Jestr

Journal of Engineering Science and Technology Review 11 (2) (2018) 153 - 159

Research Article

JOURNAL OF
Engineering Science and Technology Review

www.jestr.org

Characteristics of Bacterial Communities in High-Temperature Heavy Oil Reservoir

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Received 6 November 2017; Accepted 30 March 2018

Abstract

Revealing the characteristics of microbes inhabiting in oil reservoirs is significant for the effective application of microbial enhanced oil recovery (MEOR) technology. Many studies have been carried out to discover microbial communities in conventional oilfields, and medium- and low-temperature heavy oil reservoirs. However, few explorations on the characteristics of bacterial communities in high-temperature (>50 °C) heavy oil reservoirs are conducted. In this study, Bamianhe oilfield (China) was taken as an example and the 16S rDNA clone library technology was adopted to analyze the composition, abundance, and distribution of bacterial communities in a high-temperature (60 °C) heavy oil reservoir. A total of 715 sequences obtained from the four clone libraries were assigned to 130 operational taxonomic units (OTU) and 20 bacterial groups were identified in the oil reservoir. Results demonstrate that (1) the heavy oil reservoir has high bacterial diversity, and thermophilic bacterial communities are predominant. However, there are differences in bacterial community structure of clone libraries based on the correlation analysis. (2) The distribution of bacterial communities is consistent with the temperature, salinity, and oil properties of the oil reservoir. The findings of this study provide basic theoretical guidance for the application of MEOR in high-temperature heavy oil reservoirs.

Keywords: 16S rDNA clone library, Bacterial community, High temperature, Heavy oil

1. Introduction

Heavy oil is characterized by high viscosity and density relative to light oil [1]. With the depletion of light oil resources and the increasing global energy demand, more attention has been paid to the development of inexpensive recovery technologies for heavy oil resources [2]. Generally, enhanced oil recovery (EOR) techniques for heavy oil reservoirs include chemical and physical methods. Chemical methods include the use of solvents and surfactants. Thermal methods involve the treatment of production wells with hot fluids and steam injection, which is currently the most effective technology for improving heavy oil recovery. However, thermal methods require a high initial investment in equipment, cause water and soil pollution during application and exhibit a rapid decline in oil production [3]. Therefore, researchers have increasingly focused on the application in microbial enhanced oil recovery (MEOR) due to its simplicity, wide applicability, and economic and environmental benefits [4].

MEOR is a cost-effective technology for the exploitation of crude oil in conventional oil reservoirs, and many successful implementations of field trials have been reported [5]. Reducing the viscosity of crude oil is the key step to improve oil recovery in heavy oil reservoirs. MEOR uses biosurfactants to reduce the viscosity of heavy oil or convert heavy oil into light oil through bacterial degradation [6]. Therefore, before MEOR technology can be applied to exploit heavy oil, it is important to fully understand the ecological distribution and metabolic characteristics of microorganisms in oil reservoirs. It is believed that 99% of the microorganisms in nature cannot be identified through culture experiments. The 16s rDNA sequencing technique, a breakthrough in molecular biology, has been used to study microorganisms in oil reservoirs [7]. Although microbial communities in oil reservoirs have been reported in many studies, studies on microbial communities in heavy oil reservoirs, especially those within high-temperature oilfields are limited.

MEOR uses two main bacterial mechanisms for reducing the viscosity of heavy oil [8]. First, microorganisms can degrade asphaltene or gum and decrease the average molecular weight of heavy oil. Second, the byproducts of microbial metabolisms, such as surfactants, solvents, acids, and gases, can considerably reduce the viscosity of heavy oil [9]. Nowadays, considerable attention has been paid to the use of bacteria producing surfactants or degrading heavy oil in the complex environments of oil reservoirs [7]. Microorganisms inhabiting oil reservoirs can produce biosurfactants and/or converting heavy crude oil into light oil through microbial metabolisms [6]. Therefore, it is important to fully understand and evaluate the ecological distribution and metabolic characteristics of microorganisms in oil reservoirs before MEOR technology can be applied to exploit heavy oil.

