Energy Biosciences Institute

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EBI ASSOCIATE DIRECTOR PAUL WILLEMS (LEFT) AND BP-BRAZIL'S CAIO FORTES SURVEY HARVESTED SUGARCANE FIELD DURING SUMMER VISIT TO BRAZIL

IN THIS ISSUE

VISIT TO BRAZIL SAYS IT ALL: CELLULOSIC BIOFUELS DONE RIGHT WILL WORK

For the best example of biofuels done right, visit Brazil. We did, and we returned convinced that plant-based transportation fuels can and will provide a viable alternative to fossil fuels in the future.

By SUSAN JENKINS

After the United States, Brazil is the second largest producer of bioethanol and has the world's first sustainable biofuel economy. They produce sugarcane-sourced ethanol at a rate of more than 7 billion gallons a year, about one-third of the world's total ethanol used as fuel. So as a model for what might be done with a cellulosic ethanol industry, this nation is certainly worth studying.

Members of the EBI executive management team—Director Chris Somerville, Deputy Director Steve Long, Associate Director Paul Willems, and I—spent a week in August to see how Brazil makes it all work, albeit with the so-called "first generation" bioethanol feedstock, sugarcane. We visited a 112-year-old refinery in Sao Paolo state and toured sugarcane and bioenergy research centers. We participated in Brazil's first Bioenergy Science and Technology Conference (BBEST) and spoke with the country's leaders in the field.

And the take-away message was this: Brazil's infrastructure and its efficiently integrated processes are impressive examples of a successful agriculture-based fuel program, with potential to grow even more when they learn how to turn bagasse—that fibrous residue that remains after the stalks are crushed—-into ethanol. We planted the seeds for possible future collaborations between the EBI and the Brazilian ethanol research community and gained greater mutual understanding of our respective needs and goals.

Our visit also reinforced our belief that the things we are working on in the EBI can be applied to different crops, and that we can play a role in Brazil's energy future, just as the research going on there might be incorporated into the processes we are applying to energy grasses here in the U.S.

It is international partnerships like this that will be essential for the development of this burgeoning new industry.

It was winter in the southern hemisphere, but conditions were quite warm and a bit humid in the fields around Usina Ester, the historic sugar mill near Campinas that has been processing cane since

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EBI FELLOWSHIPS WILL SUPPORT PROMISING SCIENTISTS

The Energy Biosciences Institute, in an effort to recruit the best and brightest young researchers in bioenergy, has announced the availability of two EBI Postdoctoral Fellowships to support the awardees' expenses. In exchange for a promising research proposal that complements the EBI's mission, the Fellows will receive a salary and annual research budget for a two-year term with the possibility of extension.

"We want to discover and support individuals of outstanding talent and provide them with an opportunity to pursue independent research in the status of principal investigator," said EBI Director Chris Somerville of the University of California, Berkeley. "Successful candidates will be expected to integrate their research goals with the ongoing research of the EBI in the scientific quest to apply the systems and mechanisms of biology to the world's energy challenges."

"This is an outstanding opportunity for outstanding young investigators," said Deputy Director Steve Long of the University of Illinois. "The EBI operations at Berkeley and at Illinois are unique in providing horizontal subject integration within a single physical space, with researchers at all points in the supply chain from feedstocks and decomposition

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EBI in the News

HEAD OF EBI'S MICROBIAL HYDROCARBON RECOVERY TEAM OFF TO TENNESSEE

TERRY HAZEN

Lawrence Berkeley National Laboratory environmental biologist Terry Hazen, who has been principal investigator for the EBI's Microbially Enhanced Hydrocarbon Recovery (MEHR) program, will take his expertise to Tennessee as the newly named University of Tennessee-Oak Ridge National Laboratory Governor's Chair.

He begins on Dec. 1 as the Governor's Chair for Environmental Biotechnology and will join the faculty in the College of Engineering's civil and environmental engineering department. UT Chancellor Jimmy Cheek said Hazen "will help us build our emerging energy and environmental sciences specialty."

EBI Director Chris Somerville said Hazen "was instrumental in establishing the EBI's fossil fuel recovery program, and he helped us to assemble an outstanding team to continue our efforts in this area. We wish Terry the very best in his new venture."

Gary Andersen, who succeeded Hazen as Head of the Ecology Department in the Earth Sciences Division, will take over as principal investigator of the MEHR program at Berkeley Lab. Andersen has been a key contributor to the ecogenomics effort. He has a Ph.D. in Plant Pathology from UC Berkeley, currently specializes in microbial ecology, and is particularly well known for his co-invention of the Berkeley Phylochip, for which he and other ESD scientists received an R&D 100 Award in 2008. Earth Sciences senior scientist and Deputy Director for Programs Susan Hubbard will also be a principal investigator in MEHR at the Lab.

One of the world's foremost authorities on environmental microbiology, Hazen has been applying his expertise in his EBI program to find a more effective and ecologically friendly way of extracting oil reserves from existing wells. In MEHR, bacteria are called upon to do a variety of tasks, such as breaking down rock materials, producing substances that can improve the flow of water and oil in the reservoirs, forming biomass to improve the permeability of the rock strata, and transforming the oil itself into smoother-flowing structures. Hazen and his team in the EBI's program are using a systems biology approach to understanding the entire microbial community contained deep within the reservoir.

When the Deepwater Horizon oil spill happened in the Gulf of Mexico last year, he offered to help BP study the impacts on deep sea life. Working under a special EBI grant, he found bacteria—some never before seen—degrading the oil with unprecedented speed. The plume virtually disappeared in a matter of months.

He has also been working with the Joint BioEnergy Institute (JBEI) in Emeryville as its division head on microbial communities.

DOE GRANT FOCUSES ON IMPROVING VARIETIES OF MISCANTHUS

Researchers from the EBI are included in a recent grant from the U.S. Departments of Energy and Agriculture to broaden the genetic base for improving the efficiency of *Miscanthus x giganteus*, one of the most promising cellulosic biofuel feedstocks.

