Bioproducts and Biocatalysis Research Unit, National Center for Agricultural Utilization Research, Peoria, IL

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Project Number 3620-41000-121-00D. Microbial Catalysts to Produce Fuel Ethanol and Value Added Products

The broad goal of this research project is to develop new microorganisms and biocatalysts that can be employed in the fermentative conversion of renewable agricultural materials to fuels and other value-added products. The research entails engineering existing fermentative microorganisms to possess desirable traits for industrial fermentation of lignocellulosic material, and searching for new microorganisms that possess these traits. The specific objectives of the project are as follows: 1) create efficient xylose-fermenting *Saccharomyces cerevisiae* strains; 2) engineer lactic acid bacteria to make ethanol, and 3) determine the potential for microorganisms from extreme environments to serve as biotechnological agents in the fermentation industry.

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Key Accomplishments:

Construction of a proteomic workcell

One approach to improving biocatalysts for the fermentation industry is to use an automated high-throughput strategy to screen tens of thousands of candidates. In collaboration with Hudson Control Group, Inc., Springfield, NJ, scientists in the Bioproducts and Biocatalysis Research Unit have developed a plasmid-based proteomic workcell that integrates all of the molecular, microbiological, and biochemical techniques used for the high-throughput strategy into a single robotic platform. In 2007, construction of the workcell was completed, and the unit has been delivered and installed at NCAUR. The workcell is facilitating the development of xylose utilizing biocatalysts, improvement of enzyme function of xylanases, and mutagenesis of insecticidal peptides.

Development and characterization of novel biocatalyst strains

Lactic acid bacteria are well-suited to industrial fermentation environments and have the potential to be developed into biocatalysts for the production of ethanol from agricultural feedstocks. Scientists in the Bioproducts and Biocatalysis Research Unit have developed molecular genetic tools for the improvement of lactic acid bacteria. Strains of *Lactobacillus plantarum* and *Lactobacillus brevis* have been genetically modified to express genes required for ethanol production, which demonstrates that genetic engineering of lactic acid bacteria is a viable strategy for the development of new biocatalysts to convert agricultural materials to biofuels. Scientists in the Unit have also isolated a novel biocatalyst strain (*Lactobacillus buchneri* NRRL B30929) from a survey of bacterial contaminants inhabiting commercial ethanol plants. The strain is tolerant to

high concentrations of ethanol, and can ferment mixed sugars, like those derived from lignocellulosic biomass, to produce ethanol, lactate, and acetate. *L. bucheri* NRRL B30929 has potential industrial application in bio-based refinery platforms.

Discovery of a novel cellulase gene from a thermophilic bacterium
Thermophilic bacteria are a potential source of robust enzymes for use in the fermentation industry. Scientists in the Bioproducts and Biocatalysis Research Unit identified a thermophilic strain of *Bacillus licheniformis* that produced endoglucanase, an enzyme that helps degrade cellulose. This enzyme was purified, identified, and characterized. The gene for this enzyme was cloned, and a recombinant form of the enzyme produced in *Escherichia coli* for characterization. The catalytic properties, broad pH range and thermostability of the recombinant *B. licheniformis* endoglucanase may prove suitable for industrial application in the conversion of biomass to glucose for production of fuel ethanol or other valuable fermentation products.

Characterization of bacterial contaminants from fuel ethanol plants
Bacterial contamination of commercial fermentation cultures is a common and costly problem to the fuel ethanol industry. Scientists in the Bioproducts and Biocatalysis Research Unit surveyed bacterial contaminants from corn-based wet-mill and dry-grind ethanol plants and established for the first time that these contaminants readily form biofilms under laboratory conditions. Isolates currently are being characterized for susceptibility to penicillin and virginiamycin, two antimicrobial agents commonly used to control contamination. This information is important to ethanol plant managers and will help guide intervention strategies that control bacterial contamination.

Other Scientific Expertise or Capabilities:

Expertise in microbiology and fermentation: bench top (150 mL) to pilot plant fermentors (30-100 L), cell separation and product recovery tools.

Expertise in molecular biology: transformation of yeast and bacteria, mutagenesis of genes, protein expression and purification.

Expertise in carbohydrate chemistry and analyses: MALDI-TOFS (Matrix Assisted Laser Desorption/Ionization Time-of-Flight Spectrophotometer.