

DOE-ARRA Geologic Sequestration Training and
Research

2011 Yearly Review Meeting

Project DE-FE0002128

***Analysis of microbial activity under a
supercritical CO₂ atmosphere***

Massachusetts Institute of Technology

Prof. Janelle Thompson,

Department of Civil and Environmental Engineering

February 24-26, 2011

Project Participants

- Dr. Janelle Thompson, PhD, Assistant Professor, Civil and Environmental Engineering (MIT)
- Dr. Hector Hernandez, PhD, Martin Luther King Postdoctoral Fellow (MIT)
- Mr. Kyle Peet, doctoral student, Civil and Environmental Engineering, MIT
- Ms. Noor Al Sharif – undergraduate student (Summer 2010)
- Mr. Mathew Archer – undergraduate student (Winter 2010/2011 to present)



Janelle



Hector



Kyle

Why do microbes matter?

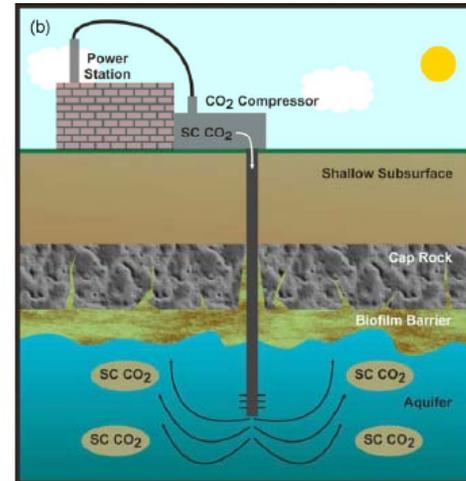
CO₂ “trapping” mechanisms

- biofilm barriers
- mineralization

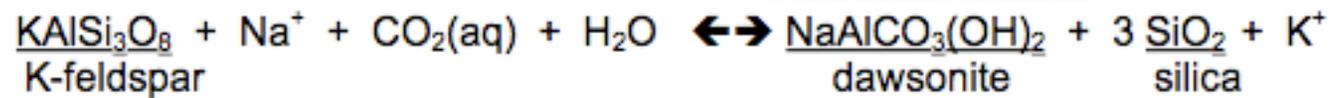
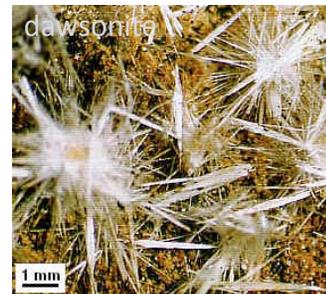
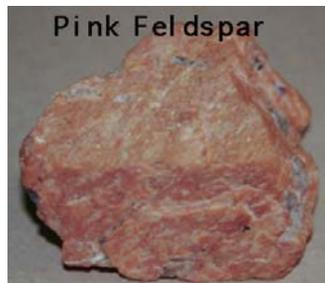
Natural and Engineered Systems

