DUSEL Extremophiles

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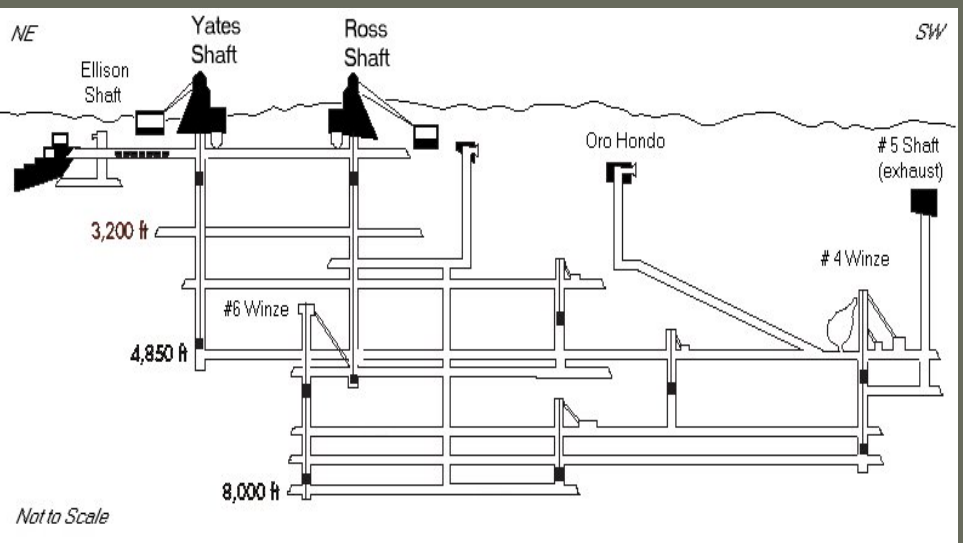
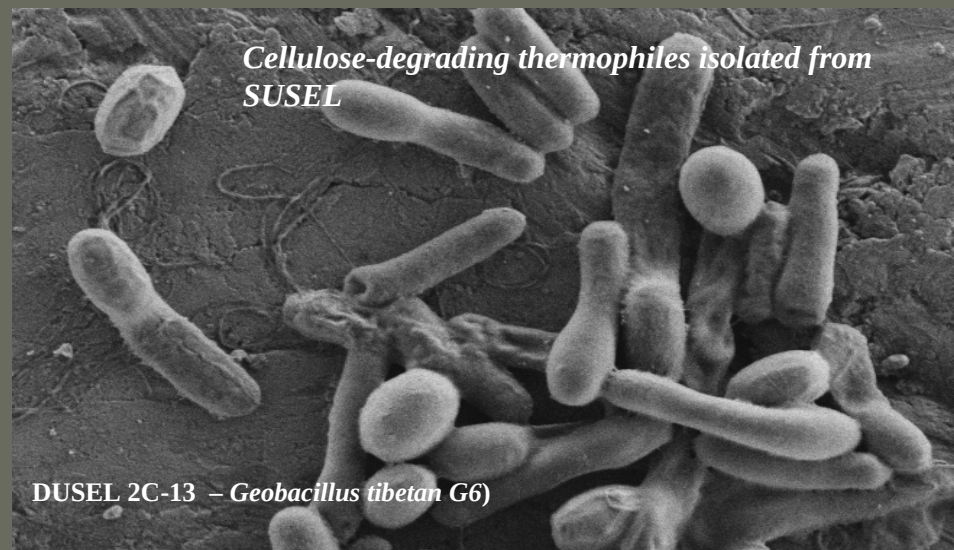




Research Thrusts

- Identification of diversity of DUSEL extremophiles
- Use of extremophiles in lignocellulose degradation
  - Enrichment and isolation of lignocellulose degrading extremophiles
  - Cloning and expression of thermostable (ligno)cellulases in yeast cells
  - Conversion of lignocellulose to bioethol by the recombinant yeast

