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Production of Recombinant GH10 Xylanase Xyl10B from *Thermotoga maritima* in Transgenic Plants Improves the Efficiency of Hydrolysis of Xylan to Fermentable Sugars for Biofuel Production

Jae Yoon Kim[1]*, Chang-Ho Kim[1], Sang Yong Park[1], Kyeong Do Min[1], Fredy Altpeter[2]

[1]Department of Plant Resources, College of Industrial Science, Kongju National University, Yesan 32439, Republic of Korea
[2]University of Florida, IFAS, Agronomy Department, Genetics Institute, Gainesville, FL 32611, USA.

[Introduction]

Sugarcane is one of the most efficient photosynthesizer in the plant kingdom, able to convert as much as 2% of incident solar energy into biomass. A large amount of lignocellulosic biomass such as leaf litter residues and bagasse are generated during the sugarcane harvest or after the sugar refining process, respectively. Therefore, lignocellulosic biomass from leaf and processing residues will likely become a valuable feedstock for biofuel production. Recent efforts focus on the integration of first and second generation bioethanol conversion technologies for sugarcane to increase biofuel yields. This integrated process will utilize both the cell wall bound sugars of the abundant lignocellulosic sugarcane residues in addition to the sucrose from stem internodes. Enzymatic hydrolysis of lignocellulosic biomass into its component sugars requires significant amounts of cell wall degrading (CWD) enzymes. *In planta* production of xylanases has the potential to reduce costs associated with enzymatic hydrolysis but has been reported to compromise plant growth and development.

[Materials & Methods]

we expressed a hyperthermostable GH10 xylanase, xyl10B in transgenic sugarcane which displays optimal catalytic activity at 105°C and only residual catalytic activity at temperatures below 70°C. Transgene integration and expression in sugarcane were confirmed by Southern blot, RT-PCR, ELISA and western blot following biolistic co-transfer of minimal expression cassettes of xyl10B and the selectable nptII.

[Results & Discussion]

Xylanase activity was detected in 17 transgenic lines with a fluorogenic xylanase activity assay. Up to 1.2% of the total soluble protein fraction of vegetative progenies with integration of chloroplast targeted expression represented the recombinant Xyl10B protein. Xyl10B activity was stable in vegetative progenies. Tissues retained 75% of the xylanase activity after drying of leaves at 35°C and a 2 month storage period. Transgenic sugarcane plants producing Xyl10B did not differ from non-transgenic sugarcane in growth and development under greenhouse conditions. Sugarcane xylan and bagasse were used as substrate for enzymatic hydrolysis with the in planta produced Xyl10B. TLC and HPLC analysis of hydrolysis products confirmed the superior catalytic activity and stability of the in planta produced Xyl10B with xylobiose as a prominent degradation product. These findings will contribute to advancing consolidated processing of lignocellulosic sugarcane biomass.

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*Corresponding aothor: Tel. 041-330-1203, E-mail. jaeyoonkim@kongju.ac.kr