# Environment and Natural Resources Trust Fund

2025 Request for Proposal

## General Information

**Proposal ID:** 2025-223

**Proposal Title:** Bioprospecting Minnesota Wetlands for Phage and Bacterial Antimicrobials

## Project Manager Information

**Name:** Brian Dingmann

**Organization:** U of MN - Crookston

**Office Telephone:** (218) 281-8249

**Email:** dingm021@umn.edu

## Project Basic Information

**Project Summary:** Antibiotic resistance represents a critical global health issue. Our innovative approach combines studentsourcing with advanced research techniques to engage the next generation of scientists in discovering potentially new antimicrobials.

**ENRTF Funds Requested:** $443,000

**Proposed Project Completion:** June 30, 2028

**LCCMR Funding Category:** Foundational Natural Resource Data and Information (A)

## Project Location

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

Region(s): NW, Central, NE,

**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

## Narrative

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

Overuse and misuse of antibiotics have contributed to a worldwide crisis in antibiotic resistance. The growing gap between how fast microbes are developing resistance and the development of new antimicrobials is a public health threat. Innovative search strategies are needed to identify new medicines to combat this health challenge.

Minnesota's wetlands are rich, biodiverse ecosystems that serve as crucial carbon sinks and play a vital role in geochemical cycling. These environments are primarily unexplored regarding their viral communities, particularly bacteriophages (phages), which could hold critical insights into novel antimicrobial agents. Our previous efforts in exploring these wetlands for bacteria have led us to explore phages in these ecosystems further. Despite the crucial role of phages in microbial ecosystems and their potential in phage therapy, their diversity and functions in Minnesota's wetlands await further exploration.

Likewise, more is needed to know about the diversity and functionality of the bacterial communities that call Minnesota wetlands home. Most antibiotics have been developed from natural products originating from soil microorganisms. However, only one percent of all soil bacteria can be grown in the laboratory. Therefore, enhancing previously unculturable soil bacteria allows for identifying new antimicrobials. Bioprospecting Minnesota's wetlands could partially address antibiotic resistance.

**What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.**

This project seeks funding to search Minnesota's wetlands for microorganisms producing antimicrobials or targeting human bacterial pathogens. The proposed research would enhance cultivating previously unculturable antimicrobial-producing bacteria and characterizing bacteriophages that target human pathogens in Minnesota's wetlands, leveraging SEA-PHAGES and Tiny Earth methodologies (see activities). We aim to use innovative isolation techniques, such as the iChip, to culture previously unculturable bacteria, potentially uncovering new antimicrobials. Streptomyces, known for its antibiotic-producing capabilities, can be targeted with these methods. The University of Minnesota Crookston (UMC) and Northwest Technical College (NTC) in Bemidji, MN, have a unique opportunity to pioneer this exploration. This project aims to provide marketable, practical skills for underrepresented students in outstate Minnesota, enhancing their scientific understanding and perceptions. This project ultimately proposes finding bacterial viruses (phages) that target bacterial human pathogens and microorganisms that produce antimicrobial products within this context. We will build a comprehensive phage discovery and characterization program by engaging UMC and NTC students, integrating phage research into broader antimicrobial discovery efforts. This approach addresses the urgent need for new antimicrobials in the face of rising antibiotic resistance and trains students in cutting-edge research techniques, fostering a new generation of scientists equipped to tackle global health challenges.

**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?**

Bioprospecting in Minnesota's wetlands will contribute to scientific knowledge and provide preliminary data to encourage exemplary stewardship/conservation of wetlands and a better understanding of ecological services. To this end:

1) The project would investigate and analyze the microbial functional diversity and community structure in Minnesota's northern wetlands and wetland phage diversity and identification.
2) Enhancement sampling for Actinomycetes would lead to potential new sources for antimicrobials.
3) UMC/NTC faculty will train undergraduates in microbiological/molecular techniques and genomics, preparing participants for impactful careers in health, biotechnology and environmental sciences. Appreciation of Minnesota's wetland resources will ultimately be emphasized.

