Accepted Manuscript

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PII: S2052-2975(18)30022-2

DOI: 10.1016/j.nmni.2018.03.002

Reference: NMNI 417

To appear in: New Microbes and New Infections

Received Date: 9 February 2018

Revised Date: 5 March 2018

Accepted Date: 9 March 2018

Please cite this article as: Bilen M, Mbogning Fonkou MD, Cadoret F, Dubourg G, Daoud Z, Raoult D, *Sanguibacter massiliensis* sp. nov., *Actinomyces minihominis* sp. nov., *Clostridium minihomine* sp. nov., *Neobittarella massiliensis* gen. nov., and *Miniphocibacter massiliensis* gen. nov., new bacterial species isolated by culturomics from human stool samples., *New Microbes and New Infections* (2018), doi: 10.1016/j.nmni.2018.03.002.

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Sanguibacter massiliensis sp. nov., Actinomyces minihominis sp. nov., Clostridium minihomine sp. nov., Neobittarella massiliensis gen. nov., and Miniphocibacter massiliensis gen. nov., new bacterial species isolated by culturomics from human stool samples.

Melhem Bilen^{1,2}, Maxime Descartes Mbogning Fonkou¹, Frédéric Cadoret¹, Gregory Dubourg ¹, Ziad Daoud², and Didier Raoult^{1,3}*

¹Aix-Marseille Université, URMITE, UM63, CNRS7278, IRD198, Inserm 1095, Institut Hospitalo-Universitaire Méditerranée-Infection, Faculté de médecine, 27 Boulevard Jean Moulin, 13385, Marseille cedex 05, France

²Clinical Microbiology Department, Faculty of Medicine and Medical sciences, University of Balamand, POBox:33, Amioun, Lebanon. ziad.daoud@balamand.edu.lb

³ Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

^{*}Corresponding author. E-mail address: didier.raoult@gmail.com

Keywords: Sanguibacter massiliensis sp. nov., Actinomyces minihominis sp. nov., Clostridium minihomine sp. nov., Neobittarella massiliensis gen. nov., and Miniphocibacter massiliensis gen. nov.

1 Abstract

Here, we are presenting the description of *Sanguibacter massiliensis* sp. nov., *Actinomyces minihominis* sp. nov., *Clostridium minihomine* sp. nov., *Neobittarella massiliensis* gen. nov., and *Miniphocibacter massiliensis* gen. nov., new bacterial species isolated by culturomics from human stool samples.

6 As part of the effort aiming to describe the human gut microbiota by the means of 7 culturomics (1), we report in the present study the isolation of four new bacterial species and two new bacterial genera Prior to the project start up, an approval from the Institut Federatif de 8 Recherche IFR48 (Marseille, France) was obtained under the number 09-022 along with a signed 9 consent from the donors. All stool samples were independently diluted with phosphate buffer 10 saline (PBS) and incubated in a blood culture bottle supplemented with 5% sheep blood and 5% 11 filtered rumen. A 30 days follow up was done in order to have an informative idea about the 12 bacterial growth and MALDI-TOF MS and 16S rRNA gene sequencing were used for strain's 13 identification as previously described (2,3). Having its spectrum missing in the present database, 14 MALDI-TOF MS failed to identify all the understudied organisms. Thus 16S rRNA gene 15 sequencing was applied and a similarity threshold of less than 98.65% between the isolated 16 strains and the phylogenetically closest species with standing in nomenclature was adapted to 17 delimitate a new species and a divergence of more than 5% was adapted to delimitate a new 18 19 genus (4).

Strain Marseille-P3815, was isolated on COS medium (BioMerieux, Marcy l'étoile, France) from the stool samples of a 12 years old healthy Pygmy female after 2 days incubation, under anaerobic conditions at 37°C. This strain is a gram-positive rod, catalase positive but oxidase negative. Its colonies have a diameter ranging between 0.2 to 0.9 mm with its cells average size being 1.0x0.4µm. It has a smooth and grey appearance after 48 hours of growth on COS medium.

Strain Marseille-P3815 exhibited a 96.96% sequence similarity with *Sanguibacter inulinus* strain
ST50 (NR_029277.1), thus its classified as a new species *Sanguibacter massileinsis*(ma.ssi.lien'sis, L. adj. neut., *massiliensis* from 'Massilia,' the antic name of Marseille, France,
where the strain was isolated) (Figure 1). The strain Marseille-P3815^T is the type strain of the
species *Sanguibacter massileinsis*.

Strain Marseille-P3850, was isolated on COS medium (BioMerieux) from the stool samples of a 30 12 years old healthy Pygmy female after 5 days incubation, under anaerobic conditions at 37°C. 31 This strain is a gram-positive rod, catalase and oxidase negative. Its colonies have a diameter 32 ranging between 0.3 to 1 mm with its cells average size being 1.0x0.5µm. It has a smooth and 33 grey appearance after 48 hours of growth on COS medium. Strain Marseille-P3850 exhibited a 34 93.36% 16S rRNA gene sequence similarity with Actinomyces marimammalium strain CCUG 35 41710 (NR 025395.1), thus its classified as a new species Actinomyces minihominis 36 (mini.ho.min'is, L. adj. neut., minihominis to refer to pygmy people from who the strain was 37 isolated and who are characterized by their small size) (Figure 2). The strain Marseille-P3850^T is 38 the type strain of the species Actinomyces minihominis. 39

