Neoactinobaculum massilliense gen. nov., a new genesis and *Pseudopropionibacterium massiliense* sp. nov., a new bacterium isolated from the human oral microbiota

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Abstract

Neoactinobaculum massilliense gen. nov., strain Marseille-P6182^T (= CSUR P6182) and *Pseudopropionibacterium massiliense* sp. nov., strain Marseille-P6184^T (= CSUR P6184) are a new bacterial genus and new bacterial species belonging to the Actinobacteria phylum that have been isolated from the human oral microbiota.

Keywords: Culturomics, *Neoactinobaculum massilliense* gen. nov., oral microbiota, *Pseudopropionibacterium massiliense* sp. nov., taxono-genomics
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Introduction

Deciphering the bacterial diversity involved in normal and pathogenic functions appears fundamental [1]. To unveil the human oral microbiota diversity, the culturomics approach, based on diversified culture conditions, has been designed to isolate species not yet cultivated and to complement 16S rRNA metagenomics [2–4]. Furthermore, a new taxonomic strategy named taxono-genomics has been developed to include the analysis of complete genome sequences in combination with phenotypic characteristics [5]. Herein, we report a short description of strain Marseille-P6182^T and strain Marseille-P6184^T that have been isolated from the human oral microbiota.

Isolation and growth conditions

In February 2018, we isolated two bacterial strains from the oral cavity of a healthy 32-year-old man that could not be identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The screening was performed on a Microflex LT spectrometer (Bruker, Daltonics, Bremen, Germany) as previously reported [6]. Spectra obtained of strain Marseille-P6182^T (Fig. 1) and of strain Marseille-P6184^T (Fig. 2) were imported and analysed using the BIOTYPER 3.0 software against the Bruker database, which was continually incremented with the MEPHI database [6]. The strain was isolated on 5% sheep blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) at 37°C in an anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France) after a 2-day pre-incubation in an anaerobic bottle supplemented with 5% sheep blood and 5% rumen fluid, previously sterilized through a 0.2-µm microfilter (Thermo Fisher Scientific, Villebon sur Yvette, France).

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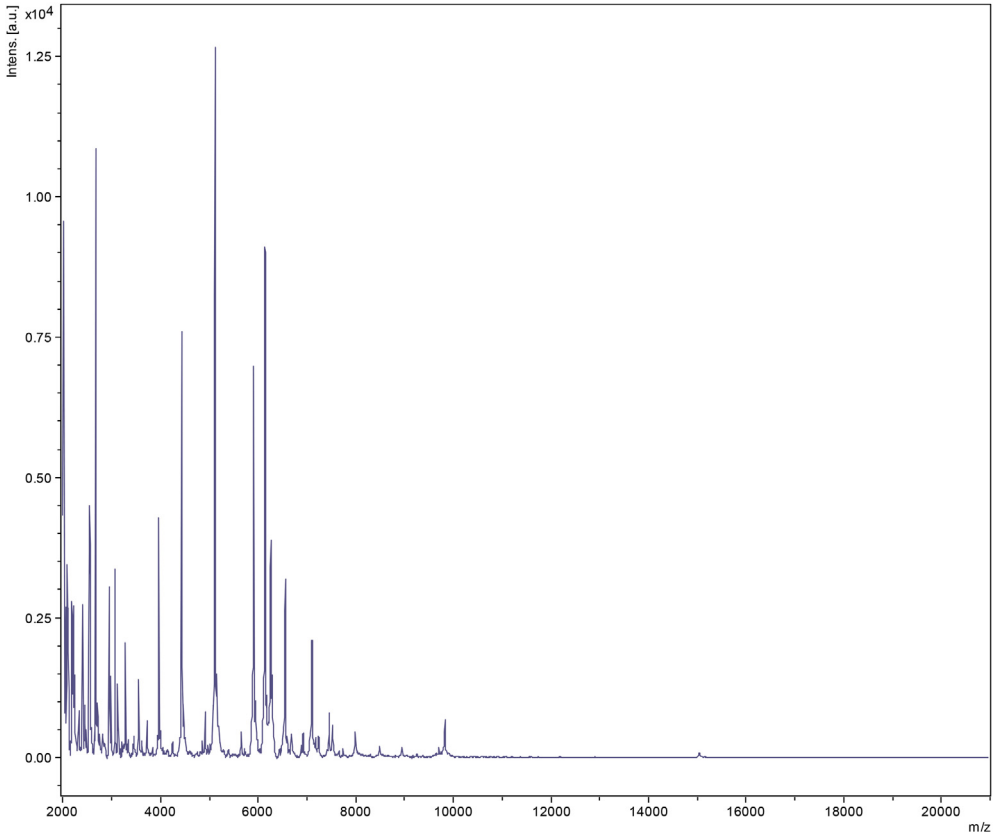

