



Environment and Natural Resources Trust Fund

2021 Request for Proposal

General Information

Proposal ID: 2021-165

Proposal Title: Microbial Degradation of Formaldehyde to Clean Polluted Waters

Project Manager Information

Name: Jannell Bazurto

Organization: U of MN - College of Biological Sciences

Office Telephone: (954) 980-6236

Email: jbazurto@umn.edu

Project Basic Information

Project Summary: We will identify environmental microbes with naturally high capacities to degrade formaldehyde and further adapt them toward enhanced formaldehyde degradation to clean contaminated water and conserve environmental waters.

Funds Requested: \$393,000

Proposed Project Completion: 2024-06-30

LCCMR Funding Category: Water Resources (B)

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

Narrative

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

Formaldehyde is a potent toxin and recognized human carcinogen. Acute formaldehyde exposure can irritate the eyes, skin, and respiratory pathways, while prolonged exposure produces chronic symptoms. In severe cases, exposure can lead to hemolytic anemia and cancers associated with nasal structures. Despite its known adverse health effects, formaldehyde is used in myriad formaldehyde-based consumer products that permeate our everyday lives, including personal care products, textiles, engineered wood products, adhesives, and paints. The Environmental Protection Agency estimates that national production of formaldehyde annually reaches 1-5 billion lbs. In Minnesota, facilities that manufacture or process formaldehyde predominately release formaldehyde-related waste into the ambient air (>500,000 lbs/year). Such widespread use brings Minnesotans into contact with formaldehyde through the use of formaldehyde-derived products and pollution of our natural resources.

In the environment, formaldehyde pollution generally arises from large-scale formaldehyde production, manufacturing of formaldehyde-based products, and combustion. Although formaldehyde pollution is predominately by air, it enters water as well, directly by wastewater and less directly because of its solubility. The high solubility of formaldehyde makes it easily transferable from the air to surface waters, putting Minnesota's water systems at risk for formaldehyde contamination.

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.

To prevent formaldehyde contamination from entering Minnesota water supplies, we propose to use formaldehyde-degrading microbes to remove formaldehyde from water sources. To achieve this, we will employ *Methylobacterium*, a family of beneficial, leaf-dwelling microbes that are prevalent in the environment. *Methylobacterium* have a natural propensity to detoxify formaldehyde that forms at low levels in the environment. To harness their ability, we will obtain a variety of natural isolates, collected from across Minnesota, and evaluate the level of formaldehyde they tolerate. We will also test their tolerance to aldehydes in general, particularly those designated as Chemicals of High Concern by the Minnesota Department of Health (acetaldehyde, propionaldehyde, glycidaldehyde, etc). Similarly, we will adapt strains with increased formaldehyde resistance and use them to degrade formaldehyde at high concentrations that would be toxic to people. As strains are adapted, they will be characterized to identify the strategies they use for increased survival on toxic compounds and tested for their ability to remove formaldehyde from synthetic, polluted water. The adapted microbes will be formulated in a mesh that can be used to treat contaminated water or air with high levels of formaldehyde.

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 will contribute natural resources by alleviating dangerous aldehyde pollutants through the enhanced microbial degradation of formaldehyde in contaminated waters. Thus, realization of this work will protect state residents from the risks conveyed by the toxic compound formaldehyde, as well as helping restore more pristine environmental waters. Use of the resulting strains may also be extended to the mitigation of formaldehyde pollution from other sources such as air-borne contamination.

Activities and Milestones

Activity 1: Characterization of Microbial Strains with Formaldehyde-Detoxifying Properties

Activity Budget: \$135,000

Activity Description:

Methylobacterium are a group of beneficial environmental microbes with a natural capacity to detoxify formaldehyde. We will characterize formaldehyde-detoxification in strains of Methylobacterium that have been engineered to tolerate formaldehyde concentrations that are lethal to most microbes as well as in natural isolates from a handful of industrial and environmental sites in Minnesota. The changes incorporated into engineered strains will be guided by our previous work, which has identified a number of genes that increase formaldehyde resistance and/or cross-resistance to numerous aldehydes. Additionally, we will improve the cell's capacity to maintain protein quality control in the face of aldehyde stress, as this has been shown to increase toxicity tolerance of other microbes in aldehyde-rich environments up to 200-fold. To obtain state-wide isolates of Methylobacterium, we will engage Minnesotans of all ages via school- and citizen-based outreach with the goal of obtaining new isolates from diverse locations in all seasons. Because Methylobacterium are plant-associated and promote plant growth, such an outreach project will be valuable for increasing scientific literacy surrounding the many positive impacts that microbes can have. Strains will be characterized in formaldehyde-media supplemented with various concentrations of formaldehyde.

