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Review

Applications of molecular biology and biotechnology in oil field microbial biodiversity research

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In recent years, with more and more attention given to the study of microorganisms in oil fields, molecular biology and biotechnology methodology has been applied prosperously. This article described the most commonly-used molecular biology techniques and related biotechnological advances in oil fields research at present, which are based mainly on 16s rRNA, such as 16S rRNA sequencing, denaturant gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), etc. The article also points out their advantages and disadvantages, respectively and introduces some of the micro-organisms, which are of largest concerns in the research.

Key words: Biotechnology, molecular biology techniques, oil field, 16s rRNA, microbial biodiversity, prospect.

INTRODUCTION

Oil field, with long-term water development, which is a relatively stable microbial community, has been formed in formation water. The vast range of substrates and metabolites present in hydrocarbon-impacted soils surely provides an environment for the development of quite a number of complex microbial community (Jonathan et al., 2003). When water is considered in relatively stable circumstances, the types and quantity of microbial community in the formation water are unchanged. In the deeper strata, due to high temperature and pressure, the microbial community structure is relatively simple, while in shallower formation where the temperature is below 100°C, microbial community are more complicated and are in large quantities, mainly for bacterial, including hydrocarbon degrading bacteria, denitrifying bacteria, methane-producing bacteria, sulfate reducing bacteria, iron bacteria, total growth bacteria, etc. (Davidova et al., 2001; Erwin et al., 2004; Jeffrey and Norman, 2007; Jutta et al., 2002; Lara et al., 2007; Nemati et al., 2001; Nielsen and Nielsen, 2002; Zheng et al., 2010; Zhang et al., 2008). With long-term survival in the formation environment, these bacteria produce a strong adaptability. The existence of these bacteria, on the one hand, have a positive role in enhancing oil recovery, while on the other hand, it may cause the corrosion and plug of injection wells and oil wells. To this end, micro-biologists are trying to find methods, which can inhibit bacterial not only to production safety and oil production, but at the same time, promote the growth and reproduction of bacterial to recovery, conducively.

For a long time, the research of beneficial or harmful microorganisms in the oil reservoir is less, either for the role of oil production (Catherine and Hilary, 1997), or for metal corrosion. At present, micro-organisms are used to raise oil output in oil reservoir, or we can say that the microbial enhanced oil recovery (MEOR) technology is popular around the world and is developed rapidly. At the same time, biocompetitive exclusion technology (BCX technology) has been successfully tested in some oil fields (Nielsen and Nielsen, 2002; Yumiko and Kazuya, 2003; Sperl and Sperl, 2010). Therefore, in oil production projects, for the use of microbial competition exclusion technology, to control the occurrence and development of hydrogen sulfide, or to use the microbial enhanced oil recovery technology to increase oil output, the analysis of

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bacterial communities in reservoir is very necessary Some useful molecular biology methods were indicated in Figures 1-4 and Tables 1-2.

TYPES OF IMPORTANT MICROBIOLOGICAL RESOURCES

Sulfate reducing bacteria

Sulfate reducing bacteria (SRB) are anaerobic microorganisms that have been found to be involved with numerous microbiologically influenced corrosion (MIC) problems affecting a variety of systems and alloys. They can survive in an aerobic environment for a period of time until a compatible environment is found. In offshore oil production, seawater is commonly injected into the reservoirs to enhance secondary oil recovery. The sulfate-rich seawater stimulates growth of sulfate reducing bacteria (SRB) in the reservoirs with subsequent H_2S production. This biogenic H_2S production, designated “reservoir souring”, is of major concern to the oil industry, as H_2S is toxic and corrosive, increases sulfur content in oil and gas, and may lead to reservoir plugging (Myhr et al., 2002).

The most common strains exist in the temperature range of 25 to 35°C, although there are some that can live at temperatures of 60°C. They can be detected through the presence of black precipitates in the liquid media or surface deposits, as well as a characteristic hydrogen sulfide smell (Richard, 2005). One report, declared by Iverson (2009), shows that in the USA, 77% corrosion of the oil well are caused by SRB, the characteristic of which is pit corrosion. Thus, the corrosion rate of steel can increase to 15 fold. Hence, the corrosion brought by SRB is one of the most important problems to be solved (Wang et al., 2004).

Nitrate reducing bacteria (NRB)

NRB can be classified on the basis of the electron donors that they use (Eckford et al., 2002). They can be chemolithotrophs that use inorganic compounds such as sulfide, thiosulfate or ferrous sulfide as electron donors (Gevertz et al., 2000; Kuenen, 1989; Sublette et al., 1994), or chemoorganotrophs (heterotrophs) that use organic compounds as electron donors (Zumft, 1992). In the presence of organics, such as nitrate and volatile fatty acids, NRB multiply rapidly. Through metabolism, they can transform the nitrate into nitrite. Further, into gases such as N_2 and N_2O, they can also remove sulfide in the system, to inhibit the growth of sulfate-reducing bacteria by surviving competition, inhibition of the formation of new sulphide, reduction of corrosion on production equipment by toxic gases such as H_2S and damage on the formation by insoluble, such as Fe (Dennis, 1998).

