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

Project Title: **ENRTF ID: 024-A**

Biogeographic Characterization of Antibiotics Produced in Minnesota Soils

Category: A. Foundational Natural Resource Data and Information

Total Project Budget: $ 171,858

Proposed Project Time Period for the Funding Requested: 2.5 years, July 2016 to October 2018

Summary:

Antibiotics produced by soil bacteria are an under-appreciated natural resource. We aim to systematically characterize the capacity of Minnesota soils to yield new antibiotics for biocontrol and clinical applications.

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Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Text description of figures included on Visual Page

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

PROJECT TITLE: Biogeographic characterization of antibiotics produced in MN soils

I. PROJECT STATEMENT

The vast majority of antibiotics used in the clinical setting are natural products, made by soil bacteria as a form of 'chemical warfare'. Unfortunately, our exploitation of antibiotics for the benefit of society in areas like medicine and agriculture has paralleled that of many other natural resources: decades of poorly managed and unregulated use were prompted by the inaccurate belief that nature would provide a limitless supply of new drugs. Recent characterization of the global antibiotic biosynthetic potential in soil bacteria has shown that *antibiotic natural products are not a limitless resource*. However, there are many still left undiscovered, and these need to be systematically characterized so they can be commercially developed responsibly with long-term management options in mind.

In addition to their commercial use, antibiotics play important roles in natural ecosystems, where they drive microbe-microbe and microbe-plant interactions. In several field research stations and agricultural plots across the state of Minnesota, communities of soil bacteria have been identified that are highly antagonistic (i.e. they have a higher-than-normal frequency of antibiotic producing bacteria). These soils are inherently *disease suppressive*, meaning that the plants that grow in them (whether they are native plants or crop species) are naturally protected against a number of microbial plant diseases. Thus, gaining a better understanding of the antibiotic biosynthetic potential in local soils addresses two goals of the ENRTF: (i) it provides a starting point for managing an important but underappreciated natural resource from our soils, namely antibiotic compounds, and (ii) it provides a mechanism to protect our native and agricultural flora from invasive disease (Figure 1).

By coupling the sequencing of environmental DNA with powerful bioinformatics tools, others have shown that antibiotic-producing bacteria are not uniformly distributed in natural soil environments. Instead, there is bio-geographic patterning of antibiotic biosynthesis, with some classes of antibiotics enriched in specific soil types. Unfortunately, there are no available data for a broad swath of the country across the upper Midwest, including Minnesota (Figure 2). The **overall goal** of the research proposed here is to identify the microenvironments in Minnesota that are enriched in the biosynthetic potential for new antibiotics and to leverage synthetic DNA technologies to characterize these compounds. The expected **outcomes** are a biogeographical map of antibiotic production across different soil types in the state and a better understanding of the mechanisms that underlie disease suppressive soils. We will accomplish these goals by completing the **specific activities** described in the next section.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Metagenomic survey to determine biogeography of MN antibiotic production. **Budget: $57,000**

Previous research has shown a distinct biogeographic pattern in antibiotic production among soil bacteria. However, the lack of sampling sites anywhere near the upper Midwest makes it impossible to extrapolate those findings to predict antibiotic production in local soil communities. In this proposal, we aim to sample soil from 60 unique soil ecotypes around the state of Minnesota, including in wetlands, farm fields, caves, pine forests, and prairies. Antibiotic biosynthetic genes will be amplified from isolated DNA with degenerate primers and sequenced using an Illumina HiSeq at the UMN Biomedical Genomics Center. This data will be processed to yield the first bio-geographical map of antibiotic production in native Minnesota ecosystems.

Outcome	Completion Date
1. Collect soil samples from 60 unique sites and classify according to USDA Soil Taxonomy	September 2016
2. Metagenome analysis of antibiotic production genes from each site	June 2017

Activity 2: Produce new antibiotics from rare Actinomycete bacteria via synthetic biology **Budget: $114,838**

We will selectively isolate rare Actinomycete bacteria from 60 soil samples using previously described enrichment techniques, with a goal of building a collection of 300 non-Streptomyces isolates. These will be identified using molecular phylogenetics and undergo preliminary screening for antibiotic production using chemical and

biological assays. We will sequence the complete genome of 5 promising strains on a PacBio sequencer and mine the genomes for novel antibiotics using our established pipeline. Genome mining will be accomplished using three complementary approaches: (i) multiplexed genome engineering will be used to modulate the genetic regulation of antibiotic production in native producing strains, (ii) antibiotic gene clusters will be cloned and mobilized to model heterologous production strains, and (iii) antibiotic biosynthesis pathways will be refactored and re-built from scratch using DNA assembly methods to ensure high-level expression of each required gene.