FIG. 1. MALDI-TOF MS reference spectrum of *Neoactinobaculum massilliense* gen. nov. The reference spectrum was obtained by comparing the spectra of 12 individual colonies.

Phenotypic characteristics

The colonies of strain Marseille-P6182^T were transparent and smooth with a mean diameter of 0.5–1 mm. Bacterial cells were Gram-positive bacilli ranging in length from 1.0 to 2.5 µm and from 0.3 to 0.5 µm in width (Fig. 3). The organism exhibits oxidase-negative and catalase–positive activities. The main characteristics of the strain Marseille-P6182^T are summarized in Table 1. Using the API ZYM (bioMérieux), positive enzymatic activities were observed for: naphthalo-AS-BI-phosphohydrolase, α-galactosidase and α-glucosidase; but not for: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Using API 50 CH strips (bioMérieux) the following carbohydrate was metabolized: D-glucose, D-fructose, D-maltose, D-saccharose, D-trehalose, D-raffinose, D-turanose and D-fucose. No acid production was observed from: glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-βD-xylopyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, *N*-acetylglucosamine, amygdaline, arbutine, esculin, ferric citrate, salicine, D-cellobiose, D-lactose, D-melibiose, inulin, D-melezitose, amidon, glycogen, xylitol, gentiobiose, D-xylose, D-tagatose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-cetogluconate and potassium 5-cetogluconate.

The colonies of strain Marseille-P6184^T were brown and smooth with a mean diameter of 1–1.5 mm. Bacterial cells were Gram-positive bacilli ranging in length from 3 to 3.5 µm and from 0.5 to 0.8 µm in width (Fig. 4). Strain Marseille-P6184^T exhibited neither catalase nor oxidase activities. The main characteristics of the strain Marseille-P6184^T are summarized in Table 2. Using the API ZYM (bioMérieux), positive enzymatic activities were observed for: alkaline phosphatase, lipase (C14), α-galactosidase, β-glucosidase; and negative enzymatic activities were observed for: esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsine, α-chymotrypsine, acid phosphatase, naphthalo-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Using API 50 CH strips