Iron/manganese oxidizing bacteria

Iron and manganese oxidizing bacteria have been found in conjunction with MIC, and are typically located in corrosion pits on steels. Some species are known to accumulate iron or manganese compounds resulting from the oxidation process. High concentrations of manganese in biofilms have been attributed to the corrosion of ferrous alloys, including pitting of stainless steels in treated water systems. Iron tubercles have also been observed as a result of the oxidation process (Richard, 2005).

Hydrocarbon degrading bacteria

Hydrocarbon degrading bacteria (HDB) refer to the bacteria groups which can use petroleum hydrocarbons as substrate of growth. This type of bacteria is the most abundant in water injection wells, close to the bottom zone, and is the start of the food chain link of microorganisms in water injection oil layer. HDB can produce catabolic enzyme through metabolism, crack heavy hydrocarbons and paraffin, reduce oil viscosity and improve the flow of oil, so as to raise oil recovery. In addition, HDB can produce surfactants, polymers, organic acids, alcohol and carbon dioxide by metabolisms, which are conducive to oil flooding. For the majority of HDB, which are aerobic bacteria, injecting air containing water that has nutrients in its formation, can greatly stimulate the growth of these bacteria so as to enhance oil recovery.

Total growth bacteria

Total growth bacteria (TGB) are all kinds of heterotrophic bacteria, that is, bacteria living on organic material. Some bacteria can produce product to improve the flow of oil by metabolism. Viscous substances and metabolites, produced by a certain group of anaerobic bacteria, as well as organism accumulation can have an impact on the formation. TGB are often referred to as Aerobic-heterotrophic bacteria. Some of these bacteria in the process of growth and reproduction can produce a large number of viscous substances. The substances attached to the pipelines and equipment, form a scale and plug in the water injection wells and filter. Once it enters into the formation, it will cause a plug in the formation, and at the same time, it will create oxygen concentration cell and cause corrosion. Sometimes, it forms local anaerobic environments which are suitable for the growth of SRB, so as to increase corrosion. However, this type of bacteria is an important control indicator in oil injected water.