The rest of this study is organized as follows. Section 2 gives the relevant background. Section 3 discusses the

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doi:10.25103/jestr.112.21

collection of experimental samples, construction of 16S rDNA clone libraries, and data analysis of clone libraries. Section 4 analyzes the composition, abundance, and distribution of bacterial communities in the high-temperature heavy oil reservoir, and potential bacteria for MEOR. Finally, Section 5 presents the conclusions of this study.

2. State of the art

The successful application of MEOR technology is based on the activity of microorganisms and the synergistic effect of their metabolites in oil reservoirs [10]. It involves many complex physiological, biochemical, and physical processes. Investigating microbial communities in oil reservoirs is a prerequisite for the application of MEOR technology. Generally, the research methods of studying microorganisms in oil reservoirs include culture, 16S rDNA clone library, second-generation DNA sequencing technology, polymerase chain reaction (PCR)/denaturing gradient gel electrophoresis, real-time PCR, and fluorescence in situ hybridization technology. The techniques for biological analysis have broken the limitation of traditional cultivation methods and the microbial communities in oil reservoirs have been objectively revealed [5]. Researchers widely use 16S rDNA clone library technology due to its simplicity, time efficiency, and inexpensiveness. This method has been used in investigating microbial communities in conventional oil reservoirs with temperatures of 20 °C–50 °C, and has promoted the successful implementations of MEOR in field trials [7]. However, few reports on microbial communities in heavy oil reservoirs have been documented. Microbial communities in the heavy oil reservoir located in Xinjiang (China) with a temperature of 30 °C were analyzed based on the 16S rDNA clone library technology. The results showed that hydrocarbon-degrading, surfactant-producing, fermentative, nitrate-reducing, and sulfate-reducing bacteria coexisted in the oilfield environment [11]. Nazina et al. [1] studied the metabolic diversity of bacterial communities in the Dagang heavy oil reservoir (China) with a temperature of 50 °C, and revealed the characteristics of several microbial communities.

The bacteria for MEOR in oil reservoirs can improve the mobility of crude oil and enhance oil production by decreasing the viscosity of crude oil through bacterial biodegradation or bioemulsification [12, 13]. The majority of studies have focused on bacteria that could enhance oil recovery in laboratories. The dominant groups of bacteria for MEOR belong to the genera of *Bacillus*, *Pseudomonas*, *Acinetobacter*, and *Clostridium*, which can metabolize hydrocarbon and produce surfactants/emulsifiers, organic solvents, acids, and gases [14-16]. The results of physical simulation in laboratories show that these strains can significantly improve oil recovery [5]. In recent years, many bacteria for MEOR have been reported to degrade or emulsify heavy oil. For example, bacteria affiliated with *Bacillus subtilis*, *Brevibacillus brevis*, *Petrobacter*, *Marinobacter*, *Geobacillus stearothermophilus*, *Geobacillus pallidus*, and *Paenibacillus ehimensis* have the potential ability to exploit crude oil or increase heavy oil recovery [17-24]. Most especially, *Bacillus subtilis* and *Bacillus licheniformis* isolated from the Omani heavy oil reservoir at 40 °C were reported to enhance oil recovery by 16% [8].

Studies on the characteristics of bacterial communities in high-temperature (60 °C) heavy oil reservoirs remain limited. Therefore, the composition, abundance, and distribution of bacterial communities in the Bamianhe oilfield with a temperature of approximately 60 °C were elucidated by using 16S rDNA clone library technology. The results can provide theoretical value for the application of MEOR to explore crude oil or enhance heavy oil recovery in high-temperature oil reservoirs.

3. Materials and methods

3.1 Sample collection

Oil–water samples were collected directly from the wellhead of four production wells in July 15, 2017, and stored in sterile 500 mL serum bottles. The four samples were transported to the laboratory as soon as possible for further analysis. The four production wells of the Bamianhe oilfield are numbered as follows: M137-7-X15 (M715), M138-7-X23 (M723), M138-9-X13 (M913), and M138-9-X15 (M915). The M715 well belongs to Block M137, and the rest belong to Block M138.