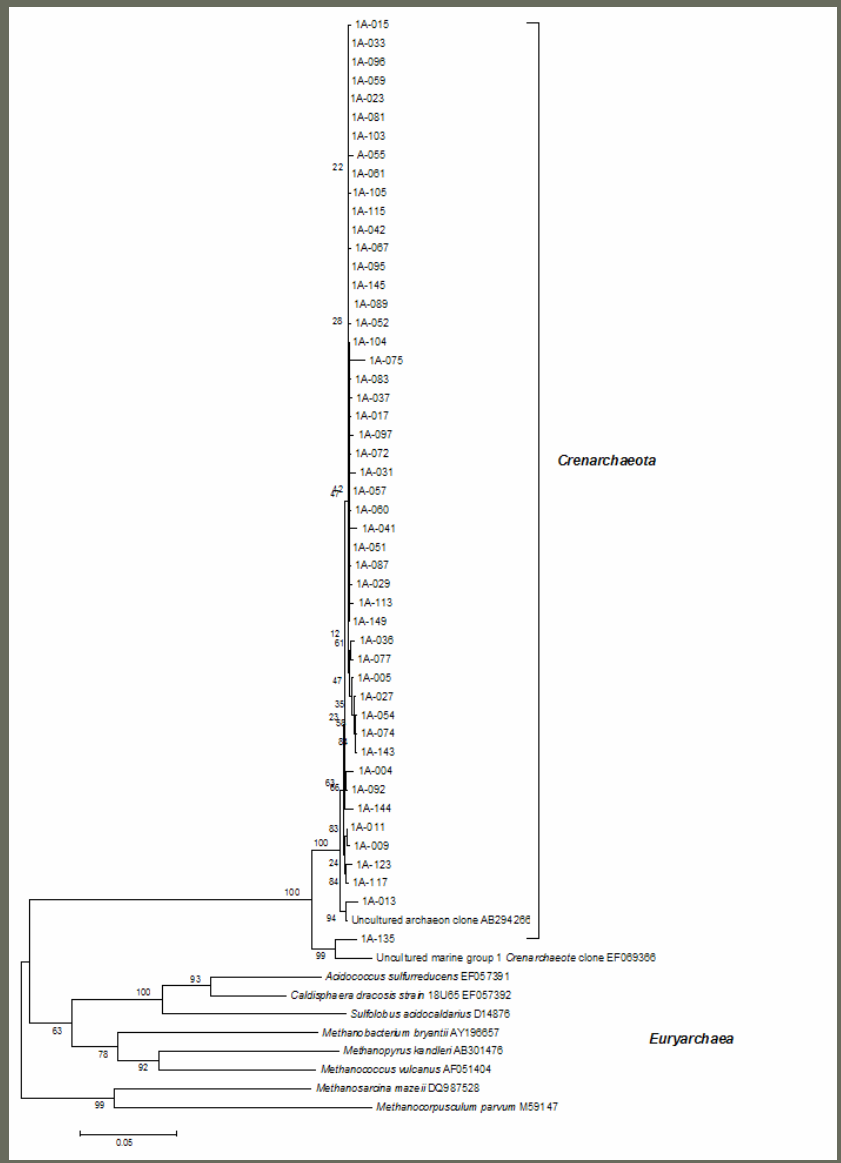
Extremophile Research

- Proposed Hypotheses
  - Extremophiles are present at the Deep subsurface at SUSEL Homestake
  - Interactions between the exogenous and indigenous microorganisms might have induced gene alteration and novel metabolic products
  - Adaptation and modification of surface-dwelling microorganisms/substrates introduced by mining activities are inevitable
  - DUSEL microorganisms may produce thermostable "Extremozymes" for lignocellulose degradation
  - It is doable to construct a recombinant yeast cell that can perform a seamless conversion of biomass to ethanol