MOLECULAR BIOLOGY METHODS

In order to achieve the bio-competitive exclusion

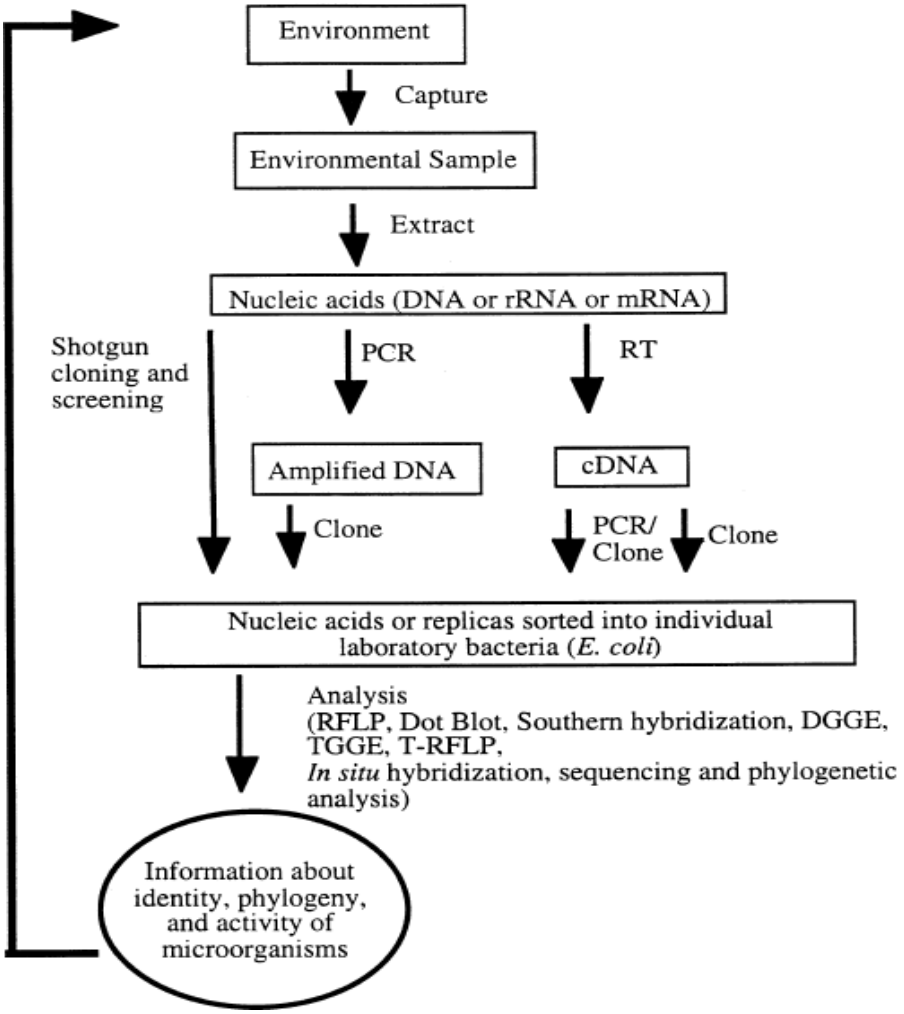

Figure 1. Steps toward nucleic-acid analysis of naturally occurring microorganisms. cDNA, complementary DNA; DGGE, denaturing gradient gel electrophoresis; TGGE, temperature gradient gel electrophoresis; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RT, reverse transcriptase; T-RFLP, terminal restriction fragment length polymorphism (modified from Madsen, 2000).

technology, we must understand the composition of microbial source in oilfield. Traditional culture techniques have yielded valuable information about microbial interactions with hydrocarbons in the environment.

However, one must keep in mind that only a small fraction of microorganisms can currently be cultured from environmental samples, and even if a microorganism is cultured, its role in a community and contribution to the ecosystem function are not necessarily revealed (Hamme et al., 2003). There are large number of uncultured microbes and cultured independent microbes in oil reservoirs which can not grow on pure culture of nutritious medium in the laboratory. At the same time, microorganisms will be inevitably placed in a new artificial manipulation environment deviating from their original small environment, which will change the original structure of microbial communities, hence, micro-organisms may be deviated from the original nature of the physical habits of the population, and even depart from their original genotype combination.

Therefore, any use of traditional microbial technology to a correct understanding of the microbial ecosystems will face considerable obstacles. A lot of microbial resources of great practical value (uncultured microbes contain about 99%) are also buried, leading to a research level of microbial diversity and ecology, lagging behind other communities in oil reservoir.

Culture-independent approaches to microbial community analyses have recently enjoyed a surge in popularity as new techniques have been developed and are available in most major research institutions. Molecular descriptions of microbial communities now dominate the literature in all areas of microbial ecology, not just petroleum microbiology (Hamme et al., 2003).

