Application of Bacterial DNA Fingerprinting in Crude Oil for Evaluating the Reservoir-Part I*

A. Hayatdavoudi[1], N. Chegenizadeh[1], A. Christoserdov[2], R. Bajpai[3], and F. Boukadi[1]

Search and Discovery Article #41127 (2013)
Posted June 17, 2013

*Adapted from extended abstract prepared in conjunction with oral presentation at AAPG Annual Convention and Exhibition, Pittsburgh, Pennsylvania, May 19-22, 2013,

[1]Petroleum Engineering, UL Lafayette, Lafayette, LA, United States (asad.hayatdavoudi@gmail.com)
[2]Department of Biology, UL Lafayette, Lafayette, LA, United States
[3]Chemical Engineering, UL Lafayette, Lafayette, LA, United States

Abstract

A novel, sensitive technique for bacterial DNA fingerprinting – denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16S rRNA genes – has been used to identify and classify bacteria found in six crude oil samples obtained from an operating company. The samples were taken from two separate wells, presumably drilled in the same reservoir. The procedure of fingerprinting bacterial communities present in the oil samples was as follows: DNA extraction from oil, DNA concentration, PCR amplification of 16S rRNA gene fragments from DNA isolated from oil, separation of the fragments by DGGE, sequencing DNA bands present in the gels, and finally identification of bacteria to which the these bands correspond. The existence of bacterial DNA in all the crude oil samples was proven after DNA concentration. Based on the bacteria identified from the two wells, it appears that one well drained oil from a fresh water environment associated with grasslands, whereas the second well drained oil from a coastal marine environment. This finding is in a sharp contrast to Chromatograms (fingerprints) which indicates both oil samples are the same.

Introduction

Oil reservoirs are excellent microbial habitats impacted by factors such as temperature, pressure, salinity, pH, and nutrient availability, whether inorganic, organic, or combination of both. Microorganisms in the water phase of petroleum reservoirs posses the ability to utilize and degrade petroleum. As a result, microbial processes have a significant impact on the oil quality and oil recovery. In fact, microbially-enhanced oil recovery (MEOR) has been suggested the fourth stage of oil production (Sen, 2008), after the three stages of oil recovery process employing mechanical, physical and chemical methods have played out. This method proposes to improve oil recovery by means of either stimulating reservoir microbes or injecting certain consortia of natural bacteria into the reservoir triggering specific metabolic events. Although MEOR is appealing and many microorganisms have been collected from oil reservoirs, it is not yet practical or of economical value due to lack of understanding of the ecology and in-situ microbial metabolic processes in oil and gas reservoirs (Kotlar, et. al., 2007).

Molecular biology techniques have only recently been used for exploring oil reservoirs. Recent achievements in molecular biology have extended the understanding of the role of microbial transformation and maturation of petroleum compounds and have enhanced our ability to investigate the microbial communities in petroleum-impacted ecosystems (Jonathan, et. al., 2003). Mostly, the analyses have focused on identifying the diversity of microbial species present using microbial 16S rRNA (a nucleic acid found in all microorganisms). To identify a microorganism, its 16S rRNA sequence information is checked against the 16S rRNA sequence data available in the GenBank database. 16S rRNA gene information is available for microorganisms found in different parts of North Sea oil fields, and oil fields in South America and Asia. Using these gene databases, microorganisms most closely related to those that have been found in oil or gas reservoirs can be identified.

The role of microbes in affecting oil production from wells has been known for a while. In the personal experience of one of us, while working over a well in South Texas-in 1970, Hayatdavoudi observed that the 150-second-funnel-viscosity HEC (hydroxyethylcellulose) work-over fluid gel practically turned into consistency of base water (28-second-funnel viscosity) overnight, rendering the expensive work-over fluid useless. His investigation of the causes of loss of viscosity in fact showed break down of the β1-4 glycosidic bonds of HEC polymer resulting in formation of single sugar molecules. Similar cellulosic derivatives are currently used in shale fracturing jobs. Again, in early 80s, while reassessing some old rocks and gravel-packed sands from a well in Bayou Blue (a South Louisiana oil field), Hayatdavoudi observed formation of damaging sludge in the samples plugging the otherwise porous and highly permeable gravel pack sand and the reservoir rock pores. Specially, the permeability of the plugged gravel pack was so low that the owner of the well thought of abandoning the field, stopping all formation damage control measures, and stating that there was no more oil left in this particular reservoir. Further laboratory experiments yielded similar plugging and sludge forming results (Hayatdavoudi and Ghalambor, 1996). This historical background motivated this research focusing on positive aspects of bacteria as a means of reservoir characterization, well connectivity analysis, reservoir classification, reservoir diagnostics, and general exploration and basin modeling tool rather than studying damaging aspects of bacteria as in reservoir formation damage research.