Activity Milestones:

Description	Completion Date
Develop formaldehyde-media based on formaldehyde concentrations in contaminated water, which fluctuate across time and location.	2021-09-30
Construct Methylobacterium strains with increased formaldehyde resistance, aldehyde resistance, and protein quality control.	2021-12-31
Characterize formaldehyde and general aldehyde resistance across lab-engineered strains and natural isolates.	2022-06-30

Activity 2: Enhanced Removal of Aldehyde Contaminants from Polluted Water

Activity Budget: \$258,000

Activity Description:

To enhance formaldehyde biodegradation in our Methylobacterium strains, we will adapt them to grow in formaldehyde-media, relying on formaldehyde as a growth substrate. As contaminated water can have exceedingly high formaldehyde concentrations and will routinely contain other contaminants, we anticipate that initially, growth will only be achieved at very low concentrations of formaldehyde. Prolonged exposure experiments will include growth on formaldehyde-media with i) ever-increasing formaldehyde concentrations, as well as ii) ever-increasing concentrations of other common contaminants (e.g., other aldehydes). The objectives of contaminated water exposure are threefold: i) increase formaldehyde-resistance, ii) increase tolerance to other common contaminants, and iii) identify robust derivatives of natural isolates that can be deployed for use in natural environments. At regular time intervals, isolates from microbial cultures that have hit particular milestones (e.g., reaching a particular number of generations or achieving growth on particularly high formaldehyde concentration) will be isolated and characterized. Characterization will include analysis of formaldehyde/general aldehyde resistance and whole genome sequencing to identify the corollary genetic determinants. High-performing isolates will be candidates for use in bioreactor experiments where they will be suspended in a mesh and evaluated for their ability to clean contaminated waters and air.

Activity Milestones:

Description	Completion Date
Adapt engineered and natural isolates of Methylobacterium to formaldehyde-media.	2022-12-31
Characterize adapted isolates for growth on formaldehyde and resistance to other common contaminants.	2023-09-30
Assemble bioreactors with adapted isolates to immobilize them and determine their potential for water decontamination.	2024-06-30
Identify genes responsible for different aspects of wastewater-adaptation through whole genome sequencing of characterized isolates.	2024-06-30

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Lawrence Wackett	University of Minnesota, Twin Cities	Professor Lawrence Wackett from the Department of Biochemistry, Molecular Biology, and Biophysics studies biodegradation as well as enzymatic mechanisms and has extensive experience with aldehydes and other toxic molecules. He will contribute to analytical chemistry and biochemistry involved in strain characterization and bioreactor design.	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 be funded?

We anticipate this project to be long-term. The chemical complexity and variability of polluted water is likely to pose additional hurdles to microbial adaptation. Once overcome, the resulting strains will be well-poised to thrive in realistic, chemically harsh conditions. This feature is particularly valuable because *Methylobacterium* strains exhibit resistance to a wide variety of toxic compounds. As formaldehyde is a widely recognized indoor air pollutant, developing strains with increased formaldehyde-detoxification properties could also eventually be utilized to improve indoor air quality. Funding for additional work will be sought after by application to federal agencies such as the Department of Energy.

Project Manager and Organization Qualifications

Project Manager Name: Jannell Bazurto

Job Title: Assistant Professor

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

I am well-suited to execute the proposed work as a microbial physiologist with extensive experience adapting microbes to stress conditions and integrating approaches that span experimental scales. I have abundant experience working with genetically tractable model microbes, including *Methylobacterium*, a key microbe in many plant communities.

My core expertise was gained in my doctoral training where I integrated in vivo genetics and biochemistry to understand metabolic integration. I was trained by Dr. Diana Downs, and rigorously studied the mechanistic basis of metabolic integration and plasticity in *Salmonella* and *Escherichia coli*. To describe the global consequences of metabolite imbalance, I began integrating additional approaches to studying metabolic stress during my postdoctoral studies in Dr. Downs's lab. This work was done in collaboration with Dr. Shawn Campagna at the University of Tennessee, where I received extensive hands-on training to perform untargeted metabolomics and subsequent data analysis. In pursuit of understanding cellular coordination surrounding metabolic toxins, I developed my research program during my postdoctoral position in the laboratory of Dr. Christopher Marx. There, I continued to integrate new approaches into my work to gain system-level insights on the stress response systems that counteract formaldehyde toxicity in *Methylobacterium*.

