Analysis of microbial activity under a supercritical CO_2 atmosphere

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U.S. Department of Energy

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Developing the Technologies and Building the Infrastructure for CO_2 Storage

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Hector Hernandez (postdoc)
Kyle Peet (PhD student)
Adam Freedman (PhD student)



http://thompsonlab.mit.edu

Collaborators:
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Prof. Roger Summons, MIT

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MITei

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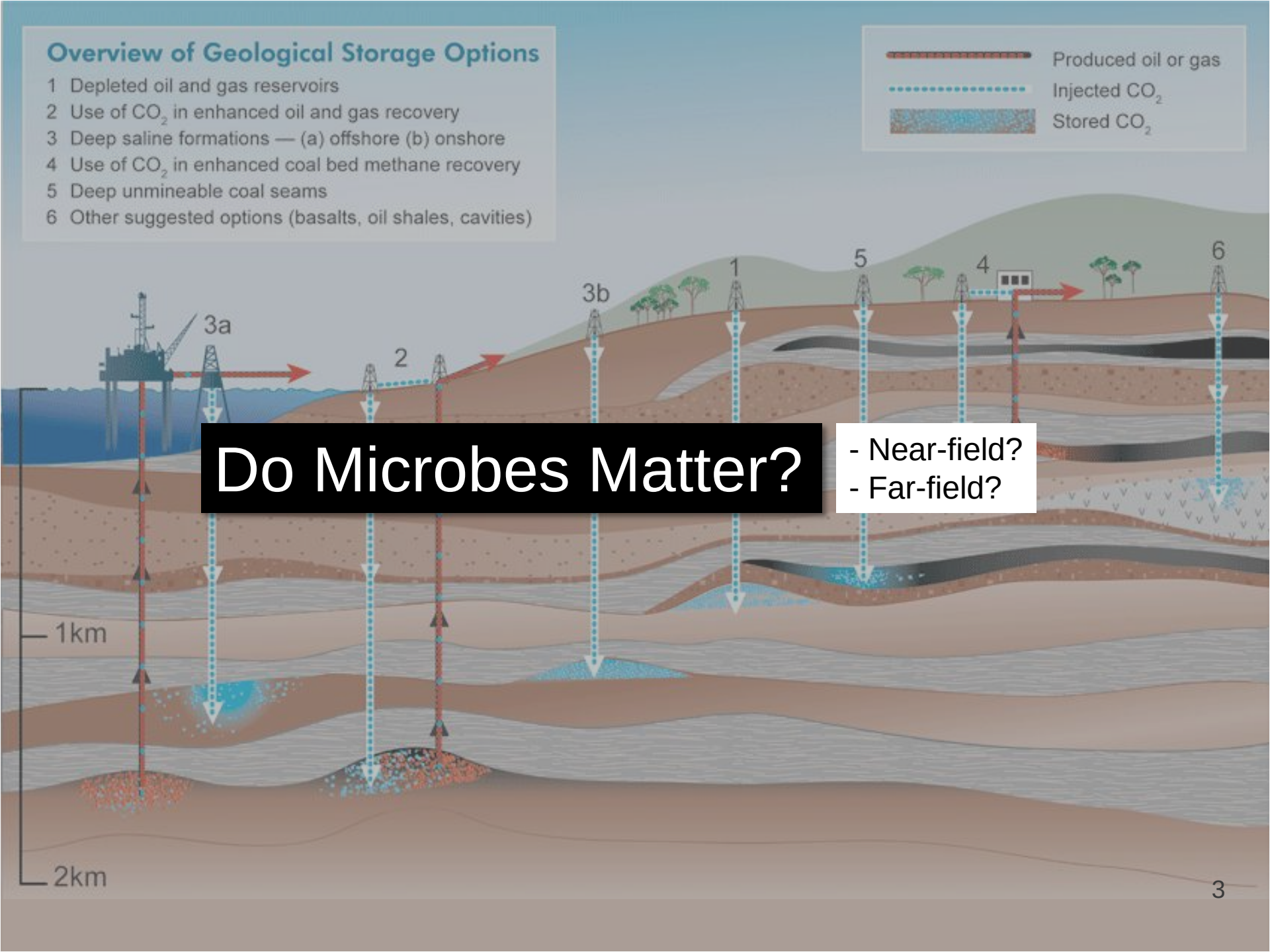
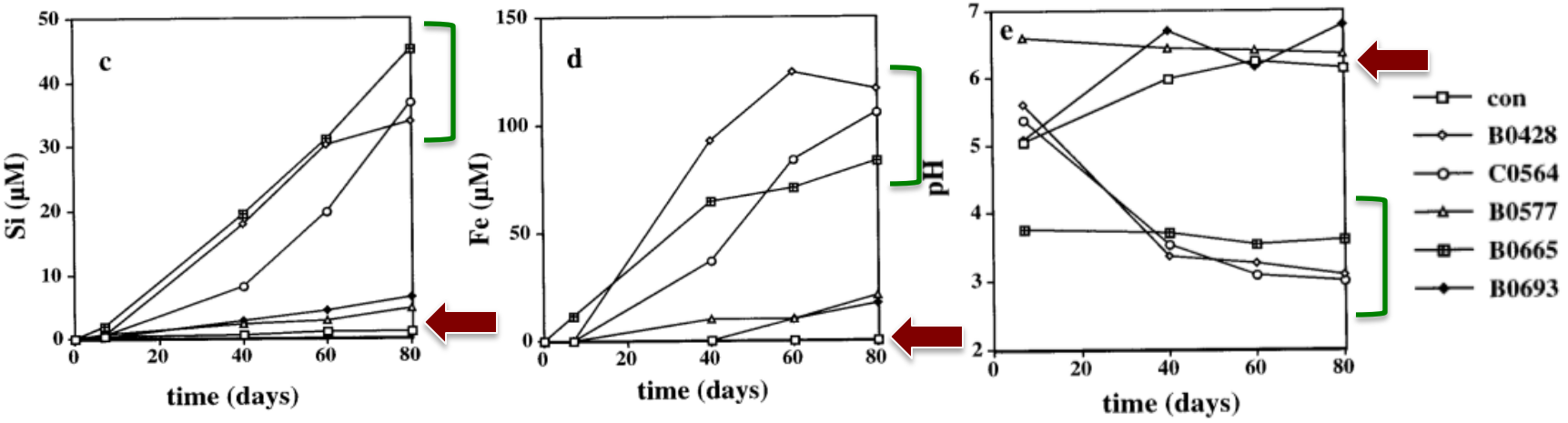

Overview of Geological Storage Options
1 Depleted oil and gas reservoirs
2 Use of CO_2 in enhanced oil and gas recovery
3 Deep saline formations — (a) offshore (b) onshore
4 Use of CO_2 in enhanced coal bed methane recovery
5 Deep unmineable coal seams
6 Other suggested options (basalts, oil shales, cavities)
Produced oil or gas
Injected CO_2
Stored CO_2
3a
2
3b
1
5
4
6
1km
2km
Do Microbes Matter?
- Near-field?
- Far-field?

1. Microbes are potentially important catalysts of geochemical reactions during GCS.



Abiotic control

Results demonstrating microbially-enhanced dissolution of Si, Fe from Biotite with concomitant acidification

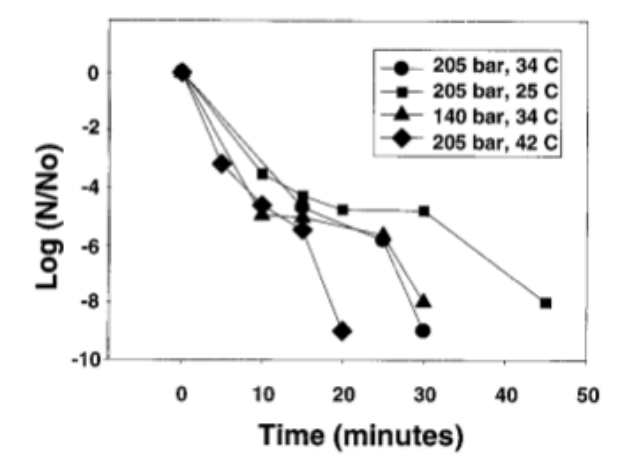
Barker, W.W. et al. (1998) Experimental observations of the effects of bacteria on aluminosilicate weathering *American Mineralogist, Volume 83, pages 1551 – 1563.*