There are other engineering application of classifying the bacteria type and their impact on oil field production systems. Driven by the high demands for oil, it is crucial to explore if there are any microorganisms in the oil, determine their source of living, their original environment and their effect on the crude oil properties. For example, industrial research shows most bacteria, either aerobic or anaerobic, have two states: planktonic and sessile. In the first state, bacteria freely float/swim around. In the second state, they attach to any surface, i.e. reservoir rock, reservoir cap rock, unconventional shale reservoirs, etc. In this research, we are concerned about the second state simply because bacteria can modify the rock grain surface thus affecting its wettability and relative permeability of oil and water, and even cause plugging of flow path of any permeable system, including porous media.

Another trait of bacteria in the oil reservoirs is to generate slime. The slime can be formed by either aerobic or anaerobic bacteria. Bacteria produce large masses of white or gray slime and one of the most common types of the slime is generated by aerobic iron bacteria, which oxidize ferrous iron to ferric iron and precipitate the iron in their insoluble sheaths. By consuming all the oxygen, the iron bacteria create a desirable condition for the growth of anaerobic bacteria. Many anaerobic bacteria cause problems too. One of the most troublesome groups of bacteria found in the oilfield is the sessile, sulfate-reducing bacteria (SRB). They reduce sulfate ions in the water to sulfide. The resulting hydrogen sulfide can cause corrosion. Iron sulfide, a product of the corrosion reaction, can plug filters, injection wells, and it can even cause more corrosion on steel because of its cathodic nature. Bacteria cause plugging of even high porosity formations and changes in oil flowing

behavior due to alterations in the oil chemistry. At times, the bacteria growth and its consequences are so severe that may render the field non-productive.

DNA fingerprinting was applied to crude oil stockpiles by researchers in Japan (Youshida, 2004). Their results, however, were not quite conclusive, possibly due to the nature of stock piling, mixing of various crude oil blends from different sources, and human contamination. Hence, careful attention was paid in this research to sampling and avoidance of human contamination. A novel, accurate, and sensitive technique for bacterial DNA fingerprinting involving denaturing gradient gel electrophoresis (DGGE) of PCR (polymerase chain reaction)-amplified 16S rRNA (PCR-DGGE) was implemented for the study. This approach has permitted us to identify (and classify) bacteria found in oil specific to the wells under consideration. The knowledge of the properties of these oil-specific bacteria can be useful for understanding microbial-dependent evolution of oils, water, and associated fluids along with their chemical content in a given reservoir. Whatever we learn from this research will provide us with a much-needed additional geostrategic information database, with firm capabilities for better mapping of the extent of reservoir and more accurate estimation of reserves.

In this research, we have pursued a three-step goal. First, we prove the existence of living bacteria inside the oil, and then in the next steps, we implement an advanced technique for bacterial DNA fingerprinting, examine, and compare the results with GC-chromatogram-(the indirect GC fingerprinting technique currently used in oil characterization). We follow the DNA isolated from oil samples, for identifying and classifying the bacteria found in oil, for determining the depositional environment of reservoir, the source rock, and the origin, chemistry, and other properties of oil in contact with the rock pore surfaces. Finally, we touch on some GC data to compare both methods of fingerprinting oil.

Methodology

a. General. Six samples of oil were carefully collected from two separate wells, supposedly drilled in the same reservoir. Precautions were taken to ensure there were no contaminations during sampling. The samples were taken 30 minutes apart to allow proper handling, tagging, and preservation of representative samples from each well. The samples were designated A1, A2, and A3 from well A, and B1, B2, and B3 from well B. All samples were placed in a cold room until analyzed.

b. GC. A few milliliter of each sample was sent to a commercial lab in Lafayette, Louisiana for gas chromatographic analysis. The GC conditions were: Column - 30 m, 0.5 micron film thickness, 0.5 mm ID. Injector - 260°C, Carrier Gas - He: 8.9 ml/min constant flow rate, Oven Temperature – Ramp from 40°C to 300°C at 20°C/min.

c. DNA. 16S rRNA in the microbial isolates was utilized to analyze bacterial diversity. This specific nucleic acid was selected because (a) this gene is present in all bacteria, (b) the function of the 16S rRNA gene has not changed over the time life exists on Earth, suggesting that random sequence changes are an accurate measure of time (evolution); and (c) the 16S rRNA gene base pair, 1500 bp, is large enough for informatics purposes. Using the sequencing system, the bacteria can be easily identified using free international gene databases.

To interpret the GC data scientists rely on certain carbon numbers cross plots of the so-called biomarkers and extensive database associated with various source rock (Ferworn, et. al., 2003). In the case of DNA, however, the cross plot interpretation and extensive petroleum database

may not be necessary as the bacteria DNA directly yields very accurate information on the crude oil and may suggest the source rock/host rock specific to certain environment in which the bacteria thrive. With this potential, DNA analysis may prove extremely useful as a second reliable source to confirm the valuable GC data or it could be used as a stand-alone method of oil and reservoir characterization, the results of which can be used for further exploration, reservoir engineering, reserve estimation, well testing, reservoir connectivity analysis without shutting-in the well(s), and other petroleum engineering purposes.

