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Synthesis of a ketone molecule that can be cracked brings researchers . . .

One Step Closer to Hydrocarbon Fuel Biosynthesis

UNIVERSITY OF MINNESOTA BioTechnology Institute

Synthetic Ecology Theme Sets the Tone for BTI

Illustration by
TIM MONTGOMERY

Over the past several decades, great advances in microbial and biological sciences have been made using reduction approaches involving single biological species. This has been fueled largely by the great wealth of information obtained from structural and functional genomics. This better understanding of microbial physiology and genomics has in turn led to a new area of biological research - synthetic biology - ultimately leading to the design and construction of new biological systems not found in nature.

While synthetic biological approaches have proven useful to engineer new biological systems, there are limitations to such approaches, including our inability to adequately construct and regulate complex pathways. Synthetic ecology is a relatively new way of getting microorganisms to work together. Rather than using genetics as a building block, cell populations are intermixed to construct a cooperative ecology of productive interactions. Sometimes enzyme catalysts are introduced into the equation to jump-start the process. Sometimes breeding is used to introduce new metabolic pathways into a mixed population through reproduction. The goal is to create cooperative relationships between microorganisms.

Researchers at the BioTechnology Institute are utilizing the concept of synthetic ecology in many ways. One group is arranging populations of bacteria to form a cooperative ecology that will produce biological hydrocarbons as the basis for creating renewable liquid fuels. Other projects focus on the biosynthesis of new drugs, the breakdown of toxic materials in the environment and the agricultural applications of symbiotic relationships between plants and soil microbes. All depend on cooperative interactions.

The cooperative interactions characteristic of synthetic ecology provide a theme for the BioTechnology Institute. Faculty members interact and work together across a range of disciplines that benefit the work of the Institute as a whole.

THE BIOTECHNOLOGY Gateway
www.bti.umn.edu
SPRING 2011

PUBLISHED TWICE A YEAR BY

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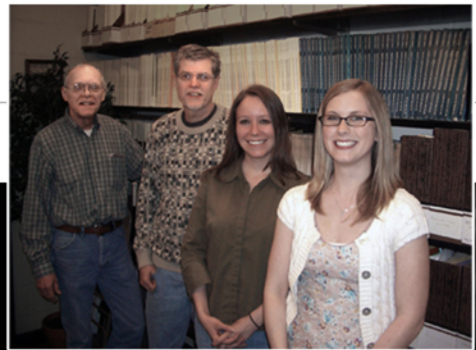
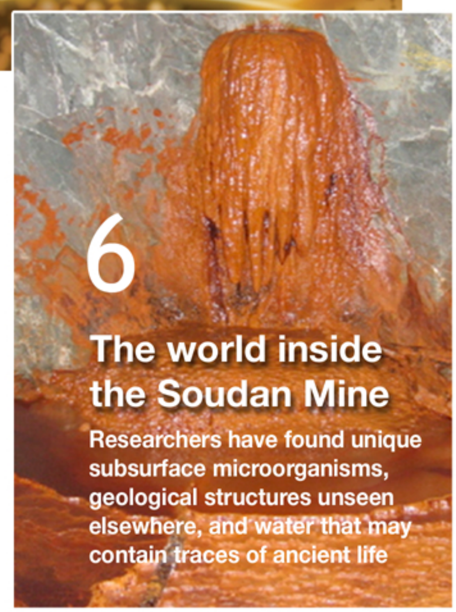
CONTENT



Left: Jack Richman, Larry Wackett, Janice Frias and Jasmine Erickson

FEATURES



SECTIONS

Amy Miller and Chris Stenland work to characterize the Nikkomycin process at small scale in preparation for a production run.

Partners Against Disease

U of M Researchers Share in $3 Million Grant to Develop an Anti-fungal Drug

Story by TIM MONTGOMERY and AMY LESLIE

Researchers from the College of Biological Science's BioTechnology Institute (BTI) and from the College of Pharmacy's Institute for Therapeutics Discovery and Development (ITDD), in partnership with the Arizona-based company Valley Fever Solutions Inc. and the University of Arizona, have been awarded a $3 million grant from the National Institutes of Health to develop and study the use of nikkomycin Z (NikZ) as a potentially curative therapy for Valley Fever.

