NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Biomass in Multifunction Crop Plants

Cooperative Research and Development Final Report

CRADA Number: CRD-05-163

NREL Technical Contact: Stephen R. Decker

NREL is a national laboratory of the U.S. Department of Energy, Office of Energy Efficiency & Renewable Energy, operated by the Alliance for Sustainable Energy, LLC.

CRADA Report
NREL/TP-7A10-52696
October 2011

Contract No. DE-AC36-08GO28308

NOTICE

Available electronically at http://www.osti.gov/bridge

Available for a processing fee to U.S. Department of Energy
and its contractors, in paper, from:

U.S. Department of Energy
Office of Scientific and Technical Information

P.O. Box 62
Oak Ridge, TN 37831-0062
phone: 865.576.8401
fax: 865.576.5728
email: mailto:reports@adonis.osti.gov

Available for sale to the public, in paper, from:

U.S. Department of Commerce
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
phone: 800.553.6847
fax: 703.605.6900
email: orders@ntis.fedworld.gov
online ordering: http://www.ntis.gov/help/ordermethods.aspx

Cover Photos: (left to right) PIX 16416, PIX 17423, PIX 16560, PIX 17613, PIX 17436, PIX 17721

Printed on paper containing at least 50% wastepaper, including 10% post consumer waste.

Cooperative Research and Development Final Report

In accordance with Requirements set forth in Article XI.A(3) of the CRADA document, this document is the final CRADA report, including a list of Subject Inventions, to be forwarded to the Office of Science and Technical Information as part of the commitment to the public to demonstrate results of federally funded research.

CRADA number: **CRD-05-163**

CRADA Title: **Biomass in Multifunction Crop Plants**

Parties to the Agreement: **Edenspace Systems Corporation**

Joint Work Statement Funding Table showing DOE commitment:

Estimated Costs	**NREL** Shared Resources
Year 1	$145982.00
Year 2	$174939.00
Year 3	$181079.00
TOTALS	$502000.00

Abstract of CRADA work:

An array of cellulase, hemicellulase, and accessory enzymes were tested for their ability to increase the conversion levels and rates of biomass to sugar after being subjected to thermochemical pretreatment. The genes were cloned by Oklahoma State University and expressed, purified, and tested at NREL. Several enzymes were noted to be effective in increasing conversion levels, however expression levels were typically very low. The overall plan was to express these enzymes in corn as a possible mechanism towards decreased recalcitrance. One enzyme, cel5A endoglucanase from Acidothermus cellulolyticus, was transformed into both tobacco and corn. The transgenic corn stover and tobacco were examined for their susceptibility to thermochemical pretreatment followed by enzymatic digestion.

Summary of Research Results:

Expression and purification of the targeted enzymes was problematic and obtaining reasonable quantities of each enzyme was difficult. The overall idea was to select a small set of enzymes that showed synergy or enhancement of biomass conversion and express these enzymes in plants to determine if in planta expression could be a viable means of enzyme production, decreased enzyme loading, or decreased thermochemical pretreatment severity. We selected cel5A (E1), an

endoglucanase from *Acidothermus cellulolyticus* for expression in plants. The detailed results can be found in the attached preprint.

Both tobacco and corn were selected as transgenic hosts and each was transformed with cel5A resulting in multiple transgenic lines of each. NREL tested two corn and one tobacco lines. Expression levels were determined using both western blotting quantitation and cel5A activity on MUL or pNPL. The E1-1 and E1-7 stover lines showed low levels of expression, with E1-7 being about 10-fold lower than E1-1 0.3 (ng cel5A/mg biomass and 0.03 ng cel5A/mg biomass, respectively). The E1-tobacco line had higher expression, approximately 10,000X higher than the E1-1 stover line at 3100 ng/mg.

Each line and wild type control biomass was milled and subjected to 3 different pretreatment severities; low, mid, and optimal. Each pretreated biomass was subjected to digestion with a commercial cellulase at both high (100 mg protein/g cellulose) and low (15 mg protein/g cellulose). The high loading is an indicator of differences in pretreatment resulting in decreased recalcitrance to digestion, while the low loading shows increased sensitivity to enzyme digestion. We also digested wild type biomass with the same commercial enzyme loadings with the addition of exogenous cel5A to approximately the same levels found in the transgenic in order to determine if E1 residual activity could be affecting the results.

The results demonstrated that transgenic cel5A-containing biomass was less recalcitrant to pretreatment and enzyme hydrolysis. At the high cellulase loading, the E1-transgenic stover from the mid-severity pretreatment was equivalent to that of the high severity pretreated wild type. This indicates a increase in susceptibility to thermochemical pretreatment in the transgenic stover. In both the high and low enzyme loading studies, the transgenic stover was always more digestible than the wild type, indicating that the biomass was more amenable to enzyme hydrolysis as well as pretreatment. Digestions with cel7A, a fungal exocellulase, showed identical patterns, though at a lower conversion level (expected for a single enzyme), indicating that the increase in digestibility is in the cellulose structure itself, likely a result of cellulose chain "nicking" by E1 when it is deposited into the cell wall. This nicking would open up more access sites for cel7A, resulting in higher activity and more conversion.

Subject Inventions listing:

The discovery that adding a cellulase to plants for the expressed purpose of increasing digestibility and susceptibility to thermochemical pretreatment was submitted as a Record of Invention (NREL ROI No. 08-30) and subsequently filed as a joint patent application with Edenspace Systems Corp.

International Application No. PCT/US2009/048153 and U.S. Application No. 12/999,590, both entitled "Processing Cellulosic Biomass".

Report Date: Responsible Technical Contact at Alliance/NREL: Stephen R. Decker

This document contains NO confidential, protectable, or proprietary information.