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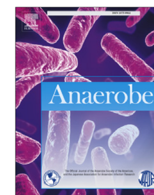

Anaerobes in the microbiome

Olegusella massiliensis gen. nov., sp. nov., strain KHD7^T, a new bacterial genus isolated from the female genital tract of a patient with bacterial vaginosis

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Strain KHD7^T, a Gram-stain-positive rod-shaped, non-sporulating, strictly anaerobic bacterium, was isolated from the vaginal swab of a woman with bacterial vaginosis. We studied its phenotypic characteristics and sequenced its complete genome. The major fatty acids were C16:0 (44%), C18:2n6 (22%), and C18:1n9 (14%). The 1,806,744 bp long genome exhibited 49.24% G+C content; 1549 protein-coding and 51 RNA genes. Strain KHD7^T exhibited a 93.5% 16S rRNA similarity with *Olsenella uli*, the phylogenetically closest species in the family *Coriobacteriaceae*. Therefore, strain KHD7^T is sufficiently distinct to represent a new genus, for which we propose the name *Olegusella massiliensis* gen. nov., sp. nov. The type strain is KHD7^T.

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1. Introduction

The female genital tract is a complex ecosystem colonized by several types of microorganisms. Its composition was described for the first time in 1892 by Doderleïn and in 1901 by Beijerink, revealing that four species of *Lactobacillus* are predominant in healthy vaginal flora: *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus jensenii*, and *Lactobacillus iners* [1,2]. The other bacteria include some anaerobic species such as *Bacteroides*, *Peptostreptococcus*, *Peptococcus*, *Corynebacterium*, and *Eubacterium* [3]. This mutualistic association maintains the stability of the vaginal environment, preventing infection by inhibiting the growth and expansion of pathogens through the production of antimicrobial molecules such as hydrogen peroxide, lactic acid, and bacteriocins [4,5].

This mutualism is disturbed in bacterial vaginosis (BV). The most common cause of vaginal discharge affecting women of child-bearing age, BV is concurrently characterized by reduced *Lactobacillus* species and increased anaerobic bacteria including *Atopobium vaginae*, *Bacteroides* spp., *Mobiluncus* spp., *Prevotella* spp., *Peptoniphilus* spp., and *Anaerococcus* spp. [6–9]. The vaginal microbiota was first studied by conventional culture methods. These methods are limited because 80% of the bacterial microbiota is considered to be fastidious or not cultivable [10]. Advances in molecular techniques, with sequencing and phylogenetic analysis of the 16S rRNA gene, enhanced understanding of the human vaginal microbiota. These molecular methods allowed the detection of fastidious and uncultured bacteria, such as bacterial vaginosis-associated bacteria type 1 (BVAB1), BVAB2, and BVAB3 [11].

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Abbreviations

AGIOS	Average of Genomic Identity of Orthologous gene Sequences
bp:	base pairs
COG	Clusters of Orthologous Groups
CSUR	Collection de souches de l'Unité des Rickettsies
DDH	DNA-DNA Hybridization
DSM	Deutsche Sammlung von Mikroorganismen
FAME	Fatty Acid Methyl Ester
GC/MS	Gas Chromatography/Mass Spectrometry
kb	kilobases
MALDI-TOF	Matrix-assisted laser-desorption/ionization time-of-flight
ORF	Open Reading Frame
TE buffer	Tris-EDTA buffer
URMITE	Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes

As part of a study on the diversity of the vaginal microbiota of patients with bacterial vaginosis using the culturomics approach, based on multiplication of culture conditions (variation of media, temperature, and atmosphere) with more rapid bacterial identification by MALDI-TOF mass spectrometry [12], we isolated a new member of the *Coriobacteriaceae* family. This family, created in 1997 by Stackebrandt, contains 35 species grouped in 13 validated genera [13,14].

Various parameters, including phenotypic and genotypic characteristics such as DNA-DNA hybridization, have been used to define a new species but they present certain limitations [15,16], so we introduced "taxono-genomics", a new approach that includes genomic analysis and proteomic information obtained by MALDI-TOF mass spectrometry analysis [17,18].