Biological catalysis
of mineral trapping

Biofilm Barriers

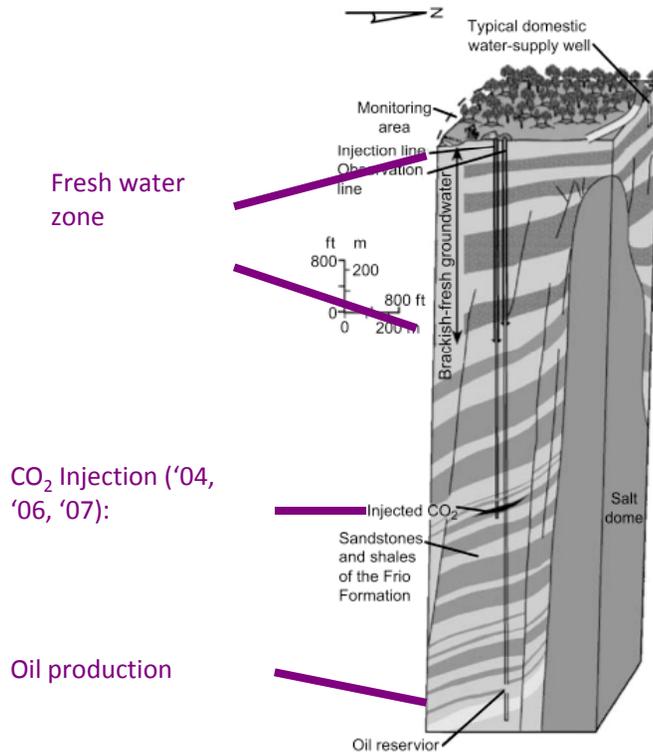


Mitchell et al, 2009



Can we recover life forms that grow in supercritical CO₂?

Frio Ridge: Pilot Geologic Sequestration of CO₂

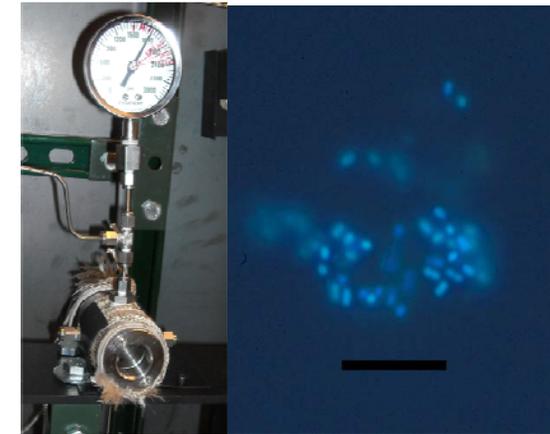


Gulf Coast Carbon Center, TX

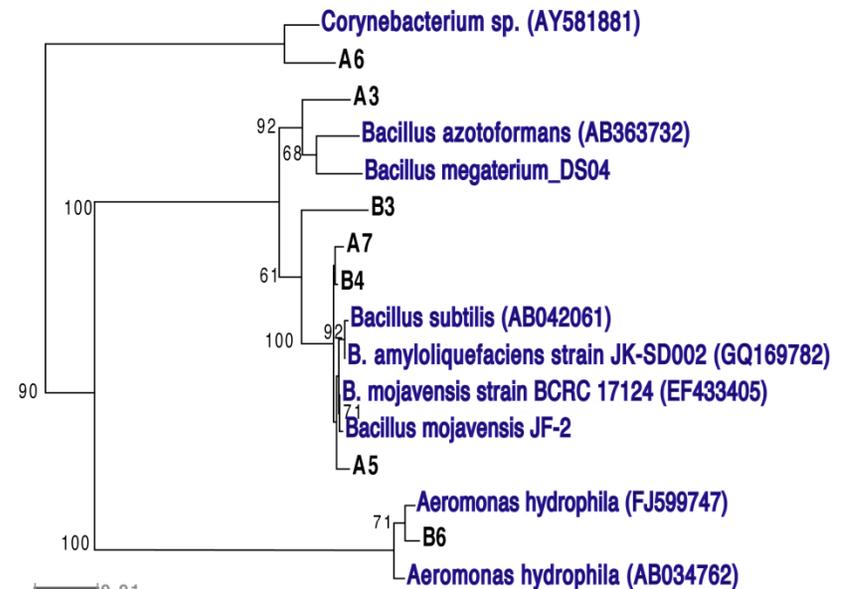
Injection well
 Observation well 30 m downfield
 Depth 1,500 to 1,657m
 Porosity 24%
 High permeability
 1,600 tons CO₂
 Pressure 150 to 165 bar
 Temperature 53-60°C

CO₂ Injection ('04, '06, '07):

Oil production



Objective: culture scCO₂ tolerant microbes, investigate use as biofilm barriers and genomic mechanisms of CO₂ tolerance.



