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THE EBInsider

HOPE (FOR BIOFUEL) SPRINGS ETERNAL AT EBI ENERGY FARM

As spring signals the emergence of farm crops in America, researchers on the University of Illinois/EBI Energy Farm have big plans for moving perennial grasses closer to commercial viability.

With the farm more than twice as large as last year, there's plenty of room for investigating the possibilities of a commercial-sized biofuel crop.

"What we're going to discover is that we're going to take it from a research scale to an agronomic scale," said Tim Mies, deputy operations director for the EBI who oversees the farm.

In the past, the facility had budgeted only small research-size plots for several different crops. But with more land for the upcoming summer, Mies said the plan is to roll out the crops on a larger scale to see how they work in a commercial scenario. The Energy Farm has the largest renewable fuels research area in the nation—more than 300 acres—planted with Miscanthus, switchgrass, corn, and prairie grasses.

Illinois researcher Rebecca Arundale, a graduate student in crop sciences, said that Miscanthus is the predominant focus in Europe, whereas switchgrass is currently getting the most attention in the United States as a prospective biofuel feedstock.

Arundale will soon begin a trial from which she hopes to determine which species is best suited for certain areas of the United States. She plans to set up 10 small research-size plots throughout the country to see which species grows best under given conditions. One of those plots is at the Energy Farm.

"With energy crops in the U.S., it's going to take a lot of different solutions,"

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PREPARING FOR THE BIG ONE–MENTALLY AND PHYSICALLY

With this issue, the Insider is debuting a new regular feature. "Safety First" will explore the many ways that EBI employees at both Berkeley and Illinois can ensure a safe and healthy workplace. This first column addresses preparation for a major earthquake in Northern California.

Zack Phillips says it's not a matter of "if," but "when."

He doesn't want to scare anyone with gloom-and-doom scenarios, but there's no escaping the facts—according to the U.S. Geological Survey, there is better than a 3-in-5 chance that the Bay Area will experience a devastating 6.7-or-above earthquake in the next 30 years. And the most likely place for that to occur is along the Hayward Fault, which runs underground a stone's throw from EBI headquarters in Calvin Laboratory.

To calibrate that, consider that the destructive 1989 Loma Prieto quake

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INSIDE THE INSIDER

EBI PEOPLE

ANTARCTICA VISITORS (FROM LEFT) EBI INVESTIGATOR NORM MILLER, EXPEDITION LEADER ROBERT SWAN, AND BERKELEY PH.D. STUDENT KELLY KARNS

EXPEDITION GIVES EBI INVESTIGATOR CLOSE-UP LOOK AT CLIMATE CHANGE

Norm Miller is a climate scientist, so it comes as no surprise that, when given a chance to go to the end of the earth to witness climate change first-hand, he jumped at it. His EBI project, which is assessing biofuel productivity potential around the world under changing climate, land use and economic conditions, would also benefit from the experience.

So it was that Miller, Berkeley Lab staff scientist and adjunct professor in UC Berkeley's Geography Department, was one of eight academic experts invited to join the BP-sponsored "Expedition Antarctica 2009" to see the planet's iciest continent and observe how the warming climate is changing it. After 11 cold April days and nights on ship and on foot, he returned to Berkeley with a renewed commitment to attack the issue.

"It was an opportunity to see real-world climate change phenomena that I've studied in models and through satellite observations," he said. "The on-ice experience was humbling, the ship interactions taught me useful social dynamics, made me aware of true corporate responsibility and next-generation efforts toward preserving our world's resources."

The corporate model was set by global energy company BP, the EBI's sponsor, who

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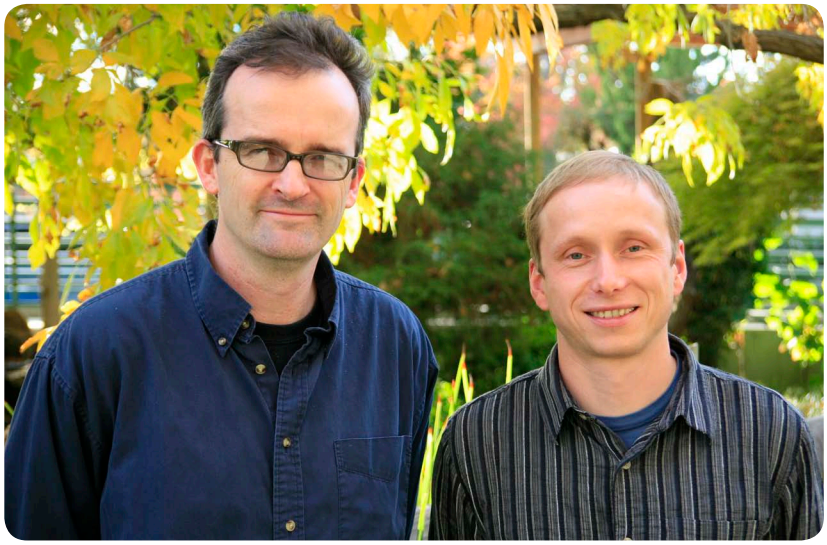
TWO FROM JGI ON 'CUTTING EDGE'



PHIL HUGENTHOLTZ AND FALK WARNECKE

Phil Hugenholtz and Falk Warnecke, EBI researchers at the Joint Genome Institute (JGI) who are studying enzymes in termites as candidates for biomass deconstruction, were among six scientists profiled by *U.S. News and World Report* magazine for their status "on the cutting edge of energy and environmental research."

The publication's April 1 issue recognized principal investigator Hugenholtz and postdoctoral researcher Warnecke for their 2007 *Nature* paper in which they identified more than 500 genes in termite guts associated with enzymes that break down cellulose. Natural enzymes like these, it notes, may offer a path to more efficient biofuels.

"The focus has turned to grass-feeding termites where discoveries might be even more applicable to commercial biofuel production," the story says. It quotes Hugenholtz: "One of the goals is to find these novel enzymes, enzymes that haven't been found before in other places."

