16S rRNA Gene Microarray Analysis of Microbial Communities in Ethanol-Stimulated Subsurface Sediment

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A high-density 16S rRNA gene microarray were used to analyze microbial communities in a slurry of ethanol-amended, uranium-contaminated subsurface sediment. Of specific interest was the extent to which the microarray could detect temporal patterns in the relative abundance of major metabolic groups (nitrate-reducing, metal-reducing, sulfate-reducing, and methanogenic taxa) that were stimulated by ethanol addition. The results show that the microarray, when used in conjunction with geochemical data and knowledge of the physiological properties of relevant taxa, provided accurate assessment of the response of key functional groups to biostimulation.

DNA microarrays are widely used to monitor and characterize microbial communities in environmental samples (15, 18, 25, 30, 34, 36, 43). Microarray technology permits simultaneous interrogation of PCR amplicons or genomic DNA across a large number of probe sequences (6, 8, 9, 20, 41). Differences as small as one nucleotide base pair can be distinguished with an oligonucleotide-based microarray, although this degree of specificity is dependent on the sequence context (e.g. local melting temperature), hybridization conditions, and detection chemistry. Under optimal conditions, DNA microarrays can be used to efficiently screen a complex mixture of different sequences (40, 41).

During in situ bioremediation of uranium, mobile U(VI) is microbiologically (enzymatically) precipitated and immobilized as the insoluble U(IV) mineral uraninite (UO_2) (1, 21-24). A diverse range of microorganisms can reduce U(VI) to U(IV) under anaerobic conditions (37). Information on the status of microbial communities involved in U(VI) reduction and other electron-accepting pathways is important to understanding the spatial/temporal dynamics and overall efficacy of in situ uranium bioremediation (1). Although 16S rRNA clone libraries and/or microarrays have been applied to various U(VI) reduction systems (2, 4, 7, 16, 27, 28, 38, 39, 42), knowledge of the quantitative coverage of these techniques, particularly in situations where multiple groups of organisms are active, remains scant.

We report here on the use of a high-density 16S rRNA gene microarray (4) for analysis of microbial communities in a slurry of ethanol-amended, uranium-contaminated subsurface sediment from Oak Ridge National Laboratory (28). The microarray results are compared with parallel 16S rRNA clone libraries. Details on the set-up and execution of the experiment, including information on the design and use of the 16S microarray for analysis of sediment microbial communities, and methods for RNA extraction, preparation of clone libraries, and

aqueous/solid-phase chemical analyses, are available elsewhere (4, 28). Both the conventional clone libraries and the 16S microarray analyses were conducted with reverse-transcribed 16S rRNA extracted from sediment samples collected at six different time points during the experiment (see Fig. 1). The goal was to assess the extent to which these two approaches could provide insight into the microbial populations responsible for catalyzing the sequence of terminal electron-accepting processes (TEAPs) observed in the slurry incubation experiment.

A clearly defined temporal pattern of TEAPs took place in the ethanol amended slurries, with NO_3^- reduction , Fe(III) reduction (Fe(II) production), SO_4^{2-} reduction, and CH_4 production proceeding in sequence until all of the added electron donor was consumed (Fig. 1A). Acetate accumulated during ethanol metabolism (Fig. 1B), and was converted to CO_2 and CH_4 during the methanogenic phase of the experiment. Approximately 60% of $NaHCO_3$-extractable U(VI) was reduced during the Fe(III) reduction phase between 4 and 12 d in the ethanol-amended slurries (Fig. 1B).

Analysis of the 16S microarray data was confined to the top 100 subfamilies (out of a total of 1608) that exhibited the greatest variation in array intensity among the different time point samples. Hierarchical cluster analysis (4, 10) was used to detect correlations between subfamilies. Overall seven major groups were detected (Fig. 2A). A complete list of array intensities for the top 100 subfamilies and their group assignments is given in Table S1. The nitrate- and metal-reducing taxa that responded (based on the 16S rRNA clone libraries) most strongly to ethanol stimulation grouped together (cluster 2, Fig. 2B) in the cluster analysis, i.e. the pattern of variability in array intensity over time resulted the clustering of these taxa into one group. Likewise, the methanogenic *Archaea* that proliferated toward the end of the experiment also formed a distinct cluster (cluster 4, Fig. 2B). Other clusters bore no specific relationship to