The Bamianhe oilfield is located in Dongying, east China's Shandong Province (Fig. 1). The reservoir temperature is approximately 60 °C. Block M137 and Block M138 of the oil reservoir have similar water formation ($CaCl_2$) and salinity content (35,000 mg/L). For the two blocks, the average buried depth is 950–1210 m with an average porosity of approximately 33.7% and permeability of approximately 0.458 μm^2. The oil viscosity is 4896 mPa·s under 50 °C conditions.

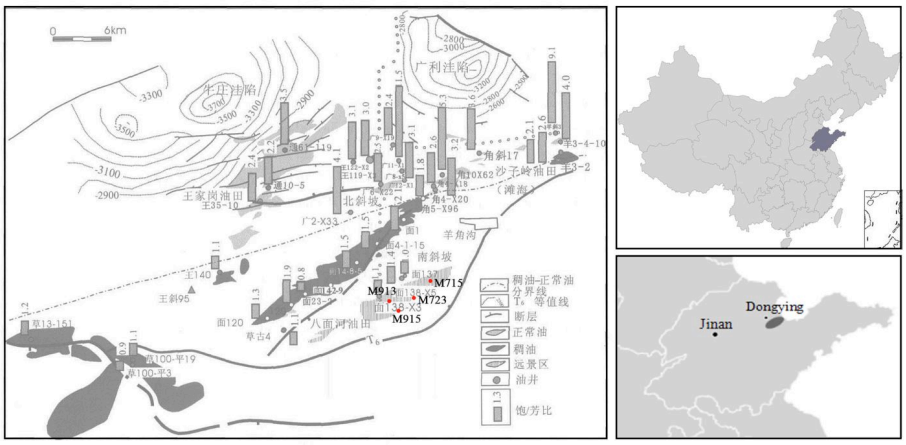

Fig. 1. Location of the Bamianhe high-temperature heavy oil reservoir (China)

3.2 Construction of 16S rDNA clone libraries

Based on the 16S rDNA clone library technology (Fig. 1), bacterial communities in the Bamianhe high-temperature heavy oil reservoir were analyzed. The experimental procedures included the collection of bacterial cells in samples, extraction of genomic DNA, amplification of 16S rDNA fragment, selection of positive clones, amplified ribosomal DNA restriction analysis, and analysis of 16S rDNA sequences.

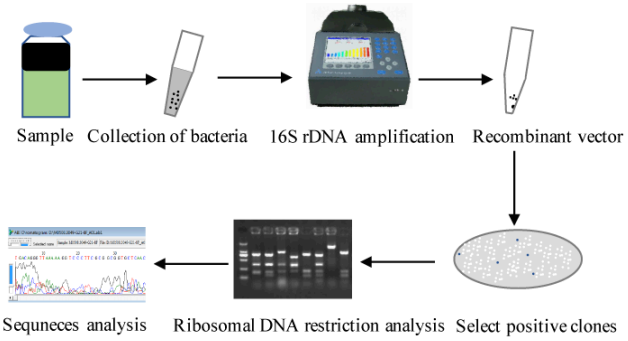

Fig. 2. Diagram illustrating the experimental procedures for the construction of 16S rDNA clone library

3.2.1 Genomic DNA extraction from samples

After the four oil–water samples were well shaken, all 500 mL samples were used to pellet cells by centrifugation at 12,000 rpm in a high-speed centrifuge (5424R, Eppendorf, Germany) for 8 min. Following the manufacturer's instructions for the TIANamp Micro DNA Kit (DP316, Tiangen Biotech (Beijing) Co., Ltd., China), genomic DNA was extracted in triplicate to avoid bias and the DNAs were mixed.

