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The Analysis of Bacterial Diversity in High Thermic-Low Salinity Oil Reservoirs Prior to Microbial Enhanced Oil Recovery Field Test

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Abstract: Nutrition injection in Microbial Enhanced Oil Recovery (MEOR) process might stimulate not only desirable microorganisms but also the undesirable ones. Therefore, studying the properties of bacterial community in reservoir prior to field test is a critical step in MEOR process. In this study, bacterial communities from high thermic and low salinity reservoirs located in Japan, China and Indonesia were characterized by 16S rDNA sequence analysis. Bacterial genomic DNAs were extracted from produced water samples and their sequences were evaluated by genetic fingerprinting based approach. PCR-DGGE analyses showed multiple bands in all produced water samples which indicated high bacterial diversities. Sequences identified in this study were mainly related to only five phyla, i.e., Bacteroidetes, Thermotogae, Defferibacteres, Firmicutes and Proteobacteria, suggesting that phylotype richness is low in oil reservoirs. One sequence of Bacteriodetes-affiliated bacteria which showed 99% similarity with Bacteriodales bacterium 5bM was retrieved from DGGE results of all oilfields. This result showed that, Bacteriodetes can grow well in Indonesia, Japan and China oilfield, making it the best bacterial candidate for MEOR field test. Results showed in this study revealed that finger printing is a considerable technique for microbial screening prior to MEOR field test application.

Key words: 16S rDNA, MEOR, DGGE, oilfield, bacterial diversity, screening

INTRODUCTION

Studies on subsurface microbiology reveal that microorganisms can grow in deep subsurface area where temperature is lower than 125°C, pH ranges between 1-11 and salinity is lower than 300 g/L with or without oxygen (Bass and Lappin-Scott, 1997). As this understanding developed, the studies on oil reservoir as a habitat for microorganism have been extensively improved. Many studies showed that those microorganisms were capable of producing a number of byproducts such as acids, solvents, gases, biosurfactants, biopolymers and emulsifiers as well as having activities on hydrocarbon metabolism and rock pore plugging that are potentially useful for oil recovery (Youssef *et al*., 2009). The technology that employs indigenous reservoir microorganism to improve oil recovery is commonly known as Microbial Enhanced Oil Recovery (MEOR).

There are three general strategies commonly applied for MEOR in oilfield industries: biostimulation by injecting selected nutrient into reservoir in order to stimulate growth of indigenous microorganisms, bioaugmentation by injection of exogenous microorganisms and nutrients into reservoir and injection of bioproduct (s) produced in bioreactor under industrial condition (*ex situ* MEOR) (Banat *et al*., 2000). The last strategy is not widely used in MEOR field test as it requires costly surface facilities such as bioreactors, extractors and purifiers of bioproduct (Moses, 1987). The introduced nutrition either during biostimulation or bioaugmentation might stimulate not only desirable microorganisms but also the undesirable ones such as Sulfate Reducing Bacteria (SRB) which are commonly known to cause reservoir souring and subsequent corrosion of reservoir facilities. Therefore, studying the properties of bacterial community in reservoir prior to MEOR field test is very important. With that information, nutrition mixture for biostimulation can be formulated correctly to avoid growth stimulation of undesirable microorganisms during MEOR process. In addition, the insight of bacterial community prior to MEOR field application will be very useful as a baseline data for monitoring after injection of microorganisms and nutrition into oil reservoir.

MEOR implementation strategy will be determined based on reservoir conditions such as temperature, pressure, pH, porosity, salinity, geological structure, organic and inorganic compositions and crude oil characteristics. Microorganisms which are suitable for MEOR field application must be able to grow under environmental conditions encountered in oil reservoirs.

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The most potent microorganisms for MEOR are commonly originated from oil reservoir because those microorganisms are well adapted to reservoir conditions. A better understanding of bacterial community structure in oil reservoir will also make the screening process for bioaugmentation microbial candidates easier.

Genetic fingerprinting technique is one of the molecular methods that can provide genetic diversity profiles of microbial community from specific environment. In petroleum microbiology, increasing application of PCR-DGGE (Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis) has allowed more complete characterization of microbial communities in oil reservoirs. Application of PCR-DGGE was performed in MEOR project in dagang oil field to monitor microbial diversity and dynamic change after biopolymer flooding (Huang *et al*., 2010). The recent analysis of 16S rRNA gene fragments by PCR-DGGE approach were conducted on four different segments core samples taken from an African oilfield. This study was carried out to get a quick insight in predominant bacteria and archaea that were found in the core and to see if the different locations (outer shell to center) in the core display different microbial community profiles (Van Dar Kraan *et al*., 2010). This study aims to analyze bacterial diversity in three oilfields located in Japan, Indonesia and China using genetic fingerprinting approach. This study describes the predominant bacteria living in different water flooded oilfield characterized with high temperature and low salinity. In addition, this study also considers the possibility to select the best candidate of bacteria for bioaugmentation based on phylotypes similarity. This study will become a reference for further laboratory study prior to MEOR field application.

