Characterization of Acetate-Utilizing Bacteria in Soil Sequences From Major Ecological Subregions of California

Brian D. Lanoil[1*] and Sukkyun Han[1]

Summary

Our goals for this project focus on characterization of organisms that consume acetate, an organic acid central to carbon transformations in anaerobic soils. To assess the acetate-utilizing bacterial community in a large breadth of California soil types, we have focused on several well-studied soil sequences from around the state. The selected soil sequences represent eight of the major California ecological subregions as designated by the USDA Natural Resources Conservation Service and are among the best-studied soils in the state. To characterize the acetate-utilizing bacterial community in these soils, we used stable isotope probing, which allows the linkage of a metabolic activity and the identity of the microorganism carrying out that activity. Using the empirically determined acetate-utilizing rate and substrate utilization efficiency for each soil under aerobic and anaerobic conditions, we incubated the soil samples with [1, 2]^{13}C-acetate for the optimal time and temperature. Following separation of labeled DNA from unlabeled DNA by CsCl gradient ultracentrifugation, amplified portions of the 16S rRNA gene and separated them by denaturing gradient gel electrophoresis. DGGE and cluster analysis of soil samples indicated low diversity of acetate utilizing organisms with no clear correlation between location and microbial community composition. Analysis of DNA sequence of clones correlated directly to DGGE bands of anaerobically incubated samples revealed a predominance of *Betaproteobacteria* of the orders Burkholdariaceae and Oxalobacteraceae and *Firmicutes* of the genera *Bacillus* or *Clostridia*. The *Firmicutes* were found in nearly all of the samples, whereas, the distribution of the *Betaproteobacteria* was more limited. Unsurprisingly, most of the closest relatives were from soils, and many were from anoxic rice paddy soils. Several closest relatives of these groups are facultative denitrifying bacteria, consistent with the incubation conditions.

Objectives

1. Collect soils from various soil sequences (climo, chrono, or bio) from around the state of California.
2. Determine gravimetric water content, pH, exchangeable cations by ICP-OES, exchangeable anions and acetate concentration by ion chromatography, and total organic carbon and total organic nitrogen by a TOC/TON analyzer for each soil.
3. Determine the optimal acetate concentrations and incubation times for SIP studies of these various soils.
4. Perform 16S rRNA denaturing gradient gel electrophoresis (DGGE) of soil samples to examine the level of diversity in the soil samples.

[1]University of California, Department of Environmental Sciences, Riverside, CA 92521
*Principal Investigator

5. Perform stable isotope probing (SIP)-DGGE with acetate as the substrate to characterize the acetate-utilizing bacterial community within each sample.
6. Compare the composition of the acetate-utilizing microbial communities from around California

Approach and Procedures

In total, 32 soil samples were collected representing seven different soil sequences from around the state (*table 1*). Multiple cores were collected from each site, combined, and homogenized by sieving. Soils were photographed and colors compared to a Munsell soil color chart (Macbeth 2000). Gravimetric water content and pH were determined by standard methods, and aliquots were removed for chemical analyses (*table 2*). The remaining soil was air dried and stored in sterile polyethylene containers with sterile cheesecloth covers.

To determine optimum incubation time and acetate concentration, the acetate utilization rates were determined in sealed vials with soils at field capacity with several different concentrations of ^{14}C-acetate (*table 3*). In a fashion analogous to a Lineweaver-Burke calculation of Michaelis-Mentin enzyme kinetics (Voet and Voet 1990), the maximum acetate utilization rate (AUR) was determined by plotting the inverse of the ^{14}C remaining in the aqueous phase after one day of incubation (i.e., 1/rate) against the inverse of the initial acetate concentration (i.e., 1/[acetate]). After linear regression, the AUR was calculated as 1/y-intercept, and the substrate concentration at ½ the AUR (i.e., the substrate utilization efficiency, or SUE) was calculated as -1/x-intercept; r^2 values for the linear regression are reported in table 3. These values were determined for samples with standard room air as headspace (aerobic condition) or oxygen-free-N_2-sparged headspace (anaerobic condition).

Following incubation at optimal concentrations and times as determined above, DNA was extracted with the FastPrep kit from Qbiogene. Labeled and unlabeled DNA were separated as described previously (Radajewski et al. 2000). Partial 16S rDNA genes were amplified from the purified ^{13}C-labeled DNAs with the bacterial specific primers 341F-GC and 518R (Muyzer et al. 1993). DGGE was performed using the Bio-Rad Dcode System (Bio-Rad, Hercules, CA, USA). The amount of PCR product loaded in each lane was normalized prior to loading by agarose gel electrophoresis and comparison to a known standard DNA. The stained DGGE gel was photographed on a UV transilluminator with a CCD system (Bio-Rad, Hercules, CA, USA).

Table 1. *Sites sampled for this study.*

Location	Number of sites in sequence	Ecological unit	Parent material /geomorphology	Soil orders	Soil moisture, temperature	Vegetation
Cima volcanic field	1	Mojave Desert	Eolian deposits on lava flow	Aridisols	Aridic, thermic	Very sparse desert shrubs on desert
Central Sierra	6 (Climo)	Sierra Nevada/Sierra Nevada Foothills	Quartz diorite, grandiorite/Granodiorite	Alfisols, Inceptisols, Entisols/ Inceptisols, Alfisols	Xeric, thermic-cryic/ xeric, thermic	Ponderosa pine, mixed conifers, true fir, lodgepole pine/ Oaks and annual grasses
Merced	5 (Chrono)	Great valley dry steppe	granitic alluvium	Entisols-Alfisols	Xeric, thermic	Annual grasses
Mt. Shasta	4 (Chrono)	Southern cascades	Andesitic mudflows	Entisols	Xeric, mesic	Ponderosa pine
Mendocino coast, Jug Handle Reserve	8 (Chrono)	Coastal steppe-mixed forest redwood	Beach sands/marine terraces	Mollisols-Alfisols-Spodosols	Udic, isomeric	grasses-redwood, Douglas firbishop pine-pine and cypress
Los Osos	4 (Chrono)	Central Coast Chaparral	Beach sands/marine terraces	Entisols-Mollisols-Alfisols	Xeric, thermic; aquic, thermic	Shrubs and annual grasses
San Dimas	4 (Bio)	Southern mountains and valleys	Diorite	Entisols	Xeric, thermic	Chamise-ceanothus - scrub oak - Coulter pine

