In planta expression of hyperthermophilic enzymes as a strategy for accelerated lignocellulosic digestion

Bilal Ahmad Mir[1,2†], Alexander A Myburg[2], Eshchar Mizrachi[2], Don A Cowan[1‡]

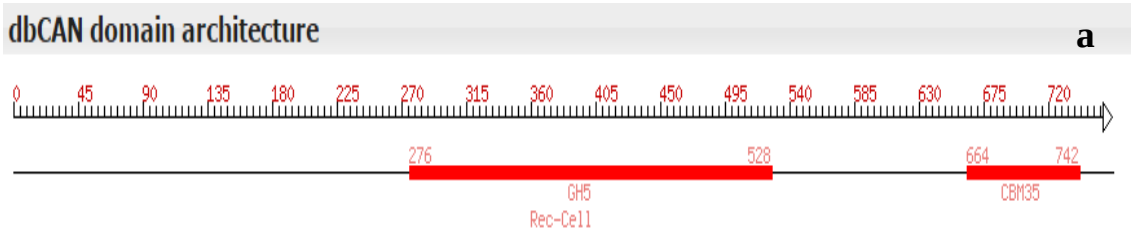
[1]Centre for Microbial Ecology and Genomics, Department of Genetics, University of Pretoria, Private Bag X20, Pretoria 0028, South Africa

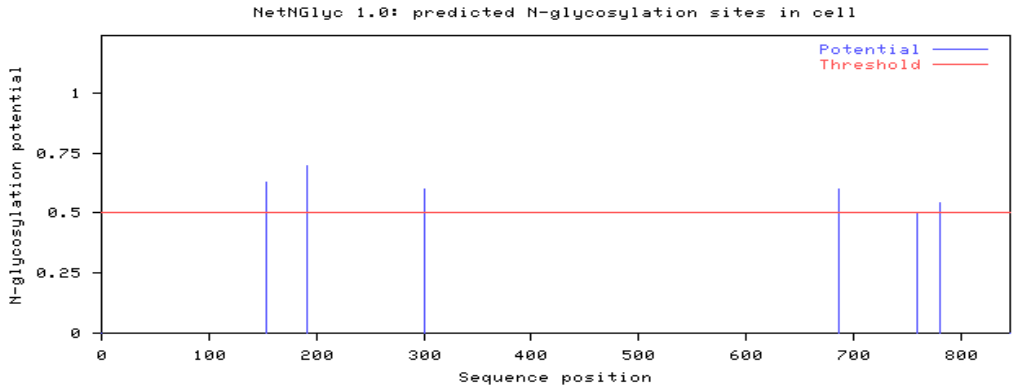
[2]Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Pretoria 0028, South Africa

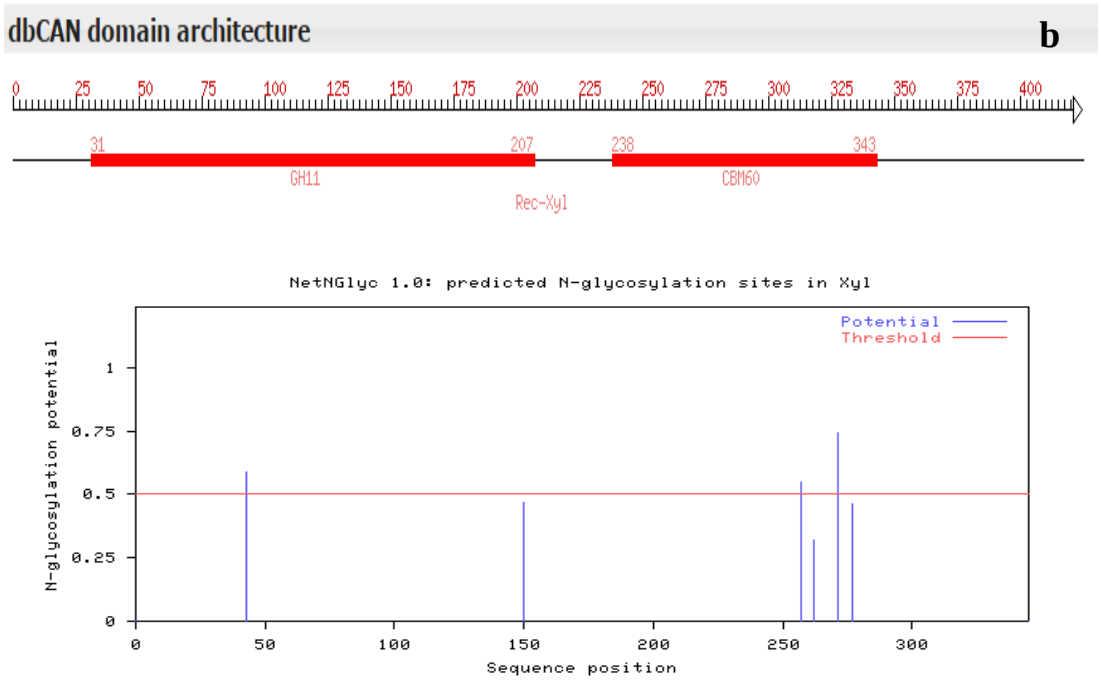
[†]Current address: Department of Botany, School of Life Sciences, University of Kashmir, Kargil Campus, Kargil, Jammu & Kashmir, India