patterns of redox metabolism, although the large initial decline in *Clostrideaceae* sequences observed in the clone libraries was reflected in the pattern of array intensity for clusters 1, 3, and 7 which included these taxa. The relatively large apparent change in *Clostrideaceae* abundance may be attributed to oxidative stress triggered by exposure of the anoxic, nitrate-depleted sediment inoculum to mM levels of nitrate. The relative intensity of the overall group signals did not change dramatically in relation to one another (maximum four-fold, Fig. 2B). Correlation analysis between array intensity for taxa detected by the 16S microarray and various geochemical parameters (e.g. ethanol, acetate, nitrate, or Fe(II) concentration; total electrons consumed; computed system redox potential) was performed within the R statistical programming environment (http://www.R-project.org) using the package 'multtest' (http://www.bepress.com/ucbbiostat/paper164) with correction for multiple observations performed using the Benjamini-Hochberg (BH) False Discovery Rate correction (3). This revealed a wealth of apparent significant correlations (R values > 0.95) (Table S2). However, none of the BH adjusted *p* values were significant due to the small number of geochemical data points relative to the large number of OTUs detected by the microarray.

Of specific interest was the extent to which the microarray detected temporal patterns in the relative abundance of major metabolic groups that might be expected to be stimulated by ethanol addition. We therefore examined average normalized array intensity for phylogenetic groups that include the major taxa that appeared (based on the chemical and clone library data) to be involved in ethanol metabolism. Analogous approaches have been used to assess the response of metal-reducing, sulfate-reducing, and methanogenic taxa during biostimulation for U(VI) and Cr(VI) immobilization (14, 33). Consistent with expectations, mean array intensities, binned at the family level, for *Geobacteraceae* (which includes *Geobacter* and related metal-reducing

organisms), *Rhodocyclaceae* (which includes *Dechloromonas* and related nitrate-reducers) and *Oxalobacteriaceae* (which includes *Herbaspirillum* and related nitrate-reducers) increased upon stimulation (Fig. 3).

A total of 90, 114, 87, 77, 104, and 134 clones were obtained from the six samples collected during the incubation experiment (28). The Greengenes (greengenes.lbl.gov) suite of tools (12) was used to reclassify the clone sequences using the same taxonomic database (G2_chip) upon which the 16S microarray is based. A complete list of the clone library sequence taxonomic assignments is given in Table S3. Approximately 50% of the 16S rRNA clones from the prestimulation (0 d) time point belonged to the family *Clostrideaceae*. Upon incubation with ethanol, sequences related to nitrate-reducing (*Herbaspirillum*, *Dechloromonas*) and metal-reducing (*Geobacteraceae*, including the genera *Geobacter*, *Pelobacter*, and *Trichlorobacter*) taxa became predominant in the 16S rRNA libraries, accounting for 66-88% of total clones (see Table 1 in ref. (28)).

The clone libraries and 16S microarray data revealed qualitatively similar temporal patterns for taxa that responded to ethanol stimulation as indicated by nitrate and Fe(III) reduction activity (Fig. 3). Correlations (r values) between family-level percent abundance in the clone libraries and mean array intensity were 0.93 ($p < 0.01$), 0.37 ($p = 0.46$), and 0.61 ($p = 0.19$) for *Oxalobacteriaceae*, *Rhodocyclaceae*, and *Geobacteraceae*, respectively. Array signals for *Rhodocyclaceae* and *Geobacteraceae* were not responsive when the relative abundance of these taxa in the clone libraries exceeded 15 %; the reason for this lack of response is unknown.

Although 16S rRNA sequences corresponding to sulfate-reducing bacteria were detected in both the clone libraries and on the 16S microarray, neither approach detected the proliferation of sulfate-reducing bacteria in conjunction with the brief period of sulfate consumption between

days 10 and 14 of the experiment (see Fig. 1) (data not shown). Sequences related to other potentially important physiological groups, e.g. metal-reducing taxa such as *Anaeromyxobacter* and *Shewanella*, which not present in the 16S rRNA libraries, were detected by the microarray, although there was little change in the array intensity for these groups over time (data not shown). Practical considerations prevented conventional 16S rRNA gene clone library analysis of archaeal taxa in this study. The 16S microarray, however, readily detected the major increase in methanogenic taxa during the latter stage of the experiment (Fig. 2D) when methane production took place (Fig. 1B).