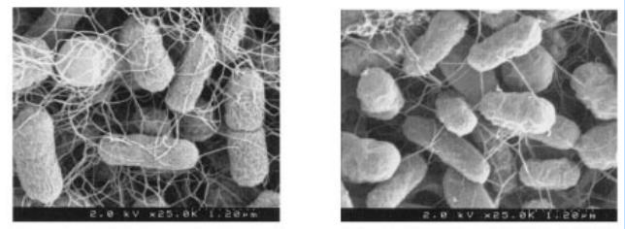
2. Supercritical CO_2 is a powerful sterilizing agent

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 10344–10348, August 1999
Medical Sciences

Bacterial inactivation by using near- and supercritical carbon dioxide

ANGELA K. DILLOW*, FARIBA DEHGHANI†, JEFFREY S. HRKACH‡§, NEIL R. FOSTER†, AND ROBERT LANGER‡¶‖

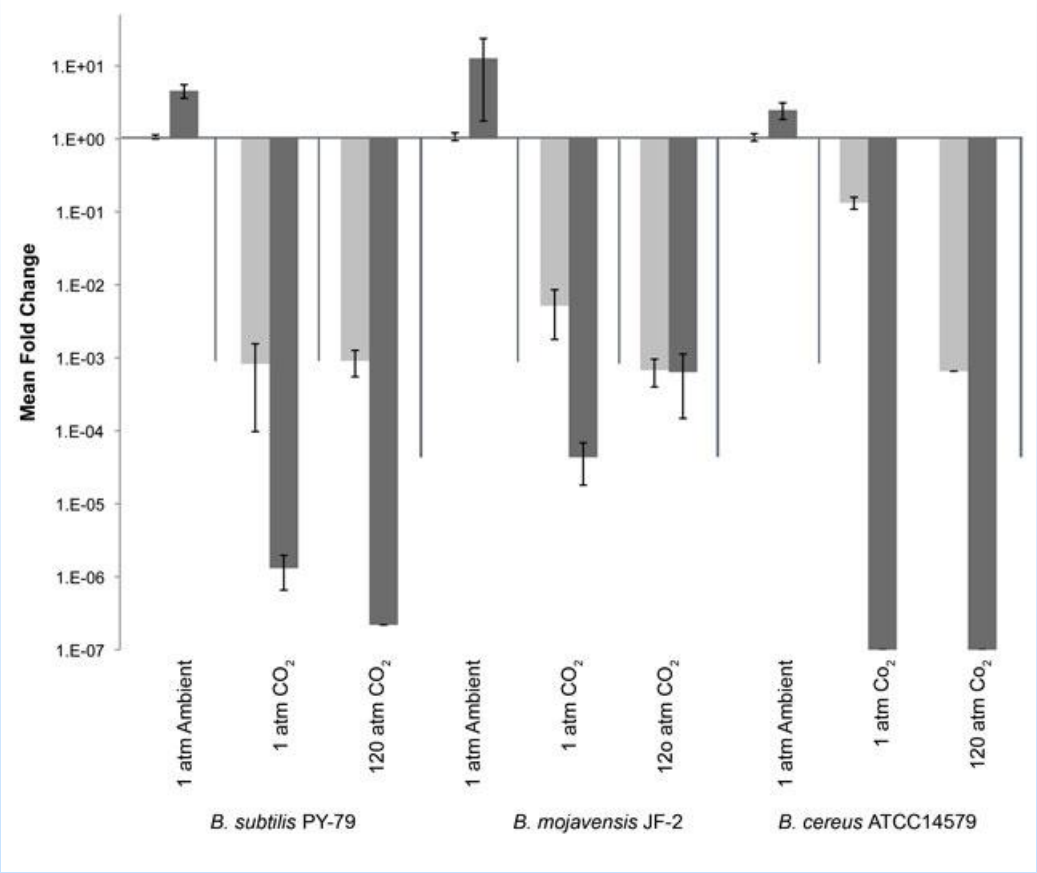




E. coli sterilization after 20-40 mins

"…a useful method for sterilization of many types of materials and pharmaceutical formulations because of the mild, non-reactive process conditions employed and the ability of SCF CO_2 to inactivate a wide variety of microorganisms."

2. Supercritical CO_2 is a powerful sterilizing agent

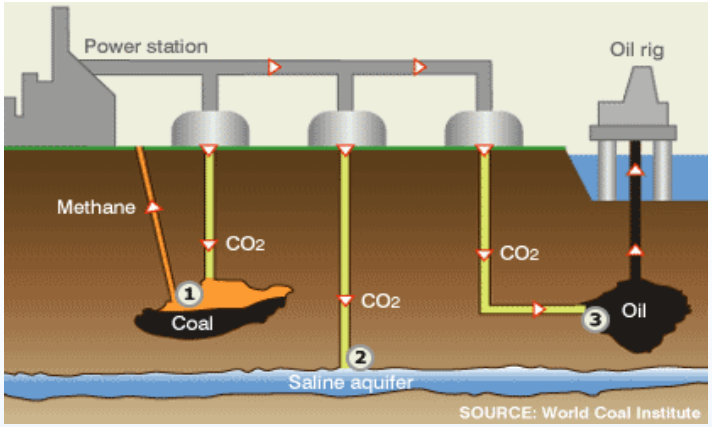


CO_2 exposure associated with 3 to 7 log fold reduction of viable cells

Viable cell counts (colony forming units/ml) for type strains incubated under 1 atm CO_2 and scCO_2 of *B. subtilis* PY-79, *B. mojavensis* JF-2 and *B. cereus* ATCC 14579 and normalized to initial values. Mean fold change in CFU cell counts of cultures grown for 6 hours (light gray) or 7 days (dark gray) under ambient conditions, atmospheric, CO_2 of 1 atm and CO_2 of 120 atm. Error bars are 1 standard deviation.

Project overview:

Do microbes matter?



Geologic sequestration of CO_2, if implemented at scales that could mitigate climate change, will result in massive perturbations to the biologically-active subsurface environment.

Questions

1. **Will subsurface microbial communities remain active under the high pCO_2 conditions associated with geological carbon sequestration?**
2. **What are the biological mechanisms of high pCO_2 tolerance?**
3. **What is their significance for the fate and transport of injected CO_2?**
4. **Can we engineer microbial systems to help improve reservoir seal integrity?**

Presentation Outline

- Project Benefits & Goals
- Results/Technical Status
  - Enrichment and isolation of CO_2 tolerant microorganisms from sequestration sites.
  - Microbial diversity in scCO_2 enrichments
  - Physiological and Genomic Characterization of strain MIT0214
  - Analysis of gene expression (transcriptomics) (in progress)
- Accomplishments
- Future Work
- Appendix

Benefits to the Program

Program goals

- Design technologies that will support industries' ability to predict CO_2 storage capacity in geologic formations
- Develop technologies to demonstrate that 99 percent of injected CO_2 remains in the injection zones.

Project benefits

- Microorganisms that tolerate sub- and super-critical CO_2 hold potential as agents of biological transformations in the deep subsurface after CO_2 injection.
- A fundamental understanding of microbial activity under supercritical CO_2, including potential for geochemical catalysis, is necessary for modeling the long term fate of injected CO_2.