Valley Fever, or coccidiomycosis, is a fungal infection that can cause fever, chest pain and coughing, and can typically last from weeks to months. It's endemic to the southwestern United States, with an estimated 50,000 to 100,000 persons developing symptoms each year - 35,000 in California alone. Some patients suffer long-term disabilities that require extensive medical treatment. At particular risk are immunosuppressed patients, including those with AIDS or recipients of organ transplants, African-Americans, Asians, pregnant women and the elderly.

Researchers at the University of Arizona initially approached the BTI in 2008 for assistance in developing a production process for NikZ to facilitate clinical testing.

"Recent advances in genetic modification of the NikZ strain made by our collaborators at the University of Arizona, allowed the deletion of an inactive metabolite called nikkomycin X," said Dr. Marc von Keitz, principal investigator on the grant and director of BTI's Biotechnology Resource Center. "By eliminating nikkomycin X, fewer steps are needed and more of the produced NikZ is recovered in the purification process, thus reducing the overall cost of the drug."

Manufacturing the material for clinical trials will be an inaugural project for a new collaborative effort between the BTI and the ITDD. The collaboration is aimed at producing new drugs from protein-based and natural product-based materials that meet government standards for clinical testing. The collaboration's production capabilities benefit from BTI's long-established Biotechnology Resource Center and ITDD's Biotherapeutic Protein Production Facility (BPPF), a new resource created by a grant from the Minnesota Partnership for Biotechnology and Medical Genomics as a joint effort of the University of Minnesota and Mayo Clinic.

"The collaboration between BTI and ITDD allowed us to successfully compete for this grant. Our goal now is to simplify scale-up of the NikZ manufacturing process and make this drug more readily available to improve the therapy for people with Valley Fever," said Dr. Vadim Gurvich, associate director of the ITDD and the other PI on the grant.

Exploring the Origins of Multicellular Life

Story by TIM MONTGOMERY

For years, researchers have been looking at organisms that transition across the divide between unicellularity and multicellularity. How do individual, single-celled organisms evolve to have the ability to cluster and live together cooperatively in groups as multicellular organisms?

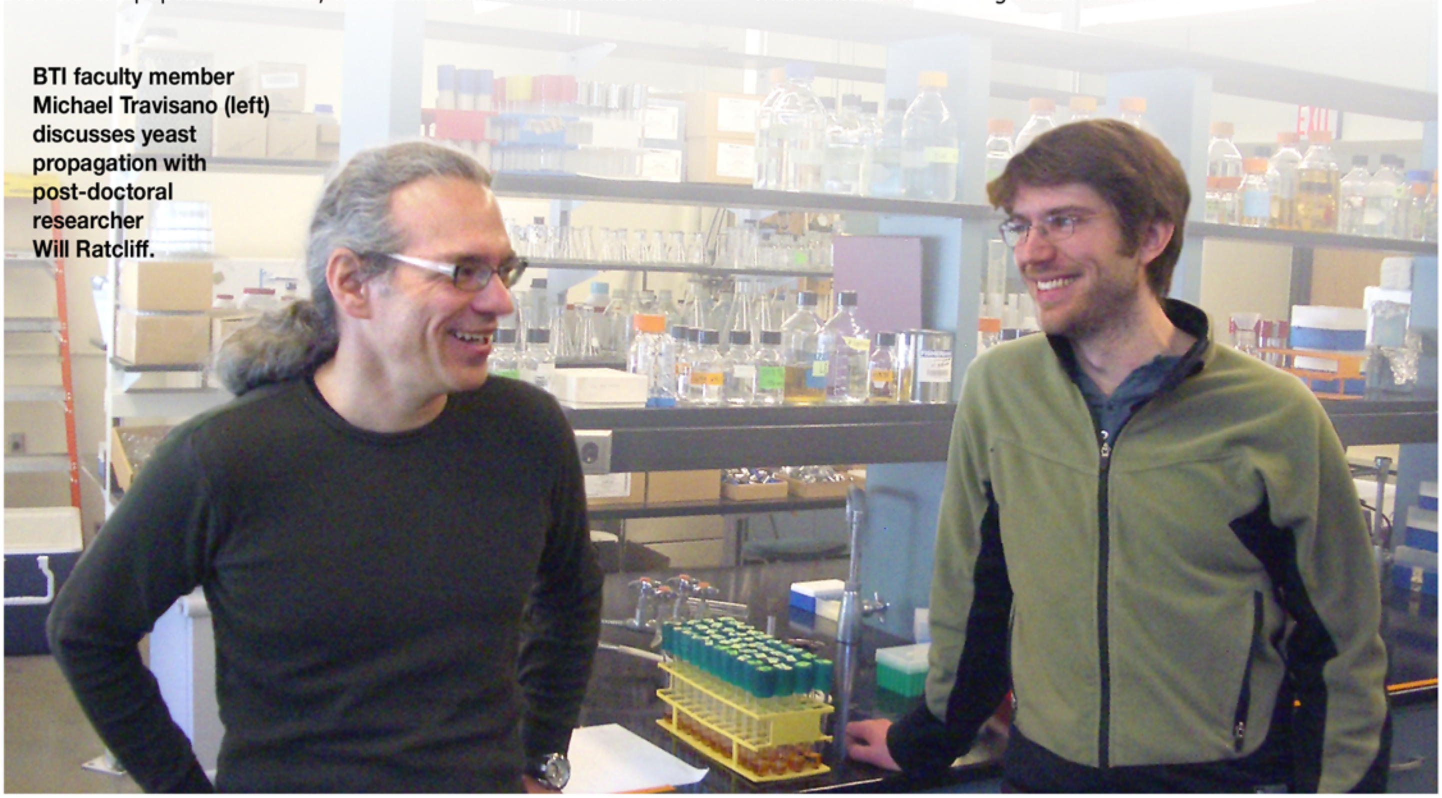
A group led by BioTechnology Institute faculty member Michael Travisano, an associate professor in the Department of Ecology, Evolution, and Behavior, have evolved novel multicellular yeast. They are examining the causes and consequences of these interdependent groupings of yeast in the hope of understanding more about multicellularity.

