Enzymatic Strategies for Solubility Control in Cellooligosaccharide Biosynthesis

Matteo Alvarez

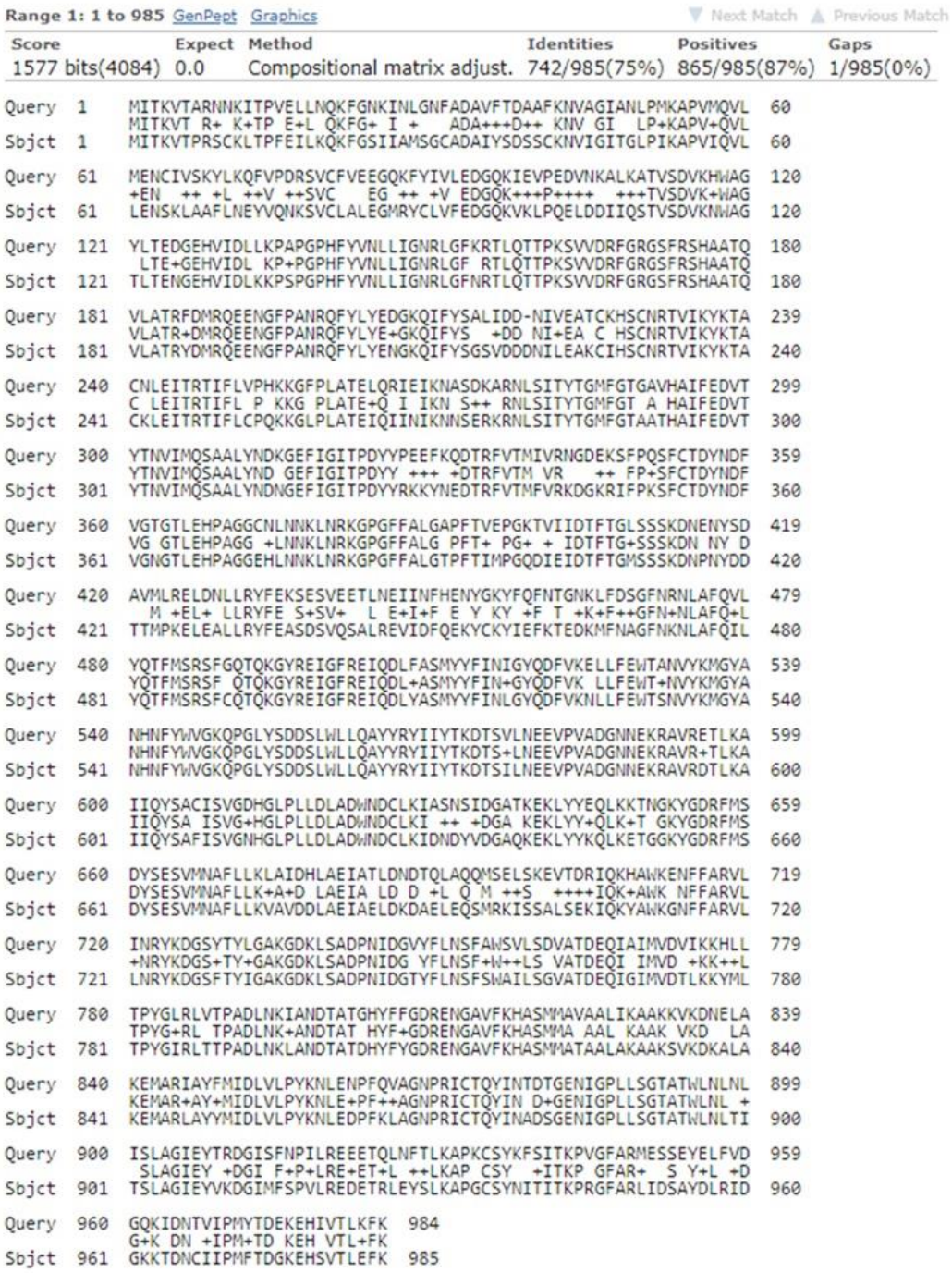
Department of Cardiovascular Research, University of Barcelona, Spain

S1 MATERIALS AND METHODS

Gene selection and cloning

Literature search revealed that cellodextrin phosphorylase (CdP) from *Clostridium stercorarium* (GenBank AAC45511) and CdP from *Ruminiclostridium thermocellum* (formerly known as *Clostridium thermocellum*, GenBank BAB71818) are patent protected. We were therefore looking for new CdP enzymes. And because CdP enzymes from *C. stercorarium* and *R. thermocellum* only share 20% amino acid sequence identity, we performed BlastP search with both CdPs. With CdP from *R. thermocellum* as query sequence, a putative glycosyltransferase from *Clostridium cellulosi* (Accession No. WP_052659865) turned out to be the most promising candidate (75% identity, subject sequence in Supporting Figure S1). The gene sequence was codon-optimized for expression in *E. coli* (Supporting Figure S3). The synthetic gene was ordered from GenScript (Piscataway, New Jersey, U.S.), sub-cloned into pQE30 from Qiagen (Hilden, Germany) and finally cloned into the self-assembled vector pC21e1 (IPTG-inducible P*tac*I promoter, ampicillin resistance) via *Eco*RI (N-terminal) and *Hind*III (C-terminal) restriction sites (see Supporting Figure S4). The recombinant enzyme, carrying an N-terminal His-tag (MRGSHHHHHHGS-), has a calculated molecular mass of 112.8 kDa and a calculated pI of 6.8. The enzyme was characterized for efficient recombinant protein production and glucosyl transfer activity from α-D-glucose 1-phosphate (αGlc1-*P*) to cellobiose at pH 7.0. The putative glycosyltransferase from *C. cellulosi* turned out to be a true cellodextrin phosphorylase. A specific activity of 3.9 µmol/min/mg in cellodextrin synthesis direction was determined from the cell-free lysate. Further biochemical and kinetic characterization studies of CdP from *C. cellulosi* (*Cc*CdP) are described in the manuscript.

