International Biodeterioration & Biodegradation 114 (2016) 164–175

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Microbial communities responsible for fixation of CO_2 revealed by using *mcrA*, *cbbM*, *cbbL*, *fthfs*, *fefe-hydrogenase* genes as molecular biomarkers in petroleum reservoirs of different temperatures

Jin-Feng Liu [a], Serge Maurice Mbadinga [a], Xiao-Bo Sun [a], Guang-Chao Yang [a], Shi-Zhong Yang [a], Ji-Dong Gu [b, **], Bo-Zhong Mu [a, *]

[a] State Key Laboratory of Bioreactor Engineering and Institute of Applied Chemistry, East China University of Science and Technology, Shanghai 200237, PR China
[b] School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, China

ARTICLE INFO

Article history:
Received 14 February 2016
Received in revised form
29 June 2016
Accepted 29 June 2016
Available online 7 July 2016

Keywords:
Microbial community
CO_2 fixation
Bioconversion
Methanogenesis
Functional gene
Oil reservoir

ABSTRACT

Sequestration of CO_2 in oil reservoir is one of the feasible options for mitigating atmospheric CO_2 building up. The *in situ* bioconversion of sequestrated CO_2 to methane by microorganisms inhabiting oil reservoirs is feasible. To evaluate the potential of *in situ* microbial fixation and conversion of CO_2 into CH_4 in oil reservoirs, a comprehensive molecular survey was performed to reveal microbial communities inhabiting four oil reservoirs with different temperatures by analysis of functional genes involved in the biochemical pathways of CO_2 fixation and CH_4 synthesis (*cbbM*, *cbbL*, *fthfs*, *[FeFe]-hydrogenase* encoding gene, and *mcrA*). A rich diversity of these functional genes was found in all the samples with both high and low temperatures and they were affiliated to members of the *Proteobacteria* (*cbbL* and *cbbM*, *fthfs*), *Firmicutes* and *Actinobacteria* (*fthfs*), uncultured bacteria (*[FeFe]*-hydrogenase), and *Methanomirobiales*, *Methanobacteriales* and *Methanosarcinales* (*mcr*A). The predominant methanogens were all identified to be hydrogenotrophic CO_2-reducing physiological types. These results showed that functional microbial communities capable of microbial fixation and bioconversion of CO_2 into methane inhabit widely in oil reservoirs, which is helpful to microbial recycling of sequestrated CO_2 to further new energy in oil reservoirs.

1. Introduction

Storage of CO_2 in deep geological formations, such as oil reservoirs, is one of the feasible solutions to reduce CO_2 emissions and further build up in the atmosphere. It is of great scientific interest and significance to understand the fate of CO_2 in the subsurface environment, which has received increasing attention due to the fact that abundant microorganisms inhabit these formations, and microbial fixation and conversion of the sequestered CO_2 into CH_4 are highly feasible.

As to the microbial involvement in CO_2 fixation, six autotrophic CO_2 fixation biochemical pathways have been documented, of which the Calvin–Benson–Bassham (CBB) cycle plays an important role in autotrophic CO_2 fixation in nature (Berg, 2011). The CBB cycle was known to occur in *Proteobacteria*, in some members of *Firmicutes*, *Actinobacteria* and *Chloroflexi* as well as in plants, algae and cyanobacteria (Berg et al., 2005; Caldwell et al., 2007; Ivanovsky et al., 1999; Lee et al., 2009; Zakharchuk et al., 2003). Another important pathway of CO_2 fixation is the reductive acetyl-CoA pathway that has been documented to occur in acetogenic prokaryotes, ammonium-oxidizing *Planctomycetes* (Strous et al., 2006), sulfidogenic bacteria (Schauder et al., 1988), and in autotrophic archaea affiliated with the order *Archaeoglobales* (Vorholt et al., 1997; Vornolt et al., 1995). This pathway is also utilized by acetogenic prokaryotes for energy conservation (Biegel and Muller, 2010; Ragsdale and Pierce, 2008; Thauer et al., 2008). Petroleum reservoirs are known to harbor diverse microorganisms including bacteria such as *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Chloroflexi* and archaea such as methanogens and *Archaeoglobales* (Li et al., 2010, 2011; Magot et al., 2000; Mbadinga et al., 2012; Wang et al., 2011) and they are expected to use effective means

