# Noncontiguous finished genome sequence and description of *Mediterranea massiliensis* gen. nov., sp. nov., a new member of the *Bacteroidaceae* family isolated from human colon

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## Abstract

Strain Marseille-P2645$^T$ was isolated in a colon sample from a Frenchwoman who underwent a colonoscopy. Bacterial cells were Gram negative, non–spore forming, mobile and strictly anaerobic. The genome of strain Marseille-P2645$^T$ is 3 950 441 bp long and contains 3374 protein-coding genes. The DNA G+C content is of 51.66 mol%. Strain Marseille-P2645$^T$ exhibited a 92.9% sequence similarity with *Bacteroides helcogenes* strain P36-108$^T$ (GenBank accession no. CP002352), the phylogenetically closest species with standing in nomenclature. Strain Marseille-P2645$^T$ (= CSUR P2645 = DSM 103034) is therefore a candidate as a type species of a new genus belonging to the *Bacteroidaceae* family, for which the name of *Mediterranea massiliensis* gen. nov., sp. nov., is proposed.
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## Introduction

The concept of culturomics [1–3], developed in our laboratory since 2010, aims at deciphering the living microbial diversity in any milieu, notably microbes that live with humans. In contrast to metagenomics, it focuses on culture, and especially the characterization of new species become available to the scientific and medical community. Indeed, culture is the first step before experimental studies, multispecies probiotics or selection of strains for specific microbiotherapy.

The combination of endoscopic sampling and microbial culturomics allowed us to isolate a new bacterial genus from the colon lavage of a 58-year-old woman without medical history, who underwent a colonoscopy because of a positive screening test for a colorectal cancer. Strain Marseille-P2645$^T$ is the type strain of *Mediterranea massiliensis* gen. nov., sp. nov., the first species of the genus *Mediterranea,* a new member of the *Bacteroidaceae* family. To describe strain Marseille-P2645$^T$, we report here the characterization of a new bacterial species using a new taxonomic strategy called taxonogenomics [4]. Taxonogenomics integrates proteomic information obtained by matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and genomic tests to describe new bacterial species [5,6]. This polyphasic approach overcomes limitations of conventional methods for new species description (genetic, phenotypic and chemotaxonomic characteristics for new species description) [7,8]. It combines phenotypic characteristics, analyses and comparison of the complete genome sequence. *Bacteroides helcogenes* strain P36-108$^T$ (GenBank accession no. CP002352) was the


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phylogenetically closest species with standing in nomenclature [9]. Study of the phenotypic, phylogenetic and genomic characteristics of strain Marseille-P2645$^T$ revealed that *M. massiliensis* was sufficiently different from *Bacteroides helcogenes* type strain (P36-108$^T$) to be classified as a new genus of bacteria of the *Bacteroides* family [10]. We previously reported the ribosomal 16S sequence and the MALDI-TOF MS spectrum [11]. Since the first report, a new strain was isolated confirming the new species. Here we present the complete description of strain Marseille-P2645$^T$ (= CSUR P2645 = DSM 103034), together with the description of the complete genomic sequencing and annotation.

## Materials and Methods

### Sample collection

Strain Marseille-P2645$^T$ was first isolated in April 2016 in a liquid colon sample from a 58-year-old woman without medical history, who underwent a colonoscopy because of a positive screening test for a colorectal cancer in the gastroenterology department of Hopital Nord, Marseille, France. Signed informed consent was collected from the patient, and the study obtained approval from the ethics committee of the Institut Fédératif de Recherche IFR48 under number 2016-010.

### Strain isolation and identification by MALDI-TOF MS

After collection using sterilized devices, the colonic sample was immediately placed in an antioxidant-enriched liquid medium [12] and transported to our laboratory (Institut Hospitalo-Universitaire Méditerranée Infection, Marseille, France). Once in the laboratory, the sample was placed under a laminar flow cabinet before being diluted in phosphate-buffered saline and seeded on different solid and liquid culture media. The initial growth of strain Marseille-P2645$^T$ was achieved after 1 day at 37°C on 5% sheep's blood–enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) in anaerobic atmosphere (anaeroGEN; Oxoid, Dardilly, France). After isolation on pure culture, the identification of all different bacterial colonies was performed using MALDI-TOF MS using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany), as previously reported [13,14]. Each colony was deposited in duplicate on a MALDI-TOF MS target to be analysed. A matrix solution of 1.5 µL (saturated solution of cyano-4-hydroxycinnamic acid diluted in 50% acetonitrile and 2.5% trifluoroacetic acid, completed with high-performance liquid chromatography water) was deposited on each spot. After the reading of the plate, the obtained protein spectra were compared by MALDI Biotyper software with those of the Bruker database (continuously updated with our recent data). When the resulting score was >2, the identification was made at the species level, while a score of <1.7 did not enable any identification.

### 16S rRNA sequencing and phylogeny

After 3 failed MALDI-TOF MS identifications, a standard 16S rRNA PCR was performed using universal primers pair fD1 and rP2 in a GeneAmp PCR System 2720 thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA). The amplified DNA was revealed by electrophoresis on 1.5% agarose gel. Once validated, the PCR product was purified and sequenced using the Big Dye Terminator Sequencing Kit and the following internal primers: 536F, 536R, 800F, 800R, 1050F, 1050R, 357F and 357R, as previously described [1]. Sequences were corrected using the Codon Code Aligner software (http://www.codoncode.com), and then a BLAST search (Basic Local Alignment Search Tool) was performed against the GenBank nucleotide collection (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A strain was considered as a candidate novel genus if the percentage of 16S rRNA similarity with the closest species with standing in nomenclature was <95% [15]. We performed a phylogenetic analysis based on the 16S rRNA of our isolate to identify its phylogenetic affiliations. Sequences were aligned using Muscle 3.8.31 [16], and phylogenetic inferences were obtained using the approximately maximum-likelihood method within FastTree software [17]. Only bootstrap values of >95% are shown, and the numbers at the nodes are the computed local values [18].

### Phenotype characteristics

Different growth conditions were tested on a 5% sheep's blood–enriched Columbia agar (bioMérieux) for strain Marseille-P2645$^T$. Five temperatures (room temperature, 28, 37, 45 and 55°C) and three atmospheres—anaerobic (anaeroGen Compact; Oxoid), microaerophilic (campyGen Compact; Oxoid) and aerobic (in a plastic pouch to maintain a humid atmosphere)—were evaluated. Tolerance of this strain to salt was tested using 5%, 7.5%, 10%, 15% and 20% of NaCl, and the pH tolerance (5, 5.5, 6, 6.5, 7, 7.5 and 8) was also tested. Individual cells of strain Marseille-P2645$^T$ were visualized using a Tecnai G20 electron microscope (FEI Company, Limeil-Brevannes, France). Gram staining was performed and observed using a photonic microscope Leica DM2500 (Leica, Wetzlar, Germany) with a 100× oil-immersion objective. Motility testing was performed by observation of a fresh colony between the blades and slats using a DM1000 photonic microscope (Leica) at 40×. To check the ability to sporulate, strain Marseille-P2645$^T$ was grown on 5% sheep's blood–

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