Bioenergy from DUSEL Extremophiles

Sookie S. Bang, South Dakota School of Mines and Technology, SD

Microbial communities in Homestake Mine have survived under nutrient-limited, extreme environmental conditions, leading to an evolutionary lineage uniquely different from surface–dwelling microorganisms. This is also contributed by the fact that subsurface life has been shielded from bombardment by cosmic rays, but exposed to various types of radioactive rocks and gases in underground environments. However, during active mining operations over the past 125 years, surface microorganisms and organic materials were introduced into the extreme environments of the subsurface. Subsequently, interactions between microorganisms were inevitable and horizontal genetic transfer might have resulted in alteration of the DNA sequences to generate novel genes.

The unique underground climate maintains constant temperature near the surface, but the temperature increases with increasing depth. Humidity also increases with depth due to the constant seepage of ground and surface waters. In more humid areas of the mine, different types of biofilms are apparent not only on the rocks but also on structural materials containing corroded metals and decaying wood. The deep subsurface environment that is typically extreme offers unique opportunities for supporting the growth of unusual microorganisms. Therefore, microorganisms in these biofilms, rocks, soil, and timbers may exhibit unique metabolic activities, producing novel biomolecules such as antibiotics, extracellular enzymes, or membrane lipids that allow them to survive in harsh environments.

It is entirely possible that genetically distinct microbes including thermophiles with diverse, novel metabolic activities might be present in the biofilms, decaying timbers, and soils of the Homestake Mine, especially at the deep levels (i.e., 8000 ft). Thus, the Homestake Deep Underground Science and Engineering Laboratory (DUSEL) may represent a potential source of high-value microbial enzymes (e.g. cellulases, xylanases, glucanases, peroxidases, amylases, proteases, and lipases) that exhibit optimal activity at high temperature (close to 65°C). Our earlier visit to the mine provided such clues and strengthened our belief that this proposed hypothesis is very likely and merits detailed research.

Currently, we are working on a research project entitled, "*Microbial Diversity in the Deep Subsurface Environment of Homestake Mine, Lead, South Dakota (SD),*" funded by the SD NASA-EPSCoR program. The main thrust of this study is to characterize the phylogenetic composition of microbial communities present in DUSEL, using culture-independent molecular (16S rDNA) analysis. Findings from our current efforts are expected to provide fundamental understanding on phylogenetic diversity of microbial communities including extremophiles in the DUSEL ecosystem.

Research Objectives:

The main objectives of the proposed research are to (1) identify thermophiles that produce high-quality thermostable lignocellulose-degrading enzymes, (2) evaluate their potential value and molecular basis of the lignocellulosic waste degradation in comparison to that of known thermostable enzymes, and (3) clone and express the thermostable cellulosic genes in thermotolerant yeast cells that can convert lignocellulose to bioethanol.

Experimental Approaches:

The proposed research will be carried out through close collaboration between SDSM&T and USDA. Following logically sequenced approaches will be employed to accomplish the proposed objectives:

- Isolation, enrichment, and characterization of thermophilic cellulose degrading bacteria from DUSEL locations. The enrichment conditions will include the variations of temperature (50 – 70°C), pH (3 – 10), and e^- acceptors (aerobic and anaerobic),

- Integrated automated high-throughput screening for expression of optimum cellulose-degrading enzymes from the DUSEL isolates, for selection of optimum cellulases from the isolates, and for identification of high-value metabolites and byproducts,
- Determination of cellulosic enzymes and comparison of their activities against known enzymes, and
- Construction of DNA expression libraries for thermotolerant cellulose-degrading enzyme(s) using novel vectors for the expression analysis in all functional open reading frames.

Expected Outcomes:

1. Isolation of lignocellulose-degrading DUSEL thermophiles,
2. Characterization of value-added chemicals from deep mine lignocellulose-degraders,
3. Construction of a novel industrial yeast strain that expresses thermostable lignocellulose-degrading enzymes obtained from deep subsurface extremophiles,
4. Production of bioethanol from biomass using the recombinant yeast, and
5. Building a strong partnership between SDSM&T and USDA to increase the efficiency of lignocellulose-based ethanol production.