

**Sulfate Reduction in the Oxic vs. the Anoxic
Zone in Sippewissett Salt Marsh
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INTRODUCTION

A recent study from Solar Lake in Sinai, Egypt showed that recognized genera associated with sulfate reduction were found to represent 30% of the population of a highly oxic cyanobacterial mat (Minz et al., 1999). This finding is surprising given the theory of the use of preferred electron acceptors with depth in bacterial mats. Many sulfate reducers have the ability to tolerate oxygen and some *Desulfvibrio* species have been shown to reduce oxygen to water, and couple this reaction to energy conservation (Cypionka, 2000). However, these oxygen-reducing SRB have not been found to divide in the presence of oxygen and so in this sense are obligate anaerobes. It is therefore interesting that they would make up such a large percentage of the bacterial population of the oxic zone of a microbial mat. The objective of this study was to determine whether a similar phenomenon also occurs in Sippewissett Salt Marsh in Woods Hole, MA, which would imply that it may be a wide-spread phenomenon.

Identification of dissimilatory sulfite reductase (*dsr*) genes within a community has been used to determine the presence of sulfate reduction. Dissimilatory sulfite reductase is a key enzyme in the sulfate reduction pathway and catalyzes the reduction of bisulfite to sulfide, which is the greatest energy-yielding step of sulfate respiration. This method is more reflective of the actual SRB community than traditional 16S analyses because of the inability to directly determine an organism's metabolism based on 16S phylogenetic relatedness. In addition, highly specific primers (DSR1F and DSR4R) are consistently used to amplify this gene, and many well-described studies have determined that they do not amplify (a) assimilatory sulfate reductase genes, (b) sulfite (but not sulfate) reducers, or (c) reverse sulfite reductase genes (Wagner et al., 1998).

16S is useful, however, in more general community analyses and is used in this study to estimate the similarities of the bacterial communities of Sippewissett and Solar Lake, and to verify the presence of sulfate reducers in each zone.

A variety of techniques including microelectrode profiling, culturing, T-RFLP community analyses, and RFLP identification of *dsr* genes were used to gain insight into the extent of sulfate reduction within the oxic and anoxic layers of this mat community.

MATERIALS AND METHODS

Sample collection. A mat sample was retrieved from Sippewissett Salt Marsh at low tide on a sunny day, from an area which appeared to have an extensive oxic layer based on coloration of the sediment. This mat was maintained on a water table at the Marine Biological Laboratory in Woods Hole, MA and sampled on Day 2.

S²⁻ and pH microelectrode analyses of the mat were used to estimate the depth of the oxic zone. 1cm-diameter cores were taken and the layers were sampled. Layers were separated for analyses from the oxic zone (0-10mm, no to little H₂S: sample A), the oxic/anoxic

transition zone (10-14mm, increasing [H₂S]: sample B), the colorless zone (14-20mm, decreasing [H₂S]: sample C), and finally the deeper black zone (20-26mm, increasing [H₂S]: sample D). First cores were immediately used for direct shake culturing and parallel cores were taken for DNA extraction from the same depth layers.

Direct Shakes. Sulfate reducing bacteria (SRB) media was made with a sea water base and autoclaved in a Widdel Flask. The media was cooled under a N₂/CO₂ atmosphere and consequently supplemented with a trace element solution and vitamin mix. 30ml/L .8M NaCO₃ was purged with CO₂ in the head space and autoclaved closed, then added to a liter of the sea water base. 1M Na₂S given a N₂ headspace and sterilized, then added to the media at 1.5ml/L. pH was adjusted to 7.3 and the media separated into 50ml bottles for later use.

Filter-sterilized FeSO₄•7H₂O was added to one shake series to 9.4μM (vs. 7.55μM in original media). Sample B was used as an inoculum for this series. These series were compared to the 7.55μM B series and percentage of black colonies/series estimated. Shakes consisted of 3 ml of a 3% agar (supplemented with 1.5%NaCl₂), 6ml SRB media, and 1ml inoculum. Lactate was added to 10mM directly before shaking was performed. Hungate tubes with the outlined ingredients were maintained at 40° C until inoculated. Approximately 1g soil from each layer was added separately to these tubes and 7 1:10 dilutions were carried out immediately. Inoculated bottles were put into room temperature water baths until solid. Each tube was then gassed with an 80/20 N₂/CO₂ mix and left upside-down at 30° C for several days. Tubes were monitored for growth.

Aerotaxis assays. "Quick-and-dirty" aerotaxis assays were performed with the help of Dr. Heribert Cypionka. Red band-forming colonies from Series B (from layer B), dilution 10⁻³ were extracted from the shake tube and suspended in fresh SRB + lactate media on a slide. An air bubble was trapped below the coverslip and formation of bands or directed motility monitored.

Community analysis. DNA was extracted for each layer with a MoBio Soil DNA kit following the manufacturer's instructions. The soil sample weight was recorded and purified DNA was quantified. PCR for 16S T-RFLP bacterial signatures was performed for each layer. PCR conditions were refined with respect to DNA and BSA concentrations and annealing temperature of the labelled bacterial 16S forward primer 8F-Hex (5'-AGAGTTTGATCCTGGCTCAG-3'), and unlabelled reverse primer 1492R (5'-GGTTACCTTGTTACGACTT-3'). Successful PCRs were performed with minimal inhibition using 1μl each primer (8F-Hex and 1492R) from 20pmol/ul stocks, 6.6-10ng DNA, 3.5μl 1% BSA, 5μl 10XPCR buffer (w/o MgCl₂), 4μl MgCl₂, 4μl dNTP mix (from 2.5mM each dNTP stock), 0.5μl Taq polymerase, and water to 50μl. DNA was denatured for 45s at 94° C, then 35 cycles of 94° C for 15s, 54° C for 30s, and 72° C for

1min. Final extension was at 72° C for 7min. Samples were maintained at 4° C until retrieved.

Bands of the appropriate size were visualized by gel electrophoresis (1% agarose) and the corresponding PCR products purified with the Microcon-100 spin column according to manufacturer's instructions. DNA was quantified and restriction digests were carried out with RSA I using 200ng purified PCR product, 1µl 10X RSA I restriction buffer, .5µl RSA I, and sterile water to 10µl. Digests were kept in a water bath at 37° C for 60min, then heat-inactivated at 80° C for 30min. Samples were sent away for T-RFLP analysis.

Dissimilatory sulfate reduction analysis. Dissimilatory sulfite reductase (*dsr*) genes were amplified from the same DNA as was used for community analysis. Each layer's *dsr* genes were amplified with the labelled forward primer DSR1F-Hex (5'-AC[C/G]CACTGGAAGCACG-3') and the unlabelled reverse primer DSR4R (5'-GTGTAGCAGTTACCGCA-3'). PCR conditions were optimized to 1µl each primer (DSR1F-Hex and DSR4R) from 20pmol/ul stocks, 6.6-20ng DNA, 2.5µl 1% BSA, 5µl 10XPCR buffer (w/o MgCl₂), 4µl MgCl₂, 4µl dNTP mix (from 2.5mM each dNTP stock), 0.5µl Taq polymerase, and water to 50µl. DNA was denatured for 45s at 94° C, then 35 cycles of 94° C for 15s, 54° C for 30s, and 72° C for 1min. Final extension was at 72° C for 7min. Samples were maintained at 4° C until retrieved. PCR products from each layer were visualized by gel electrophoresis in order to confirm the presence of the correct band size. PCR products were then cloned into a TA Cloning kit (Invitrogen) according to manufacturer's instructions. The following day the clones were used in a PCR reaction with the same concentrations of solutions as the primary reaction, except for the use of 2.5µl 1%BSA. 5 clones per layer were visualized by gel electrophoresis.