EARLY EBI SUPPORTER HEADS UC BERKELEY RESEARCH PROJECTS

Physical Bioscientist Graham Fleming, who was among the pioneer faculty who put together the concepts that eventually led to the formation of the Energy Biosciences Institute, has been appointed UC Berkeley's Vice Chancellor for Research by Chancellor Robert Birgeneau. Fleming, former Deputy Director at Berkeley Lab, succeeds Beth Burnside, another EBI facilitator and pioneer, who stepped down in September.

"I want to make the office more entrepreneurial and outward-looking, in order to maximize research opportunities for faculty and students of the university and to maximize the impact of that work on society," he said, citing the EBI as one example of such an effort, and representative of a bigger potential beyond energy research.

"One lesson we learned from EBI was that when we bring together all our strengths, all the amazing range of expertise that we have at Berkeley, we are unbeatable."

A native of England, Fleming started at UC Berkeley and the Lab in 1997 and founded the Physical Biosciences Division. He was also founding director of the UC Berkeley arm of the California Institute for Quantitative Biosciences, known as QB3.

GRAHAM FLEMING

EBI-BERKELEY RESEARCHER AMONG TOP TEACHERS

EBI researcher Max Auffhammer has received one of UC Berkeley's most prestigious recognitions: Distinguished Teacher for 2009. He and four colleagues on campus received the award, bestowed annually by the UC Berkeley division of the Academic Senate's Committee on Teaching, which is comprised of faculty members and students. It was instituted in 1959 and is considered one of the highest honors for a professor or lecturer. Only 232 professors have received the award to date.

Auffhammer, assistant professor of Agricultural and Resource Economics and International and Area Studies, is a co-principal investigator for the EBI team studying "Life Cycle Environmental and Economic Decision-Making for Alternative Biofuels."

The award noted that he "has revitalized Intermediate Microeconomics, one of the least successful courses in International Area Studies (students called it 'economics for students who hate economics'), turning it into one of the most popular and successful courses in the undergraduate program."

Students point to the fact that he manages to turn a subject to which they don't necessarily respond into something fascinating and relevant. "I have done poorly in econ before," said one student in evaluating the course. "And this is a 180-degree change for me. I now actually like econ."

As part of the screening process, committee members read at least two years' worth of the candidates' student evaluations, which can number into the thousands, observe them as they teach, and evaluate their teaching philosophy and course materials.

BP Top Scientist Named for DOE Post

BP Chief Scientist Steve Koonin and U.S. Department of Energy Secretary Steve Chu last worked together on a project when they helped put together the blueprint for what became the Energy Biosciences Institute. Now the two distinguished researchers are together again – Koonin was selected to be Chu's under secretary for science, overseeing the DOE's Office of Science in Washington, D.C.

Koonin, a physicist who has guided BP's long-range technology strategy since 2004, was one of the main architects of the EBI when Chu headed the proposal effort for Berkeley Lab in 2007. The former Caltech professor and provost is a long-time supporter of biofuels and advocated in a *Science* magazine editorial in 2006 for "a coordination of government, university and industrial R&D efforts, facilitated by responsible public policies" to address the challenges of bioenergy development.

Five Questions

...for Public Engagement Specialist Anne Heinze Silvis

For cellulosic biofuels to be successful as the renewable-of-choice in the future, the public must embrace them — from the farmers growing the feedstock to the consumers using the fuel. To explore perceptions about biofuels, Anne Heinze Silvis of the University of Illinois is leading an EBI research project on "Synergizing Engagement with Stakeholders."

ANNE HEINZE SILVIS

She and her co-principal investigators want to create a "learning community" in which farmers, consumers and researchers provide and exchange information and perspectives. The ability of these groups to assimilate information and reach common points of understanding will be critical to future decision-making and policy development.

Silvis is an Extension Specialist in program development for U of I Extension, and is Director of the Laboratory for Community and Economic Development, Department of Human and Community Development. Her work focuses on topics that include leadership development, community leadership, sustainable agriculture, and telecommunications and access in rural areas.

The EBInsider asked Silvis five questions about how this project will work toward synergy among the key stakeholders in biofuels production, processing and consumption.

1. You are sending questionnaires to 3,600 farmers across Illinois. What are you asking them, and what do you hope to find out from the data?

The questionnaire solicits information about farmers' information needs. That is, do they need information about production or marketing, or are they concerned about introducing a new crop? With this information, we will provide input to researchers as they address farmers' information needs. We also ask about how farmers want to get information. For example, would they prefer conferences or mailings or going to the Web? With those preferences, we will know how to share information in the best format for the end users.

2. Previous studies have explored farmers' perceptions of Miscanthus. What were those perceptions, and how will that inform your research for this project?

When we asked farmers specifically about Miscanthus, we found out that many Illinois farmers were positive about trying a new crop, but were concerned about the market for the crop. During the focus group discussions, farmers talked in detail about how they would adapt their production processes for a new crop—it was exciting to listen in on discussions among entrepreneurial farmers as they strategized about the potential of Miscanthus. Even while they were considering these options, they were also carefully considering their risk, and cognizant of the larger picture of energy production and consumption. We expect that farmers will approach energy and alternative crops in a similar way—cautiously optimistic, and creative in their ability to manage the production challenges.

3. You are also planning to survey farmers in Brazil and Argentina. With labor issues causing strikes in Argentina and a drought impacting both countries, how will you get the workers there to contribute to your study?

We will adapt our survey methods in Argentina and Brazil, to adjust to the political realities of the strike in Argentina and the drought. In that environment, it will probably be better for us to schedule focus group discussions to explore the complex decision-making that is required there now. Two members of our project team, German Bollero and Maria Villamil, are working there now, and thus are in a good position to explore some alternative information gathering strategies.

4. What are some of the potential barriers to consumers' acceptance and use of biofuels in the future?

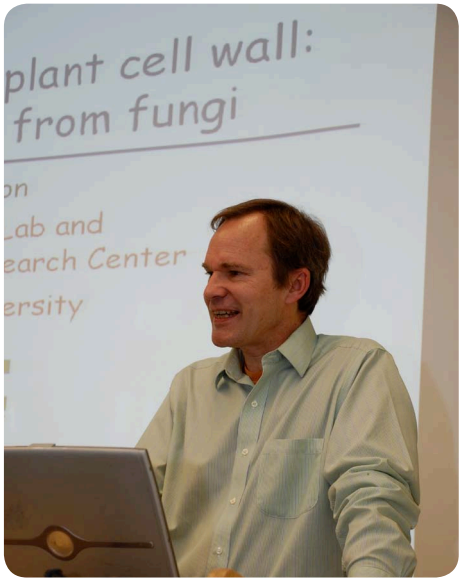
We haven't explored the consumer side of this question. Our plan is to evaluate the information we gather from the farmer surveys, and use that to create the questions for consumers. We expect to explore topics such as changes in energy consumption habits, and the food versus fuel question.

