Environment and Natural Resources Trust Fund
2019 Request for Proposals (RFP)

Project Title: **ENRTF ID: 075-B**

Combatting Antibiotic Resistance Through Bacterial Signal Manipulation

Category: B. Water Resources

Sub-Category:

Total Project Budget: $ 444,865

Proposed Project Time Period for the Funding Requested: June 30, 2022 (3 yrs)

Summary:

This project will investigate a novel solution to stop the spread of antibiotic resistance at wastewater treatment plants by thwarting bacterial signaling, thus protecting the health of Minnesotans.

Name: Justin Donato

Sponsoring Organization: University of St. Thomas

Title: Associate Professor

Department:

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Web Address

Location

Region: Metro

County Name: Ramsey

City / Township:

Alternate Text for Visual:

Bacterial signaling contributes to the spread of bacteria harboring antibiotic resistance genes. By disrupting those signaling pathways, we may be able to halt the spread of antibiotic resistance.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%
____ If under $200,000, waive presentation?			

PROJECT TITLE: Combatting Antibiotic Resistance Through Bacterial Signal Manipulation

I. PROJECT STATEMENT

The overall goal of this project is to understand the relationship between bacterial signaling and antibiotic resistance in municipal wastewater treatment, with the ultimate goal of preventing the spread of antibiotic resistance genes from municipal wastewater treatment to Minnesota's environment and Minnesota's citizens. This project arose from unexpected results revealed during a previously funded ENRTF project (M.L. 2014, Chp. 226, Sec. 2, Subd. 03c) in which we discovered that antibiotic resistance genes were often found near genes involved in cell signaling.

It is well established that municipal wastewater contains incredibly large amounts of antibiotic resistance genes that are only partially eliminated by the wastewater treatment process. Our ongoing use and misuse of antibiotics contributes to the increasing spread of antibiotic resistance genes. It is also well established that bacteria living in mixed communities, including those in wastewater treatment bioreactors, coordinate activities through chemical signaling (i.e., bacteria can "talk" to each other). In our previously ENRTF-funded project, we discovered a possible link between antibiotic resistance genes and the genes that encode cell signaling. If this link is functionally relevant, then it is possible that these bacterial communities could be manipulated to disrupt the corresponding signaling pathways and simultaneously select against antibiotic resistance genes. These bacterial "conversations" are conducted using specific chemical signals that can be destroyed. **Therefore, linking antibiotic resistance gene expression to signaling provides a vulnerability that can be exploited to prevent the expression of resistance genes.** In other words, if we can destroy the bacterial communication network, we can prevent the expression of resistance genes. To that end, this proposal aims to characterize the bacterial signaling process, its link to antibiotic resistance, and to identify factors that could be used to modify and/or to eliminate specific signals.

This project could be highly impactful in that it will include characterization of multiple aspects of signaling associated with antibiotic resistance. Particular attention will be paid to factors capable of destroying bacterial signals as these have the potential to simultaneously select against antibiotic resistance genes. This research will potentially lead to a practical approach to stop the spread of antibiotic resistance from wastewater treatment facilities.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: *Collecting and deciphering billions of bacterial DNA sequences*

The success of this project will be dependent upon the construction of a comprehensive DNA sequence database to query for genes associated with bacterial signaling. Wastewater bacterial samples containing thousands of bacteria will be collected from three municipal wastewater treatment plants. Bacterial DNA will be harvested from each sample and will be submitted for next generation sequencing at the University of Minnesota Genomics Center.

ENRTF BUDGET: $272,432

Outcome	Completion Date
1. DNA harvested from samples containing thousands of bacteria	*December 31, 2019*
2. Millions of bacterial genes prepared for DNA sequencing	*June 30, 2020*
3. Generation of billions of DNA sequences	*September 30, 2020*

Activity 2: *Identifying factors that destroy a wide range of bacterial signals*

The sequence data generated in Activity 1 will be used to identify factors predicted to produce, receive, or destroy bacterial signals. The genes encoding these factors will be individually synthesized, introduced into

bacteria, and assessed for their ability to function in signaling processes. Specific signals will be generated and factors that destroy each signal will be identified and evaluated.

