Draft Genome Sequences of 15 Bacterial Species Constituting the Stable Defined Intestinal Microbiota of the GM15 Gnotobiotic Mouse Model

Céline Elie,a Alban Mathieu,a Adrien Saliou,a Adrien Villain,a Marion Darnaud,a François Leulier,a,b Andrea Tamellini,a

aBIOASTER, Institut de Recherche Technologique, Lyon, France
bInstitut de Génomique Fonctionnelle de Lyon, Université de Lyon, École Normale Supérieure de Lyon, Centre National de la Recherche Scientifique, Université Claude Bernard Lyon 1, Lyon, France

ABSTRACT The GM15 community is a bacterial consortium used to generate a novel standardized mouse model with a simplified controlled intestinal microbiota recapitulating the specific opportunistic pathogen-free (SOPF) mouse phenotype and the potential to ensure an increased reproducibility and robustness of preclinical studies by limiting the confounding effect of microbiota composition fluctuation.

The intestinal microbiota is a complex and dynamic ecosystem largely composed of bacteria whose activity greatly impacts the health and diseases of the host (1). Associating mice with stable defined bacterial consortia reduces the complexity of the microbiota and overcomes limitations related to the variability between individuals and animal facilities (2, 3). Therefore, gnotobiotics contribute to standardization and experimental reproducibility and are a powerful tool for testing causality in host-microbiome studies (4–6). Thus, we have developed a simplified mouse microbiota that is representative of the fecal microbiota found in C57BL/6J mice on the functional level and derived a standardized gnotobiotic mouse model called GM15, which has been bred successfully for over eight generations in the gnotobiology unit of BIOASTER. All animal procedures were approved by the French Ministry of Higher Education, Research and Innovation (MESR) and the ANSES/ENV/AUPEC ethics committee (Autorisation de Projet Utilisant des Animaux à des Fins Scientifiques [APAFIS] no. 4529-2016022616404045v3, 785-2015042819315178v2, and 18918-2019020118003843v3) and were conducted in accordance with national French and European legislation on the protection of animals used for scientific purposes.

We report here the draft genome sequences of 9 bacterial strains isolated from the intestinal microbiota of C57BL/6J specific-opportunistic-pathogen-free (SOPF) mice (Charles River Laboratories, France), 2 bacterial strains isolated from C57BL/6J axenic mice recolonized with feces of the altered Schaedler flora (ASF) mouse model (Taconic, USA), and 4 bacterial strains obtained from the DSMZ collection. Then, the colonization of the axenic C57BL/6J mice with these 15 bacterial isolates resulted in the GM15 mice.