Outcome	Completion Date
1. Isolate 300 rare (non-Streptomyces) Actinomycetes from 60 collection sites	April 2017
2. Complete DNA extraction and preliminary metabolomics analysis of all 300 strains	July 2017
3. Genome sequence and annotation of 5 high-priority strains	September 2017
4. Genome mining for the production of new antibiotics in a model heterologous host	October 2018

III. PROJECT STRATEGY

A. Project Team/Partners: Described research will be led by the PI **Dr. Michael Smanski**. Dr. Smanski's roles will include tracking research progress and setting/adjusting project milestones, as well as disseminating results through yearly updates and regular publication in high-impact journals. **Suzie Hsu** and **Steven Heinsch** will perform research, analyze data, and help in the preparation of publications. PacBio sequencing and analysis will be performed in collaboration with **Dr. Bruce Eckloff**, core manager of the Mayo Clinic Molecular Biology Core. Further, we are currently conducting a pilot collaboration with the Joint Genome Institute to accelerate genome mining of new antibiotics using their DNA synthesis platform.

B. Project Impact and Long-Term Strategy

The long-term goal of this project is to create a bio-geographic map of antibiotic production in local soils to identify native soil communities that are enriched microorganisms producing novel antibiotics. We also seek to establish an efficient pipeline that will leverage the latest synthetic DNA technologies for the discovery of new antibiotics. The long-term outcomes of this research will be the discovery of new antibiotics with applications in clinical and agricultural settings.

This project is related to a long-term effort coordinated by the PI in conjunction with several other UMN faculty (**Dr. Linda Kinkel, Dr. Claudia Schmidt-Dannert, Dr. William Harcombe, Dr. Igor Libourel, Dr. Christine Salomon**) and USDA researchers (**Dr. Corby Kistler**) that aims to characterize microbial metabolites that play important roles in species-species interactions. Understanding and engineering the production of such molecules in natural and synthetic systems could provide disease-protection to crops and native flora, enhance the productivity of agricultural land, and decrease the application of synthetic fertilizers and pesticides, with associated benefits for surrounding natural resources.

We will disseminate our findings through peer-reviewed publications and (inter-)national scientific conferences. All bacterial strains isolated will be made available through the American Type Culture Collection (ATCC), and recombinant strains or plasmids will be available for interested parties for research purposes. We will coordinate any Intellectual Property pertaining to the new antibiotics discovered with the University of Minnesota's Office of Technology Commercialization, the ENRTF, and the state DNR.

C. Timeline Requirements

The research described in this proposal will be completed in 27 months, with a projected start date of June 2016. All soil samples needed to complete the proposed research will be collected during the summer of 2016. All processing of soil samples, including metagenomics analysis and isolation and taxonomy of rare Actinomycete bacteria will be concluded at the end of the first year. Finally, one full year will be dedicated to genome mining for new antibiotics. It is expected that each new genome sequence will contain the potential for 30-40 new metabolites, and these will be prioritized using bioinformatics prior to empirical characterization.

2016 Detailed Project Budget

Project Title: Biogeographic characterization of antibiotics produced in MN soils

IV. TOTAL ENRTF REQUEST BUDGET *2.25* years

BUDGET ITEM *(See "Guidance on Allowable Expenses", p. 13)*	**AMOUNT**
Personnel:	
Suzie Hsu, Graduate Research Assistant (53% salary, 47% benefits): 100% FTE for two years	$ 75,892
Stephen Heinsch, Graduate Research Assistant (53% salary, 47% benefits): 100% FTE for one year	$ 37,946
Professional/Technical/Service Contracts:	
DNA sequencing on PacBio at Mayo Clinic Molecular Biology Core	$ 5,000
DNA sequencing at UMN Biomedical Genomics Center (Illumina and Sanger)	$ 5,000
Mass Spectroscopy at UMN MS Core	$ 5,000
NMR at UMN NMR Core	$ 5,000
Equipment/Tools/Supplies:	
Supply costs (calculated as $1000 per person per month), roughly broken up into bacterial growth medium (10%), chemicals and reagents (20%), lab consumables (15%), enzymes (30%), and organic solvents (25%)	$ 36,000
Travel: *$2,000 is requested for travel to 60 state-wide sampling sites in two trips (No. Minn and So. Minn; total 2000 miles). Covers transportation costs, hotel accomodations, and meal stipend.*	$ 2,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 171,838