3.2.2 Amplification of 16S rDNA fragment

The 16S rDNA fragments of genomic DNA were amplified by using the universal bacterial primers 27F/1492R, and the amplified fragments were approximately 1500 bp [11]. The 25 mL PCR reacting system contained 2.5 mL of PCR buffer (Mg^{2+}), 10 nmol of deoxynucleotide triphosphate, 1 U Taq DNA polymerase (TaKaRa, Japan), 10 pmol of each primer, and 1 mL of genomic DNA. The thermal cycling conditions were as follows: an initial denaturation at 94 °C for 5 min; 40 cycles of 94 °C for 30 s, 56 °C for 60 s, and 72 °C for 90 s; and a final extension step of 72 °C for 10 min.

3.2.3 Genomic DNA extraction from samples

After purification with an Agarose Gel DNA Purification Kit (Tiangen Biotech (Beijing) Co., Ltd., China) and according to the manufacturer's instructions, the amplicons of 16S rRNA genes were cloned into Trans1-T1 competent cells (Tiangen Biotech (Beijing) Co., Ltd., China) using the PGEM-T Easy Vector (Promega, Madison, WI, USA). Two hundred putative clones (white) from each clone library were randomly chosen, transferred to a labelled Luria–Bertani (LB) plate with ampicillin (100 μg/mL), and cultured at 37 °C overnight. A re-amplification, with sets of vector-specific primers T7/SP6, was performed to determine positive clones. During the re-amplification procedure, a small quantity of cells of putative clones was used as template DNA in the reaction mixtures. The PCR products of positive clones were classified into different operational taxonomic units (OTUs) by using amplified ribosomal DNA restriction analysis with *Hinf*I and *Hae*III (Fermentas, Lithuania) [25].

3.2.4 Data analysis of four clone libraries

The representative clones were inoculated at 37 °C for 24 h in LB medium with ampicillin (100 μg/mL) and then were selected for 16S rDNA sequencing. Sequencing was conducted by using an ABI PRISM 3730 DNA sequencer (SinoGenoMax Co., Ltd., Beijing, China). The obtained sequences were manually checked and edited by using DNAMAN (version 5.2.2.0). Using BLAST, a representative sequence from each OTU was compared with sequences in the GenBank database to determine the most closely related sequences of bacteria. Sequences with more than 97% similarity were considered to be of the same bacterial genus. The evenness of OTU distribution of clone libraries was statistically analyzed by using the Shannon index [26]. Correlation analysis between bacterial communities and samples was evaluated by using Canoco (version 4.5).

4. Results and discussions

4.1 Statistical analysis of 16S rDNA clone libraries

To investigate bacterial communities within high-temperature heavy oil reservoirs, four oil–water samples retrieved directly from Block M137and Block M137 of the Bamianhe high-temperature heavy oil reservoir were used to construct four clone libraries (M715, M723, M913, and M915). A total of 715 positive clones were obtained from the four clone libraries (Table. 1). The 16S rDNA sequences of the positive clones were digested by *Hae*III and *Hinf*I. According to the amplified ribosomal DNA restriction analysis, the number of positive clones in the clone libraries of M715, M723, M913, and M915 were identified as 18, 28, 36 and 48 OTUs, respectively.

The Shannon index of bacterial diversity was calculated to assess the evenness of the OTU distribution. The four samples show obviously different Shannon indices (Table 1). The Shannon indices of the three samples from Block M138 are higher than that of one sample from Block M137, which suggests that the bacterial communities of Block M138 are more diverse and richer than those of Block M137. The M915 sample has the highest Shannon index, indicating the highest bacterial diversity among all four samples. By contrast, the Shannon index of the M723 sample is only 2.63. The M715 sample has the lowest Shannon index (1.74).

