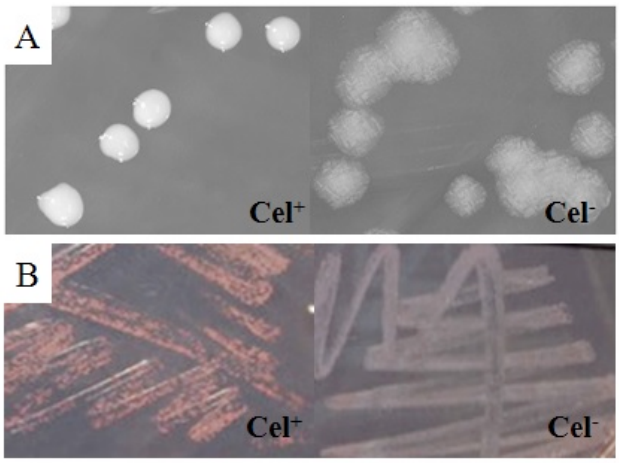

FIG S1 Colonies of stable cellulose-producing (Cel^+) and stable non-cellulose-producing (Cel^-) clones derived from a cellulose over-producing strain of *G. hansenii* ATCC23769. A: Colonies on SH agar plates. B: Colonies on SH agar plates containing 0.001% Pontamine Fast Scarlet 4B.

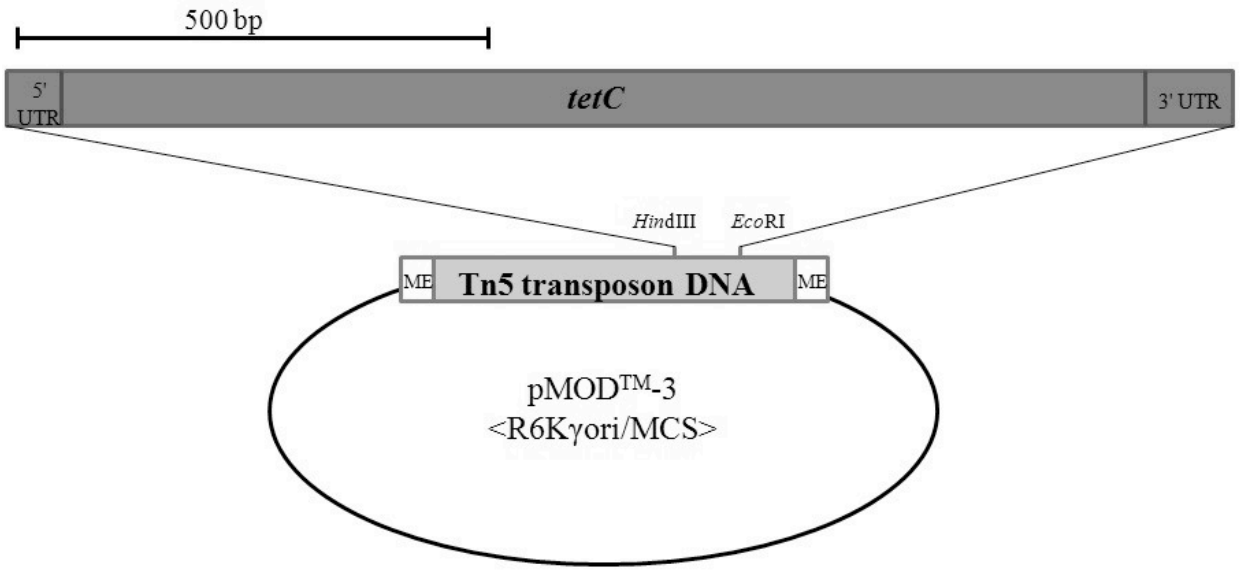

FIG S2 Schematic of the Tn5 transposon DNA used in insertion mutagenesis *of G. hansenii* ATCC23769. The tetracycline resistance gene (*tetC*) was amplified from pUCD2 by PCR and inserted at the *Hin*dIII and *Eco*RI sites of EZ-Tn5 pMOD™-3 <R6Kγori/MCS>. The resulting plasmid was amplified by PCR to generate Tn5 transposon DNA for mutagenesis. "ME" denotes the recognition sequence of EZ-Tn5 transposase.