MATERIALS AND METHODS

Sample collection: Produced water samples used in this study were collected from three oilfields located in China, Japan and Indonesia. Oilfields in China and Japan were on shore while the oilfield in Indonesia was off shore. The properties of each oilfield were described in Table 1. Produced water was extracted from wellheads of each oilfield and collected in sterile bottles. L-cysteine was added to the samples to completely eliminate oxygen from samples. Then, the entire bottle was sealed hermetically and kept at high temperature using disposable heat pack. Upon arrival at the laboratory, the samples were maintained at ambient temperature until it was used in experiments.

DNA extraction and PCR amplification: Produced water was separated from crude oil and other debris by using centrifuge at 3000 rpm for 10 min. About 100-200 mL of produced water was filtrated by membrane filter with 0.2 μm pore size to collect bacterial cells from water. The filter from which total bacterial DNA was extracted was aseptically cut with a sterile blade and purified using the Ultra Clean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., USA) according to the manufacturer's instructions.

The purified 16S rDNA gene was amplified by nested PCR using Premix Taq™Hot Start Version (Takara Bio Inc., Japan) and primers. The first PCR of the nested PCR used universal forward and reverse primers (EU27f [5′-AGA GTT TGA TCC TGG CTC AG-3′] and EU1525r [5′-AAA GGA GGT GAT CCA GCC-3′]) and amplified almost all the regions of eubacterial 16S rDNA gene. The thermal cycle profile for the first PCR was following (Sugai *et al*. 2012). The amplified DNA from the first PCR was used as templates for the second PCR of the nested PCR. The second PCR for the samples extracted from both China and Japan oilfields used universal forward and reverse primers (EU341f-GC [5′-GC clamp-(CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC TAC GGG AGG CAG CAG-3′)] and EU534r [5′-ATT ACC GCG GCT GCT GG-3′]) and amplified the variable V3 region of eubacterial 16S rDNA gene. This second PCR consisted of initial denaturation at 94°C for 2 min, 25 cycles of denaturation at 94°C for 20 sec, annealing at 63°C for 45 sec and extension at 72°C for 1 min and final extension at 72°C for 7 min. The second PCR for sample extracted from Indonesia oilfield used universal forward and reverse primers to amplify the region V1 and V2 of eubacterial 16S rDNA gene. Those primers were 27f-GC [5′-GC clamp-(CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CAG AGT TTG ATC MTG GCT CAG-3′)] while M refers to C:A at 1:1 and 342r [5′-CTG CTG CSY CCC GTA G-3′] and Y and S refers to C:T at 1:1 and C:G at 1:1, respectively. This second PCR was performed with initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec and extension at 72°C for 30 sec and final extension at 72°C for 7 min.

DGGE analysis: DGGE was performed using the D-code universal mutation system (Bio-Rad Laboratories, USA), according to the manufacturer's instructions. PCR products were loaded onto 10% polyacrylamide gels with a 30-70% denaturant gradient (100% denaturant gradient was defined as 7 mol/L of urea and 40% of deionized

Table 1: Properties of the oil reservoirs which brine samples were extracted from in this study

Oilfield location	Temp. (°C)	Salinity (%)	pH	Depth (m)	°API	Classification
China	75	1.00	8.5	1900	31	Highthermic and low salinity
Indonesia	63	0.35	8.5	700	16	
Japan	70	0.25	8.6	1240	33	

formamide) and were run at 75 V for 16 h at 60°C in 1×TAE (Tris Acetate ETDA) running buffer. After the electrophoresis, the gels were stained with 0.5 μg/mL of ethidium bromide followed by visualization of the DNA fragments with UV transilluminator. The results were photographed using UVP Imaging System (Ultra-Violet Products, Ltd., Canada). The parallel horizontal position of each band indicated species similarity of the bacteria. In order to purify the sequences, DNA extracted from each band was used as template for the second PCR-DGGE and was run in a gel with milder denaturant gradient.

