M.L. 2016, Chp. 186, Sec. 2, Subd. 06d.Project Abstract
For the Period Ending June 30, 2019

PROJECT TITLE: Biological Control of White Nose Syndrome in Bats – Phase II
PROJECT MANAGER: Christine Salomon
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 06d.

APPROPRIATION AMOUNT: $452,000
AMOUNT SPENT: $ 426,705
AMOUNT REMAINING: $ 25,295

Sound bite of Project Outcomes and Results

This project is focused on bio-control treatments for white nose syndrome (WNS) in bats. We identified microbes that inhibit the fungal pathogen, Pseudogymnoascus destructans and also quantified P. destructans along cave transects to identify best locations for treatments. This work may provide solutions to help vulnerable populations of Minnesota bats.

Overall Project Outcome and Results

White nose syndrome is a devastating disease of hibernating bats caused by the fungus, *P. destructans* (*Pd*). The primary goal of this project is to identify safe and effective bio-control treatments for WNS. We expanded our microbial strain collection and identified additional inhibitors of *Pd*, bringing our total of active strains to approximately 120. We identified the top five inhibitory strains, purified the active compounds, and determined their structures and activities. We identified approximately 15 structures new to science and 6 known compounds with antifungal activity. To determine the potential application of these active strains to substrates or bats, we developed a cell-based assay using bat skin cells derived from two bat species. By testing each compound against both the fungal pathogen and bat skin cells, we could calculate the relative potency and cytotoxicity. One of the most active and abundant compounds from an inhibitory fungus from the Soudan Iron Mine is completely nontoxic towards the cultured bat skin cells, which provides additional support for field testing with the producing strain.

Additional accomplishments include the sequencing of bacteria and fungi found throughout three distinct systems (iron mine, sandstone and calcium karst caves) from both culturable strains and mixed, non cultured microbial community samples. These taxonomic studies are significant because they allow us to see patterns of microbial communities across diverse environments, including identifying taxa that are unique or common in different areas.

We also developed tools and techniques for monitoring *P. destructans* in caves for studies going forward. Mapping of *P. destructans* along two transects in the Soudan Mine and Mystery Cave using qPCR provides a clear picture of the density and occurrence of the pathogen. This information and testing will be used to target treatments in collaboration with the DNR and managers to ultimately protect the remaining bat populations.

Project Results Use and Dissemination

The primary dissemination of the results from this project has been through numerous seminars given at academic institutions, research symposia, and at professional science society meetings. Both lectures and

posters have been presented at national conferences, and results have been shared with DNR staff through formal and informal communications. Two scientific manuscripts have been published on this work, and at least 5 more are in progress and should be published within the next 6 months. We have also participated in several outreach opportunities by having research tables at local bat week events, in collaboration with USFW staff.

The most immediate use of our results will be in collaboration with DNR staff and cave/mine park managers in locations affected by WNS. We are communicating our data about the pathogen locations to help inform any interventions and treatments, and to suggest specific areas for continued monitoring using our analytical approach.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan

Date of Report: November 18, 2019

Final report

Date of Work Plan Approval: 06/07/2016

Project Completion Date: June 30, 2019

Does this submission include an amendment request? No

PROJECT TITLE: Biological Control of White Nose Syndrome in Bats – Phase II

Project Manager: Christine Salomon

Organization: University of Minnesota, Center for Drug Design

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Location: *Ramsey County, St. Paul / Hennepin County, Minneapolis / St. Louis County, Soudan (Breitung Township)/ Fillmore County, Forestville*

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$452,000**
	Amount Spent:	**$426,705**
	Balance:	**$25,295**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06d.

Appropriation Language:

$452,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to continue research to identify, develop, and optimize biocontrol agents for white nose syndrome in bats by evaluating the biocontrol effectiveness of microbes collected at additional hibernacula throughout the state and conducting baseline characterization of the total bat microbiomes. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Biological Control of White Nose Syndrome in Bats – Phase II

II. PROJECT STATEMENT: Our primary goal is to identify, develop and optimize biological control agents for prevention and/or treatment of White Nose Bat Syndrome (WNS) in Minnesota and eventually other locations. WNS is a devastating fungal disease that has decimated bat populations throughout the Northeast and Canada, killing more than 7 million bats to date. Although diseased bats have not been found at any of the major hibernation locations in Minnesota (Soudan Iron Mine and Mystery Cave as of February 2015), WNS *is likely to develop within the next 1-3 years*. The consequences of these massive bat declines are devastating losses of biodiversity, local species extinctions, and the loss of pest control for forests and agriculture. In the state of Minnesota, the economic value of bats has been estimated to be at least $1.4 billion per year, which does not include the additional downstream "costs" of water and environmental degradation due to increased pesticide use.

This work is an extension of our current ENTRF project (Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy and Biocontrol) during which we have amassed a large collection of microbes (>500) collected from both bats and roost areas in the Soudan Mine hibernation areas to test as biocontrol agents. Additional bacterial and fungal test isolates will be obtained from bats and roosts from Mystery Cave and other hibernation areas throughout the state and assessed. We previously used non-pathogenic, faster growing fungi as "proxy" species of the real pathogen to test the biocontrol agents, but have since acquired an authentic culture of the *Pseudogymnoascus destructans* fungal pathogen for all future studies. We are especially interested in further studying and developing fast growing fungi as potential competitors or biocontrol agents and have identified ~50 non-pathogenic bacteria and fungi as candidates. An additional goal is to characterize the total microbiome of bats from each of the hibernation areas using culture-dependent and independent methods (DNA sequencing of all microbes from bat swabs). Since the disease has not yet developed among bat populations, this provides a critical window for obtaining samples from healthy bats throughout the state (which we will start to obtain now since we can't predict the arrival date of disease). This foundational data will allow us to compare how the microbial community changes over time due to either application of biocontrol agents or to WNS.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January, 2017**:**
We are continuing to sample from sediments, substrates and bats from different cave/mine environments. During this period, we isolated several hundred more fungi and bacteria and are in the process of testing them for activity against *P. destructans*. The total number of inhibitory strains is ~100, and we are growing larger cultures of the most potent strains to identify the active components. We have also developed an isolation chamber experiment to see if *P. destructans* can grow in sediments while contained in porous material *in situ*. If this is successful and quantifiable, we will expand this system to test all of our inhibitory strains directly with *P. destructans* in various substrates.

Project Status as of July, 2017**:**
The testing of bacterial and fungal isolates from multiple locations has helped us to identify additional strains that inhibit the growth of *P. destructans*. The total number of actives from all locations (Soudan Mine, Mystery Cave, Banholzer Brewery, Wabasha Cave and Heinrich Brewery) is 120, and additional testing is still in process. We are also beginning to sequence the DNA of the active microbes to determine their taxonomic relationships and closest relatives. The initial larger scale cultures of active strains have allowed us to purify and identify some of the active compounds. Nine new polyketide compounds were isolated and identified from an Oidiodendron species of fungus from the Soudan Iron Mine.

An additional finding during this period is that some of the active fungal isolates are associated with endosymbiotic bacteria (bacteria that are found inside of the fungal cell walls). We do not yet know if these

bacteria play a role in the inhibitory activity of the fungal host, but will attempt to separate each species and test them individually.

Project Status as of January, 2018**:**
We have completed the isolation and characterization of 18 pure compounds from the fungus *Oidiodendron* sp. collected from the Soudan Mine. Although most of these compounds are structurally related to each other, only three inhibit the growth of *P. destructans*. We also obtained tissue culture samples of primary fibroblast skin cells from Gray and Northern Long Eared bats to test candidate compounds for cytotoxicity in a more relevant assay system, and found that most of the isolated compounds only exhibit moderate to low toxicity towards both bat cell lines.