Results and Discussion

General GC results. Figure 1 shows a general chromatogram of non-degraded crude oil (Ferworn, et. al, 2003). The hydrocarbon range extends from C7 to approximately C40. The Biomarkers fall within Sterane Biomarkers m/z = 218 and Tricyclic Terpane Biomarkers m/z = 191. GC results from wells A and B. Figure 2 and Figure 3 exhibit the chromatograms of crude oil samples from wells A and B, respectively. An examination of these chromatograms suggests that fingerprints of different samples from each individual well are the same. In other words, there is no difference in peaks from C7 to about C40 "within" the samples from each well.

If we go a step further and examine the fingerprint data "between" the wells A and B and compare the time-line/peak relationships- that is A1, with B1, A2 with B2, and A3 with B3- the chromatogram data suggest that actually there is no difference in the oil samples from wells A and B also. Relying on this observation alone would lead us to the idea that actually wells A and B are producing oil from the same reservoir and the same source rock, especially knowing that in the Gulf Coast of Louisiana there are not many different source rocks. Interestingly, the chromatograms in Figure 2 and Figure 3 repeat the same general pattern of Figure 1 for a non-degraded crude oil

Analysis of microbial community composition. A DGGE gel of PCR-amplified 16S rRNA fragments from oil samples from wells A and B is presented in Figure 4. It shows the separation of 16S rDNA products deriving from different bacteria present in the well A and well B samples. It also confirms that bacteria are indeed present in the analyzed oil samples. A total number of the bands excised from the get were 52, which were labeled from 1 to 52 (Figure 4, Table 1). Each number in the Table 1 *represents an individual bacterium present in a respective crude oil sample*.

Two protocols for DNA isolation from oil were also compared in this experiment using samples from well B (Figure 4; Table 1). The first, originally suggested by researchers in Japan (Youshida et. al., 2004), involves an isooctane elusion stage and the second is simply a direct application of a Mo Bio kit (MO BIO Laboratories, 2007). Surprisingly, the use of isooctane decreases the number of bands that show up on the DGGE gel, suggesting that the Mo Bio kit –based DNA-isolation protocol is more efficient for DNA isolation from crude oil (Table 1).

Sequencing was completed successfully for all 16S rDNA bands. Following sequencing, a closest relative was identified in the GenBank using the BLAST program for each band sequence and closest relative of bacteria from which the bands derived were identified (shown in Table 2 and Table 3 for the well B samples and Table 4 for the well A samples.).

The microbial community of well B is dominated by various species/strains of genera *Paenibacillus* and *Magnetospirillum* or *Azospirillum brasilense*. The latter bacterium is a typical inhabitant of grasslands. Bacteria detected in well A on the other hand are dominated by *Thauera*

spp., order Pseudomonadales (*Pseudomonas*, *Acinetobacter*, *Xanthobacter* spp.), actinobacteria (*Arthrobacter* sp., *Nocardias* sp., *Rhodococcus* sp.) and marine rosebacteria (*Litoreibacter*, *Roseovarius*, *Pseudoruegeria* spp.). Thus, the microbial communities recovered from the two wells appear very different with only one group of bacteria found at both locations (*Rhodococcus* sp.)

Connecting the dots. From the data shown above, it is clear that bacteria DNA exists in crude oils from wells A and B despite the expert discouragements for the past 42 years that one should not look for DNA in crude oil. It is further clear that crude oil from well A is not the same as crude oil from well B. At this point, we should recall that chromatograms (fingerprints) of Figure 2 and Figure 3 suggested both crude oils from wells A and B were the same and typical to oil production from other fields located in Louisiana continental margins of Gulf of Mexico. Actually, the DNA data allow us to speculate that well A produces oil from marine environment or contaminated with seawater whereas well B produces oil from a terrestrial environment associated with grasslands. If this observation is correct then there must be a natural barrier like a fault separating these two wells and that these reservoirs are independent of each other. As such, the potential exist that these fields, particularly where these well reservoir are located, should be remapped for possible drilling more wells or deepening/sidetracking the existing ones.

Conclusions

Based on the results of this work we may conclude that:

1. The claims that bacteria DNA cannot exist in crude oil or DNA is fully degraded appear to be incorrect because we have shown that bacteria DNA do exist in all crude oil samples taken from wells A and B.
2. The results confirmed that based on the bacterial community composition the well A reservoir is different and independent from the well B reservoir.
3. Extraction of DNA using isooctane did not improve the recovery of DNA from the crude oil. Actually, DNA isolation without isooctane was proved to produce more DNA bands.

References Cited

Ferworn, K, J. Zumberge, and S. Brown, 2003, Integration of Geochemistry & Reservoir Fluid Properties: PTTC Workshop, 51 p.

