Update of DOE funded work at the Bear Creek FRC

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The Field Research Center. We received six samples from contaminated areas and one sample from the background area. Our initial objectives were to cultivate microaerophiles from several of the sediment samples and perform a series of culture-independent analyses including T-RFLP and 16S rDNA clone libraries. The results are as follows.

Cultivation of microaerophiles. We have purified 50 isolates from the contaminated and uncontaminated zones using both oxic and microaerophilic isolation protocols for a total of 200 isolates. These isolates have been characterized using REP-PCR and phylogenetically identified based on 16S rRNA. In general, high diversity is detected based on REP-PCR profiles and the diversity in the contaminated sample is at least as high if not higher than that detected in the uncontaminated sample. Figure 1 presents representative REP-PCR profiles of strains isolated under oxic and microaerophilic conditions. Cluster analysis (Pearson correlation) of the isolates appears below in Figure 2. No difference in diversity was detected between the oxic and microaerophilic isolates (purple and green respectively). At least one coherent cluster was detected that appeared to be specific to the impacted sediment (note the red highlighted branch that is microaerophilic exclusively). This is the only deeply branching group that is entirely microaerophilic. All other branches contain isolates from both oxic and microarophilic isolation protocols. The phylogenetic placement of the isolates was determined with sequence alignment in ARB against the Ribosomal Database Project alignment using maximum likelihood and neighbor joining. All but one of the isolates placed within the Gram +. As can be seen, (Fig. 3), the

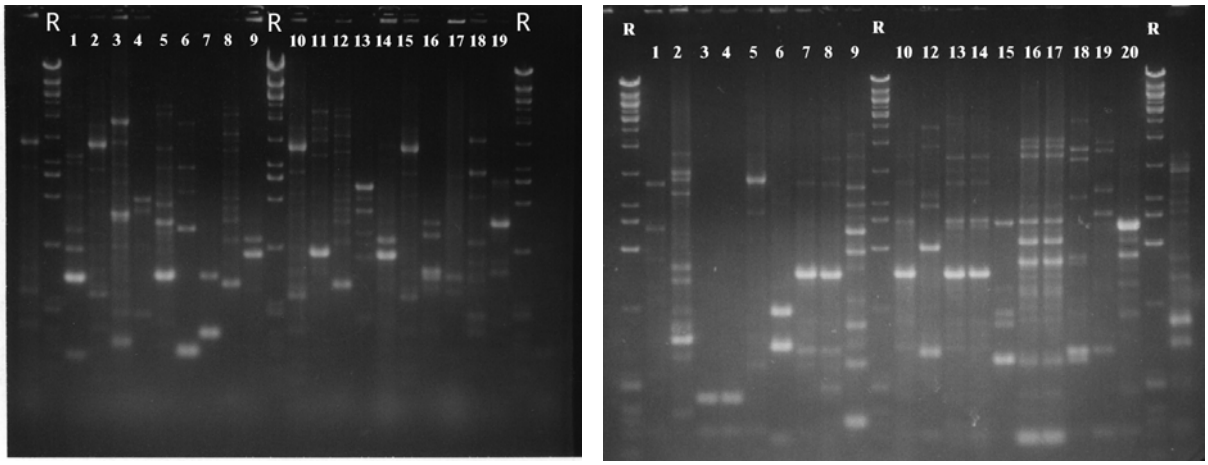

Figure 1. REP-PCR of representative isolates from FRC site. Reference lanes (R) contain size markers and numbered lanes isolates. Isolates in left panel were isolated under microaerophilic conditions and those in the right panel under oxic conditions.