## Activities and Milestones

### Activity 1: Isolate and characterize wetland phages that have antibacterial activity

**Activity Budget:** $188,000

**Activity Description:**
The growing issue of antibiotic resistance underscores the urgent necessity for novel antimicrobials. Bacteriophages, viruses that infect and lyse bacteria, present a promising avenue for novel antimicrobial strategies. The SEA-PHAGES (Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science) program has refined protocols for the discovery and detailed analysis of bacteriophages. This project focuses on isolating and characterizing bacteriophages from environmental samples, aiming to uncover phages with therapeutic potential against antibiotic-resistant pathogens. We strive to identify phages with specific antibacterial activity through meticulous screening and genomic analysis.

Faculty and undergraduate students from the University of Minnesota Crookston (UMC) and Northwest Technical College (NTC) will apply advanced molecular and microbiological techniques to uncover and enhance the cultivation of bacteriophages. This exploration will contribute to the phage therapy field and enrich our understanding of phage biodiversity in wetland ecosystems. Molecular methods will pinpoint phage genes crucial for antibacterial action and develop practical approaches to amplify these therapeutic phages. This research will be integrated into relevant courses across UMC and NTC. The deliverables include generating promising leads for future phage-based antimicrobial therapies and disseminating teaching materials to Tribal and Community Colleges through the Veden Center for Rural Development (Crookston).

**Activity Milestones:**

| Description | Approximate Completion Date |
|---|---|
| Faculty and 4-8 undergraduates will collect samples from bogs in northern Minnesota (2 seasons). | October 31, 2026 |
| Protocol development (e.g., sampling, safety, molecular and microbiology, etc.) in lab meetings and mentoring. | October 31, 2026 |
| Faculty and 4-8 undergraduate research students will be trained in phage isolation and characterization. | April 30, 2027 |
| Faculty and 4-8 undergraduate research students will compile/disseminate findings and teaching protocols. | April 30, 2027 |
| Undergraduates (teaching laboratories) will be trained in bacterial isolation and characterization (3 years; >150 students). | December 31, 2027 |

### Activity 2: Characterize the microbial community's ability to produce potential antimicrobials

**Activity Budget:** $255,000

**Activity Description:**
The antibiotic resistance crisis provides an immediate and critical need for new and novel antimicrobials. Natural products, such as secondary metabolites from microorganisms, constitute most of our antibiotics. The Tiny Earth Studentsourcing Antibiotic Discovery project has standardized the methodology for isolating and characterizing potential producers of antimicrobial bacteria. After isolating and purifying soil bacteria, we will conduct competition experiments against the so-called safe pathogens to identify likely antibiotic-producing cultures. Subsequently, chemical extraction/fractionation would lead to identifying antimicrobial compounds.

Faculty/undergraduate students at UMC/NTC will employ molecular techniques, microbiological methods, and genomics to identify and selectively enhance the culture of Actinobacteria. This group of bacteria is known to exist in wetlands and

has demonstrated the ability to produce antimicrobial products. Enhancement culturing is critical since most soil microbes fail to grow in the lab. Molecular techniques will identify Actinobacteria genes that are responsible for the production of potential antibiotics. Course-appropriate integration into suitable courses at UMC and NTC will engage at least 120 students during the grant period. The deliverables include generating promising leads for future antimicrobial drug development and disseminating teaching materials to Tribal and Community Colleges through the Veden Center for Rural Development (Crookston).

**Activity Milestones:**

| Description | Approximate Completion Date |
|---|---|
| Undergraduates (teaching laboratories) will be trained in bacterial isolation and characterization (3 years; >150 students). | December 31, 2027 |
| Faculty and 4-8 undergraduates will complete fractionation and chemical identification of potential new antimicrobials. | February 28, 2028 |
| Faculty and 4-8 undergraduates will complete microbial functional and biodiversity analyses. | May 31, 2028 |
| Faculty and undergraduate will prepare presentations/manuscript through collaborative mentorship of scientific writing. | May 31, 2028 |

## Project Partners and Collaborators

| Name | Organization | Role | Receiving Funds |
|---|---|---|---|
| Karl Anderson | University of Minnesota Crookston | Co-PI: managing students; microbiological and molecular technique guidance. | Yes |
| Venugopal Mukku | University of Minnesota Crookston | Co-PI: managing students; chemical extraction and fractionation guidance. | Yes |