5. What are some of the ways you can bridge the divide between farmers, researchers and consumers?

The overall goal of this project is to facilitate discussion among farmers, researchers and consumers. The project team doesn't expect to design a "solution" to all the energy production/energy consumption questions, but to enable the stakeholders to work together to explore alternatives and design strategies. Our role is to figure out how to facilitate that process. One of the questionnaire topics is to explore barriers to new behaviors. We ask farmers to list the barriers to producing energy crops and to using new forms of energy, and we'll ask consumers similar questions. With that information, we should be able to start some meaningful discussions about how we as a society will develop and try out new strategies that are energy smart.

MICHIGAN SCIENTIST PLACING BET ON FUNGAL DECONSTRUCTION

Researchers with the Energy Biosciences Institute and elsewhere are probing the enzymes that nature has evolved to deconstruct wood and plant fiber, searching the rumen of cows, the hindgut of termites, and the digestive system of leaf-cutting ants. Jonathan Walton thinks the answer might be found in the decay activity of fungi that degrade plant cell walls.



JONATHAN WALTON

He is not alone, of course. EBI researcher Louise Glass at UC Berkeley is devoting her attention to the fungal strain Neurospora crassa and its degradation of Miscanthus grass. But in a March seminar at Calvin Laboratory, Walton admitted that the fungal school of bioprospecting for biomass enzymes has limited enrollment.

As Associate Director of the Great Lakes Bioenergy Research Center—a U.S. Department of Energy program like the Joint Bioenergy Institute in Emeryville—Walton calls his work with the Trichoderma fungus "old school." The well-studied species is just one of the tens of thousands of fungus types that have hardly been touched for their prospects as enzyme sources.

"I think they have a lot of potential," said Walton, who is a plant biologist at Michigan State University. "Fungi are most important for lignocellulose breakdown in all ecological environments. Most industrialized enzymes come from Trichoderma. Fungal enzymes don't get the respect they deserve."

He calls those enzymes "the Swiss army knife of biomachinery." The huge diversity of fungal genomes, most of which have evolved different mechanisms for degrading biomass, makes the search for the most effective enzymes difficult. And the answer won't be with just one or two enzymes, according to Walton.

"Individual enzymes don't tell you much; they work together," he told the seminar audience. "And their substrates are complex. You can't just pluck an enzyme out of an organism and assume it will work. It takes an enzyme ecosystem."

He and his colleagues are trying to develop a "core" set of enzymes upon which to run experiments. This core—up to 20 or 30 enzymes working together—can theoretically be optimized for substrates and pretreatments, working toward a consolidated bioprocessing organism. They have already identified 80 secreted proteins from fungi as high-probability candidates. But analyzing all 80 for their impact on lignocellulose degradation will take a long time. The biggest challenge right now is evaluating enzymes for their relevance and importance to the process. For example, Trichoderma alone makes over 150 glycosyl hyrdolases.

Walton's work, and those of others seeking depolymerization enzymes, is critical for developing a competitive biofuel because enzymes are expensive. They represent the highest processing costs today. Current ethanol cellulases are complex mixtures, poorly defined and not optimized for any particular biomass, according to Walton. "They are the major bottlenecks to lignocellulose degradation," he said.

He is looking to the relatively untapped potential of fungi, in hopes that in the future their biomass enzymes can be identified and customized for specific functions.

BERKELEY LAB SCIENTIST CHRISTER JANSSON

PROBING CARBON FLUX, CYANODIESEL PROSPECTS TO ENHANCE BIOMASS DECONSTRUCTION

Berkeley Lab scientist Christer Jansson isn't an EBI researcher, but his work may influence biomass production in the biofuel supply chain. He spoke at an EBI seminar on March 31 about two initiatives he began upon arrival at LBNL's Earth Sciences Division from his native Sweden in February 2008.

In one, he is studying one-celled organisms called cyanobacteria, whose photosynthetic qualities are conducive to producing biomass. Through the biosynthesis of fatty acid methyl esters, he hopes to prove the concept of biodiesel production from microalgae.

His other research program seeks to understand the carbon distribution mechanism in plant cell walls, the objective being to increase the carbon flow into both the roots of the plant, where carbon can be sequestered, and the plant's cellulosic biomass production system. His model is an annual grass, Brachypodium distachyon.

In the Trenches:

THE PROGRAMS AND PROJECTS OF EBI

The sixth of a series of profiles about the people doing work in the Institute, designed to familiarize research teams with colleagues' efforts and thus provide potential areas of collaboration and communication.

CLAYTON RADKE: ON THE SURFACE, SMALL EBI TEAM SEEKS BIG SOLUTIONS

Clayton Radke's research team for his EBI project may be modest in size, but the importance of his work promises to be huge.