Travisano and his fellow researchers have been looking at yeast clusters in the model system of Saccharomyces cerevisiae (more commonly known as baker's yeast) and observing how clusters grow, increase in size and spew off smaller offspring that in turn propagate themselves. As the cells in new propagules multiply, they evolve to live together. In studying the origins and development of these clustered yeast groupings more closely, the Travisano group hopes to understand better how they are maintained.

One curious aspect of how these yeast colonies propagate is the role of apoptosis - that is, how cells that die are utilized in the spawning of new yeast colonies. Apoptosis is the genetically programmed death of a cell that is characteristic of multicellular organisms. Unlike traumatic cell death caused by injury, apoptosis occurs as a response to biochemical changes in the cell. Will Ratcliff, a post-doctoral researcher in the group, has found that when enough yeast cells in a colony die, it triggers the separation which creates a new colony in a form of multicellular reproduction.

"Dying can be beneficial if it produces a positive effect on the whole colony," explained Ratcliff. He believes that apoptosis in yeast cells is part of a regulation strategy in yeast cell colonies that allows them to produce more propagules and function in a multicellular way. A three-year grant from the National Science Foundation will allow Ratcliff and Travisano, Mark Borrello and R. Ford Denison to further study the role of apoptosis in the reproduction of multicellular yeast colonies.

Understanding the origins of multicellular organisms is the key to explaining biological complexity - how cells specialize and work together as a group. Prior research has identified multiple benefits to existing multicellular life, including larger body size and different cell types. But how single-celled life gave rise to complex multicellular organisms remains unclear. Travisano and his group hope to understand more about the he origins of multicellular life through their research.



BTI faculty member Michael Travisano (left) discusses yeast propagation with post-doctoral researcher Will Ratcliff.

EXPLORING THE WORLD INSIDE THE Soudan Iron

Story by TIM MONTGOMERY
Photos courtesy JEFFREY GRALNICK

University researchers have discovered an exciting world inside the Soudan Underground Mine on the iron range of northern Minnesota. Journeying down 2,341 feet to the 27th level, they viewed unique geological structures, and water that may contain traces of ancient life.

The old iron mine, which ceased operations in 1962, is part of a Minnesota state park which contains the leading deep underground science and engineering laboratory in the United States. Located on the Vermilion Range, the opening of the Soudan mine in 1884 set the stage for Minnesota's reign as the country's leading iron ore producer. Today it is the country's only underground iron ore mine open to the public and is the site of a 6,000-ton particle detector that measures the properties of neutrinos directed towards it in a high-intensity beam from the Department of Energy's Fermi National Accelerator Laboratory outside Chicago, Ill.

