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Phylogenetic Diversity of Bacterial Communities of Western India Oil Fields by PCR-DGGE

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A B S T R A C T

The diversity of bacterial communities in the formation waters of ten oil reservoirs from western India was analyzed by PCR – Denaturing Gradient Gel Electrophoresis (DGGE). 16S rRNA gene fragments of the bacterial community were PCR amplified using universal primers targeting V3-V5 eubacterial hyper-variable regions. The phylogenetic analysis revealed that formation water metagenome mainly constituted bacteria, showing similarity to *Paracoccus, Brachymonas, Hydrogenophaga, Bacillus, Pelomonas, Thalasospira* and *Pseudomonas*. Many of the bacterial sequences shared less than 95% homology with the reference sequences available in Gen-Bank database, hence; some of these could represent novel species/ genera. Application of statistical indices such as Simpson's index (1- D) and the Shannon-Weaver index (H) to the well resolved DGGE pattern of 16S rR NA gene fragments revealed more diversity of bacteria in S7 and S9 samples, whereas equitability (J) data indicated that population was not dominated by any species. It was also observed that formation water consisted of predominantly the proteobacteria capable of nitrate reduction and hydrocarbon degradation.

Introduction

Petroleum biotechnology is a very young and exciting field for the possible applications in industrial point of view. It is well-known that petroleum reservoirs contain active and diverse populations of microorganisms and their growth within oil reservoirs has usually been associated with biofouling and souring. The studies are going on to create a resource base for new genetic information achieved from the microorganisms in the reservoir. Microorganisms from the oil reservoirs can be used in the areas like exploration and production of oil as well as biological up-gradation of oil to improve quality. Extremophiles from oil reservoirs may have new thermophilic and piezophilic enzyme system which can enable new bioengineering processes and products for the oil exploration and production (Kotlar *et al*., 2004).

Formation water occurs naturally within pores of rock. There is a variation observed

in formation water composition from one reservoir to another and also within reservoirs. The possibility for living organisms to survive in oil reservoir environments depends on the physical characteristics and chemical composition of the ecosystem. Temperature is the most important limiting factor for microbial growth in oil reservoirs (Magot *et al*., 2000). Oil reservoirs harbor a wide variety of microorganisms. The study of these microorganisms is important as they may have potential to affect oil quality and geological properties of the reservoir. Oil reservoir is a distinctive habitat with astonishing diversity of microbial populations and communities. Microorganisms from petroleum reservoirs having hydrocarbon metabolizing ability play an important role in their possible industrial application such as microbial enhanced oil recovery (Wang *et al.*, 2008). Molecular techniques like 16S rRNA gene sequence analysis, restriction fragment length polymorphism (RFLP), reverse sample genome probing and oligonucleotide matrix array hybridization methods have been used extensively to study microbial diversity from oil reservoirs. PCR based finger printing technique, denaturing gradient gel electrophoresis (DGGE) is less labour-intensive approach has been used to investigate the bacterial diversity of soils and aquatic environments (Schafer and Muyzer, 2001). Such diversity studies have been done for the characterization of oil reservoirs having high temperature in Western Siberia, Russia (Bonch-Osmolovskaya *et al*., 2003), biodegraded petroleum reservoir having low temperature and low salinity from a Western Canadian Sedimentary Basin (Grabowski *et al*., 2005), water flooded oil reservoirs from western Canadian harboring reservoir (Voordouw *et al*., 1992, 1996); oil reservoirs from a southern offshore Brazilian basin (Sette *et al*., 2007) etc. Many microorganisms isolated from oil reservoirs are able to produce biosurfactants (Bryant and Douglas, 1988; Margaritis *et al*., 1979) and biopolymers (Kalish *et al*., 1964). These microorganisms are of interest in microbially enhanced oil recovery (MEOR). Therefore, it is of great interest to study microbial diversity of oil reservoirs for the exploitation of native bacteria with their positive effects (Kaster *et al.,* 2009).

Microbial communities from Indian oil reservoirs have been less studied. While India has significant reserves of coal, it is relatively poor in oil and gas resources. The petroleum reserves of India, situated in Gujarat, Bombay High (next to the seashore of Maharashtra), eastern Assam, and Rajasthan, satisfy about $1/4^{th}$ of the requirements of the nation. Its oil reserves amount to 5.9 billion barrels, (0.5% of global reserves) with total proven, probable, and possible reserves of close to 11 billion barrels (http://petroleum.nic.in). The purpose of this study was to reveal the diversity of bacteria from western India oil well formation waters by culture independent approach. 16S rDNA based PCR-DGGE technique is used for phylogenetic analysis. This study is expected to elucidate the bacterial taxonomic picture in poorly studied Indian oil reservoirs.

