Massilicoli timonensis sp. nov., a new bacterium isolated from the human microbiota

S. Ndongo1,2, M. L. Tall2,3, I. I. Ngom1,2, P.-E. Fournier1,3, A. Levasseur3, D. Raoult1,2 and S. Khelafia1,2
1 Aix-Marseille Université, IRD, APHM, MEPhi, Marseille, France, 2 Institut Hospitalo-Universitaire Méditerranéee Infection, Marseille, France and 3 UMR VITROME, Aix-Marseille Université, IRD, SSA, AP-HM, IHU–Méditerranéee Infection, Marseille, France

Abstract

Massilicoli timonensis sp. nov., strain Marseille-P3755T (= CSUR P3755 = DSM 103513) is a new bacterial species from the phylum Firmicutes and the family Clostridiales which was isolated from the human gut microbiota.

© 2019 The Authors. Published by Elsevier Ltd.

Keywords: anaerobic bacterium, Culturomics, gut microbiota, massilicoli timonensis sp. nov., taxonogenomics

Original Submission: 15 May 2019; Revised Submission: 10 July 2019; Accepted: 20 August 2019

Article published online: 27 August 2019

Introduction

Deciphering the bacterial diversity involved in normal and pathogenic functions appears fundamental [1]. In order to unveil the human gut microbial diversity, the culturomics approach, based on diversified culture conditions, was designed to isolate as yet uncultured species and to complement 16S ribosomal RNA (rRNA) metagenomics [2–4]. Furthermore, a new taxonomic strategy termed taxonogenomics was developed to include the analysis of complete genome sequences in combination with phenotypic characteristics [5]. Here we report a short description of strain Marseille-P3755T that was isolated from the human gut microbiota.

Isolation and growth conditions

As part of a culturomics study, a stool sample was collected from an 85-year-old Frenchwoman admitted in the Timone Hôpital Marseille in December 2016. A total of 0.3 g of faecal specimen was serially diluted in 900 μL of phosphate-buffered saline (Life Technologies, Carlsbad, CA, USA), and 50 μL of each dilution was seeded on 5% sheep’s blood–enriched Columbia agar (bioMérieux, Marcy l’Etoile, France). After 3 days of incubation at 37°C in an anaerobic atmosphere generated by AnaeroGen (bioMérieux), several colonies grew and were isolated. The purified isolate obtained after three subcultures from a single colony could not be identified by MALDI-TOF MS. The screening was performed on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany), as previously reported [6]. Spectra obtained (Fig. 1) were imported and analysed by Biotyper 3.0 software against the Bruker database, which is continuously updated with information from the Microbes Evolution Phylogeny and Infections (MEPhi) database [1]. This study was approved by the ethics committee of the Institut Fédératif de Recherche 48 under reference 2016-010. The patient provided written informed consent for participating in this study.

Phenotypic characteristics

The strain Marseille-P3755 colonies grown on Columbia agar plates after 3 days were circular and translucent, with a diameter of about 0.5 to 1 mm. Strain Marseille-P3755 is a strict anaerobic bacterium, has Gram-negative bacilli (0.3 μm × 2–3 μm), and is nonmotile and non–spore forming (Fig. 2). Strain
Marseille-P3755 was negative for catalase and oxidase activities. Biochemical characteristics were investigated using API ZYM, API 50CH and API 20NE strips (bioMérieux). In API ZYM, enzymatic activities were observed for phosphatase acid and naphthol-AS-BI-phosphohydrolase. A slightly positive reaction was observed for phosphatase alkaline, esterase (C4) and esterase lipase (C8); the results of the other tests were negative. Using API 50CH strips, positive reactions were observed with: L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, D-glucose, D-fructose, dulcitol, inositol, D-mannose methyl-α-D-mannopyranoside, N-acetyl-glucosamine, amygdalin, arbutin, salicin, D-maltose, D-saccharose, inulin, glycogen, xylitol, gentiobiose, Larabinose and potassium 5-keto-glucuronate. Negative reactions were observed with: negative: glycerol, erythritol, D-arabinose, methyl-β-D-mannopyranoside, D-galactose, L-sorbose, L-rhamnose, D-sorbitol, methyl-α-D-glucopyranoside, esculin ferric citrate, D-cellobiose, D-lactose, D-melibiose D-trehalose D-melezitose D-rafﬁnose, amidone, D-turanose, D-lyxose, D-tagatose D-fucose, L-fucose, D-arabitol, potassium gluconate and potassium 2-keto-glucuronate. In API 20NE, all test results were negative, including nitrate reduction, indole formation, arginine dihydrolase and hydrolysis of esculin and gelatin.
FIG. 3. Phylogenetic tree highlighting position of Massilicoli timonensis sp. nov. with regard to other closely related species. GenBank accession numbers of 16S ribosomal RNA are indicated in parentheses. Sequences were aligned using MUSCLE with default parameters; phylogenetic inferences were obtained by maximum likelihood method and MEGA 7 software. Bootstrap values were obtained by repeating analysis 1000 times to generate majority consensus tree, indicated at nodes. Scale bar indicates 2% nucleotide sequence divergence.

