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U.S. Department
of Transportation



Project Title: Development of a customized enzyme cocktail for corn stover and switchgrass saccharification

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Project Goal:

- Identify genes belonging to 12 distinct enzymatic categories that exhibited highest levels of expression on corn stover and switchgrass, respectively.
- Synthesize, clone, and express target genes in an expression vector.
- Purify products and confirm their enzymatic activity and specificity.

Project Outcomes:

- We have identified target genes belonging to 12 distinct enzymatic categories that exhibited high levels of expression on corn stover and switchgrass, respectively.
- Synthesizing, cloning, and expressing target genes in an expression vector are nearly complete. Genes with the following activities have successfully been targeted for cloning: β -glucosidase, xylanases, cellobiohydrolases, carbohydrate esterases, endoglucanases. Xylosidases, mannanases. Cloning of genes with the following activities is underway: mannosidases, α -glucuronidase, and α -N- arabinofuranosidase. In addition, enzyme assays for all 12 targeted activities has been established.
- We initially began to purify products and confirm their enzymatic activity and specificity for a few enzymes (endoglucanases and cellobiohydrolases). Purification of three distinct cellobiohydrolases through affinity chromatography is underway. Current results indicate that multiple purification steps may be necessary to purify such enzymes.



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