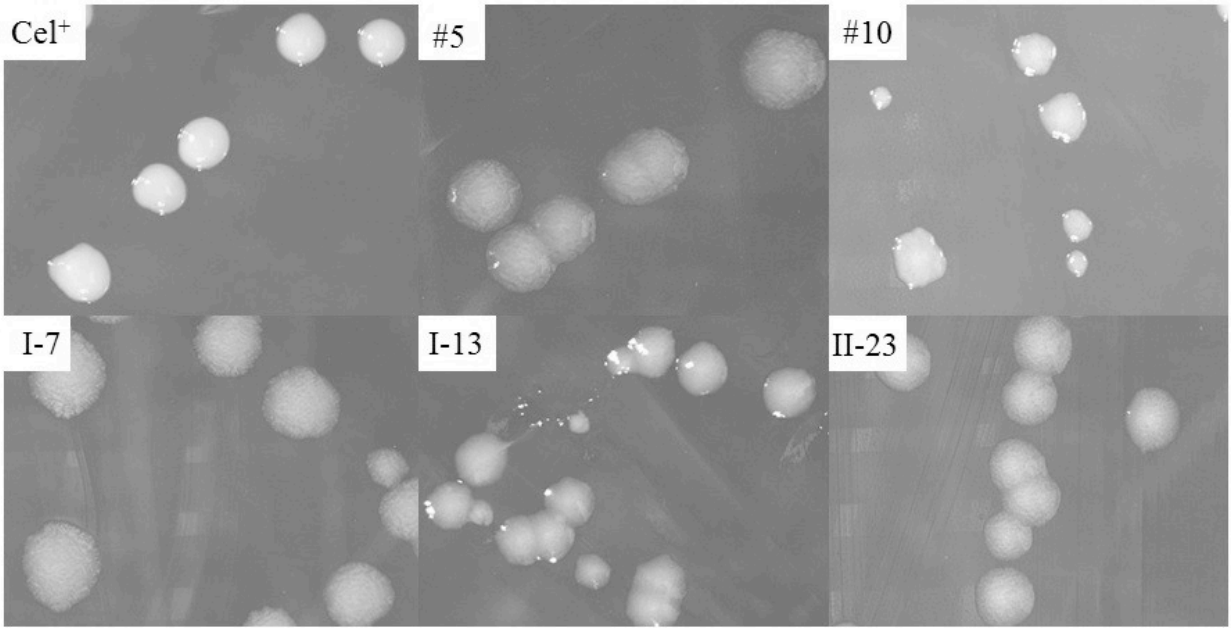

FIG S3 Morphologies of wild type cellulose-producing clone (Cel^+) and five non-cellulose-producing (Cel^-) mutants. Each mutant shown has Tn5 transposon DNA inserted in a different gene, *acsA* in mutant #5; *acsC* in mutant #10; *dgc1* in mutant I-7; ccp_{Ax} in mutant I-13; *crp-fnr* in mutant II-23 (see Table 2 for details). Cells were grown on SH agar plates containing tetracycline (20 μg/ml).

