Taxonogenomics and description of *Vaginella massiliensis* gen. nov., sp. nov., strain Marseille P2517^T, a new bacterial genus isolated from the human vagina

K. Diop[1], F. Bretelle[2], C. Michelle[1], M. Richez[1], J. Rathored[1], D. Raoult[1,3], P.-E. Fournier[1] and F. Fenollar[1]

1) *Institut hospitalo-universitaire Méditerranée-infection, Aix-Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, Inserm U1095, Faculté de médecine,* 2) *Department of Gynecology and Obstetrics, Gynépole, Aix-Marseille Université, Hôpital Nord, Assistance Publique-Hôpitaux de Marseille, Marseille, France and* 3) *Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*

Abstract

An obligate aerobic, Gram-negative, nonmotile and nonsporulating rod designated Marseille P2517 was isolated from the vaginal flora. We describe its features, annotate the genome and compare it to the closest species. The 16S rRNA analysis shows 93.03% sequence similarity with *Weeksella virosa,* the phylogenetically closest species. Its genome is 2 434 475 bp long and presents 38.16% G+C. On the basis of these data, it can be considered as a new genus in the *Flavobacteriaceae* family, for which we proposed the name *Vaginella massiliensis* gen. nov., sp. nov. The type strain is Marseille P2517^T.

Keywords: Bacterial vaginosis, *Flavobacteriaceae*, genome, vaginal flora, *Vaginella massiliensis*
Original Submission: 13 October 2016; **Accepted:** 9 November 2016
Article published online: 25 November 2016

Corresponding author: F. Fenollar, Aix-Marseille Université, Institut hospitalo-universitaire Méditerranée-infection, URMITE, UM63, CNRS 7278, IRD 198, Inserm U1095, Faculté de médecine, 27 Boulevard Jean Moulin, 13385 Marseille cedex 05, France
E-mail: florence.fenollar@univ-amu.fr

Introduction

Bacterial vaginosis is a common yet poorly understood condition affecting women of childbearing age in both industrialized and developing countries. Bacterial vaginosis is characterized simultaneously by an abnormal loss of the normal Doderleïn flora accompanied by an unexplained overgrowth of anaerobic bacteria that were previously minor in the vagina [1,2]. In pregnant women, this vaginal dysbiosis is the consequence of certain complications such as miscarriage, preterm birth or chorioamnionitis [3]. Bacterial vaginosis is mostly treated with antibiotics, mainly metronidazole and clindamycin, but treatment frequently fails; the relapse rate is estimated at 50% at 6 months [4,5].

In order to describe the vaginal flora as fully as possible and to better understand the condition in order to provide better treatment, we studied the vaginal microbiota from healthy women and patients with bacterial vaginosis using the culturomics concept. This is based on the multiplication of culture conditions (atmosphere, media, and temperature) and a rapid bacterial identification using matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) [6].

A new member of *Flavobacteriaceae* was therefore isolated. Proposed in 1985 by Jooste, it was only in 1992 that the name of this family was validated by Reichenbach. The type genus is *Flavibacterium* (http://www.bacterio.net/flavobacteriaceae.html) [7]. The family currently contains 114 genera (http://www.bacterio.net/-classifgenerafamilies.html). Some species are found in soil and the marine environment, while others are pathogens found in fish and the human urogenital tract [8].

The classical bacterial description presents some limitations. Hence, in order to describe a new bacterium, our laboratory introduced taxonogenomics, a new approach that complements classic features with the proteomic information obtained by MALDI-TOF MS and the description of the annotated whole genome [9,10].

In the following section, we describe the *Vaginella massiliensis* strain Marseille P2517^T (= DSM 102346^T = CSUR P2517), a new genus isolated from a vaginal swab taken from a healthy 22-year-old French woman without bacterial vaginosis.

Materials and Methods

Sample collection

As previously described [11], a vaginal sample was taken from a healthy 22-year-old French woman without bacterial vaginosis at

http://dx.doi.org/10.1016/j.nmni.2016.11.006

La Timone Hospital in Marseille (France) in January 2016 using a Sigma Transwab (Medical Wire, Corsham, United Kingdom). The study was authorized by the local IFR48 ethics committee (Marseille, France) under agreement number 09-022. The patient also provided written informed consent. When the sample was collected, she was not receiving any antibiotic treatment.

