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

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Abstract

Anaerococcus marasmi sp. nov. strain Marseille-P3557^T is a new species isolated from a stool of a Nigerian child with marasmus. The genome of Marseille-P3557^T 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-P3557^T as the type strain of *Anaerococcus marasmi* sp. nov., a new species within the genus *Anaerococcus*.

Keywords: *Anaerococcus marasmi* sp. nov., Culturomics, Gut microbiota, Marasmus, Taxonogenomics
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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-P3557^T (= CSUR P3557), a bacterium isolated from a stool specimen from a Nigerian child with marasmus and classified into the *Peptoniphilaceae* family.

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 (bioMérieux, 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.

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-P3557^T exhibited catalase but no oxidase activity. To reveal the biochemical properties of strain Marseille-P3557^T, the API 50 CH and ZYM strips (bioMérieux) were used under anaerobic condition at 37°C. All characteristics of the strain are summarized in Tables 1 and 2.

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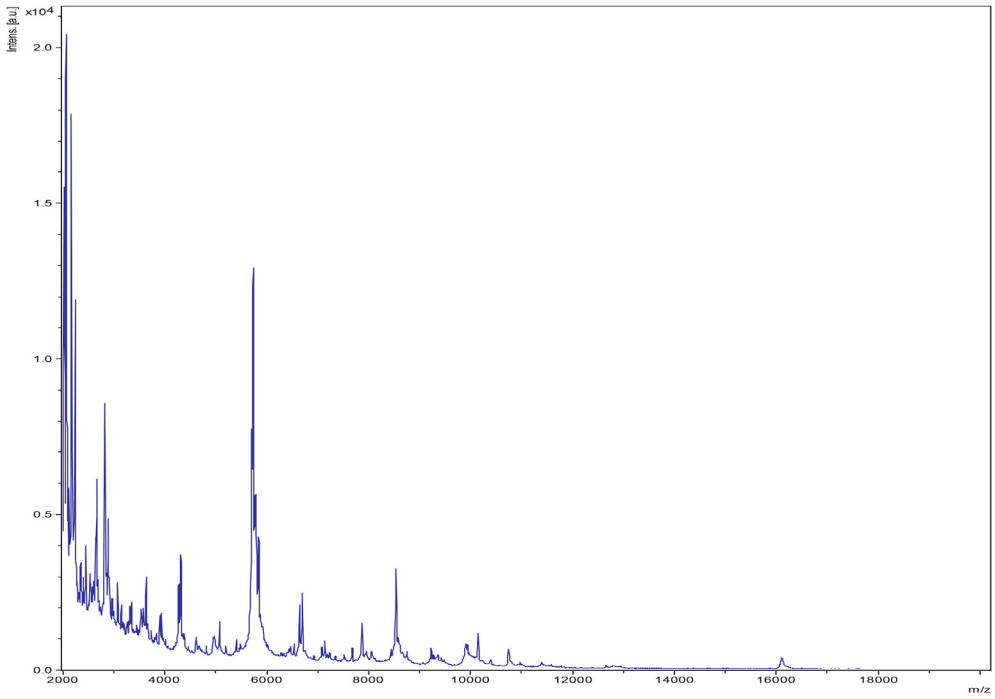

FIG. 1. MALDI-TOF MS reference spectrum of *Anaerococcus marasmi* sp. nov. Spectrum was obtained by comparing spectra of 12 individual colonies.

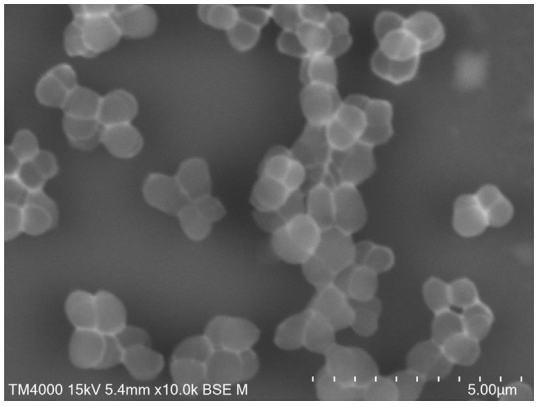

FIG. 2. SEM of stained *Anaerococcus marasmi* sp. nov. Colony was collected from agar and immersed in 2.5% glutaraldehyde fixative solution. Drop of suspension was directly deposited on poly-L-lysine–coated microscope slide for 5 minutes and treated with 1% phosphotungstic acid (PTA) aqueous solution (pH 2.0) for 2 minutes to increase SEM image contrast. Slide was gently washed in water, air dried and examined with tabletop SEM (TM4000; Hitachi, Yokohama, Japan). Scales and acquisition settings are shown. SEM, scanning electron microscope.