[‡]Corresponding author: don.cowan@up.ac.za

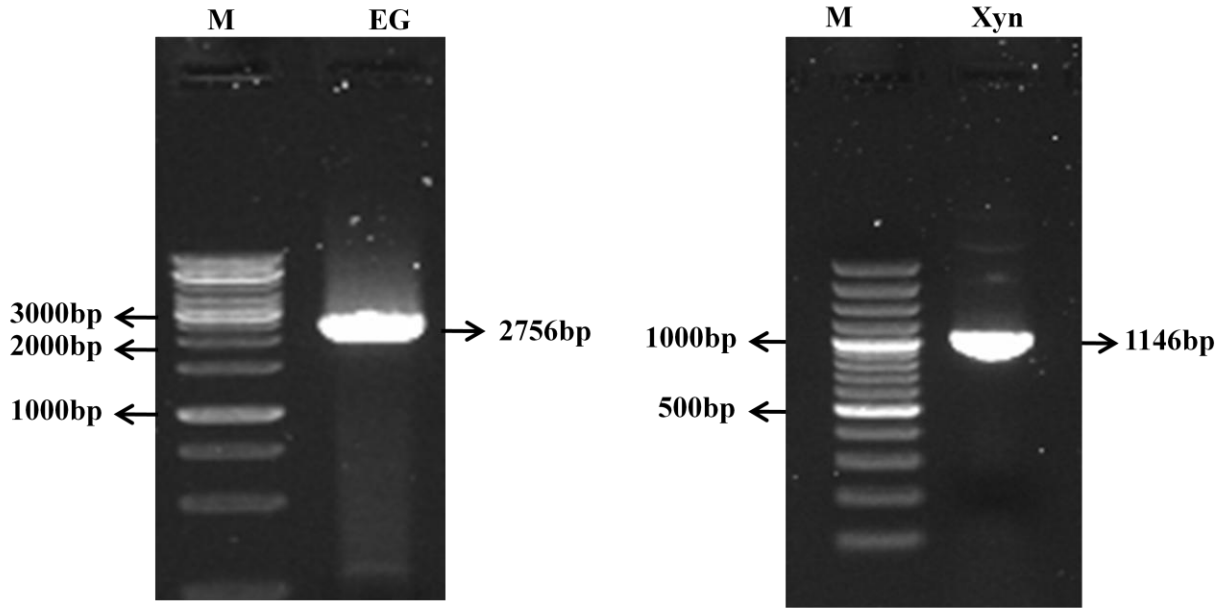
S1: Prediction of cleavage and N-glycosylation sites of recombinant endoglucanase (a) and recombinant xylanase (b). Sub-cellular localization of the recombinant proteins was predicted using TargetP (Emanuelsson et al., 2000, 2007) and N-glycosylation sites were predicted using NetNGlyc (http://www.cbs.dtu.dk/services/NetNGlyc/).







S2: Gene cloning of Rec-EG (2756bp), and Rec-Xyn (1146bp) using gene specific primers. M= marker ladders. PCR for EG gene was performed with the following conditions: 30s at 98°C; 30 cycles as follows: 10 s at 98°C; 40 s at 60°C; 1 min at 72°C; 5 min at 72°C. PCR for Xyn was performed with the following conditions: 30s at 98°C; 30 cycles as follows: 10 s at 98°C; 30 s at 60°C; 40s at 72°C; 5 min at 72°C.



