Composition of Microbial Communities in Petroleum Deposits

Jamie Scott[1], Debarati Paul[1], Magan Green[2], Lewis R. Brown[2], Mark L. Lawrence[1]*

[1]College of Veterinary Medicine, [2]Biological Sciences, Mississippi State University, Mississippi State, MS-39762

INTRODUCTION

Microbial communities in the subterranean environment are unique in that they are completely isolated from biological communities that rely on photosynthesis, earth's atmosphere, or oceans. Interestingly, bacteria found in petroleum deposits are in a dormant form known as ultramicrobacteria because petroleum deposits are very limited in nitrogenous and phosphorous-containing nutrients. These are unique populations as compared to other subsurface microbial communities and might consist of novel species, new metabolic capabilities, and undiscovered adaptive microbial mechanisms. They are cultured using methods for oligotrophic organisms in the lab. The goal of the current study was to isolate and identify cultivable UMBs based on their hydrocarbon utilizing properties and also to evaluate microbial community structure in petroleum deposits using 16S rDNA techniques such as ARDRA (amplified ribosomal DNA restriction analysis) of the environmental DNA extracted from the cores.

METHODS

Source: Cores were obtained from ~700 m below surface in an oil-bearing formation in Brookhaven, Alabama.

Processing of cores:. One-foot long sections of fresh cores were obtained, wiped with 70% ethanol, and immediately placed in BBL® GasPak®System containers under anaerobic conditions. Containers were refrigerated. These were cut into 4-inch sections using a sterile core saw in the lab. To reach the median of the core, 1 inch was cut from all sides, and the median part was placed in a stainless steel core crusher under N_2 and subjected to 20,000 psi using a hydraulic press.

DNA isolation:

(a) Genomic DNA from Cultivable bacteria: The crushed core material was mixed in simulated production water and streaked on Bacto-Tryptic Soy Agar prepared with simulated production water(1) and oil-agar. After incubation, a single colony was picked, and DNA was isolated from bacterial colonies using Wizard Genomic DNA purification kit (Promega). This DNA was quantified and used for PCR amplifications.

(b) Metagenomic DNA : The PowerMax Soil DNA Isolation Kit (Mo Bio Laboratories, CA) was adapted for use with the Pressure Cycling Technology (PCT) machine (Pressure BioSciences, MA)(2) . One gram of the crushed core material was vortexed with Power Bead buffer then mixed with lysis buffer and vortexed to mix. The PULSE tube was loaded into the Barocycler and run for 80 cycles (35 kpsi for 20 sec, atmp for 5 sec), which lysed bacteria using high pressure. DNeasy spin column (QIAGEN) was used to purify the DNA from the cell debris. The DNA was quantified and diluted with water for PCRs.

PCR, cloning and ARDRA : Universal primers were used for amplifying 16S rDNA genes from genomic and metagenmic DNA. For ARDRA, amplicons from five independent PCRs were pooled, ligated into TOPO 2.1 (Invitrogen, CA) and transformed into *E. coli* DH5α-T1^R chemically competent cells. About 200 white bacterial and 100 archaeal clones with ~1.5 kb inserts were stored in 10% glycerol at -80^{0}C. *Rsa*I and *Sau*3A were selected for restriction digestion of the clones. Restriction pattern of the clones was compared on a 2% agarose gel. Phylotypes were identified based on restriction pattern. Rarefaction analysis(3) was done and diversity indices(4) were calculated. Partial 16S rDNA gene sequence of one representative member of each phylotype was obtained, and phylogenetic trees were constructed using them. Isolates were also identified using partial 16S rDNA sequences.

RESULTS

Cultivable bacteria

Fifteen bacterial isolates were obtained from the cores on Agar enriched with oil. Partial 16S rDNA sequencing was used to identify them. Most of them looked similar. The unique ones were sequenced.

Table 1. Partial 16S rDNA sequences of some of the isolates from the core material.. The sequences are submitted to GenBank.

Isolate	GenBank ID	closest hit BLAST ID	% identity
BS2	JF963965	JF322796	98
BS65	JF963966	HM030754	99
BS67	JF963967	GU565237	99
BS67A	JF963968	HM274523	94
BS71	JF963969	FJ817481	92
BS78	JF963970	JF714216	98
BS29A	JF963971	FR851254	99
BS81A	JF963972	AY308758	99
BS66A	JF963973	EU368479	99

Unculturable bacteria

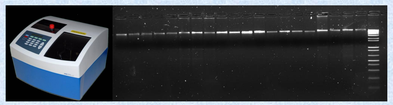

Figure 1. isolation of metagenomic DNA from crushed cores using Barocycler (PBI).

