

Brachybacterium massiliense sp. nov., a new bacterium isolated from stool from a healthy Senegalese child

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Abstract

Based on our phenotypic and genotypic analyses, *Brachybacterium massiliense* strain Marseille-P2240^T (= CSURP2240; = DSM 101766) is a new species isolated from stool of a healthy subject. The strain was stained Gram-positive. It was aerobic, catalase-positive and oxidase-negative. Its optimal growth occurs at 37°C in aerobic condition. The 16S ribosomal RNA sequence analysis revealed that strain Marseille-P2240^T shown 98.18% similarity with *Brachybacterium saurashtrense* strain JG 06, the more closely related species with standing in nomenclature.

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Here we describe, according the concept of taxonogenomics, a new *Brachybacterium massiliense* sp. nov., strain Marseille-P2240^T (= CSURP2240; = DSM 101766) isolated from the stool of a 36-month-old healthy girl living in Senegal (West Africa).

Introduction

Brachybacterium faecium is the type strain of the genus isolated from poultry deep litter in 1966 [1]. *Brachybacteria* species have been isolated from a stool sample of a healthy 3-year-old child [2] but also from environmental samples such as garden soil, salt-fermented seafood, oil-contaminated coastal sand, sediment samples and seawater [3–5].

It is crucial to understand the part of bacterial diversity in normal physiologic functions and susceptibility to disease [6]. The culturomic method, based on variable culture conditions, has made it possible to discover species never before cultivated. This new approach was invented to complete the metagenomics of 16S ribosomal RNAs (rRNAs) and to study the diversity of human gut microbiota [7–9]. In the same way, the taxonogenomic method has been elaborated to make a complete description of bacterial species by cumulating the analysis the features of the genome and the phenotypic criteria of the strain [10,11].

Isolation and growth conditions

In 2015 we isolated an unidentified bacterial strain from the stool. The study was validated by the ethics committee of IHU Méditerranée Infection (no. 2016-011). A screening was made by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [12]. The obtained spectra (Fig. 1) were imported into MALDI Biotyper 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated with the Microbes Evolution Phylogeny and Infections database). Strain Marseille-P2240 was first isolated after a 24-hour preincubation in a liquid medium containing 37 g Difco Marine Broth (Becton Dickinson, Le Pont de Claix, France) per litre of sterile water at 37°C and on 5% sheep's blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) under aerobic conditions. This strain grew temperatures ranging from 28 to 42°C, and its pH ranged from 6 to 8.

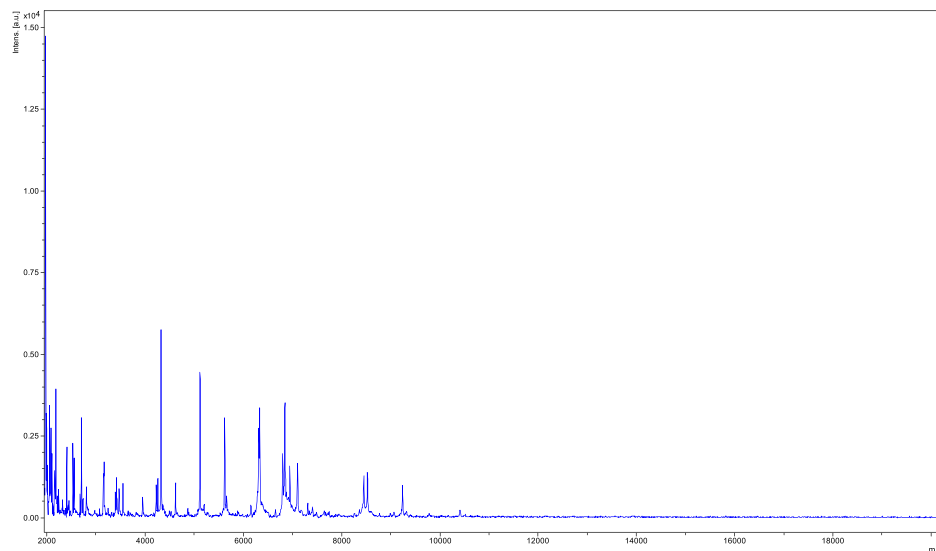


FIG. 1. MALDI-TOF MS reference mass spectrum of *Brachybacterium massiliense* sp. nov. Spectra from 12 individual colonies were compared and reference spectrum generated.