## 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 work be funded?**

We have used most of the techniques for several years in our teaching laboratories at UMC and NTC and have the existing infrastructure to train the undergraduates, as mentioned in this proposal. After the grant funding ends, we would utilize the protocols developed and lessons learned to facilitate a sustained effort to integrate techniques into various courses at the partner institutions. If we find some promising compounds or at least exciting leads for antibacterial, antifungal, or antiviral medicinal natural products, we will aggressively seek additional funding opportunities.

## Other ENRTF Appropriations Awarded in the Last Six Years

| Name | Appropriation | Amount Awarded |
|---|---|---|
| Exploring Minnesota's Wetlands: Our Resource For Future Medicine | M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 03k | $210,000 |

## Project Manager and Organization Qualifications

**Project Manager Name:** Brian Dingmann

**Job Title:** Associate Professor, University of Minnesota Crookston

**Provide description of the project manager's qualifications to manage the proposed project.**

The Tiny Earth Studentsourcing Antibiotic Discovery project has standardized the methodology for isolating and characterizing potential antibiotic-producing bacteria. Their mission is to create a worldwide crowdsourcing initiative to search for new and novel antibiotics. Additionally, the SEA-PHAGES (Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science) program has refined protocols for the discovery and detailed analysis of bacteriophages. These microbiological techniques have been used extensively in the University of Minnesota Crookston teaching laboratories. We have already trained many students in these techniques in the teaching laboratories so that this project would act as a large-scale field sampling effort.

The project manager and co-PIs have either published articles or presented at national conferences regarding the proposed techniques. The science faculty at the University of Minnesota Crookston campus seeks to empower the next generation of scientists and health science professionals. Research is not necessarily about accolades and personal achievement but rather the process of training students to think critically and problem-solve.

**Organization:** U of MN - Crookston

**Organization Description:**
The marketable laboratory-trained undergraduates and the teaching laboratory protocols produced reflect the University of Minnesota Crookston and Northwest Technical College's mission to focus on practical teaching that prepares students for good-paying jobs. This project is a partnership of two teaching institutions in northern Minnesota committed to the success of the region we serve.

Both institutions serve underrepresented populations of students pursuing science and professional degrees. Specifically, the University of Minnesota Crookston has the highest percentage of first-generation (50% of the student population) and Pell-eligible (31%) college learners within the University of Minnesota system. Likewise, Northwest Technical College has many first-generation (48%) and Pell-eligible (38%) students. High-impact retention activities such as undergraduate research engage and effectively retain more students, especially related to STEM careers. Practical training of these students has the potential to retain and empower them to seek jobs and careers that will impact their lives and extended families. Students will develop skills that can be applied to high-wage and benefit-paying situations in the region's rural communities, including the Minnesota Department of Natural Resources and the Minnesota Department of Agriculture.