CO_2-induced subsurface geochemical changes during Frio 2

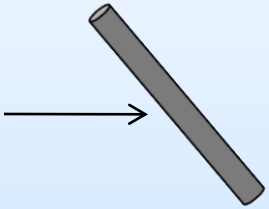
Observation Well	**Pre-CO_2**	**Post-CO_2**
pH	5.9-6.7	2 to 3
methane	93% (g)	ND
CO_2	0.03% (g)	~100%
alkalinity	100 mg/L	3000 mg/L
Fe_T	30 mg/L	1100 mg/L
Cations (Mg, Ca)		increase
Dissolved organic carbon (organic acids, toluene)	1-5 mg/L	Day 1: 5-6 mg/L Day 20: >500 mg/L Month 6: 4.5-7.5 mg/L

Hovorka et al. (2006) Measuring permanence of CO_2 storage in saline formations: the Frio experiment. Environ. Geoscience 13(2);105-121

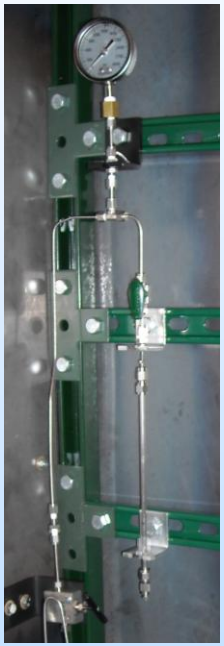
Enrichment of strains from Frio 2 formation water filters

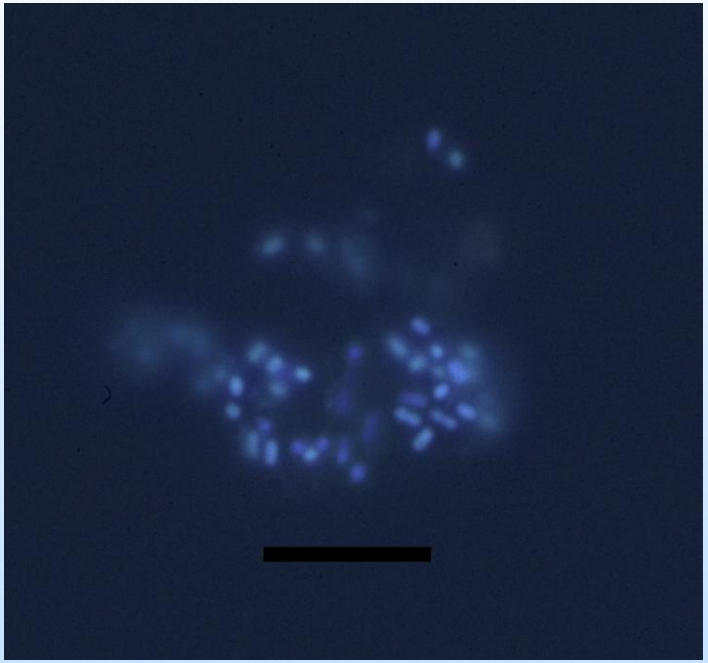
Filters courtesy of Tommy Phelps, ORNL

Incubation time = 14 days



Initial enrichment





$scCO_2$ column at 120-136 atm
6 ml culture; 4 cm^3 headspace

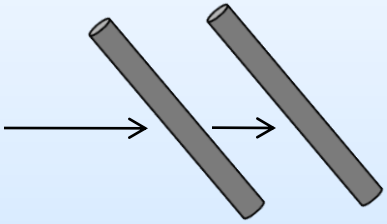
DAPI
Scale bar 10µm

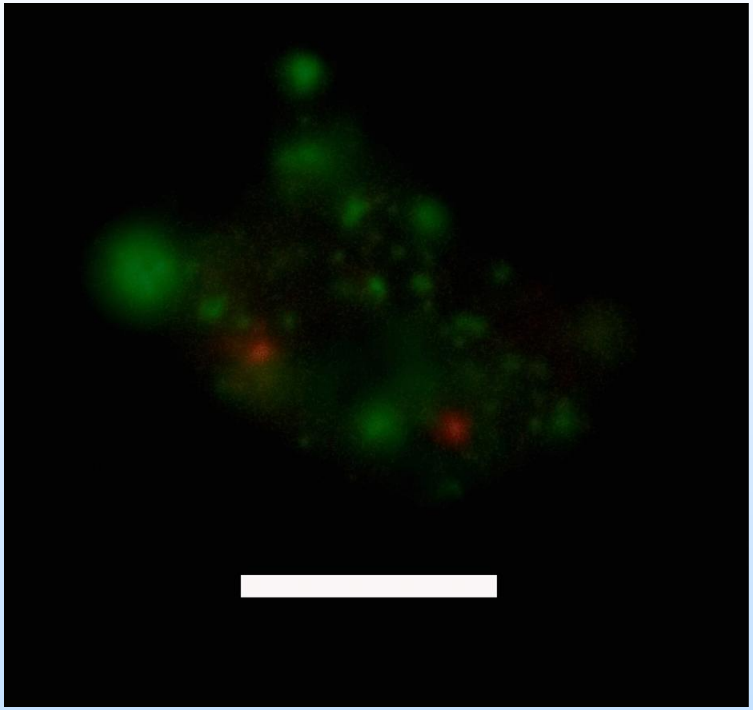
Peet, et al, in prep

Enrichment

Enrichment and isolation of supercritical CO_2-tolerant microbes

Serial passage 15% v/v
Incubation time = 16 days



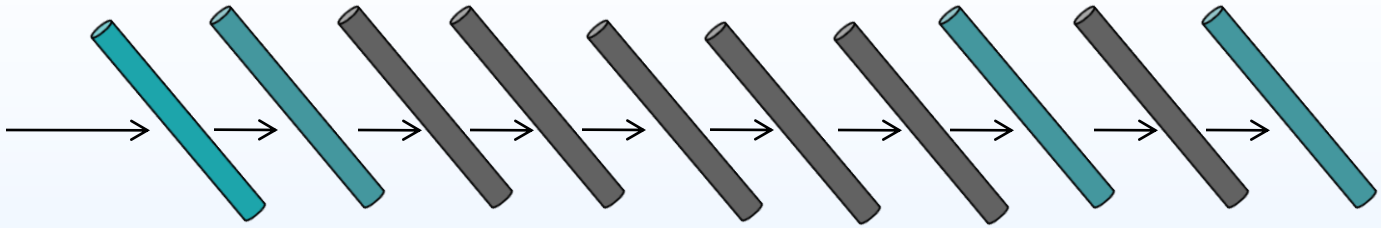


Invitrogen Live/Dead Stain
Scale bar 10 µm

Peet, et al, in prep

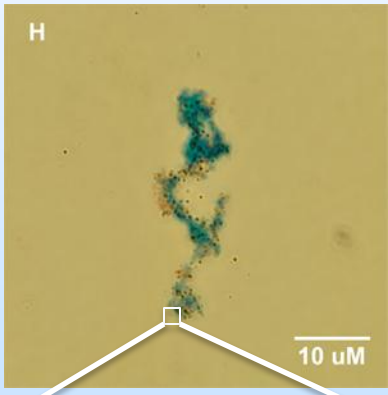
Serial passages

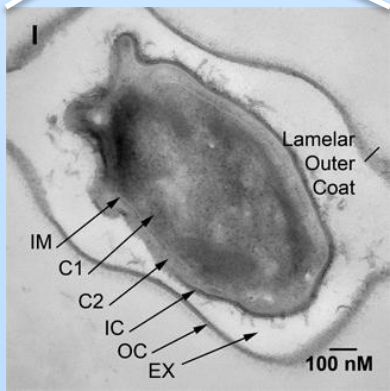
Incubation time = 9 to 16 days and 60 days



Isolation of *Bacillus* MIT0214

Passage	Duration	Amount of previous enrichment used as inoculums	Nucleic acid yield (ng/mL)	Community Analysis
Initial enrichment[a]	14 days	15 % dilution of previous	700	+
1	16 days	15 % dilution of previous	1400	+
2	15 days	10 % dilution of previous		
3	15 days	10 % dilution of previous		
4	60 days	10 % dilution of previous		
5	15 days	10 % dilution of previous		
6	12 days	10 % dilution of previous		
7	9 days	10 % dilution of previous	2300	+
8	9 days	10 % dilution of previous		
9	9 days	10 % dilution of previous	1770	+