Restriction digests were performed with 5µl PCR product, 1µl RSA I digestion buffer, .5µl RSA I, 1µl Acetylated BSA, and 2.5µl sterile water. DNA was digested for 2 hours and immediately run on a 1.7% MetaMorph agarose gel. Banding patterns were compared between samples and within each sample.

RESULTS

Microelectrode profile (Table 1) showed a sharp increase in H₂S at 10mm on Day 1 (right) and more layering on Day 2. The first column shows where samples were taken on Day 2 and a map of the H₂S profile with depth is found in the discussion. The equation for the standard curve for H₂S is $y = -112x - 788.57$ where $y = mV$ and $x = \log[S^{2-}]$. The equation for the standard curve for pH is $y = -29.35x + 370.77$ where $y = mV$ and $x = pH$. These equations were used to extrapolate the H₂S profile below.

	DAY 2			DAY 1	
Sample Layer(Day1)	Depth (mm)	pH (mV)	H2S (mV)	pH (mV)	H2S (mV)
A	0	97	-120		
	1	97	-109		
	2	98	-108	78	-75
	4	99	-105	72	-67
	6	99	-104	66	-63
	8	97	-100	63	-62
	10	95	-120	61	-401
B	12	95	-195	69	-415
	14	95	-339	71	-417
C	16	95	-171	73	-420
	18	97	-170	76	-423
	20	97	-205	79	-424
D	22	97	-326		
	24	97	-392	89	-425
	26	97	-399		
	28	97	-398		
	30	97	-399	93	-426

Table 1: Please note that Day 2 is displayed before Day one and that Sample Layer only refers to samples taken on Day 2.

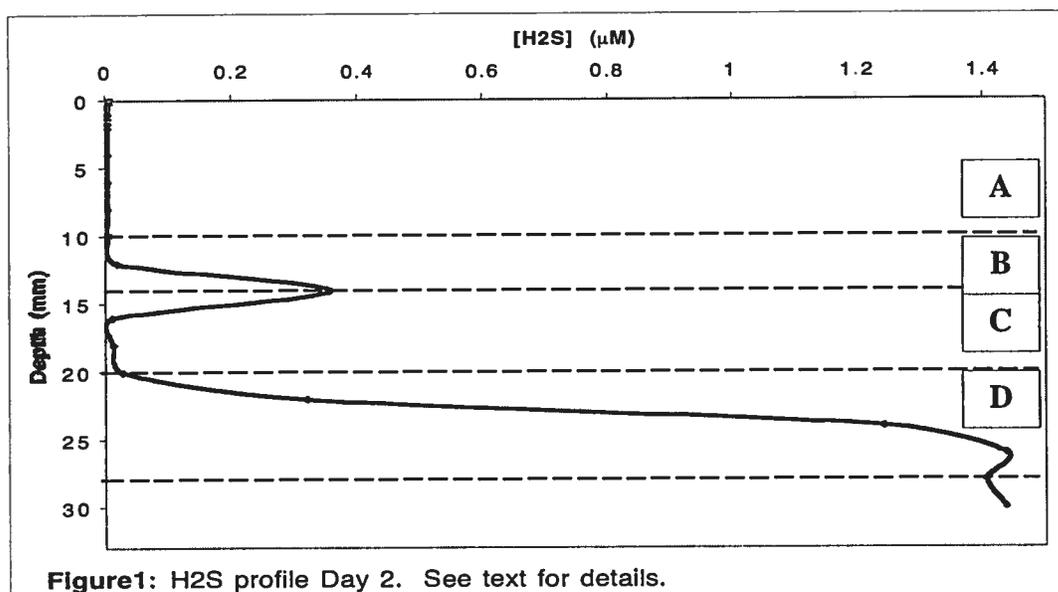


Figure 1: H₂S profile Day 2. See text for details.

Direct shakes resulted in colony formation from all layers of the mat. Band formation was observed by red colonies in shakes from Layer B but no aerotaxis was observed. 16S amplification resulted in faint bands which were purified together in order to gain enough product for T-RFLP analyses. Because of the time limitations I was unable to

reamplify any PCR products. Figure 1 (following page) shows the amplified PCR product for layer A, C and D (the product for B was obtained earlier with refining of the PCR conditions).

Figure 2 (page 6.2) shows the peaks resulting from T-RFLP analyses. Small peaks were ignored and all peaks below 35 base pair disregarded because of concern for noise in the graph. The remaining peaks were used with the Ribosomal Data Base Project's web program (<http://rdp.cme.msu.edu>) to look for sulfate reducers and any other interesting organisms. Table 2 (discussion) shows many of the microbes which came up in the database.

Amplification products from dsr primers were less successful(though bands were visualized- Figure 1, columns C, D, A, and B), and were therefore cloned into TA vectors in order to screen for the correct size product. Unfortunately I made the mistake of only screening 5 clones from each layer and so was unable to conclusively exclude any data points. Figure 3 shows amplification products of 10 of those clones.

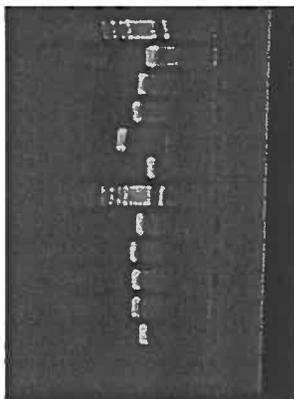


Figure 3: Amplification products from clones expected to have dsr inserts.

Figures 4 and 5 show RFLP analysis on these PCR products. Incomplete digestion occurred with the Layer D samples and redigestion was not attempted because of lack of restriction enzyme and time. These were excluded from analysis. Banding patterns were enumerated for each sample and between samples.

FIGURE 1

Lanes 16S-A,
16S-B,
16S-D

are 16S amplification
products used for
T-RFLP after
digestion.

Lanes C-DSR,
D-DSR,
A-DSR,
B-DSR

are amplification
products from
DSR primers.