Strain Marseille-P4642, was isolated on COS medium (BioMerieux) from the stool samples of a 40 39 years old healthy Pygmy male after 10 days incubation, under anaerobic conditions at 37°C. 41 This strain is a gram-positive bacilli, catalase and oxidase negative. Its colonies have a diameter 42 ranging between 0.05 to 1 mm with its cells average size being 2.2x0.43µm. It has a smooth and 43 grey appearance after 48hrs of growth on COS medium. Strain Marseille-P4642 exhibited a 44 98.13% 16S rRNA gene sequence similarity with Clostridium jeddahense strain JCD 45 (NR_144697.1), thus its classified as a new species *Clostridium minihomine* (mini.ho.min'e, L. 46 47 adj. neut., minihomine to refer to pygmy people from who the strain was isolated and who are characterized by their small size) (Figure 3). The strain Marseille-P4642^T is the type strain of the 48 species Clostridium minihomine. 49

Strain Marseille-P4047, was isolated on COS medium (BioMerieux) from the stool samples of a 50 healthy Senegalese male after 5 days incubation, under anaerobic conditions at 37°C. This strain 51 is a gram-positive coco-bacilli, catalase and oxidase negative. Its colonies have a diameter 52 ranging between 0.04 to 0.9 mm with its cells average size being 1.6x0.6um. It has a shiny grey 53 appearance after 48 hours of growth on COS medium. Strain Marseille-P4047 exhibited an 54 89.22% 16S rRNA gene sequence similarity with Acetanaerobacterium elongatum strain Z7 55 (NR_042930.1), thus its classified as a new genus Neobitarella (Neo.bita.rel'la, L. adj. fem., in 56 honor of the microbiologist Fadi Bittar). Neobitarella massiliensis is the type species of the new 57 genus Neobittarella (ma.ssi.lien'sis, L. adj. fem., massiliensis from 'Massilia,' the antic name of 58 Marseille, France, where the strain was isolated) (Figure 4). The strain Marseille-P4047^T is the 59 type strain of the species Neobittarella massiliensis. 60

Strain Marseille-P4678, was isolated on COS medium (BioMerieux) from the stool samples of a 61 39 years old healthy Pygmy male after 10 days incubation, under anaerobic conditions at 37°C. 62 This strain is a gram-positive cocci, catalase positive and oxidase negative. Its colonies have a 63 diameter ranging between 0.02 to 0.08 mm with its cells average diameter of 0.6 µm. It has a 64 smooth grey appearance after 48 hours of growth on COS medium. Strain Marseille-P4047 65 exhibited an 89.69% 16S rRNA gene sequence similarity with Parvimonas micra strain 3119B 66 (NR 036934.1), thus it is classified as a new genus Miniphocibacter (Mini.phoci.bacter, L. adj. 67 masc., miniphocibacter composed by mini referring at the small size of pygmy people from who 68 the strain was isolated and *phoci* referring at Phocae, the latine name of the city from where the 69 fundators of Marseille were). Miniphocibacter massiliensis is the type species of the genus 70 Miniphocibacter (ma.ssi.lien'sis, L. adj. masc., massiliensis from 'Massilia,' the antic name of 71 Marseille, France, where the strain was isolated) (Figure 5). The strain Marseille-P4678^T is the 72 type strain of the species Miniphocibacter massiliensis. 73

MALDI-TOF MS spectrum accession number. The MALDI-TOF MS spectrum of the
 reported organisms are available online (http://www.mediterranee infection.com/article.php?laref=256&titre=urms-database)

- 77 Nucleotide sequence accession number. The 16S rRNA gene sequence of *Sanguibacter*
- 78 massiliensis sp. nov., Actinomyces minihominis sp. nov., Clostridium minihomine sp. nov.,
- 79 Neobittarella massiliensis gen. nov., and Miniphocibacter massiliensis gen. nov., was deposited
- in Genbank under following accession numbers respectively: LT838404, LT855383, LT960605,
- 81 LT934440 and LT934441

Deposit in a culture collection. Strain Marseille-P3815, Strain Marseille-P3850, Strain Marseille-P4642, Strain Marseille-P4047 and Strain Marseille-P4678 were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number the following numbers respectively: P3815, P3850, P4642, P4047 and P4678.

- 86 **Conflict of interest:**
- 87 None to declare.
- 88 Funding sources:
- 89 This work was funded by Fondation Mediterrannée Infection.

90 Figure Legend:

91 Figure 1: This phylogenetic tree shows the position *S. massiliensis* strain Marseille-P3815 92 towards its closest species. CLUSTALW was used for alignment and MEGA software for 93 phylogenetic inferences generation with the maximum likelihood method. After 500 repeats, 94 bootstrap values are shown on the nodes with only values above 90% kept.

Figure 2: This phylogenetic tree shows the position *A. massiliensis* strain Marseille-P3850
towards its closest species. CLUSTALW was used for alignment and MEGA software for
phylogenetic inferences generation with the maximum likelihood method. After 500 repeats,
bootstrap values are shown on the nodes with only values above 90% kept.

99 Figure 3: This phylogenetic tree shows the position *C. minihomine* strain Marseille-P4042 100 towards its closest species. CLUSTALW was used for alignment and MEGA software for 101 phylogenetic inferences generation with the maximum likelihood method. After 500 repeats, 102 bootstrap values are shown on the nodes with only values above 95% kept.

Figure 4: This phylogenetic tree shows the position *N. massiliensis* strain Marseille-P4047towards its closest species. CLUSTALW was used for alignment and MEGA software for phylogenetic inferences generation with the maximum likelihood method. After 500 repeats, bootstrap values are shown on the nodes with only values above 95% kept.

Figure 5: This phylogenetic tree shows the position *M. massiliensis* strain Marseille-P4678
towards its closest species. CLUSTALW was used for alignment and MEGA software for
phylogenetic inferences generation with the maximum likelihood method. After 500 repeats,
bootstrap values are shown on the nodes with only values above 95% kept.

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112 **Reference list**

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Figure 4

