

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

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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.

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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.

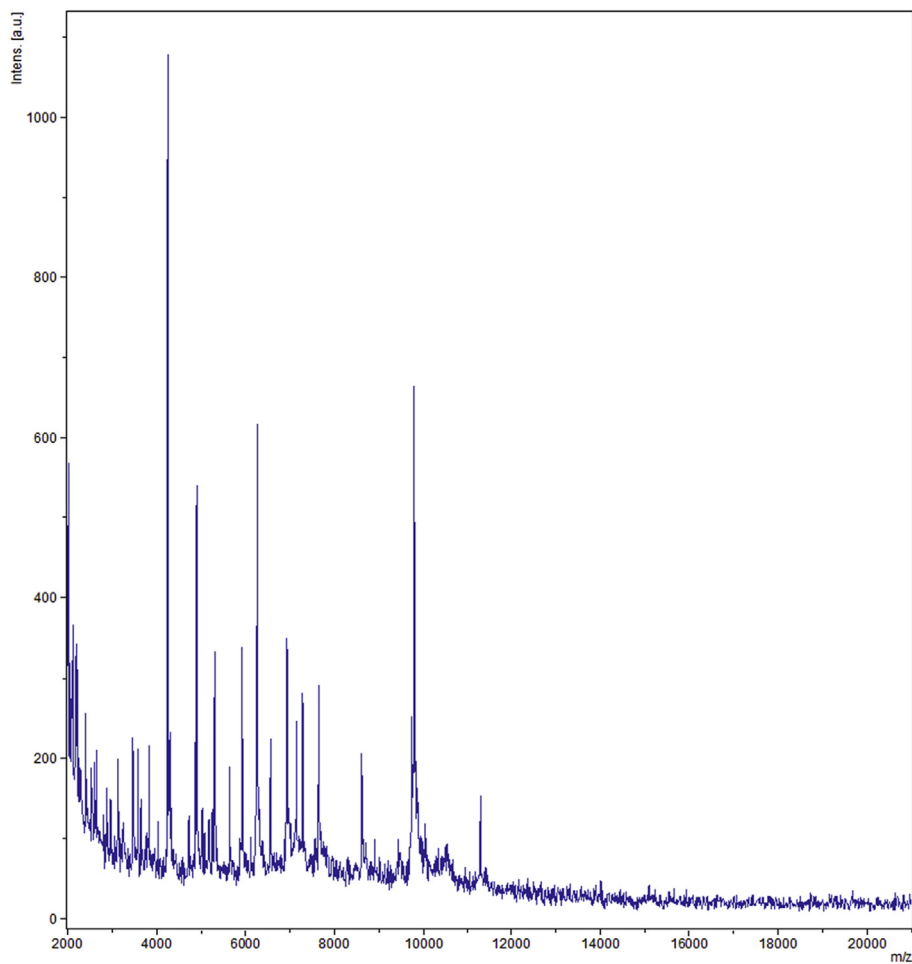


FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and reference spectrum was generated.

