

1) PROPOSAL TITLE: Bioprospecting:

2) List of participants (partial):

Sookie Bang-SDSMT

Bruce Bleakley-SDSU

James Staley-University of Washington, Seattle

Fathi Halaweish-SDSU

Mike Lehman-USDA-ARS

Ben Sayler-BHSU (education & outreach)

3) Brief Description of proposed program:

Culturable bacteria, fungi, and actinomycetes will be screened for production of antibiotics and selected extracellular enzymes using standard methods.

NOTE: In a visit to the deepest level of the Homestake Mine the year before the pumps were turned off, a strong odor of geosmin was evident over a distance of several yards in the mine, but not throughout this depth of the mine. The geosmin odor was almost certainly due to actinomycetes growing in the subsurface soil at this depth. Many actinomycetes are antibiotic producers and enzyme producers. It would not be surprising if *Bacillus spp.*, another antibiotic and extracellular enzyme-producing bacterial genus, were also present at this and other depths of the mine.

The extreme environments found at different sites (having elevated temperature, elevated pressure, alkaline pH, or elevated metal concentrations) in the mine are hypothesized to have selected for novel microorganisms. In bioprospecting studies, selected culturable microorganisms from these mine environments (both mesophiles and extremophiles) would be screened for secondary metabolites such as antibiotics. Potentially valuable, novel antibiotics could be discovered. For example, many antibiotics are inactivated at elevated temperature. One or more antibiotics produced by Homestake microorganisms in the lower regions of the mine may be more heat-stable than similar antibiotics produced by surface soil microorganisms, and have commercial value. Novel antibiotics that are stable at elevated pressure and extremes of pH or osmolarity may also be produced by the mine microflora.

**Science goals:** Goals of the work would be:

1) Obtain samples from air, water, biofilms, and solid surfaces in the Homestake mine at the 4850 foot level. Use several plating media to isolate a variety of culturable microorganisms from the samples, especially microbial types that are known to include strains that are producers of potentially useful primary and secondary metabolites. Obtain pure cultures of these isolates, and screen them for antibiotics, enzymes, and other metabolites of interest. Although mesophilic

microbial species likely dominate at the 4850 foot level, attempts will be made to culture extremeophiles as well that may produce useful metabolites.

- 2) Screen selected microbial isolates for ability to precipitate metals from aqueous solution, for possible use in production of metallic nanoparticles for industrial use.
- 3) Using same samples as in (1), use molecular techniques to screen microbial community genomes for primary and secondary metabolites of interest. If genes of interest are identified, attempt to clone them and express the genes.

**Hypothesis: Numbers and types of microbes producing antibiotics and extracellular enzymes will vary at different locations at the same depth, with more antibiotic/enzyme producers near areas rich in organic carbon (such as lignocellulose/wood) than in areas lacking such abundant organic carbon.**

It is almost certain that members of the genus *Bacillus* as well as a variety of actinomycetes are found at various sites in the mine. Some have already been found in biofilms (Bleakley). These isolates would be isolated and screened as outlined below, as would isolates of the genus *Pseudomonas* along with other selected genera.

Examined/sampled areas of the mine would include the rock surfaces of the mine shafts; mine waters in the shafts; biofilms on the rock surfaces; the soil materials that have formed on the floor of the mine shafts at different depths; the wooden ties of the rail tracks in the mine; etc.

Selected isolated microbes would be screened for antibiotic activity using standard methods such as the disc plate method. Selected Gram-positive and Gram-negative bacteria would be used in bioassay as targets.

After detection of antibiotic producers, selected strains would be studied in different growth conditions (varying nutrients, etc.) until optimal media are found for antibiotic production. Media will be extracted with a series of organic solvents. Each solvent will be processed separately under optimum condition to preserve activity. Chemical profiles of active antibiotic extract(s) will be examined using High-Performance Liquid Chromatography (HPLC) to separate individual compounds. Bioassay-guided fractionation will be used to guide the separation process. After each separation step, antibiotic assay will be conducted. Only active compound(s) will be further processed for structure determination using Nuclear Magnetic resonance (NMR) and Mass Spectroscopy (MS) techniques. Antibiotic activity of isolated/characterized compound(s) will be confirmed using bioassay.

Extracellular enzyme producers would be identified using plate screening methods for cellulose, chitinase, lipase, etc. Selected strains would be grown in broth media and enzyme production checked in different broth medium formulations. Enzyme

purification and characterization would be by standard methods, and would make use of the facilities in the SDSU Chemistry Department such as the mass spec.

4) Contact one or industrial interests that have expertise or interest in screening Homestake for potentially useful producers of antibiotics, enzymes, or other metabolites. Partner with the company in obtaining funds via grants or other sources for bioprospecting work.

5) Find funding to allow collaborators to visit the mine and each other's laboratories to have students or other personnel trained as needed in new methods and techniques.

**4) Infrastructure requirements:**

Rough estimate of space requirements and specific or unusual technical issues involved in proposal:

Some laboratory facility at BL2 level would be useful in or near the mine to allow some processing of samples, to allow some of the microbial work to be done soon after sampling. Some sample processing would take place in laboratories at university and other research sites at a distance from the mine.

Lab facilities should include running water; electricity; ice machine; refrigerators and freezers; lab cabinets and lab benches; lab furniture; sinks; toploader and analytical balances; pH meter; stir plates and hot plates; computer with Internet access; a chemical fume hood vented to the outside; at least one autoclave; gas and vacuum lines; controlled pressurized air flow in lab areas; eye wash stations, fire extinguishers, and fire blankets; chemical spill containment kits; a floor model centrifuge and one or more tabletop centrifuges; and at least one laminar flow hood for microbial culture work.

This lab facility would be available to other workers/researchers in the mine for their needs.

**5) Readiness for deployment-technology:**

Estimate of when access to underground facility would be required:

Access sometime in 2006 would be desirable, so that more materials could be obtained from the mine to attempt to isolate pure cultures that might have commercial value.

**6) Readiness for deployment-effort and funding:**

Efforts would begin in 2006 to write and submit grants to fund the proposed work. Some preliminary sampling and characterization of microbial isolates and communities could be done without major additions of new funds and resources. But new personnel (graduate students, etc.) and O & M funds to help them do their work will be needed to move this work forward.

**7) Environment, safety, & health issues/hazards:** Microbiology facilities that are built as part of the mine infrastructure should be BL2 to safely contain and allow work with the microorganisms that are most likely to be found which might pose a public health threat.

Areas of the mine that may pose chemical hazards (having concentrated amounts of toxic metals or pollutants; and gaseous hazards such as radon, methane, etc.) should be identified so that researchers are aware of their location.

Outreach activities: Will be coordinated with the research, and developed with outreach personnel.