V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ To Be Applied To Project During Project Period: *Indicate any additional non-state cash dollars secured or applied for to be spent on the project during the funding period. For each individual sum, list out the source of the funds, the amount, and indicate whether the funds are secured or pending approval.*	N/A	*Indicate: Secured or Pending*
Other State $ To Be Applied To Project During Project Period: *Indicate any additional state cash dollars (e.g., bonding, other grants) secured or applied for to be spent on the project during the funding period. For each individual sum, list out the source of the funds, the amount, and indicate whether the funds are secured or pending approval.*	N/A	*Indicate: Secured or Pending*
In-kind Services To Be Applied To Project During Project Period: In kind services will be provided by the BMBB Department and BioTechnology Institute to cover indirect costs associated with managing the research project and providing administrative support to researchers ($61,376). Salary support for the PI (10% effort, salary plus fringe; $34,584)	$ 95,960	*Secured*
Funding History: $50,000 - BTI Biocatalysis funds "Algorithmic Design of Refactored Gene Clusters"	$ 50,000	
Remaining $ From Current ENRTF Appropriation: *Specify dollar amount and year of appropriation from any current ENRTF appropriation for any directly related project of the project manager or organization that remains unspent or not yet legally obligated at the time of proposal submission. Be as specific as possible. Indicate the status of the funds.*	N/A	*Indicate: Unspent? Legally Obligated? Other?*

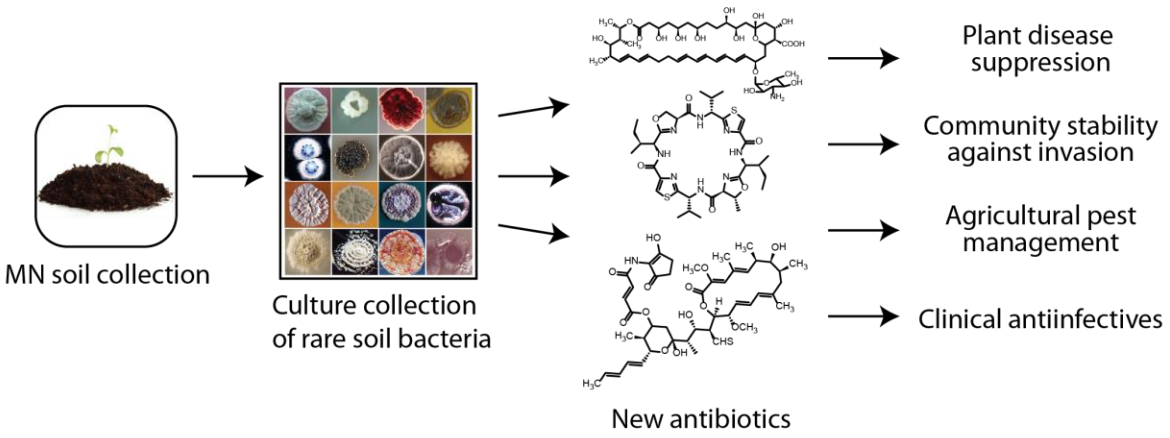

Figure 1. Overall summary figure for characterizing the unique MN soil bacteria that produce new antibiotics or other natural products.