Table. 1. Statistical analysis of four clone libraries

Production well	M715	M723	M913	M915
Block	M137	M138		
Number of positive clones	173	176	180	186
OTUs	18	28	36	48
Shannon	1.74	2.63	2.73	3.17
Simpson	0.63	0.80	0.83	0.84

4.2 Composition and abundance of bacterial communities in the high-temperature heavy oil reservoir

The composition and relative abundances of bacterial communities in four clone libraries from the Bamianhe high-temperature heavy oil reservoir are shown in Fig. 3. Similarities are found in the composition of bacterial communities in the four clone libraries at the genus level. First, groups of thermophilic bacteria are identified and dominant in all the four clone libraries. The genus *Thermanaeromonas* has predominant in the M715, M723, and M913 clone libraries, accounting for 48%, 35%, and 26%, respectively. By contrast, the genus

Thermacetogenium accounts for 30% and is predominant in the M915 clone library, and it is also detected in the M723 clone library with small quantities (8%). Second, *Pseudomonas* is identified in the four clone libraries, accounting for 36% (M715), 16% (M723), 13% (M913), and 13% (M915), respectively. Unclassified bacteria are also observed in the four clone libraries, which range from 6.36% to 18.74%. Third, *Thioclava* and *Petrobacter* coexist in the M723, M913, and M915 clone libraries. *Pannonibacter* is also found to coexist in the M715 and M915 clone libraries, whereas *Hydrogenophilus* shares in M723 and M915.

There are more bacterial communities in Block M138 than in Block M137 of the Bamianhe oilfield. The remaining bacterial sequences of the M715 clone library of Block M137 are mainly of the genera *Petrobacter*, *Pannonibacter*, and *Paenibacillaceae*. By contrast, *Acinetobacter*, *Bacillus*, *Deferribacter*, *Denitromonas*, *Desulfocaldus*, *Desulfomicrobium*, *Hydrogenophilus*, *Marinobacter*, *Oceanicaulis*, *Pannonibacter*, *Paracoccus*, *Petrobacter*, *Rhodobacter*, *Sphingomonas*, *Tepidiphilus*, and *Thioclava* are identified in the M723, M913, and M915 clone libraries of Block M138.

Additionally, the correlation relationship between bacterial communities and samples was evaluated by using Canoco. The results show that there are differences in the structure of bacterial communities of the four samples. Compared with M715, the degree of differences between the bacterial communities and samples has the following order: M913 > M915 > M723 (Fig. 4).

4.3 Analysis of the distribution of bacterial communities in the high-temperature heavy oil reservoir

The geological characterization of oil reservoirs, such as temperature, salinity and oil properties, affects microbial diversity and distribution in oil reservoirs [7]. Thermophilic genera closely related to *Thermanaeromonas*, *Thermolithobacter*, *Thermacetogenium*, *Hydrogenophilus*, *Tepidiphilus*, *Petrobacter*, *Bacillus*, and *Deferribacter* are observed in the Bamianhe oilfield, which matches with the 60 °C temperature of the oil reservoir. The halotolerant groups of *Paenibacillaceae* and *Marinobacter* are detected in the high-salinity (3.5 g/L) of oil–water samples collected from the Bamianhe oil reservoir. Moreover, the strains of *Pseudomonas*, accounting for 13% to 36%, are identified in all four clone libraries and can use heavy oil to grow, which is consistent with Bacosa's report [27].

Only a few types of bacteria have been reported because of the complexity of oilfield environments [28]. In the study, there are many 16S rDNA sequences with less than 97% similarity to the bacteria mentioned in previous studies, which indicates that new bacterial species exist in the Bamianhe oilfield with a temperature of 60 °C. For example, sequences of M723-11 and M915-15 OTUs are closely related to *Thermanaeromonas* (94%–96% identity), M723-24 and M915-18 OTUs to *Thermacetogeniu* (95% identity), and M915-38 OTU to *Deferribacter thermophilus* (94% identity). Additionally, unclassified bacteria are obtained in all four clone libraries, indicating that the oil reservoir harbors a unique community of novel bacterial species or genera.