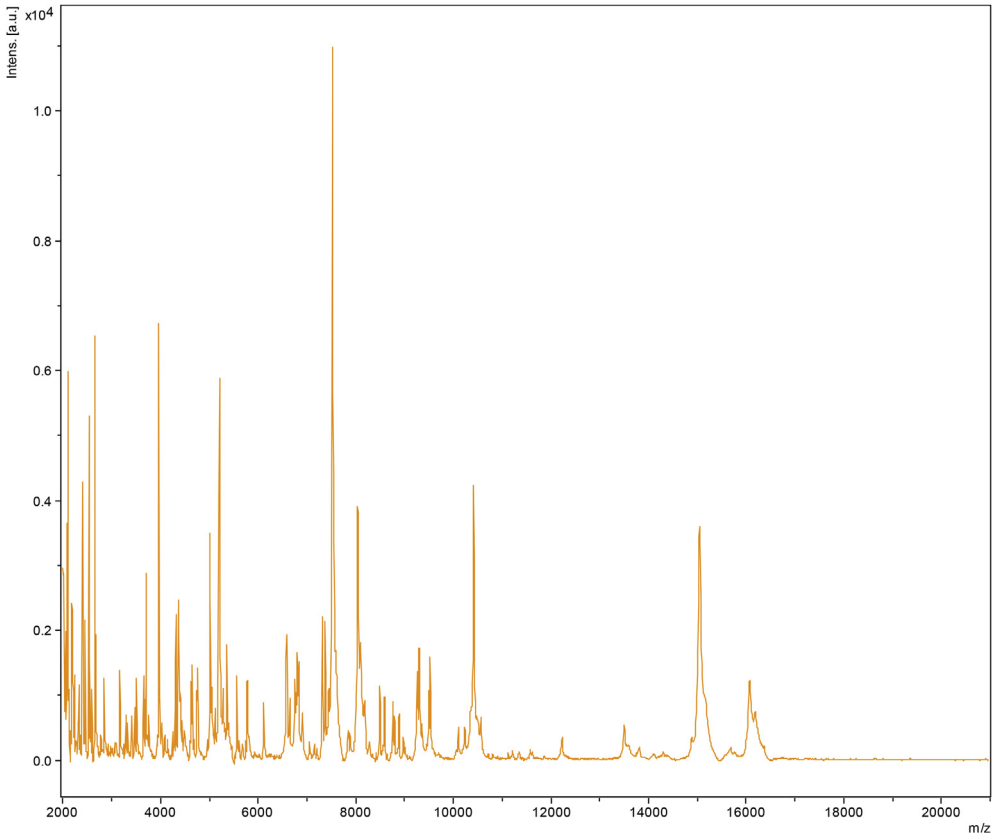

FIG. 2. MALDI-TOF MS reference spectrum of *Pseudopropionibacterium massiliense* sp. nov. The reference spectrum was obtained by comparing the spectra of 12 individual colonies.

(bioMérieux) the following carbohydrate was metabolized: erythritol, D-arabinose, D-ribose, D-adonitol, D-glucose, D-fructose, D-mannose, inositol, D-sorbitol, *N*-acetylglucosamine, D-maltose, D-lactose, D-melezitose, D-raffinose, amidon, D-turanose, L-fucose, D-arabitol, L-arabitol, potassium 5-cetogluconate. No acid production was observed from: glycerol, L-arabinose, D-xylose, L-xylose, methyl-βD-xylopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, D-mannitol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, amygdaline, arbutine, esculin, ferric citrate, salicine, D-cellobiose, D-melibiose, D-saccharose, D-trehalose, inulin, glycogen, xylitol, gentiobiose, D-xylose, D-galagtose, D-fucose, potassium gluconate and potassium 2-cetogluconate.

Strain identification

In order to classify these bacteria, the 16S rRNA gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xLGenetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France) as previously described [7]. The 16S rRNA nucleotide sequence was

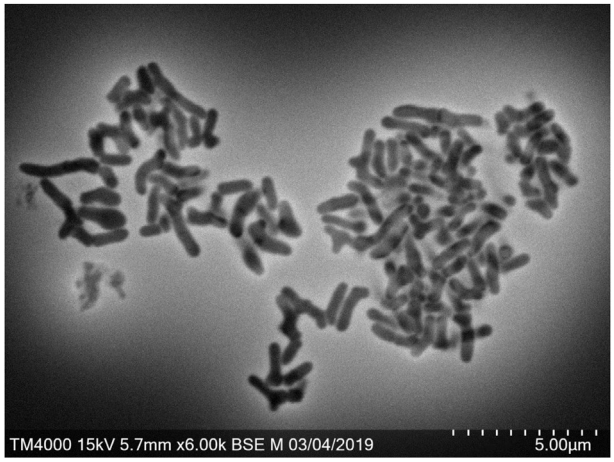

FIG. 3. Scanning electron microscopy (SEM) of stained *Neoactinobaculum massilliense* gen. nov. A colony was collected from agar and immersed in a 2.5% glutaraldehyde fixative solution. Then, a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 min and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 min to increase the SEM image contrast. The slide was gently washed in water, air-dried and examined in a tabletop SEM (Hitachi TM4000) approximately 60 cm in height and 33 cm in width to evaluate bacterial structure. The scales and acquisition parameters are presented in the figure.