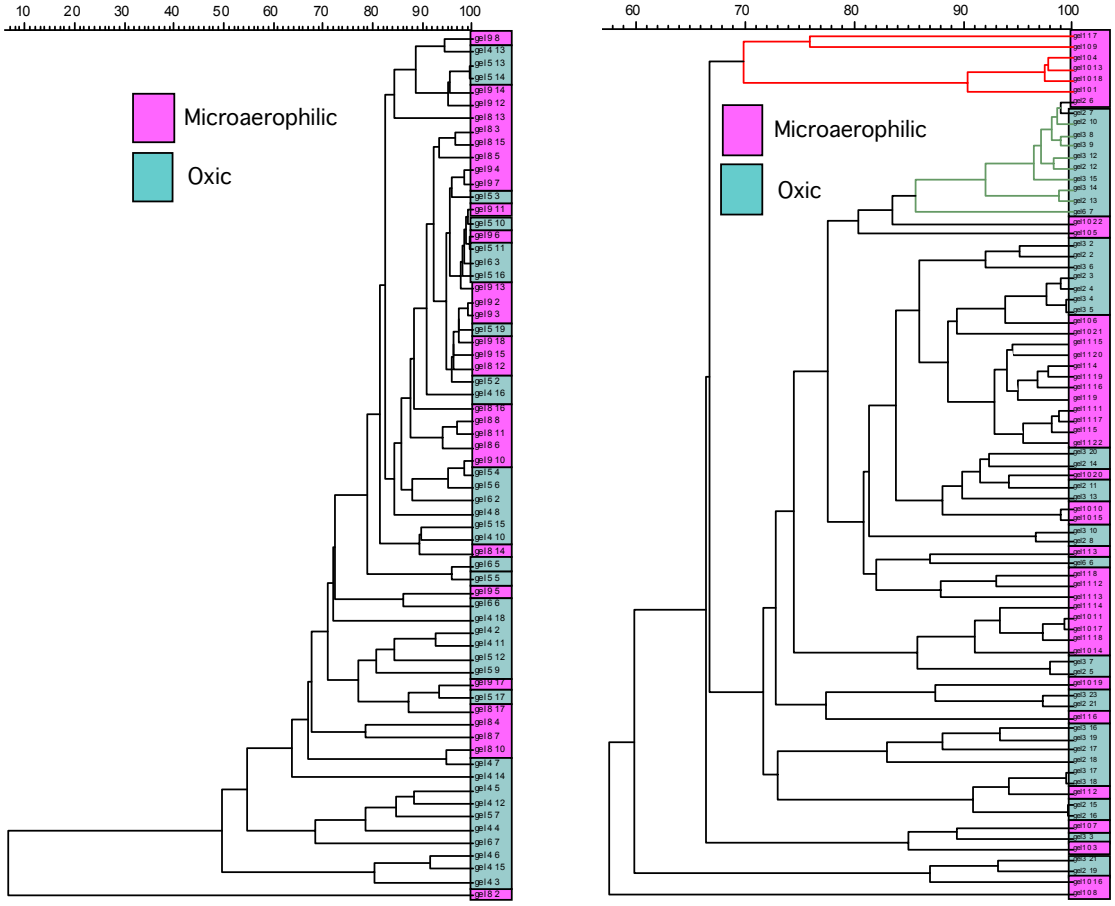

Figure 2. Pearson clustering of REP-PCR profiles from FRC isolates. Left panel, 66 isolates from FRC Background site. Right panel, 79 isolates from FRC contaminated Site 0401.Trees were cast with UPGMA using global optimization.

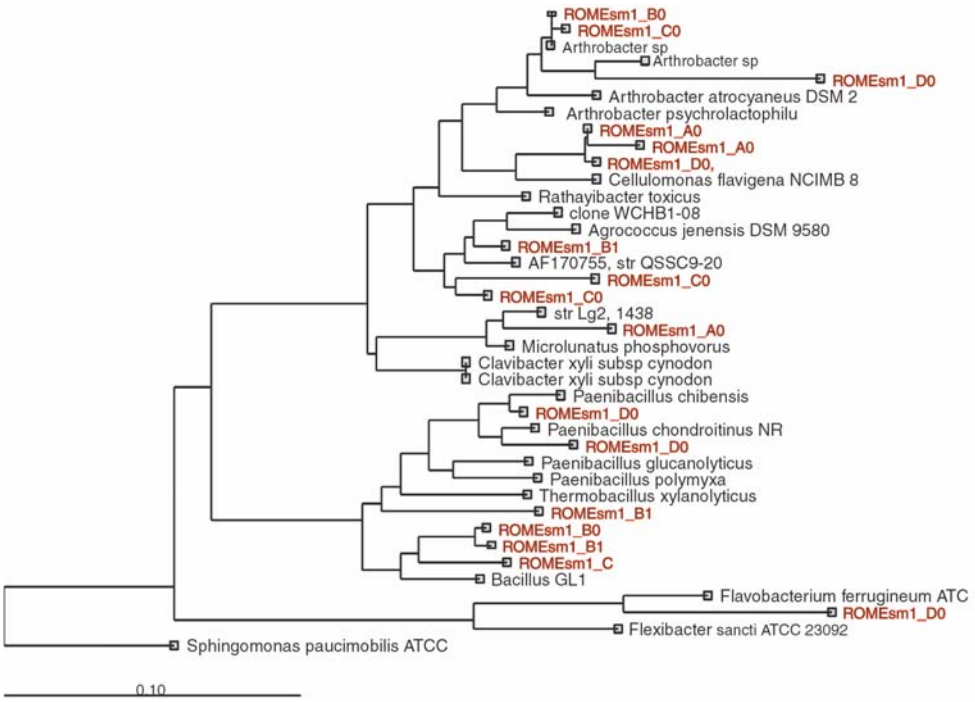

Figure 3. Phylogenetic tree of representative isolates from the Bear Crrek Valley FRC site. SSU rRNA sequences were aligned in ARB against the Ribosomal Database Project alignment. Phylogenetic inference was made with maximum likelihood.

Future work to be performed.

Culture Independent studies:

- Phase 1. We have successfully isolated DNA from all samples. Three out of the six contaminated samples as well as the background sample yielded DNA that was PCR amplifiable. However, several difficulties were encountered during the DNA isolation step. In the case of the sediment at pH 3.0-3.5, no detectable DNA was isolated. In addition, a frequently repeated observation was that the FRC DNA isolated and stored at -20°C, loses its ability to serve as a template in PCR. We have seen this symptom periodically in sediments with low density communities where the resulting community DNA was at low concentration. This DNA storage problem has pushed back the timing for the culture-independent analyses. The remaining three contaminated samples with PCR-recalcitrant DNA are being further purified in the hopes of obtaining DNA of sufficient quality for molecular analyses. These community DNAs will be prepared for analysis with T-RFLP using both general and phylogenetically focused primers as well as for the construction of rDNA libraries. These studies are currently in progress.
- Phase 2. Additional community studies will be performed on samples provided by Craig Criddle. These will include clone libraries and T-RFLP with both general and phylogenetically directed primers.