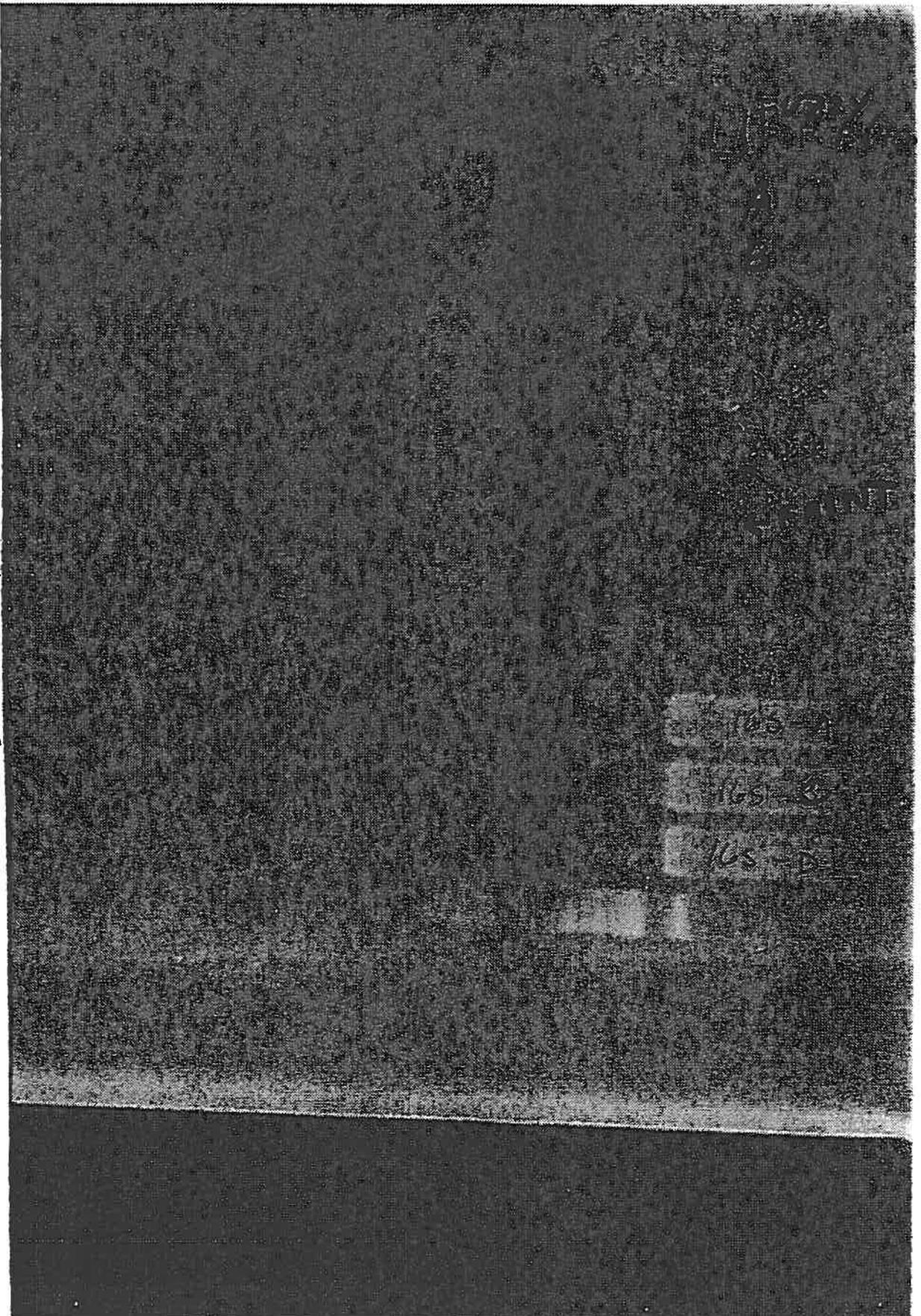


Figure 2

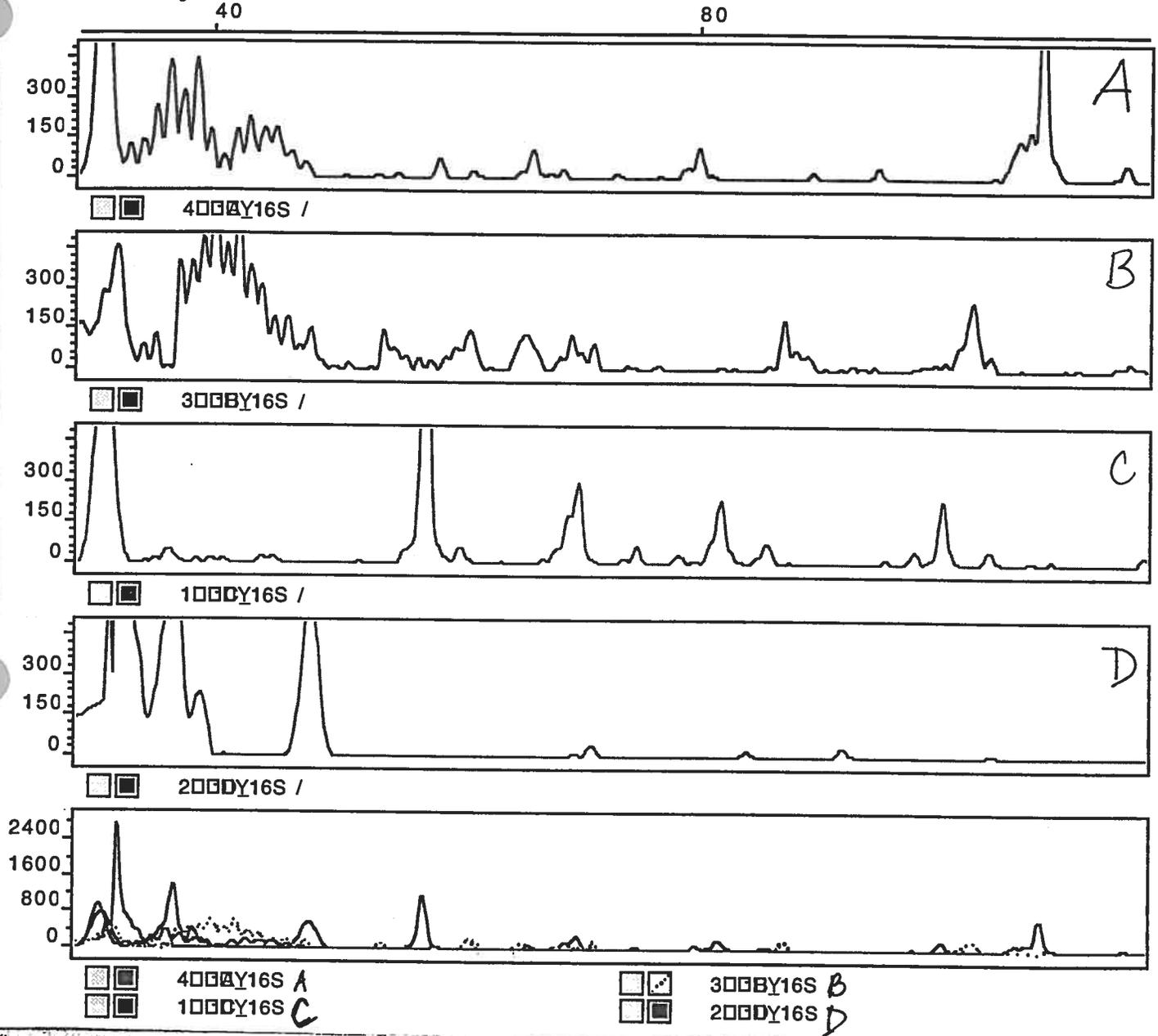


FIGURE 2: T-RFLP analysis results

"A," "B," "C," & "D" refer to those layers &
the last graph includes peaks from all layers