4.4 Potential bacteria for microbial enhanced heavy oil recovery

The effective application of MEOR technology is generally believed to involve a combination of bacteria with different physiological and metabolic abilities [5]. According to the alignment analysis of the 16S rDNA sequences of the M715, M723, M913, and M915 clone libraries in GenBank, the groups of hydrocarbon-degrading, fermentative, and nitrate-reducing bacteria for MEOR could improve oil recovery in the Bamianhe high-temperature heavy oil reservoir.

First, the groups of thermophilic hydrocarbon-degrading bacteria are identified in the high-temperature heavy oil reservoir. *Pseudomonas aeruginosa* detected in the four clone libraries was reported to be able to produce rhamnolipids that could emulsify heavy oil and could be successfully applied in high-temperature (70 °C) oil reservoir to enhance oil recovery by 20% [16, 29, 30]. *Marinobacter hydrocarbonoclasticus* identified in the M913 clone library could degrade aromatic hydrocarbons of crude oil [31]. Additionally, the bacterial sequences of the four clone libraries are closely related to the genera *Petrobacter*, *Bacillus*, *Pantoea*, *Acinetobacter*, and *Sphingomonas*, which could utilize polycyclic aromatic hydrocarbons [3, 32, 33].

Second, the strains of fermentative bacteria detected in the oil reservoir could produce acids or gases. *Thermanaeromonas toyohensis,* accounting for 35% (M715), 28% (M723), 18% (M913), and 16% (M915), was reported to be capable of fermenting glucose or xylose to produce acids [34]. The strains of *Pannonibacter phragmitetus* identified in M715 clone library could aerobiotically or anaerobiotically ferment glucose to produce acids [35]. *Thermacetogenium phaeum* detected in the M723 clone library could oxidize acetic acid to carbon dioxide [36].

Additionally, thermoresistant nitrate-reducing bacteria, such as *Denitromonas indolicum* [37], *Hydrogenophilus hirschii* [38], *Tepidiphilus margaritifer* [39], and *Petrobacter succinatimandens* [40], are observed in the M723, M913 and M915 clone libraries, and *Thermophilus Deferribacter* is identified in M915 clone library [41]. However, sulfate-reducing members of *Desulfocaldus* are identified in the M915 clone library. The strains of *Deferribacter thermophilus*, *Petrobacter succinatimandens*, and *Denitromonas indolicum* have the potential to reduce the activity of sulfate-reducing bacteria via competition, and thereby controlling the corrosion of the oil production equipment [42].

5. Conclusions

To investigate the characteristics of bacterial communities in high-temperature (>50 °C) heavy oil reservoirs, as illustrated by the example of the Bamianhe oil reservoir in this study, the 16S rDNA clone library technology was used to analyze the composition, abundance, and distribution of bacterial communities in 60 °C temperature heavy oil reservoir. The conclusions are as follows:

(1) The diversity of the bacterial communities in the high-temperature heavy oil reservoir is high. Twenty groups of bacteria were identified in the Bamianhe high-temperature heavy oil reservoir. Most especially the thermophiles groups of *Thermanaeromonas* and *Thermacetogenium* are dominant. However, there are differences in the bacterial community structure among clone libraries.

(2) The distribution of bacterial communities is consistent with the temperature, salinity, and oil properties of the oil reservoir. Thermophilic genera (*Thermanaeromonas*, *Thermolithobacter*, *Thermacetogenium*, *Hydrogenophilus*, *Tepidiphilus*, *Petrobacter*, *Bacillus,* and *Deferribacter*), unclassified bacteria, and halotolerant bacterial species (*Paenibacillaceae* and *Marinobacter*) are observed in the Bamianhe high-temperature heavy oil reservoir.

