**Anaerosphaera massiliensis** sp. nov., a new bacterium isolated from the stool of a 39-year-old Pygmy

T. Takakura1, R. Francis2,3, H. Anani2,4, M. Bilen2, D. Raoult2,3 and J. Y. Bou Khalil2

1) Hitachi High-Technologies Corporation, Analytical & Medical Solution Business Group, Ibaraki-ken, Japan, 2) Institut Hospitalo-Universitaire Méditerranée-Infection, 3) Aix-Marseille Université, Institut de Recherche pour le Développement, UMR Microbes Evolution Phylogeny and Infections (MEPHI) and 4) Aix Marseille Université, Institut de Recherche pour le Développement, Service de Santé des Armées, AP-HM, UMR Vecteurs Infections Tropicales et Méditerranéennes (VITROME), Marseille, France

**Abstract**

Anaerosphaera massiliensis strain Marseille-P4592T (= CSURP4592T; = CCUG72452T) is a new species isolated from the stool of a 39-year-old male Pygmy from the Democratic Republic of Congo.

© 2019 The Authors. Published by Elsevier Ltd.

Keywords: Anaerosphaera massiliensis, culturomics, new species, stool, taxono-genomics

Original Submission: 27 July 2019; Revised Submission: 25 November 2019; Accepted: 4 December 2019

Article published online: 12 December 2019

**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]. Once isolated, 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 and genome sequencing, to describe it [5,6].

**Isolation and growth conditions**

In 2017, we isolated from the stool sample of a healthy 39-year-old male Pygmy an unidentified bacterial strain. A screening was performed by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [7]. The obtained spectrum (Fig. 1) was imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in two databases (Bruker and the constantly updated MEPHI databases; https://www.mediterranee-infection.com/urms-data-base/). The spectrum from strain Marseille-P4592 was compared with 71 other species from the genus of Peptoniphilus and one from the genus Tissierella (Fig. 2). The study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 2016-011. The growth was obtained 24 h after culture in a Colombia agar enriched with 5% sheep blood (bioMérieux, Marcy l’Etoile, France) under anaerobic conditions at 37°C.

**Phenotypic characteristics**

Colonies were white with a mean diameter of 1 mm. Bacterial cells were Gram-positive, round-shaped, with a diameter ranging from 662 to 763 nm (Fig. 3). Strain Marseille-P4592 showed negative catalase and oxidase activities. API 50 CH and API ZYM tests were performed at 37°C under anaerobic conditions. Using an API ZYM strip, a positive reaction was observed for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase and naphthol-AS-BI-phosphohydrolase but negative reactions were obtained for alkaline and acid phosphatases, lipase, trypsine, 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-P4592 was able to metabolize esculin, D-tagatose and potassium 5-ketogluconate. However, negative reactions were obtained for glycerol, D-glucose, D-galactose, D-maltose, D-fructose, D-mannose, methyl-α-D-glucopyranoside, N-acetylglucosamine, D-lactose, D-saccharose, D-trehalose, D-turanose, erythritol, D-arabinose, L-arabinose, D-xylose, L-xylene, D-adonitol, methyl-β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-mannopyranoside, amygdalin, arbutin, salicin, D-
cellobiose, D-melibiose, inulin, D-melezitose, D-raffinose, starch, glycophen, xylitol, gentiobiose, D-lyxose, D-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 2-ketogluconate. The main biochemical characteristics of strain Marseille-P4592 and its closest species with standing in nomenclature are detailed in Table 1.

**Strain identification**

The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification and sequencing were performed using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and the Big Dye® Terminator v1.1 Cycle Sequencing Kit and ABI Prism 3130xl Genetic Analyzer capillary sequencer (Thermo Fisher, Saint-Aubin, France), respectively, as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CODONCODE ALIGNER software (http://www.codoncode.com). Strain Marseille-P4592 exhibited a 95.39% sequence identity with Anaerosphaera aminiphila strain WN036 (GenBank accession number NR041586), the phylogenetically closest species with standing in nomenclature (Fig. 4). Consequently, strain Marseille-P4592 was classified as a new member of the genus Anaerosphaera, family Peptoniphilaceae, phylum Firmicutes, with the stain Marseille P4592T as the type strain of the new species Anaerosphaera massiliensis.