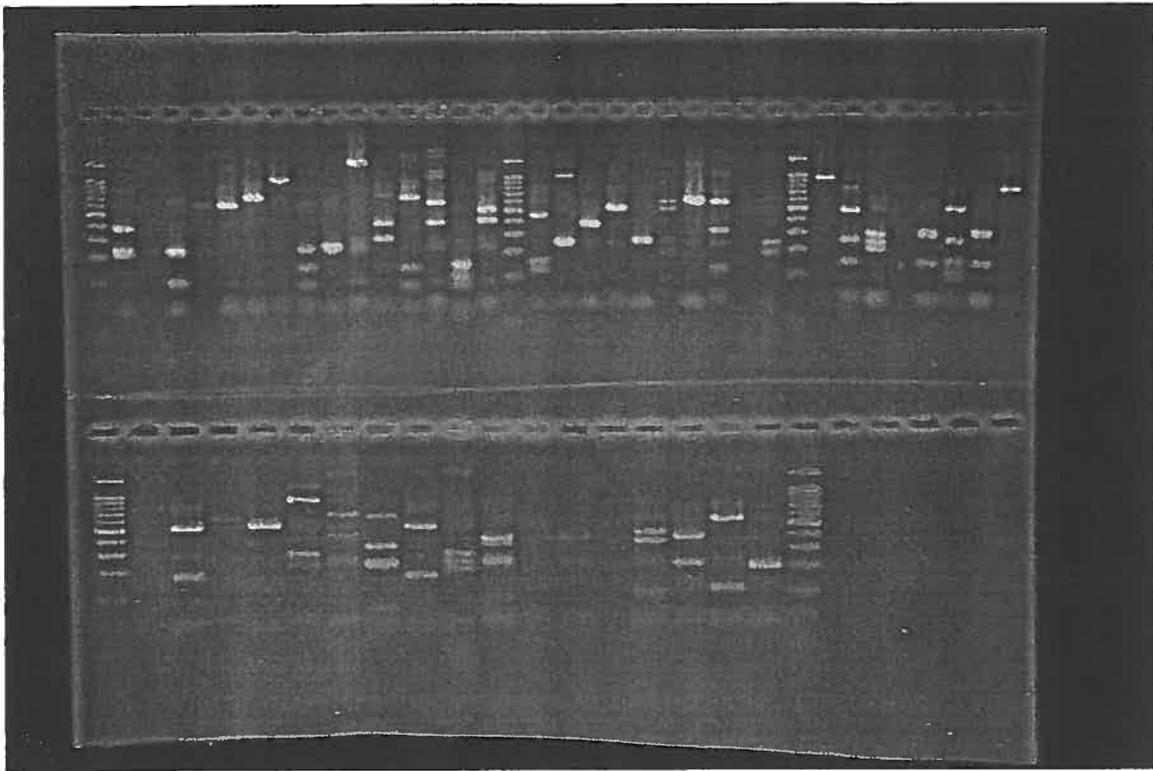


Figure 4: Restriction digests. Layer A includes all columns till the third ladder, and the following lanes are Layer B.

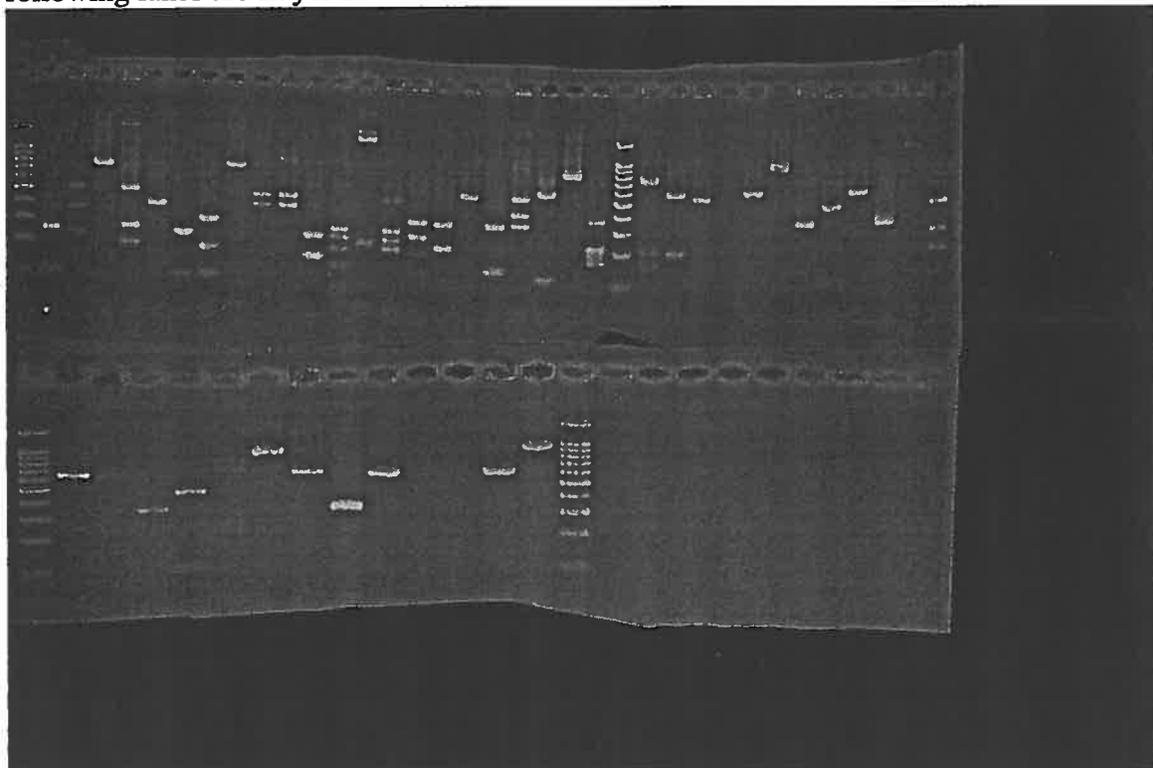


Figure 5: Restriction digests of RFLP *dsr* genes. All lanes until ladder 2 belong to layer C, the following lanes belong to Layer D.

DISCUSSION

Microelectrode analyses on Day 1 and Day 2 showed extensive migration of the anoxic zone. The Day 1 profile showed very low $[H_2S]$ until 10mm where there was a dramatic increase from virtually zero to $\sim 1.5\mu M$. The anoxic layer divided on Day 2 into 2 zones (Fig1). The profile showed that there was a high $[H_2S]$ layer just below the oxic zone in addition to the higher $[H_2S]$ zone at depth.

These zones could be distinguished by their black color- the first forming a black band followed by a colorless layer, and finally the max $[H_2S]$ zone below. Dotted lines in Figure 1 indicate breaks of sampling intervals. The first oxic zone (0-10mm) is sample A, followed by samples B, C, and D. Sample B was predicted to be one sulfate reducing zone, which is indicated by the increased sulfide. Zone C is likely the H_2S oxidation zone, but would in theory be occupied by sulfate reducers due to possible beneficial associations with sulfide oxidizers.

Direct shakes of the bacteria resulted in both white colonies and black colonies. SRB colonies would be expected to be black in color due to their H_2S production, which reacts with the Fe^{2+} in the media to precipitate black FeS . In the parallel shakes with an additional $1.85\mu M [FeSO_4]$, the percentage of black colonies was higher, indicating that some of the colonies which appeared white in color were in fact sulfate reducers. Na_2S was used as a reductant and so aerobic growth is unlikely. Due to the use of lactate as a carbon source, the growth of fermenters is possible. It would have been beneficial to do parallel shakes without SO_4 in order to determine approximately which percentage of white colonies are in fact fermenters.

A band of red colonies formed near the gas phase of the B samples with $7.55\mu M FeSO_4$. Dr. Cypionka confirmed after microscopic analysis that these colonies were likely *Desulfovibrio*'s and that the red color was due to the oxygen-reducing cytochrome c in their membranes. Cells were small, highly motile vibrios. Four attempts at aerotaxis failed, but may have been due to many factors which resulted in an abnormal environment for the cells. The formation of the band in the shakes, however, indicates aerotaxis.

T-RFLP-based community analyses were screened for the presence of any organisms known to reduce sulfate. SRB were found in all layers of the mat, though their numbers in the microbial population could not be determined with these methods because of PCR bias. The genera identified revealed a different SRB community (Table 2) in this mat than that found in Solar Lake, which primarily consisted of *Desulfonema* and *Desulfococcus*-like organisms. Given the limited number of sequences of the Ribosomal Database Project's database, it is possible that the Solar Lake sulfate reducers (or similar organisms) are in fact found here. The highest diversity of SRB was found in Layer C compared to all other zones based on percentage of peaks belonging to sulfate reducers.

Based on the decrease in sulfide in this zone, it is probably dominated by a few genera of sulfide oxidizers.

	Layer A	Layer B	LayerC	Layer D
Desulfacium	X	X	X	X
Desulfosarcina			X	
Desulfomicrobium			X	
Desulfovibrio		X		
Desulfohalobium		X		
Desulfothiovibrio		X	X	

Table2: Primary SRB found by T-RFLP

A Sorenson Index was used to analyze the respective diversity between layers. Using the equation $2[AB]/A+B$ (where AB= the number of peaks in both layers A and B, A= total number of peaks in layer A, and B= total number of peaks in Layer B), one can get an idea of which layers are made up of similar organisms. Using this index, I found similarities between sequential layers for the most part exceeded that between separated layers (Fig 6).