Materials and Methods

Sample collection

Formation water samples were collected from oil reservoirs located at Gujarat state of India from 850-1500m depth. Samples from ten different oil wells were collected in sterile poly propylene containers. Temperature and pH of the samples were recorded at the collection point. Samples

were immediately transferred to 4°C and preserved until further use.

Physico-chemical analysis of formation water

The parameters like total solids (TS), volatile solids (VS), total nitrogen (TN), ammoniacal nitrogen (TAN), heavy metal ion content of all formation water samples were determined according to standard methods (Eaton *et al*., 2005).

Extraction of nucleic acids from formation water

The metagenomic DNA from the formation water was extracted by CTAB method (Zhou *et al*., 1996). Bacterial biomass was harvested from 25ml formation water sample by centrifugation at 12,000 x g for 10 minutes and used for DNA isolation instead of 5g soil samples as described in the original method. The nucleic acid pellet was washed with cold 70% ethanol, and resuspended in sterile deionized water, to give a final volume of 40 µl.

Polymerase chain reaction for amplification of 16S rRNA gene

Nested PCR was performed to amplify V3-V5 region of 16S rDNA using universal primers (Rawlings, 1995; Muyzer *et al*., 1993; Schwieger and Tebbe, 1998). PCR amplification was performed in a 50 µl PCR mixture containing 1.5U Taq DNA polymerase, 10x buffer, 50 pmol of each primer, and 200 µM dNTP. PCR primers are listed in Table 1. PCR amplifications were performed in Mastercycler Gradient Thermal Cycler (Eppendorff, Germany) under the conditions of initial denaturation at 95°C for 5 min, 35 cycles of 94°C for 1 min, annealing at 60°C for 1 min with bacterial primers and extension at 72°C for 1 min with a final elongation step of 72°C for 5 min. The resulting 16S rRNA gene amplicons were purified using polyethylene glycol precipitation (Sambrook and Russel, 2001). The outer PCR was performed using FDD2-RPP2 primer pair whereas nested PCR was carried out using SRV3-1 and COM-2 primers. A 40 bp GC clamp was attached to the 5' end of the SRV3-1 primer (Wang *et al*., 2008; Ferris *et al*., 1996; Grabowski *et al*, 2005). The PCR products were validated by agarose gel electrophoresis and then purified to eliminate the ssDNA and heteroduplexes (Wang *et al*., 2008).

DGGE

DGGE analysis of the amplified sequences was performed on 16 x 16 cm., 6% polyacrylamide gels with a denaturing gradient of 40 to 60% (100% denaturants gels defined as 7 M urea and 40% deionized formamide). The PCR amplicons (200ng) were loaded in each well and electrophoresis was performed at a constant voltage of 130V for 200 min at 60°C for 7 hrs in the DGGE tank (Biorad, USA). Gels were stained with ethidium bromide solution (0.5 µg/ml) for 15 min, washed with distilled water and visualized by U.V. transillumination (AlphaImager, Alpha Innotech, USA).

Sequencing of DGGE bands

DGGE bands were excised from the gels and DNA was eluted in deionized distilled water. The fragments were reamplified with the SRV3-1 primers without GC clamp and purified by polyethylene glycol (PEG 6000, 8%) precipitation (Sambrook and Russel, 2001). The eluted DNA fragments were re-amplified using the set of corresponding primers. Purified PCR product was then used as template for cycle sequencing reaction using COM-2 primer and ABI

Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystem, Foster City, CA, USA). Post cycle sequencing clean-up was done by Big Dye Clean Up method, according to the manufacturer's instructions (Invitrogen, India) and subjected to sequencing in an automated sequencer (3100 Avant Gene Analyser, Applied Biosciences, USA). The sequencing output was analyzed using the accompanying DNA Sequence Analyzer computer software (Applied Biosystems).