Identification of DGGE bands: After the second PCR-DGGE, the major DNA bands on the gel were excised with fresh and sterile scalpels under UV illumination. The excised gels were each placed directly in 1.5 mL micro test tubes containing 20 μL of highly purified DNase/RNase-free sterile water and incubated at ambient temperature overnight to elute the DNA. The 1 μL of the supernatant was used as DNA template for PCR which used primers without GC-clamp. Temperature conditions of the PCR were the same with those of the second PCR as described above. The amplicons were then purified using Gen-Elute PCR clean-up filters (Sigma Aldrich) and sequenced with one of the amplification primers using a MicroSeq®500 16S rDNA Sequencing Kit (Applied Biosystems). All sequences were finally compared to those in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) using the BLAST program. Phylogenetic trees were constructed based on the partial 16S rRNA gene sequences using the neighbor-joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the maximum composite likelihood method (Tamura *et al*., 2004) and phylogenetic analyses were conducted in MEGA4 (Tamura *et al*., 2007).

Nucleotide sequence accession numbers: The nucleotide sequences obtained in this study have been deposited in the NCBI nucleotide sequence databases under accession numbers HQ439310 to HQ439328 and JN098437 to JN098445.

Diversity analysis: Shannon-Wiener index, phylotypes richness and evenness index were applied to estimate the representation of the phylotypes and to characterize the bacterial diversity of produced water samples. The Shannon-Wiener index H was calculated from the following Eq. 1:

$$H = -\sum P_i \log_n P_i \quad (1)$$

Where:

P_i = The proportion of each Phylotype
I = The relative to the total number of phylotypes

The richness of phylotypes R and evenness index E were estimated from the following Eq. 2:

$$R = (S-1)/\log N \quad (2)$$

$$E = H/\log S \quad (3)$$

Where:

S = Total number of phylotypes
N = Total Number of individual of all phyloptypes

In addition, Sorensen's index S_S was used to describe the similarity of the bacterial diversity between two sample by the following Eq. 3:

$$S_S = 2a/(2a+b+c) \quad (4)$$

Where:

a = Number of phylotypes in sample A and B (joint occurance)
b = Number of phylotypes in sample B but not in sample A
c = Number of phylotypes in sample A but not in sample B

RESULTS AND DISCUSSION

Bacterial community in Japan oilfield: In order to identify predominant bacterial species from Japan oilfield brine water, 7 bands were excised from DGGE gel to be applied in sequencing analyses (Fig. 1a). Blast N analysis revealed that bacterial sample from Japan oilfield was most closely affiliated to Bacteroidetes, Thermotogae, Defferibacteres and Firmicutes (Table 2). Phylogenetic analysis shown in Fig. 2 illustrates the relationships between each nucleotide sequence of each DNA fragment with their closest affiliates in the database. There were 4 Bacteroidetes-affiliated sequences which were closely related to known environmental sequences (98-99% similarity) with the exception of J2 sequence (95% similarity). Those three band sequences, i.e., J1, J3 and J4, were detected in formation water of Gangxi oilfield in China while J2 was detected in a Japan oilfield.

Sequence J5 was distantly related to known cultivated species AR80 (92% similarity) which has been isolated from the same oilfield while sequence J6 was