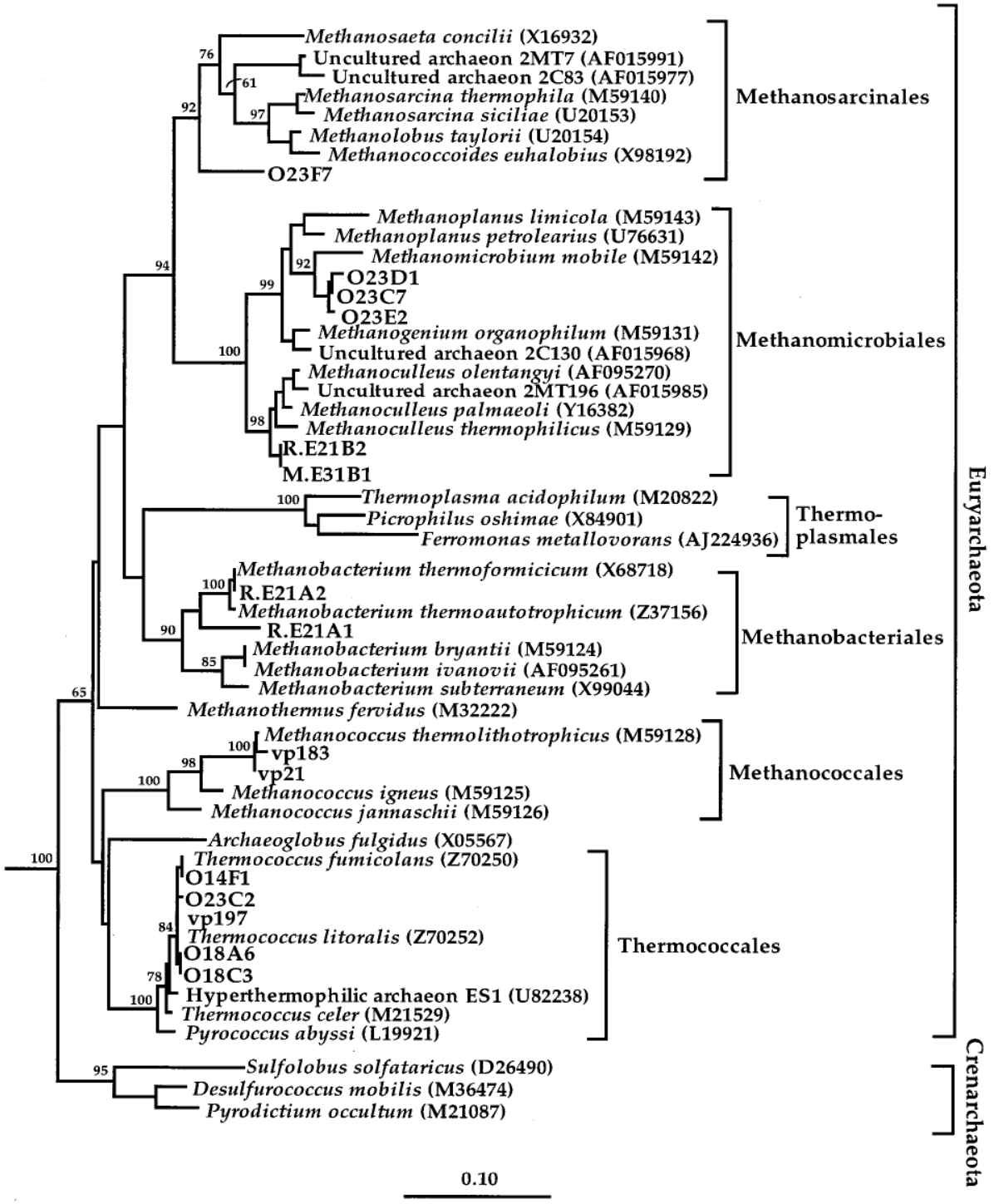

Figure 2. Example of phylogenetic tree. Phylogenetic tree of the archaeal domain and archaeal 16S rDNA phylotypes (Orphan et al., 2000). Bootstrap values (n = 1,000 replicates) of ≥ 50 are reported as percentages. The scale bar represents the number of changes per nucleotide position.

Since 1985, DNA sequencing technology was used to study the microbial ecology and evolutionary issues, whereas the research of microbial diversity has entered a new stage, and has gradually formed a development of methods and techniques of the molecular ecological technology of microorganisms. Internationally, a large number of research and development practices have proved that using molecular ecological technology to

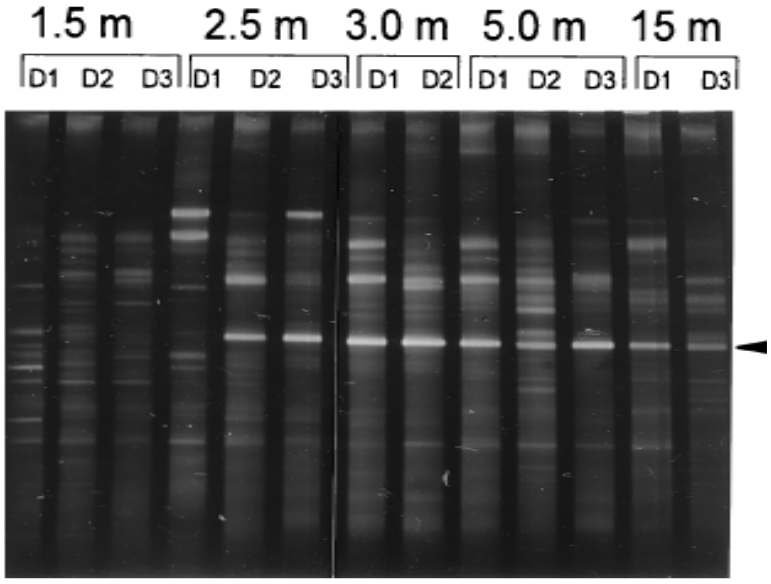

Figure 3. Example of a DGGE gel on DNA extracted from Lake Sælenvannet (Øveras et al. 1997). DGGE analysis showing variations in time and space of bacterioplankton community structure. Bacterial primers were used for PCR amplification. Five different depths and three different sampling times were processed. D1, 22 March 1995; D2, 29 January 1996; D3, 5 March 1996. The arrow labeled 17 indicates the fragment with high sequence similarity to *C. phaeovibrioides* DNA.