We also completed the first set of metagenomic sequencing of 70 microbial community samples from the Soudan Mine and Mystery Cave. Data analysis is in progress, and some of the preliminary results suggest that the microbial diversity of the caves varies by both location and type of sample. The approach has also allowed us to identify the likely species of some of the macroscopic microbial colonies present in some parts of Mystery Cave.

Project Status as of June, 2018:
We have adapted and refined a sensitive quantitative PCR (qPCR) method to detect and quantify *P. destructans* and are using this approach to map the distribution of Pd throughout the primary hibernacula caves in Minnesota (Soudan and Mystery Cave). We are also developing methods to test our best biological control candidates on rock substrates using scanning electron microscopy and a hybrid cave/lab incubation system.

Additional work is in progress to characterize and test the intracellular bacteria that were discovered associated with some of the inhibitory fungi that we previously identified. Some of these bacterial isolates have been isolated away from the fungi, and will be tested for their ability to inhibit Pd alone versus the intact fungi/bacteria association.

Project Status as of January, 2019**:**
Preliminary data shows the presence of *P.d*. DNA in most locations sampled within Mystery Cave and Soudan Mine with highest concentrations in locations where bats were observed congregating in spring 2018 as well as known bat entry/exit locations. Preliminary data from experiments undertaken on *P.d*. in culture indicate the fungus (spores and mycelia) may be capable of survival in these locations in the absence of bats for extended periods of time (>24 weeks).

We have also identified some common nutrients that may increase the growth and reproduction rate of P. destructans on various substrates, including chitin, glycogen and collagen. These results will have an impact on the types of biocontrol organisms that will be tested against P.d., since a biocontrol strategy employing fungi might inadvertently increase the local concentration of chitin, and therefore induce sporulation.

Amendment request April 17, 2019: We are requesting permission to move $6,000 from the Activity 2 column, illumina sequencing to Sanger sequencing supplies in the same activity. We are completing more individual sequence analyses using Sanger technology of new microbial strains, and require less funds for the community sequencing work (illumina).
Amendment Approved July 29, 2019

Overall Project Outcomes and Results: White nose syndrome is a devastating disease of hibernating bats caused by the fungus, *P. destructans* (*Pd*). The primary goal of this project is to identify safe and effective bio-control treatments for WNS. We expanded our microbial strain collection and identified additional inhibitors of *Pd*, bringing our total of active strains to ~120. We identified the top five inhibitory strains, purified the active compounds, and determined their structures and activities. We identified ~15 structures new to science and 6

known compounds with antifungal activity. To determine the potential application of these active strains to substrates or bats, we developed a cell-based assay using bat skin cells derived from two bat species. By testing each compound against both the fungal pathogen and bat skin cells, we could calculate the relative potency and cytotoxicity. One of the most active and abundant compounds from an inhibitory fungus from the Soudan Iron Mine is completely nontoxic towards the cultured bat skin cells, which provides additional support for field testing with the producing strain.

Additional accomplishments include the sequencing of bacteria and fungi found throughout three distinct systems (iron mine, sandstone and calcium karst caves) from both culturable strains and mixed, non cultured microbial community samples. These taxonomic studies are significant because they allow us to see patterns of microbial communities across diverse environments, including identifying taxa that are unique or common in different areas.

We also developed tools and techniques for monitoring *P. destructans* in caves for studies going forward. Mapping of *P. destructans* along two transects in the Soudan Mine and Mystery Cave using qPCR provides a clear picture of the density and occurrence of the pathogen. This information and testing will be used to target treatments in collaboration with the DNR and managers to ultimately protect the remaining bat populations.

Amendment request September 10, 2019. We are requesting permission to increase the publication budget from $500 to $2000 for a net increase of $1500 by shifting unspent funds from the travel budget. The change is needed to cover the open access publication fees for a scientific manuscript related to activity 1 (total publication costs for the 3 year period were $1703). We also request to increase the personnel budget from 151,709 to 193,756 for a net increase of 42,047 by shifting unspent funds from the travel budget by 12,000 and supplies budget by 30,047. This is need to the effort by an additional postdoctoral associate who assisted with expanded chemical extraction and analysis for activity 1. Overall we are under budget by $25,295 (returned to ENTRF).
Amendment Approved November 18, 2019

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Microbial Antagonist Library
Description: Our goal is to identify the best microbial antagonists that can be applied to roost areas and or directly to bats to provide dynamic and long lasting protection against fungal infection. Although we have successfully identified over 50 bacterial and fungal isolates from the Soudan Mine that inhibit the growth of *P. destructans* under laboratory conditions, we do not yet know which species can grow efficiently on either roost substrates or on bats themselves. We also plan to determine which microbial antagonists can successfully grow on various substrates related to bat habitats throughout Minnesota including limestone, sandstone and greenstone/banded iron (hematite, jasper, chert): See activity 3.

Bacteria and fungi will be collected from bats, roosts, and surfaces from Mystery Cave State Park and other minor hibernation areas in Minnesota that provide representatives from the various different types of substrates. We will especially focus on the non-pathogenic species of fungi found associated with bats as promising and abundant competitors of the WNS fungal pathogen *Pseudogymnoascus destructans*. Live colonies of each strain will be tested on solid media with an agar overlay spread with spores from *P. destructans* (for bacterial antagonists) or with side by side mycelial plugs (for fungal antagonists). Additional assays will include spore germination inhibition and non-contact dependent antagonism.

Summary Budget Information for Activity 1:	**ENRTF Budget:**	**$ 222,209**
	Amount Spent:	**$ 218,484**
	Balance:	**$ 3,725**

Outcome	Completion Date
1. Isolation and culture of bacteria and fungi (~500 isolates) from bats/roosts in Mystery Cave State Park and minor hibernation caves near the Twin Cities	07/01/17
2. Characterization of growth and Pd inhibition capacity of bacteria and fungi	07/01/18
3. Determination of mechanism of growth inhibition of best biocontrol agents (top 3)	07/01/19

Project Status as of January, 2017**:**
Our investigations at the Soudan Underground State Park have found unusual and unique fungi that can tolerate very extreme conditions. Over 1000 fungal cultures have been isolated from the different levels of the Soudan mine. Some of the fungi such as *Phialophora, Cadophora* and *Oidiodendron* can tolerate high concentrations of heavy metals that are being tested for bioremediation of toxic compounds. Others such as *Calocera, Coniophora, Oligoporus, Postia* are saprophytic attacking old mine timbers and any organic materials in the mine. Others found, such as several *Psuedogymnoascus* species, grow on mine sediments and rock. These isolates represent several unusual species of *Psuedogymnoascus* living saprophytically in the mine. Although these fungi are very closely related to the fungus causing white nose, *Psuedogymnoascus destructans* they apparently are not parasitic on bats. These microflora studies have provided a baseline of information on the subterranean fungi present in the mine prior to the arrival of *P. destructans*. Many of these fungi appear to be new species and are being further characterized. For the *Psuedogymnoascus* isolates, four gene regions have been sequenced and analyzed revealing several novel species (all related to but different from *P. destructans*). Additional sampling is underway to isolate fungi from caves and other bat roosts to build a more complete culture library. The saprophytic fungi are being tested for their potential antagonistic ability to *P. destructans* and for potential use as biological control agents to be used against the white nose pathogen. In addition, sample data, both sample and mine level specific, is also being examined with regard to fungal species to determine if any patterns exist based on fungal dominance of species. Subterranean microorganisms are an untapped reservoir that can be used for biological and bioprocessing technologies. Our investigations show that Minnesota has an extraordinary rich resource of these organisms and our screening investigations now underway are continuing to search for the best candidate cultures to utilize.

In addition to fungal collections, we have continued to isolate bacterial strains from bat swabs, surface swabs and various substrates collected from the Soudan Mine, Mystery Cave, Banholzer and Wabasha Caves. These isolates have been tested against *P. destructans* using overlay assays and approximately 20 show inhibitory activity.

