Neoactinobaculum massilliense gen. nov., a new genus 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-P6182T (= CSUR P6182) and Pseudopropionibacterium massiliense sp. nov., strain Marseille-P6184T (= 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.

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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-P6182T (Fig. 1) and of strain Marseille-P6184T (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).

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 taxonomics 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-P6182T and strain Marseille-P6184T that have been isolated from the human oral microbiota.
Phenotypic characteristics

The colonies of strain Marseille-P6182\textsuperscript{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\textsuperscript{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-xylene, L-xylene, D-adenitol, methyl-β-D-xylpyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, N-acetylglucamine, 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\textsuperscript{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\textsuperscript{T} exhibited neither catalase nor oxidase activities. The main characteristics of the strain Marseille-P6184\textsuperscript{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, tryspine, α-chymotrypsin, 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
the following carbohydrate was metabolized: erythritol, D-arabinose, D-ribose, D-adenitol, 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-cetoglucuronate. No acid production was observed from: glycerol, L-arabinose, D-xyllose, L-xyllose, methyl-β-D-xlyopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, D-mannitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, amygdaline, arbutine, ferric citrate, salicine, D-cellobiose, D-melibiose, D-saccharose, D-trehalose, inulin, glycogen, xylitol, gentiobiose, D-xyllose, D-galactose, D-fucose, potassium gluconate and potassium 2-cetoglucuronate.

**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 3500xL Genetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France) as previously described [7]. The 16S rRNA nucleotide sequence was
### TABLE 1. Description of Neoactinobaculum massilliense gen. nov.

| Description                                      | Value                                                                 |
|--------------------------------------------------|----------------------------------------------------------------------|
| **Taxonumber**                                   | Taxon:2364794                                                        |
| **First submission date**                        | 16 July 2019                                                         |
| **Draft number/Date**                            | UWPE01000001 11/28/2018                                              |
| **Version**                                      | NZ_UWPE01000001.1                                                   |
| **Genus name**                                   | Neoactinobaculum                                                    |
| **Specific epithet**                             | massilliense gen. nov.                                              |
| **Species status**                               | L. neut. adj. massilliense, of or pertaining to Massilia, the Latin name of Marseille, France, where the organism was first isolated) |
| **Submitter**                                    | Strain Marseille-P6182                                              |
| **E-mail of the submitter**                      | CSUR P 6182                                                         |
| **Designation of the type strain**               | LS999995                                                            |
| **Strain collection numbers**                    | UWPE00000000                                                       |
| **16S rRNA gene accession number**              | Draft                                                               |
| **Genome accession number [EMBL]**               | 1.867,681bp                                                        |
| **Genome status**                                | 62.88                                                               |
| **GC mol %**                                     | Draft                                                               |
| **Data on the origin of the sample from which the strain was isolated** | France Marseille 2018-02-20 Human oral sample 2018-02-01            |
| **Country of origin**                            | Columbia agar supplemented with 5% sheep blood, 37°C for 48h of incubation |
| **Region of origin**                             | Positive                                                            |
| **Date of isolation**                            | Bacilli                                                             |
| **Source of isolation**                          | 1.0–2.5 × 0.3–0.5 (μm)                                              |
| **Sampling date**                                | nonmotile                                                           |
| **Growth medium, incubation conditions [Temperature, pH, and further information] used for standard cultivation** | Transparent, smooth 37°C                                             |
| **Gram stain**                                   | Anaerobe                                                            |
| **Cell shape**                                   | Aerobiosis, Anaerobiosis, Microaerophilic                           |
| **Cell size (length or diameter)**               | Positive                                                            |
| **Motility**                                     | Catalase                                                            |
| **Temperature range**                            | Negative                                                            |
| **Lowest temperature for growth**                | Catalase                                                            |
| **Highest temperature for growth**               | Positive                                                            |
| **Temperature optimum**                          | Catalase                                                            |
| **Lowest pH for growth**                         | Positive                                                            |
| **Highest pH for growth**                        | Catalase                                                            |
| **Relationship to O₂**                           | Positive                                                            |
| **O₂ conditions for strain testing**             | Catalase                                                            |
| **Oxidase**                                      | Positive                                                            |
| **Catalase**                                     | Positive                                                            |
assembled and corrected using Codon Code Aligner software (http://www.codoncode.com).

Strain Marseille-P6182T 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-P6182T as a new genus within the family Actinomycetaceae in the phylum Actinobacteria.

Strain Marseille-P6184T exhibited a 98.36% 16S rRNA similarity with Pseudopropionibacterium propionicum strain NCTC1666 (GenBank accession number LR134535.1), the phylogenetically closest species with standing in nomenclature (Fig. 6). We consequently proposed to classify strain Marseille-P6184T 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 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-P6182T was 1 867 681 bp long with a 62.88 mol% G + C content. The degree of genomic similarity of strain Marseille-P6182T with closely related species was estimated using the OrthoANI software [13]. OrthoANI values among closely related species ranged from 66.12% between Trueperella bernardiae and Arca- nobacterium phoceae, to 93.84% between Trueperella bernardiae and Trueperella pyogenes. When Neoctinobaculum massiliense was compared with these closely related species, values

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

| Taxonumber          | Taxon:2220000         |
|---------------------|-----------------------|
| First submission date| 16 July 2019          |
| Draft number/Date   | UWTZ200000000 / 02/19/2019 |
| Version             | NZ_UWTZ200000000.1    |
| Species name        | Pseudopropionibacterium massiliense |
| Genus name          | Pseudopropionibacterium |
| Specific epithet     | massiliense           |
| Species status       | sp. nov.              |
| Species etymology   | L. neut. adj. massiliense, 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 | UWTZ200000000 |
| Genome accession number [EMBL] | UWTZ200000000 |
| 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 | France |
| Country of origin    | Marseille             |
| 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 |
| 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 O2   | Anaerobe              |
| O2 conditions for strain testing | Aerobiosis, Anaerobiosis, Microaerophilic |
| Oxidase              | Negative              |
| Catalase             | Negative              |

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.
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-P6184T was 4,393,662 bp long with a 54.3 mol% G + C content. The degree of genomic similarity of strain Marseille-P6184T 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 massillense was compared with the closely related species, the value was 62.81% with Tessaracoccus oleagi.

**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-P6182T as the type strain of Neoactinobaculum massilliense gen. nov. (Table 1). Strain Marseille-P6184T is the type strain of Pseudopropionibacterium massillense 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 UVPPE00000000, respectively. The 16S rRNA gene and genome sequences of Pseudopropionibacterium massillense sp. nov., were deposited in GenBank under accession number LS488977 and UWTZ00000000, respectively.

**Deposit in culture collections**

Strain Marseille-P6182T was deposited in two different strain collections under number = CSUR P6182. Strain Marseille-P6184T was deposited in two different strain collections under number = CSUR P6184.

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**Conflicts of interest**

None to declare.

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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.

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