Virgibacillus senegalensis sp. nov., a new moderately halophilic bacterium isolated from human gut

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Abstract

Virgibacillus senegalensis SK-1^T (= CSUR P1101 = DSM 28585) is the type strain of *V. senegalensis* sp. nov. It is an aerobic, Gram positive, moderately halophilic, motile bipolar flagellum isolated from a healthy Senegalese man. Here we describe the genomic and phenotypic characteristics of this isolate. The 3 755 098 bp long genome (one chromosome, no plasmid) exhibits a G + C content of 42.9% and contains 3738 protein-coding and 95 RNA genes.

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Introduction

The concept of microbial culturomics is based on the variation of physicochemical parameters of the culture conditions so as to express the maximum of microbial diversity. It is based on rapid methods for identification, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) and 16S rRNA amplification and sequencing for unidentified colonies. This concept considerably enriches the gut microbiota repertoire, including new species not previously isolated from humans [1,2].

This isolation was part of a culturomics study we undertook using high-salt-containing culture conditions to grow halophilic bacteria from human stool [1].

The typical parameters used to define bacterial species comprise 16S rRNA sequencing and phylogeny, G + C content genomic diversity and DNA-DNA hybridization (DDH). However, some limitations have been noted [3–6]. By using the availability of data in genomics through the development of new tools for sequencing DNA, we introduced a new taxonomic method for the description of new bacterial species. This concept, which we named taxonogenomics, includes their genomic features [7] and proteomic information obtained by MALDI-TOF analysis [8–17].

The genus *Virgibacillus* was first proposed by Heyndrickx in 1998 with the transfer of *Bacillus pantothenticus* to *Virgibacillus pantothenticus* [18]. To date, there are more than 25 recognized species [19]. These bacteria are positive, Gram-variable rods which are ellipsoidal to oval endospores and have DNA G + C content ranging from 36% to 43% [20]. These species were isolated from sediments of a salt lake [20–23], fermented seafood in traditional salt [24], a permafrost core collected from the Canadian high Arctic [25], a navy solar salt marsh [26,27], soil [28], seawater [29], field soil, a dairy product [30], residual wash water produced during processing wastewater, Spanish-style green table olives [31], saline sample of mud, salt

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crust [32] and Thai fermented fish [33]. Here we present a brief classification and a set of features for strain SK-1T (= CSUR P1101 = DSM 28585), with a description of the complete genome sequence and annotation. We named this new isolate *Virgibacillus senegalensis*.

Materials and Methods

Sample and culture condition

The stool sample was collected from a healthy male Senegalese volunteer patient living in N'diop, a rural village in the Guinean–Sudanian zone in Senegal. After the patient provided signed informed consent, the sample was collected in a sterile pot and transported to our laboratory. The study and the assent procedure were approved by the National Ethics Committee of Senegal and by the ethics committees of the Institut Fédératif de Recherche 48, Faculty of Medicine, Marseille, France (agreement 09-022). The salt concentration of the stool specimen was determined by a digital refractometer (Fisher Scientific, Illkirch, France) and the pH with a pH meter (Cyberscan 510PH; Eutech Instruments, Singapore).

Strain SK-1T was isolated in February 2014 by aerobic culture on a homemade culture medium consisting of a Columbia agar culture medium (Sigma-Aldrich, Saint-Quentin Fallavier, France) modified by adding (per liter): $MgCl_2$ $6H_2O$, 5 g; $MgSO_4$ $7H_2O$, 5 g; KCl, 2 g; $CaCl_2$ $2H_2O$, 1 g; NaBr, 0.5 g; $NaHCO_3$, 0.5 g; glucose, 2 g; and 100 g/L of NaCl. The pH was adjusted to 7.5 with 10 M NaOH before autoclaving.

MALDI-TOF identification

An isolated colony was deposited in duplicate on a MALDI-TOF target to be analysed. A matrix of 1.5 μL (saturated solution of α-cyano-4-hydroxycinnamic acid diluted in 500 μL acetonitrile, 250 μL of acid tri-fluoro-acetic to 10%, and 250 μL of HPLC water) was used on each spot. This solution enables ionization and desorption of the homogeneous biological sample with which it crystallizes. The analysis was performed by a Microflex (Bruker Daltonics, Leipzig, Germany) device, and protein spectra were compared with those of the hospital database. A score was assigned indicating the reliability of the identification of the bacteria; above 1.9 was considered proper identification. Conversely, if the bacterium was not referenced in the database, sequencing the 16S rRNA was used to achieve the correct identification [34].

Identification by sequencing of 16S rRNA

Colonies not identified by the MALDI-TOF after three tests were suspended in 200 μL of distilled water for DNA extraction by EZ1 DNA Tissue Kit (Qiagen, Venlo, The Netherlands). The amplification of the 16S rRNA was performed by standard PCR in a thermocycler using the universal primer pair FD1 and rp2 according to the following amplification program: activation of the polymerase (95°C for 5 minutes), followed by 40 cycles (95°C 30 seconds, 52°C 45 seconds, 72°C 2 minutes), followed by 5 minutes at 72°C. The DNA amplified by this reaction was revealed by electrophoresis on 1.5% agarose gel. Once validated, the PCR product was purified and sequenced using the Big Dye Terminator Sequencing Kit using the internal primers 536F, 536R, 800F, 800R, 1050F and 1050R, as previously described [2].

Phylogenetic analysis

Phylogenetic analysis based on 16S rRNA of our isolates was performed to identify its phylogenetic affiliations with other near isolates, including other members of the genus *Virgibacillus*. MEGA 6 software (http://www.megasoftware.net/mega.php) allowed us to construct a phylogenetic tree. Sequence alignment of the different species was performed using Clustal W (http://www.clustal.org/clustal2/), and the evolutionary distance was calculated with the Kimura two-parameter model [35].

Biochemical, atmospheric and antimicrobial susceptibility tests

Biochemical tests were performed using the commercially available Api ZYM (bioMérieux, Marcy l'Étoile, France), API 50CH (bioMérieux) and 20 NE (bioMérieux) strips. The incubation time was 48 hours for the API 50CH and 20 NE, and 4 hours for Api ZYM. Growth of strain SK-1T was tested in aerobic atmosphere, in the presence of 5% CO_2 and also in anaerobic and microaerophilic atmospheres, created using AnaeroGen (Atmosphere Generation Systems, Dardily, France). Antibiotic susceptibility was determined by Müller-Hinton agar in a petri dish (bioMérieux). The following antibiotics were tested: doxycycline, rifampicin, vancomycin, nitrofurantoin, amoxicillin, erythromycin, ampicillin, ceftriaxone, ciprofloxacin, gentamicin, penicillin, trimethoprim/sulfamethoxazole, imipenem and metronidazole.

Genome Sequencing Information

Genomic DNA preparation

We cultured our strain in the homemade culture. After 48 hours, bacteria grown on four petri dishes were resuspended in sterile water and centrifuged at 4°C at 2000 × *g* for 20 minutes. Cell pellets were resuspended in 1 mL Tris/EDTA/NaCl (10 mM Tris/HCl (pH7.0), 10 mM EDTA (pH8.0) and 300 mM

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