Sequences analysis

Sequences that differed by less than 3% were considered to belong to the same phylotype (Stackbrandt and Goebel, 1994) and each phylotype was represented by a sequence type. This sequence was compared with reference sequences available in the GenBank database using the BLAST algorithm to establish phylogenetic affiliation. Sequences alignments were performed using the software CLUSTAL W (http://www.ebi.ac.uk/) (Thompson *et al*., 1997). Software Mega 5.0 was employed to construct the phylogenetic tree based on Kimura two-parameter model (Kimura, 1980) and neighbor-joining algorithm (Saitou and Nei, 1987). Bootstrap analysis with 1,100 replicated was applied to assign confidence levels to the nodes in the trees.

Diversity Indices

DGGE profiles were statistically analysed and compared using a set of diversity indices. Gels were normalized by eliminating bands with peak height intensity of less than 2% of the most intense band in each lane as background noise. Alpha Imager software (Alpha Innotech, USA) was used to extract this information. Each band was considered as an independent OTU (Operational Taxonomic Unit) and the intensity of the bands measured in pixels was considered as population size. The diversity and relationship between the band patterns obtained in this study within the bacterial communities were evaluated by several species diversity indices (Atlas and Bartha, 1998). Here, Simpson's index (D= sum [Pi^2]) was used, where pi is the proportion of total intensity of the i^{th} band. Along with this, Simpson's index of diversity (1 - D); Simpson's reciprocal index (1/D); Shannon-Weaver index (H= - sum (Pi ln [Pi]); Equitability index (J = *H/Hmax*), where H_{max} is the theoretical maximal H for the population examined, assuming each species has only one number were also used for diversity analysis.

Results and Discussion

Physico-chemical analysis of formation Water

In the present study detailed analysis of all formation water samples was carried out. It is observed that the total solid (TS) content in all formation water samples was in the range of 0.85-0.89 g %. TS contribute the total suspended and total dissolved solids. The volatile solids (VS) contribute the organic matter which acts as a substrate for native microorganisms. The percentage of VS in all samples is less than 0.03 g %, which was very low to support the growth of the bacteria. Formation water is associated with crude oil in the reservoir. Therefore native bacteria may have developed metabolic potential to use hydrocarbons as a substrate in oil reservoirs. The pH of all formation water samples was slightly alkaline and salinity (% of NaCl) was near one percent (Table 2). The concentrations of Cu were found to be in the range of 0.08-0.3 ppb which is below the inhibitory concentration required for microorganisms (Ochoa-Herrera *et al*., 2011). MIC of Cd for

Bacteria like *Pseudomonas sp., Bacillus sp., Staphylococcus sp.* was reported to be higher than 799 mg/L (Nath *et al*., 2012). The cadmium concentration in all the formation water sample is in the range of 0.01-0.22 mg/L. The heavy metals like Hg, Pb and As were not detected in any of the sample tested. The concentration of total nitrogen (Kjeldahl nitrogen) as well as ammoniacal nitrogen was not more than 55 ppm, indicating nitrogen deficiency in any of the samples tested (Table 3). The physico-chemical analysis revealed that formation water is not inhibitory for the growth of the native bacteria.

Separation of PCR products by DGGE

The DNA of ten formation water samples was extracted with CTAB method. Even though the samples were collected from the nearby fields, in DGGE distinctly different gel pattern was observed. The pattern of bands in DGGE gel showed good resolution and separation (Fig.1). Total 36 bands were separated and after elution of each band, they were reamplified in PCR using SRV3-1 and COM-2 primers.

Nucleotide sequence accession numbers

The nucleotide sequences reported in this paper have been deposited in the NCBI nucleotide sequence databases under accession numbers JX036286 to JX036321.

Diversity indices

Diversity indices calculated using band intensities of the DGGE profiles indicated that the bacterial diversity is more in S7 and S9. Simpson's reciprocal index (1/D) and the Shannon-Weaver index (H) revealed more diversity in these samples. To quantitatively measure diversity in the ten different samples, Simpson's reciprocal index (1/D) was used, which is sensitive to the level of dominance in community. It ensures that an increase in the reciprocal index reflects an increase in diversity. Simpson's index of diversity (1 – D) measures the probability that two individuals randomly selected from a sample will belong to the same species. Therefore more is the 1-D value, more is the diversity. Samples S6 and S2 were shown relatively low values of Simpson's reciprocal index (1/D), indicating less diversity in the formation water samples. The equitability (J) data indicated that there is less variation in the communities in any of the formation water tested (Table 4).

