Environment and Natural Resources Trust Fund
2017 Request for Proposals (RFP)

Project Title: **ENRTF ID: 142-E**

Engineering Stable Microbes for Biofuels and Biodegradation

Category: E. Air Quality, Climate Change, and Renewable Energy

Total Project Budget: $ 269,000

Proposed Project Time Period for the Funding Requested: 2 years, July 2017 – June 2019

Summary:

Microbes grow faster if they lose their engineered traits. Reducing this growth advantage will stabilize them and thus, lower the cost of making butanol and cleaning up polluted sites.

Name: Romas Kazlauskas

Sponsoring Organization: U of MN

Address: 1479 Gortner Ave

St. Paul MN 55108

Telephone Number: (612) 624-5904

Email rjk@umn.edu

Web Address www.umn.edu/~rjk

Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Preventing loss of function in microbes by reducing the growth advantage of the loss will lower the cost of making butanol & waste clean-up.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Engineering stable microbes for biofuels & biodegradation

I. PROJECT STATEMENT

We propose to engineer yeast and bacteria that do not lose their abilities to make butanol and biodegrade pollutants. Our approach is to eliminate the growth advantage of losing these abilities. The outcome of these more stable microbes is to reduce cost of making butanol and cleaning-up contaminants up to ten-fold.

Microbes like yeast and bacteria can make fuels and chemicals and can degrade pollutants. Microbes are a key green technology to protect the environment and natural resources because they eliminate hazardous processes and minimize pollution and greenhouse gas emissions.

Microbe instability limits their applications in industry and the environment. Microbes evolve to grow more quickly, but quicker growth usually means losing useful abilities. Faster-growing, useless microbes (equivalent to weeds in a farm field) overrun the useful microbes. Engineered microbes can be used in tightly controlled conditions, but not in varying environments like biofuel plants and polluted sites.

For example, engineered yeast can make butanol, a useful, next-generation biofuel. Unfortunately, the efficiency of butanol production drops quickly due to the evolution of faster-growing, butanol-non-producing yeast. This inefficiency is a bottleneck that raises costs. Microbe instability similarly hinders bioremediation at polluted sites. Faster-growing, non-degrading microbes overrun the pollution-degrading microbes before they can complete their tasks.

Our goal is more stable microbes. Our hypothesis is that evolutionary pressure for faster growth causes the loss of abilities. We propose to test two strategies that reduce this evolutionary pressure and expect it to yield more stable microbes. These more stable microbes will be better suited for applications outside controlled lab environments in real world applications. In particular, our goal is to engineer stable microbes for two test cases: 1) production of butanol by yeast and 2) biodegradation of hydrocarbon pollutants by bacteria. The outcome of this work will be two-fold 1) microbe-stabilization strategies that can be applied broadly in biofuels and biodegradation applications and 2) two example engineered microbes.

II. PROJECT ACTIVITIES AND OUTCOMES

The project will test two evolution-aware principles to design more stable microbes. Our approach is to prevent the useless microbes from growing faster so they will not spread, remaining as a few isolated weeds. The two prevention strategies are: 1) add other growth-limiting traits so that even if the useful trait is lost, other growth limits prevent faster growth and 2) create many copies of the desired trait so that loss of one copy provides negligible advantage and loss of all copies is statistically unlikely. One example of a growth limiting trait to add is a defect in DNA synthesis. If a microbe loses its useful abilities, then it will still not grow faster. For the many-copies approach, we will replace a few plasmids with high expression with many plasmids with low expression to lower the growth advantage of losing a single plasmid.

Activity 1: Engineer butanol-producing yeast to be more stable. **Budget: $135,000**

This research will test the two stabilization strategies to improve a bio-based manufacturing example: the production of butanol by yeast. Efficient production of butanol could replace ethanol manufacture in the future. The production will be tested in a 20-L fermenter in the laboratory. We expect the strain stability to increase at least ten-fold.

