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ABSTRACT

The Soudan Iron Mine, located near Ely, MN in the northern portion of our state is a window into deep subsurface life. The aquatic ecosystems found on the lowest level of the mine are unlike any other studied to date where microbes appear to be thriving in an anoxic calcium chloride brine percolating through a 2.7 billion year old Banded Iron Formation over 2300 feet below the surface. This unique environment is the stage for three integrated facets of this proposal: Basic Science, Innovative Applications and Outreach and Education. Basic Science will provide a fundamental understanding of the unique environment found in the bottom of the Soudan Iron Mine. Innovative Applications will explore how novel microbes from the mine will be harnessed for discovery of novel bioactive compounds, bioenergy and bioremediation. Outreach and Education will integrate our findings to educate the general public and mine visitors and provide recommendations for protecting and preserving this resource.

BACKGROUND

The study of extreme or novel environments can sometimes lead to discoveries that change the world. In the early 1970's such a discovery was made in hot springs in Yellowstone National Park – bacteria thriving at temperatures previously thought to be inhospitable for life were identified. The commercialization of proteins from these bacteria revolutionized science, enabling an era of modern molecular genetics, including sequencing of the human genome, and is the foundation of a multi-billion dollar biotechnology industry. **Based on our preliminary analysis, a similar commercial opportunity may be found deep underground in Northern Minnesota at the bottom of an abandoned iron mine**.

The Soudan Iron Mine near Ely, MN closed in 1962 and later became the Soudan Underground Mine State Park. The Soudan Mine is both a historical site, offering the public an opportunity to reflect on past economic activities and technologies of the iron range, and also houses the High Energy Physics Lab, administered by the University of Minnesota. This state park attracts around 40,000 visitors per year; making it an important destination for both residents of Minnesota and surrounding states.

The lowest level of the Soudan Mine is home to an extraordinary environment where the fields of microbiology, geochemistry and mineralogy converge. The sedimentary iron-rich rock that was mined for 80 years at this site is known as a 'Banded Iron Formation' or BIF. BIFs contain a substantial portion of the iron found on the surface of our planet, and the Soudan BIF is estimated to be around 2.7 billion years old. Typically, oxygen is required to form rust (as we all know well in Minnesota), however the Soudan BIF was deposited ~ 400 million years before oxygen was present in significant amounts in our atmosphere. Microbiologists have suggested that iron-oxidizing bacteria could have been responsible for these ancient sedimentary formations and studying this site will provide insight into this process (23, 24).

In the lowest level of the Soudan Iron Mine water seeping from boreholes drilled in the waning days of the mine can be found (Figure 1). This water is quite unusual since it is almost three times saltier than seawater and is devoid of oxygen. Associated with many of these seeps are unique iron oxide structures and throughout this strange water are poorly characterized iron minerals and thriving bacterial communities. Some of the bacteria we have analyzed from this environment appear to be distant relatives of bacteria found in the ocean. What are bacteria from the ocean doing in water found 2341 feet underground in northern Minnesota? Are descendants of organisms that helped form the Soudan BIF still living in waters trapped within the iron formation? Are there novel microbes found here?

Fig. 1. Representative boreholes on level 27 of the Soudan Iron Mine. Left and middle images are vertical boreholes found in the west tunnel while the right image is of a horizontal borehole drilled in the east tunnel.

HYPOTHESIS / MOTIVATION

The unique environment of the lowest level of the Soudan Mine presents many exciting opportunities directly related to LCCMR funding priorities (Creative Ideas). Result 1 will help us understand the fundamental nature of this unique environment where we will characterize the microbiology, mineralogy and geochemistry of the Level 27 brine and the formations found in the mine. This information will be used to protect this unusual and exciting ecosystem and communicate our findings (result 3). In result 2 we will explore exciting innovative applications that utilize microbes isolated from the mine. Given the novelty of the microbes and environment, we expect this work will lead to new areas of research and potential revenue for the State of Minnesota in the areas of novel compound discovery, bioenergy and bioremediation. Specific hypotheses are mentioned below, where applicable.

Infrastructure for Success. The University of Minnesota's High Energy Physics Lab is located on the same floor as, and a short walk from, our proposed sample sites on Level 27 - this space is fully finished with a break room, electricity, and running water. Therefore, sample/reagent preparation and some real-time measurements (e.g. microscopy, oxygen concentrations), incubations (e.g. enrichment cultures), and experiments (e.g. iron oxidation/reduction) can be accomplished during sampling visits. The proposed research sites are accessible immediately.

METHODOLOGY / WORK PLAN

Result 1.1 Introduction: The majority of the biomass on our planet is unseen for two primary reasons. First, it is microbial, comprised primarily of archaea and bacteria. Second, it is underground and exceedingly difficult to access. Scientists estimate that up to 50% of the Earth's biomass is housed in the subsurface, an estimation primarily based on analysis of ocean sediment cores (K. Edwards, personal communication). The deep terrestrial biosphere is an extreme environment due to it being disconnected from surface input. The Soudan Underground Mine State Park offers a window into this environment, a highly unusual ecosystem to study at the convergence of an anoxic calcium chloride brine system, a 2.7 billion year old Banded Iron Formation, an iron ore mine abandoned in the early 1960's and a massive physics laboratory located over 2300 feet below ground administered by the University of Minnesota.

Hypothesis 1: geochemical differences between the east and west tunnels and between anoxic and aerobic conditions have selected for different microbial populations.

Hypothesis 2: laboratory models of cultured representatives from the Soudan brine ecosystems will give us insight into the microbiological processes occurring in the natural environment.

16s rRNA clone libraries from level 27.
Building on previous work of environmental sequencing and small-scale clone libraries of Edwards et al. (15), we will take a brute force approach to isolate DNA from a series of environments throughout the east and west tunnels from the level 27 brine communities, in both aerobic (surface water) and anaerobic (sediments and water flowing from within the boreholes) environments. We have budgeted for 1800 sequencing reactions specifically related to determining microbial community composition over the three years of this proposal. Initially, we will sample from 4 sites on the west side and 10 sites on the east side. Figure 2 shows amplifications of the 16s rRNA gene from water samples using both bacteria and archaeal primers, demonstrating the presence of both kinds of organisms at these sample sites. One observation that we will follow up is the apparent higher level of bacterial product in the aerobic sample vs higher levels of archaeal product in the anoxic sample. Our preliminary work based on cultivation (see below) suggests there is a higher degree of microbial community complexity on the east side, perhaps due to the lower concentration of calcium chloride in the water compared to the west side. We will isolate DNA from these samples using kits and protocols developed by MoBio and amplify the 16s rRNA genes using universal primer sets for bacteria and archaea and 18s rRNA genes for eukaryotes. These initial amplifications can give us a qualitative understanding of the microbial communities for each sample site. We will then construct small-scale clone libraries, where 20 individual 16s rRNA (or 18s rRNA) genes will be fully sequenced. This information will give us some idea for how diverse the specific microbial communities are within each sample set and will dictate whether we sequence additional clones from the respective sample (which may require up to 100 or more additional clones to sequence). We will collaborate with the Toner lab for phylogenetic analysis and meta-analyses that their laboratory has recently developed. This information will then be plotted against geochemical measurements from Result 1.3 and location within level 27. Knowing the full spectrum of the microbes found within these ecosystems will help formulate additional hypotheses to test either for specific constituents (using RT-PCR and species-specific primers) or to acquire additional samples for areas where the diversity is not yet fully understood. This survey will also serve to focus different cultivation techniques for isolating and growing specific representatives under laboratory conditions (see below).

