

# '*Prevotella ihumii*' sp. nov. and '*Varibaculum timonense*' sp. nov., two new bacterial species isolated from a fresh human stool specimen

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## Abstract

Here we report the main characteristics of '*Prevotella ihumii*' sp. nov., CSUR-P3385<sup>T</sup> and '*Varibaculum timonense*' sp. nov., CSUR-P3369<sup>T</sup> isolated in September 2016 from a fresh stool sample of healthy French volunteer woman.

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**Keywords:** Culturomics, human gut microbiota, '*Prevotella ihumii*', taxonogenomics, '*Varibaculum timonense*'

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Culturomics approach allowed to isolate 295 new bacterial species from the human gut [1]. As a part of culturomics study in September 2016, we isolated from a fresh stool sample of a 26-year-old healthy French woman two bacteria strains (Marseille-P3369<sup>T</sup> and Marseille-P3385<sup>T</sup>) that could not be identified by our systematic matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (<http://mediterranean-infection.com/article.php?laref=256&titre=urms-database>) on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [2]. This study was approved by the ethics committee of the Institut Hospitalo-Universitaire Méditerranée Infection under number 2016-011, and the patient provided signed informed consent.

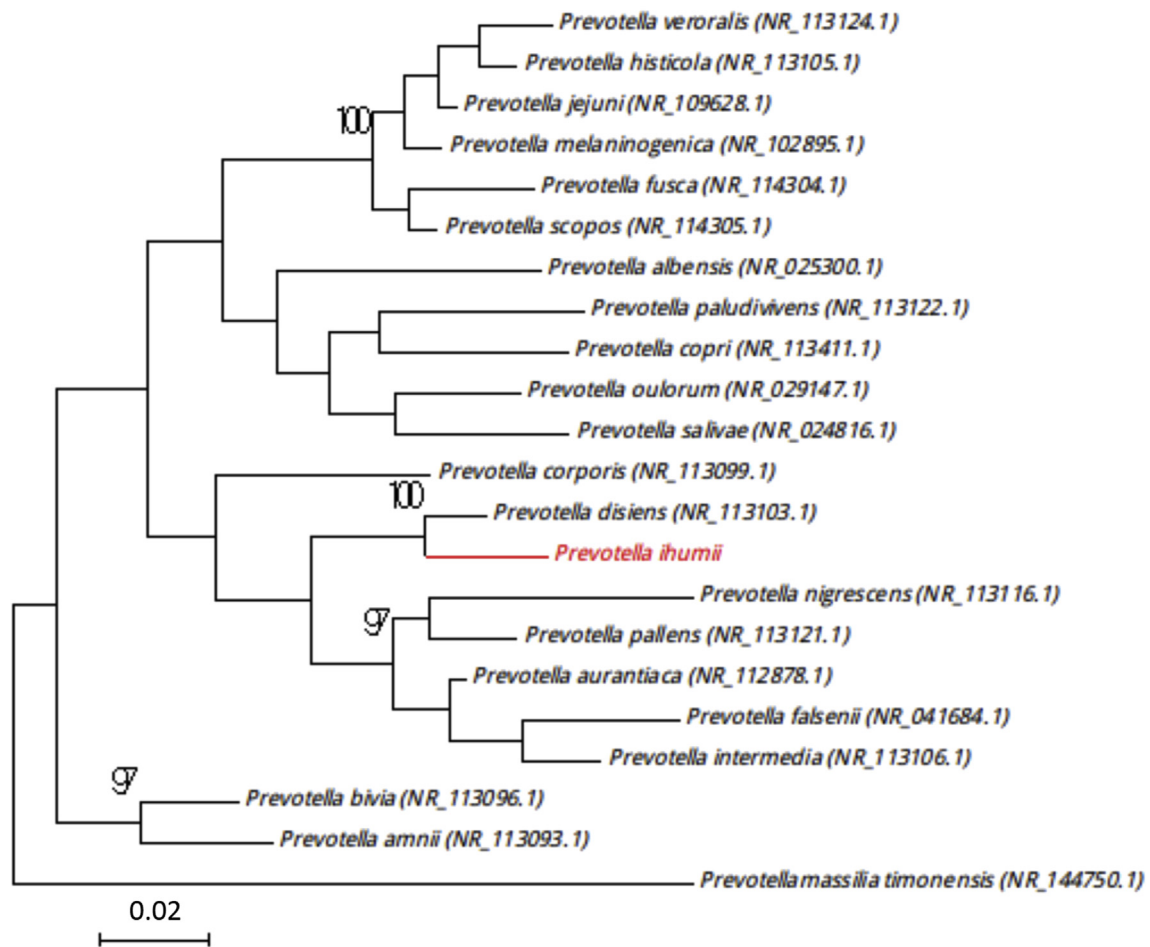
The fresh stool sample was inoculated in an anaerobic blood culture bottle (Becton Dickinson, Le Pont-de-Claix, France) incubated for 3 days at 37°C. The initial agar-grown colonies were obtained after 48 hours' incubation at 37°C on Columbia agar supplemented with 5% sheep's blood (bioMérieux, Marcy l'Etoile, France) under strict anaerobic condition generated by

anaeroGEN (Oxoid, Dardilly, France). The 16S rRNA gene was sequenced using fD1-rP2 primers as previously described, using a 3130-XL sequencer (Applied Biosciences, Saint-Aubin, France).

