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Quantitative significance of functional genes of methanotrophs and propanotrophs in soil above oil and gas fields, China

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ABSTRACT

To assess whether the anomalies of methanotrophic pmoA and propanotrophic prmA genes as target genes can reflect the existence of underlying oil and gas reservoirs, natural abundance and distribution of target genes were investigated in soil above known gas fields, oil fields and non-oil and gas fields, China. A total of 45 soil samples were collected from different depths of 2.5-m soil profiles in Xiaoquan gas field, Xiliu oil field and Beiguan non-oil and gas field and from 50 cm depth of the old oil area, the new developed area, the oil producing area and the undeveloped area of Xiliu oil field, respectively for analysis of target genes by SRBR Green-based fluorescent quantitative real-time polymerase chain reaction (RT-PCR) technique. Distributions of target gene contents in 2.5-m soil profiles of the three fields show that 50 cm was considered to be optimal sampling depth for microbial prospecting of oil and gas reservoirs. At 50 cm depth of 2.5-m soil profiles, the gas field had very high contents of methanotrophic pmoA gene (3.8×10^5 copies/g dw) and propanotrophic prmA gene (5.92×10^5 copies/g dw), the oil field had medium contents of methanotrophic pmoA gene (2.8×10^4 copies/g dw) and propanotrophic prmA gene (4.1×10^4 copies/g dw), and the non-oil and gas field had low contents of propanotrophic prmA gene (below detection limit) and relatively high contents of methanotrophic pmoA gene (1.26×10^5 copies/g dw) due to the influence of biogenic methane. The anomalies of methanotrophic pmoA gene and propanotrophic prmA gene can reflect the existence of underlying gas deposits. Different areas of the oil field had different target gene contents in the new developed area ($(1.7–2.3) \times 10^6$ copies/g dw for the prmA gene, 1.0×10^3–1.2×10^4 copies/g dw for the pmoA gene), the old oil area (below detection limit -2.0×10^6 copies/g dw for the prmA gene, below detection limit -7.1×10^4 copies/g dw for the pmoA gene), the undeveloped area (1.8×10^5–1.5×10^6 copies/g dw for the prmA gene, $5.3 \times 10^4 - 3.3 \times 10^5$ copies/g dw for the pmoA gene) and the oil producing area (below detection limit -1.0×10^6 copies/g dw for the prmA gene, 1.4×10^5–4.8×10^5 copies/g dw for the pmoA gene). Comparisons of target gene contents in different areas of Xiliu oil field indicate that oil productive activities had a large impact on contents of target genes in soil above the oil field. The anomalies of propanotrophic prmA gene with low contents of methanotrophic pmoA gene can reflect the existence of underlying oil reservoirs. Analysis of the relationships between contents of target genes may be used for identification of gas fields, oil fields and the influence of biogenic methane. Our results suggest that quantitative RT-PCR assay-based methanotrophic pmoA gene and propanotrophic prmA gene anomalies can reflect the existence of underlying oil and gas reservoirs.

1. Introduction

A large number of studies and exploration activities have demonstrated that microbial anomalies in soil can reflect the presence of subsurface oil and gas reservoirs (Tucker and Hitzman, 1994; Wagner et al., 2002; Rasheed et al., 2008; Yuan et al., 2009). These microorganisms are mainly referred to as methanotrophs and C_2–C_4 hydrocarbon-oxidizing bacteria including ethanotrophs, propanotrophs and butanotrophs. Methanotrophs can utilize methane as the sole source of carbon and energy for their growth and metabolism (Theisen and Murrell, 2005). They are generally not able to consume sugar (glucose) and C_2–C_4 hydrocarbons. Since methane is the most abundant in the microseepage hydrocarbon, and methanotrophs have strong specificity to substrates, methanotrophs are often taken as indicator bacteria for prospecting of oil and gas reservoirs.

In contrast to methanotrophs, ethanotrophs, propanotrophs and butanotrophs can utilize not only C_2–C_4 hydrocarbons, but

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also monosaccharides and polysaccharides as carbon and energy sources, but they are usually not able to metabolize methane (Wagner et al., 2002). C_2–C_4 hydrocarbons are not generated biogenically and their sole source is subsurface oil and gas reservoirs (Chan, 2011). A constant supplement of C_2–C_4 hydrocarbons from oil and gas reservoirs would result in the anomalies of ethanotrophs, propanotrophs and butanotrophs in soil overlying reservoirs. Therefore, they are also often taken as indicator bacteria for prospecting of oil and gas reservoirs.

The detection methods available for indicator bacteria are all conventional cultivation-dependent methods (Schumacher, 1999; Wagner et al., 2002; Rasheed et al., 2012; Veena Prasanna et al., 2013). Use of molecular biology technology for detection of indicator bacteria is development trends in microbial prospecting for oil and gas (Zhang et al., 2013; Wu et al., 2013), of which quantitative real-time polymerase chain reaction (RT-PCR) technique is a powerful technique for rapid quantifying environmental microorganism (Phrommanich et al., 2009). The technique detects microbial contents from environmental samples by detection of 16S rRNA genes or functional marker genes (Kolb et al., 2003; Connor et al., 2005; Rinta-Kanto et al., 2005). Methanotrophic pmoA gene encodes the enzymes of particulate methane monooxygenase (pMMO) (Himes and Karlin, 2009), and propanotrophic prmA gene encodes the large hydroxylase subunit of propane monooxygenase (Chan, 2011). The pmoA gene has been widely used for quantifying methanotrophs in soil samples (Krause et al., 2009; Akiyama et al., 2011). PCR primers for detection of the prmA gene have been reported in the literature (Chan, 2011), which make it possible for quantifying propanotrophs in soil samples. The quantitative RT-PCR technique has been applied for quantifying indicator bacteria (Zhang et al., 2010; Miqueletto et al., 2011). However, little studies systemically examine relationships between quantitative RT-PCR assay-based indicator bacteria anomalies and the presence of subsurface oil and gas reservoirs, especially less on quantifying propanotrophic prmA gene.