**TABLE 1. Comparative biochemical characteristics of (1) Anaerosphaera massiliensis, (2) Anaerosphaera aminiphila, (3) Peptoniphilus asaccharolyticus, (4) Peptoniphilus indolicus, (5) Peptoniphilus coxii, (6) Peptoniphilus duerdenii, (7) Tepidimicrobium xylanilyticum, and (8) Tissierella creatinini [19–24]**

| Characteristic            | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|---------------------------|----|----|----|----|----|----|----|----|
| Indole                    | –  | –  | w  | +  | –  | –  | +  | –  |
| Uronic                    | –  | –  | –  | –  | –  | –  | –  | –  |
| Alkaline phosphatase      | –  | –  | –  | +  | –  | –  | –  | –  |
| Coagulase                 | –  | –  | –  | –  | +  | –  | –  | –  |
| Lactose                   | –  | –  | –  | –  | –  | –  | –  | –  |
| Raffinose                 | –  | –  | –  | –  | –  | –  | –  | –  |
| Mannose                   | –  | –  | –  | –  | –  | –  | –  | –  |
| α-galactosidase           | –  | –  | –  | –  | –  | –  | –  | –  |
| β-galactosidase           | –  | –  | –  | –  | –  | –  | –  | –  |
| α-glucosidase             | –  | –  | –  | –  | –  | –  | –  | –  |
| β-glucosidase             | –  | –  | –  | –  | –  | –  | –  | –  |
| Arginine arylamidase      | –  | –  | –  | +  | +  | –  | –  | –  |
| Proline arylamidase       | –  | –  | –  | –  | –  | –  | –  | –  |
| Phenylalanine arylamidase | –  | –  | –  | –  | –  | –  | –  | –  |
| Leucine arylamidase       | –  | –  | –  | –  | –  | –  | –  | –  |
| Pyroglutamyl arylamidase  | –  | –  | –  | –  | –  | –  | –  | –  |
| Histidine arylamidase     | –  | –  | –  | –  | –  | –  | –  | –  |
| β-glucuronidase           | –  | –  | –  | –  | –  | –  | –  | –  |
| Tyrosine arylamidase      | –  | –  | –  | –  | –  | –  | –  | –  |
| W, weak                   | –  | –  | –  | –  | –  | –  | –  | –  |
Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit, then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit (Illumina), as previously described [9–12]. Genome assembly was performed with a pipeline incorporating different softwares (SPADES [13]), on trimmed (TRIMMOMATIC [14]) 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 (number of scaffolds: 56, number of contigs: 56). The genome of strain Marseille-P4592T is 2,064,271 bp long with a 28.1 mol% G + C content. Genomic characteristics of strain Marseille-P4592 and its closest species with standing in nomenclature are summarized in Table 2. The degree of genomic similarity of strain Marseille-P4592T with closely related species was estimated using the ORTHOANI software.

TABLE 2. Genomic characteristics of Anaerosphaera massiliensis sp. nov. and the seven most closely related bacterial taxa for which genome sequences are available

| Type strains                  | Accession number | Size (Mb) | GC %  | Gene content |
|-------------------------------|------------------|-----------|-------|--------------|
| Anaerosphaera aminiphila      | FQXI00000000     | 2.02      | 31.5  | 1960         |
| Anaerosphaera massiliensis    | CABHLS0100000001 | 2.06      | 28.1  | 2041         |
| Peptoniphilus asaccharolyticus| FWVR00000000     | 2.23      | 32.3  | 2268         |
| Peptoniphilus coxii           | LSDG00000000     | 1.83      | 44.6  | 1783         |
| Peptoniphilus duodenii        | AEEH00000000     | 2.08      | 34.2  | 2018         |
| Peptoniphilus indolicus       | AGBB00000000     | 2.24      | 31.6  | 2145         |
| Tepidimicrobium xylanilyticum | FNNG00000000     | 3.00      | 33.2  | 2094         |
| Tissierella creatinini        | SUSS00000000     | 2.61      | 35.7  | 2581         |

© 2019 The Authors. Published by Elsevier Ltd, NMNI, 33, 100633
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Values among closely related species (Fig. 5) ranged from 62.36% between *Peptoniphilus coxii* and *Tepidimicrobium xylanilyticum* to 82.75% between *Peptoniphilus asaccharolyticus* and *Peptoniphilus indolicus*. When the isolate was compared with these closely related species, values ranged from 63.98% with *P. coxii* to 75.69% with *Anaerosphaera aminiphila*. An average nucleotide identity value < 95% suggesting that two strains belong to distinct species. The degree of genomic similarity of strain Marseille-P4592 with closely related species was calculated also using the digital DNA–DNA hybridization software [18]. Values among closely related species (Table 3) ranged from 17.7% between strain Marseille-P4592 and *P. asaccharolyticus* to 41.8% between *P. coxii* and *P. indolicus* (Table 3). When strain Marseille-P4592 was compared with these closely related species, values ranged from 17.7% with *P. asaccharolyticus* to 27.3% with *Tissierella creatinini* (Table 3). These values are lower than the 70% threshold used for delineating prokaryotic species, which confirmed that this strain represents a new species. Core-genome-based phylogenetic relationships of strain Marseille-P4592T with the closest species with standing in nomenclature confirmed the phylogenetic position of strain Marseille-P4592T within Anaerosphaera cluster (Fig. 6). Therefore, the genomic analysis suggested that strain Marseille-P4592T was classified as a