TABLE 1. **Description of *Neoactinobaculum massilliense* gen. nov.**

Taxonumber	Taxon:2364794
First submission date	16 July 2019
Draft number/Date	UWPE01000001 11/28/2018
Version	NZ_UWPE01000001.1
Species name	*Neoactinobaculum massilliense*
Genus name	*Neoactinobaculum*
Specific epithet	*massilliense*
Species status	gen. nov.
Species etymology	L. neut. adj. *massiliense*, of or pertaining to Massilia, the Latin name of Marseille, France, where the organism was first isolated)
Submitter	
E-mail of the submitter	
Designation of the type strain	Strain Marseille-P6182
Strain collection numbers	CSUR P 6182
16S rRNA gene accession number	LS999995
Genome accession number [EMBL]	UWPE00000000
Genome status	Draft
Genome size	1,867,681bp
GC mol %	62.88
Data on the origin of the sample from which the strain was isolated	
Country of origin	France
Region of origin	Marseille
Date of isolation	2018-02-20
Source of isolation	Human oral sample
Sampling date	2018-02-01
Growth medium, incubation conditions [Temperature, pH, and further information] used for standard cultivation	Columbia agar supplemented with 5% sheep blood, 37°C for 48h of incubation
Gram stain	Positive
Cell shape	Bacilli
Cell size (length or diameter)	1.0–2.5 × 0.3–0.5 (µm)
Motility	nonmotile
Colony morphology	Transparent, smooth
Temperature range	37°C
Lowest temperature for growth	37°C
Highest temperature for growth	37°C
Temperature optimum	37°C
Lowest pH for growth	5.5
Highest pH for growth	8
Relationship to O_2	Anaerobe
O_2 conditions for strain testing	Aerobiosis, Anaerobiosis, Microaerophilic
Oxidase	Negative
Catalase	Positive

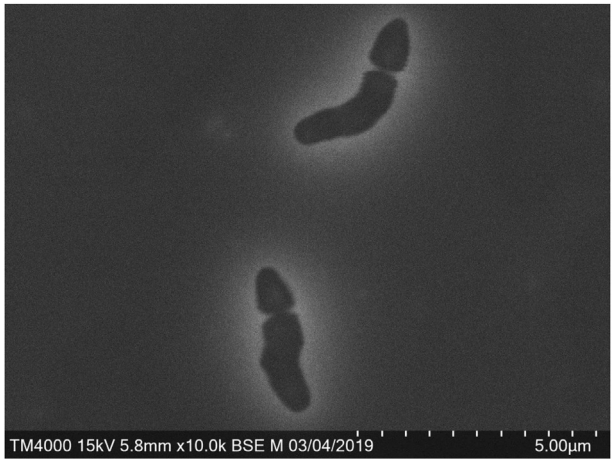

FIG. 4. Scanning electron microscopy (SEM) of stained *Pseudopropionibacterium massiliense* sp. nov. A colony was collected from agar and immersed in a 2.5% glutaraldehyde fixative solution. Then, a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 min and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 min to increase SEM image contrast. The slide was gently washed in water, air-dried and examined in a tabletop SEM (Hitachi TM4000) approximately 60 cm in height and 33 cm in width to evaluate bacterial structure. The scales and acquisition parameters are presented in the figure.

assembled and corrected using Codon Code Aligner software (http://www.codoncode.com).

