NEW SPECIES

Corynebacterium pacaense sp. nov., Alistipes megaguti sp. nov., Alistipes provencensis sp. nov., 3 new bacteria isolated from fresh human stool specimens

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Abstract

Here we describe the basic characteristics of Corynebacterium pacaense strain Marseille-P2417T (= CSUR P2417), Alistipes megaguti strain Marseille-P5997T (= CSUR P5997) and Alistipes provencensis strain Marseille-P2431T (= CSUR P2431 = DSM 102308). The phenotypic criteria, the 16S ribosomal RNA sequencing and MALDI-TOF MS spectra analysis were used to identify and characterize these new bacteria species, which were isolated from fresh human stool specimens.

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Keywords: Alistipes megaguti, Alistipes provencensis, Corynebacterium pacaense, culturomics, taxonogenomics

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Introduction

Culturomics is the concept of developing different culture conditions in order to enlarge our knowledge of the human microbiota through the discovery of previously uncultured bacteria [1–4]. However, after bacterial strain isolation, we used a taxonogenomics approach, including MALDI-TOF MS, phylogenetic analysis, main phenotypic description and genome sequencing, to describe the bacterium [5,6]. In this study, we describe three new species isolated for the first time from human stool samples.

Isolation and growth conditions

In 2016, strain Marseille-P2417T was isolated from a fresh stool sample of a 24-year-old healthy woman. In 2017, strain Marseille-P5997T was isolated from a fresh stool sample of a 25-year-old healthy woman. In 2015, strain Marseille-P2431T was isolated from a 66-year-old man with hypertension and diabetes. Screening was performed by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The spectra generated from each strain (Fig. 1) were imported and analysed by Biotype 3.0 software against the Bruker database, which is constantly updated with the Microbes Evolution Phylogeny and Infections (MEPHI) database.

The initial growth of strain Marseille-P2417T was obtained after 24 hours of culture in Colombia agar enriched with 5% sheep’s blood (bioMérieux, Marcy l’Etoile, France) in aerobic conditions at 37°C and pH 7.5. The initial growth of strains Marseille-P5997T and Marseille-P2431T was obtained after 48 hours of culture in a Colombia agar enriched with 5% sheep’s blood (bioMérieux) in anaerobic conditions at 37°C and pH 7.5.

The study of these microbes (strains Marseille-P2417, Marseille-P5997 and Marseille-P2431) was validated by the ethics committee of IHU–Méditerranée Infection under numbers 2016-010, 2016-011 and 09-022 respectively.
Strain identification

The 16S ribosomal RNA (rRNA) gene was sequenced in order to classify these bacteria. Amplification was performed using the primers fD1, rP2 and rpoB (Eurogentec, Angers, France) and was sequenced using the Big Dye Terminator v1.1 Cycle Sequencing Kit and the sequencer (Thermo Fisher Scientific, Waltham, MA, USA), as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (https://www.codoncode.com). The 16S rRNA gene (accession no. LT223574) of strain Marseille-P2417T exhibited a 97.7% sequence similarity with Corynebacterium efficiens strain YS-314T (GenBank accession no. NR_102865), the phylogenetically closest species with standing in nomenclature (Fig. 2(A)). Those of strain Marseille-P5997T (accession no. LS999984) and strain Marseille-P2431T (accession no. LT223566) showed a sequence similarity of 96.9% and 98.5% with Alistipes senegalensis strain JC50T (GenBank accession no. NR_118219) and Alistipes timonensis strain JC136T (GenBank accession no. NR_125589) respectively; these were the phylogenetically closest species with standing in nomenclature (Fig. 2(B)). These values were lower than the 98.7% 16S rRNA gene sequence threshold recommended by Meier-Kolthoff et al. [9] to delineate a new bacterial species without performing DNA–DNA hybridization.

