

Molecular Profiling of Microbial Communities from Contaminated Sources

DOE Environmental Management Project
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Introduction

Our project is to develop molecular methods for rapid characterization of microbial communities in contaminated ecosystems. We are exploring the use of 16s ribosomal DNA intergenic spacer regions (ISRs) to profile community composition. The choice proves to be a good one: there are 200-550 bases of 1 to 3 variable regions from which to choose species-specific probes, as well as 2-4 stretches of conserved sequence from which to develop universal PCR (polymerase chain reaction) primers (Fig. 1).

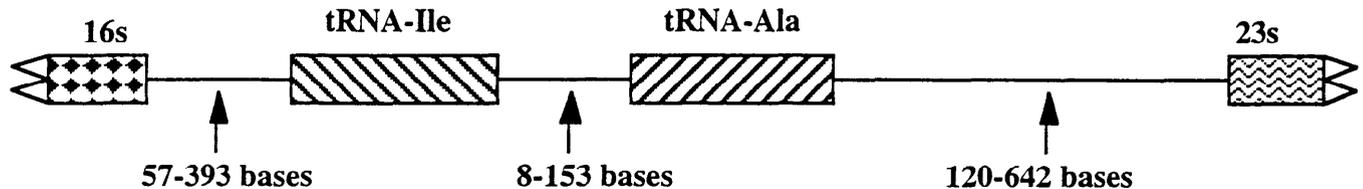


Figure 1. Structure of the most common (67%) ISRs found in Hanford groundwater samples. Only 9% of our clones have no tRNAs (ISR ~200 bases); the remaining 24% have only an Ile tRNA or Glu tRNA, or else the order of Ala tRNA and Ile tRNA is reversed.

Preliminary community characterization is complete, and several types of arrays are under development to determine the types of bacteria present and the status of the ground water. Profiling the community composition of polluted groundwater will impact the broad field of microbial ecology as well as mixed-waste bioremediation.

Results

The samples we have been analysing were provided by Dr. Fred Brockman from Pacific Northwest Laboratory, and were collected at the US DOE Hanford site, Washington state. The samples were microbial filtrates from ground water polluted with 2 mg/L carbon tetrachloride and 250 mg/L nitrate and subjected to enrichment (acetate+nitrate) and recirculation. This project is described in some detail in PNNL- 11113, Accelerated In Situ Bioremediation of Groundwater, by M.J. Truex, B.S. Hooker, and D.B. Anderson, July 1996.

We are using standard DNA isolation, PCR, cloning, and sequence analysis methods in 96-well microtiter plate formats in order to reduce the cost and time necessary for sequencing hundreds of bacterial ISRs. ISRs have been amplified by PCR using generic 16s (5 forward) and 23s (5 reverse) primers using DNA from eight well samples from the Hanford site, representing upper and lower ground water levels, early, mid. and late in enrichment/pumping procedure used in bioremediation. Libraries of these ISRs are being sequenced; sequences are being aligned and sorted into bins. Bins are described by type (best blast species) and structure of the ISR. We have 39 unique sequences (=bins) at present, after sequencing more than 200 ISR sequences. Figure 1 shows the structure of the most common type of ISR found in our samples. Other types are described relative to this model.

Hypervariable stretches of the ISRs, in the inter-tRNA (itRNA) region, have been synthesized and hybridized against synthetic, labelled templates corresponding to the opposite strand, in collaboration with Molecular Tool, Inc, in a 96-well microtiter plate hybridization format.