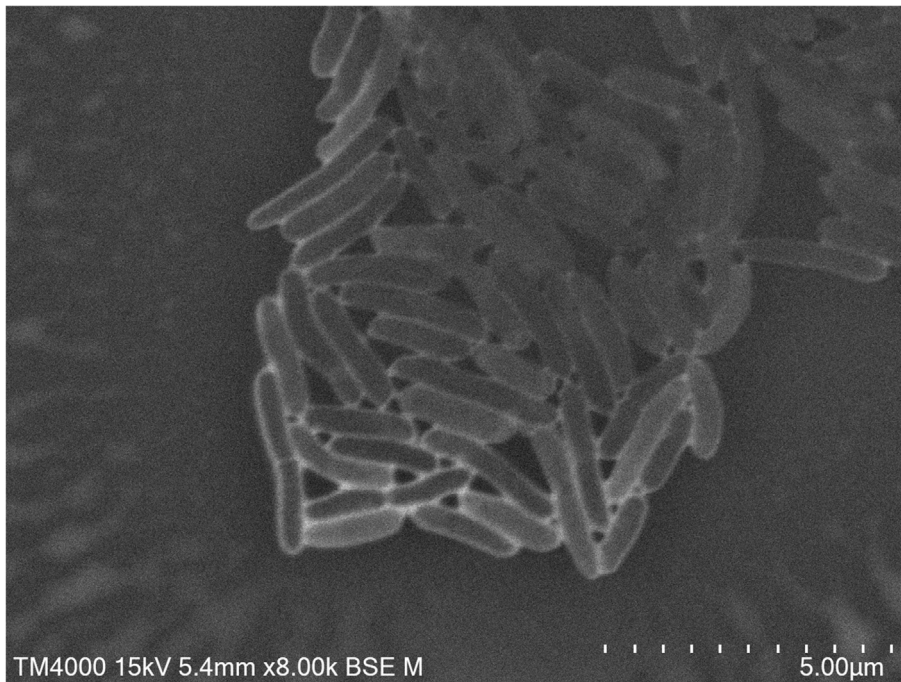


FIG. 2. Scanning electron micrograph of *Virgibacillus ndiopenis* strain Marseille P3835^T using Hitachi TM4000 microscope. Scale bar and acquisition settings are shown on original micrograph.

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-Rhamnose	—
	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	—

TABLE 1. Continued

Test	Characteristic	Result
API ZYM	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	+
	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-Galactosidase	—
	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 pantothenicus*, (4) *Virgibacillus proomii*, (5) *Virgibacillus halodentificans*, (6) *Virgibacillus salinus*, (7) *Virgibacillus necropolis*, (8) *Virgibacillus soli*.

+, positive result; —, negative result; v, variable result; w, weakly positive result; NA, data not available.

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TXNR TA00848
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 001
 Submitted
SPNA *Virgibacillus ndiopensis*
GENA *Virgibacillus*
SPEP *Virgibacillus ndiopensis*
SPST sp. nov.
SPTY ndiop.en'sis, L. masc. adj., from ndiopensis,
 related to N'Diop, a Senegalese village from
 which stool samples were collected
SUBM
EMSU raniagfrancis@gmail.com
TYPE Marseille-P3835
COLN = CSURP3835 = CCUG70388
16SR LT883149
GARE NZ_FZMZ00000000
GSTA draft
GSIZ 3853185
GGCM 36.4
COUN Senegal
REGI N'Diop
SOUR Human gut
DATS 2017-01-10
SALS 3.7
CULT halophilic modified colombia broth medium
GRAM POSITIVE
CSHA rod
CSIZ 1.54 to 3.04 um
COLM circular, pink, 2 mm diameter
TEMO 37
SALL 0.5
SALH 15
SALO 5
SALC mild halophile (optimum 1-6 % NaCl)
OREL aerobe
OXID positive
CATA positive
FAME 12-methyl-tetradecanoic acid, 15-methyl-
 Hexadecanoic acid, 12-methyl-Tridecanoic acid,
 13-methyl-tetradecanoic acid, 14-methyl-
 Pentadecanoic acid, 3-methyl-Butanoic acid,
 14-methyl-Pentadecenoic aci, 15-methyl-
 Hexadecenoic acid, Hexadecanoic acid,
 10-methyl-Dodecanoic acid, Tetradecanoic acid,
 7-Hexadecenoic acid, Pentadecanoic acid