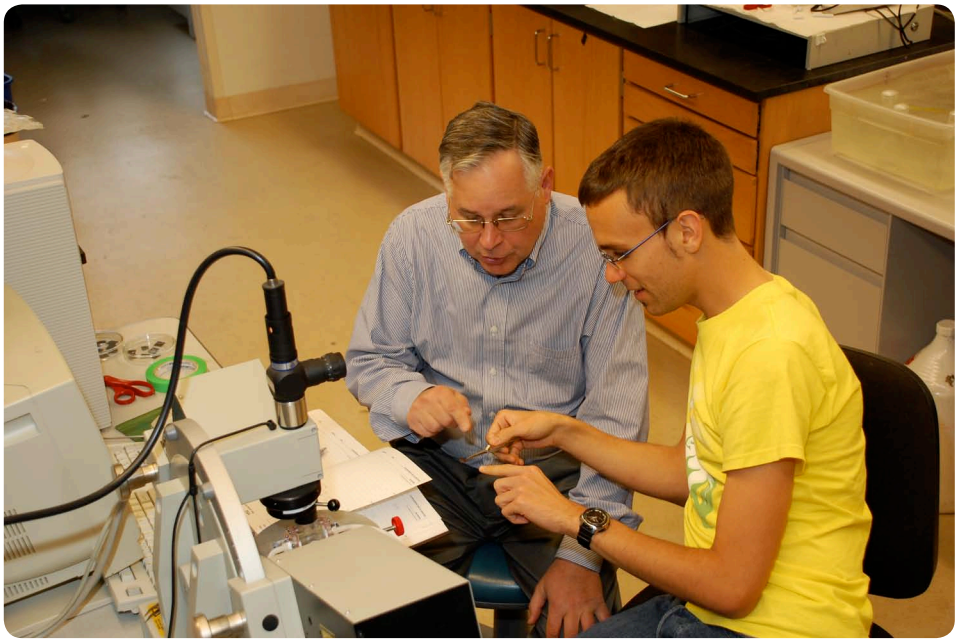
"We currently are only two people," Radke says from his UC Berkeley office in the Chemical Engineering Department, referring to himself as principal investigator and to his graduate student, Sam Maurer. "Some (EBI) programs have 15 to 20 people. We may be small, but we are focusing on the main kinetic barrier to the biological route to biofuels."

That barrier lies on the surface of cellulosic plant material, onto which enzymes adsorb to the cellulose and trigger breakdown into aqueous-soluble sugars. Depending upon the type, size and concentration of the enzyme, the temperature and other variables, the route to deconstruction will either be long, slow and expensive, or optimized for efficiency and lower cost. It is the conditions of the latter that Radke and Maurer hope to define.

"It is the enzymes, called cellulases, that do the depolymerization," says Radke. "They 'chop' the cellulose into small short-chain sugars. The mechanisms and the kinetic adsorption rates of these cellulases determine how well they will deconstruct the microfibrils of cellulose. These enzymes 'eat' away directly at the surface, not in the surrounding aqueous solution."

A natural assumption might be that the more cellulase you use, the greater its potential for a high rate of breakdown. Not true, say the early findings of Radke and Maurer. The enzyme action actually peaks and then levels, since the surface can only accommodate so much material. This result suggests that smaller enzymes are better than larger ones—"B-B's are better than marbles," all else being equal, Radke says. And smaller enzymes could reduce the cost, a critical driver in the overall production expense of biofuel.

Maurer uses an optical apparatus called a "flow ellipsometer" that's set up in a laboratory in Tan Hall. He applies a high-viscosity cellulose solution onto a tiny silicon wafer and lets it solidify. Next, Maurer contacts the cellulose coating with enzyme and, through the refraction and reflection of a laser light, records the declining thickness of the cellulose-substrate layer at various enzyme concentrations. By characterizing the aqueous/cellulose surface and the fundamental mechanisms at play during the enzyme's reversible adsorption process, they can help to define the combination most conducive to cellulosic deconstruction.



CLAYTON RADKE AND SAM MAURER WITH FLOW ELLIPSOMETER

"We are looking to create an assay that determines if a certain enzyme is better than another," says Maurer, a second-year graduate student and MIT alumnus from Pennsylvania. "By studying the thickness (of the cellulose and enzyme layers) and the refractive index, we can learn a lot about the interfacial behavior of the molecules."

Radke has studied chemical behavior on aqueous/solid surfaces for some time, focusing in the past on enzymes' interactivity with things like dishes and clothes. The laundry industry is the single largest user of commercially produced enzymes. The techniques he learned in that work, he felt, would be applicable to the study of surface kinetic mechanisms of enzymatic cellulose deconstruction—hence the EBI journey which he and Maurer are taking.

Radke says he looks forward to analyzing some of the enzymes that might be developed or discovered by other EBI research teams. He also plans to acquire something called a Quartz Crystal Microbalance tool, which he says will enhance the speed and volume throughput of assays.

"This work will have major ramifications for design (of enzymes)," he adds. "We need to think not only about improving the catalytic activity of cellulase, but also about building smaller enzymes that adsorb effectively.

"Understanding the mechanisms by which various cellulose enzymes act in concert to cleave cellulose will guide us to develop new enzymes with improved rate and adsorption constants, leading to the efficient production of liquid fuels from biomass."

MICHAEL GRAY: GOING AFTER BIOFUEL PLANTS' POTENTIAL ENEMIES

Some bioenergy crops are so new, they don't yet know who their potential enemies are. That is why the EBI has hired Mike Gray and his colleagues at the University of Illinois to find the pests and pathogens that could pose significant threats to biofuel feedstock. Gray and his colleagues, plant pathologist Carl Bradley and nematologist Terry Niblack, serve as co-principal investigators for this program.

Gray, a professor in Crop Sciences, hopes to do for Miscanthus and switchgrass what he has done for corn during the last 20-plus years—develop economical and environmentally sound crop management practices concerning the plant's ability to overcome the intrusion of pests. For corn, his research has focused primarily on the western corn rootworm. For biofuel feedstock, the pest complex may include nematodes, fungal pathogens, viruses, or insects.

"Biofuel crops are still relatively new and are grown on a very limited number of acres in the United States as compared with grain crops," Gray says. "Insect pests and plant pathogens could be a major limiting factor in the development of production-based quantities of feedstock. Knowledge of the most important insect pests, nematodes, and pathogens will help agronomists, entomologists, plant breeders, and plant pathologists develop integrated pest and pathogen management strategies to minimize losses."

Crop losses attributed to pests and pathogens are estimated to be about 30 percent worldwide. Sugarcane, a close relative of Miscanthus, has about 1,500 insect species feeding on it. So Gray's mission will interest the entire burgeoning biofuel industry.

Five postdoctoral researchers, all specialists in potential pest complexes — Monday Ahonsi (fungal pathology), Bright Agindotan (virology), Jeff Bradshaw (entomology), Tesfa Mekete Mengistu (nematology), and Jarrad Prasifka (entomology) — have been searching the country during the past year for samples of infected or diseased Miscanthus or switchgrass plants. Their survey took them throughout Illinois and to Iowa, Nebraska, Oklahoma, Tennessee, South Dakota, Louisiana and Texas. Step one has been to survey the landscape where these biofuel crops are being grown and then identify the most significant pests that may affect biomass production.

