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# *Enterococcus mediterraneensis* sp. nov., a new bacterium isolated from the stool of a 39-year-old Pygmy

T. Takakura<sup>1</sup>, R. Francis<sup>2,3</sup>, H. Anani<sup>2,4</sup>, M. Bilen<sup>2</sup>, D. Raoult<sup>2,3</sup> and J. Y. Bou Khalil<sup>2</sup>

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

*Enterococcus mediterraneensis* strain Marseille-P4358<sup>T</sup> (= CSURP4358<sup>T</sup>) is a new species isolated from the stool of a 39-year-old male Pygmy from the Democratic Republic of Congo.

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**Corresponding author:** J.Y. Bou Khalil, MEPHI, Institut Hospitalo-Universitaire Méditerranée Infection, 19–21 Boulevard Jean Moulin, 13005, Marseille, Cedex 05, France.

**E-mail:** [boukhaliljacques@gmail.com](mailto:boukhaliljacques@gmail.com)

## 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 the isolate [5,6].

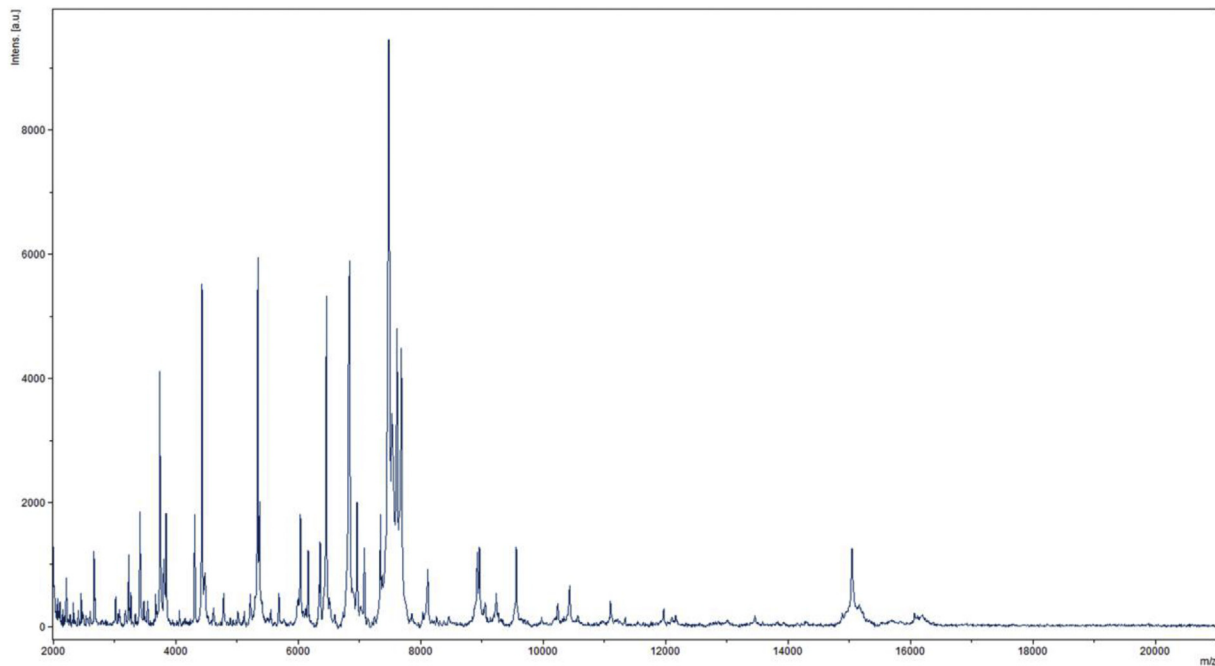
## Isolation and growth conditions

In 2017, we isolated from the stool sample of a healthy 39-year-old male Pygmy an unidentified bacterial strain. Screening was performed by MALDI-TOF MS on a Microflex LT spectrometer

(Bruker Daltonics, Bremen, Germany) as previously described [7]. 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 two databases (the Bruker and the constantly updated MEPHI databases <https://www.mediterranee-infection.com/urms-data-base/>). The study was validated by the ethics committee of the Institut Fédératif de Recherche IFR48 under number 2016-011. Initial growth was obtained 24 h after culture in a Colombia agar enriched with 5% sheep's blood (bioMérieux, Marcy l'Etoile, France) under anaerobic conditions at 37°C.