The grant is one of 10 announced in August as part of a broader effort by the Obama administration to develop domestic renewable energy and advanced biofuels. The total $12.2 million in funding is designed to improve special crops to be grown for biofuels—including selected trees and grasses—by increasing their yield, quality and ability to adapt to extreme environments.

Steve Long, Deputy Director of the EBI and a professor at the University of Illinois, and UC Berkeley postdoctoral researcher Megan Hall are among the investigators in the project "Quantifying Phenotypic and Genetic Diversity of *Miscanthus sinensis* as a Resource for Knowledge-Based Improvement of *M. x giganteus (M. sinensis x M. sacchariflorus)*." Both scientists are working on feedstock development projects with the EBI.

Hall's role in the DOE project is to do the genotyping of about 500 different Miscanthus varieties, collected throughout the native range in Asia. She has been optimizing large-throughput genotyping-by-sequencing (GBS) in Miscanthus for her current EBI project, working for UC Berkeley geneticist Dan Rokhsar on improving the genetics of bioenergy grasses for enhanced production.

"This is essentially an opportunity to use methods developed from the EBI project on a larger and more diverse dataset," Hall said. "I will lead the genotyping efforts and collaborate with others on the grant who will do phenotyping. Together, we should be able to conduct powerful association mapping to identify the locations of 'SNPs' or genetic regions controlling traits of interest to breeders."

The federal project is headed by Illinois crop scientist Erik Sacks. Its goal is to facilitate development of Miscanthus as a bioenergy crop by acquisition of fundamental information about genetic diversity and environmental adaptation. Miscanthus' improvement as a feedstock will require a broader genetic base. Identification of molecular markers associated with traits of interest will improve Miscanthus breeding efforts. Initial funding is good for up to three years.

Only a single sterile triploid genotype of *Miscanthus x giganteus* is currently available for feedstock production, which is a potential risk because a new disease or pest could cause damage to plantings. The added knowledge about Miscanthus genomics could lead to new tools for increasing the efficiency of breeding improved cultivars.

Field test sites have been identified in environmentally diverse locations in the U.S., Canada and Asia. Core genotypes will be collected for analysis from China, Japan and Korea and will be evaluated for yield potential and adaptation.

The joint DOE-USDA funding program was initiated in 2006 to support fundamental research in biomass genomics. Ultimately, the research seeks to develop and demonstrate environmentally acceptable crops and cropping systems for producing large quantities of low-cost, high-quality biomass feedstocks.

PEST/PATHOGEN EXPERT HONORED BY ENTOMOLOGY SOCIETY

MICHAEL GRAY

University of Illinois Crop Sciences Professor Michael Gray, principal investigator of the EBI program involving insect pests and pathogens, has received the Distinguished Achievement Award from the Entomological Society of America. The award is given annually to recognize outstanding contributions to the extension of entomology.

Gray was also honored by the Entomological Foundation with its Integrated Pest Management Team Award for his contributions on the European corn borer team.

"It's a great feeling," said Gray, who heads EBI's program on Assessing the Potential Impact of Insect Pests and Plant Pathogens on Biomass Production of *Miscanthus x giganteus* and Switchgrass. "I think it's very nice to be recognized by your peers. It was really kind of a humbling experience."

At Urbana-Champaign, he also serves as Crop Sciences Extension Coordinator and Assistant Dean for Agricultural and Natural Resources Extension programs.

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BP EXPANDS ITS REACH INTO BRAZIL AND ITS COMMITMENT TO BIOFUELS

With its announcement in September to increase its ownership shares and thus acquire all of the Tropical BioEnergia S.A. ethanol company in Brazil, BP has reaffirmed its commitment to the development and delivery of sustainable and competitive biofuels on a global scale.

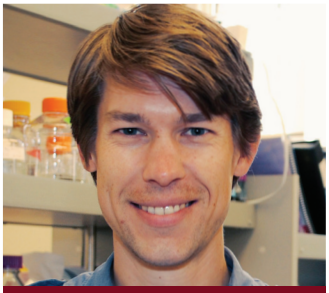
The acquisition brings the number of producing sugarcane mills in BP's Brazilian ethanol portfolio to three, all of which are located in the Goias and Minas Gerais states in the south-central part of Brazil.

"We have a major growth agenda for our biofuels business in Brazil," said Mario Lindenhayn, who heads BP Biofuels in Brazil. "This transaction, together with other recent acquisitions, gives us a strong platform from which to expand our capacity to supply both domestic and international fuels markets."

BP agreed to pay $71 million for the 50 percent of shares needed to gain full ownership of Tropical BioEnergia. The total crushing capacity of the plant, when fully developed, will be five million tons of sugarcane per year, with full mill production capacity of 450 million liters of ethanol annually.

The latest transaction brings the total amount that BP has invested in biofuels research, development and operations to more than $2 billion since 2006.

EBI TAKES TOP PRIZE, TWO 3RDS IN SYMPOSIUM POSTER COMPETITION



JOHN GALAZKA

UC Berkeley graduate student John Galazka took the $2,000 top prize for best poster in the Berkeley Energy and Resources Collaborative (BERC) 2011 Energy Symposium in October on the UC Berkeley campus. In addition, Berkeley graduate students Craig Dana and Chris Phillips placed third for their posters, each receiving $500.

The fifth annual BERC symposium attracted 84 poster entries. Galazka's entry, "A New Yeast for Improved Biofuels," featured the EBI work that also included Berkeley principal investigator Jamie Cate and University of Illinois PI Yong-Su Jin, plus UI postdoc Suk Jin Ha.

Dana's poster, "DNA Family Shifting of Cel7A for Improved Thermostability," cited Berkeley researchers Poonam Saija, Sarala Kal, and PI's Harvey Blanch and Doug Clark. Phillips' poster, "Investigation of GH61 Proteins and Their Biochemical Function," cited Berkeley graduate student Will Beeson and PI's Cate and Michael Marletta.

