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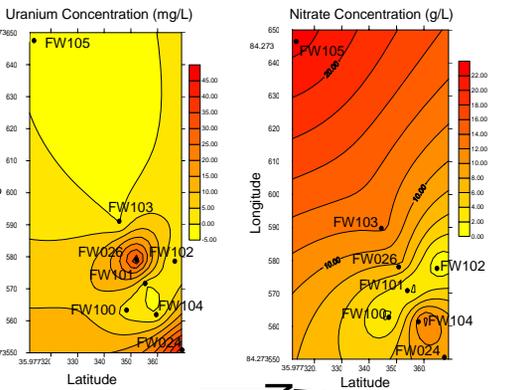
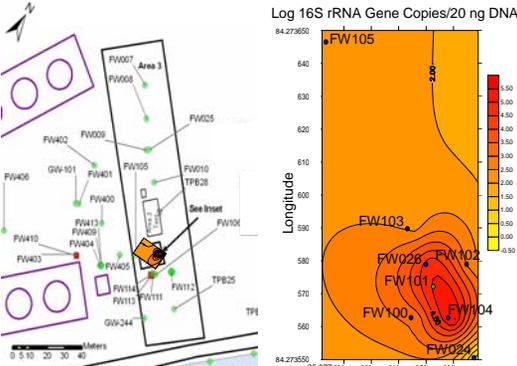
## Background

- *Anaeromyxobacter* spp. are metabolically versatile, facultative anaerobic myxobacteria.
- *Anaeromyxobacter* spp. are major contributors to the biogeochemical cycling of nitrogen and metals.
- *Anaeromyxobacter* spp. reduce soluble U(VI) to sparingly soluble and immobile U(IV).
- Multiple *Anaeromyxobacter* strains are present at the nitrate and uranium contaminated Field Research Center (FRC) in Oak Ridge, Tennessee.
- Multiple *Anaeromyxobacter* isolates were obtained from the FRC.

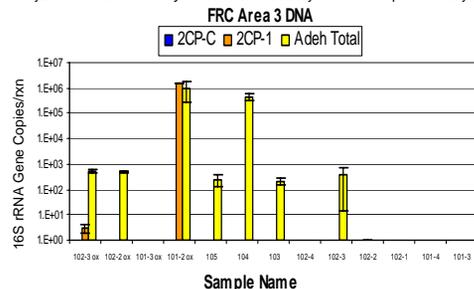
## Goals

- Characterize U(VI) reduction by *Anaeromyxobacter dehalogenans* strain 2CP-C
- Design tools to detect, distinguish, quantify and visualize *Anaeromyxobacter* strains in environmental samples (e.g., from the FRC)

## FRC Area 3: Geochemical & Microbial Characterization

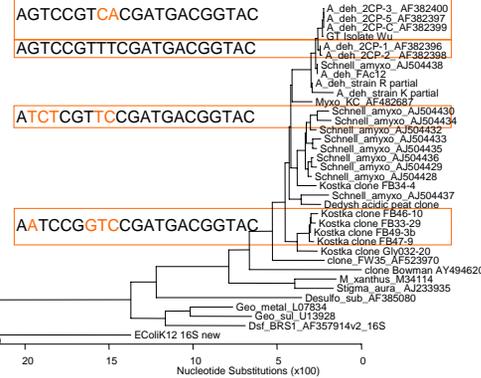


Using multiplex qPCR, community DNA samples extracted from FRC materials were analyzed for total *Anaeromyxobacter* concurrently with strain specific analyses.



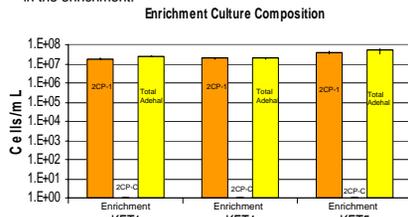
• *Anaeromyxobacter* strains in FRC Area 3 include, but are not limited to, 2CP-1-like strains

## Design of Strain-Specific Probes and Primers for Quantitative PCR



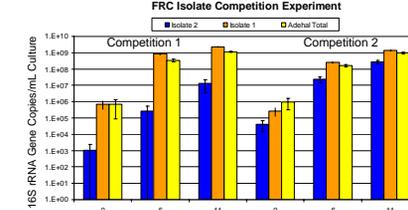
## Applications of Strain-Specific Multiplex PCR

A. Enrichment cultures using materials from the FRC. Total *Anaeromyxobacter* cell titers were compared to strain titers using multiplex TaqMan qPCR in order to estimate the *Anaeromyxobacter* strain evenness in the enrichment.