## Phenotypic characteristics

Colonies were dark green in colour with a mean diameter of 1 mm, exhibiting  $\gamma$ -haemolytic activity on sheep's blood agar. Bacterial cells were Gram-positive, round, with a diameter ranging from 812 nm to 1.21  $\mu$ m (Fig. 2). Strain Marseille-P4358<sup>T</sup> showed negative catalase and oxidase activities. Api 50 CH and Api ZYM tests were performed at 37°C under anaerobic conditions and the results are described in Table 1. The main characteristics of this strain are summarized in Fig. 3.

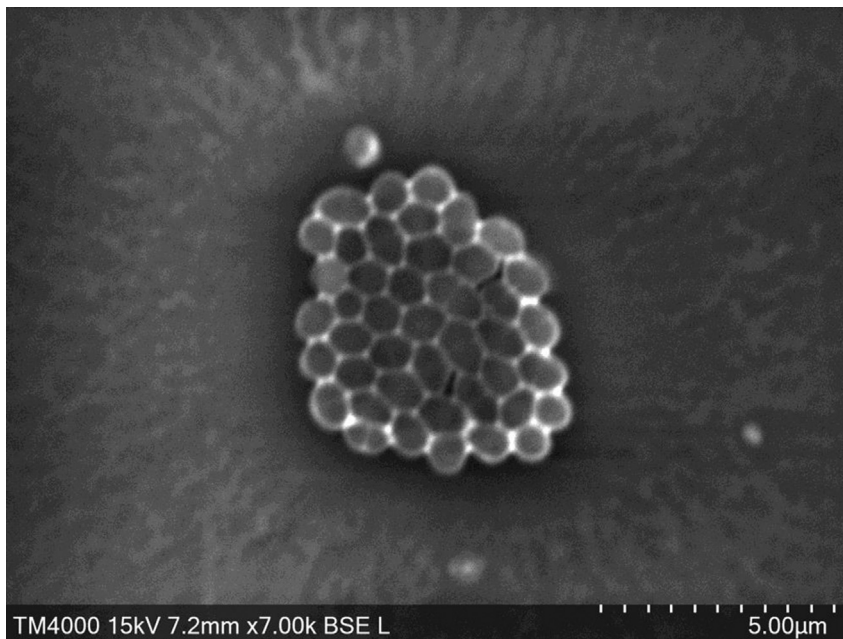


**FIG. 1.** MALDI-TOF MS reference mass spectrum. Spectra from 12 individual colonies were compared and a reference spectrum was generated.

### 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 3500xL Genetic Analyzer capillary sequencer (ThermoFisher, Saint-Aubin, France) respectively, as previously described [8]. The 16S



**FIG. 2.** Scanning electron micrograph of *Enterococcus mediterraneensis* strain Marseille P4358<sup>T</sup> using the Tabletop Microscope TM4000Plus from Hitachi. Scale bar and acquisition settings are shown on the original micrograph.

**TABLE 1.** Phenotypic characterization of *Enterococcus mediterraneensis* based on the analytical profile index: API 50 CH and API ZYM

<b>(A)</b>			
<b>Bacteria: <i>Enterococcus mediterraneensis</i></b>			
<b>api 50 CH</b>			
<b>Test</b>	<b>Results (+/-)</b>	<b>Test</b>	<b>Results (+/-)</b>
Control	-	Esculine	+
Glycerol	+	Salicine	+
Erythrol	-	D-cellobiose	+
D-arabinose	-	D-maltose	+
L-arabinose	+	D-lactose	-
D-ribose	+	D-melibiose	-
D-xylose	+	D-saccharose	+
L-xylose	-	D-trehalose	+
D-adonitol	-	Inuline	-
Methyl-βD-xylopyranoside	-	D-melezitose	-
D-galactose	+	D-raffinose	-
D-glucose	+	Amidon	-
D-fructose	+	Glycogene	-
D-mannose	+	Xylitol	-
L-sorbose	-	Gentibiose	+
L-rhamnose	-	D-turanose	-
Dulcitol	-	D-lyxose	-
Inositol	-	D-tagatose	-
D-mannitol	-	D-fucose	-
D-sorbitol	-	L-fucose	-
Methyl-αD-mannopyranoside	-	D-arabitol	-
Methyl-αD-glucopyranoside	-	L-arabitol	-
N-acetylglucosamine	+	Potassium gluconate	+
Amygdaline	+	Potassium 2-cetogluconate	-
Arbutine	+	Potassium 5-cetogluconate	-