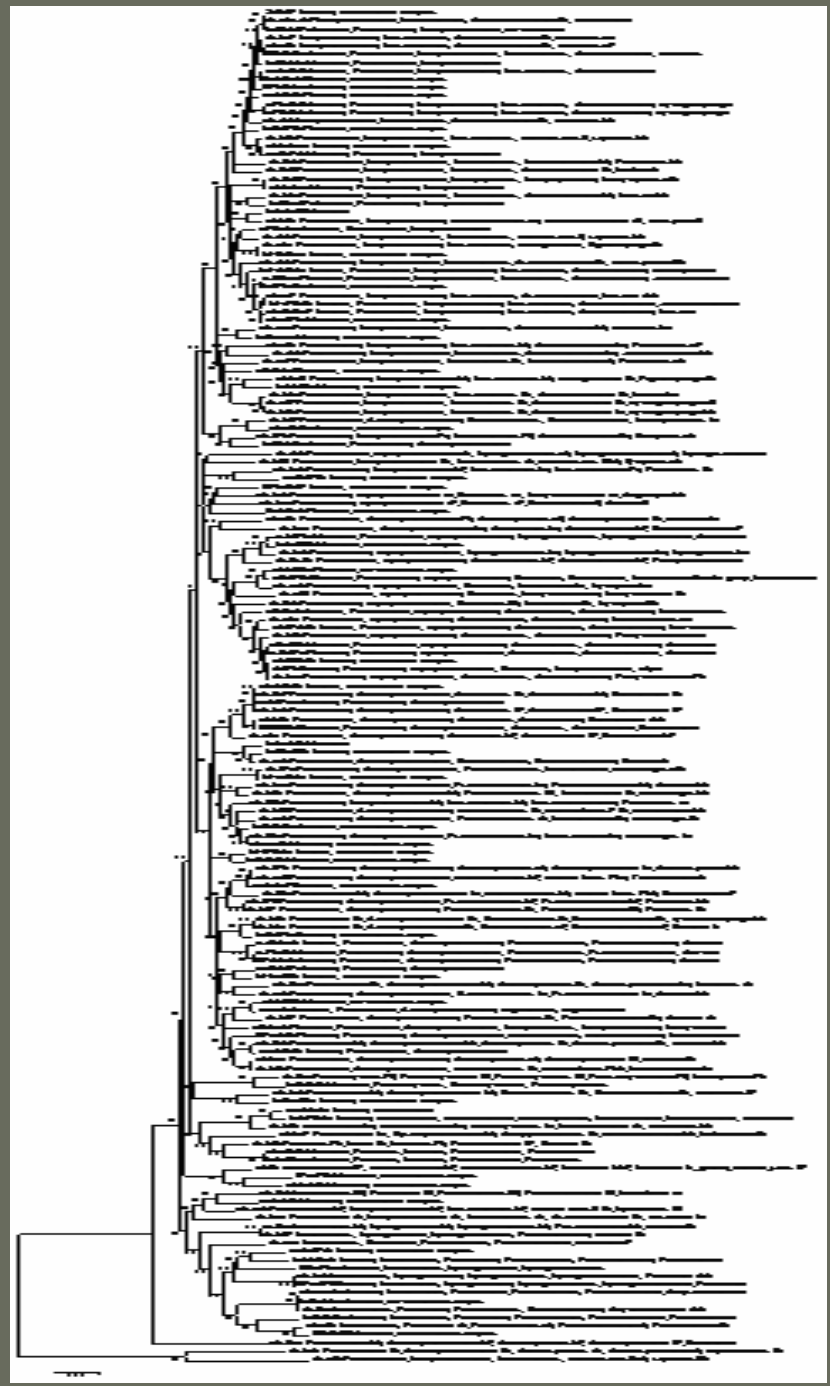
Preliminary Results

1. Phylogenetic analysis:
   - Microbial Diversity in DUSEL ecosystem with water sample (0 – 3,000 ft)
     - *Archaea* (39 unique sequences)
       - *Crenachaeota*
     - *Bacteria* (82 unique sequences)
       - *Proteobacteria* (majority)