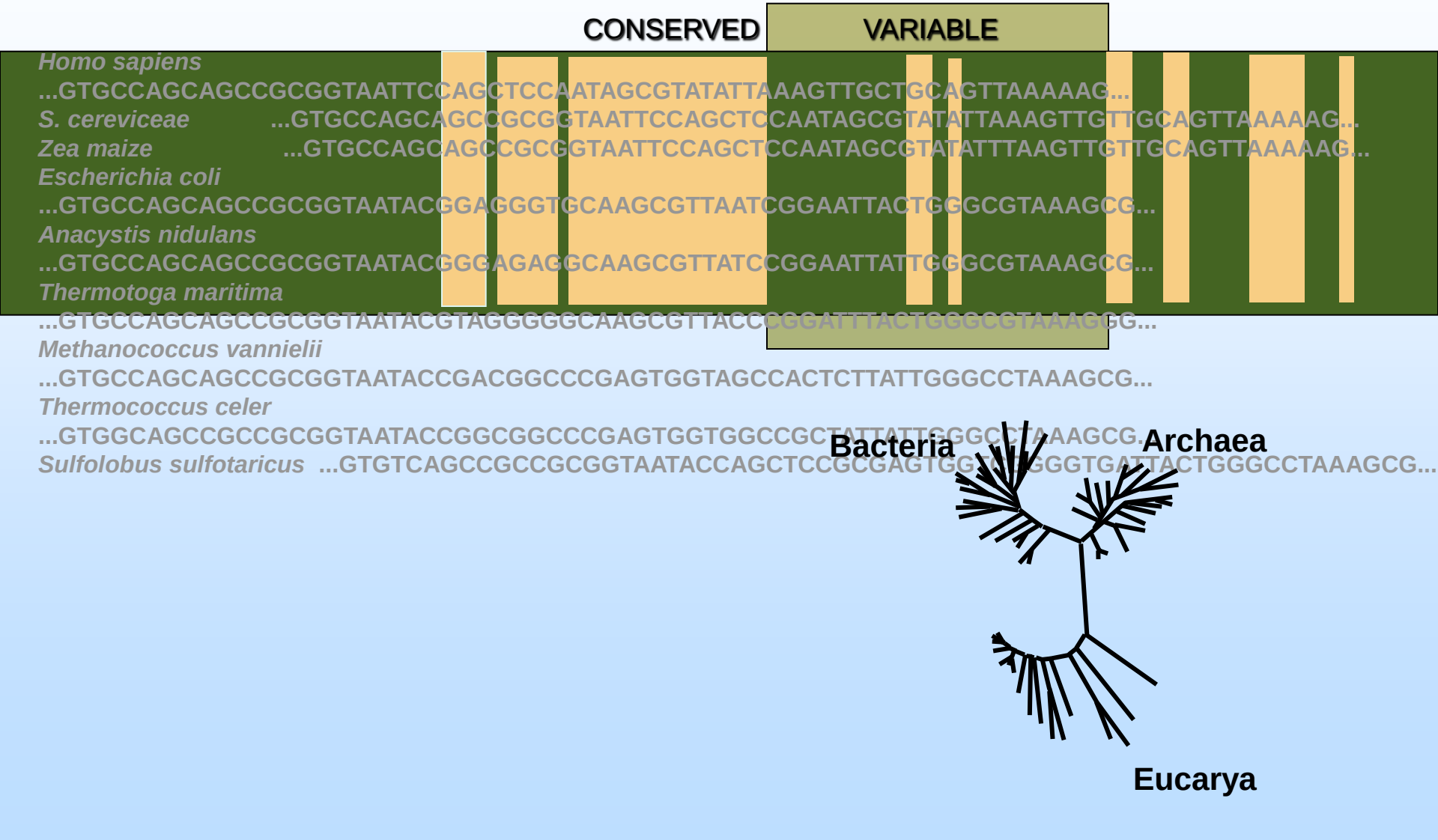


By 7th passage spores appear in aggregates

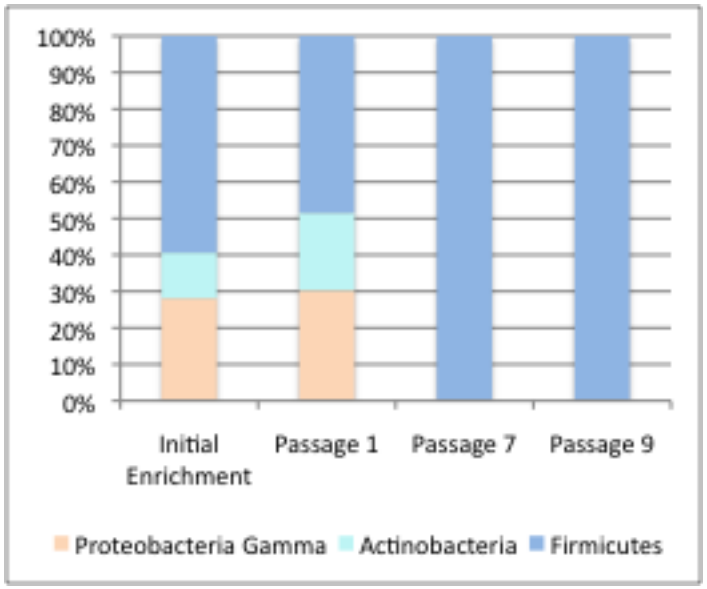
Top: Heat-fixed; stained with malachite green
Bottom: TEM of spore