S2 FIGURES AND SCHEMES



Range 1: 1 to 985 GenPept Graphics Next Match Previous Match

Score	Expect	Method	Identities	Positives	Gaps
1577 bits(4084)	0.0	Compositional matrix adjust.	742/985(75%)	865/985(87%)	1/985(0%)

```
Query  1    MITKVTARNNKITPVELLNQKFGNKINLGNFADAVFTDAAFKNVAGIANLPMKAPVMQVL  60
            MITKVT R+ K+TP E+L QKFG+ I +   ADA+++D++ KNV GI  LP+KAPV+QVL
Sbjct  1    MITKVTPRSCKLTPFEILKQKFGSIIAMSGCADAIYSDSSCKNVIGITGLPIKAPVIQVL  60

Query  61   MENCIVSKYLKQFVPDRSVCFVEEGQKFYIVLEDGQKIEVPEDVNKALKATVSDVKHWAG  120
            +EN  ++ +L ++V ++SVC   EG ++ +V EDGQK+++P++++  +++TVSDVK+WAG
Sbjct  61   LENSKLAAFLNEYVQNKSVCLALEGMRYCLVFEDGQKVKLPQELDDIIQSTVSDVKNWAG  120

Query  121  YLTEDGEHVIDLLKPAPGPHFYVNLLIGNRLGFKRTLQTTPKSVVDRFGRGSFRSHAATQ  180
             LTE+GEHVIDL KP+PGPHFYVNLLIGNRLGF RTLQTTPKSVVDRFGRGSFRSHAATQ
Sbjct  121  TLTENGEHVIDLKKPSPGPHFYVNLLIGNRLGFNRTLQTTPKSVVDRFGRGSFRSHAATQ  180

Query  181  VLATRFDMRQEENGFPANRQFYLYEDGKQIFYSALIDD-NIVEATCKHSCNRTVIKYKTA  239
            VLATR+DMRQEENGFPANRQFYLYE+GKQIFYS  +DD NI+EA C HSCNRTVIKYKTA
Sbjct  181  VLATRYDMRQEENGFPANRQFYLYENGKQIFYSGSVDDDNILEAKCIHSCNRTVIKYKTA  240

Query  240  CNLEITRTIFLVPHKKGFPLATELQRIEIKNASDKARNLSITYTGMFGTGAVHAIFEDVT  299
            C LEITRTIFL P KKG PLATE+Q I IKN S++ RNLSITYTGMFGT A HAIFEDVT
Sbjct  241  CKLEITRTIFLCPQKKGLPLATEIQIINIKNNSERKRNLSITYTGMFGTAATHAIFEDVT  300

Query  300  YTNVIMQSAALYNDKGEFIGITPDYYPEEFKQDTRFVTMIVRNGDEKSFPQSFCTDYNDF  359
            YTNVIMQSAALYND GEFIGITPDYY +++ +DTRFVTM VR    ++ FP+SFCTDYNDF
Sbjct  301  YTNVIMQSAALYNDNGEFIGITPDYYRKKYNEDTRFVTMFVRKDGKRIFPKSFCTDYNDF  360

Query  360  VGTGTLEHPAGGCNLNNKLNRKGPGFFALGAPFTVEPGKTVIIDTFTGLSSSKDNENYSD  419
            VG GTLEHPAGG +LNNKLNRKGPGFFALG PFT+ PG+ + IDTFTG+SSSKDN NY D
Sbjct  361  VGNGTLEHPAGGEHLNNKLNRKGPGFFALGTPFTIMPGQDIEIDTFTGMSSSKDNPNYDD  420

Query  420  AVMLRELDNLLRYFEKSESVEETLNEIINFHENYGKYFQFNTGNKLFDSGFNRNLAFQVL  479
              M +EL+ LLRYFE S+SV+  L E+I+F E Y KY +F T +K+F++GFN+NLAFQ+L