To obtain sufficient DNA sequence for detailed phylogenetic analysis, clone libraries were made from soil samples as previously described (Skidmore et al. 2005). Individual clones were re-amplified with DGGE primers as above and their migration position was compared to the DGGE results above. Where a clone matched a dominant band position, a partial sequence (~700 bp) was obtained from the clone. Nearest neighbors were determined by BLAST analysis to the GenBank database (*table 4*), and phylogenetic analysis was performed at the Ribosomal Database Project web site (http://rdp.cme.msu.edu/, *figs. 2-5*).

Table 2. *Soil characteristics in California soil samples and sampling site location.*

Sample number	Site	Water content (%)	pH	Temp. (°C)	Soil temp. (°C)	Latitude (North)	Longitude (West)	altitude (ft)
1	Merced1	2.51	5.7	33	29	37.44	-120.50	137.47
2	Merced2	8.81	6.5	34	30	37.52	-120.42	160.43
3	Merced3	11.38	6	35	26	37.50	-120.41	192.59
4	Merced4	2.46	6.8	39	31	37.64	-120.40	388.78
5	Merced5	3.30	7.1	40	30	37.66	-120.45	218.18
6	Sierra1	12.69	6.7	26	16	37.27	-119.11	9203.08
7	Sierra2	4.68	6.4	26	20	37.25	-119.14	7192.26
8	Sierra3	6.95	6.6	28	24	37.12	-119.31	5380.90
9	Sierra4	3.76	6.9	31	23	37.06	-119.36	4472.44
10	Sierra5	9.07	7.3	32	26	37.04	-119.43	2670.27
11	Sierra6	1.44	6.5	33	26	37.04	-119.46	1686.68
12	Shasta1	13.26	5.5	36	30	41.29	-122.05	3619.75
13	Shasta2	15.27	5.7	36	30	41.30	-122.05	3772.96
14	Shasta3	8.59	5.6	36	28	41.30	-122.06	3835.30
15	Shasta4	2.32	6.1	34	30	41.28	-122.05	3548.88
16	Mendosino1	4.66	7.2	18	17	40.41	-124.39	12.00
17	Mendosino2	1.34	6.6	18	16	40.26	-124.35	262.46
18	Mendosino3	0.51	6.5	18	18	40.26	-124.35	328.08
19	Mendosino4	0.86	6.4	18	14	40.27	-124.35	820.20
20	Mendosino6	15.29	6	16	14	39.61	-123.78	320.45
21	Mendosino7	16.74	6.1	16	15	39.61	-123.77	668.31
22	Mendosino8	16.21	6.7	16	15	39.60	-123.76	896.00
23	Mendosino9	25.25	6.2	16	13	39.35	-123.81	97.44
24	Los Osos1	1.74	6.6	16	13	35.28	-120.87	—
25	Los Osos2	9.85	6.4	16	13	35.28	-120.88	—
26	Los Osos3	13.01	6.4	16	14	35.28	-120.88	—
27	Los Osos4	15.05	6.3	16	14	35.27	-120.89	24.61
28	San DImas1	3.04	6.4	29	25	34.21	-117.76	—
29	San DImas2	4.14	6.7	31	26	34.21	-117.76	—
30	San Dimas3	3.34	6.8	31	27	34.21	-117.76	—
31	San Dimas4	2.52	6.9	32	28	34.21	-117.76	—
32	Mojave	0.05	6.5	40	34	35.20	-115.87	2296.00

Table 3. *Acetate utilization activity with oxic and anoxic conditions in California soil samples.*

Sample number	Site	Aerobic condition			Anaerobic condition		
		AUR (mmol g^{-1} day^{-1})	SUE (mmol g^{-1})	r^2	AUR (mmol g^{-1} day^{-1})	SUE (mmol g^{-1})	r^2
1	Merced1	21.22	118.04	0.99	23.950	149.523	1.00
2	Merced2	29.49	156.11	1.00	10.996	63.215	0.96
3	Merced3	21.10	126.93	1.00	3.159	29.608	0.64
4	Merced4	4.59	24.53	0.93	2.776	17.615	0.83
5	Merced5	6.67	40.15	1.00	3.453	28.730	1.00
6	Sierra1	17.50	106.70	0.99	8.201	62.476	0.82
7	Sierra2	4.27	26.93	1.00	4.195	78.953	0.90
8	Sierra3	14.96	93.29	0.99	22.102	117.306	1.00
9	Sierra4	6.41	28.93	0.93	1.866	83.943	0.73
10	Sierra5	23.38	142.25	1.00	4.069	19.217	1.00
11	Sierra6	13.19	64.08	1.00	1.964	8.977	0.57
12	Mt.Shasta1	23.76	119.03	1.00	25.402	130.227	1.00
13	Mt.Shasta2	22.79	115.01	0.99	28.213	140.294	1.00
14	Mt.Shasta3	14.86	82.27	1.00	23.039	121.103	1.00
15	Mt.Shasta4	2.62	10.98	0.80	0.484	115.521	0.52
16	Mendosino1	16.12	80.83	1.00	9.259	54.503	0.96
17	Mendosino2	-	-*	-	-	-	-
18	Mendosino3	4.93	54.59	0.64	3.999	151.975	0.99
19	Mendosino4	3.29	11.58	0.92	1.381	28.170	0.99
20	Mendosino6	4.33	53.62	0.91	2.882	11.401	0.98
21	Mendosino7	4.61	25.93	0.97	4.185	31.608	0.90
22	Mendosino8	12.00	62.16	1.00	2.457	10.610	0.86
23	Mendosino9	20.37	105.36	0.99	15.099	891.980	1.00
24	Los Osos1	11.80	61.17	1.00	8.759	48.827	1.00
25	Los Osos2	16.69	114.65	0.98	12.989	71.673	0.98
26	Los Osos3	37.40	214.39	1.00	12.844	73.977	1.00
27	Los Osos4	27.98	157.76	1.00	17.475	96.995	1.00
28	San Dimas1	4.79	20.72	0.87	1.504	10.344	0.95
29	San Dimas2	22.55	135.98	1.00	4.183	38.022	0.72
30	San Dimas3	4.37	23.48	0.90	2.748	18.393	0.84
31	San Dimas4	3.105	11.336	1.00	2.023	13.486	0.92
32	Mojave	2.942	28.748	0.95	1.968	42.905	1.00