FIG. 4. Heat map generated with OrthoANI values calculated using OAT software between Massilicoli timonensis sp. nov. and other closely related species with standing in nomenclature.
TABLE 1. Description of Massilicoli timonensis sp. nov.

| Property                                      | Value                                      |
|-----------------------------------------------|--------------------------------------------|
| Accession number                              | TA00843                                   |
| Species name                                  | Massilicoli timonensis                     |
| Genus name                                    | Massilicoli                                |
| Specific epithet                              | sp. nov.                                  |
| Species status                                | Massilicoli (mas.s.i.li.co.li, N.L. masc. n., association of Massilia, the Latin name of Marseille, France, and colon, from which the type strain was isolated) |
| Species etymology                            | Massilicoli (mas.s.i.li.co.li, N.L. masc. n., association of Massilia, the Latin name of Marseille, France, and colon, from which the type strain was isolated) |
| Designation of type strain                    | Strain Marseille-P3755                     |
| Strain collection number                      | (CSUR P3755 = DSM 103513)                 |
| 16S rRNA gene accession number                | LT899395                                  |
| Genome accession number                       | OEMR000000000                             |
| Genome status                                 | Draft                                     |
| Genome size                                   | 3 118 584 bp                              |
| GC mol%                                       | 53.0                                      |
| Data on origin of sample from which strain had been isolated | France                                    |
| Country of origin                             | France                                    |
| Region of origin                              | Marseille                                 |
| Source of isolation                           | Human stool                               |
| Gram stain                                    | Negative                                  |
| Cell shape                                    | Rod                                       |
| Motility                                      | Motile                                    |
| Colony morphology                             | On Columbia agar plates, colonies are circular and translucent, with diameter about 0.5 to 1 mm after 3 days of incubation at 37°C |
| Temperature optimum                           | 37°C                                      |
| pH optimum                                    | 7                                         |
| Oxidase                                       | Negative                                  |
| Catalase                                      | Negative                                  |
| Genome size                                   | 3 118 584 bp                              |
| GC mol%                                       | 53.0                                      |
| Data shown according to protologue TA00843 at Digital Protologue website (http://imedea.uib-csic.es/dprotologue/). |

Strain identification

In order to classify this bacterium, the 16S recombinant DNA (rDNA) gene was amplified using the primer pair F1D1 and rP2 (Eurogentec, Angers, France) and sequenced with the Big Dye Terminator v1.1 Cycle Sequencing Kit and the 3500xL Genetic Analyzer capillary sequencer (Thermo Fisher Scientific, Waltham, MA, USA) as previously described [7]. The 16S rDNA nucleotide sequence was assembled and corrected using CodonCode Aligner software (https://www.codoncode.com/).

Strain Marseille-P3755T exhibited a 95.0% 16S rDNA similarity with Dielma fastidiosa strain JC13 (GenBank accession no. NR_125593.1), the phylogenetically closest species with standing in nomenclature (Fig. 3). We consequently proposed to classify this strain as a new genus named Massilicoli within the Firmicutes phylum. Massilicoli timonensis strain Marseille-P3755T is the species type.

Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany), then sequenced on a MiSeq sequencer (Illumina, San Diego, CA, USA) with the Nextera Mate Pair prep kit and Nextera XT Paired End (Illumina), as previously described [8]. The assembly was performed using a pipeline containing several softwares (Velvet [9], Spades [10] and Soap Denovo [11]) on trimmed (MiSeq and Trimmomatic [12] softwares) or untrimmed data (only MiSeq software). GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value lower than 25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). The genome of strain Marseille-P3755T was 3 118 584 bp long with a 53.0 mol% G + C content. The degree of genomic similarity of strain Marseille-P3755T with closely related species was estimated with OrthoANI software [13]. OrthoANI values among closely related species (Fig. 4) ranged from 61.80% between Bulleidia extracto and Massilicoli timonensis to 70.36% between Clostridium innocuum and Eubacterium cylindroides. When M. timonensis was compared to these closely species, values ranged from 62.43% with Dielesia fastidio to 70.36% with Clostridium innocuum.