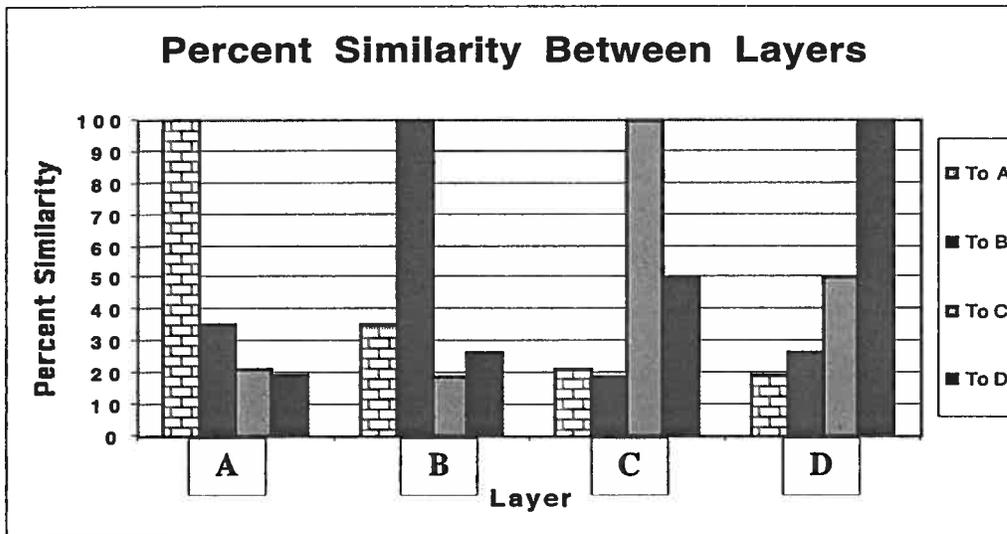


Figure 6: Sorenson Index based on T-RFLP peaks.

RFLP was used as a tool to determine whether dissimilatory sulfite reductase (dsr) genes, which are characteristic of sulfate reducing pathways, are present throughout the mat layers. RFLP is also beneficial in that it gives insight into the diversity in dsr genes within a given layer of a mat. Minz, et al. also recently found that the highest degree of diversity in dsr genes was found in the oxic layer of such mats (Minz, et al., 1999). My choice of RFLP as an aid in elucidating this diversity is a reflection of work which showed that the diversity in nitrite reductase genes could be clarified with T-RFLP

(Braker, et al., 2001). RFLP, however, provides higher resolution into the diversity of dsr genes because of its dependence on more than one restriction fragment.

An example of one of these gels (Figure 3) shows that amplification products averaged approximately 800bp in size rather than the expected 1900bp fragment. This can be explained (see below), but examples such as lane 5 cannot be convincingly explained and are possibly invalid. Unfortunately the ladders ran at different rates on this gel and the resolution is therefore not great. I believe that the last columns ran much faster than the first, based on the angle of smearing of the gel. Unfortunately Layer D did not cut completely and was excluded from analysis. I expect that this is because of inhibition from some factor from the soil, as a the same digestion solution was used as with Layer C and a high degree of inhibition resulted from PCR analyses, especially from the Layer D sample.

Analyses of fragment lengths showed the highest diversity of dsr genes to be in samples from Layer A. Table 3 gives the number of unique banding patterns within and between layers. This shows that Layer B has the highest diversity of dsr genes within a single layer, but that it has no unique dsr genes when compared to the other layers. This may be because Layer B would be considered the moore typical environment in which one would expect sulfate reducers to be found. Here they would have lower oxygen concentrations to deal with and a large supply of carbon sources from photosynthesis above. The organisms in the oxic zone are under more stress and therefore must evolve more (because of their difficulties in tolerating oxygen). The organisms below probably have less of a carbon source and this added stress may result in mutated dsr genes which are better able to maximize energy yield from carbon. However, the number of genes found is very low and so more sampling must be done before this argument can be made convincingly.

	A	B	C
WITHIN A LAYER	16/22	14/18	13/22
BETWEEN LAYERS	3/22	0/18	3/22

Table 3: Number of unique dsr genes within and between layers, based on banding patterns of digests.

An alignment of the dsr genes referred to in the Solar Lake study with the primer pair DSR1F and DSR4R resulted in the predicted amplification of a 1.9kb fragment.

However, when dsr genes from 8 organisms (*Desulfomonas pigra*, *Archaeoglobus profundus*, *Olavius algarvensis*, *Desulfovibrio africanus*, *Desulfomaculum kuznetsovi*, *Desulfomaculum aeronautica*, *Desulfomaculum putei*, and *Desulfosarcina variabilis*) were chosen at random from Genbank and aligned with DSR1F and DSR4R, half of the genes' expected products were ~800-900bp long (fig 8). This is consistent with some of the results received from PCR amplification of Sippewissett samples, where ~800-900bp

Desulfomonas_pigra [strain_Archaeoglobus_profundus_Olavius_algarvensis_sulfate_Desulfovibrio_africanus [st_Desulfotomaculum_kuznetsovi_Desulfotomaculum_aeronautic_Desulfotomaculum_putei_Desulfosarcina_variabilis_DSR1F*
DSR4R_REVCOMP

Desulfomonas_pigra [strain_

GGGACGGCAACAAGCTGTCCATCAAGACCGCCGACTGCTGCGCTGCATG
ACTGATGCATCTGGT--ATCGTGAAAGCTGTGATGGATGAACCTACGAA
ACGACGGCAAAAAGCTGTACATCGACACCGCCAACCTGACCCGCTGCATG
GGGACGGCTCCAAGCTCTCCATCAAGACCGCTGACTGTGTGCGCTGCATG
TGGAGACGGCAAGCTGGTGATCGACAACAAGGAATGCTGCGCTGCATG
TTGATGGCAACGAAGCTGACCATCGACAACCGCTAACTGACCCGTTGCATG
GGGACGGCAACGAAGCTGACCATTGACAACCGCAACTGACCCGTTGCATG
ACGAAGCGCGCAACTCGCCATCAACACAAAAGAGTGTACCCGCTGCATG
ACCCACTGGAAG

CACTGCATCAACACCATGCCTCGCGCTCTGCACATCGGTGACGAACGCGG
TACTTCACCGACCACAAGCTCCCAGCTCTGTGTA-----GA
CACTGCATCAACGTGATGCCGCGCCCTGAGAATTGGTGATGACCGCGG
CACTGCATCAACACCATGCCCGCGCTCTGCACATTGGTGACGAACGCGG
CACTGCATTAACGTGATGCCGCGAGGCGCTCAGACCTGGCACGGATACCGG
CACTGCATCAACAAAATGTGCAAAGCCCTGCGTCTGGCGATGATCGTGG
CACTGCATCAACAAGATGTGCAAAGCCCTGCGCCCTGGTGACGACCGCGG
CACTGCATCAACGTTATGCCGCGTGCACATCGGTGATGACCGCGG
CACG-----

AGCCAGCATCCTCGTGGGTGCTAAGGCCCCCGTGGTGACGGCGCT-CAG
ATTTCCCTTGCTTGCTG-TGCTAACATGTGTGGAGCAGTCCACGCTTCAG
TCTCTCCATTCGGTGGTGCCAAGGCTCCTATTCTCGACGGTGCC-CAG
CGCCAGCATCCTCGTGGGCGCAAGGCTCCCGTGGTTGACGGCGCG-CAG
CGTGAGCATCCTGGTGGGTGCAAAGCCCCATCCTGGAAGGGCGCA-CAA
TGCTACCATCTTAGTTGGTGGTAAAGCTCCCATTCTGCAAGGTGCT-ATG
CGCTACCATCCTGATCGGTGGTAAAGCTCCTATTCTCCAAGGCGCT-ATG
TTGTTCCATGCTGGTGGGCGCAAGGCTCCGATTCTCGACGGCGCT-CAA

