

'*Bacillus massiliglaciei*', a new bacterial species isolated from Siberian permafrost

P. Afouda, G. Dubourg, F. Cadoret, P.-E. Fournier and D. Raoult

Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, UM 63, CNRS 7278, IRD 198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, Aix-Marseille Université, Marseille, France

Abstract

We describe here the main characteristics of a new species isolated from Siberian permafrost dated around 10 million years. This species was named '*Bacillus massiliglaciei*' strain Marseille-P2600^T (= CSUR P2600 = DSM 102861).

© 2016 The Author(s). Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Keywords: *Bacillus massiliglaciei*, culturomics, emerging bacterium, permafrost, taxonomy

Original Submission: 26 October 2016; **Revised Submission:** 14 November 2016; **Accepted:** 18 November 2016

Article published online: 24 November 2016

Corresponding author: P.-E. Fournier, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, UM 63, CNRS 7278, IRD 198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, Aix-Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille cedex 05, France
E-mail: pierre-edouard.fournier@univ-amu.fr

Microorganisms in permafrost have been much studied in recent decades [1,2] and a large variety of bacteria, archaea, fungi and protozoa [1] were found there by using by culture-dependent and culture-independent methods [1,3].

In February 2016, using the culturomics approach [4], a new bacterial strain named Marseille-P2600^T was cultured from a Siberian permafrost sample dated around 10 million years. Growth of the strain Marseille-P2600^T was obtained after 5 days in direct culture on Columbia agar (bioMérieux, Marcy l'Étoile, France) in an aerobic atmosphere at room temperature (19 ± 5 °C).

On Columbia agar, these strain colonies appear grey and have a diameter of about 0.5 to 1.5 mm. The Gram staining shows Gram-positive rods. Electron microscopy reveals sticks ranging in length from 2 to 5 µm and 0.5 to 0.6 µm in diameter. Electron microscopy did not allow us to see whether spores formed. The catalase and oxidase tests were, respectively, positive and negative. Growth of the Marseille-P2600^T strain occurs in aerobic and anaerobic conditions. Bacterial cells tolerate a pH of 6 to 8.5 and a NaCl concentration between

0 and 100 g/L. The 16S rRNA gene sequencing was done using universal primers FD1 and RP2 (Eurogentec, Angers, France) as previously described [5] and using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France). Marseille-P2600^T showed a 97.54% sequence identity with the type strain *Bacillus foraminis* strain CV53 (sequence accession no. AJ717382). The phylogenetic tree of strain Marseille-P2600^T showing its relationship with the closest other species is provided in Fig. 1.

Marseille-P2600^T was found in Siberian permafrost and shows a 16S rRNA sequence divergence of >1.3% with its phylogenetically closest validated species [6]. Accordingly, we propose the creation of the new species '*Bacillus massiliglaciei*' sp. nov strain Marseille-P2600^T (mas.si.li', L. masc. adj. *massili*, for Massilia, the old Roman name for Marseille, where the strain was isolated, and gla.ci'ei, L. gen. n. *glaciei* 'of ice,' referring to the isolation source of the strain). '*Bacillus massiliglaciei*' sp. nov strain Marseille-P2600^T is the type strain of the new species '*Bacillus massiliglaciei*'.

matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) spectrum

The MALDI-TOF MS spectrum of '*Bacillus massiliglaciei*' Marseille-P2600^T is available online (<http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>).

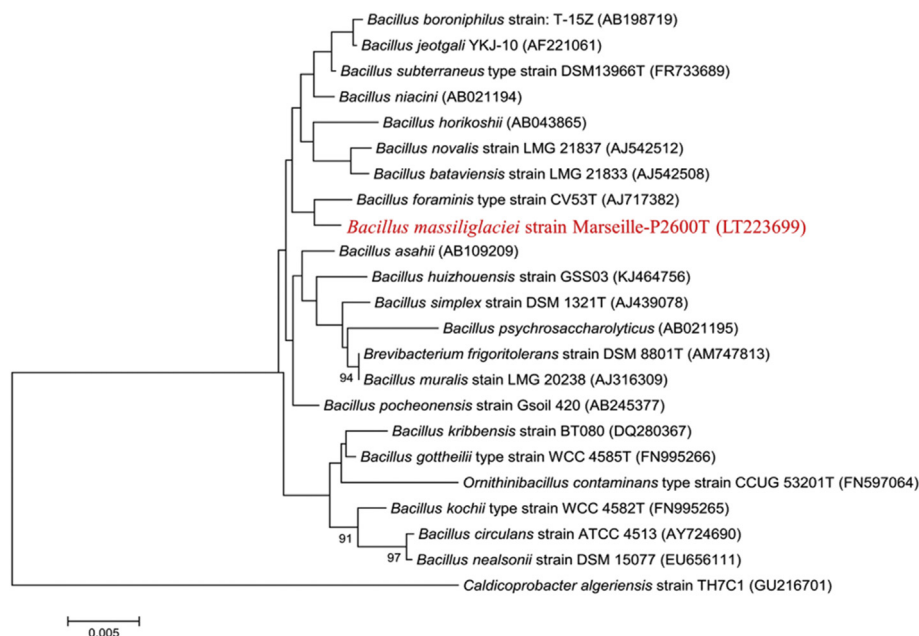


FIG. 1. Phylogenetic tree showing position of 'Bacillus massiliglaeii' strain Marseille-P3600^T relative to other phylogenetically close neighbours. Sequences were aligned using CLUSTALW, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only bootstrap scores of at least 90 were retained.

Nucleotide sequence accession number

The 16S rRNA gene sequence was deposited in GenBank under accession number LT223699.

Deposit in a culture collection

Strain Marseille-P3600^T was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number P3600 and in the Deutsche Sammlung von Mikroorganismen und Zellkulturen under number DSM102861.

Acknowledgement

This study was funded by the Fondation Méditerranée Infection.

Conflict of Interest

None declared.

References

- [1] Steven B, Leveille R, Pollard WH, Whyte LG. Microbial ecology and biodiversity in permafrost. *Extremophiles* 2006;10:259–67.
- [2] Steven B, Niederberger TD, Whyte LG. Bacterial and archaeal diversity in permafrost. In: Margesin R, editor. *Permafrost soils*. Berlin: Springer; 2009. p. 59–72.
- [3] Amann R, Ludwig W, Schleifer KH. Bacterial and archaeal diversity in permafrost, Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev* 1995;59:143–69.
- [4] Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Rober C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* 2012;18:1185–93.
- [5] Dubourg G, Lagier JC, Armougom F, Robert C, Hamad I, Brouqui P, et al. The gut microbiota of a patient with resistant tuberculosis is more comprehensively studied by culturomics than by metagenomics. *Eur J Clin Microbiol Infect Dis* 2013;32:637–45.
- [6] Huson DH, Auch AF, Qi J, Schuster SC. MEGAN analysis of metagenomic data. *Genome Res* 2010;17:377–86.