At present we have not yet saturated the sequence data from each sample. The preliminary data reveals considerable strain variation between samples (Table 1). Eighty percent of the samples have 30 or more sequences, of which 20-45% are unique. All of the sequences have been compared to those in the NCBI database using the Blastn search. Eleven of the unique sequences align with Pseudomonad ISRs. Other sequences are related to *Thiobacillus cuprinus*, *T. ferroxidans*, *Agrobacterium vitis*, *Xylophilus ampelinus*, *Bradyrhizobium japonicum*, *Bartonella bacilliformis*, *Xanthomonas*

maltophilia, *Prevotella ruminicola*, *Cryptomonas* sp., and *E. coli*. We have amplified and sequenced the ISRs of the environmental *E. coli* ribosomal operons. These differ either in tRNA present (Ile and ALA vs Glu) or in a 100 base region near the 3' end of the 16s. One other pair of ISRs differs also only in a 100 base region in the same position and has been tentatively sorted as different operons of the same organism. ISRs that are 95% identical in sequence are considered to be from the same species. ISRs that are 85% similar are tentatively assigned to different strains, and if their distribution is of interest (for example, if the occurrence of the strains is noncongruent), we examine them in more detail.

Table 1. Sequence distribution in well samples.

Isolates found in Upper (250' deep) and Lower (300' deep) zones, early (2-4 weeks), mid (6 weeks), and late (11-12 weeks) in the pumping experiment. Total number of clones sequenced, and total number of unique sequences (bins), in each time/depth sample shown.

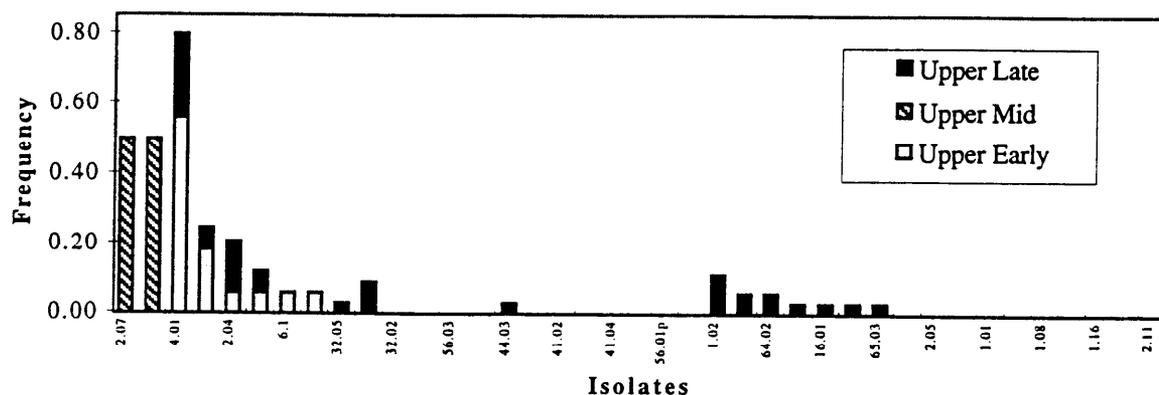
UPPER ZONE			LOWER ZONE		
Early	Late	Later	Early	Later	
30 seq/6 bins	4 seq/2bins	33 seq/17 bins	60 seq/15 bins	56 seq/16 bins	
2.02	14.01	1.02	2.04	1.01	
2.04	14.02	2.02	30.02	1.02	
4.01		2.04	32.02	1.05	
6.02		2.16	32.03	1.08	
6.1		4.01	32.05	1.15	
12.01		6.02	36.01	1.16	
		16.01	36.04	1.17	
		59.07	36.05	2.02	
		60.03	41.01	2.03	
		61.04	41.02	2.04	
		61.05	41.03	2.05	
		62.01	41.04	2.07	
		62.03	42.06	2.11	
		63.03	44.03	2.13	
		64.01	56.03	2.16	
		64.02		19.03	
		65.03			

Figure 2 shows the distribution of unique sequences, and it is obvious that there is a significant change in community composition in both the upper and lower zones when pumping is accompanied by acetate and nitrate enrichment. The upper zone is a less diverse assemblage, both early and late in the experiment, with clones 4.01, 2.04, and 1.02 being very common. The lower zone is more diverse early in the experiment; after 13 weeks of enrichment and pumping, clone 1.02 again is common. Clone 1.02 only appears late in the experiment, in both zones and in high frequency. Some clones are present only in late samples, but because only one of each has been found, more isolates will have to be sequenced before we can conclude that they also indicate late pumping/enrichment conditions.

