Environment and Natural Resources Trust Fund

M.L. 2021 Approved Work Plan

General Information

ID Number: 2021-390

Staff Lead: Rory Anderson

Date this document submitted to LCCMR: July 21, 2021

Project Title: Antibiotic Resistance And Wastewater Treatment: Problems And Solutions

Project Budget: $432,000

Project Manager Information

Name: Justin Donato

Organization: University of St. Thomas

Office Telephone: (651) 962-5580

Email: dona1145@stthomas.edu

Web Address: https://www.stthomas.edu/

Project Reporting

Date Work Plan Approved by LCCMR: July 20, 2021

Reporting Schedule: January 1 / July 1 of each year.

Project Completion: June 30, 2024

Final Report Due Date: August 14, 2024

Legal Information

Legal Citation: M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 04j

Appropriation Language: $432,000 the first year is from the trust fund to the commissioner of natural resources for an agreement with the University of St. Thomas to quantify the ability of full-scale wastewater treatment plants to eliminate antibiotic resistance genes entering or created in the water treatment process before these genes are released into the natural environment.

Appropriation End Date: June 30, 2024

Narrative

Project Summary: This project will quantify the ability of full-scale wastewater treatment plants to eliminate antibiotic resistance genes and the extent to which these genes are exchanged during the wastewater treatment process.

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Pandemic infectious diseases are an increasing threat to our daily lives. At present, more than 35,000 people die of antibiotic-resistant infections each year in the United States; some models are predicting as many as 10 million annual deaths attributable to antibiotic resistant infections (worldwide) by 2050. The scientific community now understands that untreated municipal wastewater (sewage) is especially rich in antibiotic resistant bacteria.

We postulate, therefore, that wastewater treatment is a potential solution to the problem of antibiotic resistance, although we expect different systems to perform better than others. This project, therefore, proposes to investigate full-scale wastewater treatment facilities to determine which designs are best-suited to eliminate antibiotic resistant bacteria.

Almost paradoxically, we also postulate that an unexpected consequence of centralized municipal wastewater treatment is that it facilitates the development of novel bacteria that are simultaneously resistant to multiple antibiotics. Bacteria are well-known to be able to exchange genetic material, particularly when there are dense communities of different microorganisms – which are precisely the conditions that are intentionally created during and are essential to the success of biological wastewater treatment.

What is your proposed solution to the problem or opportunity discussed above? i.e. What are you seeking funding to do? You will be asked to expand on this in Activities and Milestones.

We hypothesize that sewage sludge incineration, which is performed at the Metropolitan Wastewater Treatment Plant in St. Paul, is the best and most cost-effective technology for eliminating antibiotic resistant bacteria in untreated municipal wastewater. The application of incineration to treat sewage sludge is relatively rare in the United States but common in other countries. The reason that sewage sludge incineration is rarely practiced in the United States is due to obsolete concerns regarding air pollution; modern incinerators pose little threat to air pollution, such that a country like Switzerland (with some of the most stringent environmental regulations in the world) incinerates the majority of its sewage sludge.

Although it is inarguable that municipal wastewater treatment is necessary to protect surface water quality and public health, it is not without unanticipated consequences. The creation of a nearly ideal environment for bacteria to exchange genetic material is likely one of these collateral consequences. Common wastewater bacteria are known to exchange antibiotic resistance genes when grown as isolated cultures. However, this ability has yet to be fully explored in mixed bacterial communities present at wastewater treatment facilities. We will track resistance genes within these communities to monitor and eventually eliminate their dissemination.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

This project takes a ONE HEALTH perspective, which is defined as a collaborative, multisectoral, and transdisciplinary approach—working at the local, regional, national, and global levels—with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment. The implementation of the findings from this project will lead to more efficient removal of antibiotic resistance genes from untreated wastewater. If our hypothesis is correct, we anticipate a gradual shift towards the increased application of incineration for treated sewage sludge. This project, therefore, should lead to better environmental and public health.

Project Location

What is the best scale for describing where your work will take place?

Statewide

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

During the Project and In the Future

Activities and Milestones

Activity 1: Quantify the ability of different wastewater treatment facilities to remove/eliminate antibiotic resistance genes.