FIG. 1. MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and reference spectrum generated.
FIG. 2. Phylogenetic trees showing positions of Corynebacterium pacaense strain Marseille-P2417T (A), Alistipes megaguti strain Marseille-P5997T and Alistipes provencensis strain Marseille-P2431T (B) relative to other phylogenetically close neighbours. Respective GenBank accession numbers for 16S ribosomal RNA genes are indicated in parentheses. Sequences were aligned using Muscle v3.8.31 with default parameters, and phylogenetic inferences were obtained using maximum likelihood method within software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Only bootstrap values > 71% were retained. Scale bar indicates 2% nucleotide sequence divergence.
We consequently proposed to classify strains Marseille-P2417\textsuperscript{T}, Marseille-P5997\textsuperscript{T} and Marseille-P2431\textsuperscript{T} as new species within the genera *Corynebacterium* and *Alistipes* respectively.

**FIG. 3.** Scanning electron micrograph of (a) *Corynebacterium pacaense*, (b) *Alistipes megaguti* and (c) *Alistipes provencensis* using TM4000Plus Tabletop microscope (Hitachi, Yokohama, Japan). Scale bar and acquisition settings are shown.

**TABLE 1.** Biochemical tests of *Corynebacterium pacaense*, *Alistipes megaguti* and *Alistipes provencensis* (API 50 CH strips)

| Test                      | *C. pacaense* | *A. megaguti* | *A. provencensis* |
|---------------------------|---------------|---------------|-------------------|
| Glycerol                 | –             | +             | –                 |
| Erythrol                  | –             | –             | –                 |
| α-Arabinose               | –             | –             | w                 |
| α-Ribose                  | –             | –             | –                 |
| α-Xylose                  | –             | +             | –                 |
| α-Mannose                 | –             | +             | w                 |
| α-Sorbose                 | –             | –             | –                 |
| α-Rhamnose                | –             | –             | –                 |
| Dulcitol                  | –             | w             | –                 |
| Inositol                  | –             | +             | w                 |
| D-Mannitol                | +             | +             | –                 |
| D-Sorbitol                | –             | +             | –                 |
| Methyl-α-D-mannopyranoside| –             | –             | w                 |
| α-Galactose               | –             | –             | –                 |
| β-Glucose                 | +             | +             | –                 |
| α-Fructose                | +             | +             | –                 |
| α-Glucose                 | +             | +             | –                 |
| α-Mannose                 | +             | +             | +                 |
| α-Sorbose                 | –             | –             | w                 |
| α-Rhamnose                | –             | –             | w                 |
| Arbutin                   | w             | +             | –                 |
| Esculin                   | +             | +             | +                 |
| Salicin                   | –             | –             | –                 |
| α-Cellobiose              | –             | –             | –                 |
| D-Maltose                 | –             | +             | +                 |
| D-Lactose                 | –             | –             | +                 |
| α-Melibiose               | –             | +             | –                 |
| α-Sucrose                 | +             | –             | –                 |
| α-Trehalose               | w             | +             | –                 |
| Inulin                    | –             | –             | –                 |
| α-Melezitose              | –             | –             | +                 |
| α-Raffinose               | –             | –             | +                 |
| Starch                    | –             | w             | –                 |
| Glycogen                  | –             | –             | –                 |
| Xylitol                   | –             | –             | –                 |
| Gentibiose                | –             | +             | –                 |
| α-Turanose                | –             | –             | –                 |
| α-Lyxose                  | –             | w             | –                 |
| β-Tigecycline             | –             | +             | –                 |
| α-Fucosid                 | –             | w             | –                 |
| α-2-Methylfucosid         | –             | w             | –                 |
| α-Ribose                  | –             | –             | –                 |
| α-Xylose                  | –             | w             | –                 |
| α-Mannosidase             | –             | –             | –                 |
| α-Fucosidase              | –             | +             | –                 |
| Amygdalin                 | –             | +             | –                 |
| N-Acetylglucosamine       | –             | –             | +                 |
| Arbutin                   | w             | +             | –                 |

+, positive result; –, negative result.