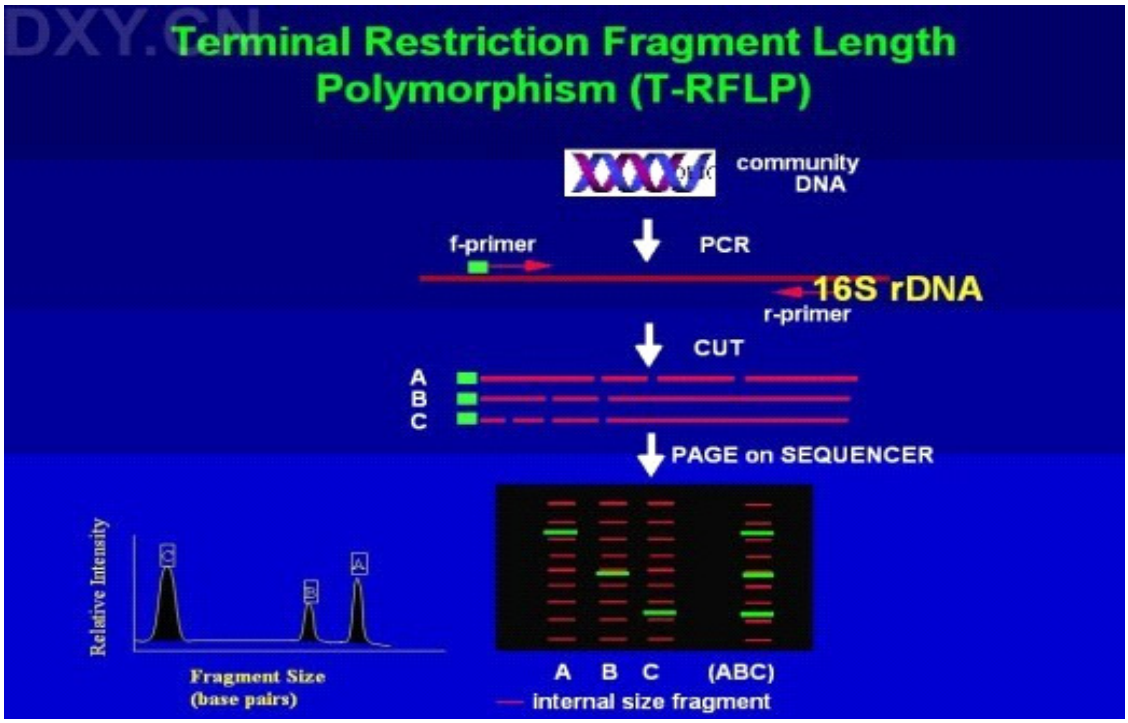

Figure 4. The flow chart of T-RFLP analysis technology (The picture is from Http://www.dxy.cn).

study microorganisms cannot be restricted by whether the microorganism can be isolated or cultured, and whether microorganism are lively in the laboratory or not. So, the complex microbial community structure can be

Table 1. Example of the closest relatives of archaeal phylotypes from 16S rDNA libraries (Orpan et al., 2000).

Division (% representation)	Type sequence	No. of RFLP types	No. of clones	Closest cultivated species	Source	Sequence similarity (%)
Methanomicrobiales (96)	O2 3C7[a]	4	145	*Methanoplanus petrolearius* *Methanomicrobium mobile*	Oil Cow rumen	95.7–96.1 93.9–94.4
Methanosarcinales (6)	O2 3F7[a]	1	7	*Methanosarcina thermophila*	Sewage	87.6
Thermococcales (1.3)	O2 3C2[a]	1	2	*Thermococcus litoralis*	Oil	99.1
Thermococcales (8.8)	O1 8A6[b]	5	14	*Thermococcus litoralis*	Oil	99.4–99.9

[a] 16S rRNA *E. coli* numbering (20 to 958). [b] 16S rRNA *E. coli* numbering (519 to 1,390).

Table 2. A summary of current techniques used to study complex microbial ecosystems (Zoetendal et al., 2004).

Methods	Uses	Limitations
Cultivation	Isolation; "the ideal"	Not representative, slow and laborious
16S rDNA sequencing	Phylogenetic Identification	Laborious and subject to PCR biases
DGGE/TGGE/TTGE	Monitoring of community/population shifts, rapid comparative analysis	Subject to PCR biases, semi-quantitative, identification requires clone library
T-RFLP	Monitoring of community shifts, rapid comparative analysis, very sensitive, potential for high throughput	Subject to PCR biases, semi-quantitative, identification requires clone library
SSCP	Monitoring of community/population shifts, rapid comparative analysis	Subject to PCR biases, semi-quantitative, identification requires clone library
FISH	Detection, enumeration, comparative analysis possible with automation	Requires sequence information, laborious at species level
Dot-blot hybridization	Detection, estimates relative abundance	Requires sequence information, laborious at species level
Quantitative PCR	Detection, estimates relative abundance	Laborious
Diversity microarrays	Detection, estimates relative abundance	In early stages of development, expensive
Non-16S rRNA profiling	Monitoring of community shifts, rapid comparative analysis	Identification requires additional 16S rRNA-based approaches

analyzed fast, accurately, roundly and truly.

