Noncontiguous finished genome sequence and description of *Virgibacillus massiliensis* sp. nov., a moderately halophilic bacterium isolated from human gut

S. Khelaifia[1], O. Croce[1], J.-C. Lagier[1], C. Robert[1], C. Couderc[1], F. Di Pinto[1], B. Davoust[1], F. Djossou[2], D. Raoult[1,3] and P.-E. Fournier[1]

1) *Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, UM 63, CNRS 7278, IRD 198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, Aix-Marseille Université, Marseille, France,* 2) *Centre Hospitalier André Rosemon, Cayenne, French Guiana and* 3) *Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*

Abstract

Strain Vm-5^T was isolated from the stool specimen of a 10-year-old Amazonian boy. This bacterium is a Gram-positive, strictly aerobic rod, motile by a polar flagellum. Here we describe its phenotypic characteristics and complete genome sequence. The 4 353 177 bp long genome exhibits a G + C content of 36.87% and contains 4394 protein-coding and 125 predicted RNA genes. Phylogenetically and genetically, strain Vm-c is a member of the genus *Virgibacillus* but is distinct enough to be classified as a new species. We propose the creation of *V. massiliensis* sp. nov., whose type strain is strain Vm-5^T (CSUR P971 = DSM 28587).

Keywords: Culturomics, genome, human gut, moderately halophilic bacteria, taxonogenomics, *Virgibacillus massiliensis*
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Corresponding author: P.-E. Fournier, URMITE, UMR CNRS 7278, L'Institut de Recherche pour le Développement 198, INSERM U1095, Faculté de Médecine, Aix-Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille Cedex 5, France
E-mail: pierre-edouard.fournier@univ-amu.fr

Introduction

Virgibacillus massiliensis strain Vm-5^T (= CSUR P971 = DSM 28587) is the type strain of *V. massiliensis* sp. nov. This bacterium is a Gram-positive, strictly aerobic rod, motile by a polar flagellum, isolated from the stool specimen of a healthy Amazonian boy as part of the culturomics study aiming at cultivating halophilic bacteria from the human feces using a high-salt-concentration medium [1].

The usual parameters used to delineate a bacterial species include 16S rRNA sequence identity and phylogeny [2,3], genomic G + C content diversity and DNA-DNA hybridization [4,5]. Nevertheless, these methods have limitations, notably because these similarity values vary greatly between species and genera [6]. In addition, chemotaxonomic analyses such as fatty acid profile, cell wall diagnostic diamino acid and sporangium morphology are only performed by a few laboratories, are only partially reproducible and thus are of no practical value to identify clinical isolates. Therefore, we deliberately decided not to use these methods but rather include parameters that could be compared among laboratories, including widely used phenotypic criteria, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) spectrum and genome sequence.

The introduction of high-throughput sequencing techniques has allowed researchers to make genomic data available for many bacterial species [6–10]. We recently proposed a new method (taxonogenomics) consisting in a polyphasic approach to describe new bacterial species [5]. This strategy combines phenotypic characteristics including MALDI-TOF spectrum and genomic analysis [6–10]. Here we present a summary classification and a set of features for the *Virgibacillus massiliensis* sp. nov., strain Vm-5^T (= CSUR P971 = DSM 28587), including the description of its complete genome sequence and annotation. These characteristics support the circumscription of the species *Virgibacillus massiliensis*.

Virgibacillus massiliensis is the first representative from the *Virgibacillus* genus to be isolated from the human intestinal microbiota. The genus *Virgibacillus* was first described by Heyndrickx *et al.* in 1998 and currently consists of mainly Gram-positive, motile, spore-forming, rod-shaped bacteria that are moderately halophilic [11]. Members of the genus *Virgibacillus* are found in various environments including sediment of a saline lake [12–15], traditional salt-fermented seafood [16], a permafrost core collected from the Canadian high Arctic [17], a marine solar saltern [18–21], biofilm formation on mural paintings [22], seawater [23,24], field soil, a dairy product sample [25], a saline mud sample [26], residual wash water

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produced during the processing of Spanish-style green table olive sewage [27], salt crust [28] and fermented fish [29].

Organism Information

Classification and features

Stool specimens were collected from a 10-year-old Amazonian boy, formed into aliquots and stored at −80°C until use. The child and his parents provided informed consent. The study and the assent procedure were approved by the ethics committees of the Institut Fédératif de Recherche 48, Faculty of Medicine, Marseille, France, under agreement 09-022. The salt concentration of the stool specimen was determined using a digital refractometer (Fisher Scientific, Illkirch, France) and the pH with a pH meter (Table 1).

Strain Vm-5^T (Table 1) was isolated in December 2013 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) the following: $MgCl_2$ $6H_2O$, 5 g; $MgSO_4$ $7H_2O$, 5 g; KCl, 2 g; $CaCl_2$ 2H2O, 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. Strain Vm-5^T (GenBank accession number HG931931) exhibited a 16S rRNA sequence identity of 97.3% with *Virgibacillus olivae* strain $E_{30}8^T$ (NR043572), its phylogenetically closest bacterial species with standing in nomenclature (Fig. 1).