Project Status as of July, 2017**:** We are continuing to collect microbial samples from several hibernacula including the Soudan Iron Mine, Mystery Cave and Heinrich Brewery Cave. We now have over 700 bacterial strains and more than 1200 fungi in our collection from various locations and substrate types. We are starting to culture the most active strains of bacteria and fungi to isolate, purify and identify the antifungal compounds responsible for their inhibitory activity. We have determined the structures of 9 new polyketide compounds from an *Oidiodendron* species of fungus collected in the Soudan Mine, and 4 of these exhibit inhibitory activity against *P. destructans*.

Project Status as of January, 2018**:** As we continue to add new microbial isolates to our collections from substrate and bat swabs, we are also scaling up cultures of the most inhibitory strains. We have completed the isolation of secondary metabolites from three species of inhibitory fungi: *Oidiodendron* sp., *Cadophora melinii*, and *Ilyonectria radicola*, all collected from the Soudan Iron Mine. For the *Oidiodendron* project, the full structural characterization of 18 compounds is complete. These compounds have been tested against *P. destructans* and other pathogenic fungi and bacteria to determine their potency and specificity. A new development in our testing protocol is that we have obtained primary cultures of fibroblast skin cells from the Northern long-eared bat (*Myotis septentrionalis)* and Gray bat (*Myotis grisescens*), courtesy of Christopher Lupfer at Missouri State University. Previously, we have been testing compounds and extracts against human skin cell lines as a measure of general cytotoxicity, but testing with bat skin cells provides a much more relevant system. Surprisingly, many of the compounds with potent antifungal activity against *P. destructans* are generally non toxic against both species of

bat cells. These results suggest that the producing strain may be a viable species as a biocontrol agent, but additional work is needed to determine if it can be applied to natural substrates.

Project Status as of July, 2018**:** Putative endobacteria have been identified in several fungal species of *Mortierella* from the Soudan mine that have previously been shown to have antagonism against PD. Previous attempts to isolate the bacteria from fungal cultures have not been successful. However, samples from which original fungal cultures were obtained were re-isolated to obtain new cultures with active bacteria. Fungal culture genomic DNA was screened with PCR using universal bacteria primers which showed the presence of bacterial DNA in the cultures. Cultures were incubated at 30C and bacterial cultures grew from the edge of the fungal hyphae and were sequenced revealing the same species that were obtained in the initial PCR screening. We are now going through the process of curing the fungus of the bacteria in order to use it in antagonism assays with PD and using microscopy to confirm bacteria are in fact residing within the fungal hyphae. Several of the species we have found have been identified as being endohyphal bacteria in other fungal species.

Project Status as of January, 2019**:** Due to new restrictions from the DNR after the change in status of the Northern Long Eared bat to "threatened", we can no longer sample from bats in underground locations in Soudan or Mystery Cave (including during or outside of hibernation periods). We are also not allowed to enter the bat areas in Soudan or Mystery Cave during the hibernation period. In order to continue to sampling from bats and associated substrates, we have turned to above ground roosting areas including large bridges. We have been working with the Department of Transportation to identify areas where bats are day or night roosting during Spring-Fall months, and have begun sampling these areas. In September 2018 we visited roost areas of 4 bridges and sampled from little brown bats and nearby substrates. These samples are being used to isolate pure cultures of bacteria and fungi to identify for testing against Pd, and for identifying the culturable microbes associated with these summer roosts. Twenty nine pure isolates have been obtained so far, and will be tested and sequenced. This brings our total number of pure isolates from Minnesota mines and caves to > 1800 strains.

Project status as of June 2019: One of our goals was to determine the mechanism of growth inhibition of the best candidate biocontrol strains that we've identified. To accomplish this, we isolated and purified the individual active components from each active strain (top 5), tested them against several fungi including *P. destructans*, and also tested them against bat skin fibroblast cells (skin). Because we identified the structures of each active (an inactive) compound, we could then compare these compounds against others in the literature for clues about mechanisms of action. The most inhibitory compound with the least toxicity towards bat cells was a compound closely related to a known fungal metabolite called LL-Z127α, which was previously identified as inhibitor of protein synthesis in the yeast *Saccharomyces cerevisiae*. An additional active compound, radicicol, was shown to be a potent inhibitor of a Heat Shock Protein (HSP90), which may explain its general toxicity towards both fungi and mammalian cells.

Final Report Summary:

Culture-Based Survey of Fungal Diversity:

Over 100 unique taxa were identified from more than 300 unique isolations of fungi from Mystery Cave and Soudan Mine. These fungi represent a library of native fungal denizens of subterranean places known to house large numbers of hibernating bats in MN. This provides valuable ecological information about the habitat of *P. destructans*, as well as providing a list of candidates for antagonistic biocontrol agents. Results of these experiments are being finalized for publication.

A phylogenetic analysis was completed on a potential biocontrol fungus *Oidiodendron* spp. This fungus had been isolated in the mine and was screened for activity against PD. Results show several taxa that match described species (*O. truncatum*, *O. nigrum*, and *O. arcticum*) as well as several isolates that form a separate clade related to *O. truncatum*. The isolate that showed activity against PD was identified as *O. truncatum*. These results

further support the further testing of *Oidiodendron* species as biocontrol candidates, which are clearly found in multiple areas of the Soudan irone mine.

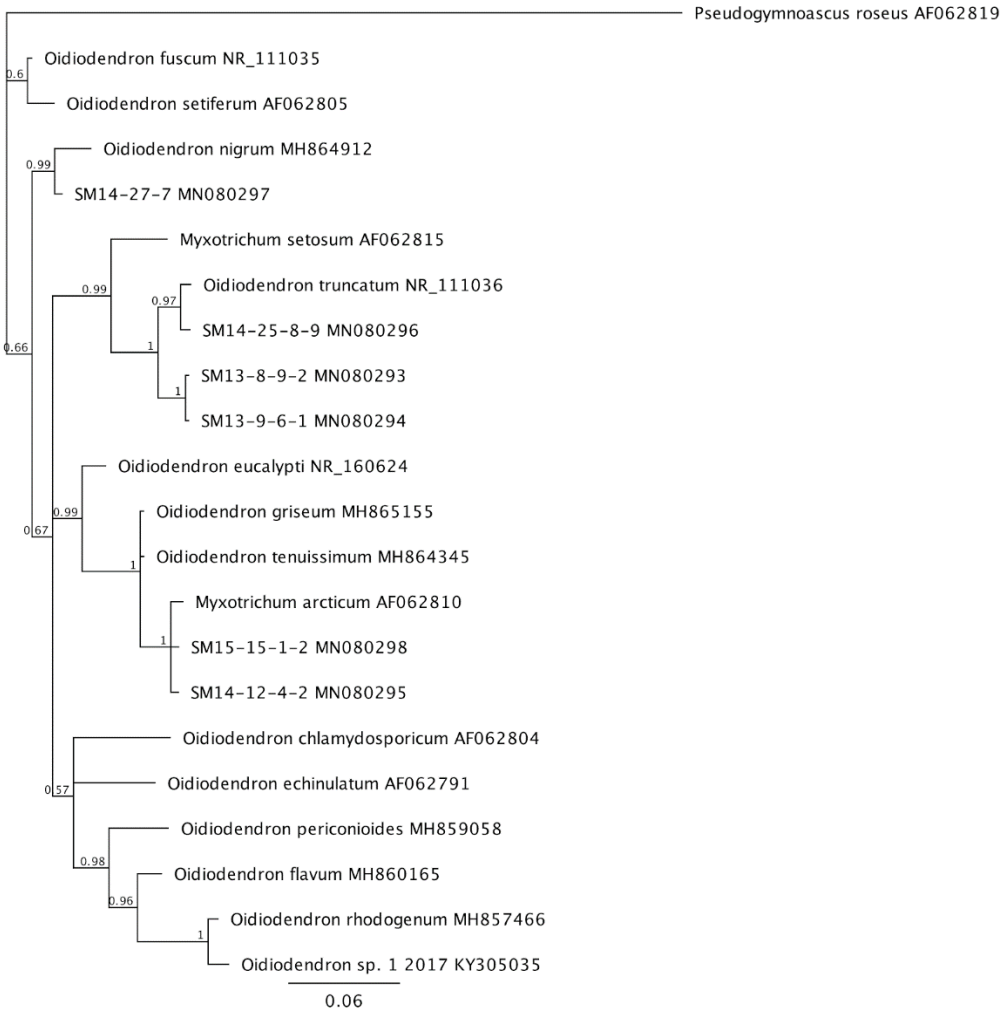


Figure 1. Analyses of sequences of potential biocontrol fungus to determine species identification.

