

Prevotella ihumii sp. nov., a new bacterium isolated from a stool specimen of a healthy woman

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Abstract

Prevotella ihumii strain Marseille-P3385^T (= CSURP3385^T; = DSM106428^T) is a new species isolated from a fresh stool specimen of a healthy woman.

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Introduction

Culturomics is a strategy combining various culture conditions to mimic as much as possible the optimal growth requirements of bacterial species and enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. Once isolated, bacteria are identified using the taxono-genomics approach that includes matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, description of the main phenotypic characteristics and genome sequencing [5,6].

Isolation and growth conditions

In 2016, we isolated from a fresh stool sample of a healthy 26-year-old French woman the bacterial strain Marseille-

P3385. The study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 2016-011. Initial growth was obtained after 72 h of culture in 5% sheep-blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) in a strict anaerobic atmosphere at 37°C. The strain could not be identified using MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained reference spectrum (Fig. 1) was imported into our database (<http://www.mediterranee-infection.com/article.php?larub=280&titre=urms-database>).

Phenotypic characteristics

Colonies were circular and black with a mean diameter of 1.2 mm. Bacterial cells were Gram-negative, rod-shaped, ranging in length from 1 to 1.5 µm and in width from 0.4 to 0.5 µm (Fig. 2). Strain Marseille-P3385 exhibited negative catalase and oxidase activities. Biochemical properties were evaluated using API 50CH and API ZYM strips (bioMérieux) in a strict anaerobic atmosphere at 37°C. Biochemical characteristics of all studied species are summarized in Table 1.

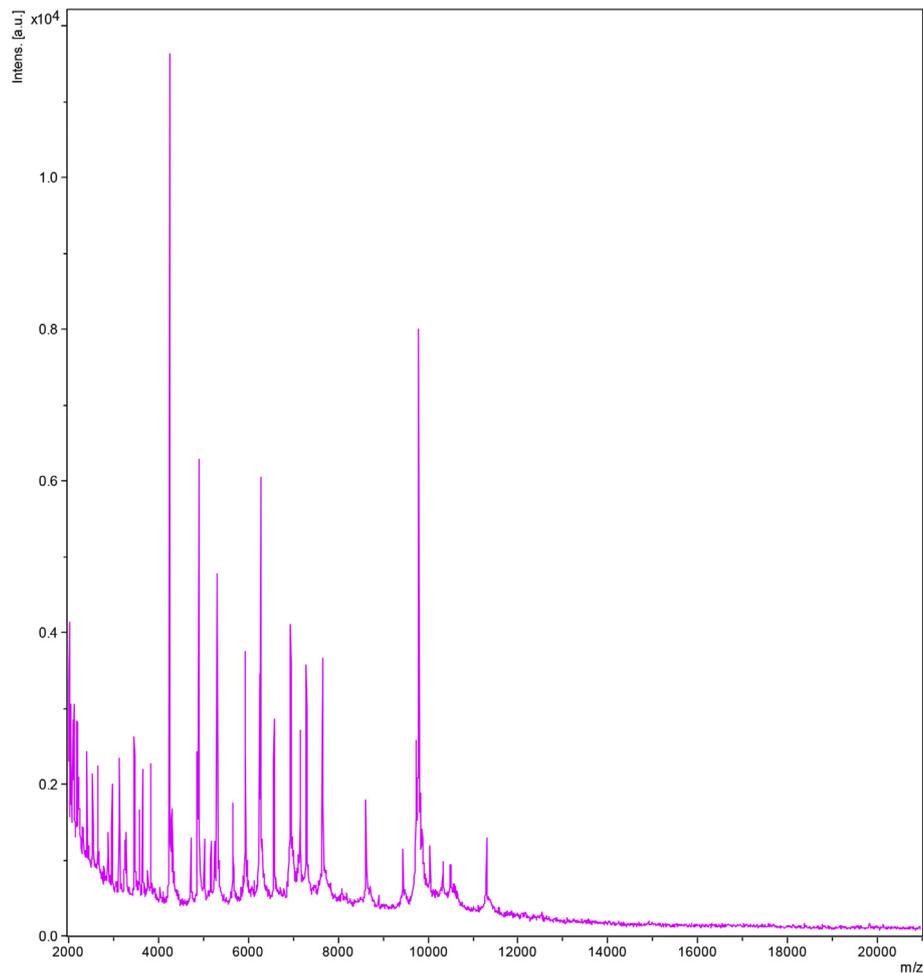
Figure 1

FIG. 1. MALDI-TOF MS reference mass spectrum from *Prevotella ihumii* sp. nov., strain Marseille-P3385^T. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