As observed in other recent studies (11), the microarray detected a much larger number of microbial groups compared to the clone libraries (Table S4). Clone libraries are known to underestimate microbial diversity due to sequencing of an insufficient number of clones (32) and/or to preferential amplification of specific sequences leading to misrepresentation of sequence abundance within genomic DNA extracts (31, 35). The 16S microarray approach is also subject to such bias, since the DNA analyzed on the array was generated by PCR. Both the 927R (used for the clone libraries) and 1492R primers (used for the 16S microarray) target all known bacteria and have been widely used in previous microbial ecological studies (5, 13, 17, 19, 26, 29). Discrepancy in assessment of bacterial diversity due to differences in the primer sets used was thus probably insignificant, especially given that all of the sequences identified in the clone libraries were detected by the array. The possibility that the high diversity detected by the array was due to nonspecific hybridization (leading to false positives) can be discounted because of the high specificity and reproducibility of hybridization between probe and 16S rDNA targets hybridization and reproducibility (4, 38). Rather, the much greater diversity detected by the

microarray can be attributed simply to the very large number of probe sequences on the array compared to the relatively small number of 16S rRNA clones sequenced (11).

In summary, 16S microarray characterization of microbial communities in the biostimulated sediment was consistent with the results of conventional 16S rRNA clone libraries. Targeted analysis of array response for bacterial and archaeal taxa likely to respond to biostimulation (based on observed geochemical data) provided evidence for a response of these groups. The array detected a wide range of taxa not recovered in the clone libraries, but did not reveal obvious trends in the relative abundance of these taxa. Collectively the results agree with those of other recent studies where the 16S microarray was used to detect and monitor microbial population response to biostimulation (4, 14, 33). Although it seems unlikely that the array can on its own gauge the quantitative response of different taxa, when used in conjunction with general knowledge of the physiological properties of key taxa together with relevant geochemical data, the array can provide accurate assessment of the response of key functional groups (14, 33).

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References

1. Anderson, R. T., and D. R. Lovley. 2002. Microbial redox interactions with uranium: an environmental perspective, p. 205-223. *In* M. J. Keith-Roach and F. R. Livens (ed.), Interactions of Microorganisms with Radionuclides. Elsevier Science Ltd.

2. Anderson, R. T., H. A. Vrionis, I. Ortiz-Bernad, et al. 2003. Stimulating in situ activity of Geobacter species to remove uranium from the groundwater of a uranium-contaminated aquifer. Appl. Environ. Microbiol. 69:5884-5891.

3. Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B-Methodol. 57:289-300.

4. Brodie, E. L., T. Z. DeSantis, D. C. Joyner, et al. 2006. Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. Appl. Environ. Microbiol. 72:6288-6298.

5. Brosius, J., M. L. Palmer, P. J. Kennedy, and H. R. Noller. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli. Proc. Natl. Acad. Sci. 75:4801-4805.

6. Call, D. R., F. J. Brockman, and D. P. Chandler. 2001. Detecting and genotyping Escherichia coli O157:H7 using multiplexed PCR and nucleic acid microarrays. Int. J. Food Microbiol. 67:71-80.

7. Cardenas, E., W.-M. Wu, M. B. Leigh, et al. 2008. Microbial communities in contaminated sediments associated with bioremediation of uranium to submicromolar levels. Appl. Environ. Microbiol. 74:3718-3729.

8. Chizhikov, V., A. Rasooly, K. Chumakov, and D. D. Levy. 2001. Microarray analysis of microbial virulence factors. Appl. Environ. Microbiol. 67:3258-3263.

9. Chizhikov, V., M. Wagner, A. Ivshina, Y. Hoshino, A. Z. Kapikian, and K., and Chumakov. 2002. Detection and genotyping of human group A rotaviruses by oligonucleotide microarray hybridization. J. Clin. Microbiol. 40:2398-2407.

10. Culhane, A. C., J. Thioulouse, G. Perriere, and D. G. Higgins. 2005. MADE4: an R package for multivariate analysis of gene expression data. Bioinformatics 21:2789-2790.

11. DeSantis, T. Z., E. L. Brodie, J. P. Moberg, I. X. Zubieta, Y. M. Piceno, and G. L. Andersen. 2007. High Density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb. Ecol. 53:371-383.