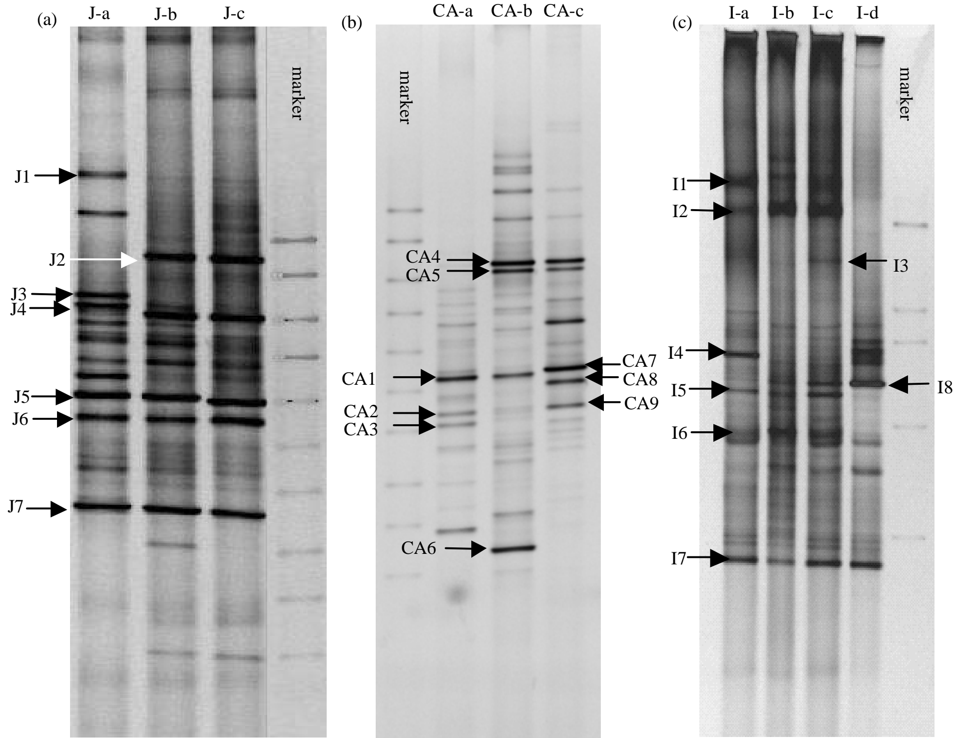

Fig. 1: PCR-DGGE profiles generated using DNA extracted and amplified from the brine of high temperature and low salinity reservoir: a) Japanese oilfield (JPN); b) Chinese oilfield (CHN) and c) Indonesian oilfield (IDN). DNA fragments shown by J1-J7; CA1-CA9; I1-I8 were identified by sequencing analyses

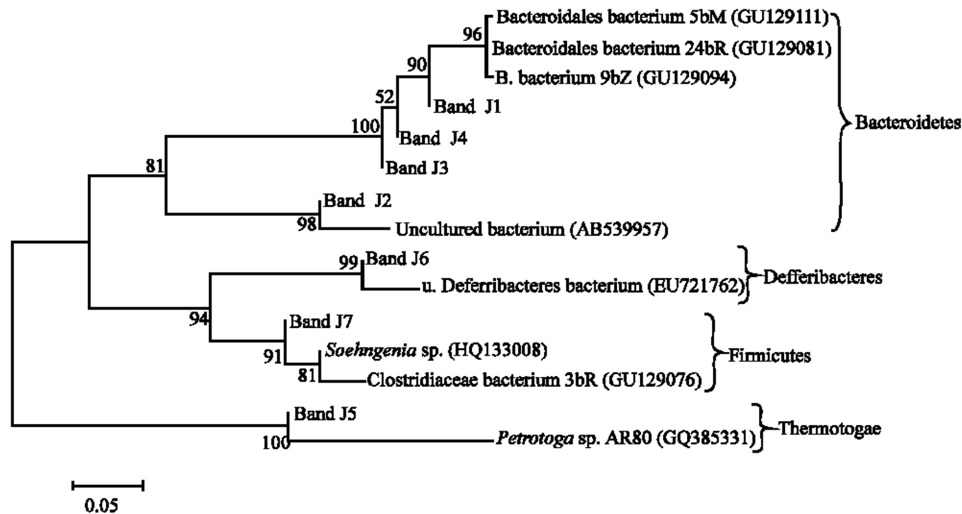

Fig. 2: Phylogenetic analysis of DNA fragments extracted from Japanese oilfield brine. Bootstrap values (expressed as percentages of 1,000 replications) >50% are shown next to the branching points. Accession numbers of 16S rDNA sequences of reference organism are included (bracket). Bar, 5 nucleotide substitutions per 100 nucleotides

closely related to uncultured defferibacteres bacterium detected at alaskan mesothermic petroleum reservoir. The remaining band, J7 was relatively identical with previously cultivated bacterium *Soehngenia* sp. (100% similarity). Based on 16S rDNA analysis, all of bands sequences extracted from the JPN oilfield were closely related with

Table 2: Closest relative species of 16S rDNA fragments extracted from the oilfield of JPN, CHN and IDN

