M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 2: Metagenomic Approaches to Develop Biological Control Strategies for Aquatic Invasive Species - Phase II: Development of Potential Microbiological Control Agents for Aquatic Invasive Species

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SUBPROJECT BUDGET AMOUNT: $303,217
AMOUNT SPENT: $286,610
AMOUNT REMAINING: $16,607

Sound bite of Subproject Outcomes and Results

This project evaluated the potential for harnessing natural microbes for use as biocontrol agents against Eurasian watermilfoil and zebra mussels. Several microorganisms were isolated that could be pathogenic to zebra mussels, but none met safety requirements for testing. EWM is associated with elevated concentrations of E. coli and human pathogens.

Overall Subproject Outcomes and Results:

Aquatic invasive species (AIS), including Eurasian watermilfoil (EWM) and zebra mussels (ZMs) pose a serious threat to the health and function of aquatic ecosystems. Traditional approaches for AIS management, including use of chemicals and manual removal, have been ineffective. This requires development of new management and eradication strategies, such as the use of (micro)biological control agents. Some microorganisms have evolved to live in close association with aquatic organisms and such relationships could be exploited to develop microbe-mediated AIS management strategies. As the first step towards the identification of potential biocontrol strategies, microbial communities associated with 'healthy' AIS were compared with that of 'diseased' AIS or to native species. Since no natural diseased mussels were available, we opted to develop an experimental model system, which allowed for the application of different intensities of stress – heat (17, 25, 33℃) and salinity (1.5, 13.5 ppt), to promote the proliferation of opportunistic pathogens. High-throughput DNA sequencing of 414 samples (providing 32 million DNA reads) resulted in the identification of several potentially 'pathogenic' microbial groups that were strongly associated with ZM mortality. These included *Aeromonas*, *Chryseobacterium*, *Flavobacterium*, *Acidaminobacter*, *Clostridiaceae* 1 sp., *Rhodobacteraceae* sp., *Acinetobacter*, *Shewanella*, and *Clostridium sensu stricto* 13. For the identification of EWM-specific microbiota, high-throughput DNA sequencing was performed on 315 samples (46 million reads) derived from leaf and root compartments of EWM and six native macrophyte species. This resulted in the identification of taxa that were significantly enriched in EWM leaves and roots compared to native plants. Though several AIS-associated microorganisms were isolated that could be pathogenic to invasive mussels (e.g. *Aeromonas*) - none of them met our safety requirements for further testing. Future studies must isolate and evaluate the efficacy of 'host-specific and pathogenic' biocontrol candidates that will only infect invasive mussel species.

Subproject Results Use and Dissemination

Our research findings were disseminated via oral and poster presentations at the following (international/ national/ local) conferences: 61st International Association for Great Lakes Research conference (Toronto, Canada), UNC Water Microbiology Conference 2019 (Chapel Hill, NC), 20th International Conference on Aquatic Invasive Species (Fort Lauderdale, FL), 5th Upper Midwest Invasive Species Conference (Rochester, MN), 119th General Meeting of the American Society for Microbiology (San Francisco, CA), and the AIS Research Management Showcase in 2017 & 2018 (St. Paul, MN). Two papers were published in the journals 'FEMS Microbiology Ecology' and 'Science of the Total Environment' during this project period. One manuscript is currently undergoing peer-review and two additional manuscripts are under preparation. All sequencing data generated in this project will be publicly available (via submission to NCBI Genbank) and all publications will list accession numbers to link to short read archive of all samples. Thus far, all sequence data mentioned in current publications is directly linked to a publicly available web site for download.