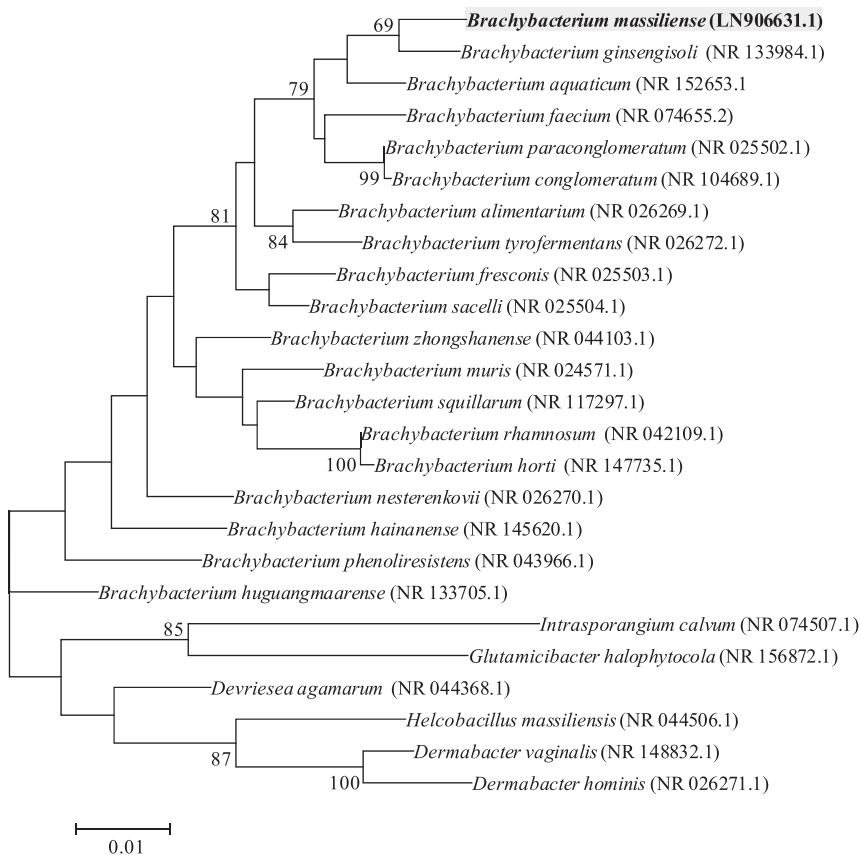


FIG. 2. Phylogenetic tree highlighting position of *Brachybacterium massiliense* sp. nov., strain Marseille-P2240^T, relative to most closely related type strains within genus *Brachybacterium*. GenBank accession numbers of 16S ribosomal RNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters. Phylogenetic inference were obtained using maximum likelihood method and MEGA7 software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Scale bar indicates 1% nucleotide sequence divergence.

Strain identification

The 16S rRNA gene was sequenced in order to classify this bacterium. Amplification was done by using the primer pair fD1 and rP2 (Eurogentec, Angers, France), and sequencing by the Big Dye Terminator v1.1 Cycle Sequencing Kit and the ABI Prism 3130xl Genetic Analyzer capillary sequencer (Thermo Fisher, Saint-Aubin, France), as previously described [13]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (<http://www.codoncode.com>). Strain Marseille-P2240^T exhibited a 98.18% sequence identity with *Brachybacterium saurashtrense* strain JG 06 (GenBank accession no. NR_116516.1), the phylogenetically closest species with standing in nomenclature (Fig. 2). We consequently classified this strain as a member of a new species within the genus *Brachybacterium*, family *Dermabacteraceae* and phylum *Actinobacteria*.

Phenotypic characteristics

Colonies were circular and opaque with a mean diameter of 0.5 to 0.9 μm . Bacterial cells were cocci shaped and Gram positive. Analysis via the Hitachi TM4000 electron microscope (Hitachi Group, Krefeld, Germany) revealed small, round bacteria

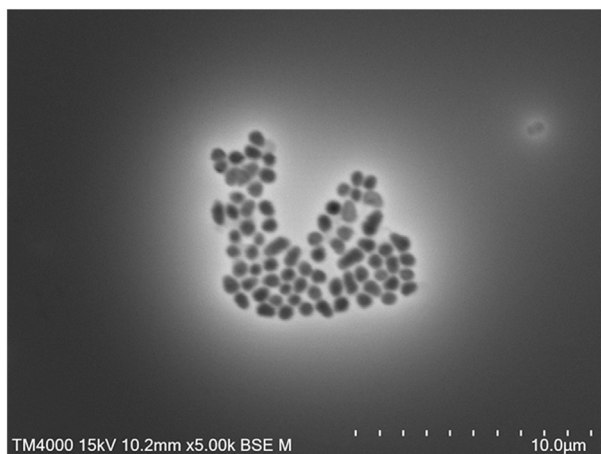


FIG. 3. Electron micrograph of stained *Brachybacterium massiliense* sp. nov., strain Marseille-P2240^T. Colony was collected from agar and immersed into 2.5% glutaraldehyde fixative solution. Drop of suspension was directly deposited on poly-L-lysine-coated microscope slide for 5 minutes and treated with 1% phosphotungstic acid 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 (Hitachi TM4000). Scales and acquisition settings are shown. SEM, scanning electron microscopy.