ATGGGTTGCTGCTGGTGCCTTC--ATCTCCTGTGAAGCTCCCTACGAT
ACATTTCCGATGGTGGAGTCCACAGAAAAGTTCCTAAGATAGATGACGAT
ATGGCGTCCCTGCTGGTTCCTTT--GTTAAGGTTGAAGAACCTTACGAT
ATGGGTTGCTGCTCGTGCCTTC--GTCTCCTGTGAAGCTCCCTACGAC
ATTTCCGAGGTGATCGTGCCTTC--ATGAAAGCGGAGCCGCTATGAC
ATGGGTTGGGTAATCGTACCCTTC--ATGAAACTAGAATCCGGCTCCGGC
ATGGGTTGGGTAATCATCCCTTC--ATGAAGCTTGAACCTCCCTACGAA
ATGGGCTCCCTGCTGGTGCCTTC--GTCAAGGTTGAAGAGCCCTACGAT

GAAA-----TCAAAGAAGTCATCGAAAAGATCTGG-GACTGGTGGATGG
GCTG-----T--AGAAGGACTTGTGAGATCCCGTCAACTGTACAGGT
GAGA-----TCAAAGAAGTTATTGAATCCATTTGG-GACTGGTGGATGG
GACG-----TCAAAGAAGTCATCGAAAAGATTGG-GACTGGTGGATGG
AACG-----TTAAGAAGTTATCGAGAAAATCTGG-GAATTTCTGGATGG
TACGAAGAACTCAAAGAGTTCTTAGAAGCTTGTGG-GAGTGGTGGGATG
GAAC-----TCAAAGAGTTCTCGAAAAGTTGG-GAATGGTGGGATG
GGAA-----TCAAAGAAGTTATCGAATCCATCTGG-AATTGGTGGGTTGG

AAGAAGGCAAGAACCGCGAACCGGTGGGCGAAACCATGAAGCGTCTTTCC
TGTCCAACCTGGTCTCTGAAGCCAGACATGAAGAGGAAGACAGTAATACT
AAGAAGGCAAGAACCGCGAACGCTCTCGGCGAGCTGATCAAGCGCCAGGGC
AAGAAGGCAAGAACCGCGAACCGGTGGGCGAAACCATGAAGCGTCTGTCC
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AAAACGGTAAAGACCGGTGAGCGTGTGGTGAGCTGATCCTCCGCTCGGGT
AAGAAGGCAAGAACCGCGAACGCTCTCGGTGAGCTGATTAAGCGTCAGGGA

TTCCAGAAGCTGCTCGAAGTCACCGACACCGAAGCCGCTCCTTACCAGT

DSR1F →

~730k
DOWNSI
FROM 0₂
DSR1F

These sequences
begin in the
dsr x sub,
approx. 730
downstream
from published
amplification
site

DSR1F →
is where
amplification
begins for
1/2 of these
genes

Archaeoglobus profundus
 Olavius algarvensis sulfate
 Desulfovibrio africanus [st
 Desulfotomaculum kuznetsovi
 Desulfotomaculum aeronautic
 Desulfotomaculum putei
 Desulfosarcina variabilis
 DSR1F*
 DSR4R_REVCOMP

CGACAAGGACAAGTGCATGTACTG---TGGAACTGTTACACAATGTGT
 TTCCAGAACTGCTTGTGCGCCACCGGTATCGACCCGGTTATCCAGCACGT
 TTCCAGAACTGCTGGAAGTCACCGACACTCCTGCTGCCGCTTACCACGT
 TTGCCAAGTTCTCTGGAGGTAATCGGCCTGCCGCTGCACCCGCAATGAT
 ATGCGTAACCTTCTTACGCGGTATTGGCCGTGAACCCATTCTCAAATGGT
 ATGCGCAACTTTCTGCGCGCTGTGACCTCGAGCCCGTTCCGCAATGGT
 TTCCAGAACTGCTCGAAGTGACCNAATCGGTCCGGTCCCGCAT---GT

Desulfomonas pigra [strain
 Archaeoglobus profundus
 Olavius algarvensis sulfate
 Desulfovibrio africanus [st
 Desulfotomaculum kuznetsovi
 Desulfotomaculum aeronautic
 Desulfotomaculum putei
 Desulfosarcina variabilis
 DSR1F*
 DSR4R_REVCOMP

CACGGCTCCCG---CTCCAACCCGTCATCTTCTTCAAGGAAGAAGAAGT
 CCGGGTATGCCAATCTTCGACCCAGAGAAT-----GACGGTGTAGCAAT
 TCAGGAACCGAG---ACACAATCCGTACATCTTCTGGAATGAAGACGAAGT
 CAAGGAACCCCG---TTCCAATCCGTACATCTTCTCAAGGAAGAAGAAGT
 CAAGGCGCCGCG---TGCCAACCCGTACATCTTCTGGAAGGAAGGATGT
 AGTTGCACCACG---TGCAAACCCCTTCTTCTTCTGGTGG-----
 CCTGGCTCCGCG---TGCAAACCCCTTCTTCTTCTGGTGGCCTGAAGAAGT
 TTTGGAACCCG---CCAACCCCGTACATCTTCTGGAAGGAAGACGAGGT

Desulfomonas pigra [strain
 Archaeoglobus profundus
 Olavius algarvensis sulfate
 Desulfovibrio africanus [st
 Desulfotomaculum kuznetsovi
 Desulfotomaculum aeronautic
 Desulfotomaculum putei
 Desulfosarcina variabilis
 DSR1F*
 DSR4R_REVCOMP

GCC-----CGGCGGCTG-GACCCGCGACCTGGCTGAATTCCGCAAACG
 ACT-----CGTGGGAGGTAAGCTATCAGATGCTAGATACCCGCCACAG
 TGA-----CGGCGGATG-GGATCGCGACATTGACGCATTCAGAAAAGA
 CCC-----CGGTGGTTG-GGATCGCGACCTCGCCGCTTACCGCAAACG
 ACCCGGCGGATTTGAAAGA-GACATTAAAGATTT-CCGGGCCAG--ACAC
 -----CCTGAAGAA-GTTATCCAAGATT-----
 -----TGTTCAAGA-CTAATTGAAAACA-TGCAGGCTG--ATGC
 -----TGCGGCTG-GGATCGCGACATCA-CGGAATTAG--AGAT

Desulfomonas pigra [strain
 Archaeoglobus profundus
 Olavius algarvensis sulfate
 Desulfovibrio africanus [st
 Desulfotomaculum kuznetsovi
 Desulfotomaculum aeronautic
 Desulfotomaculum putei
 Desulfosarcina variabilis
 DSR1F*
 DSR4R_REVCOMP

CCATCAACGCTAGTTTA-AGGGGGAGAAGAACATGGCTTTTATTTCTTCC
 CTTCGAAGGTTGT----AGTGCCATGGCTACCAAACAACCCACCAAGAT
 CCATCAGAGATAG-----GGAGGAACGACCATGGCATTGTATCCTCA
 CCATCAACGCTAGTCAA-AGGGGGAGAAAACATGGCTTTTATTTCTTCC
 AAGAGGTAATAAT----AAGGGGGTTTGTGGNATGGCTTTTCCACTG
 AATTGAAAAATGT----GCGGGATTATACTGCATAAAAATAATCAAACGG
 -CGCATAAAATAAT----GAAACGGGGAAGAGGTGAACCCACATT-----
 TCACCAGAGATAGG----AGGGGAGGATATATATGGCATTATTTCTTCA

Desulfomonas pigra [strain
 Archaeoglobus profundus
 Olavius algarvensis sulfate
 Desulfovibrio africanus [st
 Desulfotomaculum kuznetsovi
 Desulfotomaculum aeronautic
 Desulfotomaculum putei
 Desulfosarcina variabilis
 DSR1F*
 DSR4R_REVCOMP