Bands	Accession number of band	Closest relative species	Percentage of identity	Phylogenetic affiliation	Found in environment (Reference)
J1	HQ439310	Bacteroidales bacterium 5bM	99	Bacteroidetes	Formation water of Gangxi oil bed (Nazina in 2009: unpublished)
J2	HQ439317	Uncultured bacterium clone:YAB2B13	95	Bacteroidetes	High-temperature petroleum reservoir (Mayumi in 2010: unpublished)
J3	HQ439311	Bacteroidales bacterium 24bR	98	Bacteroidetes	Formation water of Gangxi oil bed (Nazina in 2009: unpublished)
J4	HQ439313	Bacteroidales bacterium 9bZ	98	Bacteroidetes	Formation water of Gangxi oil bed (Nazina in 2009: unpublished)
J5	HQ439314	*Petrotoga* sp. AR80	92	Thermotogae	Formation water of Japan oil field (Purwasena *et al*., 2014a, b)
J6	HQ439315	Uncultured deferribacteres bacterium	96	Defferibacteres	Production water from an Alaskan mesothermic petroleum reservoir (Pham in 2009)
J7	HQ439316	*Soehngenia* sp.	100	Firmicutes	Crude oil contaminated soil of Shengli oil fields (Cheng in 2010: unpublished)
CA1	HQ439319	*Halomonas* sp. G5	100	Proteobacteria	Ocean sediment (Koh and Wang in 2010: unpublished)
CA2	HQ439320	*Idiomarina* sp. S15	94	Proteobacteria	Ludaokou Salt Pan (Huang and Du in 2009: unpublished)
CA3	HQ439321	Uncultured bacterium clone Mi5A06	96	Proteobacteria	Coral reef (Castro and Kruger in 2011: unpublished)
CA4	HQ439322	Bacteroidales bacterium 5bM	99	Bacteroidetes	Formation water of Gangxi oil bed (Nazina in 2009: unpublished)
CA5	HQ439323	Bacteroidales bacterium 9bZ	100	Bacteroidetes	Formation water of Gangxi oil bed (Nazina in 2009: unpublished)
CA6	HQ439325	*Thermotoga petrophila* RKU-1	92	Thermotogae	Kubiki oil reservoir (Takahata, 2000)
CA7	HQ439326	Thermoanaero bacteriaceae bacterium	100	Firmicutes	Formation water of Gangxi oil bed (Nazina in 2009: unpublished)
CA8	HQ439327	*Sedimentibacter* sp.	97	Firmicutes	Crude oil contaminated soil of Shengli oil fields (Cheng and Lu in 2010: unpublished)
CA9	HQ439328	*Enterobacter* sp. IBP-VN6	99	Proteobacteria	Coastal sea water, NhaTrang Bay (Volova in 2010: unpublished)
I1	JN098437	Bacterium enrichment culture clone ecb8	99	Deferribacteres	Oil reservoir fluids (Li *et al*., 2007: unpublished)
I2	JN098438	Bacteroidales bacterium 5bM	99	Bacteroidetes	Formation water of Gangxi oil bed (Nazina in 2009: unpublished)
I3	JN0984345	Uncultured bacteroidetes bacterium	100	Bacteroidetes	Oil well (Pham in 2009: unpublished)
I4	JN098440	Bacterium Riz3	99	Proteobacteria	Rhizosphere (do Carmo in 2011)
I5	JN098441	Pseudomonadaceae bacterium	85	Proteobacteria	Production water of mesophilic petroleum reservoir (Li *et al*., 2007: unpublished)
I6	JN098442	*Azospirillum* sp.	92	Proteobacteria	Fuel-contaminated antarctic soils (Eckford in 2002)
I7	JN098443	Uncultured bacterium clone PW-1	100	Proteobacteria	Production well (Zhang in 2011)
I8	JN098444	*Paracoccus* sp. p1s1	100	Proteobacteria	Rocky shore (Ismail in 2010: unpublished)

environmental sequences obtained from oil field as well with the exception of band J7. *Soehngenia* sp. was isolated from crude oil contaminated soil of Shengli oilfield and there is no report regarding to its inhabitation in oil reservoir. In addition, this bacterial strain is a member of mesophilic bacteria with optimum growth at the temperature range of 30-37°C (Parshina *et al*., 2003) suggesting *Soehngenia* sp. role as a contaminant.

Bacterial community in China oilfield: Figure 2 shows the DGGE profile of 16S rDNA from China oilfield brine water. Similar with the Japan oilfield sample described above, bacterial community in China oilfield was constructed by four phylotypes (Table 2). However, Defferibacteres phylum was substituted by Proteobacteria which consisted of 4 bands, i.e., CA1, CA2, CA3 and CA9 (Fig. 3) in China oilfield and all of them was identified as Gammaproteobacteria. Sequences CA1 strongly matched with known sequence of *Halomonas* sp. (100% similarity) isolated from ocean sediment while sequences CA2 were affiliated with *Idiomarina* sp. (94% similarity).

Bacteroidetes-affiliated sequences were represented by band CA4 and CA5. Both of them strongly matched with bacteroidales bacterium 5bM and 9bZ (99 and 100% similarity) which have been detected in Japan oilfield sample. The sequences CA6 was 92% related to *Thermotoga petrophila* which was isolated from Kubiki oil reservoir in Japan. The last two bands, i.e., band CA7 and CA8 were affiliated with Firmicutes phyla. Band CA7 was identic (100% similarity) with Thermoanaero bacteriaceae bacterium isolated from formation water of Gangxi oil reservoir. Members of Thermoanaero bacteriaceae family within firmicutes are commonly encountered in oilfields and include isolates belonging to Thermoanaerobacter (Cayol *et al*., 1995), Thermoanaero bacterium (Grassia *et al*., 1996), Caldanaerobacter (Grassia *et al*., 1996; Fardeau *et al*., 2004) and Mahella genera (Salinas *et al*., 2004), all of which are thermophilic. Another Firmicutes-affiliated sequences 97% matched with the identified species *Sedimentibacter* sp. isolated from crude oil contaminated soil of Shengli oil field.