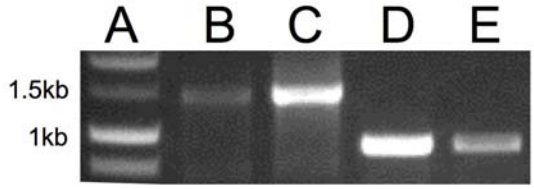

Fig. 2. 16s rRNA amplifications from level 27 far bubbler. Lane A shows a DNA standard with relevant bands marked for size. Lanes B and C show amplification products using bacterial primers while lanes D and E show amplification using archaeal primers. Lanes B and D are from the anoxic zone near the borehole mouth while lanes C and E are from the more oxidized zone several cm away from the borehole.

To date, most environmental microbiology studies do not attempt detailed microbial ecology data analysis. This is not because it is impossible, the ecological principles, statistical theory, and data analysis tools (7, 8, 35-37) are well developed and have been applied to microbial phylogenetic data in high quality studies (22), but represents the maturity of the field of research. For over a decade, the novelty of publishing phylogenetic data to describe the diversity of new environmental systems has driven a lot of environmental microbiology research. In the proposed research, we will move beyond the industry standard by:

a. Assessing microbial community composition, richness, and diversity by calculating established statistical estimators with existing software and computing resources;
b. Compare, pair-wise, all microbial community phylogenetic datasets to calculate the similarities among the Soudan Mine communities; and

c. Analyzing microbial community properties in the context of physical and geochemical data using established statistical methods, including multi-dimensional analysis

Cultivation-based approaches for characterizing specific members of the microbial community. Our initial work on the west and east side of level 27 included cultivation for heterotrophic bacteria simply by plating liquid samples from the mine on marine agar medium (a semi-defined rich medium that mimics ocean conditions). For the west side, we have isolated 22 representatives of the genus *Marinobacter* (Figure 3) and 2 representatives of the closely related *Idiomarina* genus. For the east side we have just a handful of cultured isolates from *Algoriphagus, Agrobacterium, Roseovarius, Roseobacter* and *Brevundimonas*, indicating the species diversity of the east side tunnel may be much more complex than the west side. We will develop more specific heterotrophic and chemoautotrophic media based on past and future geochemical measurements from Result 1.3 to obtain a better representation of cultivated isolates from these two ecosystems. This approach is complementary to the 16s rRNA clone library approach and has the potential to identify organisms that might be recalcitrant to the 'universal' 16s PCR primer set. Moreover, having cultivable isolates in the lab will facilitate experimentation to determine metabolic potential that cannot be accurately inferred from 16s phylogeny. Cultured organisms will be frozen in glycerol stocks at -80°C and stored by the Gralnick Lab. These stocks will serve as test cases for Results 2.2 and 2.3. Cultured representatives will be grown to determine the range of carbon source utilized, temperature range of growth, ability to grow anaerobically by fermentation or respiration and possibly other species-dependent characteristics. Initially, this work will focus on bacterial cultivation, however if significant representatives of either archaea or eukaryotic microbes appear to be present based on the molecular approach described above, we will design different cultivation approaches to try to enrich and recover representatives from these domains as well.

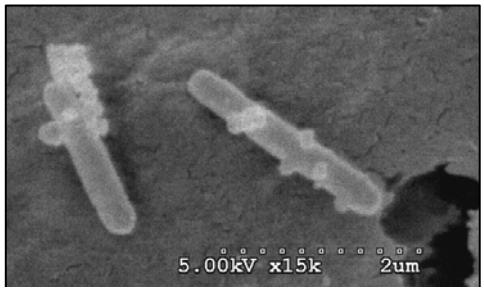

Fig. 3. Scanning electron micrograph of JG228, a *Marinobacter* isolate from the west tunnel of level 27.

Expected Results / Deliverables (Result 1.1)

We will generate a comprehensive 16s rRNA gene database for the level 27 brine communities of the Soudan Iron Mine. The 16s rRNA gene sequences will be deposited in the appropriate repositories (GENBANK, RDP, etc). To match this taxonomical survey we will have established an extensive culture collection of isolates grown in the lab from the brine communities. Knowing the distribution of microbes living in this community will allow us to develop additional hypotheses regarding their overall functionality (in combination with Results 1.2 and 1.3) to develop new proposals for research funding from organizations such as NSF and NASA. We will work to both academically publish and to broadly disseminate our findings via the interactive display and training module developed in Result 3.

Result 1.2 Introduction: The study of present-day geomicrobiological processes can assist us in interpreting past environmental conditions. The proposed research will determine whether Soudan Underground Mine State Park microorganisms leave unique signatures within the iron-bearing minerals they form and alter.

Hypothesis 3: autotrophic cycling of iron is a dominant microbial lifestyle that subsequently supports heterotrophic communities through build up of organic molecules.

Hypothesis 4: metabolic processes of microbial communities determine the mineralogy of the microbial mats by regulating the oxidation-reduction potential and particulate organic carbon content.

As part of the proposed, interdisciplinary research, the Toner lab will be responsible for describing the reactants, rates, and products of iron cycling. Through measurements of Soudan Mine iron mat properties and controlled laboratory experiments, our goal is to determine the relative influence of environmental factors and microbial activity on iron speciation. The methods we have chosen for the study of iron biogeochemistry in the Soudan Mine reflect the numerous forms of and multiple length-scales of variability in the iron present in this deep terrestrial biosphere. We will describe the mineralogy of iron-bearing phases with varying degrees of long-range structural order using bulk and micro-probe ***X-ray diffraction***. To characterize minerals with short-range order, synchrotron-radiation ***X-ray absorption spectroscopy*** will be conducted at nano- and micro-meter spatial resolution in addition to bulk measurements. ***Kinetic incubations*** with Soudan Mine iron mat materials and laboratory synthesized iron minerals will be conducted under controlled temperature, and iron and oxygen concentrations.

X-ray Diffraction. Two-dimensional, spatially resolved X-ray diffraction patterns are collected at the Advanced Light Source on beamline 10.3.2 (29). The specific sample areas of interest for XRD data collection are identified with X-ray fluorescence (XRF) mapping. The XRD patterns were collected with a CCD camera in transmission mode with 17 keV incident energy and 3-10 μm diameter spot on the sample. The two-dimensional diffraction patterns are processed using *Fit2D* software to extract the intensity versus d-spacing. The bulk mineralogy of X-ray diffracting minerals will be measured by powder X-ray diffraction and analyzed with the software program *JADE* at the Characterization Facility (UMN-TC).