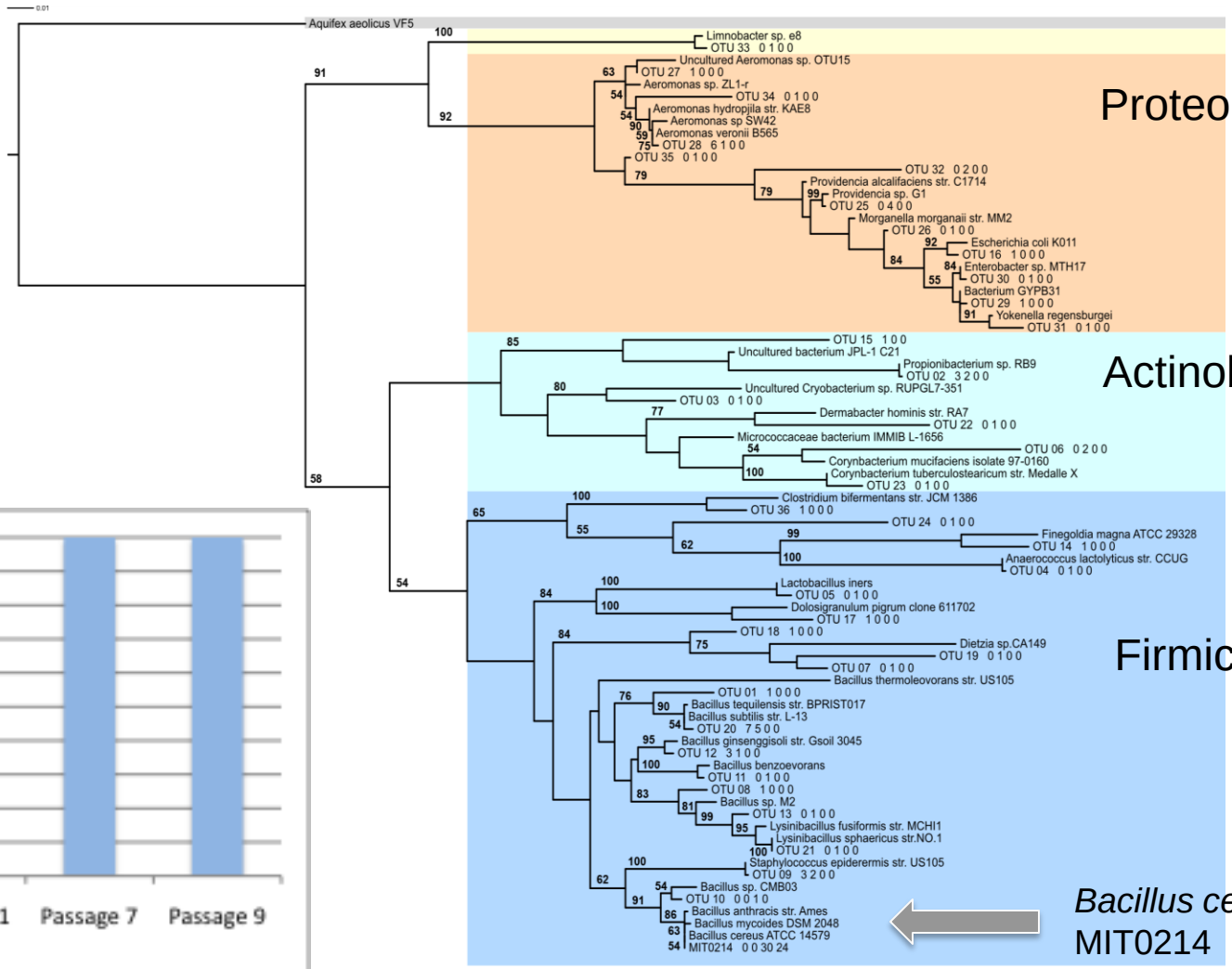
Characterization of Diversity

Alignment of 16S/18S rRNA

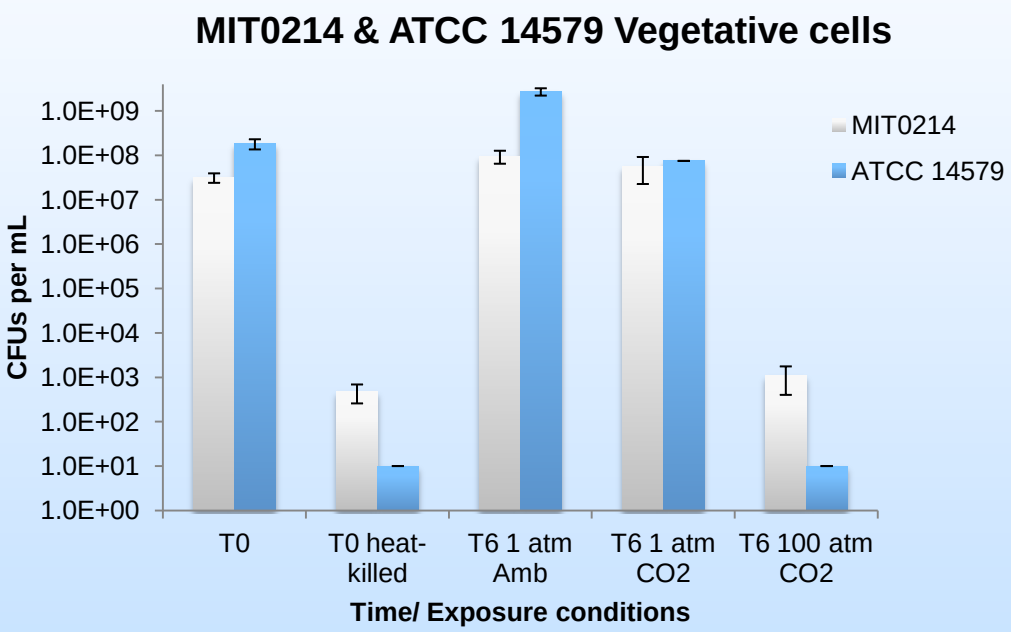


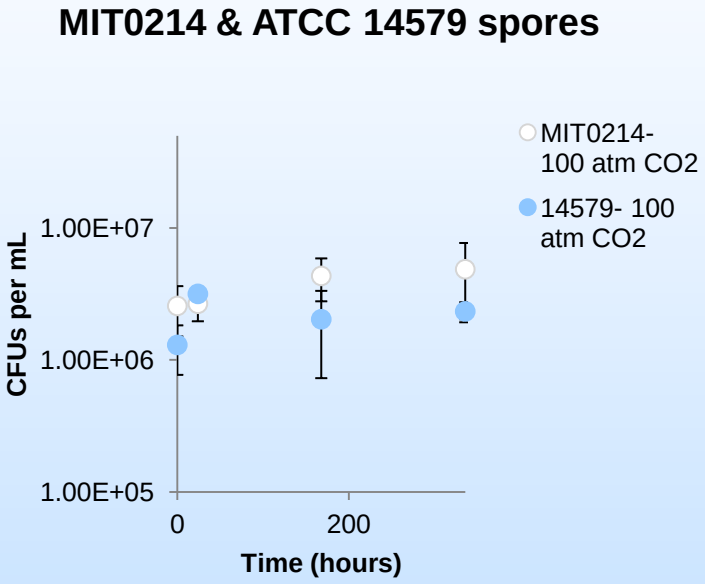
Microbial Diversity in Enrichments



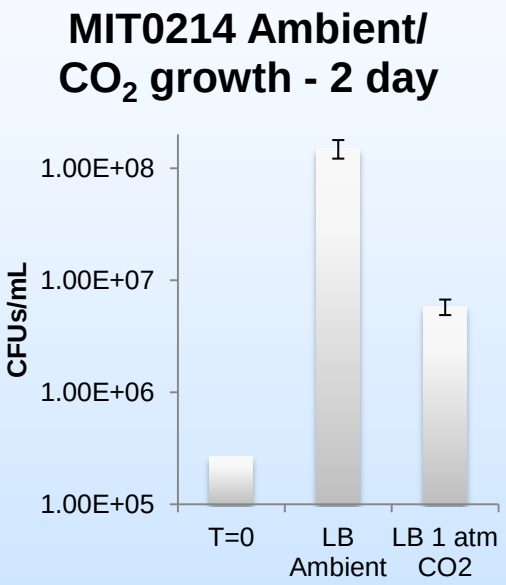


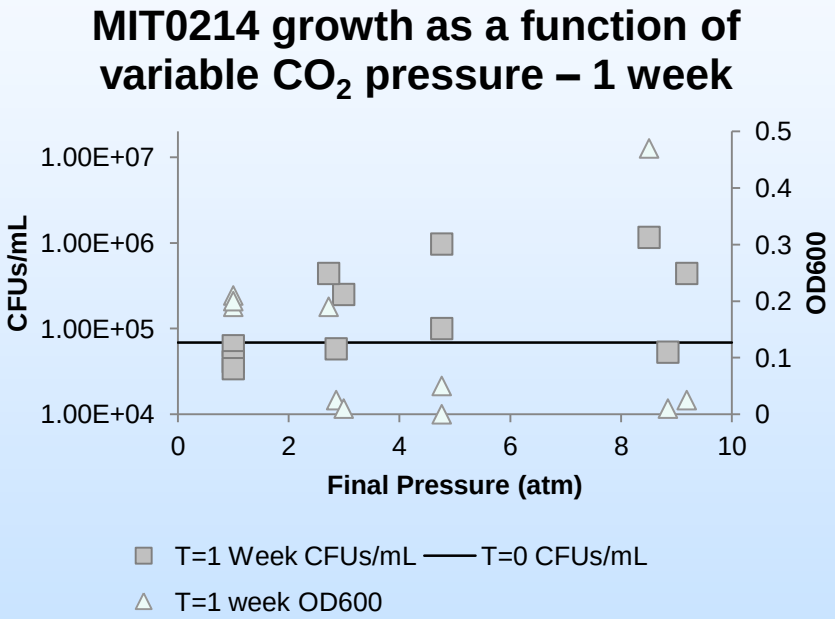
Examine MIT0214 tolerance to CO_2 in relation to closely related strains



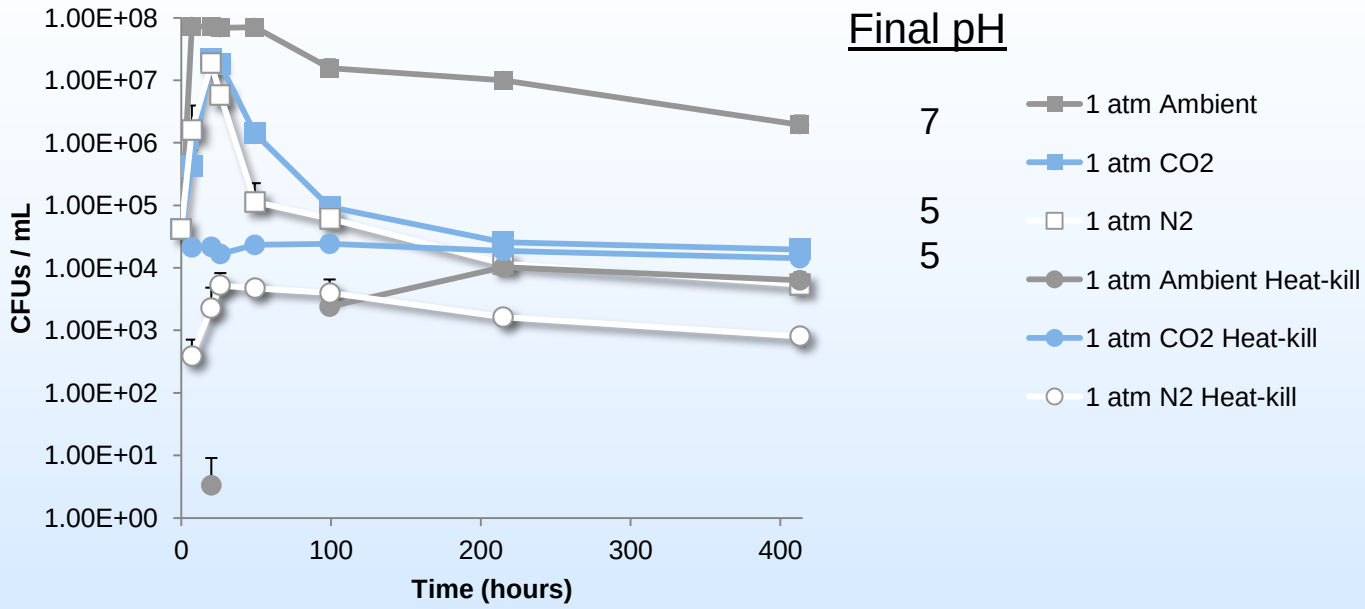


Examine MIT0214 <u>growth</u> under CO_2 (work in progress)