<b>(B)</b>	
<b>Bacteria: <i>Enterococcus mediterraneensis</i></b>	
<b>api ZYM</b>	
<b>Test</b>	<b>Results (+/-)</b>
Control	-
Alkaline phosphatase	-
Esterase (C 4)	+
Esterase Lipase (C 8)	+
Lipase (C 14)	-
Leucine arylamidase	-
Valine arylamidase	-
Cystine arylamidase	+/-
Trypsine	-
α-chymotrypsine	-
Acid phosphatase	+/-
Naphthalo-AS-BI-phosphohydrolase	+
α-galactosidase	-
β-galactosidase	+
β-glucuronidase	-
α-glucosidase	+
β-glucosidase	-
N-acetyl-β-glucosaminidase	-
α-mannosidase	-
α-fucosidase	-

rRNA nucleotide sequences were assembled and corrected using CODONCODE ALIGNER software (<http://www.codoncode.com>). Strain Marseille-P4358<sup>T</sup> exhibited a 98.26% sequence identity with *Enterococcus gallinarum* strain LMG 13129 (GenBank accession number NR104559), the phylogenetically closest species with standing in nomenclature (Fig. 4). Consequently, *Enterococcus mediterraneensis* was classified as a new member of the genus *Enterococcus*, family *Enterococcaceae*,

phylum Firmicutes, with the strain Marseille P4358<sup>T</sup> as the type strain of the new species *Enterococcus mediterraneensis*.

### Genome sequencing

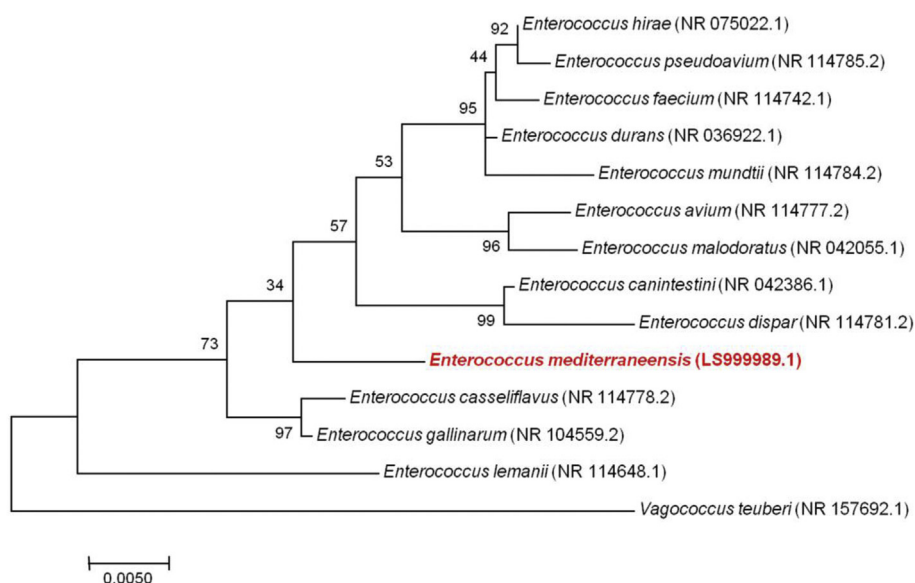
Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit, then