It was common to find aphids in most areas where Miscanthus and switchgrass was sampled. Gray points out, "We need to assess the role that aphids may play in causing direct losses in biomass production. Certainly, their role as potential vectors of disease pathogens should be determined." Thus, the interactions between pests and viruses become very important. With the diverse disciplines represented on this team, it is ideally suited to answer these intriguing questions.

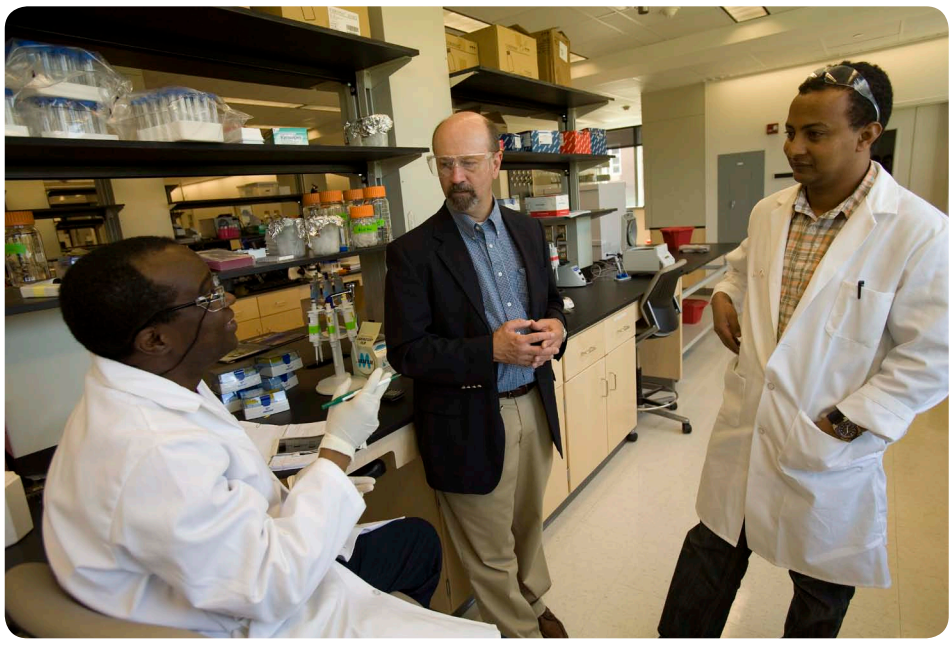
The team is establishing greenhouse experiments to determine the impact of potential key pests on the production of biomass. In addition, it will have the opportunity in multi-state surveys and at the EBI Energy Farm in Urbana to sample plots for pests and diseased plants throughout a full growing season.

"The uniqueness of our program," says Gray, "is that it goes from field biology to molecular biology. We run the spectrum from growing the plants to sequencing the DNA of the pests and pathogens." This molecular characterization will help identify the microbes responsible for infestations.

Down the road, Gray says he hopes to be able to work with other EBI researchers in developing disease-resistant strains of the biomass crops. In the meantime, his team will continue its surveys, greenhouse and small plot investigations, along with considering the strategies by which the nefarious intruders are best controlled, preferably via environmentally-friendly integrated pest management tactics such as the development of resistant plants and, as a last resort, pesticide application.

He knows from his years of research with the western corn rootworm that it's a complex ecological challenge. He recalls that for several decades the crop rotation of soybeans with corn solved the rootworm problem, until the rootworm adapted to this cultural strategy. Now most producers utilize transgenic Bt corn hybrids on rotated as well as non-rotated corn.

It is still early in the game, but Gray and his Illinois colleagues hope the knowledge they gain about pests and pathogens will enable the biofuel industry to be prepared to implement environmentally-friendly management tactics in an economically sound fashion. As more acres are devoted to Miscanthus and switchgrass, Gray and his team will continue their research to determine how an increase in the spatial scale of these biofuel crops affects potential pest densities.



BRIGHT AGINDOTAN (LEFT), MIKE GRAY (CENTER), AND TESFA MEKETE MENGISTU

ATHANASIOS LYKIDIS, NIKOS KYRPIDES: BIODIESEL FROM MICROBIAL 'FAT'?

Athanasios Lykidis is on a mission to make bacteria obese. He wants to give microbes the ability to store carbon so that they can generate lipids for biodiesel production.

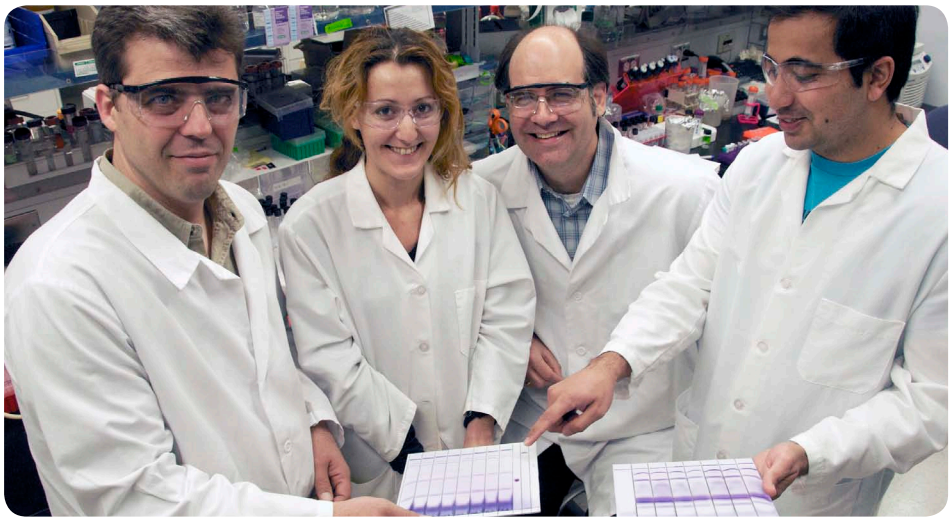
The biodiesel that fuels cars and other equipment is currently sourced from such materials as virgin soybean or rapeseed oil, or used cooking oil. Lykidis wants to add one more source by developing a commercial process for extracting fuel from bacteria.

