

Complete Genome Sequence of a *Gluconacetobacter hansenii* ATCC 23769 Isolate, AY201, Producer of Bacterial Cellulose and Important Model Organism for the Study of Cellulose Biosynthesis

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The cellulose producer and model organism used for the study of cellulose biosynthesis, *Gluconacetobacter hansenii* AY201, is a variant of *G. hansenii* ATCC 23769. We report here the complete nucleotide sequence of *G. hansenii* AY201, information which may be utilized to further the research into understanding the genes necessary for cellulose biosynthesis.

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The genus *Gluconacetobacter* contains several strains of Gram-negative bacteria that are particularly efficient producers of pure, highly crystalline cellulose, one of which is *Gluconacetobacter hansenii*, formerly known as *Acetobacter xylinum*. The export of this crystalline cellulose into the culture medium results in a membrane located at the air-liquid interface called bacterial cellulose (BC) (1–3). Because of its distinctive properties, BC is particularly well suited for medical, industrial, and commercial applications due to its ultrafine reticulated structure, high crystallinity, great mechanical strength, high water-holding capacity, moldability during formation, and biocompatibility (4–7). The results presented in this report will provide insight into the molecular mechanisms of bacterial cellulose biosynthesis and add to the study of the *Gluconacetobacter* genus.

G. hansenii AY201 (ATCC 23769) was developed from an isolate of *G. hansenii* ATCC 23796 that exhibited non-wild-type pellicle and colony morphology (unpublished data). It is an important model organism for the study of cellulose biosynthesis (8–10). However, until now, its genome had not been available for study. The DNA of *G. hansenii* AY201 was extracted and subjected to sequencing using an Illumina HiSeq 2000 PE100 system (University of Texas at Austin, ICMB Core Facility). The reads were assembled into contigs using Velvet version 1/2/02 (11) and downloaded into Geneious version 8.1.2, which revealed that it is approximately 3.35 Mbp in size with a GC content of 55.9% (12); a total of 6,443 open reading frames were predicted using Glimmer (13). Preliminary annotation data on contigs containing cellulose synthase genes were determined. The complete annotation of the full genome is in progress.

A homology comparison to *G. hansenii* ATCC 23769 (GenBank accession no. AB091060) was performed and resulted in a 95% identity to *G. hansenii* AY201. Previous studies have determined that *G. hansenii* AY201 contains at least two similar but nonidentical cellulose synthesizing regions, the *acsABCD* operon and the *acsAII* coding region (9, 10). Investigations into the genome of

G. hansenii ATCC 23769 indicated that the organism contains a total of three separate coding regions for cellulose biosynthesis: *acsABCD*, *acsAII*, and *acsABC* (9). A homology comparison of the shared cellulose-synthesizing regions revealed a sequence identity of 100%. The *acsABCD* operon is flanked by genes coding for proteins which have been determined to be essential for proper cellulose biosynthesis to occur: *cmcAx*, *ccpAx*, and *bglAx* (14–17). The genes flanking the *acsABCD* operon also shared 100% sequence identity to *G. hansenii* ATCC 23769.

Since *G. hansenii* AY201 is a model organism for genetic study, further investigations into both cellulose synthase-coding regions and why an isolate of *G. hansenii* ATCC 23769 lost a third may aid in providing a better understanding of the mechanisms necessary for cellulose biosynthesis to occur.

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number [LUCI00000000](https://www.ncbi.nlm.nih.gov/nuclink/LUCI00000000). The version described in this paper is the first version, [LUCI01000000](https://www.ncbi.nlm.nih.gov/nuclink/LUCI01000000).