Hayatdavoudi, A., and A. Ghalambor, 1996, A Study of Formation Damage of Selective Clay and other Minerals Caused by Bacterial Plugging: SPE 270006, 8 p. Web accessed 17 May 2013.
http://www.onepetro.org/mslib/app/Preview.do?paperNumber=00027006&societyCode=SPE

Kotlar, H.K., O.G. Brakstad, and S. Markussen, 2007, Use of Microbial DNA Probes as a Potential New Tool in Oil Exploration and Characterization, SPE 106418, 12 p. Web accessed 17 May 2013.
http://www.onepetro.org/mslib/app/Preview.do?paperNumber=SPE-106418-MS&societyCode=SPE

Sen., R.2008, Biotechnology in Petroleum Recovery: The Microbial EOR: Progress in Energy and Combustion Science, v. 34/6, p. 714-724.

Van Hamme, J.D., A. Singh, and O.P. Ward, 2003, Recent Advances in Petroleum Microbiology: Microbiology and Molecular Biology Review, v. 67/4, p. 503-549. doi: 10.1128/MMBR.67.4.503-549.

Youshida, N., K. Yagi, D. Sato, N. Watanable, T. Kuroishi, K. Nishimoto, A. Yanagida, T. Katsuragi, T. Kanagawa, and R. Kurane. 2005, Bacterial Communities in Petroleum Oil in Stockpiles: Journal of Bioscience & Bioengineering, v. 99/2, p. 143-149.

Website

MO BIO Laboratories, Inc., 2007, For efficiently isolating microbial DNA of high quality and purity from large quantities of soil - great for samples with low microbial load: MO BIO Laboratories, Inc. Manufacturer bulletin "PowerMaxTM Soil DNA Isolation Kit", Carlesbad, CA 2007. Web accessed 12 June 2013. http://www.mobio.com/soil-dna-isolation/powermax-soil-dna-isolation-kit.html