Sbjct  421  TTMPKELEALLRYFEASDSVQSALREVIDFQEKYCKYIEFKTEDKMFNAGFNKNLAFQIL  480

Query  480  YQTFMSRSFGQTQKGYREIGFREIQDLFASMYYFINIGYQDFVKELLFEWTANVYKMGYA  539
            YQTFMSRSF QTQKGYREIGFREIQDL+ASMYYFIN+GYQDFVK LLFEWT+NVYKMGYA
Sbjct  481  YQTFMSRSFCQTQKGYREIGFREIQDLYASMYYFINLGYQDFVKNLLFEWTSNVYKMGYA  540

Query  540  NHNFYWVGKQPGLYSDDSLWLLQAYYRYIIYTKDTSVLNEEVPVADGNNEKRAVRETLKA  599
            NHNFYWVGKQPGLYSDDSLWLLQAYYRYIIYTKDTS+LNEEVPVADGNNEKRAVR+TLKA
Sbjct  541  NHNFYWVGKQPGLYSDDSLWLLQAYYRYIIYTKDTSILNEEVPVADGNNEKRAVRDTLKA  600

Query  600  IIQYSACISVGDHGLPLLDLADWNDCLKIASNSIDGATKEKLYYEQLKKTNGKYGDRFMS  659
            IIQYSA ISVG+HGLPLLDLADWNDCLKI ++ +DGA KEKLYY+QLK+T GKYGDRFMS
Sbjct  601  IIQYSAFISVGNHGLPLLDLADWNDCLKIDNDYVDGAQKEKLYYKQLKETGGKYGDRFMS  660

Query  660  DYSESVMNAFLLKLAIDHLAEIATLDNDTQLAQQMSELSKEVTDRIQKHAWKENFFARVL  719
            DYSESVMNAFLLK+A+D LAEIA LD D +L Q M ++S  ++++IQK+AWK NFFARVL
Sbjct  661  DYSESVMNAFLLKVAVDDLAEIAELDKDAELEQSMRKISSALSEKIQKYAWKGNFFARVL  720

Query  720  INRYKDGSYTYLGAKGDKLSADPNIDGVYFLNSFAWSVLSDVATDEQIAIMVDVIKKHLL  779
            +NRYKDGS+TY+GAKGDKLSADPNIDG YFLNSF+W++LS VATDEQI IMVD +KK++L
Sbjct  721  LNRYKDGSFTYIGAKGDKLSADPNIDGTYFLNSFSWAILSGVATDEQIGIMVDTLKKYML  780

Query  780  TPYGLRLVTPADLNKIANDTATGHYFFGDRENGAVFKHASMMAVAALIKAAKKVKDNELA  839
            TPYG+RL TPADLNK+ANDTAT HYF+GDRENGAVFKHASMMA AAL KAAK VKD  LA
Sbjct  781  TPYGIRLTTPADLNKLANDTATDHYFYGDRENGAVFKHASMMATAALAKAAKSVKDKALA  840

Query  840  KEMARIAYFMIDLVLPYKNLENPFQVAGNPRICTQYINTDTGENIGPLLSGTATWLNLNL  899
            KEMAR+AY+MIDLVLPYKNLE+PF++AGNPRICTQYIN D+GENIGPLLSGTATWLNL +
Sbjct  841  KEMARLAYYMIDLVLPYKNLEDPFKLAGNPRICTQYINADSGENIGPLLSGTATWLNLTI  900

Query  900  ISLAGIEYTRDGISFNPILREEETQLNFTLKAPKCSYKFSITKPVGFARMESSEYELFVD  959
             SLAGIEY +DGI F+P+LRE+ET+L ++LKAP CSY  +ITKP GFAR+  S Y+L +D
Sbjct  901  TSLAGIEYVKDGIMFSPVLREDETRLEYSLKAPGCSYNITITKPRGFARLIDSAYDLRID  960

Query  960  GQKIDNTVIPMYTDEKEHIVTLKFK  984
            G+K DN +IPM+TD KEH VTL+FK
Sbjct  961  GKKTDNCIIPMFTDGKEHSVTLEFK  985
```

