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SEA GRANT PROJECT SUMMARY FORM (90-2)

INSTITUTION: University of Illinois Chicago
TITLE: Development of Lake Michigan actinomycete bacteria as a source for antibiotic drug leads.
PROJECT NUMBER: **INITIATION DATE:** 05/01/2012
COMPLETION DATE: 04/31/2013

PRINCIPAL INVESTIGATOR: Dr. Brian T. Murphy
AFFILIATION: Dept. Medicinal Chemistry & Pharmacognosy

BACKGROUND

Culture independent studies of freshwater bacterial communities have indicated the presence of actinobacterial clades that are exclusive to freshwater environments, and strikingly few studies have undertaken the task of exploring the chemical diversity and consequently the ability of these actinomycetes to produce biologically active secondary metabolites. To the best of our knowledge, with only one exception reports of secondary metabolites from freshwater actinomycetes are absent from the peer-reviewed literature. This is likely due to the fact that slower-growing actinomycetes are especially difficult to cultivate in the presence of several endemic Gram-negative and other unicellular bacteria in freshwater habitats; these bacteria often outgrow actinomycetes on bacterial isolation plates and require specific pretreatment techniques to abolish.

OBJECTIVE

Our objective was to grow freshwater actinomycete bacteria from sediment collected in the waters of Lake Michigan and other Great Lakes. We set out to grow approximately 50 freshwater-derived actinomycete strains with the intent of cultivating each, extracting their extracellular secondary metabolites, fractionating each set into simplified mixtures of metabolites, and screening the resulting fractions against a panel of infectious diseases in house. This study would also result in the creation of two libraries: a) cryo-preserved freshwater-derived actinomycete strains, and b) excreted secondary metabolites for use in bioassay screening.

The long-term aim of our study is to explore the hypothesis that freshwater-derived Great Lakes bacteria are taxonomically distinct from their terrestrial and marine counterparts, and as a result will have the capacity to produce unique chemical skeletons that can be used to treat infections caused by drug-resistant pathogenic bacteria.

REVISED SAMPLE COLLECTION PLAN

Since this proposal did not fall under any of the suggested topic areas, it was funded through special recommendation by the review panel. As a result, a revised proposal was submitted with a reduced budget ($10,000). Our University agreed to offer matching funds ($12,500). Part of our original plan was to hire a vessel to collect along the proposed transect out of Muskeegon (Aim 1), however this would have cost several thousand dollars in travel funds and vessel rental. This represented a substantial part of our budget. Instead, and upon the great recommendation from Carolyn Foley at IISG, we were advised to contact the RV Lake Guardian for available ship time. We did so and they offered us a few spots – first on

an extensive research voyage to collect sediment from locations spanning the entirety of Lake Huron, and second on a voyage collecting sediment in the North Channel and Georgian Bay (Figure 1). An additional collection trip was launched to acquire sediment along the Chicago River (Figure 2). The collection was integrated into an outreach program with students from Harold Washington College.

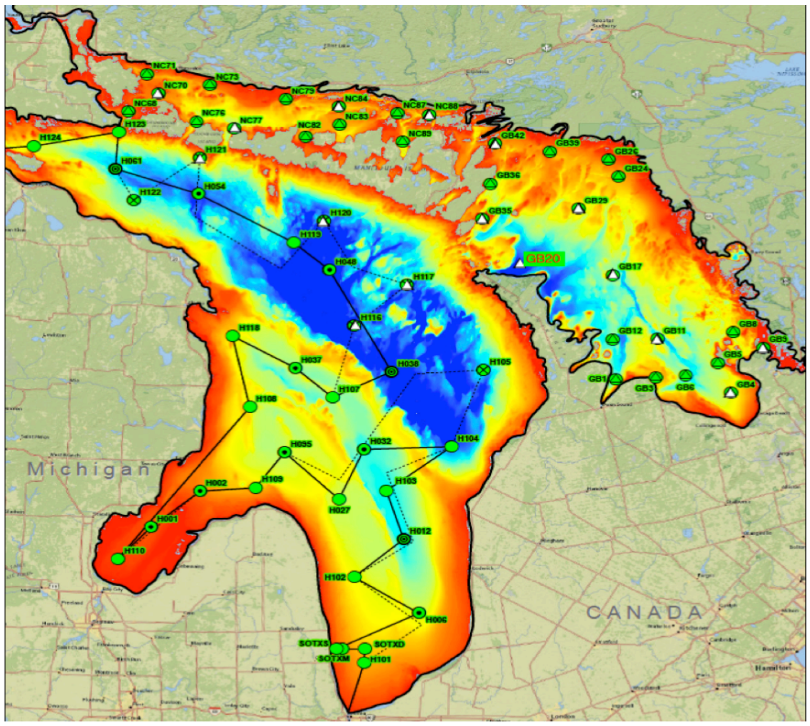

Figure 1. Lake Huron Collection Sites.