ENRTF BUDGET: $172,432

Outcome	Completion Date
1. Decoding and predicting function from DNA sequence data	*June 30, 2021*
2. Assessment of each of the predicted signaling genes in bacteria	*January 31, 2022*
3. Identification of factors that modulate or destroy bacterial signals	*June 30, 2022*

III. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role
Tim LaPara	**Professor**	**Department of Civil, Environmental, and Geo-Engineering, University of Minnesota**	**Responsible for Activity 1, collaborator on Activity 2.**

B. Partners NOT receiving ENRTF funding

Name	Title	Affiliation	Role
N/A			

IV. LONG-TERM- IMPLEMENTATION AND FUNDING:

The personnel conducting this project have collective expertise in wastewater treatment processes, antibiotic resistance, bacterial community analysis, and characterization of new bacterial enzymes. Furthermore, Drs. Donato and LaPara have successfully collaborated on the study funded by the ENRTF in 2014 that provided the impetus for this proposal. The current proposed work will further our understanding of factors contributing to the spread of antibiotic resistance (a critical problem in Minnesota that needs to be addressed) while at the same time characterizing a potential solution to the crisis in the form of new factors that can disrupt bacterial signals. These disrupting factors could become a product that is used as a bacterial management strategy that would not lead to further resistance. The outcomes of this work could lead to practical solutions to the spread of antibiotic resistance in wastewater treatment facilities and will be presented at scientific conferences, in press releases, and/or in peer-reviewed scientific journal articles.

V. TIME LINE REQUIREMENTS:

The initial stages of this study (preparing samples for DNA sequencing) will take the longest time but are absolutely critical to the success of this work. The first fifteen months of the project will entail the collection, processing, and sequencing of bacterial DNA. This will then serve as the material that is used for the computational analysis that is expected to last through the subsequent nine months. At that point, candidate genes will be identified, introduced into bacteria, and tested for their signaling properties. This work is expected to last for the remaining year in the grant period.

VI. SEE ADDITIONAL PROPOSAL COMPONENTS:

A. Proposal Budget Spreadsheet
B. Visual Component or Map
C. Project Manager Qualifications and Organization Description

2019 Proposal Budget Spreadsheet

Project Title: Combatting Antibiotic Resistance Through Bacterial Signal Manipulation

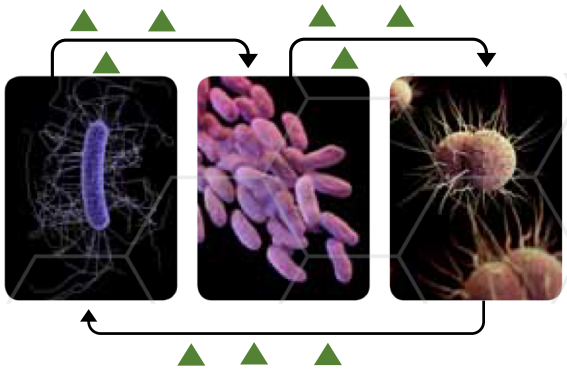
IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM (See "Guidance on Allowable Expenses")	**AMOUNT**
Personnel: Justin Donato, Project Manager, 4 weeks of salary per year plus associated fringe benefits (7.65%). Generally responsible for project completion and specifically responsible for Activities 1 and 2 ($26,379). Undergraduate students (4). These students will perform the work for Activities 1 and 2. These students will be full-time during the summer (7.65% fringe) and part-time during the academic year ($86,443). Postdoctoral researcher 100% effort plus fringe. This researcher will perform work described in Activities 1 and 2 ($183,491).	$ 296,312
Professional/Technical/Service Contracts: Personnel: Tim LaPara, Principal Investigator, 5 weeks of salary per year plus associated fringe benefits (33.6%). Spcifically responsible for Activity 1 and will actively collaborate on Activity 2 ($41,953). DNA sequencing will be performed as a fee for service by the University of MN Genomics Center ($62,500).	$ 104,453
Equipment/Tools/Supplies: This item will include expendable supplies including glassware ($2,500), chemical reagents ($16,000), DNA extraction kits ($8,000), and other consumable laboratory supplies ($12,000).	$ 37,500
Acquisition (Fee Title or Permanent Easements):	$ -
Travel: A small amount of travel will be required for this project. It will include traveling to wastewater treatment plants to obtain inoculum, travel between Universities to meet with each other, and travel to local conferences to present the results.	$ 6,600
Additional Budget Items:.	
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 444,865