Fresh cecal contents and fecal pellets of mice were resuspended (1/10 [wt/vol]) in reduced broth medium for direct dilution plating onto agar plates (same medium as the broth) and growth at 37°C under anaerobic atmosphere (90% N2, 5% H2, 5% CO2). Lactobacillus johnsonii MD006 was isolated on MRS agar. Lactobacillus murinus MD040 and Parabacteroides goldsteinii MD072 were isolated on Columbia nalidixic acid (CNA) agar with 5% sheep blood. Bacteroides acidifaciens MD185 and Lachnospiraceae sp. strain MD308 were isolated on Gifu anaerobic medium (GAM) agar. Bacteroides caecimuris MD237 and Lactobacillus reuteri MD207 were isolated on GAM agar supplemented, respectively, with 32 µg/ml vancomycin and 32 µg/ml erythromycin. Lachnospiraceae sp. strains MD335 and MD329 were isolated on M2GSC (modified Med2 of...
| Isolate      | Bacterial species                     | GenBank accession no. | SRA accession no. | Assembly size (bp) | No. of paired-end reads | GC content (%) | Genome coverage (%) | No. of contigs | N_{50}\ a (bp) | Genome completeness\ b | C (%) | S (%) | D (%) | F (%) | M (%) | n        |
|--------------|---------------------------------------|-----------------------|-------------------|-------------------|------------------------|----------------|--------------------|---------------|----------------|------------------------|-------|-------|-------|-------|-------|---------|
| MD185        | Bacteroides acidifaciens              | VIRE000000000          | SRR9696648        | 5,228,841         | 3,643,154             | 42.03          | 209                | 174           | 78,435         | 100.0                  | 99.9  | 0.2   | 0.0   | 0.0   | 0.0   | 541     |
| MD237        | Bacteroides acemicutus                | VRDO000000000          | SRR9696649        | 4,624,756         | 3,072,634             | 41.91          | 191                | 93            | 111,017        | 100.0                  | 99.8  | 0.2   | 0.0   | 0.0   | 0.0   | 541     |
| MD294        | Clostridium sp.                       | VRRC000000000          | SRR9696650        | 2,856,932         | 3,858,506             | 31.67          | 405                | 30            | 199,176        | 99.6                   | 98.5  | 1.1   | 0.0   | 0.4   | 0.0   | 264     |
| MD300        | Schaetleriella arabinosiphila         | VIRD000000000          | SRR9696651        | 6,346,609         | 2,802,282             | 43.21          | 132                | 186           | 90,162         | 100.0                  | 100.0 | 0.0   | 0.0   | 0.0   | 0.0   | 264     |
| MD335        | Unclassified Lachnospiraceae sp.      | VIRA000000000          | SRR9696652        | 5,174,357         | 3,623,726             | 42.12          | 210                | 189           | 74,501         | 100.0                  | 99.6  | 0.4   | 0.0   | 0.0   | 0.0   | 264     |
| MD306        | Lactobacillus johnsonii               | VIQY000000000          | SRR9696653        | 1,938,179         | 4,121,376             | 35.70          | 638                | 43            | 79,219         | 99.5                   | 99.0  | 0.5   | 0.0   | 0.2   | 0.3   | 402     |
| MD400        | Lactobacillus murinus                 | VIQY000000000          | SRR9696654        | 2,224,369         | 3,675,244             | 39.77          | 440                | 130           | 35,268         | 99.5                   | 99.3  | 0.2   | 0.0   | 0.3   | 0.2   | 402     |
| MD207        | Lactobacillus reuteri                 | VIQX000000000          | SRR9696655        | 2,014,265         | 3,994,740             | 39.72          | 595                | 55            | 13,090         | 99.5                   | 99.0  | 0.5   | 0.2   | 0.3   | 0.3   | 402     |
| MD072        | Parabacteroides goldsteinii          | VIQW000000000          | SRR9696656        | 6,910,815         | 3,871,748             | 42.38          | 168                | 115           | 109,394        | 100.0                  | 99.6  | 0.4   | 0.0   | 0.0   | 0.0   | 541     |
| MD329        | Unclassified Lachnospiraceae sp.      | VIQW000000000          | SRR9696657        | 2,789,948         | 3,468,474             | 39.51          | 373                | 86            | 103,195        | 98.9                   | 99.8  | 0.0   | 0.0   | 1.1   | 1.1   | 264     |
| MD308        | Unclassified Lachnospiraceae sp.      | VIQW000000000          | SRR9696644        | 4,440,317         | 2,742,248             | 40.71          | 185                | 162           | 89,086         | 98.9                   | 98.9  | 0.0   | 0.0   | 1.1   | 1.1   | 264     |
| DSM-28734    | Aerotetrus colhominis                 | VIQT000000000          | SRR9696645        | 3,502,576         | 2,478,686             | 53.63          | 193                | 37            | 200,988        | 97.3                   | 97.3  | 0.0   | 0.4   | 0.3   | 2.6   | 264     |
| DSM-26114    | Clostridium clostridioforme           | VIQS000000000          | SRR9696646        | 7,084,105         | 4,288,290             | 45.80          | 182                | 268           | 69,589         | 99.2                   | 97.3  | 1.9   | 0.4   | 0.4   | 0.4   | 264     |
| DSM-1551     | Clostridium cocleatum                | VIQR000000000          | SRR9696647        | 3,057,667         | 3,798,362             | 29.16          | 373                | 53            | 110,672        | 98.5                   | 92.0  | 0.5   | 0.0   | 0.5   | 0.5   | 218     |
| DSM-28618    | Escherichia coli                      | VIQR000000000          | SRR9696643        | 5,218,283         | 1,968,758             | 40.69          | 113                | 33            | 943,892        | 100.0                  | 99.5  | 0.5   | 0.0   | 0.0   | 0.0   | 440     |

a The N_{50} value is the length of the shortest contig for which longer and equal-length contigs cover at least 50% of the assembly.

b Genome completeness was assessed with BUSCO v4.0.6 (16) and is reported in BUSCO notation: C, complete; S, complete and single copy; D, complete and duplicated; F, fragmented; M, missing; n, total number of BUSCO groups searched.
Hobson) agar (7). All isolates mentioned were isolated from fecal pellets of SOPF mice, except for isolates MD335, MD329, and MD308, which were isolated from the cecal contents of SOPF mice. *Clostridium* sp. strain MD294 and *Schaedlerella arabinosiphila* strain MD300 were isolated on M2GSC agar (7) from the cecal contents and fecal pellets of ASF-colonized mice, respectively. Prior to genome sequencing, bacteria were identified to the genus or species level by 16S sequencing.

Genomic DNA of each bacterial isolate was extracted using the DNeasy PowerLyzer PowerSoil kit (Qiagen) from a culture inoculated with a single bacterial colony at 37°C in an anaerobic chamber (90% N₂, 5% H₂, 5% CO₂), except *Escherichia coli* DSM-28618, which was grown aerobically. The bacterial strains were grown in modified GAM broth (HyServe), except for isolates MD300, MD335, MD329, and MD308, which grew better in M2GSC medium (7), and *Anaerotruncus colihominis* DSM-28734, which was grown in *Bifidobacterium* medium (DSMZ, medium 58). DNA samples from the 15 bacterial cultures were prepared for whole-genome sequencing using the Nextera XT DNA library preparation kit (Illumina). The resulting libraries were analyzed using the high-sensitivity DNA kit on the Agilent 2100 Bioanalyzer system and quantified using the QuantiFluor One double-stranded DNA (dsDNA) kit (Promega). Paired-end (2 × 300-bp) sequencing was performed on a MiSeq sequencer using the MiSeq v3 kit (600 cycles; Illumina). The paired-end reads were assembled de novo using the A5-miseq assembly pipeline (8), comprising the following steps: adapter trimming, quality trimming and filtering, error correction, assembly, and scaffolding. The 15 de novo assemblies resulted in draft genome sequences composed of few scaffolds with high N₅₀ values. Genomes were then ordered using Mauve (9) and annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (10). Default parameters were used for all software tools.