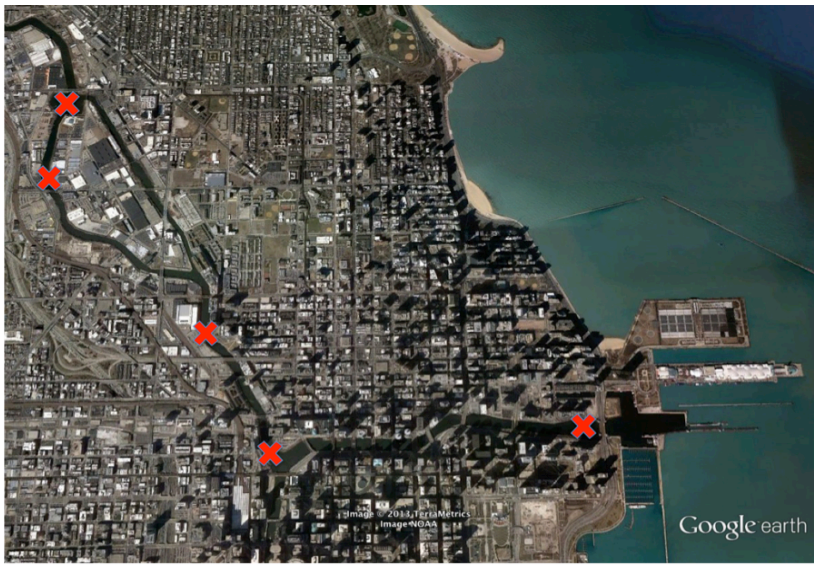

Figure 2. Lake Michigan/Chicago River Collection Sites.

SAMPLE COLLECTION DELIVERABLES

We aimed to collect approximately 20 samples and isolate ca. 50 actinomycetes (thus generating 200 metabolite fractions). Instead, we collected 85 samples and isolated ca. 600 freshwater actinomycetes (that will result in the generation of 2,400 metabolite fractions).

These numbers are far greater than we anticipated and indicate the success of our sample collection efforts. Complete processing of these samples will take several more months. However, to date we have added ca. 352 of these strains to our actinomycete library, 73 of which have already been cultivated in large scale and whose metabolites have been extracted for addition to our metabolite library (total of 292 fractions).

ONGOING STUDIES

Because the bacterial isolation phase afforded a far greater number of strains than expected, we made the decision to focus our efforts on building our library with the 600 actinomycete colonies that we isolated, since they will survive only a limited time on plates. This was a major undertaking. Because this project provided much promise, we diverted additional funds (from the PIs startup account) and personnel to the task of cultivating all 600 actinomycete strains. This required the work of one full time technician, one part time technician, and two undergraduate research volunteers. This work is ongoing.

We decided to delay the antimicrobial screening phase of the project until a significant quorum of strains were fractionated and added to the library. In the 2013 summer session we have dedicated the four personnel to working full time on library generation and screening. In approximately one month we will perform in-house screening of at least 292 fractions for their ability to inhibit the growth *Enterococcus*

facaeilis, Pseudomonas aeruginosa, and *Escherichia coli*. These assays will initially be run in single dose (100 μg/mL), and promising hits will advance to dose response screening to generate MIC values. A follow up report will be sent to IISG once the results of this screening are obtained.

IMPACT

The current study has helped to generate one of the largest freshwater-derived actinomycete libraries in the world. Overall, given the breadth of freshwater habitats in the Midwest, our actinomycete strain and metabolite libraries will provide a one of a kind source for drug leads to combat a variety of infectious diseases that are routinely screened as part of our program (see Projects section below).

TRAINING AND OUTREACH

This funding has had impact on the careers of several young scientists.

Graduate student: Skylar Carlson. Skylar and Dr. Murphy were aboard the RV Lake Guardian for one week in Lake Huron. During this time, Skylar received training in instrument operation, sample preparation and pre-treatment, and actinomycete isolation.