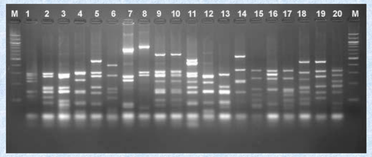

Figure 2. ARDRA gels showing *Rsa*I digestion pattern of bacterial 16S rDNA clones

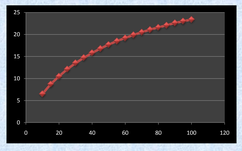

Figure 3. Rarefaction curve (no. of phylotypes vs no. of clones) indicating sufficient representation of bacterial diversity after screening about 200 clones. Clones were grouped as phylotypes on the basis of their RFLP pattern

clone	Ac. No.	closest hit BLAST ID	% identity
CVM7	JF922889	FJ870519	99
CVM8	JF963964	HQ823674	99
CVM10	JF922890	HQ585178	99
CVM13	JF922891	HM057723	99
CVM15	JF922892	FN667261	99
CVM23	JF922893	FR691516	99
CVM35	JF922895	AY753315	98
CVM27	JF922894	HM480175	98
CVM42	JF922896	EU429493	99
CVM48	JF922898	EU450347	48
CVM43	JF922897	JF202879	98
CVM49	JF922899	EU450314	98
CVM50	JF922900	HQ660803	99
CVM77	JF922901	FR774582	100
CVM91	JF922902	DQ230964	99
CVM107	JF922903	HM110470	99
CVM193	JF922912	EU148878	92
CVM121	JF922904	HM480264	99
CVM132	JF922905	HM799072	97
CVM157	JF922908	EF648091	100
CVM166	JF922909	JF346422	99
CVM184	JF922910	AY293404	99
CVM135	JF922906	HM316462	91
CVM147	JF922907	HM186821	100
CVM189	JF922911	EF658677	99

Table 2. GenBank accession numbers of representative clones for each phylotype along with GenBank IDs of the closest BLAST hit and % identity.

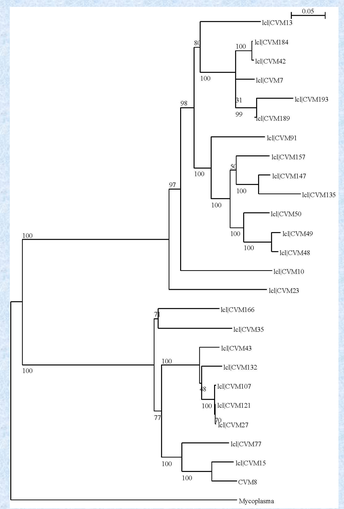

Figure 4. Dendrogram showing the phylogenetic relationships between various bacterial phylotypes. Bootstrapping values indicate the strength of each node. Distance scale indicates evolutionary distance. Mycoplasma has been used as the outgroup to root the tree.

DISCUSSION

- The core was populated with an unexpectedly diverse range of bacteria.
- PCT technology proved invaluable in isolation of metagenomic DNA.
- BLAST searches indicated much of the core population is similar to the microbial population from the Yellow Sea, followed by similarity to microbial mats of hypersaline lakes and also to some gut microflora.
- Several phylotypes belonged to α-proteobacteria, which commonly exist in all environments.

FUTURE STUDIES

- ARDRA and phylogenetic analyses of archaeal colonies are currently underway.
- Full length sequencing and polyphasic identification of isolated hydrocarbon utilizing colonies.
- Comparison of this microbial community to ones on the surface or deeper in the petroleum deposits.

REFERENCES

1. A. Azadour, L.R. Brown, and A.A. Vadie. Journal of Industrial Microbiology 16 (1996) 263.
2. F. Tao, C. Li, G. Smejkal, A. Lazarev, N. Lawrence, R.T. Schumacher. Proceedings of the 4th international conference on High Pressure Bioscience and Biotechnology 1(2007), 166-173.
3. J.C. Tipper. *Paleobiology* **5** (1979), 423–434.
4. D. Paul, G. Pandey, C. Meier, J. R. van der Meer, R. K. Jain. 2006. *FEMS Microbiol. Ecol*. 57:116-127.

Acknowledgements: Life Sciences & Biotechnology Institute and Pressure BioSciences Inc.