12. DeSantis, T. Z., P. Hugenholtz, N. Larsen, et al. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72:5069-5072.

13. Egert, M., and M. W. Friedrich. 2003. Formation of Pseudo-terminal restriction fragments, a pcr related bias affecting terminal restriction fragment length polymorphism analysis of microbial community structure. Appl. Environ. Microbiol. 69:2555-2562.

14. Faybishenko, B., T. C. Hazen, P. E. Long, et al. 2008. In situ long-term reductive bioimmobilization of Cr(VI) in groundwater using hydrogen release compound. Environ. Sci. Technol. 42:8478-8485.

15. Gentry, T., G. Wickham, C. Schadt, Z. He, and J. Zhou. 2006. Microarray Applications in Microbial Ecology Research. Microb. Ecol. 52:159-175.

16. Holmes, D. E., K. T. Finneran, R. A. O'Neil, and D. R. Lovley. 2002. Enrichment of members of the family *Geobacteraceae* associated with the stimulation of dissimilatory

metal reduction in uranium-contaminated aquifer sediments. Appl. Environ. Microbiol. 68:2300-2306.

17. Humayoun, S. B., N. Bano, and J. T. Hollibaugh. 2003. Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. Appl. Environ. Microbiol. 69:1030-1042.

18. Kyselkova, M., J. Kopecky, M. Sagova-Mareckova, G. L. Grundmann, and Y. Moenne-Loccoz. 2009. Oligonucleotide microarray methodology for taxonomic and functional monitoring of microbial community composition. Plant Soil Environ. 55:379-388.

19. Lehours, A. C., C. Bardot, A. Thenot, D. Debroas, and G. Fonty. 2005. Anaerobic microbial communities in lake Pavin, a unique meromictic lake in France. Appl. Environ. Microbiol. 71:7389-7400.

20. Li, E. S. Y., and W. T. Liu. 2003. DNA microarray technology in microbial ecology studies - Principle, applications and current limitations. Microbes Environ. 18:175-187.

21. Lloyd, J. R., and J. C. Renshaw. 2005. Bioremediation of radioactive waste: radionucleotide-microbe interactions in laborartory and field-scale studies. Curr. Opin. Biotechnol. 16:254-260.

22. Lloyd, J. R., D. R. Lovley, and L. E. Macaskie. 2003. Biotechnological application of metal-reducing microorganisms. Adv. Appl. Microbiol. 53:85-.

23. Lloyd, J. R., J. Chesnes, S. Glasauer, D. J. Bunker, F. R. Livens, and D. R. Lovley. 2002. Reduction of actinides and fission products by Fe(III)-reducing bacteria. Geomicrobiol. J. 19:103-120.

24. Lovley, D. R., P. K. Widman, J. C. Woodward, and E. J. P. Phillips. 1993. Reduction of uranium by cytochrome c_3 of *Desulfovibrio vulgaris*. Appl. Environ. Microbiol. 59:3572-3576.

25. Loy, A., C. Schulz, S. Lucker, A. Schopfer-Wendels, K. Stoecker, C. Baranyi, A. Lehner, and M. Wagner. 2005. 16S rRNA Gene-Based Oligonucleotide Microarray for Environmental Monitoring of the Betaproteobacterial Order "Rhodocyclales" 10.1128/AEM.71.3.1373-1386.2005. Appl. Environ. Microbiol. 71:1373-1386.

26. Ludemann, H., I. Arth, and W. Liesack. 2000. Spatial changes in the bacterial community structure along a vertical oxygen gradient in flooded paddy soil cores. Appl. Environ. Microbiol. 66:754-762.

27. Michalsen, M. M., A. D. Peacock, A. M. Spain, A. N. Smithgal, D. C. White, Y. Sanchez-Rosario, L. R. Krumholz, and J. D. Istok. 2007. Changes in microbial community composition and geochemistry during uranium and technetium bioimmobilization. Appl. Environ. Microbiol. 73:5885-5896.

28. Mohanty, S., B. Kollah, D. Hedrick, A. Peacock, R. K. Kukkadapu, and E. E. Roden. 2008. Biogeochemical processes in ethanol stimulated uranium-contaminated subsurface sediments. Environ. Sci. Technol. 42:4384-4390.