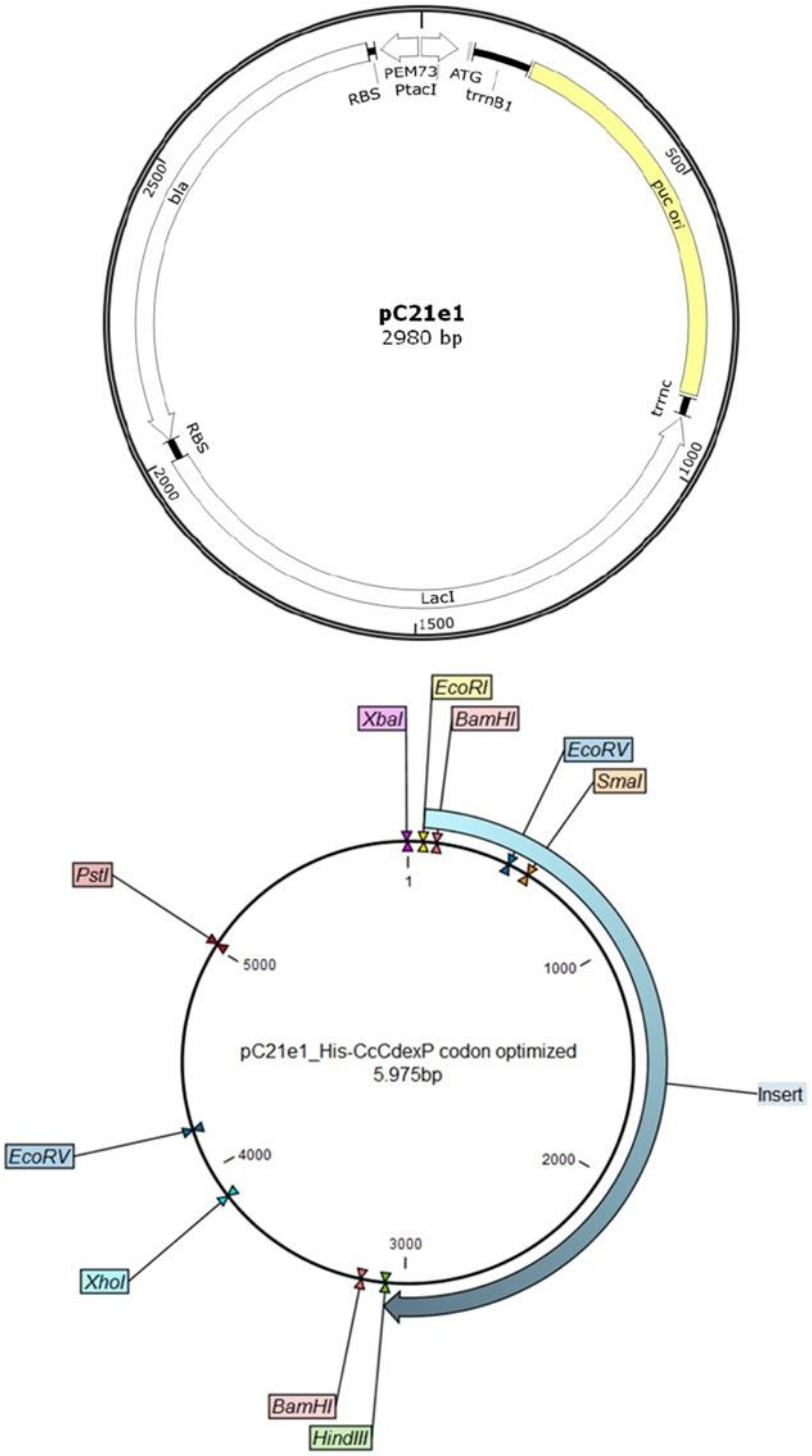
Supporting Figure S1. Result from BlastP search using CdP from *R. thermocellum* (GenBank BAB71818) as query sequence. A putative glycosyltransferase from *Clostridium cellulosi* (Accession No. WP_052659865, *Cc*CdP) turned out to be the most promising candidate (subject sequence with 75% identity).