X-ray Absorption Spectroscopy. X-ray fluorescence (XRF) elemental maps and spatially resolved X-ray absorption spectra (XAS) are also collected at the Advanced Light Source on beamline 10.3.2 with a 7-element germanium detector in fluorescence mode with a pixel size of ~ 10 × 10 μm (29). Based on the information provided by the XRF elemental maps, regions of interest are chosen for collection of Fe K-edge XAS data. The monochromator energy calibration is set with iron foil at 7110.75 eV. The spectra are collected to a reciprocal space (k) value of 14.4 ($Å^{-1}$). Individual scans collected at the same location are examined for changes in line-shape and peak position, to check for beam damage. Experimental spectra are deadtime corrected, energy calibrated, and averaged. The spectra are compared to reference spectra collected from Fe oxyhydroxide minerals. Principal component analysis, target transformation analysis, linear least squares fitting of experimental spectra with references, and shell-by-shell fitting are routinely performed by Toner (39, 40). Bulk XAS measurements provide information on the relative abundance of iron-bearing species, such as minerals. This research will be conducted at the Advanced Photon Source on BL 20-BM.

Kinetic Incubations. The rate of iron oxidation, the proportion of biotic versus abiotic iron oxidation, and the mineral products of these reactions will be measured through incubations at constant temperature, dissolved oxygen, and pH with whole microbial iron mat samples, cultured isolates, and laboratory synthesized iron minerals. The Toner lab has built a custom batch reactor system that controls pH through automated proportional additions of acid or base. These batch reaction vessels are temperature controlled by constant temperature water bath, and gas flow meters have been installed that will allow for control of dissolved oxygen concentrations. These measurements can be made in the Toner lab at UMN-TC or, in principle, in the Soudan Mine High Energy Physics Laboratory on level 27.

Expected Results / Deliverables (Result 1.2)

Iron oxidizing microbes and communities are globally distributed and have been identified as major contributors to Fe cycling and mineral formation from diverse geochemical settings – freshwater seeps (17) to deep-sea diffuse vents (16) – where ever iron-oxygen gradients prevail. Our existing measurements point toward the idea that the Soudan iron mats host large numbers of microorganisms that are actively responding to and altering the geology and geochemical conditions in this deep terrestrial biosphere. Our LCCMR research efforts will result in a complete characterization of the iron mat

mineralogy, including rates of mineral formation and alteration in the presence and absence of microbial activity. At the conclusion of our 3-year program, our team as a whole should be able to define the major characteristics of the iron mat microbial communities (abundance, distribution, diversity) and how these properties factor into the iron cycling we observe.

Result 1.3 Introduction: The study of present-day geomicrobiological processes can assist us in interpreting past environmental conditions. The proposed research will help determine how Soudan Underground Mine State Park microorganisms alter their environment.

Hypothesis 5: endogenous microbial life plays a role in the formation and sustenance of continental shield brines.

Hypothesis 6: continental shield brine formation is by abiotic, or inorganic, processes.

As part of the proposed, interdisciplinary research, the Alexander lab will be responsible for describing the chemical and isotopic processes occurring in the mine. The chemical and isotopic analyses will help determine the relative influence of environmental factors and microbial activity, as elucidated by Gralnick and Toner. The relation of brine chemistry to microbial processes can be directly investigated in this unique environment.

Knowledge gained in this research has important implications in the study of early life, development of an oxygen-rich atmosphere early in Earth's history, formation of banded iron formations, and the formation of calcium chloride rich shield brines. All of these research topics can then be applied to efforts in the exploration for life on other planets. In addition, the life that has evolved in these deep, saline environments is highly competitive, developing and honing unique attributes that may lead to new fields of bioenergy, genetics and medicine. Finally, deep saline aquifers are important candidates for long-term sequestration of CO_2.

The methods we have chosen for the study of biogeochemistry in the Soudan Mine reflect the numerous forms of and multiple length-scales of variability in the chemistry present in this deep terrestrial biosphere. Samples of the fluids and bulk rock properties will be analyzed with Ion Chromatography, Inductively Coupled Plasma/Optical Emission Spectroscopy and Inductively Coupled Plasma/Mass Spectrometry at the U of M Dept. of Geology Research Analytical Lab. Additional analyses of isotopic and radiometric properties will be conducted by outside commercial facilities.

Dissolved oxygen (DO), pH, eH, H_2 S measurements (along with conductivity and temperature) will be measured in the field using standard techniques. The samples collected for the other geochemical and isotopic analyses will be filter during collection through a 0.2 micron filter to remove any microbiology. This step eliminates many of preservation problems the reviewer alludes to. Bicarbonate will be determined by alkalinity titration with in 24 hours of collection and we may add total inorganic carbon analyses. Aqueous SiO_2 is determined as part of the ICP/OES suite. Trace metals will be determined by ICP/MS analyses. Nitrite and thiosulfate are determined as part of our IC anion determination. Ammonium is determined by IC cation analysis. We are investigating making ferrous and total iron measurements in the field and may expand these to other bioredox sensitive elements and compounds.

We plan to measure and/or arrange collaborative relationships to measure the stable O, H, C, and as many other stable isotopes in the waters, gases and dissolved compounds as possible. We plan to measure tritium and DIC/ DOC 14C in selected samples. We plan to arrange collaborations to measure ^87 Sr/^86 Sr ratios in selected waters. All of the stable and radioactive isotope will be measured by

appropriate, standard techniques.

Ion Chromatography. Anion samples will be analyzed on a Dionex ICS 2000 system with dual AS14 columns and an ASRS Ultra Suppressor in a carbonate/bicarbonate eluent following EPA method 300.0, "The Determination of Inorganic Anions in Water by Ion Chromatography." Ions to include: HCO_3, Cl, Br, NO_3, NO_2, NH_4, PO_4, SO_4, S_2O_3, F, and several small organic acids: acetate, formate, lactate, oxalate.

ICP/OES. Major cations will be analyzed by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP/OES) on a Thermo Scientific iCAP 6500 dual view ICP-OES following EPA Method: 200.7. Elements to include: Ca, Mg, Na, K, Sr, Ba, Fe, Mn, Al, Si, P.

ICP/MS. Trace elements will be analyzed by Inductively Coupled Plasma - Mass Spectrometry (ICP/MS) on an instrument being purchased under a separate project following EPA Method: 200.8. Elements to include: Li, B, Cr, Co, Ni, Cu, Zn, As, Se, Rb, Mo, Cd, Cs, W, Tl, Pb, U.

Expected Results / Deliverables (Result 1.3)

Continental shield brines occur around the world yet have contentious origins (3, 4, 18). The Soudan Underground Mine State Park offers a unique opportunity to investigate the origin of these deep fluids in rocks representative of both continental and oceanic composition. Continuous monitoring of flow, temperature and conductance will allow resolution of temporal variations in the deep waters. Characterization of the chemistry and stable isotopes (20, 38) will provide insights to the processes occurring in Minnesota. These investigations will aid the development of models for both terrestrial and extraterrestrial systems (41). At the conclusion of our 3-year program, our team as a whole should be able to define the major characteristics of the brines present in the mine and their relationship to microbial communities (abundance, distribution, diversity). These geochemical and geomicrobiological interactions will be summarized in outreach materials for the Soudan Underground Mine State Park in consultation with park management and will be integrated (when appropriate) with microbiological data for academic publication.

