

Draft Genome Sequences of the Altered Schaedler Flora, a Defined Bacterial Community from Gnotobiotic Mice

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The altered Schaedler flora (ASF) is a bacterial community that supports normal growth and development of gnotobiotic mice. We report here the draft genome sequences of the 8 bacteria that comprise the ASF.

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The importance of the gastrointestinal (GI) microbiota in the health of the host has been known for decades. Roles of the GI microbiota include nutrient acquisition, protection against pathogens, and immune system development. Recent studies have added to our understanding by providing new mechanistic insights into host-microbiota interactions $(1, 2)$ $(1, 2)$ $(1, 2)$. A significant challenge to these studies moving forward is the high abundance and diversity of bacterial species that colonize the mammalian GI tract, which is dominated by members of the phyla *Bacteroidetes* and *Firmicutes* [\(3\)](#page-1-2). Recently, there has been renewed interest in the gnotobiotic mouse model, represented by the altered Schaedler flora (ASF), for the study of how gut bacteria impact the host $(4-10)$ $(4-10)$ $(4-10)$.

The ASF mouse model is derived from the work of Schaedler et al., who colonized germfree mice with a consortium of bacteria that originated from conventional mice [\(11\)](#page-1-6). Motivated by efforts by the National Cancer Institute (NCI) to generate mice colonized with a standardized microbiota, the ASF was subsequently derived from the original Schaedler flora, which comprised eight bacterial species. The ASF also consists of eight separate bacterial species, which were isolated from Swiss outbred mice, and includes four species not present in the original community (12) . The ASF has subsequently been characterized by 16S rRNA sequence analysis to better determine the phylogeny of the members [\(13\)](#page-1-8) and by quantitative PCR to assess the abundance, stability, and spatial

distribution throughout the GI tract [\(14,](#page-1-9) [15\)](#page-1-10). To further develop the ASF model as a resource for gut microbiota studies, we have determined the genome sequences of each of the bacterial species, which represent the first genome sequences of a complete mammalian GI bacterial community.

Whole-genome shotgun sequencing was done using Illumina sequencing technology to generate draft sequences for the 8 ASF strains, as summarized in [Table 1.](#page-0-0) Genomic sequence reads were generated on an Illumina HiSeq 2000 machine. Data consisted of two libraries: one 180-bp insert paired-end library [\(16\)](#page-1-11) and a large-insert, robotically size-selected, 3- to 5-kbp jumping library [\(17\)](#page-1-12).

Genome consensus was built *de novo* using ALLPATHS-LG [\(18\)](#page-1-13) with default parameters, except for *Lactobacillus* bacterium ASF360, for which Velvet was used due to lack of jumping libraries for ASF360. Original assembly consensus was improved and corrected for *Mucispirillum schaedleri* ASF457 and *Firmicutes* bacterium ASF500 using the Pilon assembly improvement tool (D. Ward, unpublished data). Assemblies were analyzed using the GAEMR [\(http://www.broadinstitute.org/software/gaemr/\)](http://www.broadinstitute.org/software/gaemr/) assembly evaluation package and manually reviewed for quality.

Protein-coding genes were predicted with Prodigal [\(19\)](#page-1-14) and filtered to remove genes with at least 70% overlap of tRNAs or rRNAs, which were identified using tRNAscan-SE [\(20\)](#page-1-15) and RNAmmer [\(21\)](#page-1-16), respectively. Gene product names were assigned

TABLE 1 Genome features and accession numbers of the ASF bacteria

		Genome size						
ASF no.	Taxonomv	(Mb)	GC (%)	Gene count	Contig count	N_{50} (kb)	Fold coverage	GenBank accession no.
ASF356	Clostridium sp.	2.91	30.91	2,799	31	209	143	AQFQ00000000.1
ASF360	Lactobacillus sp.	2.01	35.86	1,930	244	19	47	AQFR00000000.1
ASF361	Lactobacillus murinus	2.17	39.96	2,102	78	59.7	160	AQFS00000000.1
ASF457	Mucispirillum schaedleri	2.33	31.15	2,144	39	151	142	AYGZ00000000.1
ASF492	Eubacterium plexicaudatum	6.51	42.86	6,217	248	74.4	119	AQFT00000000.1
ASF500	<i>Firmicutes</i> bacterium	3.70	58.77	3,563	42	300	137	AYJP00000000.1
ASF502	Clostridium sp.	6.48	47.90	6,062	134	137	82	AQFU00000000.1
ASF519	Parabacteroides sp.	6.87	43.45	5,477	24	584	143	AQFV00000000.1

based on top blast hits against the Swiss-Prot protein database (at least 70% identity and at least 70% query coverage) and a protein family profile search against the TIGRfam HMMER equivalogs. More detailed characterizations of the ASF genomes are forthcoming.