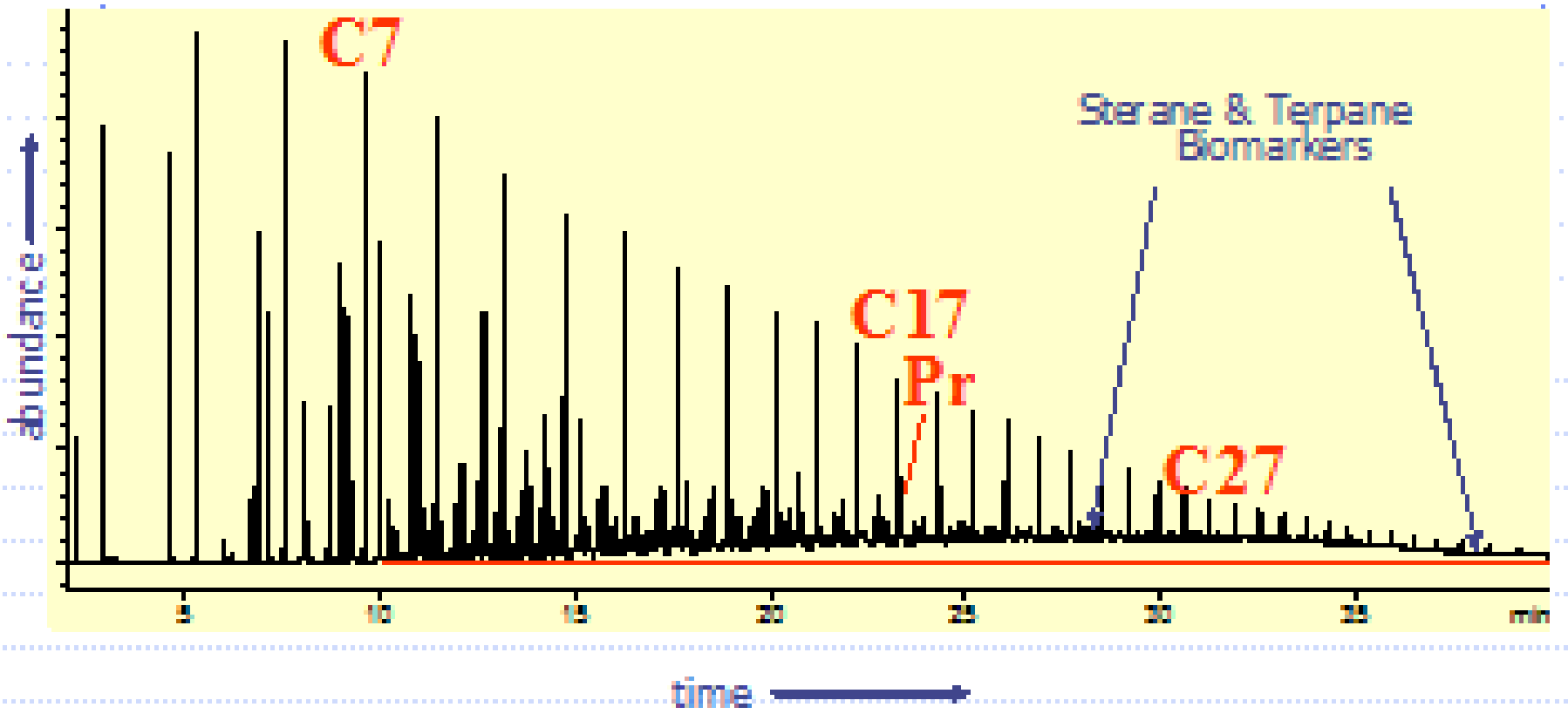

Figure 1. A general Gas Chromatogram (fingerprint) of whole crude oil. Sterane Biomarkers m/z = 218 and Tricyclic Terpane Biomarkers m/z = 191 (Ferworn et. al., 2003).

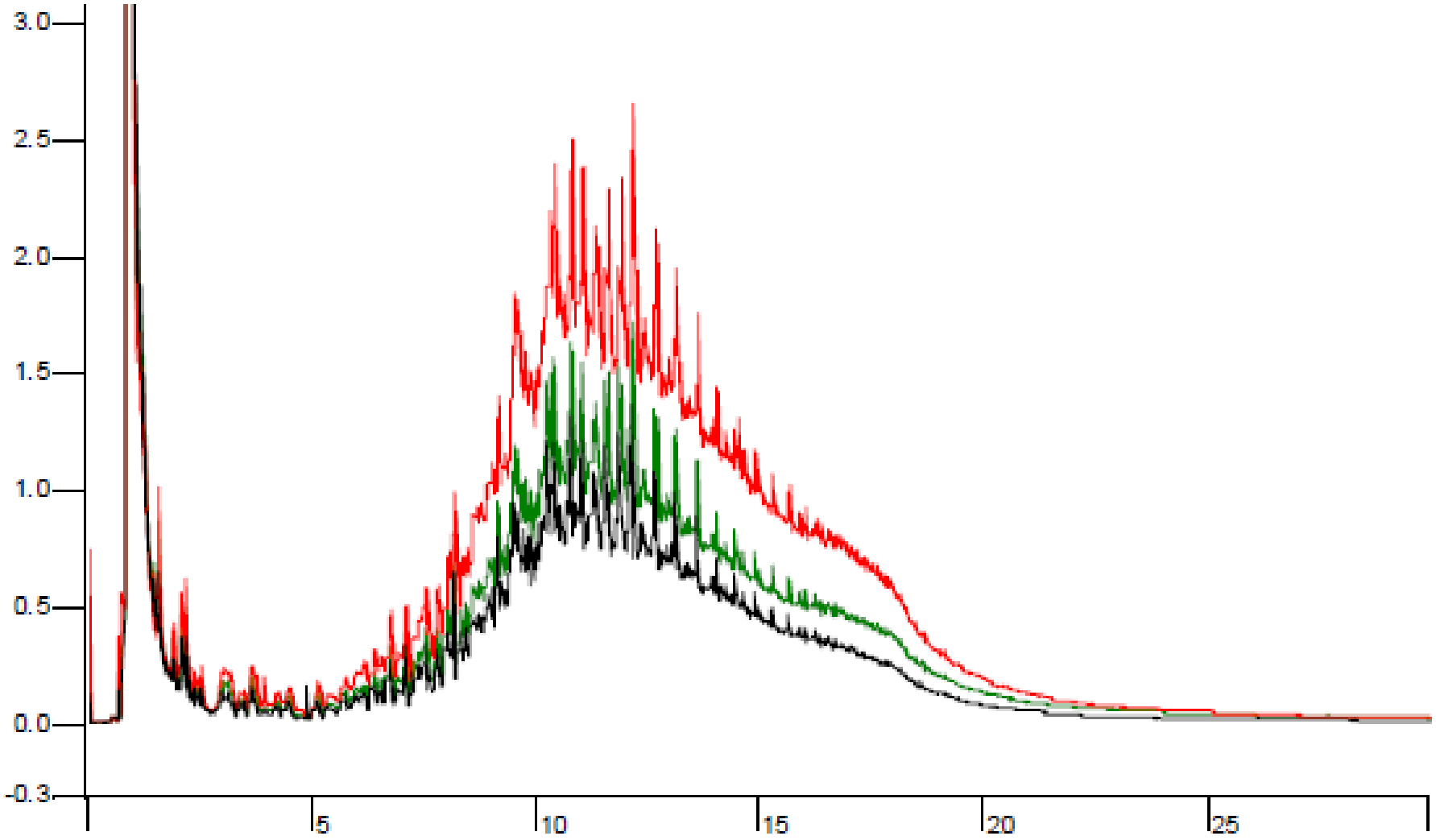

Figure 2. Compound Peak Abundance vs. Time. Chromatograms are (Black Chromatogram = Sample Crude Oil A1, Red Chromatogram = Sample Crude Oil A2, Green Chromatogram = Sample Crude Oil A3) Well A.