Result 2.1 Introduction: The Soudan Mine represents a uniquely accessible "extreme" environment for the study of unusual microbes associated with minerals and heavy metals. Previous research has demonstrated the presence of large populations of diverse bacteria in the water and sediments present in the lowest levels of the mine. However, this research is based on a metagenomics approach, which is the sequencing of the total DNA of mixed organisms in an environment without any prior cultivation of microbes. Building on this prior DNA sequence information, we propose to identify and characterize the *chemical diversity* of culturable microbes in the mine. Bacteria and fungi have proven to be extraordinarily rich in bioactive secondary metabolites or "natural products", and have provided some of the most important antibiotics and anti-tumor agents used clinically (11, 34). Microbes are particularly valuable as sources of natural products due to their rich phylogenetic diversity, structural complexity of secondary metabolites and amenability to large-scale fermentation. Among the bacteria, the Actinobacteria family of gram positive, filamentous bacteria has produced some of the most clinically relevant drugs (e.g., vancomycin, tetracycline, chloramphenicol) and is the source of two thirds of currently used antibiotics (25).

Hypothesis 7: the geochemical features of the Soudan Iron Mine provide a unique environment for diverse populations of previously unknown bacteria that may have novel biochemical pathways.

Cell counts from sediments and water in the mine demonstrated that there are significant populations of microbes (up to 1.2×10^6 per mL), and preliminary analysis of the 16s rRNA genes in the 454 sequenced library suggests that actinomycetes make up a large proportion of some samples (more than 85% based on

a small sampling of 16s rRNA sequences) (15). Our initial culturing studies of a single sample of sediment from the oxidized, low pH area of one of the outflows has provided unique strains that may represent at least one new genus of actinomycetes as well as new species of known genera. We also hypothesize that these novel microbes will produce new natural products with potential uses in agricultural and medical applications. This hypothesis is based on the premise that the Soudan mine environment is low in nutrients and that microbes compete for limited resources by producing secondary metabolites to interfere with signaling or growth of potential competitors. Preliminary analysis of extracts from the new strains obtained from the Soudan Mine (Figure 4) has demonstrated the production of natural products with potent activity against pathogenic fungi and bacteria as well as one cancer cell line.

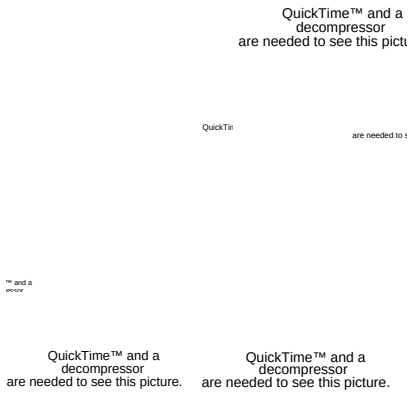

Fig. 4. Colonies of *Actinomycetes* isolates from preliminary samples from the Soudan Iron Mine.

Project Objectives: The overarching goals of our research program are to identify sources of microbial diversity and to discover novel biologically active compounds ("natural products") from new species of bacteria and fungi. Our specific objectives for this project include 1) Culture new actinomycete bacteria from different areas of the mine using the specific geochemical signatures of the environment to guide the culture conditions. 2) Sequence the 16s rRNA genes of unique species to study and compare the diversity of Soudan Mine actinomycetes 3) Test extracts from the bacteria against nine human pathogens and three cancer cell lines for biological activity 4) Identify and characterize all new compounds using bioassay guided fractionation and chemical analysis.

Design & Methods:

Microbial isolations: Water will be collected from boreholes (up to 10 mls each sample) using sterile serological pipettes. Water will be removed slowly and carefully to avoid any disruption to the outflow (ie. not more than 1 mL/min). Small quantities of sediments and rock (< 10g) will also be collected from various locations in the mine using small sterile spatulas. Small areas of mine walls and rocks (<4cm^2) will also be swabbed with sterile cotton applicators to inoculate cultivation plates directly. Samples will be transported to our microbiology lab at the University of Minnesota and will be cultured using standard techniques. Briefly, solid samples such as rocks and sediments will be dried and plated directly onto solid media or vortexed with phosphate buffer to make a microbial solution. This solution is either heat treated (55° C for 1 hour) and serially diluted or diluted and plated directly onto selective media with and without salt (artificial seawater). Preliminary data suggests that most of the strains that we have isolated require salt for growth on solid media. We will work with other researchers in this project (Drs. Calvin Alexander, Brandy Toner and Jeff Gralnick) to identify the unique geo-chemical features of the mine that may affect microbial growth and use this data to modify our culture conditions.

We will also collect larger volumes of water (1-2 liters) from one or more boreholes to incorporate directly into growth media to isolate unique strains of bacteria. In order to minimize any disruption to the outflow or water level in the borehole, a small peristaltic pump will be used to collect the water over several days. Since the measured outflow is 1 mL per minute, we would set the pump to collect water at 0.5 mL per minute (half the outflow rate). A sterile pipette is attached to the tubing and is clamped above the borehole opening to collect water at least 10 cm below the surface. The pump will run continuously for 2-3 days, collecting 720 mL per day. This water would be collected from the flask daily, sterile filtered, and stored at 4°C. A diagram of the proposed setup is shown in Figure 5.

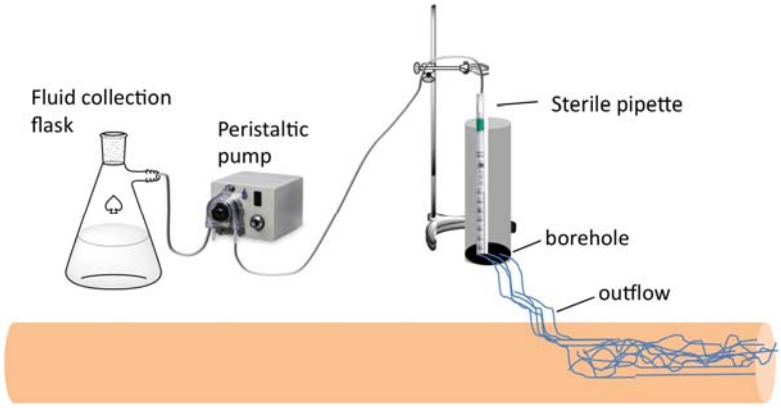

Fig. 5. Diagram of borehole water collection setup. A sterile pipette is suspended in the borehole and held in place by a clamp stand. Attached tygon tubing runs through a peristaltic pump set at half the outflow rate (0.5mL). The water is collected in a 2 liter Erlenmeyer flask that is collected and sterile filtered daily. Water would be collected for 2-3 days.

Phylogenetic analysis of strains: DNA will be isolated from each unique strain using the MolBio water DNA isolation kit. A portion of the 16s rRNA gene will be amplified from each DNA sample using PCR and a universal bacterial primer pair (27f and 1492r) (1). The DNA will be sequenced at the UMN Biomedical Genomics Center and this data will be analyzed to identify the most closely related strains using the Basic Local Alignment Search Tool (BLAST). The sequences will also be aligned and analyzed using bioinformatics software (Geneious) to build phylogenetic trees and identify the potential relatedness among strains.

Production of extracts and biological testing:
Fermentation. Each strain will be fermented under standard conditions in 50 mLs of each of the following medias: ISP2 broth, ISP2 broth supplemented with artificial seawater (ASW), M1 broth, M1 broth plus ASW, CRM broth and CRM plus ASW in 250 mL culture flasks for seven days. Cultures will be centrifuged and the supernatant will be separated from the mycelial cells. Depending on the number of hits, we may increase the number of fermentations by repeating the cultivation of each strain with different media. We have the capacity to culture 60 x 250mL flasks per week.

