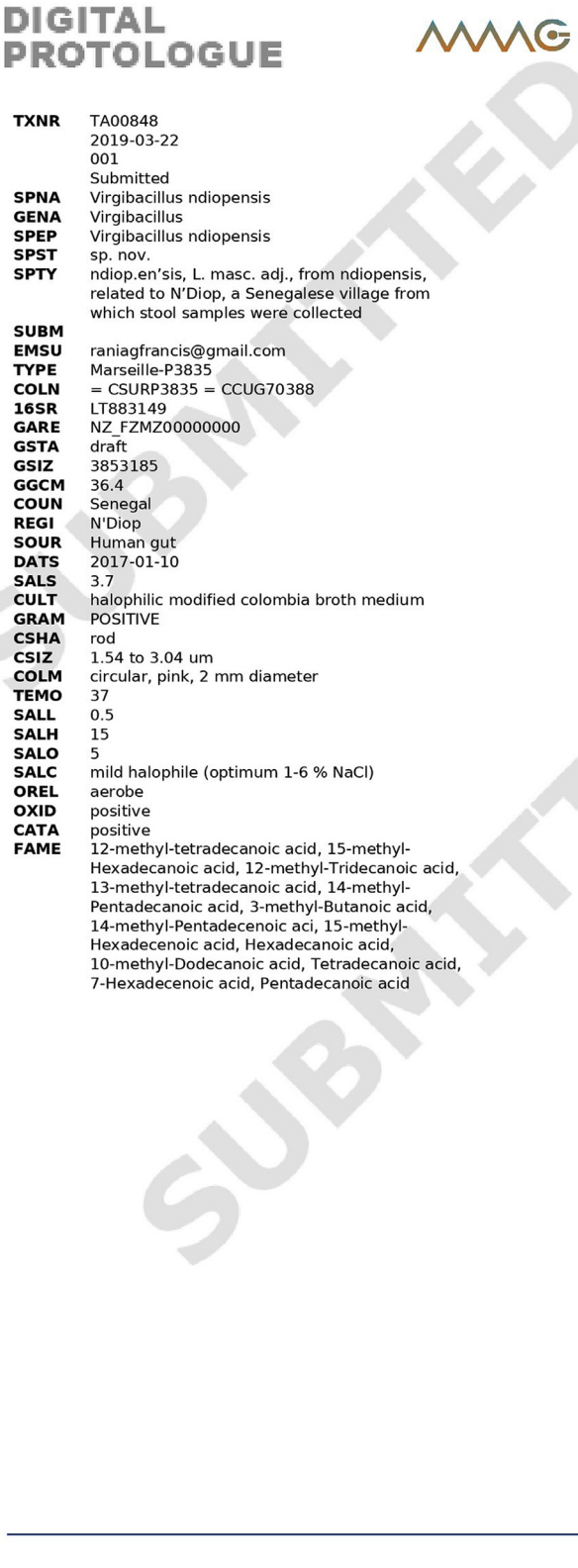

FIG. 3. Description of *Virgibacillus ndiopensis* strain Marseille P3835^T according to digital protologue TA00848 online (www.imedea.uib.es/dprotologue).

