genomeA
Journals.ASM.org

Genome Sequence of "*Candidatus* Arthromitus" sp. Strain SFB-Mouse-NL, a Commensal Bacterium with a Key Role in Postnatal Maturation of Gut Immune Functions

Alexander Bolotin,[a,b] Tomas de Wouters,[a,b] Pamela Schnupf,[c,d] Christiane Bouchier,[e] Valentin Loux,[f] Moez Rhimi,[a,b] Alexandre Jamet,[a,b] Rozenn Dervyn,[a,b] Samira Boudebbouze,[a,b] Hervé M. Blottière,[a,b] Alexei Sorokin,[a,b] Johannes Snel,[g]* Nadine Cerf-Bensussan,[c,d] Valérie Gaboriau-Routhiau,[a,b,c,d] Maarten van de Guchte,[a,b] Emmanuelle Maguin[a,b]

INRA, UMR1319 Micalis, Jouy en Josas, France[a]; AgroParisTech, UMR Micalis, Jouy en Josas, France[b]; INSERM UMR1163, Laboratory of Intestinal Immunity, Paris, France[c]; Université Paris Descartes-Sorbonne Paris Cité and Institut Imagine, Paris, France[d]; Institut Pasteur, Plate-forme Génomique, Paris, France[e]; INRA, UR1077 Mathématique Informatique et Génome, Jouy en Josas, France[f]; NIZO Food Research, Ede, The Netherlands[g]

* Present address: Johannes Snel, CTGB, Wageningen, The Netherlands.

"*Candidatus* Arthromitus" sp. strain SFB-mouse-NL (SFB, segmented filamentous bacteria) is a commensal bacterium necessary for inducing the postnatal maturation of homeostatic innate and adaptive immune responses in the mouse gut. Here, we report the genome sequence of this bacterium, which sets it apart from earlier sequenced mouse SFB isolates.

Received 19 June 2014 **Accepted** 26 June 2014 **Published** 17 July 2014

Citation Bolotin A, de Wouters T, Schnupf P, Bouchier C, Loux V, Rhimi M, Jamet A, Dervyn R, Boudebbouze S, Blottière HM, Sorokin A, Snel J, Cerf-Bensussan N, Gaboriau-Routhiau V, van de Guchte M, Maguin E. 2014. Genome sequence of "*Candidatus* Arthromitus" sp. strain SFB-mouse-NL, a commensal bacterium with a key role in postnatal maturation of gut immune functions. Genome Announc. 2(4):e00705-14. doi:10.1128/genomeA.00705-14.

Address correspondence to Emmanuelle Maguin, emmanuelle.maguin@jouy.inra.fr.

"*Candidatus* Arthromitus" spp., also known as segmented filamentous bacteria (SFB), are noncultivable, spore-forming, *Clostridia*-related commensal bacteria that colonize the digestive tracts of many animal species (1). SFB typically form filaments that are solidly anchored in gut epithelial cells (2), notably in the ileum. In mice, SFB play a key role in the postnatal maturation of gut innate and adaptive immune functions, and notably, they can induce a strong IgA response (3, 4) and the recruitment and activation of intraepithelial $CD8^+$ T lymphocytes (5) and lamina propria $CD4^+$ T cells (6, 7; reviewed in reference 8).

Here, we report the complete genome sequence of the mouse-specific isolate "*Candidatus* Arthromitus" sp. SFB-mouse-NL. While an earlier sequenced mouse SFB isolate from Japan was shown to elicit a T helper (Th) 17 immune response in the intestinal lamina propria of C57BL/6 mice (7), the present strain, an independent isolate from The Netherlands (9), was reported to not only induce a strong Th17 response but also to foster Th1, Th2, and regulatory T-cell responses in C3H/HeN mice (6). To define whether the observed differences may be related to genetic variations between the two SFB isolates, we determined the genome sequence of strain SFB-mouse-NL.

DNA was isolated from fecal material from SFB-monocolonized mice using a modified version of the protocol described in Morita et al. (10). The DNA was sequenced using Illumina paired-end sequencing technology. The sequence reads were filtered using CLC and BOWTIE (11) to eliminate mouse genome sequences, and they were assembled using CLC, ABySS (12), and SOAP*denovo* (13), yielding 40 scaffolds >1,000 bp with high BLASTn scoring to existing SFB genome sequences. Finishing was performed using GAP4 of the Staden package (14) through iterative selection of read pair mapping to scaffold extremities and independent *de novo* assembly of these reads by SOAP. The scaffolds were also ordered using Mauve aligner (15), with earlier published SFB genome sequences as the reference, and some sequence gaps bridged by PCR performed on the basis of this alignment and sequencing of the PCR products. Genome annotation was performed using RAST (16).

The genome of "*Candidatus* Arthromitus" sp. SFB-mouse-NL consists of one circular chromosome (1,654,902 bp) with 1,598 predicted coding sequences (CDS). The genome contains 4 (partial) prophages. A comparative analysis of the "*Candidatus* Arthromitus" sp. SFB-mouse-NL genome and two complete and 7 incomplete other SFB genomes retrieved from NCBI (http://www.ncbi.nlm.nih.gov/genomes/) using MUMmer (17) and Mauve (18) revealed that the SFB-mouse-NL genome is distinct (0.4% of overall nucleotide divergence) from the other genomes (which show <0.2% of overall nucleotide divergence among them).