Tree rooted with outgroup *Thermoplasma acidophilum* (NR_028235) not shown

Project Funding

- Total Project Cost: \$299,984
- DOE Share: \$299,984
- Non-DOE Cost Share: \$0
- Cost Share Provider: No required cost share
- Previous funding for enrichment of the scCO₂ tolerant consortium MIT0212 provided by the MIT Energy Initiative

Highlights of Project to Date

- Characterized the genetic diversity of scCO₂-tolerant consortium MIT0212 through 16S rRNA gene sequencing.
- Isolated scCO₂ tolerant bacterial strain MIT0214.
 - Physiological characterization of strain MIT0214.
 - Ultra-structural analysis of MIT0214 by transmission electron microscopy.
 - Genome sequencing of MIT0214 using the Illumina sequencing platform.

Tasks – Overview

Task No.	Task Description	Task Duration	Task Funding
1	Project Management and Planning	12/01/2009 – 11/30/2012	\$55,117
2	Characterization of microbial diversity in consortium MIT0212	12/01/2009-11/20/2010	\$42,300
3	Characterization of growth requirements and optima	12/01/2009 – 5/30/2011	\$60,793
4	Evaluate ability of strain to grow and reduce permeability in sandstone cores	12/01/2009 – 11/30/2011	\$56,769
5	Investigate mechanisms of supercritical-CO ₂ tolerance through genomics and genome-enabled studies	12/01/2009 – 11/30/2012	\$84,983

Scheduling: Gantt Chart from Project Management Plan

Scheduling	Year 1 (2010)				Year 2 (2011)				Year 3 (2012)				MIT Team Member(s)
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	
PHASE I:													
<u>Task 2</u>													
Task 2.1	x	A											HH, U1
Task 2.2	x	A											HH, U1
Task 2.3	x	x	x	C									HH, U1
<u>Task 3</u>													
Task 3.1	x	x	x	x	x	E							KP, HH, U1
Task 3.2		x	x	x	x	E							KP, HH, U1
<u>Task 4</u>													
Task 4.1			x	x	x	x	x	F					KP, U2
<u>Task 5</u>													
Task 5.1	x	x	B	x	x	x	x	x	x	H			KP, HH
Task 5.2				x	x	x	x	x	x	H			KP, U2
Task 5.3					D	x	x	x	G	x	x	I	KP, U2, U3
Yearly Budget	\$108,086				\$105,726				\$86,172				