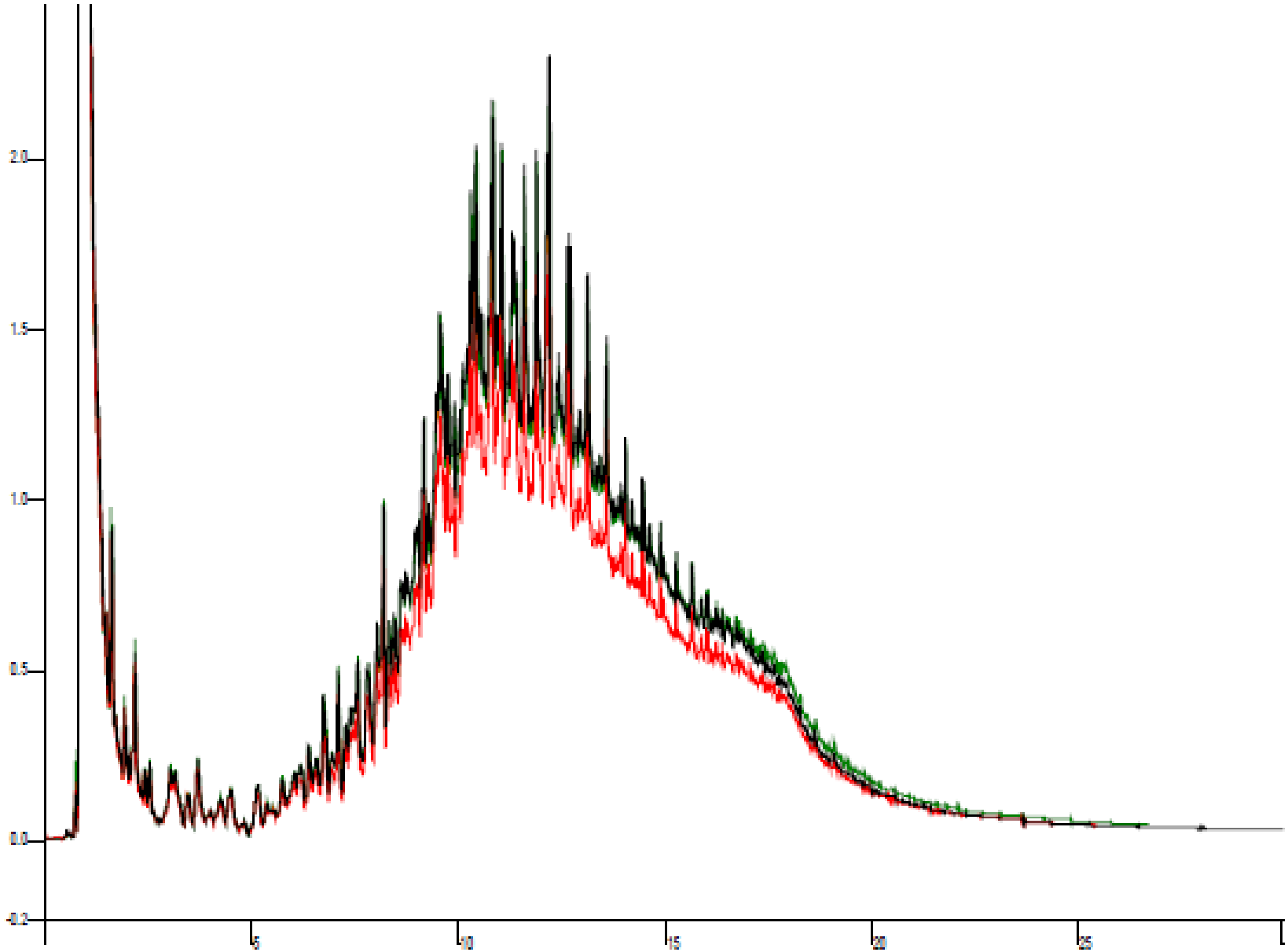

Figure 3. Compound Peak Abundance vs. Time. Chromatograms are (Black Chromatogram = Sample Crude Oil B1, Red Chromatogram = Sample Crude Oil B2, Green Chromatogram = Sample Crude Oil B3) Well B.