29. Noll, M., D. Matthies, P. Frenzel, M. Derakshani, and W. Liesack. 2005. Succession of bacterial community structure and diversity in a paddy soil oxygen gradient. Environ. Microbiol. 7:382-395.

30. Okabe, S., M. Oshiki, Y. Kamagata, et al. 2010. A great leap forward in microbial ecology. Microbes Environ. 25:230-240.

31. Polz, M. F., and C. M. Cavanaugh. 1998. Bias in template to product ratios in multitemplate PCR. Appl. Environ. Microbiol. 64:3724-3730.

32. Schloss, P. D., and J. Handelsman. 2005. Introducing DOTUR, a Computer Program for Defining Operational Taxonomic Units and Estimating Species Richness. Appl. Environ. Microbiol. 71:1501-1506.

33. Tokunaga, T. K., J. M. Wan, Y. M. Kim, R. A. Daly, E. L. Brodie, T. C. Hazen, D. Herman, and M. K. Firestone. 2008. Influences of organic carbon supply rate on uranium bioreduction in initially oxidizing, contaminated sediment. Environ. Sci. Technol. 42:8901-8907.

34. Urakawa, H., J. Matsumoto, T. Hoshino, and S. Tsuneda. 2007. Direct profiling of rRNA in saline wastewater treatment samples using an oligonucleotide Microarray. Microbes Environ. 22:116-122.

35. VonWintzingerode, F., U. B. Goebel, and E. Stackebrandt. 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol. Rev. 21:213-229.

36. Wagner, M., H. Smidt, A. Loy, and J. Zhou. 2007. Unravelling Microbial Communities with DNA-Microarrays:Challenges and Future Directions. Microb. Ecol. 53:498-506.

37. Wall, J. D., and L. R. Krumholz. 2006. Uranium reduction. Ann. Rev. Microbiol. 60:149-166.

38. Wan, J., T. K. Tokunaga, E. Brodie, Z. Wang, Z. Zheng, D. Herman, T. C. Hazen, M. K. Firestone, and S. R. Sutton. 2005. Reoxidation of bioreduced uranium under reducing conditions. Environ. Sci. Technol. 39:6162-6169.

39. Wilkins, M. J., F. R. Livens, D. J. Vaughan, I. Beadle, and J. R. Lloyd. 2007. The influence of microbial redox cycling on radionuclide mobility in the subsurface at a low-level radioactive waste storage site. Geobiology 5:293-301.

40. Wilson, K. H., W. J. Wilson, J. L. Radosevich, T. Z. DeSantis, V. S. Viswanathan, T. A. Kuczmarski, and G. L. Andersen. 2002. High-density microarray of small-subunit ribosomal DNA probes. Appl. Environ. Microbiol. 68:2535-2541.

41. Wilson, W. J., C. L. Strout, T. Z. DeSantis, J. L. Stilwell, A. V. Carrano, and G. L. Andersen. 2002. Sequence-specific identification of 18 pathogenic microorganisms using microarray technology. Mol. Cell. Probes 16:119-127.

42. Wu, W. M., J. Carley, J. Luo, et al. 2007. In situ bioreduction of uranium (VI) to submicromolar levels and reoxidation by dissolved oxygen. Environ. Sci. Technol. 41:5716-5723.

43. Zhou, J. 2003. Microarrays for bacterial detection and microbial community analysis. Curr. Opin. Microbiol. 6:288-294.

Fig. 1. Time course of microbial metabolism in ethanol-amended slurries. Arrows indicate time points when samples were obtained for 16S rRNA analysis. Data are reproduced with permission from *Environ. Sci. Technol.* 2008, *42*, 4384-4390. Copyright 2008 American Chemical Society.

Fig. 2. (A): Heatmap and cluster analysis dendrograms showing the 16S microarray response of 100 subfamilies (shown on *y* axis) exhibiting the highest standard deviation among samples from different time point during the slurry incubation (shown on *x* axis). Major taxa are indicated by colors in the left-most block (blue = *Archaea*; green = *Actinobacteria*; purple = *Bacteroidetes*; orange = Bacillus; red = *Clostridia*/*Desulfotomaculum*/*Symbiobacterales*; brown = *Alphaproteobacteria*; pink = *Betaproteobacteria*; black = *Deltaproteobacteria*; cyan = *Gammaproteobacteria*; grey = Others). The orange bar to the left of the color code block indicates the distance threshold at which cluster/groups were defined. The blue to purple color gradient in the heatmap for the different time points represents increasing array hybridization intensity. The lines and numbers to the right of the heatmap indicate taxa included in the seven major groups determined by cluster analysis. (B): Variation of mean array intensity for the major response groups detected by the cluster analysis over time.