Nucleotide sequence accession numbers.This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession numbers shown in [Table 1.](#page-0-0)

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REFERENCES

- 1. **Sommer F, Bäckhed F.** 2013. The gut microbiota—masters of host development and physiology. Nat. Rev. Microbiol. **11:**227–238. [http://dx](http://dx.doi.org/10.1038/nrmicro2974) [.doi.org/10.1038/nrmicro2974.](http://dx.doi.org/10.1038/nrmicro2974)
- 2. **Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S.** 2012. Host-gut microbiota metabolic interactions. Science **336:**1262–1267. [http://dx.doi.org/10.1126/science.1223813.](http://dx.doi.org/10.1126/science.1223813)
- 3. **Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA.** 2005. Diversity of the human intestinal microbial flora. Science **308:**1635–1638. [http://dx.doi.org/10.11](http://dx.doi.org/10.1126/science.1110591) [26/science.1110591.](http://dx.doi.org/10.1126/science.1110591)
- 4. **Collins J, Borojevic R, Verdu EF, Huizinga JD, Ratcliffe EM.** 2014. Intestinal microbiota influence the early postnatal development of the enteric nervous system. Neurogastroenterol. Motil. **26:**98 –107. [http://dx](http://dx.doi.org/10.1111/nmo.12236) [.doi.org/10.1111/nmo.12236.](http://dx.doi.org/10.1111/nmo.12236)
- 5. **Natividad JM, Hayes CL, Motta JP, Jury J, Galipeau HJ, Philip V, Garcia-Rodenas CL, Kiyama H, Bercik P, Verdu EF.** 2013. Differential induction of antimicrobial REGIII by the intestinal microbiota and *Bifidobacterium breve* NCC2950. Appl. Environ. Microbiol. **79:**7745–7754. [http://dx.doi.org/10.1128/AEM.02470-13.](http://dx.doi.org/10.1128/AEM.02470-13)
- 6. **Fremont-Rahl JJ, Ge Z, Umana C, Whary MT, Taylor NS, Muthupalani S, Carey MC, Fox JG, Maurer KJ.** 2013. An analysis of the role of the indigenous microbiota in cholesterol gallstone pathogenesis. PLoS One **8:**e70657. [http://dx.doi.org/10.1371/journal.pone.0070657.](http://dx.doi.org/10.1371/journal.pone.0070657)
- 7. **Mosconi I, Geuking MB, Zaiss MM, Massacand JC, Aschwanden C, Kwong Chung CK, McCoy KD, Harris NL.** 2013. Intestinal bacteria induce TSLP to promote mutualistic T-cell responses. Mucosal Immunol. **6:**1157–1167. [http://dx.doi.org/10.1038/mi.2013.12.](http://dx.doi.org/10.1038/mi.2013.12)
- 8. **Natividad JM, Petit V, Huang X, de Palma G, Jury J, Sanz Y, Philpott D, Garcia Rodenas CL, McCoy KD, Verdu EF.** 2012. Commensal and probiotic bacteria influence intestinal barrier function and susceptibility to colitis in Nod1-/-; Nod2-/- mice. Inflamm. Bowel Dis. **18:**1434 –1446. [http://dx.doi.org/10.1002/ibd.22848.](http://dx.doi.org/10.1002/ibd.22848)
- 9. **Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S,**

McCoy KD, Macpherson AJ. 2011. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity **34:**794 –806. [http://dx.doi.org/10.1016/j.immuni.2011.03.021.](http://dx.doi.org/10.1016/j.immuni.2011.03.021)