**TABLE 2.** Biochemical tests of *Corynebacterium pacaense*, *Alistipes megaguti* and *Alistipes provencensis* (API ZYM strips)

| Test                      | *C. pacaense* | *A. megaguti* | *A. provencensis* |
|---------------------------|---------------|---------------|-------------------|
| Alkaline phosphatase      | –             | –             | –                 |
| Esterase (C4)             | +             | +             | +                 |
| Esterase lipase (C8)      | –             | +             | –                 |
| Lipase (C14)              | –             | –             | +                 |
| Leucine arylamidase       | +             | –             | –                 |
| Valine arylamidase        | –             | +             | –                 |
| Cystine arylamidase       | –             | –             | –                 |
| Tryptophosphatase         | +             | +             | –                 |
| α-Chymotrypsin            | +             | +             | +                 |
| Phosphatase acid          | +             | +             | –                 |
| Naphthol-AS-BI-phosphohydrolase | +         | +             | +                 |
| α-Galactosidase           | –             | –             | –                 |
| β-Galactosidase           | –             | +             | +                 |
| β-Glucuronidase           | –             | –             | –                 |
| α-Glucosidase             | –             | –             | –                 |
| β-Glucosidase             | +             | –             | –                 |
| N-Acetyl-β-glucosaminidase| –             | –             | –                 |
| α-Mannosidase             | –             | +             | –                 |
| α-Fucosidase              | –             | –             | +                 |

+, positive result; –, negative result.

**TABLE 3.** Biochemical tests of *Corynebacterium pacaense* (API Coryne strips)

| Test                      | Result |
|---------------------------|--------|
| Potassium nitrate         | –      |
| Pyrazine carboxamide      | –      |
| Pyrogulatinic acid–β-naphthylamide | –      |
| 2-Naphthyl-phosphate      | –      |
| Naphthol-AS-BI-glucuronidase | –  |
| α-Galactosidase           | –      |
| β-Galactosidase           | –      |
| β-Glucuronidase           | –      |
| α-Glucosidase             | –      |
| β-Glucosidase             | –      |
| N-Acetyl-β-glucosaminidase| –      |
| α-Mannosidase             | –      |
| Urea                      | +      |
| Gelatin (bovine origin)   | –      |
| α-Glucose                 | +      |
| α-2-Methylfucosid         | +      |
| α-Xylose                  | –      |
| α-Mannosidase             | +      |
| D-Maltose                 | +      |
| D-Lactose (bovine origin) | –      |
| α-Fucosidase              | +      |
| α-Glucosidase             | –      |
| N-Acetyl-β-glucosaminidase| –      |

+, positive result; –, negative result.
Phenotypic characteristics

Strain Marseille-P2417T formed circular white colonies with a mean diameter of 1.15 mm. Bacterial cells of strain Marseille-P2417T were Gram-positive and rod shaped, and they ranged in length from 1.29 to 1.5 μm and in width from 0.5 to 0.6 μm (Fig. 3(a)). Strain Marseille-P2417T showed catalase-positive and oxidase-negative activities. Colonies of the strain Marseille-P5997T were 0.2 to 0.9 mm in diameter on blood-enriched Columbia agar. Bacterial cells of strain Marseille-P5997T were Gram negative and rod shaped, and they ranged in length from 1.36 to 3.57 μm and in width from 0.45 to 0.6 μm (Fig. 3(b)). Strain Marseille-P5997T showed catalase-negative and oxidase-negative activities. Colonies of the strain Marseille-P2431T were 0.4 to 0.64 mm in diameter on blood-enriched Columbia agar. Bacterial cells were Gram negative and rod shaped, and they ranged in length from 0.9 to 1.43 μm and in width from 0.4 to 0.5 μm (Fig. 3(c)). Strain Marseille-P2431T showed catalase-positive and oxidase-negative activities.

