

## Complete Genome of the Cellulolytic Ruminant Bacterium *Ruminococcus albus* 7

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***Ruminococcus albus* 7 is a highly cellulolytic ruminant bacterium that is a member of the phylum Firmicutes. Here, we describe the complete genome of this microbe. This genome will be useful for rumen microbiology and cellulosome biology and in biofuel production, as one of its major fermentation products is ethanol.**

The strict anaerobe *Ruminococcus albus* 7 (ATCC 27210) is a highly cellulolytic bacterium first isolated in 1951 by R. E. Hungate from cow rumen (9). *R. albus* belongs to the phylum Firmicutes, and like other cellulolytic Firmicutes, employs cellosomes to adhere to and deconstruct cellulose. *R. albus* is thought to also employ other cellulose adherence mechanisms, including Pil family proteins (11) and an exopolysaccharide glycocalyx (15). *R. albus* produces ethanol and CO<sub>2</sub> as its major fermentation products, along with lesser amounts of acetate, formate, and H<sub>2</sub> (13).

The *R. albus* 7 genome was sequenced at the DOE Joint Genome Institute (JGI) using a combination of 454 Titanium (10) and Illumina (1) technologies. Details of library construction, sequencing, and assembly can be found on the JGI website (<http://www.jgi.doe.gov/>). An Illumina GAii shotgun and three 454 Titanium libraries (one standard and two paired-end) were sequenced, generating 592 megabase pairs (Mbp) and 143 Mbp of data, respectively. All data were assembled using Velvet version 0.7.63 (16) and Newbler version 2.3 for Illumina and 454 standard data, respectively. Consensus sequences were computationally shredded into overlapping fake reads and then integrated with the 454 paired-end data using parallel Phrap, version SPS-4.24 (High Performance Software, LLC). Illumina data were used to correct potential base errors and increase consensus quality using Polisher (Alla Lapidus, unpublished data). Misassemblies were corrected using GapResolution (Cliff Han, unpublished data) or DupFinisher

(8) or by sequencing cloned bridge PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed (4, 5, 7), by PCR, and by bubble PCR (J.-F. Cheng, unpublished data) primer walks. A total of 457 additional reactions were necessary to close gaps and raise the quality of the finished sequence. Automated genome annotation was performed at the Oak Ridge National Laboratory and is available at <http://genome.ornl.gov/microbial/ralb/>.

The genome of *R. albus* 7 consists of a circular chromosome of 3,685,404 bp and four plasmids of 420,706 bp, 352,645 bp, 15,907 bp, and 7,420 bp for a total genome sequence size of 4,482,082 bp. The genome has a G+C content of 43.6%, 74 tRNAs, four 23S rRNAs, four 5S rRNAs, and four 16S rRNAs; it is predicted to encode 3,872 protein sequences. The genome encodes a variety of cellulases and hemicellulases, including 3 glycosyl hydrolase (GH) family 2s (GH2s), 4 GH3s, 13 GH5s, 1 GH8, 8 GH9s, 5 GH10s, 5 GH11s, and 7 GH43s, as reported in the Carbohydrate-Active Enzyme (CAZy) database (3). A number of these cellulase and hemicellulase genes also encode carbohydrate-binding module 37 (CBM37), which is known to aid in substrate binding and is found exclusively in *R. albus* (6). Finally, genes encoding structural components of cellosomes, such as dockerins, were found throughout the genome. The genome of *R. albus* 7 will be useful for comparative analyses with other sequenced *Ruminococcus* (2) and *Clostridium* (12, 14) genomes, and, given that ethanol is its major fermentation product, will serve as a useful model for biofuel production.

**Nucleotide sequence accession number.** The sequence of *Ruminococcus albus* 7 (ATCC 27210) is available in GenBank under accession number CP002403.1.

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#### REFERENCES

1. **Bennett, S.** 2004. Solexa Ltd. *Pharmacogenomics* **5**:433–438.
2. **Berg Miller, M. E., et al.** 2009. Diversity and strain specificity of plant cell wall degrading enzymes revealed by the draft genome of *Ruminococcus flavefaciens* FD-1. *PLoS One* **4**:e6650.
3. **Cantarel, B. L., et al.** 2009. The Carbohydrate-Active Enzymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res.* **37**:D233–238.
4. **Ewing, B., and P. Green.** 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res.* **8**:186–194.
5. **Ewing, B., L. Hillier, M. C. Wendl, and P. Green.** 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res.* **8**:175–185.
6. **Ezer, A., et al.** 2008. Cell surface enzyme attachment is mediated by family 37 carbohydrate-binding modules, unique to *Ruminococcus albus*. *J. Bacteriol.* **190**:8220–8222.
7. **Gordon, D., C. Abajian, and P. Green.** 1998. Consed: a graphical tool for sequence finishing. *Genome Res.* **8**:195–202.
8. **Han, C. S., and P. Chain.** 2006. Finishing repeat regions automatically with Dupfinisher, p. 141–146. *In* H. R. Arabnia, and H. Valafar (ed.), Proceedings of the 2006 International Conference on Bioinformatics and Computational Biology. CSREA Press, Las Vegas, NV.
9. **Hungate, R. E.** 1957. Microorganisms in the rumen of cattle fed a constant ration. *Can. J. Microbiol.* **3**:289–311.
10. **Margulies, M., et al.** 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* **437**:376–380.
11. **Morrison, M., and J. Miron.** 2000. Adhesion to cellulose by *Ruminococcus albus*: a combination of cellulosomes and Pil-proteins? *FEMS Microbiol. Lett.* **185**:109–115.
12. **Nölling, J., et al.** 2001. Genome sequence and comparative analysis of the solvent-producing bacterium *Clostridium acetobutylicum*. *J. Bacteriol.* **183**:4823–4838.
13. **Pavlostathis, S. G., T. L. Miller, and M. J. Wolin.** 1988. Fermentation of insoluble cellulose by continuous cultures of *Ruminococcus albus*. *Appl. Environ. Microbiol.* **54**:2655–2659.
14. **Tamaru, Y., et al.** 2010. Genome sequence of the cellulosome-producing mesophilic organism *Clostridium cellulovorans* 743B. *J. Bacteriol.* **192**:901–902.
15. **Weimer, P. J., et al.** 2006. Studies of the extracellular glycocalyx of the anaerobic cellulolytic bacterium *Ruminococcus albus* 7. *Appl. Environ. Microbiol.* **72**:7559–7566.
16. **Zerbino, D. R., and E. Birney.** 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* **18**:821–829.