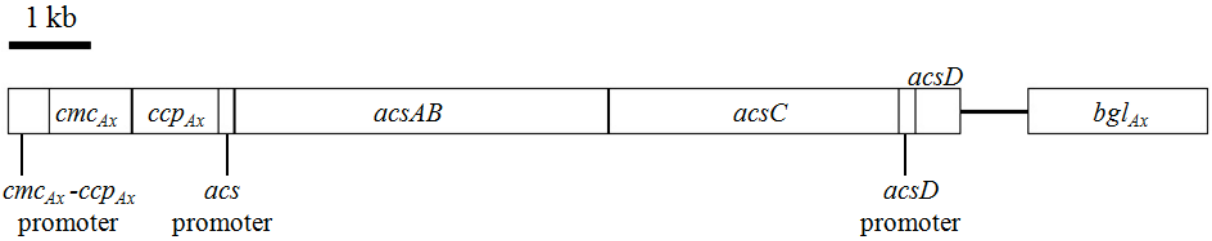

FIG S4 Schematic of the locations of the promoters identified in this study. The promoter of the operon consisting of *cmc*$_{Ax}$ and *ccp*$_{Ax}$ is located in the 504-kb region upstream from the translation initiation codon of *cmc*$_{Ax}$. The promoter of the *acs* operon, consisting of *acsAB* and *acsC*, is located in the 221-kb spacer between the translation termination codon of *ccp*$_{Ax}$ and translation initiation codon of *acsAB*. The promoter of *acsD* is located in the 321-kb spacer between the translation termination codon of *acsC* and translation initiation codon of *acsD*. *bgl*$_{Ax}$, encoding β-glucosidase, is included because its function in cellulose synthesis was examined in this study. The distance between the translation stop codon of *acsD* and the translation initiation codon of *bgl*$_{Ax}$ is 830 bp.

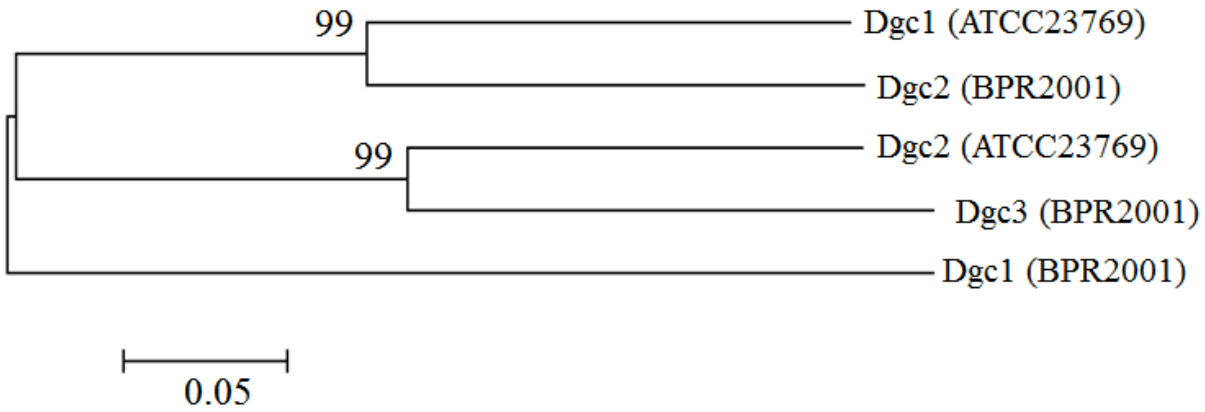

FIG S5 A phylogenetic tree of the deduced amino acid sequences of diguanylate cyclase genes in *G. hansenii* ATCC23769 and *G. xylinus* BPR2001. The tree was constructed using MEGA4 (http://www.megasoftware.net/index.html). Numbers indicate the bootstrap values derived from 1,000 replications. The GenBank accession numbers are: GXY_15447 and GXY_01661 for *dgc1* and *dgc2* of *G. hansenii* ATCC23769, respectively; AF052517, AF052518 and AF052519 for *dgc1*, *dgc2* and *dgc3* of *G. xylinus* BPR2001, respectively. The bar represents 0.05 substitutions per amino acid position.