The main phenotypic properties of the three strains were studied by using API 50 CH strips (Table 1), API ZYM strips (Table 2), API Coryne strips (Table 3) and API 20A strips (Table 4). The main characteristics of strains Marseille-P2417T, Marseille-P5997T and Marseille-P2431T are respectively summarized at the Digital Protologue website (http://imedea.uib-csic.es/dprotologue/) under the following numbers: TA00965, TA00955 and TA00966. A comparative study of the main biochemical and phenotypic features of the closest Corynebacterium species and Alistipes species is presented in Table 5.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit, then was sequenced using with the), as previously described[10]. The assembly was performed with a pipeline incorporating different

### Table 4. Biochemical tests of Alistipes megaguti and Alistipes provencensis (API 20A strips)

| Test                      | Alistipes megaguti | Alistipes provencensis |
|---------------------------|--------------------|------------------------|
| L-Tryptophane             | +                  | −                      |
| Urea                      | −                  | −                      |
| D-Glucose                 | +                  | +                      |
| D-Mannitol                | +                  | +                      |
| D-Lactose                 | +                  | +                      |
| D-Saccharose              | −                  | −                      |
| D-Maltose                 | +                  | +                      |
| Salicin                   | +                  | +                      |
| D-Xylose                  | +                  | +                      |
| D-Arabinose               | +                  | +                      |
| Gelatin (bovine origin)   | +                  | +                      |
| Esculin ferric citrate    | −                  | −                      |
| D-Cellobiose              | +                  | +                      |
| D-Mannose                 | +                  | −                      |
| D-Melezitose              | −                  | −                      |
| D-Raffinose               | −                  | −                      |
| D-Sorbitol                | −                  | −                      |
| D-Trehalose               | +                  | +                      |
| −, positive result; −, negative result.

### Table 5. Differential characteristics of Corynebacterium pacaense sp. nov. (1), Corynebacterium efficiens (2), Corynebacterium humireducens (3), Alistipes megaguti sp. nov. (4), Alistipes provencensis sp. nov. (5), Alistipes ihumii (6), Alistipes senegalensis (7) and Alistipes timonensis (8)

| Property                        | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Cell diameter (μm)              | 0.5–0.8 | 0.8–1.1 | 0.5–0.7 | 0.45–0.6 | 0.4–0.5 | 0.72 | 0.56 | 0.62 |
| Oxygen requirement              | Facultatively anaerobic | Facultatively anaerobic | Facultatively anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic | Anaerobic |
| Motility                        | +   | +   | +   | +   | +   | +   | +   | +   |
| Endospore formation             | −   | −   | −   | −   | −   | −   | −   | −   |
| Production of:                  |     |     |     |     |     |     |     |     |
| Alkaline phosphatase            | −   | −   | +   | −   | −   | NA  | NA  | NA  |
| Catalase                        | +   | +   | +   | +   | +   | +   | +   | +   |
| Oxidase                         | −   | −   | −   | −   | −   | −   | −   | −   |
| Urease                          | −   | −   | −   | −   | −   | −   | −   | −   |
| β-Galactosidase                 | −   | −   | −   | −   | −   | w   | +   | +   |
| N-Acetyl-D-glucosamine          | −   | −   | −   | −   | −   | −   | −   | −   |
| Acid from:                      |     |     |     |     |     |     |     |     |
| D-Arabinose                     | −   | −   | −   | −   | −   | −   | −   | −   |
| Ribose                          | +   | +   | +   | +   | +   | +   | +   | +   |
| Mannose                         | +   | +   | +   | +   | +   | +   | +   | +   |
| Maltose                         | +   | +   | +   | +   | +   | +   | +   | +   |
| Sucrose                         | +   | +   | +   | +   | +   | +   | +   | +   |
| D-Glucose                       | +   | +   | +   | +   | +   | +   | +   | +   |
| D-Fructose                      | +   | +   | +   | +   | +   | +   | +   | +   |
| D-Maltose                       | +   | +   | +   | +   | +   | +   | +   | +   |
| D-Lactose                       | +   | +   | +   | +   | +   | +   | +   | +   |
| G+C content (mol%)              | 63.7 | 59.0 | 59.0 | 58.6 | 58.3 | 57.9 | 58.4 | 58.8 |
| Source                          | Human gut | Soil | Microbial fuel cell | Human gut | Human gut | Human gut | Human gut | Human gut |
| +, positive result; −, negative result; w, weakly positive result; NA, data not available.
FIG. 4. Heat maps generated with OrthoANI values calculated using OAT software for Corynebacterium pacaense sp. nov. (A), Alistipes megaguti sp. nov., and Alistipes provencensis sp. nov. (B) with other closely related species with standing in nomenclature.
The genome of strain Marseille-P2417T was 3,030,201 bp long (13 scaffolds, 16 N50) with a 63.75 mol% G+C content and contains 2,820 predicted genes. The degree of genomic similarity of Marseille-P2417T with closely related species was measured about 3,270,862 and 3,805,103 bp long respectively. A genomic similarity analysis of Alistipes species was performed by OrthoANI software [15]. Values among closely related species (Fig. 4(A)) ranged from 68.40% between Corynebacterium crudilactic and Corynebacterium gultamicum. To these closely related species, values ranged from 70.22% with Alistipes putredinis to 91.39% between Alistipes ihumii and Alistipes timonensis. A list of all species and values can be found in Table S4.