TABLE 1. Classification and general features of *Virgibacillus massiliensis* strain Vm-5^T according to MIGS recommendations [30].

MIGS ID	Property	Term	Evidence code[a]
	Current classification	Domain: *Bacteria*	TAS [31]
		Phylum: *Firmicutes*	TAS [32–34]
		Class: *Bacilli*	TAS [35,36]
		Order: *Bacillales*	TAS [37–39]
		Family: *Bacillaceae*	TAS [38–41]
		Genus: *Virgibacillus*	TAS [11]
		Species: *Virgibacillus massiliensis*	IDA
		Type strain: Vm-5^T	IDA
	Gram stain	Positive	IDA
	Cell shape	Rod shaped	IDA
	Motility	Motile by polar flagellum	IDA
	Sporulation	Endospore forming	IDA
	Temperature range	Mesophile	IDA
	Optimum temperature	37°C	IDA
	pH	pH 5 to 9	
	Optimum pH	7.5	
MIGS-6.3	Salinity	0.5–20%	IDA
	Optimum salinity	5%	IDA
MIGS-22	Oxygen requirement	Aerobic	IDA
	Carbon source	Unknown	IDA
	Energy source	Unknown	IDA
MIGS-6	Habitat	Human gut	IDA
MIGS-15	Biotic relationship	Free-living	IDA
	Pathogenicity	Unknown	NAS
	Biosafety level	2	IDA
MIGS-14	Isolation	Human feces	IDA
MIGS-4	Geographic location	France	IDA
MIGS-5	Sample collection time	December 2013	IDA
MIGS-4.1	Latitude	4.916667	IDA
MIGS-4.1	Longitude	−52.316666	IDA
MIGS-4.3	Depth	Surface	IDA
MIGS-4.4	Altitude	0 m above sea level	IDA

MIGS, minimum information about a genome sequence.
[a]Evidence codes are as follows: IDA, inferred from direct assay; TAS, traceable author statement (i.e., direct report exists in the literature); NAS, nontraceable author statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species or on anecdotal evidence). These evidence codes are form the Gene Ontology project (http://www.geneontology.org/GO.evidence.shtml) [42]. If the evidence is IDA, then the property was directly observed for a live isolate by one of the authors or an expert mentioned in the acknowledgements.

Colonies were obtained on our homemade culture medium after 24 hours of incubation in aerobic conditions at 37°C. The colonies of strain Vm-5^T were circular, greyish, shiny and smooth, with a diameter of 2 to 5 mm. Cells stained Gram positive (Fig. 2). They were motile by polar flagella, were terminal spore forming and most commonly occurred as single cells or in pairs. Colonies were not haemolytic on blood-enriched agar.

Strain Vm-5 ^{T} was mesophilic and grew at temperatures ranging from 15 to 45°C, at an optimum temperature of 37°C. The isolate required NaCl for growth and grew at salinity ranging from 5 to 200 g/L of NaCl (optimum at 50 g/L). The optimal pH for growth was 7.5 (pH range 5 to 9). The growth of strain Vm-5^T was tested under aerobic atmosphere, in the presence of 5% CO_2 and in anaerobic and microaerophilic atmospheres created using GENbag anaer and GENbag microaer (bioMérieux, Marcy l'Etoile, France), respectively. The strain was strictly aerobic and grew in the presence of 5% CO_2 but did not grow in microaerophilic or anaerobic atmosphere. The size (2 to 6 µm in length and 0.5 µm in diameter) and ultrastructure of cells were determined by negative staining transmission electron microscopy (Fig. 3).

The commercially available Api ZYM, Api 20NE (bioMérieux), was used to characterize the biochemical properties of the strain according to the manufacturer's instructions. The strain was incubated at 37°C for 24 hours. Api 50 CH strips were inoculated with a bacterial suspension in Api 50CHB/E medium supplemented by 10% NaCl (w/v) and incubated at 37° C for 48 hours. *Virgibacillus massiliensis* strain Vm-5^T exhibited catalase and oxidase activities. Negative reactions were observed for alkaline phosphatase, galactosidase, N-acetyl-β-glucosaminidase and urease activities. A positive reaction was observed for nitrate reduction. Substrate oxidation and assimilation were examined using an API 50CH strip (bioMérieux) at 37°C. Negative reactions were obtained for D-lactose, L-arabinose, D-galactose and D-ribose. Positive reactions were obtained for D-glucose, D-fructose, D-mannose, D-mannitol, D-maltose and D-sucrose. Phenotypic characteristics were compared to those of the most closely related species (Table 2). *Virgibacillus massiliensis* differed from other *Virgibacillus* species based on its use of nitrate reductase (+), N-acetyl-glucosamine (−), D-mannose (+), D-sucrose (+) and D-maltose (+).