Analysis of bacterial community profile revealed in DGGE

In the DGGE, band pattern with GC Clamp and COM-2 from all the DNA samples, representatives of bands that were clear and had high intensity were excised from polyacrylamide gel. They were reamplified and subjected to sequencing. The sequences of a total of 36 DNA fragments were successfully determined. The formation of chimeric DNA fragments was not observed in those sequences. The phylogenetic relationships of the 36 sequenced bands are shown in Fig. 2.

All bands were separated into two major groups, proteobacteria and firmicutes. The total 36 sequences assigned to the 24 distinct phylogenetic groups which clustered among *Firmicutes* (10 sequenecs), *Proteobacteria* (22 sequences), *Actinobacteria* (3 sequences) and *Bacteriodetes* (1 sequence). The samples of formation water appears to harbor unique microbial communities as many of the sequences had less than or equal to 95% identity to closest matches in the GeneBank and probably represent novel genera or species. Most bacteria detected in

the PCR-DGGE library cluster are among the fermentative organisms. The ten sequences that cluster within the firmicutes lineage, indicate the presence of a large fraction of highly diverse Gram positive organisms (Table 5; Fig. 2).

It was significant that a number of bands related to hydrocarbon-degrading strains have been reported previously from oil wells and oil-contaminated soil samples. Cyclohexane degrading *Brachymonas petroleovorans* was previously isolated from oil refinery wastewater sludge, (Rouviere and Chen, 2003), C*hryseobacterium sp.* was reported as PAH degrading bacteria by Kumar *et al.,* (2011) and Guo *et al.,* (2008). *Pelobacter carbinolicus* has been detected in the clone library obtained from produced water from a high-temperature North Sea oil-field (Dahle *et al*., 2008). Species of the *Bacillus* has been reported as candidate for hydrocarbon degradation. Ahlam Al-Sharidah *et al.,* (2000), used crude oil degrading *Bacillus subtilis* for hydrocarbon degradation studies. Hydrocarbon degrading *Aeribacillus pallidus* strain MCM B-882 from western India oil reservoirs was isolated and characterized by Chitrakoti (2016). Eighteen spore-forming gram-positive bacteria were isolated from an oil reservoir located in a deep-water production basin in Brazil by Cunha *et al.,* (2006). *Fusibacter paucivorans* was isolated from an African saline oil-producing well (Ravot *et al*., 1999).

Table.1 Primers used for the amplification of 16S rRNA gene

Primer	Position*	Sequence	Reference
FDD2 (F)	8 – 27	5'CCGGATCCGTCGACAGAGTTTGATCIT GGCTCAG 3'	Rawlings *et al* (1995)
RPP2 (R)	1473-1503	5'CCAAGCTTCTAGACGGITACCTTGTTA CGACTT 3'	Rawlings *et al* (1995)
GC Clamp-SRV3-1 (F)	514-533	5'CGCCCGCCGCGCGCGGCGGGCGGGGC GGGGGCACGGGGGGGCTACGGGAGGC AGCAG 3'	Muyzer *et al*., (1993)
COM-2 (R)	907-926	5'CCGTCAATTCCTTTGAGTTT 3'	Schwieger and Tebbe (1998)

*****Positions of primers corresponding to *E. coli* 16S rDNA

Table.2 pH, salinity and organic matter content of formation water samples

Sample	TS (g %)	VS (g %)	pH	Salinity (%)
S1	0.89	0.012	7.82	0.89
S2	0.88	0.013	8.01	0.88
S3	0.86	0.013	8.00	0.87
S4	0.86	0.014	8.00	0.86
S5	0.87	0.010	8.20	0.87
S6	0.88	0.028	8.01	0.88
S7	0.86	0.020	8.02	0.86
S8	0.86	0.012	8.00	0.86
S9	0.85	0.024	8.01	0.85
S10	0.86	0.021	8.01	0.86

Table.3 Nitrogen and heavy metal ion content of formation water samples

Sample	Concentration in ppm		Concentration in ppb				
	Total N	Ammonical N	Cu	Cd	Pb	As	Hg
S1	44.06	55.00	0.08	0.14	ND	ND	ND
S2	34.59	42.00	0.08	0.01	ND	ND	ND
S3	32.54	40.00	0.18	0.08	ND	ND	ND
S4	38.38	50.00	0.10	0.80	ND	ND	ND
S5	34.92	41.00	0.08	0.09	ND	ND	ND
S6	37.30	45.00	0.06	0.22	ND	ND	ND
S7	19.68	26.00	0.15	0.09	ND	ND	ND
S8	38.59	48.00	0.29	0.08	ND	ND	ND
S9	26.00	48.11	0.07	0.09	ND	ND	ND
S10	35.68	44.00	0.13	0.08	ND	ND	ND

