catalysts

MDPI

Supplementary Material

*Calk*GH9T: A Glycoside Hydrolase Family 9 Enzyme from *Clostridium alkalicellulosi*

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ProtParam

User-provided sequence:

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        10         20         30         40         50         60
MLKKINDKRK YLVFLMIFCM LSFLFLTPPV KVSADPEYNF AKALQMSLYF FDSNKCGIGI

        70         80         90        100        110        120
TGGRLEWRGD CHVEDAEVPL IPMTEEFFGT NMSQAFIDEY RHILDPEGNG FLDLSGGYHD

       130        140        150        160        170        180
AGDHIKFGLP GTYAGSTLGW GYYEFRDSYV QTGTDDHIEE LLRWFNDFYL KVTFRDENGD

       190        200        210        220        230        240
VIAYCYQVAE GNIDHNFWNP PELQRSDVLL DFARPAYFAT AETPASDQAA GAAASLTINY

       250        260        270        280        290        300
LNFKDTDPEY AEECLDTAIA LYDFAVKHRG LGYDGGFYNS SYDYDEMSWA AVWLHIATGN

       310        320        330        340        350        360
WDYIEDIVKT DDDGNYTGYF QRIIKDTNNR WQNIWVHCWD TVWGGVFAKL APITNTERDW

       370        380        390        400        410        420
YIFRWNIEYW SGIPHEDPTD TTFLAKSPAG FSVVNPYGSA RYNTAAQLCA LVFTKETGRQ

       430        440        450        460        470        480
DFAEWSKNQM EYIMGNNPMD RSYIVGYAPN SAKHPHHRAA HGSKTLSMLD PPEHRHTLWG

       490        500        510        520        530        540
ALVGGPDLDD FHVDETYDYV YNEVAIDYNT AFVGALAGLY KYYGEGHYPL ENFPPKADPI

       550        560        570        580        590        600
DEYYIEAKLE QENKERTQVT LRLYNYSAYP PRFEEGMSVR YYFDISELLD AGQSIDDVIM

       610        620        630        640        650        660
EVYYDENKAG YDGPAEYKGP FKYDDAGTYY VEFDWTGRVV YGTREIQFAL MSGQDANWQS

       670        680        690        700        710        720
NWDPTNDYSR QGIVKDEFTL TRRVPVYLYG ELVFGEEPVP VTVTPTPTPD PNVTPGPTPI

       730        740        750        760        770        780
ESASLMVLYK SGVAISDTSD IRASINIRNT GTRPVNLSDV KIRYWFTKDG PGVQSFLCDY

       790        800        810        820        830        840
AHIESSKVTG VIREIDNPVD LADSYLEIGF TSDAGVLGAG SQTGEIQFRI EKEGFLQYDE

       850        860        870        880        890        900
TNDYSFNASA RDFIENPKIT AYVNSVLAYG VAPVETSGGN ILYGDLNGDG RINSTDYVLL

       910        920        930        940
RRYILEIIEE FPVPTEAADL NGDGRINSTD VVLMRRYILE IIPQLPR
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Number of amino acids: 947

Molecular weight: 107535.55

Theoretical pI: 4.52

Total number of negatively charged residues (Asp + Glu): 143
Total number of positively charged residues (Arg + Lys): 75

Figure S1. A translated amino acid sequence and a calculated molecular weight and a pI value of *Calk*GH9T analysed by ProtParam online tool.

