Environment and Natural Resources Trust Fund
2016 Request for Proposals (RFP)

Project Title: **ENRTF ID:** **145-E**

Expanding Biofertilizers for Responsible Nitrogen Application

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

Total Project Budget: $ 659,512

Proposed Project Time Period for the Funding Requested: 3 years, July 2016 to June 2019

Summary:

This project aims to develop a broad-application biofertilizer to accomplish in crops like corn and wheat a relationship similar to what nature evolved in soybeans to fix atmospheric-nitrogen.

Name: Brett Barney

Sponsoring Organization: U of MN

Address: 1390 Eckles Ave
St. Paul MN 55108

Telephone Number: (612) 562-3061

Email bbarney@umn.edu

Web Address http://barneybioproductslab.cfans.umn.edu/

Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Graphic shows the current symbiotic relationship between soybeans and nitrogen fixing bacteria and the biofertilizer being developed for use with corn and wheat, which is the aim of this project.

_____ Funding Priorities _____ Multiple Benefits _____ Outcomes _____ Knowledge Base

_____ Extent of Impact _____ Innovation _____ Scientific/Tech Basis _____ Urgency

_____ Capacity Readiness _____ Leverage _______ TOTAL ______%

PROJECT TITLE: Expanding Biofertilizers for Responsible Nitrogen Application

I. PROJECT STATEMENT

WHY – Agriculture requires specific resources to produce the crops that meet the needs of a growing society. Nitrogen is a key component of fertilizers, and while industrial processes such as Haber-Bosch have enabled decades of increased agriculture production by making nitrogen more available, this comes with environmental and economic costs;

- Excessive nitrogen application can result in downstream water contamination leading to eutrophication, as has been highlighted in a recent report by the Minnesota Pollution Control Agency.
- Industrial nitrogen fixation through the Haber-Bosch process is the main industrial route to ammonia. It consumes 3-5% of natural gas production and requires about 1-2% of the energy supply worldwide, resulting in substantial inputs of carbon dioxide to the atmosphere from petroleum derived fuels.
- Further drawbacks are associated with transporting nitrogen from industrial production sites to our farms, and storage of certain forms of nitrogen such as anhydrous ammonia can be a further danger to farmers and their communities, as well as to the environment.

Long before the widespread application of nitrogen fertilizers, farmers knew that rotating certain crops such as soybeans, alfalfa or clover on alternating years results in improved yields of crops such as wheat or corn the following year. Years of study have taught us that the benefit from soybean, alfalfa and clover is related to a symbiotic relationship between the plant and specific bacteria that "infect" their root systems and form a symbiotic relationship. These relationships have several benefits;

- Nitrogen fixation is coupled to photosynthesis, as the fuel required to fix nitrogen biologically by the bacterium is provided through sugars provided to the bacterium by the plant. In this manner, the symbiotic nature of this relationship is the prime example of the first true "biofertilizers."
- Nitrogen is applied directly to the plant in the "original" and natural "timed-release" manner, minimizing issues associated with over-application and wasteful migration of nitrogen to our waterways.
- Nitrogen is produced on site, requiring no transportation costs or handling of dangerous chemicals.

With all the benefits that come from growing crops such as soybeans, one might ask why we do not use symbiotic nitrogen-fixing bacteria with crops like corn or wheat? The problem is that nature evolved these very specific relationships between the nitrogen-fixing bacteria and the soybean plant, but similar associations have not evolved in corn and many other current crops grown throughout the world. Looking for solutions to overcome this drawback in these commodity crops important to Minnesota is the primary goal of this project, and success in this aim would be truly transformative in nature, and would further mitigate the release of carbon dioxide, which is tied to global warming.

GOAL – This project aims to develop a broad application biofertilizer that can achieve in crops like corn and wheat what has naturally evolved in soybeans. This will lower environmental and economic costs associated with current conventional industrially-produced fertilizers and will also mitigate excessive nitrogen over-application by providing the nitrogen directly to the crop in a timed-release fashion.

OUTCOMES – The primary outcome of the project will be a bacterium that provides excess nitrogen to support targeted crops. Early efforts aim to optimize current bacteria that already demonstrate a strong potential for success, and further development would allow the strains to more efficiently utilize various crop residues or by-products of the state's bioethanol and biodiesel production industry. The second outcome would be demonstrations of the broader application to crops such as corn or wheat.