Bacterial community in Indonesia oilfield: Sequences retrieved from DGGE of IDN oilfield sample were affiliated with some phyla to those found both in the JPN oilfield sample and the CHN oilfield sample. There were8 bands which have been sequenced successfully (Fig. 3) and it was dominated by Proteobacteria (Fig. 3 and 4, Table 2). Unlike the sequences found in China oilfield which was just clustered in Gammaproteobacteria, the Proteobacteria-affiliated sequences obtained from

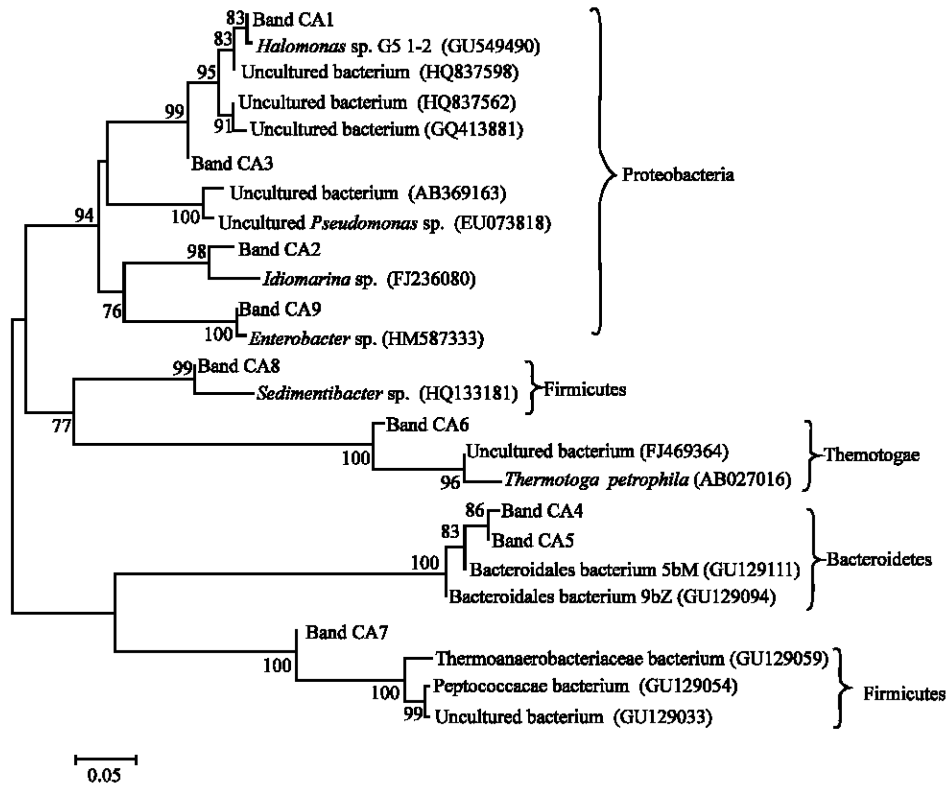

Fig. 3: Phylogenetic analysis of DNA fragments extracted from Chinese oilfield brine. Bootstrap values (expressed as percentages of 1,000 replications) >50% are shown next to the branching points. Accession numbers of 16S rDNA sequences of reference organism are included (bracket). Bar, 5 nucleotide substitutions per 100 nucleotides

