Toward more cost-effective production of biofuels from plant lignocellulosic biomass

November 16 2011

Sascha Gille (left) and Markus Pauly (right), researchers at Berkeley's Energy Bioscience Institute, are part of the team that identified a gene responsible for *O*-acetylation of a hemicellulose in *Arabidopsis*. Credit: Markus Pauly

In 1925, Henry Ford observed that fuel is present in all vegetative matter that can be fermented and predicted that Americans would some day grow their own fuel. Last year, global biofuel production reached 28 billion US gallons, and biofuel accounted for 2.7% of the world's transportation fuel. Bioethanol, a popular type of biofuel, is largely derived from sugary food crops such as corn and sugarcane. However, technologies are being developed to generate bioethanol from non-food

sources, such as the lignocellulosics present in switchgrass and trees. The sugars locked in the polymers of cell walls, i.e., cellulose, hemicellulose and lignin, can be extracted and fermented by yeast into bioethanol.

A major obstacle to this strategy is that most wall polysaccharides are *O*-acetylated (i.e., chemically bonded to acetate groups), and the acetate released from these molecules during processing inhibits the activity of the microbes that ferment sugars into alcohol. Based on techno-economical models, a 20% reduction in biomass acetylation is predicted to translate into a 10% reduction in bioethanol price. Thus, a major goal in the field of plant biofuel research is to diminish the *O*-acetate content in the cell walls of plants, possibly by blocking the enzymes that acetylate the cell wall polymers. However, little is known about the acetylation enzymes in plants.

A team of researchers at the Energy Biosciences Institute, University of California, Berkeley, set out to identify the enzymes that acetylate the polysaccharides that are present in lignocellulosic feedstocks. Their initial work focused on xyloglucan, a type of hemicellose that is abundant in plant cell walls. Using a mass spectrometric technique, the scientists isolated a mutant from amongst a mutagenized population of the model plant Arabidopsis (a member of the mustard and cabbage family) that exhibited a 20-45% reduction in xyloglucan *O*-acetylation. The researchers mapped the mutation to a physical location in the Arabidopsis genome, and named the gene locus *ALTERED HEMICELLULOSE XYLOGLUCAN 4* (*AXY4*). Blocking the expression of *AXY4* in Arabidopsis eliminates xyloglucan *O*-acetylation.

A natural variety of Arabidopsis growing in northern Scotland also has low levels of xyloglucan *O*-acetylation. Intriguingly, this variety was found to have a natural mutation in the same gene - *AXY4*. This finding demonstrates that lack of xyloglucan *O*-acetylation does not represent a selective disadvantage for the plant, and supports the feasibility of

genetically blocking the expression of the protein that controls *O*-acetylation in plants destined for biofuel production.

"The identification of the first gene to encode a polysaccharide *O*-acetyltransferase opens the door for identifying similar genes in bioenergy crop feedstocks, such as miscanthus or other energy-grasses. These genes can be used as genetic markers to facilitate breeding programs that aim to generate biofuel feedstocks with reduced lignocellulosic acetate content," says Markus Pauly, a plant biologist at Berkeley's Energy Biosciences Institute.

More information: Sascha Gille, Amancio de Souza, Guangyan Xiong, Monique Benz, Kun Cheng, Alex Schultink, Ida–Barbara Reca, and Markus Pauly. 2011. O-Acetylation of Arabidopsis Hemicellulose Xyloglucan Requires AXY4 or AXY4L, Proteins with a TBL and DUF231 Domain. The Plant Cell, November 2011, tpc.111.091728. www.plantcell.org/cgi/content/short/tpc.111.091728

Provided by American Society of Plant Biologists