ACTIVITY 2: Characterization of total microbiome associated with bats and roosts throughout Minnesota (culturable and culture-independent).

Description: We will compare the diversity and biocontrol characteristics of the new microbial library (~500 strains) obtained in activity 1 to those already obtained from the Soudan Iron Mine. Each isolate will be characterized using DNA sequencing (16s rRNA gene for bacteria and the ITS region for fungi). These data, together with the morphological and growth inhibition characteristics, will be compared and analyzed to identify any potential patterns of exceptional bioactivity. In addition to the comparing the culturable microbial communities associated with bats and roosts, we will use culture-independent methods to obtain a more complete picture of bacterial and fungal species associated with bats from different locations. DNA will be isolated from bat swabs taken from each location and purified and submitted to the UMN Genomics center for sequencing. We will begin obtaining samples immediately so that we can ideally have a pre-WNS sample set. We will also use these methods to eventually compare the outcomes of treatments on animals and roost materials. The timing of subsequent sampling for full microbiome sequencing will depend on the arrival of WNS disease to the hibernacula. These data will also allow us to compare the microbial communities between bat species and among the different habitats and geographic regions of the state.

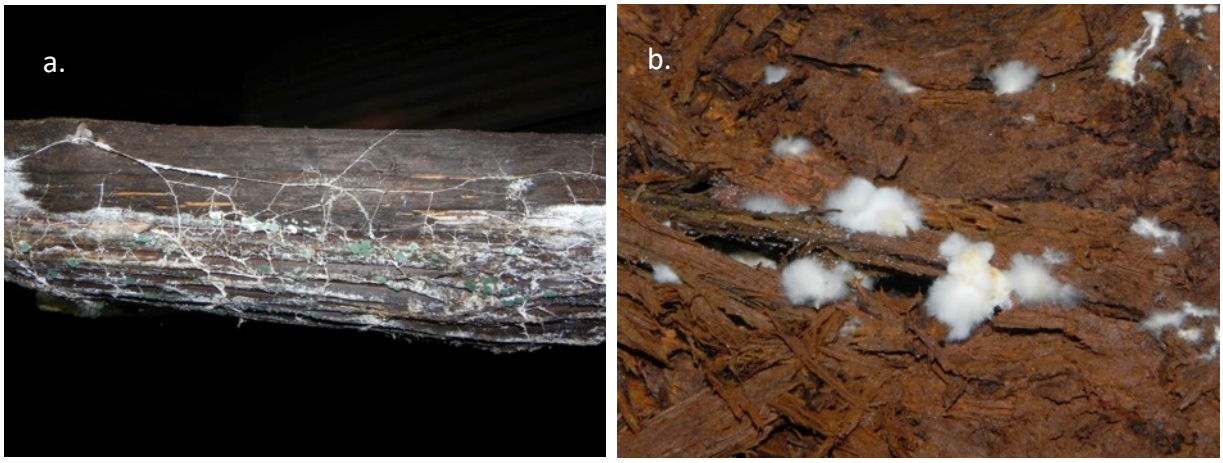
Summary Budget Information for Activity 2: **ENRTF Budget: $ 130,922**

Amount Spent: $ 118,692
Balance: $ 12,230

Outcome	Completion Date
1. Collection of microbial samples from bats in Soudan, Mystery Cave and other locations for DNA analysis	07/01/17
2. DNA isolation and sequencing from samples	07/01/18
3. DNA analysis of total microbial communities and comparison with cultured populations and pre-disease samples	07/01/19

Project Status as of January, 2017**:** Some of our most antagonistic fungi are members of the *Pseudogymnoascus* genus, but are different species than the pathogen *P. destructans*. We studied a subset of 6 strains of *Pseudogymnoascus* collected from the Soudan Iron Mine and compared their growth rates, susceptibility to several antifungal compounds and level of resistance to antifungals under different temperatures. We found that temperature had a significant effect on antifungal susceptibility, which is an important finding that suggests that laboratory testing should be done under conditions as similar to the relevant environment as possible. We also tested the ability of *P. destructans* and the non-pathogenic *Pseudogymnoascus* species to utilize individual nutrients and found that the non-pathogens are generalists and could consume nearly all of the 95 different substrates tested. *P. destructans* was more selective in its nutrient use, but if given enough time could utilize more (but not all) of the same substrates as the non-pathogens. These results suggest that a combination of antagonism and nutrient limitation might be a possible strategy for controlling *P. destructans* on substrates.

Project Status as of July, 2017**:** Samples from the Soudan Mine and Mystery Cave were provided by the Salomon lab, collected when researchers accompanied the DNR on the annual bat census, in February and March of 2017 respectively. After receiving the samples, we successfully isolated and identified fungi from: cotton swabs of diseased bats, various substrates, sediments, and dead bat carcasses. DNA was extracted from 149 fungal cultures and BLAST searches of the sequences revealed 63 distinct taxonomic units from three fungal phyla. 43 of the taxa are members of the Ascomycota, 15 are from the Mucoromycota, and the remaining 5 are basidiomycetes. The most heavily represented and diverse genera are *Penicillium* and *Mortierella*. Four taxonomically distinct cultures of *Pseudogymnoascus spp.* were recovered from the samples, but thus far *P. destructans* was not found in the samples we isolated from.



Fungal mycelia growing on timbers (a) and sediments (b) in the Soudan Iron Mine

Additional sampling is underway in Mystery Cave and the Soudan Mine. In addition, sterile flocked swabs samples were taken from walls and ceilings of cave/mine at heights of 1m and 2m. Sampling areas include

areas known to harbor hibernating bats, as well as locations in which bats are not known to roost and hibernate. In the Soudan Mine, sampling is being done from the same locations the Blanchette lab sampled from pre-WNS to allow for comparison of pre-WNS and post-WNS fungal communities. Samples have been collected from Mystery Cave in July and additional sampling will be done in the Soudan Mine in early August 2017. DNA extractions and sequencing will take place as previously described.

Project Status as of January, 2018**:** Samples were collected from Forestville Mystery Cave in July, 2017, and from the Soudan Underground Mine in August, 2017. Research is underway to investigate the non-culture dependent diversity of fungi and bacteria in these locations. The investigation into fungal diversity utilized a two-pronged approach; a culture-based survey of fungal diversity, and a molecular approach for fungal and bacterial diversity. Culturing and ITS sequence-based identification of samples from Mystery Cave have recently been completed, and Soudan Underground Mine is in progress. DNA extractions and Illumina sequencing for the molecular-based approach has been completed, and data analysis is in progress. Some interesting preliminary results are that we see most of the same clades (major phylogenetic groups) of bacteria across the two collection locations, and between wall swabs and sediment samples (Figure 1). Additionally, we have preliminary phylogenetic information about the major microbial species that make up the visible "colonies" on the wall of the first entry room of Mystery Cave.