Extractions. Five grams of adsorbent resin (XAD-4) will be added to each fermentation broth in a porous nylon bag to concentrate secondary metabolites, as well as potentially increase the yield of compound production (6). By placing the resin in an enclosed "pillow" made with a heat sealer, this removes an additional handling step of separating the resin from the media and cells. We have had good success using this technique with the 50 mL cultures, which allows the use of 50 mL conical tubes to make the extracts efficiently and quickly.

The separated cell pellets and XAD-4 resin pillows will be extracted in methanol in 50 mL conical tubes. The extracts will be made at room temperature by placing the resin bags and cell pellets with solvent in tubes on a shaker for one hour. Culture broths will also be partitioned with ethyl acetate (50 mL) to extract any compounds not adsorbed to the resin. We anticipate the initial production of six culture conditions x three extracts for a total of 18 extracts per strain.

Bioactivity assays. Each extract will be tested against nine microbial pathogens and three cancer cells lines in a 96 well plate format. The standard broth dilution assay will be used for the microbial assays with the following microorganisms: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 2523 (methicillin resistant, MRSA), *Enterococcus faecalis,* ATCC 51299 (vancomycin resistant, VRE), *Escherichia coli* ATCC 25922 , *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 10145, *Klebsiella pneumonia*, ATCC 13883, *Cryptococcus neoformans* ATCC 66031 and *Candida*

albicans ATCC 10231 (9). The microtiter MTT assay will be used to identify anti-cancer activities with the following cell lines: Human Colon Tumor HCT116, breast MCF7 and leukemia CCRF-CEM (12, 31). Each extract will initially be tested at 250 mg/mL in duplicate and active extracts will be purified by bioassay-guided fractionation.

Purification and structural elucidation of active compounds:

Once the fermentation and extraction conditions have been optimized, the compounds will be purified using a combination of solvent fractionation, LH-20 gel filtration, normal and reverse phase flash or vacuum chromatography and HPLC.

Pure novel compounds will be analyzed by standard spectroscopic methods to determine their structures and stereochemistry. High-resolution mass spectrometry will be used to determine the chemical formula, and IR and UV spectroscopy will provide information about functional groups. The structures will be determined primarily by NMR spectroscopy using 1 and 2D experiments such as COSY, HMQC and HMBC. Although it is anticipated that most compounds will be analyzed using the 600 MHz NMR at the Center for Drug Design, even higher resolution and sensitivity can be obtained with the 800 MHz Varian instrument (with cryoprobe) in the Biochemistry department at UMN in the case of extremely small samples (< 1 mg) or complex spectra. The relative and absolute configuration of new compounds will be determined using a variety of methods including NMR chiral shift analysis as well as coupling constant analysis, circular dichroism (CD), chemical degradation and semi-synthesis.

Storage and curation of specimens. We anticipate collecting 10-20 samples of water and sediment per collection trip. Because we are interested in identifying diverse microbes, we will sample from as many different environments in the mine as possible. Once the microbes are cultured, we will sequence the 16s ribosomal genes of unique strains and deposit these sequences into Genbank, the National Institute of Health genetic sequence database. We will primarily be searching for actinomycete bacteria, but may identify other interesting bacteria or fungi during the culturing studies. If we identify new genera of bacteria, we will fully characterize the type strains and deposit a culture into the American Type Culture Collection (ATCC) for archival and dissemination, ensuring beforehand that the University, LCCMR and DNR retain some rights to compound discovery. We will also keep stocks of spores and mycelia from all isolated strains in our laboratory culture collection at UMN, which is stored in glycerol solutions at -80 degrees.

Equipment. The standard sampling equipment includes 50 mL conical tubes, sterile serological pipettes, sterile soil borers, plastic sample bags and spatulas. We will also have a small peristaltic pump that can operate by battery, tygon tubing and collection bottles. We use a digital camera to document sampling sites. We will need to store approximately 2-3 liters of water at refrigeration temperatures during a 3-day collection period. (Alternatively, this could be done in coolers with icepacks).

Expected Results / Deliverables (Result 2.1)

Initially, we will be focusing on actinomycete bacteria due to their well-known production of diverse, biologically active natural products, initial sequencing data as well as preliminary culturing experiments in our lab. However, if time allows, we will also expand our approach to include other novel microbes such as anaerobes, autotrophs, acidophiles and fungi. We expect to isolate and screen at least 50 different isolates over the course of the three-year project. The exact number screened will be dependent on two specific factors, 1) ability to cultivate different strains from throughout the mine and 2) if specific isolates are found to have particularly interesting compounds we will focus more specifically on characterizing the compound(s) rather than continuing to isolate new organisms. Our preliminary work suggests there are not only novel isolates to be found in the level 27 brine, but also that they might be making novel compounds. For novel compounds identified with activities of interest, preliminary patent disclosures will be discussed in addition to academic publications.

Results 2.2 and 2.3 Introduction: Biotechnological advances can come from the most unexpected places, as seen in the discovery of Taq DNA polymerase from bacteria living in hot springs in Yellowstone National Park. These two sections are related in that we will survey the Soudan Iron Mine for microbes with two specific properties that may have biotechnological applications. In Result 2.2 we will explore the use of iron oxidizing bacteria found in the Level 27 brine in microbial fuel cells (MFCs). In Result 2.3 we will take a more general survey of organisms cultured from several levels of the mine (including Level 27) for the ability to precipitate the toxic metals copper and cobalt. These results have been combined because we expect to have a single Microbial Engineering MS student working on this project.

Microbial Fuel Cells (Result 2.2): Some bacteria have the natural ability to respire insoluble substrates such as iron and manganese oxide in the environment (28). Electrons from a carbon source are passed through respiratory proteins to the outside of these cells and ultimately, through a variety of controversial mechanisms, can be transferred to an insoluble electron acceptor. It has been demonstrated that many microbes that can carry out this metabolism with iron oxide as the electron acceptor can also utilize carbon or gold electrodes to the same ultimate outcome. Those electrons can then be utilized to do work, as presented in Figure 6. Once electrons have done work, a final electron acceptor must accept them. In the diagram below, and in our own mitochondria, oxygen is the terminal electron acceptor to form water.

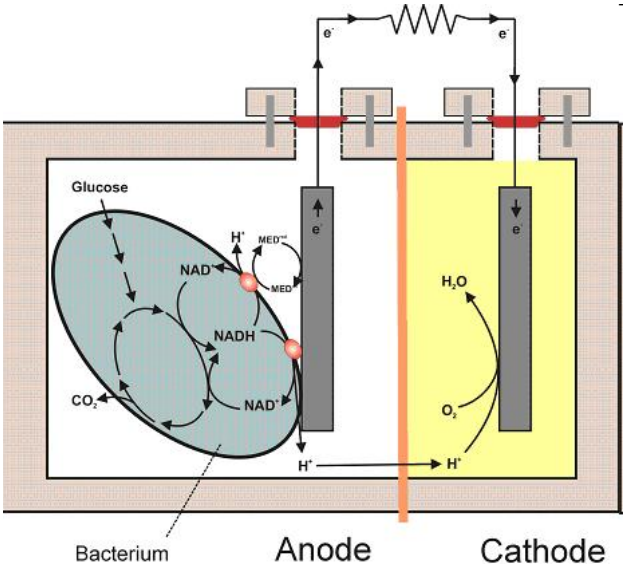

Fig. 6. Schematic of a microbial fuel cell. (microbialfuelcell.org)

Electrons enter the fuel cell at the anode and are accepted at the cathode. In principal, iron oxidizing bacteria, which use Fe(II) as a source of electrons for metabolism, could act as a terminal electron acceptor for electrons on the cathode. Iron oxidizing bacteria actively working to accept electrons from the cathode in a microbial fuel cell would allow for biology to both 'push' and 'pull' a microbial fuel cell simultaneously, perhaps leading to an increase in workload for the fuel cell and perhaps leading to bioengineerable traits on both sides of the reaction. Electrochemical analysis also can help us understand how metabolic processes occur (2, 10, 30) and may provide some insight into the mechanism for iron oxidation in bacteria from the level 27 brine.