"This happens to be a place with highly unusual geology," said BTI faculty member Jeffrey Gralnick, an assistant professor of Microbiology who first visited the deep recesses of the mine in 2006. "The water found on the lowest level is more salty than sea water and contains no oxygen as it exits places where the rock has been drilled."

Gralnick and other scientists believe the salty water is being pressured out from even further below. More importantly, the ancient brine contains microorganisms whose adaptations to life in an extreme environment may hold a key to the development of new technology and novel drugs.

"It's possible that we will find unique metabolisms in organisms isolated from this unusual

place," commented Gralnick. "We've already cultured iron-oxidizing bacteria from the Level 27 brine - bacteria that accelerate rust formation and could be used to precipitate toxic metals, facilitating remediation."

Mine

In their characterization of microbes living in the mine, Gralnick and other researchers such as fellow BioTechnology Institute faculty member Christine Salomon of the University's Center for Drug Design, have also isolated bacteria from the group Streptomyces. This type of bacteria is used to produce most natural antibiotics used in clinical trials, and studying these Streptomyces isolates could lead to the development of new drugs.

Underlying bedrock formations in the Soudan Mine date back over 2.7 billion years. The minerology is fascinating to Gralnick, who has observed drips, bubblers, and bearded rust formations. Studying life in the Soudan Mine presents not only a rare opportunity to access an ancient subsurface environment, but may provide a parallel to life on other planets.

ELECTROSYNTHESIS

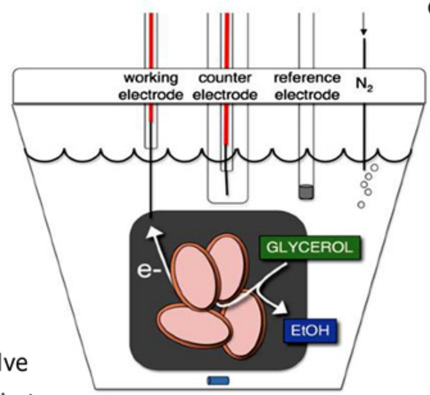
Stimulates new reactions

Story by TIM MONTGOMERY

University researchers have found that bacterial metabolism can be manipulated and enhanced through an interchange of electrons. Using bacteria linked to electrodes, they recently demonstrated that waste products from biodiesel production such as glycerol can be converted to biofuels or bioproducts, with electricity as the only waste product.

“It turns out that if we use an electrode to guide and facilitate certain reactions that happen inside the cell,” explained BioTechnology Institute faculty member Jeffrey Gralnick, “we can make things more effectively.”

Most approaches to microbial engineering involve controlling the amount of food, or air available to microbes in order to affect what they metabolize. But Gralnick and fellow BioTechnology Institute faculty member Daniel Bond have been able to manipulate the metabolism of certain microbes through the use of electrodes.



Courtesy DANIEL BOND

By pulling excess electrons out of Shewanella oneidensis and into an electrode, using pathways normally harnessed by the cells to reduce metals in the environment, they are able to enable new reactions within. Going one step further, Gralnick and Bond concentrated on how they could introduce new biochemical pathways to harness this electron exchange, and create new or better products.

"The idea that Bond came up with originally was to turn glucose into Shikimic Acid," said Gralnick. "This is a compound that is essential in the synthesis of the anti-flu drug called Tamiflu."

Gralnick and Bond believe that using electrode-interfaced bacteria such as Shewanella oneidensis creates new possibilities for metabolic engineering and synthetic biology strategies in producing other biofuels and materials.

Making Connections

Biotechnology is a global enterprise, and BTI has established a formal exchange program that allows students and faculty to experience different cultures and approaches to biotechnology.

Each year, 3 to 4 students from BTI and the Nara Institute of Science and Technology (NAIST) in western Japan, participate in an exchange visit. Students are assigned to a specific laboratory, based on their research interests, with the intent of learning new skills and techniques. Students from the host laboratory also become cultural mentors for the visitors.