TTGATAACTAAAGTTACACCCAGAAGCTGCAAATTGACACCTTTTGAAATTTTAAAACAAAAATTCGGCAGCATTATTGCGA
TGTCAGGCTGCGCCGACGCAATTTACAGTGATTCATCATGCAAGAATGTGATTGGCATAACGGGACTGCCGATTAAGGCT
CCGGTTATCCAGGTGCTGCTTGAAAACAGTAAGTTGGCCGCGTTTTTAAATGAATATGTCCAAAATAAATCGGTTTGCCTG
GCATTAGAGGGAATGCGTTATTGCCTTGTCTTTGAGGACGGGCAAAAAGTGAAGCTGCCCCAAGAATTAGACGACATTAT
CCAGTCAACTGTCAGCGACGTCAAAAATTGGGCCGGTACATTGACTGAAAACGGAGAGCATGTCATCGACCTTAAAAAGC
CCTCTCCCGGGCCGCATTTTTATGTCAATCTCTTGATTGGAAACAGGCTCGGTTTCAACAGAACTTTGCAGACAACGCCAAA
GAGCGTAGTCGACCGGTTCGGCAGAGGTTCATTCAGGTCGCACGCTGCAACACAGGTGCTTGCAACAAGATATGACATGC
GCCAAGAGGAAAACGGGTTCCCGGCAAACAGGCAATTTTATTTATATGAAAACGGCAAGCAGATTTTCTATTCCGGGTCC
GTTGACGACGACAATATCCTCGAAGCAAAGTGCATCCATTCCTGCAACAGAACGGTTATAAAATATAAAACGGCATGCAA
ACTCGAAATCACCAGAACAATTTTCCTCTGCCCTCAAAAGAAAGGGCTGCCCCTTGCAACTGAAATACAGATAATCAACAT
AAAGAATAATTCCGAACGGAAAAGGAACTTGAGCATTACATATACCGGCATGTTCGGAACAGCCGCGACACACGCGATTT
TTGAAGACGTCACCTATACCAATGTTATTATGCAAAGCGCCGCCCTTTATAATGACAACGGGGAATTTATTGGCATTACGCC
CGACTATTACCGTAAAAAGTATAATGAAGATACGCGCTTTGTGACAATGTTTGTCCGTAAGGATGGTAAGCGTATATTCCC
GAAGAGCTTCTGCACAGATTACAATGATTTTGTCGGCAACGGGACATTGGAACATCCGGCGGGCGGCGAGCATTTAAACA
ATAAGCTGAACCGCAAAGGCCCGGGTTTCTTCGCTTTGGGAACGCCTTTCACCATTATGCCCGGACAAGACATCGAGATAG
ACACGTTTACTGGAATGTCTTCAAGCAAAGATAACCCGAACTACGACGACACTACCATGCCGAAGGAGCTTGAAGCCCTGC
TCCGCTATTTTGAAGCCAGTGACTCCGTGCAGTCGGCACTGCGAGAAGTTATTGATTTTCAGGAAAAATACTGCAAATACA
TCGAATTCAAAACTGAAGACAAGATGTTTAATGCCGGATTTAATAAAAATCTGGCGTTCCAGATACTTTACCAGACCTTTAT
GTCGCGCTCATTCTGCCAGACCCAGAAAGGCTACCGCGAGATTGGTTTCAGGGAGATACAGGACCTTTACGCGTCCATGT
ACTACTTTATCAATCTCGGCTATCAGGATTTTGTGAAGAACCTGCTGTTCGAGTGGACATCAAACGTCTACAAGATGGGAT
ACGCGAACCATAATTTCTACTGGGTCGGCAAACAGCCGGGACTCTATTCTGACGACAGCCTGTGGTTATTGCAGGCTTATT
ACCGCTATATTATCTATACTAAGGACACATCAATCTTGAACGAAGAAGTGCCGGTCGCTGACGGCAACAACGAAAAGAGG
GCTGTCCGCGATACTCTTAAGGCGATAATCCAGTATTCCGCCTTTATATCTGTCGGAAACCATGGTCTCCCGCTCCTTGACTT
AGCGGACTGGAACGATTGCCTGAAGATAGACAATGACTATGTCGACGGCGCACAAAAGGAAAAGCTTTATTACAAGCAAC
TAAAAGAAACCGGCGGCAAATACGGTGACCGCTTTATGAGTGATTACTCCGAGAGCGTTATGAATGCGTTTCTTCTGAAA
GTCGCCGTAGACGATCTTGCCGAAATAGCGGAACTTGACAAAGACGCCGAACTTGAGCAGAGCATGAGGAAAATCTCCA
GCGCCCTTTCAGAAAAAATCCAAAAATATGCTTGGAAAGGCAATTTCTTCGCGCGTGTGCTGCTTAACCGCTATAAAGACG
GCTCTTTCACTTACATCGGCGCAAAAGGCGATAAACTTTCGGCCGACCCGAATATCGACGGCACATACTTCCTCAACAGTTT
TTCCTGGGCAATACTGTCCGGAGTCGCAACCGACGAGCAGATAGGCATTATGGTCGATACGCTTAAAAAGTATATGCTGA
CCCCCTACGGAATCCGCCTTACAACCCCTGCTGACTTAAACAAGCTTGCAAATGATACGGCAACTGACCACTATTTCTATGG
CGACAGAGAAAACGGAGCCGTTTTCAAACACGCCTCGATGATGGCGACTGCTGCGCTTGCAAAAGCGGCTAAAAGCGTTA
AGGACAAGGCGCTTGCCAAGGAAATGGCAAGGTTAGCCTACTATATGATTGACCTCGTCTTGCCTTACAAAAACCTTGAAG
ACCCGTTTAAACTCGCTGGAAACCCCAGAATATGCACTCAGTATATCAACGCTGATTCCGGTGAGAATATCGGGCCTCTGC
TCAGCGGAACCGCAACGTGGCTCAACCTTACAATCACTTCGCTGGCAGGCATTGAATATGTAAAAGACGGCATTATGTTCA
GCCCCGTTTTGCGCGAGGATGAAACACGCCTTGAGTATTCTCTGAAAGCGCCGGGCTGCTCTTACAACATAACTATCACTA
AGCCCCGCGGTTTTGCAAGGCTCATTGATTCTGCCTACGACTTACGTATTGACGGCAAGAAAACAGACAATTGCATAATTC
CTATGTTCACTGACGGCAAAGAGCACTCTGTAACCCTTGAATTCAAA**TAA**

