Prevotella marseillensis sp. nov., a new bacterium isolated from a patient with recurrent Clostridium difficile infection

E. Kuete Yimagou[1], F. Mekhalif[1], M. Lamine Tall[1], J. P. Baudoin[1], D. Raoult[1,2] and J. Y. Bou Khalil[1]

1) Aix Marseille Université, IRD, AP-HM, MEPHI, IHU-Méditerranée Infection, Marseille, France and 2) Special Infectious Agents Unit, King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

Prevotella marseillensis strain Marseille-P8229[T] (= CSURP8229) is a new species isolated from a patient with recurrent Clostridium difficile infection. It is an anaerobic, non-motile, non-spore-forming Gram-negative coccobacillus isolated from the stool of patient with recurrent Clostridium difficile infection in Marseille. We present herein its phenotypic description together with MALDI-TOF mass spectrometry analysis and genome sequencing and comparison. The genome of P. marseillensis is 4.1607 Mbp long with 45.80 mol% of G+C content, and it contains 3078 protein-coding genes.
© 2019 Published by Elsevier Ltd.

Corresponding author: J.Y. Bou Khalil, MEPHI, Institut Hospitalo-Universitaire Méditerranée Infection, 19-21 Boulevard Jean Moulin, 13385, Marseille Cedex 05, France.
E-mail: boukhaliljacques@gmail.com

Introduction

Culturomics is a concept developing different culture conditions to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1−4]. Once the isolate was obtained, we used a taxono-genomics approach including matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), phylogenetic analysis, main phenotypic description (Table 1) and genome sequencing to describe it [5,6].

Isolation and growth conditions

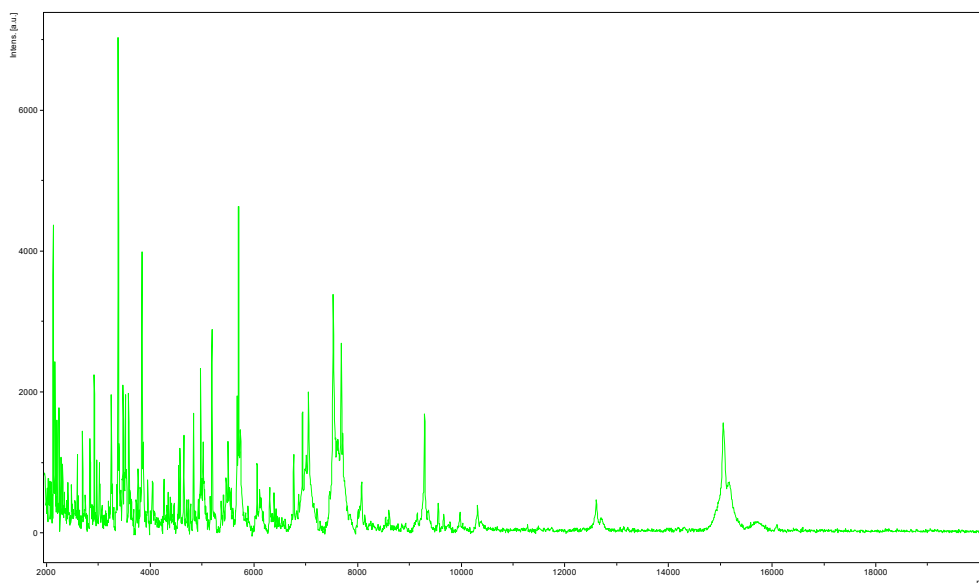In 2018, we isolated from the human stool an unidentified bacterial strain. The study was validated by the ethics committee of IHU Méditerranée Infection under number 2016-011. Screening was performed using MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectra (Fig. 1) were imported into MALDI BIOTYPER 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database, constantly updated with MEPHI database https://www.mediterranee-infection.com/urms-data-base/). Initial growth was obtained after 48 h of culture on Columbia agar with 5% sheep blood in anaerobic conditions at 37°C, pH 7.5.