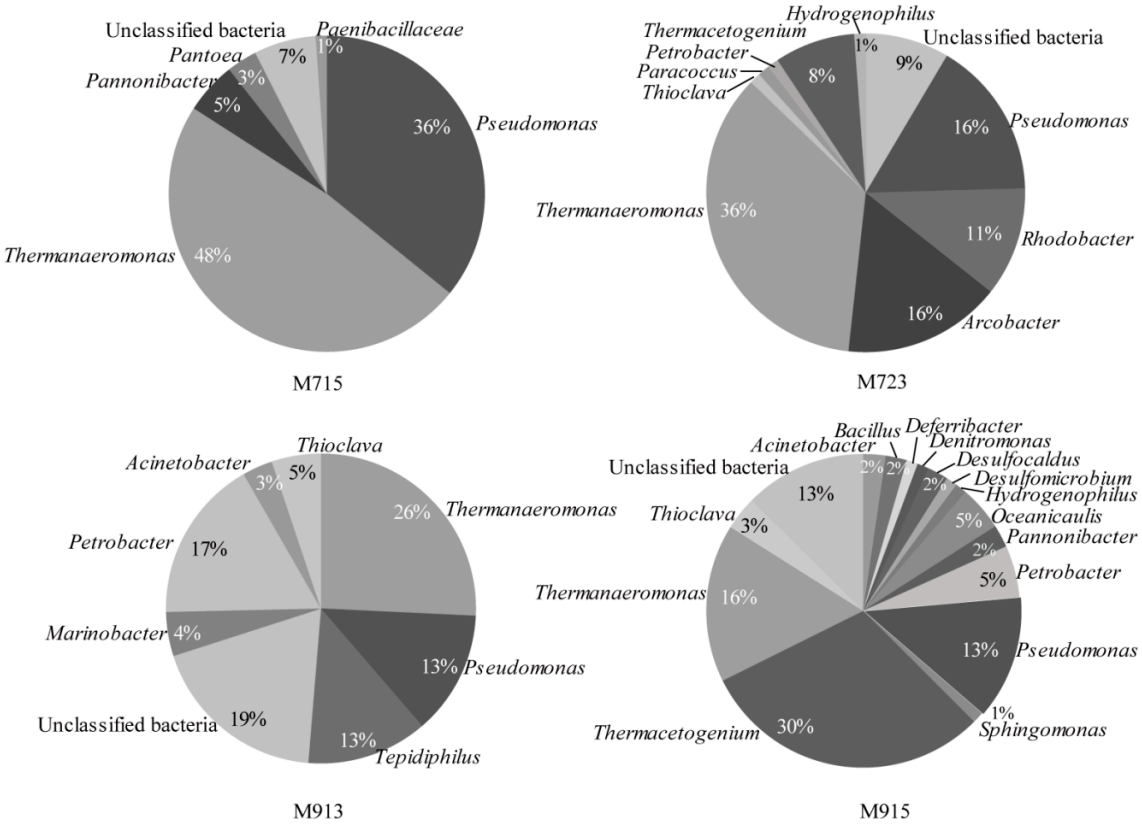

Fig. 3. Composition and relative abundances of bacterial communities in M715, M723, M913, and M915 clone libraries