ND- Not detected

Table.4 Diversity indices to analyze bacterial community data in oil reservoirs

Sample	D	1-D	1/D	H	J
S1	0.14	0.86	7.14	2.28	0.84
S2	0.43	0.62	2.32	2.34	0.84
S3	0.24	0.76	4.16	1.80	0.75
S4	0.20	0.80	5.00	1.75	0.90
S5	0.17	0.83	5.88	2.35	0.74
S6	0.45	0.55	2.22	2.22	0.84
S7	0.09	0.91	10.86	2.59	0.88
S8	0.12	0.88	8.33	2.27	0.86
S9	0.10	0.90	10.10	2.53	0.87
S10	0.12	0.88	8.06	2.39	0.83

Table.5 Identification of 16S rRNA gene sequences from bacterial communities of formation water samples

Sample	DGGE band code (accession no.)	Closest cultivable species (% identity) [accession no]	Maximum identity to GenBank (% identity) [accession no.]
S1	H1(JX036286)	*Pelobacter carbinolicus* (97) [NC007498.2]	*Pelobacter carbinolicus* (97) [NC007498.2]
	H2 (JX036287)	*Fusibacter paucivorans* (96) [NR024886.1]	*Fusibacter paucivorans* (96) (NR024886.1)
S2	F1 (JX036288)	*Brachymonas petroleovorans* (97) [AY275432.1]	Uncultured bacterium (97) (FN4294OO.1)
	F2 (JX036289)	*Hydrogenophaga sp.* (98) [AB681449.1]	Uncultured bacterium (99) [EF459884.1]
S3	1a (JX036290)	*Hydrogenophaga sp.* (97) [EF179863.1]	Uncultured bacterium (99) [EF459884.1]
	1b (JX036291)	*Brachymonas petroleovorans* (94) [AY275432.1]	Uncultured bacterium (95) [FN429400.1]
	1c (JX036292)	*Nitrospira sp.* (97) [AJ224041.1]	Uncultured bacterium (98) [JQ003189.1]

Table.5 continued..

