

## UNIVERSITY OF FLORIDA

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

**PI:** K.T. Shanmugam

**Students:** Brelan Moritz (Ph. D.)

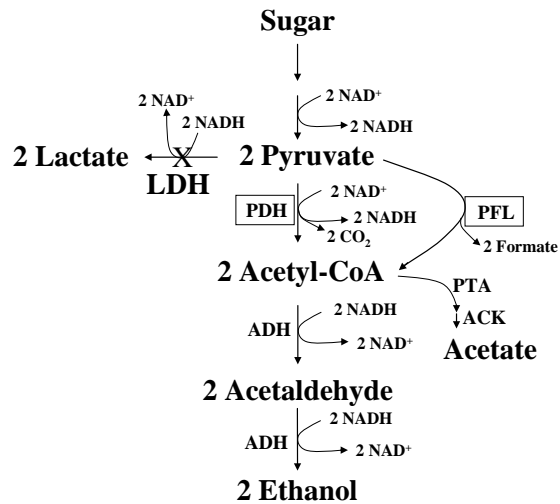
**Description:** Biomass is an attractive source of sugars for a state like Florida that produces very limited amount of corn for fermentation to produce ethanol as transportation fuel or other products such as lactic acid that can be converted to bioplastics. Florida currently generates about 8.7 million 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 °C to 50-55 °C, 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

**Universities:** UF

### Progress Summary

As a first step towards developing thermotolerant *B. coagulans* as an ethanologenic microbial biocatalyst, activity of the primary fermentation enzyme L-lactate dehydrogenase was removed by mutation (strain Suy27). Strain Suy27 produced ethanol as the main fermentation product from glucose during growth at pH 7.0 (0.39 g ethanol per g glucose fermented). Pyruvate dehydrogenase (PDH) and alcohol dehydrogenase (ADH) acting in series contributed to about 55% of the ethanol produced by this mutant while pyruvate formate-lyase and ADH were responsible for the remainder. Due to the absence of PDH activity in *B. coagulans* during fermentative growth at pH 5.0, the *l-ldh* mutant failed to grow anaerobically at pH 5.0. Strain Suy27-13, a derivative of the *l-ldh* mutant strain Suy27, that produced PDH activity during anaerobic growth at pH 5.0 grew at this pH and also produced ethanol as the fermentation product. These results show that construction of an ethanologenic *B. coagulans* requires optimal expression of PDH activity in addition to removal of LDH activity to support growth and ethanol production.



Fermentation pathways of *B. coagulans l-ldh* mutants.

PDH, pyruvate dehydrogenase complex; ADH, alcohol dehydrogenase; PFL, pyruvate formate-lyase; PTA, phosphotransacetylase; ACK, acetate kinase; LDH, lactate dehydrogenase

*B. coagulans* strains Suy27 and Suy27-13 lack LDH activity and produce ethanol using the indicated pathways.

We also evaluated the potential of *B. coagulans* for production of lactic acid from non-food carbohydrates. Lactic acid is used as an additive in foods, pharmaceuticals and cosmetics as well as an industrial chemical. Optically pure lactic acid is increasingly used as a renewable bio-based product to replace petroleum-based plastics. However, current production of lactic acid depends on carbohydrate feedstocks that have alternate uses as foods. The use of non-food feedstocks by current commercial biocatalysts is limited by inefficient pathways for pentose utilization. *B. coagulans* strain 36D1 is a thermotolerant bacterium that can grow and efficiently ferment pentoses using pentose-phosphate pathway and all other sugar constituents of lignocellulosic biomass at 50°C and pH 5.0, conditions that also favor simultaneous enzymatic saccharification and fermentation (SSF) of cellulose. Using this bacterial biocatalyst, high levels (150-180 g L<sup>-1</sup>) of lactic acid was produced from xylose and glucose by trapping the lactic acid as calcium salt. In a fed-batch SSF of crystalline cellulose, CaCO<sub>3</sub> addition also improved lactic acid production by *B. coagulans* with a yield of near 80% based on a final titer of about 80 g L<sup>-1</sup>. These results demonstrate that *B. coagulans* can effectively ferment non-food carbohydrates from lignocellulose to L(+)-lactic acid at sufficient concentrations for commercial application.