Here, we describe *Olegusella massiliensis* strain KHD7^T (= CSUR P2268 = DSM 101849), with its complete annotated genome, a new member of the *Coriobacteriaceae* family isolated in the vaginal flora of a patient with bacterial vaginosis.

2. Materials and methods

2.1. Sample collection

In October 2015, the vaginal sample of a French 33 year-old woman was collected at Hôpital Nord in Marseille (France). The patient was suffering from bacterial vaginosis, which was diagnosed as previously reported [19]. At the time of sample collection, she was not being treated with any antibiotics. She gave her written consent. This study was authorized by the local IFR48 ethics committee (Marseille, France) under agreement number 09-022. The sample was collected and transported using a Sigma Transwab (Medical Wire, Corsham, United Kingdom).

2.2. Strain identification by MALDI-TOF MS

After collection, the sample was first inoculated in a blood culture bottle (BD Diagnostics, Le Pont-de-Claix, France) supplemented with 4 mL of rumen that was filter-sterilized through a 0.2 μm pore filter (Thermo Fisher Scientific, Villebon-sur-Yvette, France), and 3 mL of sheep blood (bioMérieux, Marcy l'Etoile, France). The supernatant was then inoculated on 5% sheep blood-enriched CNA agar (BD Diagnostics) under anaerobic conditions at 37 °C. Isolated colonies were deposited in duplicate on a MTP 96 MALDI-TOF target plate (Bruker Daltonics, Leipzig, Germany) for identification with a microflex spectrometer (Bruker) [20]. Briefly, 1.5 μL of matrix solution, containing solution of α-cyano-4-hydroxycinnamic acid diluted in 500 μL acetonitrile, 250 μL 10% trifluoroacetic acid and 250 μL HPLC water was deposited on each spot for ionization and crystallization. All protein spectra obtained were compared with those in the MALDI-TOF database. If the score was greater than or equal to 1.9, the strain was considered identified. Otherwise, the identification failed.

2.3. Strain identification by 16S rRNA sequencing

For unidentified strains using MALDI-TOF MS, 16S rRNA sequencing was used to achieve identification [21]. As Stackebrandt and Ebers suggested, if the 16S rRNA sequence similarity value was lower than 98.7% or 95%, the strain was defined as a new species or genus, respectively [22–24].

2.4. Morphologic observation and growth conditions

Optimal strain growth was also tested at different temperatures (25, 28, 37, 45, and 56 °C) in an aerobic atmosphere with or without 5% CO_2, and in anaerobic and microaerophilic atmospheres using GENbag Anaer and GENbag miroaer systems (bioMérieux).

For electron microscopy, detection formvar-coated grids were dropped onto a 40 μL bacterial suspension before incubation at 37 °C for 30 min. Then, the grids were incubated on 1% ammonium molybdate for 10 s, dried on blotting paper and finally observed using a Tecnai G20 transmission electron microscope (FEI, Limeil-Brevannes, France) at an operating voltage of 60 Kv. Standard procedures were used to perform Gram-staining, motility, sporulation as well as oxidase and catalase tests [25].

2.5. Biochemical analysis and antibiotic susceptibility tests

Cellular fatty acid methyl ester (FAME) analysis was performed by GC/MS. Strain KHD7^T was grown on Columbia agar enriched with 5% sheep blood (bioMérieux). Then, two samples were prepared with approximately 30 mg of bacterial biomass per tube harvested from several culture plates. Fatty acid methyl esters were prepared as described by Sasser [26]. GC/MS analyses were realized by using a Clarus 500 gas chromatograph equipped with a SQ8S MS detector (Perkin Elmer, Courtaboeuf, France). 2 μL of FAME extracts 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), allowing the detection of C4 to C24 fatty acid methyl esters. Helium flowing at 1.2 mL/min was used as 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 by 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 indexes from the NIST database were obtained using a 37-component FAME mix (Supelco; Sigma-Aldrich, Saint-Quentin Fallavier, France); FAME identifications were confirmed using this index).

API ZYM, API 20A, and API 50CH strips (bioMérieux) were used

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