*: not determined

Table 4. *DGGE band's sequences, listed with nearest neighbor from NCBI database, percent similarity based on alignable base pairs, and the affiliation.*

Site	DGGE band	Nearest neighbor in BLAST search	Genbank accession number	Similarity (sequence length)	Phylogenetic Affiliation
Merced 2	1	Uncultured soil bacterium clone W4Ba103 16S ribosomal RNA gene, partial sequence	DQ643674	91% (788/864)	*Bacteroidetes*
Merced 2	2	Uncultured gamma proteobacterium clone AKYH1244 16S ribosomal RNA gene, partial sequence	AY922098	100% (848/850)	*Gammaproteobacteria*
Merced 3	7	Uncultured bacterium clone AKIW790 16S ribosomal RNA gene, partial sequence	DQ129269	96% (743/776)	*Firmicutes/Clostridia*
Merced 3	9	Uncultured bacterium clone AKIW991 16S ribosomal RNA gene, partial sequence	DQ129277	96% (809/840)	*Firmicutes/Clostridia*
Merced 3	13	Unidentified eubacterium from anoxic bulk soil 16S rRNA gene (clone BSV61)	AJ229210	96% (798/827)	*Firmicutes/Clostridia*
Merced 4	3	Uncultured soil bacterium clone TD12 16S ribosomal RNA gene, partial sequence	DQ248265	97% (852/879)	*Betaproteo/Burkholderiaceae*
Sierra 1	14	Uncultured beta proteobacterium clone G09_WMSP1 16S ribosomal RNA gene, partial sequence	DQ450777	96% (820/851)	*Betaproteo/Burkholderiales/Incertae*
Sierra 1	15	Burkholderiaceae bacterium KVD-1700-31 16S ribosomal RNA gene, partial sequence	DQ490294	98% (848/862)	*Betaproteo/Burkholderiaceae*
Sierra 1	16	Janthinobacterium sp. IC161 gene for 16S ribosomal RNA, complete sequence, strain: IC161	AB196254	100% (856/858)	*Betaproteo/Oxalobacteraceae*
Sierra 1	17	Uncultured Ralstonia sp. bacterium 16S rRNA gene, clone HrhB81	AM159296	96% (825/863)	*Betaproteo/Burkholderiaceae*
Sierra 1	20	Janthinobacterium sp. IC161 gene for 16S ribosomal RNA, complete sequence, strain: IC161	AB196254	99% (848/854)	*Betaproteo/Oxalobacteraceae*
Sierra 1	22	Uncultured soil bacterium clone F27_Pitesti 16S ribosomal RNA gene, complete sequence	DQ378191	100% (859/860)	*Betaproteo/Burkholderiaceae*
Sierra 2	25	Janthinobacterium sp. IC161 gene for 16S ribosomal RNA, complete sequence, strain: IC161	AB196254	100% (852/856)	*Betaproteo/Oxalobacteraceae*
Sierra 2	29	Janthinobacterium sp. IC161 gene for 16S ribosomal RNA, complete sequence, strain: IC161	AB196254	99% (855/860)	*Betaproteo/Oxalobacteraceae*
Sierra 3	33	Uncultured Bacilli bacterium clone M10Ba75 small subunit ribosomal RNA gene, partial sequence	AY360662	98% (868/890)	*Firmicutes/Bacilli*
Sierra 3	36	Uncultured Bacilli bacterium clone M10Ba45 small subunit ribosomal RNA gene, partial sequence	AY360634	97% (858/887)	*Firmicutes/Bacilli*
Sierra 5	24	Burkholderia sp. CC-S-L25 16S ribosomal RNA gene, partial sequence	DQ835011	99% (854/859)	*Betaproteo/Burkholderiaceae*
Mt. Shasta 1	37	Janthinobacterium sp. IC161 gene for 16S ribosomal RNA, complete sequence, strain: IC161	AB196254	99% (862/873)	*Betaproteo/Oxalobacteraceae*
Mt. Shasta 1	38	Uncultured Bacilli bacterium clone M10Ba81 small subunit ribosomal RNA gene, partial sequence	AY360668	97% (867/890)	*Firmicutes/Bacilli*
Mt. Shasta 1	41	Uncultured bacterium clone AKIW1067 16S ribosomal RNA gene, partial sequence	DQ129549	96% (833/867)	*Firmicutes/Bacilli*
Mt. Shasta 1	44	Janthinobacterium sp. IC161 gene for 16S ribosomal RNA, complete sequence, strain: IC161	AB196254	99% (850/856)	*Betaproteo/Oxalobacteraceae*

Results

Chemical and physical characteristics were determined for soil samples from around the state of California (*tables 1 and 2*). To determine the optimum substrate concentration and incubation time, we determined the acetate utilization rate of each soil by assuming Michaelis-Mentin-like kinetics of substrate utilization (*table 3*). We determined the maximum acetate utilization rate (AUR; equivalent to V_{max}) and substrate utilization efficiency (SUE; equivalent to K_s) for each soil (*table 3*). Ideally, soils would be incubated at a concentration ½ of the AUR, thereby maximizing DNA labeling with concerns about maintaining limiting substrate concentrations, and would be incubated for a period of time sufficient to utilize all of that substrate were the system at the AUR, thereby balancing labeling with concerns about cross feeding Furthermore, comparisons of the acetate concentration at ½ the AUR (i.e., the SUE) can indicate the efficiency with which the community utilizes acetate: higher values means lower efficiency.