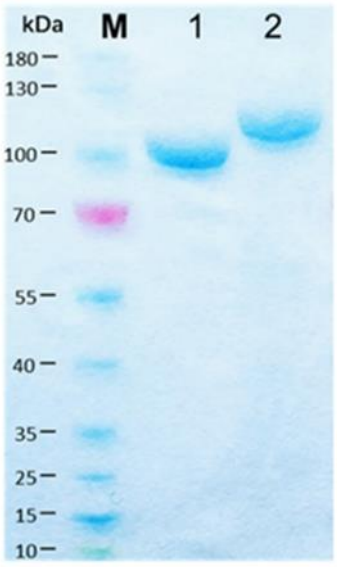
Supporting Figure S2. Wildtype gene sequences of *Cc*CdP (GenBank CDZ24361.1). Start and stop codon are marked in bold.

ATGATTACCAAAGTGACCCCGCGTAGCTGCAAACTGACCCCGTTCGAGATTCTGAAACAAAAATTCGGTAGCATTATTGCG
ATGAGCGGCTGCGCGGATGCGATCTACAGCGACAGCAGCTGCAAGAACGTGATCGGTATTACCGGCCTGCCGATCAAAG
CGCCGGTGATTCAGGTTCTGCTGGAGAACAGCAAGCTGGCGGCGTTCCTGAACGAATACGTGCAAAACAAAAGCGTTTGC
CTGGCGCTGGAGGGTATGCGTTATTGCCTGGTGTTTGAAGACGGCCAGAAGGTTAAACTGCCGCAAGAGCTGGACGATAT
CATTCAGAGCACCGTGAGCGATGTTAAAAACTGGGCGGGTACCCTGACCGAGAACGGCGAACACGTGATCGACCTGAAG
AAACCGAGCCCGGGTCCGCACTTCTACGTTAACCTGCTGATTGGTAACCGTCTGGGCTTTAACCGTACCCTGCAAACCACC
CCGAAGAGCGTGGTTGATCGTTTCGGTCGTGGCAGCTTTCGTAGCCATGCGGCGACCCAGGTTCTGGCGACCCGTTACGA
CATGCGTCAAGAGGAAAACGGCTTCCCGGCGAACCGTCAGTTTTACCTGTATGAGAACGGCAAGCAAATCTTCTACAGCG
GCAGCGTGGACGATGACAACATCCTGGAAGCGAAATGCATTCACAGCTGCAACCGTACCGTTATTAAGTATAAAACCGCG
TGCAAGCTGGAGATCACCCGTACCATTTTTCTGTGCCCGCAGAAGAAAGGTCTGCCGCTGGCGACCGAGATCCAAATCATC
AACATCAAGAACAACAGCGAACGTAAACGTAACCTGAGCATCACCTACACCGGCATGTTTGGTACCGCGGCGACCCACGC
GATCTTTGAAGATGTGACCTACACCAACGTTATTATGCAGAGCGCGGCGCTGTATAACGACAACGGCGAGTTCATCGGCA
TTACCCCGGATTACTATCGTAAGAAATACAACGAAGACACCCGTTTCGTGACCATGTTTGTTCGTAAGGACGGTAAACGTA
TCTTCCCGAAGAGCTTCTGCACCGATTATAACGACTTTGTGGGTAACGGTACCCTGGAGCACCCGGCGGGTGGCGAACAC
CTGAACAACAAGCTGAACCGTAAAGGTCCGGGCTTCTTTGCGCTGGGTACCCCGTTCACCATTATGCCGGGCCAAGACATC
GAGATTGATACCTTTACCGGTATGAGCAGCAGCAAGGATAACCCGAACTACGATGACACCACCATGCCGAAAGAGCTGGA
AGCGCTGCTGCGTTATTTCGAAGCGAGCGATAGCGTGCAGAGCGCGCTGCGTGAGGTTATCGACTTCCAAGAAAAGTACT
GCAAGTACATCGAGTTCAAGACCGAAGACAAGATGTTCAACGCGGGTTTTAACAAAAACCTGGCGTTTCAGATCCTGTACC
AAACCTTCATGAGCCGTAGCTTTTGCCAGACCCAAAAGGGTTATCGTGAGATCGGCTTCCGTGAAATTCAGGATCTGTACG
CGAGCATGTACTACTTCATCAACCTGGGTTACCAAGACTTCGTGAAGAACCTGCTGTTTGAGTGGACCAGCAACGTTTACA
AAATGGGCTATGCGAACCACAACTTCTACTGGGTTGGTAAACAGCCGGGCCTGTATAGCGATGACAGCCTGTGGCTGCTG
CAAGCGTACTATCGTTACATCATTTATACCAAGGATACCAGCATCCTGAACGAGGAAGTGCCGGTTGCGGATGGTAACAAC
GAAAAGCGTGCGGTGCGTGACACCCTGAAAGCGATCATTCAGTACAGCGCGTTCATCAGCGTTGGTAACCATGGTCTGCC
GCTGCTGGATCTGGCGGACTGGAACGATTGCCTGAAGATTGATAACGACTATGTGGACGGTGCGCAGAAGGAGAAACTG
TACTATAAGCAACTGAAAGAAACCGGTGGCAAATACGGCGATCGTTTCATGAGCGACTATAGCGAGAGCGTTATGAACGC
GTTTCTGCTGAAGGTGGCGGTTGATGACCTGGCGGAGATCGCGGAACTGGATAAGGACGCGGAGCTGGAACAGAGCAT
GCGTAAAATCAGCAGCGCGCTGAGCGAAAAGATTCAAAAATACGCGTGGAAGGGTAACTTCTTTGCGCGTGTGCTGCTGA
ACCGTTACAAAGATGGCAGCTTCACCTATGTTGGTGCGAAGGGCGACAAACTGAGCGCGGACCCGAACATCGATGGTACC
TATTTCCTGAACAGCTTTAGCTGGGCGATTCTGAGCGGTGTGGCGACCGATGAGCAGATCGGCATTATGGTTGACACCCTG
AAGAAATACATGCTGACCCCGTATGGCATCCGTCTGACCACCCCGGCGGATCTGAACAAACTGGCGAACGACACCGCGAC
CGATCACTACTTCTATGGTGACCGTGAAAACGGCGCGGTGTTTAAGCATGCGAGCATGATGGCGACCGCGGCGCTGGCGA
AGGCGGCGAAAAGCGTTAAGGATAAAGCGCTGGCGAAAGAGATGGCGCGTCTGGCGTACTATATGATCGATCTGGTGCT
GCCGTACAAGAACCTGGAAGACCCGTTCAAACTGGCGGGTAACCCGCGTATCTGCACCCAGTATATTAACGCGGACAGCG
GCGAGAACATTGGTCCGCTGCTGAGCGGTACCGCGACCTGGCTGAACCTGACCATCACCAGCCTGGCGGGTATTGAGTAC
GTGAAGGATGGCATTATGTTCAGCCCGGTTCTGCGTGAGGACGAAACCCGTCTGGAATACAGCCTGAAGGCGCCGGGTT
GCAGCTATAACATCACCATTACCAAACCGCGTGGCTTTGCGCGTCTGATCGACAGCGCGTACGATCTGCGTATTGACGGTA
AGAAAACCGACAACTGCATTATCCCGATGTTCACCGATGGTAAAGAGCATAGCGTGACCCTGGAGTTCAAG**TAA**