Outcome	Completion Date
1. Yeast strain that produces butanol (control strain)	31 Dec 2017

2. Yeast strain with other growth-limiting traits (strategy 1)	30 Jun 2018
3. Yeast strain with many copies of butanol genes (strategy 2)	31 Dec 2018
4. Compare stability and butanol production of all three strains	30 Jun 2019

Activity 2: Engineer hydrocabon-degrading *Pseudomonas* bacteria to be more stable **Budget: $ 134,000**

This research will test two similar stabilization strategies to improve a bioremediation example: degradation of hydrocarbons, such as spilled fuel, by *Pseudomonas* bacteria. All tests will be done in the laboratory, no field trials are planned for this project stage. Bacteria will include a 'kill-switch' (a special food requirement) to ensure that they grow only where and when desired.

Outcome	Completion Date
1. Bacterial strain that degrades hydrocarbons (control strain)	31 Dec 2017
2. Bacterial strain with other growth limiting traits (strategy 1)	30 Jun 2018
3. Bacterial strain with many copies of degradation genes (strategy 2)	31 Dec 2018
4. Compare of stability hydrocarbon degradation of all three strains	30 Jun 2019

III. PROJECT STRATEGY

A. Project Team/Partners

No funding requested for the two professors responsible for the project:

Prof. Romas Kazlauskas, Professor of Biochemistry, Molecular Biology and Biophysics

Prof. Michael Travisano, Professor of Ecology, Evolution and Behavior

Funding requested for salary and benefits for two postdoctoral fellows:

Postdoctoral Fellow #1: engineering and characterization of the yeast

Postdoctoral Fellow #2: engineering and characterization of bacteria

B. Project Impact and Long-Term Strategy

This work will generate a proof of concept for the transition of engineered microbes from the tightly controlled laboratory environments to the real world. The work will be disseminated by publication of journal articles and patents. In addition, we will contact current bio-based manufacturing companies (Cargill, Agri-Energy and ethanol manufacturers) to share our findings and yeast strains for testing in pilot plants. We will also contact bioremediation researchers (Wackett at the U of Minnesota) and the Department of Natural Resources to share our finding and bacterial strains.

In the longer term, this work can be extended to engineer other more stable microbes for bio-manufacturing and bioremediation. Funding for this additional work will be solicited from industrial partners and from government funding agencies in the future.