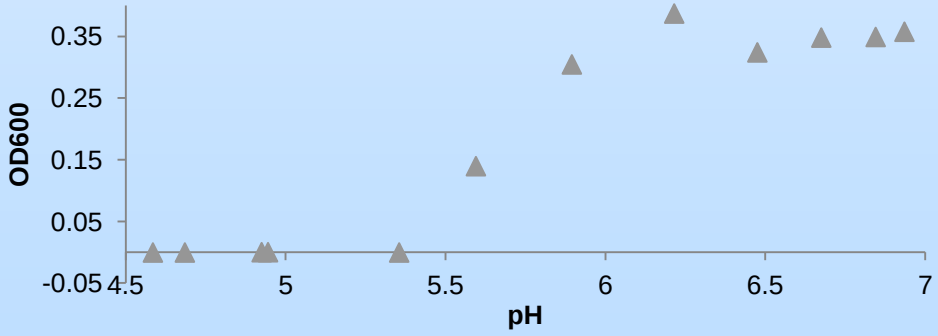




MIT0214 Growth and sporulation vs headspace



MIT0214 OD600 vs. pH



Physiological Characterization

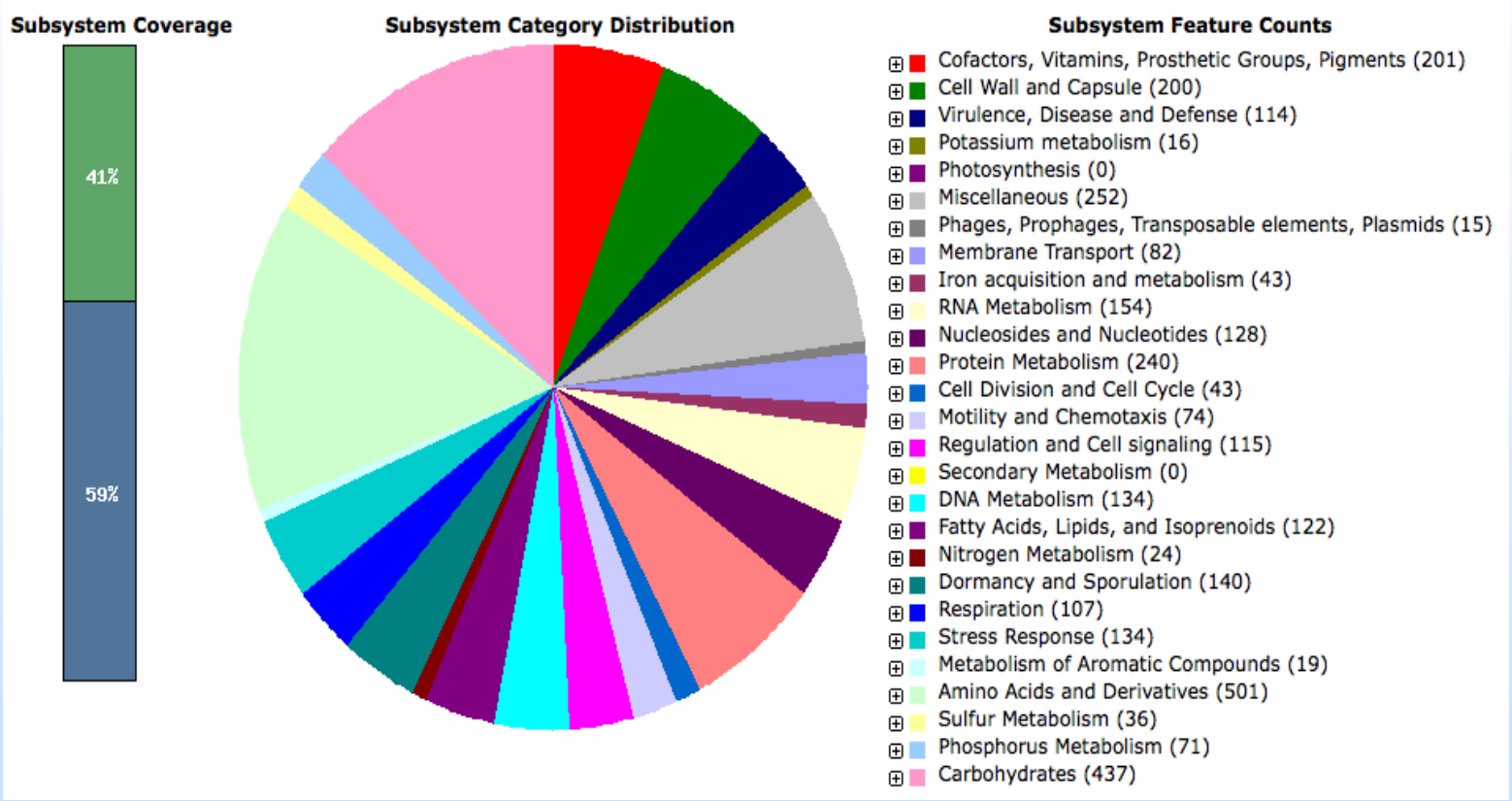
- MIT0214 isolated from Frio Enrichment Culture.
- Spores tolerate supercritical CO_2
- Growth occurs under 100% CO_2 at ambient and elevated pressures (up to 10 atm confirmed).
- pH tolerance to 5.5
- Observation of *Bacillus*-like lipid profiles in Frio site (Tommy Phelps and Susan Pfiffner pers. Comm.)

What adaptations allow the strain to grow under CO_2?

Genome Sequencing

- Illumina “next-generation” sequencing technology
- MIT MicroBioCenter – core facility
- Illumina GAII machine
- 100 bp paired end reads
- Assembled into contigs 50kb to 250kb using CLC Genomics Workbench.
- Annotated using MG-RAST

Genome annotation



Highlights – genes for aerobic and anaerobic respiration; catabolism of proteins, sugars and aromatic compounds; antibiotic resistance.

Comparative genomic analysis of MIT0214

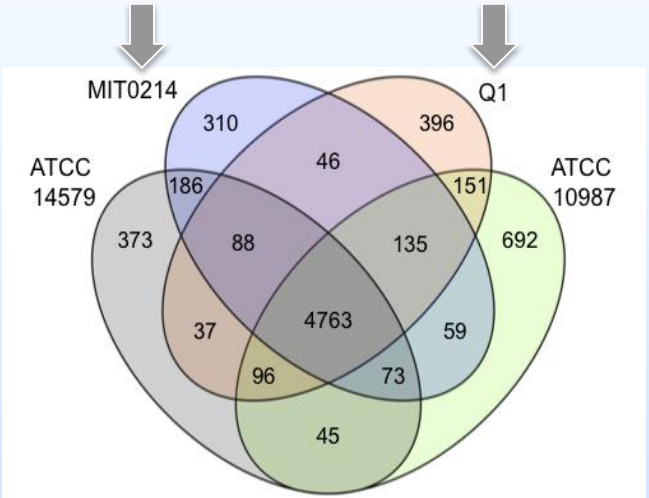
	B. cereus MIT0214	*B. cereus* Q1	*B. anthracis* Ames	*B. cereus* ATCC 14579	*B. cereus* ATCC 10987
GC content (%)	34.9%	35.5%	35.4%	35.3%	35.5%
No. of plasmids (size)	TBD	2 (53kb & 239kb)	2 (95kb & 182kb)	1 (15kb)	1 (208kb)
Genome size (Mb)	5.62 Mb	5.51 Mb	5.23 Mb	5.43 Mb	5.43 Mb
No. of Coding Sequences	5640	5646	5667	5561	5924
Genes shared with MIT0214	-	5032 (89.2%)	5001 (88.7%)	5086 (90.2%)	5034 (89.3%)
Average Nucleotide Identity with MIT0214	-	93.6%	93.2%	97.9%	93.3%