Strain identification

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 3500xLGenetic 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 Trimmomatic [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

TABLE 1. Biochemical tests of *Anaerococcus marasmi* strain Marseille-P3557^T by API 50 CH and API ZYM

Test	Variable	Result
API 50 CH	Control	+
	Glycerol	+
	Erythrol	—
	D-Arabinose	—
	L-Arabinose	—
	D-Ribose	—
	D-Xylose	—
	L-Xylose	+
	D-Adonitol	—
	Methyl β-D-xylopyranoside	—
	D-Galactose	+
	D-Glucose	+
	D-Fructose	+
	D-Mannose	+
	L-Sorbose	—
	L-Rhamnose	—
	Dulcitol	—
	Inositol	—
	D-Mannitol	—
	D-Sorbitol	+
	Methyl α-D-mannopyranoside	—
	Methyl α-D-glucopyranoside	—
	N-Acetyl-glucosamine	+
	Amygdalin	—
	Arbutin	—
	Esculin	—
	Salicin	+
	D-Cellobiose	+
	D-Maltose	+
	D-Lactose	+
	D-Melibiose	+
	D-Saccharose	+
	D-Trehalose	+
	Inulin	—
	D-Melezitose	—
	D-Raffinose	+
	Starch	—
	Glycogen	—
	Xylitol	—
	Gentiobiose	—
	D-Turanose	+
	D-Lyxose	—
	D-Tagatose	+
	D-Fucose	—
	L-Fucose	+
	D-Arabitol	—
	L-Arabitol	+
	Potassium gluconate	—
	Potassium 2-ketogluconate	+
	Potassium 5- ketogluconate	+
API ZYM	Alkaline phosphatase	—
	Esterase (C4)	—
	Esterase lipase (C8)	—
	Lipase (C14)	—
	Leucine arylamidase	+
	Valine arylamidase	—
	Cystine arylamidase	—
	Trypsin	—
	α-Chymotrypsin	—
	Acid phosphatase	+
	Naphtalo-AS-BI-phosphohydrolase	+
	α-Galactosidase	—
	β-Galactosidase	—
	β-Glucuronidase	+
	α-Glucosidase	+
	β-Glucosidase	—
	N-Acetyl-β-glucosaminidase	—
	α-Mannosidase	—
	α-Fucosidase	—

API, Analytical Profile Index; 50 CH, CarboHydrate; ZYM, Enzymes.

TABLE 2. Classification and general features of *Anaerococcus marasmi* strain Marseille-P3557^T

Features	Term
Species name	*Anaerococcus marasmi*
Genus name	*Anaerococcus*
Specific epithet	*marasmi*
Species status	sp. nov.
Species etymology	mar'as'mi
Designation of type strain	Strain Marseille-P3557^T
Strain collection number	CSURP3557
16S rRNA gene accession number	LT966068
Genome accession number	OLMF01000000
Genome status	Whole genome
Genome size	2 130 060 bp
GC mol%	35.4
Data on origin of sample from which strain was isolated	
Country of origin	Niger
Region of origin	Niamey
Date of isolation	2016-04
Source of isolation	Human stool sample
Growth medium, incubation conditions used for standard cultivation	Columbia agar supplemented with 5% sheep's blood, 37°C for 48 hours of incubation
Gram stain	Positive
Cell shape	Rod
Cell size (length or diameter)	1.5–2.1 × 0.5–0.7 µm
Motility	Nonmotile
Colony morphology	White, smooth, circular, crateriform
Temperature range	28°C to 56°C
Lowest temperature for growth	28°C
Highest temperature for growth	56°C
Temperature optimum	37°C
Lowest pH for growth	6.5
Highest pH for growth	8
Relationship to O2	Strictly anaerobic
O2 conditions for strain testing	Aerobiosis, anaerobiosis, microaerophilic
Oxidase	Negative
Catalase	Positive

removed. The best assembly was selected using different criteria (number of scaffolds, N50, number of N). The genome of strain Marseille-P3557^T was 2 130 060 bp long with a 35.4 mol% G + C content. The degree of genomic similarity of strain Marseille-P3557^T 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-P3557^T 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-

