# Bacterial Remediation of Microcystin-HAB Toxins

**Lead PI:** Carina M. Jung, PhD, ERDC, Carina.M.Jung@usace.army.mil
**Co-PI:** Lyndsay Carrigee, PhD, ERDC, Lyndsay.A.Carrigee@usace.army.mil

**USACE Harmful Algal Bloom Research & Development Initiative**

*Delivering scalable freshwater HAB prevention, detection and management technologies through collaboration, partnership and cutting-edge science*

**Problem** Cyanobacteria containing toxins have been documented in most states and are a priority concern for inland waterways. Microcystins (MCs) are the most reported freshwater toxins with ~100 analogues and are potent hepatotoxins. Conventional methods for water treatment (boiling, chlorination, and UV treatment), are unsuccessful at destroying these toxins. Naturally occurring bacteria can degrade MC toxins typically through the mlrABCD cluster. The MlrA enzyme opens the cyclic structure, and subsequently linear toxin is ≥ 160 times less toxic. MlrB further degrades the linear MC.

**Objective** The goal of this project is to reengineer and produce a shelf-stable MC-degrading enzyme that can be used in the field to deactivate harmful toxins while cleanup personnel remediate MC-HAB events in a safe environment.

**Approach** The project team will develop a synthetically derived, bacterial-produced enzyme(s) capable of degrading the harmful algal bloom toxin, microcystin. By producing a bicistronic MC-degrading clone, the MlrA enzyme which linearizes the highly toxic cyclic MC, thus significantly reducing its toxicity, is coupled with the MlrB enzyme which begins the natural degradation process by further cleaving the MC. The resultant clone expels the enzyme(s) and allows for high level production and eventual purification of the enzyme(s). The MlrAB will be purified and its effectiveness against MC in a variety of real-world matrices will be evaluated. The final product of this project is anticipated to be a shelf-stable, enzyme powder that can be safely administered to MC-impacted bloom areas to reduce toxicity to wildlife and cleanup crews.