It is important to note that Dr. Daniel Bond (Department of Microbiology and BioTechnology Institute, University of Minnesota) is listed as a collaborator for this portion of the proposal. Dr. Bond is one of the world's experts in MFC technology and bioelectrochemistry. The Bond Lab has developed a controlled potentiostat system where electrodes can be poised to act as either an anode or cathode, as shown in Figure 7.

Hypothesis 8: bacteria isolated from the Soudan Iron Mine can utilize cathodes as electron donors.

Hypothesis 9: bacteria isolated from the Soudan Iron Mine can directly mediate the oxidation of Co(II) and can generate highly reactive iron oxide minerals.

Cathode enrichments. Samples from sites throughout level 27 will be diluted with synthetic Soudan Mine medium (SSMM) and used to inoculate bioreactors where the electrode is poised to provide electrons to organisms in contact with the electrode surface. SSMM will be constructed based on geochemical measurements determined in Result 1.3 and also based partly on previous reported brine composition from the far west side tunnel (15). Low levels of oxygen (1-2%) will be mixed with a balance of nitrogen gas to provide microaerobic conditions. The flow rate and composition of the gas can be adjusted as needed. As microbes capable of drawing electrons off the electrode colonize and begin to actively metabolize, current leaving the electrode will be monitored using the potentiostat system. We will enrich for microbes capable of growing under such conditions from the Soudan Iron Mine. When significant current is monitored leaving, the electrode can be harvested and bacteria isolated from the electrode surface. We will determine the range of organisms present on active electrodes, plate for isolation and also reinoculate a fresh reactor to enrich for cathode reactivity a second time.

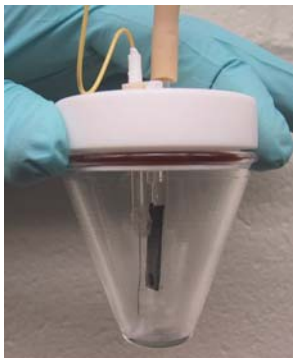

Fig. 7. Electrobioreactor for cathode experiments.

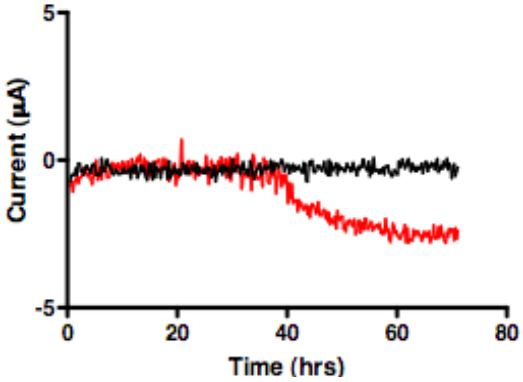

Fig. 8. *Marinobacter* from Soudan Iron Mine (JG228) is able to facilitate current leaving a cathode (red line) while a non iron-oxidizing *Marinobacter* isolate (black) cannot.

Marinobacter activity on cathodes. Previous work by the Gralnick Lab has established that *Marinobacter* is a very abundant organism in the aerobic brine environments of the west side tunnel on level 27. Preliminary work by the Gralnick Lab has shown that the *Marinobacter* isolates are capable of mediating iron oxidation at low oxygen concentrations, consistent with some recent reports of several *Marinobacter* species isolated from oceanic environments (13, 14). We will use pure culture isolates from the Soudan Iron Mine to determine if they are able to be metabolic active on cathode electrodes. Prelimiary results from experiments with *Marinobacter* sp. strain JG228, an isolate from the West tunnel on level 27, suggest that this organisms can utilize electrons from a glassy carbon electrode (Figure 8).

Characterization of electroactive cathode oxidizing bacteria. Cyclic voltammetry will be used to determine redox properties of cells attached to electrodes from either set of experiments above. This process can help identify mechanisms of electron transfer from electrode to the microbial cell and determine specific redox potentials that allow the reaction to proceed. It can also help identify if there is a soluble compound facilitating the reaction or if there is a direct connection between the cells and the surface of the electrode (2).

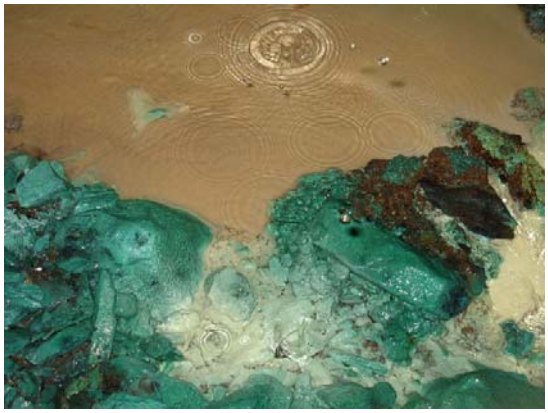

Fig. 9. Copper precipitation on level 10 of the Soudan Iron Mine.

Microbial Mineral Precipitation (Result 2.3): Many bacteria are known to influence redox chemistry of metals (19). In many cases the redox property of a metal directly influences its solubility. Level 10 of the Soudan Iron Mine (Figure 9) has water that contains significant concentrations of copper and cobalt. As a consequence, the water pumped from the mine is treated by anion exchange chromatography before water is released to the surrounding

environment. While this system is highly effective, it is also very costly. In this section of the proposal we will test two specific ideas related to bioremediation of copper and cobalt. If suitable natural isolates are identified we can imagine deploying biofiltration units upstream of the anion exchange chromatography system that would serve to remove a significant portion of the contaminant in a renewable way, possibly decreasing the maintenance / replacement frequency of the chromatography system.

Cobalt precipitation / removal. We will test the ability of bacterial isolates from throughout the mine for their ability to mediate the precipitation of Co(II) to Co(III), two metals specifically of interest to the park staff. Co(III) then can react with oxygen to form a cobalt oxide mineral. The Gralnick Lab has experience working with chelated cobalt, having demonstrated the mechanism of cobalt reduction by *Shewanella oneidensis* strain MR-1 (21). Previous experience with Co reduction assays, synthesis and analysis of chelated Co complexes will be important to the success of this section of the result. Co(II) oxidation is seen in some aquatic environments, and is thought to be linked to Mn(II) oxidation (32). Our screens of isolates from the Soudan Iron Mine (including isolates from Results 1.1 and Result 2.1) will include both Co(II) oxidation and Mn(II) oxidation. These screens will be performed in a high-throughput manner utilizing 96-well plate formats and assays compatible with plate readers available in the Gralnick Lab. Promising candidates will be further analyzed for Co over time with either spectophotometric analysis or elemental analysis using ICP-MS (21), Mn will be measured spectrophotometrically using a previously described method (32). Since reactive nanoparticles of Mn(IV) may act as a catalyst for Co(II) oxidation in some systems (32) we think it is important to monitor strains for both activities.