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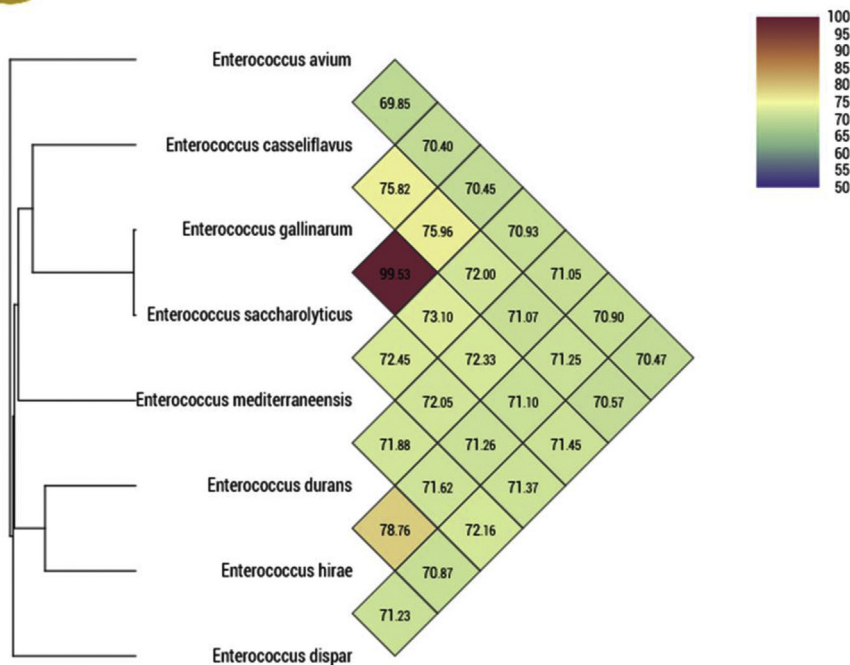
**TXNR** TA01008  
 2019-07-10  
 2019-07-10  
 002  
 Submitted  
**SPNA** Enterococcus mediterraneensis  
**GENA** Enterococcus  
**SPEP** Enterococcus mediterraneensis  
**SPST** sp. nov.  
**SPTY** mediterrane.en'sis, L. masc. adj., from  
 mediterranean, related to the mediterranean  
 region from where the sample was collected  
**SUBM** RANIA FRANCIS  
**EMSU** raniagfrancis@gmail.com  
**TYPE** Marseille P4358  
**COLN** CSURP4358T  
**16SR** LS99989  
**GARE** UWOP00000000  
**GSIZ** 2699190  
**GGCM** 40.90  
**COUN** Democratic Republic of Congo  
**REGI** Democratic Republic of Congo  
**DATU** < 2017  
**SOUR** Human gut  
**DATS** 2017-01-10  
**CULT** Columbia agar enriched with 5% sheep's blood  
**GRAM** POSITIVE  
**CSHA** coccus  
**CSIZ** 1  
**MOTI** nonmotile  
**COLM** green colonies, diameter of 1 mm  
**TEMO** 37  
**OREL** anaerobe  
**OXID** negative  
**CATA** negative

**FIG. 3.** Description of *Enterococcus mediterraneensis* strain Marseille P4358<sup>T</sup> according to the digital protologue TA01008 on the [www.imedeauib.es/dprotologue](http://www.imedeauib.es/dprotologue) website.

**FIG. 4.** Phylogenetic tree showing the position of *Enterococcus mediterraneensis* strain Marseille P4358<sup>T</sup> relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequence alignment and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree.



Heatmap generated with OrthoANI values calculated from the OAT software. Please cite Lee et al. 2015.



**FIG. 5.** Heatmap generated with ORTHOANI values calculated using the OAT software between *Enterococcus mediterraneensis* and other closely related species with standing in nomenclature.

sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit (Illumina), as previously described [9]. Genome assembly was performed with a pipeline incorporating different softwares (SPADES [10]), on trimmed (TRIMMOMATIC [11]) or raw data. GAPPLOTTER 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: 3, number of contigs: 3). The genome of strain Marseille-P4358<sup>T</sup> is 2 699 190 bp long with a 40.90 mol% G+C content. The degree of genomic similarity of Marseille-P4358<sup>T</sup> with closely related species was estimated using the ORTHOANI software [12]. Values among closely related species (Fig. 5) ranged from 69.85% between *Enterococcus avium* and *Enterococcus casseliflavus* to 99.53% between *Enterococcus gallinarum* and *Enterococcus saccharolyticus*. When the isolate was compared with these closely related species, values ranged from 70.93% with *Enterococcus avium* to 73.10% with *Enterococcus gallinarum*.

## Conclusion

Strain Marseille-P4358<sup>T</sup>, exhibiting a 16S rRNA sequence divergence <98.65 % with its phylogenetically closest species with standing in nomenclature, is consequently proposed as the type strain of the new species *Enterococcus mediterraneensis* sp. nov.

## Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LS999989 and NZ\_UWOP00000000, respectively.

## Deposit in culture collections

Strain Marseille-P4358<sup>T</sup> was deposited in two different strain collections under number (= CSURP4358<sup>T</sup>).

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Méditerranée-Infection, and for the installation and services on the Tabletop Microscope TM4000Plus from Hitachi in our facility.

## Conflict of interest

The authors have no conflict of interest to declare.

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