Nucleotide sequence accession number. The genome sequence of "*Candidatus* Arthromitus" sp. SFB-mouse-NL has been deposited in GenBank under the accession no. CP008713.

ACKNOWLEDGMENTS

We thank Laurence Ma for technical assistance in DNA sequencing, as well as Jean-Michel Batto and Pierre Leonard for help in computing.

This project was supported by the French National Research Agency (ANR) (contract ANR-10-BLAN-1317) and INRA AIP Bio-Resources.

REFERENCES

1. **Snel J, Heinen PP, Blok HJ, Carman RJ, Duncan AJ, Allen PC, Collins MD.** 1995. Comparison of 16S rRNA sequences of segmented filamentous bacteria isolated from mice, rats, and chickens and proposal of "Candida-

tus Arthromitus." Int. J. Syst. Bacteriol. **45:**780–782. http://dx.doi.org/10.1099/00207713-45-4-780.
2. **Klaasen HL, Koopman JP, Poelma FG, Beynen AC.** 1992. Intestinal, segmented, filamentous bacteria. FEMS Microbiol. Rev. **8:**165–180.
3. **Klaasen HL, Van der Heijden PJ, Stok W, Poelma FG, Koopman JP, Van den Brink ME, Bakker MH, Eling WM, Beynen AC.** 1993. Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. Infect. Immun. **61:**303–306.
4. **Lécuyer E, Rakotobe S, Lengliné-Garnier H, Lebreton C, Picard M, Juste C, Fritzen R, Eberl G, McCoy KD, Macpherson AJ, Reynaud CA, Cerf-Bensussan N, Gaboriau-Routhiau V.** 2014. Segmented filamentous bacterium uses secondary and tertiary lymphoid tissues to induce gut IgA and specific T helper 17-cell responses. Immunity **40:**608–620. http://dx.doi.org/10.1016/j.immuni.2014.03.009.
5. **Umesaki Y, Okada Y, Matsumoto S, Imaoka A, Setoyama H.** 1995. Segmented filamentous bacteria are indigenous intestinal bacteria that activate intraepithelial lymphocytes and induce MHC class II molecules and fucosyl asialo GM1 glycolipids on the small intestinal epithelial cells in the ex-germ-free mouse. Microbiol. Immunol. **39:**555–562. http://dx.doi.org/10.1111/j.1348-0421.1995.tb02242.x.
6. **Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, Eberl G, Snel J, Kelly D, Cerf-Bensussan N.** 2009. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity **31:**677–689. http://dx.doi.org/10.1016/j.immuni.2009.08.020.
7. **Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR.** 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell **139:**485–498. http://dx.doi.org/10.1016/j.cell.2009.09.033.
8. **Schnupf P, Gaboriau-Routhiau V, Cerf-Bensussan N.** 2013. Host interactions with segmented filamentous bacteria: an unusual trade-off that drives the post-natal maturation of the gut immune system. Semin. Immunol. **25:**342–351. http://dx.doi.org/10.1016/j.smim.2013.09.001.
9. **Klaasen HL, Koopman JP, Van den Brink ME, Van Wezel HP, Beynen AC.** 1991. Mono-association of mice with non-cultivable, intestinal, segmented, filamentous bacteria. Arch. Microbiol. **156:**148–151. http://dx.doi.org/10.1007/BF00290989.
10. **Morita H, Kuwahara T, Ohshima K, Sasamoto H, Itoh K, Hattori M, Hayashi T, Takami H.** 2007. An improved DNA isolation method for metagenomic analysis of the microbial flora of the human intestine. Microbes Environ. **22:**214–222. http://dx.doi.org/10.1264/jsme2.22.214.
11. **Langmead B, Trapnell C, Pop M, Salzberg SL.** 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. **10:**R25. http://dx.doi.org/10.1186/gb-2009-10-3-r25.
12. **Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I.** 2009. ABySS: a parallel assembler for short read sequence data. Genome Res. **19:**1117–1123. http://dx.doi.org/10.1101/gr.089532.108.
13. **Luo R, Liu B, Xie Y, Li Z, Huang W, Yuan J, He G, Chen Y, Pan Q, Liu Y, Tang J, Wu G, Zhang H, Shi Y, Liu Y, Yu C, Wang B, Lu Y, Han C, Cheung DW, Yiu SM, Peng S, Xiaoqian Z, Liu G, Liao X, Li Y, Yang H, Wang J, Lam TW, Wang J.** 2012. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. GigaScience. **1:**18. http://dx.doi.org/10.1186/2047-217X-1-18.
14. **Staden R, Judge DP, Bonfield JK.** 2003. Managing sequencing projects in the GAP4 Environment. *In* Krawetz SA, Womble DD (ed), Introduction to bioinformatics. a theoretical and practical approach. Humana Press, Inc., Totowa, NJ.
15. **Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT.** 2009. Reordering contigs of draft genomes using the Mauve aligner. Bioinformatics **25:**2071–2073. http://dx.doi.org/10.1093/bioinformatics/btp356.
16. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O.** 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics **9:**75. http://dx.doi.org/10.1186/1471-2164-9-75.
17. **Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL.** 2004. Versatile and open software for comparing large genomes. Genome Biol. **5:**R12. http://dx.doi.org/10.1186/gb-2004-5-6-p12.
18. **Darling AE, Mau B, Perna NT.** 2010. ProgressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One **5:**e11147. http://dx.doi.org/10.1371/journal.pone.0011147.