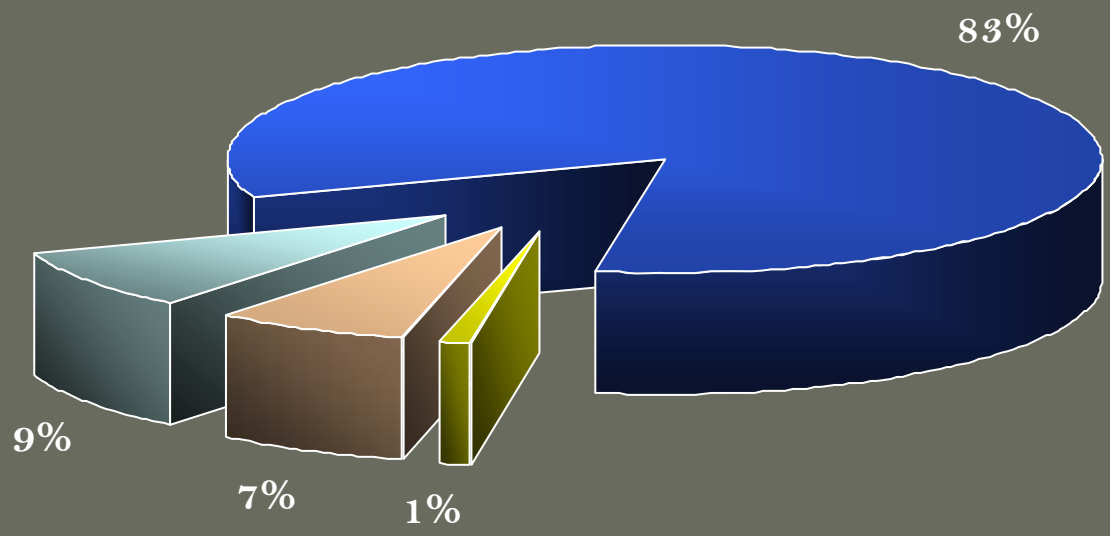
Archaea:


1A-015
1A-033
1A-096
1A-059
1A-023
1A-081
1A-103
A-055
1A-061
1A-105
1A-115
1A-042
1A-067
1A-095
1A-145
1A-089
1A-052
1A-104
1A-075
1A-083
1A-037
1A-017
1A-097
1A-072
1A-031
1A-057
1A-060
1A-041
1A-051
1A-087
1A-029
1A-113
1A-149
1A-036
1A-077
1A-005
1A-027
1A-054
1A-074
1A-143
1A-004
1A-092
1A-144
1A-011
1A-009
1A-123
1A-117
1A-013
Uncultured archaeon clone AB294266
1A-135
Uncultured marine group 1 Crenarchaeote clone EF069366
Acidococcus sulfurreducens EF057391
Caldisphaera draconis strain 18U65 EF057392
Sulfolobus acidocaldarius D14876
Methanobacterium bryantii AY196657
Methanopyrus kandleri AB301476
Methanococcus vulcanus AF051404
Methanosarcina mazeii DQ987528
Methanocorpusculum parvum M59147
Crenarchaeota
Euryarchaea
0.05