At present, the microbial molecular ecology research focused on 16S rRNA sequencing and analysis, DGGE, T-RFLP, FISH, PCR, etc. Here are several major research methods in molecular biology and their application in oil microbial biodiversity research.

16S rRNA sequencing and analysis

16S rRNA sequencing and analysis method is an important way to infer phylogeny of bacteria and their evolution relationship, which comparatively analyze the homology of 16S rRNA sequence of different bacteria. The basic principle is as follows: 16S rRNA gene fragments are extracted from microbial samples, after which they are cloned, sequenced or digested. The probe hybridizations are used to gain 16S rRNA sequence information, and then the 16S rRNA sequence data in the database are compared with other data.

The evolutionary distance between them is calculated by the differences in sequence, whereas their position is determined in the evolutionary tree, so as to determine the types of microbes that may exist in samples.

For example, database searches with 16S rDNA sequences were conducted by using the BLAST program and the GenBank database. The profile alignment technique of ClustalW version 1.7 was used to align the

sequences, and the alignments were refined by visual inspection. Secondary structures were considered for the refinement analysis. A phylogenetic tree was constructed by the neighbor-joining method using the njplot software in ClustalW version 1.7. Nucleotide positions at which any sequence had an ambiguous base were not included in the phylogenetic calculations. Checks for chimeric sequences were conducted by using the chimera check in the RDP database (Watanabe et al., 2002).

One report by Orphan et al. (2000) shows that two 16S rRNA gene libraries were generated from the total community DNA collected from high-temperature, sulfur-rich oil reservoirs in California, using either archaeal or universal oligonucleotide primer sets. Sequence analysis of the universal library indicated that a large percentage of clones were highly similar to known bacterial and archaeal isolates recovered from similar habitats.

Gevertz et al. (2000) isolated two novel nitrate-reducing, sulfide-oxidizing bacteria strains, CVO and FWKO B, from the produced brine at the Coleville oil field in Saskatchewan, Canada. The present 16s rRNA analysis suggests that both strains are members of the epsilon subdivision of the *Proteobacteria* division, with CVO most closely related to *Thiomicrospira denitrifcans,* and FWKO B most closely related to members of the genus *Arcobacter*.

Li et al. (2007) analyzed the diversity of an archaeal community in the water from a continental high-temperature, long-term water-flooded petroleum reservoir in Huabei Oilfield in China. The archaea were characterized by their 16S rRNA genes. Phylogenetic analysis of these sequences indicated that the dominant members of the archaeal phylotypes were affiliated with the Methanomicrobiales, although some had been previously isolated from a number of high-temperature petroleum reservoirs worldwide. So, they are speculated to exhibit adaptations to the environments and the common habitants of geothermally heated subsurface environments.

Denaturant gradient gel electrophoresis (DGGE)

DGGE is a method used to separate DNA samples multiplied by PCR. DNA samples are pulled through a gel, containing a gradient of denaturant using an electrical field. Depending on which base pairs the DNA contains, it stops at specific denaturant concen-trations. A sample containing a number of bacterial strains thus gives a corresponding number of bands on a DGGE gel. The position and number of bands may itself be sufficient information in comparing different samples. The bands may also be cut out and the base pair sequence decoded by direct gene sequencing. Denaturing gradient gel electrophoresis (DGGE) has been used to resolve PCR-amplified regions of genes coding for 16S rRNA (16S rDNA), based solely on differences in nucleotide sequence (Øvreas et al., 1997; Ferris et al., 1996). This has proven to be a simple approach to obtain profiles of microbial communities that can be used to identify temporal or spatial differences in community structure or to monitor shifts in the structure that occurs in response to environmental perturbations (Øvreas et al., 1997; Ferris et al., 1996; Muyzer et al., 1993; Wawer et al., 1995). Moreover, since each DNA fragment in the profile is likely to be derived from one (or few) phylogenetically distinct population, one can readily obtain an estimate of species number and abundance, based on the number and intensity of amplified fragments in the profile. It has also been possible to infer the phylogeny of community members by DNA sequence analysis of amplified fragments after they have been excised (Øvreas et al., 1997; Muyzer et al., 1993, 1995; Rolleke et al., 1996; Teske et al., 1996).

Zang et al. (2008) monitored changes in exogenous bacteria and investigated the diversity of indigenous bacteria during a field trial of microbial enhanced oil recovery using DGGE. DGGE profiles indicated that the exogenous strains were retrieved in the production of water samples, and indigenous strains could also be detected. Sequence analysis of the DGGE bands revealed that Proteobacteria were a major component of the predominant bacteria. Their experiment confirmed that DGGE analysis was a successful approach to investigate the changes in microorganisms used for enhancing oil recovery.