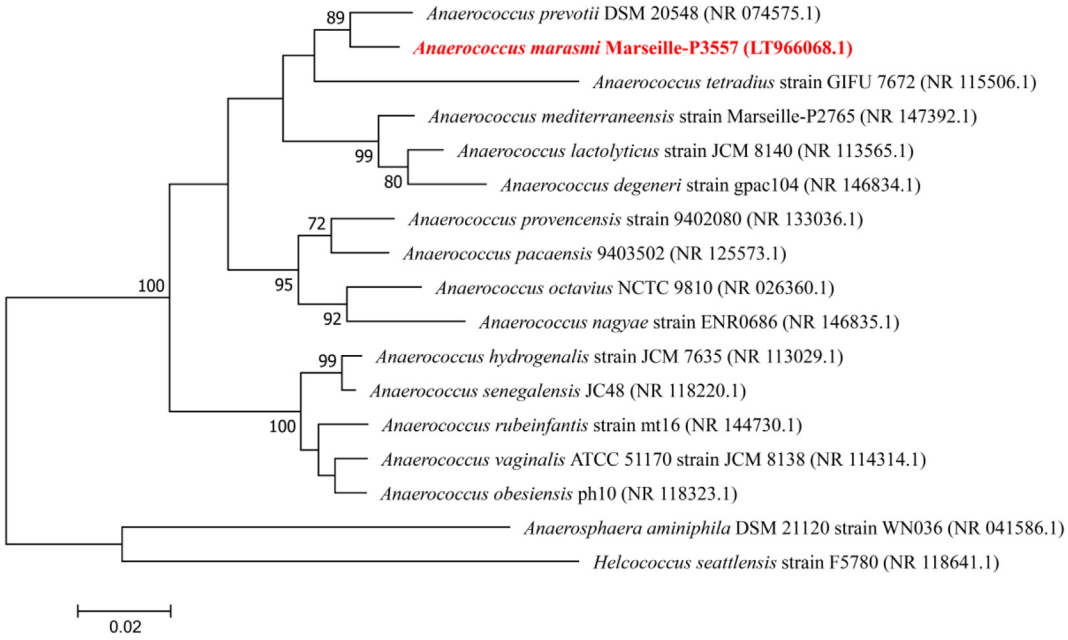

FIG. 3. Phylogenetic tree highlighting position of *Anaerococcus marasmi* sp. nov. with regard to other closely related species. GenBank accession numbers of 16S ribosomal RNA provided in parentheses. Sequences were aligned using MUSCLE with default parameters; phylogenetic inference was obtained by 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.

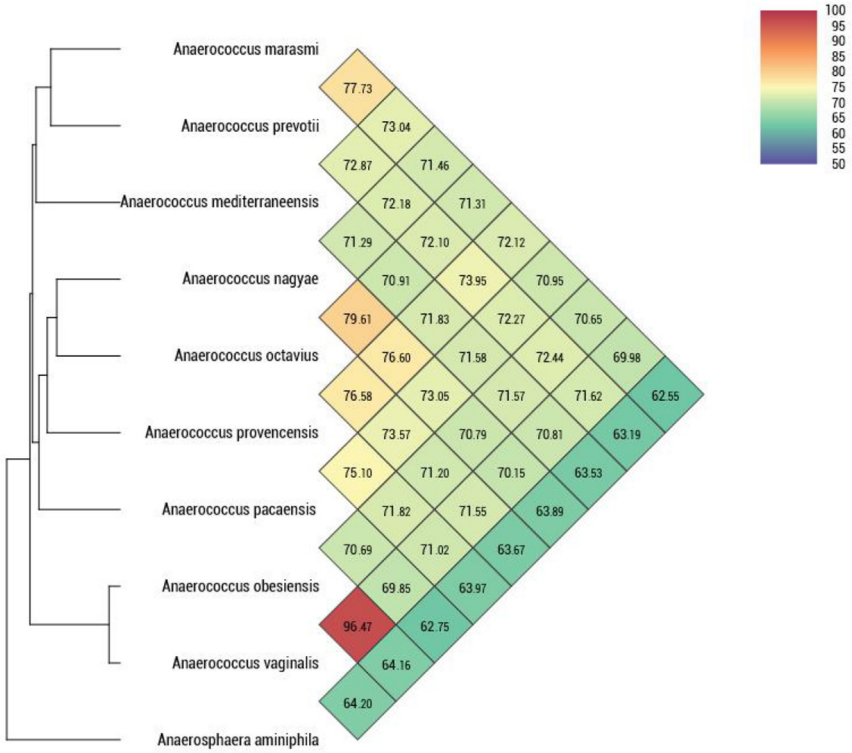

FIG. 4. Heat map generated with OrthoANI values calculated using OAT software between *Anaerococcus marasmi*. sp. nov. and other closely related species with standing in nomenclature.

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, glycerol, glucose, 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.

Acknowledgements

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