Galazka was also a finalist for the Deloitte QB3 Award for Innovation, which recognizes research that has the potential to improve human health. He was one of 38 initial nominees and among the final five honored at a ceremony on Oct. 27 in San Francisco. His entry, "Yeast Engineered for Biofuels," described his role in the EBI research that led to engineering a strain of yeast that digests two types of sugar through the introduction of a cellobiose transport pathway.

The award was developed from a partnership between Deloitte, a biotechnology consulting company, and QB3—the California Institute for Quantitative Biosciences—to honor the work of young reseachers at the three participation campuses (UC Berkeley, UC San Francisco and UC Santa Cruz).

EBI CHEMIST CHANG IS NIH INNOVATION AWARD RECIPIENT

MICHELLE CHANG

EBI Chemist Michelle Chang of UC Berkeley had added yet another honor to her growing portfolio of awards. The latest was announced by the National Institutes of Health, which in September named Chang as one of its Director's New Innovator Award recipients.

"The NIH Director's Award programs reinvigorate the biomedical work force by providing unique opportunities to conduct research that is neither incremental nor conventional," said James Anderson, who directs the NIH Common Fund from which the grants are drawn. "The awards are intended to catalyze giant leaps forward for any area of biomedical research, allowing researchers to go in entirely new directions.

Chang received one of 79 awards totaling $143.8 million in the latest round of NIH innovation funding. In addition to her work for the EBI researching lignin-degrading enzymes and microbial fuel synthesis, she is working on developing designer enzymes as synthetic tools for drug discovery and development.

Since 2008, Chang has won six national research honors, including support grants from the Dreyfuss Foundation, Agilent Technologies, the National Science Foundation and the Beckman Foundation.

NEW EBI PROJECTS SEEK CHEMICAL UNDERSTANDING OF BIOFUEL PATHWAYS

Two University of California, Berkeley, biochemists have been added to the roster of Energy Biosciences Institute researchers seeking a molecular understanding of what happens when organisms initiate the complex biochemical synthesis that results in the production of fuel from plants and microbes.

Complementing investigators of other projects in the EBI portfolio designed to discover the systems necessary to develop a non-petroleum-based transportation fuel industry, assistant professors Wenjun Zhang and Dave Savage both seek answers to research questions that might lead to the increased yield and efficiency of biofuel compounds.

Zhang's project will analyze the biosynthetic machinery involved in engineering so-called "volatile" compounds such as alkanes, alkenes, and alcohols, which are promising prospects to replace or supplement fossil fuels. Produced by bacteria, these volatile compounds would be options to the traditional bioethanol and plant oil-derived biodiesel.

She is particularly interested in a compound called 1-undecene due to its superior physical properties and its potential as both a fuel and an industrial chemical. Her goal is to fully characterize the enzymatic mechanisms of biosynthesis that occur in the production of 1-undecene by microbes, such that engineering similar pathways might increase the yield and efficiency of the hosts.

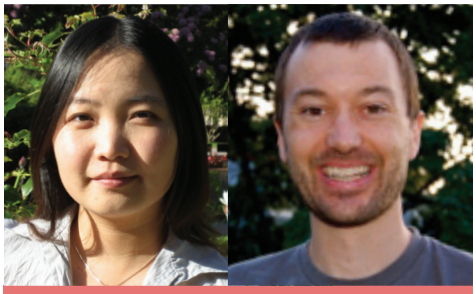
Zhang says the results "will expand our molecular toolbox for hydrocarbon biosynthesis and facilitate the production of new microbial fuels."

Savage also wants to add to the toolbox by engineering fluorescent protein biosensors that will allow scientists to identify important chemicals involved in biofuel synthesis. The ability to assay the presence of most small molecules is essential in the rapid construction of metabolically engineered organisms that will produce significant quantities of fuel.

He proposes to identify and characterize proteins capable of specifically binding biofuels and their metabolic intermediates and then use this protein toolkit to evolve fluorescent proteins capable of acting as biosensors.

A small number of the best biosensors will then be optimized and used to screen for novel biofuel-producing microbes with improved efficiencies.

The two new projects bring the total number of funded activities in the EBI to 87 since it began operations in 2007. Work continues at the three public partner institutions—UC Berkeley, the University of Illinois at Urbana-Champaign, and Lawrence Berkeley National Laboratory—with the support of industry partner BP.



WENJUN ZHANG AND DAVE SAVAGE

Investigations are being conducted in five focus areas —feedstock development, biomass depolymerization, biofuel production, enhanced fossil fuel recovery, and the impacts of a biofuel industry on the economic, social and environmental status of individuals and nations. Information on all programs and projects in the EBI can be found on the institute's web site, **www.energybiosciencesinstitute.org.**

ILLINOIS MODEL SHOWS BEST TIME, WEATHER TO HARVEST ENERGY CROPS

YOGENDRA SHASTRI

BioFeed is the name of a mathematical computer model that has helped Energy Biosciences Institute scientists to determine the best production systems for energy crops—things like types of harvesters to use, where to store the feedstock, and how much should be stored at one time. Now, they have taken BioFeed one step further and have incorporated weather data to determine what impact weather can have on production and harvest.

And what they found has changed conventional wisdom. Agronomy and crop studies have indicated that winter is the optimal time to harvest Miscanthus, one of the more promising of the energy grasses, in Illinois. But researchers are now discovering a different story.

"Winter in Illinois can be very difficult," said Yogendra Shastri, a visiting research assistant professor in the Department of Agricultural and Biological Engineering (ABE) at the University of Illinois and a member of K. C. Ting's team studying feedstock engineering systems in the EBI. "And you cannot ignore its impact on the feedstock harvest system."

He explained that BioFeed now includes the probability of working day (PWD) parameter. The PWD defines the fraction of days in a specific period (such as two weeks) that are suitable for field operations, and its value depends on a number of weather-related parameters, such as rainfall, snow depth, soil temperature, and soil moisture content. Researchers found that the average value of PWD for Illinois in winter is about 30 percent. Model simulations were conducted for Miscanthus for intended biorefinery capacity of 3,000 megagrams per day (about 90 million gallons per year of ethanol). The impact on total cost and farm machinery requirements was then quantified.

