**Anaerococcus marasmi** sp. nov., a new bacterium isolated from human gut microbiota

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**Abstract**

*Anaerococcus marasmi* sp. nov. strain Marseille-P3557T is a new species isolated from a stool of a Nigerian child with marasmus. The genome of Marseille-P3557T was 2 130 060 bp long (35.4% G + C content). The closest species based on 16S ribosomal RNA sequence was *Anaerococcus prevotii* strain 20548, with 97.6% sequence similarity. Considering phenotypic features and comparative genome studies, we propose the strain Marseille-P3557T as the type strain of *Anaerococcus marasmi* sp. nov., a new species within the genus *Anaerococcus*.

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**Phenotypic characteristics**

Colonies were white, smooth and crateriform with a mean diameter of 1 to 4 mm. Bacterial cells were Gram-positive bacilli ranging in length from 1.5 to 2.1 μm and 0.5 to 0.7 μm in width (Fig. 2). Strain Marseille-P3557T exhibited catalase but no oxidase activity. To reveal the biochemical properties of strain Marseille-P3557T, the API 50 CH and ZYM strips (bio-Mérieux, Marcy l’Etoile, France) at 37°C in an anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France) after a 15-day preincubation in an anaerobic bottle containing a *Brucella* medium.

The study was approved by the ethics committee of the Institut Federatif de Recherche 48 under reference 2016-010. The patient provided signed informed consent before participating in this study.

**Isolation and growth conditions**

In April 2016 we isolated in a stool sample from a Nigerian child with marasmus a bacterial strain that could not be identified by MALDI-TOF MS. The screening was performed on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany), as previously reported [7]. The spectra obtained (Fig. 1) were imported and analysed using the Biotyper 3.0 software against the Bruker database, which was continually incremented with the laboratory MEPHI database. The strain was isolated on 5% sheep’s blood-enriched Columbia agar (bio-Mérieux, Marcy l’Etoile, France) at 37°C in an anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France) after a 15-day preincubation in an anaerobic bottle containing a *Brucella* medium.

The culturomics concept has recently been developed in our laboratory as an alternative method to expand the human gut repertoire through the multiplication of culture conditions with a rapid identification method by MALDI-TOF MS [1–3]. As a result, new bacterial genera and species may be found for the first time using this technique [4,5]. Thereafter, a new taxonomic strategy, termed taxonogenomics, was developed to include proteomic information obtained by MALDI-TOF MS, complete genomic tests and phenotypic characteristics [6]. Here we present an analysis of the characteristics that allowed us to describe *Anaerococcus marasmi* strain Marseille-P3557T (= CSUR P3557), a bacterium isolated from a stool specimen from a Nigerian child with marasmus and classified into the *Peptoniphilaceae* family.
To classify this bacterium, the 16S ribosomal RNA (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 (Thermo-Fisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequence was assembled and corrected by CodonCode Aligner software (https://www.codoncode.com/). Strain Marseille-P3557\(^T\) exhibited a 97.6% 16S rRNA similarity with Anaerococcus prevotii strain 20548 (GenBank accession no. NR_074575.1), the phylogenetically closest species with standing in nomenclature (Fig. 3). We consequently proposed to classify strain Marseille-P3557\(^T\) as a new species within the genus Anaerococcus in the phylum Firmicutes.

### 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, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT Paired End (Illumina), as previously described [9]. The assembly was performed using a pipeline containing several different software packages (Velvet [10], Spades [11] and Soap Denovo [12]) and trimmed (MiSeq and Trimomatic [13] software) or untrimmed data (only MiSeq software). GapCloser was used to reduce assembly gaps. Scaffolds of <800 bp and scaffolds with a depth value < 25% of the mean depth were
Biochemical tests of Marseille-P3557T with closely related species was estimated mol% G + C content. The degree of genomic similarity of strain Marseille-P3557T by API 50 CH and API ZYM of strain Marseille-P3557T was 2 130 060 bp long with a 35.4 criteria (number of scaffolds, N50, number of N). The genome removed. The best assembly was selected using different criteria (number of scaffolds, N50, number of N). The genome of strain Marseille-P3557T was 2 130 060 bp long with a 35.4 mol% G + C content. The degree of genomic similarity of strain Marseille-P3557T with closely related species was estimated using the OrthoANI software [14]. OrthoANI values among closely related species (Fig. 4) ranged from 62.55% between Anaerococcus marasmi and Anaerosphaera aminiphila to 96.47% between Anaerococcus obesiensis and Anaerococcus vaginalis. When A. marasmi was compared to these closely related species, values ranged from 62.55% with Anaerosphaera aminiphila to 77.73% with A. prevotii.

### Conclusion

On the basis of unique phenotypic features, including the MALDI-TOF spectrum, a 16S rRNA sequence divergence of >1.3% and an OrthoANI value of <95% of the phylogenetically closest species with standing in nomenclature, we formally propose strain Marseille-P3557T as the type strain of A. marasmi sp. nov., a new species within the genus Anaerococcus.

### Description of Anaerococcus marasmi sp. nov

Anaerococcus marasmi (ma.ras.mi, L. adj. fem., referring to marasmus, the patient’s disease) comprises anaerobic Gram-
**FIG. 3.** Phylogenetic tree highlighting position of *Anaerococcus marasmi* sp. nov. with regard to other closely related species. GenBank accession numbers of 16S ribosomal RNA provided in parentheses. Sequences were aligned using MUSCLE with default parameters; phylogenetic inference was obtained by maximum likelihood method and MEGA 7 software. Bootstrap values were obtained by repeating analysis 1000 times to generate majority consensus tree and are indicated at nodes. Scale bar indicates 2% nucleotide sequence divergence.

**FIG. 4.** Heat map generated with OrthoANI values calculated using OAT software between *Anaerococcus marasmi* sp. nov. and other closely related species with standing in nomenclature.
positive bacilli ranging in length from 1.5 to 2.1 μm and 0.5 to 0.7 μm in width. Colonies grown on 5% sheep's blood–enriched Columbia agar (bioMérieux) are circular, smooth and white after 72 hours of incubation in a strict anaerobic atmosphere and have a mean diameter of 1.2 mm. Growth occurs at 37°C. Positive reactions are observed for leucine arylamidase, phosphatase acid, β-glucuronidase, galactosidase, fructose, mannose, salicin, D-lactose, D-melibiose, sucrose, and potassium 5-ketogluconate; negative reactions were detected with arabinose, ribose, rhamnose, methyl β-D-xylopyranoside, inositol, mannitol, methyl α-D-glucopyranoside, amygdalin, esculin, starch, glycogen, D-fucose and D-arabitol.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT966068 and OLMF01000000 respectively.

Conflict of Interest

None declared.

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