* Corresponding author.
** Corresponding author.
E-mail addresses: jdgu@hku.hk (J.-D. Gu), bzmu@ecust.edu.cn (B.-Z. Mu).

http://dx.doi.org/10.1016/j.ibiod.2016.06.019
0964-8305/

for CO_2 fixation and bioconversion.

To investigate whether oil reservoirs have the potential capability of CO_2 fixation and conversion of CO_2 into CH_4, and to have a better knowledge on microorganisms involved in this process, studies from a viewpoint of functional genes are necessary. Functional genes involved in CO_2 fixation and conversion into CH_4 have been shown to be valuable biomarkers for detecting members in the microbial communities in both environments and enrichment cultures. The genes *cbbL* and *cbbM* respectively encoding the key enzymes ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) form I and II of the CBB cycle for CO_2 fixation have been applied to study microbial communities from hydrothermal vents of the Logatchev field (Hugler et al., 2010). The gene *fthfs* encoding formyltetrahydrofolate synthetase, a key enzyme in the reductive acetyl-CoA pathway, has been used to investigate the diversity of homoacetogenic bacteria in thermophilic and mesophilic anaerobic sludge (Ryan et al., 2008). Methyl-coenzyme M reductase (*mcr*) is vital for CH_4 formation, and the α-subunit of MCR (*mcrA* gene) is commonly used for the detection of specific groups of methanogens (Juottonen et al., 2006). In addition, H_2 should be supplied in the process of CO_2 bioconversion into CH_4. However, H_2-producing bacteria are polyphyletic. *[Fe-Fe]*-hydrogenases are known to catalyze H_2 production in fermentative microorganisms. Thus, gene encoding for *[Fe-Fe]*-hydrogenases represent a useful marker gene for the detection of H_2-producing anaerobes (Schmidt et al., 2010).

Microbial fixation of CO_2 and conversion of CO_2 to CH_4 in oil reservoirs is becoming increasingly recognized. Although the potential of *in situ* microbial conversion of sequestered CO_2 into CH_4 by hydrogenotrophic methanogens has been observed in a laboratory test by Sugai et al. (2012) and some studies on the effects and feasibility of CO_2 injection and storage in a deep saline aquifer was examined (Wandrey et al., 2011), information on the functional microbial communities involved in microbial fixation and conversion of CO_2 into CH_4 in different oil reservoirs is still very limited. The objective of this study was to evaluate the potential of *in situ* microbial fixation and conversion of CO_2 into CH_4 in subsurface oil reservoirs through analysis of functional genes (*cbbM*, *cbbL*, *fthfs*, *[FeFe]-hydrogenase* encoding gene and *mcrA*) by characterization of the functional microbial communities involved in this process which inhabited in the production waters.

2. Material and methods

2.1. Sampling sites and production water samples

Production water samples were collected from four oil fields in China with different temperatures, including Kelamayi Oilfield (XJ6, 21 °C; XJ7: 32 °C), Huabei Oilfield (45 °C), and Daqing Oilfield (90 °C). Five liters of production water samples from each production oil well were collected directly from the production valve of the pipeline at the well head into sterile bottles after initial flushing for 10–15 min. The sterilized bottles were completely filled with oil/water mixture, tightly sealed, cooled with chilled water and immediately transported back to laboratory within 4 h for treatment. The physicochemical properties of these production waters from these oilfields are listed in Table 1. Aliquot, after separation oil from the oil/water mixture, was used to concentrate the microbial cells directly through filtration (membrane filter 0.2-μm-pore-size).