Discussion

The obvious differences in the bacterial community composition and structure between early and late pumping times is a significant result, even in preliminary form. The organisms appearing late may be more sensitive to carbon tetrachloride and therefore only appear when the levels have dropped below 1.5 mg/L, perhaps in response to degradation by the species in the early samples. Ongoing analysis of the t=0 sample will help to identify the carbon tetrachloride metabolizers and differentiate the normal flora of unpolluted groundwater. The use of extensive sequences from new sites will provide a comparative basis for correlation of pollutant content and presence or absence of strains.

Upper Zone Community Composition Change



Lower Zone Community Composition Change

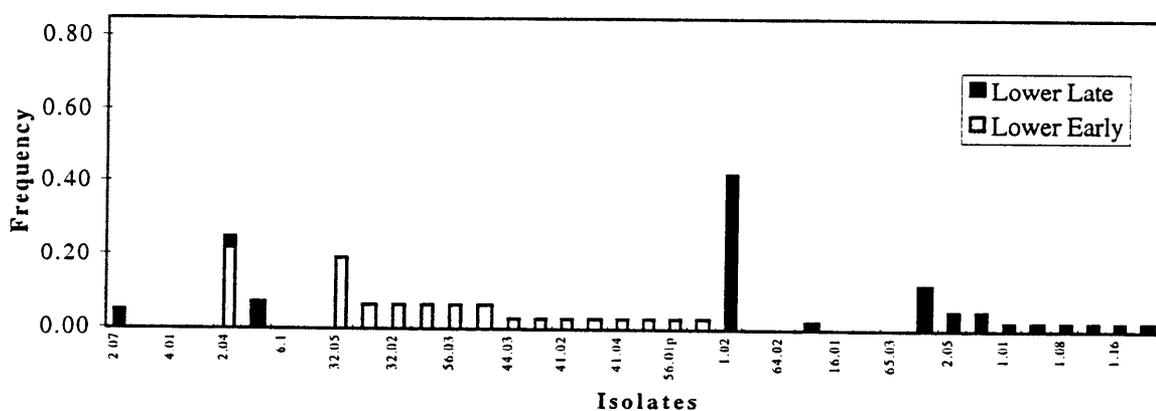


Figure 2. Community composition in upper zone (A: 250' deep) and lower zone (B: 300' deep) ground water through the course of acetate+nitrate enrichment and recirculation.

Future Work

After cross-reactivity and stringency issues have been addressed, we will hybridize PCR products from environmental samples against the diagnostic primers. We will screen sequences against the diagnostic primers to dereplicate and “bin” the new sequences quickly. We will sequence at least 50 isolates from each of the 9 samples from the Hanford bioremediation test (some of which are zone and time replicates), for a total of at least 450 sequences, produced by at least 3 pairs of per primers. Once we have sequenced an estimated 95% of the species in these samples, we will produce and test an ISR sequence array designed to detect species indicating higher and lower levels of carbon tetrachloride. We will then take a second sample set, provided by Joanne Horn at Lawrence Livermore Laboratory, and screen it against the carbon tetrachloride array before analysing the community composition in those samples. This will provide both a test of an ISR array and an expanded database of the types of bacteria in groundwater.

Publications

Robb, F.T. and R.T. Hill. 1998. Bacterial viruses and hosts: Influence of culturable state. In Nonculturable Microorganisms in the Environment, R.R. Colwell, ed. In press.

Brown, D.C, R.H. Shanks, D. Maeder, M.L. Lowe, G. Souza, and Robb, F.T. Profiling microbial communities in polluted ground water: Effects of bioremediation. In prep.

Collaborators

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