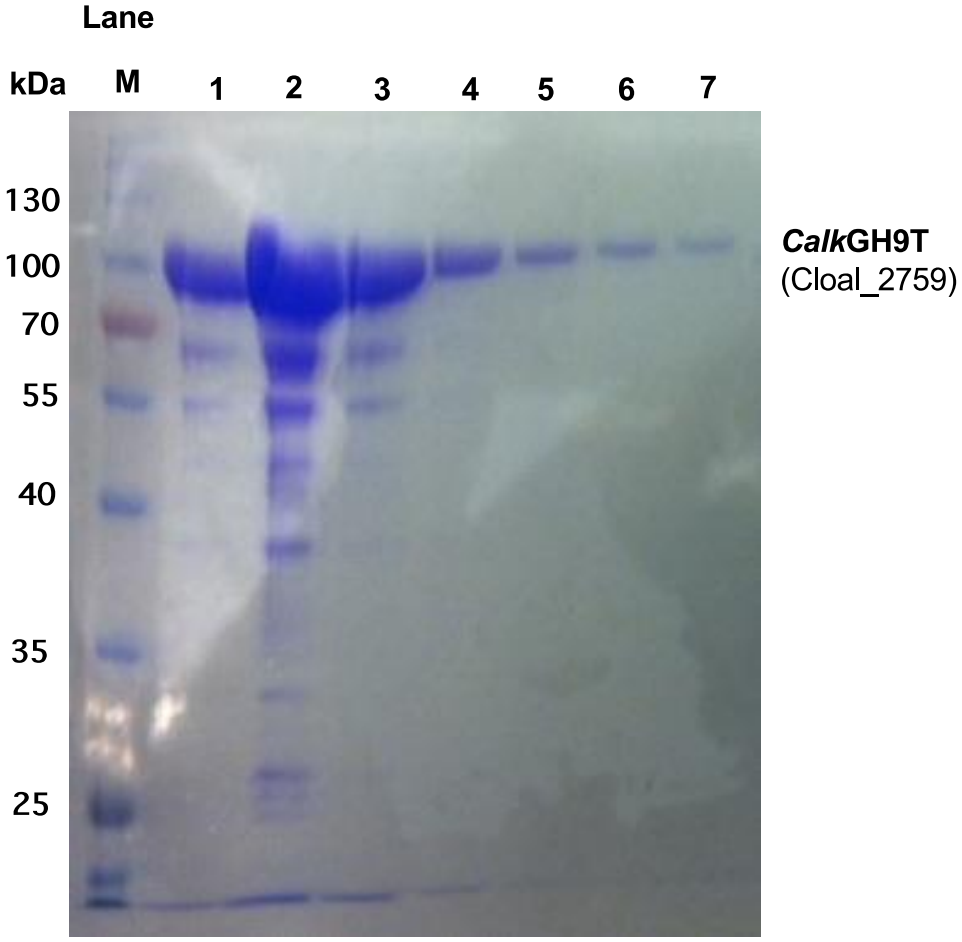

Figure S2. 10% SDS-PAGE analysis of *Calk*GH9T expression and purification. Lane: M, molecular weight marker (PageRulerTM prestained protein ladder, 10 to 180 kDa). Lane: 1-7, the eluted proteins from 2-mL collected fractions. Each well was loaded with 20 μL of individual eluted protein fractions.