The average AUR under aerobic conditions was 13.7 ± 9.5 μmol g soil^{-1} day^{-1} and under anaerobic conditions was 8.6 ± 8.4 μmol g soil^{-1} day^{-1}; these values are significantly different (two-tailed T-test = 0.001) (*table 3*). These values ranged over more than an order of magnitude from 2.6 to 37.4 μmol g soil^{-1} day^{-1} under aerobic conditions and nearly two orders of magnitude from 0.48 to 28.2 μmol g soil^{-1} day^{-1} under anaerobic conditions. The average SUE is 78.0 ± 53.1 μmol g soil^{-1} under aerobic conditions and 89.1 ± 155.8 μmol g soil^{-1} under anaerobic conditions; these values are not significantly different (two-tailed T-test = 0.69). These values ranged ~20 fold from 11.0 to 214.4 μmol g soil^{-1} under aerobic conditions and two orders of magnitude from 9.0 to 892.0 μmol g soil^{-1} under anaerobic conditions, although the high value for anaerobic conditions appears anomalous. However, even with the anomalously high value removed, the values are still not significantly different. Together, these data indicate that aerobic or anaerobic conditions don't affect the efficiency of acetate utilization in soils, but the soils are significantly slower at utilizing acetate under anaerobic conditions.

Following incubation with [1,2]^{13}C-acetate at the empirically determined optimal acetate concentration and optimal time for each sample and ultracentrifugation (i.e., SIP), 16S rRNA DGGE analysis revealed low diversity of acetate-utilizing microbes in soils from around the state (*fig. 1*). No clear correlation was observed between community composition and location in that samples from within the same soil series showed highly different patterns. However, indications based on cluster analysis of DGGE banding patterns are that some samples from different soil sequences have similar acetate-utilizing community composition. Thus, it is likely that some physical and/or chemical forcing factors (e.g., climate, plant cover, soil quality, etc.) are a more dominant control of acetate-utilizing community composition than is physical proximity.

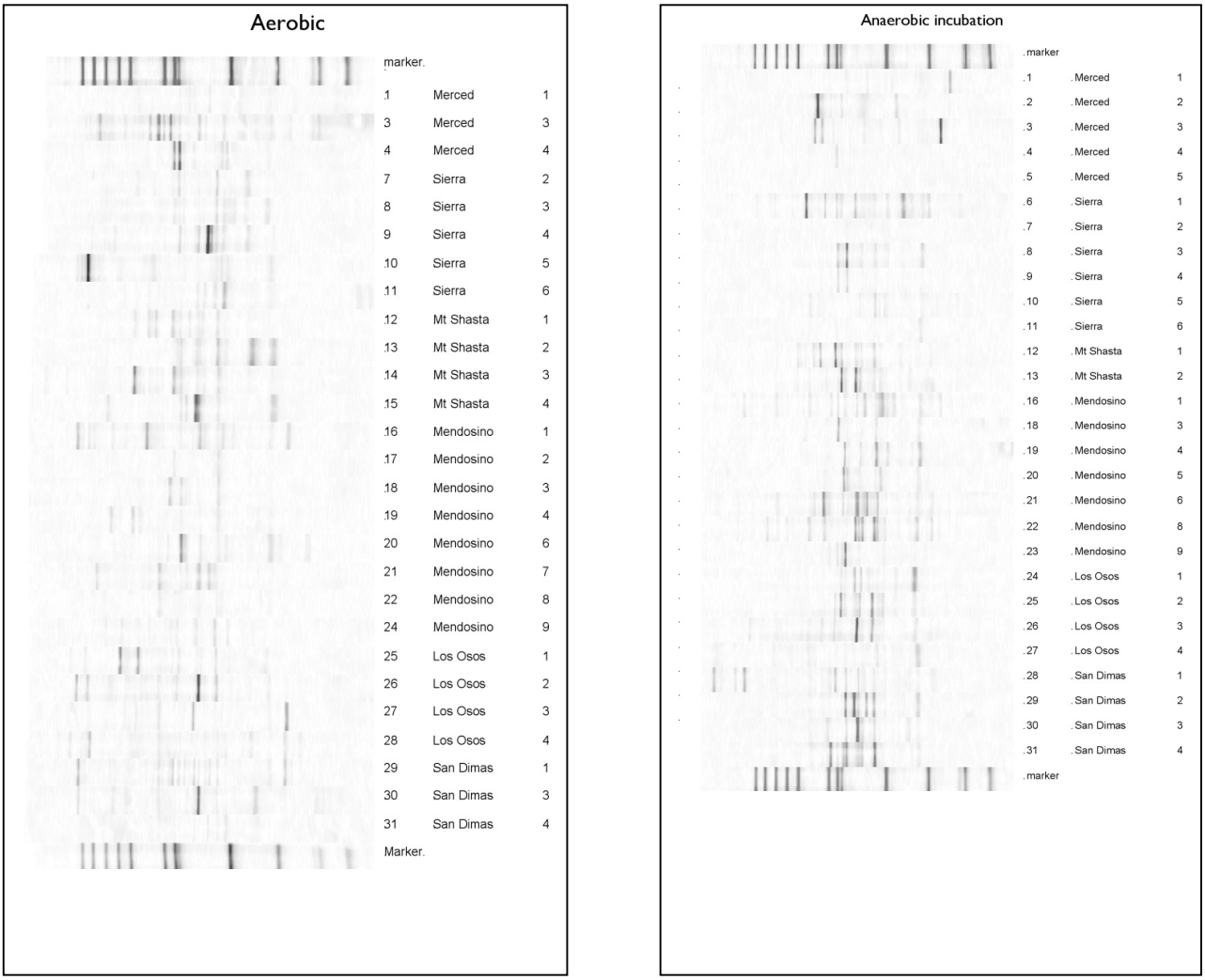

Figure 1. 16S rRNA-DGGE patterns of ^{13}C-acetate labeled DNA from California soils.