S3: Endoglucanase and xylanase activity of protein extracts from C108 and X7 plants expressing Rec-ebi244Opt and Rec-rMxylOpt respectively. A) Observation of clear zone around the protein extracts on 0.1% RBB-xylan (Sigma) plate using 0.1% Congo Red. B-C) Observation of clear zone around the protein extracts on 1% CMC (Sigma) plates using 0.1% Congo Red. The plates were incubated at 75°C for 30 min.

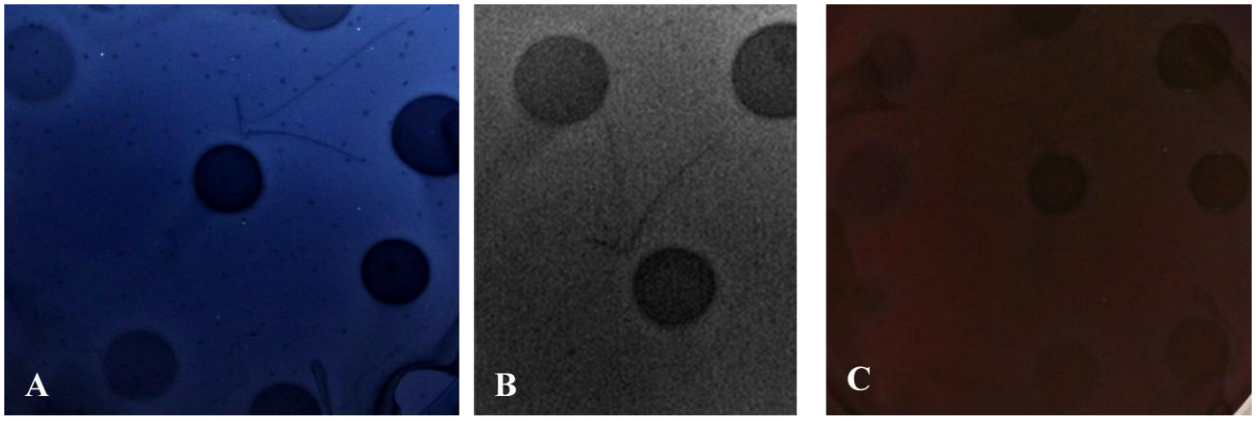


Table 1: Primers used in this work

Primer name	Sequence (5'-3')
EGFW1	CAGCTGGCCACCATGGGATTTGTTCTCTT
EGRev1	CAGCTGTTAGCCAGCCCTAGTGATTTCG
XynFW1	CAGCTGGCCACCATGGGATTTGTTC
XynRev1	CAGCTGTTACGGCGTGTTTCCGTAG
EGFW2	CAGCTGGCCACCATGGGATTTGTT
EGRev2	CAGCTGTTAGCCAGCCCTAGTGAT
XynFW2	CAGCTGGCCACCATGGGATTTGTT
XynRev2	CAGCTGTTACGGCGTGTTTCCGTA
EGFW3	CAGCTGGCCACCATGGGATTCGTG
EGRev3	CAGCTGTTATCCTGCCCTAGTAAT
XynFW3	CAGCTGGCCACCATGGGTTTCGT
XynRev3	CAGCTGTTAAGGTGTATTACCATATCC
EGFW4	TGTCAGAAATTAGAGAGTTTATGGTTA
EGRev4	TAATTGTAGTGGTAGGTGTCTGAGGTG
EGFW5	GATACGGACCAGAAGGTTTTAAGGATA
XynFW4	CTTCATCAAGTTCTTCAAGTTCTGGAG
XynRev3	AAAGGGTTTGTAGTCCATCCATACAAT
RT-PCR/qRT-PCR-Xyn-FW	CAGACCTCCAGGAGGTCAAGGGGTCCAGTT
RT-PCR/qRT-PCR-Xyn-Rev	AGTACCTCCGGTCCTTCTCTGTTCGGCATGC
RT-PCR/qRT-PCR-EG-FW	GAATAGGATTGGTTCCATGGGCTCCGTCGGA
RT-PCR/qRT-PCR-EG-Rev	TATCTCCCCAATTATCTGCGAGCGTTGCGA