Strain identification

The 16S rRNA gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing was performed using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and a choose sequencer3500xLGenetic Analyzer capillary sequencer (ThermoFisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequence was assembled and corrected using the CODONCODE ALIGNER software (<http://www.codoncode.com>). Strain Marseille-P3385 exhibited a 96.35% sequence identity with *Prevotella disiens* strain JCM-6334^T (GenBank accession number L16483), its phylogenetically closest species with standing in nomenclature (Fig. 3). Consequently, we considered this strain as a member of a putative new

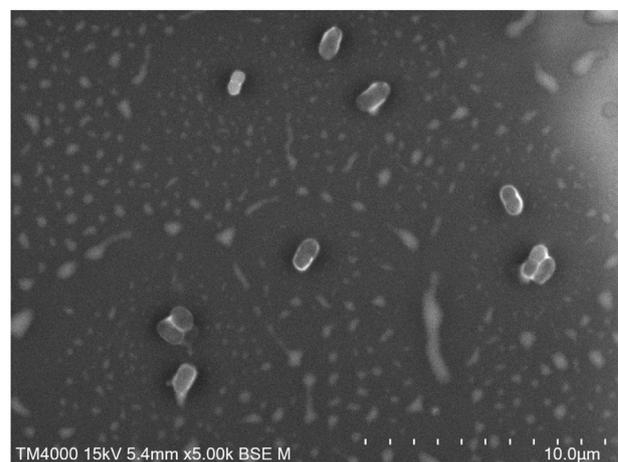


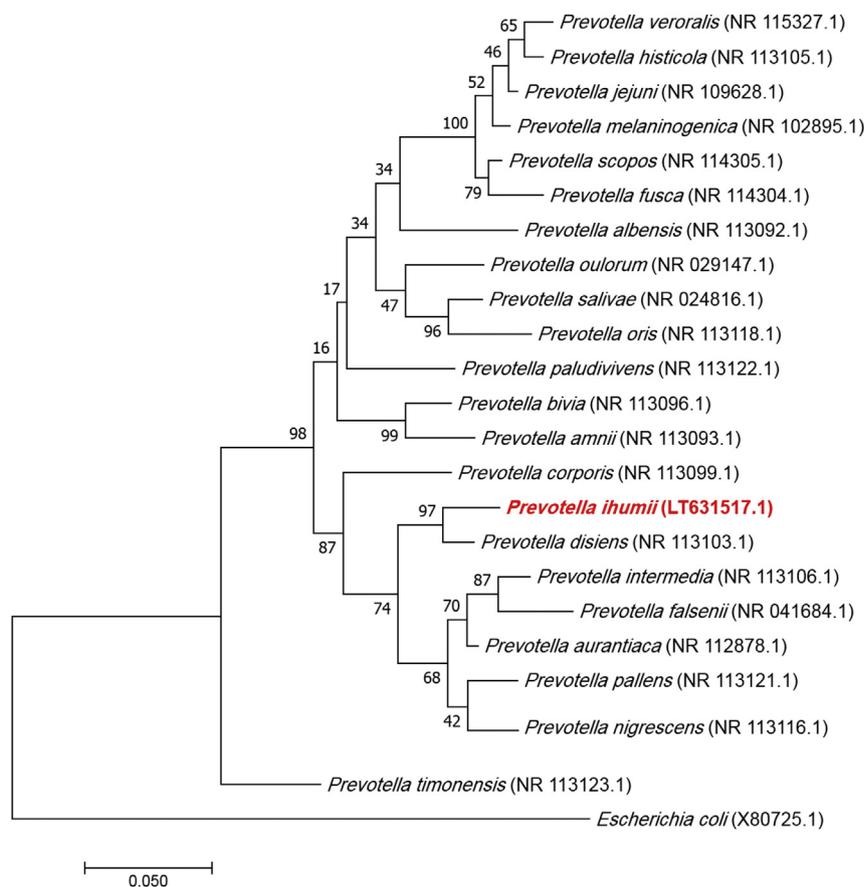
FIG. 2. Scanning electron micrograph of *Prevotella ihumii* sp. nov., strain Marseille-P3385^T, using a TM4000 microscope (Hitachi, Tokyo, Japan). The scale bar and acquisition settings are displayed in the figure.