Strain Marseille-P6182^T exhibited a 92.49% 16S rRNA similarity with *Actinotignum urinale* strain R9242 (GenBank accession number NR_028978.1), the phylogenetically closest species with standing in nomenclature (Fig. 5). We consequently proposed to classify strain Marseille-P6182^T as a new genus within the family *Actinomycetaceae* in the phylum Actinobacteria.

Strain Marseille-P6184^T exhibited a 98.36% 16S rRNA similarity with *Pseudopropionibacterium propionicum* strain NCTC11666 (GenBank accession number LR134535.1), the phylogenetically closest species with standing in nomenclature (Fig. 6). We consequently proposed to classify strain Marseille-P6184^T as a new species within the genus *Pseudopropionibacterium* in the phylum Actinobacteria.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) and then sequenced on a MiSeq sequencer (Illumina Inc., San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT Paired End (Illumina), as previously described [8]. The assembly was performed using a pipeline

TABLE 2. Description of *Pseudopropionibacterium massiliense* sp. nov.

Taxonumber	Taxon:2220000
First submission date	16 July 2019
Draft number/Date	UWTZ00000000 / 02/28/2019
Version	NZ_UWTZ00000000.1
Species name	*Pseudopropionibacterium massiliense*
Genus name	*Pseudopropionibacterium*
Specific epithet	massiliense
Species status	sp. nov.
Species etymology	L. neut. adj. *massiliense*, of or pertaining to Massilia, the Latin name of Marseille, France, where the organism was first isolated
Submitter	
E-mail of the submitter	
Designation of the type strain	Strain Marseille- P6184
Strain collection numbers	CSUR P6184
16S rRNA gene accession number	LS488977
Genome accession number [EMBL]	UWTZ00000000
Genome status	Draft
Genome size	4,393,662 bp
GC mol %	54.3
Data on the origin of the sample from which the strain was isolated	
Country of origin	France
Region of origin	Marseille
Date of isolation	2018-04-20
Source of isolation	Human stool sample
Sampling date	2018-04-01
Growth medium, incubation conditions [Temperature, pH, and further information] used for standard cultivation	Columbia agar supplemented with 5% sheep blood, 37°C for 48h of incubation
Gram stain	Positive
Cell shape	Rod
Cell size (length or diameter)	3.0-3.5 X 0.5-0.8 (µm)
Motility	motile
Colony morphology	Brown, smooth
Temperature range	37°C
Lowest temperature for growth	37°C
Highest temperature for growth	37°C
Temperature optimum	37°C
Lowest pH for growth	6
Highest pH for growth	8
Relationship to O_2	Anaerobe
O_2 conditions for strain testing	Aerobiosis, Anaerobiosis, Microaerophilic
Oxidase	Negative
Catalase	Negative

containing several softwares (Velvet [9], Spades [10] and Soap Denovo [11]) on trimmed data (MiSeq and Trimmomatic [12] softwares) or untrimmed data (only MiSeq software). 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 using different criteria (number of scaffolds, N50, number of N).

The genome of strain Marseille-P6182^T was 1 867 681 bp long with a 62.88 mol% G + C content. The degree of genomic similarity of strain Marseille-P6182^T with closely related species was estimated using the OrthoANI software [13]. OrthoANI values among closely related species (Fig. 7) ranged from 66.12% between *Actinotignum schaalii* and *Arcanobacterium phocae*, to 93.84% between *Trueperella bernardiae* and *Trueperella pyogenes*. When *Neoactinobaculum massilliense* was compared with these closely related species, values