**FIG. 5.** Heatmap generated with OrthoANI values calculated using the OAT software between Anaerosphaera massiliensis sp. nov. and other closely related species with standing in nomenclature.

**TABLE 3.** Digital DNA–DNA hybridization values (%) obtained by comparison of all studied genomess

|            | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  |
|------------|----|----|----|----|----|----|----|----|
| 1-Anaerosphaera aminiphila | 100 | 19.2 | 19.2 | 32.3 | 33.2 | 18.9 | 22.6 | 30.7 |
| 2-Anaerosphaera massiliensis | 100 | 17.7 | 17.7 | 25.5 | 20.6 | 18.1 | 24.8 | 27.3 |
| 3-Peptoniphilus asaccharolyticus | 100 | 100 | 35.4 | 35.4 | 32.9 | 26.4 | 20.2 | 33.1 |
| 4-Peptoniphilus coxii | 100 | 100 | 38.3 | 38.3 | 41.8 | 31.4 | 25.4 | 17.5 |
| 5-Peptoniphilus duerdenii | 100 | 100 | 25.4 | 25.4 | 25.9 | 25.9 | 25.9 | 35.7 |
| 6-Peptoniphilus indolicus | 100 | 100 | 100 | 100 | 20.2 | 20.2 | 85.1 | 31.1 |
| 7-Tepidimicrobium xylanilyticum | 100 | 100 | 100 | 100 | 100 | 18.8 | 100 | 100 |
| 8-Tissierella creatinini | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
member of a new species within the genus *Anaerosphaera*, family *Peptoniphilaceae*, phylum *Firmicutes*.

**Conclusion**

Strain Marseille-P4592<sup>T</sup> exhibiting a 16S rRNA sequence identity <98.65%, an average nucleotide identity value < 95% and a digital DNA–DNA hybridization value < 70% with its phylogenetically closest species with standing in nomenclature, is consequently proposed as the type strain of the new species *Anaerosphaera massiliensis* sp. nov.

**Description of *Anaerosphaera massiliensis* sp. nov**

*Anaerosphaera massiliensis* (mas.si.li.en’sis; L. masc. adj. massiliensis for Massilia, the Roman name of Marseille, where strain Marseille-P4592 was first isolated). Cells are anaerobic, Gram-positive, with a mean diameter of 1 mm. Catalase and oxidase activities are negative. Cells are round-shaped with a diameter ranging from 662 to 763 nm. Colonies grown on 5% sheep blood-enriched Columbia agar (bioMérieux) are white after 24 hours of incubation in a strict anaerobic atmosphere. Growth occurs at 37°C. Cells grow anaerobically only. Using an API ZYM strip, a positive reaction was observed for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase and naphthol-AS-BI-phosphohydrolase but negative reactions were obtained for alkaline and acid phosphatases, lipase, 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-P4592<sup>T</sup> was able to metabolize esculin, D-tagatose and potassium 5-ketogluconate. However, negative reactions were obtained for glycerol, D-glucose, D-galactose, D-maltose, D-fructose, D-mannose, methyl-D-glucopyranoside, N-acetylgalosamine, D-lactose, D-saccharose, D-trehalose, D-turanose erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adenitol, methyl-β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-D-mannopyranoside, amygdalin, arbutin, salicin, D-cellobiose, D-melibiose, inulin, D-
melezitose, d-raffinose, starch, glycogen, xylitol, gentiobiose, d-xylose, d-fucose, l-fucose, d-arabitol, l-arabitol, potassium gluconate and potassium 2-ketogluconate. The genome is 2 064 271 bp long and its G + C content is 28.1%.

The type strain, Marseille-P4592T, isolated from a stool sample of a healthy 39-year-old male Pygmy, was deposited in the CSUR and CCUG collections under accession numbers CSUR P4592 and CCUG 72 452, respectively. The 16S rRNA and genome sequences are available in GenBank under accession numbers LT934438 and CABHLS010000001 – CABHLS010000061, respectively.