"We found that if you assume every day is available for field work, lower PWD value could increase your actual costs by as much as 38 percent. You lose biomass, and that has an impact on all the subsequent operations," said Shastri. "A way to compensate for that is to buy more machinery, so that you are still able to complete all the operations in the limited amount of time you have. However, this leads to about 34 percent more investment in machinery. So it's a trade-off."

Shastri and his colleagues have recommended that instead of starting the harvest in January, begin in late October or early November. That will have a significant impact on reducing the cost.

Researchers have also found that switchgrass, another candidate energy crop, is not impacted as much by weather since it is harvested in late fall when the weather is much more conducive.

"These results emphasized that the consideration of weather impacts on farm production activities is extremely important when selecting the appropriate regional energy crop," Shastri concluded.

This study, "Impact of Weather on Biomass Feedstock Harvest System Operations and Cost," was presented at the American Society of Agricultural and Biological Engineers Annual International Meeting in Louisville, KY in August. Researchers include Shastri, Alan Hansen, Luis Rodriguez and Ting.

— Leanne Lucas

BIOMASS CONVERSION: CAUTIOUS OPTIMISM IN THE FACE OF COMPLEX CHALLENGES

The First Symposium on Biomass Conversion, held this fall and sponsored by the EBI, delved deeply into the challenges of engineering microbes to process a myriad of feedstocks into biofuels at an industrial scale.

Jamie Cate of UC Berkeley, an EBI principal investigator and one of the presenters, summarized the problem simply: "The plant cell wall is an abundant source of recalcitrant sugar. The big issue is how to extract those sugars."

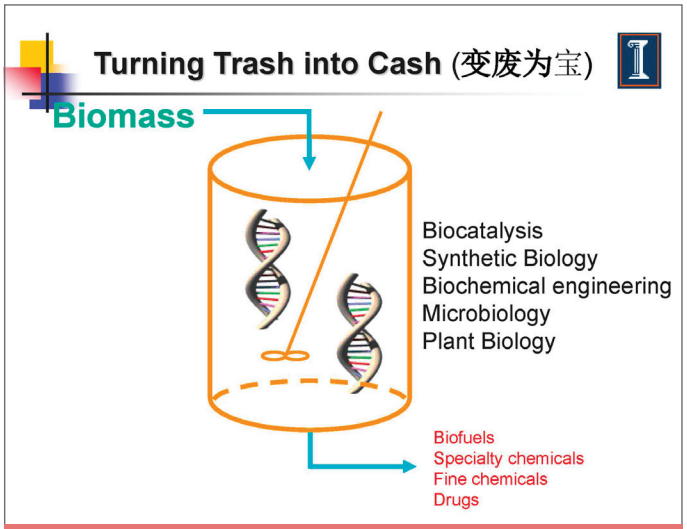
Most of the over 100 scientists at the conference, some who have worked on this problem for 30 years, knew that success will be anything but simple. Nevertheless, a feeling of optimism, despite the enormous challenges, seemed to pervade the gathering. Huimin Zhao, professor of chemical and biomolecular engineering at the University of Illinois and an EBI program leader, organized the event, which was held at the University's iHotel and Conference Center. He hoped the conference would spark collaborations and increase the flow of information between those working on biomass conversion issues.

"It went better than I even expected," he said. "There were many well-established experts; it is very rare to get them all in one room. EBI is doing exciting research and that is a big draw. In addition, the conference was more focused than others that I have been to. This was totally on engineering organisms to produce chemicals."

Conference speakers presented data that elucidated the complexities of the enzymes and pathways underlying biomass conversion. They also reported on efforts to engineer organisms, mainly yeast and *E. coli*, to optimize production of ethanol, and to identify other organisms that might be natively better able to process biomass and could be harnessed for that task.

Several speakers addressed challenges inherent in optimizing biomass conversion. The enzymatic processes that break down lignocellulose are complex and dependent on factors such as the specific feedstock, specific enzyme(s) used by a given organism, temperature and the presence or absence of oxygen. Optimizing those already complex processes and then scaling up to industrial production levels present major challenges.

Isaac Cann, another EBI investigator and professor of microbiology and animal sciences at Illinois, described the synergistic activity of enzymes in cow rumen. He explained that, because the plant cell wall is so complex, many enzymes are needed to deconstruct it. His work showed that multiple members of a given family of enzymes may have subtle differences that lead to enhanced depolymerization of the plant cell wall, if they are all present. It appears that the enzymes are working in conjunction with one another and the conversion is not nearly as efficient if only a single enzyme from that family is used.



CONFERENCE SLIDE SHOWS OTHER BYPRODUCTS OF BIOMASS CONVERSION

Saccharomyces cerevisiae and *E. coli* continue to be the workhorses of microbial engineering. One of the biggest challenges has been to modify microbes to process, not just the glucose in feedstocks, but also the xylose, another major source of sugar. For example, Nancy Ho, professor of chemical engineering at Purdue University, has worked since 1980 to develop strains of *S. cerevisae* that are highly efficient at fermenting glucose, xylose and several minor sugars on various substrates, from corn stover to wood.

Several EBI and BP researchers, including Yong Su Jin, Zhao, Cate and BP engineer Xiaomin Yang, have addressed this challenge by engineering a yeast strain that digests cellobiose (a dimer of glucose) and xylose simultaneously. The advantage to this approach is that it removes a step; pretreatment to break the cellobiose into a monomer (glucose) is not required.

Thomas Jeffries, professor of microbiology at the University of Wisconsin-Madison, advocated identifying "unconventional" yeasts that have beneficial properties. For example, several different yeast species can metabolize xylose preferentially compared to glucose. His approach has been to find these native organisms, identify and use those genes that ferment xylose. By selecting for these genes, along with genes for better sugar transportation and working within industrial constraints, Jeffries also is advancing efforts to use *S. cerevisiae* to co-ferment xylose, cellobiose and glucose. He also has identified a yeast strain that has a high tolerance for acetic acid, a common byproduct of fermentation that is toxic to yeast.