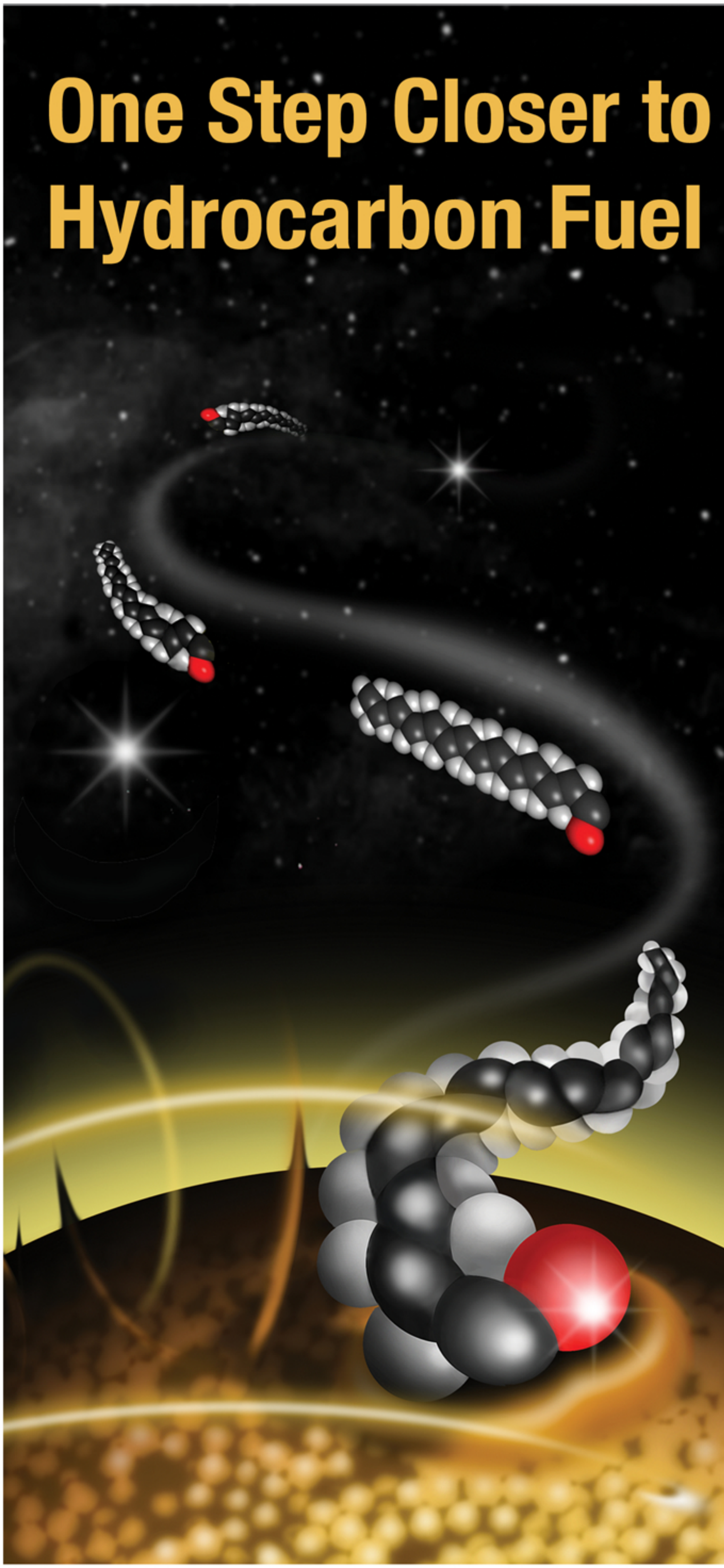
These international connections provide an opportunity for technological as well as cultural learning experiences.

Photo courtesy MAGGIE LINAK

Above, BTI exchange students Ilya Tikh, Sarah Bloch and Maggie Linak in Nara, Nov. 2010. Right, Sarah and Maggie with Mt. Fuji in the background.

Photo by ILYA TIKH

One Step Closer to Hydrocarbon Fuel Biosynthesis



MINNEAPOLIS / ST. PAUL — University of Minnesota researchers are a key step closer to making renewable petroleum fuels using bacteria, sunlight and carbon dioxide, a goal funded by a $2.2 million United States Department of Energy grant.

Graduate student Janice Frias, who earned her doctorate in January, made the critical step by figuring out how to use a protein to transform fatty acids produced by the bacteria into ketones, which can be cracked to make hydrocarbon fuels. The university is filing patents on the process.