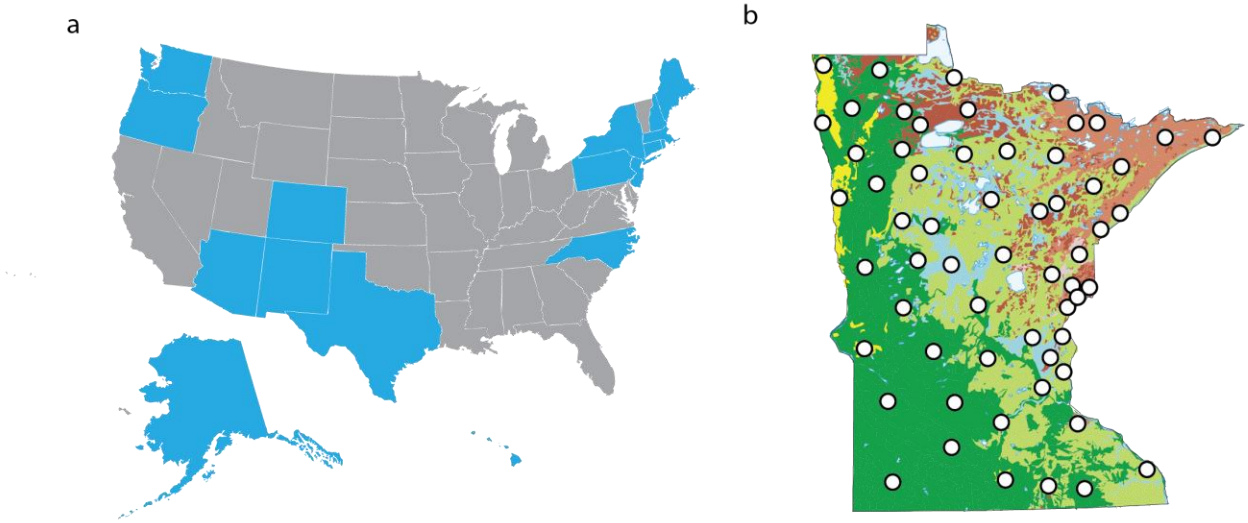

Figure 2. Past and proposed sampling sites for biogeographic studies. (a) Previous domestic sampling sites for antibiotic bio-geographic studies (adapted from drugsfromdirt.org).. Blue states have been previously sampled and grey states have not. (b) Soil type map of MN, with colors denoting major soil taxonomic groups (detaild map and key available at http://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/survey/class/taxonomy/) and points indicating proposed sampling sites.

PROJECT MANAGER QUALIFICATIONS: *Michael J. Smanski (PI)*

PROFESSIONAL PREPARATION

University of California, San Diego	Biochemistry	B.S., 2006
University of Wisconsin, Madison	Microbiology	Ph.D., 2011
Massachusetts Institute of Technology	Biological Engineering	2011-2014

APPOINTMENTS

Since 2014 Assistant Professor, Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Twin Cities

QUALIFICATION STATEMENT

Dr. Smanski's research involves understanding the distribution of small molecules, a.k.a. 'specialized metabolites' or 'natural products', present in nature and engineering their biosynthesis in model laboratory strains. Additionally, he is asking questions about the natural roles that these molecules play, including in stabilizing soil and plant communities from invasive species or pathogens. The Smanski lab combines 'omics'-level analyses and powerful computational algorithms with the latest synthetic DNA technologies to address these questions. He has extensive research experience in natural products biosynthesis and synthetic biology that make him well-suited to lead the research described in this proposal. His group is building on these experiences and leveraging the world-class facilities at the University of Minnesota, including the Minnesota Supercomputing Institute, the Biomedical Genomics Center, and the Metabolomics facilities at the UMN BioTechnology Institute, to accomplish their current research goals.

HONORS AND AWARDS

HHMI Fellow of the Damon Runyon Cancer Research Foundation	2012-2014
Dale F. Frey Award for Breakthrough Scientists	2014

FIVE MOST CLOSELY RELATED PUBLICATIONS

1. Smanski MJ, Bhatia S, Zhao D, Park YJ, Woodruff L, Giannoukos G, Ciulla D, Busby M, Calderon J, Nicol R, Gordon DB, Densmore D, Voigt CA. (2014) Functional optimization of gene clusters by combinatorial design and assembly. Nat. Biotechnol. 32:1241-1249.
2. Smanski MJ, Casper J, Peterson RM, Yu Z, Rajski SR, Shen B (2012) Expression of the platencin biosynthetic gene cluster in heterologous hosts yielding new platencin congeners. J. Nat. Prod. 75:2158-2167.
3. Smanski MJ, Yu Z, Casper J, Lin S, Peterson RM, Chen Y, Wendt-Pienkowski E, Rajski SR, Shen B (2011) Dedicated ent-kaurene and ent-atiserene synthases for platensimycin and platencin biosynthesis. Proc. Natl. Acad. Sci. USA 108:13498-13503.
4. Yu Z, Smanski MJ, Peterson RM, Marchillo K, Andes D, Rajski SR, Shen B (2010) Engineering of Streptomyces platensis MA7339 for overproduction of platencin and congeners. Org. Lett. 12:1744-1747.
5. Smanski MJ, Peterson RM, Rajski SR, Shen B (2009) Engineered Streptomyces platensis strains that overproduce antibiotics platensimycin and platencin. *Antimicrob. Agents Chemother.* **53**:1299-1304.