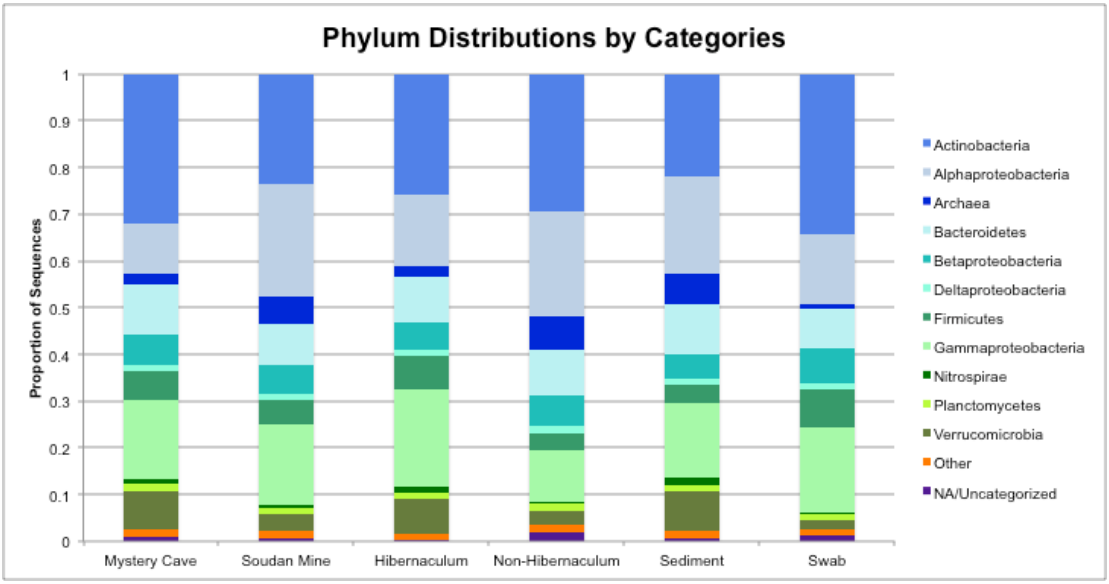


Figure 1. Proportional distributions of phyla by categories from metadata. Hibernaculum status was determined by whether or not hibernating bats have ever been observed at the specific in-cave location of each sample. "Other" includes the phyla acidobacteria, armatimonadetes, chlamydiae, chloroflexi, deferribacterales, deinococcus-thermus, elusimicrobia, fusobacteria, gemmatimonadetes, spirochaetes, and tenericutes. "NA/Uncategorized" indicates sequences which do not match any previously characterized phylum.

Project Status as of July, 2018**:** We have been detecting the presence of *P. destructans*, the causal agent of WNS, in the Soudan Mine and Mystery Cave using culture-based methods and also an extremely sensitive and specific detection assay utilizing quantitative PCR (qPCR). The assay was found to detect *P. destructans* DNA in very small quantities, representing less than a single-spore level of sensitivity. Sampling in 2018 at Mystery Cave and Soudan Mine has focused on determining the spatial distribution of *P. destructans* by utilizing this qPCR approach. This data will inform any control strategy proposed in the future by helping to determine the spatial constraints of pathogen spread. Analysis of previous metabarcoding data from Soudan Mine and Mystery Cave is being supplemented by data obtained from recent sampling, and by already completed additional testing of

prior samples using the qPCR protocol. Rotary spore trappers are being tested for use in the Soudan Mine to detect the airborne presence of the pathogen, which has previously been reported in bat hibernacula in Poland. Culture-based surveys of fungal diversity in Soudan Mine and Mystery cave are being finalized and the results of both culture-based and metabarcoding approaches are being prepared for publication.

Project Status as of January, 2019**:** In 2018, new environmental sampling efforts were focused on determining the spatial distribution of *P.d.* in the Forestville Mystery Cave and the Soudan Underground mine. Mystery cave was sampled in July 2018, and Soudan Mine was sampled in August of 2018. In each location samples consisted of ~100 swabs of walls and ceilings, and ~30 small vials of sediment. Samples were collected from 10 different sub-locations at Mystery Cave and Soudan Mine, and analyzed using a sensitive and specific DNA amplification protocol (qPCR) previously validated in our lab. Preliminary data shows the presence of *P.d*. DNA in most locations sampled within Mystery Cave and Soudan Mine with the highest concentrations in locations where bats were observed congregating in spring 2018, as well as known bat entry/exit locations. Data from these experiments are currently being analyzed and prepared for publication.

Laboratory culture-based experiments were started in October 2018 to determine the duration of *P.d.* survival across a range of temperatures and remain in progress. The pathogen is being incubated at 9C, 20C, 30C, and 40C with replicates for 24 weeks. Previous work has established upper and lower temperature thresholds for *P.d.* survival. This experiment aims to provide novel information about the duration of survival of *P.d*. at temperatures approximating bat fur after bats have left the hibernacula in the spring. Data from this experiment will be informative in developing a further understanding of disease epidemiology, specifically by providing an estimate of how long *P.d.* could remain viable on bats in the Spring to infect unexposed bats or be transferred to unexposed hibernacula. These experiments will be completed and the results prepared for publication in 2019.

Final Report Summary:
Environmental Metabarcoding Survey of Fungal Diversity: Environmental samples from Mystery Cave and Soudan Mine were submitted for community sequencing utilizing the Illumina MiSeq platform and fungal specific primers. Results of these experiments will provide novel information about the broader fungal community present in MN bat hibernacula. This information will broaden understanding and complement culture-based approaches to increase understanding of microbial diversity in subterranean places. Knowledge of the broader fungal community will aid in the measure of potential off-target effects of any White Nose Syndrome management intervention. Results of these experiments are being finalized for publication.

Our collective work on identifying both culturable and uncultured bacteria and fungi across three distinct cave types (iron mine, sandstone and calcium karst caves) provides useful fundamental information about which taxa are common or unique to different ecosystems. We can also use this information for continued monitoring of microbial communities over time as bat populations decrease or move throughout caves

ACTIVITY 3: : Development of dissemination methods for application of biocontrol agents
Description: Understanding the life cycle of *P. destructans* (Pd) is key to developing the best treatments for WNS. Pd is infamous for producing tough spores (conidia) that can lie dormant in caves for many years until they find their way onto a hibernating bat. It is unknown if Pd can successfully reproduce on natural substrates outside of its bat hosts, which could mean that this pathogen may persist indefinitely in a cave. We need to develop treatments for both bats and their cave environments to successfully combat WNS, as a treatment strategy that only attacks Pd in one place is likely to fail. We propose to screen our Soudan mine microbes that were active against Pd in our initial tests for their ability to combat Pd on bat skin punches and in Minnesota cave roosts and sediments.

Once the most potent biological control microbes are identified (Activity 1), they will be tested for efficacy and specificity under different application environments. The first assay will involve growing each candidate on medias

made from each of the three major roost materials: Soudan Mine rock material, limestone (Mystery Cave) and sandstone (Metro area caves). We will test the ability of strains to grow in both liquid and solid medias made from extracts of each substrate type. Once we have identified growth-positive antagonists, they will be tested against Pd in natural substrate materials. Pd-inoculated soils/substrates will be challenged with inoculations of Soudan mine microbes and changes in Pd growth will be compared with the previously established baseline. Similar studies will be conducted on bat wing punch explants to identify microbes that could be used directly on bats. Microbes that inhibit Pd and grow at an acceptable rate will be considered for scaled-up testing in natural environments once their environmental safety is evaluated. This component will require optimization of formulation (how the materials will be physically used and applied to roosts and/or bats). Future studies will incorporate these findings for direct testing with live bats.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 98,869
Amount Spent: $ 89,685
Balance: $ 9,340

Outcome	Completion Date
1. Test the growth of *P. destructans* on each representative substrate material (Soudan Iron Mine material, Mystery Cave limestone and Metro area sandstone)	07/01/2017
2. Test the growth of best antagonist strains on representative substrate materials and bat wing punch explants	07/30/2018
3. Measure inhibition of Pd growth by antagonist strains on substrates and bat wing punch explants	01/30/2019
4. Optimize treatment formulation for best inhibitors that can grow on each kind of substrate.	07/01/2019

Project Status as of January, 2017**:**
We are developing new methods to test the ability of the best strains to inhibit *P. destructans* in the natural environment (caves and mines). One method involves making "microcosms" of sediment/rocks inside of mesh packets that allow the exchange of water, humidity and air but prevent any microbes from traveling into or our of the packets. We added sediment to the packets on two different levels in the Soudan Mine, added *P. destructans* spores, and then sealed the packets closed with a heat sealer. These packets were then placed on the surface of the sediment and covered with a milk crate for easy identification. Subsamples of these packets will be collected over the next two years to determine the growth rate, and community composition of the inoculated sediments. We anticipate that these data will allow us to determine if this is a viable method for testing the best antagonistic strains in a controlled but native environment.