DNA sequence analysis revealed an extraordinarily low diversity, with nearly all sequences falling into one of five major groups: the Betaproteobacteria orders Burkholdariaceae and Oxalobacteraceae, the Firmicutes genera *Bacillus* and *Clostridium*, Acidobacteria, or Bacteroidetes (*figs. 2-5 and table 4*). The cultured members of the Burkholdariaceae are versatile heterotrophs that utilize a variety of organic carbon substrates and are often facultative aerobes that can denitrify. The cultured members of the Oxalobacteraceae obligately degrade oxalate to formate and CO_2, but get at least some of their carbon from acetate (*fig. 4*) (Cornick and Allison 1996). The cultured members of the *Bacillus* are metabolically similar to the Burkholdariaceae (*fig. 2*). The cultured members of the *Clostridia* are primarily fermentative organisms that are strict anaerobes (*fig. 3*). Since acetate is a non-fermentable carbon source, it is likely that the *Clostridium* relatives in our samples do not have the same physiology as their cultivated relatives. Most Acidobacteria are uncultured (and thus we do not know their physiology, but are widespread globally, especially in soils (fig. 5) (Barns et al. 1999). The Bacteroidetes are versatile heterotrophs found globally and many are facultative anaerobes (fig. 5) (Kirchman 2002). Most of the sequences were related to environmental clones or isolates obtained from

soils, and many were related to sequences from anoxic rice paddy soils or other anoxic environments.

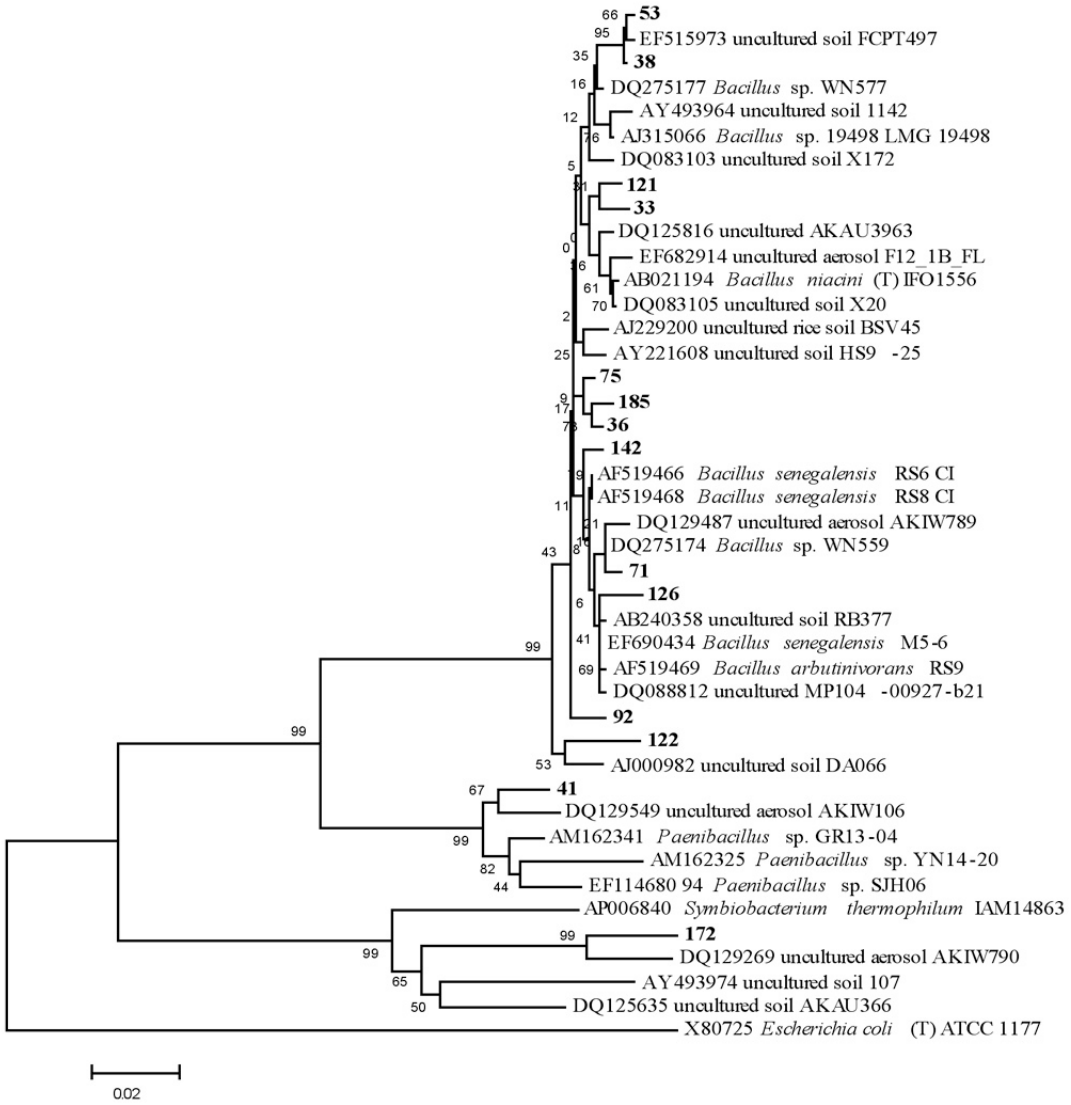

Figure 2. Phylogenetic tree of 16S rRNA gene sequences related to the Firmicutes/Bacillus obtained from ^{13}C-acetate labeled DNA extracted from California soils. Numbers correspond to DGGE bands as listed in Table 4.

