"*Ezakiella massiliensis*" sp. nov., a new bacterial species isolated from human female genital tract

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Abstract

We report the primary characteristics of "*Ezakiella massiliensis*" strain Marseille P2951 (= DSM 103122 = CSUR P2951), a new member of the *Ezakiella* genus. Strain Marseille P2951 was isolated from a vaginal sample taken from an asymptomatic 20-year-old woman who had sex with another woman who had bacterial vaginosis.

Keywords: Bacterial vaginosis, culturomics, *Ezakiella massiliensis*, vaginal flora
Original Submission: 4 September 2016; **Revised Submission:** 20 September 2016; **Accepted:** 23 September 2016
Article published online: 4 October 2016

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As part of the study of the human vaginal microbiota by the concept of microbial culturomics [1], we isolated from the vaginal swab of an asymptomatic 20-year-old woman who had sex with another woman with bacterial vaginosis [2] a bacterium for which identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Microflex spectrometer, Bruker Daltonics, Bremen, Germany) [3] had failed. The patient provided written consent, and the study was also authorized by the local ethics committee of the IFR48 (Marseille, France; agreement 09-022).

Strain Marseille P2951 was first isolated after 20 days of preincubation of the vaginal sample at 37°C under anaerobic condition in a blood culture bottle (BD Diagnostics, Le Pont-de-Claix, France) supplemented with 4 mL rumen that was filter sterilized through a 0.2 µm pore filter (Thermo Fisher Scientific, Villebon-sur-Yvette, France) and 3 mL of sheep's blood (bioMérieux, Marcy l'Etoile, France). After this preincubation, the supernatant was inoculated on colistin nalidixic acid agar (BD Diagnostics), and the agar plates were incubated for 7 days at 37°C under anaerobic condition. On Columbia agar supplemented with 5% sheep's blood (bioMérieux), colonies were clear and grey, circular and convex with a diameter of 0.8 mm. Strain Marseille P2951 is strictly anaerobic. Bacterial cells were Gram-positive cocci with a diameter ranging from 0.6 to 0.8 µm. They were also positive for catalase and oxidase activities.

The 16S rRNA gene sequence was obtained after amplification using the universal primer pair (fD1 and rp2) and a 3130-XL sequencer (Applied Biosciences, Saint-Aubin, France), as previously reported [4]. 16S rRNA gene sequence–based identification of strain Marseille P2951 showed 98.5% of identity with *Ezakiella peruensis* strain M6.X2 (GenBank accession no. KJ469554.1), the phylogenetically closest bacterium with a validly published name (Fig. 1). Because the sequence similarity was lower than the 98.7% threshold set by Stackebrandt and Ebers [5] to define a new species without carrying out DNA-DNA hybridization, strain Marseille P2951 was classified as a new member of the *Ezakiella* genus belonging to the phylum *Firmicutes* [6].

Described by Patel *et al.* [7] in 2015, *Ezakiella peruensis* is a Gram-positive coccus that is strictly anaerobic, nonmotile, and non–spore forming; it does not exhibit catalase activity. *Ezakiella peruensis* was isolated from a faecal sample of an individual residing in a traditional coastal community in Peru [7].

Strain Marseille P2951 presents a 16S rRNA gene sequence divergence of approximately 1.5% from its phylogenetically closest species [8]. We propose that strain Marseille P2951 may be the representative strain of a new species named "*Ezakiella massiliensis*" (mas.il'ien'sis, L. gen. fem. n. *massiliensis*, "of Massilia," the Latin name for Marseille, where strain Marseille P2951 was first cultivated). Strain Marseille P2951 is the type strain of "*Ezakiella massiliensis*" sp. nov.

MALDI-TOF MS spectrum accession number

The MALDI-TOF MS spectrum of "*Ezakiella massiliensis*" is available at http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database.

http://dx.doi.org/10.1016/j.nmni.2016.09.011

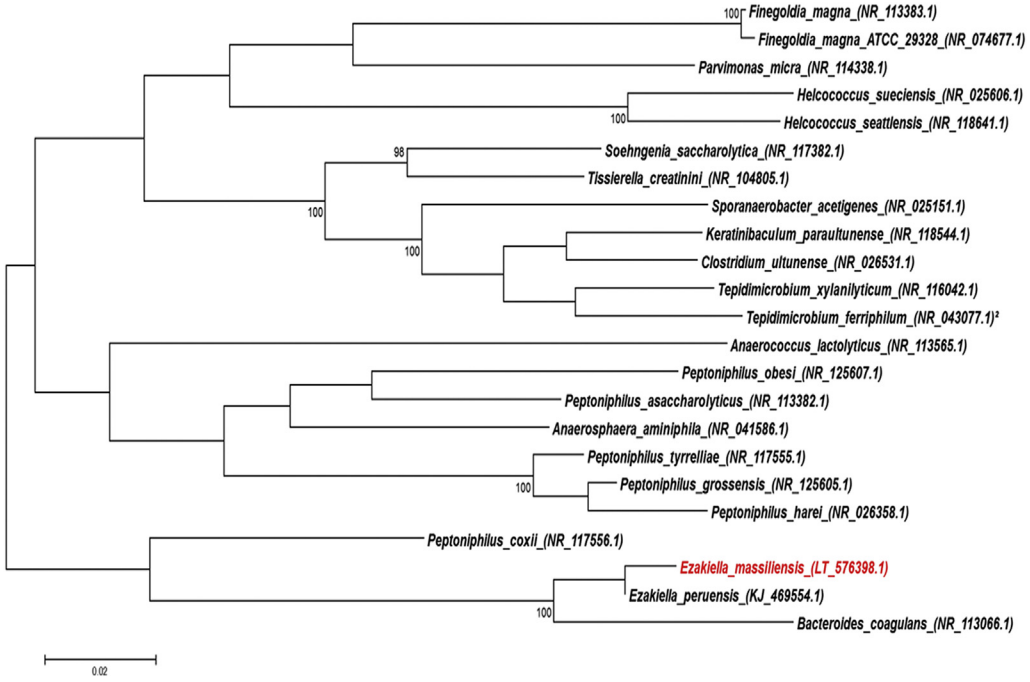

FIG. 1. Phylogenetic tree highlighting position of "*Ezakiella massiliensis*" strain Marseille P2951^T relative to other closest species. GenBank accession numbers are indicated after names. Sequences were aligned using CLUSTALW and phylogenetic inferences obtained using approximately maximum-likelihood method within MEGA6 software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstrap scores of $\geq$95% were retained. Scale bar indicates 2% nucleotide sequence divergence.

Nucleotide sequence accession number

The 16S rRNA gene sequence was deposited in European Molecular Biology Laboratory–European Bioinformatics Institute under accession number LT576398.1

Deposit in culture collection

Strain Marseille P2951 was deposited both in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) and in Deutsche Sammlung von Mikroorganismen (DSM) under numbers P2951 and 103122, respectively.

Acknowledgement

This study was funded by the Fondation Méditerranée Infection.

Conflict of Interest

None declared.

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