Theme 3: Metabolism, Survival & Adaptation

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Theme 3: Objectives

**Theme 3: Metabolism, Survival, and Adaptation**—concentrating on the actions and traits of individual microbial species.

3.1 Isolate and characterize novel bacteria and archaea from diverse subseafloor habitats.

3.2 Examine fundamental physiology of subseafloor microbes under conditions of low growth rates and low energy flux.

3.3 Perform adaptive evolution and long-term survival experiments with subseafloor microbes to characterize molecular genetic signatures associated with particular phenotypes.
Outline

• Introduction to the “problem.” Cell density as a function of depth & time in model sediments.

• Models of survival and selection.

• Current experiments.
• Determine cells/cm³
• Measure metabolic activities
• Identify species/OTUs
Concept sketch of Long-Term Survival
“Adaptive Evolution” vs. “Ecological Selection”

After Lever et al. *FEMS Microbiol Rev* 2015
Concept sketch of Long-Term Survival
“Adaptive Evolution” vs. “Ecological Selection”

After Lever et al. FEMS Microbiol Rev 2015
• At any given depth, what are the microbes “doing”?

• What are the physiological states of the microbes?
Concept sketch of microbial populations in different physiological states

Growing

Maintenance

Survival

(A)

(B)  

(C)

1 μm

Mark A. Lever et al. *FEMS Microbiol Rev* 2015
Concept sketch of microbial populations in different physiological states

Growing **VS.** Maintenance **VS.** Survival

Mark A. Lever et al. *FEMS Microbiol Rev* 2015
Concept sketch of microbial populations in different physiological states

Growing          Maintenance          VS.          Survival

(A)          (B)          (C)

Mark A. Lever et al. *FEMS Microbiol Rev* 2015
Concept sketch of microbial populations in different physiological states

Growing

Maintenance

Survival

Subpopulations Growing

Mark A. Lever et al. *FEMS Microbiol Rev* 2015
• Do microbes change as a function of depth/time?
Different depths within a model sediment
Different depths within a model sediment
Different depths within a model sediment
Mechanisms of Survival

- Do surviving microbes grow slowly (or not at all) because (a) that is all they can ever do or (b) that is what they have been selected to do, perhaps modifying existing functions?

- What are the metabolisms and selective pressures that maintain slow growth phenotypes?

- Are there “cryptic” activities that can be “activated” by one or very few mutations (or environmental signals)?
One Mutation – Significant Change in Phenotype
Ongoing Experiments

• Slow growth & starvation of *Dehalococcoides*

• Pore Water Nutrient Bioassay with *Pseudomonas*

• *Halomonas* adaptive evolution
Studying the metabolism of slow-growing *Dehalococcidiodes mccartyi*

- 6 reactors in parallel
- doubling time: 1 week - 6 month
- 2 different media

- Currently growing enriched *Dehalococcoides mccartyi* consortia on formate and 1,2-dichloroethane

Fincker & Spormann
Proteome dynamics during slow and no-growth

- *D. mccartyi* cells are still metabolically active after 3 months of starvation
- BONCAT signal is clearer with extracted protein PAGE gels than with microscopy
- After 1 week of starvation and upon substrate reintroduction, protein biosynthesis resumes within 1-4 h

PAGE gel of BONCAT tagged proteins extracted from the *D. mccartyi* consortium
1 week starvation - 1 day incubation with methionine homolog

Fincker & Spormann
Pore Water Nutrient Bioassay with *Pseudomonas*
Pore Water Nutrient Bioassay

Ray, D'Hondt & Finkel
Halomonas adaptive evolution
Halomonas strains isolated from North Pond

Huber Lab
16S rRNA Tree of *Halomonas* Strains

Huber Lab
Long-Term Growth & Survival of *Halomonas* Strains

Ray, Huber & Finkel