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REFERENCES

- Nishi Y, Uryu M, Yamanaka S, Watanabe K, Kitamura N, Iguchi M, Mitsuhashi S. 1990. The structure and mechanical properties of sheets prepared from bacterial cellulose: part 2, improvement of the mechanical properties of sheets and their applicability to diaphragms of electroacoustic transducers. *J Mater Sci* 25:2997–3001. <http://dx.doi.org/10.1007/BF00584917>.
- Cousins SK, Brown RM, Jr. 1997. Photoisomerization of a dye-altered β -1,4 glucan sheet induces the crystallization of a cellulose-composite. *Polymer* 38:903–912. [http://dx.doi.org/10.1016/S0032-3861\(96\)00588-5](http://dx.doi.org/10.1016/S0032-3861(96)00588-5).
- Nobles DR, Brown RM, Jr. 2008. Transgenic expression of *Gluconacetobacter hansenii* strain ATCC 53582 cellulose synthase genes in the cyanobacterium *Synechococcus leopoliensis* strain UTCC 100. *Cellulose* 15: 691–701. <http://dx.doi.org/10.1007/s10570-008-9217-5>.

4. Yamanaka S, Watanabe K, Kitamura N, Iguchi M, Mitsuhashi S, Nishi Y, Uryu M. 1989. The structure and mechanical properties of sheets prepared from bacterial cellulose. *J Mater Sci* 24:3141–3145. <http://dx.doi.org/10.1007/BF01139032>.
5. Ross P, Mayer R, Benziman M. 1991. Cellulose biosynthesis and function in bacteria. *Microbiol Rev* 55:35–58.
6. Yoshinaga F, Tonouchi N, Watanabe K. 1997. Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material. *Biosci Biotechnol Biochem* 61: 219–224. <http://dx.doi.org/10.1271/bbb.61.219>.
7. Czaja W, Romanovicz D, Brown RM, Jr. 2004. Structural investigations of microbial cellulose produced in stationary and agitated culture. *Cellulose* 11:403–411. <http://dx.doi.org/10.1023/B:CELL.0000046412.11983.61>.
8. Brown RM, Jr, Willison JH, Richardson CL. 1976. Cellulose biosynthesis in *Acetobacter xylinum*: visualization of the site of synthesis and direct measurement of the *in vivo* process. *Proc Natl Acad Sci U S A* 73: 4565–4569. <http://dx.doi.org/10.1073/pnas.73.12.4565>.
9. Saxena IM, Kudlicka K, Okuda K, Brown RM, Jr. 1994. Characterization of genes in the cellulose-synthesizing operon (*acs* operon) of *Acetobacter xylinum*: implications for cellulose crystallization. *J Bacteriol* 176: 5735–5752.
10. Saxena IM, Brown RM, Jr. 1995. Identification of a second cellulose synthase gene (*acsAII*) in *Acetobacter xylinum*. *J Bacteriol* 177:5276–5283.
11. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
12. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <http://dx.doi.org/10.1093/bioinformatics/bts199>.
13. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679. <http://dx.doi.org/10.1093/bioinformatics/btm009>.
14. Standal R, Iversen TG, Coucheron DH, Fjaervik E, Blatny JM, Valla S. 1994. A new gene required for cellulose production and a gene encoding cellulolytic activity in *Acetobacter xylinum* are colocalized with the *bcs* operon. *J Bacteriol* 176:665–672.
15. Nakai T, Sugano Y, Shoda M, Sakakibara H, Oiwa K, Tuzi S, Imai T, Sugiyama J, Takeuchi M, Yamauchi D, Mineyuki Y. 2013. Formation of a highly twisted ribbons in a carboxymethylcellulase gene-disrupted strain of a cellulose-producing bacterium. *J Bacteriol* 195:958–964. <http://dx.doi.org/10.1128/JB.01473-12>.
16. Sunagawa N, Fujiwara T, Yoda T, Kawano S, Satoh Y, Yao M, Tajima K, Dairi T. 2013. Cellulose complementing factor (Ccp) is a new member of the cellulose synthase complex (terminal complex) in *Acetobacter xylinum*. *J Biosci Bioeng* 115:607–612. <http://dx.doi.org/10.1016/j.jbiosc.2012.12.021>.
17. Deng Y, Nagachar N, Xiao C, Tien M, Kao TH. 2013. Identification and characterization of non-cellulose-producing mutants of *Gluconacetobacter hansenii* generated by Tn5 transposon mutagenesis. *J Bacteriol* 195: 5072–5083. <http://dx.doi.org/10.1128/JB.00767-13>.