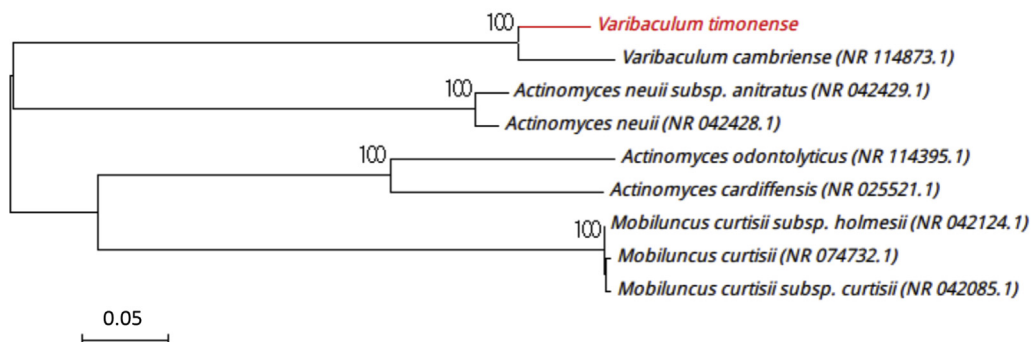
Strain Marseille-P3385<sup>T</sup> is a nonmotile and non-spore-forming bacteria. Agar-grown colonies are dark and circular, with a mean diameter of 1.2 mm. Cells were Gram negative and rod shaped ranging in length from 1 to 1.5 µm and large from 0.4 to 0.5 µm. Strain Marseille-P3385<sup>T</sup> exhibit negative catalase and oxidase reaction and grow optimally at 37°C, pH 7; NaCl is not required for growth.

Strain Marseille-P3369<sup>T</sup> is a nonmotile and non-spore-forming bacteria. The colonies are white and circular, and 0.5 mm in diameter. Cells were Gram positive, small rod shaped, and slightly curved, ranging in diameter from 350 to 400 nm. Strain Marseille-P3369<sup>T</sup> did not exhibit catalase or oxidase activities and grew optimally at 37°C and pH 7.5.

16S rRNA gene sequence-based identification demonstrated that strain Marseille-P3385<sup>T</sup> exhibited 96.31% sequence identity with *Prevotella disiens* JCM 6334<sup>T</sup> (GenBank accession no. NR\_113103.1) [3] (Fig. 1), and strain Marseille-P3369<sup>T</sup> exhibited a 16S rRNA gene sequence similarity of 98.25% with *Varibaculum cambriense* strain CCUG 44998 (GenBank accession no. NR\_114873.1), its closest related species with standing in nomenclature [4] (Fig. 2). These values are under the thresholds of 98.7% separating new species established by Stackebrandt and Ebers [5]. Consequently, we propose the



**FIG. 1.** Phylogenetic tree showing position of '*Prevotella ihumii*' Marseille-P3385<sup>T</sup> relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained by maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstraps score at least 95 were retained. Scale bar indicates 0.02% nucleotide sequence divergence.



**FIG. 2.** Phylogenetic tree showing position of '*Varibaculum timonense*' Marseille-P3369<sup>T</sup> relative to other phylogenetically close neighbours. Sequence alignment and phylogenetic inferences were obtained by maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstraps score at least 95 were retained. Scale bar indicates 0.05% nucleotide sequence divergence.

creation of *Prevotella ihumii* sp. nov., and *Varibaculum timonense* sp. nov., two new bacterial species belonging to *Prevotella* and *Varibaculum* genera respectively.

Strain Marseille-P3369<sup>T</sup> is the type strain of *Varibaculum timonense* sp. nov. (ti.mo.nen'se, N.L. neut. adj. *timonense*, related to Timone, the name of the main university hospital in Marseille, France, from where the strain was isolated); and strain Marseille-P3385<sup>T</sup> is the type strain of *Prevotella ihumii* sp. nov. (i.hum.i'i, N.L. gen. n. *ihumii*, based on the acronym IHUMI, the Institut hospitalo-universitaire Méditerranée-infection, where the type strain was isolated).

### MALDI-TOF MS spectra

The MALDI-TOF-MS spectra of *Prevotella ihumii* and *Varibaculum timonense* are available online (<http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>).

### Nucleotide sequence accession numbers

The 16S rRNA gene sequences of *Prevotella ihumii* and *Varibaculum timonense* were deposited in GenBank under accession numbers LT631517 and LT797538 respectively.

### Deposit in a culture collection

Strains Marseille-P3385<sup>T</sup> and Marseille-P3369<sup>T</sup> were deposited in the Collection de Souches de l'Unité des

Rickettsies (CSUR) under numbers P3385 and P3369 respectively.

### Acknowledgement

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### Conflict of Interest

None declared.

### References

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