Sample	DGGE band code (accession no.)	Closest cultivable species (% identity) [accession no]	Maximum identity to GenBank (% identity) [accession no.]
S4	M1 (JX036293)	*Bacillus thioparans* (97) [JN999834.1]	*Bacillus thioparans* (97) [JN999834.1]
	M2 (JX036294)	*Thalassospira sp.* (99) [EU440812.1]	*Thalassospira sp.* (99) [EU440812.1]
	M3 (JX036295)	*Thalassospira sp.* (92) [EU440812.1]	*Thalassospira sp*. (92) [EU440812.1]
	M4 (JX036296)	*Thalassospira sp*. (99) [EU440812.1]	*Thalassospira sp.* (99) [EU440812.1]
S5	C1 (JX036297)	*Sphingomonas panni* (97) [HQ739092.1]	Uncultured bacterium (97) [GQ158668.1]
	C2 (JX036298)	*Paracoccus sp.* (99) [681877.1]	Paracoccus sp. (99) [681877.1]
	C3 (JX036299)	*Paracoccus pantotrophus* (96) [JQ246875.1]	Uncultured bacterium (96) [JF189197.1]
	C4 (JX036300)	*Paracoccus sp.* (99) [JN681877.1]	*Paracoccus sp.* (99) [JN681877.1]
S6	A1 (JX036301)	*Brachymonas petroleovorans* (97) [AY275432.1]	Uncultured bacterium (98) [EF459884.1]
	A2 (JX036302)	*Sulfurospirillum sp.* (94) [AF357199.1]	Uncultured bacterium (98) [FJ469320.1]
	A3 (JX036303)	*Pelomonas puraquae* (94) [JQ660112.1]	Uncultured bacterium (95) [AB240287.1]
	A4 (JX036304)	*Thauera sp.* (97) [AY570693.1]	Uncultured Thauera sp. (97) (JN648270.1)
S7	a1 (JX036305)	*Pelomonas saccharophila* (90) [AB681917.1]	Uncultured bacterium (91) [AB240287.1]
	a2 (JX036306)	*Pelomonas puraquae* (93) [JQ660112.1]	Uncultured bacterium (91) [AB240287.1]
	a3 (JX036307)	*Paracoccus sp.* (99) [JQ691539.1]	Uncultured bacterium (99) [HM314606.1]
	a4 (JX036308)	*Propionibacterium sp.* (99) [AB084066.1]	Uncultured organism (99) [HQ749197.1]
S8	b1 (JX036309)	*Paracoccus sp.* (99) [JQ691539.1]	*Paracoccus sp.* (99) [JQ691539.1]
	b2 (JX036310)	*Staphylococcus sp*. (97) [JQ361085.1]	Uncultured bacterium (97) [JF199400.1]
	b3 (JX036311)	*Staphylococcus sp.* (97) [JQ361085.1]	Uncultured bacterium (97) [JF199400.1]
	b4 (JX036312)	*Thermoanaerobacter brockii* (100) [HE601764.1]	Uncultured bacterium (100) [JQ612532.1]
	b5 (JX036313)	*Propionibacterium sp.* (99) [AB084066.1]	Uncultured organism (99) [HQ749197.1]
S9	c1 (JX036314)	*Enterococcus sp.* (94) [JQ028132.1]	Uncultured bacterium (95) [HM821143.1]
	c2 (JX036315)	*Bacillus sp.* (98) [AY159884.1]	*Bacillaceae* bacterium (98) [DQ490410.1]
	c3 (JX036316)	*Bacillus circulans* (95) [JQ246876.1]	*Bacillus circulans* (95) [JQ246876.1]
	c4 (JX036317)	*Propionibacterium sp.* (99) [AB084066.1]	Uncultured bacterium (99) [JF185156.1]
S10	d1 (JX036318)	*Chryseobacterium sp.* (95) [HQ396899.1]	*Chryseobacterium sp.* (95) [HQ396899.1]
	d2 (JX036319)	Uncultured bacterium (91) [JF208716.1]	Uncultured bacterium (91) [JF208716.1]
	d3 (JX0363120)	*Lysobacter sp.* (97) [EU780693.1]	Uncultured bacterium (97) [DQ532277.1]
	d4 (JX036309)	*Propionibacterium sp.* (99) [AB084066.1]	Uncultured organism (99) [HQ749197.1]

Fig.1 DGGE patterns of the PCR products amplified from primers GC Clamp and COM- 2. Lane 1- S1, Lane 2-S2, Lane 3- S3, Lane 4- S5, Lane 5- S4, Lane 6- S9, Lane 7-S10, Lane 8- S7, Lane9 -S8, Lane10 - S6