Cate, professor of chemistry and molecular and cell biology at Berkeley, described work his group has done using "unconventional" fungi, including *Neurospora crassa*, a model biomass degrader grown on burnt grasslands and burnt trees. His group also worked to remove the need to pretreat feedstocks by finding a way to produce ethanol from cellobiose instead of glucose.

William Metcalf, professor of microbiology at the University of Illinois, made a case for using methanogens rather than yeast or *E. coli* because they can produce methane gas, which already has a distribution network in place, unlike other fuels. Metcalf is working to develop methanogens that can produce methane from residual biomass present in the waste streams of biofuel production.

In addition to discussing various approaches to optimize biomass conversion, conference attendees also heard about several commercially successful ventures in bio-derived chemicals.

Doug Cameron of the Alberti Group, a venture capital company, opened the conference by noting that, with the current blend wall for ethanol, the market for fuel is almost saturated. But because of a strong and growing market for bio-derived chemicals that replace petrochemicals, there are many reasons to be optimistic about the demand for products made from biomass conversion. Bio-derived chemicals have higher profit margins and lower capital investment than biofuels, he said.

The business plans of many biofuels startup companies, such as Amyris, Gevo and Solazyme, include producing not only biofuels but also specialty chemicals, Zhao said. And, while "bio-derived" chemicals

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EBI STUDY: UNRAVELING THE MECHANISM OF ACETYLATION

Lignocellulosic biomass—such as energy grasses and wood residues—contains bound acetate in addition to the coveted sugars in cellulose and its rigid partners, hemicellulose and lignin. The acetate is released upon processing and is a major inhibitor of microbial fermentation of sugars into bioethanol, a popular type of biofuel largely derived from sugary food crops such as corn and sugarcane.

A study at the EBI in Berkeley has identified a plant gene responsible for adding acetate to a sugar-laden hemicellulose in plant cells, thus providing a new avenue for reducing the level of acetylation in plant feedstocks and thereby potentially lowering the cost of biofuel production.

According to the study, published in *The Plant Cell*, mutation of the gene, accomplished in the model plant Arabidopsis, a member of the mustard and cabbage family, eliminates acetylation of a hemicellulose.

A team of researchers at the EBI, headed by Markus Pauly, a plant biologist at the University of California, Berkeley, set out to identify the enzymes that acetylate the polysaccharides, which contain multiple sugars, that are present in lignocellulosic feedstocks. Their initial work focused on xyloglucan, a type of hemicellulose that is abundant in plant cell walls.

Using a mass spectrometric technique, the scientists isolated a mutant from among a mutagenized population of Arabidopsis plants that exhibited a 20 to 45 percent reduction in acetate bound to xyloglucan. The researchers mapped the mutation to a physical location in the Arabidopsis genome and named the gene locus "Altered Hemicellulose Xyloglucan 4," or AXY4. Blocking the expression of AXY4 in Arabidopsis leads to an elimination of the bound acetate.

"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," said Pauly. "These genes can be used as genetic markers to facilitate breeding programs that aim to generate biofuel feedstocks with reduced lignocellulosic acetate content."

SASCHA GILLE AND MARKUS PAULY

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 composed of cellulose and hemicellulose packed in lignin can be extracted and fermented by yeast into bioethanol.

A major obstacle to this strategy is that most wall polysaccharides contain 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 percent reduction in biomass acetylation is predicted to translate into a 10 percent 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 has been known about the acetylation enzymes in plants.

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 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.

In addition to Pauly, other EBI authors on the *Plant Cell* paper include Sascha Gille, Amancio de Souza, Guangyan Xiong, Monique Benz, Kun Cheng, and Alex Schultink. Also participating was Ida-Barbara Reca of the DOE Great Lakes Bioenergy Center at Michigan State University.

The paper, "*O*-acetylation of the Hemicellulose Xyloglucan Requires AXY4/AXY4L Proteins with a TBL and DUF231 Domain," can be accessed at **http://www.plantcell.org/cgi/content/short/tpc.111.091728?keytype=ref&ijkey=XdR7NTjE6reEzPL**. *Plant Cell* is the journal of the American Society of Plant Biologists.

RENEWABLE FUEL STANDARDS: 'CORNERSTONE LEGISLATION' FOR BIOFUELS


JAY KESAN

The Energy Biosciences Institute's lead faculty member on biofuel law and regulation told a Congressional subcommittee that the federal Renewable Fuel Standards (RFS), currently under review for renewal and possible modification, are essential elements to the further expansion of advanced biofuels.

"The RFS is a cornerstone piece of legislation for the biofuel industry," University of Illinois law professor Jay Kesan told members of the House Subcommittee on Energy and the Environment earlier this month. "The RFS mandates will accelerate the production of advanced biofuels and lead to more cumulative experience and promote the innovation needed to lower production costs in the future."

The hearing in Washington D.C. was held to discuss "Conflicts and Unintended Consequences of Motor Fuel Standards." Kesan was one of seven witnesses invited to speak by subcommittee chair Andy Harris (R-MD) and his colleagues.

Kesan, a principal investigator for the EBI, cited evidence that the RFS has already yielded tangible results "on the ground," with companies like BP, POET, Abengoa and INEOS breaking ground on new plants and projects for advanced biofuels.

"I am an engineer and a lawyer," he told the panel. "But my esteemed colleagues at the Energy Biosciences Institute, who are world-class experts in the plant sciences, tell me that scientific advancements have already solved the problem of obtaining sugars from lignocellulosic biomass many times. Therefore, it is now only a matter of technological effort and time, together with a foundational policy such as the RFS, before we see large-scale production of advanced biofuels."

The RFS requires the production of 36 billion gallons of renewable transportation fuels annually by 2022. Kesan said such a mandate encourages commercial producers to develop large-scale operations and induces them to invest in research activities that involve cost-saving innovation. He said it also removes a degree of uncertainty and risk from investors who now see a guarantee of market demand in the future.