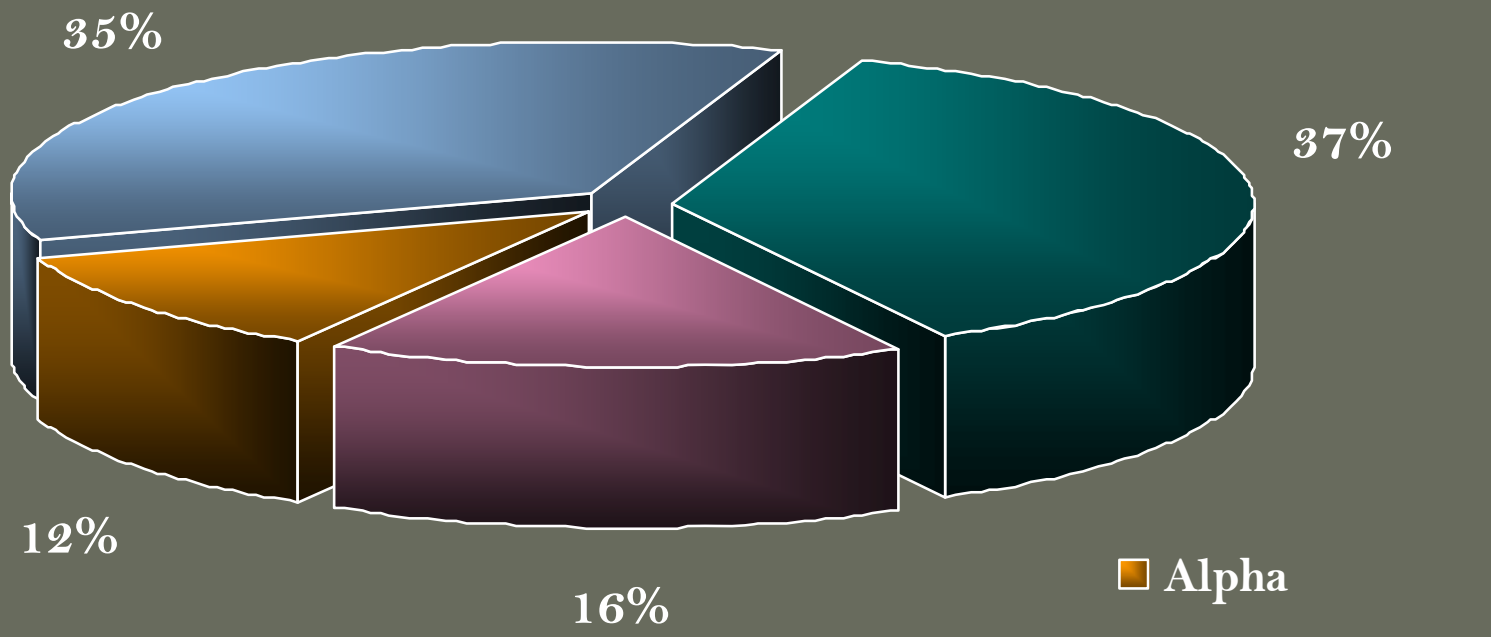
Bacteria:



Phylogenetic Composition of *Bacteria*



Proteobacteria

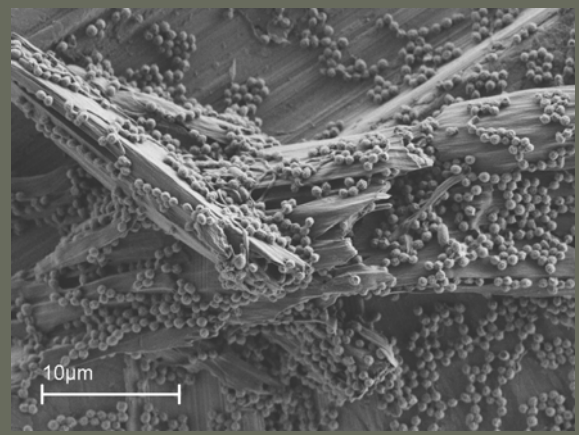


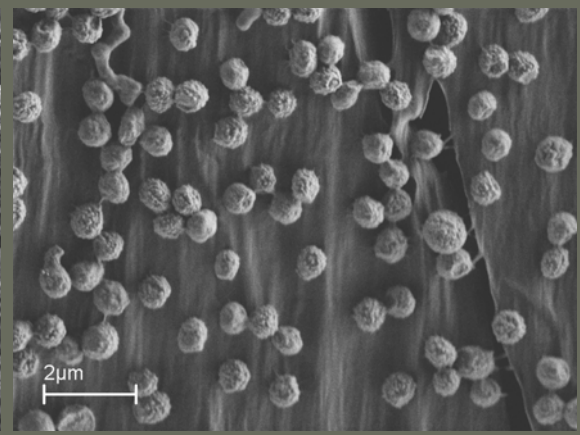
- Findings from Phylogenetic Analysis (from water sample; 0 - 3,000 ft)
  - ***Archaea***:
    - There is allelic diversity, although no significant phylogenetic diversity
    - More diversity is expected in deeper mine sites
  - ***Bacteria***:
    - Significant phylogenic diversity
    - More Gram positives including *Geobacillus* and *Clostridium* are expected in deeper mine sites