Discussion

Our goals for this project focus on characterization of organisms that consume acetate, a carbon molecule central to overall soil carbon cycling. In order to understand the mechanisms and processes that govern the storage and flow of carbon in the soils that support California's diverse ecosystems, it is necessary to understand not only the processes themselves but the microbial assemblages that underlie those processes. Acetate is central to carbon transformations in anaerobic soils, as it is an end product of fermentation of more complex organic material, a

product of acetogenesis from H_2 and CO_2, and a carbon and energy source for a number of aerobic and anaerobic metabolisms.

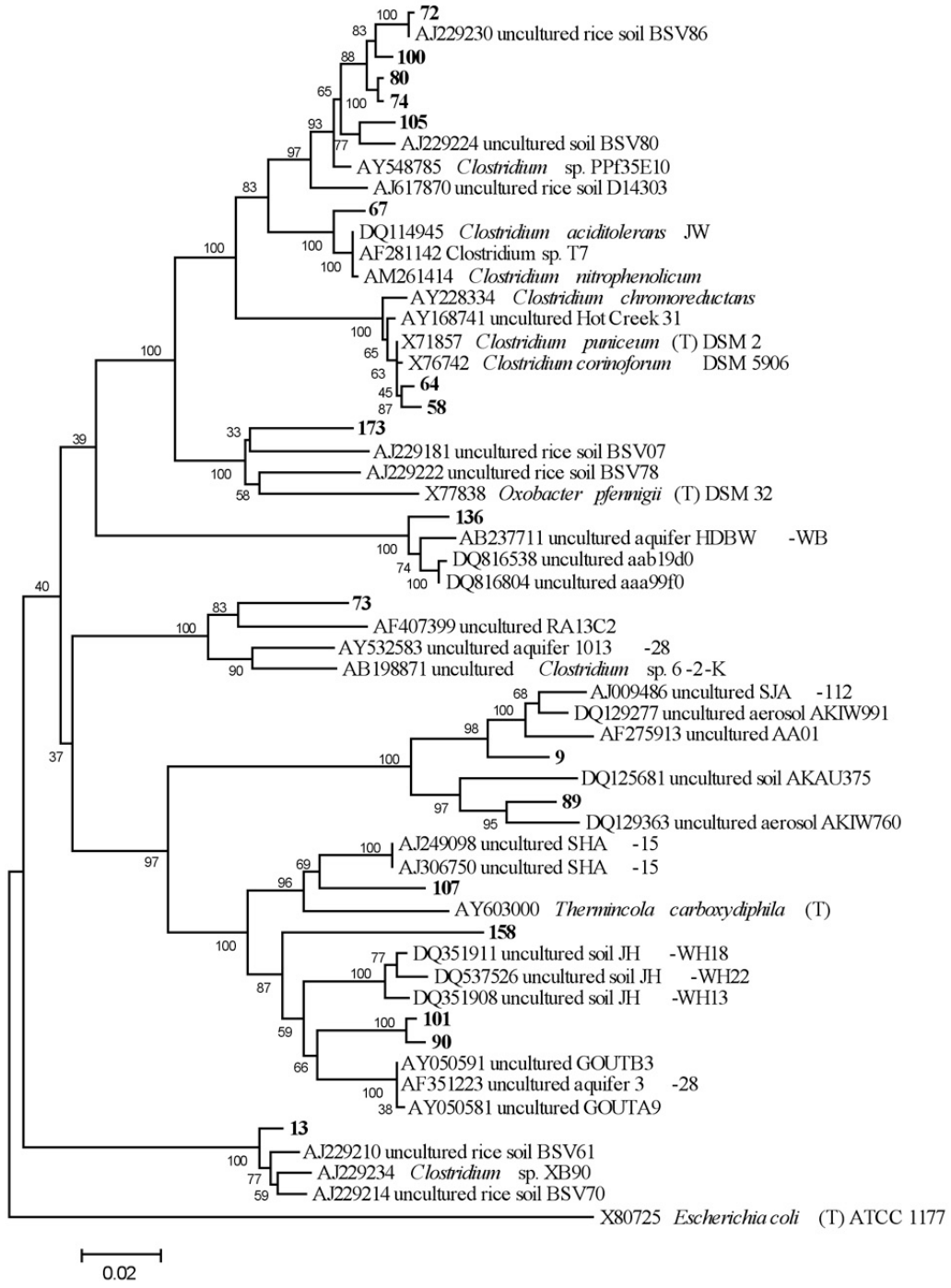

Figure 3. Phylogenetic tree of 16S rRNA gene sequences related to the Firmicutes/Clostridium obtained from ^{13}C-acetate labeled DNA extracted from California soils. Numbers correspond to DGGE bands as listed in Table 4.