Biogenic iron oxide minerals for Cu(II) and Co(II) removal. Biogenic iron oxide minerals are known to have a dramatically higher surface reactivity compared to inorganic iron oxide minerals and have been the subject of many studies showing an increased capacity to bind a wide variety of cationic metals including arsenic, lead, uranium and chromium (26, 27, 33). This property has lead to many studies of using iron oxide minerals as natural ‘sponges’ for these kinds of toxic metals and is the rationale for adding large amounts of ferrous (Fe(II)) chloride to the Haiwee Reservoir in California where the formation of iron oxide minerals facilitate the retention of large concentrations of arsenic found at this aquifer (5). The Gralnick and Toner labs will collaborate to test the effectiveness of iron oxide minerals formed by iron oxidizing bacteria from the Soudan Iron Mine to retain Cu(II) and Co(II) and other cationic metals as directed by the park manager. We will test isolates from Result 1.1 to determine their rates of Fe(II) oxidation with the goal of identifying strains that can robustly catalyze the formation of biogenic iron oxide under laboratory conditions. The biogenic iron oxidation that appears to be occurring in the level 27 brine systems where *Marinobacter* is present results in a very strange mineral precipatate with biogenic materials (proteins and/or polysaccharides) yielding orange ‘foam’ as depicted in Figure 10. We suspect this material contains significant levels of biomolecules (the subject of some of our research efforts in result 1.2) that may enhance its activity in retaining toxic metals.

Fig. 10. Iron oxide ‘foam’ precipated downstream from boreholes in the west tunnel of level 27.

Expected Results / Deliverables (Results 2.2 and 2.3)

At the conclusion of Results 2.2 and 2.3, we will have attempted to identify microbes from the Soudan Iron Mine that can utilize electrons from cathode electrodes – a metabolic process that has not yet

been attributed to any specific organism. The microbes living in the brine ecosystem appear to have the appropriate metabolic process (iron oxidation) that could facilitate electrode harvesting from a cathode, but experimentation is required. We will also screen through microbes isolated as part of Result 1.1 to determine if any can robustly accelerate the oxidation of Fe, Co and Mn using high-throughput 96-well format screening. We will also test biogenic iron oxide synthesized in the lab by Soudan Mine isolates and biogenic iron oxide produced *in situ* by the natural brine microbial communities for their ability to sequester Cu(II) and Co(II). These experiments are highly exploratory in nature, but will result in critical preliminary information that can lead to additional academic or industrial support. We expect to also publish results from these studies, and we will also take the appropriate intellectual property considerations as recommended by the Office of Technology Commercialization (U of Minnesota).

Result 3 Introduction: One critical aspect of this proposal is to provide public outreach, not only to convey to the citizens of the state of Minnesota how the LCCMR has invested their money but also to educate and excite people about science. This section will be developed with help from Jim Essig (Park Manager) and his team at the Soudan Underground Mine Sate Park.

Training module development. As we begin to integrate results from our research Drs. Gralnick and Toner, with input from Drs. Salomon and Alexander will work to construct a training module to bring park staff up to speed with our science and subsurface microbiology, mineralogy and geochemistry. The goal is to have park staff learn about these areas to sufficiently answer questions from people taking the general mine tour. We will also develop a short guide based on the training module that the park staff can help use to answer questions during their tour. The module will be comprised of the basic areas of microbiology, mineralogy and geochemistry and also directly integrate knowledge generated from Result 1 of this proposal.

Develop a best practices policy for displaying, preserving and science. We will work with the park staff to develop a policy related to public access, preservation and scientific sampling of the environments we are proposing to study on level 27 of the mine. We will explore the possibility of remote display technology to allow park visitors to see some of the brine environments via the interactive display to be developed. This is of special interest for the east side features (see Figure 1, right picture) given their limited accessibility. We will discuss the possibility of a limited Micro-oriented tour where visitors with specific interest in the extremophiles could be taken to visit some of these environments on level 27. Most importantly, we will determine a best practices policy for preserving these features and develop specific guidelines for scientific sampling. Much of what we learn in Result 1 will be critical to making informed decisions regarding preservation, as it will provide us with an excellent working knowledge of what is there today.

Develop an interactive display for the Visitor Center at the surface of the mine. Funds will be used to purchase and set up a computer with a large flat panel display (possibly a touch screen display). The PIs will work with park staff to develop a user-friendly interactive display where visitors can explore the microbiology, mineralogy, geochemistry and biotechnology aspects of our LCCMR proposal, in addition to learn about subsurface microbiology and life in extreme environments. This will be a combination of pictures, videos and clickable slideshows.

TIMETABLES

Result 1.1 – Microbiology

Year 1

- Sample collection / DNA extraction / 16s rRNA Library construction
- Library DNA Sequencing
- Preliminary isolations on new medium compositions

Year 2

- Secondary sample collection / DNA extraction 16s rRNA library
- Library DNA Sequencing
- Continue organism isolation with 16s rRNA library information
- Characterize isolates with 16s rRNA DNA sequencing

Year 3

- Final sample collections as driven by cultivation, 16s rRNA, geochemistry data
- 16s rRNA gene sequence deposition in databases
- Continue organism characterization / growth properties
- Prepare manuscript(s) / new grant proposal

Result 1.2 – Mineralogy

Year 1

- Bulk iron XAS at Advanced Photon Source (3 visits to BL 20-BM)
- Bulk XRD at Characterization Facility
- Develop/conduct laboratory experiments with synthetic iron oxide minerals (abiotic)

Year 2

- Micro-probe XAS at Advanced Light Source (2 visits to BL 10.3.2)
- Micro-probe XRD at Advanced Light Source (2 visits to BL 10.3.2)
- Develop/conduct laboratory experiments with iron mat samples

Year 3

- Synthesis of XAS and XRD
- Geochemical modeling of mineral formation (collaboration with Alexander)
- Develop/conduct experiments with microbial isolates (collaboration with Gralnick)
- Develop/conduct experiments with microbial exudates (collaboration with Salomon)
- Prepare manuscript(s) / new grant proposal

Result 1.3 – Geochemistry

Year 1

- Install temperature and conductance monitoring equipment at one location on the 27 West Level
- Collect field measurements of all existing diamond drill holes accessible on the 27^{th} level
- Collect and analyze major and trace element samples from a representative selection of sites on the 27^{th} level
- Collect samples of bulk rock materials representative of the mine environment

Year 2

- Continue temperature and conductance monitoring
- Use chemical data from Year 1 select sites for more detailed isotopic analysis and radiometric dating
- Collect and analyze additional rock and mineral samples

Year 3

- Combine results of Year 1 and Year 2 analyses to collect final chemical, isotopic and radiometric dating samples
- Geochemical modeling of brine formation and fluid chemistry
- Develop models of isotopic processes in the mine
- Develop models of the ground water ages based on radiometric dating
- Prepare manuscript(s) / new grant proposal