Fig. 1. *In situ* inoculation experiments with *P. destructans*. Packets are filled with sediment and inoculated with fungal conidia. Growth and reproduction of *P. destructans* will be monitored over two years.

Project Status as of July, 2017**:**
Endobacteria are bacteria living in active fungal cells. Endobacterial symbioses have been found in several Ascomycota and Basidiomycota phyla and many in the Mucormycota. Very little is known about the function and role of these bacteria, but there is evidence of metabolic complementation with their fungal hosts. We screened

fungi that were antagonistic to PD and other *Pseudogymnoascus* sp. from prior studies to determine if bacterial DNA could be amplified and possibly have a role in antagonism or adaptation to the mine environment. All cultures had been isolated on media with antibiotics and several cultures were examined under a light microscope reducing the possibility of bacteria on hyphal surfaces being amplified vs endobacteria. Bacterial primers were used on DNA extracted from 54 fungal cultures and amplified. Seven cultures (all *Mortierella* in the Mucormycota) amplified bacterial DNA, which were sequenced. One *Pseudogymnoascus* species had amplification but sequencing results were mixed. The best BLAST matches to the bacterial DNA showed matches to *Pseudomonas* sp. and one endosymbiont of *Mortierella elongata*. These are interesting results which will be studied further to attempt to isolate endobacterial strains and study growth effects on the its host and potentially against PD. These studies will be critical for determining if we should develop the endosymbionts alone as biocontrol agents, the fungi alone or the symbiotic partnership.

Figure 1. Rock slabs cut from materials collected in hibernation areas

Project Status as of January, 2018**:**
We are developing additional methods to test inhibitory strains on more natural substrates. We collected rocks from the Heinrich Cave in Minneapolis, disinfected the rock and used a diamond saw to cut the rock into small (~1x1x 0.25 cm) slices (Figure 1). These samples were then placed back into the cave for two months to develop a natural coating of nutrients and microbes. The samples were then collected and will be used for paired inoculation with *P. destructans* spores and the most inhibitory biocontrol strains. These inoculated substrates will be incubated in the lab, and monitored using scanning electron microscopy (SEM) and DNA analysis (quantitative PCR).

Project Status as of July, 2018**:** The rock slabs that were allowed to incubate in Heinrich cave were analyzed by scanning electron microscopy (SEM) microscopy using a technique that preserves most microbial "biofilm" structures. This imaging indicates that complex assemblages of both bacteria and fungi rapidly colonize the rock surfaces, even in relatively dry and protected areas of the cave (Figure 2). These results will have implications for the next tests with inoculations of these substrates with Pd plus antagonists.

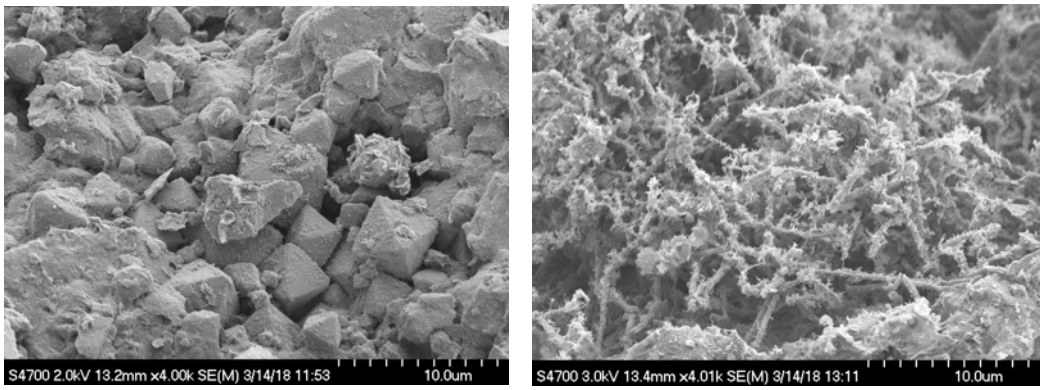


Figure 2. Scanning electron microscopy (SEM) images of rock slabs, 4,000X magnification a. control slab, no treatment b. rock slab incubated in Heinrich cave for 2 months showing bacterial and fungal cell growth .

Project Status as of January, 2019**:** We are developing the list of top inhibitory strains (and pure compounds) to test against Pd on relevant substrates. The testing of pure compounds against bat skin cells (above) has also provided critical information about which metabolites might be useful as applied anti-fungals. As part of this process, we conducted a preliminary experiment to study the role of individual carbon sources on both growth

and reproduction of *P. destructans*. One of the goals of this work was to determine if any nutrients associated with our potential biocontrol strains might inadvertently increase the growth or reproduction rate (spore number) of *P. destructans*. Mycelial growth was not very different among the various substrates, but the presence of chitin, glycogen and collagen significantly increased spore production. These experiments need to be repeated with varied concentrations of the nutrients, but preliminary analysis suggests that the close proximity of other fungi on substrates could increase the rate of *P. destructans* spore production.

Project Status as of June, 2019

QPCR-Based Survey of Spatial Distribution of *P.d.*

Intensive sampling of Soudan Mine and Mystery Cave was undertaken to better understand the spatial distribution of *P.d.* in these major MN bat hibernacula. These samples were subjected to an extremely sensitive quantitative-PCR assay capable of detecting a single spore's worth of *P.d*. DNA while also determining the quantity of DNA present. *P.d*. DNA was detected in the majority of samples taken at both locations. The greatest quantity of *P.d.* DNA, and the greatest proportion of positive samples, was detected in late hibernation gathering areas and known exit points. In particular, small exit areas or portals from the hibernacula showed the highest levels of *P.d*. DNA, thousands of times more abundant than positive samples from locations distal to the entrances. This knowledge, and methods developed to obtain it, will be extremely useful for any WNS management strategy undertaken. Similar surveys can inform the necessary scope of control measures as well as identify "low-hanging fruit" targets, such as entry/exit points, where bats are likely to encounter high levels of inoculum when entering hibernacula in the late fall.

Temperature Survival Study

Spores and mycelial plugs of *P.d*. were incubated at different temperatures for 24 weeks to determine the feasible duration of survival for *P.d*. inoculum on bat fur and hibernacula entry/exit points. Results showed that inoculum can remain viable at temperatures similar to those found on bat fur for 12 weeks, long enough for bats to spread *P.d*. to uninfected bats or hibernacula. Spores and mycelial plugs remained viable for the entire duration of the experiment (24 weeks) at temperatures similar to those found in hibernacula entry/exit points, indicating that these are likely sources of inoculum as bats enter subterranean locations for their hibernation period. This information is informing how and where we will focus any disinfection and monitoring activities in all locations.

Final Report Summary: One of the early determinations for this project was that we wanted to focus on substrate specific biocontrol agents, rather than applications on bats directly. We identified several top candidate biocontrol strains and purified the active compounds (see above, activity 1) and tested them against bat skin cells for cytotoxicity, but we decided that we did not want to test the biocontrol strains on bat wing samples because we focused on substrate specific inhibitors. All of the top candidate biocontrol strains are spore forming fungi and bacteria, which allows for a more stable formulation. We inoculated several different cave substrates with *P. destructans* and *Oidiodendron truncatum* and growth has been extremely slow, so these experiments are still in progress. We plan to test additional substrates, including wood, which might provide for a mechanism of installing in hibernacula areas.