Fattening up microbes is one of the efforts currently funded by the EBI at the U.S. Department of Energy (DOE) Joint Genome Institute (JGI). At the DOE JGI, Lykidis can sequence the genome of an organism—in this case, bacteria—and study its metabolic systems at a molecular level.

Nikos Kyrpides, head of the DOE JGI's Genome Biology group in Walnut Creek, Calif., and Lykidis' colleague, said the EBI collaboration gives their program the opportunity to go beyond sequencing genomes and into working out practical applications.

Lykidis started with E. coli, a bacteria often used by researchers as a model organism because it's been extensively studied. Being used to an environment where food is plentiful, E. coli doesn't store lipids. Humans, on the other hand, are very good at storing the pasta they've just eaten, according to Lykidis.

He and his team, which includes researcher Parwez Nawabi and postdoctoral researcher Maria Billini, want to turn E. coli into a biodiesel-producing factory before applying the lessons learned to other bacteria that may prove to be more productive biodiesel generators.



THE JOINT GENOME INSTITUTE TEAM LOOKING TO EXTRACT FUEL FROM BACTERIA FOR THE EBI (FROM LEFT): ATHANASIOS LYKIDIS, MARIA BILLINI, NIKOS KYRPIDES, AND PARWEZ NAWABI.

"It all depends on the relative yields," Lykidis said. "E. coli doubles every 30 to 35 minutes and if you have a bacterium that doubles every three days, even if it accumulates more lipids, it's useless because you're going to need much more time to reach the same biomass as E. coli."

Kyrpides said the bacteria's relative size compared to plants leads many people to erroneously conclude that plants have more biomass. Size is trumped by the sheer quantity of microbes and their biomass.

"There are more microbes on Earth than there are stars in the universe," he said. "It's not that you're going to get all those microbes to produce energy, but we can get way more biomass from microbes than we can from plants."

As an analogy he said that a string of bacteriophages—viruses that live in bacteria—from every microbe would span 10 times the diameter of the Milky Way.

With so many microbes out there, finding the right bacteria for commercial biodiesel production can be tricky. Lykidis ticked off other unknown factors in the equation such as identifying the right pathways that allow bacteria to store lipids rather than use them and identifying what signals trigger the pathways' activation.

In the undertaking he calls a "reverse engineering of obesity," Lykidis and his team have already deleted some pathways in E. coli's system that divert lipids toward cell functions and incorporated new pathways from other organisms. They're now reviewing the E. coli strains they've engineered so far to see how the tinkering has affected the organism as a whole.

—Massie Santos Bollon

EBI PROMINENT AT JGI ANNUAL MEETING

Biofuels and biomass degradation were important themes at the 2009 DOE Joint Genome Institute User Meeting in March at JGI's Walnut Creek, CA facility. Nearly 500 participants heard speakers from research institutions and industry speak on a variety of subjects, including genome evolution, adaptation and new technologies.

In his keynote address, EBI Director Chris Somerville pointed out that while scientists at the EBI investigate the most productive feedstock and new ways to convert cellulosic biomass to liquid fuels, they are also spending a great deal of time on an issue that at first may seem more political or economic than scientific: land use.

The problem, he said, is that an acre of corn or any other food crop diverted to producing feedstock for fuel has a good chance of resulting in an acre of non-agricultural land being deforested or otherwise developed elsewhere in the world, causing large carbon dioxide emissions from burning and soil emissions.

Somerville also remarked on several potential biofuels sources, such as sugarcane, Miscanthus, and algae.

EBI biochemist Jamie Cate also spoke at the meeting, presenting on "Reverse-engineering Cellulosomes in Clostridia." Clostridia are bacteria that degrade cellulose; gaining a better understanding of how they work will help achieve the ultimate goal of creating a "designer cellulosome" whose enzymes can work as well as or better than nature to break down plant biomass into fermentable sugars.

A video from the meeting, including Somerville's presentation, can be viewed at http://jgi.doe.gov/meetings/usermtg09/agenda.html.

IT WILL ALL COME TOGETHER AT THE EBI RETREAT IN ILLINOIS

It is a broad and extensive portfolio—51 programs and projects spanning more than a dozen academic disciplines. The work is also spread geographically, across three campuses and at field sites throughout the world. But for the Energy Biosciences Institute, there is still one common mission—bringing together biological processes, materials and mechanisms to create a new sustainable, responsible source of energy.

On a historic weekend in June, the EBI will bring those disparate elements together in a first-time all-hands retreat. From those interactions and collaborations between more than 200 researchers, students and staff, the EBI's goals will be focused, the directions fine-tuned.

"The retreat will provide an opportunity for everyone affiliated with the EBI to develop an understanding of the full scope of the research portfolio," said Institute Director Chris Somerville of the June 19-22 conference at the University of Illinois at Urbana-Champaign. "I hope the meeting will help EBI investigators to broaden their understanding of energy biosciences and how the various research topics relate to the big picture."

Beginning with a welcome dinner and poster session on Friday evening, through the following Monday's tour of a 100-million-gallon corn ethanol plant, the retreat promises to be an information-packed experience. Presentations on every aspect of EBI's research program will fill the agenda, leaving plenty of time for questions and discussions.

One of the highlights, especially for those 100-plus people traveling from Berkeley, will be a visit to the EBI Energy Farm, where the prospective biofuel feedstock is being grown and monitored.

"Although most EBI investigators have become familiar with pictures of energy crops such as Miscanthus, experiencing the plants in the field provides a more tangible understanding of why perennial grasses have emerged as a promising path from photons to fuels," Somerville said.

Attendees will be briefed on all the major investigation areas—feedstocks, deconstruction, ecosystem services, fuel synthesis, socioeconomics, and microbially enhanced hydrocarbon recovery—and will hear from EBI and BP leadership. Speakers will include Somerville, EBI Deputy Director Steve Long, Associate Director Paul Willems of BP, BP technology vice president David Eyton, and BP biofuels vice president Phil New.

Poster sessions will conclude the day's activities on Friday and Saturday evenings.

"I am particularly looking forward to a focus group session on Sunday afternoon in which the EBI community will have an opportunity to propose new research directions to the management group," Somerville said.