TABLE 1. Compared phenotypic characteristics of studied species

Characteristics	PIh	PIin	PAm	PP	PO	PC	PD	PF	PB	PAu
Gram stain	-	-	-	-	-	-	-	-	-	-
Production of catalase	-	na	-	na	na	na	na	-	na	-
Oxidase	-	na	-	na						
Arabinose	-	-	-	-	-	-	-	-	-	-
β-Glucosidase	-	-	+	-	+	-	-	-	-	-
Glycine aminopeptidase	-	-	-	-	-	-	+	-	+	-
Rhamnose	-	-	-	-	-	-	-	-	-	-
Salicin	-	-	-	-	-	-	-	-	-	-
Cellobiose	-	-	-	-	-	-	-	-	-	-
N-acetyl-β-glucosaminidase	-	-	+	-	+	+	-	-	+	-
Lactose	-	-	+	-	+	-	-	-	-	-
Indole	-	+	-	+	-	-	-	+	-	+
Sucrose	-	+	-	+	-	-	-	+	-	+
α-Fucosidase	-	+	-	+	+	-	-	+	+	+
β-Xylosidase	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-

PIh, *Prevotella ihumii*; PIin, *Prevotella intermedia*; PAm, *Prevotella amnii*; PP, *Prevotella pallens*; PO, *Prevotella oulorum*; PC, *Prevotella corporis*; PD, *Prevotella disiens*; PF, *Prevotella falsenii*; PB, *Prevotella bivia*; PAu, *Prevotella aurantiaca*; +, positive reaction; -, negative reaction; na, data not available.
Data for reference strains [13–17].

FIG. 3. Phylogenetic tree showing the position of *Prevotella ihumii* sp. nov., strain Marseille-P3385^T relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Sequences were aligned using MUSCLE v3.8.31 with default parameters and phylogenetic inferences were obtained using the maximum likelihood method within the MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 5% nucleotide sequence divergence.



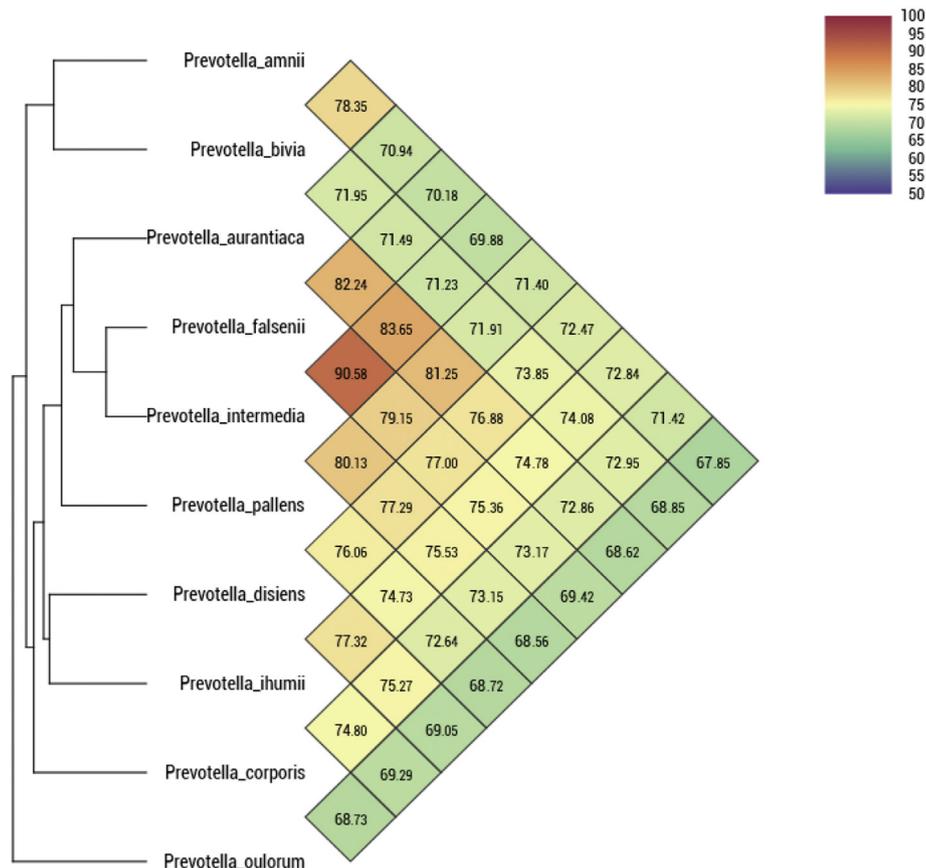


FIG. 4. Heatmap generated with ORTHOANI values calculated using the OAT software between *Preotella ihumii* sp. nov., strain Marseille-P3385^T and other closely related species with standing in nomenclature.

species within the genus *Preotella* (family *Prevotellaceae*, phylum Bacteroidetes).