(Fig. 3). Strain Marseille-P2240^T showed catalase-positive and oxidase-negative activities. Biochemical characteristics of the Marseille-P2241^T strain obtained using the API ZYM and 50 CH strips (bioMérieux) are provided in Table 1. The results of a

TABLE 1. Phenotypic characterization of *Brachybacterium massiliense* sp. nov., based on analytical profile index (API) tests

Test	Result
API 50 CH	
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-acetylglucosamine	–
Amygdalin	–
Arbutin	+
Esculin	+
Salicin	–
D-Cellobiose	+
D-Maltose	+
D-Lactose	+
D-Melibiose	+
D-Sucrose	+
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	–
Phosphatase acid	–
Naphthol-AS-BI-phosphohydrolase	+
α -Galactosidase	+
β -Galactosidase	–
β -Glucuronidase	–
α -Glucosidase	+
β -Glucosidase	–
N-Acetyl- β -glucosaminidase	–
α -Mannosidase	–
α -Fucosidase	+

TABLE 2. Differential characteristics of *Brachybacterium massiliense* sp. nov. strain Marseille-P2240^T (1) with its closely related species *Brachybacterium saurashtrense* sp. nov. strain JG 06 (2)

Property	1	2
Cell diameter (µm)	0.5–0.9	0.3–0.75
Oxygen requirement	Aerobic	Aerobic
Growth temperature	28–45°C	10–45°C
Gram stain	+	+
Motility	Nonmotile	Nonmotile
Endospore forming	–	–
pH	6–8	6–11
NaCl concentration	Up to 10% (w/v)	Up to 15% (w/v)
G + C content (mol%)	70.7	73.0
Production of:		
Catalase	+	+
Oxidase	–	–
Hydrolysis of:		
α-Glucosidase	+	NA
Gelatin	–	+
Tween 80	+	+
Assimilation of:		
Adonitol	+	+
Arabinose	+	+
Fructose	+	+
Galactose	+	+
Lactose	+	+
Mannose	–	+
Ribose	+	+
Xylose	–	+

+, positive reaction; –, negative reaction; NA, not available.

comparative study of the biochemical characteristics of this strain with its closely related *Brachybacterium saurashtrense* strain JG 06^T are detailed in Table 2.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany), 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 [14]. The assembly was performed using a pipeline containing several different softwares (Velvet [15], Spades [16] and Soap Denovo [17]) and trimmed (MiSeq and Trimmomatic [18] softwares) or untrimmed data (only MiSeq software). GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value of <25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). The genome of strain Marseille-P2240^T is 3<thinsp>865<thinsp>488 bp long with 70.7 mol% G + C content, and it contains 3574 predicted genes. The degree of genomic similarity of strain Marseille-P2240^T with closely related species was estimated by OrthoANI software [19]. Values among closely related species (Fig. 4) ranged from 75.58% between *Brachybacterium alimentarium* and *Brachybacterium nesterenkovi* to 83.79% between *Brachybacterium*

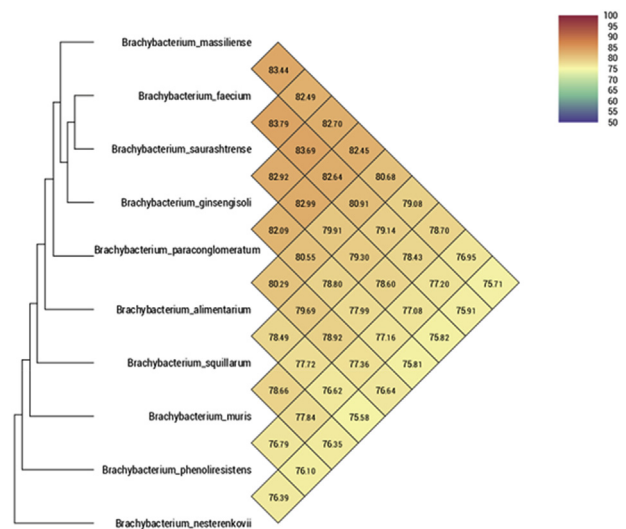


FIG. 4. Heat map generated by OrthoANI values calculated by OAT software between *Brachybacterium massiliense* sp. nov. and other closely related species with standing in nomenclature.

faecium and *Brachybacterium saurashtrense*. When the isolate was compared to these closely species, values ranged from 75.7% with *Brachybacterium nesterenkovi* to 83.44% with *Brachybacterium faecium*.

Conclusion

Strain Marseille-P2240^T exhibited a 16S rRNA sequence divergence of >1.3% and an OrthoANI value < 95% with its phylogenetically closest species with standing in nomenclature; it also had unique phenotypic features. We consequently propose *Brachybacterium massiliense* sp. nov. strain Marseille-P2240^T as the type strain of a new species, *Brachybacterium massiliense* sp. nov.

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LN906631 and FXXB00000000, respectively.

Deposit in culture collections

Strain Marseille-P2240^T was deposited in two different strain collections under numbers CSURP2240 and DSM 101766.

Conflict of Interest

None declared.

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