Kesan reiterated the rationale behind the RFS legislation—to enhance national energy security, to reduce greenhouse gas emissions, and to promote economic development, especially in rural areas. "All of these drivers are definitely still with us today and will continue to remain important in the foreseeable future," he told the group.

Critics of the RFS also testified, including Chairman Harris, who referred to "heavy handed mandates" when he said "the picking of energy winners and losers by government fiat is an exercise in futility destined to fail miserably."

DOE'S KOONIN HIGHLIGHTS BIOMASS AND ENERGY CROPS IV CONFERENCE

EBI ENERGY FARM FIELDS, AND STEVE KOONIN

When it comes to energy, the United States can do better, said Steven Koonin, Undersecretary for Science in the U.S. Department of Energy, at the 2011 Biomass and Energy Crops IV Conference in Champaign last week.

"Scientists play an important role in advocating for basic research to help solve our energy challenges," Koonin said during the conference, co-hosted by the Association of Applied Biologists (AAB), University of Illinois, and the Energy Biosciences Institute (EBI) on Sept. 21-23. "We need scientists to understand the bigger problems—both as citizens and as scientists. We also need their help to advocate for sensible policies and to become more involved from a technical standpoint to make sure the science can be applied commercially."

He defined three major U.S. energy challenges and painted a six-picture strategy to provide a framework to overcome these challenges. "Each strategy involves a mix of policies, economics and technologies," Koonin said.

The transport strategies include deploying alternative fuels, progressively electrifying the fleet, and increasing vehicle efficiency. The three stationary strategies include deploying clean electricity, modernizing the grid, and increasing building and use efficiency.

Koonin, who was instrumental in the design and development of the Energy Biosciences Institute during his time as Chief Scientist for BP, also encouraged more research into the cost of energy crop production.

"In order to bring energy crops to scale on unused land, two things have to happen," he said. "You have to drive the cost of production down to be competitive with fossil fuel. And second, land mandates are necessary. No one will do it if they can't make money. Once you demonstrate that it's competitive, it will take off. Miracles are already happening—you just have to make it cheaper."

Tom Voigt, associate professor in the Department of Crop Sciences and principal investigator for the EBI's agronomy program, said Koonin played an instrumental role in helping biomass and energy crops research increase in the United States through his former role at BP.

In addition to hearing Koonin's address on energy, attendees engaged in three days of presentations about the latest in biomass and energy crops research throughout the United States and Europe. Miscanthus and willow generated the most discussion time from both sides of the Atlantic, Voigt said.

"The Association of Applied Biologists is primarily a UK-based group," Voigt said. "They are interested in growing membership worldwide and sharing information on a much broader basis. We decided to co-host the meeting this year to help build relationships."

In addition to fostering future collaborations, the conference provided attendees from across the world the opportunity to tour EBI's Energy Farm on the U of I campus. Tour hosts included EBI researchers Tim Mies, D.K. Lee, Gary Kling, Pat Brown, Erik Sacks and Voigt.

"I was really impressed with the Energy Farm," said Richard Weightman of ADAS in the United Kingdom. "I admire the way they have integrated the environmental monitoring with biodiversity and conservation aspects, as well as energy crop production. The Energy Farm is a fantastic and unique facility that I hope will continue for a long time."

Trevor Hocking, AAB president, said the input from EBI researchers was extremely beneficial for participants. "We hope to organize another meeting in the United States in the foreseeable future to build on the base that we have established and to bring in other major players in applied biology and plant agriculture," Hocking said.

— Jennifer Shike

EBI IN BRAZIL (CLOCKWISE): FIELD WORKER LAYS STALKS IN FURROW, FROM WHICH NEW CANE WILL GROW, TOURING THE REFINERY, VINASSE BEING SPREAD WITH HIGH-PRESSURE HOSES ONTO NEW CANE GROWTH, MECHANICAL HARVEST OF SUGARCANE, EBI ENTOURAGE READY TO TOUR SUGARCANE REFINERY, LOADED CANE TRUCK ENTERS REFINERY GROUNDS, THOUSANDS OF YOUNG SHOOTS AT SUGARCANE BREEDING CENTER

VISIT TO BRAZIL SAYS IT ALL: CELLULOSIC BIOFUELS DONE RIGHT WILL WORK

(cont. from page 1)

1898. They annually crush nearly 2 million tons of cane, producing three different grades of ethanol and sugar for food.

As we walked the dusty landscape and watched the harvesting machines slice through the fields of cane and shoot the cut-up stalks into trucks to be hauled to conveyor belts at the mill, I was impressed with how everything in the process is integrated. The bagasse, cake and vinasse generated in the steps from cleaning the cane to producing the ethanol are all re-incorporated into the overall system. The dried bagasse is burned to generate steam and electricity for the mill, while the vinasse and cake are both returned to the field to provide irrigation and nutrients for the next generation of cane.

At Usina Ester, the vinasse is returned to the field by pipelines where pressurized cannons spray it over the crops. The residual cake that's left when the purified sugar is removed is collected and dried, then spread over new field plantings for nourishment. Meanwhile, on the periphery of the fields, workers were hand-cutting the cane into long pieces that will be planted in rows of newly plowed fields. The long stalks are laid overlapping one other in the furrow, the nutrient cake is added and the plants get a fresh start for what will be 5 to 7 years of continuous growth and harvest.

The refinery and distillery itself shows remnants of its age but seems quite contemporary in its operation. It runs 24 hours a day, 8 months out of the year. Its centrifuges, crystallization tanks, and distillation equipment are all state-of-the-art, but in an era that demands minimizing steps and maximizing output, we sensed opportunities for improvement and for some producers a willingness to discover them.

The Brazilian governments – both federal and state – are quite supportive of the research enterprise, as are the biofuel industries there. We toured one of the top scientific centers dedicated to bioenergy research, the Brazilian Bioethanol Science and Technology Laboratory (CTBE). As Scientific Director Marcos Buckeridge told us, CTBE was established to investigate an impressive range of technical and non-technical issues related to biofuel production, similar to that supported in the EBI. We were also led by Strategic Development Director Jaime Finguerut through a leading sugarcane technology center (CTC) in Piracicaba, where they breed 70 varieties of cane, tested in plots five times the size of the EBI Energy Farm.