Activity Budget: $307,000

Activity Description:

Samples will be collected from multiple locations within municipal wastewater treatment facilities to allow us to determine the fraction of antibiotic resistant bacteria that enter the facility (i.e., with raw sewage), exit the facility with the treated effluent (i.e., the "clean" wastewater that is usually released to a river), and the treated sewage solids (i.e., either incinerated, applied to agricultural land, or landfilled). DNA will be extracted and purified from these samples and we will use techniques pioneered at the University of Minnesota to quantify numerous genes known to encode antibiotic resistance. We would like to work with at least 10 different wastewater treatment plants and to collect at least triplicate samples from each facility (i.e., samples collected on different dates). We anticipate collect from 6 different locations within each treatment facilities (i.e., we anticipate collecting at least 180 samples; 10 facilities x 3 sample dates x 6 locations = 180). We anticipate quantifying at least 20 different antibiotic resistance genes.

Activity Milestones:

Description	Completion Date
Sample collection and processing	December 31, 2022
Quantification of antibiotic resistance genes	August 31, 2023
Data Analysis	December 31, 2023

Activity 2: Recognizable antibiotic resistance genes from each stage of wastewater treatment will be identified and tracked as they spread between bacteria.

Activity Budget: $125,000

Activity Description:

Antibiotic resistance genes will be identified by analyzing all DNA sequences present in wastewater-derived samples. A subset of the samples harvested in Activity 1 will be subjected to a more comprehensive, cutting-edge DNA sequencing technology that is commercially available from Phase Genomics. Antibiotic resistance genes are often located on small DNA segments that are exchanged between bacteria. Therefore, identifying which bacterial species harbors a given resistance gene by simply analyzing the isolated DNA fragments is often impossible. The technology that will be used here links resistance gene sequences to their bacterial hosts, facilitating identification of (1) all resistance genes in the samples and (2) which bacteria harbor those resistance genes. Samples will be taken from four points within four treatment facilities. All of the DNA within each sample will be fully sequenced, yielding billions of base pairs of genetic data. The resistance genes within this dataset will be linked to the specific identities of bacteria in each original sample. Since samples will be taken from multiple points in the treatment process, changes in the identities of the bacteria associated with specific resistance genes will indicate spread of those resistance genes during wastewater processing.

Activity Milestones:

Description	Completion Date
DNA processing and submission for sequencing	June 30, 2022
Antibiotic resistance gene determination	June 30, 2023
Spread of resistance gene analysis complete	June 30, 2024

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
George Sprouse and Larry Rogacki	Metropolitan Council, Environmental Services	MCES will provide access to samples from the largest municipal wastewater facilities in the State of Minnesota.	No
Timothy LaPara	University of Minnesota - Twin Cities	Co-project manager; Dr. LaPara will co-supervisor the students and post-doc working on this project.	Yes

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.
Findings will be shared among the project personnel. This includes the Metropolitan Council Environmental Services, so the information will be available to wastewater facility managers they oversee. The findings will be included in reports to LCCMR, peer-reviewed publications, conference presentations, and potentially press releases to the media. In presentations with a visual component (e.g. conference presentations with slides and poster presentations), the ENRTF logo will be displayed. In those instances, ENRTF will also be acknowledged orally. In print media (e.g. peer-reviewed publications), a statement acknowledging ENRTF will be included per the ENRTF guidelines. The DNA sequence data generated from this work will be deposited in GenBank, a publicly available repository. Where appropriate, the associated metadata and sequence data will also be made publicly available in databases such as CARDLive for use by other researchers. The intended audience includes the public, wastewater treatment managers, and scientists.