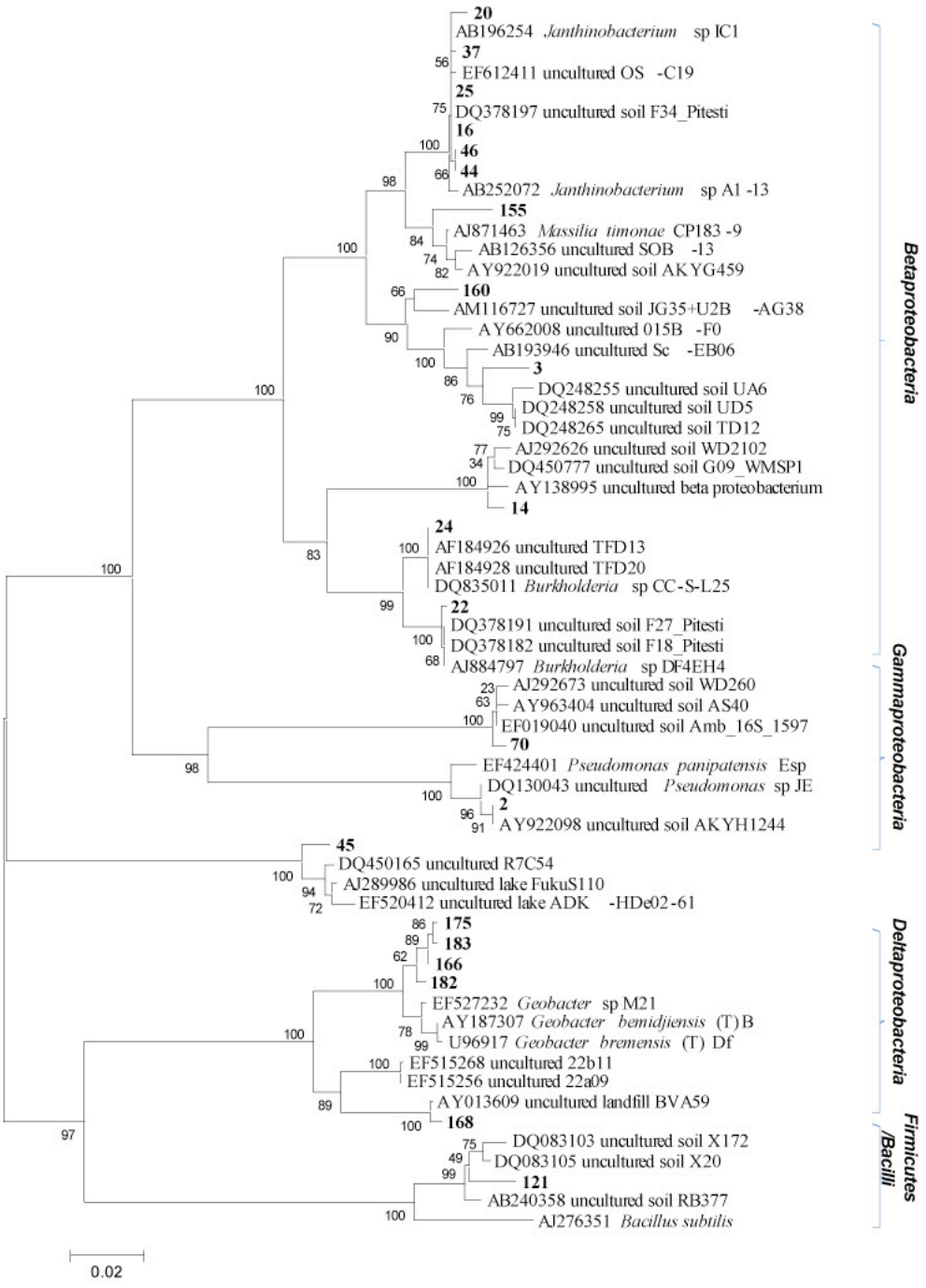

Figure 4. Phylogenetic tree of 16S rRNA gene sequences related to the Proteobacteria obtained from ^{13}C-acetate labeled DNA extracted from California soils. Numbers correspond to DGGE bands as listed in Table 4.

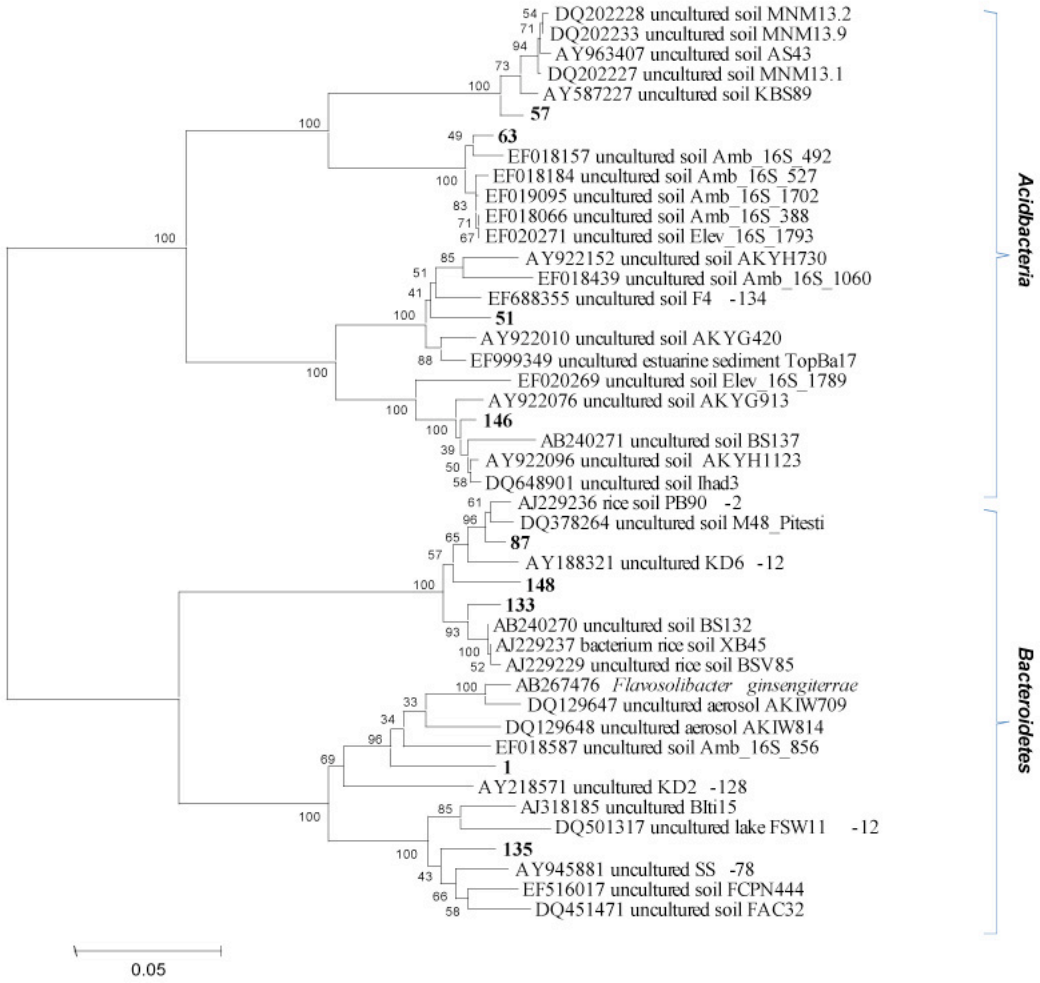

Figure 5. Phylogenetic tree of 16S rRNA gene sequences related to the Acidobacteria and Bacteroidetes obtained from ^{13}C-acetate labeled DNA extracted from California soils. Numbers correspond to DGGE bands as listed in Table 4.