The optional tour on June 22 to the Archer Daniel Midlands ethanol plant in Decatur, IL, will also be a highlight for those who can go. According to Somerville, a trip to the plant, which includes injection and monitoring wells to be used for carbon sequestration tests, "should be an unforgettable experience for anyone who has not been to an industrial production facility.

Although the EBI is not engaged in corn ethanol, the production of cellulosic fuels is expected to involve a similar scale of process engineering."

The wells will also be the site of research activities involving EBI investigators looking at microbially enhanced hydrocarbon recovery prospects and microbial biorefining.

All meetings and lodging will be at the iHotel, the University's new conference center located in the research park adjacent to the campus. Illinois attendees with questions about arrangements should contact Becky Heid at heid@illinois.edu. Participants from Berkeley should contact Trisha Togonon at trishatogonon@berkeley.edu.

ENERGY FARM *(CONT'D FROM PAGE 1)*

Arundale said, referring to the fact that one species will not become the sole biofuel feedstock.

Interest in Miscanthus as biomass is growing quickly, Mies said, but there are still obstacles to overcome before the plant can be seen as a commercial enterprise.

"Every new crop has its challenges," Mies said.

It can take three years or more to see a full yield from a Miscanthus crop, whereas traditional crops will turn a full yield each year of growth. Still, the biggest problem limiting Miscanthus' commercial potential is propagation. Miscanthus is sterile, so its rhizomes—its underground plant structure—must be harvested manually to enable reproduction.

And that's one area where the Energy Farm comes in. Researchers are working with manufacturers to develop machinery that can efficiently harvest the rhizomes. That now is a four or five-person job, Mies said, and can be complicated by the weather. Because the target is underground, harvesting can only be done during the winter, after the grass is harvested but before it starts growing in the spring.

Researchers are continually refining the machinery and the rhizome harvesting process, and Mies' goal is to get the ordeal down to a one or two-person job by the end of the year. "We're getting close," he said. "At this point it's working out the small details and running field trials to find improvements."

With the first full year of cultivation and growth ahead at the EBI Energy Farm, Arundale anticipates a virtual flowering of information from the verdant fields. "It's important that before things get too big, you have the answers to (some) fundamental questions," she said.

And in those answers may lie the future of bioenergy as a significant fuel source.

SYMPOSIUM SHOWCASES 'CONTINUING EVOLUTION OF THE INDUSTRY' AND 'PROMISE FOR THE FUTURE'

If Chris Somerville were to give a report card on the biofuel industry based on the 31st annual Society of Industrial Microbiology's Symposium on Fuels and Chemicals this month, he would probably write "making good progress" and "shows much promise for the future."

The San Francisco meeting of more than 800 scientists, entrepreneurs, researchers, students, and industrial representatives featured four days of rapid-fire half-hour presentations on all aspects of developing cellulosic and algal fuels as renewable alternatives to oil and gasoline. EBI Director Somerville moderated one session and summarized his views of the meeting at a news media forum on May 5.

"We're beginning to see the first real commercialization of the field," he said in the ballroom of the InterContinental Hotel, site of this year's symposium.

He said he was excited to hear about promising non-ethanolic biofuels, with renewable hydrocarbon components of diesel and gasoline, citing the presentations by companies

LS9, Gevo and Amyris and previously stated intentions by DuPont and BP regarding butanol. "In addition to ethanol, it seems likely that we will soon see companies producing next generation biofuels such as butanol, alkanes, and terpenes, opening up many other possibilities," Somerville noted. "Another new theme was the emerging idea that we may see competition for biomass feedstock between the power and transportation industries." A paper in *Science* by Chris Field and colleagues at Stanford outlined the improved transportation efficiency that may be obtained with electric cars fueled by biomass-derived electricity.

But most encouraging, he said, was the "continuing evolution of the new industry." He pointed to a flurry of research presentations concerning the use of ionic liquids as a novel and potentially effective pretreatment strategy (Sasi Padmanabhan and Jerome Fox of EBI both presented posters on this subject), large-scale DNA sequencing for finer insight into biological diversity (Somerville said that EBI investigators sequenced 17 billion base pairs last year through the DOE Joint Genome Institute), and novel organisms like the brown rot fungus that suggest deconstruction pathways not heretofore studied.

Several EBI researchers shared their results and their insights with conferees during the featured technical program. They included:

Tom McKone, of UC Berkeley and Berkeley Lab, who with Arpad Horvath is leading an EBI effort to track the life cycle impacts of emerging biofuels. He told his audience that making an accurate assessment is complicated by insufficient data, unreliable current models, and predictive uncertainties. The goal is to track the biofuel process from extraction to production to end use, evaluating impacts on health, security, costs, resources, climate, etc., in both space (location) and time.

Frank Robb, of the University of Maryland Technology Institute, who discussed the EBI program's work in studying extreme thermophiles—cellulose-degrading microorganisms drawn from high-temperature hot springs in Nevada and Northern California. A co-investigator with UC Berkeley's Doug Clark and Harvey Blanch, Robb explained how they enriched a variety of thermophilic bacteria, exposed them to ground-up Miscanthus, and with a filter paper process discovered several cellulolytic organisms and archae that could be candidate enzymes for cellulose deconstruction.

Matthias Hess of the JGI, who reported results of his team's search for promising enzymes for biomass degradation in the

MATTHIAS HESS

rumen of cows. Through transcriptome sequencing, they have determined that organisms within the bovine system are different based upon the type of feedstock degraded. They also identified numerous glycosyl hydrolases and 4,000 genes without known function that may encode novel proteins involved in biomass deconstruction.

Doug Clark of UC Berkeley, who explained his EBI team's efforts to improve the speed and effectiveness of cellulases. He illustrated his talk with slides of preliminary results from mathematical modeling of cellulase mechanisms, which will help to identify the properties most important to modify. A discovery tool known as cell-free protein expression is being used to screen for new enzymes, and Clark said they have sequenced 800 mutant enzymes so far, some with promising activity levels.

In addition to Padmanabhan and Fox, others explaining EBI posters included Purbasha Sarkar (cell wall modeling), Chaoguang Tian and Jianping Sun (Neurospora crassa fungus as deconstruction model), and Bin Wang (hydrosylate inhibitors). Clark also displayed a poster about cellulase activity on Avicel microcrystalline cellulose and Miscanthus giganteus.