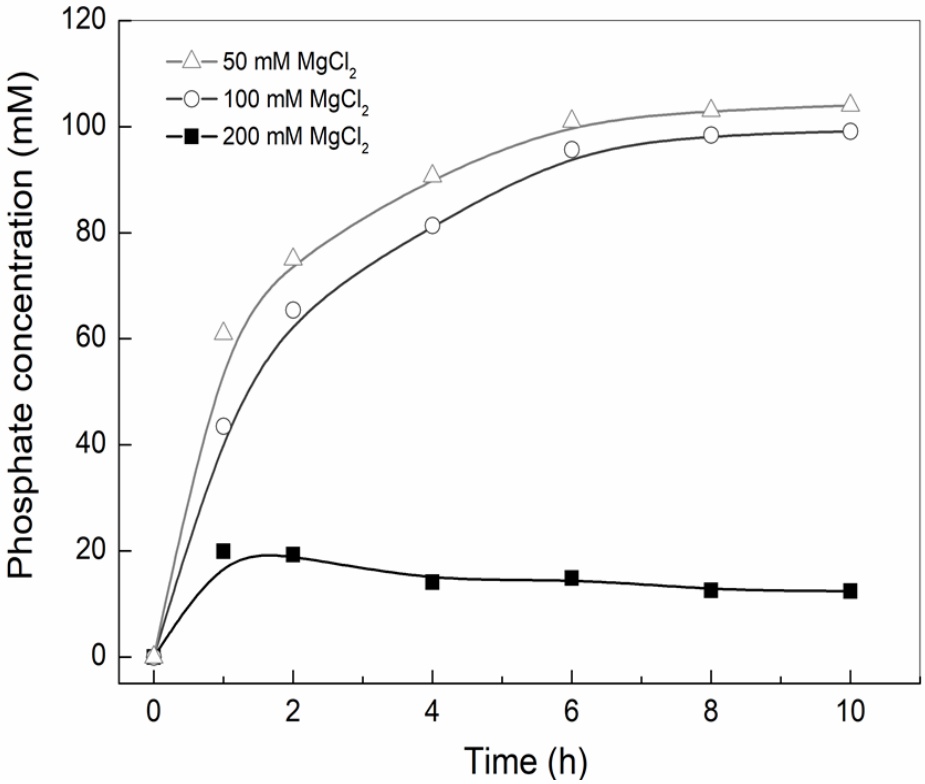
Supporting Figure S3. Codon-optimized gene sequence of *Cc*CdP. Start and stop codon are marked in bold. The synthetic gene was ordered from GenScript (Piscataway, New Jersey, U.S.) carrying an N-terminal *BamH*I and a C-terminal *Hind*III restriction site for sub-cloning into pQE30 from Qiagen (Hilden, Germany).



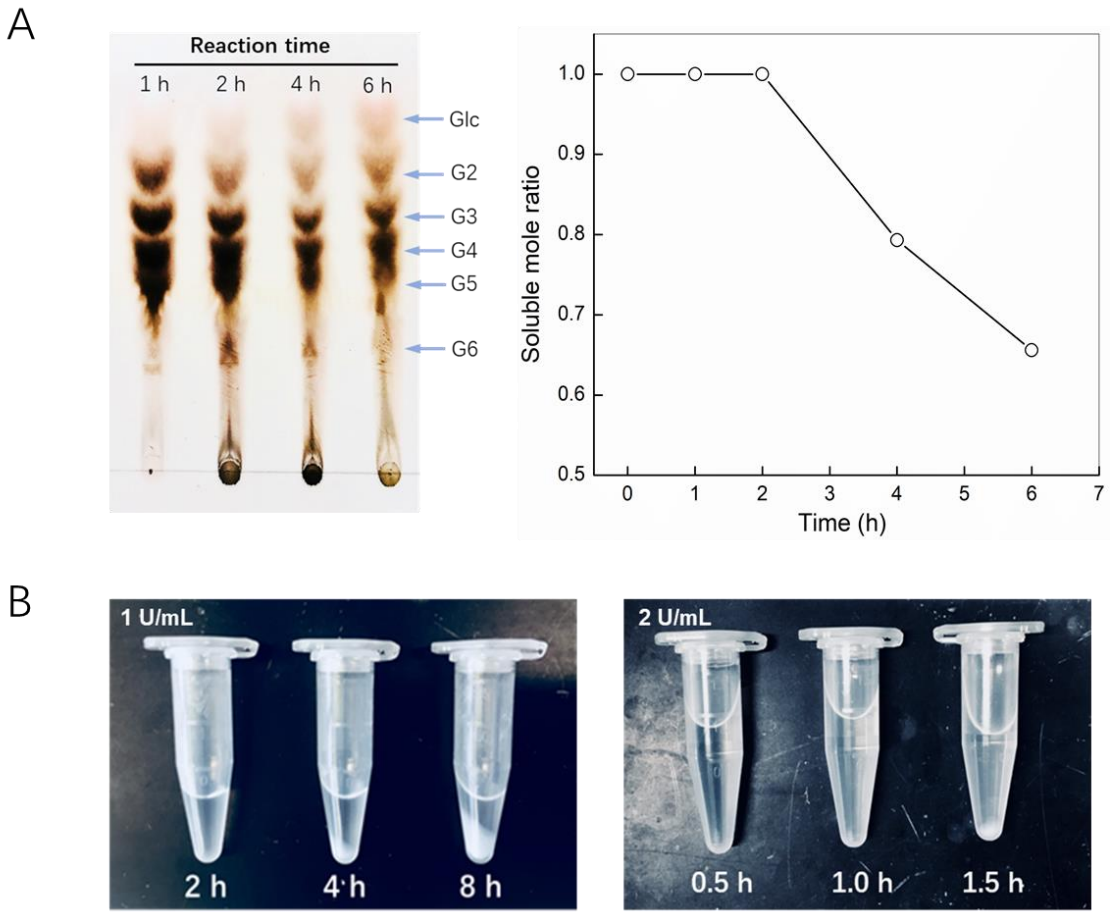
Supporting Figure S4. Genetic map of the self-assembled vector pC21e1 with IPTG-inducible P*tac*I promoter and ampicillin resistance (upper panel) and of the expression plasmid pC21e1_His-*Cc*CdP (lower panel).