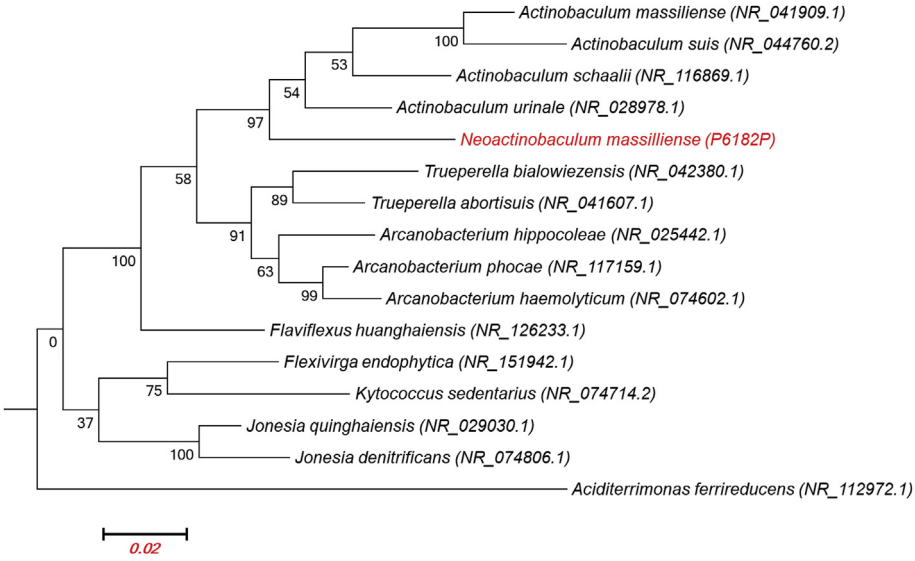

FIG. 5. Phylogenetic tree highlighting the position of *Neoactinobaculum massilliense* gen. nov., with regard to others closely related species. GenBank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inference was obtained using the maximum likelihood method and MEGA 7 software. Bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree are indicated at the nodes. The scale bar indicates a 2% nucleotide sequence divergence.

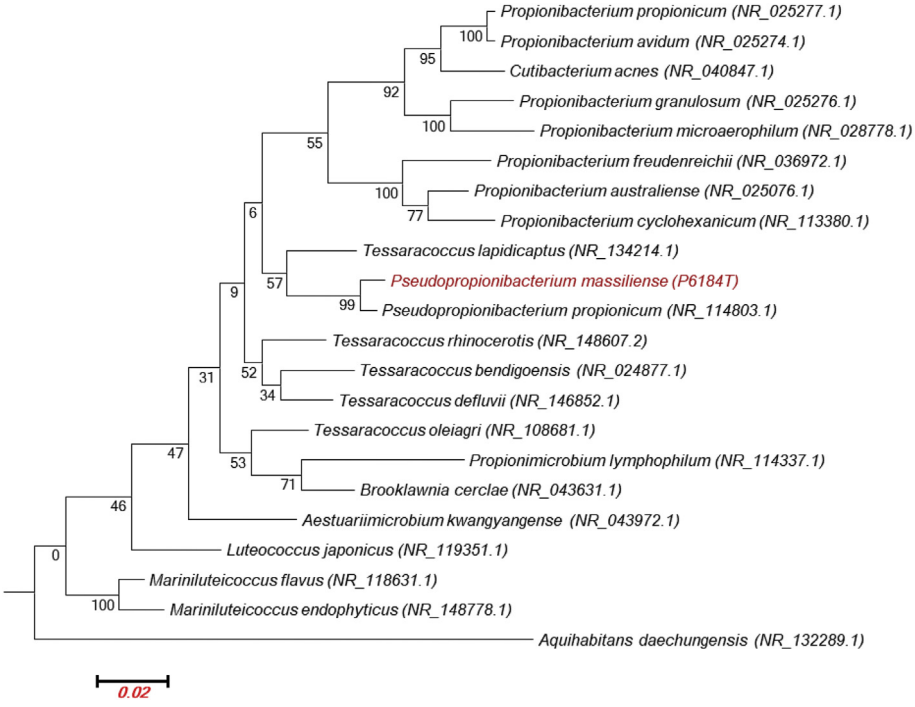

FIG. 6. Phylogenetic tree highlighting the position of *Pseudopropionibacterium massiliense* sp. nov., with regard to others closely related species. GenBank accession numbers of 16S rRNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters, phylogenetic inference were obtained using the maximum likelihood method and MEGA 7 software. Bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree are indicated at the nodes. The scale bar indicates a 2% nucleotide sequence divergence.