To assess a large breadth of California soil types, we have focused on several well-studied soil sequences from around the state. Soil sequences, i.e., soils with a similar developmental background subjected to different environmental conditions, have received great attention because they allow for natural experiments that would be impossible on a laboratory scale. For example, chronosequences can be used to examine changes in soil development over timescales well beyond a single human lifespan, in the range of tens to millions of years. Climosequences, or elevational gradients, allow studies of weather effects and other elevational parameters on soil development. Biosequences allow studies of different plant cover effects on soil development.

Such systems have been used extensively in California, allowing studies on such varied aspects of soils as formation and pedogenesis mechanisms (Anderson et al. 2002; Dahlgren et al. 1997; Merritts et al. 1991; Moody et al. 1995; Wood et al., 2005), weathering processes (Harden 1988; Ulery et al. 1995; White et al. 1996), organic matter accretion (Lilienfein et al. 2004; Lilienfein et al. 2003; Sollins et al. 1983), variation in soil nitrogen abundance and/or composition (Brenner et al. 2001; Lilienfein et al. 2004; Ulery et al. 1995; Yu et al. 2003), phosphorous adsorption (Lilienfein et al. 2004), and temperature-driven exchange of soil and atmospheric carbon (Trumbore et al. 1996). Soil sequences have also been the focus of several microbiological studies (Burke et al. 2003; Crews et al. 2000, 2001; Dunfield and King 2004; King 2003; Kitayama 1996; Ohtonen et al. 1999; Wardle et al. 2004; Yu et al. 2003). These studies have provided insight into the influence of environmental factors on microbial community composition and function. However, few of these studies utilized the less biased non-culture based methods described here, and none have used such methods to link microbial community composition and function.

The selected soil sequences represent eight of the major California ecological subregions (Sierra Nevada and Sierra Nevada foothills are considered two ecological subregions) as designated by the USDA Natural Resources Conservation Service (Goudey and Smith 1994; Miles and Goudey 1997) and are among the best studied soils in the state (*table 1*). As such, they provide two major advantages: 1) soils within a sequence originated from the same parent material and have other environmental features in common, thereby minimizing variation between the soils and allowing us to focus on fewer variables, and 2) they are among the best studied soils in the state and there is significant background literature and information on each. We plan to use collected and published data on soil physical and chemical parameters (e.g., soil age, parent material composition, particle size distribution, pH, TOC, TON, TIC, exchangeable ions, weather and climate, and plant cover) to examine their effects on acetate-utilizing microbial community composition. Significant physical, chemical, soil taxonomic, and other studies have been performed on these soils, providing a significant data source to assist with interpretation of our data.

An advanced molecular biology method, which uses stable isotope labeling combined with rRNA gene methods, allows the linkage of a metabolic activity and the identity of the microorganism carrying out that activity. In this approach, known as stable-isotope probing (SIP), the culture or unknown population is fed a substrate labeled with ^{13}C, the heavy stable isotope of carbon (Lueders, et al. 2004; Manefield et al. 2002; Murrell and Radajewski 2000; Radajewski et al. 2000; Whitby et al. 2001). Cells, and their constituent components, which utilize the substrate as a carbon source become labeled. DNA or RNA is extracted from the cells or sample and centrifuged in a CsCl density gradient. Because the labeled nucleic acid is denser than unlabeled nucleic acid, it migrates to a different position in the gradient. Nucleic acids from each portion of the gradient are isolated and the rRNA genes are characterized, allowing for identification of community members that are the source of ^{13}C-labeled DNA. Thus, use of SIP can identify individual microbial species responsible for assimilating a particular substrate in the environment. To date, this approach has been used with substrates metabolized by only a small portion of the community, such as methane, methanol, or phenol (Hill et al. 2000; Lueders et al. 2004; Manfield et al. 2002; Murrell and Radajewski 2000; Radajewski et al. 2000). In a previous study, we developed SIP for use with acetate, a compound that is theoretically metabolized by nearly all heterotrophic organisms.

In this study, we collected the soils, characterized their physical and chemical characteristics, determined the AUR and SUE, extracted DNA, and performed 16S rDNA DGGE, incubated soils for the optimal time and at the optimal acetate concentrations, separated labeled from unlabeled DNA, and characterized the acetate utilizing bacterial community by DGGE on 32 soils from seven different soil sequences. We also completed the sequence and phylogenetic analyses of the aerobic incubations. These analyses indicate that 1) no direct correlation between location and acetate utilizing microbial community composition is apparent, 2) diversity of acetate utilizing microbes is limited, 3) members of the Firmicutes genera *Bacillus* and *Clostridium* are primary acetate utilizing microbes in all (or nearly all) soils, 4) members of the Betaproteobacteria orders Burkholdariaceae and Oxalobacteraceae are primary acetate utilizers in many, but clearly not all, California soils, and 5) in a few soils, Acidobacteria and Bacteroidetes also are primary acetate utilizers. It appears that in upland soils (which all of the studied soils are), denitrification is a major pathway by which acetate is consumed under suboxic conditions, thereby directly linking the carbon and nitrogen cycles in unmanaged California soils. We plan more detailed statistical analyses of these data in the future to clarify these results further.

These results will directly address the goals of the Kearney Mission and will provide important information regarding bacterial community distribution across the regional and landscape scale in California. As critical as it is to have inventories of soil carbon in California to understand the relationship of the California carbon cycle to global climate change, as stated in the Kearney Mission, it is perhaps equally critical to have inventories of the organisms and processes affecting the composition, size, and liability of that carbon. To our knowledge, this study is the first regional survey of specific bacterial functional groups using non-culture based methods.

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