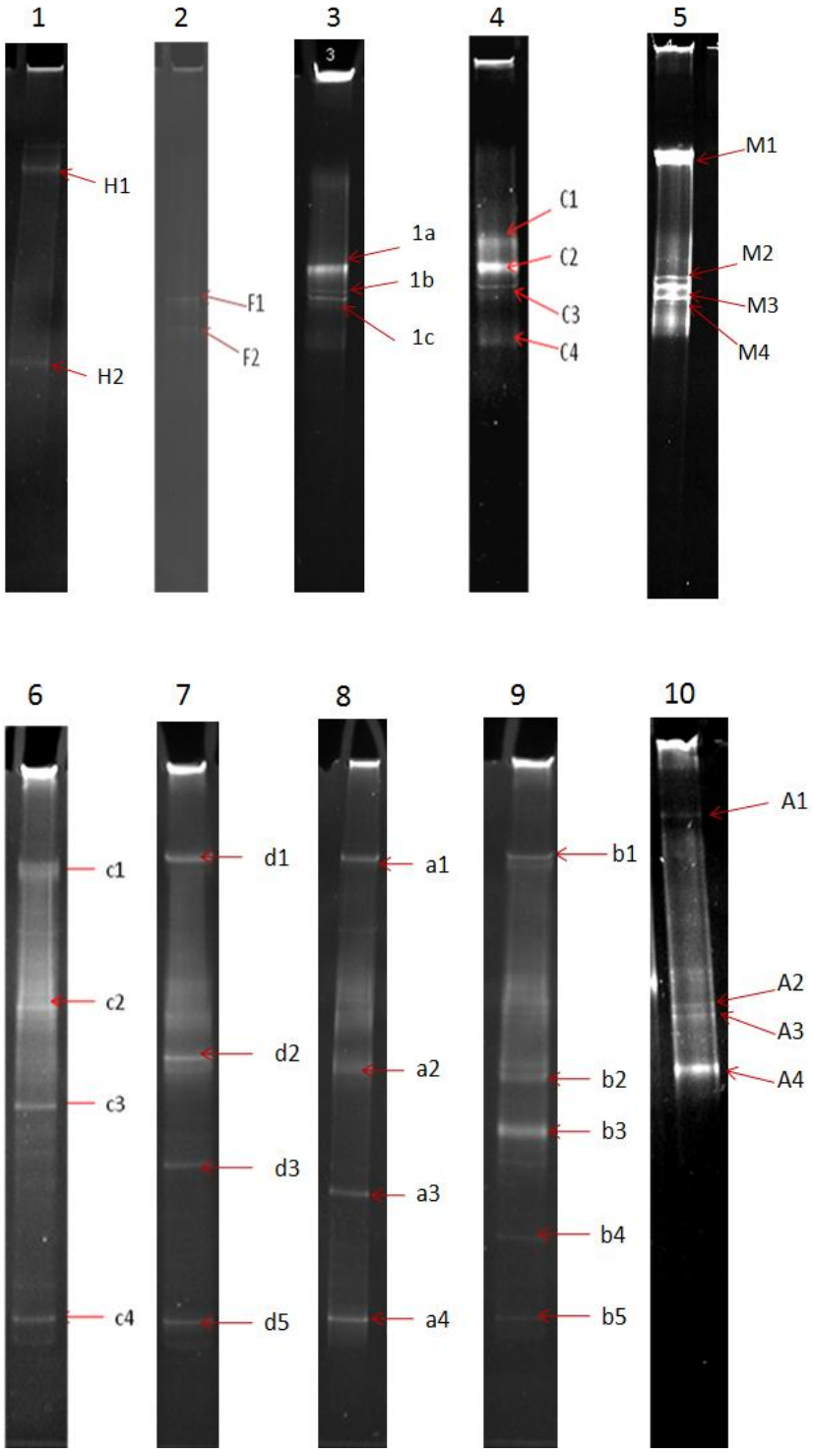
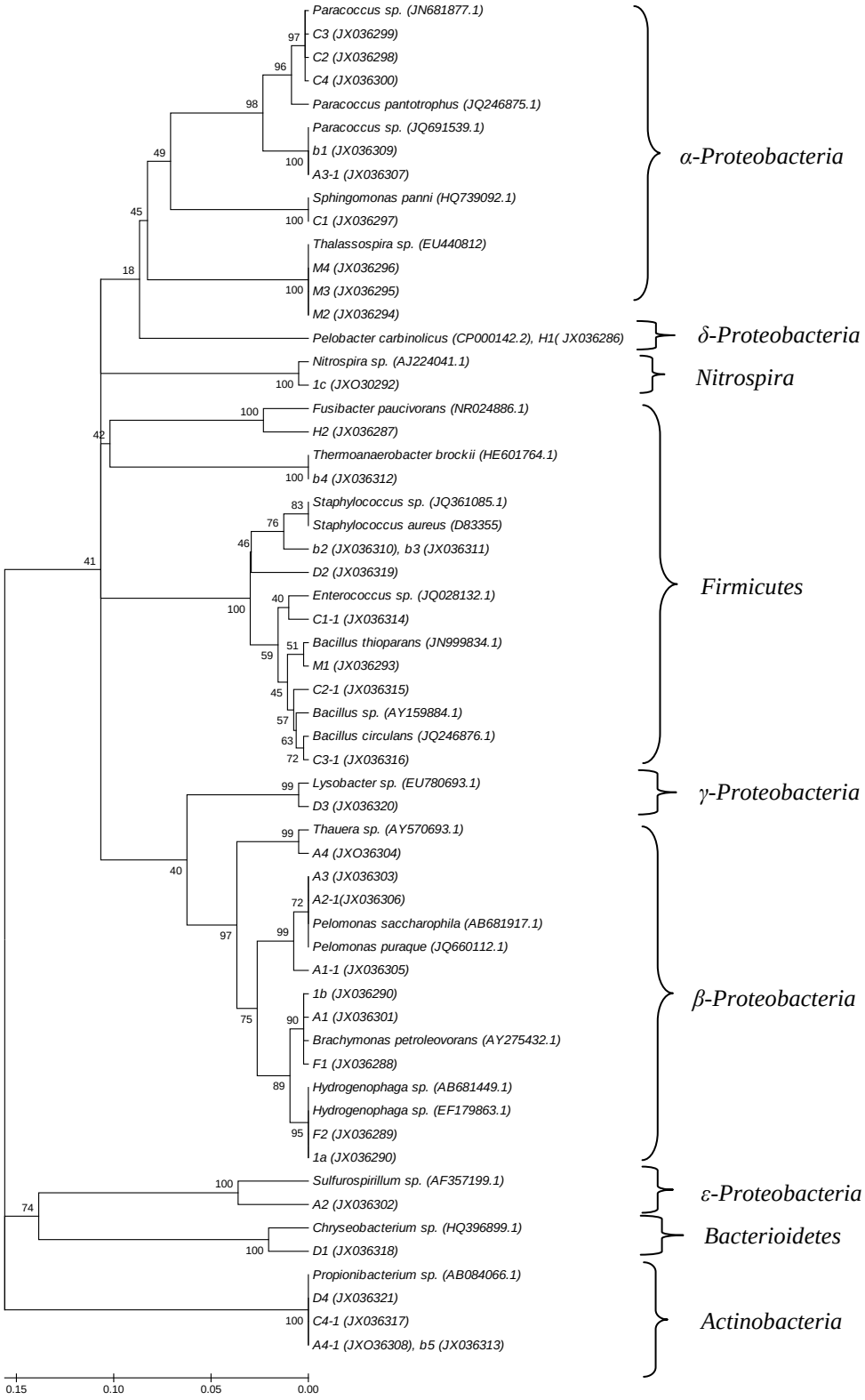