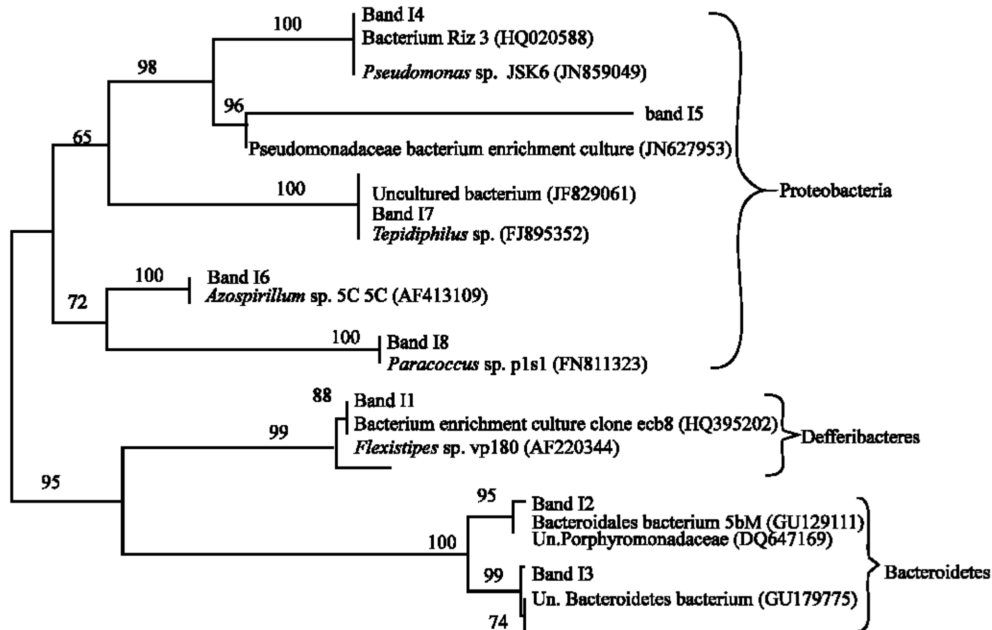

Fig. 4: Phylogenetic analysis of DNA fragments extracted from Indonesian oilfield brine. Bootstrap values (expressed as percentages of 1,000 replications) >50% are shown next to the branching points. Accession numbers of 16S rDNA sequences of reference organism are included (bracket). Bar, 5 nucleotide substitutions per 100 nucleotides

Table 3: The biodiversity index of phylotype based on phylogenetic affiliation

Countries	Shanon wiener index	Phylotypes richness	Evenness index
JPN	1.15	3.53	0.690
CHN	1.27	3.16	0.762
IDN	0.90	2.22	0.432

Sorensen's index: JPN-CHN, 0.57; JPN-IDN, 0.57; CHN-IDN, 0.75

Indonesia oilfield strongly matched with Gammaproteo bacteria (I4, I5), Alphaproteo bacteria (I6, I8) and Betaproteo bacteria (I7). However, only sequences I5 and I7 were found from oil reservoir which indicated that the other sequences might be present as contaminants. Band I5 was distantly related to Pseudomonadaceae bacterium (85% similarity) while band I7 completely matched with uncultured bacterium clone PW-1. The sequences I6 matched with *Azospirillum* sp. retrieved from fuel contaminated antarctic soils, sequences I8 matched with *Paracoccus* sp., retrieved from rocky shore and sequence I4 matched with bacterium Riz 3 retrieved from rhizosphere.

A bacteroidetes phylum appeared in two DGGE bands of the Indonesia oilfield i.e., band I2 and I3. Band I2 was 99% similar to Bacteroidales bacterium 5bM sequences which was isolated from formation water of Gangxi oilfield. Band I3 had 100% similarity with uncultured Bacteroidetes bacterium which was isolated from oilfield. Deferribacteres-affiliated sequence only showed in band I1 which was closely related to bacterium enrichment culture clone ecb8 (99% similarity).

Diversity indices: Diversity indices were calculated based on phylogenetic affiliation data obtained from each sample. Table 3 shows the diversity indices obtained along with the similarity index of the bacterial community. All indices except phylotypes richness indicated that the bacterial diversity in China oilfield was slightly greater than the diversity in Japan and Indonesia oilfield. The diversity indices of the Indonesian sample had the lowest number compared to the others. However, the similarity index showed that the highest phylotypes similarity was obtained for Indonesia and China oilfield while the similarity index of Japan oilfield with either Indonesia or China oilfield showed the same number.

MEOR technology has many advantages and it has become one of the promising technologies of enhanced oil recovery. However, the technology still have some issues to be solved, particularly the ones related to the inconsistent technical performances in several field tests. This typical issues usually arise due to the lack in monitoring system of microbial conditions during MEOR process. Consequently, the relationship between microbial performance and oil yields is still ill-defined. Therefore, analysis of microbial structure and diversity before MEOR field test is very important for monitoring purpose.

DGGE analyses of PCR amplified 16S rDNA fragments showed many bands in all produced water samples which indicated high bacterial diversities. However, the sequences identified in this study were mainly related to only fourth phyla, i.e., Bacteroidetes, Thermotogae, Defferibacteres, Firmicutes and Proteobacteria, suggesting that phylotype richness is low in oil reservoir. The apparently low diversity may be attributed to the extreme condition in the subsurface area.

