UNIVERSITY OF FLORIDA

Thermophilic Biocatalysts for the Conversion of Cellulosic Substrates to Fuels and Chemicals

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Description: Biomass is an attractive source of sugars for a state like Florida that produces very limited amount of corn for fermentation to produce ethanol as transportation fuel or other products such as lactic acid that can be converted to bioplastics. Florida currently generates about 8.7 million tons of dry cellulosic biomass per year (US-DOE) that can be converted to about 0.7 billion gallons of ethanol. With specific energy crops and short rotation trees cultivated for energy production using the abundant sunshine and water resources, the ethanol produced from biomass can be significantly increased to meet the demand for transportation fuel in the State of Florida. Before biomass-based fuels and chemicals become an economic reality, several key steps in the depolymerization of biomass to constituent sugars need to be addressed. One is depolymerization of cellulose to glucose by fungal cellulases before fermentation to ethanol by microbes. The current estimated cost of fungal cellulases is $0.32 per gallon ethanol produced and this cost is targeted for reduction to $0.10 or less by year 2012 (DOE). We have demonstrated that by increasing the temperature of Simultaneous Saccharification and Fermentation (SSF) of cellulose from 30-35 ºC to 50-55 ºC, the amount (and associated cost) of cellulases can be reduced by the required 3-fold with the current commercial enzyme preparations. A microbial biocatalyst that produces ethanol or other chemicals as the main fermentation product and can also function at this higher temperature and pH 5.0 in conjunction with the fungal cellulases in the SSF process is a critical component of this process. We have identified a thermophilic facultative anaerobe, *Bacillus coagulans*, with versatile metabolic capability as the microbial platform for the SSF of biomass to products and engineering this L(+)-lactic acid producing bacterium to produce ethanol. *The primary objective of this proposed study is to construct a B. coagulans derivative that produces ethanol as primary product of fermentation and to enhance the ethanol productivity of the engineered derivative.*

Budget: $192,000.00
Universities: UF

Progress Summary

Optically pure lactic acid is an attractive chemical for production of bio-based, renewable, polylactide-derived plastics and this is currently produced by microbial fermentation of sugars at temperatures below 40°C. Fermentations at 50-55 °C is expected to enhance the use of non-food carbohydrates for production of optically pure lactic acid while also reducing potential contamination that could lower the optical purity. Biodegradable plastics of varying thermal and physical properties can be produced by judicious mixing of D(-)- and L(+)- lactic acid derived polylactides. This requires optically pure lactic acid and only microbial fermentation is known to produce such a product. We have engineered a thermotolerant bacterium, *Bacillus coagulans* that grows optimally at 50-55 °C and produces (L+)-lactic acid as the primary fermentation product to produce D(-)-lactic acid by deleting the genes in the competing pathways: *ldh* (L-lactate dehydrogenase) and *alsS* (acetolactate synthase). Neither a single (*ldh*) (strain QZ4) or a double (*ldh, alsS*) (strain QZ5) mutant produced D(-)-lactic acid although a native *ldhA* encoding D-LDH is present in the chromosome. Upon metabolic evolution of strain QZ5 for anaerobic growth at pH 5.0 and D(-)-lactate production, a derivative, strain QZ19, was selected. Strain QZ19

produced about 90 g/L of optically pure D(-)-lactic acid in less than 48 hours in batch fermentations at 50°C. The wild type *B. coagulans* and the mutant strain QZ19 can each provide the necessary optical isomer of lactic acid at high titer and yield from biomass-derived non-food carbohydrates at 50-55 °C for production of polylactides and bio-based plastics.

The double mutant is currently being evolved for ethanol production from lignocellulosic biomass derived sugars at 50-55°C.

A provisional patent application with the US Patent Office was filed on the development of the thermotolerant bacterium that produces D(-)-lactic acid.

Proposals						
Title	**Agency**	**Ref. #**	**Investigators/ collaborators**	**Funding requested**	**Duration**	**Date submitted**
Next-generation sweet sorghums: sustainable production of feedstocks for fuels, chemicals and value-added products	DOE-USDA (BRDI)	2010-05340	K. T. Shanmugam (Co-PI) W. Vermerris (PI)	$5,430,439	4 years	October 2010