Fig.2 Phylogenetic tree demonstrating the relationship of the thirty six predominant band sequences based on 16S rRNA gene V3-V5 region from the petroleum reservoir with other sequences obtained from Blast searches. The scale bar represents number of substitutions per site.



In bacterial community analysis of the Huabei Oilfield China, in a clone library, few OTUs were related to *Hydrogenophaga, Sphingomonas* and *Brachymonas* species (Tang *et al*., 2012). In the study of composition of bacterial communities in oil reservoirs from a southern offshore Brazilian basin, 16S rRNA gene clones were related to the genera *Propionibacterium, Bacillus* etc. (Sette *et al*., 2007). Bacterial phylotypes detected by PCR-DGGE of high temperature oil reservoir contained the sequence related to *Thermoanaerobacter brockii* (Lan *et al*., 2011). *Thauera sp*. was reported to be toluene degrader under denitrifying conditions (Van Ham *et al*., 2003). *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus sp.* were isolated from hydrocarbon-polluted site in Ilaje, Ondo state, Nigeria (Boboye *et al*., 2010). Sequence of sulfate reducing genus *Nitrospira* was detected in 16S rRNA clone library of Hubei oil field, China (Li *et al*., 2006). Two novel species of genus *Thalosospira* were isolated from surface water of waste oil pool (Liu *et al*., 2007). *Sulfurospirillum sp*. was isolated from up-flow, packed-bed bioreactors inoculated with water produced from an oil field (Hubert and Voordouw, 2007). *Paracoccus sp*. was isolated from *Microcoleus* consortium and was able to grow in the extreme conditions such as acid and alkaline pH, high salinity, high and low temperature, and in the presence of petroleum and heavy metals like Cu and Pb (Diestra *et al*., 2007). *Pelomonas sp*. was found to degrade benzene anaerobically (Weelink, 2008). Carazole degradaing *Lysobacter sp.* was isolated from onshore and freshwater sites (Maeda *et al.*, 2010). As all these bacteria are living in the extreme habitats they are of prime importance in metagenomics study.

In the present study, molecular analysis is used to characterize the diversity of bacterial community in the formation water from petroleum reservoirs of western India. Analysis showed four major bacterial classes: Firmicutes, gamma-proteobacteria, beta-proteobacteria and actinobacteria, revealing a great microbial diversity in these formation water samples. Sequence analysis of excised DGGE bands revealed phylogenetic relationships with 16S rRNA gene sequences from many microorganisms previously isolated or identified from petroleum or soil contaminated with petroleum habitats. Further studies are necessary in order to isolate functional bacteria and promote the applications in microbial enhanced oil recovery as well as in bioremediation. Although the level of efforts expended in this study did not profoundly sample the bacterial diversity, we can conclude that it is sufficient to understand the types of bacterial communities in western India oil fields which are otherwise not reported anywhere.

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