Result 2.1 – Novel Compound Isolation

Year 1

- Sample collection
- Microbial cultivation
- 16s rRNA sequencing for actinobacteria
- Preparation of extracts / compounds

Year 2

- Sample collection
- Microbial cultivation
- 16s rRNA sequencing for actinobacteria / fungal isolates
- Preparation of extracts / compounds
- Antimicrobial / cancer screening
- Active compound purification and identification

Year 3

- Microbial cultivation
- 16s rRNA sequencing for actinobacteria / fungal isolates
- Preparation of extracts / compounds
- Antimicrobial / cancer screening
- Active compound purification and identification
- Prepare manuscript(s) / new grant proposal

Results 2.2 and 2.3 – MFC and Metal Oxidation / Sequestration

Year 1

(No work planned)

Year 2

- Enrichments tested in cathode bioreactor system
- Pure cultures from Result 1.1 tested for metal oxidation
- Pure culture system for biotic iron oxide formation developed

Year 3

- Isolates from enrichment cultures identified and retested
- Pure cultures of metal oxidizers tested in cathode bioreactor system
- Cyclic votammetry on working bioreactors
- Test metal retention of laboratory and natural biogenic iron oxide

Result 3 – Public Outreach and Education

Year 1

- Meetings with park staff on outreach and preservation

- Begin developing training module with emphasis on basic microbiology and chemistry of the deep subsurface

Year 2

- Meetings with park staff on outreach and preservation
- Begin integrating results into training module
- Work with park staff on interactive display design / content

Year 3

- Meetings with park staff on outreach and preservation
- Teach training module (2 sessions may be required), develop reference guide for park staff on LCCMR project and basic materials
- Review content for interactive display with park staff, DNR
- Deploy interactive display station for visitor center

BUDGET

Result 1: Basic Science - $366,860 total / 3 years

1.1

Jeffrey Gralnick PI 5% effort no salary requested	
TBN Grad RA (Microbiology) 50% effort	73,506
TBN Grad RA (Microbiology) 64.5% fringe ($0 retirement & $4126 health insurance)	38,100
Molecular biology reagents (PCR reagents, DNA extraction kits, plasmid purification kits ($250 ea., ~12/year), enzymes, chemicals, microbiology consumables (agar, media), general lab supplies (tubes, tips, gloves etc.), cultivation supplies, sterile sampling supplies - Micro PhD Student - Years 1-3	26,344
Sequencing for phylogentic analysis of microbial communities - bacteria, fungi and archaea (AGAC Sequencing facility on UM campus $3.50 / reaction, estimate 600 reactions / year over 3 years)	6,300

1.2

Brandy Toner Co-PI 8% effort	26,667
Brandy Toner Co-PI 32.3% fringe ($1182 retirement & $944 health insurance)	8,613
TBN Grad RA (Soil,Water,Climate) 50% effort	57,690
TBN Grad RA (Soil,Water,Climate) 77% fringe ($0 retirement & $3238 health insurance)	44,400
Laboratory supplies and consumables (chemicals, sample storage, sample preparation, general lab supplies) - SWC PhD Student - Years 1-3	15,000
Microscopy (SEM, Light - User fees at CBS Biological Imaging Facility)	2,500
Advanced Photon Source at Argonne National Labs (Chicago - travel, lodging, user fees) for mineralogical analyses (years 1-3)	10,500

1.3

E. Calvin Alexander Co-PI 1% effort no salary requested	
Scott Alexander Scientist 8% effort	10,353
Scott Alexander Scientist 32.7% fringe ($172 retirement & $563 health insurance)	3,387
Chemical, stable isotopic, gas analysis and radiometric dating (reagents for extraction / preservation, measurements, user facility fees) Years 1-3	30,000

Result 2: Innovative Applications - $168,140 total / 3 years

2.1

Christine Salomon Co-PI 1% effort no salary requested	
TBN Post-doc Assoc (Center for Drug Design) 50% effort	57,000
TBN Post-doc Assoc (Center for Drug Design) 19.75% fringe ($0 retirement & $2018 health insurance)	11,250
Lab supplies for Center for Drug Design - chemicals and glassware for culturing microbes, DNA isolation and sequencing for strain identification, solvents for compound isolation, HPLC and MS time for compound identification and structural characterization, laboratory consumables for Postdoc (50% time) - Years 1-3	15,000

2.2, 2.3 (Years 2-3)

TBN Grad RA (Microbial Engineering) 50% effort	40,000
TBN Grad RA (Microbial Engineering) 74.7% fringe ($0 retirement & $3368 health insurance)	29,890
Bioremediation experiments - Heavy metal quantitation, pure and mixed culture screening for bioreduction, characterization of strains, laboratory consumables. Bioenergy experiments - electrode maintence, new reactor design for Fe oxidizers, media preparation for MS student - Years 2-3	15,000

Result 3: Public Outreach and Education - $10,000 / 3 years

Outreach development (display, content development, education, implementation, content updates)	10,000

Shared Expenses / 3 years

Publication fees (~ 3 total, $500/publication - page charges required to make scientific discoveries available to other scientists and the public)	1,500
Travel: In-state travel to/from mine (+ lodging), in/out of mine - estimate 5-6 trips / year	12,000

CREDENTIALS (Please also see CVs Attached):

Program Manager / PI: Jeffrey A. Gralnick – Dr. Gralnick is an Assistant Professor of Microbiology at the University of Minnesota and a member of the BioTechnology Institute, located on the St. Paul Campus. Gralnick was trained in Bacteriology at the University of Wisconsin – Madison where he specialized in microbial physiology. He then spent three years at the California Institute of Technology (Caltech) in the Division of Geological and Planetary Sciences where he studied Geomicrobiology – specifically how bacteria directly influence the fate of minerals. His training in both microbiology and geomicrobiology will provide cohesiveness for our interdisciplinary team.

Co-PI: Brandy Toner – Dr. Toner is an Assistant Professor of Environmental Geochemistry in the Department of Soil, Water and Climate at the University of Minnesota and has extensive experience working with synchnotron-based analysis of biogenic and natural minerals and in characterizing microbial populations.

Co-PI: Christine Salomon – Dr. Salomon is an Assistant Professor and Assistant Director of the Center for Drug Design at the University of Minnesota and will isolate and screen microbes for production of medically relevant compounds. Her group will be involved in screening for antibacterial and anticancer activities and will purify and characterize novel compounds.

Co-PI: E. Calvin Alexander, Jr. – Dr. Alexander is a Morse Alumni Professor of Geology and Geophysics at the University of Minnesota and will be responsible for overseeing all geochemical and isotopic characterizations related to the waters on Levels 27 and 10.

James Essig – Minnesota Department of Natural Resources, Park Manager – Soudan Underground Mine State Park – will help coordinate research trips to the mine, outreach activities and consult on future technology commercialization possibilities directly relating to operations at the mine.

DISSEMINATION AND USE

- Publications to primary scientific journals will be submitted covering all aspects of this proposal. 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 / Patent Strategies will be coordinated by the University of Minnesota Office of Technology Commercialization, LCCMR and the DNR.

- Results will also be communicated to the general public through the interactive display to be developed as a part of Result 3 in this proposal, serving the public of the State of Minnesota and visitors to the Soudan Underground Mine State Park.

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