

Discovery of a new species within *Bacillus* genus in a stool sample from Gabon: “*Bacillus massiliogabonensis*” sp. nov.

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Abstract

The discovery of new bacteria from the human gut using a culturomics method is a novel field of increasing interest in microbiology. Here the main characteristics of “*Bacillus massiliogabonensis*” strain Marseille P2639, a new Gram-negative bacterium isolated from the stool sample of a healthy 16-year-old Gabonese boy, are reported.

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Keywords: 16S rRNA gene, “*Bacillus massiliogabonensis*”, culturomics, human gut microbiota, taxonomy

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Recent advances in microbiology enhance the culture strategies of bacteria through a new method called culturomics, which is based on the multiplication of bacterial culture conditions [1,2]. After receiving the approval of the National Ethics Committee of Gabon (no. 0023/2013/SG/CNE) and IFR48 of Marseille, France (no. 09-022), a stool sample was collected from a healthy 16-year-old Gabonese boy (body mass index, 19.03 kg·m⁻²) (in Lebamba, Gabon) in January 2015 and sent to Unité des Maladies Infectieuses et Tropicales Emergentes (URMITE, Marseille, France) for microbiologic analysis. Coupled with taxonogenomics, culturomics detected and isolated strain Marseille P2639. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) failed to identify this bacterial strain using a Microflex spectrometer (Bruker Daltonics, Bremen, Germany).

Succinctly, strain Marseille P2639 was isolated using a Marine medium culture bottle (bioMérieux, Marcy l'Etoile, France)

followed by subculture in 5% sheep's blood–enriched Columbia agar (bioMérieux) in an aerobic atmosphere at 37°C [3–5]. The Gram-negative bacillus, strain Marseille P2639, is motile and catalase positive. It does not exhibit oxidase activity. Endospore forming, individual colonies of this bacterium are whitish with a diameter of 3 mm on a 5% sheep's blood–enriched Columbia agar plate. Additionally, its individual cell is 3.7 µm (range, 3.3–4.2 µm) in length with a diameter of 0.8 µm (range, 0.7–1 µm). Strain Marseille P2639 is strictly aerobic and grows within 24 hours. Its growth temperature ranges from 23°C to 45°C with an optimum temperature of 37°C. Strain Marseille P2639 can grow under salinity ranging from 0 to 50 g/L and pH varying from 6 to 10.

To complete the identification of strain Marseille P2639, 16S rRNA gene sequencing was performed using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). The set of primers used included fD1-rP2 [3–5]. Correction and assembly of the 16S rRNA sequences were done using CodonCode Aligner. Nucleotide Basic Local Alignment Search Tool (BLAST) analysis performed using the National Center for Biotechnology Information database showed that the 16S rRNA gene sequence of strain Marseille P2639 has a 97.2% similarity with the closest species, *Bacillus acidicola* strain I05-2 [6]. This similarity value is lower than the percentage of 16S rRNA gene sequence threshold recommended by Meier-Kolthoff et al. [7] for *Firmicutes* to

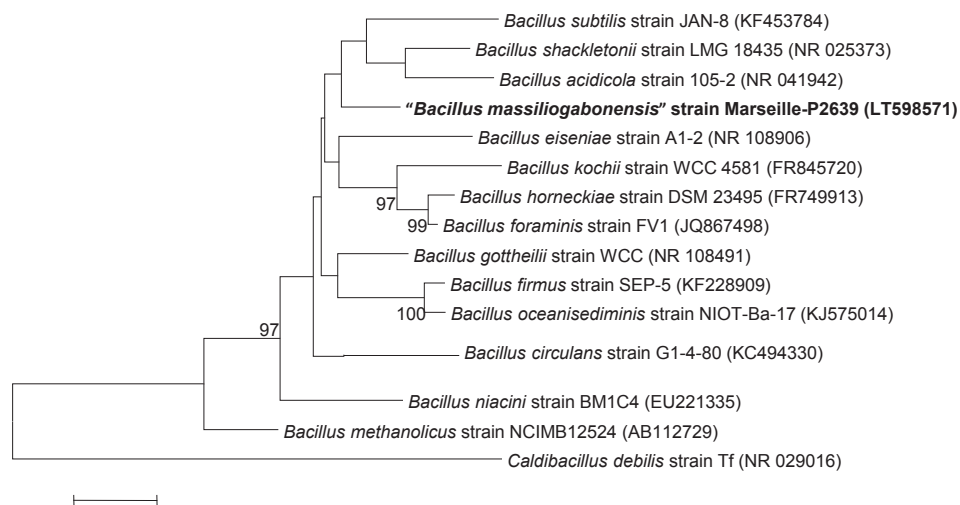


FIG. 1. Phylogenetic tree showing position of “*Bacillus massiliogabonensis*” strain Marseille P2639 relative to other phylogenetically closest species. Sequences were aligned using CLUSTALW and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes depict bootstrap percentages values obtained by repeating analysis 500 times to yield consensus tree. Only bootstrap values >95% were retained. Scale bar indicates 1% nucleotide sequence divergence.

delineate a new bacterial species without carrying out DNA-DNA hybridization, with a maximum error probability of 0.01%. Therefore, strain Marseille P2639 was putatively considered to be representative of a new bacterial species named “*Bacillus massiliogabonensis*” strain Marseille P2639 (Fig. 1).

The *Bacillus* genus was created in 1872 by Ferdinand Julius Cohn [8]. The closest neighbouring species to “*Bacillus massiliogabonensis*” sp. nov. by 16S rRNA gene sequence include *Bacillus acidicola* [6], *Bacillus eiseniae* [9], *Bacillus shackletonii* [10], *Bacillus horneckiae* [11] and *Bacillus subtilis* [12]. They are aerobic, catalase positive, motile and endospore forming. Of these species, Gram staining is negative for “*Bacillus massiliogabonensis*” sp. nov. and *B. eiseniae*. Oxidase activity is positive only for *B. subtilis* and *B. shackletonii*.

We propose the creation of “*Bacillus massiliogabonensis*” sp. nov. (named for Marseille and Gabon, the city and the country where the stool specimen was analysed and collected, respectively) as a new bacterial species. Strain Marseille P2639 is the type strain of this new species.

MALDI-TOF MS spectrum accession number

The MALDI-TOF MS spectrum of “*Bacillus massiliogabonensis*” is available at <http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>.

Nucleotide sequence accession number

The 16S rRNA gene sequence of strain Marseille P2639 was deposited in GenBank database under accession number LT598571.

Deposit in culture collection

Strain Marseille P2639 was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR) and registered under number P2639.

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Conflict of Interest

None declared.

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