**Nucleotide sequence accession number**

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT934438 and CABHLS010000001 – CABHLS010000061, respectively.

**Deposit in culture collections**

Strain Marseille-P4592T was deposited in two different strain collections under numbers (= CSURP4592T; = CCUG72452T).

**Conflict of interest**

We have no conflict of interest to declare.

**Funding sources**

The study was supported by the Méditerranée Infection foundation, the National Research Agency under the program *Investissements d’avenir*, reference ANR-10-IAHU-03 and by Région Provence Alpes Côte d’Azur and European funding FEDER PRIMI.

**Acknowledgements**

We sincerely thank Taku Sakazume, Takashi Irie, Yusuke Ominami, Kyoko Imai, Shigeki Matsubara, Akiko Hisada, and all the Hitachi Team in Japan for the collaborative study we are conducting together between the Hitachi High-Technologies Corporation and the Institut Hospitalo-Universitaire Méditerranée-Infection, and for the installation and services on the Tabletop Microscope TM4000Plus from Hitachi in our facility.

**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 JC, Hugon P, Khelifa S, Fournier PE, 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 JC, Khelifa 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 J-C, Edouard S, Pagnier I, Medianikov 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 JC, Padmanabhan R, Rossi M, Sentaux 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, 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-SS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta J-PP, 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, Khelifa S, Armstrong N, Labas N, Fournier P-E, Raoult D, et al. Microbial culturomics unravels the halophilic microbiota repertoire of table salt: description of Grucllobilus massiliensis sp. nov. Microb Ecol Health Dis 2016;27.

[10] Anani H, Haani I, Zgheib R, Fadlane A, Fourien P-E. Whole-genome sequence of French clinical Peptoniphilus catoniae Strain PB546. Microbiol Resour Announc 2019;8(45).

[11] Anani H, Khodor M, Raoult D, Fournier P-E. Whole-genome sequence of French clinical Oxilabacter jiluni Strain PB502. Microbiol Resour Announc 2019;8(31).

[12] Anani H, Raoult D, Fournier P-E. Whole-genome sequence of Halcomads isolongiprae strain PB956. Microbiol Resour Announc 2019;8(43).

[13] 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.

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

[15] Lee I, Kim YQ, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.

[16] Anani H, Bou Abdallah R, Chekhna N, Fontanini A, Ricaboni D, Mailhe M, et al. Draft genome and description of Merbactera massiliensis gen. nov., sp. nov., a new bacterium genus isolated from the human ileum. Sci Rep 2019;9:7931.

[17] Anani H, Guilhot E, Andreu C, Fontanini A, Raoult D, Fournier PE. Prevotella ihumii sp. nov., a new bacterium isolated from a stool specimen of a healthy woman. New Microb New Infect 2019;32:100607.

[18] Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 2013;14:60.

[19] Ueki A, Abe K, Suzuki D, Kaku N, Watanabe K, Ueki K. Ominamibacter oimpatiens gen. nov., sp. nov., a glutamate-degrading, Gram-positive anaerobic coccus isolated from a methanogenic reactor treating cattle waste. Int J Syst Evol Microbiol 2009;59:1361–7.

[20] Ezaki T, Li N, Shu S, Zhao L, Kawamura Y, Li ZY. Proposal of the genera Anaeroacoccus gen. nov., Peptoniphilus gen. nov. and Galkiola gen. nov. for members of the genus Peptostreptococcus. Int J Syst Evol Microbiol 2001;51:1521–8.
[21] Ulger-Toprak N, Lawson PA, Summanen P, O’Neal L, Finegold SM. Peptoniphilus duerdenii sp. nov. and Peptoniphilus koenoveniae sp. nov., isolated from human clinical specimens. Int J Syst Evol Microbiol 2012;62:2336–41.

[22] Citron DM, Tyrrell KL, Goldstein EJC. Peptoniphilus coxii sp. nov. and Peptoniphilus tyrrelliae sp. nov. isolated from human clinical infections. Anaerobe 2012;18:244–8.

[23] Harms C, Schleicher A, Collins MD, Andreesen JR. Tissierella creatinophila sp. nov., a Gram-positive, anaerobic, non-spore-forming, creatinine-fermenting organism. Int J Syst Bacteriol 1998;48: 983–93.

[24] Niu L, Song L, Liu X, Dong X. Tepidimicrobium xylanilyticum sp. nov., an anaerobic xylanolytic bacterium, and emended description of the genus Tepidimicrobium. Int J Syst Evol Microbiol 2009;59:2698–701.