



BIOTORK AND NCERC: PAVING THE WAY FOR ECONOMICALLY VIABLE ETHANOL PRODUCTION FROM HEMICELLULOSE

Using Adaptive Evolution to Optimize the Growth Rate of Yeast Strains Genetically Engineered to Ferment Sugar D-Xylose

Gainesville, Florida and Edwardsville, Illinois– May 7, 2012 – BioTork and The NCERC at SIUE announce today the successful completion of the first step in a joint development program intended to improve the processes and economics of ethanol production.

During the first step of this program, BioTork used proprietary technology developed by Evolugate to facilitate, via adaptive evolution, growth optimization of yeast genetically engineered by the United States Department of Agriculture (USDA). The result is a strain of yeast that can grow on xylose as the sole source of carbon with ~3 fold faster growth rate. "While improvements to the growth rate and initial scale up of its performance in an industrial setting are underway, this strain has the potential to be one of the first economically viable xylose-fermenting strains, and represents a fruitful combination of genetic engineering and adaptive evolution" says Tom Lyons, Chief Scientific Officer of BioTork.

"This project is crucial for corn farmers, ethanol producers and for gas prices at the pump" says Sabrina Trupia, Director of Biological Research at NCERC. Dr. Trupia adds: "During the last decade, the ethanol industry has been focusing on the use of ligno-cellulosic biomass as low cost and abundant feedstock. Different agricultural residues have been considered such as corn fiber, corn stover, straw and bagasse but the stumbling block to commercial success has been the inability of most yeast strains to ferment the complex sugars in the ligno-cellulosic biomass".

Xylose is the second most abundant sugar in ligno-cellulosic biomass after glucose, accounting for up to 30% of the dry weight of biomass. The yeast *Saccharomyces cerevisiae* has been a workhorse for high-yield ethanol production for centuries. Yet, this yeast cannot ferment xylose. The USDA genetically engineered a strain of *S. cerevisiae* to be able to ferment xylose. However, as is often the case with genetic engineering, the act of genetically modifying the strain left it growth-attenuated, with a generation time of >20 hr on xylose. Thus, even though the strain can convert xylose to ethanol, it cannot do so with a high enough time-space yield to be of economic value. "The reason for this is simple" says Dr Lyons, "in many instances genetic

engineering produces strains that are 'competent' to do the job for which they were engineered at a laboratory scale, but incapable of doing so at a commercially viable scale".

The achievement of this first step presents several opportunities for the biofuel industry. First, it can generate immediate improvements for ethanol producers: With the glucose and xylose available in dried distiller's grains (a main by-product of corn ethanol production), ethanol production in the US can be increased by approximately 10% or 1.3 billion gallons with an estimated value of \$3.2 bn. With the right microorganisms, all the sugars in dried distiller's grains as well as other lingo-cellulosic corn residues can be fermented yielding up to 16 billion gallons of additional ethanol with the existing corn harvest. Second, since as much as 30% of all cellulosic biomass is comprised of the pentose sugar D-xylose, this proof of principle will open the door to the use a variety of non-corn biomass for ethanol production. And third, it illustrates the complementarity of genetic engineering and adaptive evolution: This is a proof of principle that genetically engineered microbes could be optimized through adaptive evolution for performance in the real world.

Created in 2008, BioTork LLC is a biotechnology company developing microbial strains to be used to produce biofuels and bio-renewable chemicals. The mission of BioTork is to achieve complete replacement of petroleum oil with biomass derived equivalents. BioTork is based in Gainesville, FL. Further information on BioTork is available on the internet at <u>www.biotork.com</u>

Founded in 2003, The NCERC is a not-for-profit research center that conducts research and findings for industrial and institutional clients. The center's mission is to facilitate the commercialization of new technologies for producing biofuels more effectively. NCERC is located on the Southern Illinois University campus in Edwardsville, IL. Further information on NCERC is available on the internet at http://www.ethanolresearch.com/

For more information contact

Ziad Ghanimi **Public Relations Manager** ziad.ghanimi@biotork.com Phone: (352) 505-8611 Mobile: (352) 213-7492 Skype ID: ziadghanimi

Courtney Breckenridge *Marketing and Policy Assistant* cbreckenridge@ethanolresearch.com Phone: 618-401-9218