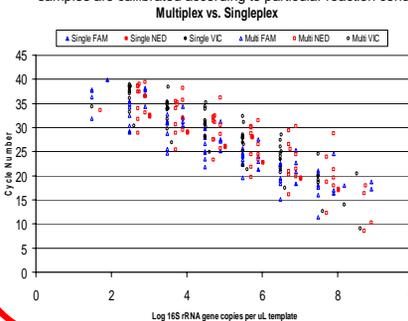
• FRC enrichment cultures contain primarily 2CP-1-like *Anaeromyxobacter* strains

B. Competition experiments using two different FRC isolates in minimal media with acetate and fumarate. Each strain was then monitored over time using multiplex qPCR.

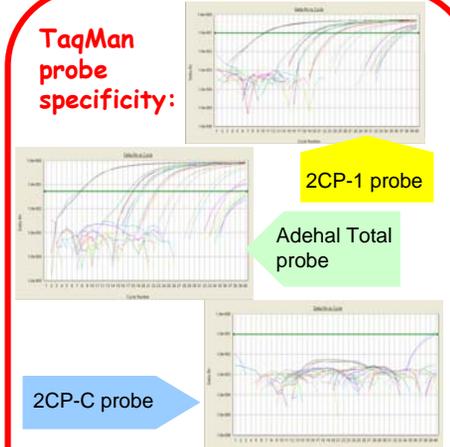


• Two individual *Anaeromyxobacter* strains were tracked concurrently using multiplex qPCR

C. While maximum fluorescence changes from singleplex to multiplex PCR occur, standard curves included with each run ensure that samples are calibrated according to particular reaction conditions.



## TaqMan probe specificity:



• TaqMan probes are able to distinguish genes with only a two-base-pair-difference in sequence

## Developing a FISH Protocol

Further elucidation of the role and distribution of *Anaeromyxobacter* in the environment can be obtained by combining qPCR techniques with fluorescent *in situ* hybridization.

### Synthetic mixture consisting of:

*Anaeromyxobacter dehalogenans* 2CP-C, *Desulfobacter postgatei*, *Mycococcus xanthus* (GJV1), filament affiliated with  $\beta$ -proteobacteria

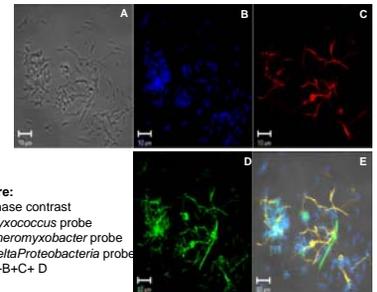


Figure:  
A: Phase contrast  
B: *Myxococcus* probe  
C: *Anaeromyxobacter* probe  
D: *DeltaProteobacteria* probe  
E: A+B+C+D

• FISH is a promising tool for *Anaeromyxobacter* monitoring

## Conclusions

- The FRC harbors a diversity of *Anaeromyxobacter* organisms.
- Field studies are warranted to establish cause-and-effect relationships between treatment (e.g., biostimulation) and the U(VI)-reducing *Anaeromyxobacter* community.

## Future Efforts

- Continue the design of qPCR tools for quantitative analysis of the *Anaeromyxobacter* community
- Apply strain 2CP-C whole genome microarrays to identify genes involved in metal and radionuclide reduction
- Expand the qPCR approach to the quantitative assessment of biomarker gene transcripts (e.g., c-type cytochrome genes)
- Link the approach with FISH and high-throughput proteomics (MALDI-TOF-MS)

JGI has completed the genome sequence for *A. dehalogenans* strain 2CP-C. The genomes of three additional isolates are currently being sequenced.

