





## Complete Genome Sequence of Komagataeibacter hansenii Strain SC-3B

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**ABSTRACT** This study reports the release of the complete nucleotide sequence of *Komagataeibacter hansenii* SC-3B, a new efficient producer of cellulose. Elucidation of the genome may provide more information to aid in understanding the genes necessary for cellulose biosynthesis.

*comagataeibacter hansenii* strains (formerly *Gluconacetobacter xylinus* and *Acetobacter xylinum*) have been found to be efficient producers of a pure-form cellulose synthesized through a hierarchical cell-directed self-assembly process (1–4). As a result of this process, the subsequent membrane formed at the air–liquid interface possesses unique properties. Its ultrafine reticulated structure, high crystallinity, great mechanical strength, high water-holding capacity, moldability during formation, and biocompatibility make it well suited for medical, industrial, and commercial applications (5–8). To aid in the understanding of the mechanisms needed to guide this assembly process, this study reports the release of the complete nucleotide sequence of a novel strain, *K. hansenii* SC-3B.

K. hansenii SC-3B was isolated from Kombucha tea (Kombucha Kamp, Beverly Hills, CA, USA), and from initial observations we determined that it is an efficient producer of bacterial cellulose. DNA was extracted and subjected to sequencing using an Illumina HiSeq 2000 PE100 system (University of Texas at Austin, ICMB Core Facility). The reads were downloaded into Geneious version 8.1.2 and assembled into contigs using Velvet version 1/2/02 (9), where it was revealed that the genome is approximately 3.64 Mb in size with a G+C content of 59.6% (10). A total of 3,792 open reading frames were predicted using Glimmer (11). Preliminary annotation data on contigs containing cellulose synthase genes were determined.

Preliminary phylogenetic analysis using 16S rRNA genes determined that this new strain is closely related to *K. hansenii* ATCC 23769. A homology comparison to the *acsABCD* operon of *K. hansenii* ATCC 23769 (GenBank accession no. AB091060) was performed and resulted in a 99.7% identity to *acsAB*, 99.4% identity to *acsC*, and 100% identity to *acsD*. Further investigations into the genome indicated that *K. hansenii* SC-3B contains a total of three separate coding regions for cellulose biosynthesis: *acsABCD*, *acsAll*, and *acsABC*. These three operons are also found in *K. hansenii* ATCC 23769. A homology comparison of the shared cellulose-synthesizing regions revealed a sequence identity of 76.6% identity to *acsAll* and 99.1% identity to *acsABC*. 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* (12–15). These genes shared, respectively, 99.5%, 99.7%, and 98.9% sequence identities to *K. hansenii* ATCC 23769.

Further investigations into the genome of *K. hansenii* SC-3B may provide more insight into the mechanisms necessary for cellulose biosynthesis.

**Accession number(s).** This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession number MJMF00000000. The version described in this paper is the first version, MJMF01000000.

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## **REFERENCES**

- 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. https://doi.org/10.1016/S0032-3861(96)00588-5.
- 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. https://doi.org/10 .1007/BF00584917.
- Nobles D, 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. https://doi.org/10.1007/s10570-008-9217-5.
- Haigler CH, White AR, Brown RM, Jr, Cooper KM. 1982. Alteration of in vivo cellulose ribbon assembly by carboxymethylcellulose and other cellulose derivatives. J Cell Biol 94:64–69. https://doi.org/10.1083/jcb.94 .1.64.
- 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. https://doi.org/ 10.1007/BF01139032.
- 6. Ross P, Mayer R, Benziman M. 1991. Cellulose biosynthesis and function in bacteria. Microbiol Rev 55:35–58.
- 7. 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. https://doi.org/10.1271/bbb.61.219.
- Czaja W, Romanovicz D, Brown, RM, Jr. 2004. Structural investigations of microbial cellulose produced in stationary and agitated culture. Cellulose 11:403–411. https://doi.org/10.1023/B:CELL.0000046412.11983.61.
- 9. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read

- assembly using de Bruijn graphs. Genome Res 18:821–829. https://doi.org/10.1101/gr.074492.107.
- 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. https://doi.org/10.1093/ bioinformatics/bts199.
- Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with glimmer. Bioinformatics 23: 673–679. https://doi.org/10.1093/bioinformatics/btm009.
- 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. https://doi.org/10.1128/jb.176.3.665-672.1994.
- 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. https://doi.org/10.1128/JB.01473-12.
- 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 xyli-num*. J Biosci Bioeng 115:607–612. https://doi.org/10.1016/j.jbiosc.2012 12 021
- Deng Y, Nagachar N, Xiao C, Tien M, Kao TH. 2013. Identification and characterization of non-cellulose-producing mutants of *Gluconacetobac*ter hansenii generated by Tn 5 transposon mutagenesis. J Bacteriol 195:5072–5083. https://doi.org/10.1128/JB.00767-13.

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