Forney et al. (2007) applied the DGGE method to determine the relative genetic complexity of microbial communities at different depths in the meromictic Lake Sælenvannet. The technique was also used to monitor community changes in space and time (Øveras et al., 1997). The results showed that among the dominant populations, the representatives were related to *Chlorobium phaeovibrioides*, chloroplasts from eukaryotic algae, and unidentified *Archaea.*

Fluorescent *in situ* hybridization (FISH)

The genetic material (DNA, RNA) inside the bacteria is unique for all individual species. Using, as an example, the sequences of base pairs in the 16S rRNA, probes can be designed to target individual groups, generas or strains of bacteria. Adding a fluorophore (due to fluorescent) to the probe enables detection of cells to which the specific gene probe is attached. Though, the preparation steps are different, the cells are counted as in direct bacterial counts. Using different fluorophores, it is possible to stain more than one group at a time, and including a general stain like DAPI, it is possible to obtain quantitative information (% specific group of the total population) (Hojris et al., 2000).

Kleikemper et al. (2002) characterized the SRB population in a PHC-contaminated aquifer by using FISH. For *in situ* hybridization, they used the indocarbocyanine (Cy3)-labeled 16S rRNA oligonucleotide probes (all

purchased from MWG Biotech, Ebersberg, Germany) EUB338 to target bacteria, Arch915 for Archaea, SRB385 plus SRB385-Db for SRB, DSV698 plus DSV1292 for *Desulfovibrio*, DSB985 for *Desulfobacter*, and probe 660 for *Desulfobulbus*. The result showed that a large fraction of suspended bacteria is hybridized with SRB-targeting probes SRB385 plus SRB385-Db (11 to 24% of the total cells).

Terminal restriction fragment length polymorphism (T-RFLP)

T-RFLP is also known as the 16 S rRNA gene terminal restriction fragment (TRF) analysis technology, which is a recently emerging molecular biology technique of the microbial polymorphism research. When compared to other methods of molecular biology, there are some distinct advantages in T-RFLP technology. Sequence database with direct reference value, that is, all size of end fragments obtained from the digestion can be compared to end fragments in sequence database; hence, phylogeny can be inferred. When compared with DGGE relying on electrophoresis system, the results of DNA sequencing technology are more reliable. Analysis of capillary gel electrophoresis in T-RFLP is quicker, and the output of the results is in form of dates. Distinct advantages of T-RFLP make it an ideal community comparative analysis method. Therefore, T-RFLP has drawn the attention of the research staff more.

Yuan et al. (2007) used T-RFLP technique to analyze the microbial diversity of an injection well (S122ZHU) and three related production wells (S1224, S1225 and S12219) in the ShengLi oilfield in China. The Shannon-Wiener Diversity index, based on the T-RFLP profiles, indicated that the bacterial and archaeal species richness in the injection well was higher than those of the production wells. This study indicates that T-RFLP is useful for the analysis of the microbial diversity in petroleum reservoirs.

Quantitative dot blot

Information of abundance for specific DNA sequence can be obtained by using quantitative dot blot for the extraction of DNA from environment samples. The main principle is as follows: Separating the hybridization of specific probe (such as, specific 16 S rRNA gene probe) and universal probe (such as, the total 16 S rRNA gene probe) from the total DNA of environmental samples, the relative abundance of the specific DNA (such as, the 16 S rRNA gene sequences) can be determined by the ratio of two hybridization signals of the two types of probes, so as to reflect the number of cells of specific microbial or the relative physical activity of specific populations.

Quantitative polymerase chain reaction (qPCR)

Genetic material may be extracted from the total population in a sample. Though, such extracts contain information on all cells present, the concentrations of genetic material is too low to be analyzed. The polymerase chain reaction enables exponential multiplication of the DNA, thereby increasing the concentration to measurable amounts (~10^9 gene copies). With a few adjustments, the PCR method can be made quantitatively, and is called real time quantitative PCR (abbreviated as qPCR). In the qPCR method, a fluorescent signal is measured after each cycle in the PCR. This fluorescent signal corresponds with the amount of DNA produced in the PCR, which again corresponds with the initial number of specific bacterial cells in the sample. Most qPCR assays have a linear range spanning more than six orders of magnitude regarding DNA concentration. The method can detect very few (theoretically only one) gene copies in the initial sample. However, the detection limit (sensitivity) and target specificity has to be carefully evaluated in each new qPCR application (Skovhus et al., 2004).