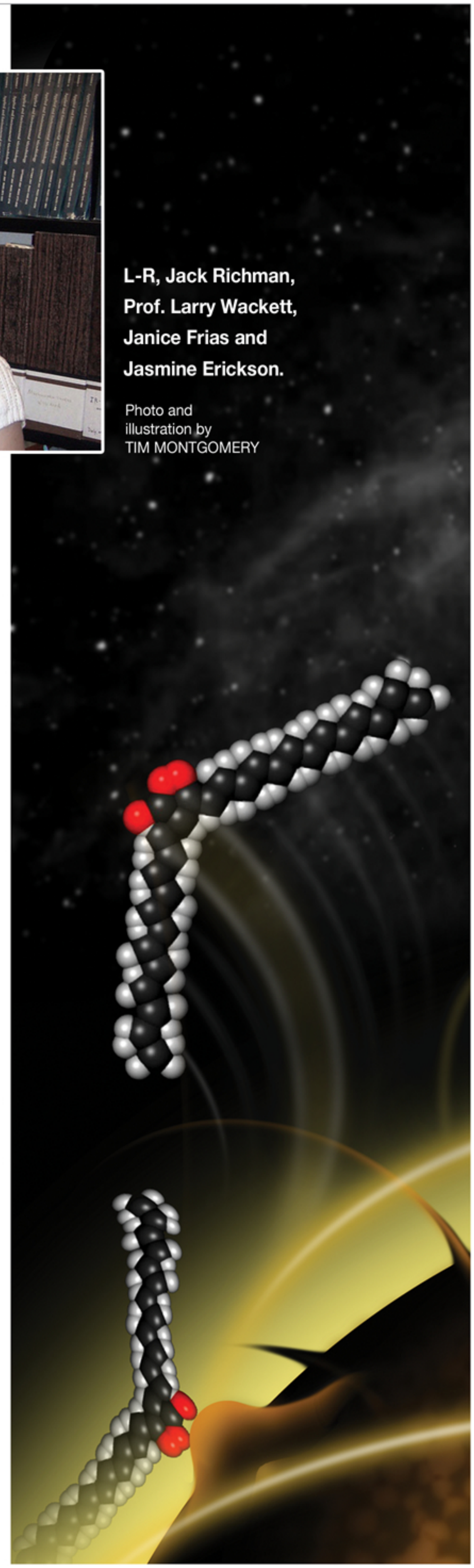
The research is published in the April 1 issue of the Journal of Biological Chemistry. Frias, whose advisor was Larry Wackett, Distinguished McKnight Professor of Biochemistry, is lead author. Other team members include organic chemist Jack Richman, a researcher in the College of Biological Sciences' Department of Biochemistry, Molecular Biology and Biophysics, and undergraduate Jasmine Erickson, a junior in the College of Biological Sciences. Wackett, who is senior author, is a faculty member in the College of Biological Sciences and the university's BioTechnology Institute.

"Janice Frias is a very capable and hard-working young scientist," Wackett says. "She exemplifies the valuable role graduate students play at a public research university."

Aditya Bhan and Lanny Schmidt, chemical engineering professors in the College of Science and Engineering, are turning the ke-

L-R, Jack Richman, Prof. Larry Wackett, Janice Frias and Jasmine Erickson.

Photo and illustration by TIM MONTGOMERY



tones into diesel fuel using catalytic technology they have developed. The ability to produce ketones opens the door to making petroleum-like hydrocarbon fuels using only bacteria, sunlight and carbon dioxide.

"There is enormous interest in using carbon dioxide to make hydrocarbon fuels," Wackett says. "CO2 is the major greenhouse gas mediating global climate change, so removing it from the atmosphere is good for the environment. It's also free. And we can use the same infrastructure to process and transport this new hydrocarbon fuel we use for fossil fuels."