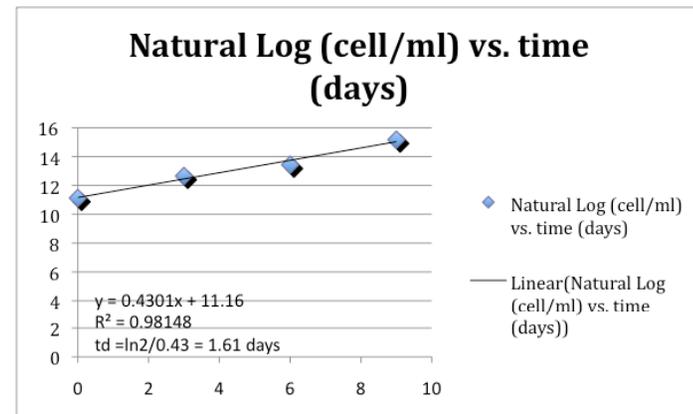
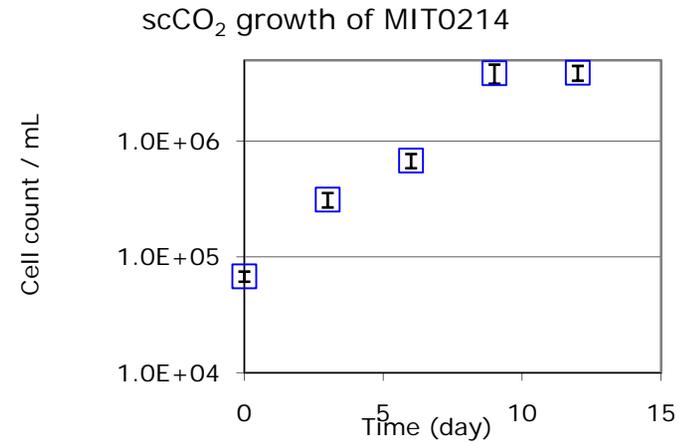
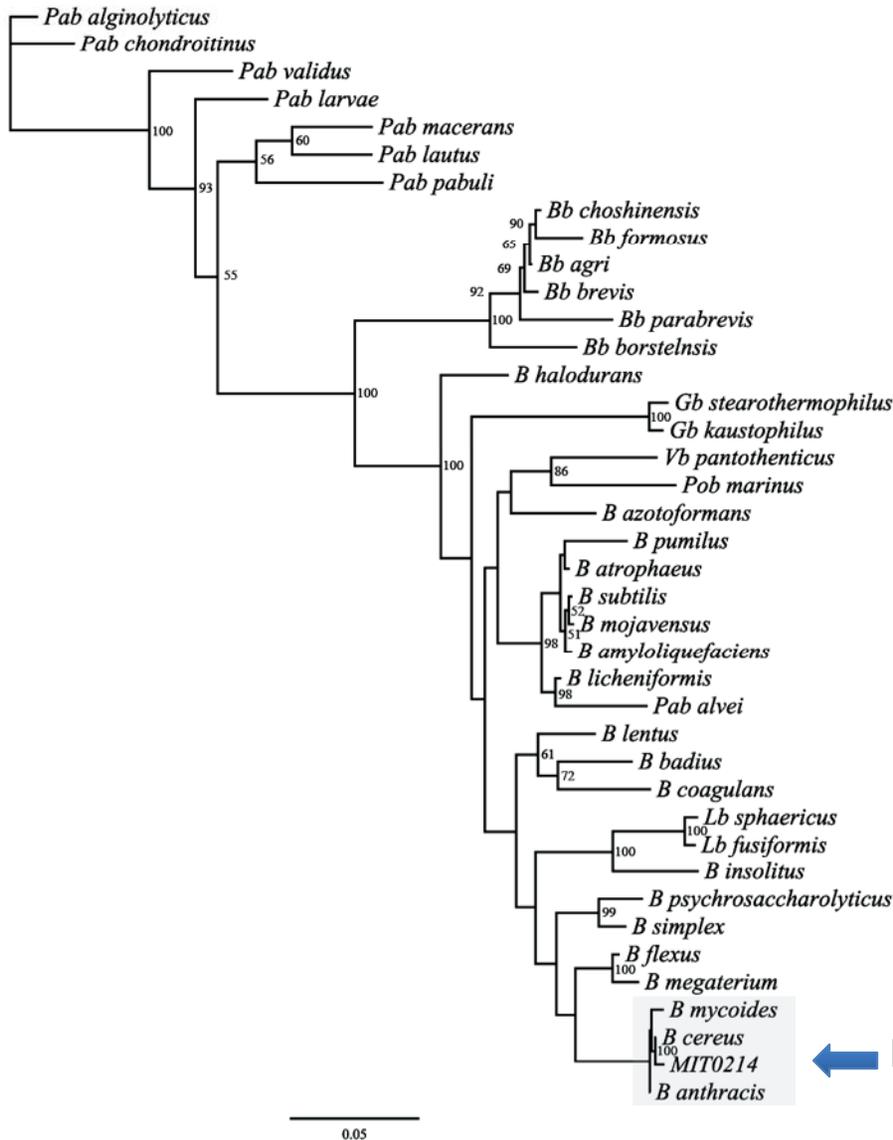
Letters in timeline refer to milestones identified in milestone log (Section D)

