

Letter of Intent

Title: Investigation of Microbial Diversity in Subsurface Ecosystems

Investigator: Sookie S. Bang
South Dakota School of Mines and Technology
501 E. Saint Joseph Street
Rapid City SD 57701

Overview

Microorganisms are ubiquitous and abundant in ecosystems including extreme environments. In nature, they behave as catalysts during the course of biogeochemical cycling. Mounting evidence has been documented on how microorganisms interact with environment and what the consequences are. The characteristics of microorganisms depend on those of environment whose changes further affect the microbial behavior. The research objective of the subsurface microbial initiative at SDSM&T is to explore the phylogenetic diversity of microbial communities present at different depths and geologic formations in Homestake Mine, SD, which is currently proposed to be a deep underground science and engineering laboratory (DUSEL).

In collaboration with researchers at BHSU, the investigator currently conducts research on the microbial diversity of calcareous mats formed on rocks in Spearfish Creek and adjacent areas of the Black Hills, which is located downstream of Homestake Mine. DNA-based techniques are being employed to identify prokaryotic microflora, *Bacteria* and *Archaea*, from calcareous mats differentiating apparent phylogenetic distinction. Wide spectra of microorganisms are involved in the formation of calcareous matrices as well as the utilization or even dissolution of the mats for their own survival. Microbial metabolic activity in relation to the evolution of the surrounding environment will be further examined by analyzing organic and inorganic components of mats, soil, and water. In particular, the interactive role of microflora in the environment will be evaluated in terms of its biological complexity. Outcomes of this ongoing research will provide a database for phylogenetic diversities of rDNA sequence of microorganisms potentially present at DUSEL.

The proposed research is to identify microbial diversity in subsurface environments of Homestake Mine. As part of multidisciplinary and comprehensive scientific approaches that are planned for DUSEL, this initial attempt focuses on microbial diversity studies based on the following hypotheses:

- Microbial communities indigenous to Homestake Mine are diverse, mainly because microorganisms utilize the resources uniquely available at the site. Further, the uniqueness is more distinct as the site becomes deeper, where the rock formation is more primitive and temperature increases accordingly.
- Surface (exogenous) microorganisms may have been introduced in the areas where human activities were engaged. It is therefore possible that the presence of the surface microorganisms will diminish with depth. Adaptation and modification of exogenous microorganisms are inevitable in the extreme environments found in DUSEL.
- The interaction between the exogenous and indigenous microorganisms may increase the chance of microbial mutations and modifications.
- Microbial communities found in calcareous mats from Spearfish Creek are related to those at DUSEL due to the fact that DUSEL is not only in geographically close proximity but also an ancient geological environment for evolutionary conserved microbial populations.

Proposed Research Plan

1. Research Objectives – Identification of Microbial Diversity isolated from:
 - a. different geological formations in the presence and absence of oxygen
 - b. different depths and temperatures in the presence and absence of oxygen

2. Methodology:
 - a. Molecular techniques – DNA extraction, PCR, subcloning, sequencing
 - b. Microbiological techniques – Microbial cultures in selective media
 - c. Biochemical techniques – Enzyme assays, end-product identification

The proposed microbial investigation will help us understand the range of microbial communities and their interactions with the unique subsurface environment at Homestake Mine. Findings from this study are expected to provide fundamental information on subsurface microbial communities, which can be related to those found in other extreme environments and further dovetail ongoing studies on the Black Hills microbial ecology between BHSU and SDSMT.

Infrastructure Requirements:

Equipment: Real-Time PCR (\$28K) for molecular cloning and manipulation; Deep Freezer (\$5K) for storage of microbial and DNA samples

Personnel: One Post doc and one MS student (\$67K per year)