The objective of this study was to systemically investigate natural abundance and distribution of methanotrophic pmoA and propanotrophic prmA gene as target genes in known gas fields, oil fields and non-oil and gas fields as well as in the old oil area and the new developed area, the oil producing area and the undeveloped area of the oil field using SRBR Green-based fluorescent quantitative RT-PCR technique to assess whether the anomalies of target genes can reflect the presence of underlying oil and gas reservoirs.

2. Materials and methods

2.1. Site description

Sampling sites are located in Xiaoquan (XQ) gas field (31°14′51″ N, 104°19′38″ E), Xiliu (XL) oil field (38°40′50″N, 115°50′52″E) and Beiguan (BG) non-oil and gas field (38°09′52″N, 114°34′27″ E), China (Fig. 1). Xiaoquan gas field is in the southwest of China with a mean annual temperature of 16 °C and rainfall of 893 mm, being of one of the western Sichuan gas fields. Gas-bearing formations are mainly upper Triassic and middle-upper Jurassic sandstone layers. Methane contents in natural gas range from 90.11% to 97.86%, ethane ranging from 0.51% to 4.97%, propane ranging from 0.04% to 1.36% and other alkane being less than 0.5%. The genetic type of the gas reservoir is of typical coal-type gas.

Xiliu oil field is situated in northern China with a mean annual temperature of 12 °C and rainfall of 515 mm, belonging to one of the North China oil fields. Oil-bearing formations are mainly Lower Tertiary sandstone layers. Crude oil had characteristics of high viscosity, high density, high asphaltene with a mean viscosity of 680 mPa s, density of 0.927 g/cm^3, asphaltene contents of 47.4%, belonging to medium crude oil. Oil was firstly struck in 1985. Annual oil production amount was about 2×10^5 t in 2009.

Beiguan non-oil and gas field lies about 150 km to the southwest of Xiliu oil field. It is situated in the middle-upper part of the Hutuo River alluvial fan, Taihang Mountain Piedmont. There is no oil producing well in the surrounding of sampling sites.

2.2. Sample collection

45 Soil samples were collected from Xiaoquan gas field, Xiliu oil field and Beiguan non-oil and gas field by Luoyang shovels and manual drilling. Among these samples, 16 soil samples were collected from different depths of two 2.5-m soil profiles of Xiaoquan gas field and Xiliu oil field. Sampling sites were located in farmland where there were 50 m away from gas or oil pumping wells in order to avoid ground crude oil contamination, sampling depth being 5, 20, 40, 50, 100, 150, 200 and 250 cm for each of the soil profiles. The 2.5-m soil profile in Xiaoquan gas field had lithology of clay at 100 and 150 cm depth with high water contents of 31.8% and 32.74%, respectively; medium coarse sand occurring at 200 and 250 cm and other horizontal layers being of silty clay layers. The 2.5-m soil profile, located in the old oil area, in Xiliu oil field had relatively uniform lithology with silty clay and clayey loam; silty clay occurring at 50 cm depth. 22 Soil samples were separately taken from 50 cm depth in the old oil area and the new developed area (12 soil samples), the oil producing area and the undeveloped area (10 soil samples) of Xiliu oil field along east to west sampling lines. Each of the sampling sites was 50 m away from the corresponding oil pumping well for the A–B sampling line, and sampling intervals were all 250 m for the C–D sampling line (Fig. 1). 7 Soil samples were collected from different depths of 2.5-m soil profiles located in farmland of Beiguan non-oil and gas field, the sampling depth being 5, 20, 50, 100, 150, 200 and 250 cm. The soil profile had relatively coarser grain than that of Xiliu oil field, silt being present at 50 cm depth and other horizontal layers

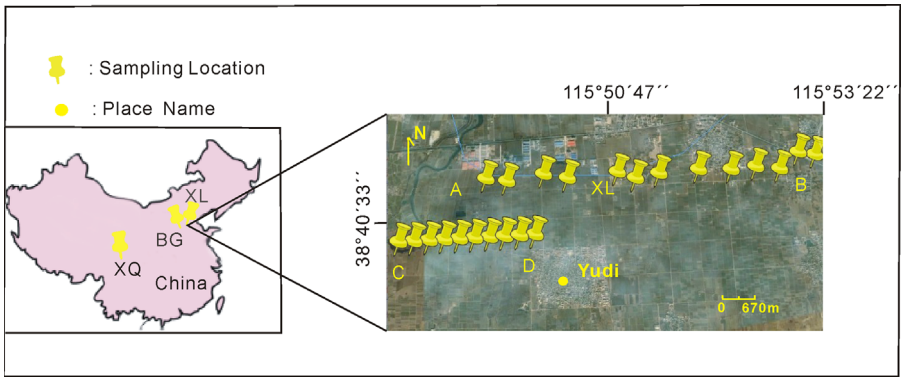

Fig. 1. Location of sampling sites.