HH: Hector Hernandez; KP: Kyle Peet; U: Undergraduate Students

Discussion – Task 2

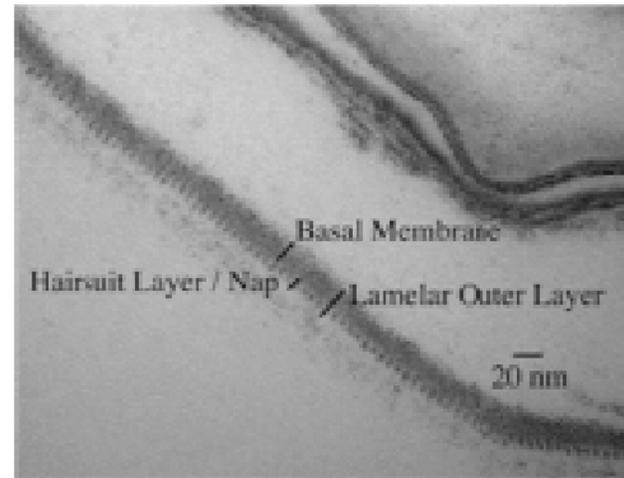
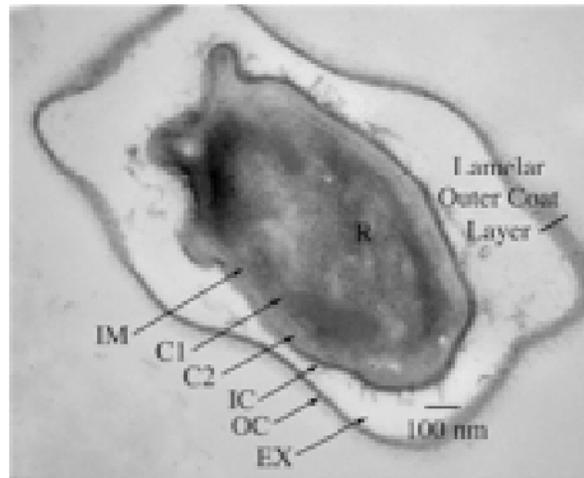
- Characterization of microbial diversity in consortium MIT0212
 - 2.1: Characterize 16S ribosomal RNA gene diversity and consortium stability under routine subculture under a scCO₂ atmosphere
 - 2.2: Visualization and localization of specific microbial populations in scCO₂-bioreactor grown biofilms using fluorescence *in situ* hybridization
 - 2.3: Isolation and identification of pure cultures from scCO₂-tolerant consortium using dilution subculture and selection for spores
- Dr. Hector Hernandez and Graduate Student Kyle Peet
- Task Status (80-90% complete – FISH optimization for *B. cereus* probe is still in progress however imaging with EUB338 is complete)
- Major accomplishment:
 - Demonstrated growth under scCO₂ (doubling time of 1.4 to 1.6 days)
 - Observed single cells and spores by FISH, Invitrogen Live/Dead staining, and TEM
 - Isolation of scCO₂ tolerant strain and genetic identification as *Bacillus cereus*
- Major issues/problems: None. Manuscript in progress.

Task 2.3: Supercritical CO₂ tolerant Isolate *B. cereus* MIT0214



Phylogenetic relationship of strain MIT0214 to 39 *Bacillus* (*B*), *Brevibacillus* (*Bb*), *Paenibacillus* (*Pab*), *Virgibacillus* (*Vb*), *Geobacillus* (*Gb*), *Pontibacillus* (*Pob*), and *Lysinibacillus* (*Lb*) species inferred from the alignment of the 1465 bp 5' 27-1492 rRNA coding region.

Transmission Electron Microscopy: MIT0214 spores



Spore ultrastructure matches *B. cereus* type strains.

Discussion – Task 3

- Characterize the growth requirements and optima of the supercritical CO₂-tolerant consortia or isolated strains
 - 3.1: Quantify growth as a function of physiochemical variables
 - 3.2: Characterize extracellular polymer production
- Dr. Hector Hernandez and Graduate Student Kyle Peet
- Task Status (50% complete)
- Major accomplishment(s)
 - Physiological characterization reveals *B. cereus* strain MIT0214 is a thermophile, acidophile and a barophile
- Some issues
 - Physiological experiments had lag due to backordered bioreactor parts.
 - Several methods have been evaluated to quantify extracellular polymer (EPS) production in high pressure bioreactors
 - Crystal violet based staining of adherent cells on removable bead media (ruled out – low sensitivity)
 - conA fluorescent based tagging of cells (semi-quantitative)
 - Chemical extraction of EPS from cultures using phenol/sulfuric acid... (quantitative, optimization and validation in progress)