My overall research goal is to understand novel mechanisms used to maintain cellular homeostasis when confronted with stress and how they impact organisms in their natural environments. By studying a microbe that generates copious quantities of formaldehyde as a metabolic intermediate, my research program is poised to make exciting contributions to the field of microbial stress response, and to understand cellular strategies for managing metabolic stress, specifically. My findings have led to exciting revelations related to metabolic control, and the adaptive dynamics of organisms encountering transient stress.

Organization: U of MN - College of Biological Sciences

Organization Description:

The mission of the College of Biological Sciences is to deliver cutting-edge, internationally recognized research and teaching at all levels of biological organization from molecules to ecosystems. While preparing today's students to create the biology of tomorrow, CBS promotes collaborative research within and beyond the University to advance knowledge and find solutions that improve human health and the environment locally, nationally and globally.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
Personnel								
2 Research Associates		The research associates will perform chemical/biochemical analyses to characterize degradation and develop the bioreactor.			27%	2.31		\$174,000
1 Graduate Student		will perform strain engineering, adaptation, and subsequent characterization of adapted strains.			39%	1.5		\$129,000
1 Undergraduate Student		undergrad student will assist with strain characterization			0%	0.75		\$15,000
							Sub Total	\$318,000
Contracts and Services								
							Sub Total	-
Equipment, Tools, and Supplies								
	Tools and Supplies	reagents (i.e. oligonucleotides, DNA polymerase, HiFi cloning kits), growth (i.e. media, chemicals), and consumables, (i.e. pipet tips, Petri dishes, centrifuge tubes, 96-well plates)	molecular work, growth experiments					\$48,000
	Tools and Supplies	sequencing, mass spectrometry	Next-generation sequencing needs for whole genome sequencing of adapted populations and individual isolates, mass spectrometry needs to analyze chemical reactions					\$25,000
							Sub Total	\$73,000
Capital Expenditures								
							Sub Total	-
Acquisitions and Stewardship								

							Sub Total	-
Travel In Minnesota								
	Miles/ Meals/ Lodging	car rental, gas, lodging	to obtain natural bacterial isolates from different sites in Minnesota, including publicly owned treatment works (POTW) and to obtain formaldehyde contaminated water samples from publicly owned treatment works (POTW) and industrial sites.					\$2,000
							Sub Total	\$2,000
Travel Outside Minnesota								
							Sub Total	-
Printing and Publication								
							Sub Total	-
Other Expenses								
							Sub Total	-
							Grand Total	\$393,000

Classified Staff or Generally Ineligible Expenses

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

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	-
Non-State				
In-Kind	Indirect costs for this proposal, though not allowed, are listed as in-kind contribution of 55% MTDC	To pay for administrative and facility expenses for this project	Secured	\$198,048
			Non State Sub Total	\$198,048
			Funds Total	\$198,048

Attachments

Required Attachments

Visual Component

File: [6e15f165-278.pdf](#)

Alternate Text for Visual Component

Three-panel figure. The left vertical panel is entitled "Contamination of MN Water Systems." Here, formaldehyde-polluted smoke is emitted from a factory and car exhaust. An arrow from these air pollution sources depicts the formaldehyde entering otherwise clean water.

The top horizontal panel is entitled, "Characterization of Microbial Strains with Formaldehyde-Detoxifying Properties." This panel shows a series of 3 sets of microbes: the first set is captioned "Native Microbes" and there are a few formaldehyde molecules around them because they naturally encounter and degrade formaldehyde, the second set is captioned "Engineered Microbes" and they are a different color to represent they have been modified and they are surrounded by more formaldehyde, the third set is captioned "Adapted Microbes" and they are a third color to represent further change and are completely surrounded by formaldehyde.

The bottom horizontal panel is entitled, "Enhanced Removal of Aldehyde Contaminants from Polluted Water." This panel shows an arrow that reads "Contaminated Water In" and leads to an image of "Bioreactor of Adapted Microbes" and a second arrow that reads "Clean Water Out" that leads to an outdoor scene with clean lake water surrounded by trees.

Optional Attachments

Support Letter or Other

Title	File
Authorization to submit	dfdb3b05-cb0.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

Does your project have patent, royalties, or revenue potential?

No

Does your project include research?

N/A

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