- 10. **Jergens AE, Wilson-Welder JH, Dorn A, Henderson A, Liu Z, Evans RB, Hostetter J, Wannemuehler MJ.** 2007. *Helicobacter bilis* triggers persistent immune reactivity to antigens derived from the commensal bacteria in gnotobiotic C3H/HeN mice. Gut **56:**934 –940. [http://dx.doi.org/10.11](http://dx.doi.org/10.1136/gut.2006.099242) [36/gut.2006.099242.](http://dx.doi.org/10.1136/gut.2006.099242)
- 11. **Schaedler RW, Dubos R, Costello R.** 1965. Association of germfree mice with bacteria isolated from normal mice. J. Exp. Med. **122:**77–82. [http:](http://dx.doi.org/10.1084/jem.122.1.77) [//dx.doi.org/10.1084/jem.122.1.77.](http://dx.doi.org/10.1084/jem.122.1.77)
- 12. **Orcutt RP, Gianni FJ, Judge RJ.** 1987. Development of an "altered Schaedler flora" for NCI gnotobiotic rodents. Microecol. Ther. **17:**59.
- 13. **Dewhirst FE, Chien CC, Paster BJ, Ericson RL, Orcutt RP, Schauer DB, Fox JG.** 1999. Phylogeny of the defined murine microbiota: altered Schaedler flora. Appl. Environ. Microbiol. **65:**3287–3292.
- 14. **Sarma-Rupavtarm RB, Ge Z, Schauer DB, Fox JG, Polz MF.** 2004. Spatial distribution and stability of the eight microbial species of the altered Schaedler flora in the mouse gastrointestinal tract. Appl. Environ. Microbiol. **70:**2791–2800. [http://dx.doi.org/10.1128/AEM.70.5.2791-280](http://dx.doi.org/10.1128/AEM.70.5.2791-2800.2004) [0.2004.](http://dx.doi.org/10.1128/AEM.70.5.2791-2800.2004)
- 15. **Ge Z, Feng Y, Taylor NS, Ohtani M, Polz MF, Schauer DB, Fox JG.** 2006. Colonization dynamics of altered Schaedler flora is influenced by gender, aging, and *Helicobacter hepaticus* infection in the intestines of Swiss Webster mice. Appl. Environ. Microbiol. **72:**5100 –5103. [http://dx](http://dx.doi.org/10.1128/AEM.01934-05) [.doi.org/10.1128/AEM.01934-05.](http://dx.doi.org/10.1128/AEM.01934-05)
- 16. **Ribeiro FJ, Przybylski D, Yin S, Sharpe T, Gnerre S, Abouelleil A, Berlin AM, Montmayeur A, Shea TP, Walker BJ, Young SK, Russ C, Nusbaum C, MacCallum I, Jaffe DB.** 2012. Finished bacterial genomes from shotgun sequence data. Genome Res. **22:**2270 –2277. [http://dx.doi.org/10.110](http://dx.doi.org/10.1101/gr.141515.112) [1/gr.141515.112.](http://dx.doi.org/10.1101/gr.141515.112)
- 17. **Fisher S, Barry A, Abreu J, Minie B, Nolan J, Delorey TM, Young G, Fennell TJ, Allen A, Ambrogio L, Berlin AM, Blumenstiel B, Cibulskis K, Friedrich D, Johnson R, Juhn F, Reilly B, Shammas R, Stalker J, Sykes SM, Thompson J, Walsh J, Zimmer A, Zwirko Z, Gabriel S, Nicol R, Nusbaum C.** 2011. A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. Genome Biol. **12:**R1. [http://dx.doi.org/10.1186/gb-2011-12-1-r1.](http://dx.doi.org/10.1186/gb-2011-12-1-r1)
- 18. **Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Gnirke A, Nusbaum C, Lander ES, Jaffe DB.** 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc. Natl. Acad. Sci. U. S. A. **108:** 1513–1518. [http://dx.doi.org/10.1073/pnas.1017351108.](http://dx.doi.org/10.1073/pnas.1017351108)
- 19. **Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ.** 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics **11:**119. [http://dx.doi.org/10.1186/14](http://dx.doi.org/10.1186/1471-2105-11-119) [71-2105-11-119.](http://dx.doi.org/10.1186/1471-2105-11-119)
- 20. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25:** 955–964. [http://dx.doi.org/10.1093/nar/25.5.0955.](http://dx.doi.org/10.1093/nar/25.5.0955)
- 21. **Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. **35:**3100 –3108. [http://dx.doi.org/10.1093](http://dx.doi.org/10.1093/nar/gkm160) [/nar/gkm160.](http://dx.doi.org/10.1093/nar/gkm160)