Micro auto radiography (MAR)

To determine if a bacterium is capable of utilizing a specific substrate (for example, glucose or acetate), the bacteria can be exposed to a media, containing a radioactive variation of the substrate in question. If the bacteria can utilize the substrate, it becomes slightly radioactive. Within a complex sample of various strains, some bacteria may become radioactive and some may not. The sample can be developed like a photographic film, using silver grains as indicators for radioactivity and an image of cells capable or not of utilizing the specific substrate may be obtained (Nielsen et al., 2002).

Bio-chip (biochip)

Due to the integration of DNA blotting hybridization technology, a revolution is taking place in molecular biology techniques, which is bio-chip (biochip) technology, also known as gene chip or DNA arrays. Biochips are composed of tens of thousands of gene probes closely aligned as networks. Through combining the DNA fragments (whose base sequences have been marked DNA or RNA), which possess complementary sequence of base pairs, we can determine the corresponding categories and infer microorganism groups in environmental samples. The current view of the 16S rRNA gene chip is still in the stage of basic research; however, with the wider use of 16 S rRNA / DNA technology, such products in the market are an inevitable trend.

ISSUES AND FUTURE PROSPECTS

The nature and diversity of bacteria in oil field ecosystems is still poorly understood. However, recent incorporation of molecular methods has allowed a broader characterization of microbial assemblages in this type of environment (Sette et al., 2007). However, any method used to study the composition of a microbial community has its limitations. Madsen (2000) reviewed many of the major nucleic acid - based methods used for characterizing naturally occurring microorganisms, and he listed limitations for each procedure (Table 2).

During 16S rRNA sequencing and analysis, DNA of microorganisms may have been missed due to PCR biases when using community DNA, such as preferential amplification, or differential lysis efficiency may have occurred when purifying DNA from the samples prior to 16S rRNA gene library assembly.

A study by Watanabe et al. (2002) shows that methods employed in the molecular ecological approaches largely affect the results obtained. In one report, Kazuya et al. (2002) pointed out that they did not use FISH, because a possible bias that causes the underestimation of slow-growing bacteria has been suggested. Besides, in the present study, FISH with the *Bacteria*- and *Archaea*-specific probes detected only 60% in the total of DAPI-stained cells. In addition, a large portion of labeled cells exhibited weak signals.

Isabel et al. (2003) point out that, while using PCR-DGGE to examine the bacteria in wine fermentations, they noted that several commonly used PCR primers for amplifying bacterial 16S rDNA also coamplified yeast, fungal, or plant DNA present in samples. Amplification of nonbacterial DNA can result in a masking of bacterial populations in DGGE profiles. Coamplification of nonbacterial DNA is problematic since it can result in an overestimation of the bacterial content of any particular niche. Moreover, competition between bacterial and non-target templates during PCR may mask lower bacterial populations. This work demonstrates the importance of testing purportedly bacterium-specific PCR primers on potential eukaryotic DNAs that might co-purify with bacterial DNA in environmental samples prior to embarking on a detailed analysis.

Zang et al. (2008) also pointed out the shortcomings of the use of PCR-DGGE. First, PCR-DGGE profiles could only detect the predominant population in the environmental samples. Secondly, the reported sensitivity of DGGE was 1% of the template DNA. Thirdly, many problems might arise during the sample collection, DNA extraction, PCR amplification or DGGE steps (gel resolution and gel staining). Only microorganism populations with high concentrations could be detected by DGGE fingerprinting.

Although, the PCR and DNA extraction used in investigating the microbial ecosystems would introduce biases (Polz et al., 1998; Suzuki and Giovannoni, 1996; Von Wintzingerode et al., 1997), they are the primary sources of information available to assess the phylogenetic richness and complexity of microbial communities. Additionally, when compared to other molecular techniques, the RNA approach has a great advantage, in that the generation of sequence data can be used to design group-specific probes and primers for further studies such as microarray and real-time PCR, which have not been used to characterize the microbial diversity in the petroleum reservoir (Li et al., 2007).

The use of T-RFLP and DGGE on the microbial community relations in reservoirs rose from the traditional research methods to the molecular level. Researches show that the DGGE technology can be used to find the advantage bacteria groups and the changes on bacterial diversity, and through T - RFLP technology, we can find the main species in the reservoir and the situation of abundance. The combination of the two kinds of methods can reflect the relation of microbial community in reservoir truly.

Some scholars suggest that a more comprehensive assessment of microbial diversity in oil, and probably other environments, can be obtained by using a combination of culture- and molecular-based techniques than by using either method alone (Sette et al., 2007), based on both molecular and cultivation techniques, allowing researchers insights into the microorganisms that might be involved in the biogeochemical transformations that take place in these environments.

Although, there is much to be desired, molecular biology technology and research strategy will be the main approach to reveal the true level of diversity and species composition of microbial community in oilfield. Its development will promote the great progress of oil and environmental microbiology technology, and is of great practical significance.

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