HOW – This project is divided into two primary activities; (i) improving nitrogen-fixing bacteria for broader application to expanded agriculturally relevant crops and (ii) laboratory and greenhouse feasibility evaluations of these enhanced bacteria with target crops. These studies can be pursued simultaneously and will provide valuable information as to the application of this technology in future field studies, while also evaluating the potential to scale-up the process for broad crop applications.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Improve Nitrogen-Fixing Bacteria for Application to Expanded Crops Budget: $409,512

The aim of this activity is to optimize a model nitrogen-fixing bacterium that has the potential for broad application and is found ubiquitously in the environment. Our laboratory has worked with this bacterium for more than a decade, focusing on the development of the strain into a suitable biofertilizer for the past four years. We have developed methods that allow us to screen the effects on nitrogen productivity by probing and eliminating each gene in the genome. Additional methods would evaluate the effect of regulating (increasing or decreasing levels of the gene products) using alterations of the techniques already employed. This approach is important, as it allows us to develop a strain that is only altered in a manner that either removes or reorganizes the genes already present in the bacterium, so that the strains are not "transgenic," and can be broadly applied.

Outcome	Completion Date
1. Screen nitrogen production in several thousand strains following gene deletions	*Sep 1st, 2017*
2. Screen nitrogen production in several thousand strains following differential regulation	*Feb 1st, 2018*
3. Final construction of optimized nitrogen production strains	*Aug 1st, 2018*

Activity 2: Feasibility Studies of Improved Bacteria with Agricultural Crops Budget: $250,000

The objective of the second activity is to evaluate currently developed strains – and additionally those that become available as a result of Activity 1 – to provide the requisite nitrogen to various target agricultural crops. These studies will be pursued under controlled conditions in greenhouse studies using conventional approaches or aquaponics with an eventual goal of pilot field studies. Further studies will evaluate the potential of crop by-products, crop residues remaining following harvest, and alternative industrial waste streams to aid in the growth of bacteria for expanded application.

Outcome	Completion Date
4. Complete Preliminary Laboratory Plant Growth Screens with Modified Bacteria	*March 1st, 2018*
5. Complete Greenhouse Feasibility Studies with Commodity Agricultural Crops	*Feb 1st, 2019*
6. Initiate Pilot Scale Field Studies	*May 31st, 2019*

III. PROJECT STRATEGY

A. Project Team/Partners

The research team will include Professor Brett Barney and Dr. Velmurugan Natarajan from the Department of Bioproducts and Biosystems Engineering and the Biotechnology Institute, Professor Craig Sheaffer from the Department of Agronomy and Plant Genetics and Professor Neil Olszewski from the Department of Plant Biology at the University of Minnesota. Brett is an expert in biological nitrogen fixation. Craig is an expert in sustainable cropping systems. Neil is an expert in plant genetics. The team is also supported by a range of undergraduate students, and would include additional graduate students based on this support.

B. Project Impact and Long-Term Strategy

Looking to future application, one key benefit of a biofertilizer is that widespread use of developed strains could begin immediately following successful demonstration. Biofertilizers are already commonly utilized based on various crop and agricultural by-products. Our efforts aim to improve upon current approaches to apply improved strains with broader application to crops that generally require industrial nitrogen inputs. Future funding needs may be required for promotion and educational opportunities to facilitate broader dissemination, or to launch extensive pilot-scale field studies. However, the potential implications of success in this project would be transformative in nature, and would likely lead to substantial interest by agricultural firms.

C. Timeline Requirements

The demonstration aspect of the project will be completed within 3 years. Successful completion of these aims will generate intense interest based on both economic and environmental incentives.

2016 Detailed Project Budget

Project Title: Expanding Biofertilizers for Responsible Nitrogen Application

IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM	AMOUNT	
Personnel:		
Brett Barney, Project Manager (75% salary, 25% benefits), Assistant Professor, 9 Month Appointment, Summer Salary; 10% FTE for 3 years	$	49,361
Velmurugan Natarajan, Postdoctoral Microbiologist (82% salary, 18% benefits); 75% FTE for 3 years	$	115,515
Research Scientist, Greenhouse Study Management and Maintenance (78.5% salary, 21.5% benefits); 50% FTE for 3 years	$	118,947
Marnie Plunkett, Junior Scientist, Laboratory Experiment Data Analysis (78.5% Salary, 21.5% Benefits); 100% FTE for 3 years		130,367
1 Graduate Research Assistant, Laboratory Experiment Data Analysis (58% salary, 42% benefits); 50% FTE for 3 years each	$	134,322
3 Undergraduate Technicians, Laboratory and Field Data Collection (100% salary, 0% benefits); 10% FTE for 3 years (generally rotating 1 year appointments)	$	50,000
Contracts:		
DNA Sequencing Analysis, Sequencing of Modifications to Nitrogen Fixing Bacteria, Locally sourced through either the University of Minnesota Sequencing Center or Local Companies		10,000
Equipment/Tools/Supplies:		
Laboratory Supplies: General Laboratory Chemicals, Media, Reagents and Kits for Performing Routine Molecular Biology, Analytical Reagents, DNA Synthesis of Primers, Liquid Nitrogen for Strain Storage (Based on historical costs of approximately $1250 a month)	$	45,000
Publication Charges: Costs associated with the broad dissemination of research findings in journals that are largely accessible to the broader public.	$	3,000
Travel:		
Travel to study sites within Minnesota for sample collection or to manage potential field studies at experiment stations or evaluate potential future studies.	$	3,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$	659,512