**Conclusion**

Strains Marseille-P2417T, Marseille-P5997T and Marseille-P2431T exhibited a 16S rRNA sequence similarity of <98.65% and an OrthoANI value < 95% with its phylogenetically closest species with standing in nomenclature, and we consequently propose them to be the type strains of the new Corynebacterium pacense sp. nov., Alistipes megaguti sp. nov. and Alistipes provencensis sp. nov. respectively.

**Description of Alistipes megaguti sp. nov.**

Alistipes megaguti (meg.a.gu’ti, N.L. masc. adj. megaguti, ‘mega-gut,’ the name of a project aiming to individually cultivate all species from the digestive tract of healthy humans). Cells were anaerobic, Gram-negative, nonmotile and asporogenous rods. Catalase and oxidase activities were negative. Bacterial cells had a length of 1.36 to 3.57 μm and a width of 0.5 to 0.8 μm. Colonies growing on 5% sheep’s blood–enriched Columbia agar (bioMérieux) were circular and white after 72 hours of incubation in aerobic atmosphere at 37°C, and they showed a mean diameter of 1.15 mm. Positive reactions were observed for D-glucose, D-mannitol, D-sucrose, D-fructose, D-mannose, esculin, salicin, esterase (C4), leucine arylamidase, phosphatase acid, naphtaloyl-AS-BI-phosphohydrolase and β-glucosidase. Negative reactions were obtained with glycerol, D-maltose, D-ribose, D-xyllose, glycogen, amygdalin, D-adonitol, D-arabinose, D-arabitol, D-cellobiose, D-fucose, D-galactose, D-lactose, D-lyxose, D-melezitose, D-melibiose, D-raffinose, D-sorbitol, D-tagatose, D-turanose, dulcitol, erythrol, gentiobiase, inulin, L-arabinose, L-arabitol, L-lucose, L-rhamnose, L-sorbitose, L-xyllose, methyl-D-glucopyranoside, methyl-D-xylopyranoside, N-acetylglucosamine, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium glutonate, starch, xylitol, alkaline phosphatase, esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

The genome of strain Marseille-P2417T was 3,030,201 bp long, and its G+C content was 63.75 mol%. The strain Marseille-P2417T was isolated from a fresh stool sample from a 24-year-old healthy woman. It was deposited in the Collection de l’Unité des Rickettsies (CSUR) collection under accession number CSUR P2417. The 16S rRNA, rpoB genes and genome sequences are available in GenBank under accession numbers LT223574, LT223580 and FWCI00000000 respectively.