Strain identification

The 16S rRNA gene was sequenced to classify this bacterium. Amplification used the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing used the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France) as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CODON-CODE ALIGNER software (http://www.codoncode.com). Strain Prevotella marseillensis exhibited a 91.12% sequence identity with

TABLE 1. Description of *Prevotella marseillensis* according to the digitalized protologue TA00970 on the www.imedea.uib.es/dprotologue website

Taxonumber	TA00970
Date of the entry	2019-05-28
Draft number/date	001
Version	Submitted
Species name	*Prevotella marseillensis*
Genus name	*Prevotella*
Specific epithet	*Prevotella marseillensis*
Species status	sp. nov.
Species etymology	*Prevotella*, named after the French microbiologist, A. R. Prévot, a pioneer in anaerobic microbiology, and *marseillensis*, pertaining to Marseille, the name of the French territory where strain Marseille-P8229T was isolated
E-mail of the corresponding author	edmondkuete@yahoo.fr
Submitter	KUETE YIMAGOU EDMOND
E-mail of the submitter	edmondkuete@yahoo.fr
Designation of the type strain	Marseille-P8229T
Strain collection numbers	CSURP8229
16S rRNA gene accession number	LR031296
Genome accession number (EMBL)	UYXY00000000
Genome size	4.1607
GC mol %	45.80
Data on the origin of the sample from which the strain had been isolated	
Country of origin	France
Region of origin	Bouches du Rhône
Source of isolation	Stool
Sampling date	2018-03-17
Source of isolation of non-type strains	gut
Growth medium, incubation conditions (temperature, pH and further information) used for standard cultivation	Columbia agar with 5% sheep blood in anaerobic conditions at 37°C and pH 7.5.
Gram stain	negative
Cell shape	coccobacillus
Motility	non-motile
Sporulation (resting cells)	none
Lowest temperature for growth	25°C
Highest temperature for growth	45°C
Temperature optimum	37°C
pH optimum	7.5
Oxidase	negative
Catalase	negative
Biosafety level	2
Habitat	human

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

Prevotella shahii strain EHS11 (GenBank accession number NR_024815.1) the phylogenetically closest species with standing in nomenclature (Fig. 2). We consequently classify this strain as a member of a new species within the genus *Prevotella*, family *Prevotellaceae*, phylum Bacteroidetes.

Phenotypic characteristics

Colonies were coccobacilli with a mean diameter of 2.81 μm. Bacterial cells were Gram-negative and rod-shaped (Fig. 3). Strain Marseille-P8229^T showed catalase-negative and oxidase-negative activities. Characteristics of the strain are summarized in Table 1. API 50CH and API ZYM tests were performed at 37°C under anaerobic conditions and the results are summarized in Table 2.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit and then sequenced on MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera XT Paired end (Illumina), as previously described [9]. The assembly was performed with a pipeline incorporating different softwares (Velvet [10], Spades [11] and
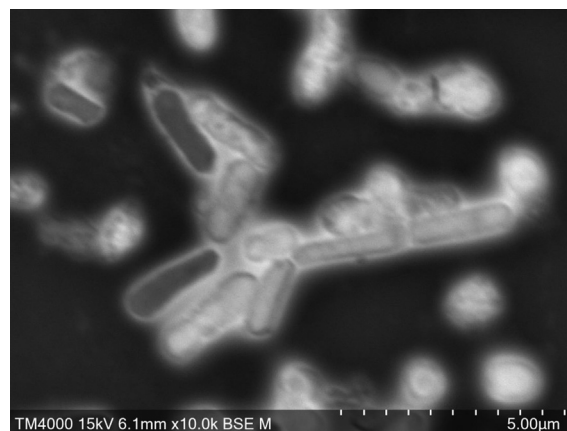

FIG. 3. Electron micrograph of *Prevotella marseillensis* strain Marseille-P8229^T was acquired with a Hitachi TM4000Plus tabletop scanning electron microscope.