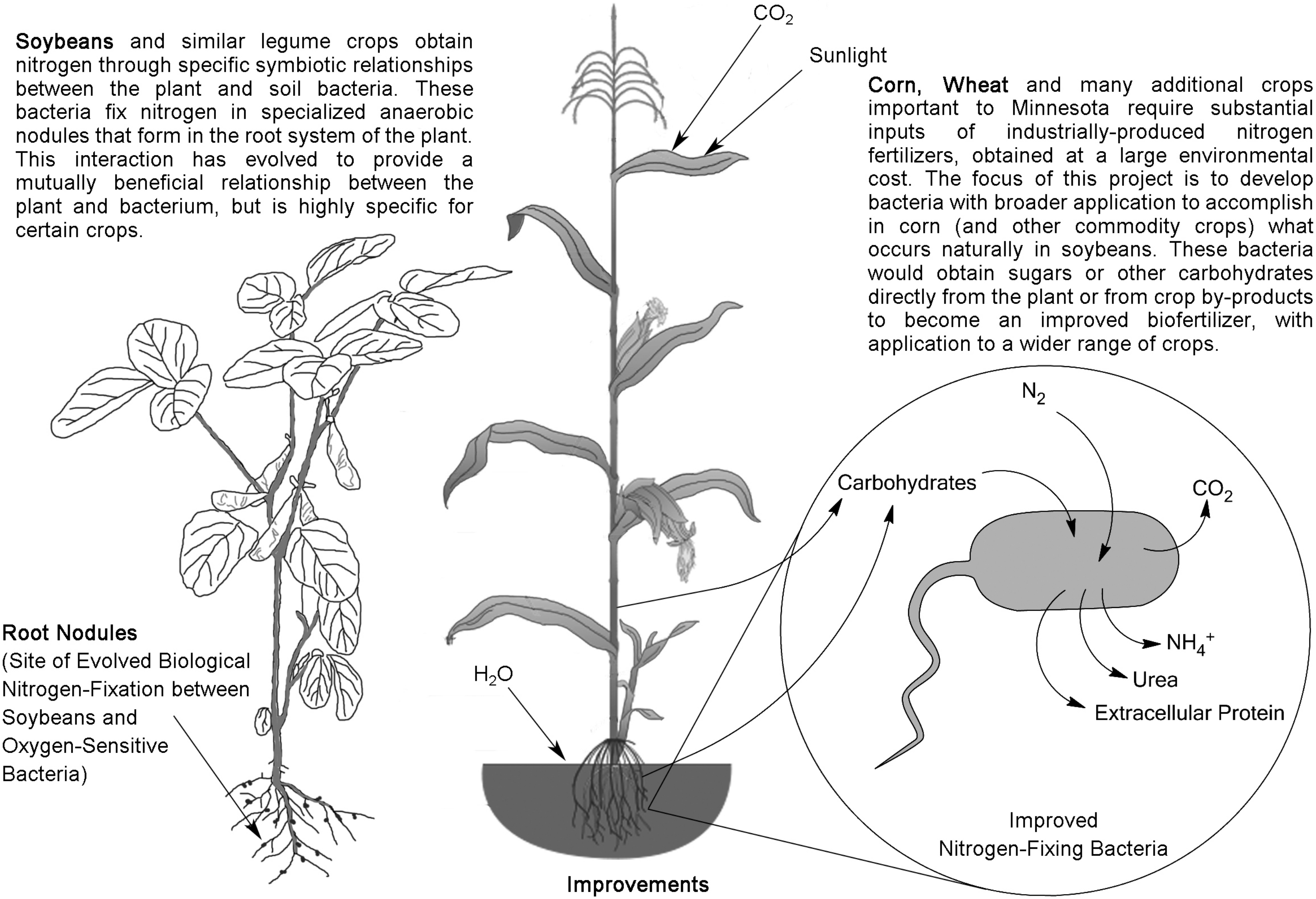
V. OTHER FUNDS

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:		
Indirect Costs/Facilities and Administration (52%)	$ 315,184	
Funding History:	$ 650,000	
$500,000 - MnDRIVE Transdisciplinary Research Program: Enhancement of Microbial Biofertilizers for Sustainable Food Systems. This grant funded advanced studies to complete laboratory demonstration projects, showing that developed strains could provide sufficient nitrogen to support plant cells (algae based system).		
$150,000 - IREE Career Award: Microbial Communities for Enhanced Biofuel Feedstock Production; This proposal funded initial studies into beneficial nitrogen-fixing bacteria and their application as a biofertilizer.		
Remaining $ From Current ENRTF Appropriation:	N/A	

Expanding Biofertilizers for Responsible Nitrogen Application

Soybeans and similar legume crops obtain nitrogen through specific symbiotic relationships between the plant and soil bacteria. These bacteria fix nitrogen in specialized anaerobic nodules that form in the root system of the plant. This interaction has evolved to provide a mutually beneficial relationship between the plant and bacterium, but is highly specific for certain crops.