TABLE S1 Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype or characteristics	Source/reference
Strain		
Gluconacetobacter hansenii ATCC23769	Wild type, cellulose-overproducing isolate	Candace Haigler (1982)
Escherichia coli DH5α	fhuA2Δ(argF-lacZ)U169, phoA, glnV44 Φ80Δ, (lacZ)M15, gyrA96 recA1, relA1, endA1, thi-1 hsdR17	New England BioLabs
Stellar™	Used for standard DNA manipulations; F-, endA1, supE44, thi-1,recA1, relA1, gyrA96, phoA, Φ80d lacZΔ M15, Δ(laZYA –arF) U169, Δ(mrr –hsdRMS –mcrBC), mcrA, λ-	Clontech
Plasmid		
pMOD™-3	EZ-Tn5™ transposon construction vector, ampR, R6Kγ ori/MCS	Epicentre Biotechnology
pUCD2	Shuttle vector, ampR, kanR, tetC, spcR,	ATCC 37342
pGEM®-T	TA cloning vector, ampR, lacZ/MCS	Promega
pUC18	Integrated vector, ampR	Clontech

TABLE S2 Primers used in this study

Primer	Sequence (5′-3′)[a]	Note
TetC F1	agtcaagcttAGCTTTAATGCGGTAGT (*Hind*III)	amplification of *tetC* for cloning into pMOD-3
TetC R1	agtcgaattcGGTTGGTTTGCGCATTCACA (*EcoR*I)	
ME plus 9-3' primer	CTGTCTCTTATACACATCTCAACCATCA	For amplification of Tn5 transposon DNA
ME plus 9-5' primer	CTGTCTCTTATACACATCTCAACCCTGA	
TSP1	GAACGGGTTGGCATGGATTGT	for genomic DNA walking PCR
TSP2	GGCCACCTCGACCTGAATGGAA	
AcsA-PF	aagggcatcggtcgacCCTATATTCAGGCGACCGCCTGT (*Sal*I)	for complementation of *acsAB* and *acsC* in mutant #5
AcsC-CER	ttcggtcctccgatcgATACACAGGATTGGTCGGGAAGA (*Pvu*I)	
Dgc1-CEF	aagggcatcggtcgacTGATGATACCGGTCGTCGTACTGT (*Sal*I)	for complementation of *dcg1* in mutant I-7
Dgc1-CER	ttcggtcctccgatcgGAACATGCATGATGCGTCGCGTA (*Pvu*I)	
Crp-CEF	aagggcatcggtcgacATATCAAGGGGTCAGACATAGTGT (*Sal*I)	for complementation *crp-fnr* in mutant II-23
Crp-CER	ttcggtcctccgatcgATCCTGGAATTTTCATGCAGGGAT (*Pvu*I)	
AcsC-CEF	ATGACCCATAAACGATATGCTTCGT	for complementation of *acsC* in mutant #10
AcsC-CER	ttcggtcctccgatcgATACACAGGATTGGTCGGGAAGA (*Pvu*I) (repeat)	

AcsD-PF	aagggcatcggtcgacTGGCCTGTTTGACTGGGATGTCA (*Sal*I)	for amplifying DNA fragment containing *acsD* promoter
AcsD-PR	attgttagatttcatTACTGGTCCATAATAAGATAGTGA	
AcsA-PF	aagggcatcggtcgacCCTATATTCAGGCGACCGCCTGT (*Sal*I)	for amplifying DNA fragment containing promoter of *acs* operon
AcsA-PR	attgttagatttcatAAACAACTCGTCCGAAATGCTGT	
Cmc_{Ax}-PF	aagggcatcggtcgacTTGCGGTTTGGGAAGTGTCATTGA	for amplifying DNA fragment containing promoter of cmc_{Ax}-ccp_{Ax} operon
Cmc_{Ax}-PR	attgttagatttcatAAAGGAACGTCGCCCGACC	
TetC pro-F	ATGAAATCTAACAATGCGCTCATCGT	for amplifying the coding sequence of *tetC*
TetC pro-R	ttcggtcctccgatcgGGTTGGTTTGCGCATTCACA (*Pvu*I)	
Ccp_{Ax}-CER	ttcggtcctccgatcgACCCAGGCCAAAGACGGAATAGA (*Pvu*I)	for amplifying DNA fragment containing promoter of cmc_{Ax}-ccp_{Ax} operon used in complementation of mutant I-13
AcsA-PR2	tcgtttatgggtcatAAACAACTCGTCCGAAATGCTGT	for amplifying DNA fragment containing promoter of *acs* operon used in complementation of mutant #10
Bgl_{Ax}-F	cgactctagaggatccCGGTATCCAGTCGCAACATGTGA *(Bam*HI)	amplification of flanking regions of bgl_{Ax} for making knockout construct
Bgl_{Ax}-R	tgtcaaacatgagaaGGTTTCCACTGTTCTTCAGCAGCA	