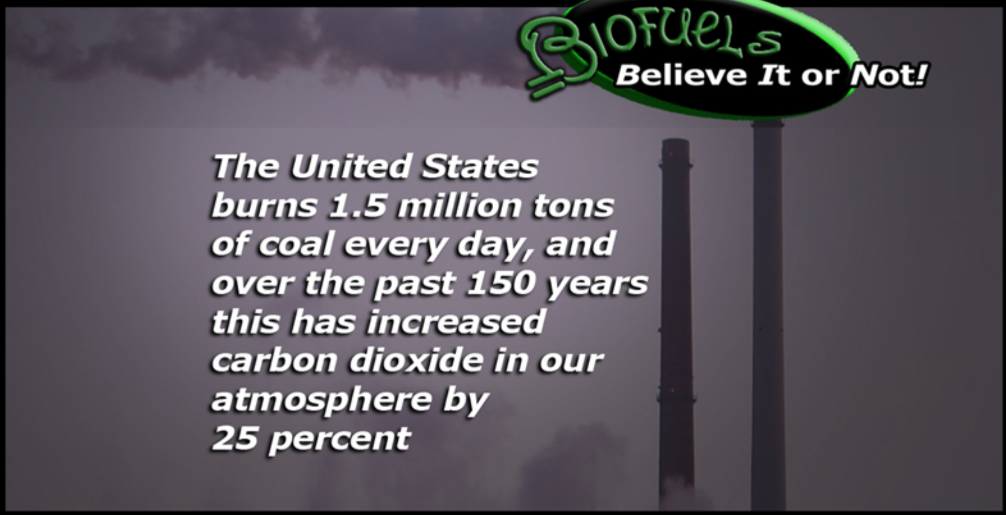
The research is funded by a $2.2 million grant from the U.S. Department of Energy's Advanced Research Projects Agency-energy (ARPA-e) program, created to stimulate American leadership in renewable energy technology.

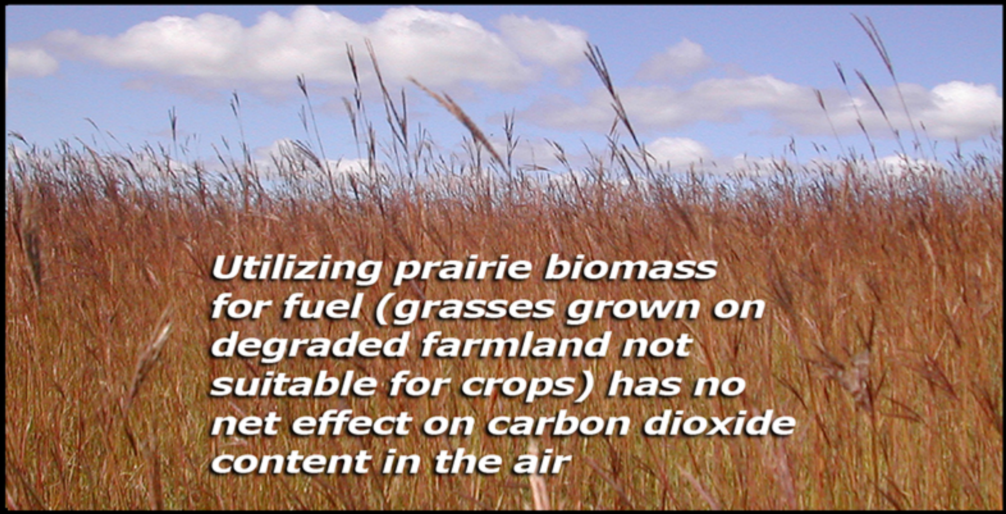
The U of M proposal was one of only 37 selected from 3,700 and one of only three featured in the New York Times when the grants were announced in October 2009. The University of Minnesota's Initiative for Renewable Energy and the Environment (IREE) and the College of Biological Sciences also provided funding.

Wackett is principal investigator for the ARPA-e grant. His team of co-investigators includes Jeffrey Gralnick, assistant professor of microbiology and Marc von Keitz, chief technical officer of BioCee, as well as Bhan and Schmidt. They are the only group using a photosynthetic bacterium and a hydrocarbon-producing bacterium together to make hydrocarbons from carbon dioxide.

The U of M team is using Synechococcus, a bacterium that fixes carbon dioxide in sunlight and converts CO2 to sugars. Next, they feed the sugars to Shewanella, a bacterium that produces hydrocarbons. This turns CO2, a greenhouse gas produced by combustion of fossil fuel petroleum, into hydrocarbons.

Hydrocarbons (made from carbon and hydrogen) are the main component of fossil fuels. It took millions of years of heat and compression to produce fossil fuels, which experts expect to be largely depleted within 50 years.





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can make a difference

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