Fig. 3. Abundance of selected sequences, binned at the family level, from the 16S rRNA clones libraries (filled bars) and normalized array intensity for analogous taxa detected by the 16S microarray (open bars) over time during the sediment slurry incubation experiment.

Fig. 1

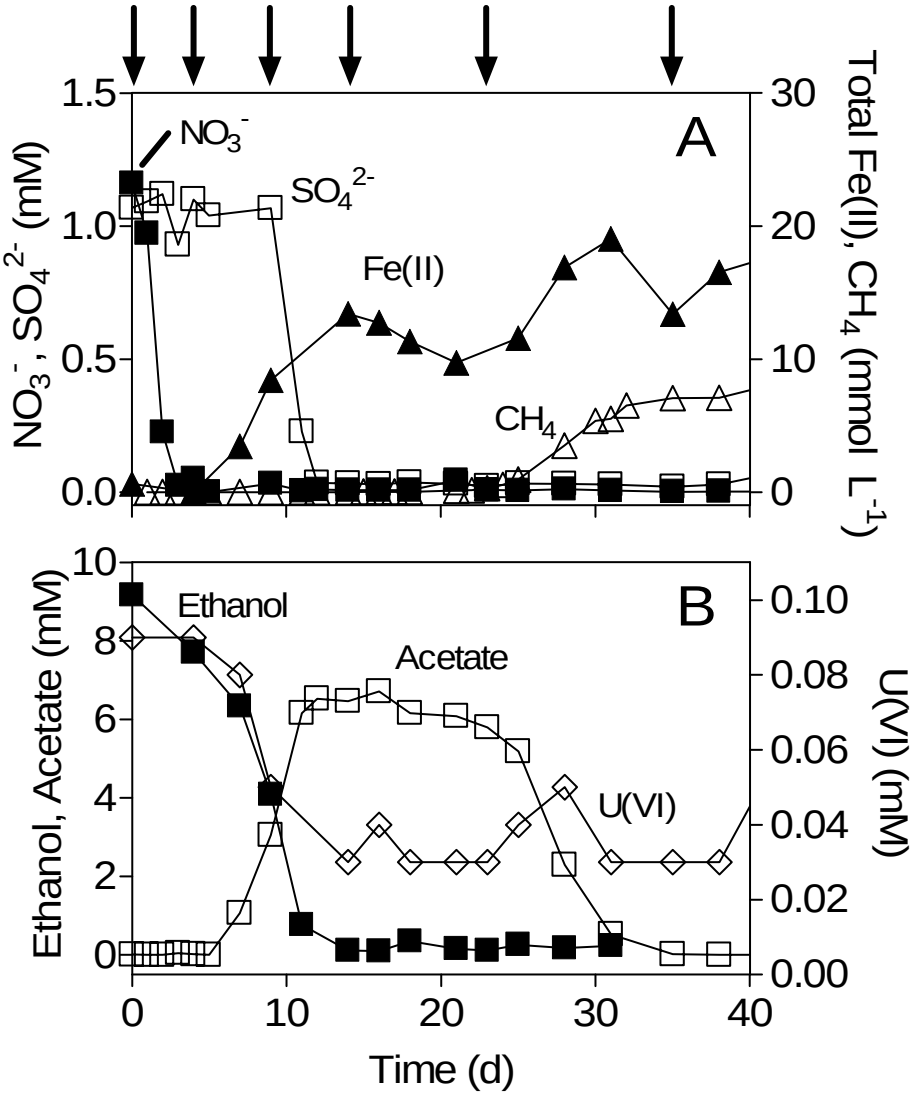

A
B
NO_3^-
SO_4^{2-}
Fe(II)
CH_4
NO_3^-, SO_4^{2-} (mM)
Total Fe(II), CH_4 (mmol L^{-1})
Ethanol
Acetate
U(VI)
Ethanol, Acetate (mM)
U(VI) (mM)
Time (d)
1.5
1.0
0.5
0.0
30
20
10
0
10
8
6
4
2
0
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40