## Budget Summary

| Category / Name | Subcategory or Type | Description | Purpose | Gen. Ineligible | % Benefits | # FTE | Classified Staff? | $ Amount |
|---|---|---|---|---|---|---|---|---|
| **Personnel** | | | | | | | | |
| Undergraduate Researchers | | Undergraduate research; 4-5 students per summer hired by UMC | | | 8% | 0.39 | | $101,087 |
| co-PI | | Venugopal Mukku (UMC research collaborator) | | | 36% | 0.52 | | $24,548 |
| co-PI | | Karl Anderson (UMC research fellow) | | | 36% | 0.78 | | $22,968 |
| Project Manager/PI | | Brian Dingmann (project manager) | | | 36% | 0.78 | | $38,397 |
| | | | | | | | **Sub Total** | **$187,000** |
| **Contracts and Services** | | | | | | | | |
| TBD | Internal services or fees (uncommon) | Identification and characterization of untargeted metabolic analysis. | | | | - | | $46,000 |
| TBD | Professional or Technical Service Contract | Service contract for HPLC (2 years); machine needs to be in optimal condition for everyday use | | | | - | | $12,000 |
| | | | | | | | **Sub Total** | **$58,000** |
| **Equipment, Tools, and Supplies** | | | | | | | | |
| | Tools and Supplies | Chemical reagents | Various chemical reagents will be used that are considered general chemical consumables. | | | | | $6,000 |
| | Tools and Supplies | Chemical extraction | Extraction, and fractionation is necessary to identify potential antimicrobial products from the bog microbes. | | | | | $20,000 |
| | Tools and Supplies | DNA sequencing lab supplies | DNA sequencing is necessary to identify microbial strains and phages in the bog environment (>600 samples) | | | | | $60,000 |
| | Tools and Supplies | Microbiolgical lab supplies | Microbiolgical culturing and manipulating soil microbes. | | | | | $15,000 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Tools and Supplies | QPCR on 200 samples | To analyze and quantify the microbial community structure and function we will investigate 200 samples across the state. | | | | | $21,000 |
| | Tools and Supplies | General use Polymerase Chain Reaction (PCR) reagents | To analyze micobial structure and function we will investigate 200 samples across the state. General PCR will allow us to gauge structure and funtion to focus research. | | | | | $18,000 |
| | Tools and Supplies | Phage Research Supplies | Phage isolation, enhancement, characterization from soil samples. | | | | | $22,000 |
| | | | | | | | **Sub Total** | **$162,000** |
| **Capital Expenditures** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| **Acquisitions and Stewardship** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| **Travel In Minnesota** | | | | | | | | |
| | Miles/ Meals/ Lodging | Travel to sample bogs with no overnight stays. Bog sampling will require 2 summers with the bog sampling "clustered" to reduce travel/trips. | Travel ($0.67 per mile of travel, 6500 miles sampling around state, $4355; per diem for 3 faculty, 4-5 students per summer (2 summers)) | | | | | $10,000 |
| | | | | | | | **Sub Total** | **$10,000** |
| **Travel Outside Minnesota** | | | | | | | | |
| | Conference Registration Miles/ Meals/ Lodging | Annual conference presentations (2 years) for 4 students and 2 faculty to disseminate grant findings, airfare, lodging as allowed in travel policy. | National Conference of Undergraduate Research (NCUR) presentations for four students. | | | | | $16,000 |
| | | | | | | | **Sub Total** | **$16,000** |
| **Printing and Publication** | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Printing | Printing at UMC and WETCC for students and dissemination student posters to promote project. | There will be required student presentations, article publication fees to disseminate research manuscripts. | | | | | $10,000 |
| | | | | | | | **Sub Total** | **$10,000** |
| **Other Expenses** | | | | | | | | |
| | | | | | | | **Sub Total** | **-** |
| | | | | | | | **Grand Total** | **$443,000** |

## 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** | | | | |
| | | | **Non State Sub Total** | **-** |
| | | | **Funds Total** | **-** |

**Total Project Cost: $443,000**

**This amount accurately reflects total project cost?**

Yes

## Attachments

### Required Attachments

*Visual Component*

File: b6b973d4-f37.pdf

*Alternate Text for Visual Component*

The attached picture provides an overview of the proposed research. Bioprospecting Minnesota Wetlands for Phage and Bacterial Antimicrobials....

### Supplemental Attachments

*Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other*

| Title | File |
|---|---|
| Northwest Technical College Support Letter | bda129f6-fee.pdf |
| Approval to Submit Letter | 0f76aa72-643.docx |

## Administrative Use

**Does your project include restoration or acquisition of land rights?**

No

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

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?**

Yes, Sponsored Projects Administration

**Does your project include the pre-design, design, construction, or renovation of a building, trail, campground, or other fixed capital asset costing $10,000 or more or large-scale stream or wetland restoration?**

No

**Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services (as defined in Minnesota Statutes section 299C.61 Subd.7 as "the provision of care, treatment, education, training, instruction, or recreation to children")?**

No

**Provide the name(s) and organization(s) of additional individuals assisting in the completion of this proposal:**

University of Minnesota Sponsored Projects Administration and University of Minnesota Crookston Academic Affairs Office.