Following Edgar’s recommendation (11), a full-length 16S rRNA sequence identity ≥99% using either the BLAST (12), RDP (13), or EzTaxon (14) programs allowed the identification of 12 isolates at the species level. Despite phylogenetic placements and average nucleotide identity (ANI) calculations performed with GTDB-Tk (15), isolates MD329, MD335, and MD308 could only be assigned to the *Lachnospiraceae* family.

These 15 bacteria cover 7 of the most representative and prevalent families of the intestinal microbiota of C57BL/6J SOPF mice (3).

**Data availability.** The assembled sequences and sequencing reads have been deposited in DDBJ/ENA/GenBank and SRA, respectively (BioProject accession no. PRJNA551571), under the accession numbers listed in Table 1. The versions described in this paper are the first versions.

**ACKNOWLEDGMENT**

This work was supported by the French Government as part of the Programme des Investissements d’Avenir (PIA; grant no. ANR-10-AIRT-03). The funder had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

**REFERENCES**

1. Lynch SV, Pedersen O. 2016. The human intestinal microbiome in health and disease. N Engl J Med 375:2369–2379. [https://doi.org/10.1056/NEJMra1600266](https://doi.org/10.1056/NEJMra1600266).

2. Laukens D, Brinkman BM, Raes J, De Vos M, Vandenberghe P. 2016. Heterogeneity of the gut microbiome in mice: guidelines for optimizing experimental design. FEMS Microbiol Rev 40:117–132. [https://doi.org/10.1093/femsre/fuv036](https://doi.org/10.1093/femsre/fuv036).

3. Clavel T, Lagkouvardos I, Blaut M, Stecher B. 2016. The mouse gut microbiome revisited: from complex diversity to model ecosystems. Int J Med Microbiol 306:316–327. [https://doi.org/10.1016/j.ijmm.2016.03.002](https://doi.org/10.1016/j.ijmm.2016.03.002).

4. Stappenbeck TS, Virgin HW. 2016. Accounting for reciprocal host-microbiome interactions in experimental science. Nature 534:191–199. [https://doi.org/10.1038/nature18285](https://doi.org/10.1038/nature18285).

5. McCoy KD, Geuking MB, Ronchi F. 2017. Gut microbiome standardization in control and experimental mice. Curr Protoc Immunol 117:1–13. [https://doi.org/10.1002/cpim.25](https://doi.org/10.1002/cpim.25).

6. Mooser C, Gomez de Aguero M, Ganal-Vonarburg SC. 2018. Standardization in host-microbiota interactions studies: challenges, gnotobiology as a tool, and perspective. Curr Opin Microbiol 44:50–60. [https://doi.org/10.1016/j.mib.2018.07.007](https://doi.org/10.1016/j.mib.2018.07.007).

7. Barcenilla A, Pryde SE, Martin JC, Duncan SH, Stewart CS, Henderson C, Flint HJ. 2000. Phylogenetic relationships of butyrate-producing bacteria from the human gut. Appl Environ Microbiol 66:1654–1661. [https://doi.org/10.1128/aem.66.4.1654-1661.2000](https://doi.org/10.1128/aem.66.4.1654-1661.2000).

8. Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. [https://doi.org/10.1093/bioinformatics/btu661](https://doi.org/10.1093/bioinformatics/btu661).
9. Darling AE, Tritt A, Eisen JA, Facciotti MT. 2011. Mauve assembly metrics. Bioinformatics 27:2756–2757. https://doi.org/10.1093/bioinformatics/btr451.

10. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.

11. Edgar RC. 2018. Updating the 97% identity threshold for 16S ribosomal RNA OTUs. Bioinformatics 34:2371–2375. https://doi.org/10.1093/bioinformatics/bty113.

12. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

13. Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267. https://doi.org/10.1128/AEM.00062-07.

14. Kim O-S, Cho Y-J, Lee K, Yoon S-H, Kim M, Na H, Park S-C, Jeon YS, Lee J-H, Yi H, Won S, Chun J. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721. https://doi.org/10.1099/ijs.0.038075-0.

15. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics 36:1925–1927. https://doi.org/10.1093/bioinformatics/btz848.

16. Seppey M, Manni M, Zdobnov EM. 2019. BUSCO: assessing genome assembly and annotation completeness. Methods Mol Biol 1962:227–245. https://doi.org/10.1007/978-1-4939-9173-0_14.