Strain identification by MALDI-TOF MS and 16S rRNA sequencing

The vaginal sample was first preincubated in a blood culture bottle (BD Diagnostics, Le Pont-de-Claix, France) supplemented with 4 mL rumen and filtered at 0.2 µm using a pore filter (Thermo Fisher Scientific, Villebon-sur-Yvette, France) and 3 mL sheep's blood (bioMérieux, Marcy l'Etoile, France). After 7 days of preincubation, 50 µL of the supernatant was inoculated on Chocolat PolyViteX (PVX) agar (BD Diagnostics). After 2 days of incubation at 37°C in aerobic conditions, purified colonies were deposited in duplicate on a MSP 96 MALDI-TOF MS target plate (Bruker Daltonics, Leipzig, Germany), and, as previously described, 1.5 µL of matrix solution was added to each spot. Identification was carried out using a Microflex spectrometer (Bruker) [12], which compares the protein spectra found to those in the Bruker database (constantly updated with spectra of new species discovered in our laboratory). If the score is greater than 1.9, the bacterium is correctly identified. In contrast, if no spectra match the database, and for unidentified bacteria with a clear spectrum, 16S rRNA gene sequencing is performed [13]. As Stackebrandt and Ebers suggested [14], if the 16S rRNA sequence similarity value is lower than 95% or 98.7%, the strain is defined as a new genus or species, respectively.

Phylogenetic tree

A custom Python script was used to automatically retrieve all species from the same order of the new genus and to download 16S sequences from National Center for Biotechnology Information (NCBI) by parsing NCBI eUtils results and the NCBI taxonomy page. This only retains sequences from type strains. In the event of multiple sequences for one type strain, it selects the sequence obtaining the best identity rate from the BLASTn alignment with our sequence. The script then separates 16S sequences into two groups: one containing the sequences of strains from the same family (group A) and one containing the others (group B). Finally, it only retains the 15 closest strains from group A and the closest one from group B. If it is impossible to retrieve 15 sequences from group A, the script selects more sequences from group B to achieve at least nine strains from both groups.

Growth conditions

The ideal growth was tested by cultivating the strain Marseille P2517^T on Colombia agar with 5% sheep's blood incubated at different temperatures (25, 28, 37, 45 and 56°C) and different atmospheres (anaerobic, microaerophilic and aerobic). The anaerobic and microaerophilic atmospheres were generated using, respectively, GENbag anaer and GENbag microaer systems (bioMérieux). Salinity and pH conditions were also tested at different concentrations of NaCl (0, 5, 15 and 45%) and different pH (5, 6, 6.5, 7 and 8.5).

Morphological, biochemical and antibiotic susceptibility tests

Sporulation, motility, Gram stain, catalase and oxidase tests were performed using standard test procedures (https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi#test-procedures). In order to observe cell morphology, samples were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for at least an hour at 4°C. One drop of cell suspension was deposited for approximately 5 minutes on glow-discharged formvar carbon film on 400 mesh nickel grids (FCF400-Ni, EMS). The grids were dried on blotting paper, and cells were negatively stained for 10 seconds with 1% ammonium molybdate solution in filtered water at room temperature. Electron micrographs were acquired using a Tecnai G^{20} Cryo (FEI Company, Limeil-Brevannes, France) transmission electron microscope operated at 200 keV. Biochemical characteristics were studied using API ZYM, API 20NE and API 50CH strips (bioMérieux) according to the manufacturer's instructions.

A cellular fatty acid methyl ester (FAME) analysis was performed by gas chromatography mass spectrometry (GC/MS). Two samples were prepared with approximately 90 mg of bacterial biomass per tube collected from several culture plates. FAMEs were prepared as described by Sasser (http://www.midi-inc.com/pdf/MIS_Technote_101.pdf). GC/MS analyses were carried out using a Clarus 500 gas chromatograph equipped with a SQ8S MS detector (Perkin Elmer, Courtaboeuf, France). FAME extracts (2 mL) were volatilized at 250°C (split 20 mL/min) in a Focus liner with wool and separated on an Elite-5MS column (30 m, 0.25 mm i.d., 0.25 mm film thickness) using a linear temperature gradient (70–290°C at 6°C/min), enabling the detection of C4 to C24 fatty acid methyl esters. Helium flowing at 1.2 mL/min was used as a carrier gas. The MS inlet line was set at 250°C and EI source at 200°C. Full scan monitoring was performed from 45 to 500 *m/z*. All data were collected and processed using Turbomass 6.1 (Perkin Elmer). FAMEs were identified through a spectral database search using MS Search 2.0 operated with the Standard Reference Database 1A (National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) and the FAMEs mass spectral database (Wiley, Chichester, UK). Retention time correlations with estimated nonpolar retention