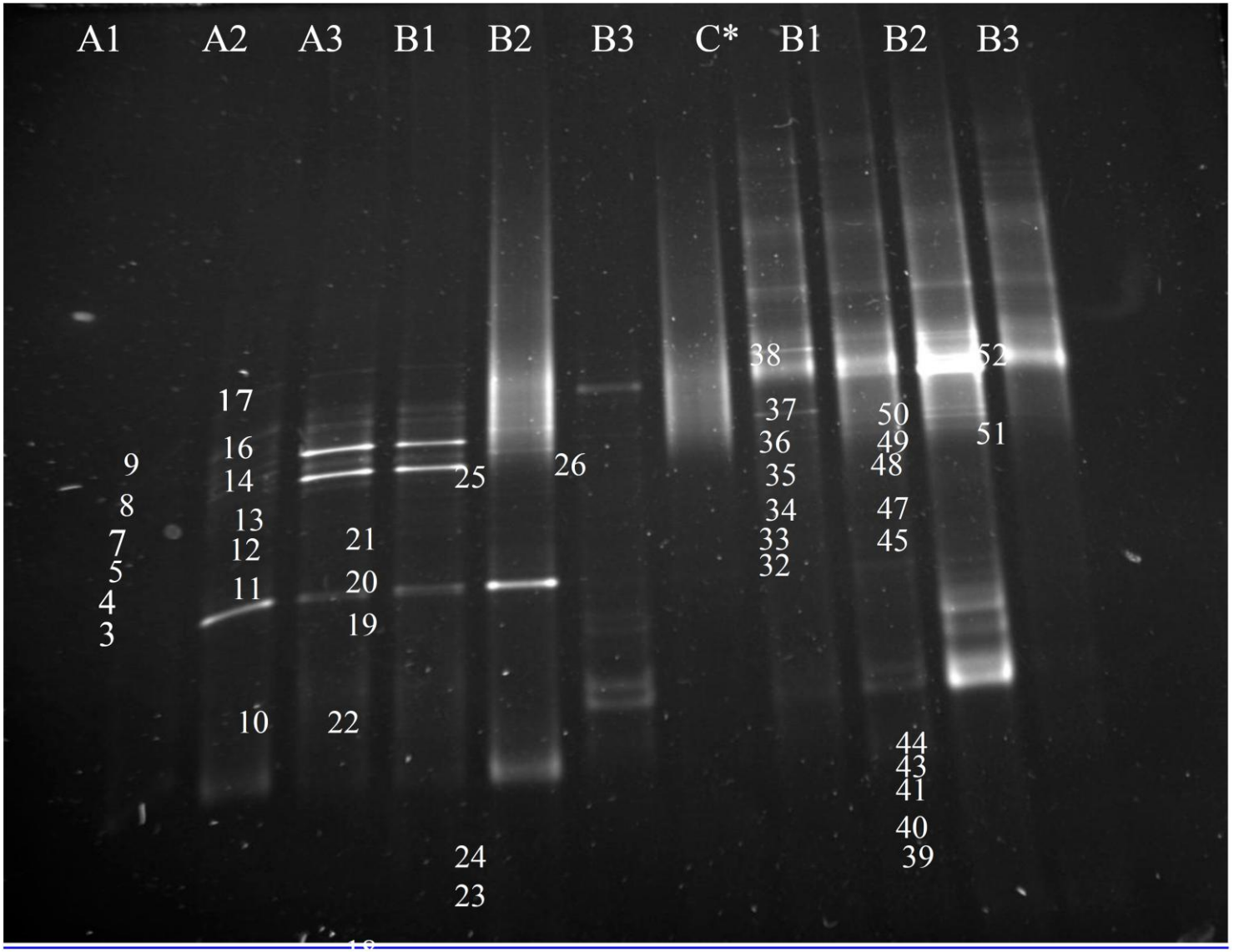

Figure 4. Photograph of a DGGE gel with bands corresponding to PCR amplified products of a portion of the 16S rRNA gene from bacteria inhabiting oil from Wells A and B. Lanes are labeled as follows: first six sample lanes were loaded with PCR products derived from samples extracted with isooctane (A1, A2, A3, B1, B2, B3), the last three sample lanes were loaded with PCR products derived from samples extracted without isooctane (B1, B2, B3); a contaminated samples (C*), which was not used in the analyses. Several analyzed bands are not shown (i.e., 6 15, 27-31, 42, 46).

Samples, DNA Extracted with isooctane						Samples, DNA Extracted without isooctane		
Sample A1	Sample A2	Sample A3	Sample B1	Sample B2	Sample B3	Sample B1	Sample B2	Sample B3
2	3 4 5 6 7 8 9	10 11 12 13 14 15 16 17	18 19 20 21 22	23 24 25	26	32 33 34 35 36 37 38	39 40 41 42 43 44 45 46 50	52 51

Table 1. Distribution of sequenced bands among the samples.