**Description of Corynebacterium pacense sp. nov.**

Corynebacterium pacense (pa.ca.en’se, L. adj. fem., ‘PACA,’ the name of the region Provence-Alpes-Côte d’Azur, France, where the strain was isolated). Cells were aerobic, Gram positive, rod shaped, nonmotile and non–spore forming, and catalase and oxidase. Cells had a length of 1.29 to 1.5 μm and a width of 0.5 to 0.8 μm. Colonies growing on 5% sheep’s blood–enriched Columbia agar (bioMérieux) were circular and white after 72 hours of incubation in aerobic atmosphere at 37°C, and they showed a mean diameter of 1.15 mm. Positive reactions were observed for D-glucose, D-mannitol, D-sucrose, D-fructose, D-mannose, esculin, salicin, esterase (C4), leucine arylamidase, phosphatase acid, naphtaloyl-AS-BI-phosphohydrolase and β-glucosidase. Negative reactions were obtained with glycerol, D-maltose, D-ribose, D-xyllose, glycogen, amygdalin, D-adonitol, D-arabinose, D-arabitol, D-cellobiose, D-fucose, D-galactose, D-lactose, D-lyxose, D-melezitose, D-melibiose, D-raffinose, D-sorbitol, D-tagatose, D-turanose, dulcitol, erythrol, gentiobiase, inulin, L-arabinose, L-arabitol, L-lucose, L-rhamnose, L-sorbitose, L-xyllose, methyl-D-glucopyranoside, methyl-D-xylopyranoside, N-acetylglucosamine, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium glutonate, starch, xylitol, alkaline phosphatase, esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase.

The genome of strain Marseille-P2417T was 3,030,201 bp long, and its G+C content was 63.75 mol%. The strain Marseille-P2417T was isolated from a fresh stool sample from a 24-year-old healthy woman. It was deposited in the Collection de Souches de l’Unité des Rickettsies (CSUR) collection under accession number CSUR P2417. The 16S rRNA, rpoB genes and genome sequences are available in GenBank under accession numbers LT223574, LT223580 and FWCI00000000 respectively.
Negative reactions were observed for alkaline phosphatase, leucine arylamidase, α-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β glucosaminidase, d-arabinose, l-xyllose, methyl β-D-xlylopyranoside, L-rhamnose, d-sorbitol, methyl α-D-mannopyranoside, sucrose, glycogen and potassium 2-ketogluconate. The most abundant fatty acid by far was hexadecanoic acid (53%), followed by 13-methyltetradecanoic acid (16%) and 9-octadecenoic acid (8%).

The genome of strain Marseille-P5997T was 3 270 862 bp long, and its G+C content was 58.6 mol%. Strain Marseille-P5997T was isolated from a fresh stool sample from a 25-year-old healthy woman and was deposited in the CSUR collection under accession number CSUR P5997. The 16S rRNA and genome sequences are available in GenBank under accession numbers LS999984 and LR027382 respectively.

**Description of Alistipes provencensis sp. nov.**

Alistipes provencensis (pro.ven.cen’sis, N.L. adj. neut., from Provence, the region in France, where the strain was isolated). Cells were anaerobic, Gram-negative, nonmotile, asporogenous rods that were catalase positive and oxidase negative. Cells had a length of 0.9 to 1.43 μm and a width of 0.4 to 0.5 μm. Colonies of the strain Marseille-P2431T were 0.4 to 0.64 mm in diameter on blood-enriched Columbia agar. Growth occurred at 37°C under anaerobic conditions. Positive reactions were observed for esterase (C4), lipase (C14), β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, D-xyllose, D-glucose, D-mannose, esculin, D-lactose, D-melezitose and D-raffinose. Negative reactions were observed with alkaline phosphatase, esterase lipase (C8), α-chymotrypsin, phosphatase acid, α-galactosidase, N-acetyl-β-glucosaminidase, glycerol, erythrol, L-arabinose, D-ribose, D-galactose, D-fructose, dulcitol, D-mannitol, amygdalin, arbutin, D-cellobiose, D-maltose, D-sucrose, D-trehalose, inulin, starch, glycogen, D-fucose and potassium 2-ketogluconate.

The genome of strain Marseille-P2431T was 3 805 103 bp long, and its G+C content was 58.3 mol%. Strain Marseille-P2431T was isolated from a 66-year-old man with diabetes and was deposited in the CSUR collection under accession number CSUR P2431. The 16S rRNA and genome sequences of the strain Marseille-P5997T and strain Marseille-P2431T were respectively deposited in the CSUR collection under the following numbers: CSUR 2417T, CSUR P5997T and CSUR 2431T = DSM 102308.

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**Conflict of Interest**

None declared.

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