

## '*Peptoniphilus pacaensis*' sp. nov., a new species isolated from human female genital tract

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### Abstract

We relate here the primary characteristics of '*Peptoniphilus pacaensis*' strain KHD5 (= CSUR P2271), a new member of the *Peptoniphilus* genus. Strain KHD5 was isolated from a vaginal sample of a 33-year-old woman exhibiting a bacterial vaginosis.

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**Keywords:** Bacterial vaginosis, culturomics, human microbiota, *Peptoniphilus pacaensis*, vaginal flora

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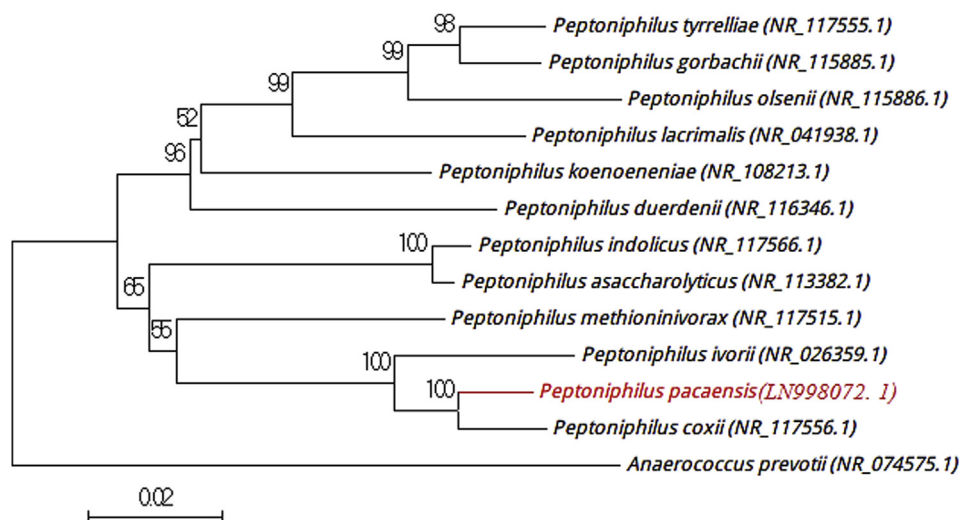
We are currently studying the human microbiota by culturomics in our laboratory in Marseille, France [1]. As part of this study, we isolated in the vaginal flora of a 33-year-old French woman with bacterial vaginosis [2], a new species of the *Peptoniphilaceae* family that could not be identified using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) and a MicroFlex spectrometer (Bruker Daltonics, Bremen, Germany) [3]. The agreement number of the National Ethics Committee of the IFR48 (Marseille, France) for this study is 09-022. The patient has also given her written consent.

First, the vaginal sample was pre-incubated at 37°C for 15 days 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 blood (bioMérieux, Marcy l'Etoile, France). Then, after these 15 days of pre-incubation, the sample was inoculated on colistin nalidixic acid (CNA) agar (BD Diagnostics) and trypticase soy

agar (BD Diagnostics). After 4 days of incubation at 37°C in anaerobic conditions, strain KHD5 was isolated on both CNA and trypticase soy agar plates. On Columbia agar with 5% sheep blood (bioMérieux), colonies were translucent and grey; they exhibited also a diameter of 1–1.5 mm. Bacterial cells were Gram-positive cocci with a diameter from 0.6 to 0.8 µm; they were also negative for catalase and oxidase.

The 16S rRNA gene was sequenced using the universal eubacterial primers fD1 and rp2 as previously reported [4] with a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Strain KHD5 exhibited a sequence similarity of 97.2% with *Peptoniphilus coxii* (GenBank NR\_117556.1), the phylogenetically closest validated species (Fig. 1).

Consequently, as this value is < 98.7% of the 16S rRNA sequence threshold recommended to delineate a new species within the *Firmicutes* phylum without carrying out DNA–DNA hybridization [5], we propose to classify strain KHD5 as the representative strain of a novel species within the genus *Peptoniphilus* in the *Peptoniphilaceae* family. Created in 2001, by Ezaki *et al.*, after a subdivision of the *Peptostreptococcus* genus into three genera, the *Peptoniphilus* genus contains only strictly anaerobic bacteria [6]. This genus includes 13 species isolated from diverse human clinical specimens [7]. Of note, *Peptoniphilus coxii*, the phylogenetically closest validated species of strain KHD5 has been already cultured from a leg infection, an infected ischial pressure wound, and perirectal abscesses [8].



**FIG. 1.** Phylogenetic tree showing the phylogenetic position of ‘*Peptoniphilus pacaensis*’ strain KHD5<sup>T</sup> relative to other species that are close members of *Peptoniphilaceae*. GenBank accession numbers are indicated after the name. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using the approximately maximum likelihood method within the MEGA6 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. Only the bootstrap scores  $\geq 95\%$  were retained. The scale bar indicates a 2% nucleotide sequence divergence.

Strain KHD5 is  $>2.8\%$  divergent in the 16S rRNA gene sequence compared with its closest phylogenetic neighbour [9]. Hence, we consequently propose that it may be the representative strain of a novel species named ‘*Peptoniphilus pacaensis*’ sp. nov. (*pa.ca’en.sis* N.L. masc. adj. *pacaensis*, derived from the abbreviation PACA, for the region of Provence Alpes Côte d’Azur, where the strain was first isolated). Strain KHD5 is the type strain of the new species of ‘*Peptoniphilus pacaensis*’ sp. nov.

### MALDI-TOF-MS Spectrum Accession Number

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

### Nucleotide Sequence Accession Number

The 16S rRNA gene sequence of strain KHD5 was deposited in EMBL-EBI under the accession number LN9980072.1.

### Deposit in Culture Collection

The isolate ‘*Peptoniphilus pacaensis*’ was deposited in the ‘Collection de Souches de l’Unité des Rickettsies’ (CSUR, WDCM 875) under number P2271.

### Acknowledgements

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### Conflict of Interest

The authors have no conflicts of interest to declare.

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