The qPCR quantification of *P. destructans* throughout the different cave environments has proved to be a valuable tool for assessing pathogen reservoirs and areas for potential treatments. We will be collaborating with cave managers and the DNR to continue to monitor caves and test areas before and after any treatments or interventions.

V. DISSEMINATION:

- Publications to primary scientific journals will be submitted covering all aspects of this proposal. Seminars and lectures will be given at scientific conferences and to local stakeholders. Strains of interest will be made available through the American Type Culture Collection (ATCC, with appropriate usage restrictions agreed to by the University of Minnesota, LCCMR and the DNR).

- Intellectual Property will be kept confidential so that patent protection can be coordinated by the University of Minnesota Office of Technology Commercialization, LCCMR and the DNR. This will be done in accordance with Statute 116p.10, "royalties, copyrights, patents, and sale of products and assets".

- Relevant results will also be communicated to the general public through an interactive display at the Soudan Mine Visitor Center that was developed as part of our previous ENRTF project. We are also pursuing the development of a small exhibit at the Science Museum of Minnesota to educate the public about the threat of White Nose Bat Syndrome as well as related current research efforts.

Project Status as of January, 2017**:**
Seminars:
Health and Biology Research news club (Salomon), May 2016
WNS National Workshop (Salomon), June 2016

Project Status as of July, 2017**:**
Seminars:
UMycoNet (Salomon), February 2017
UMN MicrobeTech seminar (Salomon), March 2017
BTI Tokyo Biotechnology Symposium (Salomon) July 2017

Posters:
WNS National Workshop (Salomon), Nashville, TN, May 2017

Publications:
Resource capture and competitive ability of non-pathogenic *Pseudogymnoascus* spp. and *P. destructans*, the cause of white-nose syndrome in bats. Wilson MB, Held BW, Freiborg AH, Blanchette RA, **Salomon CE**. *PLoSONE* 2017, 12(6): e0178968.

Project Status as of January, 2018**:**
Seminars:
American Society of Pharmacognosy annual meeting, Portland, OR, August 2017
AHC mini Medical School, UMN, October 16, 2017 Using Nature's Toolbox to treat infectious disease
Bat Week, DNR, Mississippi Wildlife Refuge, Bloomington, MN October 2017
American Association of University Women, Minneapolis, MN November 2017

Outreach activities:
Minnesota Bat Festival, Salomon lab table, august 2017

Project Status as of June, 2018**:**
Seminars:
Chemical Ecology guest lecture/discussion (remote), Central Washington University, WA, Feb 2018
GCC 3016/3015 Science and Society: Working Together to Avoid the Antibiotic Resistance Apocalypse, University of Minnesota, MN, Feb 2018
Chemicals in the Environment, Civil Engineering, University of Minnesota, MN, March 2018
Guest lecture for Graduate Women in Science, Minneapolis, MN, March 2018
Perlman Symposium on Antibiotics, University of Wisconsin, Madison, WI, May 2018

Project Status as of January, 2019**:**
Seminars:
Microbiology Club seminar, University of Minnesota, MN, September 2018
Syngenta Science Symposium , University of North Carolina, Greensboro, NC, November 2018
North Carolina Bat Working Group Annual Meeting, Haw River State Park, NC, November 2018

Carleton College, Carleton, MN, November 2018
International Symposium on the Chemistry of Natural Products, Athens, Greece, November 2018

Outreach:
Minnesota Bat Festival, Salomon research table on WNS, August 2018

Project Status as of: June, 2019
Seminars:
Biology Department Lecture Randolph Macon College, Virginia, February 2019
Bug club Salomon research presentation, UMN, March 2019
WNS update presentation for DNR resource managers, March 2019
UMN SciSpark presentation, April 2019

Publications:
Rusman, Y, Wilson, MB, Williams, JM, Held, B, Blanchette, RA, Anderson, BN, Lupfer, CR, Salomon, CE. Antifungal norditerpene oidiolactones from the fungus *Oidiodendron truncatum*, a potential bio-control agent for white-nose syndrome in bats. In review, Journal of Natural Products.

Outreach: Posters and model objects provided to staff at the Soudan Iron Mine for development of a new research tour

Final report summary:
The primary dissemination of the results from this project has been through numerous seminars given at academic institutions, research symposia, and at professional science society meetings. Both lectures and posters have been presented at national conferences, and results have been shared with DNR staff through formal and informal communications. Two scientific manuscripts have been published on this work, and at least 5 more are in progress and should be published within the next 6 months. We have also participated in several outreach opportunities by having research tables at local bat week events, in collaboration with USFW staff.

The most immediate use of our results will be in collaboration with DNR staff and cave/mine park managers in locations affected by WNS. We are communicating our data about the pathogen locations to help inform any interventions and treatments, and to suggest specific areas for continued monitoring using our analytical approach.

VI. PROJECT BUDGET SUMMARY:
A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel		
Christine Salomon,	$ 19,836	Project Manager and Principle Investigator (75% salary, 25% benefits): 5% FTE for each of 3 years
1 postdoctoral Research Associate	$ 165,388	(82% salary, 18% benefits): 100% FTE for each of 3 years, sample collections, testing, assay development, biocontrol formulation and optimization, data/statistical analysis
1 Technician	$ 70,881	(79% salary, 21% benefits): 50% FTE for each of 3 years, sample collections, DNA extractions and analysis, biological assays, media and reagent preparations, data organization
1 Research Scientist	$ 64,427	(75% salary, 25% benefits): 25% FTE for each of 3 years, sample collections with focus on fungi,

		fungal taxonomy and characterization, data analysis and management
1 undergraduate student technician	$ 21,000	50% FTE for each of 3 years, media and sample prep, sample management, fungal cultivations, general lab support
Equipment/Tools/Supplies:	$	
Activity 1		
Supplies for microbial isolations and characterization	$ 50,000	growth media, reagents, antibiotics, petri dishes, tubes, DNA isolation supplies (extraction kits $350 per kit x 4 per year) general lab supplies (gloves, tips, tubes, etc.), chemicals, solvents, glassware. For 2 FTE scientists for 3 years.
Microscopy	$ 2,500	Scanning electron, light, confocal microscopes-hourly instrument fees at CBS Biological Imaging Facility $25-37 per hour plus specimen preparation fees, ~20 hours per year
Activity 2:		
DNA and sequencing supplies	$ 3,000	DNA amplification reagents and consumables, DNA cleanup kits (for ~ 500 strains)
DNA sequencing (Sanger sequencing)	$ 10,968	Sequencing for phylogenetic analysis of bacterial and fungal isolates (AGAC sequencing facilities, $3.60 per reaction x ~1000 reactions per year x 3 years)
DNA sequencing (MiSeq)	$ 11,000	DNA library preparation and amplification services (10.95 x ~ 150 samples per run), MiSeq sequencing paired-end single lane, 300 cycles ($1,968 per lane) x 3 runs over 3 years.
Activity 3:		
Bioassays (antifungal testing)	$ 10,000	Reagents, compounds and consumables (microbiology supplies, antibiotics, plasticware) for biological testing, general lab supplies, glassware. For 0.5 FTE scientists over 2 years
Instrumentation/core facility fees for chemical analysis	$ 2,000	Fees for core facilities for chemical analysis of active strains (NMR spectroscopy, gas chromatography, mass spectrometry). Hourly charages of $10-40 per hour or per sample, estimated at $1000 per year x 2 years
Other expenses (all activities)		
Repair of equipment and instrumentation	$ 3,001	Repair for instruments such as vacuum pumps, water baths, incubators, shakers, etc. and replacement of glassware/components due to inevitable breakage. Estimated at $1000 per year x 3 years.
Publication fees	$ 1,500	~3 total publications, $500 per publication charge for open access journals
Travel Expenses in MN:	$16,500	In-state round trip travel between St. Paul and Soudan Mine Park, Mystery Cave and metro area caves: room/board for 2-4 researchers, mileage, est. 5-6 trips/yr (0.5-2 days each trip) for 3 yrs
TOTAL ENRTF BUDGET:	**$ 452,000**	