2. Cellulose degradation using DUSEL:

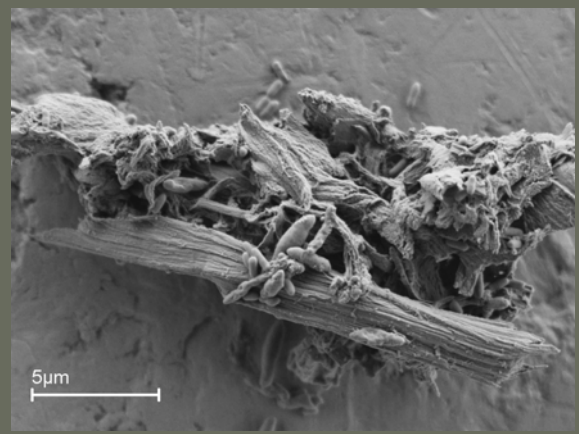
- Isolation of meosphiles (37°C) and thermophiles (60°C) using cellulose as a sole energy source
- SEM analysis

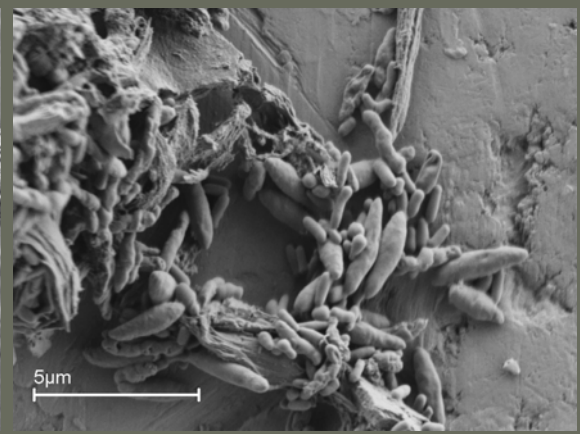
at 37°C





at 60°C





3. Construction of phylogenetic dendrograms

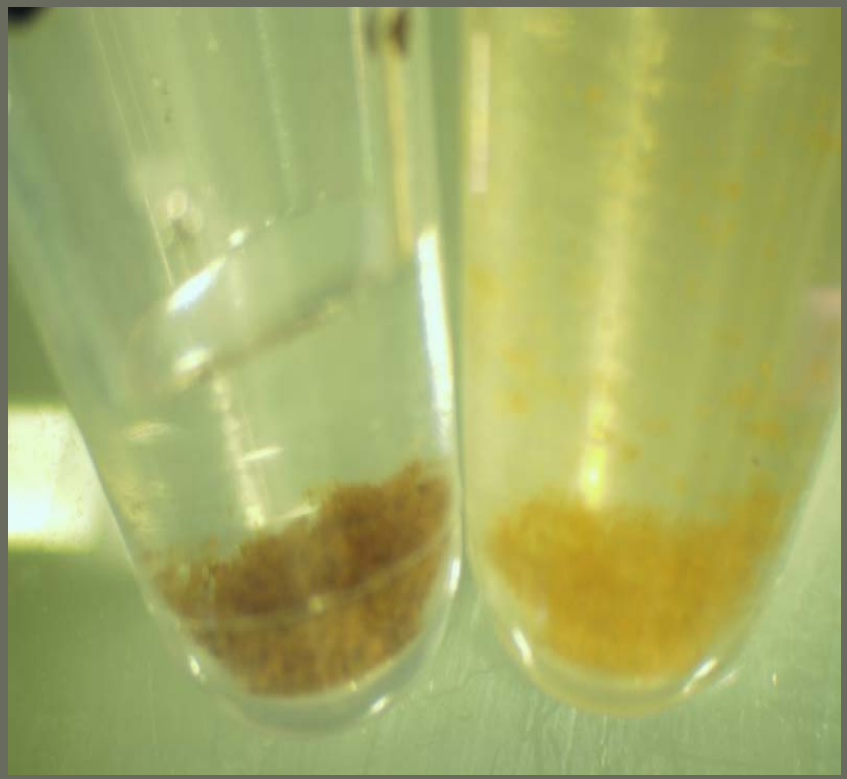
- Cellulose-degrading SUSEL mesophiles and thermophiles:
  - *Bacillales* and *Clostridiales* for mesophiles
  - *Bacillales* for thermophiles