This study

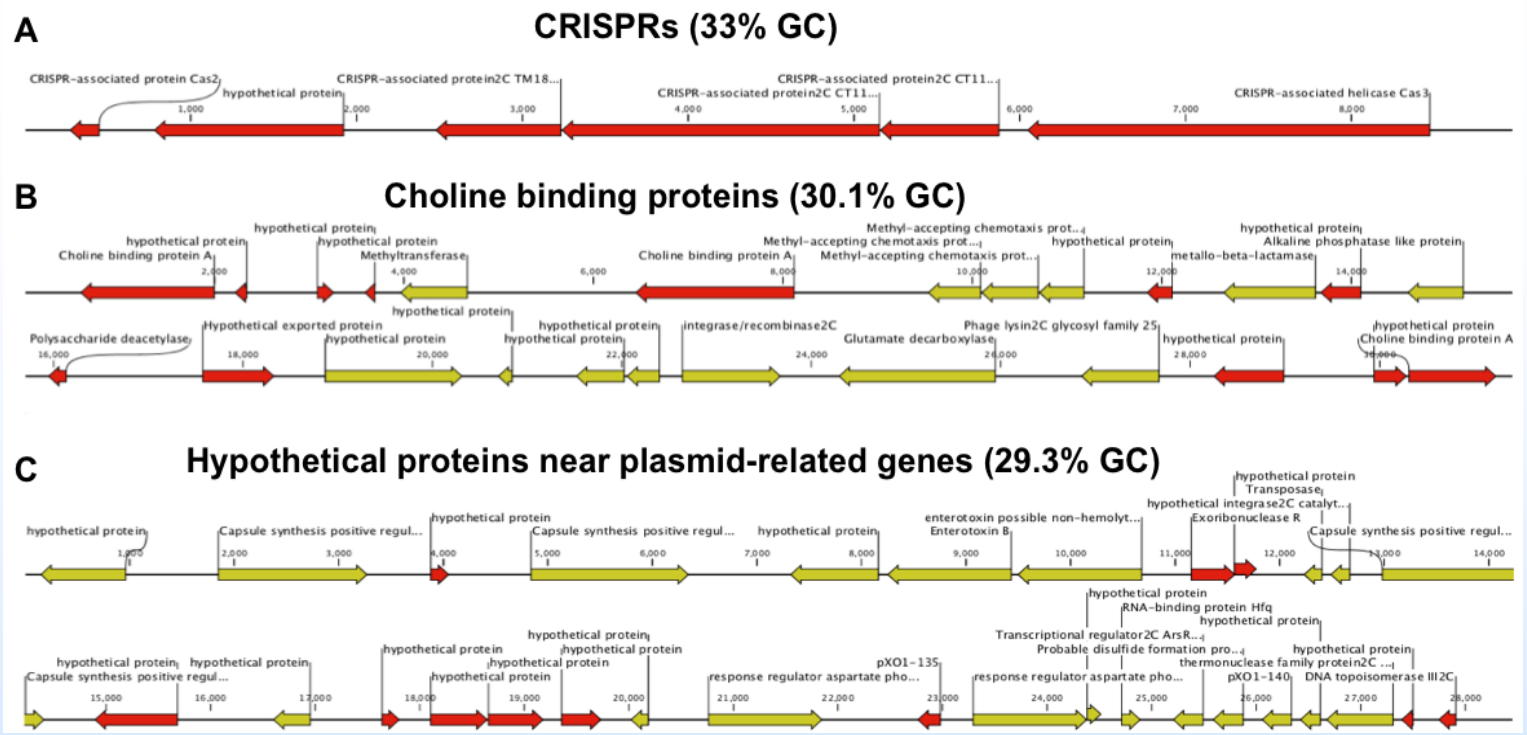
Oil field (China)

Comparison of the percent of total SEED subsystems of both MIT0214 and *B. cereus* Q1 reveals enrichment of three subsystem that show more than 2 standard deviations from the mean of 6 surface strains. These may represent important functions for adaptation to the subsurface

6 Surface isolates % (Std. Dev.)	MIT0214 %	Q1 %	SEED Level 1 Subsystems
7.59 (0.32)	5.89	6.27	Cofactors, Vitamins, Prosthetic Groups, Pigments
4.52 (0.43)	**5.86**	**5.68**	Cell Wall and Capsule
0.972 (0.09)	0.47	0.48	Potassium metabolism
9.67 (0.45)	7.38	8.35	Miscellaneous
5.99 (0.11)	4.51	3.66	RNA Metabolism
3.34 (0.14)	**3.93**	**3.86**	Stress Response
0.397 (0.047)	**0.56**	**0.51**	Metabolism of Aromatic Compounds



1. cell wall and capsule,
2. stress response and
3. metabolism of aromatic compounds.



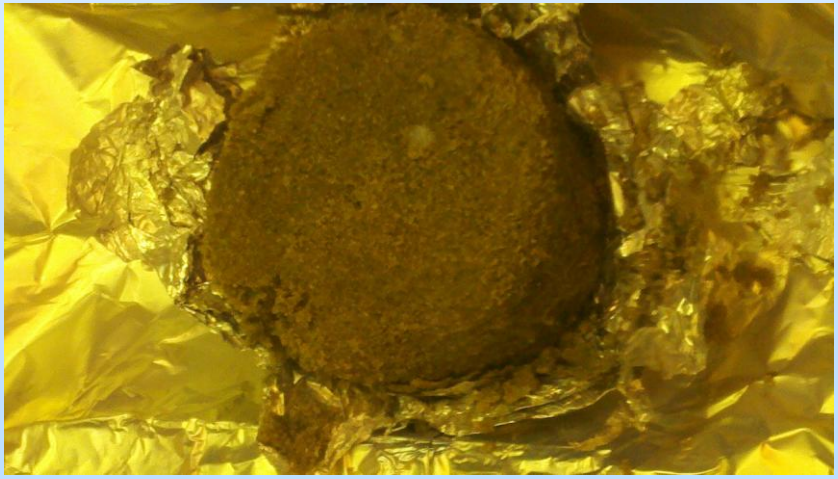
- **Genes unique to MIT0214 are indicated in red while yellow indicates the gene is shared with another closely related *Bacillus* strain.**
- **A)** Contig 28 annotates as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) genes, indicating MIT0214 may have been challenged with phage recently in its history.
- **B)** Contig 49 reveals several unique Choline binding proteins, a protein involved in cell adhesion and hydrophobicity of the cell surface[4]. GC content is 5% lower than the genome average suggesting acquisition of these genes via HGT.
- **C)** Contig 284 contains unique hypothetical proteins and a pXO1 protein, possibly indicating novel gene content transferred through plasmid vectors to MIT0214.

Accomplishments to Date

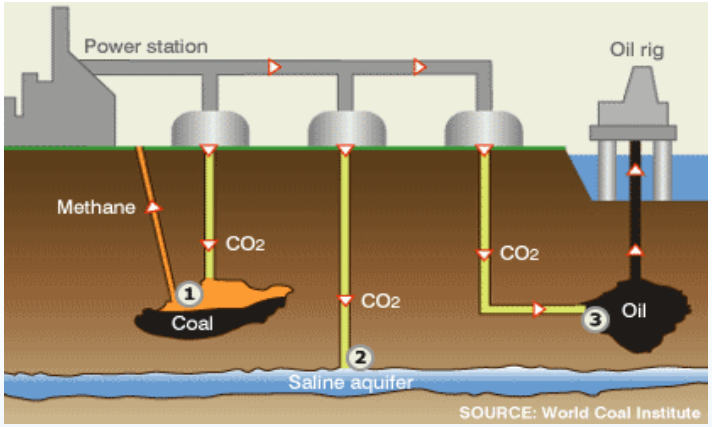
- High pressure cultivation system constructed
- Enrichment and Isolation of scCO_2-tolerant bacteria from 3 sites.
- Established bioinformatics pipeline for comparative genomics analysis
- Sequencing and analysis of MIT0214 genome
- Sequencing of MITOT1 genome (in progress)
- Funded training of 4 undergraduates (including 2 women and 3 URM) and 1 full-time PhD student.
- Launched AGU session: Microbiology of Geologic Carbon Sequestration

Work in Progress

- Cultivation of scCO_2 tolerant organisms
  - WestCARB core (started 4/4); 1599 m depth
  - Otway Basin Australia
  - McElmo Dome formation waters
- Analysis of gene expression under N_2 and CO_2 to identify genes up-regulated under CO_2 that mediate acclimation to high pCO_2.