C. Timeline Requirements

2 years; start 1 July 2017; end 30 June 2019

2017 Detailed Project Budget

Project Title: Domesticating microbes for biofuels & biodegradation

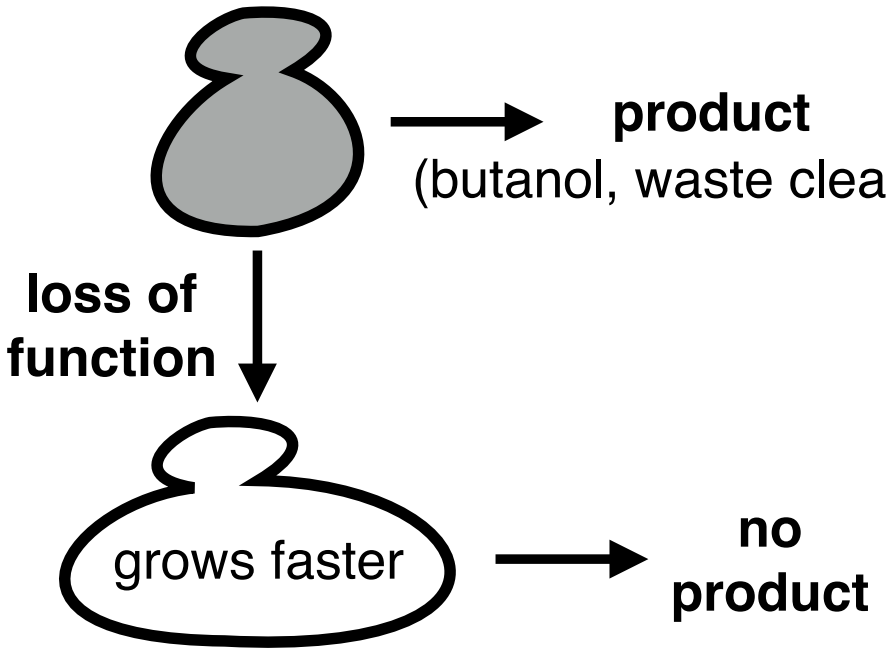
IV. TOTAL ENRTF REQUEST BUDGET 2 years

BUDGET ITEM	AMOUNT
Personnel: 2 Postdocs (82% salary, 18% benefits) 100% FTE; postdoc 1 will focus on activity 1 (domestication of yeast for production butanol) and postdoc 2 will focus on activity 2 (domestication of Pseudomonas bacteria for biodegradation of hydrocarbons). Salaries are $45,000 each for the first year, with 3% inflation for year 2.	$ 224,000
Equipment/Tools/Supplies: Laboratory supplies, incl. molecular biology reagents ($15,000 per year), Laboratory services, incl. DNA sequencing & gene synthesis ($5,000 per year), estimates based on experience with similar research	$ 40,000
Additional Budget Items: Publication fees $5,000 in year two.	$ 5,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 269,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period: N/A	$ -	
Other State $ To Be Applied To Project During Project Period: N/A	$ -	
In-kind Services To Be Applied To Project During Project Period: The University of Minnesota charges funders amounts in addition to direct costs in order to pay for the indirect costs of research (shared services such as the library, physical plant operation, maintenance, utility costs, administrative expenses, and depreciation for buildings and equipment). Since LCCMR does not allow charging indirect costs, the University is foregoing this charge and contributing it to the	$ 140,000	*Secured*
Funding History: N/A	$ -	
Remaining $ From Current ENRTF Appropriation: N/A	$ -	

Stabilize microbes



- **what** = prevent loss of function
- **how** = reduce growth advantage of loss
- **why** = lower cost of making butanol & waste clean-up

Project Manager Qualifications

Romas Kazlauskas earned his Ph.D. in chemistry at MIT in 1982. After postdoctoral work at Harvard University and three years of industrial research at General Electric, he taught chemistry at McGill University (Montréal, Canada) for fifteen years. He currently teaches biochemistry at the University of Minnesota (Twin Cities, USA). He has been a visiting professor in Germany, Sweden and South Korea. His favorite course is protein engineering for which he is currently writing a textbook. He is an expert in biotechnology and has published three books, >100 research papers and six patents. He has been honored with a Biocat Prize in Hamburg, Germany in 2004 and an honorary doctorate in Stockholm, Sweden in 2012.

Organization Description

The University of Minnesota-Twin Cities is the flagship research university in Minnesota and one of the top public research universities in the United States.

The research will be done in 174 Gortner Laboratory. This is a 2000 sq. ft. laboratory fully equipped for organic synthesis, biochemical, molecular biological, analytical chemical and microbiological work. Specific items include: shaker with temperature control, incubators, rotary evaporator, two UV/Vis plate reader (SpectraMax Plus384, Molecular Devices) capable of continuous wavelength scanning and cuvette accommodation, a fluorescence plate reader (SpectraMax Gemini XS, Molecular Devices), multichannel pipettors, balances, pH-meter, refrigerators and freezers (-80 and -20 °C), thermal cycler, high-speed centrifuges including one capable of centrifuging well plates, a high-throughput HPLC (Agilent HP1100) equipped with a binary pump, thermostated column compartment and well-plate sampler and a photo diode array detector and an Agilent HP6890 gas chromatograph with automatic injector and flame ionization detector. Next to the laboratory are cold and constant temperature rooms and a 300-MHz NMR spectrometer. A shared autoclave facility is upstairs.

Some of the fermentation experiment will be done in Biotechnology Resource Center Pilot Plant, also in the Gortner Laboratory. This is a 4,000 square foot laboratory/pilot plant facility with state-of-the-art equipment for research and development in fermentation, protein expression, and separation of a wide range of biological molecules.