Graduate student: Maryam Elfeki. Maryam is an incoming UIC graduate student (Class of 2014) and she has agreed to work in the Murphy laboratory (Maryam has also been an undergraduate volunteer researcher in this group since September 2010). Her entire dissertation will be focused on Projects 2 and 3, described below. She will be co-advised by Dr. Stefan Green, the Director of UICs DNA Sequencing Facility. She has been thoroughly trained in freshwater actinomycete microbiology and is currently using the sediment and actinomycete samples generated from this study to acquire expertise in both chemical separation of secondary metabolites and metagenomic studies of microbial populations.

Technician: Mark Sadek. Mark was aboard the RV Lake Guardian for one week in the North Channel and the Georgian Bay. During this time, Mark received training in instrument operation, sample preparation and pre-treatment, and actinomycete isolation. He now oversees the generation of our actinomycete secondary metabolite library.

Technician: Ying Gao. Ying received training in actinomycete isolation, fermentation, and metabolite extraction. She now oversees the generation of our actinomycete strain library.

Harold Washington College outreach: Dr. Murphy recently gave a lecture on this topic to a group at Harold Washington College in downtown Chicago, and following the lecture a group of three students, including Dr. Murphy, and Dr. Farahnaz Zadeh, rented a small boat and collected sediment at various locations along the Chicago River (Figure 2). Students were trained in field work, sample processing and are in the process of attending sample processing workshops at UIC.

High school student: Matthew Hennings, Sean Hickey. Matthew was a summer 2012 volunteer in the Murphy Laboratory and assisted with actinomycete strain library development. Sean is a volunteer for the summer of 2013. Sean will focus on actinomycete metabolite and strain library development.

PROJECTS EMANATING FROM THIS STUDY

Several projects will be the direct result of this pilot study. A brief synopsis of each is provided below.

Project 1. Drug-lead Discovery from freshwater actinomycetes. Using a number of collaborations at and outside UIC, we will screen the fraction library generated from this study against diseases such as tuberculosis, ovarian cancer, hepatitis B, etc. After prioritizong each bioactive strain for its ability to produce promising drug-leads, we will ferment the actinomycete on a large scale (20 to 40 L) and isolate and identify the molecule of interest. Such molecules can be used for further drug-lead development, or can be used to probe the mechanisms of the disease. *Target funding agency: NIH*

Project 2. Creation of microbial map of Great Lakes Sediment. In collaboration with Dr. Green, we will seek NSF funding to map actinomycete occurrence and abundance throughout Great Lakes sediment. We have already initiated preliminary studies on the 85 sediment samples collected in Lake Huron. This will be the first comprehensive study of actinomycete occurrence in the Great Lakes of its kind. Such data will also guide drug discovery researchers of the benefits of sampling multiple locations and the resulting possibility of isolating novel actinomycete taxa. *Target funding agency: NSF*

Project 3. Use of Imaging Mass Spectrometry (IMS) to correlate microbial taxonomy with secondary metabolite production. We plan on performing agar-based IMS on all 600 actinomycete strains that we have isolated; IMS will generate a unique and comprehensive secondary metabolite profile of each colony that will act as a small molecule 'fingerprint.' We will then perform clustering analysis on these fingerprints and assess whether they correlate to 16S rRNA gene sequence data. These data will inform us on the extent of horizontal gene transfer among actinomycete species and suggest whether taxonomic classification is a sufficient indicator of endemic secondary metabolite diversity. *Target funding agency: NIH, NSF*

PRESENTATIONS AND PRESS

Articles featuring our drug-lead discovery effort from freshwater actinomycetes:

- Drug-discovery program featured in Science Daily (03/07/13): http://www.sciencedaily.com/releases/2013/03/130307190524.htm
- Drug-discovery program featured in The Columbia Chronicle (03/18/13): http://columbiachronicle.com/researchers-search-for-tb-treatment-underwater/
- *UIC News*, Cover story (4/17/2013): http://news.uic.edu/key-to-tb-cure-could-lie-underwater
- Currently in communication with science reporter from in the Toronto Star. Upcoming story will focus on search through the Great Lakes and other sources for new antibiotics.

Anticipated publications highlighting Great Lakes as a potential source of drug-leads:

- Newsome, A.; Mullowney, M.; O'Hainmire, E., Wei, X., Tanouye, U., Burdette, J., and **Murphy, B. T.** Diazaquinomycins E and F – novel secondary metabolites from a freshwater-derived *Micromonospora* sp. Manuscript in preparation for submission to *Journal of Natural Products*.
- Hwang, C.H., Wei, X., Tanouye, U., Wang, Y., Cho, S.; Franzblau, S., and **Murphy, B.T.** Anti-tuberculosis activity of the freshwater-derived diazaquinomycin antibiotic class. Manuscript in preparation for submission to *Antimicrobial Agents and Chemotherapy*.