

'*Anaerococcus mediterraneensis*' sp. nov., a new species isolated from human female genital tract

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Abstract

We report the principal characteristics of '*Anaerococcus mediterraneensis*' strain Marseille P2765, a new member of the *Anaerococcus* genus. Strain Marseille P2765 was isolated in a vaginal sample of a 26-year-old patient with bacterial vaginosis.

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Keywords: *Anaerococcus mediterraneensis*, bacterial vaginosis, culturomics, human microbiota, vaginal flora

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We are currently studying the human microbiota by culturomics in our laboratory in Marseille, France [1]. As part of this study, we isolated in the vaginal flora of a 26-year-old French woman with bacterial vaginosis [2] a bacterium which could not be identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) performed with a MicroFlex spectrometer (Bruker Daltonics, Leipzig, Germany) [3]. The agreement number of the National Ethics Committee of the IFR48 (Marseille, France) for this study is 09-022. The patient provided written consent.

First, the vaginal sample was preincubated at 37°C for 21 days in a blood culture bottle (BD Diagnostics, Le Pont-de-Claix, France) enriched 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 21 days of preincubation, the sample was inoculated on Schaedler agar enriched with sheep's blood and vitamin K (BD Diagnostics) and incubated for 7 days in anaerobic conditions at 37°C. On sheep's blood agar (bioMérieux), colonies were white with a mean diameter of

2 mm. Bacterial cells were Gram-positive cocci. Catalase activity was positive; oxidase activity was negative.

The 16S rRNA gene was amplified and sequenced using the universal primers (fD1 and rp2) and a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France), as described elsewhere [4]. Strain Marseille P2765 exhibited a 97.2% sequence identity with *Anaerococcus lactolyticus* strain JCM 8140 (GenBank accession no. NR_113565.1), the phylogenetically closest validated species (Fig. 1). This degree of similarity was lower than the 98.7% threshold to define a new species [5], and we propose that strain Marseille P2765 be considered representative of a new species within the *Anaerococcus* genus in the phylum *Firmicutes*. The *Anaerococcus* genus was created by Ezaki et al. [6]. This genus is one of the three genera obtained after the subdivision of the *Peptostreptococcus* genus [6]. Bacterial species from the *Anaerococcus* genus have been already reported from diverse human clinical specimens [6]. *Anaerococcus lactolyticus*, the phylogenetically closest validated species, was first isolated from vaginal discharges [6] like strain Marseille P2765^T.

Because strain Marseille P2765 is more than 2.8% divergent in the 16S rRNA gene sequence with its closest phylogenetic neighbour [7], we propose that it may be the representative strain of a novel species named '*Anaerococcus mediterraneensis*' (me.di.ter.ra.ne.en'sis, L. masc. adj., *mediterraneensis*, 'of Mediterranean,' the Latin name of the Mediterranean Sea by which Marseille, where strain P2765 was isolated, is located). Strain

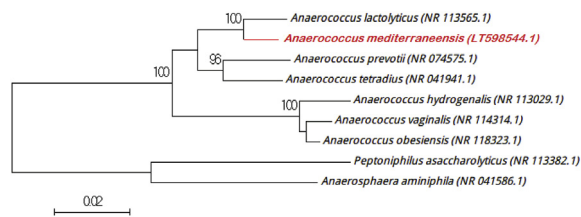


FIG. 1. Phylogenetic tree showing phylogenetic position of ‘*Anaerococcus mediterraneensis*’ strain Marseille 2765^T relative to close species in genus *Anaerococcus*. GenBank accession numbers are indicated after name. Sequences were aligned using Muscle v3.8.31 with default parameters, and phylogenetic inferences were obtained using neighbour-joining method with 500 bootstrap replicates within MEGA6 software. Only bootstrap values >95% are shown. Scale bar represents 2% nucleotide sequence divergence.

Marseille P2765^T is the type strain of the new species ‘*Anaerococcus mediterraneensis*’ sp. nov.

MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of ‘*Anaerococcus mediterraneensis*’ is available online (<http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>).

Nucleotide sequence accession number

The 16S rRNA gene sequence was deposited in European Molecular Biology Laboratory–European Bioinformatics Institute under accession number LN598544.1.

Deposit in a culture collection

The type isolate of ‘*Anaerococcus mediterraneensis*’ was deposited in the collection Deutsche Sammlung von Mikroorganismen

(DSM 103343) and the Collection de Souches de l’Unité des Rickettsies (CSUR, WDCM 875) under number P2765.

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Conflict of Interest

None declared.

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