TetC-HF	TTCTCATGTTTGACAGCTTATCA	amplification of *tetC* for making bgl_{Ax} knockout construct
TetC-HR	ccatgattacgaattcGGTTGGTTTGCGCATTCACA (*Eco*RI)	
Bgl-CF	CATGACGCTTCAGGACAAACTGT	for PCR confirmation of *tetC* insertion in bgl_{Ax}
TSP4	GGCATAACCAAGCCTATGCCTACA	
Cmc_{Ax}-qF	CCCGACAAGAACAATGCCACAGA	for quantitative Real-Time PCR analysis of cmc_{Ax} transcript
Cmc_{Ax}-qR	CATCAGGTTCAGCACATCGCCAT	
Ccp_{Ax}-qF	AGGATGACCAGCATGACCAAGACA	for quantitative Real-Time PCR analysis of ccp_{Ax} transcript
Ccp_{Ax}-qR	GACCACCCAGCGTCATGAACA	
Bgl_{Ax}-qF	TATTGCAGGTGTCACGGCCAAC	for quantitative Real-Time PCR analysis of bgl_{Ax} transcript
Bgl_{Ax}-qR	ATGGTCGAACCAGCGATAGCCA	
Dgc1-qF	GAAAGCGTGGAGGACATGCAGA	for quantitative Real-Time PCR analysis of *dgc1* transcript
Dgc1-qR	CTGCCCGTTCTTCACACCACT	
Crp-fnr-TF qF	TCTCGAAGAGGCATCCAACGAACT	for quantitative Real-Time PCR analysis of *crp-fnr* transcript
Crp-fnr-TF qR	GCGCTGTTGAGTTCACCGTGA	
16S rRNA-qF	GGTCAAACCAACTCCCATGGTGT	for quantitative Real-Time PCR analysis of 16S rRNA
16S rRNA-qR	CCGTCTCAGTTCGGATTGCACTCT	

[a] Lower-case letters of a primer sequence indicate the sequence designed for overlap between the resulting PCR fragment and another PCR fragment, or a cloning vector, for in-fusion cloning; upper-case letters of a primer sequence indicate the sequence corresponding to the gene to be amplified; the underlined nucleotides in a primer sequence indicates the recognition sequence of the restriction enzyme shown in parentheses.

TABLE S3 Promoter activity assayed by tetracycline resistance of transformants

Plasmid construct	Promoter	Transformed into *E. coli*[1]	Transformed into *G. hasseni* ATCC23769[1]
pUCD2 (*tetCp*:*tetC*[2])	tetC	+	+
pUCD2(*tetC*)	none (*tetC* promoter deleted)	-	-
pUCD2:$cmc_{Ax}p$: *tetC*	cmc_{Ax}-ccp_{Ax} operon	+	+
pUCD2: *acsp*:*tetC*	*acs* operon	-	+
pUCD2:*acsDp*:*tetC*	*acsD*	-	+

[1]Transformed cells streaked out on LB (for *E. coli*) or SH (for *G. hansenii*) agar plates containing tetracycline (20 μg/ml); "+" indicating cell growth and "-" indicating no cell growth
[2]*tetC* denotes the coding region of the *tetC* gene from pUCD2.