Fig. 2

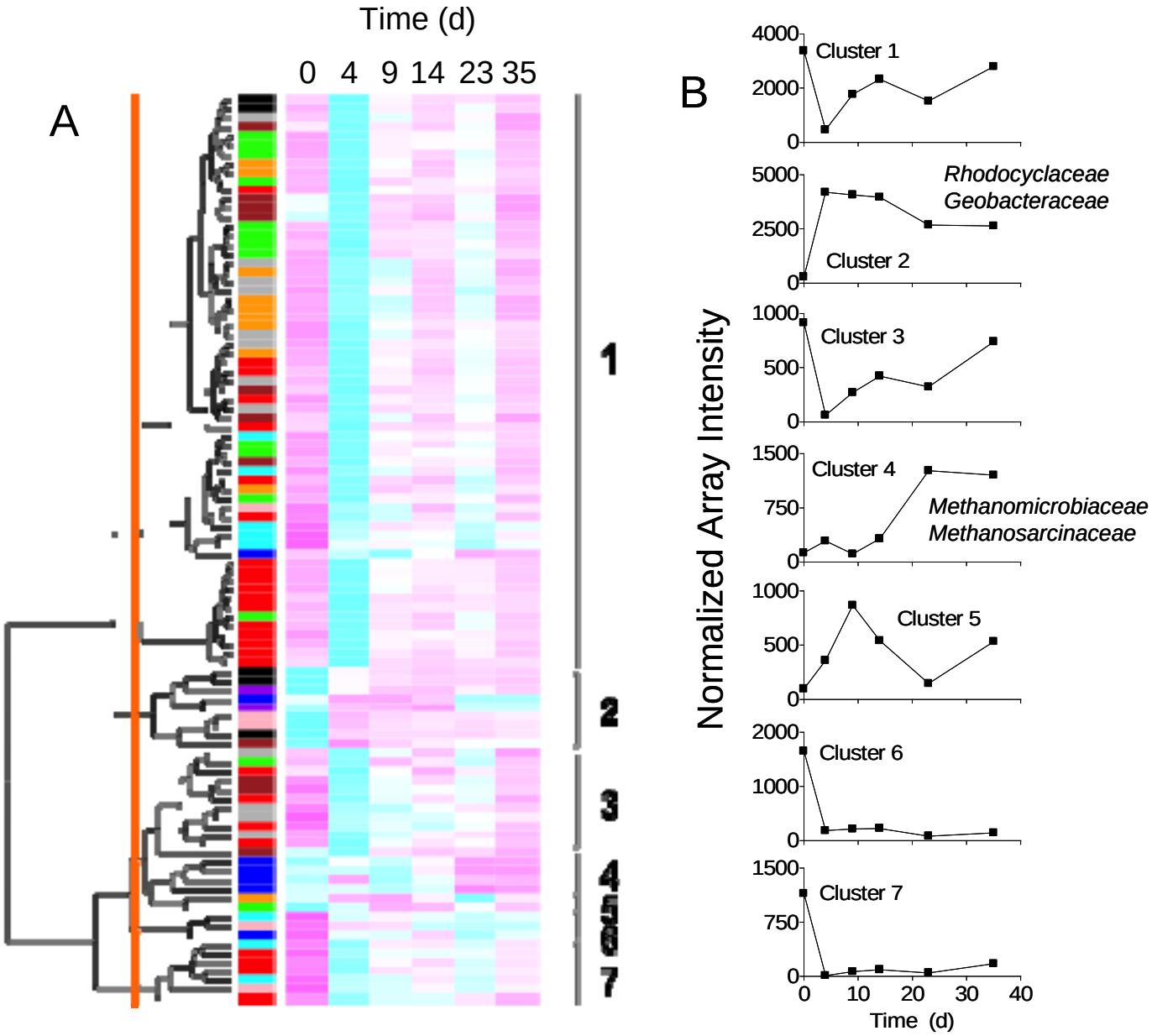
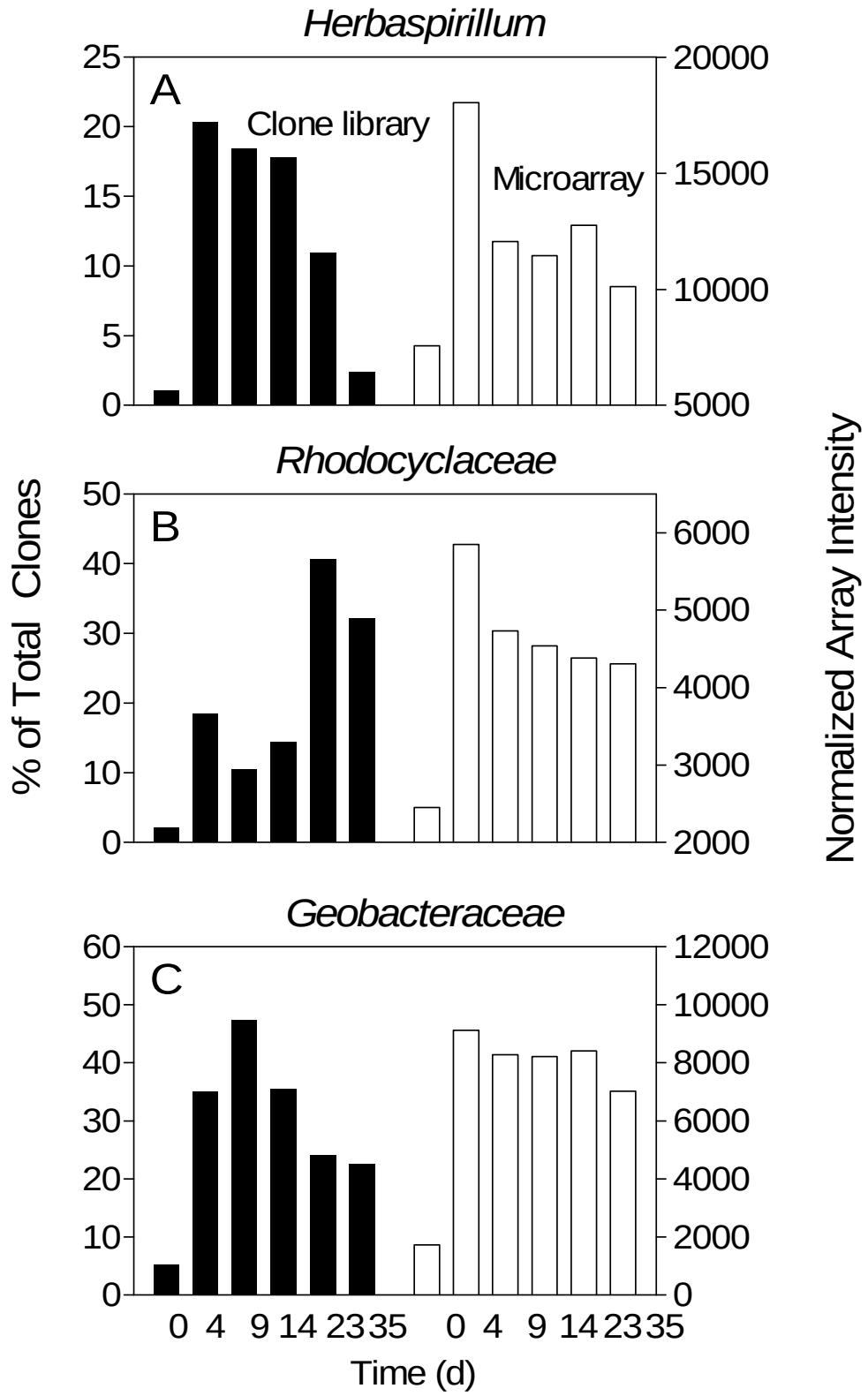

A
Time (d)
0 4 9 14 23 35
1
2
3
4
5
6
7
B
Normalized Array Intensity
Cluster 1
4000
2000
0
Cluster 2
5000
2500
0
Rhodocyclaceae
Geobacteraceae
Cluster 3
1000
500
0
Cluster 4
1500
750
0
Methanomicrobiaceae
Methanosarcinaceae
Cluster 5
1000
500
0
Cluster 6
2000
1000
0
Cluster 7
1500
750
0
0 10 20 30 40
Time (d)

Fig. 3


Herbaspirillum
A
Clone library
Microarray
Rhodocyclaceae
B
Geobacteraceae
C
% of Total Clones
Normalized Array Intensity
Time (d)
0 4 9 14 23 35 0 4 9 14 23 35

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