Explanation of Use of Classified Staff:

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 2.3 FTE per year x 3 years

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
US Fish and Wildlife	$ 240,000	200,000	Related project on White Nose Bat syndrome—sharing of sampling data and strains between projects.
State			
University of Minnesota (Dept. Center for Drug Design)	$ 39,672	$13224	In kind PI salary support, 3 years at 10% FTE
University of Minnesota (Dept. Center for Drug Design)	$ 239,560	79,853	In kind overhead/indirect costs: U of M rate of 53% of direct costs for 3 years
TOTAL OTHER FUNDS:	**$519,232**	**$293,077**	

VII. PROJECT STRATEGY:

A. Project Partners:

Dr. Christine Salomon (UMN) Associate Professor, BioTechnology Institute and Center for Drug Design is an expert in microbial culturing, testing and characterization and will oversee the project and contribute to all activities.

Dr. Robert Blanchette (UMN) is a Professor in Plant Pathology and an expert in fungal biology. He will lead the fungal collections and characterizations in all activities.

Additional partners (not funded by ENRTF) include Jim Essig (DNR Park Manager of Soudan Mine State Park) and Dr. Gerda Nordquist (DNR, State Mammologist) who will help coordinate research activities and provide logistical support for sampling and experiments. We are also in communication with key managers with the US Fish and Wildlife Service: Richard Geboy, Midwest Regional WNS Coordinator and Jonathan Reichard, National WNS Assistant Coordinator and participate in their hosted monthly national conference calls.

B. Project Impact and Long-term Strategy:

At the very minimum, our work will provide foundational information about the diversity, abundance andgeographical characteristics of microbial communities associated with both healthy and sick bats (anticipated in the near future) throughout the state of Minnesota. If we are successful at identifying biocontrol agents that inhibit the pathogen, these could be developed into therapeutic tools for disease management in Minnesotaand other affected states. We are also applying for additional grants from the US Fish and Wildlife Federation to expand this work. Due to the rapid spread of the disease and dynamic nature of how diseases change the microbial landscape of their hosts, we anticipate needing to change our focus in the future to characterizing the microbes of surviving bats. We may also need to apply for "Phase 3" round of funding to support the testing of treatments or preventative measures in live bats during hibernation periods, in collaboration with bat disease experts.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount

ENTRF 2013- 2016 to conduct research on White Nose Syndrome as a sub- aim of a larger Soudan Mine Microbe project ("Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy and Biocontrol", ML 2013- 03F) This investment has led directly to the applied work in the proposed application. 100% obligated	July 2013-July 2016	$838,000
		$
		$

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): See attached

X. RESEARCH ADDENDUM: Continuation from previous ENTRF 2013 Research Addendum

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than January 2017, July 2017, January 2018, July 2018 and January 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

Project Title: *Biological Control of White Nose Bat Syndrome-Phase 2*
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06d
Project Manager: *Christine Salomon*
Organization: *University of Minnesota, Center for Drug Design*
M.L. 2016 ENRTF Appropriation: $ *452,000*
Project Length and Completion Date: *3 Years, July 1, 2016-June 30, 2019*
Date of Report: November 18, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	revised activity 1 budget	Amount Spent	Activity 1 Balance	Revised Activity 2 budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	***Fill in your activity title here.***			***Fill in your activity title here.***			***Fill in your activity title here.***				
Personnel (Wages and Benefits)	$193,756	$193,756	$0	$104,454	$104,454	$0	$85,368	$85,368	$0	$383,578	$0
Christine Salomon, Project Manager and Principle Investigator (75% salary, 25% benefits): 5% FTE for each of 3 years, $19,836		$6,647			$6,647			$6,647			
Postdoctoral researcher (82% salary, 18% benefits): 100% FTE for each of 3 years, 100% FTE for 1 year		$158,704			$32,855			$54,352			
1 technician (79% salary, 21% benefits): 50% FTE for each of 3 years, $70,881		$7,946			$7,514			$15,770			
1 Research Scientist (75% salary, 25% benefits): 25% FTE for each of 3 years, $64,427		$10,572			$52,079			$2,257			
1 Undergraduate student technicians (100% salary): 50% FTE for each of 3 years, $21,000		$9,887			$5,359			$6,343			
Equipment/Tools/Supplies											
Supplies for microbiology collections, isolations and purifications: growth media, reagents, antibiotics, petri dishes, tubes, DNA isolation supplies (extraction kits $350 per kit x 4 per year) general lab supplies (gloves, tips, tubes, etc.), chemicals, solvents, glassware. For 2 FTE scientists for 3 years (approximately $8000 per scientist per year)	$19,953	$18,880	$1,073							$19,953	$1,073
microscopy fees/core facilities: Scanning electron, light, confocal microscopes-hourly instrument fees at CBS Biological Imaging Facility $25-37 per hour plus specimen preparation fees, ~20 hours per year	$2,500	$267	$2,233							$2,500	$2,233
DNA sequencing and supplie: DNA amplification reagents and consumables, DNA cleanup kits (for ~ 500 strains)				$9,000	$5,501	$3,499				$9,000	$3,499
DNA sequencing (Sanger sequencing): Sequencing for phylogenetic analysis of bacterial and fungal isolates (AGAC sequencing facilities, $3.60 per reaction x ~1000 reactions per year x 3 years)				$10,968	$7,365	$3,603				$10,968	$3,603
DNA sequencing (MiSeq Illumina sequencing) DNA library preparation and amplification services (10.95 x ~ 150 samples per run), MiSeq sequencing paired-end single lane, 300 cycles ($1,968 per lane) x 3 runs over 3 years.				$5,000	$1,372	$3,628				$5,000	$3,628
Bioassay supplies: Reagents, compounds and consumables (microbiology supplies, antibiotics, plasticware) for biological testing, general lab supplies, glassware. For 0.5 FTE scientists over 2 years							$10,000	$4,161	$5,839	$10,000	$5,839
Instrument and core facilty fees for chemical analysis: Fees for core facilities for chemical analysis of active strains (NMR spectroscopy, gas chromatography, mass spectrometry). Hourly charages of $10-40 per hour or per sample, estimated at $1000 per year x 2 years							$2,000	$0	$2,000	$2,000	$2,000
Travel expenses in Minnesota											
Travel between St.Paul and: Soudan Mine (444 miles round trip), Mystery Cave (240 miles) at $.52 per mile. Metro area cave trips (~10-20 miles round trip). Lodging for 2-4 researchers for 1-2 days per trip, plus meals. Estimated 2 trips to Soudan, 2 trips to Mystery Cave and 3-4 trips to Metro area caves per year x 3 years	$3,000	$2,945	$55							$3,000	$55
Other											
publication fees ($500 per manuscript for open access publication x 3)	$2,000	$1,703	$297	$500	$0	$500	$500	$0	$500	$3,000	$1,297
Repair for instruments such as vacuum pumps, water baths, incubators, shakers, etc. and replacement of glassware/components due to inevitable breakage. Estimated at $1000 per year x 3 years.	$1,000	$932	$68	$1,000	$0	$1,000	$1,001	$0	$1,001	$3,001	$2,069
COLUMN TOTAL	**$222,209**	**$218,483**	**$3,726**	**$130,922**	**$118,692**	**$12,230**	**$98,869**	**$89,529**	**$9,340**	**$452,000**	**$25,295**