Task 3.1: Physiological Characterization

		<i>B. cereus</i> MIT0214	<i>B. cereus</i> ATCC 14570
Temperature (°C) ^b			
	37	+	+
	45	+	+
	50	+	-
	60	+/-	-
	80	-	-
Atmosphere and Pressure			
	1 atm - Ambient conditions	-	+
	1 atm - 95%CO ₂ /5%H ₂	-	-
	1 atm - N ₂ / pH 7.0	-	+
	1 atm - N ₂ / pH 3.7	-	- ^c
	120 atm - CO ₂ / pH ~3.9 ^d	+	-
	120 atm - N ₂ / pH 7.0	+	-
	120 atm - N ₂ / pH 3.7	+	-
NaCl concentration (ppt) ^b			
	0.1	+	+
	1	+	+
	5	+	+
	10	+	+
	35	+	+
	50	+	-
	75	-	-
	100	-	-

Hernandez, et al, in preparation

MIT0214 -pressure tolerant
 -moderately halophilic, thermophilic
 -acidophilic

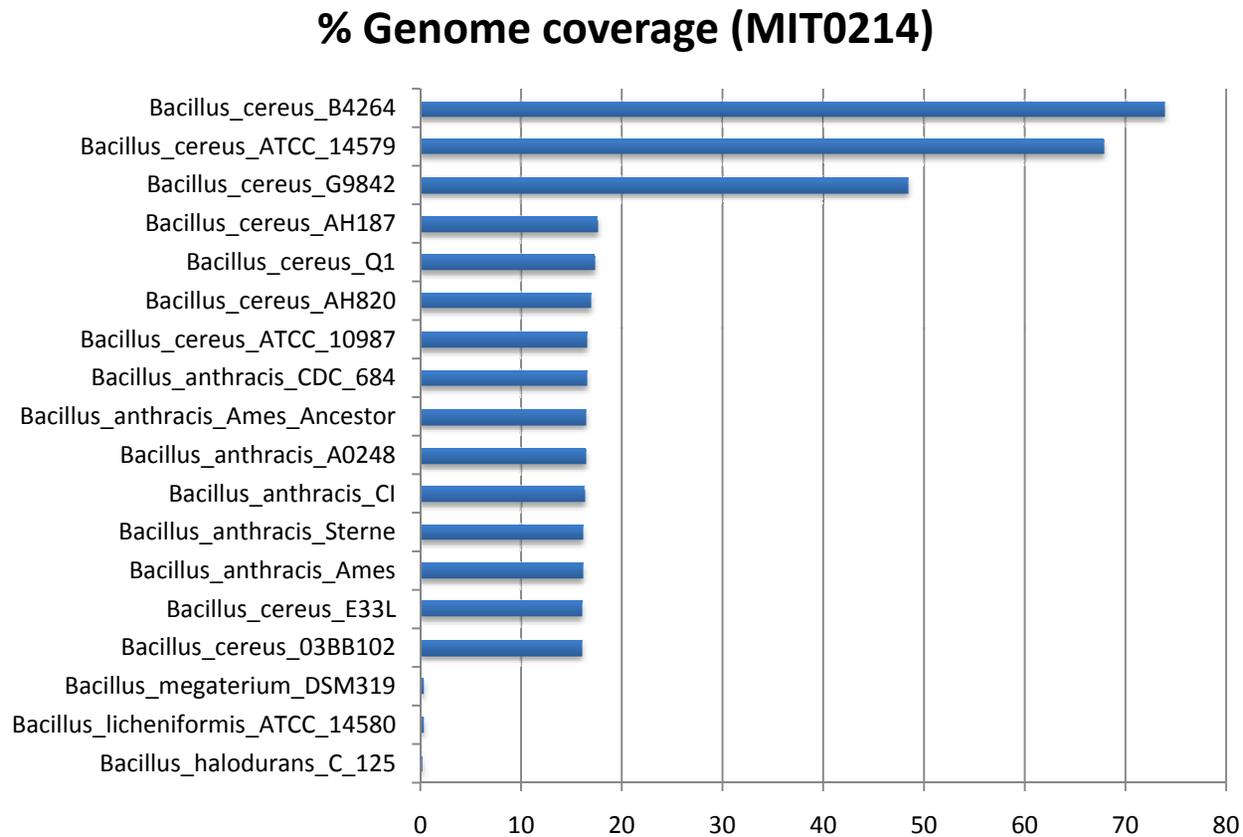
Discussion – Task 4

- Evaluate the ability of the MIT0212 consortium (or isolated strains) to form biofilm barriers in sandstone cores under supercritical CO₂ conditions.
- Task Status (0% complete)
- Work with MIT0214 is planned for summer 2011 to be on target for completion by December 2011.

Discussion – Task 5

- Investigate the mechanisms of supercritical CO₂ tolerance through genome-enabled studies
 - 5.1: Sequence genome/metagenome of supercritical CO₂ tolerant consortium or strain
 - 5.2: Comparative genomic analysis of scCO₂-grown bacteria to identify genetic differences that may correspond to molecular adaptations enabling persistence under high pCO₂.
 - 5.3: Transcriptomic profiling of scCO₂-grown bacteria to identify differentially expressed genes under scCO₂.
- Dr. Hector Hernandez and Graduate Student Kyle Peet
- Task Status (33% complete)
- Major accomplishment:
 - Work is now focused on strain *B. cereus* MIT0214 isolated from the MIT0212 consortium.
 - Sequenced the genome of *B. cereus* MIT0214 to >50X coverage using Illumina sequencing technology.
 - Preliminary comparative genomics using Bowtie software

Task 5.2: Preliminary comparative genomic characterization



- Ongoing: what genomic changes underpin scCO₂ tolerance?