Conclusion

On the basis of unique phenotypic features, including MALDI-TOF MS spectrum, a 16S rRNA sequence divergence greater than >1.3% and an OrthoANI value < 95% with the phylogenetically closest species with standing in nomenclature, we formally propose the creation of the new genus ‘Massilicoli’ gen. nov., and Massilicoli timonensis sp. nov., strain Marseille-P3755T is the type strain.

Description of Massilicoli gen. nov.

Massilicoli (mas.s.i.li.co.li, N.L. masc. n., association of Massilia, the Latin name of Marseille, France, and colon, from which the type strain was isolated).

Description of Massilicoli timonensis strain Marseille-P3755T gen. nov., sp. nov.

Massilicoli timonensis (ti.mo.nen’sis, L. masc. adj., timonensis from Timone, the name of the university hospital in Marseille, France, where the strain type was isolated).

The characteristics of the species are listed in Table 1. The type strain is Marseille-P3755T (= CSUR P3755 = DSM 103513).

Nucleotide sequence accession number

The 16S rRNA gene and genome sequences were deposited in GenBank under accession numbers LT899395 and OEMR00000000 respectively.

© 2019 The Authors. Published by Elsevier Ltd. NMNI, 32, 100592
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0).
Deposit in a culture collection

Strain Marseille-P3755T was deposited in the Collection de Souches de l’Unité des Rickettsies (CSUR) under P3755 and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) under DSM 103513.

MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of ‘Massilicoli timonensis’ Marseille-P3755T is available online (http://backup.mediterranee-infection.com/article.php?larub=280&titre=urms-database).

Acknowledgements

Supported in part by the Méditerranée Infection Foundation and the French National Research Agency under the programme ‘Investissements d’Avenir’, reference ANR-10-IAHU-03. The authors thank C. Robert (1 Aix-Marseille Université,IRD, APHM, MEPHI, Marseille, France) for sequencing the genome, A. Caputo (1 Aix-Marseille Université,IRD, APHM, MEPHI, Marseille, France) for submitting the genomic sequence to GenBank and M. Lardiére (1 Aix-Marseille Université,IRD, APHM, MEPHI, Marseille, France) for English-language review. We also thank T. Irie, K. Imai, S. Matsubara, T. Sakazume, Y. Ominami, H. Akiko and the Hitachi team in Japan (Hitachi High-Technologies Corporation, Science & Medical Systems Business Group, Tokyo, Japan) for the collaborative study conducted with the IHU – Méditerranée Infection, and for the installation of a TM4000 microscope at the IHU – Méditerranée Infection facility.

Conflict of interest

None declared.

References

[1] Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007;449:804–10.
[2] Lagier JC, Armougom F, Million M, Hugon P, Pagnier I, Robert C, et al. Microbial culturomics: paradigm shift in the human gut microbiome study. Clin Microbiol Infect 2012;18:1185–93.
[3] Lagier JC, Hugon P, Khelaifi S, Fournier PE, La Scola B, Raoult D. The rebirth of culture in microbiology through the example of culturomics to study human gut microbiota. Clin Microbiol Rev 2015;28:237–64.
[4] Lagier JC, Khelaifi S, Alou MT, Ndongo S, Dione N, Hugon P, et al. Culture of previously uncultured members of the human gut microbiota by culturomics. Nat Microbiol 2016;1:16203.
[5] Ramasamy D, Mishra AK, Lagier JC, Padmanabhan R, Rossi M, Sentausa E, et al. A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. Int J Syst Evol Microbiol 2014;64:384–91.
[6] Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 2009;49:543–51.
[7] Morel AS, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta JP, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis 2015;34:561–70.
[8] Ndongo S, Bittar F, Beye M, Robert C, Di Pinto F, Fournier PE, et al. ‘Cellulomonas timonensis’ sp. nov., a taxonomics description of the new bacterial species isolated from the human gut. New Microbe New Infect 2018;23:7–16.
[9] Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18:821–9.
[10] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.
[11] Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, et al. SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. GigScience 2012;1:18.
[12] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
[13] Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100–3.