We were accompanied on much of our trip by BP employees Wesley Ambrosio, Caio Fortes, Marco Souza, Daniel Atala and Americo Ferraz. This team is gearing up for BP's newest venture in Brazil, acquiring two producing ethanol mills and another un-

FROM LEFT (ABOVE): RAFAEL VALAMEDI, AGRICULTURAL SUPERVISOR FOR THE USINA ESTER MILL; MARCO SOUZA, WESLEY AMBROSIO AND DANIEL ATALA OF BP-BRAZIL; AND THE EBI'S SUSAN JENKINS, STEVE LONG, CHRIS SOMERVILLE AND PAUL WILLEMS.

der development in the Cerrado region. Included are over 100,000 acres of sugarcane fields.

Our trip began in Campos do Jordao outside of Sao Paolo, where the BBEST conference was held. The EBI was well represented, with Chris giving a plenary talk on second-generation biofuels, Steve offering a tutorial on cellulosic ethanol and a talk on biomass and climate change, and UC Berkeley microbiologist Jamie Cate presenting "A Tale of Two Fungi: Improving Biofuel Production from Plant Biomass."

So the learning that week was two-way: EBI expertise offered perspectives on biofuels for the Brazil ethanol community looking to lignocellulosic options, and our team gained a much deeper appreciation for the process and the infrastructure required to manage commercial production. BP will also benefit as a new corporate partner in Brazil.

The EBI has established several useful ties to the country. One project in our portfolio, headed by UC Berkeley environmental scientist Alistair Iles, is focusing on land use issues and the impacts of cattle intensification on feedstock growth. Resource economists Madhu Khanna of the University of Illinois and David Zilberman of UC Berkeley will be engaging in discussions with counterparts in Brazil on the economic dimensions of biofuels and fuel markets, while law and policy expert Jody Endres, also at Illinois, has begun to study Brazilian biofuel policies. Through exchange programs, student interns from Brazil have been contributing to EBI programs on economics, policies and regulations.

Clearly, we have much to share. Brazil and the United States lead the world in sustainable fuel development and production, and as we progress toward a global bioenergy future, these two nations will be the beacons for a successful, responsible biofuels market. The doors are open for meaningful dialogue and collaboration.

Susan Jenkins is Managing Director for the Energy Biosciences Institute. She oversees administrative activities for the EBI and in addition, as manager of the institute's outreach and education programs, she represents the program as invited speaker at various conferences and meetings, including this fall's Ecopetrol forum in Columbia and at BioRefine, a meeting of the New Biomass Products Programme in Finland. Currently she serves as the president of the Washington DC-based Council on Energy Research and Education Leaders, a consortium of 35 universities.

CARBON SEQUESTRATION POLICY MUST BALANCE PRIVATE, PUBLIC INTERESTS


BRYAN ENDRES

The lack of a settled legal framework that balances private property rights while maximizing the public good ultimately hinders the large-scale commercial deployment of geologic carbon sequestration, according to published research by EBI renewable energy law expert A. Bryan Endres.

In order to justify major investment by firms, issues with the property rights of the subsurface pore space that would permanently house the captured carbon dioxide must be resolved first, says Endres, a professor of agricultural law at the University of Illinois.

"You have a new technology that requires a lot of upfront capital investment, but you don't have a legal framework for how you're going to be able to implement this technology with regard to property rights," said Endres. "What's unique about property rights is they're usually pretty well settled, and yet here we are dealing with a situation where ownership isn't quite so clear. That's a key question, because a firm isn't going to invest money in a carbon sequestration plant before they are confident about who owns the area underneath."

According to his study, published in the University of Illinois Law Review, ownership of the pore space at the depths necessary for permanent geologic carbon sequestration is still an open question in the vast majority of states.

"Right now, only Wyoming, Montana and North Dakota have assigned the property rights of the pore space to the surface property owner," Endres said. "While that might make good political sense, I don't think that makes good policy sense because it creates a patchwork of small landholdings. With carbon sequestration, the geology is going to determine the limits, not some grid-based property system. This is why we need to have legislative involvement to clarify the situation."

Endres says sequestration operations implicate a unique set of property rights issues, one that's analogous to a plane flying over a house at 30,000 feet.

"Do you own the airspace above your house?" he said "Well, no, and the reason we know the answer to that question is that there was a court case that settled the issue. And that was one of the things that allowed the airline industry to develop, so that planes didn't have to weave around an easement, like railroads do. Similarly, picture a really deep hole that may start on your land but goes down 7,000 feet. Who owns that? One argument is that a property owner does not have a reasonable expectation of ever using the pore space at such extreme depths."

Like air transport, carbon sequestration should be thought of as a public good—one that has the added potential to reduce carbon dioxide emissions and curb global climate change.

"It makes more sense to treat it as you would airspace for an airplane, in that it belongs to the state and they can decide who's going to access it," Endres said. "It would be a much more efficient system if the state had ownership of it."

Endres notes that there's also the potential for states to generate a significant amount of revenue from carbon sequestration, either through an auction or a royalty system.

While this isn't necessarily the silver bullet to reverse carbon dioxide emissions, Endres says it's one of many ready-made and already available tools that could slow the growth rate of global climate change. "This is a technology that will allow us to utilize natural resources like coal while also shrinking its carbon footprint," he said. "So it's important to get this framework in place so the industry can really take off, because now you just have a lot of speculation, experimental labs and pilot projects. This is something that needs to get developed sooner rather than later."

— Phil Ciciora

DEFINING MOMENTS FOR POLICY-MAKERS IN NEW BIOFUELS


JODY ENDRES

Making policies to regulate biofuels is like shooting at a moving target, and until various rule-making agencies come up with some common definitions on what is "sustainable" and "renewable," the fledging industry will be less able to develop globally in a responsible way.

That is one of the conclusions by Energy Biosciences Institute senior regulatory associate Jody Endres in her article "No Free Pass: Putting the 'Bio' in 'Biomass'," in the summer 2011 issue of the American Bar Association's *Natural Resources and Environment*.