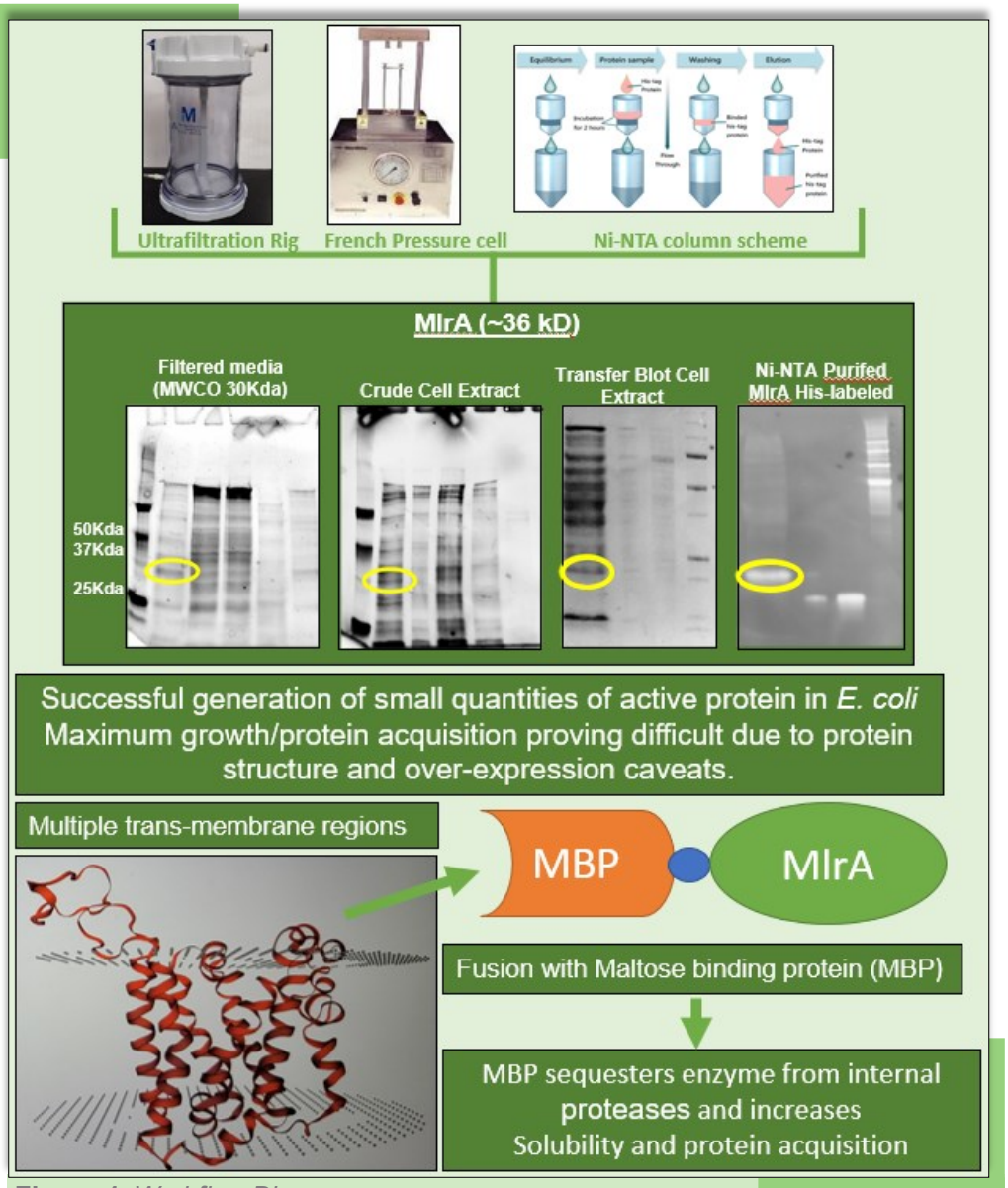


**Figure 1:** *Workflow Diagram*

# Bacterial Remediation of Microcystin-HAB Toxins

**Lead PI:** Carina M. Jung, PhD, ERDC, Carina.M.Jung@usace.army.mil
**Co-PI:** Lyndsay Carrigee, PhD, ERDC, Lyndsay.A.Carrigee@usace.army.mil

**USACE Harmful Algal Bloom Research & Development Initiative**

*Delivering scalable freshwater HAB prevention, detection and management technologies through collaboration, partnership and cutting-edge science*

## Major Milestones

| Date | Milestone |
|---|---|
| FY20, Q4 | Project initiation, including development of PMP and ordering supplies |
| FY21, Q1 | Production of an MlrA hyper-producing bacterium (Patent disclosure initiated) |
| FY21, Q2 | LC-MS method development to quantify microcystin |
| FY21, Q3 | Purified MlrA will be generated by recombinant E. coli |
| FY21, Q4 | Assess MlrA activity against MC in various matrices |
| FY21, Q4 | Presentation – Society for Industrial Microbiology and Biotechnology |
| FY21, Q4 | Patent application # 17405012 |
| FY22, Q1-2 | MlrA will be generated by recombinant E. coli host strain in a bench-scale trial, whereby maximum cell growth and maximum protein production will first be assessed. |
| FY22, Q2-3 | MlrA activity against MC (spiked at different levels) in various matrices will be assessed. Kinetics of the enzyme will be determined (ie, reaction rates, upper and lower concentrations of substrate interactions, how matrix interference affects these rates) through the following experimental variables |
| FY22, Q3-4 | Optimization - Protein production will occur stepwise: assessing stability under different storage conditions with appropriate protein functionality maintained over time, implementing lessons learned |
| FY22, Q4 | Go-No Go – If the attempts to produce active enzyme(s) fail or alternate attempts to use naturally occurring bacterial strains for enzyme production also fail to yield measurable decreases in MC toxin in water samples the project will not progress to Task 3. |
| FY22, Q4 | Presentation- ERDC RD22 |
| FY22, Q4 | Journal Article/Technical Report (Begin draft) |
| FY22, Q4 | Possible transition to partner (Allonia, LLC) |
| FY23 | Meso/field-scale demo and cost/feasibility assessment for scale-up of MlrA application |
| FY23 | Presentation/Technical Report/Journal Article/Patent Application |
| **Costs** | **FY20:**$35k **FY21:**$130K **FY22:**$200K **FY23:**$125k **TOTAL:**$490K |

## Partnership/Leveraging Opportunities

This work will leverage multiple collaborations and other work units. This project involves collaboration with multiple USACE district liaisons from Omaha, Jacksonville and Buffalo District. In the future this project may be transferred to a partner, Allonia, LLC.

## Value to USACE Mission

Once a bloom event occurs, detoxifying the water would be hugely beneficial to animal and human health and allow for safety of HAB response team personnel.

***USACE District Liaison:*** John Hargrave and Brent Dinkel of Omaha District, Chelsea Bohaty and Jon Lane of Jacksonville District, and Mike Greer and Karen Keil of Buffalo District