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit and then sequenced on a MiSeq sequencer (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit (Illumina), as previously described [9]. The assembly was performed with the SPADes software [10], on trimmed (MiSeq and TRIMMOMATIC [11] softwares) or untrimmed (only MiSeq software) data. GAPP-CLOSER was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a read depth value <25% of the mean depth were removed. The best assembly was selected by using various criteria (number of scaffolds, N50, number of Ns). The genome of strain Marseille-P3385 is 3 311 176 bp long with a 42.1 mol% G + C content and 47 contigs. The degree of genomic similarity of Marseille-P3385 with closely related species was estimated using the ORTHOANI software [12]. Values among closely

related species (Fig. 4) ranged from 67.85% between *Preotella amnii* and *Preotella oulorum* to 90.58% between *Preotella falsenii* and *Preotella intermedia*. When strain Marseille-P3385 was compared with these closely related species, values were in the same interspecies range, from 69.28% with *Preotella oulorum* to 77.32% with *Preotella disiens*.

Conclusion

Strain Marseille-P3385^T, exhibiting 16S rRNA sequence and ORTHOANI similarity values <98.65% and <95.0%, respectively, with the phylogenetically closest species with standing in nomenclature, is proposed as the type strain of the new species *Preotella ihumii* sp. nov.

Description of *Preotella ihumii* sp. nov.

Preotella ihumii (i.hum.i'i, N.L. gen. n. *ihumii*, based on the acronym IHUMI, the Institut hospitalo-universitaire Méditerranée-infection, in Marseille, France, where the type strain was isolated). Cells are anaerobic, Gram-negative, non-

motile and asporogenous rods. Catalase and oxidase activities are negative. Cells have a length of 1–1.5 μm and a width of 0.4–0.5 μm . Colonies grown on 5% sheep-blood-enriched Columbia agar (bioMérieux) are circular and black after 72 h of incubation in a strict anaerobic atmosphere, with a mean diameter of 1.2 mm. Growth occurs at 37°C. Cells grow anaerobically only. Using an API ZYM strip, a positive reaction was observed for leucine arylamidase, naphthol-AS-BI-phosphohydrolase and alkaline and acid phosphatases but negative reactions were obtained for esterase, esterase lipase, lipase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase enzymes. Using an API 50 CH strip, strain Marseille-P3385^T was able to metabolize *D*-galactose, *D*-maltose, *D*-tagatose and potassium 5-Ketogluconate. However, negative reactions were obtained for glycerol, *D*-glucose, *D*-fructose, *D*-mannose, methyl- α -D-glucopyranoside, *N*-acetylglucosamine, *D*-lactose, *D*-saccharose, *D*-trehalose, *D*-turanose erythritol, *D*-arabinose, *L*-arabinose, *D*-ribose, *D*-xylose, *L*-xylose, *D*-adonitol, methyl- β -D-xylopyranoside, *L*-sorbitol, *L*-rhamnose, dulcitol, inositol, *D*-mannitol, *D*-sorbitol, methyl- α -D-mannopyranoside, amygdalin, arbutin, esculin, salicin, *D*-cellobiose, *D*-melibiose, inulin, *D*-melezitose, *D*-raffinose, starch, glycogen, xylitol, gentiobiose, *D*-lyxose, *D*-fucose, *L*-fucose, *D*-arabitol, *L*-arabitol, potassium gluconate and potassium 2-ketogluconate. The most abundant fatty acids are 3-hydroxy-15-methyl-hexadecanoic acid, hexadecanoic acid and 12-methyl-tetradecanoic acid. The genome is 3 311 176 bp long and its G + C content is 42.1%.

The type strain, Marseille-P3385^T, isolated from a fresh stool sample of a healthy 26-year-old French woman, was deposited in the CSUR and DSMZ collections under accession numbers CSUR P3385 and DSM 106428, respectively. The 16S rRNA and genome sequences are available in GenBank under accession numbers LT631517 and FTLS00000000, respectively.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT631517 and FTLS00000000, respectively.

Deposit in culture collections

Strain Marseille-P3385^T was deposited in the CSUR and DSMZ collections under numbers = CSURP3385^T and = DSM106428^T.

Conflict of interest

None to declare.

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