1 / 1

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

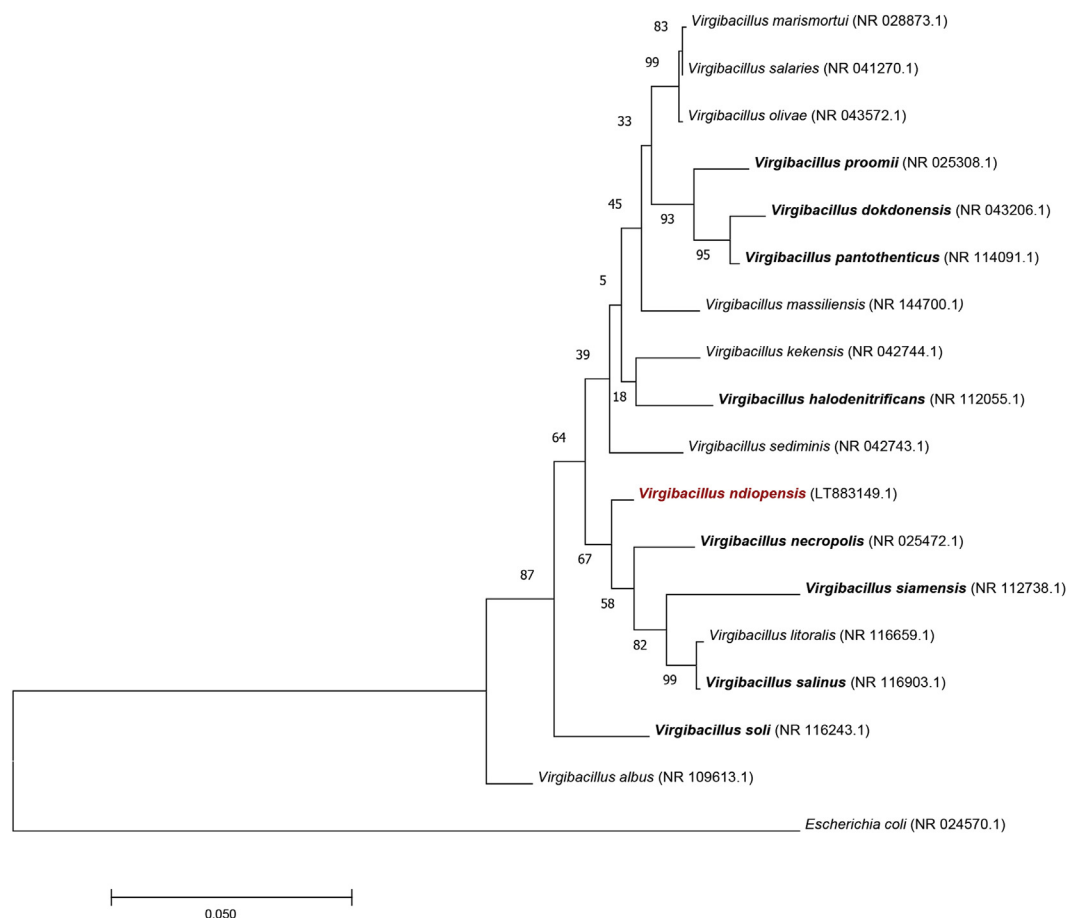


FIG. 4. Phylogenetic tree showing position of *Virgibacillus ndiopensis* strain Marseille P3835^T relative to other phylogenetically close neighbours. Respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences alignment and phylogenetic inferences were obtained using 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 strain Marseille P3835^T as the type strain of the new species *Virgibacillus ndiopensis*.

Genome Sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qia-gen, 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 pantothenicus*. When the isolate was compared to these closely species, values ranged from 67.00% with *Virgibacillus soli* to 73.79% with *Virgibacillus salinus*.

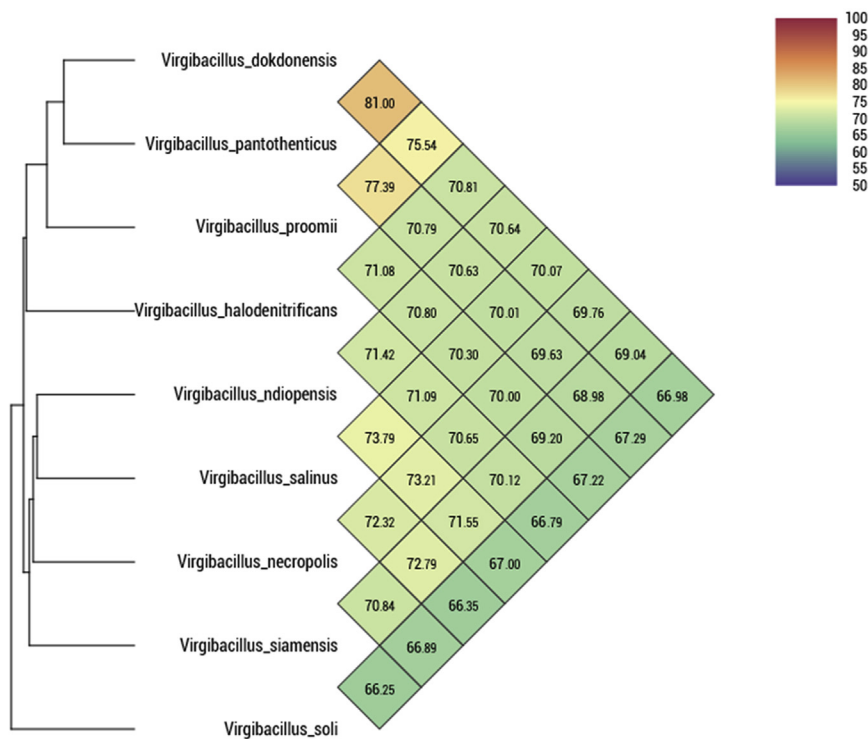


FIG. 5. Heat map generated with OrthoANI values calculated using OAT software between *Virgibacillus ndiopensis* and other closely related species with standing in nomenclature.

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

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