Soap Denovo [12]) on trimmed data (Trimmomatic [13]) or raw data. GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value < 25% of the mean depth were removed. The best assembly was selected by using different criteria (17 scaffolds, 19 contigs). The genome of strain Marseille-P8229^T is 4.1607 Mbp long with a 45.80 mol% G+C content and contains 3078 predicted genes. The degree of
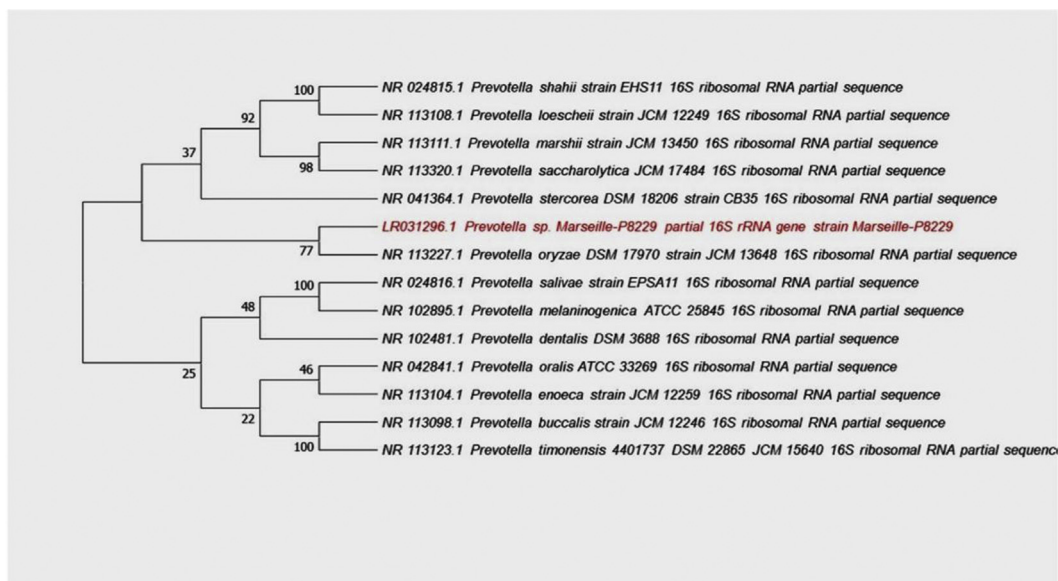

FIG. 2. Phylogenetic tree showing the position of *Prevotella marseillensis* strain Marseille- P8229^T relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using Muscle v3.8.31 with default parameters, and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 100 times to generate a majority consensus tree. The scale bar indicates a 5% nucleotide sequence divergence.

TABLE 2. **Phenotypic characterization of *Prevotella marseillensis* based on the biochemical tests**

Bacteria: *Prevotella marseillensis*	
Test	**Results (+/−)**
API 50 CH	
Control	−
Glycerol	+
Erythrol	+
D-arabinose	+
L-arabinose	+
D-ribose	+
D-xylose	+
L-xylose	+
D-adonitol	+
Methyl-βD-xylopyranoside	+
D-galactose	+
D-glucose	+
D-fructose	+
D-mannose	+
L-sorbose	+
L-rhammose	+
Dulcitol	+
Inositol	+
D-mannitol	+
D-sorbitol	+
Methyl-αD-mannopyranoside	+
Methyl-αD-glucopyranoside	+
|N-acetylglucosamine	+
Amygdaline	+
Arbutine	+
Esculine	+
Salicine	−
D-cellobiose	+
D-maltose	+
D-lactose	+
D-melibiose	+
D-saccharose	+
D-trehalose	+
Inuline	+
D-melezitose	+
D-raffinose	+
Amidon	+
Glycogene	+
Xylitol	+
Gentibiose	+
D-turanose	+
D-lyxose	+
D-tagatose	+
D-fucose	+
L-fucose	+
D-arabitol	+
L-arabitol	+
Potassium gluconate	+
Potassium 2-cetogluconate	−
Potassium 5-cetogluconate	+
API ZYM	
Control	−
Alkaline phosphatase	+
Esterase (C 4)	−
Esterase Lipase (C 8)	−
Lipase (C 14)	−
Leucine arylamidase	−
Valine arylamidase	−
Cystine arylamidase	−
Trypsine	−
α-chymotrypsine	−
Acid phosphatase	+
Naphthalo-AS-BI-phosphohydrolase	+
α-galactosidase	−
β-galactosodase	−
β-glucuronidase	−
α-glucosidase	−
β-glucosidase	−
N-acetyl-β-glucosaminidase	−
α-mannosidase	−
α-fucosidase	−