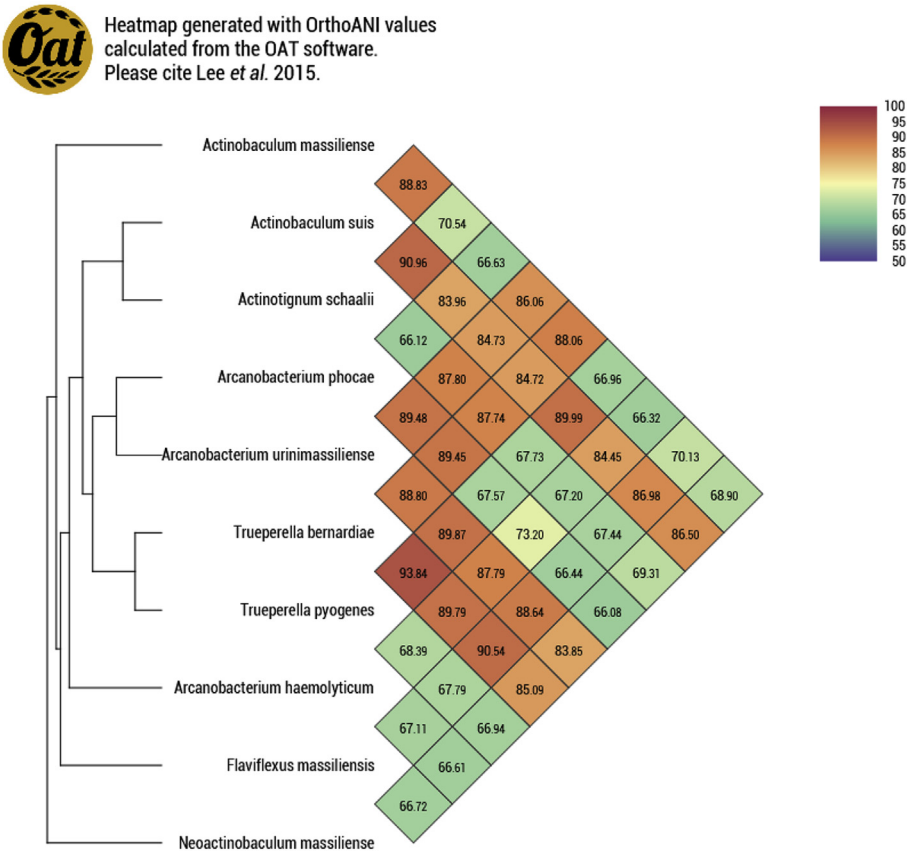

FIG. 7. Heatmap generated with OrthoANI values calculated using the OAT software between *Neoactinobaculum massilliense* gen. nov., and other closely related species with standing in nomenclature.