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this be funded?
The findings from this project will inform the best practices for eliminating antibiotic resistance genes during the wastewater treatment process. The processes being analyzed in this project are already in place at different municipal wastewater treatment facilities. Once the most efficient processes have been established, the information will be shared with facility managers to use in determining their preferred methods of antibiotic resistance gene elimination. We anticipate new lines of research may be stimulated by the proposed project; we will seek funding for these ideas from appropriate sources, such as the National Science Foundation and the LCCMR/MN ENTRF.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Triclosan Impacts on Wastewater Treatment	M.L. 2014, Chp. 226, Sec. 2, Subd. 03c	$380,000

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Undergraduate Researcher 2		Researcher			8%	0.99		$33,000
Undergraduate Researcher 1		Researcher			8%	0.99		$33,000
Postdoctoral Researcher		Researcher			33%	3		$210,000
Justin Donato		Project Manager			8%	0.24		$27,400
							Sub Total	**$303,400**
Contracts and Services								
Phase Genomics	Professional or Technical Service Contract	This project is dependent upon the generation of DNA sequence datasets using a technology pioneered by Phase Genomics. We plan to generate these data as a fee for service. Parts of the data that will be generated by the University of Minnesota Genomics Center to reduce costs if possible.				0		$50,000
University of Minnesota - Twin Cities	Sub award	This sub-award will fund the activities conducted by Dr. Timothy LaPara, the co-PI on this project. These funds will cover expenses associated with compensation for Dr. LaPara and one researcher, lab supplies, and travel within MN for sample collection.				0.15		$59,600
							Sub Total	**$109,600**
Equipment, Tools, and Supplies								
	Tools and Supplies	Reagents for molecular biology analysis of antibiotic resistance genes	Antibiotic resistance genes will be detected and quantified using standard techniques and reagents.					$6,000
							Sub Total	**$6,000**
Capital Expenditures								

		One autoclave	The majority of tools and reagents to complete this project need to be sterilized in an autoclave.					$8,000
							Sub Total	**$8,000**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
							Sub Total	**-**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
	Publication	Open access journal article publishing	Dissemination of findings					$5,000
							Sub Total	**$5,000**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$432,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	**-**
Non-State				
In-Kind	University of Minnesota - Twin Cities	These are overhead contributions that would otherwise be requested as indirect costs.	Secured	$32,200
In-Kind	University of St. Thomas	These are overhead contributions that would otherwise be requested as indirect costs.	Secured	$164,400
			Non State Sub Total	**$196,600**
			Funds Total	**$196,600**

Attachments

Required Attachments

Visual Component

File: aea5dc97-e00.pdf

Alternate Text for Visual Component

The problem of antibiotic resistance gene spread among wastewater bacteria can be eliminated through effective treatment strategies. Technologies including incineration can be implemented to destroy antibiotic resistant bacteria....

Financial Capacity

File: 81d06e83-756.pdf

Optional Attachments

Support Letter or Other

Title	File
Research Addendum	34e24645-d73.docx
Signed Background Check	0b967eda-b4b.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

To meet the reduced budget, one undergraduate was removed from the project, the project manager compensation was reduced, and the subaward amount was reduced.

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?

Yes

Do you agree travel expenses must follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

N/A

Does your project have potential for royalties, copyrights, patents, or sale of products and assets?

No

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

N/A

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

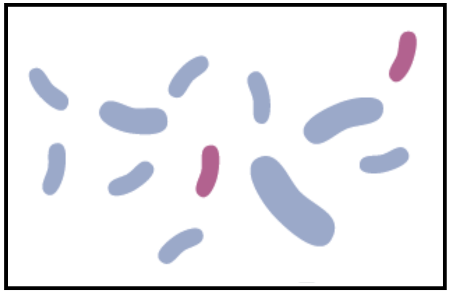
N/A

Does your project include original, hypothesis-driven research?

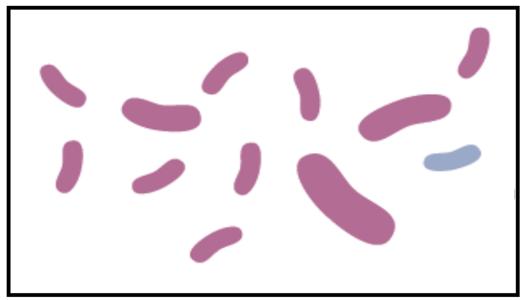
Yes

Does the organization have a fiscal agent for this project?

No



The Problem:
Bacteria can exchange antibiotic resistance genes. Wastewater treatment bioreactors are ideal locations for resistance gene swapping to occur.



The Solution:
Wastewater treatment technologies can be used to destroy antibiotic resistant bacteria, especially when sewage sludge is incinerated.