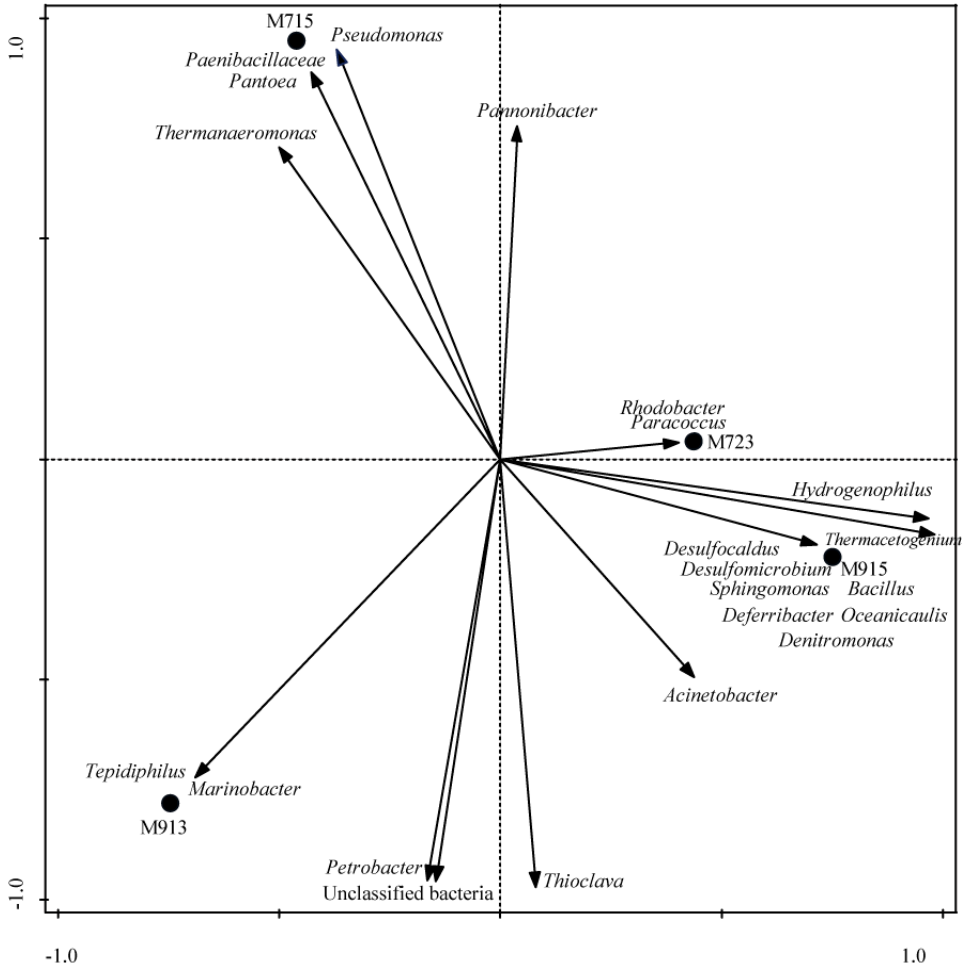

Fig. 4. Correlation analysis of bacterial communities and samples

(3) Potential bacteria for MEOR are identified in the high-temperature heavy oil reservoir. Hydrocarbon-degrading bacteria (*Pseudomonas*, *Acinetobacter*, *Marinobacter*, *Bacillus*, *Petrobacter*, *Pantoea*, and *Sphingomonas*) and fermentative bacteria (*Thermanaeromonas*, *Thermacetogenium*, and *Pannonibacte*) are identified and can reduce the viscosity of heavy oil to improve oil recovery. In addition, thermoresistant nitrate-reducing groups of *Petrobacter succinatimandens* and *Denitromonas indolicum* could be used to reduce the damage of sulfate-reducing bacteria *Desulfocaldus* to oil production.

On the one hand, although the 16S rDNA clone library technology used in this study detected fewer sequences than that generated by modern high-throughput sequencing technology, it still could effectively reveal the characteristics of bacterial communities with different physiological and metabolic abilities in the Bamianhe high-temperature heavy oil reservoir. The results of this study provide a theoretical reference value for the application of MEOR in high-temperature heavy oil reservoirs. On the other hand, the inadequacy of the study was that the number of samples was insufficient. Therefore, it is necessary to collect a large number of samples from high-temperature heavy oil reservoirs for further analysis in order to fully understand the characteristics of the bacterial communities in the special oil reservoir environments.

On the basis of this study, the future research directions are to 1) further study the metabolic functions of bacteria for MEOR, e.g., the structure of biosurfactants, changes of crude oil composition before and after bacterial biodegradation, and evaluation of oil recovery in the laboratory and oilfield, and 2) comprehensively evaluate the bacterial interactions for MEOR. The successful application of MEOR technology often requires the cooperation of bacterial groups, which is a research trend in comprehensively evaluating the oil recovery of multi-functional bacterial communities in the oil reservoirs.

Acknowledgements

The study was supported by National Science and Technology Major Project (No. 2016ZX05007-003) and the National Natural Science Foundation of China (No. 41673047, 41572125).

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