GGGTA----CAATCCCG-----CAAACCGATGGAAGGCCGCATCAC-
 GGCCA----GAAGTCGT-----TCAGACAGTTAAGAAGATACTTGAGA
 GGTTA----CAATCCTG-----ACAAACCGATGGAACCCGATCTC-
 GGGTA----CAATCCCG-----CAAACCGATGGAAGGCCGTATCAG-
 GGAAATACGGTAAATTCGATCCGCAGAAATCCAACCAAGACAGGATTAC-
 GGAGA----GGTGAACC-----ATTTGGCACAAGCAAGA--AC-
 -GGCA----CAAGC-----AAGAAGTATGAT----ATCGGACCGC-
 GGATA----CAATCCCG-----ACAAACCGATGGAACCCGATCAC-

Desulfomonas pigra [strain
 Archaeoglobus profundus
 Olavius algarvensis sulfate
 Desulfovibrio africanus [st
 Desulfotomaculum kuznetsovi
 Desulfotomaculum aeronautic
 Desulfotomaculum putei
 Desulfosarcina variabilis
 DSR1F*
 DSR4R_REVCOMP

CGACATCGGCCCCACAAGTATG-ACGA-ATACTTCCCGCCGGTCAATCAA
 CTTGGGCAGCCACGCAGAGAAGTACGAGAGAGTTGCTG--AGTGGATAC
 GGACATCGGACTCAGAAAATTTG-ACGA-ATTTTATCCGCGCGTCAATGC
 TGACATCGGCCCCACAAGTATG-ATGA-ATACTTCCCTCGGTTATCAA
 GGATATTGGTCCGCGCCACTACT-GGGA-AACTTCCCGCCAGCAATCA
 CGATATCGGACCGCCGCTCTATA-AAGA-AATGTTGCCGCGCCGTTATCCT
 CGCT-----CTATAAAGACATG-TTA-----CCGCTATTATCAA
 CGATATTGGTCCGAGAAATACG-ATAA-ATTTTATCCGCGCCGTCATCGC

Desulfomonas_pigra [strain_Archaeoglobus_profundus
 Olavius_algarvensis_sulfate
 Desulfovibrio_africanus [st
 Desulfotomaculum_kuznetsovi
 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

AAAGAATTTTCGGCAAGTGGCTGTACCACGAAATTTGAGCCCGGCGT--
 ACAGAAT--CGGCTGGGAGAGATTCCTTCGAG--TTGACAGGCTTGGAGTTC
 CAAAAATAAAGGCAAATGGCTTTATCACGAAATTTGAGCCCGGCGC--
 AAAGAACTTCGGCAAGTGGCTCTATCATGAAATTTGAGCCCGGCGT--
 AAAGAACTACGGCAAGTGGCTGTACCACGAGATCCTCGAGCCCGGCGT--
 AGAAAACTATGGAAAATGGCGTTATCACGAAATTTGTAAGCCCGGCGT--
 AGAAAACTACGGCAAGTGGCGTTATCATGAAATCTTAAAGCCCGGCGT--
 CAAAAACAAAGGCAAATGGCTGTATCACGAAATCATTAAACCCGGCGT--

Desulfomonas_pigra [strain_Archaeoglobus_profundus
 Olavius_algarvensis_sulfate
 Desulfovibrio_africanus [st
 Desulfotomaculum_kuznetsovi
 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

GCTGATGCACGTGGCCGAAGGTGGCGACAAGGTGTACACCGTCCGCGTGG
 ACTGA-GAAGCTGATCGAAGACTACCACA----GAACACCGTACCTCTAC
 GTTGGTGCACGTGGCTGAGTCCGGCGACGAAGTCTACACGGTTCGCTGGC
 GCTCATGCACGTGGCCGAAAGCGGCGACAAGTGTACACCGTCCGCGTGG
 GCTGGTACACGTTTCCGAAACGGGGGACAAGGTCTACACCGTACAGGTGG
 TCTGGTACACGTGTCCGAATCCGGAGCCAAGCTGTACACTGTACGTGCTG
 GCTCGTACACGTATCTGAAACCGGAGCCAAGCTGTACACCGTACGCGCTG
 ACTTGTTCACGTGGCCGAAAGCGGCGACGAAGTCTACACCGTACGCTGTG

Desulfomonas_pigra [strain_Archaeoglobus_profundus
 Olavius_algarvensis_sulfate
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 Desulfotomaculum_kuznetsovi
 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

GCGGCACCCGCACCATGTCCATCACCCACATCCGCGAAGTGTGGCAGATC
 ACGACATTCAGAACATCCGCAGCTTTCAGATG-GTAAAGTGTAT-ATACT
 GCGCGCGCGGCTGATGAGCGTCACCCACATCCGCGAAATCTGCGACATT
 GTGGTACTCGTACCATGTCTGTACGCTGATCCGTGAAGTGTGGCAGATC
 GTTCGGCCCGCTTGATGACCGTACAGCAGTGCGGGAAATCTGCGATATT
 GTTCTCCGCGCTAATCAGTATCTTTTTCATTTCGCGACATCTGCGATCTA
 GTTCTCCGCGTCTGATCTCTATCTACTTCATTCTGAAATCTGTGATCTG
 GCGGCGCCGCTGTGATGAGCGTCACCCATATCCGCGAAATCTGCGAAATC

Desulfomonas_pigra [strain_Archaeoglobus_profundus
 Olavius_algarvensis_sulfate
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 Desulfotomaculum_kuznetsovi
 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

GCCGACAAGTA-CTGCGGCGGTTACCTGCGCTGGACCACGTAACAACA
 ACTTTAACCTAATTTTTTTGGTGACCTAAAATGGCCGA--GGAAATAAAT
 GCCGGCGCCA CTGCGACGGGTACCTGCGCTTACCACCCGTAACAATA
 GCCGACAAGTA-TTGCGAAGGCTGTCTGCGTTGGACCACCCGTAACAACA
 GCCGACAATA-CTGCGACGGCTATGTGCGTTTACCACCCGCAACAACA
 GCTGACAATA TTGCGATGGCTACCTGAGATTTACCAGCCGTCACAACG
 GCAGACAATA-CTGCGATGGTTACCTGAGATTTACCAGCCGCCACAACA
 GCCGACAAGCA TTGTGACGGGCATCTGCGCTTACCACCCGTAACAACA

Desulfomonas_pigra [strain_Archaeoglobus_profundus
 Olavius_algarvensis_sulfate
 Desulfovibrio_africanus [st
 Desulfotomaculum_kuznetsovi
 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

TCGAATTCATGGTGAAGACGAAGAAACCATGAAGGCCCTGCGGACGAC
 AAGGA-----CGAGCTAAAGCAGAAAGTTATAGAATAC TTGCA-GAAGA-
 TCGAGTTCATGGTGGATTCTAAAGACAAAGTCCAGCCGCTGATCGACGAC
 TTGAATTCATGGTTCGACAGCGAAGAGGGCATGAAAGCCCTGCGGACGAC
 TCGAGTTTATCGTGGACAGCCTTGAGAAGGTGGAAGCCCTCAAGAAGAT
 TGAATTCCTT-----GCTTCCGA---CGAAAG---CAAGTTGAT
 TCGAGTTCCTGTTAAGCGATGAAACCAAATGATCCCTGATTGCTGAC
 TCGAATTCATGGTTCGACGACAAATCAAAGTGGACCCGCTGATCAAGGAT

Desulfomonas_pigra [strain_Archaeoglobus_profundus
 Olavius_algarvensis_sulfate
 Desulfovibrio_africanus [st
 Desulfotomaculum_kuznetsovi
 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