Will microbial processes influence storage of CO_2?



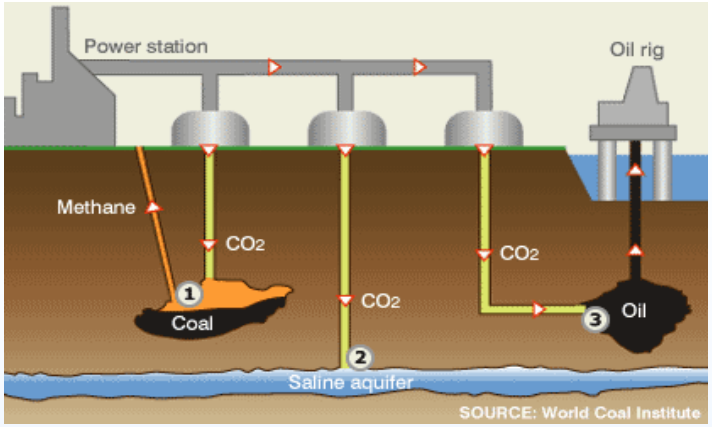
Geologic sequestration of CO_2, if implemented at scales that could mitigate climate change, will result in massive perturbations to the biologically-active subsurface environment.

Questions

1. **Will subsurface microbial communities remain active under the high pCO_2 conditions associated with geological carbon sequestration?**

EVIDENCE THAT MICROBES CAN SURVIVE (GROW!) UNDER $scCO_2$ CONDITIONS

Will microbial processes influence storage of CO_2?



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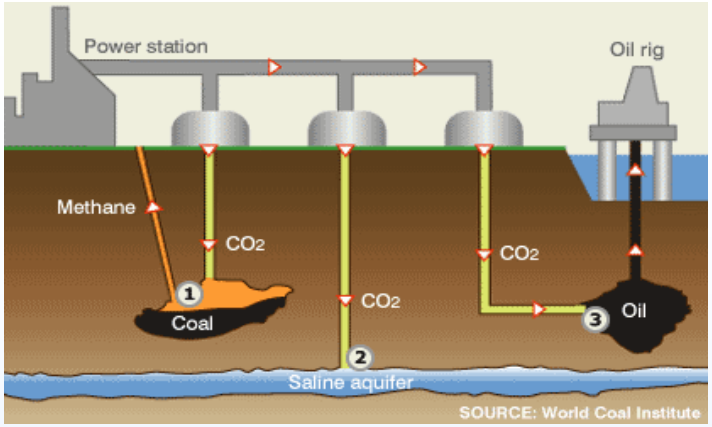
Future insights via "-OMIC's"

Do microbes matter?
--Yes! Near and Far field



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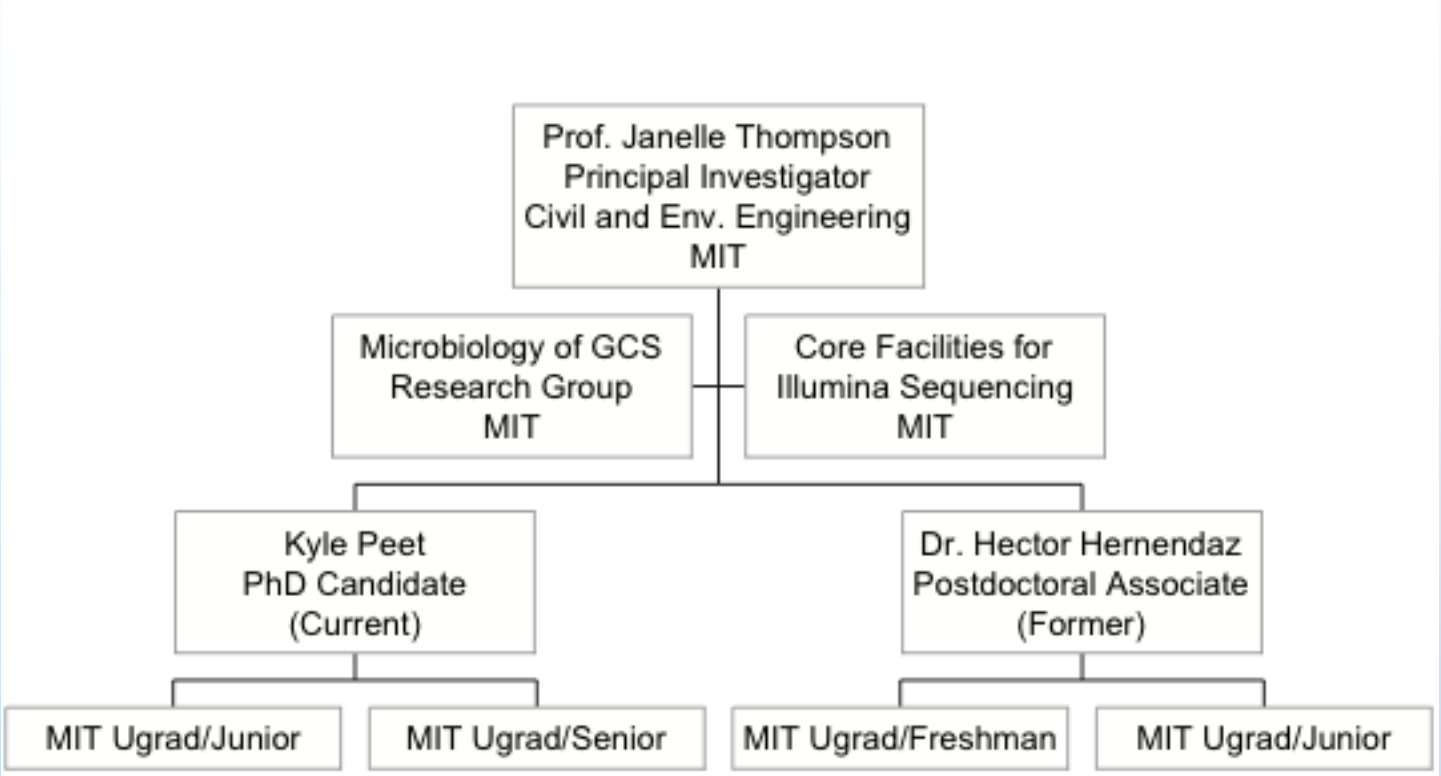
3. ***What is their significance for the fate and transport of injected CO_2?***
4. ***Can we engineer microbial systems to help improve reservoir seal integrity?***

Appendix

– These slides will not be discussed during the presentation, but are mandatory

Organization Chart



Gantt Chart

Task/SubTask			Project Year 1				Project Year 2				Project Year 3			
		'09	(2010)				(2011)				(2012)			
		Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	**Q3**	Q4
Task 2: Characterization of microbial diversity in consortium MIT0212														
Task 2.1	Describe 16 rRNA gene diversity	-	x	A										
Task 2.2	Microscopy of scCO2-bioreactor biomass.	-	x	A										
Task 2.3	Isolation and identification of pure cultures.	-	x	x	x	C								
Task 3: Characterize the growth requirements and optima of the supercritical CO2-tolerant consortium and isolated strains														
Task 3.1	Quantify growth under different environments	-	x	x	x	x	x	x	**x**	E				
Task 5: Investigate the mechanisms of supercritical CO2 tolerance in isolated strains and the consortium MIT0212 through genome-enabled and metagenomic studies														
Task 5.1	Prepare total nucleic acids and sequence the (m	-	x	x	B	x	x	x	**x**	x	x	x	x	H
Task 5.2	Comparative genomic analysis of scCO2-atmos	-	-			x	x	x	**x**	x	x	x	x	H
Task 5.3	Transcriptome profiling of the MIT0212 isolate	-	-				D	x	**x**	x	x	x	x	G, I

Note: Task 4 (Seeding sandstone cores) was dropped from the grant due to time/personnel constraints and budgeted time was re-allocated to bioinformatic analysis of microbial genomes

Bibliography

- Manuscripts are currently in preparation.

- Peet KC, Freedman A, Hernandez HH, Thompson JR. 2011. Genomic insights into growth and survival of supercritical-CO2 tolerant bacterium MIT0214 under conditions associated with geologic carbon dioxide sequestration. American Geophysical Union Fall Meeting: San Francisco.