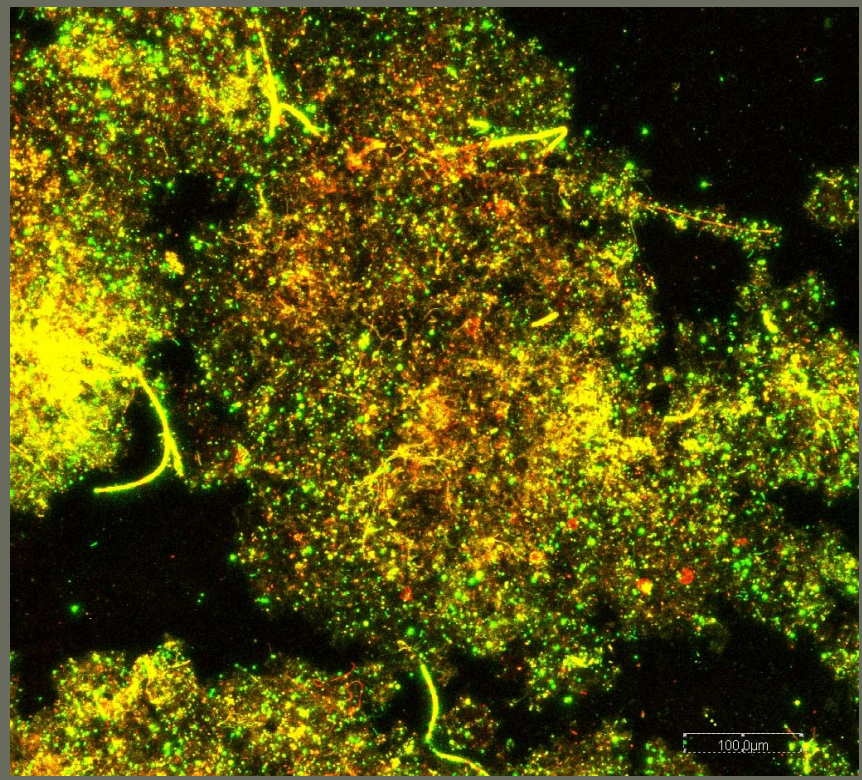
3. Biofilm material used as inoculum:

- Inoculum: Older stored biofilm material from 4850 foot depth; stored at 23° C for 5 years
- Used to inoculate stationary broth enrichments with particulate cellulose at 50° and 60° C
- Little macroscopic evidence of turbidity in broth column; but cellulosic solids at bottom sometimes showed macroscopic evidence of growth (change in color)

- Several pure cultures obtained that grow at 50-60° C, which digest carboxy-methylcellulose in agar plate assays
- Show tendency to swarm on agar surface; need elevated agar concentration to obtain isolated colonies
- Do not seem to tolerate elevated salt concentration
- Partial 16S rDNA analysis indicates isolates are *Bacillaceae*

Macroscopic view of biofilm from 4850 ft depth (left); and sample viewed using BacLight stain (right)





Sampling at SUSEL Homestake (*Dynatec*)

Research Plans

- Culture-dependent Studies:
  1. Isolation/enrichment of lignocellulose degrading thermophiles
  2. Automated screening for quality cellulosic enzymes
  3. Construction of genomic DNA and cDNA libraries using automated expression paradigms for novel thermotolerant cellulase and hemicellulase genes

Research Plans

- Culture-independent Studies:
  1. Genomics –
     - Phylogenetic diversity of microbial communities present in DUSEL, using molecular (16S rDNA) analysis
  2. Metagenomics of DUSEL microbes
     - Pyrosequencing/Construction of metagenomic library
     - Analysis of metabolic subsystems using the SEED database
  3. Functional (heterologous) gene expression
     - Novel cellulosic enzymes
     - Degradative enzymes (for value added chemicals)

- Long-Term Goal:

  Development of recombinant microbes expressing thermostable cellulosic enzymes

Expected Outcomes

1. Identification of *genomic/metagenomic diversity* of the deep mine microbes
2. Identification of *lignocellulose-degrading DUSEL extremophiles*
3. *Construction of the recombinant yeast expressing DUSEL extremozymes*
4. *Building strong partnerships between SDSM&T, SDSU, USDA, and DOE* to increase the efficiency of lignocellulose based ethanol production

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