genomic similarity of strain Marseille-P8229[T] with closely related species was estimated using the ORTHOANI software [14]. Values among closely related species (Fig. 4) ranged from 65.78% between *Prevotella oryzae* and *Prevotella dentalis* to 81.75% between *Prevotella loescheii* and *Prevotella shahii*. When the isolate was compared with these closely related species, values ranged from 68.64% with *Prevotella saccharolytica* to 69.98% with *Prevotella buccalis*.
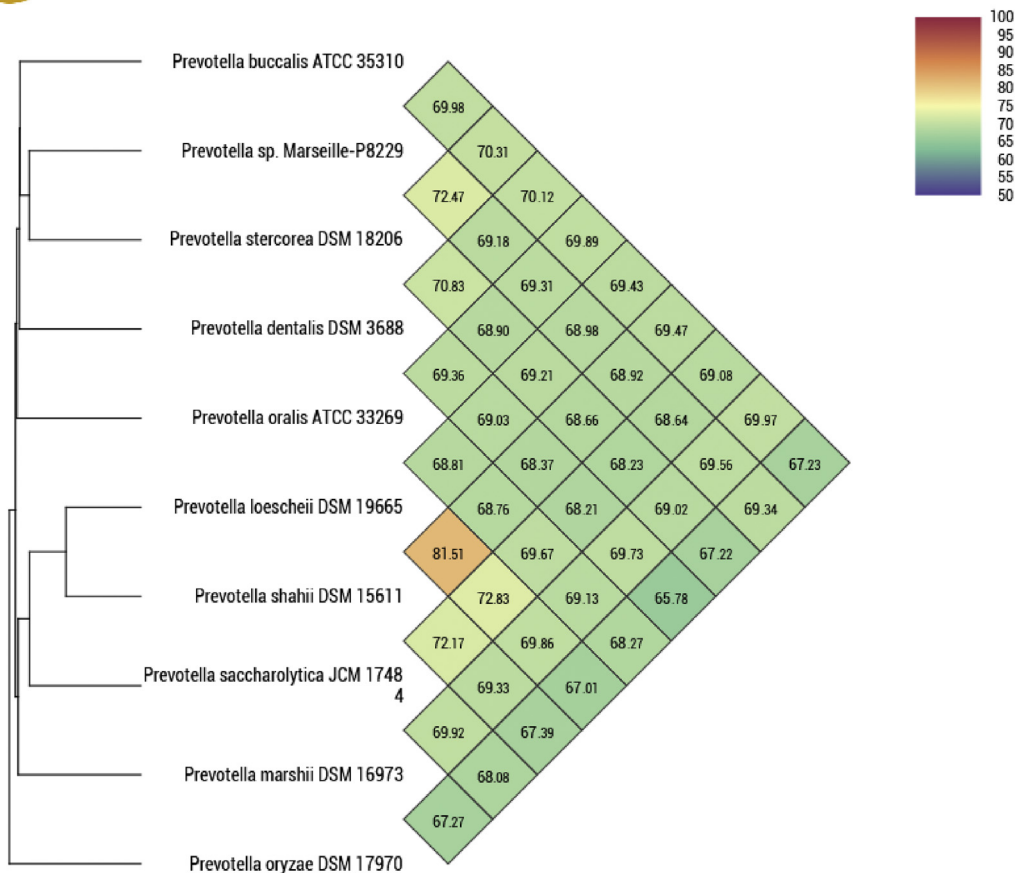
FIG. 4. Heatmap generated with ORTHOANI values calculated using the OAT software between *Prevotella marseillensis* sp. nov. and other closely related species with standing in nomenclature.

Conclusion

Strain *Prevotella marseillensis* exhibited a 16S rRNA sequence identity of 91.12% with *Prevotella shahii*. This value falls outside the 95%–98.65% threshold of 16S rRNA similarity to delineate a new species, suggesting that it belongs to a new genus. However, the phylogenetic tree shows its classification with other species of the *Prevotella* genus. So, for this strain, the 95%–98.65% cut-off does not apply. The OrthoANI value was <95% with its phylogenetically closest species with standing in nomenclature, together with unique phenotypic features. It is consequently proposed as the type strain of the new species: *Prevotella marseillensis* sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession number LR031296 and UYXY00000000, respectively.

Deposit in culture collections

Strain Marseille-P8229^T was deposited in two different strain collections under number CSURP8229.

Conflicts of interest

None to declare.

Acknowledgements

References

[1] Lagier J-C, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185–93.

[2] Lagier J-C, Hugon P, Khelaifia S, Fournier P-E, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 2015;28: 237–64.

[3] Lagier J-C, Khelaifia S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016;1:16203.

[4] Lagier JC, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev 2015;28:208–36.

[5] Fournier PE, Lagier JC, Dubourg G, Raoult D. From culturomics to taxonomogenomics: a need to change the taxonomy of prokaryotes in clinical microbiology. Anaerobe 2015;36:73–8.

[6] Ramasamy D, Mishra AK, Lagier J-C, Padhmanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 2014;64:384–91.

[7] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51.

[8] Morel A-S, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta J-P, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis 2015;34:561–70.

[9] Diop A, Khelaifia S, Armstrong N, Labas N, Fournier P-E, Raoult D, et al. Microbial culturomics unravels the halophilic microbiota repertoire of table salt: description of *Gracilibacillus massiliensis* sp. nov. Microb Ecol Health Dis 2016;27.

[10] Zerbino DR, Birney E. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18:821–9.

[11] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.

[12] Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. Gigascience 2012;1:18.

[13] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.

[14] Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.