PREPARING FOR THE BIG ONE *(CONT'D FROM PAGE 1)*

was a 6.9 on the Richter Scale, and it was centered across the bay, 60 miles south of San Francisco. Phillips, EBI's building manager and health and safety officer in Berkeley, says there is some good news here—both buildings occupied by EBI researchers, Calvin and Hildebrand Hall, are structurally sound. Calvin is not a high-rise and is made of concrete. Hildebrand has undergone seismic upgrades and features internal cross-bracing. That means the structures will withstand a lot of shaking.

Nonetheless, personal safety cannot be assured in every instance, so he suggests the following precautions to be taken by all EBI employees:

- *Know the evacuation routes and outside assembly areas for the buildings. They are posted on every floor at stairways and elevators and at main entrances.*
- *If an earthquake hits, staff inside the buildings should immediately brace themselves under a heavy object, like a desk, conference table or lab bench, to avoid falling debris. Hold there until the shaking stops, then proceed in an orderly fashion outside the building. Take essentials like glasses and prescription medication with you.*
- *Those engaged at the time in processes that could result in secondary hazards—for example, using a Bunsen burner or working with volatile chemicals under a fume hood—should take mitigating measures, like turning off burners or pulling down the fume hood sash, before leaving the area.*
- *Be careful of imploding glass from windows and other airborne objects.*
- *Once at the assembly area outside, let administrators know you are safe. Phillips will be the on-site emergency coordinator.*

He has two more pieces of advice in preparing for the next big one: sign up for the Berkeley campus' "WarnMe" electronic warning system, and store at least three days' worth of supplies in an emergency kit at home and in your vehicle. Calvin and Hildebrand buildings

SAFETY FIRST

are stocked with both 20-person survival kits and 4-person backpack kits on all floors, in conference rooms, in select offices and in the EBI vehicle. Food, water, sanitary items and medical supplies are included.

To assemble a home emergency kit, go to http://www.72hours.org/build_kit.html

"The WarnMe system is a campus-wide way to make announcements about large-scale emergencies," Phillips explains. "Signup is voluntary, but I encourage all EBI personnel to do it. You can receive messages via e-mail or text, including the most up-to-date information and recommended actions from the incident command center."

To get on the system, go to http://warnme.berkeley.edu

"We can expect after a significant earthquake that reoccupation of the buildings will not be a quick process," Phillips also cautioned. "The campus procedure for screening the buildings' structural integrity and checking for secondary hazards (like spills) will take some time."

If exiting from campus is impossible or difficult, Phillips notes the campus-wide emergency response plan includes provisions for temporary sheltering of employees and students. A warning siren system, tested every month, will signal an emergency situation. A public address system is also available in the buildings for instruction and updates.

CALVIN LAB SAFETY MANAGER ZACK PHILLIPS (CENTER) POINTS OUT EMERGENCY ESCAPE ROUTES FOR POSTDOC SASI PADMANABHAN (LEFT) AND LABORATORY MANAGER MARA BRYAN.

EXPEDITION *(CONT'D FROM PAGE 2)*

arranged this voyage under the guidance of noted polar explorer and environmental pioneer Robert Swan. The purpose was to expose 60 students chosen from 30 universities in 20 countries to real-time energy issues and to encourage their discussion of the challenges with experts in climate and energy, like Miller.

The social dynamics to which Miller referred developed from the close interactions among this group, which also included 10 of Swan's staff from his "2041" company, on the 110-meter, six-deck academic research vessel, Akademik Ioffe. During the round trip from Ushuaia, Argentina, to Antarctica, students attended lectures and joined the professors in working groups on subjects like leadership, collaborations and interdependencies. Miller led the latter group and discussions on biofuels, meteorology and climate change.

Among the team was first-year UC Berkeley graduate student Kelly Karns, who is pursuing a Ph.D. in bioengineering. Although her career path runs more toward medical biotechnology, she said she wanted to observe the impact of climate change.

"It opened my eyes," said Karns, whose upbringing in her native Hawaii is about as far as you can get from life in Antarctica. "Robert Swan made a great impression on me, and I know I experienced personal growth. I think of transportation a lot more now, and I'm sure there will be some lifestyle changes, too, related to saving the planet's resources."

She and Miller will surely remember the adventure, which wasn't always smooth sailing. In fact, within 12 hours from departure from Tierra del Fuego at the tip of South America, the ship entered the dreaded Drake's Passage, known to have perhaps the roughest seas in the world. For 24 hours the vessel pitched and rolled, the captain had to "heave to" the vessel for 20 hours waiting out a fierce storm that tossed waves against Miller's sixth-deck cabin window, and half the voyagers got sick.

But at the other end, the views were spectacular — of humpback whales breeching in the waters, penguins skittering on ice flows, and thunderous chunks of ice breaking off glaciers into the ocean. One night was spent camping on ice in below-freezing temperatures — Karns said her two sleeping bags and a bivy bag kept her reasonably comfortable.

They also helped to resupply a Ukrainian research station in Verdansky, formerly a British station where the first-ever measurements of the ozone hole were made. As the Akademik Ioffe left for the return voyage, a 400-square-kilometer section of Wilkins Ice Shelf broke free just south of the Verdansky station. The National Ice and Snow Data Center released a statement that the ice bridge calving was absolutely due to global warming.

Swan, the dashing British explorer and motivational speaker, presided over it all "like a mother hen," according to Miller, who also called him "very compassionate and devoted to his cause." That cause is "informing, engaging and inspiring the next generation of leaders to take responsibility, to be sustainable, and to know that now is the time for action in policy development, business generation and future technologies," according to his Web site.

"He gave me a private note thanking me for being part of the expedition," Miller said of Swan. "It said 'Carry On. Stay Relevant. Attack at Once.' I put it up in my office, so I don't lose sight of this wonderful experience."

Note: An 18-minute film of the expedition can be viewed on Berkeley Lab's Earth Sciences Department Web site: http://esd.lbl.gov. Photos, a diary and expedition maps can be seen at http://www.expedition-antarctic-2009.com/.

THE EBInsider

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