2.2. DNA extractions

Microbial biomass in the oil/water samples was concentrated onto membrane filters as described above and previously by Wang et al. (2012). Total genomic DNA was extracted from 2.0 L of production water samples using AxyPrep™ Bacterial Genomic DNA Miniprep Kit (Axygen Biosciences, Inc., CA, USA) according to the manufacturer's DNA Miniprep spin protocol after concentration onto membrane filters. The genomic DNAs obtained were purified with a DNA purification kit (U-gene, China) according to the manufacturer's instructions. The extracted DNAs were stored at −20 °C until PCR amplification of functional genes.

Table 1
The physicochemical properties of production water samples from the oilfields of this study.

Parameter	Xinjiang J6	Xinjiang J7	Hubei	Daqing
Depth (m)	480.0	1088.0	1101.0	1500.0
Temperature (°C)	21.0	32.0	45.0	90.0
pH	7.0	7.1	7.2	6.0
Salinity (mg L^{-1})	15,728.0	4212.0	4091.0	3900.0
Cl^- (mg L^{-1})	5336.0	2000.0	819.0	1914.0
SO_4^{2-} (mg L^{-1})	124.8	7.7	32.4	731.3
PO_4^{3-} (mg L^{-1})	ND	ND	ND	ND
NO_3^- (mg L^{-1})	0.8	1.4	ND	ND
K^+ (mg L^{-1})	35.1	45.6	22.3	6.3
Na^+ (mg L^{-1})	4196.0	5399.0	1064.0	1110.0
Ca^{2+} (mg L^{-1})	103.3	128.2	53.0	97.9
Mg^{2+} (mg L^{-1})	44.7	64.0	17.6	9.2
Mn^{2+} (mg L^{-1})	0.3	0.4	0.1	ND
formate (mg L^{-1})	ND	ND	ND	ND
acetate (mg L^{-1})	344.0	7.0	57.9	56.2
Propionate (mg L^{-1})	ND	ND	ND	ND
Butyrate (mg L^{-1})	ND	ND	0.5	ND
Isobutyrate (mg L^{-1})	32.7	ND	ND	4.9
Oil density (ground), g/cm^3	0.912	0.867	0.907	0.858
Oil viscosity (ground), mPa.s	80	5.6	198	3.3

Xinjiang, Huabei and Dqqing, XJ, HB, DQ; ND, Not Detected.

2.3. PCR amplifications

Amplifications of the *cbbL* gene fragment (771 bp) and the *cbbM* gene fragment (328 bp) were carried out under the conditions according to Campbell and Cary (2004). For amplification of a portion (1102 bp) of the *fthfs* gene, the PCR conditions used were those described previously by Leaphart and Lovell (2001). For amplification of a fragment (620 bp) of *[Fe-Fe]*-hydrogenase encoding gene, the primer set HydH1f/HydH3r was applied using the conditions described by Oliver Schmidt et al. (2010). A fragment (470 bp) of the *mcrA* genes was amplified using the primer set MLf/MLr (Luton et al., 2002), applying the conditions as reported previously (Galand et al., 2005). The primer information used for PCR is summarized in Table 2. Functional gene fragments were all amplified in five parallel PCR reactions in a Peltier thermal cycler (Bio-Rad, USA), which was subsequently pooled for cloning exercises and construction of the respective gene libraries.

2.4. Construction of functional genes clone libraries

The amplified and pooled PCR products were gel-purified using the Gel Extraction Kit (U-gene, China) and then cloned into *Escherichia coli* using a pMD19®-T simple vector kit (Takara, Japan) following the instructions of the manufacturer. For each gene clone library, the white colonies obtained were randomly picked and cultured overnight at 37 °C in 0.8 mL Luria broth (LB) medium supplemented with ampicillin (50 mg L^{-1}). The inserted DNAs were amplified by using M13-47 (5′-CGCCAGGGTTTTCCCAGTCACGAC-3′) and RV-M (5′-GAGCGGATAACAATTTCACA CAGG-3′) primers targeting the flanking vector sequence, followed by agarose gel electrophoresis with ethidium bromide staining (Guan et al., 2013).