Bioenergy and Energy Alternatives National Program
FY2005 Annual Report

Introduction

Research is conducted under this national program to develop technology that will create jobs and economic activity in America, improve energy security by reducing the Nation's dependence on foreign oil, and improve the environment by developing alternate energy sources and increasing the use of agricultural crops as feedstocks for biofuels.

Selected Accomplishments by National Program Component for Fiscal Year 2005

Ethanol

Better understanding of ethanol-producing bacteria. The process of cellulose degradation (a key process in producing ethanol from cellulosic biomass) is poorly understood at the level of gene expression. Four genes involved in cellulose degradation by Clostridium thermocellum, a bacterium that grows at high temperature in the absence of oxygen, were shown by ARS scientists at the U.S. Dairy Forage Research Center to be regulated in a manner that varied in response to the conditions under which the bacterium is grown. This research -- the most complete study to date in any organism of the relationship between cell growth rate and gene expression -- provides new information on the regulation of cellulose degradation by an organism that shows particular promise for converting cellulosic biomass to both ethanol and a fermentation residue having desirable properties as a wood adhesive. The results provide new strategies for improving cellulose degradation and ethanol production.

Improved organisms for ethanol production: Inhibitors formed during pretreatment of lignocellulosic material reduce the performance of ethanol producing fermentation organisms. Due to the synergistic effect of combined inhibitors, development of organisms tolerant to multiple inhibitors even at low concentrations has been more difficult. Scientists with the Crop Bioprotection Research Unit at Peoria, IL using a method called directed adaptation, developed strains of organisms (Saccharomyces cerevisiae and Pichia stipitis) that have enhanced ability to convert toxic compounds (furfural and 5-hydroxymethylfurfural (HMF)) into less toxic compounds. These improved strains grow normally in the presence of both furfural and

HMF and produce normal ethanol yields. Development of these more tolerant organisms is a significant step toward achieving the technology necessary for commercial production of ethanol from cellulosic plant material.

An improved enzyme producing fungi. Improved technology for conversion of lignocellulosic biomass into liquid fuels is necessary for economic competitiveness with petroleum fuels. Enzyme technology for converting the hemicellulose portion of the biomass is especially needed. Scientists with the Fermentation biotechnology Research Unit in Peoria, IL expressed a highly active enzyme (a xylanase protein) in a fungus (Trichoderma reesei) used for producing industrial enzyme. The genetically modified fungal strain produced elevated yields of xylanase. This new strain will be of interest to biotechnology companies researching or marketing enzymes for conversion of biomass to ethanol and also enzymes to improve animal feed.

A bacteria modified to produce more ethanol. Lactic acid bacteria are a group of bacteria that survive and function well in industrial fermentation environments, and that have the potential to be developed into new biocatalysts for the efficient production of ethanol and other value-added products from agricultural feedstocks. ARS scientists in the Bioproducts and Biocatalysis Research Unit at Peoria, IL, have genetically modified a strain of Lactobacillus plantarum to express a gene encoding PDC, which is involved in the production of ethanol. Evaluation of this modified strain in flask fermentations demonstrated that the PDC enzyme was expressed in an active form and that the modified strain produced increased levels of ethanol in comparison to the parent strain. Although significant optimization of the recombinant strain remains to be explored, these results demonstrate that metabolic engineering of lactic acid bacteria is a viable strategy for the development of new biocatalysts to convert agricultural materials to biofuels.

Field pea; a viable fuel ethanol feedstock. Large quantities of renewable feedstocks are needed to offset the large amount of non-renewable petroleum based liquid fuels used in the U.S. Field pea production in northern U.S. is growing and has expanded market potential. Field pea is high in starch and, as such, represents a potential ethanol feedstock. Scientists with the Fermentation biotechnology Research Unit in Peoria, IL developed processes for dry fractionation of field pea into enriched protein and starch streams and for fermenting the pea starch to ethanol. Ethanol yields from pea starch were comparable to that from corn starch, and the enriched protein was similar in protein content to high-protein soy meal, with a well balanced amino acid profile. Both farmers and ethanol producers can benefit from the fuel ethanol potential of this alternate feedstock.

Enzyme reduces energy to produce biofuel. Bioconversion of biomass such as corn stover is not efficiently converted to chemicals such as ethanol due to a lack of efficient enzymes. Engineers and scientists with the Bioproduct Chemistry and Engineering Research Unit at the Western Regional Research Center in Albany, CA through screening of multiple genomic DNA libraries of xylan-degrading enzymes discovered an enzyme that functions effectively at relatively low temperatures. This enzyme characteristic will make biological conversion of biomass more efficient and economically competitive by reducing the heat necessary for production of chemicals and biofuels.

Biodiesel

Measuring blends of biodiesel in petrodiesel. Biodiesel is most commonly utilized as a 20% blend in petrodiesel (B20), but other blend levels, such as B5 and B2, also are used. Fuel distributors have had a problem maintaining consistent biodiesel blend levels. As the marketing of biodiesel blends grows, easy and rapid methods are needed for determining or verifying the portion of biodiesel in a fuel blend. ARS scientists in the Fats, Oils and Animal Coproducts Research Unit at Wyndmoor, PA developed a high performance liquid chromatographic method for quantifying biodiesel blend levels of from 1 to 30% in petrodiesel that can be completed within 20 minutes. This blend-level measurement procedure could become a much needed standard to help maintain quality control and remove a barrier to biodiesel use.

Contaminants improve lubricating properties of biodiesel fuel. The properties of biodiesel are affected not only by the fatty acid alkyl esters which are its prime components but also by remaining contaminants. Biodiesel has been shown to have better lubricating properties than petrodiesel, however, the nature of biodiesel's lubricity has not been known. ARS scientists with the Food and Industrial Oils Research Unit at Peoria, IL found that contaminants of biodiesel, such as free fatty acids and monoglycerides, possess better lubricity than neat alkyl esters and are largely responsible for the lubricity of low-level blends of biodiesel with petrodiesel. These results aid the design of lubricity-enhancing components of fuels and can increase biodiesel use.

Feedstock affects biodiesel fuel emissions and performance. Atmospheric emissions from burning transportation fuels are an environmental concern. Use of vegetable oil-derived biodiesel when compared with petroleum diesel consistently reduces emissions of concern except for nitrous oxides (NOx). These increased NOx emissions are a barrier to increased biodiesel use. ARS scientists in the Fats, Oils and Animal Coproducts Research Unit at Wyndmoor, PA found that, when blended at 20% with petroleum diesel, biodiesel from animal fats had lower nitrous oxide emissions than did biodiesel from soybean oil. The lubricity and oxidative stability were also better for biodiesel from animal fats; however, the cold temperature properties were poorer. These findings will enhance biodiesel use by helping to properly select and use biodiesel fuels based upon the feedstock from which they were made.

Energy Crops

Key gene in cell wall biosynthesis identified. There is a need to identify genes that regulate cell wall composition of alfalfa so that new varieties can be developed that have greater potential as biofuel feedstocks. ARS scientists with the Plant Science Research Unit in St. Paul, MN identified and characterized a gene, UDP-sugar pyrophosphorylase (USP), that plays an important role in cell wall biosynthesis in plants. The USP gene, found to be widely expressed in plant tissues, was cloned and the properties of the protein it produces were determined. The isolation of the USP gene and new knowledge learned about the protein it produces will allow cell walls of alfalfa plants to be modifies to improve the value of this crop as a bioenergy feedstock.