CTGAACAGCCGCAAGTTTCGACGGCGGTTCTTCAAGT-TCCCCGTGGGCG
 ---AGCCTTGGCAAGTTAGA---GATTTGGCGAAAA-TAC---TAAGAG
 CTGGAAAGCCGCAAGTTTCGACGGCGGATCCTTCAAGT-TCCCCGTGGGCG
 TTGAACAGCCGCAAGTTTTCGACGGCGGTTCTTCAAGT-TCCCCGTGGGCG
 CTGTGGAGCCGCAATTTGTTTCCGGGAGCTACAAGT-TCCCCATCGGCG
 CC-TTTAATTTGAAGACCTG-----AAAGCTATGGGCGTTCCCGTTGGTG
 CTGAA---TGCAA---TGGGTG-----TTCCCGTTGGCG
 CTGGAAAGCCGCAAGTTTTCGACGGCGGAGCTTCAAGT-TCCCCATCGGCG

Desulfomonas_pigra_[strain_Archaeoglobus_profundus
 Olavius_algarvensis_sulfate
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 Desulfotomaculum_kuznetsovi
 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

GCACCGGGCTGGTATCAGCAACATGGTGCACA--CCCAGGGCTGGGTGC
 TCAAGAAGAAAGAACTTGATAAGATAGTGCAGGAGCTTATAAATGAAGGA
 GCACCGGTGCCGGCGTGACCAACATCGTGCACA--CCCAGGGCTGGATCC
 GCACCGGGCTGGCATCAGCAACATGGTGCACA--CCCAGGGTTGGGTGC
 GCACTGGGGCCAGCATCACCAATATCGTGCACA--CGCAGGGGTACGTGC
 GTACTGGTAACCTCCATTACCAACATCGTTCACA--CCCAAGGCTGGGTAC
 GTACCGGTAACCTCCATTACCAACATCGTTCACA--CCCAGGGTTGGGTAC
 GCACCGGTGCCGGCGTAACCAACATCATCCATA--CCCAGGGCTGGATCC

-----TGCGGTA

DSR4R

Desulfomonas_pigra_[strain_Archaeoglobus_profundus
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 Desulfovibrio_africanus_[st
 Desulfotomaculum_kuznetsovi
 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

ACTGCCACACCCCCGCCACCGACGCCTCCGGCCCGGTGAAGTGCCTGATG
 AAAGCAGCATATTGGTCGTCTGGTTCACACATATATAACTACTCCAGA
 ACTGCCACACCCCCGCCACCGACGCCTCCGGCCCGGTGAAGGCGACCATG
 ACTGCCACACCCCCGCCACCGACGCCTCCGGCCCGGTGAAGGCTGTGATG
 ACTGCCACACTCCGGCCACTGACGCCTTCCTCCCTGGTCAAGGCCGTGATG
 ACTGCCATACCCGGCTACCGATGCTTCGGGTATCGTTAAAGCCGTTATG
 ACTGCCATACTCCGGCTACCGATGCTTCGGGTATCGTGAAGCCGTCATG
 ACTGCCACACCCCCGCCACCGACGCCTCCGGTCCGGTCAAGGCCACCATG

-----ACTGCTACAC-----

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 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

GACGCCATCTTTGATGACTTC-AAGGATATGCGTCTGCCCGCTCCCGTGC
 GAAGCTCAAGGAGATGG-----AAGAAAAGAGATCAGGAATTTGATTTTT
 GACGTCTGTTTCGACGACTTTAAACAGCACAGA-ATGCCGGCCAGATAC
 GACGCCGTGTTTGAAGACTTCAAGTCCATGCGT-ATGCCCGCTCCCGTGC
 GATGACCTGTTTACTACTTC-ACCGCATGACCCTGCCGGCCAAGGTAC
 GATGAGTTGTTTCGAGTACTTC-ACCAGCATGAAGCTGCCGCAAGATGA
 GATGAGCTGTTTCGAGTACTTC-ACCACCATGAAACTGCCTGCCAAGCTGA
 GACGAGCTGTTTCGCCGACTTCCAGAATCATCGT-CTGCCGGCCAGCTGC

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 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

GCATCGCCCTGGCTGCTGCATCAACATGTGGGGCGCGGTGCACTGCTCC
 ACTTCATTTTTAAC--CTATTTTGTTTTATTCGGT--CATACCTTACTGA
 GGGTTTCCCTGGCTGCTGTCTGAACATGTGGGGCGCGGTGCACTGCTCC
 GCATCGCCCTGGCTGCTGCATCAACATGTGGGGTCCCGTGCATGCTCG
 GGGTGGCGGTGGCTGCTGCCTGAACATGTGGGGTCCCGTGCCTGCTTCG
 GAATTGCTTTGGCTGCTGCCTGAACATGTGGGGTGTGTACTGCTCTC
 GAATTGCTTGGCTGCTGCCTGAACATGTGGGGTGTGTACTGCTCTC
 GTGTTTCCCTGGCTGCTGCCTGAACATGTGGGGCGCGGTGCACTGCTCT

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 Desulfotomaculum_aeronautic
 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

GACATCGGCTGTTGGGTATCCACCGCCAAACCGCCCATGATC CCACGA
 GAGACTCG-CGAGTGATTTAAAA-----
 GATATCGCCATTCGGGTACCACCGAAACCGCCCTGCTTGACCATGA
 GACATCGGCCCTTGTGGGTATCCACCGCCAAGCCCCCATGA CCACGA
 GACATCGCCCTGCTGGCATTACCGGAAGCCCCGATCATTGACCACGA
 GACATCGCTATCTGGGTATTCACAGAACCCTACCCAAGATTGACCATGA
 GACATCGCTATCTGGGTATCCACAGAACCCTGCCAGAAATCGACCATGA
 GATATCGCCATACTCGGCTATCACCGTAAACCGCCGCTGCTGGATCATGA

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 Desulfotomaculum_putei
 Desulfosarcina_variabilis
 DSR1F*
 DSR4R_REVCOMP

ATGGGCTGACCAGCTGTGCGAAATTCCTCTGGCTGTGGCCGCTGCCCA

 GTACCTGGACAAGATGTGTGAAATTCGGTGGCCATCGCGGCCGCCCCA
 ATGGGCTGACCAGCTGTGCGAAATTCCTCTGGCTGTGGCCGCTGCCCA
 GGTCAATTGATCAGGTCTGCGAGCTTCCCTGGTCAATTTCTTCTGCCCA
 AAACCTGCACAACTGTGCGAAATTCCTACTTTGACTGTAGCTGCCCA
 AAACCTGCACAAAGTTTGGCGAAATTCCTACTTCTGACTGTAGCTGCCCA
 ATATCTGGACAAGATGTGCGAAATTCCTGCTTGCATCGCTGCCCTGCCCA

fragments were amplified (Fig 3). Unfortunately the *dsr* genes for *Desulfacinum infernum* and *Desulfohalobium retbaense* (which can be expected from my samples based on T-RFLP patterns), or their congeners weren't available in genbank. It is highly possible that their *dsr* genes are the types which result in a ~900bp amplified fragment. In addition, confidence in these primers' specificity for *dsr* genes is high based on the literature (Wagner et al., 1998; Minz, et al. 1999a, Minz et al., 1999b). If this is the case, since I used the same PCR conditions as they published (but a higher [BSA]) the only PCR products from the original digestion should be the *dsr* genes. Since TA cloning only inserts fragments with A-overhangs which result from polymerization by Taq polymerase, these should be the only ones amplified from the clones. In addition to the screening of all clones prior to digestion, this study would be much more conclusive if more samples amplifications were performed at all levels, and if more than one digest was performed on each sample. Higher resolution is required for many of these analyses to be conclusive. Based on *dsr* analyses however, sulfate reduction was found in all layers and this follows what was found in the Solar Lake study.

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