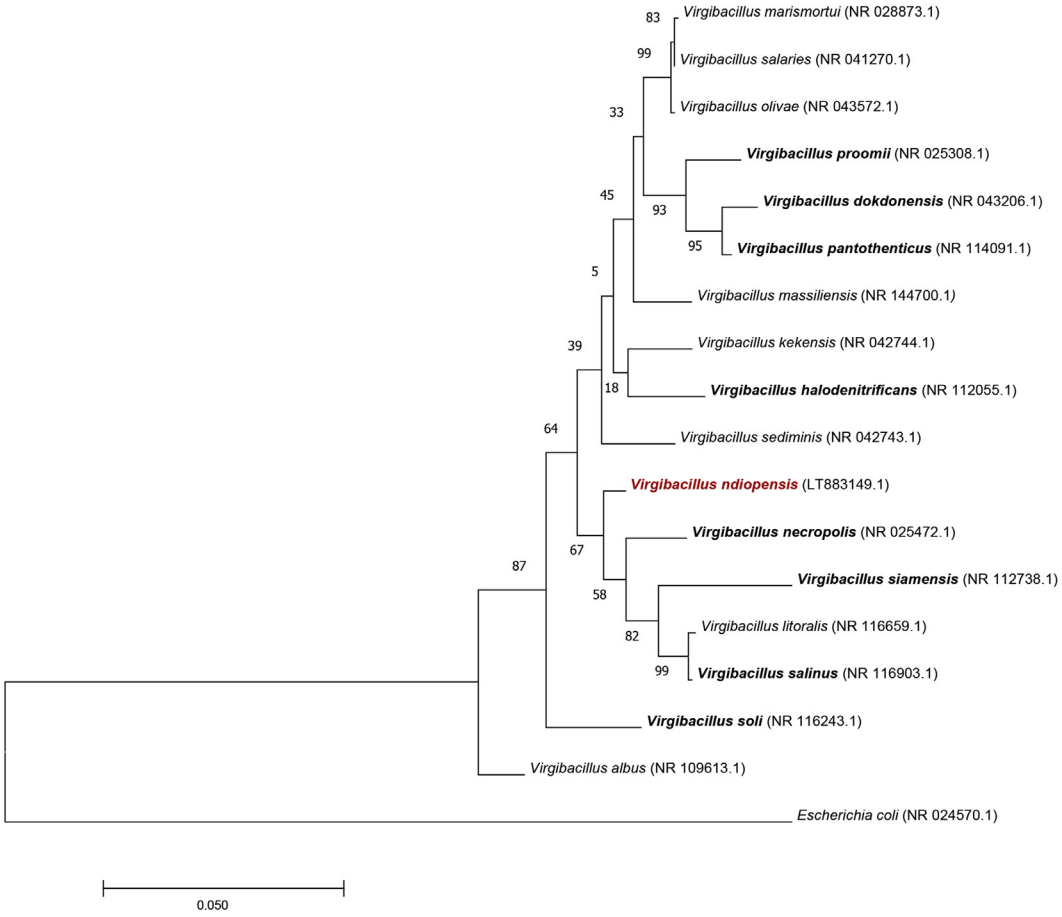

FIG. 4. Phylogenetic tree showing position of *Virgibacillus ndiopensis* strain Marseille P3835^T relative to other phylogenetically close neighbours. Respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences alignment and phylogenetic inferences were obtained using maximum likelihood method within MEGA 7 software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree.

of the genus *Virgibacillus,* family *Bacillaceae,* phylum *Firmicutes,* with the stain Marseille P3835^T as the type strain of the new species *Virgibacillus ndiopensis.*

Genome Sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit, then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit (Illumina), as previously described [10]. Genome assembly was performed with a pipeline incorporating different software packages (Spades [11]), on trimmed (Trimmomatic [12]) or raw data. GapCloser was used to reduce assembly gaps. Scaffolds of <800 bp and scaffolds with a depth value lower than 25% of the mean depth were removed. The best assembly was selected by using different criteria (six scaffolds, six contigs). The genome of strain Marseille-P3835^T is 3 853 185 bp long with a 36.4 mol% G+C content. The degree of genomic similarity of Marseille-P3835^T with closely related species was estimated by OrthoANI software [13]. Values among closely related species (Fig. 5) ranged from 66.25% between *Virgibacillus soli* and *Virgibacillus siamensis* to 81.00% between *Virgibacillus dokdonensis* and *Virgibacillus pantothenticus.* When the isolate was compared to these closely species, values ranged from 67.00% with *Virgibacillus soli* to 73.79% with *Virgibacillus salinus.*