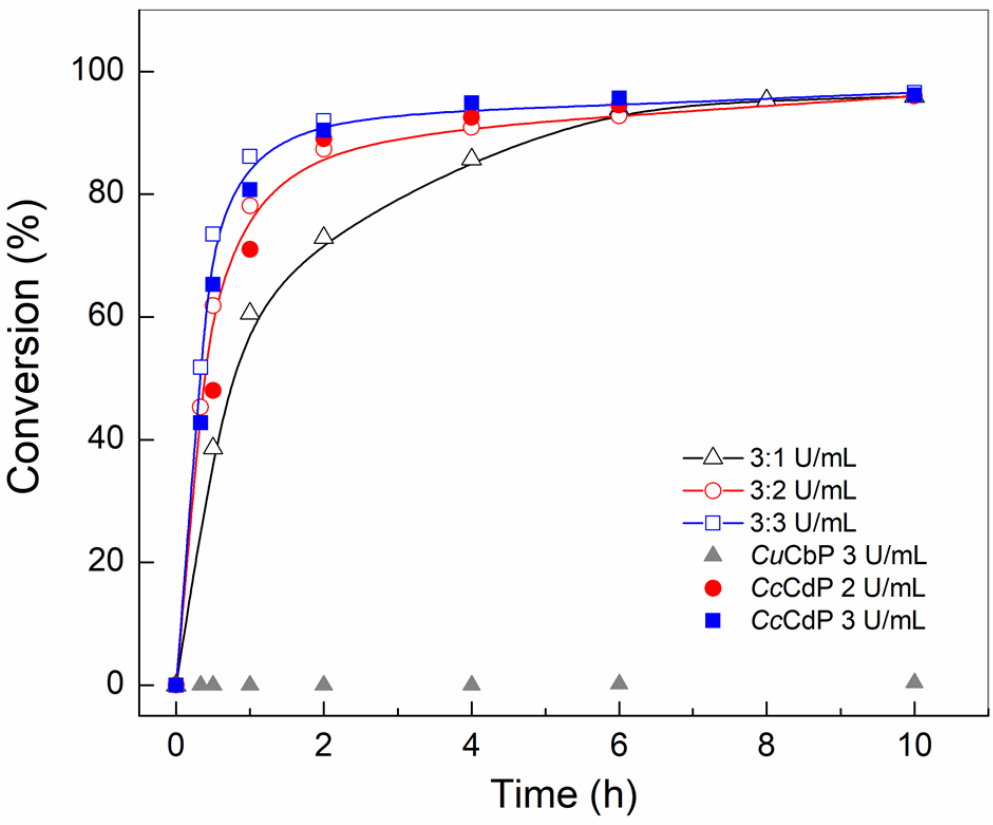
Supporting Figure S5. SDS polyacrylamide gel showing purified recombinant enzymes, expressed in *E. coli*. **M**, PageRuler™ Prestained Protein Ladder (10-180 kDa); **1**, purified CbP from *Cellulomonas uda* (*Cu*CbP); **2**, purified CdP from *C. cellulosi* (*Cc*CdP).



Supporting Figure S6. Time courses of phosphate release during cellodextrin synthesis by coupled *Cu*CbP and *Cc*CdP (200 mM αGlc1-*P*, 50 mM glucose; 3 U/mL *Cu*CbP, 1 U/mL *Cc*CdP, pH 7.0, 45 °C) in the presence of 50 mM (Δ), 100 mM (○) or 200 mM (■) of $MgCl_2$.



Supporting Figure S7. (A) Time courses of product formation during cellodextrin synthesis by coupled *Cu*CbP and *Cc*CdP (200 mM αGlc1-*P*, 200 mM $MgCl_2$, 50 mM glucose, 3 U/mL *Cu*CbP, 1 U/mL *Cc*CdP, pH 7.0, 45 °C) using analysis by TLC (left) and determination of the soluble mole ratio of product (right). The soluble mole ratio of product is defined as the ratio of the moles of glucosyl units in the soluble cellodextrins, including cellobiose, to the moles of glucosyl units transferred from αGlc1-*P* at the time indicated. A soluble mole ratio of 1 indicates that all cellodextrin products were soluble. (B) Accumulation of insoluble cellodextrins over time using different *Cc*CdP concentrations (1 and 2 U/mL) under otherwise identical reaction conditions (200 mM αGlc1-*P*, 200 mM $MgCl_2$, 50 mM glucose, 3 U/mL *Cu*CbP, pH 7.0, 45 °C).



Supporting Figure S8. Time courses of αGlc1-*P* conversion during cellodextrin synthesis. Coupled *Cu*CbP and *Cc*CdP reactions (open symbols) were started from 50 mM glucose while individual reactions of *Cu*CbP and *Cc*CdP reactions (filled symbols) were started from 50 mM cellobiose under otherwise identical conditions (200 mM αGlc1-*P*, 200 mM $MgCl_2$, pH 7.0, 45 °C). Different volumetric activities of 3:1 U/mL *Cu*CbP:*Cc*CdP (Δ), 3:2 U/mL *Cu*CbP:*Cc*CdP (○), 3:3 U/mL *Cu*CbP:*Cc*CdP (□), 3 U/mL *Cu*CbP (▲), 2 U/mL *Cc*CdP (●) and 3 U/mL *Cc*CdP (■) were used. The reaction using *Cu*CbP alone confirms that the enzyme does not elongate cellobiose.