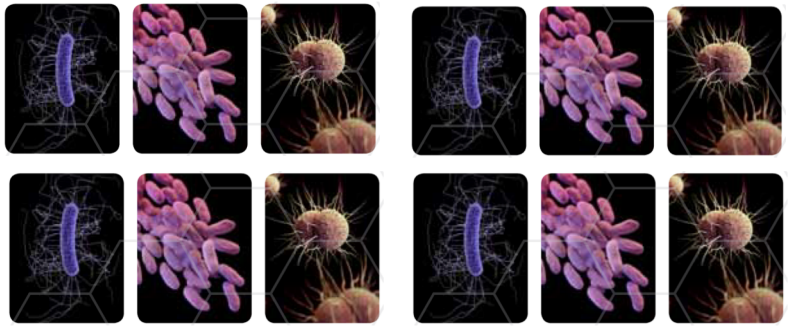
V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ To Be Applied To Project During Project Period: 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: Overhead contributions that would otherwise be requested as indirect costs**.**	$ 199,810	
Past and Current ENRTF Appropriation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03c	$ 380,000	
Other Funding History: N/A	$ -	

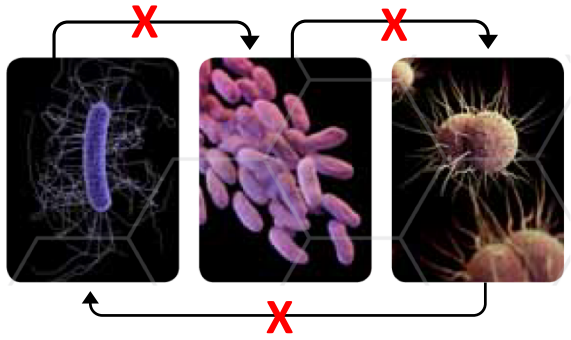
Bacteria (black boxes) communicate using chemical signals (green triangles).



Successful signaling leads to the spread of antibiotic resistance.



Destruction of chemical signals renders bacteria unable to communicate.



Disruption of bacterial signaling blocks the spread of antibiotic resistance.

Project Title: Combatting Antibiotic Resistance Through Bacterial Signal Manipulation

Project Manager Qualifications

Justin J. Donato

Education: **B.S.** 1999, Chemistry, University of Delaware; **Ph.D**., 2006, Biochemistry, Cornell University.

Employment: **Associate Professor**, 2016 - present, Department of Chemistry; **Assistant Professor**, 2010 – 2016, Department of Chemistry, University of St. Thomas; **Post-doctoral Fellow**, 2006 - 2010, Department of Bacteriology, University of Wisconsin - Madison.

Research
Dr. Donato's research focuses on identifying and characterizing new genes responsible for conferring important traits on their bacterial hosts. His group's current interests focus on the use of cutting edge genomic technology to analyze antibacterial resistance genes from diverse habitats and the factors that lead to their expression.

Timothy M. LaPara

Education: **B.S.C.E.,** 1995, Civil Engineering, University of Notre Dame; **Ph.D**., 1999, Environmental Engineering, Purdue University.

Employment: **Professor,** 2013-present**,** Department of Civil Engineering, University of Minnesota; **Associate Professor**, 2006-2013, Department of Civil Engineering, University of Minnesota; **Assistant Professor**, 2000-2006, Department of Civil Engineering, University of Minnesota; **Post-doctoral Research Associate**, 2000, Department of Biological Sciences, Purdue University.

Research
Dr. LaPara's research is focused on the role of municipal and industrial wastewater treatment plants in preserving environmental quality and in protecting public health. His research has a strong interdisciplinary nature, stemming from his unique background in both environmental engineering and microbiology.

See both the main proposal and the project budget for a description of the specific responsibilities of each project manager within this proposed project.

Organization Descriptions
Dr. Donato is in the Chemistry Department at the University of St. Thomas (St. Paul, MN). Dr. LaPara is in the Civil Engineering department at the University of Minnesota-Twin Cities, which is the state of Minnesota's largest institution of higher education.