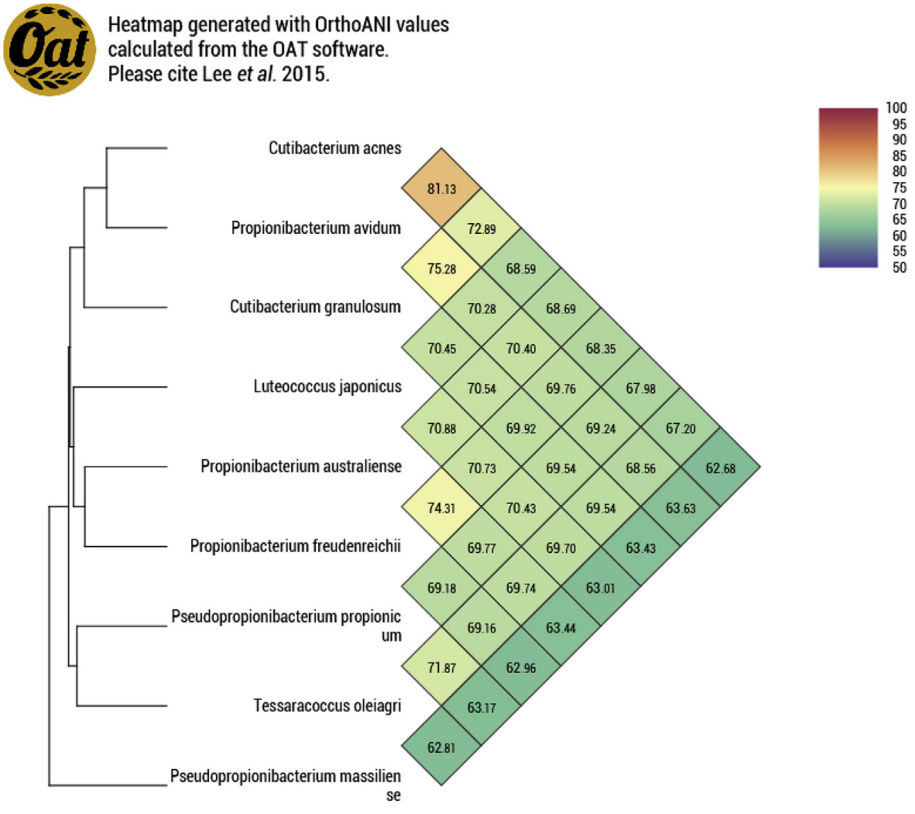

FIG. 8. Heatmap generated with OrthoANI values calculated using the OAT software between *Pseudopropionibacterium massiliense* sp. nov., and other closely related species with standing in nomenclature.

ranged from 66.08% with *Arcanobacterium phocae*, to 86.50% with *Actinobaculum suis*.

The genome of strain Marseille-P6184^T was 4 393 662 bp long with a 543 mol% G + C content. The degree of genomic similarity of strain Marseille-P6184^T with closely related species was estimated using the OrthoANI software [13]. OrthoANI values among closely related species (Fig. 8) ranged from 62.68% between *Cutibacterium acnes* and *Propionibacterium australiensis* to 81.13% between *Cutibacterium acnes* and *Propionibacterium avidum*. When *Pseudopropionibacterium massiliense* was compared with the closely related species, the value was 62.81% with *Tessaracoccus oleiagri*.

Conclusion

On the basis of unique phenotypic features, including MALDI-TOF spectrum, 16S rRNA sequence divergence >1.3% and an OrthoANI value <95% with the phylogenetically closest species with standing in nomenclature, we have formally proposed strain Marseille-P6182^T as the type strain of *Neoactinobaculum massilliense* gen. nov. (Table 1). Strain Marseille-P6184^T is the type strain of *Pseudopropionibacterium massiliense* sp. nov. (Table 2), a new species within the genus *Pseudopropionibacterium*.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences of *Neoactinobaculum massilliense* gen. nov., were deposited in GenBank under accession number LS999995 and UWPE00000000, respectively. The 16S rRNA gene and genome sequences of *Pseudopropionibacterium massiliense* sp. nov., were deposited in GenBank under accession number LS488977 and UWTZ00000000, respectively.

Deposit in culture collections

Strain Marseille-P6182^T was deposited in two different strain collections under number = CSUR P6182. Strain Marseille-P6184^T was deposited in two different strain collections under number = CSUR P6184.

Acknowledgements

The authors thank Catherine Robert for sequencing the genome and Aurelia Caputo for submitting the genomic sequence to GenBank.

Conflicts of interest

None to declare.

Funding sources

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Ethics and consent

The study was approved by the ethics committee from the local ethics committee of the IHU Mediterranée Infection (Marseille, France; agreement no. 2016-010). The patient gave and signed consent to participate in this study.

References

[1] Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007;449(7164):804–10.

[2] 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.

[3] 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.

[4] 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.

[5] 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.

[6] 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.

[7] 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.

[8] 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.

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

[10] 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.

[11] 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.

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

[13] 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.