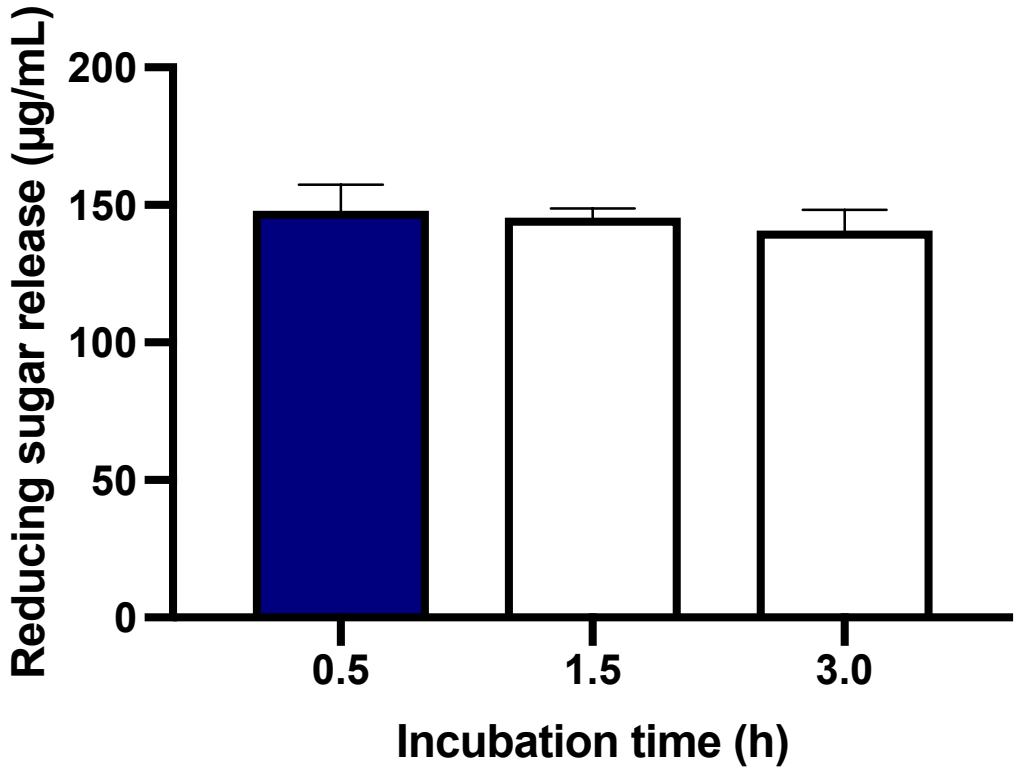

Figure S3. Reducing sugar release from RAC by *Calk*GH9T. Values are means and bars represent the standard deviations for three independent experiments.

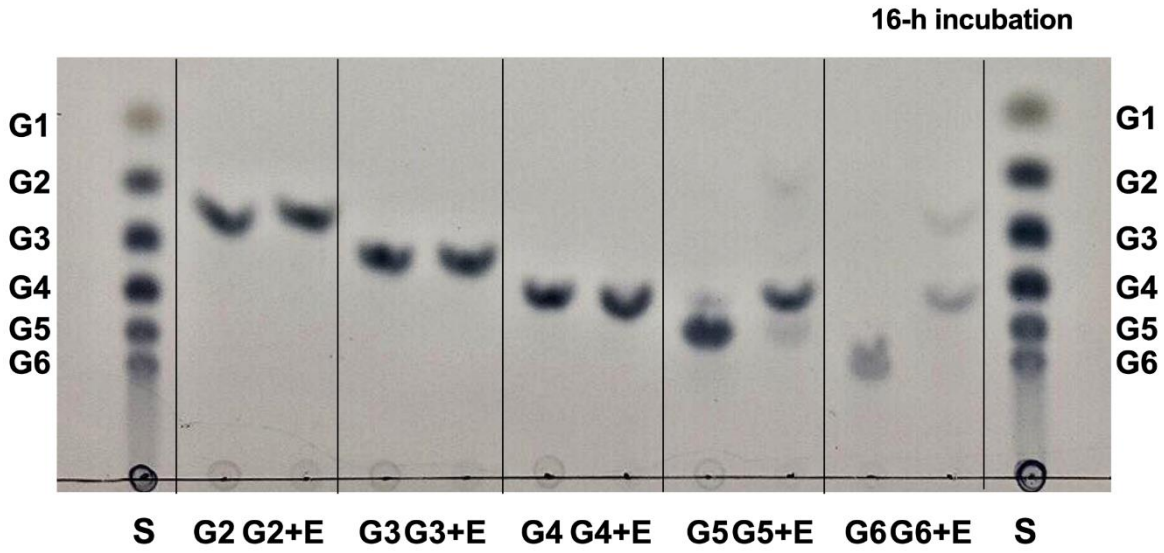

Figure S4. TLC analysis of hydrolysis products generated by 0.25 μM *Calk*GH9T using cellodextrins with a degree of polymerization of 2-6 (G2-G6) as the substrates for 16-h incubation. S, standards of glucose and cellodextrins with a degree of polymerization of 2-6 (G2-G6). E, *Calk*GH9T. G2, G3, G4, G5, and G6 represent a reaction control (a reaction mixture containing a substrate and a denatured enzyme). G2+E, G3+E, G4+E, G5+E, and G6+E represent enzymatic reactions. The enzymatic reactions were performed at pH 7.4 and 55 °C.