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Complete Genome Sequence of NBRC 3288, a Unique Cellulose-Nonproducing Strain of *Gluconacetobacter xylinus* Isolated from Vinegar

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***Gluconacetobacter xylinus* is involved in the industrial production of cellulose. We have determined the genome sequence of *G. xylinus* NBRC 3288, a cellulose-nonproducing strain. Comparative analysis of genomes of *G. xylinus* NBRC 3288 with those of the cellulose-producing strains clarified the genes important for cellulose production in *Gluconacetobacter*.**

Several *Gluconacetobacter* species produce cellulose as a structural polysaccharide (3). Until recently, only partial sequences of the cellulose-synthesizing operon in the cellulose-producing strains had been reported (4, 6), and currently the whole genome sequence of *Gluconacetobacter hansenii* ATCC 23769, one of these strains, has been determined (2). Among *Gluconacetobacter* species, *G. xylinus* is involved in the industrial production of cellulose. We have found a strain of *G. xylinus* that cannot produce cellulose. In this study, we determined the whole genome sequence of *G. xylinus* NBRC 3288, a cellulose-nonproducing strain, isolated from vinegar in Japan and obtained from NITE Biological Resource Center of Japan (NBRC). The complete genome sequence of this strain was determined by the conventional whole-genome shotgun strategy using dye terminator chemistry on an ABI Prism 3730XL sequencer (ABI, Foster City, CA), with an average sequencing depth of 9.4, as described previously (1). The genome of *G. xylinus* NBRC 3288 consists of a single circular chromosome of 3,136,818 bp with 60.92% GC content and seven distinct plasmids (255,866 bp, 76,071 bp, 28,572 bp, 4,776 bp, 4,615 bp, 4,255 bp, and 2,218 bp). The chromosome is predicted to contain 3,195 protein-encoding open reading frames (ORFs), five copies of rRNA operons, and 60 genes encoding tRNAs. Of 3,195 predicted protein-coding genes, putative functions were assigned to 73.9% (2,358 genes), while the remaining 26.1% (837 genes) were annotated as hypothetical genes. The *in silico* analysis of the *G. xylinus* NBRC 3288 genome identified 11 genes related to cellulose synthesis within two operons. The cellulose-synthesizing operon contained the endoglucanase (GLX_25040 and GLX_25050) and the cellulose synthase catalytic subunit (GLX_25060, GLX_25070, GLX_25080, GLX_25090, and GLX_25100). GLX_25070 and GLX_25080 were annotated as fragmented genes. Detailed analysis of the GLX_25070 sequence revealed that a nonsense mutation (a TGA stop codon at position number 514) was identified in GLX_25070. A BLAST search indicated that the GLX_25070 gene product was likely to be a part of the catalytic subunit of cellulose synthase (BcsB1). Alignment of the full-length sequence of GLX_25070 to GLX_25080 showed high sequence identity to *G. xylinus* JCM 7664 BAA77586.1 (*bcsB1*) (5). A nonsense mutation caused splitting of *bcsB* into GLX_25070 and GLX_25080. The biological significance of *bcsB* in cellulose production has been reported previously (6), suggesting that this gene is indispensable for cellulose production. We concluded that this single mutation of GLX_25070 might affect the cellulose synthesis of this strain. GLX_25060, GLX_25070, and GLX_25080 of *G. xylinus* NBRC 3288 were fused together into one gene, GXY_04277 (*acsAB*) in *G. hansenii* ATCC 23769. The operon involved in synthesis of cyclic di-GMP, an allosteric activator of cellulase synthase, was identified as GLX_19900 to GLX_19850. GLX_19860 encoded a c-di-GMP phosphodiesterase, and GLX_19850 encoded a diguanylate cyclase. In contrast, a BLAST search against the *G. xylinus* NBRC 3288 genome and GenBank sequence database could not find any homologous gene of GXY_04272, a gene of unknown function in *G. hansenii* ATCC 23769. Any other gene required for cellulose production has not been assigned in this bacterium.

Nucleotide sequence accession numbers. Complete nucleotide sequences of the chromosome and 7 plasmids of the *G. xylinus* NBRC 3288 have been deposited in the DDBJ under accession no. AP012159 to -12166.

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