Corn, Wheat and many additional crops important to Minnesota require substantial inputs of industrially-produced nitrogen fertilizers, obtained at a large environmental cost. The focus of this project is to develop bacteria with broader application to accomplish in corn (and other commodity crops) what occurs naturally in soybeans. These bacteria would obtain sugars or other carbohydrates directly from the plant or from crop by-products to become an improved biofertilizer, with application to a wider range of crops.



Improvements

Broader Appication Potential (Not Restricted to Certain Crops)

Naturally Aerobic Bacterium (Not Sensitive to Oxygen)

Increased Nitrogen Output

Project Manager Qualifications

The research team is composed of three faculty members, a postdoctoral fellow, and two junior scientists who assist with laboratory and greenhouse studies. Dr. Brett Barney is a Professor in the Department of Bioproducts and Biosystems Engineering and also a faculty member of the Biotechnology Institute at the University of Minnesota. Dr. Craig Shaeffer is a professor in the Department of Agronomy and Plant Genetics at the University of Minnesota. Dr. Neil Olszewski is a professor in the Department of Plant Biology at the University of Minnesota. Additional graduate students and undergraduate students will be hired as part of this project and jointly advised.

Dr. Barney is currently an Associate Professor in the Department of Bioproducts and Biosystems Engineering at the University of Minnesota. He is also a faculty member in the Biotechnology Institute at the University of Minnesota, and a member of the Microbial and Plant Genomics Institute at the University of Minnesota. He has served as a Laboratory Manager in multiple appointments, including both academic and industry settings for over 25 years. His extensive research experience includes enzymology, biological nitrogen fixation, hazardous waste site treatment methodologies, algal lipid production, genetic engineering and analytical chemistry for the quantification of a range of chemicals.

Dr. Barney's laboratory has been focused on two primary research aims, including biological oil production for the biofuel industry, and biological fertilizers (biofertilizers) for minimizing costs associated with biofuel costs, as well as environmental impacts related to biofuels and agriculture in general. Previous research funding has come from sources such as the National Science Foundation (NSF), the United States Department of Agriculture (USDA), the United States Department of Energy (DOE), the Defense Advanced Research Projects Agency (DARPA), Minnesota's Discover, Research and InnoVation Economy (MnDRIVE) and the Initiative for Renewable Energy and the Environment (IREE).

The Barney laboratory is housed on the second floor of the Cargill building for Microbial and Plant Genomics at the University of Minnesota. The Cargill building was designed with the intention to promote interdisciplinary collaborations and provide a shared lab space for each floor, which facilitates flexible group sizes. This large laboratory space is designed around a shared communal format, with various rooms available for utilization for specific experiments. The laboratory contains the primary equipment to perform this research project, including facilities to cultivate various bacteria, autoclaves, analytical instrumentation for analysis (gas chromatography, spectrophotometers, and balances), thermocyclers for PCR reactions, centrifuges, electrophoresis equipment and various incubators. Additional facilities include the Biotechnology Resource Center, the Genomic Sequencing Center and a broad range of additional analytical laboratories which are available as pay services.

Organization Description

Dr. Brett Barney has been a professor with the Department of Bioproducts and Biosystems Engineering at the University of Minnesota since 2009. The Bioproducts and Biosystems Engineering Department serves as a core department combining Agricultural Engineering, Biological Engineering and Environmental and Ecological Engineering. Numerous faculty members from the department have received support from the LCCMR program in the past. Our collaboration with Craig Sheaffer from the Department of Agronomy and Plant Genetics at the University of Minnesota brings expertise in plant systems that are important components of agriculture in Minnesota, while collaboration with Neil Olszewski from the Department of Plant Biology provides opportunities to study plant and bacterial interactions. The University of Minnesota provides a range of facilities and sufficient laboratory space to perform each of the activities described in this proposal. Additionally, controlled environments including greenhouse space sufficient for this work is conveniently located next door to Dr. Barney's laboratory space. UMN Sponsored Projects Administration (SPA) is the entity authorized by the Board of Regents to manage project agreements with LCCMR program.