DNA Band	Bacterial matches found in GeBank	Number of bands in Fig. 4
DNA Band 18 in sample B1	*Arthrobacter* sp., *Nocardias* sp., *Rhodococcus* sp.	1
DNA Band 19 in sample B1	*Azospirillum* sp., *Dechlorospirillum* sp., *Magnetospirillum* sp.,*Caenispirillum* sp.	1
DNA Band 20 in sample B1	*Azospirillum* sp., *Caenispirillum* sp., *Dechlorospirillum* sp., *Magnetospirillum* sp.	1
DNA Band 21 in sample B1	*Azospirillum* sp., *Magnetospirillum* sp.	1
DNA Band 22 in sample B1	*Bacillus* sp., *Paenibacillus* sp.	1
DNA Band 23 in sample B2	*Paenibacillus* sp.	1
DNA Band 24 in sample B2	*Aquaspirillum* sp., *Azospirillum* sp., *Phaeospirillum* sp., *Kiloniella* sp., *Magnetospirillum* sp., *Rhodospirillum* sp., *R.rubrum* sp.	1
DNA Band 25 in sample B2	*Paenibacillus* sp.	2 (sample B2, sample B3 band 26)
DNA Band 26 in sample B3	*Paenibacillus* sp.	2 (sample B2 band 25, sample B3)

Table 2. Summary of closest relatives to the oil bacteria found in Well B using DNA isolation procedure with isooctane.

DNA Bands	Bacterial matches found in GenBank	Number of bands in Fig.4
DNA Band 32 in sample B1	*Paenibacillus* sp.	1
DNA Band 33 in sample B1	*Paenibacillus* sp.	2(sample B1, sample B2 band 45)
DNA Band 34 in sample B1	*Paenibacillus* sp.	1
DNA Band 35 in sample B1	*Paenibacillus* sp.	2 (sample B1, sample B2 band 47)
DNA Band 36 in sample B1	*Paenibacillus sp.*	3 (sample B1, sample B2 band 48, sample B3 band 51)
DNA Band 37 in sample B1	*Paenibacillus* sp. *Bacillus* sp., *Siboglinum* sp.	2 (sample B1, sample B2 band 49)
DNA Band 38 in sample B1	*Paenibacillus* sp., *Bacillus* sp.	3 (sample B1, sample B2 band 50, sample B3 band 52)
DNA Band 39 in sample B2	*Magnetospirillum* sp., *Azospirillum brasilense, A. magnetotacticum*	1
DNA Band 40 in sample B2	*Oscillatoria spongeliae, Magnetospirillum* sp., *Azospirillum brasilense, Phaeospirillum cystidoformans*	1
DNA Band 41 in sample B2	Uncultured bacterium clone F1Q32TO05GHG32 16S ribosomal RNA gene,	1
DNA Band 43 in sample B2	Uncultured *Streptomyces* sp., *Paenibacillus* sp., *Bacillus* sp.	1
DNA Band 44 in sample B2	*Paenibacillus* sp., *Bacillus* sp.	1
DNA Band 45 in sample B2	*Paenibacillus* sp.	2(sample B1 band 33, sample B2)
DNA Band 47 in sample B2	*Paenibacillus* sp.	2 (sample B1 band 35, sample B2)
DNA Band 48 in sample B2	*Paenibacillus* sp., *Bacillus* sp.	3(sample B1 band 36, sample B2, sample B3 band 51)
DNA Band 49 in sample B2	*Paenibacillus* sp., *Bacillus* sp.	2 (sample B1 band 37, sample B2)
DNA Band 50 in sample B2	*Paenibacillus* sp., *Bacillus* sp.	3(sample B1 band 38, sample B2, sample B3 band 52)
DNA Band 51 in sample B3	*Paenibacillus* sp., *Bacillus* sp.	3 (sample B1 band 36, sample B2 band 48, sample B3)
DNA Band 52 in sample B3	*Paenibacillus* sp.	3 (in sample B1 band 38, sample B2 band 50 , sample B3)

Table 3. Summary of closest relatives to the oil bacteria found in Well B using DNA isolation procedure without isooctane.

DNA Band	Bacterial matches found in Gene Bank	Number of bands in Fig. 4
DNA Band 1 in sample A1	*Arthrobacter* sp., *Nocardias* sp., *Rhodococcus* sp	1
DNA Band 2 in sample A1	*Acinetobacter* sp.	3 (sample A1, sample A2, sample A3 band 10)
DNA Band 3 in sample A2	*Thauera* sp.	1
DNA Band 4 in sample A2	*Thauera* sp	3 (sample A1, sample A2, sample A3 band 11)
DNA Band 5 in sample A2	*Pseudomonas* sp.	3 (sample A1, sample A2, sample A3 band 12)
DNA Band 6 in sample A2	Uncultured betaproteobacterium shui5-6 16	3 (sample A1, sample A2, sample A3)
DNA Band 7 in sample A2	*Xanthobacter autotrophicus*	3 (sample A1, sample A2, sample A3 band13)
DNA Band 8 in sample A2	*Rhodococcus* sp.	2 (sample A2, sample A3 band 14)
DNA Band 9 in sample A2	*Litoreibacter* sp., *Roseovarius* sp., *Pseudoruegeria* sp.	2 (sample A2, sample A3 band 16)
DNA Band 17 in sample A3	*Litoreibacter* sp., *Roseovarius* sp., *Pseudoruegeria* sp	1

Table 4. Summary of closest relatives to the oil bacteria found in well A using DNA isolation procedure without isooctane.