Endres first provides the history of "sustainability" definition in U.S. agricultural and energy policies, noting that the reduction of greenhouse gas (GHG) emissions has emerged as the primary denominator in definition development. She predicts that the next "decade promises further refinement of what it means for biomass-based energy to be 'renewable,' 'green,' 'alternative,' 'advanced,' 'next generation,' or 'sustainable.'"

"Sustainability" definitions, she says, will also affect budget decisions involving incentive payments that encourage biofuels development, land use decisions and their environmental impacts, and the ongoing "food vs. fuel" debate that has raised questions about the efficacy of an agriculture-based energy industry in the face of continuing world poverty.

And within the land use category itself, defining the terms "degraded," "marginal," "abandoned" and "idle" will be critical in both the U.S. and Europe as policies and standards proscribe biofuels' future course.

Endres, based at the University of Illinois, draws upon her experience leading U.S. standards-drafting efforts to conclude that science will play a major role in resolving the questions. "Whether government-sponsored or private in nature," she says, "any sustainability standard for energy biomass relies critically upon scientific research to gauge achievements." She opines, however, that such research is in its embryonic stages; "…measuring the costs, benefits, and barriers and enforcing different levels of sustainability will be critical to development of the biomass sector."

Biofuels' impact on food production continues to be a contentious issue—food price spikes like those of 2008 had some blaming the corn ethanol industry, while others cited factors unrelated to biofuels' growth. "This poses particular challenges for standards development, as policies must hone mechanisms to measure and mitigate any adverse impacts biomass cropping may have on food prices, while at the same time addressing GHG impacts from direct and indirect land conversion," Endres writes.

Governments and agencies have been struggling with a proper balance of incentives and direct regulation for years. Policy solutions, she says, will require innovative thinking about global resource pressures and consistent accounting methodologies that measure economic, social and environmental costs and benefits.

As governments reconsider land use policies in an attempt to better balance humankind's social and economic needs with those of natural systems, the refinement of the definition of 'sustainable' renewable energy will structure development of the biomass sector.

She adds that producers who embrace the biomass sustainability standards could reap economic opportunities as early movers and serve as leaders in fashioning "a new sustainability paradigm" in agricultural landscapes.

FALL 2011 EBI PUBLICATIONS

The following journal articles featuring EBI research were published online in August, September and October this year. Papers can be accessed via the EBI web site —**www.energybiosciencesinstitute.org** —under Publications in the "Resources" category.

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BIOMASS CONVERSION: CAUTIOUS OPTIMISM IN THE FACE OF COMPLEX CHALLENGES

(cont. from page 5)

are not biofuels, they do fit with the overarching goal to displace petroleum as a feedstock.

Michael Lynch, chief scientific officer of OPX Biotechnologies, described how his company has engineered *E. coli* to produce acrylic acid, which can be used in paints, diapers (the material is super absorbent), water treatment, detergents, soaps and shampoos. OPXBIO focuses on acrylic because it is an $8 billion-per-year market and growing. Bioacrylic is less expensive than petroleum-derived acrylic and is sustainable.

Like bioacrylic, succinic acid is a "platform chemical" that can be used in a wide range of products, and Myriant Technologies' Jan Pero explained its utility. Sundeep Vani of Archer Daniels Midland described a joint venture with Metabolix to make biodegradable plastic that can be thermoformed, extruded in either sheets or film (plastic bags), or injection molded.

At this stage, it appears that no single feedstock and no single enzyme will address every biomass conversion need. The conference showed that conversion is a challenge that — at least in the short term — will be solved, not by a silver bullet, but by flexibility, perseverance and creativity.

— Deb Aronson

EBI FELLOWSHIPS WILL SUPPORT PROMISING SCIENTISTS

(cont. from page 1)

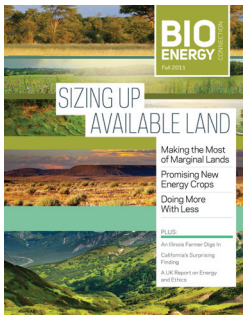
to fuel synthesis and life-cycle analysis. Within this context the Fellow has the rare opportunity to devote 100 percent time to developing their own idea and team to solve the renewable energy challenge."

Fellowship candidates cannot apply; they must be nominated by their research advisor or other distinguished scientist from the candidate's field or institution. To be eligible, candidates must have, or be about to receive, a doctoral degree. EBI Fellows will be selected on the basis of their academic achievement, the promise of their research, and the relevance of their research interests to EBI's mission. Preference will be given to candidates whose research spans multiple labs at either Berkeley or Illinois campuses.

Nominations, in the form of one- to two-page letters, should assess the candidate's research accomplishments and future potential. Once the EBI review committee decides upon "candidates of interest," those individuals will be asked to submit a two-page proposal describing the research they would like to pursue, a completed application form, a current curriculum vitae, and two additional letters of reference by people familiar with the candidate's research and academic accomplishments.

Review of applications began on Dec. 1 and will continue until the position is filled. The final application deadline is Feb. 28, 2012. Nomination deadline is Jan. 17, 2012. Selected Fellows will start their work no later than six months after the offer date.

Information on the EBI Fellows Program, as well as nomination and application forms, can be found on the EBI web site at **http://www.energybiosciencesinstitute.org/**. Questions about the program should be directed to **ebifellows@lists.berkeley.edu.**



COMING SOON

EBI Fall magazine

An estimated 20,000 people showed up at the San Francisco Giants' home stadium in San Francisco on Nov. 6 to hear about regional science achievements at the first Bay Area Science Festival. The EBI was one of more than 200 organizations to staff booths and share information, like Berkeley graduate student Padma Gunda (above), who talked biofuels with a visiting guest on the crowded ballpark promenade. Also carrying EBI's message were Will Beeson, John Galazka, Mike Fisher, Philipp Benz, Fernanda Haffner, Dacia Leon, and Mark Shaw.

EBIBULLETIN
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