Thermophilic bacteria found in the particular environment in this study were present in the genera Thermoanaerobacter and Thermotoga. Thermoanaerobacter related sequences were detected only in the China oilfield while Thermotogae-related sequences were found both in China and Japan oilfields. Thermotogae isolates have been shown to be members of high temperature oil reservoir communities, suggesting an indigenous nature of these microorganisms to oil reservoirs. Two genera of this phylum, i.e., Geotoga and Petrotoga were exclusively isolated from oil reservoirs (Youssef *et al*., 2009). Thermotogae-affiliated bacteria in Japan oilfield and China oilfield was most closely related to *Petrotoga* sp. and *Thermotoga petrophila* which were found in environmental sample of formation water extracted from high temperature oilfield in Japan (Purwasena *et al*., 2014a, b). In addition, this *Petrotoga* sp. has been described as bacterial species which has the potential to be applied in MEOR technology (Purwasena *et al*., 2014a, b).

Deferribacteres has been found in limited number in high temperature oil reservoir, so far (Gittel *et al*., 2012). The phylum of Deferribacteres has a deep-branching lineage of obligate thermophiles bacteria that consisted of the genera Deferribacter, Flexistipes, Denitrovibrio, Geovibrio and Mucispirillum (Jumas-Bilak *et al*., 2009). Deferribacteres bacteria detected in this study were closely related with unidentified Deferribacteres bacterium which were found in petroleum reservoir, indicating the possibility that those sequences were acquired from bacteria inhabiting the oil reservoir.

This study revealed that, although, the sequence analyses showed high similarity index for the phylum of identified band sequences, almost all of these closely related species are unique to each produced water sample. The predominant bacteria in each oilfield are therefore shall be different. In this study, Bacteroidetes-affiliated bacteria were found in all oilfields. The phylum of Bacteroidetes has been detected in high temperature and low salinity reservoir in Troll oil formation in North Sea (Dahle *et al*., 2008) and in multiple California oilfields (Orphan *et al*., 2000). This phylotype was also detected in low temperature Pelican lake oilfield in Canada (Grabowski *et al*., 2005) suggesting that Bacteroidetes might appear as common inhabitants both in low and

high temperature oilfield. Interestingly, one sequence of Bacteriodetes-affiliated bacteria retrieved from DGGE band of all samples showed 99% similarity with Bacteriodales bacterium 5bM. This result showed that this bacterium has the ability to grow well in Indonesia, Japan and China oilfield making it the best bacterial candidate for MEOR process. In the other word if Bacteriodales bacterium 5bM can be isolated and cultivated successfully from one of those oilfields, it can be applied in other oilfield as well. The effect of this bacterium activity both in the fluids and rock reservoir should obviously be observed thoughtfully prior to field application. This result showed that finger printing is a considerable technique for microbial screening prior to MEOR field test application.

SRB has always been intriguing petroleum microbiologists and oil industries due to its threatening effect on the oil production. Thus, SRB is one of the undesirable bacteria which is needed to be eliminated from oil reservoir. However, as a key player in anaerobic environment, SRB is commonly found in oil reservoir (Birkeland, 2005). Surprisingly, there is no SRB detected at the entire oilfields in this study. The existence of SRB in this study might be not detected due to the limitation of DGGE methods. Only high concentration of microbial population could be detected by DGGE fingerprinting (Wang *et al*., 2008). Regardless of the biased data obtained in this study, MEOR can be applied in Indonesia, China and Japan oilfields either by biostimulation or bioaugmentation method, since, SRB might be present in only low quantities that we need not to be concerned about.

Some sequences were characterized to be related with mesophilic bacteria such as Soehngenia, *Pseudomonas Halomonas* and *Streptococcus* sp. These mesophilic bacterial species probably do not inhabit oilfield as indigenous microorganisms. These results could be linked to the reservoir system from which the produced water has been extracted in this study. All of the samples were obtained from high temperature water flooded oilfield into which water has been continuously injected. It has been hypothesized that the identified mesophilic bacteria from high temperature oil reservoir may originate from microorganisms that are able to colonize the upper cooler parts of well tubings (Kaster *et al*., 2009). Together with the unsterilized water recycled from injection wells to production wells, large numbers of microorganisms could be introduced into the oil reservoir. Some may reside in the cooler portions of the reservoir and along the walls or openings of production well tubing which may results in the detection of mesophilic microorganisms in production water (Li *et al*., 2007).

CONCLUSION

The analyses of bacterial diversity in Indonesia, China and Japan suggest that phylotype richness is low in oil reservoirs. However, one sequence of Bacteriodetes-affiliated bacteria with 99% similarity with Bacteriodales bacterium 5 bM was present at all tested oilfields, indicating its survivability in high temperature environment and its potential to be successfully augmented to oil reservoir for MEOR process. The results showed in this study reveal that finger printing can be a considerable technique for microbial screening prior to MEOR field test application.

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