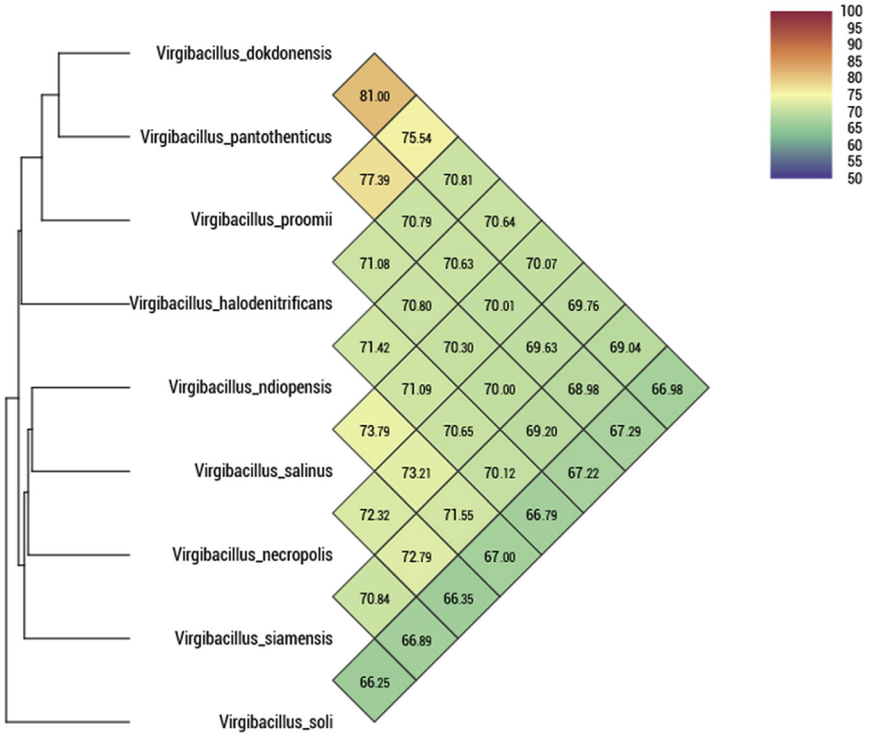

FIG. 5. Heat map generated with OrthoANI values calculated using OAT software between *Virgibacillus ndiopensis* and other closely related species with standing in nomenclature.

Conclusion

Strain Marseille-P3835^T, exhibiting a 16S rRNA sequence divergence < 98.65% with its phylogenetically closest species with standing in nomenclature, is consequently proposed as the type strain of the new species *Virgibacillus ndiopensis* sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession number LT883149 and FZMZ00000000, respectively.

Deposit in a culture collection

Strain Marseille-P3835^T was deposited in two different strain collections (= CSURP3835 = CCUG70388).

Conflict of Interest

None declared.

Acknowledgements

We sincerely thank S. Taku, T. Irie, K. Imai, S. Matsubara, A. Hisada and all Hitachi team members in Japan for the collaborative study with HHT and IHU, and for the installation of and services on the TM4000 microscope in our facility. The study was supported by the Méditerranée Infection foundation, the National Research Agency under the programme 'Investissements d'avenir' (ANR-10-IAHU-03) and by Région Provence Alpes Côte d'Azur and European funding (FEDER PRIMI).

References

[1] Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185–93.
[2] Lagier JC, Hugon P, Khelaifia S, Fournier PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 2015;28:237–64.

[3] 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.
[4] Lagier JC, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev 2015;28:208–36.
[5] Fournier PE, Lagier JC, Dubourg G, Raoult D. From culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. Anaerobe 2015;36:73–8.
[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(pt 2):384–91.
[7] Senghor B, Khelaifia S, Bassène H, Seck EH, Fournier PE, Sokhna C, 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 Microbe New Infect 2017;20:51–4.
[8] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51.
[9] Morel AS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta JP, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis 2015;34:561–70.
[10] Diop A, Khelaifia S, Armstrong N, Labas N, Fournier PE, Raoult D, et al. Microbial culturomics unravels the halophilic microbiota repertoire of table salt: description of *Gracilibacillus massiliensis* sp. nov. Microb Ecol Health Dis 2016;27:32049.
[11] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.
[12] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
[13] Ouk Kim Y, Chun J, Lee I, Park SC. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.