Project Milestones

Milestone	Planned Completion Date	Actual Completion Date
Analysis of 16S ribosomal RNA gene diversity in scCO ₂ bioreactors	July 2010	July 2010
Isolation of a supercritical CO ₂ tolerant microbial strain	December 2010	December 2010
Genome sequencing of a supercritical CO ₂ tolerant microbial strain or metagenome	December 2010	December 2010
Identify environmental gradient for differential gene expression study – scCO ₂ vs. N ₂	March 2011	Feb. 2011
Complete analysis of optimal conditions for growth and extracellular polysaccharide production for consortium and/or isolated strains.	July 2011	In progress for MIT0214

Project Milestones, continued...

Milestone	Planned Completion Date	Actual Completion Date
Analysis of ability of MIT0214 to form a biofilm barrier in sandstone cores	Dec. 2011	-
Transcriptome sequencing of differential gene expression study	Jan. 2012	-
Complete bioinformatics analysis of genome sequence	Sept. 2012	In progress
Complete bioinformatic analysis of differential gene expression	Nov. 2012	-

(-) Indicates work has not yet been initiated towards specific milestone goal

Anticipated Efforts for the Coming Year

- Task 2.2: Optimization and visualization of cells in reactor grown biomass using *B. cereus*-specific FISH probe. What proportion of initial MIT0212 consortium corresponded to *B. cereus* MIT0214?
- Task 3.2: Characterization of extracellular polymer production by MIT0214 - does this vary with pCO₂?
- Task 4.1: Test potential for using MIT0214 to form biofilm barriers in sandstone cores under scCO₂ conditions.
- Task 5.2: Comparative genomic analysis of MIT0214
- Task 5.3: Isolation of mRNA from MIT0214 under high pressure N₂ and CO₂ for study of differential gene expression.

PI Contact Information

- If you have any questions or would be interested in collaboration please contact
- jthompson@mit.edu